Naoyuki Taniguchi · Tamao Endo
Jun Hirabayashi · Shoko Nishihara
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Kiyoko F. Aoki-Kinoshita *Editors*

Glycoscience: Basic Science to Applications

Insights from the Japan Consortium for Glycobiology and Glycotechnology (JCGG)

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This book is dedicated to the late Professor Tamio Yamakawa, one of the pioneers of glycosphingolipid who passed away on October 7, 2018, at the age of 96, and the late Professor Yoshitaka Nagai who was the founding president of JCGG.

Preface

It is our great pleasure and honor to have this opportunity to publish a book entitled *Glycoscience: Basic Science to Applications Subtitle: Insights from the Japan Consortium for Glycobiology and Glycotechnology* (JCGG).

As you may know, glycosylation is one of the major posttranslational modifications of proteins, and this research area designated as glycoscience which focuses on elucidation of structure and function of glycans is one of the important fields in life science and material science as well. Many, but not all, scientists, students, stakeholders, persons in funding agencies, and company persons feel glycoscience is important in life science area, but due to the complexity and diversity of glycans, they feel it is difficult or not easy to understand. As compared to genome and proteome research, however, most of the people feel difficulty in pursuing glycoscience or glycome research because glycans are so heterogeneous and diverse and contain many multiple-branching structures. Moreover, at present, there is still no simple method for amplification like PCR or synthetic apparatus such as DNA sequencer or peptide synthesizer, which also makes most of the researchers feel complicated.

Therefore, it is our responsibility as researchers involved in glycoscience to try to explain and inform more easily to those people about the present situation and the future problems regarding basic science and applied science including medical science.

In 2010, the National Academy of Sciences, USA, published an excellent proposal designated as *Transforming Glycoscience: A Roadmap for the Future* and gave a strong impact on glycoscientists and stakeholders and funding agencies including NIH. In Japan, however, many glycoscientists and glycochemists are actively involved in glycoscience and glycotechnology, and in 2003, the Japan Consortium for Glycoscience and Glycotechnology (JCGG) was established in order to exchange scientific information among glycoscientists as well as other fields of research in academia and research institutes and companies in Japan. I would like to take this opportunity to explain about the JCGG which was founded 13 years ago. Instead of receiving direct official support from the Japanese government for this operation, scientists took the initiative for creating and supporting the consortium by using their own existing governmental research grants and support

from donation from JCGG members, industries, and public foundations. The activity of JCGG is to organize annual JCGG symposium on glycoscience. JCGG is a nonprofit organization supported by glycoscientists, companies, research foundations, etc., and 3 years ago, we have established the Tamio Yamakawa Award, which awards internationally renowned scientists. Last year, we had the 16th JCGG symposium in Tokyo, and JCGG has published a book in Japanese entitled *Glycoscience Creating Future: A Roadmap in Japan*, but it is not commercially available, and its distribution is very limited. This book has been originally planned to be published in Japanese, and this book is just distributed inside of Japan glycoscience community without any commercial purposes.

In this book, we have asked over 125 people in Japan who originally developed his/her research in Japan to sum up the glycoscience in the future to describe the (1) significance in the field of glycoscience and its current situation; (2) impact on other fields of research; (3) significance as fundamental research; (4) possible application for industry and medicine, if any; and (5) future perspectives. We sincerely hope this book will facilitate and motivate the people who are interested in it or may be interested in the future.

We gratefully acknowledge all those who contributed to this book despite their busy schedules. Special thanks are due to Mr. Keiichi Yoshida, the former secretary general of JCGG; Ms. Fumi Ohta, the former technical assistant at Systems Glycobiology Group at RIKEN; and Ms. Keiko Fujikawa, science writer, for their skillful assistance in editing when we published the original reports in Japanese. Thanks also go to editorial staff members at Springer, especially Dr. Sue Lee, Mr. Sivachandran Ravanan, Mr. Balaji Padmanaban and Ms. Coral Zhou, for their continuous support, patience regarding the deadline for the manuscript, and skill in directing the production of this book. We also thank Dr. Milton Feather and Mr. Nicholas Halewood for their English editing of our manuscripts.

The former President of JCGG
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On behalf of all of the editors
Naoyuki Taniguchi MD., PhD.

Introduction

In recent years, great progress has been made in the field of life sciences, particularly the innovation of genome technologies that include next-generation sequencing and genome editing technology. Glycans have long been regarded as the third bio-macromolecules as important as nucleic acids and proteins; however, it is difficult to study glycans because basic infrastructure for their analysis has not yet been well established. However, it can be argued whether we really have gained a complete understanding of complex life system without them. It was at the end of the twentieth century that genome technologies made great advancement, and by early twenty-first century, the entire human genome was sequenced. With the obtained information, the mechanisms of diverse biological phenomena, including development and various diseases, such as cancer, have been elucidated. As a result, new approaches for drug discovery have also been pioneered. At the same time, genome-associated large data have been produced, the analysis and utilization of which will require the use of artificial intelligence, as opposed to human participation. However, more fundamental issues, which are related to cellular mechanisms, remain almost unanswered, considering that most of these events are based on cell–cell recognition involving glycans. In other words, advanced genome science produced a large number of unknown than known. Briefly, cell community-based life systems are elucidated with the combination of genome and glycosciences.

To describe nucleic acids, protein, and glycans, all are composed of a relatively few members of elements (i.e., nucleotides, amino acids, and monosaccharides), which are linked to each other using the same dehydration condensation principle to build up much larger molecules with structural diversity. Unlike the former two chains, glycans have a unique property, which is linkage. This is because two monosaccharides are linked by various kinds of glycoside bonds. Moreover, there are anomers, with hemiacetal as their reaction center. Thus, glycans are often branched at multiple sites, and they can produce an extremely large number of structural diversity (as many as 1.05×10^{12} in cases of six components according to R.A. Laine, Glycobiology, 1994). Such feature is never shared with other biomolecules. Therefore, the biological systems are considered to have two different types of informative molecules: one is rigorous molecular recognition system comprising

nucleic acids and proteins, and the other is a system involving glycans. Obviously, the former system works essentially inside the cell, while the latter works substantially outside of the cell. However, the meaning of individual glycans largely remains to be elucidated, because the selection of particular glycan structure is often made with no particular direction of functionality, but based on random selection, i.e., by-chance theory. However, the physicochemical properties of many glycans include hydration property found in mucin and hyaluronan, and increased solubility of secreted glycoproteins. We are still far from the complete understanding of life systems because of lack of solution methods for "glycocode."

It is noteworthy that glycans usually exist as glyco-conjugates such as glycoproteins and glycolipids. This is closely associated with the fact that both secreted and membranous proteins are biosynthesized in the lumen of endoplasmic reticulum and Golgi apparatus, where series of enzymes involved in glycan synthesis reside and collaborate. However, we realized recently through the discovery of calnexin/calreticulin that protein glycosylation is critical for quality control of these secreted and membranous glycoproteins. The occurrence of glycocalyx is common to all life systems by contributing as a physical barrier to the outside stimuli. However, its profile is unique to individual cell types and states. Thus, it is often utilized as a specific gate for infectious microorganisms and their toxins. Specific contact to cells is made at various levels, e.g., biomolecules, exosomes, bacteria, and viruses, from both endogenous and exogenous origins. Such interactions include free milk oligosaccharides found in mammalian milk. Interestingly, infants cannot utilize most oligosaccharides as energy source, but bacterial flora are thought to metabolize them. Though still speculative, some oligosaccharides may contribute to infection defense and immunity as well as to symbiosis. Thus, the roles of glycans in cell communications are quite diverse and important.

Definitively, genome codes proteins, but their functions largely remain unknown. Consistent with this fact, the number of rare and incurable diseases is increasing. In this context, it is expected that many of these diseases be attributed to impaired glycosylation as in the case of congenital disorder of glycosylation (CDG) and the recently identified NGly1 disease. If this is the case, many other rare diseases may be related but significantly different in glycan metabolism with their curative procedures provided. In a large timescale, such change in solution methods is seen in many areas of social sciences including the environment, energy, education, and food, of which glycoscience has greater concerns than before. In this context, our future goal should be in the same framework as sustainable developmental goals (SDGs), where "game-changing technologies" are required for this achievement.

Under the current situation of glycoscience described above, the book entitled "Glycoscience: Basic Science to Applications: Insights from the Japan Consortium for Glycobiology and Glycotechnology (JCGG)" is edited. They are composed of six parts, referring to European version of glycoscience roadmap. The six parts are Part I: Future Technological Advances to Elucidate the Structures and Functions of Glycans (edited by Tamao Endo). Part II: Glycans and Biopharmaceuticals (edited by Jun Hirabayashi). Part III: Sugar Chains (glycans) Involved in Medical Science and Medical Care (edited by Shoko Nishihara and Naoyuki Taniguchi). Part IV: Food Implicated in Glycans and Its Function (edited by Naoyuki Taniguchi). Part V: Glycan-Related Materials and Their Use for Biomaterials (edited by Kazunari

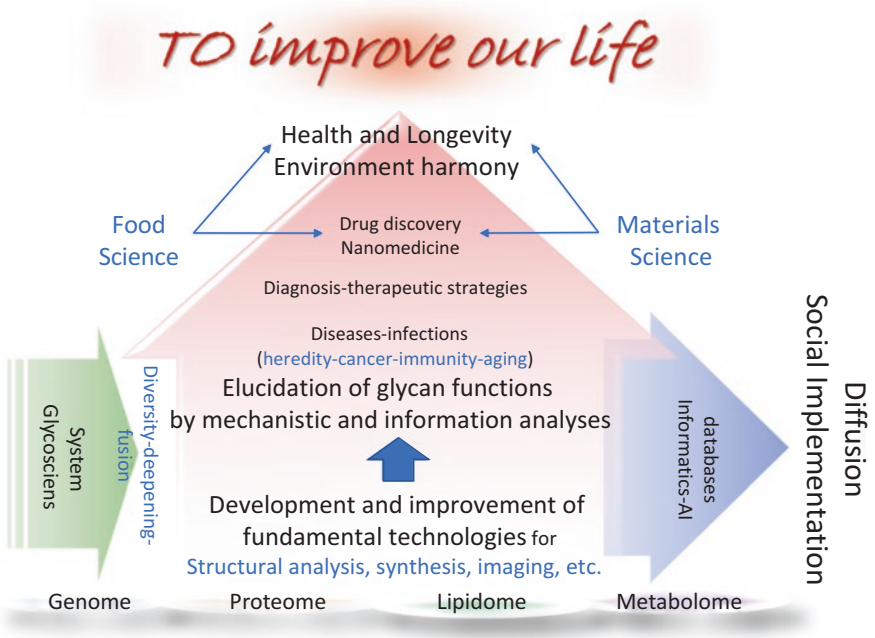

Fig. 1 The concept and future perspectives of glycoscience in our life

Akiyoshi). Part VI: Educational Materials and Training for Glycosciences (edited by Kiyoko F. Aoki-Kinoshita). Each chapter contained in the above six parts is contributed by expert scientists of the field selected by the editors. The expert briefly describes the six themes previously questioned by the editors, which are, (1) significance in the field of glycoscience and its current situation, (2) impact on other fields of research, (3) significance as fundamental research, (4) possible application for industry and medicine, (5) future perspectives, and (6) problems to be solved and years to solve the problem.

As described, range of fields involve glycoscience both in basic and in applied sciences, which include medical sciences represented by drug discovery, regenerative medicine, cancer, life style-related diseases, aging, and rare diseases, and sciences related to materials, food and energy considering the presence of biomass polysaccharides, and the importance of plant-derived starch as food and energy sources. It is a keen issue to develop a worldwide network system to share diverse information derived from the ongoing glycoscience, and further collaborate for better understanding of complex life systems based on cell society by combining all the omics technologies, i.e., genome, proteome, glycome, and lipidome. This finally reaches SDGs for our happiness and welfare. An overall scheme for this goal is illustrated in Fig. 1.

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Contents

Part I
Future Technological Advances to Elucidate the Structures and Functions of Glycans

Foreword

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Glycans are highly associated with various diseases, medicines, foods, and material sciences. However, our understanding and use of the full functions of glycans are far from complete. We already know many important functions and roles of glycans, but such findings are obtained by individual researchers using limited tools and technologies developed by themselves. In other words, at the present time, the study of glycan function requires highly specialized expertise in synthesis and analysis of glycans. On the other hand, fast and reliable methods have been developed in the research fields of DNA and proteins, other biopolymers in our body, and analytical and synthetic technologies for them have been high-throughput automated and then generalized in many laboratories, making it much easier to study genomes and proteins, and to apply their functions to human life. In contrast, general synthetic and analytical methods have not been developed sufficiently in the field of glycoscience. More facile tools and technologies that will allow general researchers, without specialized skills, to study glycoscience. In this chapter, tools and technologies frequently used so far for glycoscience and future expected useful methods will be summarized. We added Boxe(s) for readers to understand their significance in related to Glycoscience and its application which we could not include. Please see page 25 Box 1.1: Lectin Microarray and page 111 Box 4.1: Gene Editing (CRISPR/Cas9).

Chapter 1
Structural Analysis of Glycans (Analytical and Detection Methods)

Kazuki Nakajima, Kazuo Takahashi, Yoshiki Yamaguchi, Yasuro Shinohara, Hiroyuki Kaji, Jun-ichi Furukawa, Akemi Suzuki, Yoshimi Haga, Koji Ueda, Yasuo Suda, Yoshio Hirabayashi, Kiyoshi Furukawa, Kazuo Yamamoto, Toshisuke Kawasaki, and Koichi Honke

1.1 Liquid Chromatography/Mass Spectrometry (LC-MS)

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Keywords Glycoprotein, Glycolipid, Structural characterization

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1. Significance in the field of glycoscience and its current situation
   Liquid chromatography (LC)-mass spectrometry (MS) is a powerful tool used for the separation and structural characterization of glycoproteins and glycolipids. Recent technical developments, such as nano-LC, multistage tandem MS(MS^n), high-resolution MS, and electro transfer dissociation, have enabled the characterization of glycans in small amounts of materials. Glycopeptide analysis provides information regarding the amino acid sequences and attachment sites, and compositions of glycans [1]. In particular, clustered *O*-glycopeptides can be characterized by a combination of ETD-tandem MS [2, 3]. In contrast, structural characterization of glycolipids can be performed using LC-MS systems with repeated high-speed polarity and MS^n switching [4]. Currently, glycomics approaches involving LC-MS have been applied in various medical fields.
2. Impact on the other fields of research
   LC-MS analysis of glycan structural alterations is useful for the development and quality control of new glycoprotein biopharmaceuticals.

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3. Significance as the fundamental research
   LC-MS will contribute to the understanding of the biological importance of glycans. It is desirable to carry out glycomics research using cells or mice harboring altered glycosyltransferase genes. Such research is based on glycan profiling and identification of glycan-carrier proteins, which can clarify functional changes of glycans.
4. Possible application for industry and medicine, if any
   LC-MS analysis of glycans in body systems such as serum and urine is useful for the discovery and validation of glycan-related disease biomarkers.
5. Future perspectives
   Because tissue samples in biopsy sections are heterogeneous, specific pathological cell populations must be isolated before sample analysis. Approaches involving laser microdissection will efficiently reveal glycan structural alterations in diseases. Additionally, mass isotopomer analysis of stable isotope-labeled glycans may be advantageous for systematically assessing glycan turnover, which governs glycan synthetic speed and structural alterations [5].
6. Problems to be solved
   It is necessary to develop an automated program to rapidly identify and quantify glycopeptides. From a long-term perspective, further development of high-sensitive mass spectrometry is expected (Fig. 1.1).

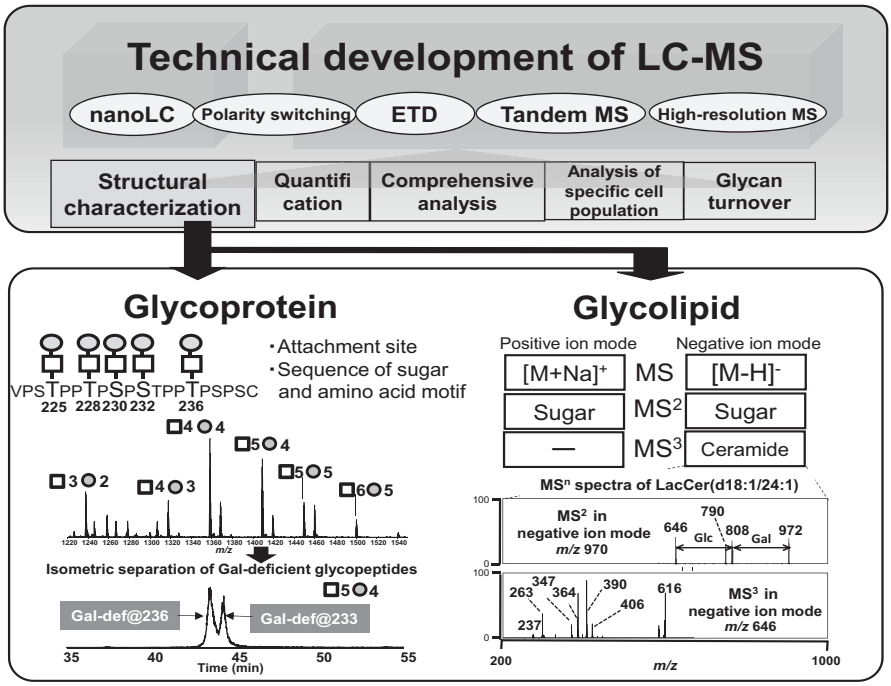

Fig. 1.1 Outline of workflow and information obtained on glycan-based LC-MS/MS analysis. (Left: glycoprotein) MS analysis of *O*-glycopeptides in IgA hinge-regions. This figure is adapted from Kondo et al. [3], with slight modification, with permission. (Right: glycolipid) MS^2 and MS^3 analysis of LacCer (d18:1/24:1) in the negative ion modes. This figure is adapted from Ito et al. [4], with slight modification, with permission from Springer

1.2 Ion Mobility-Mass Spectrometry(IM-MS)

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Keywords Ion mobility, Isomer, Collision cross section (CCS), Theoretical calculation, Mixture

1. Significance in the field of glycoscience and its current situation
   A central issue in glycan mass analysis is the ambiguity of structural assignments due to the heterogeneity and complexity of glycan structures. Although detailed tandem mass analysis does yield the glycan structure, the process is often time-consuming and is not suited for handling many glycan mixtures in glycomics studies. In most cases, therefore, the separation of isomeric glycans has been accomplished by the use of liquid chromatography (LC). Ion mobility mass spectrometry (IM-MS), a technique that separates ions based on their mass, charge, size and shape, has the potential to separate isomeric glycans depending on their unique collisional cross sections. In the last decade, IM-MS has emerged as a powerful alternative for isomer discrimination [6, 7].
2. Impact on the other fields of research
   IM-MS is now applied to glycan samples and other biomolecules including proteins and lipids. Further, IM-MS can be applied not only to identify specific isomers, but can also serve as a separation technique to reduce the complexity of acquired data [8].
3. Significance as the fundamental research
   CCSs acquired on IM-MS analysis are dependent on the size and shape of the ions in the gas phase. Basic research is now in progress to calculate a glycan structure and predict the CCS theoretically [6].
4. Possible application for industry and medicine, if any
   IM-MS will play a significant role in quality control of biopharmaceuticals such as antibodies and erythropoietin. It can also contribute to metabolite analysis and biomarker discovery by simplifying the complex data.
5. Future perspectives
   IM-MS can be coupled to other methods such as LC, which is eminently suitable for the confident and rapid distinction of glycan structures within a defined mixture [6]. More accurate and precise CCS values with higher resolution will pave the way for the analysis of more complex glycan samples.
6. Problems to be solved
   Tools are necessary to identify glycan structures quickly and accurately from a huge amount of IM-MS data. The CCS values of intact and fragmented ions are essential for the identification of glycan structures, and the development of a database with a defined format is necessary [9, 10] (Fig. 1.2).

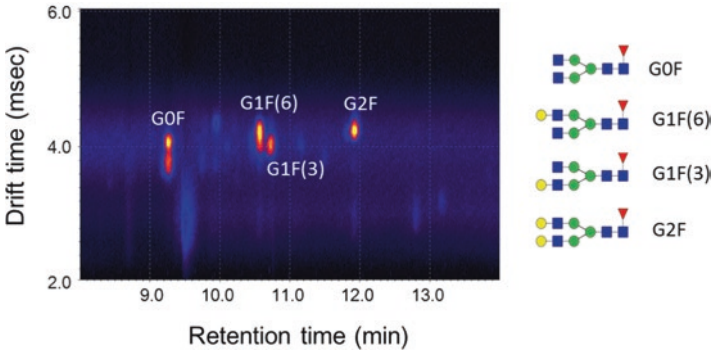

Fig. 1.2 Separation and identification of pyridylaminated glycan mixtures with a tandemly connected LC-IM-MS system. A two dimensional spectrum is shown with reference to retention time (min) and drift time (msec). The spectrum includes all *m/z* data

1.3 Current Status and Future of the Automated Glycan Analysis Technologies

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Keywords Specialized equipment, General purpose equipment, Sensitivity, Throughput, Cost reduction

1. Significance in the field of glycoscience and its current situation
   The development of a glycan sequencer is a demanding task as glycans cannot be amplified like DNA and no database is available to specify structures obtained on MS/MS analysis as is possible, like proteins. On the other hand, automation of glycan profiling has been partially achieved already. Existing solid technologies such as liquid handling robots [11], microfluidics and magnetic beads [12] are used for sample preparation in combination with various types of resin to purify target molecules. Glycans are derivatized, separated by liquid chromatography (UPLC, etc.) or multiplexed capillary electrophoresis if necessary, and detected by MS or fluorescence spectrometry [13, 14]. Automation of profiling for the purposes of quality control of biopharmaceuticals as well as comparison between biosimilars and innovator drugs is realistic because the purpose is clear and sufficient amounts of sample are available. However, the development of a generalized glycan profiling system is not straightforward because demands for the performance of the systems vary depending on the studies/researchers.
2. Impact on the other fields of research
   Many researchers are interested in analyzing glycoforms/glycoconjugates if information is easily available. Like automated technologies for the analysis of DNA and proteins, which are widely used in clinical and basic research laboratories, an automated glycan analysis system of high quality will become a core technology.
3. Significance as the fundamental research
   Though most technologies required to achieve automation may be formed from existing conventional ones, it is anticipated that novel methodologies will be established based on novel concept(s) utilizing the unique chemical and physical properties of glycans.
4. Possible application for industry and medicine, if any
   Demand for monitoring of glycosylation during biopharmaceutical production already exists in pharmaceutical industries because glycosylation often reflects the effects of process conditions [15]. Real-time monitoring of glycosylation of biopharmaceuticals may be demanded. It will also be applicable to clinical purposes such as health examinations and intraoperative diagnosis, as well as a tool for basic research.

5. Future perspectives
   To make the analysis of low abundant species possible, the detection sensitivity needs to be drastically improved. As analyses of various types of glycoconjugates themselves will be improved, such analyses will become automated. Targets will increase and quantitative accuracy will be improved in the field of imaging analysis such as MALDI-imaging.
6. Problems to be solved
   It is important to realize that a non-expert can operate and obtain high quality data. This will increase the number of researchers in the field, refine the purpose of the analysis, and lead to the development of next generation system(s) with clearer purpose and higher demand. As existing technologies are generally expensive, cost reduction is also important (Fig. 1.3).

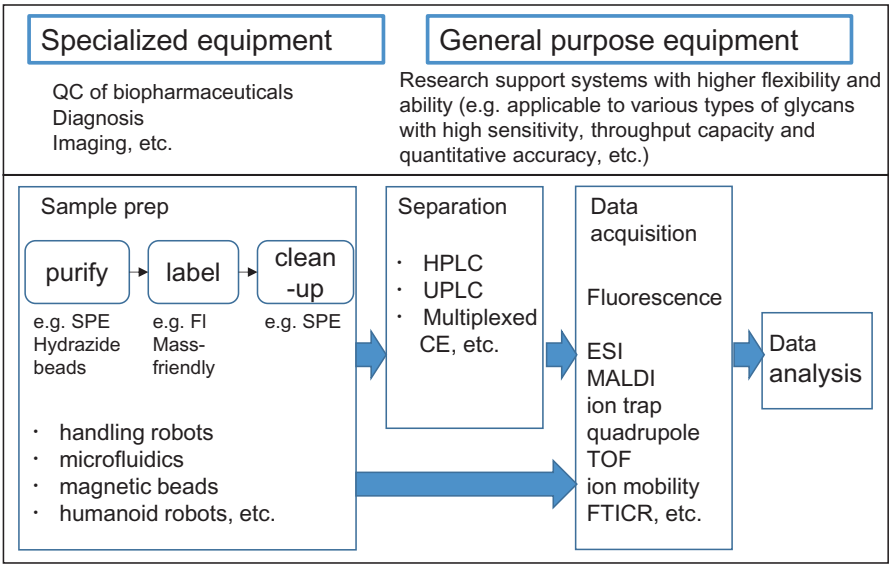

Fig. 1.3 Purposes and typical processes of automated glycan analysis. Specifications may differ greatly depending whether specialized or general purpose equipment is needed. The process may be mainly composed of sample preparation, separation, data acquisition and data analysis, and they are not necessarily integrated. Useful technologies already exist and are rapidly advancing

1.4 Glycoproteomics

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Keywords Glycopeptide, Glycan heterogeneity, Mass spectrometry, Glycoproteomics, Biomarker

1. Significance in the field of glycoscience and its current situation
   Posttranslational modification plays an important role in protein function control, and glycosylation is also deeply involved in various biological phenomena. Glycoproteomics aims at understanding of the functions of glycans in biological phenomena, through comprehensive elucidation of what kind of glycan binds to what kind of protein in what proportion and how it changes under what circumstances. Because glycan structure is diverse and heterogeneous, due to the technical difficulties of the analysis, initially, glycopeptides are collected using the affinity of a lectin with a glycan or by means of a glycan-specific chemical reaction, and after removal of the glycan, the peptide portion is systematically identified by the LC/MS method, which only identifies the actual glycoproteins in the sample. Recently, direct analysis of glycopeptides is shifting to a new phase of provision of both peptide sequence and glycan composition as linked information [16–20].
2. Impact on the other fields of research
   To utilize glycans that change in association with cellular lesions such as inflammation and carcinogenesis as biomarkers to facilitate diagnosis, it is necessary to combine the glycans with proteins specifically expressed in target cells. Glycoproteomics is a powerful tool for discovering such glycoproteins.
3. Significance as the fundamental research
   It is thought that glycan variety (glycomes) differs for each cell, for each protein, and furthermore for each glycosylation site. Glycoproteome analysis technology based on direct analysis of glycopeptides can comprehensively reveal this status and provide important information for glycan function elucidation.
4. Possible application for industry and medicine, if any
   In addition to biomarker development, glycoproteomics can be used for monitoring the glycosylation states of biopharmaceuticals during their manufacture and is useful for quality control. This technology will also enable identification of cell-specific glycoproteins and accelerate the development of drug targets.

5. Future perspectives
   With the improvement of mass spectrometers, analytical methods and sample preparation (pretreatment) methods, it will become possible to analyze *O*-glycosylation in addition to analysis of *N*-glycosylation, which is currently mainly performed. Not only the glycan composition but also more detailed structural information might be obtained.
6. Problems to be solved
   Since glycans are diverse and heterogeneous, the complexity of a glycopeptide sample is remarkably high. Therefore, the detection sensitivity on mass spectrometry is low. The ionization efficiency of glycopeptides is intrinsically low, and their sialylation and sulfation suppress the ionization, and thus the coverage and depth of the analysis are further lowered. Analysis of mucins with many glycans will be extremely difficult (Fig. 1.4).

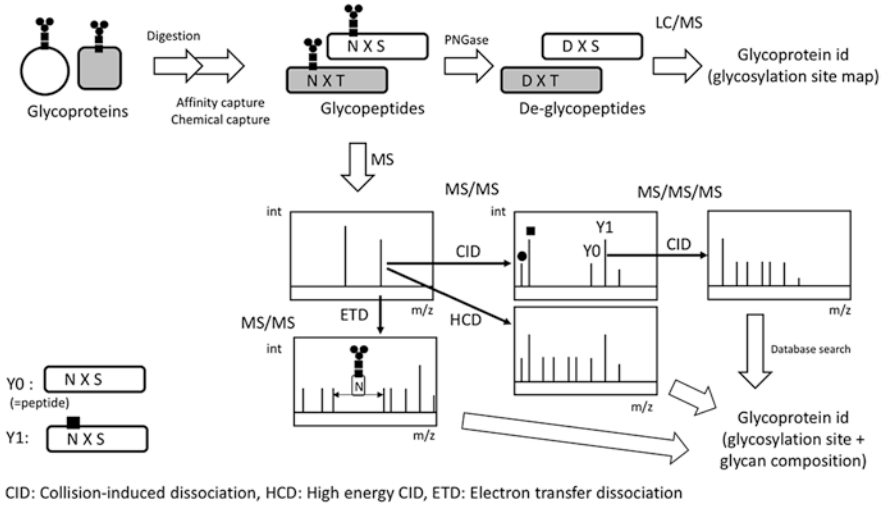

Fig. 1.4 Major procedure of glycoproteome analysis (for *N*-glycosylation). A (glyco) protein mixture is digested with a protease and glycopeptides are collected by means of the affinity toward a glycan or a glycan-specific chemical reaction. Initially, the glycan is excised with PNGase, and the peptide portion is identified by LC / MS. Recently, glycans and peptides have been identified by direct analysis of glycopeptides

1.5 *O*-Glycome Analysis of Glycoproteins

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Keywords Glycomics, Proteomics, *O*-glycan, β-elimination, Mass spectrometry

1. Significance in the field of glycoscience and its current situation
   Glycoconjugates such as glycoproteins and glycolipids play important roles in various biological events on the cell surface [21]. *O*-Glycans attached to serine/threonine residues of glycoproteins have been mostly analyzed by means of chemical digestion because analogous endoglycosidases for such glycoproteins are currently unavailable [22]. β-Elimination in the presence of pyrazolone analogues (BEP) is one of the novel methods for the analysis of *O*-glycans derived from glycoproteins [23]. BEP allows simultaneous release and labeling of *O*-glycans with pyrazolone analogs without any significant side reaction (peeling reaction, less than 3%). Since pyrazolone is a carbon-carbon bond-forming as Michael donor, peptides formerly modified with *O*-glycans are concomitantly labeled with the same reagent, thus BEP is unique in that it allows labeling of both the released *O*-glycan and the deglycosylated peptide. Furthermore, a MW-assisted BEP reaction substantially improved the recovery of total *O*-glycans and the reaction time was reduced [24].
2. Impact on the other fields of research
   O-Glycome and glycosylation site analyses of various biological samples such as cells and tissues would be useful for elucidating a pathological mechanism.
3. Significance as the fundamental research
   Labeling of both the released *O*-glycans and the deglycosylated peptides by the BEP method is very important, leading to the identification of the glycoproteins.
4. Possible application for industry and medicine, if any
   A *O*-glycomic technique based on BEP allows the discovery of diagnostic markers and drug targets.
5. Future perspectives
   O-Glycome and glycosylation site analyses remain extremely difficult tasks. The MW-assisted BEP protocol was proven to be applicable to *O*-glycomic analysis of various biological samples such as cells, serum, and tissues. A versatile analytical procedure would allow elucidation of the mechanisms of various biological phenomena.
6. Problems to be solved
   It is necessary to establish a routine protocol based on BEP for the analysis of both *O*-glycans and *O*-glycosylation sites (Fig. 1.5).

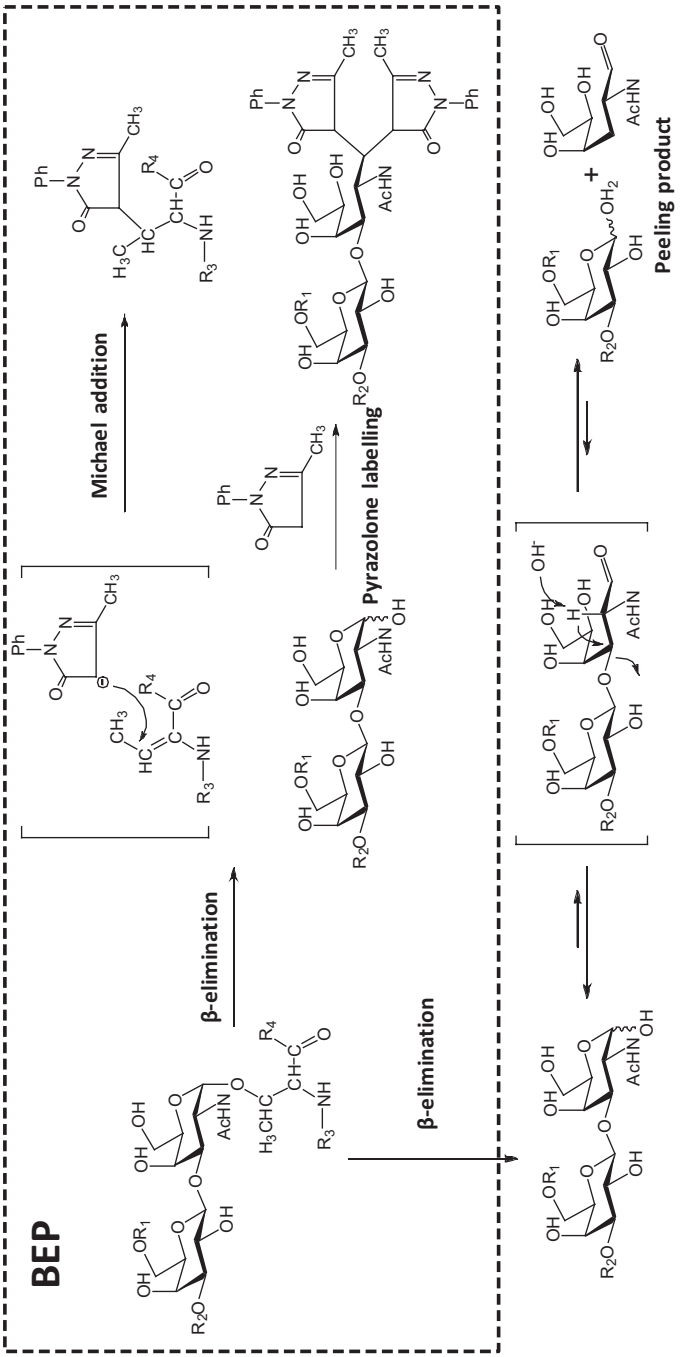

Fig. 1.5 *O*-Glycan and glycosylation site analysis through β-elimination in the presence of pyrazolone analogues

1.6 Glycolipidomics

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Keywords Glycolipid, Raft, Microdomain, Mass spectrometry, Database

1. Significance in the field of glycoscience and its current situation
   Glycolipids (GLs), glycoproteins (GPs), and glycosaminoglycans (GAGs) comprise three major families of glycoconjugates in living organisms. The protein cores of GPs and GAGs carry glycans and primarily determine the functions of these molecules. Structural information on the core proteins is indispensable for the understanding of their glycan functions, and the development of proteomics has greatly contributed to GP and GAG research. The same viewpoint can be applied to GL research, clearly indicating how the development of glycolipidomics will have a great impact on GL research [25]. Glycolipidomics at present is very primitive and requires much effort for maturation. Two activities in Japan are worth mentioning, one is the LipidBank which is open to the public and stores the structures, biological functions, and references for 696 GL molecules [26], and the other is the Glycolipid Library distributed by Shimadzu Co., which covers the MS spectra of 300 GL molecules and a search engine for obtained MS/MS spectra [27].
2. Impact on the other fields of research
   GLs are localized at biomembranes, therefore GLs must function in the trafficking of functional membrane molecules such as receptors, channel proteins, transporters, etc., and in modulation of the functions of these molecules at their destined sites on the membranes [28]. Thus, GL research can have an impact on the research of such membrane functions.
3. Significance as the fundamental research
   GL research has not received critical recognition yet, and this will be realized if actual functions in and contributions to biological activities are revealed. Until then, GL research will remain basic and fundamental.
4. Possible application for industry and medicine, if any
   Modification of membrane functions can be achieved if the presence and functions of GLs at the microdomains on the membranes are artificially modulated. Several papers have reported that GLs are involved at the site on membranes where proteins are aggregated and become toxic to the cells, like amyloid β and α-synuclein, and insulin receptors can transduce insulin signals into the cells.

5. Future perspectives
   In the 1980s, GL research attracted attention in relation to congenital disorders of metabolism and cancer-associated antigens, and then the membrane raft or microdomain concept became another focus. However, critical development of analytical methods applicable to molecule complexes forming rafts [29] or microdomains, and their functions is difficult and has not been realized. If this is resolved by the support of glycolipidomics, exciting and interesting views of cellar functions will be realized.
6. Problems to be solved
   The establishment of glycolipidomics requires the development of analytical methods, refinement of sample preparation methods suitable for further analysis, chemical synthesis of isotope-labeled standards, compilation of obtained data, development of databases and data-search algorisms, etc. (Fig. 1.6).

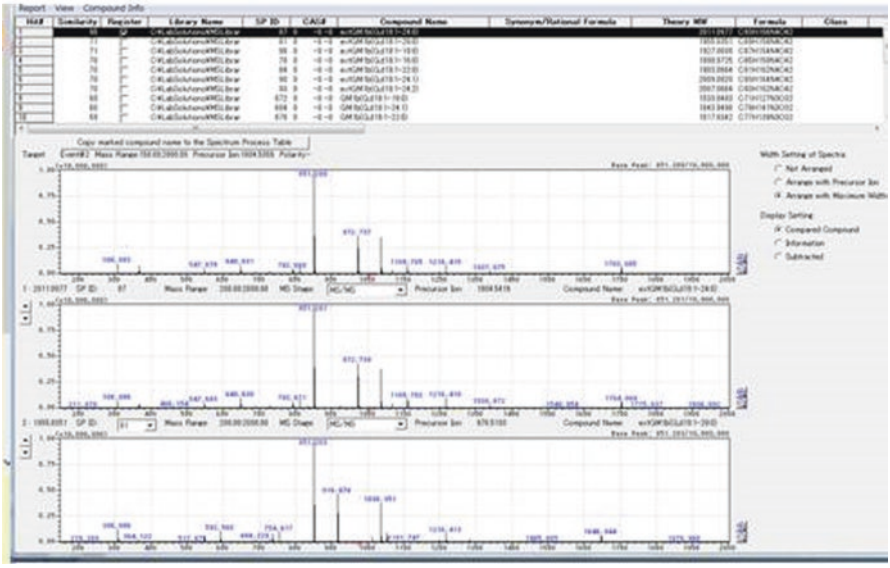

Fig. 1.6 An example of search results of an obtained MS/MS spectrum (top) using the Glycolipid Library distributed by Shimadzu Co., which picks up similar spectra, the middle one having the highest match-score the bottom one the lower score

1.7 Glycometabolome

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Keywords Mass spectrometry, Glycometabolome, Glycan profiling

1. Significance in the field of glycoscience and its current situation
   Biological activity in a living cell is controlled through the expression levels of proteins and post-translational modifications of proteins such as phosphorylation and glycosylation. Therefore, comprehensive analysis of metabolites (e.g., sugars, lipids and amino acids) allows observation of biological phenomena directly and comprehensively. Not only a monosaccharide, an oligosaccharide and a phosphate sugar, which have been already registered with Human Metabolome Database, but also a free oligosaccharide released from *N*-glycoproteins can be assumed as a metabolite of a glycan. When a lysosomal enzyme involved in carbohydrate metabolism has been lost, lysosomal storage disease develops. An excessive amount of free oligosaccharide is accumulated in the cytosol or urine of a patient [30].

   With recent advancement of mass spectrometry technology, sensitivity of metabolome analysis has been greatly enhanced. On the other hand, glycometabolome analysis is lagging due to the microheterogeneity and low detectivity of glycans. Therefore, the development of a novel analytical method is desired.
2. Impact on the other fields of research
   N-glycome of serum proteins changes in various diseases including cancer and diabetes [31, 32]. Development of a novel diagnostic method using peripheral blood or urine would be useful for a less invasive diagnosis.
3. Significance as the fundamental research
   A change of metabolites directly reflects an event occurring in the cell. Since it is difficult to quantify all metabolites only by means of a biochemical technique, a high-throughput and comprehensive analytical method could be the core technology for understanding a biological system.
4. Possible application for industry and medicine, if any
   When a metabolite that is characteristic of a patient group is found on analysis of glycometabolites in body fluid of a patient and compared with that of the healthy person, it could be a powerful biomarker.

5. Future perspectives
   The application to early diagnosis or prognosis of a disease, and a appropriate checking of the drug together with metabolite monitoring after drug administration are expected.
6. Problems to be solved
   (1) Improvement of purification and analysis methods for glycometabolites, (2) improvement of the sensitivity of analytical instruments, (3) improvement of the database, and (4) enhancement of authentic samples of glycometabolites are essential (Fig. 1.7).

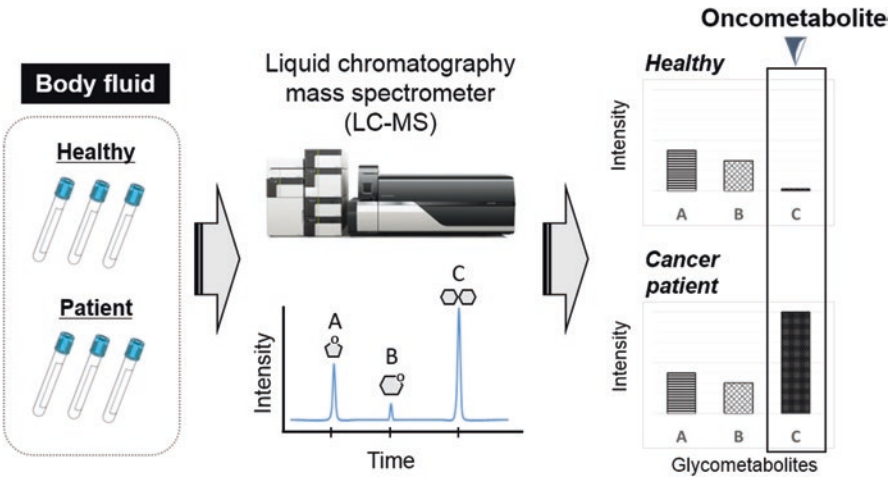

Fig. 1.7 Outline of glycometabolome analysis. When a metabolite that is characteristic of a patient group is found on analysis of glycometabolites in the body fluid of a patient and compared with that in a healthy person, it could be a powerful biomarker

1.8 Glyco-array

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Keywords Sugar chain, Immobilization, Binding interaction, On-time analysis, Organic synthesis

1. Significance in the field of glycoscience and its current situation
   For the functional analysis of sugar chains, the first critical step is to analyze the binding interaction between the sugar chains and proteins. According to the recent progress of micro-array technology, glyco-arrays in which many kinds of sugar chain are immobilized on a device become an important analytical tool not only for high throughput analysis of the binding interaction with proteins, but also more accurate and highly sensitive analytical systems in combination with quantitative kinetic calculation or with mass spectrometry. Recently, more applications for the binding of viruses, bacteria and even whole living cells have become available. Furthermore, glyco-arrays are used for on-time analysis using surface Plasmon resonance, resulting in a more valuable analytical tool [33–37].
2. Impact on the other fields of research
   Using glyco-arrays, biological reactions mediated by sugar chains can be observed more easily and precisely, and therefore they will have a big impact on biochemical research on infectious diseases, such as virology and bacteriology, developmental biology, signal transduction or cancer metastasis, and so on.
3. Significance as the fundamental research
   More developments are needed, as follows: sugar chain synthesis for preparation of oligosaccharides immobilized on glyco-arrays, stable immobilization reactions without side reactions, and various on-time analysis technologies that can recognize weak affinity binding interactions. Close fundamental collaborative research with the chemistry and physics fields is needed.
4. Possible application for industry and medicine, if any
   In the field of infectious diseases such as ones caused by viruses and bacteria, the measurement of binding affinity can be used for the screening of new drug targets, such as novel low molecular compounds and antibodies, and for an effective diagnostic tool.
5. Future perspectives
   It is obvious that more researchers will use glyco-arrays in the future not only for proteins but also for viruses, bacteria, and living cells in combination with mass spectrometry and on-time measurement technologies.

6. Problems to be solved
   Since an unlimited number of structurally defined oligosaccharides are necessary for determination of the precise structure-activity relationship of sugar chains, the field of organic synthesis must contribute more. In the future, automation of synthesis or robotic synthesis, like a peptide synthesizer, must be an important goal (Fig. 1.8).

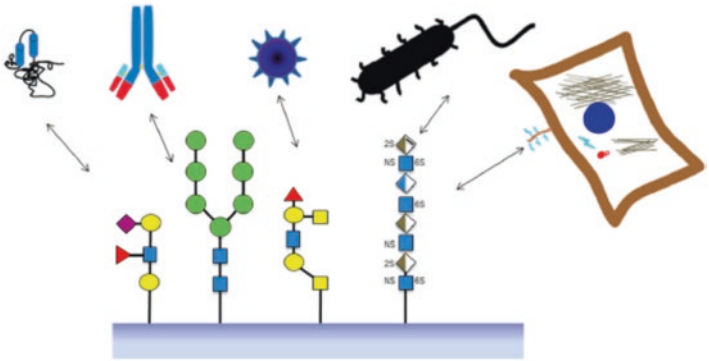

Fig. 1.8 The various applications of glyco-arrays including screening of proteins or antibodies, viruses, bacteria and living cells

1.9 Glycolipid Array

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Keywords Glycolipid, Microbiota, Lipid rafts, Antibodies, Chemical biology

1. Significance in the field of glycoscience and its current situation
   Microbiota have been found to be crucial for immunological, hormonal and metabolic homeostasis, and even the brain physiology of their hosts [38]. Both the sugar chain diversity and lipid chain heterogeneity of glycolipids are critical for the bacteria and host responses. Recent studies have shown the presence of new glycolipids in the brains and the glycolipids in rafts are immunogenic to humans [39]. The novel type of "glycolipid array" will provide a new strategy for the diagnosis of aging-related neuronal diseases such as the Parkinson and Alzheimer diseases.
2. Impact on the other fields of research
   Glycolipid-arrays allow us to determine the interaction between glycolipids and proteins/microorganisms under conditions close to those of the native membrane structures. Thus, they will have a significant impact on basic as well as translational medical research fields [40].
3. Significance as the fundamental research
   To establish a glycolipid-array system, it is essential to develop a method to immobilize glycolipids under conditions close to those of the native form in the cells [41]. Tight collaboration with synthetic chemists and chemical biologists is needed.
4. Possible application for industry and medicine, if any
   A novel type of "glycolipid array" will provide a new strategy for the diagnosis of aging-related neuronal diseases such as the Parkinson and Alzheimer diseases.
5. Future perspectives
   Glycolipid-array will provide a chance to discover novel receptor proteins involved in inter- and intra-cellular interactions by which a variety of biological functions based upon glycolipid/protein interactions will be elucidated.
6. Problems to be solved
   It is critical to establish a systematic method for the preparation of a large number of glycolipids for the arrays (Fig. 1.9).

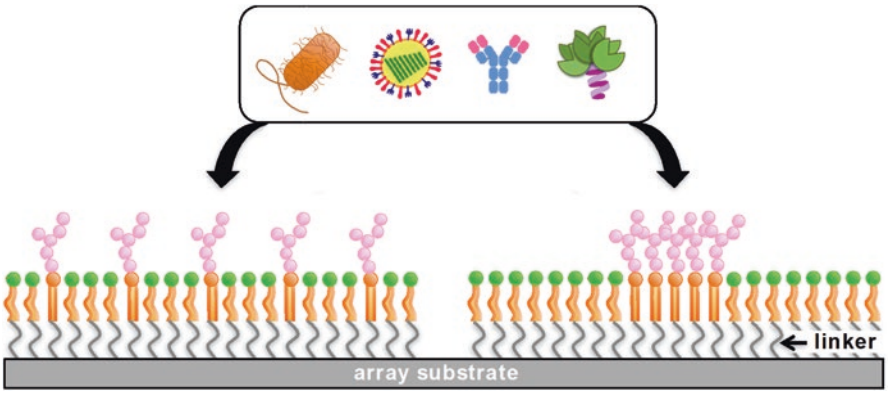

Fig. 1.9 A glycolipid-array makes it possible to detect and identify proteins (toxins, antibodies, etc) and microorganisms

1.10 Lectin Blotting

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Keywords Glycoproteins, Electrophoresis, Lectins, Glycosylation, Structural estimation

1. Significance in the field of glycoscience and its current situation
   Many proteins are glycosylated and glycan moieties are important not only for proper folding of proteins but also for their functions. Glycan structures are always accompanied by microheterogeneity, and species- and organ-specificities, and alter upon changes in the physiological conditions of cells including initiation of several diseases [42, 43]. The fine structures of glycans can be now determined by mass-spectrometry in spite of their small amounts, though it takes time for the preparation of good samples and the equipment itself is expensive. Routinely, we simply need information on what types of glycans are attached to proteins and the presence or absence of carbohydrate antigens expressed at the non-reducing termini rather than their whole fine structures. To obtain such information instantly, lectin blotting is a simple and effective tool [44, 45].
2. Impact on the other fields of research
   The variation of glycan structures in glycoproteins often makes it hard for researchers outside to deal with glycans. However, once one learns their basic structures, lectin blotting is a simple and easy method to get basic structural information about glycans attached to proteins after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [44].
3. Significance as the fundamental research
   A number of studies have shown that alteration of protein glycosylation is one of the serious causes of developmental abnormality and diseases in man, and can be used for detection of the diseases as markers. Lectin blotting can reveal such changes in glycans attached to proteins simply and effectively [42, 46].
4. Possible application for industry and medicine, if any
   As a congenital disorder of glycosylation (CDG) was revealed initially by changes in the molecular weights and isoelectric points of proteins in patients' sera due to the decreased N-glycosylation, lectin blotting is a simple and rapid tool to check whether glycans are associated with abnormal conditions and diseases.
5. Future perspectives
   The structures of glycans attached to proteins are important for their half-lives in the blood circulation, and targeting to tissues and organs, particularly for cytokines and glycohormones. Terminal and sub-terminal glycosylation of proteins

often differ among cell types produced. Lectin blotting can easily detect such differences in recombinant glycoproteins, and will contribute to the production of them with high quality.

6. Problems to be solved
   The binding specificities of lectins toward glycans have not been fully established. More detailed structural requirements for binding of lectins to glycans including their branching and their avidity in a glycoprotein have to be further studied. It would be ideal if more lectins that can discriminate minute differences in glycan structures are isolated for estimating fine glycan structures by the present method (Fig. 1.10).

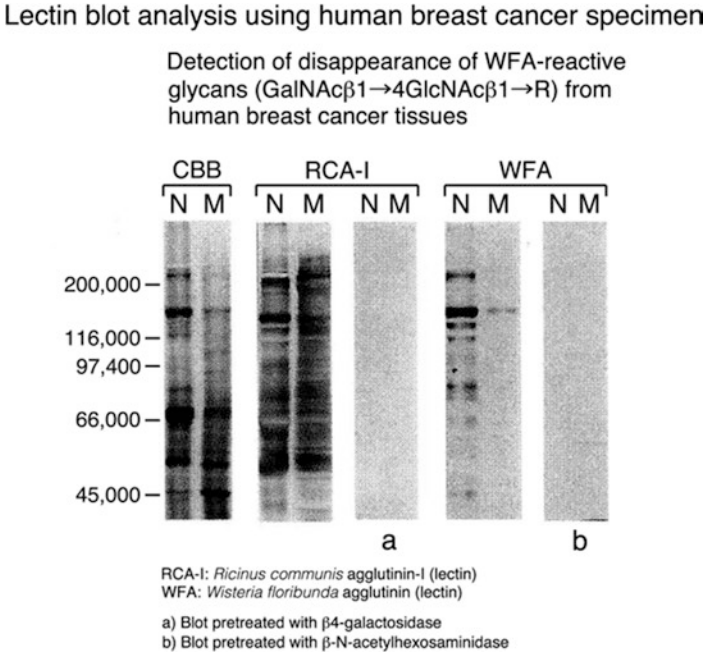

Fig. 1.10 Membrane proteins prepared from malignant (M) and non-malignant (N) regions of a human breast cancer specimen at stage II were subjected to lectin blot analysis. Proteins were detected by CBB staining, and terminal galactose (Galβ1 → 4GlcNAc) and its competitive N-acetylgalactosamine (GalNAcβ1 → 4GlcNAc) were detected with RCA-I and WFA lectins, respectively. CBB, Coomassie Brilliant Blue

1.11 Lectin Engineering

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Keywords Lectin, Specificity, Engineering

1. Significance in the field of glycoscience and its current situation
   It is an important issue as to whether glycan structures can be specifically distinguished during the monitoring of structural changes of glycans. Immunoglobulin is widely used to identify proteins, however, it has potential drawbacks including that establishment of monoclonal or polyclonal antibodies against specific glycan structures is quite difficult. Lectins are proteins that can recognize distinct glycan structures and are thought to function as self-defense molecules, especially in plants, although lectin genes do not undergo any recombinational events. One of the goals of lectin engineering is to establish mutated lectins to distinguish specific glycan structures by introducing mutations into their sugar-recognition domains [47, 48], which will allow us to apply such engineered lectin probes to diagnosis and therapeutics [49, 50].
2. Impact on the other fields of research
   Glycosylation regulates cell behavior and glycoprotein function. Thus, lectin engineering to produce probes to distinguish glycan structures is necessary for understanding cell and protein functions. Such techniques are widely used for diagnosis and therapeutics together with regenerative medicine associated with cell differentiation and development.
3. Significance as the fundamental research
   Lectin-glycan interactions have several distinct characteristics compared with those of protein-protein interactions. In lectin-glycan interactions, several kinds of glycan structures are produced and polymerization and dissociation of lectin receptors causes switching of signal transduction from glycan ligands. These characteristics are mandatory for understanding biological events in both cells and individuals.
4. Possible application for industry and medicine, if any
   Antibodies against specific glycan structures are widely used for diagnosis and therapeutics. In addition, probes to discriminate sugar structures are required for understanding several pathological conditions. Targeting of cancer cells using engineered lectin-conjugated drugs may be applicable for therapeutics. Lectin engineering is a technology that will become popular in the future.
5. Future perspectives
   Potential application of engineered lectins to medical practice will occur gradually.
6. Problems to be solved
   Glycosaminoglycans on proteoglycans provide an microenvironment as an extracellular matrix. This kind of glycans directly regulates the proliferation and development of neighboring cells. However, the precise structures of glycosaminoglycans have not been revealed yet and standards for their fragments are not available (Fig. 1.11).

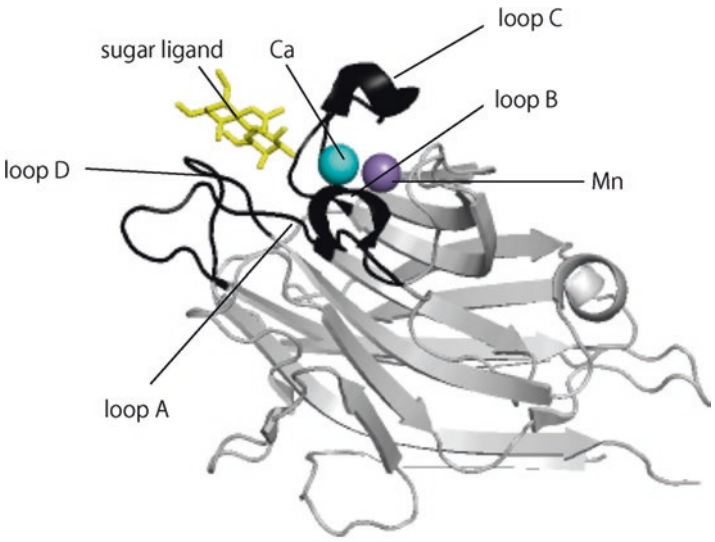

Fig. 1.11 Sugar-binding region of Leguminous lectins consists of 4 loops, especially loop C being greatly involved in sugar-binding specificity. We constructed engineered lectins with unique sugar-binding specificities from a lectin library that was randomly mutated as to loop C

Box 1.1: Lectin Microarray

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This technology, also called lectin array and lectin chip, used for glycan profiling in various contexts involves a slide glass, to which an array of different lectins are immobilized to contact with various forms of glycoconjugates. In general, determination of complex glycan structures is very difficult and laborious. So, for the sake of rapid investigation of glycan profiles, which depend on cell types and states, the technology was developed by a few groups at the beginning of the twenty-first century. In Japan, a group of the National Institute of Advanced Industrial Science and Technology developed a system combining a lectin microarray plate and a specialized scanner, which is based on an evanescent-field activated fluorescent detection principle in the framework of the NEDO project in 2005, and the next year was instrumented by a relevant company. This technology has proved very useful for investigation of glycan-related biomarkers for various diseases as well as quality control of various cells. More recently, the FDA introduced this system for a rapid analysis of various biological drugs, almost all of which are glycoproteins. Glycome analysis targeting the exosome is also a matter of keen attention.

1.12 Recognition of iPS/ES Cells by Glycan-Binding Antibodies

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Keywords TRA-1-60, SSEA-3, SSEA-4, R-10G, R-17F

1. Significance in the field of glycoscience and its current situation
   Carbohydrate-recognizing antibodies in conjugation with high performance optical instruments including a flow cytometer and a confocal laser fluorescence microscope are tremendously useful experimental tools for monitoring the changes of glycan structures as well as for identification of specific glycans, on a specific cell type, with high sensitivity and strict specificity. Most marker antibodies to human embryonic stem (hES) and human-induced pluripotent stem (hiPS) cells are carbohydrate-recognizing ones, which include stage specific embryonic antigen (SSEA)-3 and SSEA-4 recognizing globosides, and tumor rejection antigen (TRA)-1-60 and TRA-1-81 recognizing keratan sulfates. However, these antibodies are not specific to hiPS/ES cells, rather, they recognize those glycans that are common to hiPS/ES cells and human embryonal carcinoma (hEC) cells. With this background, generation of monoclonal antibodies that bind specifically to hiPS/hES cells but exhibit little or no binding to hEC cells has been desired. We have developed two monoclonal antibodies, R-10G [51] and R-17F [52], both of which are glycan-recognizing hiPS/ES specific antibodies of the IgG1 subtype.
2. Impact on the other fields of research
   The antibodies, R-10G and R-17F, had a powerful impact on glycobiology and surrounding area. Requests and proposals for an invited speaker, submission of articles, and editing of a book and Journals have been made to me in the last few years.
3. Significance as the fundamental research
   These antibodies can be very useful tools to study fundamental questions in basic biology such as how are pluripotent stem cells able to keep the infinite proliferation ability in the undifferentiated state, and what are the critical differences between iPS cells and tumor cells.
4. Possible application for industry and medicine, if any
   (i) Antibody micro-arrays made of anti-iPS cell antibodies with slightly different binding specificities would be very useful for the quality control and standardization of hiPS cells.
   (ii) Ubiquitous staining of all hiPS/ES cells with R-17F together with its strong cytotoxic activity toward these cells make this antibody very special. The antibody may be very valuable as a tool to remove undifferentiated stem cells from developing cells.

5. Future perspectives
   This is indeed a rapidly developing new area, and a lively and prosperous future wait us not only in basic research but also in medical and industrial applications.
6. Problems to be solved
   (i) Elucidation of the epitope structure of TRA-1-60 and its carrier molecules on hiPS cells, which is still controversial.
   (ii) Elucidation of the molecular mechanism of cytotoxicity of R-17F towards hiPS/ES cells.
   (iii) Development of novel glycan-recognizing marker antibodies to hiPS/ES cells (Fig. 1.12).

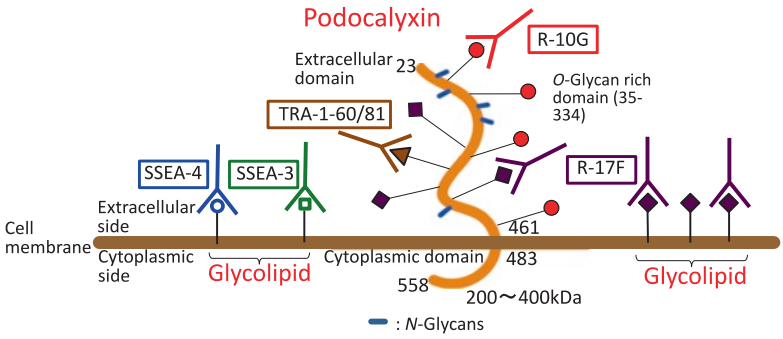

Fig. 1.12 Membrane topology of major marker glycan-epitopes on hiPS cells. The TRA-1-60/81 and R-10G epitopes are expressed on a membrane glycoprotein (podocalyxin). The SSEA-3 and SSEA-4 epitopes are expressed on membrane glycolipids. The R-17F epitope is expressed predominantly on glycolipids but also on podocalyxin and other glycoproteins [53]

1.13 The EMARS Method

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Keywords Membrane microdomain, Horseradish peroxidase (HRP), Proteomics, Molecular interaction

1. Significance in the field of glycoscience and its current situation
   The EMARS(Enzyme-Mediated Activation of Radical Sources)system is a method for identification of molecules in the vicinity within 300 nm from a given molecule [56]. Applications of this method in the field of glycoscience are as follows: (1) to find molecules localized in a particular glycolipid microdomain; (2) to find molecules co-localized with a particular GPI-anchored protein; (3) to find molecules near the glycoprotein to which a lectin binds. As an example, signal molecules in the GD3 microdomain have been identified in human cancer cells. It is found that different GPI-anchored proteins form distinct membrane microdomains. The study on the effects of a lectin on the membrane microdomains is underway.
2. Impact on the other fields of research
   The advantages of the EMARS method are as follows; (i) easy, high throughput, and without the need for special equipment, (ii) applicable to systematic approaches such as proteomic analyses [57], (iii) applicable to studies on not only proteins but also carbohydrate chains and membrane lipids. Thus this method is useful for a wide range of research concerning molecular interactions in membrane domains.
3. Significance as the fundamental research
   The EMARS method using expressed HRP fusion proteins can identify co-clustering molecules in microdomains under a living condition. This new approach will provide a useful tool for a wide range of research concerning molecular interactions in the cells as well as on the cell surface.
4. Possible application for industry and medicine, if any
   The EMARS method is applicable for elucidation of molecular mechanisms underlying the pharmacological actions of therapeutic antibodies. As an example, rituximab-stimulated interaction between CD20 and FGFR3 was discovered using this method [58].
5. Future perspectives
   We will apply the EMARS method at organismal level using a transgenic mouse in which an HRP-fused protein is expressed.
6. Problems to be solved
   How can we deliver the labeling reagent for the EMARS reaction to the target cells? (Fig. 1.13)

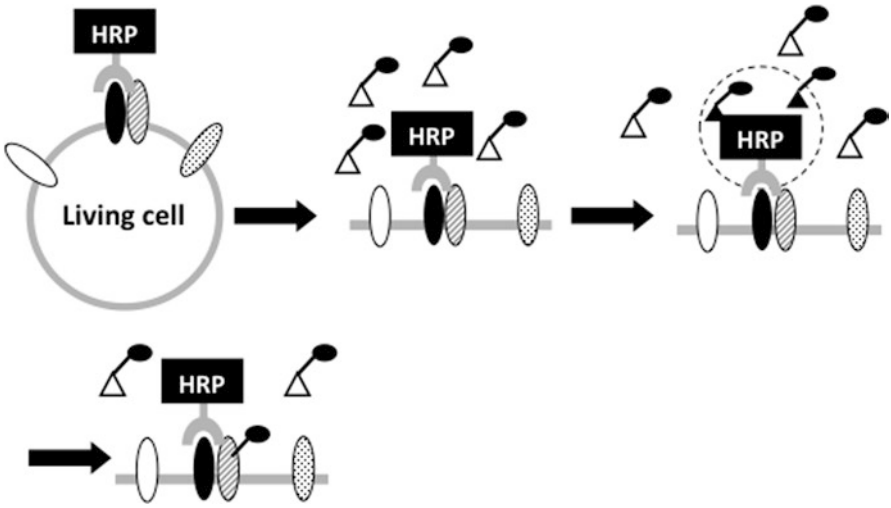

Fig. 1.13 First, an HRP-conjugated antibody or lectin is bound to the probed molecule on the cell surface of living cells. Next, a labeling reagent is added to the medium. The active group is converted to a radical by HRP. The radical attacks adjacent molecules and form a covalent bond with the tag

References

References for Section 1.1

1. Kawasaki N et al (2009) LC/MS^n for glycoprotein analysis: N-linked glycosylation analysis and peptide sequencing of glycopeptides. Methods Mol Biol 534:239–248
2. Takahashi K et al (2010) Clustered *O*-glycans of IgA1: defining macro- and microheterogeneity by use of electron capture/transfer dissociation. Mol Cell Proteomics 11:2545–2557
3. Kondo A et al (2018) Mass spectrometry-based approach for development of biomarkers in IgA nephropathy: a pilot trial. Fujita Med J 4:36–41
4. Ito E et al (2013) Structural characterization of neutral glycosphingolipids using high-performance liquid chromatography-electrospray ionization mass spectrometry with a repeated high-speed polarity and MS^n switching system. Glycoconj J 30:881–888
5. Nakajima K et al (2013) Mass isotopomer analysis of metabolically labeled nucleotide sugars and *N*- and *O*-glycans for tracing nucleotide sugar metabolisms. Mol Cell Proteomics 12:2468–2480

References for Section 1.2

6. Yamaguchi Y et al (2012) Confident identification of isomeric *N*-glycan structures by combined ion mobility mass spectrometry and hydrophilic interaction liquid chromatography. Rapid Commun Mass Spectrom 26:2877–2884
7. Hofmann J et al (2015) Identification of carbohydrate anomers using ion mobility-mass spectrometry. Nature 526:241–244
8. Hofmann J, Pagel K (2017) Glycan analysis by ion mobility-mass spectrometry. Angew Chem Int Ed 56:8342–8349
9. Hinneburg H et al (2016) Distinguishing N-acetylneuraminic acid linkage isomers on glycopeptides by ion mobility-mass spectrometry. Chem Commun 52:4381–4384
10. Struwe WB et al (2016) GlycoMob: an ion mobility-mass spectrometry collision cross section database for glycomics. Glycoconj J 33:399–404

References for Section 1.3

11. Stöckmann H et al (2013) Automated, high-throughput IgG-antibody glycoprofiling platform. Anal Chem 85:8841–8849
12. Váradi C et al (2014) Rapid magnetic bead based sample preparation for automated and high throughput N-glycan analysis of therapeutic antibodies. Anal Chem 86:5682–5687
13. Duke R, Taron CH (2015) N-glycan composition profiling for quality testing of biotherapeutics. BioPharm Int 28:59–64
14. Shubhakar A et al (2015) High-throughput analysis and automation for glycomics studies. Chromatographia 78:321–333
15. DePalma A (2017) Glycosylation: window into quality glycosylation. Genet Eng Biotechn N 37:16–17

References for Section 1.4

16. Yang Y et al (2017) Glycoproteomics: a balance between high-throughput and in-depth analysis. Trends Biotechnol 35:598–609
17. Thaysen-Andersen M et al (2016) Maturing glycoproteomics technologies provide unique structural insights into the N-glycoproteome and its regulation in health and disease. Mol Cell Proteomics 15:1773–1790
18. Sun S et al (2016) Comprehensive analysis of protein glycosylation by solid-phase extraction of *N*-linked glycans and glycosite-containing peptides. Nat Biotechnol 34:84–88
19. Chen M et al (2017) An engineered high affinity Fbs1 carbohydrate binding protein for selective capture of *N*-glycans and *N*-glycopeptides. Nat Commun 8:15487
20. Noro E et al (2015) Large-scale identification of *N*-glycan glycoproteins carrying lewis x and site-specific *N*-glycan alterations in Fut9 knockout mice. J Proteome Res 14:3823–3834

References for Section 1.5

21. Stanley P, Cummings RD (2009) Structures common to different glycans. In: Varki A et al (eds) Essentials of glycobiology, 2nd edn. Cold Spring Harbor Laboratory Press, New York, pp 175–198
22. Wada Y et al (2010) Comparison of methods for profiling *O*-glycosylation: human proteome organisation human disease glycomics/proteome initiative multi-institutional study of IgA1. Mol Cell Proteomics 9:719–727
23. Furukawa J-i et al (2011) A versatile method for analysis of serine/threonine posttranslational modifications by β-elimination in the presence of pyrazolone analogues. Anal Chem 83:9060–9067
24. Furukawa J-i et al (2015) Quantitative *O*-glycomics by microwave-assisted β-elimination in the Presence of pyrazolone analogues. Anal Chem 87:7524–7528

References for Section 1.6

25. Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature 387:569–572
26. Merrill AH, Sullards M (2017) Opinion article on lipidomics: inherent challenges of lipidomic analysis of sphingolipids. Biochim Biophys Acta 1862:774–776
27. LipidBank: http://lipidbank.jp/
28. Glycolipid library: http://www.an.shimadzu.co.jp/lcms/ittof-option/glycolipid-library.htm
29. Yoshikawa M et al (2015) Ganglioside GM3 is essential for the structural integrity and function of cochlear hair cells. Hum Mol Genet 24:2796–2807

References for Section 1.7

30. Winchester B (2005) Lysosomal metabolism of glycoproteins. Glycobiology 15:1R–15R
31. Pinho SS, Reis CA (2015) Glycosylation in cancer: mechanisms and clinical implications. Nat Rev Cancer 15:540–555
32. Testa R et al (2015) *N*-glycomic changes in serum proteins in Type 2 diabetes mellitus correlate with complications and with metabolic syndrome parameters. PLoS One 10:e0119983

References for Section 1.8

33. Puvirajesingh TM, Turnbull JE (2016) Glycoarray technologies: deciphering Interactions from proteins to live cell responses. Microarrays 5:3
34. Miyachi K et al (2015) Syntheses of chondroitin sulfate tetrasaccharide structures containing 4,6-disulfate patterns and analysis of their interaction with glycosaminoglycan-binding protein. Bioorg Med Chem Lett 25:1552–1555
35. Suda Y et al (2014) Conventional and easy analysis of virus-binding GAG structure using array-type sugar chips. In: Glycoscience: biology and medicine. Springer, Tokyo, pp 163–174
36. Suda Y et al (2013) Discrimination of influenza virus strains and super high sensitive detection of viruses using sugar chip and sugar-chain immobilized gold nanoparticles. ACS Symp Ser 1135:331–350
37. Jacob F et al (2011) Serum antiglycan antibody detection of nonmucinous ovarian cancers by using a printed glycan array. Int J Cancer 130:138–146

References for Section 1.9

38. Donia MS, Fischbach MA (2015) Human microbiota. Small molecule from the human microbiota. Science 349:1254766
39. Shima S et al (2014) Anti-neutral glycolipid antibodies in encephalomyeloradiculoneuropathy. Neurology 82:114–118
40. Shi J et al (2006) GM1 clustering inhibits cholera toxin binding in supported phospholipid membranes. J Am Chem Soc 129:5954–5961
41. Guy AT et al (2015) Neuronal development. Glycerophospholipid regulation of modality-specific sensory axon guidance in the spinal cord. Science 349:974–977

References for Section 1.10

42. Hirano K et al (2015) Enhanced expression of the β4-*N*-acetylgalactosaminyltransferase 4 gene impairs tumor growth of human breast cancer cells. Biochem Biophys Res Commun 461:80–85
43. Tagawa M et al (2014) Enhanced expression of the β4-galactosyltransferase 2 gene impairs mammalian tumor growth. Cancer Gene Ther 21:219–227
44. Kumagai T et al (2010) Involvement of murine β-1,4-galactosyltransferase V in lactosylceramide biosynthase. Glycoconj J 27:685–695
45. Tadokoro T et al (2009) Involvement of galectin-3 with vascular cell adhesion molecule-1 in growth regulation of mouse Balb/3T3 cells. J Biol Chem 284:35556–35563
46. Kitamura N et al (2003) Prognostic significance of reduced expression of β-N-acetylgalactosaminylated *N*-linked oligosaccharides in human breast cancer. Int J Cancer 105:533–541

References for Section 1.11

47. Yamamoto K et al (1991) Purification and characterization of a carbohydrate-binding peptide from *Bauhinia purpurea* lectin. FEBS Lett 281:258–262
48. Yamamoto K et al (1992) Alteration of carbohydrate-binding specificity of *Bauhinia purpurea* lectin through the construction of chimeric lectin. J Biochem 111:87–90
49. Soga K et al (2015) Mammalian cell surface display as a novel method for developing engineered lectins with novel characteristics. Biomolecules 5:1540–1562
50. Abo H et al (2015) Mutated leguminous lectin containing a heparin-binding like motif in a carbohydrate-binding loop specifically binds to heparin. PLoS One 10:e0145834

References for Section 1.12

51. Kawabe K et al (2013) A novel antibody for human induced pluripotent stem cells and embryonic stem cells recognizes a type of keratan sulfate lacking oversulfated structures. Glycobiology 23:322–336
52. Matsumoto S et al (2015) A cytotoxic antibody recognizing lacto-*N*-fucopentaose I (LNFP I) on human induced pluripotent stem (hiPS) cells. J Biol Chem 290:20071–20085
53. Nakao H et al (2017) Characterization of glycoproteins expressing the blood group H type 1 epitope on human induced pluripotent stem (hiPS) cells. Glycoconj J 34:779–787

References for Section 1.13

54. Kotani N et al (2008) Biochemical visualization of cell surface molecular clustering in living cells. Proc Natl Acad Sci U S A 105:7405–7409
55. Jiang S et al (2012) A proteomics approach to the cell-surface interactome using the enzyme-mediated activation of radical sources reaction. Proteomics 12:54–62
56. Kotani N et al (2012) Fibroblast growth factor receptor 3 (FGFR3) associated with the CD20 antigen regulates the rituximab-induced proliferation inhibition in B-cell lymphoma cells. J Biol Chem 287:37109–37118
57. Miyagawa-Yamaguchi A et al (2015) Each GPI-anchored protein species forms a specific lipid raft depending on its GPI attachment signal. Glycoconj J 32:531–540
58. Kaneko K et al (2016) Neogenin, defined as a GD3-associated molecule by enzyme-mediated activation of radical sources, Confers malignant properties via intracytoplasmic domain in melanoma cells. J Biol Chem 291:16630–16643

Chapter 2
Structural Biology of Glycans

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2.1 Study of Glycan Structure and Its Recognition Mechanism by Cryo-electron Microscope Single Particle Analysis

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Keywords Cryo-electron microscopy, Single particle reconstruction, Drug design, Glycan-protein complex, Infectious diseases

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1. Significance in the field of glycoscience and its current situation
   Recent development in single particle reconstruction using cryo-electron microscopy has enabled structure determination of glycans [1], which has been difficult due to their structural variations and soft nature. Single particle reconstruction determines a structure by averaging, then only the core 3D structure necessary for its function can be reconstructed. This will enable drug design and further induce development of glycan-related industries.
2. Impact on the other fields of research
   Single particle reconstruction is suitable to determine molecular complexes, and can determine multiple structures, leading to understanding of molecular movement. Such information would be valuable for structure-oriented drug design and antibody production, and yield various applications in medicine, agriculture, food science and material science.
3. Significance as the fundamental research
   Structure determination of glycans using X-ray crystallography has been difficult due to their structural variations, which sometimes precludes crystal formation. Recent developments in single particle cryo-electron microscopy have a potential to overcome this difficulty by reaching the core structure determination by 3D averaging. Such approaches and results have a big impact in basic biology and biochemistry.

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4. Possible application for industry and medicine, if any
   Since single particle reconstruction might enable determination of soft glycan structures that are recognized by infectious bacteria and virus; the core 3D structure necessary for its function and recognition could be reconstructed using the glycoproteins docked with their receptors. This will provide missing information for the drug design and development of glycan related technologies.
5. Future perspectives
   Single particle reconstruction has been developed to determine large molecular complexes, and a big potential to determine multiple structures, that reveal molecular movements. It is advantageous in the study of receptor-glycoprotein complex study for cancer metastasis and embryonic development, in combination with other microscopies for glycoscience [2–5].
6. Problems to be solved
   The best resolution of single particle reconstruction for glycans is 4.2 Å by Lee et al. (Science 2016) [1]. This is a great milestone, but resolution should be improved because present resolution is not enough for drug design. For this purpose, single particle reconstruction microscopy focused on glycan should be intensively developed and enforced, including sample preparation methods and algorithms as a inter-national project (Fig. 2.1).

Fig. 2.1 Single particle reconstruction is a promising method for the structure determination of glycans. In this method, ice-embedded biological samples are imaged using cryo-electron microscope. An example of cryo-TEM is shown here. Further methodological development including biochemistry, electron microscopy and reconstruction software is required for high-throughput reconstructions of glycans at atomic resolution

2.2 Conformational Analysis of Oligosaccharides

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Keywords NMR spectroscopy, Molecular dynamics simulation, Conformational fluctuation, Molecular recognition

1. Significance in the field of glycoscience and its current situation
   The versatile functions of oligosaccharides are exerted primarily through their interactions with cognate proteins. To gain a deeper understanding of carbohydrate recognition by proteins, it is essential to elucidate the conformations of the oligosaccharides in detail. However, conformational fluctuations of the oligosaccharides due to their high degrees of freedom as to internal motion hamper detailed conformational analyses. To address this, NMR spectroscopy can provide atomic-level information regarding the dynamic structures of biomacromolecules in solution and therefore will play an indispensable role in conformational analyses of oligosaccharides [6]. In particular, the recently developed paramagnetism-assisted NMR method, when combined with molecular dynamics simulation, has enabled accurate description of conformational spaces occupied by dynamic oligosaccharides [7–9].
2. Impact on the other fields of research
   The methodology developed for conformational analysis of oligosaccharides will be applicable to structural studies of flexible biomacromolecules, including intrinsically disordered proteins, and therefore will promote biomolecular science in general.
3. Significance as the fundamental research
   Exploration of conformational spaces of oligosaccharides will contribute to a quantitative understanding of the energetics of carbohydrate-protein interactions [9]. Deeper insights into the physicochemical bases of molecular recognition involving oligosaccharides are essential for elucidating glycofunction mechanisms.
4. Possible application for industry and medicine, if any
   Needless to say, conformational analyses of oligosaccharides are important for developing drugs targeting carbohydrate recognition systems. Moreover, most biopharmaceuticals are modified with oligosaccharides and their functional roles are defined through conformational analyses [10].

5. Future perspectives
   It is expected that current analytical techniques will be developed to allow conformational analyses of more complicated glycoconjugates and dynamic supramolecular complexes exemplified by microdomains. This methodological development will provide molecular bases for a variety of biological processes and enable conformational analyses of artificial oligosaccharides, thereby contributing to drug discovery research.
6. Problems to be solved
   For further advancement of carbohydrate conformational analyses, it is necessary to develop experimental and theoretical approaches for observing the dynamics of water molecules surrounding glycans and for charactering the dynamic structures of glycans in heterogeneous environments (Fig. 2.2).

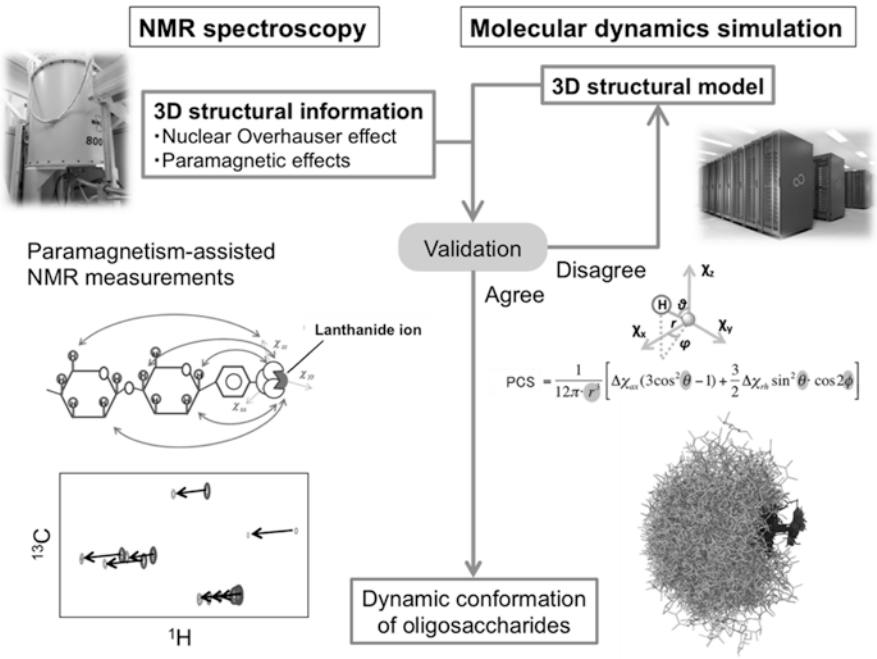

Fig. 2.2 Conformational analysis of oligosaccharides by a hybrid approach combining NMR spectroscopy and molecular dynamics simulation. Part of the figure was adapted from Kato et al. (2018) [Modern Magnetic Resonance, Webb G. (eds), 2018, pp 737–754 with the permission of Springer

2.3 Conformational Analysis of Glycans

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Keywords NMR, Molecular dynamics simulation, Lectin, Dynamics, Affinity

1. Significance in the field of glycoscience and its current situation
   Glycans attached to proteins contribute to the maintenance of protein structure and protein stability through intramolecular interactions [11]. Glycans are also involved in signal transduction via intermolecular interactions with lectin receptors. In order to understand these biological events, it is essential to analyze the conformations and interactions of glycans. Currently, conformational analysis of glycans is being performed by means of experimental (e.g. NMR or X-ray) [12] or theoretical (e.g. molecular dynamics simulation) [13, 14] methods. However, it is often difficult to define the conformations experimentally because glycans are inherently flexible. Theoretical approaches have an advantage in considering the dynamics, but the output is highly dependent on the force field and theory applied.
2. Impact on the other fields of research
   The techniques used in glycan analysis will be applicable to other molecules such as polysaccharides and glycoproteins. Similarities and differences will be discussed by comparison with other flexible biomolecules such as intrinsically disordered proteins.
3. Significance as the fundamental research
   It is currently impossible to describe the conformations and dynamics of glycans based solely on experimental data. Further efforts are necessary to develop experimental methods. Theoretical approaches need improvement of the force field and its parameters by considering experimental data.
4. Possible application for industry and medicine, if any
   Conformational analysis of glycans will play a significant role when a lectin receptor is a target of drug development. Furthermore, it is essential to analyze the structure-function relationships of biopharmaceuticals such as antibody therapeutics and to develop an inhibitor of glycosidases.
5. Future perspectives
   Through accumulation of knowledge on the conformations and dynamics of glycans, it will be possible to predict the binding affinities between glycans and proteins. Currently, qualitative estimation of binding free energy is being successfully performed.
6. Problems to be solved
   It is challenging to accurately estimate the binding free energies of lectin-glycan complexes. It is important to develop a suitable method to predict the effect of a "non-epitopic" glycan region on the binding to lectin molecules [15] (Fig. 2.3).

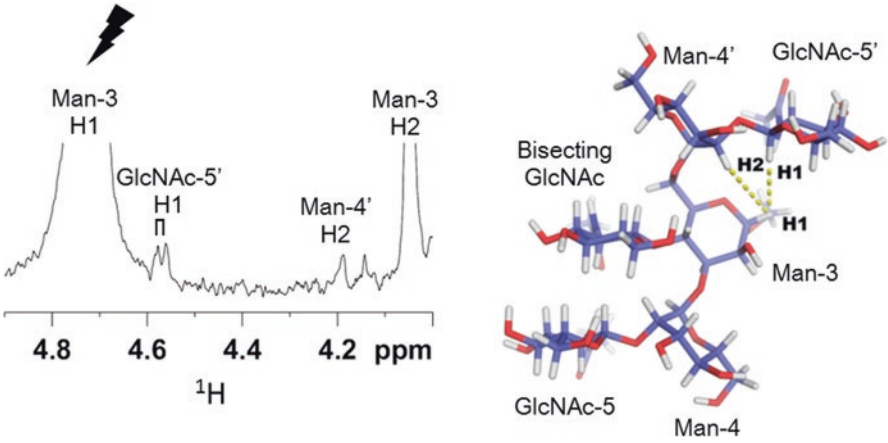

Fig. 2.3 Analysis of lectin-bound glycan conformation by solution NMR spectroscopy. A 1D selective NOESY spectrum was obtained for the glycan in the presence Calsepa lectin, inverting the Man-3 H1 signal. Inter-residue TR-NOE signals were detected to define a flipping-back conformation

2.4 Computational Science (Supercomputer) and AI (Artificial Intelligence)

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Keywords Supercomputer, Computational science, AI, Machine learning, Black box

1. Significance in the field of glycoscience and its current situation
   Computational sciences using supercomputers is now prevailing in all scientific fields. In life science, supercomputers are used for molecular dynamics simulation of macromolecules in a cell [16, 17], rapid sequencing of whole genome data [18], and drug-receptor interaction analysis [19]. RIKEN's K computer belongs to the highest level of supercomputers in the world and is utilized in every area from basic science to industrial application. Meanwhile, artificial intelligence (AI) is highly dependent on the performance of computers and is now attracting the attention of the public [20]. Computational science including AI will play a significant role in the glycoscience field, however, the applications are currently limited.
2. Impact on the other fields of research
   In order to solve the issues in glycoscience using computational science or AI, a database must be organized with a proper ontology format. In particular, glycan structures are often ambiguous and its incorporation into a database is not straightforward. However, once this is resolved, the glycoscience field will be totally open to many other scientists.
3. Significance as the fundamental research
   AI is now mostly utilized for industrial applications by IT companies. The use of AI for basic research is rather limited. Some of the important issues in basic science might be solved by AI.
4. Possible application for industry and medicine, if any
   By utilizing computational science and AI, many advances will be possible such as the development of glycan-related drug (glycomimetics) or finding of some correlations between glycan structures and a certain disease (glycan biomarker).
5. Future perspectives
   AI can facilitate early diagnosis and personalized care of particular diseases by analyzing the patterns of glycan structures.
6. Problems to be solved
   The computational thinking is a black box to us. One critical issue is to open the black box. Another important issue is to collect high-quality, well-annotated data for machine learning (Fig. 2.4).

Fig. 2.4 A supercomputer with a massively parallel computing system

2.5 Structural Study of Proteins in the Glycoscience Field

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Keywords Structural biology, X-ray crystallography, Nuclear magnetic resonance, Cryo-electron microscopy, Single particle analysis

1. Significance in the field of glycoscience and its current situation
   Molecular insights into protein structures are essential for understanding the functions of biomolecules in glycoscience, as in other life science fields. The targets of structure determination are diverse, including biosynthetic and degrading enzymes for glycans, and proteins that bind to and transport glycans. Whole-genome analysis will reveal many candidate genes for glycosyltransferases and glycosidases, but their precise biological roles cannot be logically inferred from the amino acid sequences. The three-dimensional structures could provide critical clues as to their biological functions. Usually, proteins from eukaryotic organisms are chosen as targets, but ones from prokaryotic organisms are also useful as model systems of eukaryotic proteins, since eubacteria and archaea also utilize glycans as an essential component of their cells.
2. Impact on the other fields of research
   The three-dimensional structures of monosaccharides and oligosaccharides have similar chemical properties and only differ in stereochemistry. Elucidation of the recognition mechanisms of monosaccharides and oligosaccharides by proteins is a very difficult task, but it will be very useful for clinical and industrial applications.
3. Significance as the fundamental research
   The atomic coordinates of proteins are very useful information in basic sciences. Almost all are deposited in the Protein Data Bank and available for public use.
4. Possible application for industry and medicine, if any
   For commercial application of glycoproteins, such as erythropoietin, blood-clotting factors, anti-coagulants, immunoglobulins, gonadotropins and interferons, fully occupied glycosylation sites and homogeneous glycan structures are crucial. Structural studies of glycoproteins of interest and related enzymes will provide useful hints for the quality improvement of glycoprotein production.
5. Future perspectives
   X-ray crystallography and cryoEM single particle analysis are the methods of choice for structural determination of proteins. However, due to the intrinsic flexibility of glycans, crystallization of glycoproteins is difficult and cryoEM single particle analysis only provides information on protein portions, i.e., not on glycan portions. NMR spectroscopy is indispensable for the structural and dynamical analyses of glycans themselves. Technical developments for in-depth characterization of glycoproteins will be beneficial in the glycoscience field.

6. Problems to be solved
   Low-cost and efficient large-scale preparation of glycans and glycoproteins is required for structural biology [21]. In particular, a low-cost and efficient stable isotope labeling method for glycans is essential for NMR spectroscopy [22, 23]. Molecular simulation calculation of oligosaccharide structures should be developed [24] (Fig. 2.5).

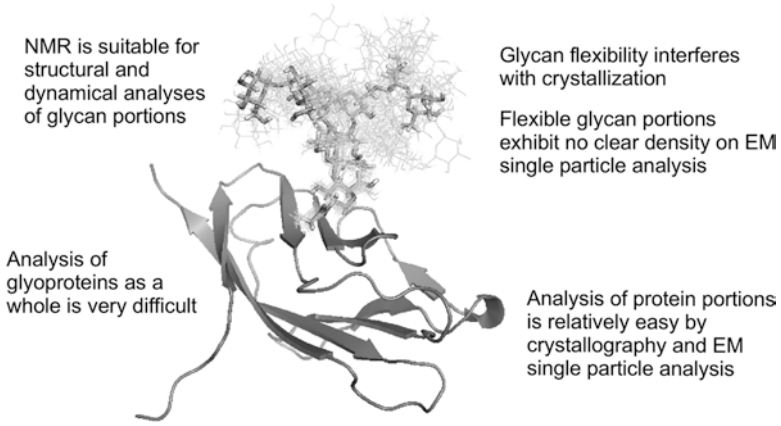

Fig. 2.5 The flexibility of glycan portions makes structural analysis of glycoproteins very difficult. Frequently, the trimming or removal of glycans is performed prior to structural determination of protein portions. NMR is suitable for the structural and dynamical analyses of glycan portions

2.6 Structural Analysis of Sugar Related Proteins

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Keywords Glycosyltransferase, Lectin, Three dimensional structure, Molecular recognition

1. Significance in the field of glycoscience and its current situation
   Since sugar chains are extremely diverse, research on how they are biosynthesized, and how they are recognized, etc. has not progressed much. However, along with the progress of research in the field, it is being revealed that protein groups recognize only a part of sugar chains and consequently bind multiple sugar chains, and that other protein groups specifically recognize specific sugar chains. These proteins range from ones showing simple sugar-binding activity to ones showing glycosyltransferase activity. Also, their physiological activities are also being analyzed. As an example, analysis of the three dimensional structure of the glycosyltransferase POMGnT1 and glycopeptide complexes revealed the molecular mechanism of onset of muscular dystrophy [25]. In summary, in order to clarify the molecular recognition mechanism between sugar chains and proteins, three-dimensional structure information in these complexes is indispensable.
2. Impact on the other fields of research
   Most tumor markers are sugar-binding proteins [26], and some of the targets of antiviral drugs are glycosylases [27]. In addition, a genetic disease, muscular dystrophy, is a glycosyltransferase abnormality [28]. As in these examples, research on sugar chain-related proteins is directly linked to the medical and medicine fields.
3. Significance as the fundamental research
   Recently, a sugar that has not been found in mammals was found in sugar chains involved in muscular dystrophy [29]. Research on biosynthetic enzymes for the sugar and elucidation of the molecular function is awaited. As in this example, fundamental scientific research in this field remains to performed.
4. Possible application for industry and medicine, if any
   Improvements and new development of tumor markers, development of anticancer drugs expected to develop from those, etc. are expected. In addition, sugar-related proteins could be widely used as a research tool for sugar chains themselves.

5. Future perspectives
   Sugar chains and sugar-related proteins remain unknown. As research progresses, not only contribution to fundamental science but also possibilities for application are expected.
6. Problems to be solved
   It is necessary to clarify the molecular mechanisms of how various sugar chains are synthesized and how they are recognized by proteins. It is also important to clarify the relationship between the mechanisms and physiological functions (Fig. 2.6).

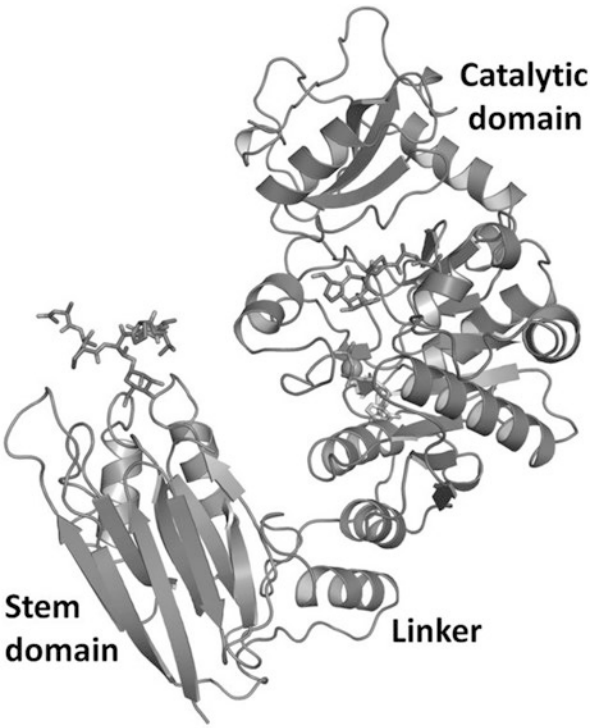

Fig. 2.6 Structure of a complex of POMGnT1 and a glycopeptide. The glycopeptide shown by the rod model binds not only to the catalytic domain but also to the stem domain. This explains well the molecular mechanism of biosynthesis of the core M1 and core M3 sugar chains, which are related to muscular dystrophy

2.7 Simulation and Imaging of Membranes

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Keywords Fluorescence labeling, Click reaction, Efficient synthesis of glycan, Fluorescence resonance energy transfer, Single-molecule imaging

1. Significance in the field of glycoscience and its current situation
   It is necessary to carry out molecular imaging of glycans in living cell membranes to determine their functions. To fluorescently label glycans in cell membranes, a method with which modified glycans are taken into cells and fluorescent dyes are conjugated with the modified glycans in cell membranes has been used [30]. However, this method is not suitable for function analysis because all the modified glycans are detected. To avoid loss of the function of glycolipids after labeling with fluorophores, a highly efficient synthesis technique is required. For these reasons, molecular imaging of glycans has not been necessarily performed in a variety of systems. Coarse grained and all atom molecular dynamics simulation of glycans in membranes has been recently reported [31].
2. Impact on the other fields of research
   Improvement of the imaging (fluorescent labeling) technique for glycans will allow us to detect glycan-protein interactions by the fluorescence resonance energy transfer (FRET) method [32], and increase understanding of the interaction mechanisms.
3. Significance as the fundamental research
   It has been proposed that lipid rafts are the platform for signal transduction, and enriched in glycolipids such as gangliosides. Raft mechanisms may be unraveled by molecular imaging and simulation of glycans.
4. Possible application for industry and medicine, if any
   Interactions between glycans and proteins are involved in invasion of pathogens into cells, and carcinoma cell metastasis. It may be possible to unravel the mechanisms by the methods mentioned above. Elucidation of the mechanisms may also facilitate drug discovery.
5. Future perspectives
   As mentioned above, the method involving labeling of modified glycans with fluorophores is not appropriate for functional analysis of glycans. However, specific glycans can be visualized by observing FRET between GFP fused with the proteins and the fluorescently labeled modified glycans [32]. Furthermore, improvement of the techniques of glycan synthesis and single-molecule imaging may allow us to detect weak interactions of glycans.

6. Problems to be solved
   As mentioned in 1), it is impossible to specifically label glycans with fluorophores by labeling modified glycans that are taken up by cells. Furthermore, highly efficient synthesis techniques are required for fluorescence labeling of glycolipids such as gangliosides [33, 34]. These issues should be solved (Fig. 2.7).

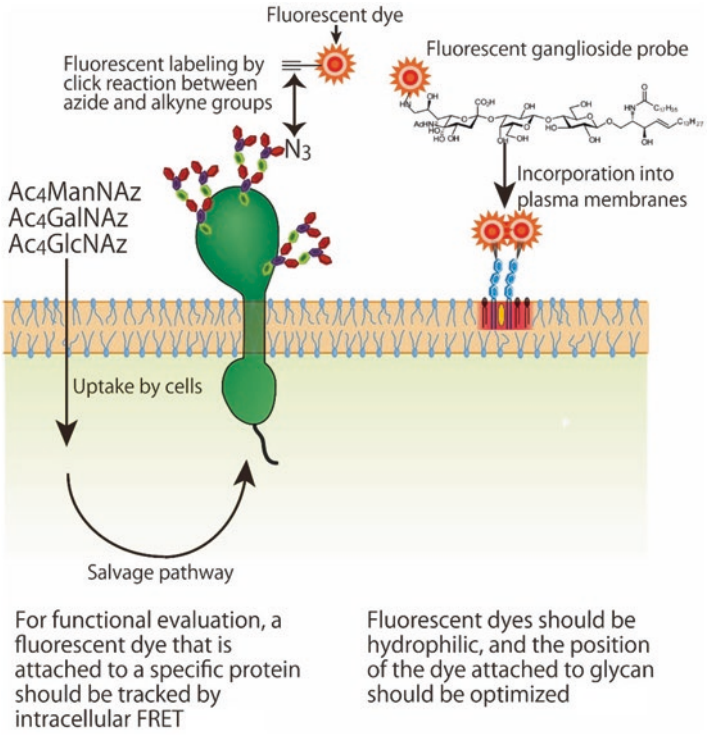

Fig. 2.7 Metabolic labeling of glycans of membrane proteins with fluorescent dyes and synthesis of fluorescent ganglioside probes that behave like the parental molecules. In both cases, we need to devise a way to avoid losing the glycan functions

2.8 Simulation/Imaging of Membranes

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Keywords Imaging, Membrane protein, Lipid, Trafficking, Labeling

1. Significance in the field of glycoscience and its current situation
   Imaging techniques involving fluorescence and electron microscopes provide valuable information to elucidate the roles of saccharides in the functions and trafficking of membrane proteins and lipids. To date, it has been verified that glycan addition to membrane proteins is significant for endocytosis or localization of the proteins [35, 36]. In addition, previous studies have shown that some lipids with glycans accumulate in lipid rafts that are involved in regulation of signal transduction [37]. Disorders of glycoconjugates cause various diseases including cancer and diabetes [37, 38]. Thus, imaging analyses of saccharide function will contribute to elucidation of biological phenomena or pathogenic mechanisms related to trafficking of membrane proteins and lipids, and thus become increasingly important.
2. Impact on the other fields of research
   Combination of imaging analyses with various techniques in the field of chemical biology, omics, informatics and so on will lead to identification of molecules in biological membranes whose functions are regulated by saccharides. It is presumed that saccharides are involved in a wide range of biological phenomena and pathogeneses, since there are a lot of molecules modified with saccharides in biological membranes.
3. Significance as the fundamental research
   It is becoming clear that saccharides control various biological phenomena in biological membranes. It is thus important to detect the interactions of saccharides with membrane proteins and lipids, and image them in real time. Information on these interactions is meaningful for understanding the regulation mechanisms of life.
4. Possible application for industry and medicine, if any
   Much research has shown that saccharide-bound molecules on biological membranes are involved in cancer, diabetes, and neurological disorders. Findings obtained on imaging research will be useful for the diagnosis and treatment of these diseases.
5. Future perspectives
   Imaging-based screening analyses will promote development of molecules that target interactions between saccharides and other biomolecules in membranes. These molecules will be useful for treatment of various diseases involving saccharides or related factors [39].

6. Problems to be solved
   It is highly necessary to develop novel techniques for specific labeling and sensitive detection of membrane proteins and lipids modified with saccharides. Moreover, in addition to the use of imaging techniques such as super-resolution imaging and correlative light electron microscopy (CLEM), a technological breakthrough in the imaging field will be required to analyze the functions and trafficking of biomolecules in membranes with higher temporal and spatial resolution (Fig. 2.8).

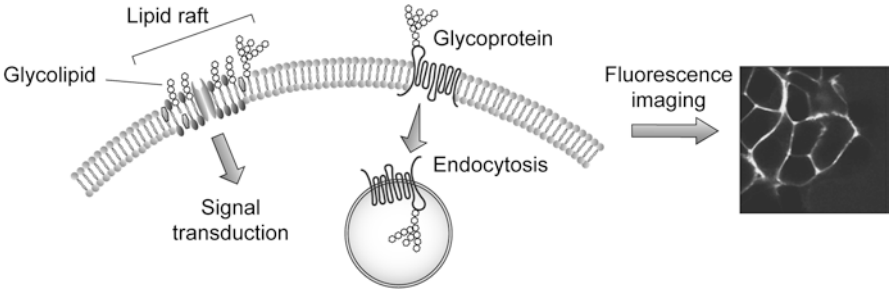

Fig. 2.8 Functions and trafficking of glycolipids and glycoproteins in biological membranes and imaging

2.9 Molecular Imaging of Cells and Organisms Using Labeled Glycans

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Keywords Metabolic labeling, Bioorthogonal reactions, Protein modification, Molecular imaging, Glycosyltransferases

1. Significance in the field of glycoscience and its current situation
   Molecular imaging of glycans is an important topic in glyco-science. The key to this technique is the preparation of labeled glyco-conjugates. This approach can be roughly categorized into two methods: analysis of the localization and behavior of labeled glyco-conjugates analogues prepared by organic synthesis [40, 41], and visualization of the expression of target glycans prepared by insertion of labeled monosaccharide analogues into glyco-conjugates through metabolic processes [42, 43]. These methods continue to evolve, with new chemical tools being developed for *in vivo* imaging [44, 45] in a model system mimicking the heterogeneity of glyco-conjugates [40, 41] or of sialic acid analogues capable of passing through the blood brain barrier [46]. Recent advances include the detection of a specific glycosylation pattern on a specific protein using FRET analysis [47, 48].
2. Impact on the other fields of research
   Since the progress of this field is highly relevant to the development of protein modification methods such as the RIKEN click reaction, and bioorthogonal organic reactions such as the copper-catalyzed azide-alkyne click reaction or the Bertozzi ligation, methods for site-specific/selective chemical modification of biomolecules applicable in cells or *in vivo* are being actively developed.
3. Significance as the fundamental research
   Unlike the molecular imaging of proteins, glycans are difficult to visualize by genetic engineering procedures. Although this field has dramatically advanced after the breakthrough reports of bioorthogonal organic reactions, it should be mentioned that it is still in the process of development. Thus, further fundamental research will be highly significant.
4. Possible application for industry and medicine, if any
   Molecular imaging of a specific glyco-conjugate related to a certain disorder will be applicable as a diagnostic method. Application to drug delivery is expected by using artificial glyco-conjugate analogues with high accumulation properties as to specific tissues/tumors.
5. Future perspectives
   Development of imaging technologies for a specific glycosylation pattern on a specific protein (especially intracellular proteins) as well as *in vivo* imaging,

which is currently limited to several organisms, will enable the determination of the precise functions of glyco-conjugates more spatiotemporally and in real time. Utilization of recently developed imaging technologies (such as Raman microscopy and photo-acoustic effect) is considered to be effective.

6. Problems to be solved
   Preparation of glyco-conjugates by metabolic labeling methods is still limited, and chemical synthesis of these molecules is a rather time-consuming and complicated process. Modification of glycan chains via the degradation of prepared labeled glyco-conjugates by glycohydrolases needs to be taken into account, and could be overcome by the development of metabolically stable glyco-conjugate analogues (Fig. 2.9).

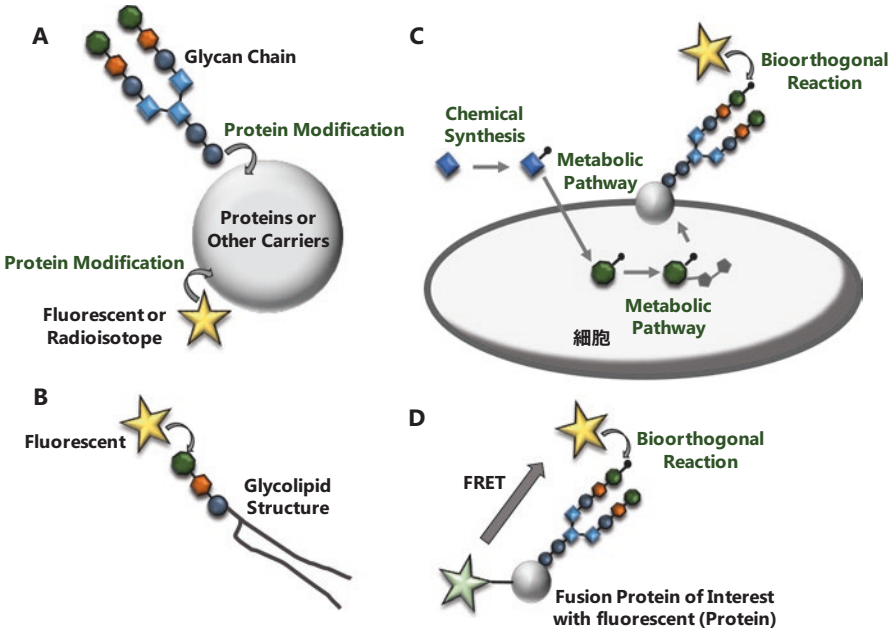

Fig. 2.9 (**A**) Preparation of labeled glyco-conjugates; (**B**) Preparation of labeled glycolipids by chemical synthesis; (**C**) Comprehensive labeling of glyco-conjugates through metabolic processes; (**D**) FRET imaging for the detection of a specific glycosylation pattern on a specific protein

2.10 Diagnosis and Imaging Using Labeled Glycans in Cells and Organisms

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Keywords Click chemistry, Sugar analog, Imaging, Biomarker

1. Significance in the field of glycoscience and its current situation
   As particular changes in glycan structure were revealed to cause the development and progression of diseases by altering the functions of proteins and cells, visualization (imaging) of the particular glycans will allow us to clarify the functions and states of proteins, cells and organisms, and to diagnose diseases. Currently, two methods are commonly used; one is metabolic glycan labeling in combination with sugar analog and click chemistry [49–52], and the other is incorporation of modified glycolipids and glycoproteins having chemically labeled glycans [53].
2. Impact on the other fields of research
   Visualizing glycans is critically important for both neuroscience and medical science, which favor visual and spatial information of molecules and easy analysis without complex steps and expertise, respectively. Development of easy imaging tools for glycans would contribute to the spread of glycobiology to these fields.
3. Significance as the fundamental research
   To understand the functions, localization and metabolic pathways of a given glycan, it is indispensable to label and trace it in living cells and organisms. Currently, the lack of good imaging tools hampers our examination of the detailed spatial information of glycans, and thus development of novel tools is strongly desired.
4. Possible application for industry and medicine, if any
   Advance in this technology would lead to development of novel glycan biomarkers and methods for detection of those markers. In particular, development of new ways to detect glycan markers is expected, which will be useful for the diagnosis and prognosis of diseases including cancer.
5. Future perspectives
   Visualizing specific glycans and glycoforms can contribute to elucidation of the genuine functions of glycans that have so far been obscured by the term “heterogeneity”. In addition, visualization of specific glycoforms would lead to development of novel glycan biomarkers that have been overlooked and buried in the vast glycoform forest.

6. Problems to be solved
   The three major challenges to be tackled; first is the development of methods to label and detect a specific glycan on a specific glycoprotein. Second is development of methods to detect sugar analogs in labeled glycans without using click chemistry. Third is development of novel ways to visualize sugars to which current labeling methods are not applicable, such as glucuronic acid, xylose and mannose (Fig. 2.10).

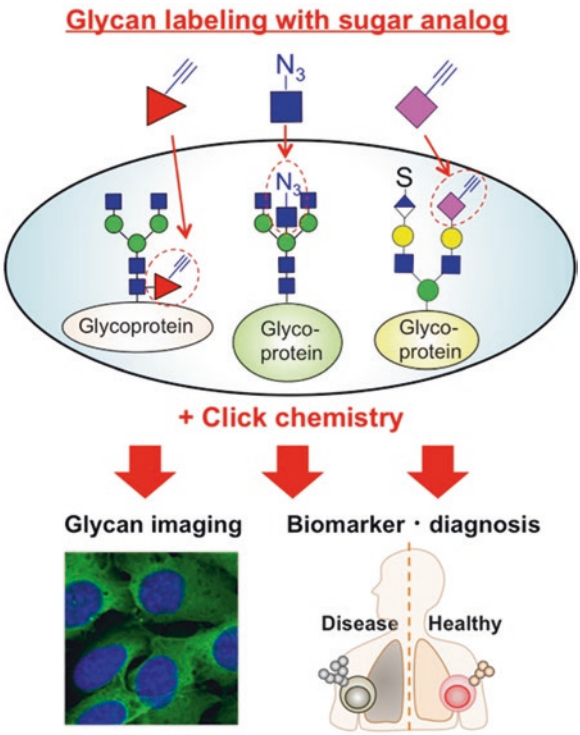

Fig. 2.10 Living cells are treated with sugar analogs, which are incorporated into glycans. The glycans can be easily labeled by click chemistry. This approach can be applied to glycan imaging and biomarker discovery

2.11 Glycoconjugates and Glycoclusters as New Drug Delivery Molecules for In Vivo Molecular Imaging and Theranostics

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Keywords Glycoconjugate, Glycocluster, Heterogeneity, Pattern recognition, Molecular imaging

1. Significance in the field of glycoscience and its current situation
   New drug delivery molecules, which target diseased cells or control excretion pathways in vivo, are desirable to develop an efficient theranostic (diagnosis & therapy) strategy. The interaction of a single molecule of glycan to lectin is generally weak, but can be significantly enhanced when the glycan molecules are conjugated to other biomolecules or arranged to construct a glycocluster environment [54–56]. In particular, heterogeneous glycoclusters could interact with specifically arranged molecules on the cell surface through "pattern recognition mechanisms" [55, 57], and hence result in a strong and selective interaction with target cells in vivo.
2. Impact on the other fields of research
   Glycoconjugates or glycoclusters could be used as new drug delivery molecules for a diagnostic [54, 56] and therapeutic [58] strategy (theranostics). These molecules could be more efficient than conventional antibodies or peptides.
3. Significance as the fundamental research
   Studying "heterogeneous glycan pattern recognition" [55, 57] could lead to new mechanisms of biological significance through glycan interactions.
4. Possible application for industry and medicine, if any
   Glycoconjugates or glycoclusters could be applied to PET (positron emission tomography) [54, 56] and MRI (magnetic resonance imaging), as well as to various carrier-based therapies with RI and anti-tumor drugs.
5. Future perspectives
   Innovative chemical and biological "molecular technologies" for (1) supplying various *N*- and *O*-glycans, and (2) preparing "structurally well-defined" hererogeneous glycococlusters [57] (Fig. 2.11).

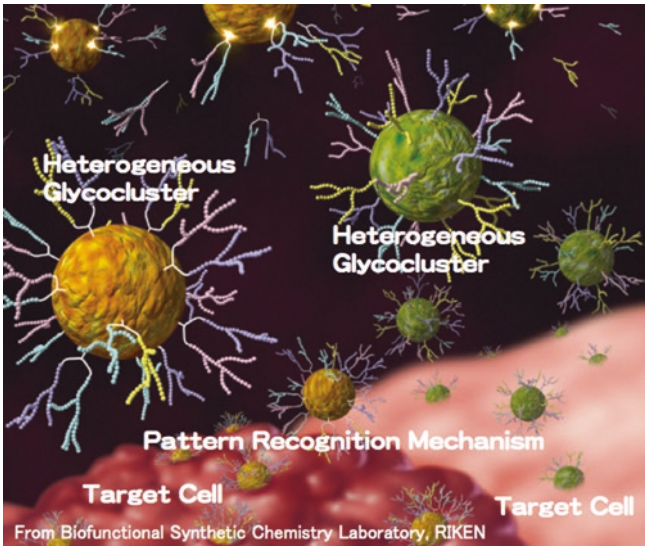

Fig. 2.11 Heterogeneous glycoclusters selectively interact with several molecules on the target cell surface through a "pattern recognition mechanism"

2.12 Imaging Mass Spectrometry (IMS)

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Keywords Imaging mass spectrometry (IMS), Glycolipids, Glycotherapeutics

1. Significance in the field of glycoscience and its current situation
   Imaging mass spectrometry (IMS) is a two-dimensional mass spectrometric method for visualizing the spatial distribution of biomolecules. The method does not require separation or purification of target molecules, and it can be applied, not only to the identification of unknown molecules, but also the localization of numerous molecules simultaneously. Japanese groups have already reported on numerous studies of glycolipids using IMS [59–63]. For example, in ganglioside studies, molecules of the same class that contain fatty acids with different lengths such as GM1 (d18: 1/18: 0) and GM1 (d20: 1/18: 0) can be analyzed simultaneously by IMS although it is difficult when other methods are used [60, 63]. IMS will contribute to the future development of the glycoscience.
2. Significance as the fundamental research
   IMS can distinguish different glycolipid molecular species by simultaneously measuring the difference in mass-to-charge ratio (m / z). In addition, the use of tandem mass spectrometry (MS^n) to examine the tissue surface permits the visualized molecule to be identified and further provides detailed information on its structure.
3. Possible application for industry and medicine, if any
   It is important to determine how candidate drugs such as glycotherapeutics are distributed and metabolized in the body at an early stage of drug discovery. IMS is gaining great interest in monitoring drug delivery and metabolism. Since this emerging technology allows for the simultaneous imaging of many types of metabolite molecules, IMS can be used to visualize and distinguish parent drugs and their metabolites.
4. Future perspectives
   The fundamental contribution of IMS to science makes it a powerful tool for use in the early detection and characterization of cellular processes, both in health and disease states, to understand and treat diseases very effectively. Hopefully we can expect that this approach will lead to the development of glycotherapeutics.

5. Problems to be solved
   Many significant advances have been made in IMS to characterize a variety of molecular species in various types of biological samples, but there is still room for improvements in the areas of sample preparation, ionization and instrumentation (Fig. 2.12).

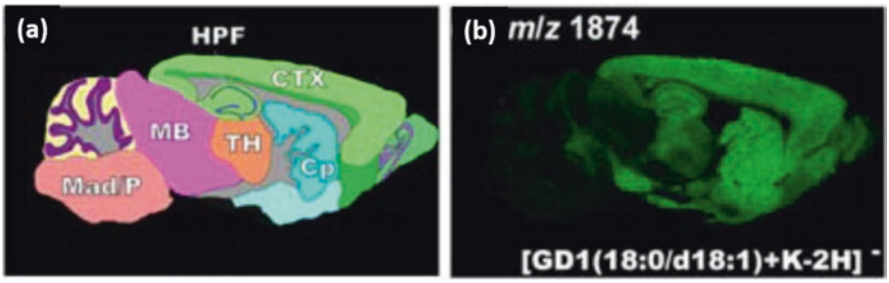

Fig. 2.12 Schematic diagram of the brain section (**a**). A representative result of the brain ganglioside obtained by IMS (**b**). These data were cited with modifications from Ref. [60]

References

References for Section 2.1

1. Lee JH et al (2016) Cryo-EM structure of a native, fully glycosylated, cleaved HIV-1 envelope trimer. Science 351:1043–1048
2. Kinoshita T et al (2017) Short stop mediates axonal compartmentalization of mucin-type core 1 glycans. Sci Rep 7:41455
3. Itoh K et al (2016) Mucin-type core 1 glycans regulate the localization of neuromuscular junctions and establishment of muscle cell architecture in *Drosophila*. Dev Biol 412:114–127
4. Kinoshita T et al (2014) Immuno-electron microscopy of primary cell cultures from genetically modified animals in liquid by atmospheric scanning electron microscopy. Microsc Microanal 20:469–483
5. Murai T et al (2011) Low cholesterol triggers membrane microdomain-dependent CD44 shedding and suppresses tumor cell migration. J Biol Chem 286:1999–2007

References for Section 2.2

6. Yamaguchi Y et al (2014) Structural analysis of oligosaccharides and glycoconjugates using NMR. Adv Neurobiol 9:165–183
7. Yamaguchi T et al (2014) Exploration of conformational spaces of high-mannose-type oligosaccharides by an NMR-validated simulation. Angew Chem Int Ed 53:10941–10944
8. Kato K, Yamaguchi T (2015) Paramagnetic NMR probes for characterization of the dynamic conformations and interactions of oligosaccharides. Glycoconj J 32:505–513
9. Suzuki T et al (2017) Conformational analysis of a high-mannose-type oligosaccharide displaying glucosyl determinant recognised by molecular chaperones using NMR-validated molecular dynamics simulation. ChemBioChem 18:396–410
10. Kamiya Y et al (2014) Recent advances in glycoprotein production for structural biology: toward tailored design of glycoforms. Curr Opin Struct Biol 26:44–53

References for Section 2.3

11. Nagae M, Yamaguchi Y (2012) Function and 3D structure of the *N*-glycans on glycoproteins. Int J Mol Sci 13:8398–8429
12. Nagae M et al (2016) Atomic visualization of a flipped-back conformation of bisected glycans bound to specific lectins. Sci Rep 6:22973
13. Re S et al (2011) Structural diversity and changes in conformational equilibria of biantennary complex-type *N*-glycans in water revealed by replica-exchange molecular dynamics simulation. Biophys J 101:L44–L46
14. Nishima W et al (2012) Effect of bisecting GlcNAc and core fucosylation on conformational properties of biantennary complex-type *N*-glycans in solution. J Phys Chem B 116:8504–8512
15. Navarra G et al (2017) Carbohydrate-lectin interactions: an unexpected contribution to affinity. Chembiochem 18:539–544

References for Section 2.4

16. Yu I et al (2016) Biomolecular interactions modulate macromolecular structure and dynamics in atomistic model of a bacterial cytoplasm. elife 5:e19274
17. Kobayashi C et al (2017) GENESIS 1.1: a hybrid-parallel molecular dynamics simulator with enhanced sampling algorithms on multiple computational platforms. J Comput Chem 38:2193–2206
18. Puckelwartz MJ et al (2014) Supercomputing for the parallelization of whole genome analysis. Bioinformatics 30:1508–1513
19. Dror RO et al (2013) Structural basis for modulation of a G-protein-coupled receptor by allosteric drugs. Nature 503:295–299
20. Musib M et al (2017) Artificial intelligence in research. Science 357:28–30

References for Section 2.5

21. Kamiya Y et al (2014) Recent advances in glycoprotein production for structural biology: toward tailored design of glycoforms. Curr Opin Struct Biol 26:44–53
22. Kato K et al (2010) Stable-isotope-assisted NMR approaches to glycoproteins using immunoglobulin G as a model system. Prog Nucl Magn Reson Spectrosc 56:346–359
23. Fujinami D et al (2017) Asn-linked oligosaccharide chain of a crenarchaeon, *Pyrobaculum calidifontis*, is reminiscent of the eukaryotic high-mannose-type glycan. Glycobiology 27:701–712
24. Galvelis R et al (2017) Enhanced conformational sampling of *N*-glycans in solution with replica state exchange metadynamics. J Chem Theory Comput 13:1934–1942

References for Section 2.6

25. Kuwabara N et al (2016) Carbohydrate-binding domain of the POMGnT1 stem region modulates *O*-mannosylation sites of α-dystroglycan. Proc Natl Acad Sci U S A 113:9280–9285
26. Romano G (2015) Tumor markers currently utilized in cancer care. Mater Methods 5:1456
27. Smith BJ et al (2001) Analysis of inhibitor binding in influenza virus neuraminidase. Protein Sci 10:689–696
28. Endo T (2015) Glycobiology of α-dystroglycan and muscular dystrophy. J Biochem 157:1–12
29. Manya H et al (2016) The muscular dystrophy gene TMEM5 encodes a ribitol β1-4 xylosyltransferase required for the functional glycosylation of dystroglycan. J Biol Chem 291:24618–24627

References for Section 2.7

30. Saxon E, Bertozzi CR (2000) Cell surface engineering by a modified Staudinger reaction. Science 287:2007–2010
31. Gu RX et al (2017) Ganglioside-lipid and ganglioside-protein interactions revealed by coarse-grained and atomistic molecular dynamics simulations. J Phys Chem B 121:3262–3275

32. Haga Y et al (2012) Visualizing specific protein glycoforms by transmembrane fluorescence resonance energy transfer. Nat Commun 3:907
33. Komura N et al (2016) Raft-based interactions of gangliosides with a GPI-anchored receptor. Nat Chem Biol 12:402–410
34. Tamai H et al (2011) The total synthesis of neurogenic ganglioside LLG-3 isolated from the starfish Linckia laevigata. Angew Chem Int Ed 50:2330–2333

References for Section 2.8

35. Hirayama S et al (2016) Fluorogenic probes reveal a role of GLUT4 *N*-glycosylation in intracellular trafficking. Nat Chem Biol 12:853–859
36. Rabinovich GA et al (2007) Functions of cell surface galectin-glycoprotein lattices. Curr Opin Struct Biol 17:513–520
37. Kopitz J (2017) Lipid glycosylation: a primer for histochemists and cell biologists. Histochem Cell Biol 147:175–198
38. Ohtsubo K et al (2011) Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. Nat Med 17:1067–1075
39. Yau T et al (2015) Lectins with potential for anti-cancer therapy. Molecules 20:3791–3810

References for Section 2.9

40. Ogura A et al (2016) Visualizing trimming dependence of biodistribution and kinetics with homo- and heterogeneous N-glycoclusters on fluorescent albumin. Sci Rep 6:21797
41. Komura N et al (2016) Raft-based interactions of gangliosides with a GPI-anchored receptor. Nat Chem Biol 12:402–410
42. Ovryn B et al (2017) Visualizing glycans on single cells and tissues. Curr Opin Chem Biol 39:39–45
43. Lopez Aguilar A et al (2017) Tools for studying glycans: recent advances in chemoenzymatic glycan labeling. ACS Chem Biol 12:611–621
44. Agarwal P et al (2015) Systemic fluorescence imaging of zebrafish glycans with bioorthogonal chemistry. Angew Chem Int Ed Eng 54:11504–11510
45. Laughlin ST et al (2008) In vivo imaging of membrane-associated glycans in developing zebrafish. Science 320:664–667
46. Xie R et al (2016) In vivo metabolic labeling of sialoglycans in the mouse brain by using a liposome-assisted bioorthogonal reporter strategy. Proc Natl Acad Sci 113:5173–5178
47. Haga Y et al (2012) Visualizing specific protein glycoforms by transmembrane fluorescence resonance energy transfer. Nat Commun 3:907
48. Doll F et al (2016) Visualization of protein-specific glycosylation inside living cells. Angew Chem Int Ed Engl 55:2262–2266

References for Section 2.10

49. Agard NJ et al (2004) A strain-promoted [3 + 2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems. J Am Chem Soc 126:15046–150467
50. Chang PV et al (2009) Metabolic labeling of sialic acids in living animals with alkynyl sugars. Angew Chem Int Ed Engl 48:4030–4033

51. Sawa M et al (2006) Glycoproteomic probes for fluorescent imaging of fucosylated glycans *in vivo*. Proc Natl Acad Sci U S A 103:12371–12376
52. Kizuka Y et al (2016) High-sensitivity and low-toxicity fucose probe for glycan imaging and biomarker discovery. Cell Chem Biol 23:782–792
53. Komura N et al (2016) Raft-based interactions of gangliosides with a GPI-anchored receptor. Nat Chem Biol 12:402–410

References for Section 2.11

54. Tanaka K (2016) Chemically synthesized glycoconjugates on proteins: effects of multivalency and glycoform in vivo. Org Biomol Chem 14:7610–7621
55. Ogura A et al (2016) Visualizing trimming dependence of biodistribution and kinetics with homo- and heterogeneous *N*-glycoclusters on fluorescent albumin. Sci Rep 6:21797
56. Tanaka K et al (2010) Noninvasive imaging of dendrimer-type *N*-glycan clusters: in vivo dynamics dependence on oligosaccharides structure. Angew Chem Int Ed 49:8195–8200
57. Latypova L et al (2017) Sequential double "clicks" toward structurally well-defined heterogeneous *N*-glycoclusters: The importance of cluster heterogeneity on pattern recognition in vivo. Adv Sci 4:1600394
58. Tsubokura K et al (2017) In vivo gold complex catalysis within live mice. Angew Chem Int Ed 56:3579–3584

References for Section 2.12

59. Shimma S et al (2008) Mass imaging and identification of biomolecules with MALDI-QIT-TOF-based system. Anal Chem 80:878–885
60. Sugiura Y et al (2008) Imaging mass spectrometry technology and application on ganglioside study; visualization of age-dependent accumulation of C20-ganglioside molecular species in the mouse hippocampus. PLoS One 3:e3232
61. Goto-Inoue N et al (2010) The Detection of glycosphingolipids in braintissue sections by imaging mass spectrometry using gold nanoparticles. J Am Soc Mass Spectrom 21:1940–1943
62. Russo D et al (2017) Glycosphingolipid metabolic reprogramming drives neural differentiation. EMBO J 37:e97674
63. Sugiyama E, Setou M (2018) Visualization of brain gangliosides using MALDI imaging mass spectrometry. Methods Mol Biol 1804:223–229

Chapter 3
Chemical and Enzymatic Synthesis and Production of Glycans

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3.1 Synthetic Glycans

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Keywords Oligosaccharide, Glycoconjugates, Glycosylation, Stereoselectivity

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1. General background
   Oligosaccharides derived from naturally occurring glycoconjugates are quite often heterogeneous and difficult to isolate in large amounts (Fig. 3.1). Synthetic methods are considered to be promising to solve these problems. Therefore, oligosaccharide synthesis by chemical glycosylation has been very actively studied in the last four decades. As a result, it is now possible to synthesize complex glycans with high efficiency [1, 2]. In addition, construction of conceptually difficult glycosidic linkages those in sialic acid glycosides and β-mannosides is quite feasible now. However, it is often necessary to examine reaction conditions in a try-and-error manner to obtain satisfactory results.
2. Significance in glycoscience
   Synthetic glycans are expected to promote biological sciences, especially glycobiology. Participation of researchers from mainstream synthetic organic chemistry has pioneered new directions in oligosaccharide synthesis [3]. Accordingly, glycan chain synthesis is expected to continuously stimulate the chemistry.
3. Major problems
   Glycosylations are key reactions in glycan chain synthesis. They provide important subjects in pure chemistry as the behavior of cationic species is not well understood. Continuous research that aims to develop completely selective glycosylation that proceeds with quantitative yields would be of great value.
4. Future perspectives
   The future would be hard to predict, as it strongly depends on if glycan-derived drug candidate molecules are found.
5. Conceptual breakthroughs for future developments
   Understanding the mechanism of glycosylation is a great challenge in pure chemistry. New directions will be opened by new disciplines, such as activation of hydroxyl groups [4].
6. Practical issues that need to be addressed
   It is extremely important to establish standardized methods for glycan chain synthesis. Speeding-up or creating kits for monosaccharide components is needs to be studied extensively (Fig. 3.1).

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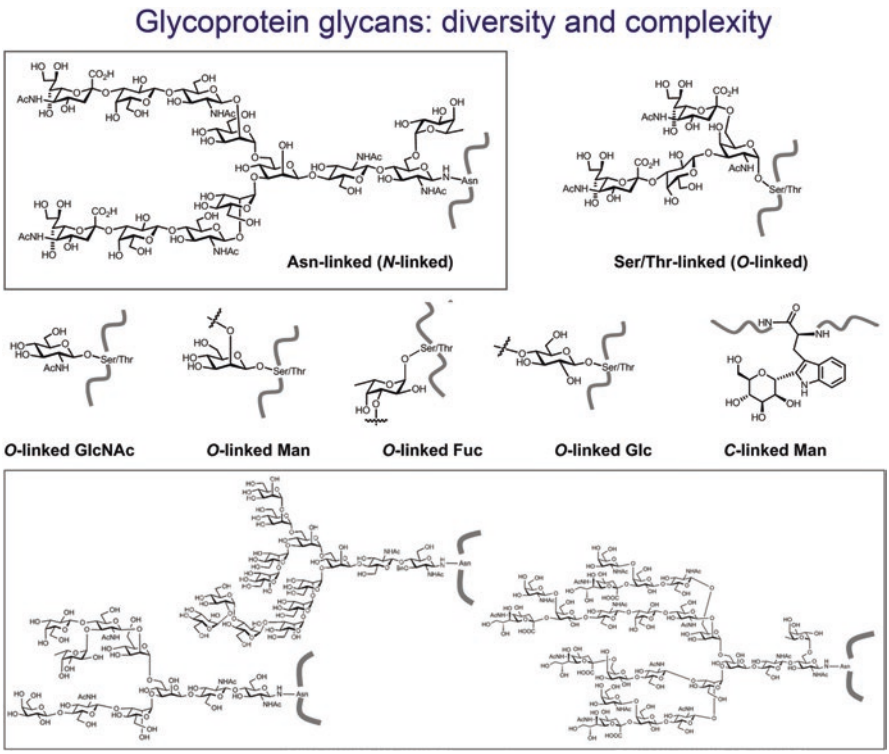

Fig. 3.1 The diversity and complexity of the structures found in glycoproteins. In order to investigate their functions, it is important to supply a sugar chain having a uniform structure. Various synthetic methods have been developed for such a purpose, and it is now possible to synthesize even highly complex sugar chains

3.2 Recent Progress and Perspective on Automated Synthesis of Oligosaccharides

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Keywords Automated synthesis, Solid-phase synthesis, Chemical synthesis, Enzymatic synthesis oligosaccharide

1. Significance in the field of glycoscience and its current situation
   Automated synthesis of oligosaccharides has been recognized as a crucial technique for glycoscience; however, it is still one of the missing technologies in our scientific community. The first automated synthesis of oligosaccharides based on solid-phase synthesis was reported in 2001 [5]. More sophisticated and easily accessible machines have been reported independently [6, 7]. Although solid-phase synthesis is a powerful method to make many compounds in short period of time, it is hard to make a compound in a large scale. An automated synthesizer based on an enzymatic method was also developed in the last decade [8]. Scientists have developed several types of automated synthesizer for oligosaccharides; however, these technologies have not been widespread yet. Now we are facing possible barriers such as low versatility of the machines and carbohydrate building blocks and high prices. To solve these problems development of an automated electrochemical synthesizer for solution-phase synthesis of oligosaccharide is in progress [9].
2. Impact on the other fields of research
   Novel oligosaccharides with unique structures will attract scientists of biochemistry as biocompatible materials. Oligosaccharides with flexible or rigid three dimensional structures may be useful for physical chemists to prepare nanomaterials with structural regularity.
3. Significance as the fundamental research
   Numbers of structurally pure oligosaccharides, which can be isolated from natural sources, are limited. Therefore, automated synthesis of oligosaccharides is a crucial and fundamental technique of glycosciene, which will reveal functions of natural oligosaccharides.
4. Possible application for industry and medicine, if any
   Within 5 years, libraries of oligosaccharides for drug discovery, development of functional foods, and diagnosis using sugar tips will be provided by automated synthesis.

5. Future perspectives
   Technology and knowledge for development of automated synthesis of oligosaccharides will be useful for automated synthesis of functional materials. On demand synthesis of useful compounds is crucial technology for sustainable future of our society.
6. Problems to be solved
   Time efficiency, high yield, and sustainability are all required for automated synthesis of oligosaccharides. A novel technology which fills all these requirements will be highly appreciated as a truly innovative synthetic method (Fig. 3.2).

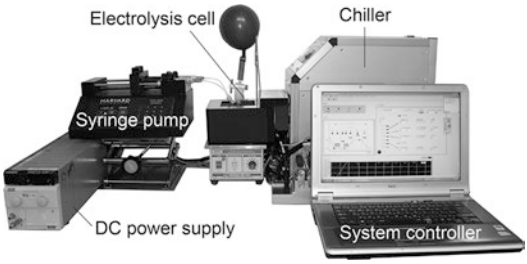

Fig. 3.2 The first generation automated electrochemical synthesizer for solution-phase synthesis of oligosaccharide

3.3 New Synthetic Methods for Standard Glycans: Glycoproteins

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Keywords Glycopeptide, Glycoprotein, Native chemical ligation, Homogeneous glycoproteins

1. Significance in the field of glycoscience and its current situation
   We can prepare glycoproteins in mammalian cells and this method has been used for the preparation of glycoprotein drugs and for basic scientific research. The N-glycosylation positions of glycoproteins can be changed by DNA manipulation, while the O-glycosylation positions cannot be regulated. In addition to this, regulation of the structures of N- and O-glycans is still impossible. Therefore, we cannot identify which oligosaccharide structure is critical for an individual biological event. Under these circumstances, the development of chemical synthesis of glycoproteins bearing homogeneous oligosaccharides has been expected. Five groups have demonstrated chemical syntheses of glycoproteins [10–14].
2. Impact on the other fields of research
   Almost all proteins on the cell surface and in body fluids are glycosylated. The non-glycosylated proteins cannot exhibit their biological functions. Understanding of oligosaccharide functions will reveal the necessity of oligosaccharides to society of glycoprotein pharmaceutical drugs and other research fields of biology.
3. Significance as the fundamental research
   If we can change oligosaccharide structure by chemical synthesis, we can evaluate oligosaccharide functions depending on their structures through several specific biological experiments.
4. Possible application for industry and medicine, if any
   Glycoproteins bearing heterogeneous oligosaccharides have been used as pharmaceutical drugs. Glycoproteins bearing homogeneous oligosaccharides are ideal, because potent activity has been confirmed when glycoproteins have homogeneous sialyloligosaccharides. Actually, a company dealing with glycopeptide custom synthesis in Europe reported chemical synthesis can be practically used for the preparation of glycoprotein drugs.
5. Future perspectives
   We expect the application of chemical synthesis to the preparation of glycoprotein drugs.
6. Problems to be solved
   Chemical synthesis needs multi chemical conversion steps. We need improvement involving a method combing chemical synthesis of glycopeptides and *E. coli* expression of non-glycosylated long peptides. Chemical synthesis of varieties of oligosaccharyl-amino acids is essential (Fig. 3.3).

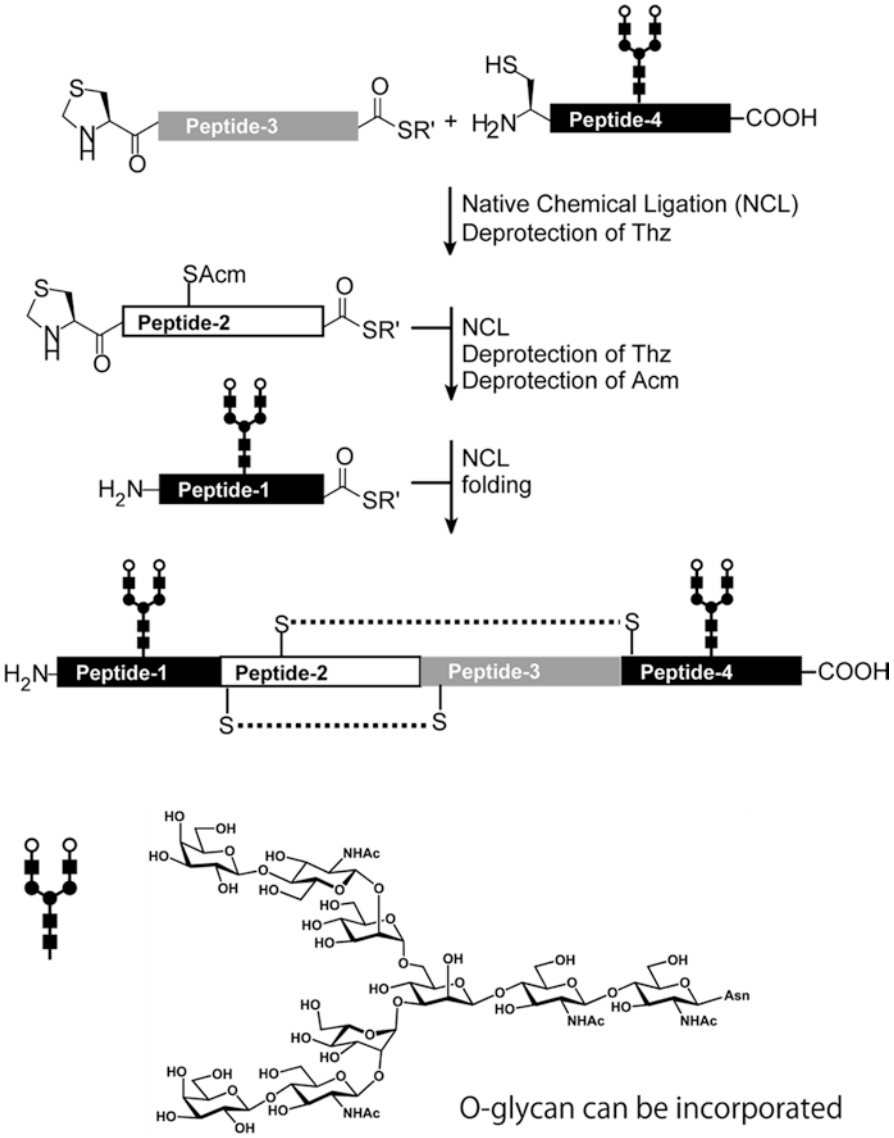

Fig. 3.3 Chemically synthesized glycopeptides and non-glycosylated peptide can be coupled by native chemical ligation or other coupling methods can give glycosyl-full length polypeptides. Subsequent folding experiments give correctly folded glycoproteins

3.4 New Synthetic Methods for Standard Glycans: *N*-Glycans

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Keywords Chemical synthesis, Glycosylation, Convergent synthesis, Chemoenzymatic synthesis, *N*-glycan

1. Significance in the field of glycoscience and its current situation
   In order to clarify glycoprotein glycan functions at the molecular level, structurally defined glycan samples are needed. For this reason, development of an efficient synthetic route for glycans is important. Synthesis of *N*-glycans through a convergent route is an excellent method that can conveniently yield large sugar chains [15]. With the aim to greatly simplify the overall synthetic processes, a semi-chemical synthetic method involving complex type *N*-glycans from biological samples [16] has also been developed [17]. In contrast, biochemists can readily prepare target high-mannose type glycans employing a top-down approach [18]. However, not all glycan samples necessary for biological research can be synthesized using recent synthetic methods. Therefore, there is an urgent need to develop an efficient and stereoselective glycosylation reaction for synthesizing complex glycan structures. Furthermore, in order to study and elucidate the functions of sugar chains, development of practical methods for large-scale synthesis are greatly needed.
2. Impact on the other fields of research
   It is possible to develop highly active biopharmaceuticals by remodeling *N*-glycan containing synthetic glycans. These synthetic glycans can be used as materials for obtaining glycan recognition molecules such as cancer-related antigens and affinity chromatography carriers.
3. Significance as the fundamental research
   Structurally defined glycans are essential for the understanding of glycan functions.
4. Possible application for industry and medicine, if any
   Such research is also important as basic organic synthetic chemistry, including the development of selective glycosylation reactions and new protective groups.
5. Future perspectives
   Highly active antibody drugs can be obtained through remodeling of the *N*-glycans on antibodies (industry and medicine).

6. Problems to be solved
   By determining the essential structures of highly functional oligosaccharides, drug discovery can be accelerated.
   Development of efficient and stereoselective glycosylation reactions is essential for synthesizing complex glycan structures, elucidating the functions of complex carbohydrate chains, and the development of practical methods for large-scale synthesis (Fig. 3.4).

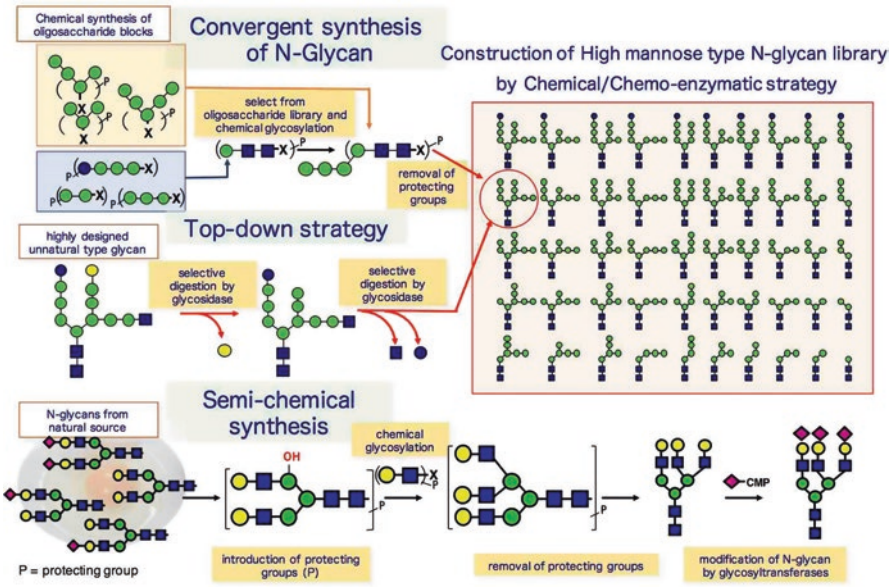

Fig. 3.4 Schemes for chemical synthesis of *N*-glycans

3.5 New Synthetic Methods for Standard Glycans: Glycolipids

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Keywords Sphingoglycolipids, Glyceroglycolipids, Gangliosides, Glycosylation, Rafts

1. Significance in the field of glycoscience and its current situation
   Glycolipids are classified into glycosphingolipids and glyceroglycolipids, both of which exhibit structural diversity in their glycan and lipid moieties. The provision of chemically synthesized, standard products has been strongly desired because they are minor components and exhibit heterogeneity in their structures. From the viewpoint of biological importance, the chemical synthesis of glycosphingolipids, particularly gangliosides, which are glycosphingolipids having sialic acids, has been intensively studied, and chemical synthesis of mammalian gangliosides is almost covered by improving the efficiency of introducing sialic acids using a new sialic acid donor and establishing a lipid introduction method involving a glucosylceramide as a cassette [19]. On the other hand, although the structure of a glyceroglycolipid is relatively simple, there are inherent problems such as having a chemically unstable ester bond in the molecule and having two kinds of linkages, α and β, between glycans and lipids.
2. Impact on the other fields of research
   A supply of chemically synthesized standards in which the structures of the glycan and lipid moieties have been established and no contamination by other biological components (proteins, etc.) is indispensable for elucidation of the biological functions of glycolipids. Establishment of a chemical synthesis method will also lead to the development of probes for biochemical research [20].
3. Significance as the fundamental research
   Glycolipids are minor components possessing biological activities, but are also important constituents of plasma membranes. The supply of a glycolipid with a defined structure can contribute to the elucidation of biological phenomena related to cell membranes, such as raft function [21].
4. Possible application for industry and medicine, if any
   In the study of glycolipids, the structural diversity of sugar chains has attracted attention, and libraries focusing on sugar chain structures have been developed. Because the importance of the lipid moiety is being clarified, new functions of glycolipids will be found in the future by constructing and utilizing a library that covers the diversity of the lipid moiety as well as glycans.
5. Future perspectives
   It is possible to cope with the diversity of their structures, but it is still difficult to synthesize glycolipids in large amounts. This issue should be solved when new compounds are found as seeds for drug discovery in the future (Fig. 3.5).

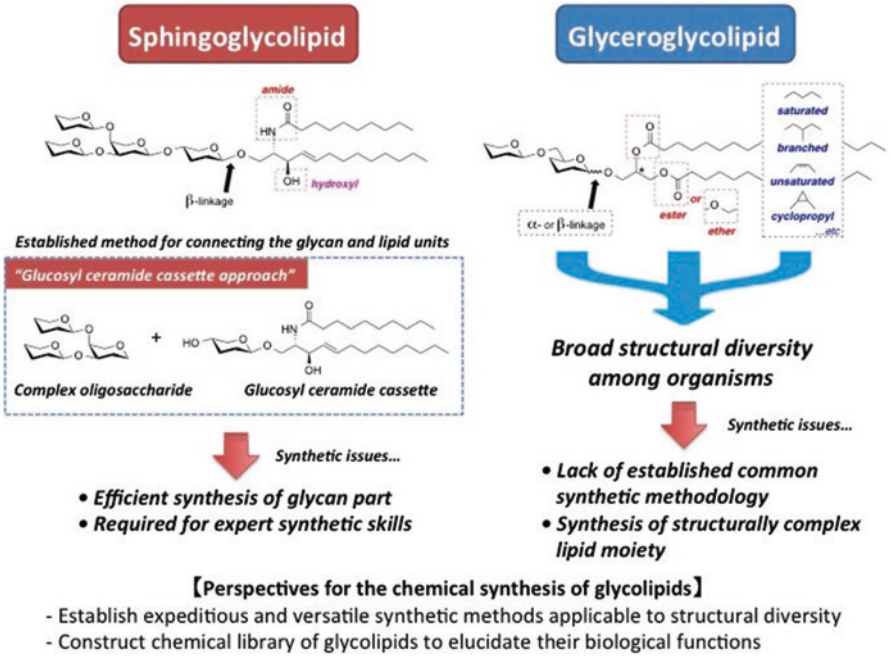

Fig. 3.5 Synthesis of sphingoglycolipids and glyceroglycolipids

3.6 New Synthetic Methods for Standard Glycans: Neo-Glycoconjugates

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Keywords Neo-glycoconjugate, Clustering, Glycan-protein interaction

1. General background
   Due to the involvement of glycan-protein interactions in various biological events, the medicinal application of glycans has been a subject of intensive effort. In the application of glycan functions, amplifying relatively weak glycan-protein interactions is a critical issue, thereby making the technology for glycan clustering very important. Vigorous studies on multivalent glycoconjugates, which are oriented to bio-imaging, drug delivery, and vaccine development (cancer cells, bacteria, and viruses) [22, 23] have led to the development of the conjugates of glycans with a variety of molecules and materials such as proteins, nucleic acids, fullerenes, nano-particles, liposomes etc [24–26].
2. Significance in glycoscience
   The development of multivalent glycoconjugates with bioactive molecules would fulfill crucial roles in pharmacokinetic control.
3. Major Problems
   Investigations on the conjugation of homo- or heterogeneous glycan clusters with functional molecules are of great importance for understanding the biological functions of natural glycoconjugates that are present in cell membranes as heterogeneous molecular architectures.
4. Future perspectives
   Harnessing glycan clustering technology would be of great advantage for developing bio-imaging technology, drug delivery systems and cell culture system.
5. Conceptual breakthroughs for future developments
   The advancement of the synthesis methods for diverse glycans and the development of new molecules and materials as conjugation partners will expand the repertoire of neo-glycoconjugates, promoting application studies of glycan functions.
6. Practical issues that need to be addressed
   Speed-up, automation and cost reduction of glycan synthesis.
   Expansion of the method of chemo-selective conjugation of glycan with other molecules and materials (Fig. 3.6).

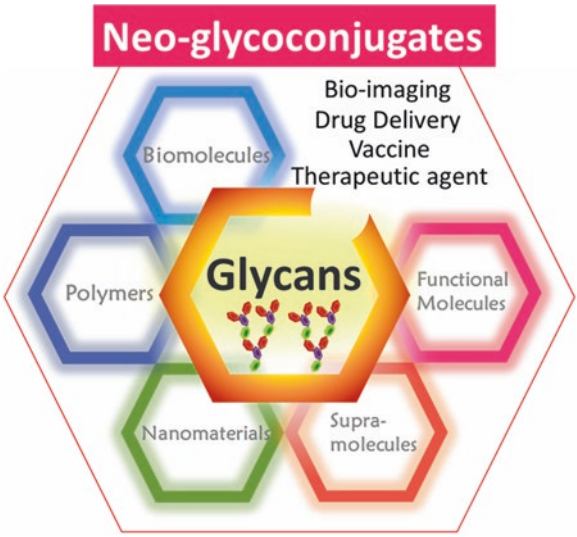

Fig. 3.6 Outline of the components of neo-glycoconjugates

3.7 New Synthetic Methods of Standard Glycans: GPI

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Keywords Glycosyl phosphatidylinositol, GPI anchor, Protozoa, CD1d, Immunomodulation

1. Significance in the field of glycoscience and its current situation
   Glycosyl phosphatidylinositols (GPIs) widely exist in eukaryotes as GPI anchors. GPIs from parasitic protozoa such as the malaria and Entamoeba parasites have characteristic structures and therefore show antigenicity and CD1d- or TLR-dependent immunomodulating activity. Synthetic study of GPIs has been a critical issue, since GPIs from natural sources are heterogeneous in general and possible contamination by other immunomodulating molecules of the GPI fraction cannot be excluded even on extensive purification. Chemical synthesis can afford pure and homogenous compounds for precise functional analysis [27–31]. GPIs have complex structures composed of sugar, lipid, and phosphoric acid and therefore GPIs are attractive synthetic targets for organic chemists. Syntheses of GPIs from the malaria and Entamoeba parasites have already been accomplished, and their application to vaccines and adjuvants has been under investigation [27–31].
2. Impact on the other fields of research
   Since protozoa and nematode derived GPIs are related to their parasitism, providing uniform GPIs and their partial structures by chemical synthesis will lead to elucidation of the role of parasite-derived GPI in the host immune system [27–29, 31]. It will also contribute to the analysis of the in vivo functions of GPIs of higher animals.
3. Significance as the fundamental research
   Since GPIs have a complex structure composed of sugar, lipid, and phosphoric acid, establishing an efficient synthesis is an important task in organic chemistry. Moreover, GPIs derived from parasites are promising sources for their vaccines [27]. Elucidation of their immunomodulating activity is important from the viewpoint of treatment of infectious diseases.
4. Possible application for industry and medicine, if any
   Many neglected tropical diseases (NTDs) are parasitic diseases caused by protozoa and parasites. Parasite-derived molecules should regulate the immune system of the hosts and affect the pathogenicity. The carbohydrate moieties of GPIs are promising targets as vaccines, and the immunomodulatory action of GPIs is also applicable to immunotherapies [27–29, 31].
5. Future perspectives
   Synthetic GPIs are expected to be immunological adjuvants and vaccine therapy against protists. Functional analysis of endogenous GPIs, especially in immunity, is an important task in physiology. GPI synthesis will also contribute to functional analysis of proteins incorporating GPI anchors.

6. Problems to be solved
   Progress of glycosylation and protection/deprotection methods has enabled the synthesis of complex GPIs. However, further improvements are required for their practical use. Furthermore, in order to realize the synthesis of GPI proteins, it is necessary to develop a new method for introducing the GPI moiety into a protein (Fig. 3.7).

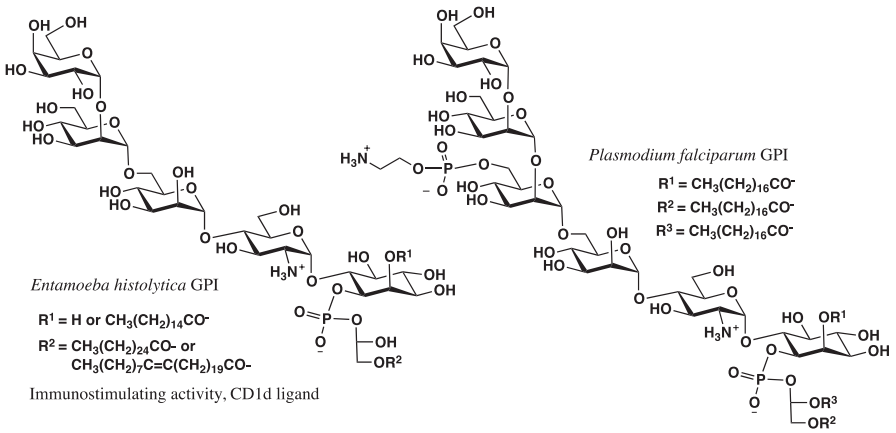

Fig. 3.7 Structures of synthesized GPIs

3.8 New Synthetic Methods for Standard Glycans: GAG

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Keywords Glycosaminoglycan, Glycosylation, Sulfated sugars, Oligosaccharides

1. Significance in the field of glycoscience and its current situation
   Due to the structural heterogeneity of naturally occurring GAG, we need structurally defined synthetic GAG glycans to elucidate the relationship between the fine structure of a GAG and its biological activity. Synthesized glycans could be a powerful tool to resolve such problems. In addition, chemical synthesis could yield required amounts of glycans, and their analogues could also be obtained. GAG repeating oligosaccharides, such as chondroitin sulfate [32, 33]/dermatan sulfate, heparin/heparan sulfate, keratan sulfate [34], and hyaluronic acid, as well as complexes of linkage tetrasaccharides and core peptides have been synthesized [35, 36]. For GAG glycan synthesis we have been developing new synthetic methods and accumulating invaluable information. The mechanisms of interaction between GAG oligosaccharides and specific signal proteins are going to be clarified. The importance of GAG synthesis is increasing.
2. Impact on the other fields of research
   We can obtain the information about the interactions between GAG oligosaccharides and specific signal proteins, for example, by using exactly designed and synthesized GAG oligosaccharides, which will contribute to medical and pharmaceutical purposes. An analogue library is available to develop glyco-medicines.
3. Significance as the fundamental research
   Although companies tend to avoid GAG synthesis due to the multisteps, we can obtain new synthetic information and develop techniques for the synthesis of GAG glycans exhibiting high degrees of synthetic difficulty.
4. Possible application for industry and medicine
   Many biological properties of GAG are still hidden. Synthetic technology for GAG is indispensable for developing glyco-medicines, and will be a useful tool to enter the market.
5. Future perspectives
   Synthetic technology for GAG is indispensable to develop glyco-science in various spheres, i. e., not only for medicinal purposes. GAG synthesis needs advanced techniques due to many functional groups containing sulfate groups, therefore demand and possibilities are anticipated in future.

6. Problems to be solved
   The multistep synthesis of GAG, which takes longer than common organic synthesis, has become the bottleneck for development in industry. New methods for effective synthesis of GAG with short-steps, high-yields, and high-selectivity are needed incorporating one-pot as well as automated reactions (Fig. 3.8).

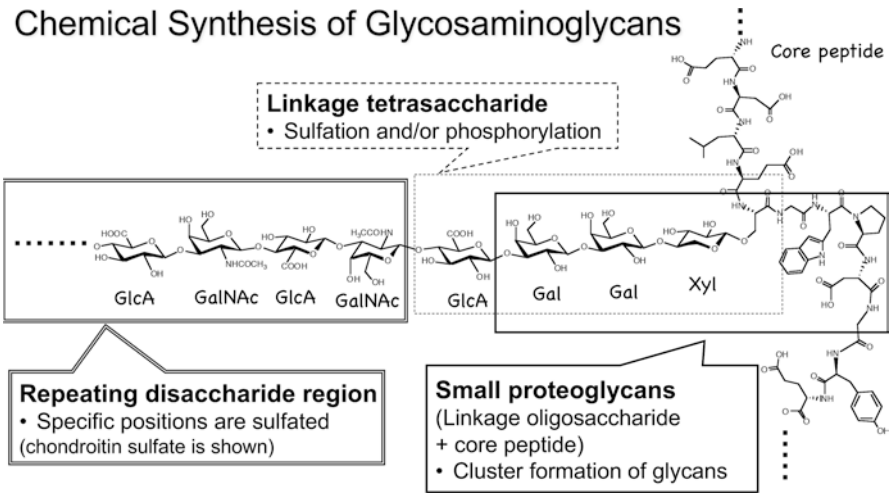

Fig. 3.8 Glycosaminoglycans have been synthesized as (1) linkage tetrasaccharides, (2) small proteoglycans, and (3) oligomers of the repeating disaccharide region

3.9 New Synthetic Methods of Standard Glycans: Glycopeptides

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Keywords Synthesis of glycopeptide, Glycosyl amino acid derivative, Solid-phase glycopeptide synthesis, Ligation, Transglycosylation reaction

1. Significance in the field of glycoscience and its current situation
   To synthesize glycopeptides, which are partial structures of glycoproteins, a technique that is compatible with the two conflicting synthetic fields, peptide chemistry and carbohydrate chemistry, is required. There are two synthetic strategies, i.e., a method without protection of the hydroxyl functions of sugar moieties, and a method using protected sugar hydroxyl groups [37]. In addition, it is also possible to synthesize a glycoprotein by segment condensation (ligation) [38]. Furthermore, a glycopeptide having a natural sugar chain has been synthesized by transglycosylation reaction using endo-N-glycosidases [39].
2. Impact on the other fields of research
   Glycopeptides will be key compounds for middle molecular pharmaceuticals. Discovery of a novel glycosyltransferase using a synthetic glycopeptide as a substrate will lead to the development of new medical treatments. The discovery of POMGnT1 is a good example [40]. Synthetic glycopeptides can also be used as standards to ensure the structural homogeneity of glycoprotein pharmaceuticals.
3. Significance as the fundamental research
   Study on methodology for glycopeptides could contribute to the development of both synthetic carbohydrate chemistry and peptide synthesis. The design and synthesis of carbohydrate moieties and peptide moieties freely are the next tasks. Also, the synthesis of artificial glycopeptides will become an active area of research.
4. Possible application for industry and medicine, if any
   Use as a standard substance for quality control in the manufacture of glycoprotein pharmaceuticals and so on. Use as a substrate for study of the effective chemical structure in drug discovery.
5. Future perspectives
   Developments in synthetic organic chemistry can be expected such as a new method with which anyone can synthesize glycopeptides more easily.
6. Problems to be solved
   The supply of various types of glycopeptides, such as not only natural O- and N-glycopeptides, etc. but also artificial glycopeptides, is expected (Fig. 3.9).

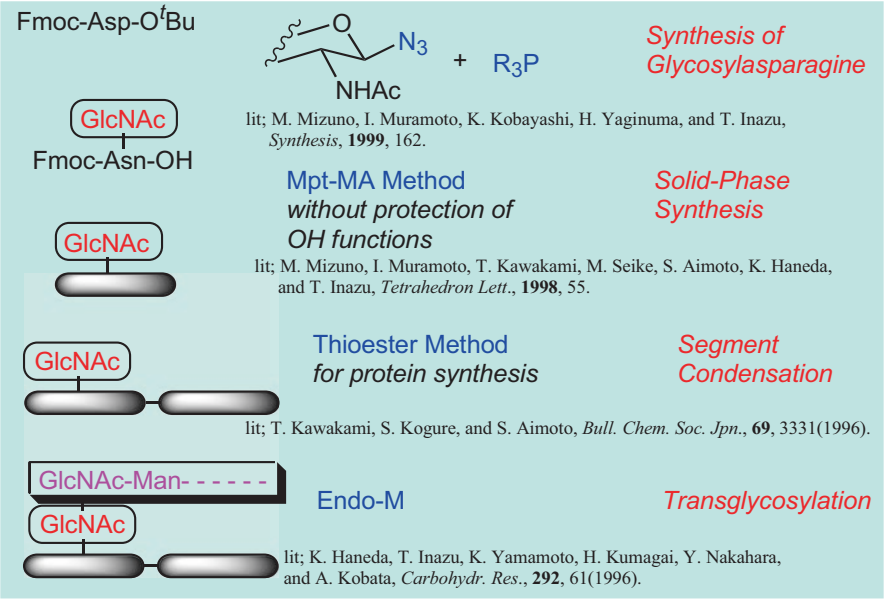

Fig. 3.9 A typical glycopeptide (glycoprotein) synthesis: (1) Synthesis of glycosyl amino acid derivatives, (2) elongation of peptide chains by solid phase synthesis, (3) extension of peptide chains by the ligation method, and (4) extension of sugar chains by the transglycosylation reaction using an endo-N-glycosidase

References

References for Section 3.1

1. Lowary TLL (2013) Context and complexity: the next big thing in synthetic glycobiology. Curr Opin Chem Biol 17:990–996
2. Wu Y et al (2017) Total synthesis of mycobacterial arabinogalactan containing 92 monosaccharide units. Nat Commun 8:14851
3. Ito Y et al (2015) Functional anaylsis of endoplasmic reticulum glucosyltransferase (UGGT): synthetic chemistry's initiative in glycobiology. Semin Cell Dev Biol 41:90–98
4. Park Y et al (2017) Macrocyclic Bis-Thioureas catalyze stereospecific glycosylation reactions. Science 355:162–166

References for Section 3.2

5. Plante OJ et al (2001) Automated solid-phase synthesis of oligosaccharides. Science 291:1523–1527
6. Kröck L et al (2012) Streamlined access to conjugation-ready glycans by automated synthesis. Chem Sci 3:1617–1622
7. Pistorio SG et al (2016) HPLC-assisted automated oligosaccharide synthesis: implementation of the autosampler as a mode of the reagent delivery. J Org Chem 81:8796–8805
8. Fumoto M et al (2005) Combinatorial synthesis of MUC1 glycopeptides: polymer blotting facilitates chemical and enzymatic synthesis of highly complicated mucin glycopeptides. J Am Chem Soc 127:11804–11818
9. Nokami T et al (2013) Automated solution-phase synthesis of oligosaccharides via iterative electrochemical assembly of thioglycosides. Org Lett 15:4520–4523

References Section for 3.3

10. Gamblin DP et al (2009) Glycoprotein synthesis: an update. Chem Rev 109:131–163
11. Payne RJ, Wong CH (2010) Advances in chemical ligation strategies for the synthesis of glycopeptides and glycoproteins. Chem Commun 46:21–43
12. Unverzagt C, Kajihara Y (2013) Chemical assembly of *N*-glycoproteins: a refined toolbox to address a ubiquitous posttranslational modification. Chem Soc Rev 42:4408–4420
13. Sakamoto I et al (2012) Chemical synthesis of homogeneous human Glycosyl-interferon-beta that exhibits potent antitumor activity in vivo. J Am Chem Soc 134:5428–5431
14. Murakami M et al (2016) Chemical synthesis of erythropoietin glycoforms for insights into the relationship between glycosylation pattern and bioactivity. Sci Adv 2:e1500678

References for Section 3.4

15. Ito Y, Takeda Y (2012) Analysis of glycoprotein processing in the endoplasmic reticulum using synthetic oligosaccharide. Proc Jpn Acad Ser B Phys Biol Sci 88:31–40
16. Seko A et al (1997) Occurence of a sialylglycopeptide and free sialylglycans in hen's egg yolk. Biochim Biophys Acta 1335:23–32
17. Maki Y et al (2016) Semisynthesis of intact complex-type triantennary oligosaccharides from a biantennary oligosaccharide isolated from a natural source by selective chemical and enzymatic glycosylation. J Am Chem Soc 138:3461–3468
18. Koizumi A et al (2013) Top-down chemoenzymatic approach to a high-mannose-type glycan library: synthesis of a common precursor and its enzymatic trimming. Angew Chem Int Ed 52:7426–7431

References for Section 3.5

19. Ando H et al (2010) Renewed synthetic approach to gangliosides exploiting versatile and powerful synthetic units. Meth Enzymol 478:522–540
20. Imamura A et al (2009) Ganglioside GQ1b: efficient total synthesis and the expansion to synthetic derivatives to elucidate its biological roles. J Org Chem 74:3009–3023
21. Komura N et al (2016) Raft-based interactions of gangliosides with a GPI-anchored receptor. Nat Chem Biol 12:402–410

References Section for 3.6

22. Ogura A et al (2016) Visualizing trimming dependence of biodistribution and kinetics with homo- and heterogeneous *N*-glycoclusters on fluorescent albumin. Sci Rep 6:21797
23. Adamo R et al (2013) Synthetically defined glycoprotein vaccines: current status and future directions. Chem Sci 4:2995–3008
24. Marradi M et al (2013) Glyconanoparticles as multifunctional and multimodal carbohydrate systems. Chem Soc Rev 42:4728–4745
25. Muñoz A et al (2016) Synthesis of giant globular multivalent glycofullerenes as potent inhibitors in a model of Ebola virus infection. Nat Chem 8:50–57
26. Broecker F et al (2016) Multivalent display of minimal Clostridium difficile glycan epitopes mimics antigenic properties of larger glycans. Nat Commun 7:11224

References Section for 3.7

27. Schofield L et al (2002) Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. Nature 418:785–789
28. Liu X et al (2005) Convergent synthesis of a fully lipidated glycosylphosphatidylinositol anchor of *Plasmodium falciparum*. J Am Chem Soc 127:5004–5005

29. Götze S et al (2014) Diagnosis of toxoplasmosis using a synthetic glycosylphosphatidylinositol glycan. Angew Chem Int Ed Engl 53:13701–13705
30. Aiba T et al (2016) Regioselective phosphorylation of myo-inositol with BINOL-derived phosphoramidites and its application for protozoan lysophosphatidylinositol. Org Biomol Chem 14:6672–6675
31. Aiba T et al (2017) Employing BINOL-Phosphoroselenoyl chloride for selective inositol phosphorylation and synthesis of Glycosyl inositol phospholipid from Entamoeba histolytica. Chem Eur J 23:8304–8308

References Section for 3.8

32. Tamura J et al (2008) Synthesis of chondroitin sulfate E octasaccharide in a repeating region involving an acetamide auxiliary. Carbohydr Res 343:39–47
33. Tamura J et al (2012) Synthesis and interaction with midkine of biotinylated chondroitin sulfate tetrasaccharides. Bioorg Med Chem Lett 22:1371–1374
34. Takeda N, Tamura J (2014) Synthesis of biotinylated keratan sulfate repeating disaccharides. Biosci Biotechnol Biochem 78:29–37
35. Tamura J (2001) Recent advances in the synthetic studies of glycosaminoglycans. Trends Glycosci Glycotechnol 13:65–88
36. Mende M et al (2016) Chemical synthesis of glycosaminoglycans. Chem Rev 116:8193–8255

References Section for 3.9

37. Mizuno M (2001) Recent trends in glycopeptide synthesis. Trends Glycosci Glycotechnol 13:11–30
38. Hojo H et al (2010) Progress in the ligation chemistry for glycoprotein synthesis. Transglycosylation. Trends Glycosci Glycotechnol 22:269–279
39. Fairbanks AJ (2017) The ENGases: versatile biocatalysts for the production of homogeneous *N*-linked glycopeptides and glycoproteins. Chem Soc Rev 46:5128–5146
40. Yoshida A et al (2001) Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. Dev Cell 1:714–724

Chapter 4
Technologies to Elucidate Functions of Glycans

Koichi Furukawa, Yuhsuke Ohmi, Yuji Kondo, Yuki Ohkawa, Orie Tajima, Keiko Furukawa, Koichi Honke, Jin-ichi Inokuchi, Jianguo Gu, Kenji Kadomatsu, Satomi Nadanaka, Hiroshi Kitagawa, Shoko Nishihara, Kazuya Nomura, Shogo Oka, Makoto Ito, Ken Kitajima, Shunji Natsuka, Motoi Kanagawa, Takeshi Ishimizu, Kazuhito Fujiyama, Yasunori Chiba, and Hiroyuki Osada

4.1 Genetically Modified Mice: Glycolipids

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Keywords Knockout, Transgenic, Neurodegeneration, Maintenance of homeostasis, Lipid rafts

1. Significance in the field of glycoscience and its current situation
   Glycolipids are expressed on the cell membranes of the majority of tissues, and expected to play unique roles in individual organs due to their differentiation-specific expression patterns. As an efficient approach to investigate glycolipid functions *in vivo*, knockout/transgenic mouse lines have been established, and their abnormal phenotypes have been analyzed [1]. So far, important functions of glycolipids in maintenance and regeneration of nervous tissues have been demonstrated, and their roles in immune systems, endocrine-metabolism [2], the cardio-vascular system, and genital organs have also been analyzed. In addition, their roles in malignant tumors have been clarified [3].
2. Impact on the other fields of research
   Since glycolipids have been reported to play important roles mainly in systemic regulatory organs, findings obtained would have a strong impact on the progress of medical, physiological and pathological researches, basic biology studies,

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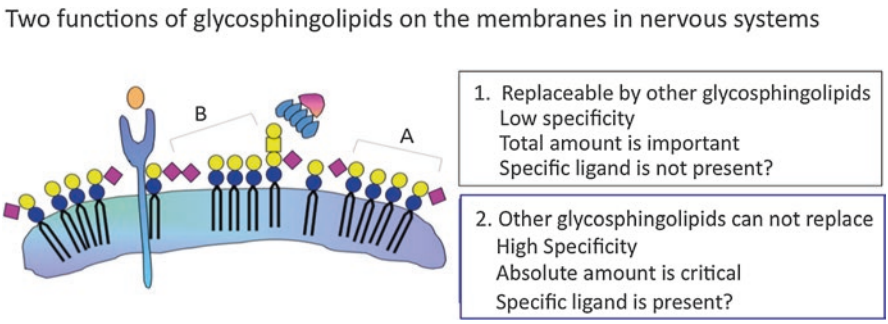

Fig. 4.1 Based on the results of analysis of abnormal phenotypes exhibited by knockout mouse lines of ganglioside synthase genes, glycosphingolipids could be classified into two groups

and also the development of novel diagnostic and therapeutic reagents in the future.

3. Significance as the fundamental research
   The functions and action modes of amphopathic molecules, glycolipids have been a puzzling issue in basic biology [3]. Gene-engineered mice would be helpful for further understanding of those issues [4, 5].
4. Possible application for industry and medicine, if any
   As gene-engineered mice, complex knockout mice and tissue/cell-specific knockout mice would be useful. They are also useful as sources of glycosylation-modified cells.
5. Future perspectives
   The genome editing technique should be applied for establishment of knockout/transgenic mice with short term and low cost (Fig. 4.1).

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4.2 Genetically Modified Mouse:Glycolipids

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Keywords Development, Immunity, Nervous system, Spermatogenesis, Infection

1. Significance in the field of glycoscience and its current situation
   Generation of knockout (KO) mice of glycogenes is very important because it allows us to elucidate the biological role of a particular glycolipid by analyzing the mutant mice [6]. KO mice have been generated for most enzymes involved in the biosynthesis of major glycolipids [7]. These mutant mice are deposited in a resource center somewhere.
2. Impact on the other fields of research
   The analysis of KO mice of glycogenes sometimes provides an insight into common diseases such as diabetes mellitus and cancer [8]. For example, insulin sensitivity is enhanced in skeletal muscle of GM3 synthase KO mice, suggesting that inhibition of GM3 synthesis could prevent the development of type2 diabetes [9].
3. Significance as the fundamental research
   Disruption of a specific glycogene in mice has proved that some glycolipids are essential for important life events including development, immune system, nervous system, reproduction system, cancer progression, establishment of infection, and so on [10].
4. Possible application for industry and medicine, if any
   The phenotype of KO mice may provide a symptomatic clue in a search for human deficiencies that are poorly understood at present. KO mice also may serve as a test animal model for the newly developed treatment.
5. Future perspectives
   Since humans share many genes with mice, the phenotype observed in KO mice may give hints to understand how the orthologs cause diseases in humans. New kinds of KO mice including conditional KO mice can be easily generated by using genome editing technology with the CRISPR/Cas system.
6. Problems to be solved
   The functions of glycans are intrinsically entangled, and our knowledge and idea regarding the glycan functionality are shortsighted. KO mice of glycogenes are valuable resources to untangle the complex functions of glycans, although it will require a great deal of time and labor to fully understand the roles of individual carbohydrate chains by using KO mice (Table 4.1).

Table 4.1 Phenotypes of KO mice and molecular functions of glycolipid glycan-synthesizing enzymes

Glycogenes and enzymes	Phenotypes of KO mice	Molecular functions
GleCer synthase	embryonic lethality	unknown
GalCer synthase (CGT)	tremor, ataxia, paralysis, male sterility	myelin formation and maintenance, spermatogenesis
sulfatide synthase (CST)	tremor, ataxia, paralysis, male sterility	myelin maintenance, spermatogenesis
GM3 synthase	enhanced insulin sensitivity, deaf	regulation of the insulin receptor signal
GD3 synthase	reduced nerve regeneration	unknown
GM2/GD2 synthase	late-onset neuronal degeneration, male sterility	maintenance of neurons, transportation of testosterone
Gb3(CD77) synthase	normal, resistant to verotoxin	production of the verotoxin receptor

4.3 Genetically Modified Mice: Glycosphingolipid Synthases

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Keywords Glycosphingolipids (GSLs), GSL synthases, Knockout mouse, GM3 synthase deficiency

1. Significance in the field of glycoscience and its current situation
   The glycosphingolipid family (GSLs) members are composed of various sugar chains attached to ceramide and facilitate microdomain formation (lipid rafts) in the plasma membrane, contributing to cell-cell interaction and receptor-mediated signal transduction. The functions of individual GSL have been investigated using gene targeted mice of various GSL synthases, as summarized in Fig. 4.2 [11]. Deficiencies of GM3 synthase (ST3GAL5) [12] and GM2/GD2 synthase (B4GALNT1) [13] in humans have been reported. The common pathological feature of GM3 synthase deficiency in man and mouse is deafness [14].
2. Impact on the other fields of research
   GSLs can be classified into pro- and anti-inflammatory molecular species but the details of the mechanisms remain to be elucidated. It is important to elucidate the involvement of GSLs molecular species in various diseases involving chronic inflammation.
3. Significance as the fundamental research
   GM3 synthase deficiency in humans exhibits severe symptoms such as epilepsy and blindness [12], but GM3 synthase KO mice do not show such a phenotype [13]. Elucidation of the differences in GSLs metabolism and specific functions between man and mouse could lead to an understanding human diseases mentioned above.
4. Possible application for industry and medicine, if any
   Since GM3 synthase KO mice are resistant to high fat diet (HFD) induced diabetic phenotypes including insulin resistance [15], it is expected that GM3 synthase could be a novel target for therapy for metabolic syndrome.
5. Future perspectives
   GSLs molecular species are expected to become novel biomarkers and therapeutic targets for various diseases caused by chronic inflammation.
6. Problems to be solved
   Diverse and complex glycan structures in GSLs have been well analyzed, however, the diversity of the lipid portion of GSLs, which should contribute to important functions, remains obscure. Therefore, it is important to perform highly sensitive LC-MS/MS analysis to establish a comprehensive database of glycosphingolipidomics.

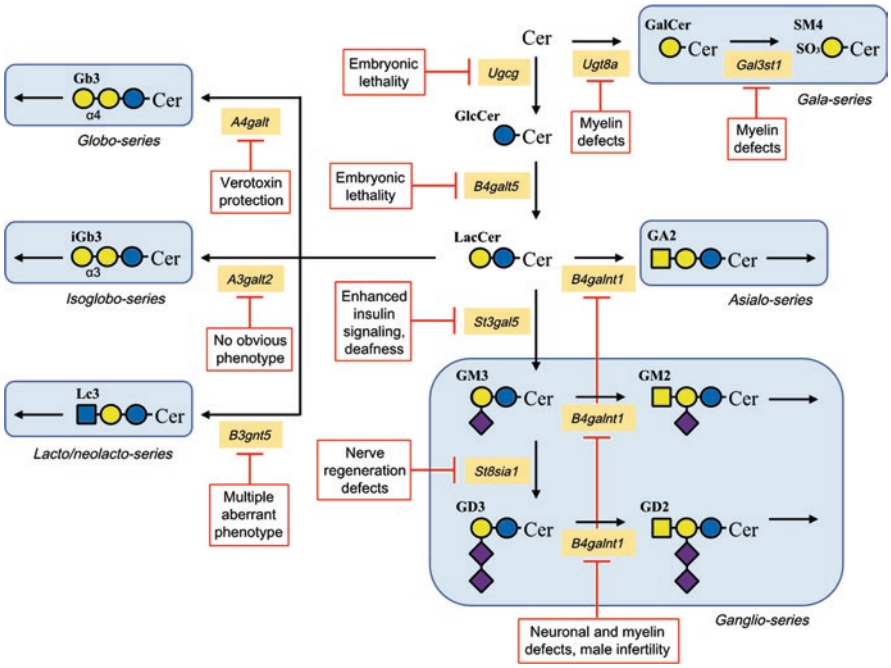

Fig. 4.2 Biosynthetic pathway for glycosphingolipids(GSLs), and the major phenotypes of systemic KO mice deleted with a single GSL synthase gene

4.4 Genetically Modified Mice: Glycoproteins

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Keywords *N*-glycan, *O*-glycan, Receptor, Interaction, Supramolecular complex

1. Significance in the field of glycoscience and its current situation
   Glycoproteins are biomolecules that contain oligosaccharides (glycans) covalently attached to proteins. Oligosaccharides of glycoproteins are generally classified as *N*-linked glycans (*N*-glycans) and *O*-linked glycans (*O*-glycans), depending on the linkage bonds. *N*-Glycans contain an *N*-acetylglucosamine residue at the reducing terminal and are linked to an amide group of an Asn residue of a polypeptide, which carries a specific subset residing in the Asn-X-Ser/Thr motif. *N*-Glycans, based on their structural features, have been classified roughly into three kinds: high mannose type, hybrid type and complex type. On the other hand, *O*-glycan is a general term for glycans, which are linked to the hydroxyl group of either a Ser or Thr residue of a polypeptide, and includes *O*-GalNAc, *O*-Fuc, *O*-Man, *O*-GlcNAc, *O*-Gal or *O*-Glc, etc. Even in the same glycoprotein, the glycan is known to vary based on the developmental stage and the various tissue or cell type [16]. Therefore, the glycan of a glycoprotein should play an important role in its functional expression.
2. Impact on the other fields of research
   Glycosylation is the most common posttranslational modification of proteins (more than 50%), and their structural diversity participates in various biological processes [17]. Even with a single glycan, dysplasia may cause various diseases such as infection, cancer, muscular dystrophy, a neurological disorder, etc.
3. Significance as the fundamental research
   In fact, more than 90% of membrane proteins on the cell surface are believed to be modified by glycans. However, it remains unclear how glycans control the function of a receptor and its interaction with other molecules. Using α5β1integrin as a model molecule, we performed functional analysis of *N*-glycans, and found that they regulate integrin-mediated functions, but also modulate specific interaction with other membrane proteins, and downstream signaling [18].
4. Possible application for industry and medicine, if any
   Congenital disorders of glycosylation (CDG) are genetic diseases due to defective glycosylation of proteins, and more than 100 types have been reported [19]. These diseases are caused by a defect in the transfer of a glycan to a protein, glycan synthesis, or transplantation of glycoproteins. Using glycomics, more diseases should be detected in the future.

5. Future perspectives
   Unlike that of a protein, the biosynthesis of a glycan, without a template, can be influenced by various factors including the expression levels of enzymes and substrates, and also depend on the cell type, development stage and pathological conditions. At present, it is extremely difficult to obtain a precise glycome from an endogenous protein due to the difficulty in its purification and analysis of its multiple glycosylation sites occupied by various glycan structures. An interdisciplinary study to adopt multidisciplinary approaches in glycoscience will be indispensable for the understanding of a living entity in future.
6. Problems to be solved
   Concerning the functions of glycans on glycoproteins expressed on the cell surface, there are many questions and mysteries to be solved. If the significance and molecular mechanisms underlying the diversity and specificity of glycans are clarified, it should facilitate elucidation of the structures and functions of the complicated supramolecular complexes on the cell membrane (Fig. 4.3).

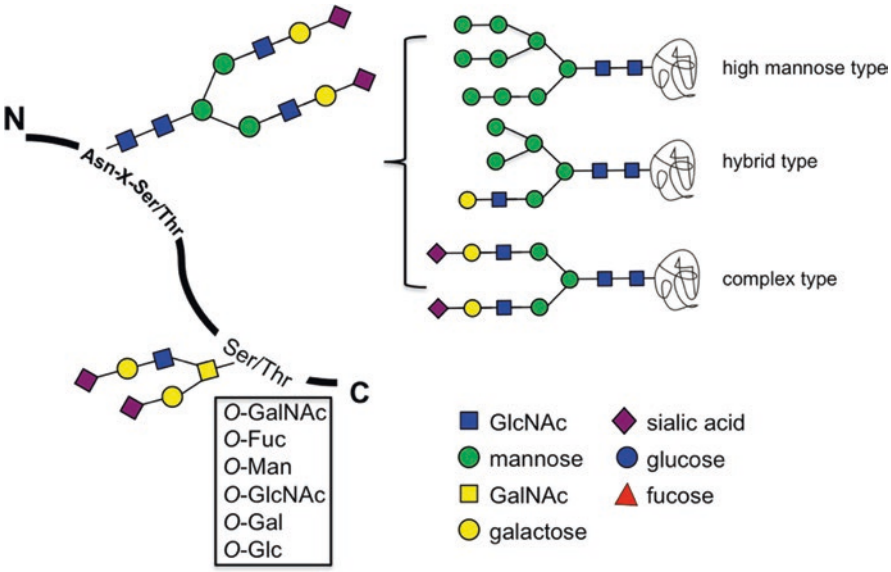

Fig. 4.3 Oligosaccharides of glycoproteins. The three main types of *N*-glycan structures and a representative structure of *O*-glycans (mucin-type, *O*-GalNAc) are shown

4.5 Genetically Modified Mice: Glycosaminoglycans (GAGs)

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Keywords Glycosaminoglycan, Mucopolysaccharidosis, Neural plasticity, Enzyme replacement therapy

1. Significance in the field of glycoscience and its current situation
   Hereditary disorders related to glycosaminoglycans (GAGs) are mainly due to mutations or deletions of enzymes responsible for their synthesis or degradation. Gene-manipulated mice with such defects revealed the cause-consequence relationships for these diseases. Some mucopolysaccharidoses that involve defects in GAG degradation can be ameliorated by enzyme replacement therapy, and further new approaches, such as transplantation of genetically engineered hematopoietic stem cells or production of enzymes by genetically engineered silkworms, have been developed. Moreover, genetically engineered mice have revealed unexpected functions of GAGs. For example, chondroitin sulfate (CS) suppresses ocular dominance plasticity and synaptic plasticity. While CS and keratan sulfate inhibit axon regeneration, heparan sulfate (HS) promotes it. At least, CS and HS exert their functions through binding to specific receptors (receptor-type protein tyrosine phosphatases).
2. Impact on the other fields of research
   Genetically manipulated animals as to GAGs have contributed to understanding of the roles of GAGs in cartilage and connective tissues [20, 21]. Also, they have provided new findings regarding GAG functions in the nervous system, such as neural plasticity and axon regeneration [22–24].
3. Significance as the fundamental research
   Physical interactions with other molecules and ions are thought to be an essential mechanism of the GAG's action. However, a new mechanism whereby GAGs bind to their specific membrane-bound receptors and mediate intracellular signaling has been proposed. Such new mechanisms of action may be uncovered through investigations through genetically engineered animals, and will strikingly influence research in other fields.
4. Possible application for industry and medicine, if any
   Enzyme replacement therapy is currently clinically used for the treatment of mucopolysaccharidoses that are caused by defects in the degradation of GAGs. It is expected that new therapeutic strategies will be also developed for diseases caused by defects in the biosynthesis of GAGs, through elucidation of the mechanisms of the GAGs' action using genetically engineered animals.

5. Future perspectives
   Mechanisms of action for GAGs should be fully elucidated. Genetically engineered animals will be useful for finding new meaning or significance of GAGs in some disorders, e.g., neural disorders and cancer.
6. Problems to be solved
   (1) Mechanisms of action, (2) mechanisms of biosynthesis and degradation, and (3) roles in patho/physiological conditions. These are not fully understood. To address these problems, genetically engineered animals, such as conditional knockout mice, will serve as important tools for studies (Fig. 4.4).

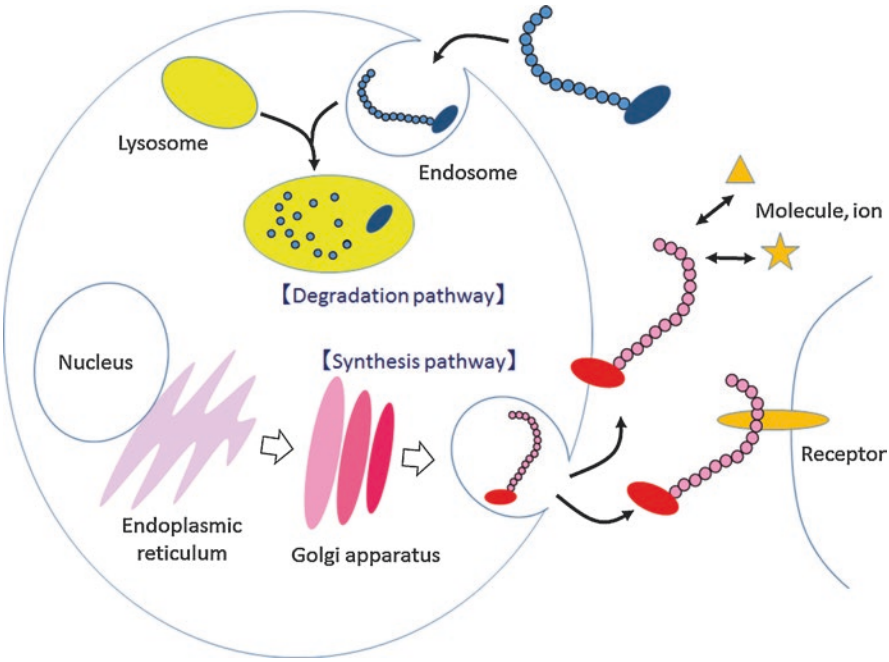

Fig. 4.4 Defects in degradation or synthesis of GAGs cause hereditary disorders. Genetically engineered animals have revealed not only the cause-consequence relationships of such diseases, but also novel mechanisms of action for GAGs. For example, GAGs serve as ligands of specific receptors on axons to regulate axon regeneration

4.6 Mice with Genetic Mutations in the Glycosaminoglycan Biosynthetic Pathway

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Keywords Glycosaminoglycan (GAG), GAG-related disorders, Chondroitin sulfate, Heparan sulfate, Proteoglycan

1. Significance in the field of glycoscience and its current situation
   Studies on cells and organisms of genetically altered biosynthetic and/or catabolic enzymes for sulfated glycosaminoglycans (GAGs) such as chondroitin sulfate (CS) and heparan sulfate (HS) have revealed the roles of GAGs in the context of human development and disorders [25]. For example, gene ablation of *Ext1,* which encodes an enzyme essential for elongation of HS chains, has demonstrated the important role of HS in gastrulation, nervous system development, and bone formation [26, 27]. In addition, conditional *Ext1*-knockout mice show autism-like socio-communicative deficits and osteochondromagenesis [28, 29]. Thus, structural alterations in GAGs can be the causes of and/or confounding factors for human disorders by characterizing the phenotypes of mutant mice lacking individual GAG biosynthetic enzyme genes. Moreover, the genetically-engineered mice can be useful as models for elucidating the molecular mechanisms of human disorders and for developing new medical treatments.
2. Impact on the other fields of research
   Monitoring of the pathophysiological changes that affect GAG metabolic gene expression, GAG quantity, and GAG structure can be useful for diagnosis, and these disease markers will have an impact on personalized medicine.
3. Significance as the fundamental research
   Genetically modified mice, in conjunction with genome wide association studies in humans, are powerful tools for identifying affected GAGs as causes and/or confounding factors, and for elucidating the mechanisms underlying GAG-related disorders.
4. Possible application for industry and medicine, if any
   Cell lines with genetic mutations in the glycosaminoglycan biosynthetic pathway are powerful tools for producing proteoglycans with tailor-made GAG chains.
5. Future perspectives
   Small compounds that directly regulate disease-associated biosynthetic and/or catabolic enzymes for GAG chains would be promising candidates for therapeutic use in the treatment of various GAG-related disorders.
6. Problems to be solved
   To develop effective glycotherapeutic intervention for GAG-related disorders through manipulation of GAG structure, identification of disease-associated GAG structures and related biosynthetic genes (or gene clusters), and techniques to spatiotemporally control the target genes for desired goals will be required (Fig. 4.5).

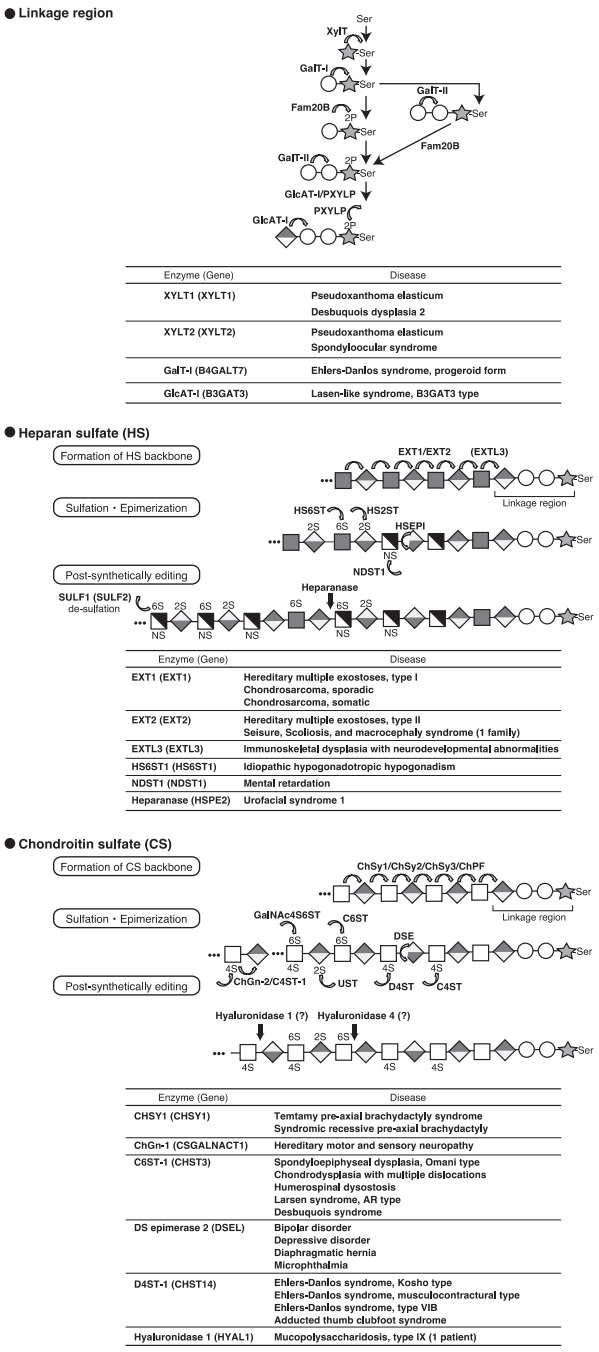

Fig. 4.5 Biosynthetic pathway for GAGs and genetic disorders caused by mutations affecting the biosynthesis of glycosaminoglycan chains. By using genetically modified mice, we will clarify the biological roles of GAGs in various cellular responses and understand the relationship between failure of GAGs and disease

4.7 *Drosophila melanogaster*

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Keywords *Drosophila*, Mutant, Genetics, Glycosyltransferase, Basic function

1. Significance in the field of glycoscience and its current situation
   More than 200 glycosyltransfearses are expressed in mammalian cells. Although the number of *Drosophila* glycosyltransferases is half that in humans, *Drosophila* orthologs synthesize similar glycan structures to those synthesized by the corresponding human glycosyltransferases. Heparan sulfate, chondroitin sulfate, *O*-Fuc, *O*-Man and *O*-GlcNAc are found in both *Drosophila* and humans. *Drosophila* stock centers inside and outside of Japan possess mutant and RNAi lines covering all genes including those of glycosyltransferases. By using these lines, comprehensive analysis of glycosyltransferases and detailed analyses of each glycosyltransferase have been performed [30–34]. The glycan functions discovered in *Drosophila,* for example, the role of heparan sulfate in Wnt signaling and the function of *O*-Fuc on Notch, were also found in mammalians later. Therefore, *Drosophila* is an indispensable tool for the analysis of basic glycan function (Fig. 4.6).
2. Impact on the other fields of research
   By using *Drosophila* mutants, the developmental process, the nervous system, innate immunity, behavior, lifespan and so on can be analyzed. The glycan function, found in each analysis, provides a novel insight into the glycan function in each corresponding field.
3. Significance as the fundamental research
   Drosophila is an important model organism that can be used as a powerful genetic tool to analyze the physiological functions of various molecules. The *Drosophila* life cycle is completed within 14 or 15 days. As genetic screening is easy to perform, unexpected glycan functions could be found by using the *Drosophila* model system.
4. Possible application for industry and medicine, if any
   Drosophila remains alive, even if a disease-like phenotype is induced in the eyes or wings. Therefore, *Drosophila,* in which eyes a disease-like phenotype is induced, is used for drug screening.
5. Future perspectives
   Genetic screening is easy to perform with *Drosophila*, and therefore unexpected glycan functions could be found by using *Drosophila*. It is essential how to take advantage of this.
6. Problems to be solved
   Drosophila has various glycosyltransferases, which are homologs of human ones. However, many of their activities have not been determined yet. Also, there are many glycan structures synthesized by undefined glycosyltransferases.

***Drosophila* stock center maintains mutant and RNAi lines**

Stock center	RNAi lines	Gal4 driver lines	P element Insertion lines
Bloomington *Drosophila* stock center (BDSC)	12990	7096	34170
Vienna *Drosophila* resource center (VDRC)	32933	8457	-
Kyoto stock center	-	8612	-
Fly stocks of national institute of genetics (NIG-Fly)	17780	-	-

Analysis of basic glycan function by using *Dorophil*a mutant and RNAi lines

- **Comprehensive analysis**
- **Functional analysis of each glycosyltransferase**

Fig. 4.6 Functional analysis of glycans by using *Drosophila* mutants and RNAi lines

4.8 *Caenorhabditis elegans*, the Nematode Worm

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Keywords Single gene disorder, Polygenic disease, Humanized *C. elegans*, Balancer, Genome editing

1. Significance in the field of glycoscience and its current situation
   Caenorhabditis elegans is an ideal model organism for studying glycoscience. Because over 60% of its genes are orthologs of human ones, many human disease (single-gene and polygenic) models have been introduced and widely studied [35, 36]. RNAi and efficient gene KO techniques combined with forward/reverse genetics analyses have accelerated such studies. By studying orthologs of human glycogenes, novel roles of glycogenes have been highlighted (See [37, 38]). National Bioresource Project Japan::C. elegans (NBRP) provides us with more than 8000 gene KO strains, and the isolation of KO strains is quick and easy. This is especially useful for producing glycogene disease models through genome editing. Phenotypes can be monitored with sophisticated imaging technology. Maintaining sterile and lethal KO strains is often difficult with other model organisms, but for this nematode, a newly constructed balancer tool kit covering over 83% of the genome (NBRP) [39] makes this easily possible. The nematode is a genome editing friendly animal, and with well-characterized gene regulatory elements, control of expression of the genes (e.g., optogenetics) is easy, and this will accelerate the functional analysis of glycogenes at a single cell level upwards.
2. Impact on the other fields of research
   Single or simultaneous multiple gene modification is possible with this model organism. Introduction of a human gene/gene network (associated with gangliosides, sialic acids, etc.) into the nematode genome will shed light on the roles of gene functions, gene regulatory networks and biochemical pathways of multicellular organisms including humans.
3. Significance as the fundamental research
   The phenotypes after gene editing/modification can be monitored from the germline formation stage to the adult stage afterwards. Thus, the effects of gene modification at any stage of development can be resolved with this model organism. This will greatly facilitate the understanding of mechanisms of the gene regulation network as well as the roles of glycogenes in development and behavior.
4. Possible application for industry and medicine, if any
   Gene editing/modification in the nematode is quite useful for producing (single-gene/polygenic) disease models. These disease models can be used for analysis of pathogenic mechanisms, and for drug screening. Analysis of the disturbed

gene network in a disease model will shed light on the gene networks associated with a particular disease. Humanized *C. elegans* could also be a useful model for pharmaceutical development.

5. Future perspectives
   To maintain lethal and sterile mutations in heterozygotes, balancer chromosomes are essential for further analyses. A newly constructed balancer chromosome tool kit (covering c.a. 89% of the coding genes) made by CRISPR-Cas9 is available from NBRP. Making use of this tool with gene editing techniques will greatly facilitate analyses of glycogene functions [39].
6. Problems to be solved
   For using a gene edited/gene modified animal as a disease model or a tool for studying gene regulatory mechanisms, the animal should be stably maintained under laboratory conditions. The gene regulation techniques involving specific regulatory DNAs and RNAs should be improved for further study in addition to techniques for constructing humanized *C. elegans* (Fig. 4.7).

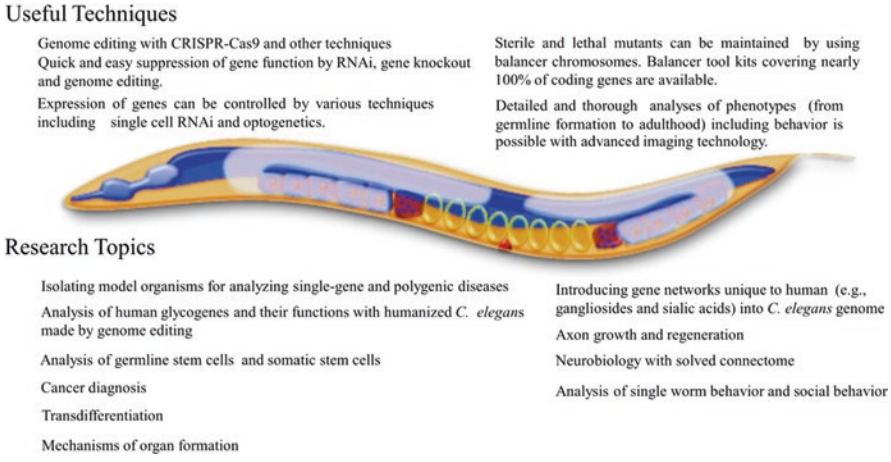

Fig. 4.7 *C. elegans* is an ideal organism for conducting breakthrough studies in glycoscience. Its life cycle is about 3 days. Handling of a genetically homogeneous population is easy, and phenotypes can be monitored in detail by means of sophisticated imaging techniques. Application of forward/reverse genetics with genome editing techniques will enable us to perform analyses effectively

4.9 Medaka and Zebrafish (Small Fish as Experimental Models)

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Keywords Medaka (Japanese medaka), Zebrafish, Embryonic development, Transparency, Ac4GalNAz

1. Significance in the field of glycoscience and its current situation
   Small fish commonly used as experimental models are medaka and zebrafish. They have many eggs and are easy to breed. In addition, since the embryos are transparent and develop rapidly, the developmental process can be easily observed. Currently, strains having mutations introduced into various genes have been prepared [40, 41], suggesting the possibility that they are useful for elucidating the roles of glycans during the development process. In fact, peracetylated *N*-azidoacetylgalactosamine (Ac4GalNAz) was metabolically incorporated into zebrafish glycans, and then the incorporated glycans were fluorescently labeled by means of the click chemistry reaction and the change in the embryonic development process was observed [42]. This paper was recently cited in Science under the title "Looking for a Sugar Rush" [43].
2. Impact on the other fields of research
   Recently, transgenic zebrafish have been established allowing visualization of axonal extension of specific neurons in real time using various fluorescent proteins [40]. In combination with mutants as to glycan-modifying enzymes, there is a high possibility that the glycan functions in neural circuit formation will become clear.
3. Significance as the fundamental research
   Medaka and zebrafish have almost the same organs and tissues as mammals, and their developmental process can also be observed outside in detail, so these small fish are useful as experimental models. In addition, because the growth temperature of medaka ranges from 4 to 40 °C, it is possible to create temperature-sensitive mutant strains.
4. Possible application for industry and medicine, if any
   It is possible to observe detailed changes of glycan-dependent diseases in the process of embryonic development. These fish may also be used as screening systems for therapeutic agents.
5. Future perspectives
   It would be essential to develop tools for visualizing specific glycans taking full advantage of medaka and zebrafish.

4.10 Gene Modification: Small Fish (Zebrafish and Medaka)

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Keywords Vertebrate model, Genome editing, Early development, Lysosomal disease, Neurodegeneration

1. Significance in the field of glycoscience and its current situation
   Small fish such as zebrafish and medaka are prevalent as models for the early development of vertebrates because they can be generated quickly, and observation of their organs is easy due to the transparent nature of their embryos. In addition to the development of genome databases, genetic modification technologies involving small fish have rapidly advanced [44]. Large-scale mutation screening using chemical mutagenesis (ENU) was the mainstream in the 1990s, and a knockdown method using morpholino oligo (MO) was introduced in the early 2000s. Since 2009, genome editing techniques such as Zinc Finger Nucleases (ZFNs), TAL-Effector Nucleases (TALENs), and CRISPER/Cas9 have been successfully applied to small fish. In the field of glycoscience, the knockdown of glycoconjugate-related genes (glycogenes) by MO in small fish has been carried out in order to elucidate the roles of sugar chains in the neurogenesis and angiogenesis of vertebrates [45]. In addition, medaka [46] and zebrafish [47] models of a lysosomal disease, Gauche disease (GBA1 KO), have been generated. In recent years, zebrafish models of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases have also been produced [48], and elucidation of the functions of glycogenes in these neurodegenerative diseases is important.
2. Impact on the other fields of research
   If the distinct roles of sugar chains can be elucidated in angiogenesis, neurogenesis, and neurodegeneration using small fish, it will have a marked impact on other fields such as developmental biology and neuroscience.
3. Significance as the fundamental research
   Small fish are regarded as vertebrate models complementing mice, because they allow observation of the entire process of embryogenesis in a short period and genomic editing techniques can be easily applied.
4. Possible application for industry and medicine, if any
   As model organisms of neurodegenerative and lysosomal diseases, small fish will contribute to the development of therapeutic methods and drugs for these diseases. It is possible to respond to the international principle of experimental animal welfare (regulation of mammalian use based on 3R principles) by replacing mice with small fish.

5. Future perspectives
   With the progress of genome editing technology, we can now easily knockout as well as knock-in target genes in small fish. From now on, small fish will become powerful models in the glycoscience field for analyzing the roles of glycogenes in the early development of vertebrates and neurodegenerative diseases.
6. Problems to be solved
   Identification of the glycogenes of small fish and functional analysis of these genes using MO have been conventionally performed. However, systematic research that collectively analyzes glycogene mutants obtained by a genome-editing approach such as CRISPER/Cas9 has yet to be conducted in Japan (Fig. 4.8).

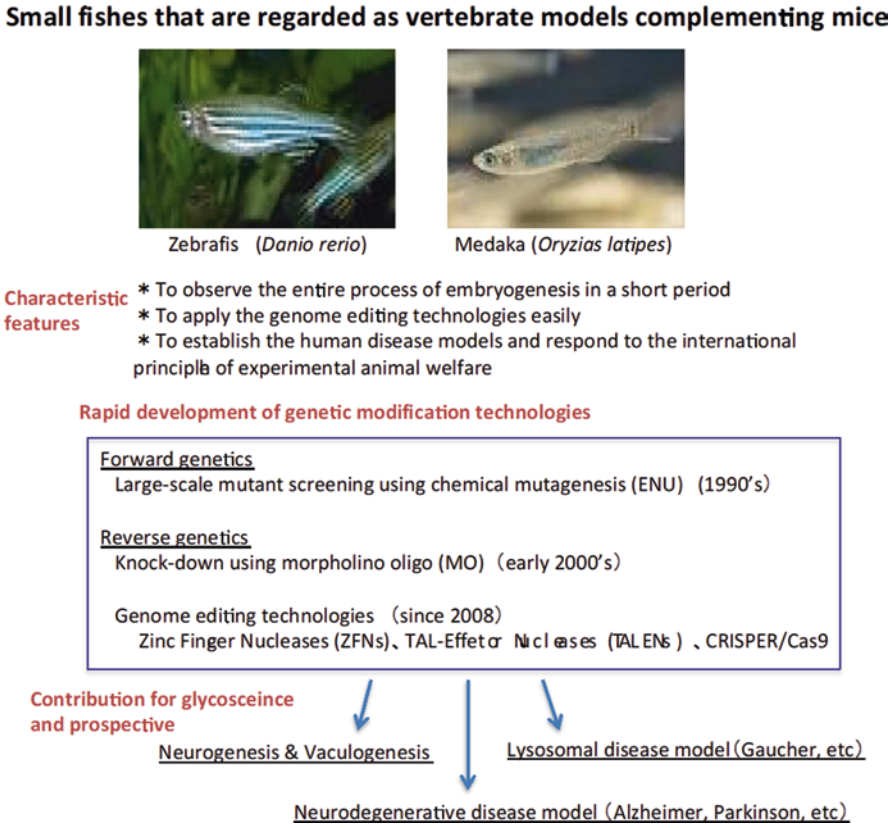

Fig. 4.8 Small fish such as zebrafish and medaka are establishing their position as vertebrate models complementing mice due to the progress of genome editing technology such as CRISPER/Cas9

4.11 Genetically Modified Small Fish (Zebrafish and Medaka)

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Keywords Vertebrate, Developmental biology, Genetics, Neurosciences, Model animal for human disease

1. Significance in the field of glycoscience and its current situation
   Zebrafish and medaka are being used as model vertebrates in basic science fields (developmental biology and genetics), and medical and pharmaceutical fields [49, 50]. The prominent merits of using small experimental fish are the easiness of genetic manipulation, and availability of enriched sources of strains including inbred and wild-type, mutated, transgenic, or Target Induced Local Lesion In Genome (TILLING) ones. In Japan, collection, stocking, and provision of small fish are carried out under the National BioResource Project. In the glycoscience field, studies on elucidation of the significance of glycolipids and glycoproteins in fertilization, embryogenesis, and neurogenesis are in progress [51, 52]. In addition, their usefulness as model animals for human diseases such as muscle dystrophy is also recognized [53]. Study on zebrafish greatly outnumbers those of medaka in the world; however, research on medaka, which was established as an experimental animal in Japan, is increasing. Both models will be more and more demanded as experimental animals for basic and medical and pharmacological research areas in future.
2. Impact on the other fields of research
   The developmental biology and genetics of small fish are well advanced as to the biology of vertebrates. Therefore, the significance of glycosciences in basic and applied sciences will be quickly recognized as soon as various kinds of knowledge about the effects of impaired and modified glycosylation are accumulated.
3. Significance as the fundamental research
   Small fish are already well-used in the research areas of developmental biology, genetics, and neuroscience, and utilization of these animals in the glycoscience area is expected to be fruitful with the exchange of mutual information. As biochemical analytical methods advancing, mechanistic studies will reveal the underlying phenotypes displayed by genetically modified animals.
4. Possible application for industry and medicine, if any
   Zebrafish and medaka are vertebrates like humans, and are being utilized as model animals for human diseases and as test animals for environment monitoring.

5. Future perspectives
   Zebrafish and medaka have merits in technical feasibility and low cost for genetic modification, and are expected to be widely used in basic and applied areas of research. In particular, utilization as vertebrate models for human diseases will further increase.
6. Problems to be solved
   Of experimental animals that can be genetically modified, these small fish have been successfully utilized for biological studies. Biochemical approaches to understand the structures and properties of glycoconjugates should be more developed and applied (Fig. 4.9).

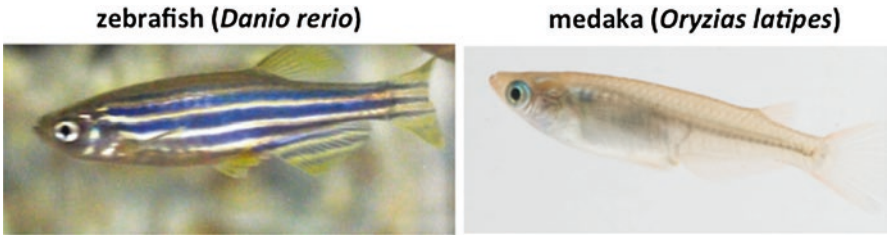

Fig. 4.9 Adult zebrafish (left) and medaka (right). The body length is 4–5 cm for zebrafish and 3 cm for medaka. The photos were provided by Dr. Tomoko Kurata (NIBB) for zebrafish, and Dr. Masahiko Hibi (Nagoya University) for medaka

4.12 Genetic Modification: Small Fish (Zebrafish and Medaka)

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Keywords Zebrafish, Medaka, Embryogenesis, Vertebrate, Genome editing

1. Significance in the field of glycoscience and its current situation
   Small fish, especially zebrafish and medaka, are widely used as model animals of vertebrates [54]. Genome information is available and gene suppression technology involving morpholino RNA has been widely used. Furthermore, genetic modification by genome editing has recently been established [55–57]. Thus, they are excellent organisms for research on the functions of glycan-related genes. In addition, structural studies of their glycan molecules are progressing, and it has already been shown that the basic structures of their glycans are similar to those of mammals [58], so they are excellent as model animals for the glycoscience of higher animals. On the other hand, the glycans on glycoproteins have various terminal structures and are suitable for analysis of the species-specific functions of the glycans.
2. Impact on the other fields of research
   Since embryogenesis of small fish is basically the same as in mammals, it is possible to obtain compatible knowledge for human and/or mouse glycan functions in morphogenesis and the cranial nervous system.
3. Significance as the fundamental research
   Small fish are model animals of developmental biology and genetics, and are important for studies on vertebrate morphogenesis. Their embryogenesis can be easily observed because they are oviparious. Since it is easy to prepare many embryos, they can also be used for biochemical analysis.
4. Possible application for industry and medicine, if any
   Research to utilize small fish for drug discovery is advancing, and they are being applied to pharmacology, toxicity, and drug screening. In particular, they are attracting attention as targets for the whole-animal drug screening for compound screening.
5. Future perspectives
   Glycobiology of small fish can also contribute to human life sciences. Its significance in the fields of human diseases and drug discovery will further increase in the future.

6. Problems to be solved
   As with other species, it is essential to analyze glycan structure comprehensively in every developmental stage and whole organs, and to create a database, i.e., Glycan Atlas. In addition, it is necessary to modify comprehensively glycan-related genes by genome editing and to associate them with phenotypic changes (Fig. 4.10).

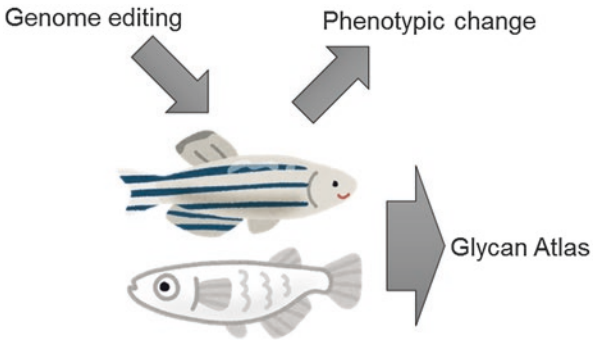

Fig. 4.10 Combination of studies on phenotypic changes resulting from alteration of glycan-related genes by genome editing, and on the glycan-structure changes before and after genetic modification by creating the Glycan Atlas

Box 4.1: Gene Editing (CRISPR/Cas9)

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Genome editing is a technology used to modify genes through deletion, insertion, or replacement of DNA in the genome by employing engineered DNA nucleases that target specific DNA sequences in a living organism. This technology is widely used in various fields with applications that include analysis of gene function in plants and animals, and generation of model cells and animals for human diseases. The nucleases that are used are ZFN, TALEN, and Cas9. CRISPR/Cas9, which is the most widely used genome editing system, consists of a guide RNA and the Cas9 protein. The guide RNA binds to a targeted DNA sequence, leading the Cas9 protein to the target DNA. The two strands of the target DNA are then cleaved, which can be repaired through intrinsic cellular DNA double-strand break repair mechanisms. The nonhomologous end joining mechanism is error-prone, and mutations that result in a frame shift or a premature stop codon cause disruption of the targeted gene. On the other hand, the homology-directed repair mechanism allows the introduction of a desired sequence that exhibits homology to the region where a double-strand break has occurred, which can then create the desired changes within the target gene.

4.13 Plant and Glycans

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Keywords Plant cell wall, Polysaccharides, Carbohydrate-active enzymes, Biofuel, Recombinant therapeutic glycoproteins

1. Significance in the field of glycoscience and its current situation
   Plants synthesize a wide variety of unique glycans such as cell wall polysaccharides as structural components, glycoprotein or glycolipids as functional molecules, and secondary metabolic glycosides as molecules against infectious pathogens [59]. Such plant glycans are essential sources of food and energy. Plant genomes encode more than twice the number of carbohydrate-active enzymes than those encoded by animal genomes [60]. However, functions of many genes have not yet been identified. Understanding of glycan biosynthesis/degradation mechanisms is an important area of research because plant glycans can potentially be used as biofuel sources to reduce carbon dioxide emissions [61, 62]. In addition, plant cells are expected to be used as host cells for the production of recombinant therapeutic glycoproteins because they are free from the risk of animal virus contamination. Currently, plant cells engineered to produce glycoproteins bearing the human-type glycans have been developed [63].
2. Impact on the other fields of research
   Glycan content in plants is much higher than that in other living organisms. Therefore, basic research on plant glycans promotes plant research.
3. Significance as the fundamental research
   Determination of biosynthesis/degradation mechanism of plant polysaccharides, which have complex structures, is a target for system biology. The structures of plant glycans are different from those of other organisms. Therefore, research on plant glycans shall lead to the elucidation of conserved and diverse functions of glycans.
4. Possible application for industry and medicine, if any
   Elucidation of biosynthesis/degradation of plant glycans contributes to the development of efficient methods of production of biofuels from plant sources. Plant-produced therapeutic glycoprotein with human-type glycans contributes to treatment of diseases.
5. Future perspectives
   Although plant glycan research lags behind as compared to mammalian glycan research, it is a key area for development of useful products like plant-derived biofuels or plant-produced therapeutic glycoproteins, especially in light of the fact that people are increasingly becoming sensitive to global environment and human health.

6. Problems to be solved
   Functional identification of all the carbohydrate-active enzymes encoded by the plant genomes, estimated to be more than 1000 in number, is needed (Fig. 4.11).

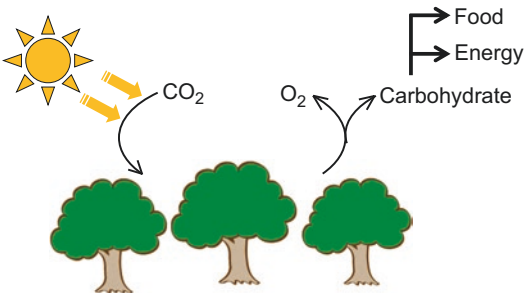

Fig. 4.11 Plant glycans are essential sources of food and energy

4.14 Plant and Glycan

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Keywords Plant-made pharmaceuticals, Plant immunity, Cell wall, Protein trafficking

1. Significance in the field of glycoscience and its current situation
   Analysis of N-glycosylation genes in model plant Arabidopsis has been completed. However, crop plants including rice have not yet been analyzed well [64]. In addition, the contribution of glycosylation to physiological functions in plant cells should be intensively studied and revealed. Allergenicity has been postulated, but careful studies have not been performed yet.
2. Impact on the other fields of research
   The effect of plant glyosylation on the cell wall would be very complexed. Most proteins are glycosylated during intracellular trafficking and show functions at the final locations. Mutation of glycogenes will change protein localization, which should be identified and examined more. Allergenicity studies would contribute to immune responses in humans [65].
3. Significance as the fundamental research
   Fundamental knowledge on plant glycosylation will facilitate understanding of cell wall biosynthesis, plant immunity and defense, and intracellular and intercellular protein trafficking.
4. Possible application for industry and medicine, if any
   Plant-made pharmaceuticals will gain more attention. So glycoengineered pharmaceuticals would be expected [66]. Understanding of intracellular and intercellular protein trafficking will contribute to more efficient pharmaceutical production. Extension to cell wall biosynthesis of plant glycosylation research will also help study on biomass utilization and development of cell wall engineering [67]. Plant immunity and defense can contribute to the development of stress-tolerant plants. Studies on allergenicity will facilitate development of low allergenic foods.
5. Future perspectives
   Successful research outputs from plant glycosylation research will further develop agriculture, such as plant breeding, and medical technology, such as human-friendly pharmaceuticals [66].
6. Problems to be solved
   Further analysis of glycogenes in other crop plants, and the relationships between glycogenes and phenotypes is necessary [67]. Plant glycans are considered as allergens, but studies on plant glycans have not yet been carefully performed (Fig. 4.12).

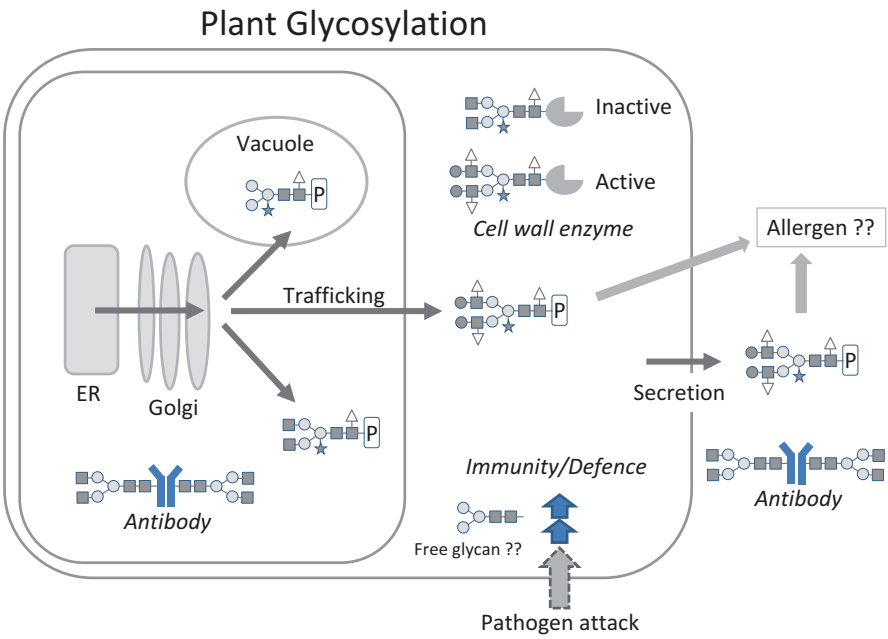

Fig. 4.12 Plant glycosylation critical for pharmaceuticals, cell wall synthesis, and many others

4.15 Genetic Modification of Yeast

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Keywords Yeast, Glycan remodeling, Glycosyltransferase, Gene editing

1. Significance in the field of glycoscience and its current situation
   In order to produce glycoproteins for therapeutics in yeast, glycan modification to mammalian type is required. So far, glycan remodeling through gene disruption and introduction of glycan-related genes has been attempted in *S. cerevisiae* [68], followed by several methylotrophic yeast strains [69–71]. Since yeast does not have a biosynthetic pathway for CMP-sialic acid, GDP-fucose and so on, it is necessary to introduce a large number of genes for their biosynthesis in addition to introduction of the glycosyltransferase genes. A means of introduction of a large number of genes without the need for recyclable markers or strains with multiple auxotrophies by combining two amino acid synthetic pathway genes has been reported [71]. Other strategies consisting of the disruption of an endogenous glycosyltransferase gene and the introduction of the heterologous glycan-related gene through antibiotic resistance has been reported [72]. In order to localize a glycosyltransferase in the Golgi apparatus, construction of a fusion protein consisting of the transmembrane domain of a yeast protein and the catalytic domain of a glycosyltransferase has been investigated [69].
2. Impact on the other fields of research
   Since yeast has been used as a model organism of eukaryotes, modification of the glycan-related genes of yeast will contribute to the application of glycan remodeling of other species. Genetic modification techniques are also useful for advanced improvement of the cells using synthetic biology.
3. Significance as fundamental research
   Gene disruption and introduction methods in yeast are useful for genome editing. They will also contribute to elucidation of the biosynthesis pathway, and the difference between the mammalian and yeast glycosylation mechanisms.
4. Possible application for industry and medicine
   It is not only being used for the production of glyco-biologics, such as antibodies for therapeutics [69], lysosomal enzymes [70] and cytokines, but also as a host for the production of glycoproteins used as reagents and diagnostic reagents with lower production costs.
5. Future perspectives
   The genetic modification technology for yeast can be applied not only to glycoprotein synthesis but also to the synthesis of glycolipids and secondary metabolites such as glycosides. By applying genome editing technology, it will also contribute to the development of productive yeasts.

6. Problems to be solved
   To modify *N*-linked and *O*-linked glycans, a technique for introducing multiple genes coding glycan-related proteins is required. Screening techniques to isolate several gene promoters and to optimize the expression of numerous glycan-modifying proteins are essential (Fig. 4.13).

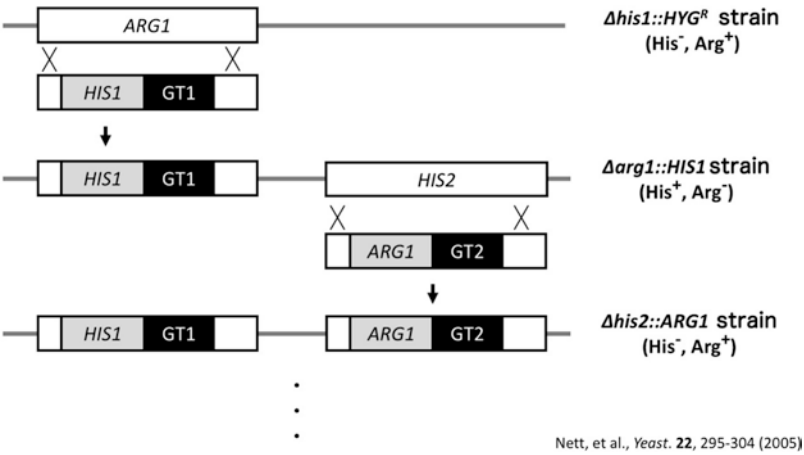

Fig. 4.13 By combining two amino acid (histidine and arginine) synthetic pathway genes, many genes can be indroduced into yeast without the need for recyclable markers or strains with multiple auxotrophies. GT: Glycan-related gene

4.16 Chemical Library

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Keywords Chemical biology, Inhibitor, Natural product, NPDepo

1. Significance in the field of glycoscience and its current situation
   The aim of chemical biology is to elucidate the complex phenomena of biological systems by using small molecule compounds [73]. For example, studies on post-translational modification of proteins and degradation of proteins are topics of chemical biology. Inhibitors of the enzymes involved in important biological functions are needed not only as reagents for basic research but also as medicines.

   Originally tunicamycin was found as an inhibitor of glycoprotein synthesis and it has become an indispensable reagent for studies of ER stress. Recently, the chemical library is being utilized for the screening of glycosyltransferase inhibitors.
2. Impact on the other fields of research
   The 2016 Nobel Prize for Physiology or Medicine was conferred on a study of autophagy. Tunicamycin is known to induce autophagy in mammalian cells. As new autophagy regulators are required, the screening of compounds from the chemical library is attracting researchers.
3. Significance as the fundamental research
   The utilization of the chemical library is a wise approach to screen for inhibitors of important enzymes [74–76].
4. Possible application for industry and medicine, if any
   Selective inhibitors may be applied not only as reagents but also as medicines.
5. Future perspectives
   The screening of medicines or pesticides could be performed only in a company having a compound library. Nowadays, compound libraries are available in public institutions. Using such chemical libraries, researchers in academia can perform the screening of the medicines or pesticides [77].
6. Problems to be solved
   Personnel training and funding are necessary to maintain a compound library of high quality (Fig. 4.14).

Fig. 4.14 Microbial metabolites and synthesized derivatives are deposited in RIKEN Natural Products Depository (NPDepo). The compounds shown in the picture are sold by companies as "bioprobes (reagents)", useful probes to elucidate the biological phenomena

References

References for Section 4.1

1. Furukawa K et al (2017) Glycolipids: essential regulator of neuro-inflammation, metabolism and gliomagenesis. Biochim Biophys Acta 1861:2479–2484
2. Ji S et al (2016) Increased a-series gangliosides positively regulate leptin/Ob receptor-mediated signals in hypothalamus of GD3 synthase-deficient mice. Biochem Biophys Res Commun 479:453–460
3. Furukawa K et al (2016) Roles of glycosphingolipids in the regulation of the membrane organization and cell signaling in lipid rafts. In: Lipid/rafts. Nova Science Publishers, London, pp 129–146
4. Furukawa K et al (2014) Glycosphingolipids in the regulation of the nervous system. Adv Neurobiol 9:307–320
5. Ohmi Y et al (2014) Ganglioside deficiency causes inflammation and neurodegeneration via the activation of complement system in the spinal cord. J Neuroinflammation 11:61

References for Section 4.2

6. Lowe JB, Marth JD (2003) A genetic approach to mammalian glycan function. Annu Rev Biochem 72:643–691
7. Furukawa K et al (2007) Knockout mice and glycolipids. In: Kamerling JP et al (eds) Comprehensive glycoscience from chemistry to systems biology, vol 4. Elsevier, Oxford, pp 149–157
8. Honke K, Taniguchi N (2009) Animal models to delineate glycan functionality. In: Gabius H-J (ed) The sugar code, fundamentals of glycosciences. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 385–401
9. Inokuchi J (2011) Physiopathological function of hematoside (GM3 ganglioside). Proc Jpn Acad Ser B Phys Biol Sci 87:179–198
10. Furukawa K et al (2017) Glycolipids: essential regulator of neuro-inflammation, metabolism and gliomagenesis. Biochim Biophys Acta 1861:2479–2484

References for Section 4.3

11. Laura M, Proia RL (2014) Simplifying complexity: genetically resculpting glycosphingolipid synthesis pathways in mice to reveal function. Glycoconj J 31:613–622
12. Simpson MA et al (2004) Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. Nat Genet 36:1225–1229
13. Boukhris A et al (2013) Alteration of ganglioside biosynthesis responsible for complex hereditary spastic paraplegia. Am J Hum Genet 93:118–123
14. Yoshikawa M et al (2015) Ganglioside GM3 is essential for the structural integrity and function of cochlear hair cells. Hum Mol Genet 24:2796–2807
15. Nagafuku M et al (2015) Control of homeostatic and pathogenic balance in adipose tissue by ganglioside GM3. Glycobiology 25:303–318

References for Section 4.4

16. Haltiwanger RS, Lowe JB (2004) Role of glycosylation in development. Annu Rev Biochem 73:491–537
17. Apweiler R et al (1999) On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. Biochim Biophys Acta 1473:4–8
18. Hang Q et al (2016) *N*-Glycosylation of integrin α5 acts as a switch for EGFR-mediated complex formation of integrin α5β1 to α6β4. Sci Rep 6:33507
19. Jaeken J, Peanne R (2017) What is new in CDG? J Inherit Metab Dis 40:569–586

References for Section 4.5

20. Mizumoto S et al (2014) Human genetic disorders and knockout mice deficient in glycosaminoglycan. Biomed Res Int 2014:495764
21. Kitakaze K et al (2016) Protease-resistant modified human β-hexosaminidase B ameliorates symptoms in GM2 gangliosidosis model. J Clin Invest 126:1691–1703
22. Ito Z et al (2010) *N*-acetylglucosamine 6-*O*-sulfotransferase-1-deficient mice show better functional recovery after spinal cord injury. J Neurosci 30:5937–5947
23. Coles CH et al (2011) Proteoglycan-specific molecular switch for RPTPσ clustering and neuronal extension. Science 332:484–488
24. Miyata S et al (2012) Persistent cortical plasticity by upregulation of chondroitin 6-sulfation. Nat Neurosci 15:414–422

References for Section 4.6

25. Mizumoto S et al (2013) Human genetic disorders caused by mutations in genes encoding biosynthetic enzymes for sulfated glycosaminoglycans. J Biol Chem 288:10953–10961
26. Inatani M et al (2003) Mammalian brain morphogenesis and midline axon guidance require heparan sulfate. Science 302:1044–1046
27. Forsberg E, Kjellen L (2001) Heparan sulfate: lessons from knockout mice. J Clin Invest 108:175–180
28. Irie F et al (2012) Autism-like socio-communicative deficits and stereotypies in mice lacking heparan sulfate. Proc Natl Acad Sci U S A 109:5052–5056
29. Jones KB et al (2009) A mouse model of osteochondromagenesis from clonal inactivation of Ext1 in chondrocytes. Proc Natl Acad Sci U S A 107:2054–2059

References for Section 4.7

30. Itoh K et al (2016) Mucin-type core 1 glycans regulate the localization of neuromuscular junctions and establishment of muscle cell architecture in *Drosophila*. Dev Biol 412: 114–127
31. Yamamoto-Hino M et al (2015) Phenotype-based clustering of glycosylation-related genes by RNAi-mediated gene silencing. Genes Cells 20: 521–542

32. Nishihara S (2010) Glycosyltransferases and transporters that contribute to proteoglycan synthesis in *Drosophila*: identification and functional analyses using the heritable and inducible RNAi system. Methods Enzymol 480: 323–51
33. Yamamoto-Hino M et al (2010) Identification of genes required for neural-specific glycosylation using functional genomics. PLoS Genet 23: e1001254
34. Ueyama M et al (2010) Increased apoptosis of myoblasts in *Drosophila* model for the Walker-Warburg syndrome. PLoS One 5: e11557

References for Section 4.8

35. McGary KL et al (2010) Systematic discovery of nonobvious human disease models through orthologous phenotypes. Proc Natl Acad Sci U S A 107:6544–6549
36. McWhite CD et al (2015) Applications of comparative evolution to human disease genetics. Curr Opin Genet Dev 35:16–24
37. Akiyoshi S et al (2015) RNAi screening of human glycogene orthologs in the nematode *Caenorhabditis elegans* and the construction of the *C. elegans* glycogene database. Glycobiology 25:8–20
38. Mizuguchi S et al (2003) Chondroitin proteoglycans are involved in cell division of *Caenorhabditis elegans*. Nature 423:443–448
39. Dejima K et al (2018) An aneuploidy-free and structurally defined balancer chromosome toolkit for *Caenorhabditis elegans*. Cell Rep 22:232–241

References for Section 4.9

40. NBRP Zebrafish: http://shigen.nig.ac.jp/zebra/
41. NBRP Medaka: https://shigen.nig.ac.jp/medaka/
42. Laughlin ST et al (2008) In vivo imaging of membrane-associated glycans in developing zebrafish. Science 320:664–667
43. Service RF (2012) Looking for a sugar rush. Science 338:321–323

References for Section 4.10

44. Liu J et al (2017) CRISPR/Cas9 in zebrafish: an efficient combination for human genetic diseases modeling. Hum Genet 136:1–12
45. Avsar-Ban E et al (2010) Protein *O*-mannosylation is necessary for normal embryonic development in zebrafish. Glycobiology 20:1089–1102
46. Uemura N et al (2015) Viable neuronopathic Gaucher disease model in Medaka (*Oryzias latipes*) displays axonal accumulation of alpha-synuclein. PLoS Genet 11:e1005065
47. Keatinge M et al (2015) Glucocerebrosidase 1 deficient *Danio rerio* mirror key pathological aspects of human Gaucher disease and provide evidence of early microglial activation preceding alpha-synuclein-independent neuronal cell death. Hum Mol Genet 24:6640–6652
48. Newman M et al (2014) Using the zebrafish model for Alzheimer's disease research. Front Genet 5:Article 189

References for Section 4.11

49. Westerfield M (2007) The Zebrafish book, 5th Edition; a guide for the laboratory use of zebrafish (*Danio rerio*), Eugene, University of Oregon Press. Distributed by the Institute of Neuroscience, University of Oregon, Copyright 1993 by Monte Westerfield, Edition 3; For on-line Edition 4, http://zfin.org/zf_info/zfbook/zfbk.html
50. Kinoshita M et al (2009) Medaka: biology, management, and experimental protocols. Willey-Blackwell, Ames
51. Tonoyama Y et al (2009) Essential role of β-1,4-galactosyltransferase 2 during medaka (*Oryzias latipes*) gastrulation. Mech Dev 126:580–594
52. Avsar-Ban E et al (2010) Protein *O*-mannosylation is necessary for normal embryonic development in zebrafish. Glycobiology 20:1089–1102
53. Moore CJ et al (2008) Genes required for functional glycosylation of dystroglycan are conserved in zebrafish. Genomics 92:159–167

References for Section 4.12

54. Westerfield M (2007) The Zebrafish book, 5th Edition; A guide for the laboratory use of zebrafish (*Danio rerio*). University of Oregon Press, Eugene
55. Hisano Y et al (2015) Precise in-frame integration of exogenous DNA mediated by CRISPR/Cas9 system in zebrafish. Sci Rep 5:8841
56. Hwang WY et al (2013) Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 31:227–229
57. Ansai S, Kinoshita M (2017) Genome Editing of Medaka. Methods Mol Biol 1630:175–188
58. Hanzawa K et al (2017) Structures and developmental alterations of *N*-glycans of zebrafish embryos. Glycobiology 27:228–245

References for Section 4.13

59. Buchanan BB et al (2000) Biochemistry & molecular biology of plants. American Society of Plant Physiologists, Rockville
60. Lombard V et al (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495
61. Tan HT et al (2016) Emerging technologies for the production of renewable liquid transport fuels from biomass sources enriched in plant cell walls. Front Plant Sci 7:1854
62. Albersheim P et al (2011) Plant cell walls, Garland Science
63. Dicker M, Strasser R (2015) Using glyco-engineering to produce therapeutic proteins. Expert Opin Biol Ther 15:1501–1516

References for Section 4.14

64. Pedersen CT et al (2017) *N*-glycan maturation mutants in *Lotus japonicus* for basic and applied glycoprotein research. Plant J 91:394–497

65. Mercx S et al (2017) Inactivation of the β(1,2)-xylosyltransferase and the α(1,3)-fucosyltransferase genes in *Nicotiana tabacum* BY-2 cells by a multiplex CRISPR/Cas9 strategy results in glycoproteins without plant-specific glycans. Front Plant Sci 8:403
66. Limkul J et al (2016) The production of human glucocerebrosidase in glyco-engineered Nicotiana benthamiana plants. Plant Biotechnol J 14:1682–1694
67. von Schaewen A et al (2015) Arabidopsis thaliana KORRIGAN1 protein: *N*-glycan modification, localization, and function in cellulose biosynthesis and osmotic stress responses. Plant Signal Behav 10:e1024397

References for Section 4.15

68. Chiba Y et al (1998) Production of human compatible high mannose-type (Man5GlcNAc2) sugar chains in *Saccharomyces cerevisiae*. J Biol Chem 273:26298–26304
69. Choi BK et al (2003) Use of combinatorial genetic libraries to humanize *N*-linked glycosylation in the yeast *Pichia pastoris*. Proc Natl Acad Sci U S A 100:5022–5027
70. Kuroda K et al (2008) Efficient antibody production upon suppression of O mannosylation in the yeast *Ogataea minuta*. Appl Environ Microbiol 74:446–453
71. Nett JH et al (2005) Cloning and disruption of the *Pichia pastoris ARG1, ARG2, ARG3, HIS1, HIS2, HIS5, HIS6* genes and their use as auxotrophic markers. Yeast 22:295–304
72. Jacobs PP et al (2009) Engineering complex-type *N*-glycosylation in *Pichia pastoris* using GlycoSwitch technology. Nat Protoc 4:58–70

References for Section 4.16

73. Osada H (2010) Introduction of new tools for chemical biology research on microbial metabolites. Biosci Biotechnol Biochem 74:1135–1140
74. Miyazaki I et al (2010) A small-molecule inhibitor shows that pirin regulates migration of melanoma cells. Nat Chem Biol 6:667–673
75. Kato N et al (2012) Construction of a microbial natural product library for chemical biology studies. Curr Opin Chem Biol 16:101–108
76. Kawatani M et al (2015) Identification of matrix metalloproteinase inhibitors by chemical arrays. Biosci Biotechnol Biochem 79:1597–1602
77. Piotrowski JS et al (2017) Functional annotation of chemical libraries across diverse biologocal processes. Nat Chem Biol 13:982–993. (errata, 13, 1286)

Part II
Glycans and Biopharmaceuticals

Foreword

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Now that it is becoming more and more difficult develop new drugs, accompanied by the emergence of the potency of their side effects as well as the patent-off issue, pharmaceutical companies are spending much time on the development of biopharmaceutical drugs represented by antibody drugs and hormone agents. However, another emerging issue is apparent; most of these biopharmaceutical drugs are originally glycoproteins exhibiting considerably heterogenous complexity, of which assessment is quite difficult. With such a situation, emerging countries including China, India and Korea, are eager to find a new market for biosimilar drugs, in analogy to generic drugs, which will intensify international competition. On the other hand, in general, development of such complex biopharmaceutical drugs involves much cost and technique for high quality control of their production processes. Thus, development of high cost-effective drugs of medium size between low molecular drugs and biopharmaceuticals is also attracting attention. Such medium molecular medicines include glycomimetic drugs, represented by Relenza-Tamiflu (glycosidase inhibitors) and Rivipansel (selectin inhibitor), albeit they are not defined as biopharmaceutical drugs. Glycans are also extensively related to immunity to pathogenic microbes as well as cancer, where a new approach for chemical synthesis of structure-defined glycoconjugates as adjuvants and vaccines is also emerging as innovative way of converting conventional therapeutics to future preventive medicines. Please see page 142 Box 7.1: Expression of glycoproteins in CHO cells, page 162 Box 8.1: Erythropoietin, page 163 Box 8.2: Understanding the Molecular Basis that Governs Glycan Patter Recognition of glycoclusters and applying Drug Delivery System, page 176 Box 9.1: High Throughput Screening Targeting Glycosyltransferase, page 176 Box 9.2: Heparinoid.

Chapter 5
Antibody Pharmaceuticals

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5.1 Biosimilar/Follow-On and Biobetter Biologics

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Keywords Biological products, Biosimilar, Biobetter, Quality, Efficacy, Safety

1. Significance in the field of glycoscience and its current situation
   Many different recombinant proteins, including monoclonal antibodies, have been developed as biological products. More than 100 biological products have been approved in Japan, most of which are glycosylated proteins (Fig. 5.1). It is known that glycosylation affects the efficacy, safety, and quality of glycoprotein products; therefore, glycosylation analysis is essential for glycoprotein drug development. Some off-patent biological products have become targets of biosimilar/follow-on biologics (BS) development. A BS is a biotechnological drug product developed to be comparable with regard to quality, safety, and efficacy to an already approved biotechnology-derived product (original biologic) from a different company [1, 2]. A glycoprotein BS needs to be developed by evaluating the similarity of glycosylation based on the roles of glycans in the efficacy and safety of the drug products [3]. A biobetter biologic is often used as a new active pharmaceutical ingredient drug, which is developed by improving the original

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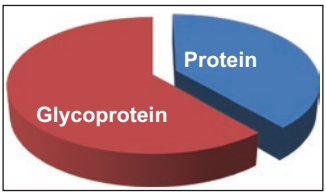

Fig. 5.1 Ratio of glycoproteins to biotechnological products approved in Japan

biologic to enhance its efficacy and safety [4]. In the glycoscience field, glycoengineering is expected to yield biobetter products, which are characterized by improved features, such as an elongated blood half-life and enhanced biological activity.

2. Impact on other fields of research
   Therapeutic measures for various intractable diseases and adult diseases have been changed by the development of novel glycoprotein products, including *nivolumab*, *evolocumab*, and lysosome enzyme products. We can expect additional innovation of glycoprotein products that solve unmet medical needs and have an impact on medical care.
3. Significance as fundamental research
   It is crucial to clarify glycan structure and heterogeneity, and the impact of glycans on safety and efficacy needs to be evaluated for the development of glycoprotein products. Innovation regarding glycoprotein products is dependent on the progress of glycoscience.
4. Possible application for industry and medicine
   With the aging society, the growth rate of annual sales of ethical drugs, including biotechnological products, is estimated to be over 6% (2014–2020) [5]. The distribution of BS products is expected to be a concrete measure for reducing healthcare cost.
5. Future perspectives
   The market for biological drugs in 2020 is estimated to be approximately tens of trillions yen. Because the patents on a nine trillion biopharmaceutical market will be expired soon, the BS market in 2020 is estimated to be one to three trillion yen [5]. Development of biobetter and biosimilar products is expected to accelerate in the near future.
6. Problems to be solved
   A common problem to be solved for original, biobetter, and BS products is the development of efficient, rapid and easy methods for the evaluation and quality control of glycosylation on biotechnological products (10 years).

5.2 Quality Evaluation of Glycoprotein Products Using Glycoprotein with Homogeneous Glycans

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Keywords Glycoengineering, Transglycosylation technology, Glycoproteins with homogeneous glycans, Glycoprotein products, Quality evaluation

1. Significance in the field of glycoscience and its current situation
   Transglycosylation technology enables the preparation of glycoproteins with homogeneous glycans [6, 7]. During the development of therapeutic glycoprotein products, it is difficult to assess the impact of each glycan on the efficacy and safety of products because glycoprotein glycosylations are heterogeneous [8, 9]. However, by using glycoproteins with homogeneous glycans, we can investigate the functions of each glycan on glycoprotein products in detail (Fig. 5.2). On the other hand, glycans that are useful for transglycosylation are limited, and one of the important challenges is to increase the types of glycans applicable to transglycosylation.
2. Impact on other fields of research
   Generalization of the technology to prepare glycoproteins with homogeneous glycans will be useful for studies concerning quality evaluation of glycoprotein products as well as research on developing new drugs.
3. Significance as fundamental research
   Usage of glycoproteins with homogeneous glycans will be useful to investigate the impact of glycoprotein glycans on structural stabilities, biological activities, and pharmacokinetics.
4. Possible application for industry and medicine (not relevant)
5. Future perspectives
   Development of glycoproteins with homogeneous glycans will lead to the production of novel therapeutic glycoproteins with improved functions and pharmacokinetics.
6. Problems to be solved
   For quality evaluation of glycoprotein products, glycans that are useful for transglycosylation are limited, and one of the important challenges is to increase the types of glycans applicable to transglycosylation (5 years).

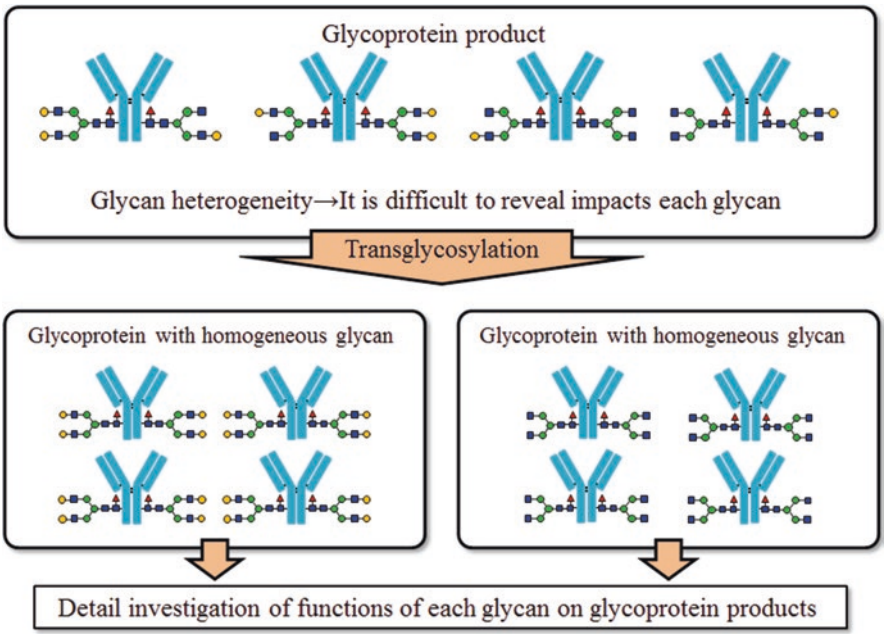

Fig. 5.2 Quality evaluation using glycoproteins with homologues glycans produced by glycoengineering

References

References for Section 5.1

1. Lodford H (2015) First biosimilar drug set to enter US market. Nature 517:253–254
2. Schneider CK et al. (2012) Working party on similar biological (Biosimilar) medicinal products (BMWP); biologicals working party (BWP) of the committee for medicinal products for Human use (CHMP). Nat Biotechnol 30:745–748; author reply 748–749
3. Schiestl M et al (2011) Acceptable changes in quality attributes of glycosylated biopharmaceuticals. Nat Biotechnol 29:310–312
4. Beck A (2011) Biosimilar, biobetter and next generation therapeutic antibodies. MAbs 3:107–110
5. EvaluatePharma (2017)

References for Section 5.2

6. Huang W et al (2012) Chemoenzymatic glycoengineering of intact IgG antibodies for gain of functions. J Am Chem Soc 134:12308–12318
7. Kurogochi M et al (2015) Glycoengineered monoclonal antibodies with homogeneous glycan (M3, G0, G2, and A2) using a chemoenzymatic approach have different affinities for FcγRIIIa and variable antibody-dependent cellular cytotoxicity activities. PLoS One 10:e0132848
8. Harazono A et al (2013) Mass spectrometric glycoform profiling of the innovator and biosimilar erythropoietin and darbepoetin by LC/ESI-MS. J Pharm Biomed Anal 83:65–74
9. Hashii N et al (2014) Characterization of N-glycan heterogeneities of erythropoietin products by liquid chromatography/mass spectrometry and multivariate analysis. Rapid Commun Mass Spectrom 28:921–932

Chapter 6
Standard Glycan Library

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1.1 Human-Type Glycan Library

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Keywords Glycan, Lectin, Virus, Infection

1. Significance in the field of glycoscience and its current situation
   Human-type glycans are defined as glycans with the same chemical structures as those expressed in human organs [1]. A collection of these glycans can be called a human-type glycan library. These glycans can be used as ligands for lectins, and substrates for glycosidases and glycosyltransferases [2, 3]. In addition, it is also useful to identify tissue and stage-specific glycans as authentic standards [4]. However, information on human-type glycans is based on the results of glycan structure analysis of glycoproteins performed in the past, and thus only limited information is available as to the expression levels of individual glycans in specified tissues and stages. Another issue is the difficulty in providing substantial amounts of a large variety of human-type glycans required for extensive analysis of the substrate specificities of lectins, glycosidases, and glycosyltransferases.

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2. Impact on other fields of research
   Glycans cover the surface of all types of cells. These glycans are thought to be involved in cell recognition and cell adhesion. In particular, if a human-type glycan library becomes more easily available, viral infection mechanisms through specific glycan recognition will be elucidated [5].
3. Significance as fundamental research
   A human-type glycan library does not simply provide a collection of human glycans, but also provides useful information based on certain indexes (e.g., organ, stage, and expression level) (Fig. 6.1).
4. Possible application for industry and medicine
   If glycans such as those particularly related to influenza virus infection can be detected, surveillance of pandemics caused by virus mutations will be possible more effectively. Application to biopharmaceuticals is also promising by providing substantial amounts of homogenous glyco-materials.
5. Future perspectives
   If a human-type glycan library is constructed, it will become possible to easily investigate molecular mechanisms by which cell adhesion and recognition occur in vivo.
6. Problems to be solved
   There is only limited information on the precise chemical structures of human glycans expressed in various tissues. They must be clarified with their quantification. It is also important to establish a methodology to produce and provide extensive human-type glycans by all means (combination of organic synthesis, enzymatic synthesis, and utilization of natural products) (5 years).

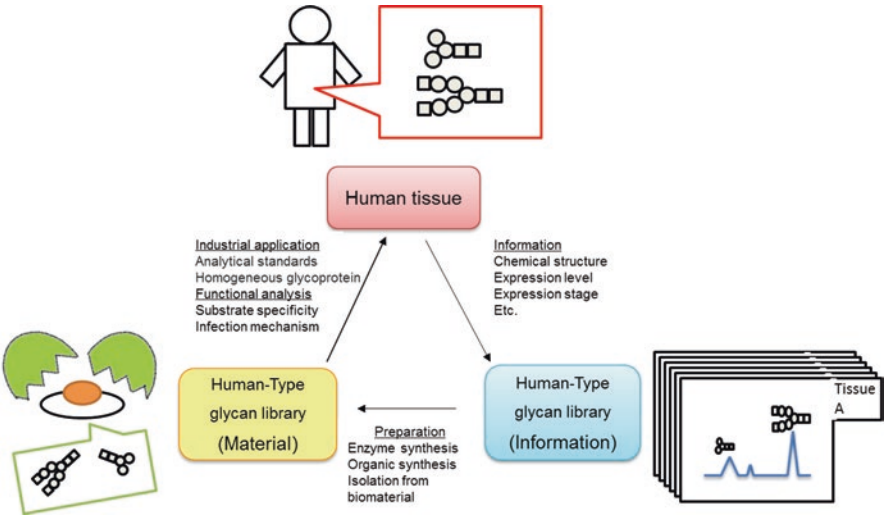

Fig. 6.1 The concept of a human type glycan library. Human-type glycans were prepared

References

1. Sumiyoshi W et al (2009) Strategic glycan elution map for the production of human-type *N*-linked oligosaccharides: the case of hen egg yolk and white. Biosci Biotechnol Biochem 73:543–551
2. Nakakita S et al (1999) beta1-4galactosyltransferase activity of mouse brain as revealed by analysis of brain-specific complex-type *N*-linked sugar chains. J Biochem 126:1161–1169
3. Okamoto Y et al (1999) Conversion of brain-specific complex type sugar chains by *N*-acetyl-beta-*D*-hexosaminidase B. J Biochem 125:537–540
4. Nakakita S et al (1998) Development-dependent expression of complex-type sugar chains specific to mouse brain. J Biochem 123:1164–1168
5. Kubota M et al (2016) Trisaccharide containing α2,3-linked sialic acid is a receptor for mumps virus. Proc Natl Acad Sci U S A 113:11579–11584

Chapter 7
Mass Production in Silk Worm and Yeast

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7.1 Glycoprotein Production with Yeast

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Keywords Yeast, Glycan remodeling, Glycosyltransferase, Glyco-biologics

1. Significance in the field of glycoscience and its current situation
   Since yeast has an excellent secretion system, it has been used as a production system for simple proteins such as growth hormones and vaccines for a long time. In recent years, due to ease of handling and inexpensive medium, it is expected to become a production system for antibodies as therapeutic agents. It is necessary to modify the glycan to eliminate the antigenicity of yeast-specific glycan structures [1]. It has been reported that production of antibodies with complex-type glycans [2] and of lysosomal enzymes with mannose-6-phosphate type glycan [3] by disruption of yeast-specific glycosyltransferase genes and

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introduction of glycan-related genes required for remodeling to human-type. In addition, mucin-type [4] and dystroglycan-type [5] glycoprotein production by yeast have been reported (Fig. 7.1).

2. Impact on other fields of research
   Glycan remodeling is also promoted in plant cells by the same method as that in yeast. Since modification of the glycan biosynthetic pathway is an advanced cell-modification technique involving synthetic biology, it will contribute to the development of material production by microorganisms.
3. Significance as fundamental research
   It is useful for elucidating the biosynthesis pathways and physiological roles of glycans in yeast and for elucidating the difference between mammalian and yeast glycosylation mechanisms. It will also contribute to the development of yeast gene disruption/introduction technology.
4. Possible application for industry and medicine
   It is not only being used for the production of glyco-biologics, such as the antibodies for therapeutics [2], lysosomal enzymes [3], and cytokines, but also as a host for the production of glycoproteins used as reagents and diagnostic reagents requiring lower production costs.
5. Future perspectives
   It is expected to be applied not only to the production of antibodies as therapeutic agents but also to the production of other biopharmaceuticals such as antibody alternatives and vaccines using viral surface proteins.
6. Problems to be solved
   Since complete inhibition of yeast-specific *O*-mannosylated modification, which is essential for yeast, has not been achieved, engineering of yeast cells based on a new concept is required (5 years).

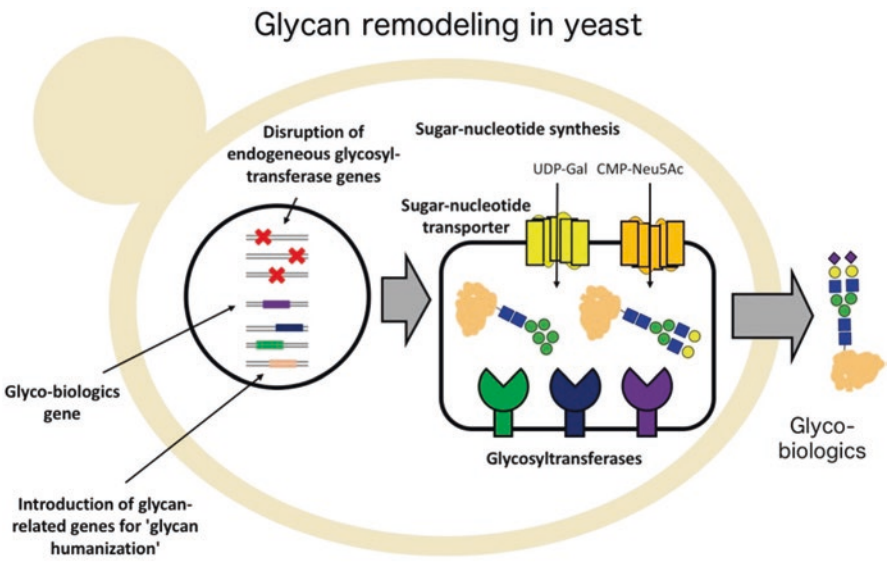

Fig. 7.1 Glycan remodeling of yeast requires disruption of the glycosyltransferase genes that synthesize yeast-specific glycans and introduction of genes necessary for human- type glycan synthesis, such as sugar nucleotide syntheses, sugar nucleotide transporters and glycosyltransferases

7.2 Mass Production of Glycoproteins Using Silkworms

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Keywords Silkworms, Yeasts, Recombinant protein, Human-type glycans

1. Significance in the field of glycoscience and its current situation
   Most biologics including therapeutic antibodies are glycoproteins. Glycan chains attached to therapeutic glycoproteins need to be of a human-type, because the glycan chains have great effects on the activity and biokinetics of glycoproteins. On the other hand, the high production cost of biologics is now a social problem, and therefore new technologies are required for the production of large amounts of therapeutic proteins at low cost. Thus, we need to develop low cost mass production systems for glycoproteins with human-type glycan chains using silkworms or yeasts as hosts [6–8] (Fig. 7.2).
2. Impact on other fields of research
   In addition to the medical field, mass production systems for glycoproteins will also be useful in various industrial fields including research-use and diagnostic reagents or cosmetics.
3. Significance as fundamental research
   It will possibly lead to the elucidation of the glycan structures and activities of glycoproteins.
4. Possible application for industry and medicine
   It is necessary for medical uses of glycoproteins.
5. Future perspectives
   Various glycoproteins will be produced by such systems, and the creation of new industries is expected.
6. Problems to be solved
   It is now difficult to produce glycoproteins with perfectly human-types glycans in silkworms or yeasts. Host organisms such as silkworms or yeasts need to be improved by introducing the genes for glycosyltransferases and other glycosylation-related proteins (5 years).

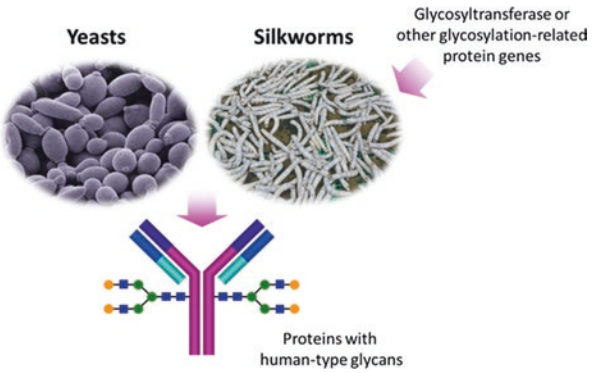

Fig. 7.2 We will develop mass production systems for glycoproteins with human-type glycans using yeasts or silkworms as hosts, which can be improved by introducing the genes for glycosyltransferases and other glycosylation-related proteins

Box 7.1: Expression of Glycoproteins in CHO Cells

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A wide variety of recombinant glycoprotein therapeutics, such as monoclonal antibodies, growth factors and cytokines, as well as traditional non-recombinant biopharmaceuticals such as vaccines and blood products are now clinically available. Because glycosylation is often required for their biological activities, it is necessary to use eukaryotic cells to express these recombinant glycoproteins. Since the first approved recombinant glycoprotein therapeutic, tissue plasminogen activator, was produced using Chinese Hamster Ovary cells (CHO) as the host, CHO cells have now become the most widely used system for their commercial production. In addition to the lower risk in the approval by regulatory authorities compared to the use of alternate cell hosts, there are several reasons and technical advantages due to being well characterized. High viability of CHO cells can be maintained for a long period, thereby leading to an increased production of recombinant glycoproteins, and the cells can grow in suspension cultures using chemically defined media. The absence of receptiveness to human viruses is also advantageous in terms of the production of therapeutic glycoproteins for humans, because the risk of viral infections is minimized. Gene amplification systems, for example, involving dihydrofolate reductase and glutamine synthetase, have been established in CHO cells, allowing a high copy number of the integrated transgene and a high level of the desired protein. As indicated by the expression of IgG in CHO cells, the cells biosynthesize a typical type of mammalian glycan, namely a fucosylated biantennary structure. While sialylation of glycoproteins, which are important for achieving a prolonged half-life in the circulation, occurs in CHO cells, α2,3-linked sialylation occurs exclusively, but α2,6-linkages are lacking. The overexpression of a sialyltransferase along with a galactosyltransferase results in an improved sialic acid content in glycoproteins that are expressed, which is probably a contributing factor to the beneficial increase in therapeutic efficacy. CHO cells also lack α1,3/4 fucose and bisecting GlcNAc, both of which are formed in humans. To produce glycoproteins with these glycan structures, CHO cells must be genetically re-engineered so as to produce human-like glycan structures. It is known that non-core fucosylated glycan structures of the Fc portion of a monoclonal antibody enhances therapeutic activity via antibody-dependent-cell-mediated-cytotoxicity. The engineered CHO cells in which FUT8 is disrupted in both alleles, as well as a variant CHO cell line such as Lec13 cells that are incapable of synthesizing GDP-fucose, are used to produce non-fucosylated glycoproteins. On the other hand, CHO cells express non-human glycan epitopes such as Galα1,3-Gal and Neu5Gc, both of which are potentially immunogenic to human and may thus reduce the efficacy of the produced glycoprotein therapeutics.

References

References for Section 7.1

1. Chiba Y et al (1998) Production of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae. J Biol Chem 273:26298–26304
2. Li H et al (2006) Optimization of humanized IgGs in glycoengineered *Pichia pastoris*. Nat Biotechnol 24:210–215
3. Akeboshi H et al (2009) Production of human beta-hexosaminidase A with highly phosphorylated *N*-glycans by the overexpression of the *Ogataea minuta* MNN4 gene. Glycobiology 19:1002–1009
4. Amano K et al (2008) Engineering of mucin-type human glycoproteins in yeast cells. Proc Natl Acad Sci USA 105:3232–3237
5. Hamilton SR et al (2013) Production of sialylated *O*-linked glycans in *Pichia pastoris*. Glycobiology 23:1192–1203

References for Section 7.2

6. Iizuka M et al (2009) Production of a recombinant mouse monoclonal antibody in transgenic silkworm cocoons. FEBS J 276:5806–5820
7. Tada M et al (2015) Characterization of anti-CD20 monoclonal antibody produced by transgenic silkworms (Bombyx mori). MAbs 7:1138–1150
8. Kurogochi M et al (2015) Glycoengineered Monoclonal Antibodies with Homogeneous Glycan (M3, G0, G2, and A2) using a chemoenzymatic approach have different affinities for FcγRIIIa and variable antibody-dependent cellular cytotoxicity activities. PLoS One 10:e0132848

Chapter 8
Glycoengineering

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8.1 Chemistry-Based Engineering

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Keywords Glycosynthase, Oligosaccharyl transfer, Homogeneous glycoproteins

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1. Significance in the field of glycoscience and its current situation:
   We can prepare glycoproteins in mammalian cells and this method has been used for the preparation of glycoprotein-drugs. The *N*-glycosylation position on a glycoprotein can be regulated by DNA manipulation, while the *O*-glycosylation position cannot be regulated. In addition to this, regulation of the structures of *N*- and *O*-glycans is still impossible. In terms of *N*-glycans, conversion of heterogeneous *N*-glycans into homogeneous ones can be performed with endo-β-*N*-acetyl glucosaminidases and glycosynthases (Fig. 8.1). Chemical synthesis of glycoproteins bearing homogeneous glycans is also possible. However, the structures of *N* and *O*-glycans cannot be regulated in a cell expression system. Therefore, we cannot identify which oligosaccharide structure is critical for an individual biological event.
2. Impact on other fields of research
   Almost all proteins on the cell surface and in body fluids are glycosylated. When non-glycosylated, these proteins cannot perform their biological functions. Understanding of oligosaccharide functions will highlight the necessity of oligosaccharides to society for glycoprotein pharmaceutical drugs.
3. Significance as fundamental research
   If we can change oligosaccharide structure by a mammalian cell expression method, we can evaluate oligosaccharide functions depending on their structure through several specific biological experiments.
4. Possible application for industry and medicine
   Glycoproteins bearing heterogeneous oligosaccharides have been used as pharmaceutical drugs. Actually, glycoproteins bearing homogeneous oligosaccharides are ideal, because potent activity is confirmed when glycoproteins have homogeneous sialyloligosaccharides.

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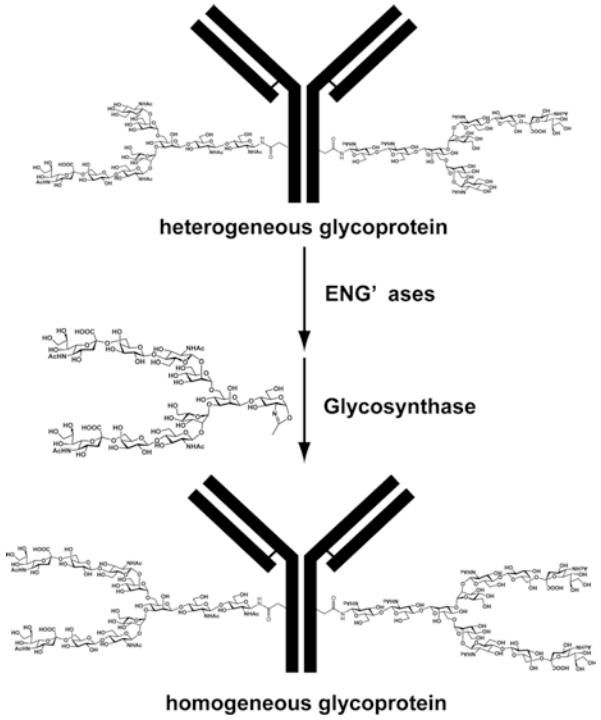

Fig. 8.1 Conversion of a heterogeneous glycan into a homogeneous glycan with a glycosynthase

5. Future perspectives
   Regulation of oligosaccharide structure should be well regulated in mammalian cell expression. Conversion of heterogeneous oligosaccharides into homogeneous ones by glycosynthases [1–3] should be performed on a large scale and chemical synthesis of homogeneous glycoproteins [4] should also be expanded on a large scale.
6. Problems to be solved
   In order to obtain glycoengineered glycoproteins, a large amount of enzymes, glycosynthases should be easily obtained cheaply. Chemical synthesis should be performed cheaply (5 years).

8.2 Tools for Glycoengineering: Key Enzymes

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Keywords Endoglycosidase, Transglycosylation activity, Glycosylation reaction, Remodeling, Glycoengineering

1. Significance in the field of glycoscience and its current situation
   In a similar manner to endonucleases (restriction enzymes) or proteases, which freely cut and paste genes or proteins, and are used in the field of gene or protein engineering, respectively, endoglycosidases are used as tools to cut and paste sugar chains in the field of glycotechnology. Among these enzymes, endo-β-*N*-acetylglucosaminidase is a unique enzyme that hydrolytically cleaves the core region of an *N*-linked oligosaccharide attached to a protein, leaving a single *N*-acetylglucosamine moiety bound to the protein. This key enzyme has attracted attention in glycotechnology because it can cleave, ligate and modify sugar chains. In particular, the transglycosylation activity of the enzyme is useful for binding an *N*-linked oligosaccharide to a protein: the oligosaccharide is transferred to an *N*-acetylglucosamine residue attached to a protein as an acceptor, which is produced after hydrolysis by the same endo-β-*N*-acetylglucosaminidase [5] (Fig. 8.2). In addition, both peptide-*N*-glycanase, which liberates *N*-linked sugar chains from proteins, and endo-α-*N*-acetylgalactosaminidase, which liberates *O*-linked sugar chains from proteins, are very important enzymes in the field of glycoengineering.
2. Impact on other fields of research
   Endo-β-*N*-acetylglucosaminidase can attach a sugar chain to a protein, and this is an important tool in the production of biomedicines. Because sugar chains are important for providing stability, solubility and effectiveness of biomedicines, this enzyme has the potential to be developed as a practical and convenient tool in biomedicine production [6]. Additionally, the remodeling of a sugar chain in biomedicines is possible using this enzyme.
3. Significance as fundamental research
   As to various phenomena in the living body, it is very significant in fundamental research to elucidate the functional roles of sugar chains in various glycoconjugates. Endo-β-*N*-acetylglucosaminidase can be used as an important tool to elucidate the structures and functions of sugar chains, because this enzyme can cleave and attach sugar chains to proteins without damaging either the sugar chain or protein.
4. Possible application for industry and medicine
   The sugar chain attached to the Fc domain in human immunoglobulin G (IgG) plays a critical role in the effectiveness of antibody drugs [7]. The structure of this N-linked oligosaccharide affects the levels of antibody-dependent cellular

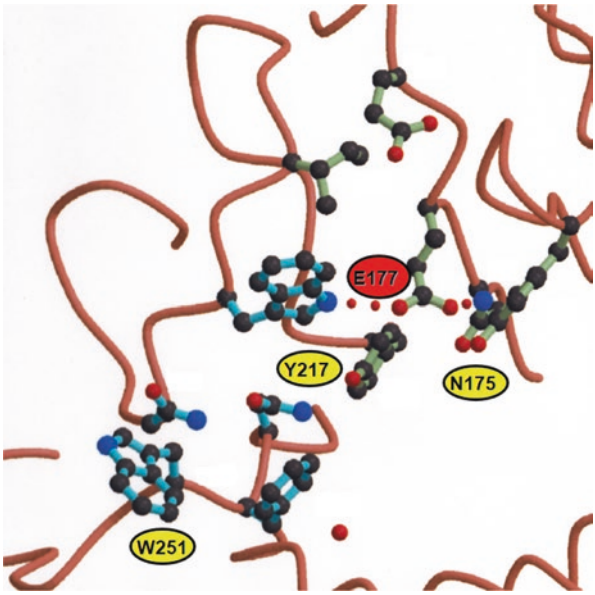

Fig. 8.2 Critical amino acid residues around the active center (E177) of *Mucor hiemalis* endoglycosidase (Endo-M). Mutant enzymes as to Y217F and N175Q possess enhanced transglycosylation activity and glycosynthase-like activity with sugar oxazoline as a donor, respectively. The W251N mutant can act on core-fucosylated N-glycans. Mutants of other similar endoglycosidases, in which the critical residue is replaced by the corresponding residue of Endo-M mutant, show the same activity as that of each Endo-M mutant

cytotoxicity (ADCC) activity and complement-dependent cytotoxicity (CDC) activity *in vitro*, as well as anticancer effects *in vivo*. Therefore, application of endoglycosidases for modifying the sugar chain of IgG will contribute greatly to the development of new medicines [8].

5. Future perspectives
   Enzymatic methods are more useful for glycoengineering than organic methods because an enzyme reaction can be performed under mild-to-moderate conditions, which differs from the case of organic methods. The substrate specificity of the enzyme can be altered using various gene engineering and protein engineering techniques. Using these techniques, enzymes can be designed to exhibit activity toward a range of substrates [9].
6. Problems to be solved
   One of the main problems when using enzymes as tools in engineering is their instability. There are various factors, such as heat, pH and the presence of protein-degrading enzymes that can decrease the activity of an enzyme. It is necessary to find an enzyme that exhibit high activity over a range of conditions. Moreover, the narrow substrate specificity and the limitation of the reaction conditions are also problems when considering enzyme applications (5 years).

8.3 Therapeutic Antibody (ADCC)

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Keywords Therapeutic antibodies, Antibody-dependent cell-mediated cytotoxicity (ADCC), Glycoengineering, Fucosylation, POTELLIGENT® technologies

1. Significance in the field of glycoscience and its current situation
   Antibody-dependent cellular cytotoxicity (ADCC) is defined as the cytotoxic activity through an antibody bound to a target cell mediated by lymphocytes such as NK cells and macrophages as effector cells, eliciting target cell injury in an antibody-dependent manner. ADCC is one of the mechanisms of the major antitumor effect of therapeutic antibodies, and the *N*-linked complex type oligosaccharide structure bound to the Fc region of an antibody greatly influences the strength of its physiological activity (Fig. 8.3). Removal of fucose in the oligosaccharides markedly enhances ADCC and the non-fucosylated antibody exhibits high efficacy [10]. A manufacturing process for therapeutic antibodies whose fucosylation (Potelligent®) is well controlled has been developed in Japan [11], is and utilized worldwide, resulting in the launch of products as a next generation therapeutic antibodies [12].
2. Impact on other fields of research
   Potelligent® technology is also applied to the industrial production of other glycoprotein medicines whose oligosaccharide structures have a great influence on their physiological activity, and recombinant antithrombin (AT), enabling the replacement of blood products, have been launched in Japan [13].
3. Significance as fundamental research
   The influence of the oligosaccharide structures of therapeutic antibodies on ADCC has been comprehensively analyzed, and their optimal structure as a drug has been clarified [14].
4. Possible application for industry and medicine
   Approved biologics license application (BLA) drugs such as therapeutic antibodies and glycoprotein, manufactured by means of the fucosylation-controlled technology Potelligent®, are as follows.

   - Anti-CCR4 Humanized Antibody (Mogamulizumab) was approved in Japan for relapsed or refractory CCR4-positive ATL in March 2012.
   - Recombinant Human Antithrombin (Antithrombin Gamma (Genetical Recombination)) was approved in Japan for thrombophilia due to congenital antithrombin deficiency and disseminated intravascular coagulation accompanied by a decrease in antithrombin in July 2015.
   - Anti-IL-5 Receptor Humanized Antibody (Benralizumab) was approved in US, EU and Japan for severe asthma as of June 2017.

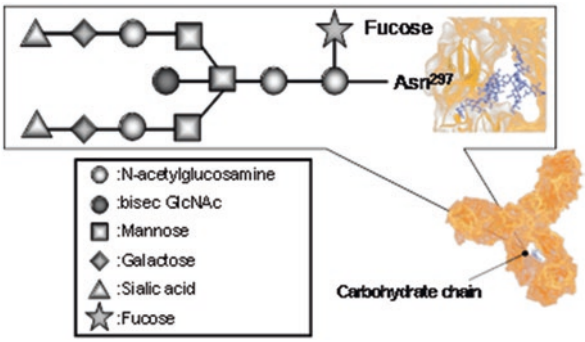

Fig. 8.3 The oligosaccharide structure of a therapeutic antibody: ADCC can be dramatically enhanced by removing fucose residues at the non-reducing terminal of the N-linked oligosaccharides

5. Future perspectives
   As of June 2017, 14 companies are licensed to use the fucosylation-controlled technology Potelligent® and the development of 22 therapeutic antibodies using the technology were clinically on going.
6. Problems to be solved
   Elucidation of the structures of antibody oligosaccharides exhibiting the strongest ADCC and development of therapeutic antibodies utilizing their structures has been progressed. However, the reason why antibody function is physiologically subjected to control by oligosaccharide structures remains to be determined (10 years).

8.4 Antibody-Drug Conjugates (ADCs)

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Keywords Antibody-drug conjugate, EPR (enhanced permeability and retention) effect, Drug-to-antibody ratio (DAR), Theranostics, Linker technology

1. Significance in the field of glycoscience and its current situation
   Antibody-drug conjugates (ADCs) are used to deliver cytotoxic agents to tumor sites. These conjugates, which leak from neovascular vessels via an enhanced permeability and retention (EPR) effect, bind to cell surface antigens and are thus internalized, releasing the cytotoxic agents site-specifically. Thus, ADCs expand the therapeutic window of cytotoxic agents. As the conjugation-site and number of drug-molecules affect the pharmacokinetics/pharmacodynamics and efficacy of ADCs, preparation of homogeneous ADCs is important (Fig. 8.4). Several examples of homogeneous ADCs prepared using an *N*-glycan in the Fc region as a conjugation-site using glycosyltransferases and glycosidase mutants with chemically modified glycans as donors have been reported. Such modifications can prevent the conjugation of drugs to variable regions of antibodies [15].
2. Impact on other fields of research
   ADC development spans various research disciplines including organic chemistry, which is involved in linker technology and bio-orthogonal reaction development, and structural biology, which is involved in ADC purification and analysis. Antigen identification, enzymology, new radioisotope generation, and development of diagnostic devices and therapeutic apparatuses could therefore lead to innovations in these fields.
3. Significance as fundamental research
   Investigations involving glycan-modified antibodies will likely contribute to improved understanding of the structural biology of glycans and their role in antibody function. Furthermore, this technology may also be applied to glycoprotein preparation. Identification of biomarkers, and an understanding of the relationship between diseases and glycan structure are required.
4. Possible application for industry and medicine
   ADCs are effective in treating relapsed and refractory cancers, and are expected to contribute to the next generation of antibody therapies. Two ADCs are currently clinically approved [16, 17], with more than 70 in the pipeline. The Ministry of Health, Labour and Welfare guided control of glycan structure is necessary. ADC preparation is in progress for a humanized glyco-optimize monoclonal antibody to a novel tumour-specific MUC1 glycopeptide epitope [18].

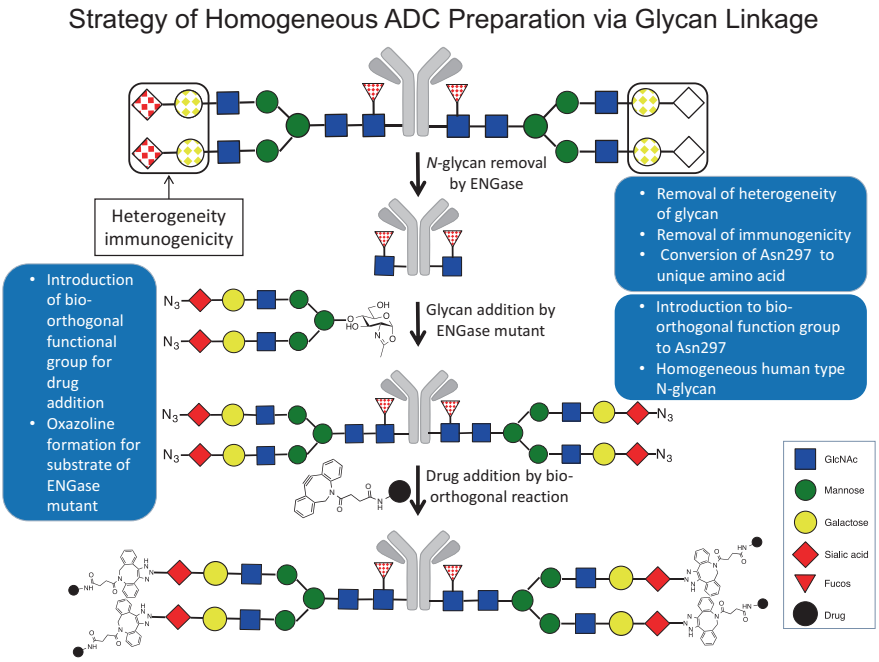

Fig. 8.4 Strategy of homogeneous ADC preparation via glycan linkage

5. Future perspectives
   An about 7 billion dollar market is expected in 2020. Although cancer therapies will become increasingly important in a rapidly-aging society, the application of ADCs will not be limited to cancer treatment. Targeting of antibiotic-resistant bacteria with an ADC containing an antibody against bacterial surface glycans has already been reported [19].
6. Problems to be solved
   Cost-reduction is essential. Highly efficient enzymes and glycan donors that reduce side-reactions during the glycan addition reaction step are required. Additionally, the current linker technology requires further development for improving target-specific drug release. Moreover, efficient protocols need to be developed for the preparation and analysis of glycan-modified antibodies and ADCs, and label-free imaging of the released drug (5 years).

8.5 Lectin-Drug Conjugates (LDCs)

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Keywords Lectin-drug conjugate (LDC), Glycan, Drug delivery, Haemagglutination

1. Significance in the field of glycoscience and its current situation
   Thus far, the potential of lectins as in vivo drug carriers has not been well tested, likely due to the common belief that all lectins mediate harmful haemagglutination [20]. However, their attractiveness is being highlighted by an accumulated evidence that several lectins are actually intravitally administrable safely [21–23].

   The recombinant lectin rBC2LCN has notable advantages, as follows [24]: (i) target glycans are located in the outermost coatings of cancer cells, known as the glycocalyx, conferring easy access for targeting drugs; (ii) cancer cells possess abundant glycan sites for interaction with rBC2LCN, including branched glycan chains attached to multiple different core proteins; (iii) the small size of a lectin should be beneficial for drug endocytosis; and (iv) glycolipids are also binding targets of rBC2LCN, and the very close proximity of glycolipids to the cell membrane should be favorable for drug endocytosis (Fig. 8.5).
2. Impact on other fields of research
   Although cell surfaces are covered by a glycan layer that is referred to as the glycocalyx, development of drugs that target glycan has not been well examined. The reason may due to the complexity of glycan structure and the immaturity of glycan analysis technologies, and thus the lack of exploration of appropriate target glycans. Since LDCs will play a crucial role in drug development in the near future, searches for specific glycan-lectin pairs in diseases will be attractive research fields.
3. Significance as fundamental research
   The cell surfaceome is an emerging research field that intends to comprehensively reveal the structural organization of cell surfaces. This approach is quite important from both the viewpoints of understanding cell-cell interactions and cell surface targeting surveys, and characteristic glycans should be the main components of the cell surfaceome. LDCs, that have various pharmacological effects, could be a key tool also in the basic scientific research field of cell surface function analysis.

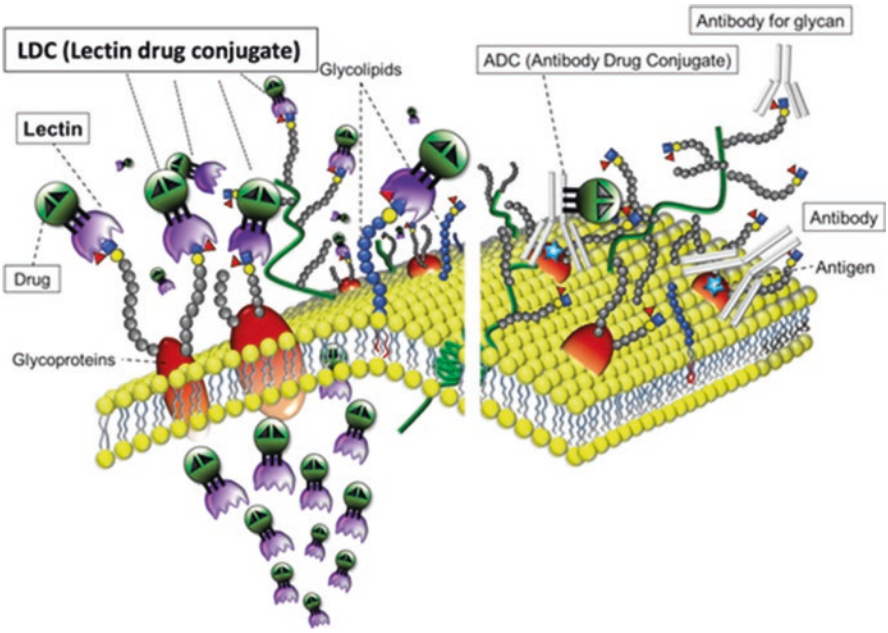

Fig. 8.5 (right) The previous failure of antibody-drug conjugates (ADC) strategies may be due to the one-to-one relationship between a cancer cell surface peptide antigen (blue star in brown glycoprotein) and an antibody, which results in the delivery of an insufficient amount of the drug to cancer cells. (left) In contrast, binding sites for rBC2LCN could consist of multiple glycans on various glycoproteins and glycolipids, resulting in the delivery of more drug to the surface of target cells. In addition, the relatively large size of immunoglobulins tends to prevent effective internalization of antibody-based drugs; however, the relatively small size of lectins would facilitate effective endocytosis and the prominent cytocidal effect of rBC2LCN (5 years)

4. Possible application for industry and medicine
   The development of targeting treatments has been largely based on the principal of antigen-antibody affinities, but the effect appears to be limited and its high cost will cause medico-economical difficulties. A new strategy that does not rely on antigen-antibody affinities is urgently desired. Lectins have several characteristic advantages against antibodies, and thus LDC could be attractive key components of a next generation strategy for drug development.
5. Future perspectives
   The rapid improvement in lectin engineering technologies will allow control over the harmful adverse effects of some lectins, and synthetic lectin technologies will permit the development of *de novo* lectins with ideal characteristics as drug carriers. The concept of utilizing lectins as drug carriers appears to be realistic with such lectin engineering technologies.
6. Problems to be solved
   The biggest concerns in intravital administration of LDCs are (1) haemagglutination toxicity and (2) immunogenic toxicity. Another problem to be overcome may be off target toxicity in non-target organs (5 years).

8.6 Polysaccharide-Based Drug Delivery System: A New Approach Involving Use of β-Glucan/DNA Complexes

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Keywords Polysaccharide/polynucleotide complexes, Dectin-1, Therapeutic nucleotide delivery, Immunocytes, Targeting

1. Significance in the field of glycoscience and its current situation
   Natural β-(1 → 3)-D-glucan and homo-polynucleotides such as poly(dA) form a stoichiometric complex [25, 26]. The complex can bind to an immunocyte receptor, Dectin-1, and is ingested [27, 28]. This complex provides a new strategy for specifically transporting therapeutic oligodeoxynucleotides (ODNs) including antisense-DNA, CpG-DNA, and siRNA to immunocytes [29] (Fig. 8.6).
2. Impact on other fields of research
   This complex is one of the most powerful immunocyte-targeting delivery tools [28, 29].
3. Significance as fundamental research
   The finding of this novel polysaccharide/polynucleotide complex may suggest that the complex may be involved in chemical evolution and provide a new method to construct supramolecular biologics.
4. Possible application for industry and medicine
   The application of this technology has already been initiated. Delivery of CpG by use of β-glucans is useful for developing low-dose and thus safer vaccines, and that of antisense or siRNA is good for controling immunocytes [25, 27–29].
5. Future perspectives
   Possibility to extend this DDS tool to antigen peptides, and low-molecular drugs, and protein delivery to immunocytes.
6. Problems to be solved
   The main target molecule is Decitn-1, but the binding mechanism and affinity for other β-glucan receptors remain unknown (10 years).

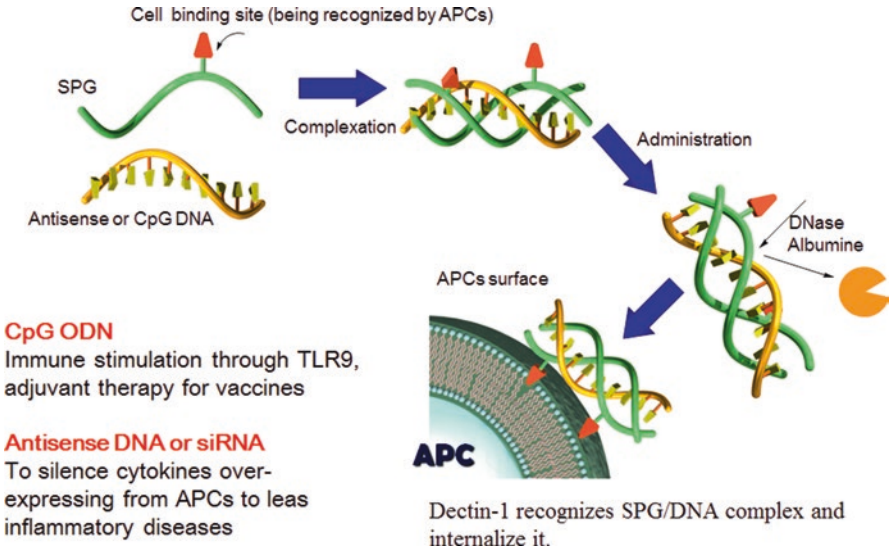

Fig. 8.6 Schematic illustration of therapeutic ODN delivery by use of the complex to immunocytes

8.7 Remodeling of Glycoantigens in Bio-Artificial Organs/Tissues

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Keywords Xenotransplantation, Bio-artificial organ/tissue, Glycoantigen, Remodeling

1. Significance in the field of glycoscience and its current situation
   Bio-artificial islet transplantation using microencapsulated or macroencapsulated islets attempts to cure diabetes of mellitus (DM) patients. However, the clinical results have not been positive so far. The cause of this is mainly their high antigenicity related to pig glycoantigens [30]. Regarding pig glycoantigens, the α-Gal, Hanganutziu-Deicher (H-D) (NeuGc), and some blood antigens have been identified.

   In addition, in spite of the fact that a newborn pig cell cluster (NPCC) is much easier to handle compared with adult pig islets (API) [31], it shows high antigenicity to humans, besides clearly expressing the α-Gal [32, 33]. Thus, gene-modified pigs such as α-Gal/H-D-knockout (KO) ones have already been produced [34] (Fig. 8.7). However, the double KO pigs still show clear antigenicity that can have a substantial effect on clinical results. Therefore, the discovery of other new glycoantigens or strategies for modifying the remaining antigens is an important issue [30, 34].
2. Impact on other fields of research
   Not only in pancreatic islet transplantation but also in other transplantation fields, gene-modified pigs that have less antigenicity to humans can be used as sources of organs for transplantation.
3. Significance as fundamental research
   The field of glycotechnology is focusing not only species specificity in humans and pigs but also the changes in antigenicity from NPCC to API. Therefore, investigation of the changes in glycosylation according to development may be helpful in regeneration studies.
4. Possible application for industry and medicine
   Bio-artificial islets can be directly used in therapy for type I and some type II DM patients as a medical tool. In addition, such tools including any kinds of bio-artificial organs/tissues, can be used not only for domestic patients but also worldwide.

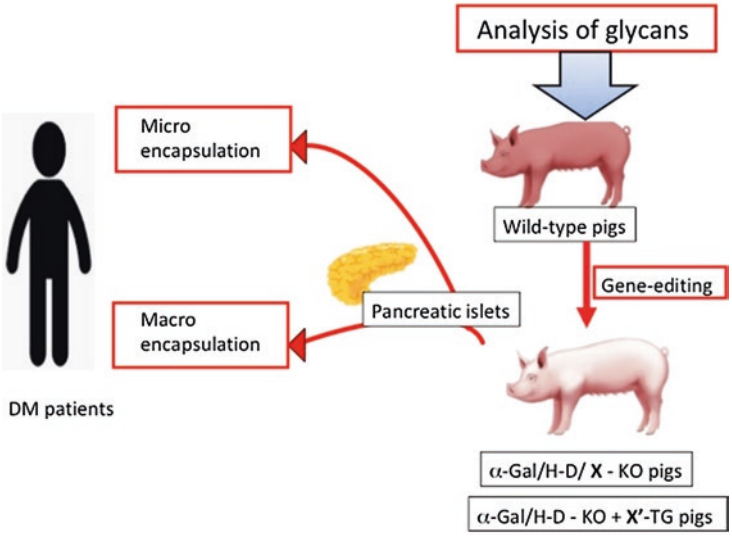

Fig. 8.7 Procedure for establishing bio-artificial islets. X indicates a gene for a new glycoantigen for knockout. X' indicates a gene that is overexpressed

5. Future perspectives
   Once these tools derived from gene-modified pigs become popular in therapy for DM patients, a significant number of patients who are now undergoing insulin therapy could be cured. In addition, bio-artificial kidneys, hearts and livers will become available for patients who have kidney failure, small children who face heart failure and subjects with fulminant hepatitis, respectively.
6. Problems to be solved
   The first challenge is to identify other pig glycoantigens to humans, in addition to the α-Gal and H-D antigens. The second is to develop other methods that reduce the residual antigenicity to humans after double knockout. This would include its remodeling (5 years).

8.8 Therapeutic Potential of Carbohydrate Mimetic Peptides Composed of D-Type Amino Acids

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Keywords Peptide display phage library, Selectin, Annexin A1, Tumor vasculature, Angiogenesis

1. Significance in the field of glycoscience and its current situation
   While carbohydrates play numerous roles in the human body, few carbohydrate-based therapeutics have been proposed, in part because complex carbohydrates with specific function are difficult to synthesize. In this regard, we have taken advantage of peptide display phage technology to identify carbohydrate mimetic peptides [35, 36]. This technology also provides phage clones, which are equivalent to amplifiable carbohydrates. Furthermore, protease-resistant D-type peptides are stable in vivo and reportedly non-immunogenic, minimizing potential toxicity if applied as therapies (Fig. 8.8). Carbohydrate-mimetic peptides have been successfully identified by screening peptide-display phage libraries with anti-carbohydrate antibodies. D-type carbohydrate mimetics can be designed either by generating a retro-inverso peptide [37] or by mirror-image phage library screening [38]. One example of a carbohydrate-mimetic therapeutic is the peptide IF7, which functions as a highly efficient drug delivery vehicle to malignant tumors through binding to annexin 1, the most specific tumor vasculature surface marker to date [39].
2. Impact on the other fields of research
   Peptide display phage technology is superior to other screening systems in terms of the diversity, as it comprises more than one billion random peptide patterns. This technology is applicable to numerous diseases associated alterations in carbohydrate and/or carbohydrate-binding proteins.
3. Significance as the fundamental research
   In vivo, most proteins are modified with carbohydrates, and that modification can significantly alter protein function and disease onset. Carbohydrate-mimetic peptides can also be used as reagents for basic biological research, as exemplified by identification of annexin 1 as a carbohydrate-binding protein [2].
4. Possible application for industry and medicine, if any
   A D-type version of IF7 is easily synthesized, can be modified chemically, and is well-suited for development as a therapeutic to treat malignant tumors.

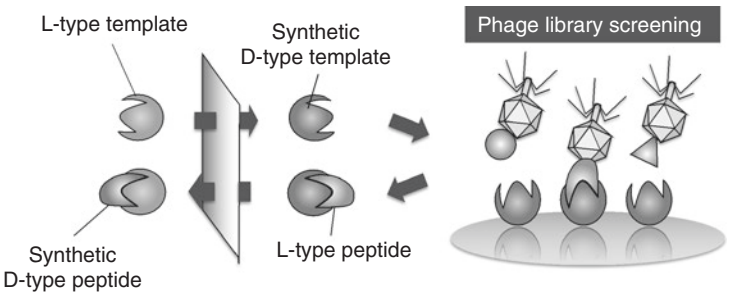

Fig. 8.8 Schematic presentation of mirror-image peptide displaying phage library screening

5. Future perspectives
   Phage display is an emerging approach useful for combinatorial drug discovery. Carbohydrate mimetic peptides may be developed to recognize factors previously difficult to target using more conventional methods. Despite this utility, application of this technology to glycobiology is in early stages, and many potentials remain untapped. Use of carbohydrate mimetic peptides including D-type peptides could lead to unprecedented drug discovery opportunities relevant to numerous human diseases.
6. Problems to be solved
   The therapeutic activity of peptide-based therapeutics depends in part on peptide solubility. In addition, formulation of therapeutics to minimize toxicity and optimize drug stability should be determined.

Box 8.1: Erythropoietin

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Erythropoietin (EPO) is a hematopoietic hormone produced mainly in kidney. Its molecular mass is 34,000 albeit comprising only 165 amino acids, with almost half size attributed to glycans. Natural EPO contains 3 sites for *N*-glycosylation and 1 for *O*-glycosylation. Glycosylation is necessary for the maintenance of EPO structure, while in vitro it acts in a rather suppressive manner for the binding to its receptor. On the other hand, in vivo, glycosylation plays a important role in stability in blood, largely dependent on the number of terminal Sia residues which is due to branching features. EPO from kidney is supposed to express α2–6 Sia in N-glycans, while those produced as recombinant glycoproteins in CHO cells are exclusively of α2–3 form. EPO often becomes a hot topic as a matter of doping for athletes involved in long-distance competition. If discriminating precisely the glycoforms difference, it becomes a useful method to inspect doping, but this has not been realized yet, because of difficulty to detect an extremely low concentration of the recombinant hormone in blood and urine if any. Several engineered EPOs are also developed, which acquired improved blood stability by introducing either methoxy-polyethyleneglycol chemically or additional N-glycosylation sites by amino acid subsitution, which are called bio-better.

Box 8.2: Understanding the Molecular Basis that Governs Glycan Pattern Recognition of Glycoclusters and Applying to Drug Delivery System

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On the surface of most cells is a carbohydrate-enriched coating known as the glycocalyx. Due to the wide variance in glycoprotein/glycolipid/proteoglycan assemblies among differing cell types, the glycocalyx is known to play crucial roles for cell-to-cell interactions, differentiation, intracellular trafficking, and immune modulation. One key component that facilitates these interactions is lectins, a class of highly specific carbohydrate-binding proteins. Individually, lectin-glycan interactions are poor (millimolar K_D level), such that their one-to-one interactions have little biological selectivity. However, due to the enormous presence of lectin isoforms, the combined interactions of clustered (two or more) sugars allow for strong and selective cell binding in nature; the phenomena referred as a "glycan pattern recognition". Exploiting the glycocalyx as an alternative path for disease-targeting, e.g., cancer, has been the research focus for many groups, whom have synthesized and/or tested various neoglycoconjugates (synthetic glycan-linked biomolecules) with templates based on dendrimers, nanoparticles, liposomes, etc. In general, these studies broadly fall into two categories with different focuses. One field of study prioritizes the comprehensive *in vitro* screening of different glycan assemblies, for example using microarrays. Another field of study prioritizes biological cell- and animal-based assays. Combining these two approaches is important to further our understanding of the molecular basis that governs *in vivo* glycan pattern recognition, as well as to applying the glycoclusters to next generation of drug delivery system (DDS).

Box 8.3: Anti-diabetes Drug

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α-Glycosidase inhibitors are a class of antidiabetic drugs. They inhibit α-glycosidases in small intestinal mucosal epithelial cells, and prevent glucose absorption. Structurally, α-glycosidase inhibitors are aza-sugars or transition state-mimic compounds.

Recently, several sodium glucose cotransporter 2 (SGLT 2) inhibitors have been developed as antidiabetic drugs. The drugs are egests glucose through suppressing the re-absorption of glucose in the proximal renal tubule by SGLT 2 inhibition. Phlorhizin, a natural product found in the bark of apple and pear plants, was used as a lead compound in the current study. The *O*-glycoside structure of phlorhizin was converted to a *C*-aryl glucoside skeleton to avoid degradation by glycosidases, thereby increasing the *in vivo* stability of the compound.

References

References for Section 8.1

1. Kurogochi M et al (2015) Glycoengineered monoclonal antibodies with homogeneous glycan (M3, G0, G2, and A2) using a chemoenzymatic approach have different affinities for FcγRIIIa and variable antibody-dependent cellular cytotoxicity activities. PLoS One 10:e0132848
2. Yamamoto K et al (1998) Chemoenzymatic synthesis of a novel glycopeptide using a microbial endoglycosidase. Carbohydr Res 305:415–422
3. Wang L-X, Lomino JV (2012) Emerging technologies for making glycan-defined glycoproteins. ACS Chem Biol 7:110–122
4. Murakami M et al (2016) Chemical synthesis of erythropoietin glycoforms for insights into the relationship between glycosylation pattern and bioactivity. Sci Adv 2:e1500678

References for Section 8.2

5. Wang LX (2011) The amazing transglycosylation activity of endo-beta-N-acetylglucosaminidases. Trends Glycosci Glycotechnol 23:33–52
6. Parsons TB et al (2016) Optimal synthetic glycosylation of a therapeutic antibody. Angew Chem Int Ed 55:2361–2367
7. Huang W et al (2012) Chemoenzymatic glycoengineering of intact IgG antibodies for gain of functions. J Am Chem Soc 134:12308–12318
8. Katoh T et al (2016) Generation of a mutant *Mucor hiemalis* endoglycosidase that acts on core-fucosylated N-glycans. J Biol Chem 291:23305–23317

9. Yamamoto K (2013) Recent advances in glycotechnology for glycoconjugate synthesis using microbial endoglycosidases. Biotechnol Lett 35:1733–1743

References for Section 8.3

10. Shinkawa T et al (2003) Absence of fucose but not presence of galactose or bisecting *N*-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466–3473
11. Yamane-Ohnuki N et al (2004) Establishment of FUT8 knockout chinese hamster ovary cells: an ideal host cell line for producing completely defucosylated antibodies with enhanced antibody-dependent cellular cytotoxicity. Biotechnol Bioeng 87:614–622
12. Ishii T et al (2010) Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. Clin Cancer Res 16:1520–1531
13. Yamada T et al (2016) Comparison of biological activities of human antithrombins with high-mannose or complex-type nonfucosylated *N*-linked oligosaccharides. Glycobiology 26:482–492
14. Kanda Y et al (2007) Comparison of biological activity among non-fucosylated therapeutic IgG1 antibodies with three different *N*-linked fc oligosaccharides: the high-mannose, hybrid, and complex types. Glycobiology 17:104–118

References for Section 8.4

15. Parsons TB et al (2016) Optimal synthetic glycosylation of a therapeutic antibody. Angew Chem Int Ed 55:2361–2367
16. Wu AM, Senter PD (2005) Arming antibodies: prospects and challenges for immunoconjugates. Nat Biotechnol 23:1137–1146
17. Verma S et al (2012) Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 367:1783–1791
18. Fiedler W et al (2016) A phase I study of PankoMab-GEX, a humanized glyco-optimized monoclonal antibody to a novel tumour-specific MUC1 glycopeptide epitope in patients with advanced carcinomas. Eur J Cancer 63:55–63
19. Lehar SM et al (2015) Novel antibody-antibiotic conjugate eliminates intracellular *S. aureus*. Nature 527:323–328

References for Section 8.5

20. Bies C et al (2004) Lectin-mediated drug targeting: history and applications. Adv Drug Deliv Rev 56:425–435
21. Della Giovampaola C et al (2017) Formulation of liposomes functionalized with Lotus lectin and effective in targeting highly proliferative cells. Biochim Biophys Acta 1861:860–870
22. de Oliveira Figueiroa E et al (2017) Lectin-carbohydrate interactions: implications for the development of new anticancer agents. Curr Med Chem 24:3667–3680
23. Ikemoto K et al (2016) Bauhinia purprea agglutinin-modified liposomes for human prostate cancer treatment. Cancer Sci 107:53–59
24. Shimomura T et al (2017) A novel therapeutic strategy for pancreatic cancer targeting cell surface glycan using rBC2LC-N lectin. Mol Cancer Therap 17:183–195

References for Section 8.6

25. Sakurai K, Shinkai S (2000) Molecular recognition of adenine, cytosine, and uracil in a single-stranded RNA by a natural polysaccharide: Schizophyllan. J Am Chem Soc 122:4520–4521
26. Mizu M et al (2004) A polysaccharide carrier for immunostimulatory CpG DNAs to enhance cytokine secretion. J Am Chem Soc 126:8372–8373
27. Mochizuki S, Sakurai K (2011) Dectin-1 targeting delivery of TNF-a antisense ODNs complexed with beta-1,3-glucan protects mice from LPS-induced hepatitis. J Control Release 151:155–161
28. Kobiyama K et al (2014) Nonagonistic dectin-1 ligand transforms CpG into a multitask nanoparticulate TLR9 agonist. Proc Natl Acad Sci U S A 111:3086–3091
29. Sanada Y et al (2012) β-1,3-d-Glucan schizophyllan/poly(dA) triple-helical complex in dilute solution. J Phys Chem B 116:87–94

References for Section 8.7

30. Miyagawa S et al (2001) Remodeling of the major pig xenoantigen by N-acetylglucosaminyltransferase III in transgenic pig. J Biol Chem 276:39310–39319
31. Miyagawa S et al (2015) Generation of α1,3-galactosyltransferase and cytidine monophospho-N-acetylneuraminic acid hydroxylase gene double-knockout pigs. J Reprod Dev 61:449–457
32. Komoda H et al (2004) A study of the xenoantigenicity of adult pig islets cells. Xenotransplantation 11:237–246
33. Miyagawa S et al (2010) Survey of glycoantigens in cells from alpha1-3galactosyltransferase knockout pig using a lectin microarray. Xenotransplantation 17:61–70
34. Miyagawa S et al (2014) A comparison of the main structures of N-glycans of porcine islets with those from humans. Glycobiology 24:125–138

References for Section 8.8

35. Fukuda MN (2012) Peptide-displaying phage technology in glycobiology. Glycobiology 22:318–325
36. Hatakeyama S et al (2011) Targeted drug delivery to tumor vasculature by a carbohydrate mimetic peptide. Proc Natl Acad Sci U S A 108:19587–19592
37. Chen X, Fan Z, Chen Y, Fang X, Sha X (2013) Retro-inverso carbohydrate mimetic peptides with annexin1-binding selectivity, are stable in vivo, and target tumor vasculature. PLoS One 8:e80390
38. Funke SA, Willbold D (2009) Mirror image phage display–a method to generate D-peptide ligands for use in diagnostic or therapeutical applications. Mol BioSyst 5:783–786
39. Oh P et al (2004) Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. Nature 429:629–635

Chapter 9
Glycomimetics

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9.1 Selectin Inhibitors

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Keywords Glycomimetics, Selectin, Sickle cell anemia, Vaso-occlusive crisis

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1. Significance in the field of glycoscience and its current situation
   Glycomimetics are drugs that mimic the structures of glycans. Among such drugs, development of inhibitors of selectins that specifically block cell rolling along blood vessels is preceding. A pan-selectin inhibitor that blocks all three selectins (Fig. 9.1) is now being used in a phase III trial for vaso-occlusive crisis associated with sickle cell anemia [1, 2]. An E-selectin-selective inhibitor [3], an E-selectin and chemokine receptor CXCR4 dual inhibitor, and a galactin-3 and galectin-9 inhibitor are now in the process of development.
2. Impact on other fields of research
   Approaches to block glycan-lectin interactions by glycomimetics will have a great impact on both life science and disease treatment because of their applicability to a wide variety of lectins involved in various phenomena in life and diseases.
3. Significance as fundamental research
   Glycomimetics should be useful for the determination of the significance of glycan-carbohydrate interactions in life.
4. Possible application for industry and medicine
   Selectin inhibitors that are being developed will be useful for the treatment of vaso-occlusive crisis associated with sickle cell anemia, acute myeloid leukemia and multiple myeloma.
5. Future perspectives
   It is highly expected that successful glycomimetic development of selectin inhibitors will lead to the development of new glycomimetics targeting various lectins. For example, the abovementioned glycomimetics that inhibit galectins will have the potential to block tumor evasion from immunity.
6. Problems to be solved
   Elongation of the serum half-life of glycomimetics is expected (5 years).

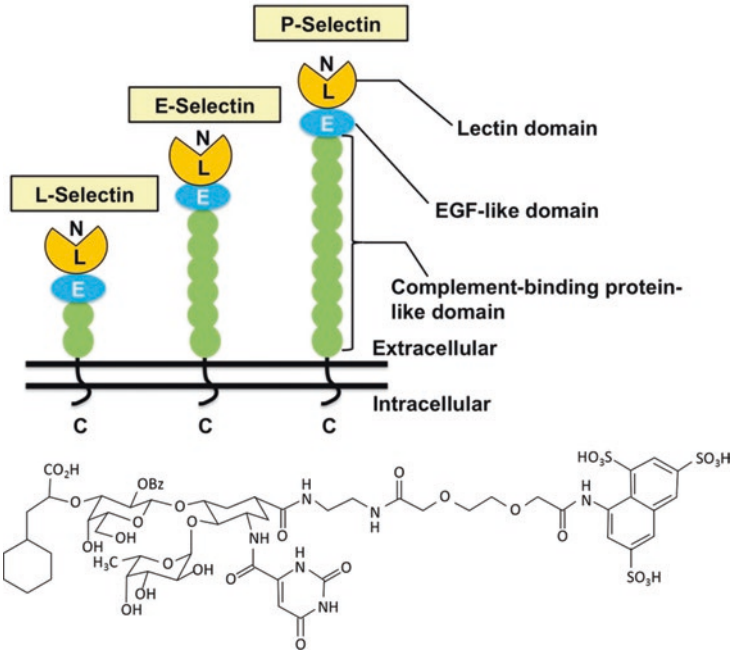

Fig. 9.1 Protein structures of selectins (above) and chemical structure of a pan-selectin inhibitor (below)

9.2 Development of Siglec Regulators

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Keywords Siglecs, autoimmune diseases, inflammatory diseases, sialic acid, drug discovery

1. Significance in the field of glycoscience and its current situation
   Sialic acid-binding immunoglobulin-like lectins (Siglecs) number around 10 in both mice and humans, and many of them have a signaling function. Siglecs are expressed in various cell types especially immune cells, and each member is expressed in a specific cell type [4]. Thus, development of chemical compounds that regulate Siglecs will facilitate development of novel drugs for immune regulation (Fig. 9.2). The molecular structure of Siglecs does not include a pocket suitable for accommodating chemical compounds. Nonetheless, synthetic sialosides that bind to CD22 (also known as Siglec-2) have been synthesized [5–7], indicating that development of chemical compounds that regulate Siglecs is feasible.
2. Impact on other fields of research
   Because Siglecs do not contain a molecular pocket suitable for accommodating chemical compounds, successful development of chemical compounds that regulate Siglecs would alter the processes of drug discovery, and considerably expand the number of drug targets [8].
3. Significance as fundamental research
   Membrane-bound lectins including Siglecs are known to interact with glycan ligands expressed on the same cell (cis-ligands). The sialosides that block binding of membrane-bound lectins to the cis-ligands will be useful for elucidating the roles of the cis-ligands in cellular functions.
4. Possible application for industry and medicine
   Because Siglecs have signaling functions and are expressed in various immune cell types, chemical compounds that regulate Siglecs will facilitate the development of novel drugs for autoimmune diseases and inflammatory diseases.
5. Future perspectives
   Development of chemical compounds that regulate various Siglecs will facilitate elucidation of the function of Siglecs and their glycan ligands, and development of novel drugs for autoimmune and inflammatory diseases.
6. Problems to be solved
   Development of Siglecs regulators that can be easily synthesized, and exhibit both high activity and good pharmacokinetics is required (5 years).

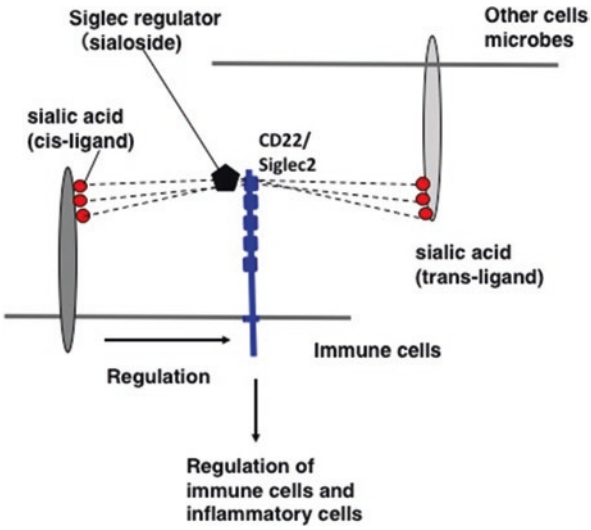

Fig. 9.2 Siglec regulators such as Siglec-binding sialosides regulate immune cells and inflammatory cells through mechanisms including inhibition of the binding of Siglecs to their glycan ligands

9.3 Galectin Inhibitors

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Keywords Galectin, ligand, inhibitor, drug design

1. Significance in the field of glycoscience and its current situation
   Galectins are involved in a variety of biological phenomena and 15 galectins are known in the mammalian system [9]. Although the basic recognition unit for galectins is β-galactosides, the sugar binding specificity differs among galectins [10]. Galectins have been targeted for drug design [11], and some inhibitors have entered clinical trial phases. While some galectin inhibitors are designed to have more specific and strong affinity for the sugar-binding sites of galectins [12] (Fig. 9.3), there are inhibitors that have allosteric effects that do not bind to sugar-binding sites [13].
2. Impact on other fields of research
   Since galectins are involved in a variety of biological phenomena, analysis of their functions using specific galectin inhibitors could lead to the discovery of novel biological processes that are not known yet for galectin involvement.
3. Significance as fundamental research
   Since galectins are involved in a variety of biological phenomena and have functions in many tissues and cells, analysis of their functions using specific galectin inhibitors could lead to more detailed knowledge of galectin functions in these processes.
4. Possible application for industry and medicine
   Developing a highly specific inhibitor for each member of the galectin family should lead to the development of new drugs that interact with galectins in various cells and tissues.
5. Future perspectives
   Highly specific galectin inhibitors should contribute to analysis of the role and function of each galectin in more detail. Furthermore, since galectins are known to be involved in a variety of biological functions, specific galectin inhibitors could become new drugs with new mechanisms that are different from existing drugs.
6. Problems to be solved
   For galectin-1 and galectin-3, some inhibitors have been developed as possible drugs (for example, galectin-3 inhibitor for lung or liver fibriosis in clinical studies), however, highly specific inhibitors for other galectins are also needed. Furthermore, since galectins function in a variety of cells and tissues, designing of these inhibitors to be properly adsorbed by or distributed to the desired tissues or cells after administration will also be very important (10 years).

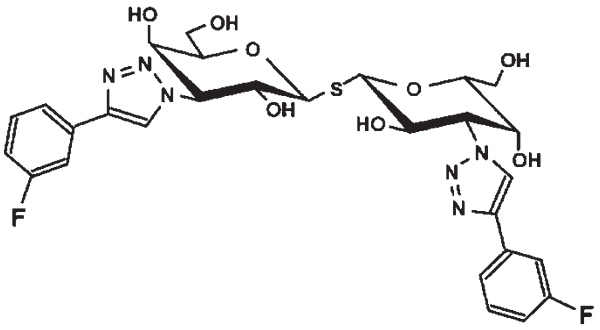

Fig. 9.3 Structure of galectin-3 inhibitor TD139, undergoing a clinical trial in the U. K. for inhaled therapy for idiopathic pulmonary fibriosis (IPF) patients. Modification of the C3 position of the galactose moiety of thiodigalactoside (TDG) with an aromatic substituent through a triazole linkage resulted in a galectin-3 inhibitor with increased binding affinity

9.4 Inhibitors of Glycolsyltransferases

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Keywords Glycosyltransferases, Glucosylceramide (GlcCer) synthase, Inhibitor, Gaucher's disease, Substrate reduction therapy (SRT)

1. Significance in the field of glycoscience and its current situation
   Development of specific inhibitors of glycolsyltransferases is expected to be very useful not only for studies on the physiclogical significance of glycoconjugates but also the treatment of patients showing excessive accumulation of specific glycolconjugates. For example, for Gaucher's disease caused by great accumulation of glucosylceramide (GlcCer) due to a genetic defect of glucocerebrosidase. Eliglustat, which is a specific inhibitor of GlcCer synthase, has been authorized for the treatment of Gaucher's disease as a substrate reduction therapy (SRT) [14, 15] (Fig. 9.4).
2. Impact on other fields of research
   Ganglioside GM3 has been suggested to be involved in the pathogenesis of various metabolic diseases caused by chronic inflammation [16]. Inhibitors of GlcCer synthase have been proved to have a curative effect on diabetic animal models suggesting another possible clinical application [17].
3. Significance as fundamental research
   Development of highly specific inhibitors of glycolsyltransferases is highly expected to provide a practical strategy for elucidation of the physiological roles of glycoconjugates.
4. Possible application for industry and medicine
   Development of specific inhibitors of glycolsyltransferases is expected to be very useful not only for studies on the physiological significance of glycoconjugates but also the treatment of patients showing excessive accumulation of specific glycolconjugates.
5. Future perspectives
   When specific inhibitors of glycolsyltransferases are developed, they are expected to be useful for the treatment of various diseases including diabetes, cancer and infectious diseases.
6. Problems to be solved
   So far, except for GlcCer synthase inhibitors [16, 17], specific glycolsyltransferase inhibitors functioning efficiently at the cellular level have not yet been developed (5 years) (Boxes 9.1 and 9.2).

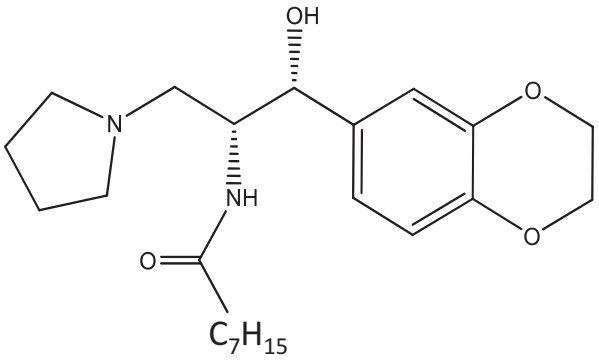

Fig. 9.4 N-[(1R,2R)-1-(2,3-Dihydro-1,4-benzodioxin-6-yl)-1-hydroxy-3-(1-pyrrolidinyl)-2-propanyl]octanamide

Box 9.1: High Throughput Screening Targeting Glycosyltransferase

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Because many glycosyltransferases were found to be drug targets for various diseases, including Alzheimer's disease and cancer, it is desirable to develop inhibitors/activators of them. Currently, however, typical methods for glycosyltransferase assaying involve an RI- or fluorescence-labeled substrate and require a complex detection system such as HPLC, which are not suitable for large-scale screening. So far, two types of assays have been developed for application to high-throughput screening, both of which detect nucleotides (UDP, etc.), byproducts of the glycosylation reaction. For example, a phosphate is enzymatically released from UDP, or UDP is converted to ATP. These compounds can be detected by measuring absorbance or chemi-luminescence in a plate-based format, which enables large-scale screening for glycosyltransferase inhibitors/activators.

Box 9.2: Heparinoid

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Heparin is a member of the glycosaminoglycan family that consists of a variably sulfated repeating disaccharide unit comprising glucuronic acid/iduronic acid and glucosamine. Heparin has anticoagulant activity, which is exerted by binding to antithrombin III (AT), thereby altering the conformation of AT and its inhibitory action on thrombin or activated factor X.

Unfractionated heparin (molecular weight 5,000-20,000 Da) is isolated from porcine small intestine, and has been shown to be clinically problematic. For example, contamination of oversulfated chondroitin sulfate (OSCS) causes death. Additionally, heparin-induced thrombocytopenia and a desire for Halal compliance further limit the use of unfractionated heparin.

In an effort to ameliorate these issues, use of fractionated heparin (approximately 5,000 Da) and synthetic low molecular weight heparin (1726.77 Da) has increased. Fractionated heparin and synthetic low molecular weight heparin have longer half-lives in blood and fewer bleeding side effects than unfractionated heparin does. The anti-factor Xa/thrombin ratio increases as the molecular weight of heparin decreases.

References

References for Section 9.1

1. Wun T et al (2014) Phase 1 study of the E-selectin inhibitor GMI 1070 in patients with sickle cell anemia. PLoS One 9: e10130
2. Telen MJ et al (2015) Randomized phase 2 study of GMI-1070 in SCD: reduction in time to resolution of vaso-occlusive events and decreased opioid use. Blood 125:2656–2664
3. Dutta P et al (2016) E-selectin inhibition mitigates splenic HSC activation and myelopoiesis in hypercholesterolemic mice with myocardial infarction. Arterioscler Thromb Vasc Biol. 36:1802–1808

References for Section 9.2

4. Macauley MS et al (2014) Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol 14:653–666
5. Abdu-Allah HH et al (2011) CD22-antagonists with nanomolar potency: the synergistic effect of hydrophobic groups at C-2 and C-9 of sialic acid scaffold. Bioorg Med Chem 19:1966–1971
6. Matsubara N et al (2018) CD22-binding synthetic sialosides regulate B lymphocyte proliferation through CD22 ligand-dependent and independent pathways, and enhance antibody production in mice. Front Immunol 9:820
7. Bull C et al (2016) Sialic acid mimetics to target the sialic acid-siglec axis. Trends Biochem Sci 41:519–531
8. Abi Hussein H et al (2017) Global vision of druggability issues: applications and perspectives. Drug Discov Today 22:404–415

References for Section 9.3

9. Barondes SH et al (1994) Galectins: A family of animal β-galactoside-binding lectins. Cell 76:597–598
10. Hirabayashi J et al (2002) Oligosaccharide specificity of galectins: a search by frontal affinity chromatography. Biochim Biophys Acta 1572:232–254
11. Klyosov AA, Traber PG (2012) Galectins in disease and potential therapeutic approaches. In: Klyosov AA, Traber PG (eds) Galectins and disease implications for targeted therapeutics. American Chemical Society, vol 1115. American Chemical Society, Washington, DC, pp 3–43
12. Sörme P et al (2005) Structural and thermodynamic studies on cation-Pi interactions in lectin-ligand complexes: high-affinity galectin-3 inhibitors through fine-tuning of an arginine-arene interaction. J Am Chem Soc 127:1737–1743
13. Dings RPM et al (2012) Antitumor agent calixarene 0118 targets human galectin-1 as an allosteric inhibitor of carbohydrate binding. J Med Chem 55:5121–5129

References for Section 9.4

14. Shayman JA (2010) ELIGLUSTAT TARTRATE: Glucosylceramide synthase inhibitor treatment of type 1 Gaucher disease. Drugs Future 35:613–620
15. Cox TM et al (2015) Eliglustat compared with imiglucerase in patients with Gaucher's disease type 1 stabilised on enzyme replacement therapy: a phase 3, randomised, open-label, non-inferiority trial. Lancet 385:2355–2362
16. Nagafuku M et al (2015) Control of homeostatic and pathogenic balance in adipose tissue by ganglioside GM3. Glycobiology 25:303–318
17. Zhao H et al (2007) Inhibiting glycosphingolipid synthesis improves glycemic control and insulin sensitivity in animal models of type 2 diabetes. Diabetes 56:1210–1218

Chapter 10
Glycan Vaccine

Tsukasa Seya, Sho Yamasaki, and Koichi Fukase

10.1 Microbial Glycans and Immune-Response

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Keywords Toll-like receptor, Inflammation, Immune system, Adjuvant

1. Significance in the field of glycoscience and its current situation
   Glycosylated material of microbacteria induces an immune response through recognition of its unique patterns by the human innate immune system [1, 2]. Dendritic cells respond to cross-present antigens if the antigens occur with the glycosylation. Acquired immune responses, including antibody production and T cell proliferation, occur in an antigen-specific manner irrespective of the ligand species in innate immunity [2]. However, the mechanism by which sugars accelerate class-switching and cross-priming of T cells molecularly remains undetermined. The signals of the innate receptors converge on the activation of transcription factors, NF-kB, AP-1 and IRF3 (Fig. 10.1). The signal pathway appears to develop only in vertebrates including humans [3, 4]. Important sugar structures for host defense need to be addressed.

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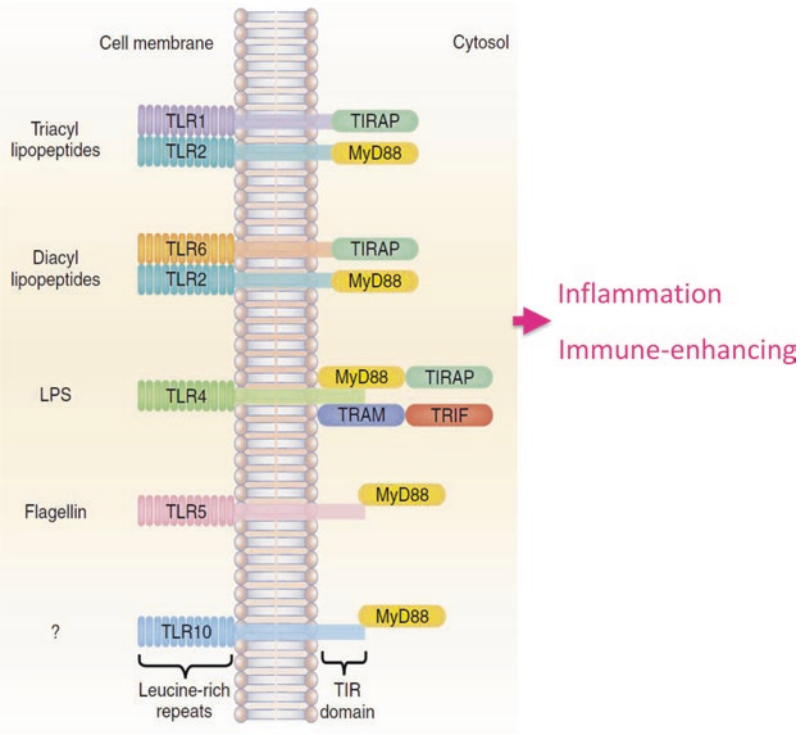

Fig. 10.1 TLR family their ligands for glycolipids. Fllagelin is a protein. The ligand for TLR10 is unknown [3]

2. Impact on other fields of research
   In the fields of vaccines and tumor immunology, peptidoglycans and lipopeptides are regarded as immune-enhancing adjuvants with possible clinical use.
3. Significance as fundamental research
   Structural analysis of interactions between adjuvants and receptors by means of crystal studies will enable us to test molecular design for drug screening.
4. Possible application for industry and medicine
   The goal for the design of immune-enhancing glycans is clinical use for patients with cancer and infections. The elderly usually have inflammatory bases, which promote lifestyle diseases. Immune enhancer with less inflammation would be applicable to elderly patients with lifestyle diseases.
5. Future perspectives
   Immune-enhancing adjuvants are essentially required for antitumor peptide vaccines. Cytokinemia is a major side effect of adjuvant, which will be improved by use of non-inflammatory adjuvant.
6. Problems to be solved
   So far, most adjuvants induce inflammation, which is a main side effect. Inflammation is separable from immune-enhancing. Side effects should be overcome before clinical use (5 years).

10.2 Pathogen-Derived Glycolipids

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Keywords Glycolipids, Polysaccharides, PAMPs (pathogen-associated molecular patterns), PRR (pattern recognition receptors)

1. Significance in the field of glycoscience and its current situation
   Rational design of adjuvants and delivery systems will promote development of next-generation vaccines to control emerging and re-emerging diseases. To accomplish this purpose, understanding the immune enhancing properties of new adjuvants relative to those induced by natural infections will facilitate the development of pathogens mimicking materials that will effectively initiate innate immune signaling cascades (Fig. 10.2).
   Pathogens possess various glycans and glycolipids that have potent immunostimulatory activities. However, the molecular mechanisms behind this regulation are not fully determined.
2. Impact on other fields of research
   Provide valuable information toward vaccine development against infections disease or cancer.
3. Significance as fundamental research
   Precise molecular mechanisms of immunity against pathogens will be clarified.
4. Possible application for industry and medicine
   Application to vaccine development.
5. Future perspectives
   Rational design of multiple adjuvants and delivery systems will promote development of next-generation vaccines to control emerging and re-emerging diseases.
6. Problems to be solved
   To dissect beneficial and adverse effects induced by adjuvants (5 years).

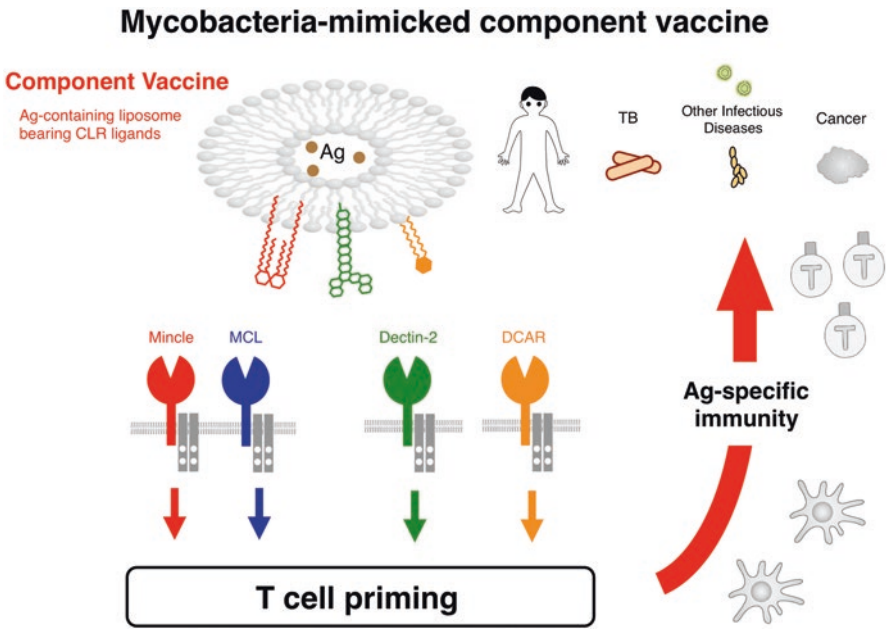

Fig. 10.2 Development of novel adjuvants by decorating the surfaces of particles with immunostimulatory glycolipids to confer "pathogen-like" properties and enhance adjuvanticity

10.3 Carbohydrate-Based Vaccines

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Keywords Carbohydrate antigen, Vaccine, Adjuvant, Tumor-associated carbohydrate antigen, Self-adjuvanting vaccine

1. Significance in the field of glycoscience and its current situation
   Vaccines are the most inexpensive and effective drugs for the prevention of infectious diseases and pandemics caused by pathogens such as bacteria and viruses. The effectiveness of vaccines against various infectious diseases has been historically demonstrated. Currently, vaccines are used for 26 infectious diseases (see WHO website [8]).
   Live, attenuated vaccines can elicit strong cellular and antibody responses. Inactivated vaccines, produced by killing the pathogens, are more stable and safer but less effective than live vaccines. These vaccines contain various antigens and adjuvants that effectively trigger immunity to produce antibodies. However, side reactions such as infection by the vaccine strain, induction of inflammation and autoimmunity are serious issues.
   Improvement of safety has been an essential task for vaccine development, since many vaccines are prophylactic agents. Therefore, various vaccines such as purified antigen vaccines, component vaccines (subunit vaccines), recombinant vaccines, and toxoids have been developed to improve safety and quality control. Recently, chemically synthesized vaccines that involve minimal antigenic epitopes have been extensively studied to develop safer vaccines, since they can trigger the desired and specific immune responses. In addition, a chemical process can eliminate contamination by pathogens and other antigens, and the purification of products easily affords highly purified homogeneous antigens to enable easier quality control. Hence, a GMP-compliant manufacturing process can be more readily established.
   Carbohydrate antigens (glycan antigens) derived from pathogens such as bacteria and protozoa are candidates for promising vaccines. Since the antigenicity of low molecular weight carbohydrate antigens is generally weak, conjugated vaccines composed of carbohydrate antigens and carrier proteins with an adjuvant have been put to practical use in order to induce an effective immune response to carbohydrate antigens [9].
   Tumor-associated carbohydrate antigens (TACAs) have been actively investigated for cancer active immunotherapy (cancer vaccine) [9]. However, TACA-carrier proteinconjugated vaccines have not been approved for practical use yet. For example, treatment of metastatic breast cancer with Theratope vaccine (STn keyhole-limpet hemocyanin (KLH) conjugate) failed in a Phase III trial. A self-adjuvanting strategy involving the use of chemically synthesized conjugated

vaccines, in which antigens and adjuvants are combined, is promising in order to overcome the low immunogenicity of TACAs and to induce effective immune responses against TACAs (Fig. 10.3).
Technology for the synthesis of self-adjuvanting vaccines has already been realized, and its effectiveness and safety are being studied.

2. Impact on other fields of research
Pipeline vaccines are currently under development for 24 infectious diseases (WHO). On the other hand, many studies are underway to develop effective cancer vaccines. So far, Provenge® consisting of autologous dendritic cells, primed with a recombinant fusion protein of prostatic acid phosphatase (PAP) and a granulocyte-macrophage colony-stimulating factor as an antigen, has been developed for immunotherapy against prostate cancer. Vaccines against protein aggregation diseases such as Alzheimer's disease have also been investigated. Development of effective vaccines against these diseases will tremendously contribute to the medical treatment and health of modern society. Infectious diseases in the tropics are impediments to the social development of developing countries. Developing vaccines against these diseases is a major challenge for modern society. Vaccines against malaria, leishmaniasis, Chagas disease, schistosomiasis, etc. are already in the pipeline. It is of crucial importance to develop effective vaccines against a number of diseases with no available vaccines such as Toxoplasma (one third of the world population is infected).
3. Significance as fundamental research
Optimizing potency while minimizing toxicity has been a critical issue for vaccine development and improvement. The self-adjuvating strategy involving conjugates of adjuvants and neutralizing epitopes may solve these contradictory problems by inducing specific immunoresponses. In this strategy, adjuvants can activate the immune system and hence no additional adjuvants are required, and recruitment to and uptake of vaccines by APCs and B cells are promoted. In addition, synthetic vaccines enable the fine-tuning of interactions of antigens and adjuvants with immune cells.
Carbohydrate antigens are promising as vaccines against infectious diseases and cancers. Conjugated vaccines involving carbohydrate antigens loaded on carrier proteins have been used for protection against infectious bacteria such as *Haemophilus Influenzae* Type b, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. More efficient and safer vaccines are being developed through the conjugation of synthetic epitopes with synthetic adjuvants. The pure vaccines can improve the safety and quality control. Conjugation methods, linkers used for conjugation, and carbohydrate antigen-adjuvant combinations are also important subjects for developing carbohydrate antigen-adjuvant conjugated vaccines. Immunoregulation methods to overcome the low antigenicity of TACAs while securing safety, i.e., development of safe adjuvants, are critical subjects for cancer vaccines.
4. Possible application for industry and medicine
Vaccines have been used for prevention against infectious diseases. Vaccines contribute to the avoidance of economic and social losses. *Haemophilus influenzae* type b, *Neisseria meningitidis*, *Streptococcus pneumoniae*, etc. are used for

glycoconjugate vaccines. Anticancer vaccines will be expected to have a large economic impact. Chemically synthesized vaccines are superior from the viewpoints of safety, process control, quality control, etc., and are promising as next generation vaccines.

5. Future perspectives
   Development of prophylactic vaccines with high efficiency, high safety, and low cost is becoming increasingly important for preventing infectious diseases including emerging diseases. Combating parasitic diseases and neglected tropical diseases (NTD) is a critical issue in modern society. Glycoconjugate vaccines will be explored for the world's major protozoan parasitic diseases such as malaria, toxoplasmosis, and leishmaniasis. Vaccination will be a novel therapeutic strategy for chronic diseases including cancers, obesity, neurodegenerative diseases such as Alzheimer disease, addictions and atherosclerosis to extend the life span with an improved QOL, although development of prophylactic vaccines for these diseases is still very difficult. Development of therapeutic vaccines will lead to new treatments for these diseases in addition to the present immunotherapies such as cancer passive immunotherapy using monoclonal antibodies. Cancer vaccine therapy using TACAs will be developed as one of these immunotherapies. Immunoregulation methods will be the key technologies for overcoming the low antigenicity of TACAs and the immune evading system of cancer cells.
6. Problems to be solved
   As described, optimizing potency while minimizing toxicity has been a critical issue for vaccine development and improvement. Extensive studies are required to accelerate the development of potent and safe vaccines against bacteria, viruses, protozoa, cancer, etc. In the present glycoconjugate vaccines, a carbohydrate antigen is conjugated with a carrier protein and an adjuvant is required as an additive to induce an effective immune response. Self-adjuvanting glycoconjugate vaccines, in which a carbohydrate antigen and an adjuvant are bound to each other, are highly promising as next generation vaccines from the viewpoint of potency, safety, and quality control. Thus, the development of both antigens and adjuvants as well as selection of appropriate combinations will lead to potent and safe vaccines.
   So far, no TACA-based cancer vaccine has been approved for clinical use. Self-adjuvanting synthetic vaccines incorporating TACAs and adjuvants such as T cell epitopes and immunostimulants will be further elucidated for the discovery of effective vaccines.
   The efficacy of a cancer vaccine may vary from patient to patient, since the relevant antigens may differ among patients. Development of companion diagnostics is desired to test the efficacy of a cancer vaccine in a particular cancer patient. Evaluation of safety as well as evaluation of efficacy is required for any carbohydrate antigen, adjuvant, and TACA-adjuvant conjugated vaccine (Within 10 years).

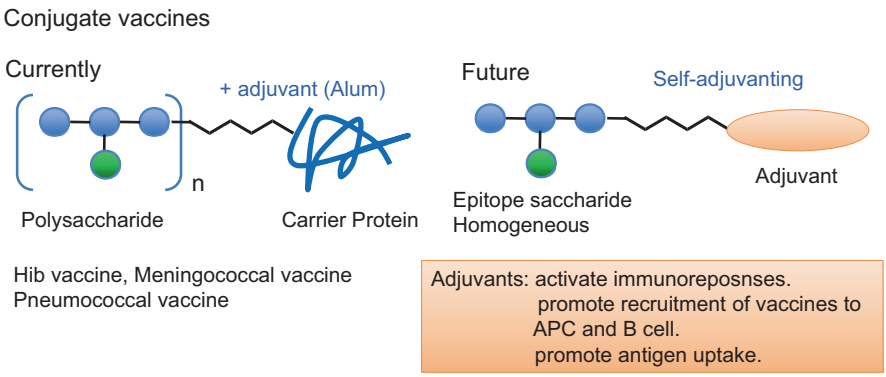

Fig. 10.3 Structures of self-adjuvanting anti-cancer vaccine candidates

References

References for Section 10.1

1. Medzhitov R et al (1997) A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. Nature 388:394–397
2. Iwasaki A, Medzhitov R (2004) Toll-like receptor control of the adaptive immune responses. Nat Immunol 5:987–995
3. Takeda K, Akira S (2005) Toll-like receptors in innate immunity. Int Immunol 17:1–14
4. Seya T et al (2009) Functional evolution of the TICAM-1 (TRIF) pathway for extrinsic RNA sensing. Immunol Rev 227:44–53
5. Ishii A et al (2007) Phylogenetic and expression analysis of amphibian Xenopus Toll-like receptors. Immunogenetics 59:281–293

References for Section 10.2

6. Ishikawa E et al (2017) Recognition of mycobacterial lipids by immune receptors. Trends Immunol 38:66–76
7. Petrovsky N, Cooper PD (2015) Advax™, a novel microcrystalline polysaccharide particle engineered from delta inulin, provides robust adjuvant potency together with tolerability and safety. Vaccine 33:5920–5926

References for Section 10.3

8. http://www.who.int/immunization/diseases/en/
9. Nishat S, Andreana PR (2016) Entirely carbohydrate-based vaccines: an emerging field for specific and selective immune responses. Vaccines 4:19

Part III
Sugar Chains (Glycans) Involved in Medical Science and Medical Care

Foreword

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Glycosylation is one of the most important post-translational modifications. Sugar chains (glycans) are synthesized on proteins and lipids by various glycosyltransferases that are localized in the endoplasmic reticulum and Golgi apparatus. As almost all membrane proteins and secretory proteins are glycosylated, it is not too much to say that glycans on proteins play roles in various biological events in a wide range of medical fields, such as histogenesis, stem cell differentiation, neurogenesis, immunity, and infection. In addition, glycolipids on the cell surface are also glycosylated and play roles in a wide range of medical fields. Expression of glycosyltransferases is regulated by transcription factors, microRNAs, and epigenetic factors. Glycan structures change in various ways and function during normal development and progression of diseases including cancer. Nowadays, some glycan structures are used as tumor markers and stem cell markers for quality control of cells. In the future, many relationships between glycan changes and diseases will be clarified, and then glycans will be used for monitoring health and aging, presymptomatic diagnosis, and liquid biopsy. Meanwhile, viruses and bacteria bind to specific glycans on the cell surface and then enter host cells. In host immunity against them, lectins play various important roles. Moreover, the roles of glycans in many

kinds of signal transduction have been clarified. Glycans will provide us with novel therapeutic targets for various diseases such as spinal cord injuries, bone diseases, osteoarthritis, mental disorders, autoimmune diseases, infectious diseases, as well as their biomarkers.

It is difficult for people to understand how glycans are involved in disease. This is compounded by that among scientists and clinicians there exists a prejudice that glycan research is difficult. In fact, biochemical textbooks have described starch and glycogen from long ago, but the biological significance of glycans has been restricted to blood type and glycans. Regarding disease implications, the development of a next generation high-speed DNA sequencer identified many genetic disorders, and some of which are classified as rare diseases that are one of the scientific topics worldwide. In particular the different kinds of Congenital Disorders of Glycosylation (CDG) have been reported.

Since the first case of CDG was reported in 1981 the number of species of CDG has exponentially increased and currently over 100 kinds of CDG which include α-dystroglycanopathy and *Ngly* 1 disease. Cancer, and life-style related diseases such as diabetes and COPD are also reported to involve glycans.

Cancer biomarker for hepatocellular carcinoma based on glycan changes is well known but recently cancer biomarker for fibrosis was found. We added Boxe(s) for readers to understand their significance in related to Glycoscience and its application which we could not include. Please see page 232 Box 14.1: Influenza Drug Formulation, page 233 Box 14.2: Dengue Virus, page 276 Box 16.1: Interstitial Pneumonia, page 307 Box 18.1: Blood Group Antigen and Glycans.

Chapter 11
Glycan Function in Development and its Regulation

Shoko Nishihara, Masashi Toyoda, Yasuhiko Kizuka, Yuki I. Kawamura, Miyako Nakano, Yoshimi Haga, and Koji Ueda

11.1 Roles of Glycans in Development, Evolution and Stem Cells

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Keywords Glycans conserved across species, Early embryogenesis, Embryonic stem cells, Human embryogenesis, Essential signals

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1. Significance in the field of glycoscience and its current situation
   Molecular evolutionary studies showed that a prototype set of glycosyltransferases existed before the protostome-deuterostome split, suggesting conservation of glycan function from *Drosophila* to mammals. In *Drosophila* development, conserved glycan structures regulate important signals of morphogenesis, such as Notch, FGF, Wnt, BMP and Hedgehog signals. These signals are also important for various phases in *Drosophila* and mammalian development including cell-fate determination, differentiation and so on, as well as morphogenesis. Moreover, these signals facilitate the maintenance and differentiation of stem cells, such as embryonic stem cells and tissue stem cells, via conserved glycan structures [1, 2]. Therefore, this field is basic and important in glycoscience.
2. Impact on the other fields of research
   Notch, FGF, Wnt, BMP and Hedgehog signals, which are regulated by conserved glycan structures from *Dosophila* to mammals, are essential signals in every field of life science including cancer science and immunology, as well as development. Because the glycan function in development is also found in other fields, it could have a marked impact on other fields.
3. Significance as the fundamental research
   Glycan structures conserved during evolution are considered to have essential roles. Actually these glycans regulate the essential signals, including Notch, FGF, Wnt, BMP and Hedgehog signals, that are used repeatedly in various phases of biological phenomena. These glycans contribute to the maintenance and differentiation of stem cells, and also to cell-fate determination in the developmental process, via these signals [3–6] (Fig. 11.1).
4. Possible application for industry and medicine, if any
   Human early embryogenesis is not the same as in mouse and is largely unknown. Studies on human embryonic stem cells will promote a better understanding of human embryogenesis. Clarification of glycan function in human embryonic stem cells will facilitate understanding of genetic diseases and the application of glycans to regenerative medicine.
5. Future perspectives
   Human and mouse embryonic stem cells are at different developmental stages; namely, they corresponding to pre- and post-implantation embryos, respectively. The reason why human and mouse embryonic stem cells are at different developmental stages is an important issue in developmental biology and stem cell biology. The function of glycans might provide an answer to this fundamental question [1, 2].
6. Problems to be solved
   The functional analysis of glycans in early embryogenesis and embryonic stem cells in mammals including human should be performed. This should take ten years.

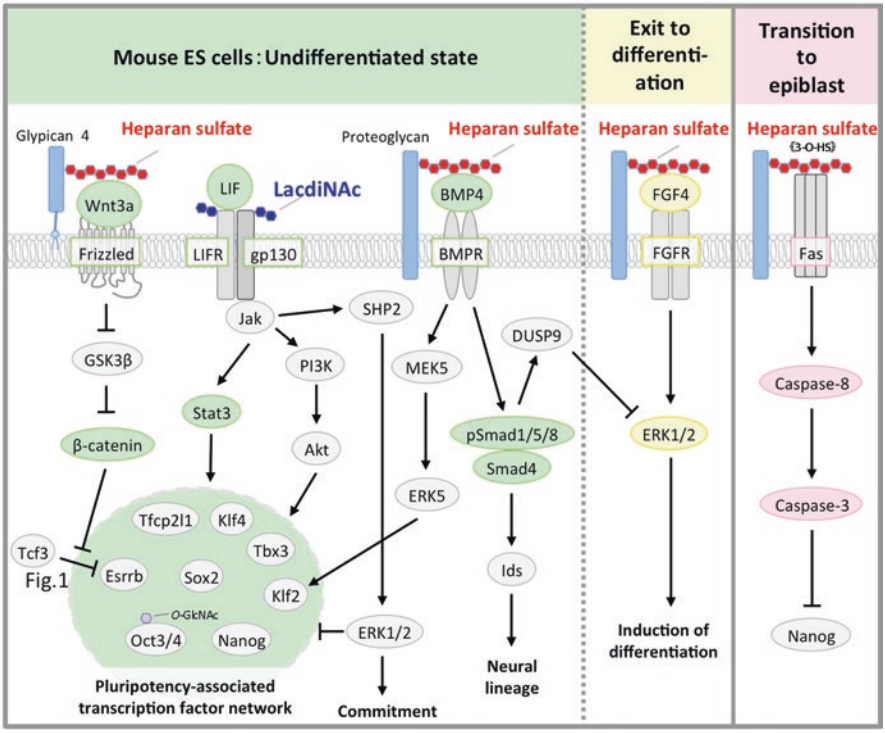

Fig. 11.1 Glycan structures conserved during evolution regulate the main signaling pathways (Wnt, LIF, BMP, FGF) for the maintenance and differentiation of mouse ES cells

11.2 A Characteristic Glycan Profile of Stem Cells

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Keywords Stem cells, Differentiation, Glycan profile, Lectin, Validation

1. Significance in the field of glycoscience and its current situation
   Human hematopoietic stem cells, which are used in the treatment of leukemia, are defined as being positive for the CD-34 antibody that recognizes the sugar chain form of the CD-34 glycoprotein as an antigen. Human pluripotent stem (hPS) cells (ES/iPS cells) are defined as being positive for TRA-1-60/80, which recognizes keratan sulfate antigen, and SSEA 4/3, which recognizes glycolipid antigens. Also, hPS cells have specific glycan profiles different from those of somatic cells [7–9]. In this way, sugar chains are utilized as markers to define various stem cells used in basic to clinical research. Although novel stem cells, and new technology and methods for inducing differentiation into target cells from stem cells have been reported, the reproducibility and equivalence of each cell type has not been fixed yet. Validation by means of glycans is required for future basic and clinical research on stem cell transplantation medicine.
2. Impact on the other fields of research
   Validation of stem cells and differentiated cells will be useful for elucidating the mechanisms of ontogeny and disease onset. Interaction of stem cells and the surrounding microenvironment (niche) has been reported to maintain homeostasis and destruction, and it is expected that sugar chains play an important role in intercellular communication.
3. Significance as the fundamental research
   By clarifying the lineage of differentiation from stem cells, it will become possible to clarify the mechanism of human ontogeny at the molecular/cellular levels, and it will be useful for elucidating the functions of tissues and organs. It will also lead to elucidation of the mechanisms of aging and diseases related to it [10].
4. Possible application for industry and medicine, if any
   Validation of stem cells by means of glycans will be useful for safe implementation of regenerative medicine and application to industries related to quality evaluation of stem cell products for cellular transplantation. It can also be applied to technology for 3D functional tissues derived from stem cells, for drug discovery screening and for quality control in device development.

5. Future perspectives
   It will be useful for application to verification and validation for cellular and gene therapies, device development for drug discovery screening, and for three-dimensional tissue (mini-organ) construction with tissue engineering.
6. Problems to be solved
   It is not enough to characterize and identify various (novel) stem cells and their induced cells. Each cell type will be validated based on the glycan profile, gene expression data and so on, that is, identification of markers. Also, functional analysis of glycans is required for stem cell biology. These will be performed within five years (Fig. 11.2).

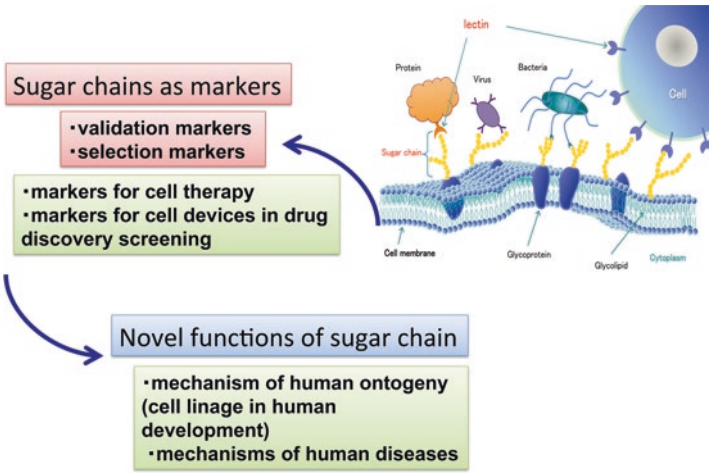

Fig. 11.2 Roles of sugar chains in medical science

11.3 Epigenetics of Glyco-Genes

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Keywords Epigenetics, Glyco-genes, DNA methylation, Histone modification, miRNA

1. Significance in the field of glycoscience and its current situation
   Epigenetics, mainly comprising DNA methylation, histone modifications and miRNAs, is now known to be an indispensable factor for gene regulation, and epigenetic changes have been revealed to be a basis for both physiological phenomena and disease development [11]. Although the importance of epigenetic regulation of glyco-genes is widely accepted, the underlying mechanisms are still poorly understood except for some examples on DNA methylation [12, 13]. In recent years, reports on miRNA-mediated regulation of glyco-genes have been rapidly increasing [14, 15], and have drawn attention to mechanisms underlying disease-associated alterations of glycan expression and function particularly in the cancer context.
2. Impact on the other fields of research
   Knowledge of epigenetic regulation of glyco-genes would spread glycoscience to the genome/epigenome field. In addition, it would contribute to elucidation of the expression mechanisms of not only glycans but other secondary gene products like lipids and metabolites.
3. Significance as the fundamental research
   Detailed mechanisms have not yet been clarified as to how cell-type- and time-dependent glycan expression is achieved, and how glycan expression is altered by stimuli or diseases. In addition to the amount, the localization and activity of glyco-related enzymes, genetic (including transcription factors), and epigenetic regulation of the corresponding glyco-genes are also key factors. Unless we unveil epigenetic mechanisms, we will not fully understand the mechanisms of glycan expression.
4. Possible application for industry and medicine, if any
   Disease-associated changes in glycans are profoundly involved in disease development and progression through functional alterations of cells and proteins. Therefore, elucidation of epigenetic mechanisms of glycan expression would lead to novel therapeutic strategies targeting epigenetic factors, even though a glycan itself is hard to target.
5. Future perspectives
   A novel scientific approach will be possibly developed in which glycan expression is controlled by manipulating epigenomes.

6. Problems to be solved
   Genome-wide analyses focusing on glyco-genes have barely been done. In contrast, a large number of genome-wide analyses focusing on epigenome factors have been done. In such analyses, however, if glyco-genes are hit, deeper analysis tends to be avoided probably due to technical difficulties of glycan analysis. Genome-wide analyses of glyco-genes will be performed within five years (Fig. 11.3).

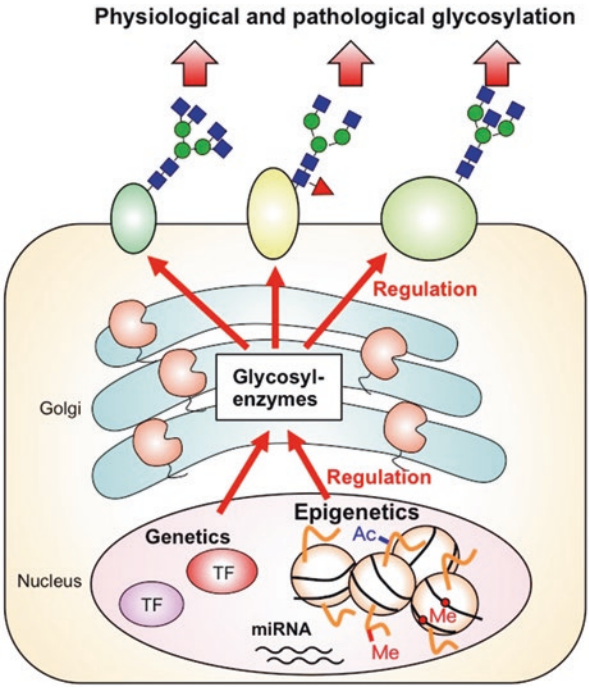

Fig. 11.3 Genetic factors including transfection factors (TFs), and epigenetic factors including modifications of DNA and histones, and miRNAs both regulate the expression of glyco-genes. Then, the expressed glycosyl enzymes regulate glycan expression. This mechanism alters glycan levels, leading to various physiological and pathological functions

11.4 Epigenetic Regulation of Glyco-Genes

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Keywords Epigenetics, DNA methylation, Histone modification, Glyco-genes

1. Significance in the field of glycoscience and its current situation
   Carbohydrate antigens reflect the cell states of differentiation and carcinogenesis [16]. Environmental stimuli (infection, inflammation, alcohol consumption and smoking) induce epigenetic changes such as aberrant DNA methylation and histone modification, which sometimes cause aberrant gene expression without changing the DNA sequence [17, 18]. Such epigenetic modifications may play a significant role in the transcriptional dysregulation of glyco-genes, which results in aberrant glycosylation.
2. Impact on the other fields of research
   Epigenetic modifications are reversible processes, therefore, the normalization of carbohydrate structure via epigenetic remodeling may be a therapeutic target for diseases in which disruption of the carbohydrate structures plays an important role in their pathogenesis, natural history or progression.
3. Significance as the fundamental research
   It has been known that many genes are involved in the synthesis of carbohydrate antigens. Previous studies revealed that, when tumor-related carbohydrate antigens emerge, the numbers of associated glyco-genes are epigenetically and simultaneously dysregulated [19, 20]. Study on epigenetic regulation of glyco-genes may contribute to elucidation of the selection mechanism for target genes in aberrant epigenetic changes.
4. Possible application for industry and medicine, if any
   Carbohydrate antigens have been clinically proven to be useful markers of a variety of carcinomas and ailments. Therefore, epigenetic modifications that trigger pathological changes in carbohydrate structures might be more advantageous in terms of assessing the condition of a disease and therapeutic effects than currently used markers.
5. Future perspectives
   Identification and clarification of the epigenetic modifications in glyco-genes may enable prediction of the changes in carbohydrate structures corresponding to altered gene expression. Furthermore, the production of antibodies against the emerging carbohydrate structures may contribute to the progress of applicable diagnostic measures.

6. Problems to be solved
   In order to comprehend the very complex nature of multiple epigenetic modifications in over 250 glyco-genes, a systematic method for bioinformatics analysis should be established immediately. This will be carried out within five years (Fig. 11.4).

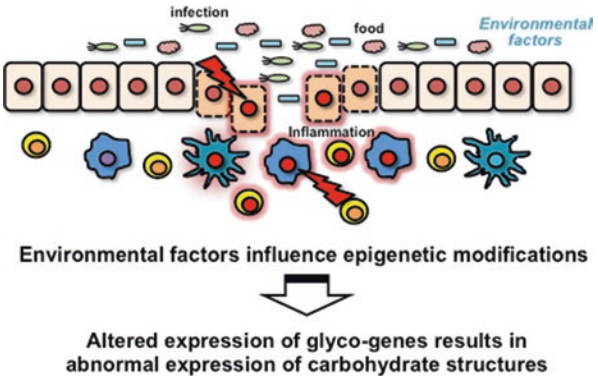

Fig. 11.4 Epigenetic alterations induced by environmental factors influence the expression of glyco-genes and carbohydrate determinants

11.5 Posttranscriptional Regulation of Glycan Expression by MicroRNA

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Keywords MicroRNA, Regulation of glycan expression, Post-transcriptional regulation of glycan-related genes

1. Significance in the field of glycoscience and its current situation
   MicroRNA (miRNA) is a single-stranded noncoding RNA of about 20–25 nucleotides in length. More than 1500 miRNAs have already been reported in human. miRNAs bind to the 3'-untranslated region of a target mRNA, destabilize it, and inhibit its translation. They can suppress the translation of many target mRNAs, including glycosyltransferases such as fucosyltransferases, sialyltransferases and polypeptide *N*-acetylglucosaminyltransferases, concurrently. Therefore, it is possible for an miRNA to regulate the expression of one glycan effectively by suppressing every step of its biosynthetic pathway or the expression of different kinds of glycans coincidentally. miRNA is considered to play important roles in the regulation of glycan expression [21–25] (Fig. 11.5).
2. Impact on the other fields of research
   The patterns of miRNA expression change markedly in developmental processes or in malignant transformation, and then miRNAs regulate the translation of target mRNAs. The contribution of miRNAs to glycan changes in these processes provides novel insight into the biological roles of glycans in development and cancer.
3. Significance as the fundamental research
   Because miRNAs suppress the translation of many target mRNAs concurrently, miRNA comprise an important post-transcriptional regulation system inhibiting the expression of various proteins rapidly at the same time. It is also noteworthy that miRNAs are secreted via exosomes, thereby contributing to long-distance cell-cell communication.
4. Possible application for industry and medicine, if any
   Nucleic acid medicine targeted at miRNAs might become that targeted at glycans.
5. Future perspectives
   The regulation of glycan expression by miRNAs is just beginning to be studied. In the future, key miRNAs causing diseases will regulate the expression of glycans that play an important role in the progression of the disease.

6. Problems to be solved
   The seed sequence of a miRNA binds to the 3'-untranslated region of the target mRNA. A database of miRNAs and glycan-related genes that bind to each other is required. In addition, the actual regulation of glycan-related molecule expression by miRNAs should be proved by means of experiments. This will be performed within five years.

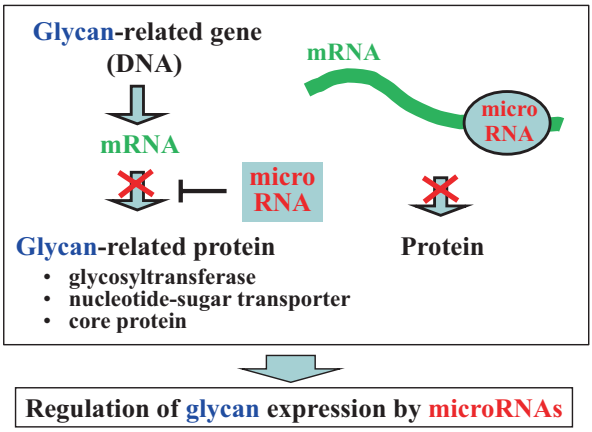

Fig. 11.5 Schematic diagram of regulation of glycan expression by microRNAs. MicroRNAs suppresse the expression of glycan-related proteins such as glycosyltransferase, nucleotide-sugar transporters and core proteins. Consequently, microRNAs regulate glycan expression

11.6 Drug Resistance

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Keywords Antitumor drug, Acquisition of resistance, Leukemia, Sensitivity/resistivity, Diagnostic marker of acquired resistance

1. Significance in the field of glycoscience and its current situation
   Drug (antitumor agent)-resistance acquisition leads to a worse prognosis. The mechanism of drug-resistance acquisition has been extensively studied, but the results of fundamental research have not led to clinical applications such as an acquisition diagnosis or sensitivity recovery. On this fundamental research, there have been many reports suggesting that changes of glycans on proteins are one of the causes of the resistance acquisition. High mannose type glycans in an epidermoid carcinoma cell line with drug (platinum agent) resistance were increased and might be involved in ERAD (degradation system of glycoprotein) [26]. *N*-Glycans in p-glycoproteins on the cell membrane of breast cancer cell line with drug (microtubule-depolymerization inhibitor) resistance were increased and might have enhanced the pump-out function of p-glycoproteins [27, 28]. α2-6 sialylated glycans in all proteins on the cell membrane of leukemia cell line with drug (microtubule depolymerization inhibitor) resistance were decreased [29]. The decrease of α2-6 sialylated glycans on the resistant cells might be regarded as a means of escape from the apoptosis of the cancer cells [30]. Based on these results, the development of diagnostic markers for drug-resistance acquisition and the development of treatments targeting molecules to recover the sensitivity are most important.
2. Impact on the other fields of research
   Previously, we believed that drug resistance occurred due to variation of genes (the first life chain) and variation of proteins (the second life chain), but we recently discovered that variation of glycans (the third life chain) was one of the causes based on the results of fundamental research, as shown in 1) and references. This is very interesting.
3. Significance as the fundamental research
   This fundamental research is important for the development of simple and quick means of measurement of diagnostic markers for acquired drug resistance and the development of diagnostic targeting molecules to recover the sensitivity of tumor cells.
4. Possible application for industry and medicine, if any
   Development of simple and quick means of measurement of diagnostic markers for acquired drug resistance. Development of diagnostic targeting molecules to recover the sensitivity of tumor cells.
5. Future perspectives
   There are no useful diagnosis kits for measuring drug resistivity and no useful medicine to enhance the drug sensitivity so far. Therefore, this has caused suffering

for patients and doctors in clinical on-site antitumor treatment. However, when 4) can be realized, drug resistance will disappear and cancer will be exterminated.

6. Problems to be solved
   We must elucidate individual glycan-related resistance mechanisms for a variety of antitumor drugs for a variety of tumor cell types. It is necessary to develop a method to obtain tumor cells derived from cancer patients with both drug sensitivity and drug resistivity. This will be performed within 10 years (Fig. 11.6).

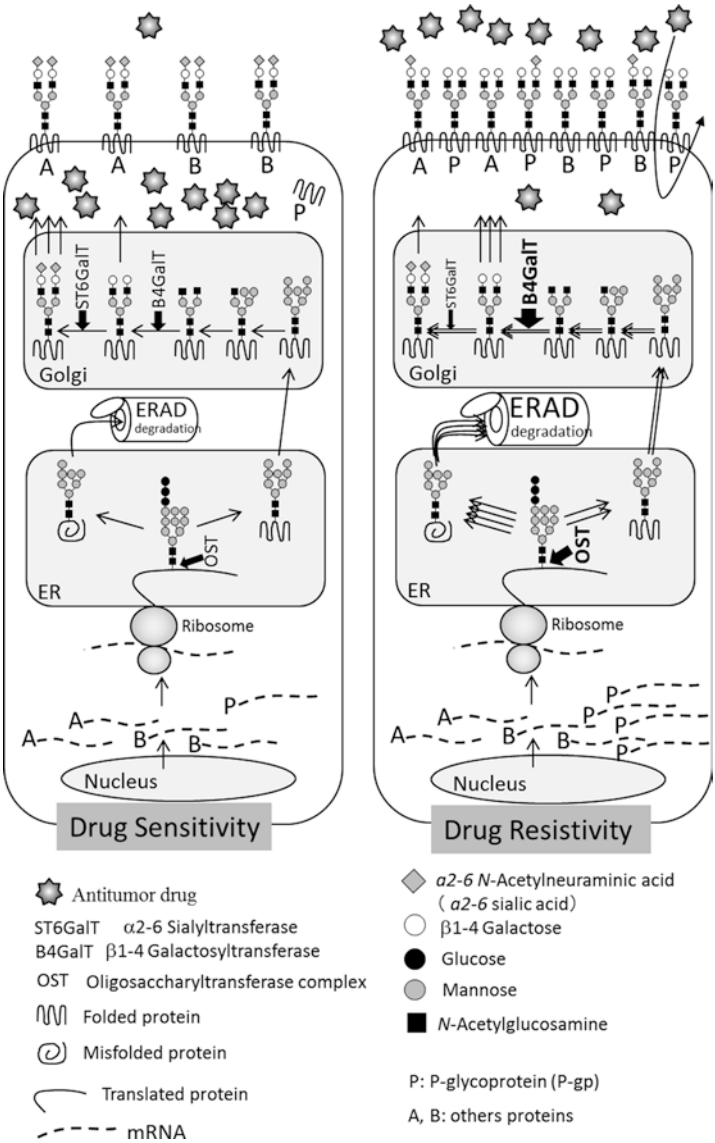

Fig. 11.6 View of several drug resistance mechanisms related with glycans

11.7 Secretion and Uptake of Exosomes Via Glycan

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Keywords Exosome, Targeting, Liquid biopsy

1. Significance in the field of glycoscience and its current situation
   The exosome is a kind of vesicle secreted to the outside of a cell in a process of vesicle transportation, and contains the same glycoconjugates as the parent cell [31]. Exosomes released into body fluid are uptaken by a recipient cell, and release their contents and function in the resident cells. However, the mechanism targeting exosomes to a specific cell type remains unclear. Since the discovery that microRNAs exist in exosomes and mediate cell-cell communication [32], analysis of components, such as nucleic acids and proteins, has greatly advanced. On the other hand, glycan profiling of exosomes is lagging due to the technical difficulties. Therefore the establishment of a comprehensive analytical method for glycans on exosomes is necessary.
2. Impact on the other fields of research
   Clarification of the mechanism of novel vesicular transport that mediates the communication between the cells would be important knowledge for understanding a biological system. In addition, the development of clinical applications is expected, as described below.
3. Significance as the fundamental research
   Recently, Hoshino et al. showed that tumor-derived exosomes are taken up by organ-specific cells and form a pre-metastatic niche [33]. Analysis of glycoproteins in exosomes would lead to the discovery of a factor determining the organ specificity of cancer metastasis that has not been unveiled previously.
4. Possible application for industry and medicine, if any
   Many applications are expected, as follows; (1) development of a less invasive diagnosis method by liquid biopsy using body fluid derived exosomes, (2) development of innovative therapeutics by suppressing a function of exosomes derived from a pathogenetic cell, and (3) development of DDS using artificial exosomes equipped with a small molecule therapeutic agent and a nucleic acid therapeutic agent.
5. Future perspectives
   Exosomes seem to play a key role in not only cancer but also Alzheimer's disease and other diseases. Analysis of exosome glycans would lead to elucidation of the mechanisms of these diseases and the development of diagnostic agents.
6. Problems to be solved
   In order to analyze complex glycan structures on small amounts of exosomes in body fluids, improvement of the purification and analysis methods are essential. This will occur within 5 years (Fig. 11.7).

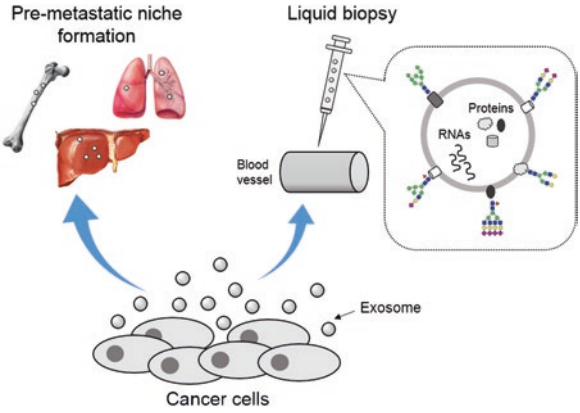

Fig. 11.7 An exosome is a "replica" of a exosome-producing cell, and contains the same glycoconjugate same as the parent cell. Exosomes released into body fluid are taken up by a recipient cell, and release contents and function in the resident cells

References

References for Section 11.1

1. Nishihara S (2018) Glycans in stem cell regulation: from *Drosophila* tissue stem cells to mammalian pluripotent stem cells. FEBS Lett 592: 3773–3790
2. Nishihara S (2017) Glycans define the stemness of naïve and primed pluripotent stem cells. Glycoconj J 34: 737–747
3. Miura T et al (2018) *O*-GlcNAc on PKCζ inhibits the FGF4-PKCζ-MEK-ERK1/2 pathway via inhibition of PKCζ phosphorylation in mouse embryonic stem cells. Stem Cell Reports 10: 272–286
4. Hirano K et al (2012) 3-*O*-sulfated heparan sulfate recognized by the antibody HS4C3 contributes to the differentiation of mouse embryonic stem cells via fas signaling. PLoS One 7: e43440
5. Sasaki N et al (2011) LacdiNAc (GalNAcβ1-4GlcNAc) contributes to self-renewal of mouse embryonic stem cells by regulating leukemia inhibitory factor/STAT3 signaling. Stem Cells 29: 641–650
6. Sasaki N et al (2008) Heparan sulfate regulates self-renewal and pluripotency of embryonic stem cells. J Biol Chem 283: 3594–3606

References for Section 11.2

7. Toyoda M et al (2011) Lectin microarray analysis of pluripotent and multipotent stem cells. Genes Cells 16:1–11
8. Tateno H et al (2011) Glycome diagnosis of human induced pluripotent stem cells using lectin microarray. J Biol Chem 286:20345–20353
9. Ojima T et al (2015) Glycolipid dynamics in generation and differentiation of induced pluripotent stem cells. Sci Rep 5:14988
10. Itakura Y et al (2016) *N*- and *O*-glycan cell surface protein modifications associated with cellular senescence and human aging. Cell Biosci 6:14

References for Section 11.3

11. Bonasio R et al (2010) Genomic comparison of the ants *Camponotus floridanus* and *Harpegnathos saltator*. Science 330:612–616
12. Lauc G et al (2014) Epigenetic regulation of glycosylation is the quantum mechanics of biology. Biochim Biophys Acta 1840:65–70
13. Kawamura YI et al (2008) DNA hypermethylation contributes to incomplete synthesis of carbohydrate determinants in gastrointestinal cancer. Gastroenterology 135:142–151
14. Kurcon T et al (2015) miRNA proxy approach reveals hidden functions of glycosylation. Proc Natl Acad Sci USA 112:7327–7332
15. Gaziel-Sovran A et al (2011) miR-30b/30d regulation of GalNAc transferases enhances invasion and immunosuppression during metastasis. Cancer Cell 20:104–118

References for Section 11.4

16. Hakomori S (2002) Glycosylation defining cancer malignancy: new wine in an old bottle. Proc Natl Acad Sci USA 99:10231–10233
17. Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genet 3:415–428
18. Alegria-Torres JA et al (2011) Epigenetics and lifestyle. Epigenomics 3:267–277
19. Kawamura YI et al (2008) DNA hypermethylation contributes to incomplete synthesis of carbohydrate determinants in gastrointestinal cancer. Gastroenterology 135:142–151
20. Dohi T, Kawamura YI (2008) Incomplete synthesis of the Sda/Cad blood group carbohydrate in gastrointestinal cancer. Biochemica Biophysica Acta 1780:467–471

References for Section 11.5

21. Agrawal P et al (2014) Mapping posttranscriptional regulation of the human glycome uncovers microRNA defining the glycocode. Proc Natl Acad Sci U. S. A. 18: 4338–4343
22. Pedersen ME et al (2013) An epidermal microRNA regulates neuronal migration through control of the cellular glycosylation state. Science 341: 1404–1408
23. Nakamura S et al (2015) Influenza A virus-induced expression of a GalNAc transferase, GALNT3, via microRNAs is required for enhanced viral replication. J Virol 90: 1788–1801
24. Ibrahim SA et al (2014) MicroRNA regulation of proteoglycan function in cancer. FEBS J 281: 5009–5022
25. Minami A et al (2013) Reduction of the ST6 β-galactosamide α-2,6-sialyltransferase 1 (ST6GAL1)-catalyzed sialylation of nectin-like molecule 2/cell adhesion molecule 1 and enhancement of ErbB2/ErbB3 signaling by microRNA-199a. J Biol Chem 288: 11845–11853

References for Section 11.6

26. Nakagawa H et al (2008) Alterations in the glycoform of cisplatin-resistant human carcinoma cells are caused by defects in the endoplasmic reticulum-associated degradation system. Cancer Lett 270:295–301
27. Honma K et al (2008) RPN2 gene confers docetaxel resistance in breast cancer. Nat Med 14:939–948
28. Zhou H et al (2013) B4GALT family mediates the multidrug resistance of human leukemia cells by regulating the hedgehog pathway and the expression of p-glycoprotein and multidrug resistance-associated protein 1. Cell Death Dis 4:e654
29. Nakano M et al (2011) Identification of glycan structure alterations on cell membrane proteins in desoxyepothilone B resistant leukemia cells. Mol Cell Proteomics 10:M111.009001
30. Malagolini N et al (2009) Exposure of alpha2,6-sialylated lactosaminic chains marks apoptotic and necrotic death in different cell types. Glycobiology 19:172–181

References for Section 11.7

31. Costa J (2017) Glycoconjugates from extracellular vesicles: structures, functions and emerging potential as cancer biomarkers. Biochim Biophys Acta 1868:157–166
32. Valadi H et al (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9:654–659
33. Hoshino A et al (2015) Tumour exosome integrins determine organotropic metastasis. Nature 527:329–335

Chapter 12
Glycans in Nervous System

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12.1 Schizophrenia (Polysialic Acid)

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Keywords Polysialic acid (polySia, PSA), Polysialyltransferase, Prefrontal cortex, Hippocampus, Bipolar disorder

1. Significance in the field of glycoscience and its current situation
   Schizophrenia (SZ) is a severe disease from which 1% of the world-wide population is suffering. Because the patients have difficulties in spending a normal life, the economic loss for a country is a big problem. Therefore, drugs for the treatment and measurement of disease state are important. The cause of the disease remains unknown; however, impairments of the prefrontal cortex (large ventricle), mossy fiber in hippocampus and a small volume of olfactory bulb have been reported. As for glycosylation, the polySia-expressing cells in the prefrontal

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cortex and hippocampus have been reported to be impaired. In genome-related studies, several significant SNPs have been found in the polysialyltransferase, *ST8SIA2*, gene [1], and some of these SNPs were shown to lead to impairment of the polySia structure and function by biochemical analyses [2, 3]. *ST8Sia2*-KO mice were shown to be SZ-model mice [4]. Interestingly, chlorpromazine, which is an anti-schizophrenia reagent, influenced the polySia-expression in human neural cells and mouse brain, especially in the prefrontal cortex [5]. Therefore, understanding of the mechanism for regulated expression of polySia in the brain might be a key step for the development of diagnosis and treatment of SZ.

2. Impact on the other fields of research
   PolySia is well known as a marker of adult neurogenesis. Therefore, understanding of polySia expression will have a big impact on the brain and neurology fields. In addition, polySia is a marker for cancer cells. Therefore, big impacts on the pharmacological and medical fields are also predicted.
3. Significance as the fundamental research
   It is possible that study of the mechanism for fine-tuning of polySia expression will lead to understanding of the causes of psychiatric disorders such as SZ, BD and autism (Fig. 12.1). In addition, analysis of the changes of cell surface glycosylation caused by genetic and environmental factors is important for basic study.
4. Possible application for industry and medicine, if any
   PolySia is known to be an onco-developmental antigen and is used as a marker for several cancer cells. Recently, polySia chains were shown to bind to biologically active molecules such as FGF2, BDNF and dopamine. Therefore, polySia can be used not only as a marker for cancer cells, but also for DDS. In addition, it will be used as a marker of psychiatric disorders.
5. Future perspectives
   There are few diagnostic markers for the psychiatric disorders. As polySia expression was shown to decrease in the prefrontal cortex and increase after anti-SZ reagent administration, a probe for polySia might be a good diagnostic marker. In addition, because polySia can bind to biologically active molecules with high affinity, it might be used for DDS.
6. Problems to be solved
   Development of specific probes for fine-tuned polySia chains is required. In addition, a precise study to understand the synthesis of polySia by polysialyltransferases is required in vitro and in vivo. These unique glycosylations that are considered to be related to SZ should be analyzed. This will be performed within 10 years.

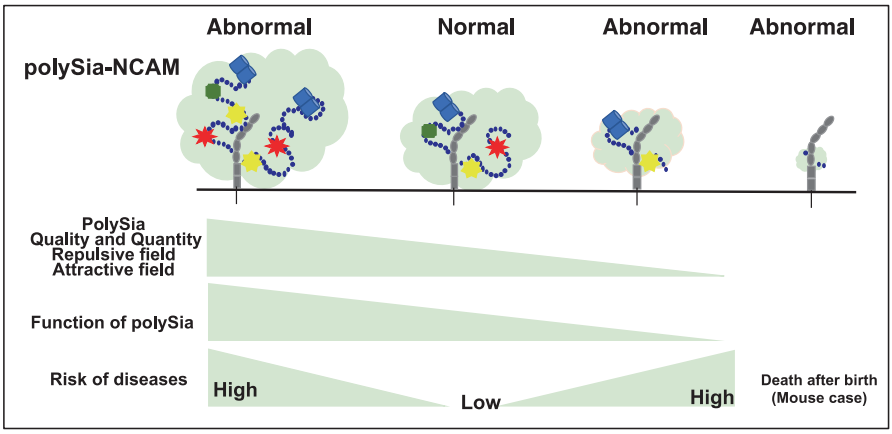

Fig. 12.1 Fine-tuned polySia expression: The expression of polySia is highly regulated spacio-temporally. Therefore, impairment of the quantity and quality of polySia lead to impairment of the functions of polySia. Finally, the risk and the conditions of the diseases (psychiatric disorders and cancers) will be high. The molecules on the polySia are biologically active molecules. The cloud around polySia is the repulsive and attractive fields of polySia

12.2 Schizophrenia

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Keywords α1,6-fucosyltransferase, Fut8, Polysialic acid, α2,8-sialyltransferase 8B (ST8SIA2/STX)

1. Significance in the field of glycoscience and its current situation
   Schizophrenia is a common, chronic, and severe brain disorder that ranks as one of the leading causes of disability worldwide because it afflicts 1% of the world's population. The etiology of schizophrenia is only partially understood, but a breakdown in the communication across neurotransmitter systems through intracellular signaling pathways. Interestingly, a decrease in the expression of α1,6-fucosyltransferase (Fut8) has been observed in the superior temporal gyrus of elderly patients with schizophrenia [6]. In addition, abnormality in *N*-glycosylation of the GluA2 subunit of AMPARs, an ionotropic receptor for glutamate, is an integral to plasticity and synaptic transmission in schizophrenia patients [7]. Consistently, Fut8 KO mice clearly show similar schizophrenia-like phenotypes [8] and the long-term potentials of the hippocampal neuron are impaired due to an abnormal formation of AMPARs in the postsynapses [9]. Also association between polysialyltransferase ST8SIA2/STX and the risk of schizophrenia has been reported [10]. Therefore, these facts emphasize the importance of glycans, and may contribute to develop novel therapeutic approaches for schizophrenia.
2. Impact on the other fields of research
   Alteration in functions of each target protein for a glycosyltransferase is too small to make a schizophrenic disorder. But, these small alterations can add up to make a big effect. To elucidate the roles of α1,6-fucose and polysialic acid in the brain nerve system is required for understanding and treatment for either the schizophrenia or other mental disorder such as depression and Bipolar disorder.
3. Significance as the fundamental research
   The cause of schizophrenia is still unclear that many different risk genes are concerned with schizophrenia. It is not yet possible to use genetic information to predict who will develop schizophrenia. Unique glycosylation can affect many proteins features simultaneously. Therefore, it may simplify the underlying molecular mechanisms to use a specific carbohydrate structure deficient murine model, and which may make a breakthrough in unraveling the communication between neurons and glia cells, as well as their functions.

4. Possible application for industry and medicine, if any
   GDP-fucose is synthesized in the salvage and de novo pathways. Upregulation of α1,6-fucose would be likely candidates for the therapeutics of Schizophrenia via the salvage pathway for GDP-fucose synthesis using L-fucose. Fucoidan known as functional food is sulfated L-fucose isomers. In addition, sialic acids are available at functional food. L-fucose and sialic acids would be likely candidates for the glycotherapeutics for the Schizophrenia.
5. Future perspectives
   α1,6 Fucosylation play important roles in several growth factor receptors and adhesion molecules. Also, it has been well known to relate with ADCC. Therefore, a comprehensive analysis of the biological functions for α1,6-fucose will accelerate the development for glycotherapeutics.
6. Problems to be solved
   It needs to reveal the underlying molecular mechanisms in detail, e.g. how does the α1,6-fucose regulate the neuron network; and why does the brain tissue highly express α1,6-fucose? (Fig. 12.2).

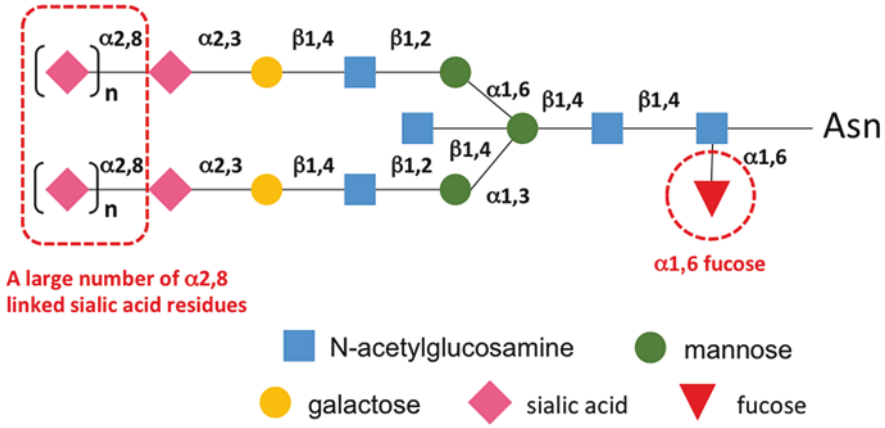

Fig. 12.2 Structure of α1,6-fucose and polysialic acid. Fut8 catalyzes the transfer of a fucose residue from GDP-fucose to the innermost GlcNAc residue to form α1,6-fucose in *N*-linked oligosaccharides of glycoproteins. ST8SIA2 catalyzes a large number of α2,8-linked sialic acid residues through α2,3-linked sialic acid on N-glycan chains

12.3 Spinal Cord Injury

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Keywords Spinal cord injury, Chondroitin sulfate, Heparan sulfate, Keratan sulfate, Hyaluronan

1. Significance in the field of glycoscience and its current situation
   Axon regeneration is important for functional recovery after spinal cord injury (SCI). Glycans have been recently paid attention among many important factors regulating axon regeneration. Chondroitin sulfate (CS) and keratan sulfate (KS) inhibit axon regeneration, and heparan sulfate (HS) promotes it [11–15]. Through investigation of the mechanisms of action of these glycans, novel receptors, i.e., protein tyrosine phosphatases, were identified [14]. A new concept that glycans play a main role in cellular signaling was established, while core proteins rather play an assistant role. In addition, CS and hyaluronan (HA) regulate synaptic plasticity. Together, glycosaminoglycans, e.g., CS, KS, HS and HA, are now accepted as regulators of neural plasticity. Furthermore, these findings may lead to new therapies for SCI and other neural injuries.
2. Impact on the other fields of research
   The research of SCI is an example on research that successfully combines glycoscience and other research fields. The discovery of glycan receptors and the finding of the regulation of neuronal functions by glycans will expand the importance of glycans in neurology and other biology fields, and promote collaboration involving glycoscience.
3. Significance as the fundamental research
   Signaling through receptor-type protein tyrosine phosphatases by glycans will trigger research on these receptors, whose mechanisms of action remain elusive.
4. Possible application for industry and medicine, if any
   CS, KS, HS and HA are important factors regulating axon regeneration and synaptic plasticity. Therefore, their synthetic forms, their mimetics, degrading enzymes or synthetic enzymes could be candidate therapeutics for SCI or other neural injuries.
5. Future perspectives
   Most expected is elucidation of the action mechanisms of glycans and their application to medicine. In addition, since glycan structures can change more easily in response to environmental changes than other biopolymers, such as nucleic acids and proteins, do. Therefore, glycans may reflect more sensitively senescence and chronic inflammation that cause many neurological disorders (neurodegenerative diseases, psychiatric diseases, and a decline in resilience against neuronal dysfunctions, etc.).

6. Problems to be solved
   It is unlikely that the whole length of a glycosaminoglycan is required for signaling. So-called functional domains may exist in it. Identification of such domains and the stoichiometry of glycan-receptor binding, and elucidation of downstream signaling are needed to elucidate the mechanisms of glycans' action. This will be resolved within 5 years (Fig. 12.3).

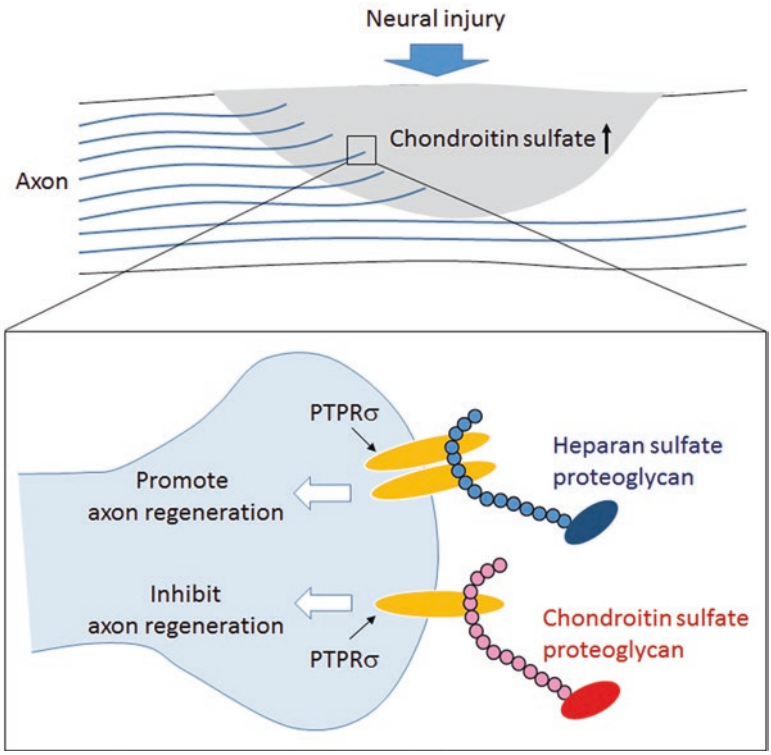

Fig. 12.3 Regulation of axon regeneration in spinal cord injury: HS and CS share a common receptor, PTPRσ. HS dimerizes PTPRσ, suppresses its phosphatase activity and promotes axon regeneration. In contrast, CS monomerizes PTPRσ, activates its phosphatase activity and inhibits axon regeneration

12.4 Microglia

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Keywords Microglial activation, Siglec, Sialylated glycan

1. Significance in the field of glycoscience and its current situation
   Microglia are considered to be immune cells of the brain, and they are involved in removal of invading cells and injured brain cells. Accordingly, they are always scanning the entire brain, searching for abnormal cells. It was shown recently that they make use of cell surface glycans to recognize any abnormality [16, 17]. For example, siglecs on the microglial cell surface contribute by activating (inducing phagocytotic activity) them upon interaction with the cell surface sialylated glycans, and knockout mouse to microglial siglecs exhibits an immune abnormality [18, 19]. Therefore, it is highly expected that the analysis of glycan structure will contribute to clarification of the immunological function of microglia in the brain.
2. Impact on the other fields of research
   Understanding of the immunological function of microglial siglecs is expected to provide useful information for clarification of the regulation of other immune systems as well.
3. Significance as the fundamental research
   The brain has been believed to be “immune privileged” and thus to have little immune activity. However, the ability to recognize and eliminate invading/injured cells have been conserved in microglia, and thus the glycoscientific study on microglia will greatly add to our understanding of general immunology.
4. Possible application for industry and medicine, if any
   Stimulation of development of treatment for brain inflammation by regulating microglial siglecs or modifying the partner sialylated glycan structures is expected.
5. Future perspectives
   Microglia are involved in synaptic elimination in addition to the elimination of invading/injured cells. Impaired recognition of unwanted synapses leads to a dysfunction of synaptic elimination, which could be the cause of autism or schizophrenia. Thus, such study may lead to the development of treatment for psychiatric diseases.

6. Problems to be solved
   Microglial siglecs recognize glycans expressed on a single cell, which leads to selective elimination of an invading/injured cell. In the case of syanaptic elimination, the recognized regions are even smaller. Immunohistochemistry or lectin histochemistry are the only methods for analyzing glycan structures within these small areas. However, these methods only provide us with partial structural information. Thus, development of an analytical method to determine the glycan structures expressed in a small area (single cell or single synapse) is essential. This will occur within 10 years (Fig. 12.4).

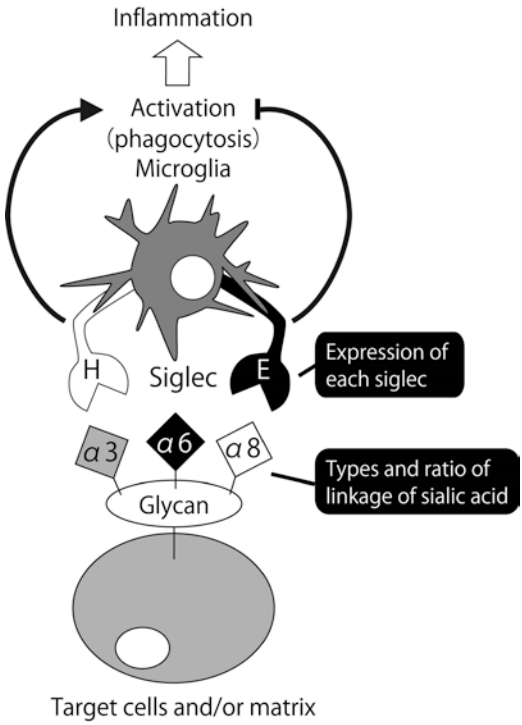

Fig. 12.4 Cells to be eliminated by microglia express various sialylated glycans, which are recognized by the siglecs expressed by microglia to induce phagocytosis

References

References for Section 12.1

1. Sato C et al (2016) Relationship between ST8SIA2, polysialic acid and its binding molecules, and psychiatric disorders. Biochim Biophys Acta 1860:1739–1752
2. Isomura R et al (2011) Structural and functional impairments of polysialic acid by a mutated polysialyltransferase found in schizophrenia. J Biol Chem 286:21535–21545
3. Hane M et al (2015) Protective effects of polysialic acid on proteolytic cleavage of FGF2 and proBDNF/BDNF. Glycobiology 10:1112–1124
4. Kröcher T et al (2015) Schizophrenia-like phenotype of polysialyltransferase ST8SIA2- deficient mice. Brain Struct Funct 220:71–83
5. Abe C et al (2017) Chlorpromazine increases the expression of polysialic acid (PolySia) in human neuroblastoma cells and mouse prefrontal cortex. Int J Mol Sci 18:1123

References for Section 12.2

6. Mueller TM et al (2017) Altered fucosyltransferase expression in the superior temporal gyrus of elderly patients with schizophrenia. Schizophr Res 182:66–73
7. Tucholski J et al (2013) Abnormal *N*-linked glycosylation of cortical AMPA receptor subunits in schizophrenia. Schizophr Res 146:177–183
8. Fukuda T et al (2011) α1,6-fucosyltransferase-deficient mice exhibit multiple behavioral abnormalities associated with a schizophrenia-like phenotype: importance of the balance between the dopamine and serotonin systems. J Biol Chem 286:18434–18443
9. Gu W et al (2015) Loss of α1,6-fucosyltransferase decreases hippocampal long term potentiation: implications for core fucosylation in the regulation of AMPA receptor heteromerization and cellular signaling. J Biol Chem 290:17566–17575
10. Sato C et al (2016) Relationship between ST8SIA2, polysialic acid and its binding molecules, and psychiatric disorders. Biochim Biophys Acta 1860:1739–1752

References for Section 12.3

11. Bradbury EJ et al (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636–640
12. Ito Z et al (2010) *N*-acetylglucosamine 6-*O*-sulfotransferase-1-deficient mice show better functional recovery after spinal cord injury. J Neurosci 30:5937–5947
13. Imagama S et al (2011) Keratan sulfate restricts neural plasticity after spinal cord injury. J Neurosci 31:17091–17102
14. Coles CH et al (2011) Proteoglycan-specific molecular switch for RPTPσ clustering and neuronal extension. Science 332:484–488
15. Takeuchi K et al (2013) Chondroitin sulphate *N*-acetylgalactosaminyl-transferase-1 inhibits recovery from neural injury. Nat Commun 4:2740

References for Section 12.4

16. Claude J et al (2013) Microglial CD33-related Siglec-E inhibits neurotoxicity by preventing the phagocytosis-associated oxidative burst. J Neurosci 33:18270–18276
17. Kopatz J et al (2013) Siglec-h on activated microglia for recognition and engulfment of glioma cells. Glia 61:1122–1133
18. Läubli H et al (2014) Engagement of myelomonocytic Siglecs by tumor-associated ligands modulates the innate immune response to cancer. Proc Natl Acad Sci USA 111:14211–14216
19. Schmitt H et al (2016) Siglec-H protects from virus-triggered severe systemic autoimmunity. J Exp Med 213:1627–1644

Chapter 13
Glycans in Osseous Tissue and Articulation

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13.1 Bone Diseases

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Keywords Chondroitin sulfate, Heparan sulfate, Proteoglycan, Skeletal dysplasias, Bone remodeling

1. Significance in the field of glycoscience and its current situation
   Bone diseases cover a wide spectrum of disorders, ranging from heterogeneous congenital "skeletal dysplasias" to metabolic bone diseases including osteoporosis. Age-related osteoporosis is an especially common and increasingly growing problem worldwide. Because of their abundance in the bone matrix, proteoglycans and their sugar moieties, sulfated glycosaminoglycans (GAG) chains such as chondroitin sulfate (CS) and heparan sulfate (HS), are thought to be involved in bone formation and homeostasis. Indeed, increasing evidence points towards genetic defects of biosynthetic enzymes for GAG chains as being responsible for various skeletal dysplasias [1, 2]. Notably, CS-E, a highly sulfated CS subtype, promotes osteoblast differentiation [3], and mediates estrogen-induced osteoanabolism [4]. In contrast, it can also participate in non-cell autonomous

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inhibition of osteoclastogenesis. Moreover, overproduction of CS chains in the aorta increases aortic calcification in murine chronic kidney disease [5]. These findings indicate the therapeutic potential of fine-tuning of GAG chains in myriad bone diseases.

2. Impact on the other fields of research
   A quantitative and/or qualitative change in GAG chains in the bone matrix could be a critical determinant controlling the balance between bone resorption and bone formation. This concept would be applicable to bone diseases in which heterotopic ossification occurs as a result of either congenital gene defects or metabolic complications.
3. Significance as the fundamental research
   Such glycobiological approaches would provide valuable new insights into the underlying mechanisms of metabolic bone diseases, especially age-related osteoporosis, and would also be useful for further identification of genes responsible for rare but severe forms of skeletal dysplasias.
4. Possible application for industry and medicine, if any
   Small compounds that directly regulate distinct biosynthetic and/or catabolic enzymes for GAG chains would be promising candidates for therapeutic use in the treatment of various bone disorders.
5. Future perspectives
   Understanding the pleiotropic effects of GAG chains in bone microenvironments will open new avenues for therapeutic control of most of the nonhealing bone diseases, with resultant improvement of a patient quality of life.
6. Problems to be solved
   To develop effective glycotherapeutic intervention for bone diseases, obtaining a comprehensive understanding of the functional involvement of individual enzymes in GAG production and degradation in the underlying etiologies using genetically modified cells and mice remains an important undertaking. This will take 10 years (Fig. 13.1).

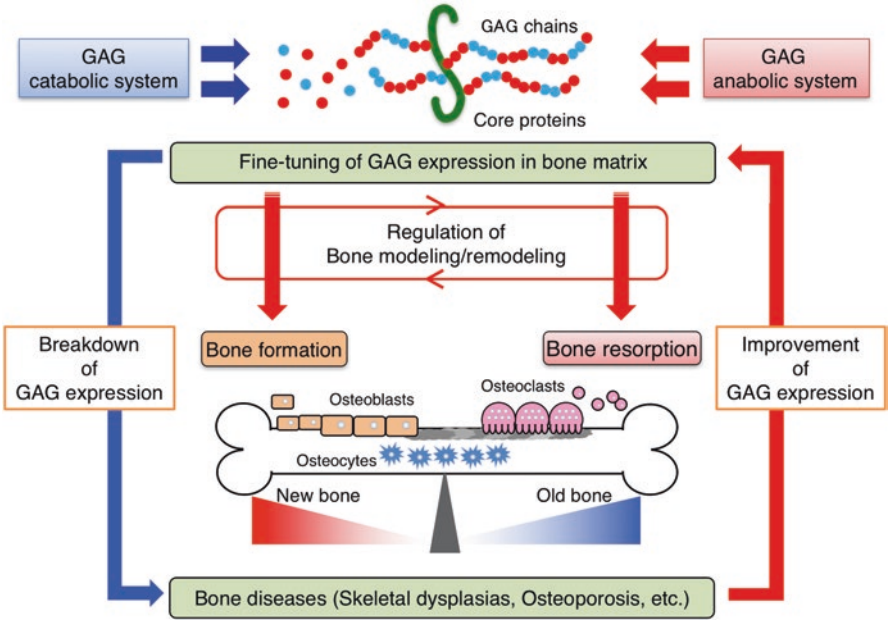

Fig. 13.1 Functional involvement of GAG chains in bone development and homeostasis. Breakdown of normal GAG expression causes various bone diseases. Thus, strategies aimed at improving GAG expression are promising therapies for bone diseases

13.2 Arthritis

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Keywords Osteoarthritis (OA), High-mannose type glycan, Glycosphingolipid, Musculoskeletal tissue

1. Significance in the field of glycoscience and its current situation
   Osteoarthritis (OA) is the most common joint disease. Over 70% of the world population between the ages of 55 and 70 are affected by OA. This disease is characterized by degradation of articular cartilage that results in disability as to performing daily activities among the elderly. However, therapeutic options that fundamentally alter the natural course of OA are lacking. The difficulty in establishing OA management highlights the requirement of elucidation of OA pathogenesis.
   The homeostasis of articular cartilage is primarily maintained by chondrocytes, which are the only cells responsible for the synthesis and degradation of the extracellular matrix (ECM). Although a large number of gene- and protein-based studies have been performed to clarify the mechanism of cartilage degradation, it remains unclear. Therefore, the development of OA therapies requires the identification of novel molecular targets involved in the degradation mechanism. To date, I have elucidated the functional roles of *N*-glycans and glycosphingolipids in OA pathogenesis [6–10].
2. Impact on the other fields of research
   High-mannose type *N*-glycans and glycosphingolipids in chondrocytes regulate IL-1 signal transduction [7–9]. This will be applied to elucidation of the disease pathogenesis or development of novel treatments.
3. Significance as the fundamental research
   Although the research field associated between musculoskeletal biology and glycobiology has only just started, this field has the potential of developing a novel approach for musculoskeletal science.
4. Possible application for industry and medicine, if any
   The glycobiological approach allows the possibility of developing novel biomarkers, drug discovery, and scaffold materials in the orthopaedic field.
5. Future perspectives
   The extra-cellular matrix of musculoskeletal tissues contains abundant glycans. Therefore, it is considered that elucidation of their functional roles will promote musculoskeletal research.
6. Problems to be solved
   The detailed functions of glycans in the regulation of cartilage metabolism and cartilage repair remain unclear. These functions must be clarified to develop novel treatments for OA. This will be resolved within 5 years (Fig. 13.2).

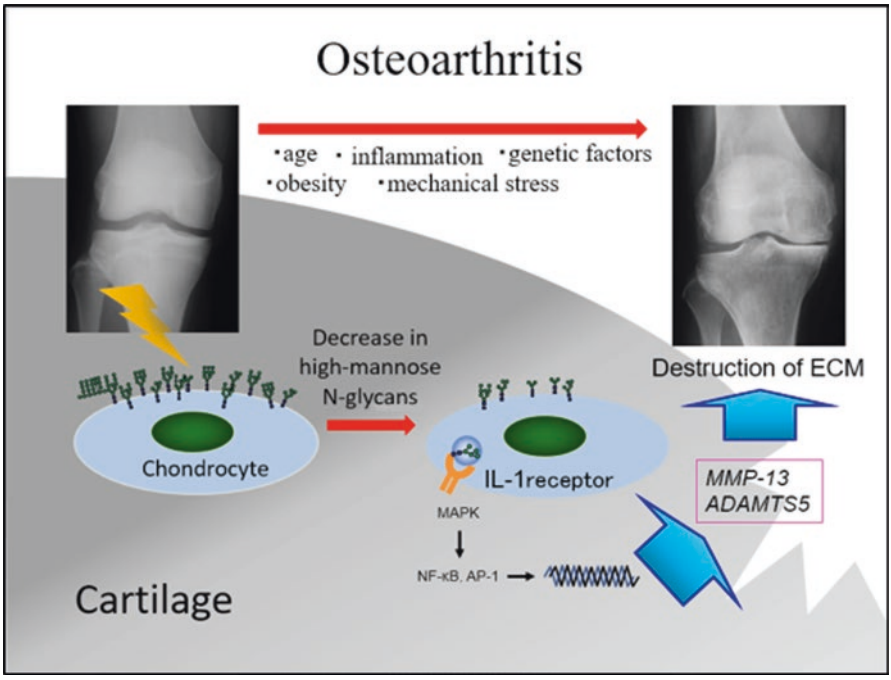

Fig. 13.2 Glycobiological pathogenesis of osteoarthritis

References

References for Section 13.1

1. Mikami T, Kitagawa H (2013) Biosynthesis and function of chondroitin sulfate. Biochim Biophys Acta 1830:4719–4733
2. Thiele H et al (2004) Loss of chondroitin 6-*O*-sulfotransferase-1 function results in severe human chondrodysplasia with progressive spinal involvement. Proc Natl Acad Sci USA 101:10155–10160
3. Koike T et al (2014) Chondroitin sulfate-E fine-tunes osteoblast differentiation via ERK1/2, Smad3 and Smad1/5/8 signaling by binding to *N*-cadherin and cadherin-11. Biochem Biophys Res Commun 420:523–529
4. Koike T et al (2015) Chondroitin sulfate-E mediates estrogen-induced osteoanabolism. Sci Rep 5:8994
5. Purnomo E et al (2013) Glycosaminoglycan overproduction in the aorta increases aortic calcification in murine chronic kidney disease. J Am Heart Assoc 2:e00040523985378

References for Section 13.2

6. Matsuhashi T et al (2008) Alteration of *N*-glycans related to articular cartilage deterioration after anterior cruciate ligament transaction in rabbits. Osteoarthr Cartil 16:772–778
7. Urita A et al (2011) Alterations of high-mannose type *N*-glycosylation in human and mouse osteoarthritis cartilage. Arthritis Rheum 63:3428–3438
8. Seito N et al (2012) Interruption of glycosphingolipid synthesis enhances osteoarthritis development in mice. Arthritis Rheum 64:2579–2588
9. Sasazawa F et al (2014) Depletion of gangliosides enhances cartilage degradation in mice. Osteoarthr Cartil 22:313–322
10. Matsuoka M et al (2017) Depletion of gangliosides enhances articular cartilage repair in mice. Sci Rep 7:43729

Chapter 14
Glycans in Infection and Immunity

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14.1 Influenza Virus

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Keywords Influenza virus, Hemagglutin, Neuraminidase, Sialidase, Sulfatide

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1. Significance in the field of glycoscience and its current situation
   Influenza viruses are contagious respiratory pathogens and the viral infection is initiated by attachment of the viruses to sialic acid-containing sugar chains. The most severe influenza pandemic of 1918–19 caused over 50 million deaths worldwide. The influenza A and B viruses have two spike glycoproteins, hemagglutinin (HA) and neuraminidase (NA). A difference in the recognition of HA for molecular species of terminal sialic acids and sialic acid-galactose linkages, which are expressed on cells of the viral host, is a critical factor in the viral host range restriction. NA, which has sialidase enzymatic activity, plays an important role in the initiation of influenza virus infection and the budding of progeny virions from the host cell surface [1, 2]. Sialidase inhibitors based on the crystal structure of NA have been developed as anti-influenza drugs [3].
2. Impact on the other fields of research
   Anti-influenza drugs (sialidase inhibitors) have been developed using computer simulation based on the crystal structure of influenza virus NA. The interaction of HA with sialo-sugar chains would be interesting for the protein-glycan interaction.
3. Significance as the fundamental research
   Elucidation of the mechanisms of influenza virus replication, pathogenicity and transmission will be useful.
4. Possible application for industry and medicine, if any
   Sialic acid analogs (sialidase inhibitors) are widely used as anti-influenza drugs [3, 4].

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5. Future perspectives
   Other virus pathogens with sialidase activity including the human parainfluenza virus and mumps virus will also be useful. Compounds that inhibit binding of HA to sulfatide [5] would be likely candidates for anti-influenza drugs.
6. Problems to be solved
   Since there are no data regarding influenza virus receptors in host target cells, identification for the receptor(s) would be essential. This will take 10 years (Fig. 14.1).

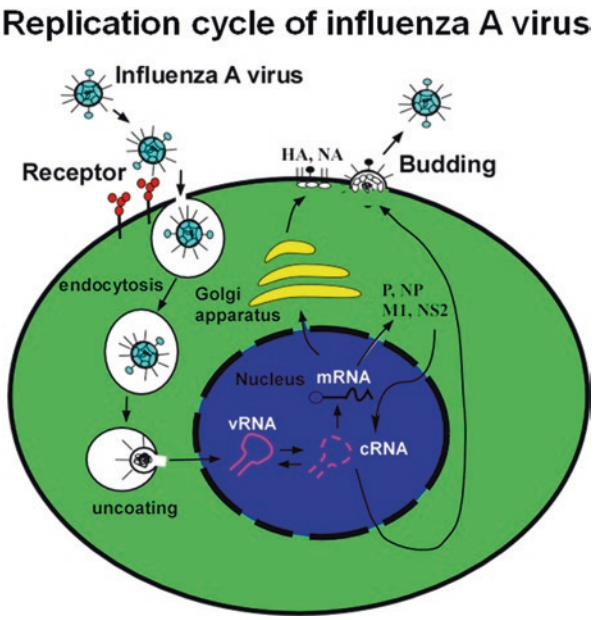

Fig. 14.1 Replication cycle of the influenza virus

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14.2 Recent Research Topics of Influenza

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Keywords Influenza virus, Hemagglutinin, Sialogycans, Interspecies transmission, Antiviral drug

1. Significance in the field of glycoscience and its current situation
   One of the viruses that have been known to use glycans as receptors is the influenza virus [6]. Human infections of animal influenza viruses has been reported, such as H5Nx highly pathogenic avian influenza viruses since 1997, H1N1 pandemic influenza in 2009, and recent H7N9 influenza virus infections since 2013 [7]. These cases cannot be explained only by relationship between the receptor specificity of viruses and sialoglycans in the host. Since it is becoming clear that there is a more complicated relationship between them, it is desirable to identify the true receptor of the virus and to predict virus transmission among hosts to control human and animal influenza.
2. Impact on the other fields of research
   By analyzing the expression profile of glycans in the host and sialoglycans recognized or used by the viruses, it is possible to predict virus transmission among hosts, especially the emergence of pandemic influenza viruses. These results can be applied to other virus infections in which the causative viruses use glycans as receptors.
3. Significance as the fundamental research
   Clarification of the diversity of sialoglycans expressed in humans and animals, and the expression kinetics before and after the infections will lead understanding of other infectious diseases. It is important to determine how virus binding is affected by sialic acids [8], and sulphated [9] and fucosylated [10] sialoglycans.
4. Possible application for industry and medicine, if any
   Neuraminidase inhibitors have been widely used as anti-influenza drugs, but ones that inhibit virus binding or internalization have not been successfully developed for clinical use. The search for sialic acid sugar chain derivatives strongly binding to hemagglutinin has the potential to reveal a new antiviral drugs.
5. Future perspectives
   Hemagglutinin is a lectin. It provides important knowledge for determining the specificity of other lectins, and hemagglutinin recognizes different glycans with several mutations in virus replication and contributes to host specificity.
6. Problems to be solved
   It is necessary to clarify the expression and dynamics of glycans of an influenza virus-infected host. In addition, it is necessary to clearly identify the glycans expressed in the host and the sugar chains really involved in the infection. This will be performed within 5 years (Fig. 14.2).

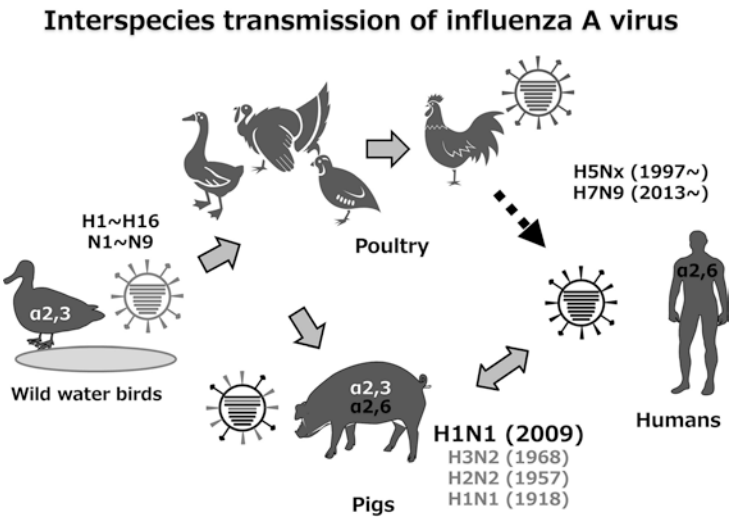

Fig. 14.2 Interspecies transmission of influenza A viruses. Human infections with animal influenza viruses have been reported in recent years, but it is not possible to explain this interspecies transmission by the receptor specificity of viruses and glycans expressed in host tissues. So the true receptors should be determined

Box 14.1: Influenza Drug Formulation

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Influenza viruses are classified as RNA viruses possessing negative-sense viral RNAs. They have hemagglutinin (HA) and neuraminidase (NA) on the surface of the viral particles. Influenza viruses bind through HA to sialic acid on the surfaces of target epithelial cells and then enter the cells. After replication, a large amount of influenza viruses is released from infected cells. NA is involved in the release of progeny virus from infected cells, by cleaving sialic acid on the cells binding to HA on the mature progeny viral particles. Therefore, NA is one of the targets for antiviral drugs. Typical NA inhibitors used against influenza are as follows; Oseltamivir (Tamiflu), Zanamivir (Relenza) and Laninamivir octanoate hydrate (Inavir). These drugs are used to treat flu symptoms caused by influenza virus in patients who have had symptoms for less than 2 days.

Box 14.2: Dengue Virus

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Dengue virus (DENV) is a single-stranded, positive-sense RNA flavivirus, which has three structural envelope (E), matrix (M) and capsid (C), and seven nonstructural (NS) proteins. Based on antigenic differences in the E protein, there are four serotypes of DENV. Dengue is a major arbovirus-borne infectious disease in tropical and subtropical regions of the world. About 390 million cases of dengue fever are estimated to occur each year globally. DENV cause a variable spectrum of disease that ranges from an undifferentiated fever to the potentially fatal dengue shock syndrome (DSS). Dengue hemorrhagic fever (DHF)/DSS is characterized by capillary permeability and a bleeding diathesis. DHF/DSS is an important cause of morbidity in Southeast Asia and occur almost exclusively in young children. Unlike the neighboring areas, most of the Taiwanese dengue patients are adults. The adult dengue has high morbidity and mortality. In 2015, Taiwan experienced its largest dengue outbreak since 1981. A cumulative total of 43,784 dengue cases, including 212 deaths have been confirmed. More effective and efficient integrated dengue control programs that include various surveillance systems, a network of rapid diagnostic laboratories, and rapid responses would be needed for better control of dengue. Especially early diagnosis of dengue is crucial to provide timely evidence-based case management because initial symptoms are often nonspecific.

14.3 Pathogens and GPI Anchors

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Keywords African trypanosome, Malaria parasites, Candida, Variant surface glycoproteins

1. Significance in the field of glycoscience and its current situation
   Trypanosomes, malaria parasites and other protozoan pathogens are unicellular organisms whose cell surfaces are covered by GPI-anchored proteins and/or protein-free GPI, which act to protect the organisms from the host immune system [11]. GPI-anchored proteins are also major components of the cell walls of pathogenic fungi such as Candida. In prokaryotic cells, there are no known GPI-anchored proteins, however, Mycobacteria, such as Tuberculosis bacilli, have abundant GPI-like glycolipids on their cell walls [12]. Because GPI-anchors and GPI-like glycolipids are essential for the growth of these pathogens or cell wall integrity, their biosynthetic pathways are promising targets for anti-pathogen drug development [13]. Therefore, studies on GPI-anchors in pathogens are important for infectious disease control. Because a host has similar GPI-anchors, it is critical to target pathogen-specific enzymes for selective toxicity. Drugs inhibitory for such targets are under screening [14].
2. Impact on other fields of research
   Protozoan parasites cause many diseases not only in humans but also in domestic animals. Knowledge obtained through research on human pathogens and GPI should have a big impact on veterinary medicine.
3. Significance as fundamental research
   GPI is a membrane-anchor of proteins ubiquitously used by eukaryotes. In particular, GPI is the major type of protein membrane anchor in single cell organisms. Research on GPI is therefore important for fundamental scientific research [11].
4. Possible application for industry and medicine, if any
   Protozoan diseases are prevalent mainly in developing countries and are often called neglected diseases [13]. They are not very attractive targets of pharmaceutical companies in developed countries, however, they may provide business opportunities for companies in developing countries.
5. Future perspectives
   Once a drug that effectively inhibits GPI biosynthesis in one particular pathogen is developed, similar drugs may be developed for inhibition of GPI biosynthesis in other pathogens.

6. Problems to be solved
   GPI biosynthesis is critical for the human body. To use inhibitors of GPI biosynthesis as anti-infection drugs, drugs that specifically inhibit GPI biosynthesis in pathogens but not in the host need to be developed [14]. This will occur within 10 years (Fig. 14.3).

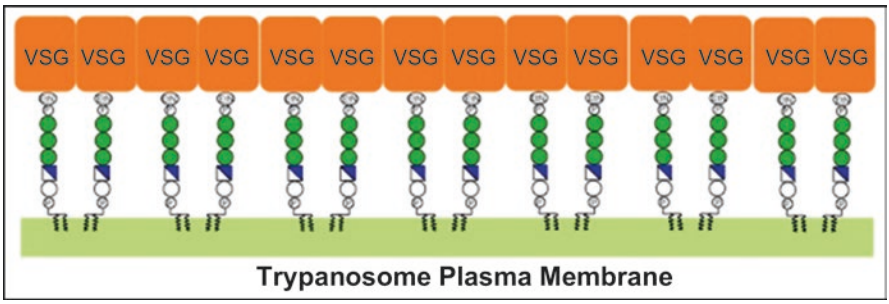

Fig. 14.3 The surface of African trypanosome, a unicellular parasite that causes sleeping sickness, is protected from the host immune system by a coat of GPI-anchored proteins, termed Variant Surface Glycoproteins (VSG)

14.4 Sugar Chain-Mediated Interaction Between Microbes and Higher Animals

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Keywords Symbiosis, Milk oligosaccharides, Mucin-type *O*-glycans

1. Significance in the field of glycoscience and its current situation
   Previous studies on sugar chain-mediated interactions between microbes and animals mainly focused on elucidation of how bacterial adhesions (sugar-binding proteins) recognize host sugar chain receptors and thereby trigger invasion into host cells. On the other hand, many researchers have recently been focusing on how host-derived sugar chains are involved in establishment of symbiosis between gut microbes and a host. Mucin glycoproteins and milk oligosaccharides, both of which are secreted into intestinal lumen of hosts, play important roles in shaping the gut microbiota [15, 19], which significantly influences host health and disease. Elucidation of sugar chain-driven symbiosis and dysbiosis will lead to a better understanding of the physiology as well as pathology of higher animals including humans.
2. Impact on the other fields of research
   Immunologists have significantly contributed to elucidation of the molecular basis of symbiosis between gut microbes and hosts. They approached this topic from the host (animal) side [16, 18]; however, undoubtedly, approaches from the microbe side are equally required. Fortunately, current studies on sugar chain (mucin glycoprotein and milk oligosaccharid)-mediated symbiosis and dysbiosis are being mainly conducted by microbiologists. Thus, researchers in both fields can effectively complement each other to obtain a comprehensive understanding of interactions between gut microbes and hosts.
3. Significance as the fundamental research
   Comprehensive understanding of how host-derived glycans affect the gut microbiota composition is anticipated. In addition, because gut microbial enzymes acting on host-derived glycans show very high substrate specificity, they can be utilized for precise determination of sugar chain structures.
4. Possible application for industry and medicine, if any
   Mucin sugar chains and milk oligosaccharides are promising targets for the development of functional foods and pharmaceuticals. Quite recently, Western countries put milk oligosaccharide 2′-fucosyllactose to practical use, i.e., they started to add the compound to formula milk for fortification.

5. Future perspectives
   Recent regeneration medical techniques have made it possible to differentiate iPSC from gut organoids [17]. Construction of a symbiosis model using individual intestinal cells and gut microbes may allow precise understanding of the sugar chain-mediated interactions between gut microbes and hosts.
6. Problems to be solved
   The number of genetically amenable gut microbes is very limited, despite that more than hundred bacterial species inhabit the intestines. The lack of available genetic tools for gut microbes has significantly hampered a detailed experimental approach from the microbe side in animal models. This will be resolved within 5 years (Fig. 14.4).

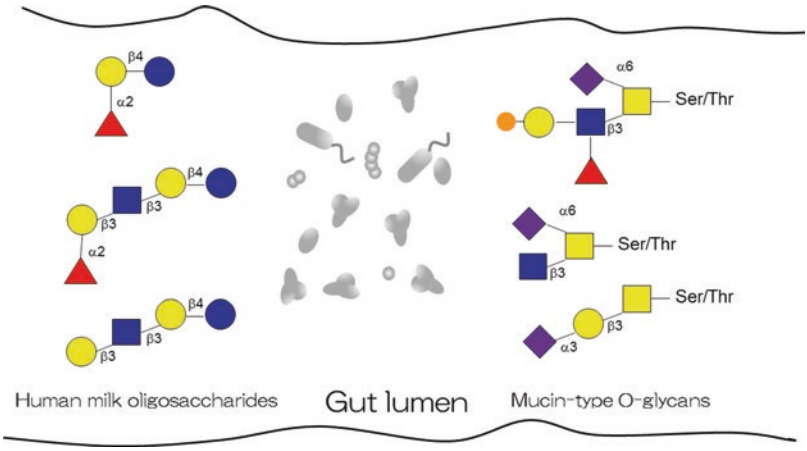

Fig. 14.4 Host-derived glycans significantly contribute to shaping of the gut microbiota

14.5 Current Status and Future Directions of Study on Glycosylation for Gut Microbiota and the Mucosal Immune Network

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Keywords Fut2, α1,2-fucose, Commensal bacteria, Autoimmune diseases, Mucosal immunity

1. Significance in the field of glycoscience and its current situation
   The study of the gut microbiota is now one of the hottest research fields in life science, in particular, the sugar chains expressed on host intestinal epithelial cells interact with microorganisms including commensal bacteria. The sugar chains on epithelial cells not only construct a symbiotic environment in the intestinal tract but also provide an opportunity for infection by pathogenic microorganisms, such as *Helicobacter pylori* and norovirus [20]. It has been reported that an inactive type gene polymorphism in Fut2, which is one of the glycosyltransferases, causes disturbance of the gut microbiota, and development of various infections and autoimmune diseases, such as Crohn's disease and type I diabetes [21]. Therefore, it is extremely important for glycoscience to understand the roles of glycochains and their regulation in the gut microbiota and mucosal immune network in the fields of biology and medicine.
2. Impact on the other fields of research
   The sugar chains, α1,2-fucose, expressed on host intestinal epithelial cells are closely related to regulation of the gut microflora. The host-bacterial interaction mediated by α1,2-fucose has a great impact on many research fields, such as microbiology, immunology, and cell biology. In the context of medical application, α1,2-fucose has been reported to be associated with various diseases, such as autoimmune diseases, cancer, and metabolic diseases as well as infectious diseases.
3. Significance as the fundamental research
   Numerous bacteria constantly colonize the intestinal tract and establish a peaceful symbiotic relationship with the host. Most of the mechanism of this symbiotic relationship between the host and gut microbiota remains unknown. It is biologically important to clarify the biological role of glycosylation in the symbiotic mechanism in the intestines.

4. Possible application for industry and medicine, if any
   As represented by viable bacterial drugs, research on the roles of glycosylation in the gut microflora and mucosal immunity will lead to novel industrial and medical applications. In addition, the sugar chains expressed on intestinal epithelial cells are closely related to infectious diseases involving pathogenic microorganisms and autoimmune diseases such as inflammatory bowel disease. Understanding of gut glycosylation will lead to the development of new preventive and therapeutic approaches for these diseases.
5. Future perspectives
   Sugar chains that interact with commensal bacteria are limited to specific molecules such as α1,2–fucose. The roles of other sugar chains in the regulation of the gut microbiota are expected to be clarified in future studies. Although it has been reported that immune cells are involved in the induction and regulation of α1,2-fucose [22, 23], more detailed elucidation of the role of glycosylation in the gut microbiota-immune network should be performed.
6. Problems to be solved
   Molecules derived from gut microflora for the induction of sugar chains such as α1,2-fucose expressed on intestinal epithelial cells remain unknown. In addition, the mechanism by which Fut2 and α1,2-fucose-deficient humans are susceptible to autoimmune diseases such as inflammatory bowel diseases should be clarified. This will take 10 years (Fig. 14.5).

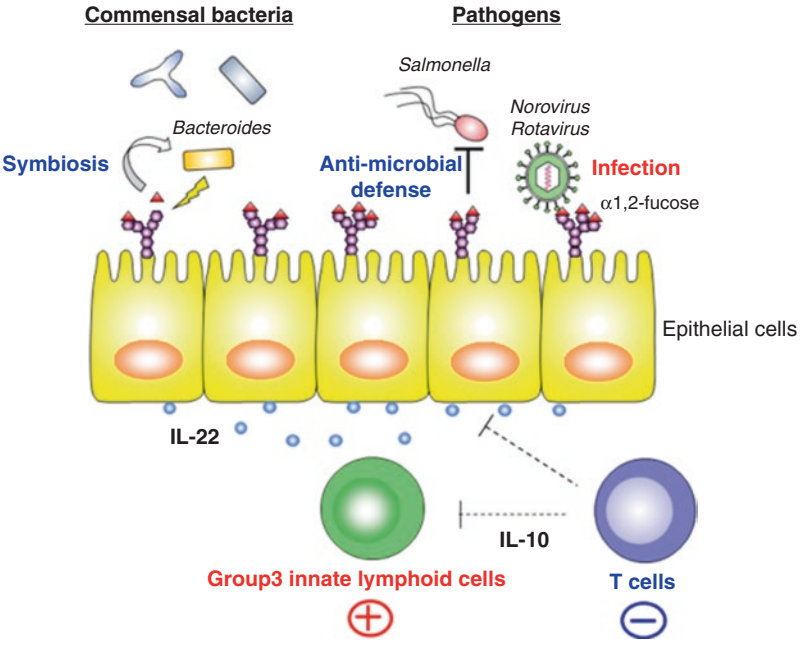

Fig. 14.5 α1,2-fucose is added to the end of a sugar chain expressed on the intestinal epithelial cells. α1,2-fucose creates a symbiotic environment for intestinal bacteria and eliminate pathogenic bacteria, but provides an opportunity for virus infection. α1,2-fucose is induced and regulated by immune cells such as group 3 innate lymphoid cells and T cells

14.6 Carbohydrate-Mediated Interactions Between Microbial and Higher Animal Cells (Fungi)

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Keywords Opportunistic infection, Fungi, Yeast, Glucan, Innate immune system

1. Significance in the field of glycoscience and its current situation
   Invasive aspergillosis and invasive candidiasis are major life-threatening nosocomial invasive fungal infections. Difficulty in the biological detection of invasive fungal infections is related to the low yields of culture-based methods. There is extensive literature on the diagnostic value of fungal polysaccharide detection, including that of β-(1,3)-glucan, and mannan or galactomannan, found in Aspergillus and Candida, respectively. In contrast, the innate immune system of mammals constituents the first line of defense against invading microbes. Fungal cell wall components (β-glucans, *N*- and *O*-linked mannans) are recognized by host receptors like C-type lectins (dectins, mannose receptor, etc.) and Toll-like receptors [24–28]. Such recognition leads to activation of a variety of host signaling cascades and production of anti-microbial compounds. Early diagnosis of a fungal infection has been shown to significantly increase the survival rate of the patient. Optimal exploitation of glucans and mannans in anti-cancer treatments, vaccines, and in priming host defenses will require a mechanistic understanding of how each receptor works.
2. Impact on the other fields of research
   A better understanding of the recognition mechanism by fungal cell wall components will allow not only improvement of the host defense against fungal pathogens but also analysis of the protein (enzyme or lectin)-carbohydrate interactions.
3. Significance as the fundamental research
   It is very important to examine the oligosaccharide structures and the existence of key enzymes (genes) of pathogenic fungi in the phylogenetic tree.
4. Possible application for industry and medicine, if any
   The development of new drugs and therapies to control fungal diseases, and immune strength-ameliorating food.
5. Future perspectives
   Understanding the recognition mechanisms for fungal cell wall components in mammals may facilitate the development of new drugs and therapies to control fungal diseases.
6. Problems to be solved
   Since there are no data regarding possible receptors for galactofuranose-containing oligosaccharides from Aspergillus sp., identification of the receptor(s) in vivo such as C-type and X-type lectins is essential. This will be performed within 5 years (Fig. 14.6).

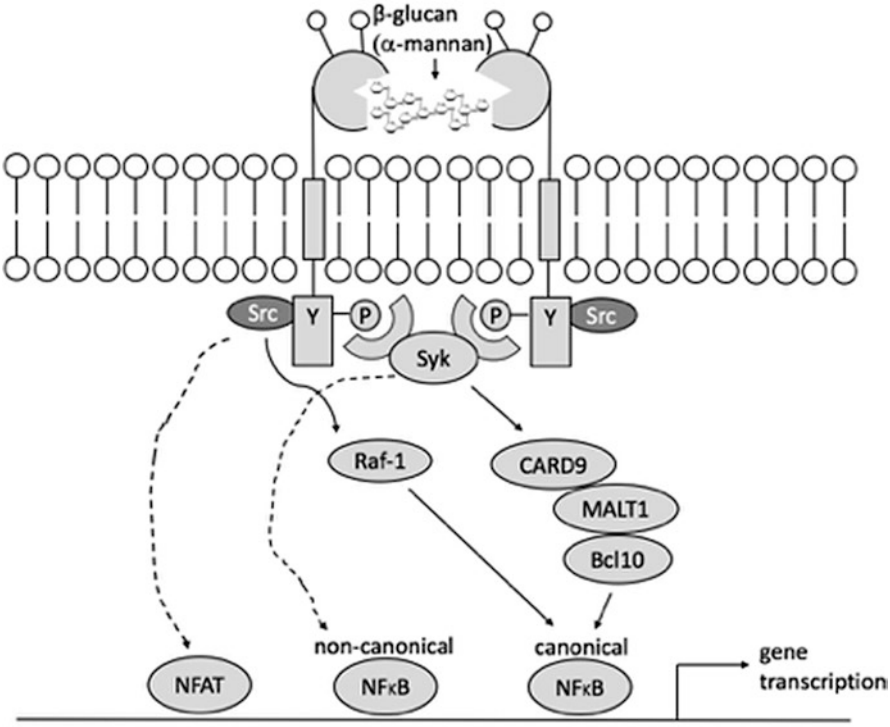

Fig. 14.6 Dectin-mediated signal transduction. On ligand binding, dectins become tyrosine phosphorylated, and induce an intracellular signaling cascade that results in various cell-specific responses. (Modified from Marakalala et al. [29])

14.7 *Plasmodium* and Protozoa in General

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Keywords *Plasmodium*, *Toxoplasma*, *Babesia*, Sialic acid, Heparan sulfate

1. Significance in the field of glycoscience and its current situation
   Protozoa are eukaryotic unicellular organisms with functions and structures similar to mammalian cells. Some protozoan species infect humans and animals, and induce clinical diseases with various degrees of pathogenicity. Therefore, protozoan parasites are often considered to be pathogens of veterinary and medical importance. At present, effective and safe therapeutic drugs and vaccines are not available for most protozoan diseases. Many pathogenic protozoan species invade the host cells, where they can survive and proliferate. Invasion of protozoa into the host cells is mediated by surface or secretory molecules of the parasites and receptors on the host cell membrane (sialic acids, heparan sulfate, etc.) [30–32]. Compounds similar to these carbohydrate receptors were found to block the cellular invasion by several protozoan parasites, including *Plasmodium*, *Toxoplasma*, *Babesia*, and *Cryptosporidium* [33, 34]. Thus, elucidation of the mechanisms associated with the receptor-ligand interaction will facilitate the development of novel therapeutic agents and vaccines.
2. Impact on the other fields of research
   Several pathogens, such as protozoa, viruses, bacteria, and Rickettsia, share conserved mechanisms for the recognition of carbohydrate receptors. Therefore, the findings of investigations on carbohydrate recognition by protozoa will be useful for elucidating the mechanisms adopted by other pathogens as well.
3. Significance as the fundamental research
   The mechanisms of parasitism and pathogenesis of protozoa will be revealed through elucidation of the molecular mechanisms associated with host cell recognition, invasion, internalization, replication, and egress.
4. Possible application for industry and medicine, if any
   Sugar chain derivatives with structures similar to the sugar moieties on the surface of host cells are potential inhibitors of protozoa growth. Thus, therapeutic agents can be developed based on sugar chain derivatives. Additionally, the protozoan molecules that are involved in host cell recognition could be potential candidates for subunit vaccines.

5. Future perspectives
   Babesia parasites do not infect erythrocytes with reduced sialic acid levels on their membranes. Similarly, a low heparan sulfate content in host cells results in a decreased infection rate of *Toxoplasma* parasites. Therefore, genetically engineered livestock animals resistant to *Babesia* and *Toxoplasma* infections could be created.
6. Problems to be solved
   Identification of sugar chain receptors on the host cell membrane and analyses of their structures are essential. After such structural analyses, the synthesis of sugar chain derivatives and their analogues, safety and toxicity testing, and evaluation as therapeutic agents are important before the novel compounds can be considered effective and safe. This will be accomplished within 5 years (Fig. 14.7).

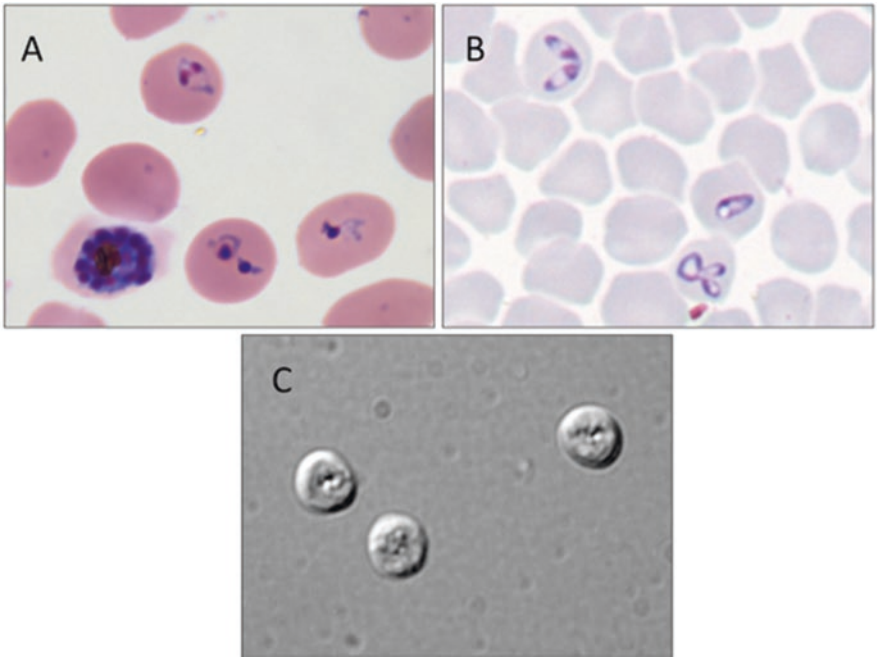

Fig. 14.7 Microscopic images of *Plasmodium* (**a**), *Babesia* (**b**), and *Cryptosporidium* (**c**)

14.8 GPI Anchor Deficiencies

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Keywords Paroxysmal nocturnal hemoglobinuria, Inherited glycosylphosphatidylinositol deficiency, Rare diseases, Intractable diseases, Anti-complement drugs

1. Significance in the field of glycoscience and its current situation
   There are two forms of GPI anchor deficiencies: paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disease whereas inherited glycosylphosphatidylinositol deficiency (IGD) is inherited [35–38]. Both are rare and designated intractable diseases by the Ministry of Health, Labour and Welfare of Japan. PNH is known for over 100 years and the estimated number of patients in Japan is approximately 1000 or bigger, whereas IGD was discovered in 2006 and the current numbers of patients in Japan and worldwide are approximately 30 and 200, respectively [39]. The number of reported IGD patients is quickly increasing with application of whole exome analysis for diagnosis of rare genetic diseases. Significance of studies on GPI deficiencies is that they help understanding physiological roles of GPI-anchored proteins in vivo.
2. Impact on other fields of research
   PNH is characterized by hemolysis and thrombosis caused by dysregulation of complement activation. Eculizumab, anti-C5 monoclonal antibody, is effective in preventing hemolysis and thrombosis in patients with PNH [37]. The successful use of eculizumab in PNH triggered trials on other diseases involving complement activation, which proved effectiveness of complement inhibition in atypical hemolytic uremic syndrome and other diseases, leading to active development of various anti-complement drugs.
3. Significance as fundamental research
   Research on PNH and IGD will demonstrate clinical symptoms that occur when levels of 150 or more GPI-anchored proteins are reduced to various extents. These findings will lead to understanding of physiological roles of various GPI-anchored proteins.
4. Possible application for industry and medicine, if any
   Eculizumab, an anti-C5 monoclonal antibody, has been used effectively and safely for many patients with PNH [37]. This stimulated development of various anti-complement drugs for a number of diseases, in which complement-activation is suspected to play roles in clinical symptoms.
5. Future perspectives
   Clinical symptoms of IGD vary in the severity and the affected organs/tissues, dependent upon what gene is affected and how deeply the mutations affect functions of the gene. IGD therefore is a typical disease that requires development of patient-specific therapeutic measures [38, 39]. Progress in development of therapies of IGD will contribute to advance precision medicine.

6. Problems to be solved
   Development of therapeutic measures for IGD is a current problem. In particular, drugs that restore reduced biosynthesis of GPI in patients' cells need to be developed (Fig. 14.8).

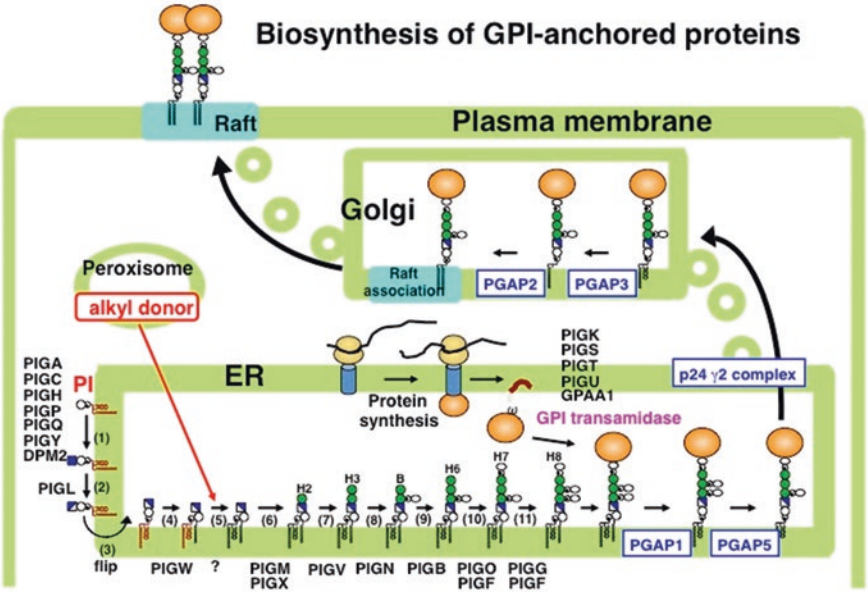

Fig. 14.8 More than 25 proteins working in the endoplasmic reticulum and the Golgi apparatus are involved in biosynthesis of GPI-anchored proteins. Mutations in genes for those proteins cause PNH and IGD. Genes for biosynthesis and protein attachment of GPI are termed PIG genes whereas those involved in maturation of GPI after protein attachment are termed PGAP genes

14.9 Defense Mechanism

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Keywords Defense mechanism, Innate immunity, Microorganism, Pattern recognition receptors (PRRs)

1. Significance in the field of glycoscience and its current situation
   In the biological defense mechanism against infectious diseases, microbial cell-surface molecules, especially glycans and glycoconjugates, play an important role in activating the immune system [40–44]. Namely, these sugar chains and glycoconjugates from various microorganisms are recognized by innate immunity receptors such as TLR, CLR, NLR, etc., which leads to activation of the immune system against infection, and to modulation of the balance of cellular and humoral immunity. It also leads to activation of the acquired immune system, in which the antibody production is enhanced. These research fields in Japan are one of the front lines in the world, especially regarding the discovery of innate immune receptors and their ligands, and also their important functions.
2. Impact on the other fields of research
   The defense mechanism against infectious diseases, especially activation of the immune system and modulation of the immune balance, is closely related to the immunity in the anti-cancer mecahnism, transplant tolerance and rejection, and also allergies, etc. Therefore, the findings in this research field have great impacts on the development in these other related fields.
3. Significance as the fundamental research
   Understanding the molecular basis of the defense mechanism, which includes the structures and functions of cell-surface sugar chains and glycoconjugates, is fundamental for the comprehension of and development of therapies for various diseases including infectious ones.
4. Possible application for industry and medicine, if any
   Immunomodulatory carbohydrate compounds derived from microorganisms have been the key compounds as to understanding the defense system and also for developing medicines (including immune adjuvants) for infections, cancer, transplant rejection and allergies.
5. Future perspectives
   Understanding of microbial glycans/glycoconjugates will lead to comprehensive understanding of our own endogenous glycans/glycoconjugates, based on the molecular structural similarities and differences, which would lead to comprehension of the defense system for various diseases.
6. Problems to be solved
   Because of the difficulty in obtaining or synthesizing microbial glycans and glycoconjugates comprehensively, many structure-activity relationships have not been revealed yet. This will take 10 years (Fig. 14.9).

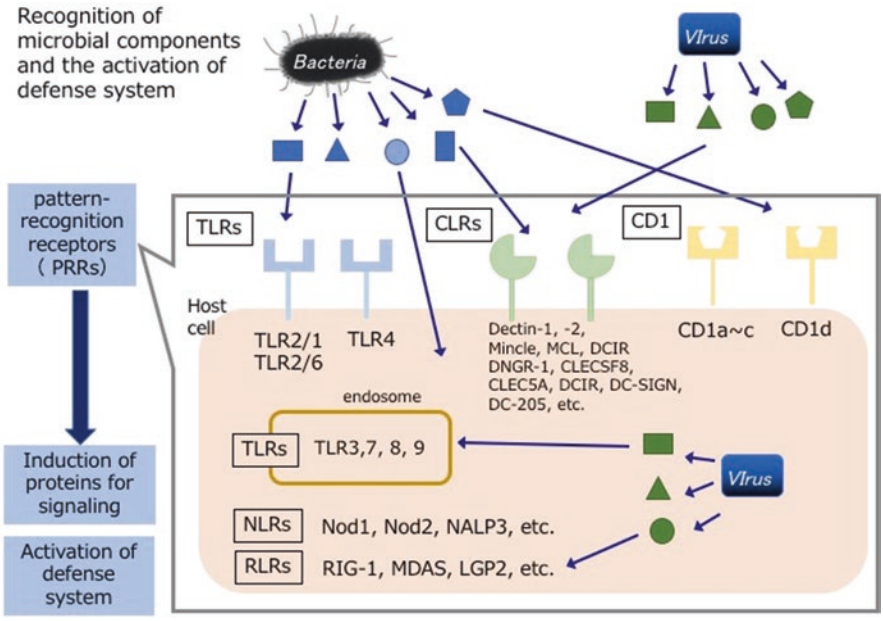

Fig. 14.9 Microbial molecules are recognized by pattern recognition receptors (PPRs) of the host cells, and activate the defense mechanism via the induction of proteins for the signaling

14.10 Siglecs

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Keywords Sialic acids, Self-nonself discrimination, Immune checkpoint molecule

1. Significance in the field of glycoscience and its current situation
   Siglecs comprise a family of receptor-type lectins that recognize glycans containing sialic acids [45–47]. The distribution of sialic acids in nature is limited to deuterostomes (including vertebrates and echinoderms), microbes that express sialic acids being rare. Therefore, sialic acids can be a molecular marker of "self" for the immune system of vertebrates. Siglecs are a part of the immune recognition machinery that recognizes sialic acids as a marker of self, and contribute to the self-nonself discrimination by the immune system. Siglecs are as important glycan-related molecules as C-type lectins and galectins. The basic functions of Siglecs have been elucidated through studies on the knockout mice that lack a Siglec gene. Although there are notable differences between human and mouse Siglecs, knowledge on the functions of human Siglecs is also accumulating though studies of genetic polymorphisms in human Siglec genes and human phenotypes, such as disease susceptibility.
2. Impact on the other fields of research
   As a group of molecules that recognize glycans and participate in self-nonself discrimination, studies of the Siglec family may make as essential contributions as those on C-type lectin-like receptors to the understanding of the immune system.
3. Significance as the fundamental research
   Discovery of the Siglec family has provided an answer to a long-standing question, i.e., "why do sialic acids exist?"
4. Possible application for industry and medicine, if any
   Antibody therapies targeting some Siglecs to treat leukemia/lymphoma are already in the advanced stages of clinical development. Recent studies have revealed that some Siglecs may play similar roles as immune checkpoint molecules, and thus it is implied that blockade of these Siglecs may be beneficial for the treatment of some types of cancer.
5. Future perspectives
   It is suggested that Siglecs may be useful as targets of drug and/or antigen delivery.

6. Problems to be solved, and the years that will take to solve the problems
   It is already possible to target Siglecs with antibodies, whereas the targeting of Siglecs using glycans still requires further study [48]. Development of glycan-based ligands with high affinity and selectivity is awaited. This will take 10 years (Fig. 14.10).

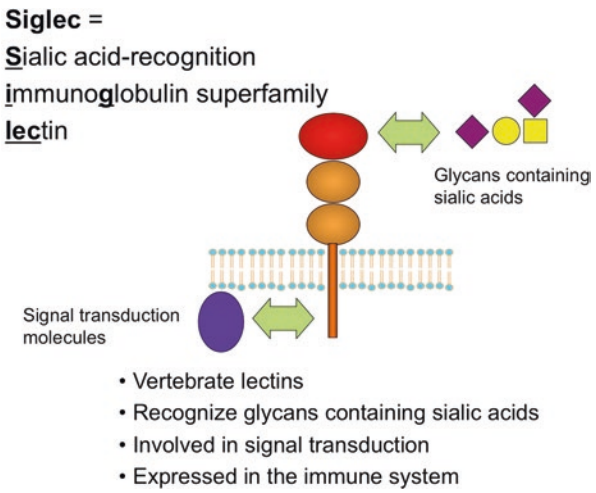

Fig. 14.10 The extracellular domain of Siglecs consists of multiple immunoglobulin-like folds, and the amino-terminal domain is primarily responsible for their interaction with glycans. Most Siglecs also associate with signal transduction molecules at the intracellular or transmembrane domain, and participate in signal transduction

14.11 Collectins

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Keywords Lectin, Collagen, Complement, Host defense, Neuronal maturation

1. Significance in the field of glycoscience and its current situation
   Collectins comprise a family of C-type lectins possessing a collagen like-region in their structure. Six types of collectins are found in humans [49]. Pulmonary collectins (SP-A and SP-D) have emerged in terrestrial animals with opsonizing activation as their main function with no complement system activation activity. The other four types of collectins (MBL (MBP), CL-K1, CL-L1 and CL-P1) all activate the complement system through the recognition of bacterial surface carbohydrates. Once bacteria or any other microorganisms are recognized, they are either phagocytized via opsonization or led to cell lysis through disruption of the cell wall via the complement cascade as a part of the biological defense system [49]. Therefore, the bacterial surface carbohydrate pattern recognition function of collectins is suggested to play an important role in the innate immune system [50].
2. Impact on the other fields of research
   Mutation in the *CL-K1/L1* or *MASP3* gene has been determined to cause 3MC syndrome, and CL-K1/L1 induced complement activation has been found to play a role in an organism's development [51]. This complement activation is reported to be involved in neuronal maturation in neonatal animal models [52].
3. Significance as the fundamental research
   The lancelets, the oldest vertebrates have more than 60 collectin genes. Six of these genes are conserved in humans [53]. Collectins in humans are assumed to have become indispensable for survival by linking the carbohydrate recognition function to many biological functions.
4. Possible application for industry and medicine, if any
   MBL and SP-D are possible drug candidates for direct virus inhibitors or antibiotics. CL-K1, CL-L1 and CL-P1 are possible drug candidates for neuronal maturation factors.
5. Future perspectives
   There have been many reports about the role of collectins in neuronal maturation or migration in animal models, and they may allow a better understanding of the pathologies of neurological disorders. This may lead to the early diagnosis of the disorders or the development of new drugs.
6. Problems to be solved
   The current understanding is based on the studies on model organisms. The functions of collectins in humans need to be analyzed. This will be performed within 5 years (Fig. 14.11).

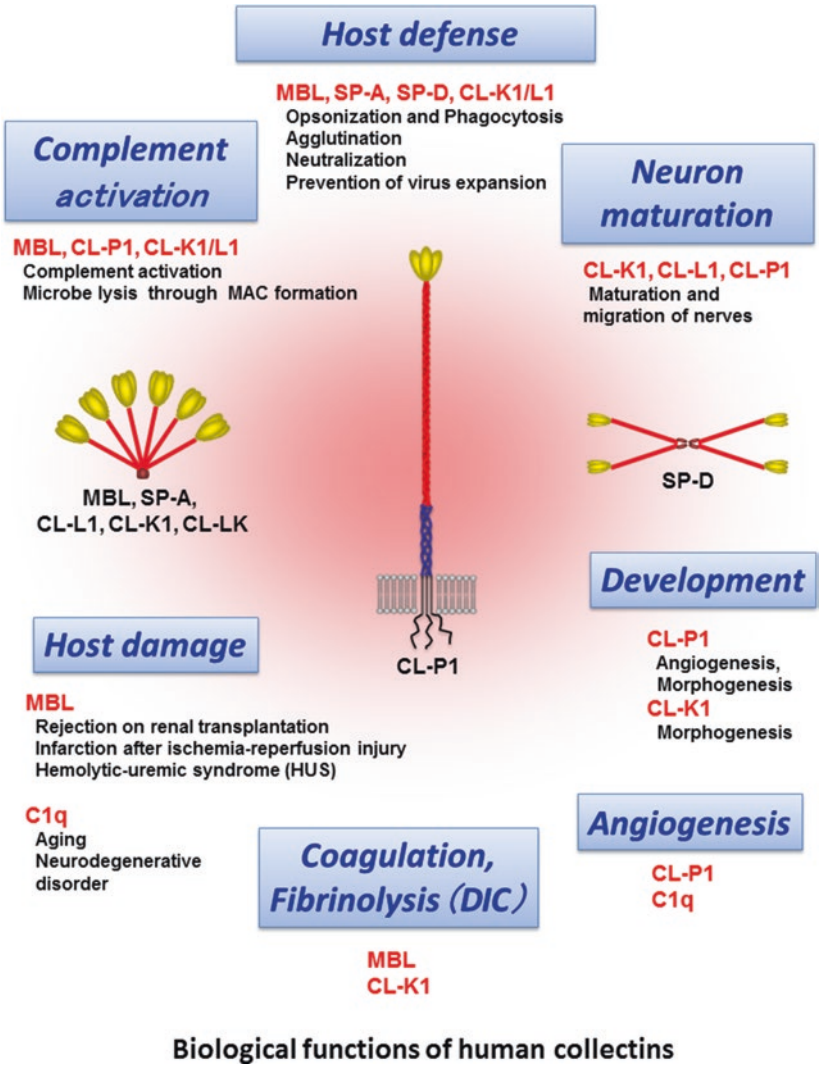

Fig. 14.11 Biological functions of human collectins

14.12 Autoimmune Disease, Immunosuppressive Drugs

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Keywords Inhibitory receptor, Inflammation, Autoimmunity, Immune suppression

1. Significance in the field of glycoscience and its current situation
   Rheumatoid arthritis, systemic lupus erythematosus and type I diabetes are representative autoimmune diseases. Although they are thought to be caused by an immune response against self-antigens, the mechanisms of autoimmune diseases have remained unclear [54]. On the other hand, immune cells express a variety of inhibitory receptors such as PILR and Siglec that recognize sugar chain structures, and these receptors play an important role in regulation of immune responses [55–57]. Therefore, it is important to develop certain molecules that are specifically directed forward sugar chain-recognizing inhibitory receptors. However, such a molecule has not been identified yet. Therefore, development of regulatory molecule targeting sugar chain-recognizing receptors would be important for immune regulation.
2. Impact on other fields of research
   Sugar chain-recognizing receptors are involved in herpes simplex virus infection and varicella zoster virus infection [58]. In addition, some cancers show immune evasion via sugar chain-recognizing inhibitory receptors. Therefore, development of a method to regulate sugar chain-recognizing inhibitory receptors is important not only for autoimmune diseases but also for viral infections and cancers.
3. Significance as fundamental research
   Sugar chains are present on all cells in the body. Therefore, elucidation of how these sugar chains are involved in immune responses is important to understand the immune regulatory mechanism and immune cell development.
4. Possible application for industry and medicine, if any
   Because sugar chain-recognizing inhibitory receptors are involved in immune regulation as well as viral infection, certain molecules that regulate sugar chain-recognizing inhibitory receptors could be candidates for immune regulatory drugs, anti-virus drugs and anti-tumor drugs.

5. Future perspectives
   Studies on sugar chain recognition by immune cells have not focused on immunology so much. However, considering that there are a lot of sugar chain-recognizing inhibitory receptors, further analyses of these sugar chain-recognizing inhibitory receptors would be important.
6. Problems to be solved
   The exact binding specificity of many sugar chain-recognizing inhibitory receptors has remained unclear. Sugar chain-recognizing inhibitory receptors have been thought to recognize only the sugar chain structure. However, some receptors such as PILRα recognize both sugar chain structure and the protein structure. Further analyses of recognition by these receptors would be important (Fig. 14.12).

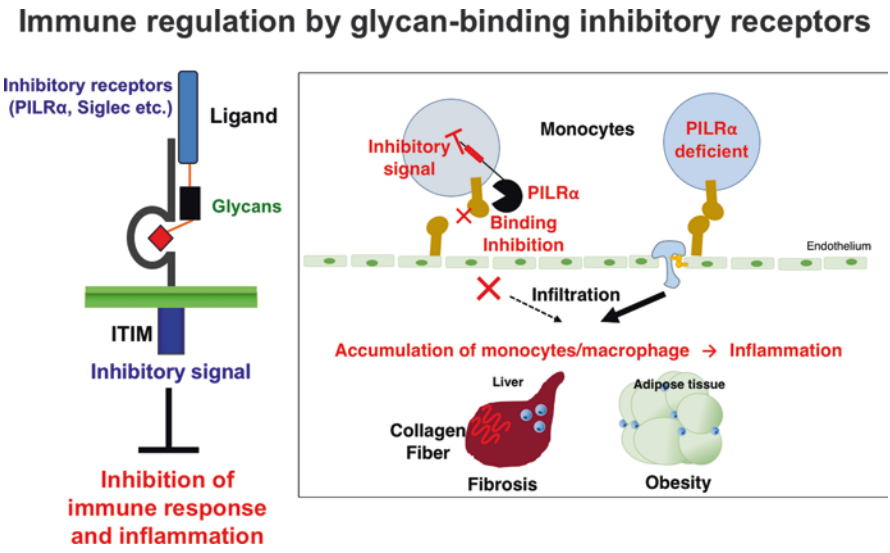

Fig. 14.12 Immune receptors express a variety of inhibitory receptors that recognize the sugar chain structure. In particular, PILRα, which is mainly expressed on monocytes and macrophages, plays an important role in the regulation of inflammation [55–57]

References

References for Section 14.1

1. Wright PF et al (2013) Orthomyxoviruses. In: Knipe DM, Howley PM (eds) Fields virology sixth edition. Lippincott Williams & Wilkins, Philadelphia, pp 1186–1243
2. Suzuki T et al (2005) Sialidase activity of influenza a virus in an endocytic pathway enhances viral replication. J Virol 79:11705–11715
3. Laborda P et al (2016) Influenza neuraminidase inhibitors: synthetic approaches, derivatives and biological activity. Molecules 21:1513
4. von Itzstein M et al (1993) Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature 363:418–423
5. Takahashi T et al (2008) Sulfatide is required for efficient replication of influenza a virus. J Virol 82:5940–5950

References for Section 14.2

6. Rogers GN, Paulson JC (1983) Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin. Virology 127:361–373
7. Webster RG, Govorkoba EA (2014) Continuing challenges in influenza. Ann N Y Acad Sci 1323:1115–1139
8. Takahashi T et al (2014) *N*-glycolylneuraminic acid on human epithelial cells prevents entry of influenza a viruses that possess *N*-glycolylneuraminic acid binding ability. J Virol 88:8445–8456
9. Ichimiya T et al (2014) Frequent glycan structure mining of influenza virus data revealed a sulfated glycan motif that increased viral infection. Bioinfomatics 30:706–711
10. Hiono T et al (2014) A chicken influenza virus recognizes fucosylated α2,3sialoglycan receptors on the epithelial cells lining upper respiratory tracts of chickens. Virology 456–457:131–138

References for Section 14.3

11. Ferguson MAJ et al (2017) Glycosylphosphatidylinositol anchors. In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Darvill A, Kinoshita T, Packer NJ, Prestegard J, Schnaar R, Seeberger P (eds) Essentials of Glycobiology, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
12. Morita YS et al (2011) Inositol lipid metabolism in mycobacteria: biosynthesis and regulatory mechanisms. Biochim Biophys Acta 1810:630–641
13. Field MC et al (2017) Anti-trypanosomatid drug discovery: an ongoing challenge and a continuing need. Nat Rev Microbiol 15:217–231
14. Smith TK et al (2004) Chemical validation of GPI biosynthesis as a drug target against African sleeping sickness. EMBO J 23:4701–4708

References for Section 14.4

15. Yamada C et al (2017) Molecular insight into evolution of symbiosis between breast-fed infants and a member of the human gut microbiome Bifidobacterium longum. Cell Chem Biol 24:515–524
16. Tsuboi K et al (2015) Autophagy protects against colitis by the maintenance of normal gut microflora and secretion of mucus. J Biol Chem 290:20511–20526
17. Ozawa T (2015) Generation of enterocyte-like cells from human induced pluripotent stem cells for drug absorption and metabolism studies in human small intestine. Sci Rep 5:16479
18. Kawashima H (2012) Roles of the gel-forming MUC2 mucin and its *O*-glycosylation in the protection against colitis and colorectal cancer. Biol Pharm Bull 35:1637–1641
19. Fujita K et al (2005) Identification and molecular cloning of a novel glycoside hydrolase family of core 1 type *O*-glycan-specific endo-α-*N*-acetylgalactosaminidase from *Bifidobacterium longum*. J Biol Chem 280:37415–37422

References for Section 14.5

20. Goto Y et al (2016) Epithelial glycosylation in gut homeostasis and inflammation. Nat Immunol 17:1244–1251
21. Maroni L et al (2015) Fucosyltransferase 2: a genetic risk factor for primary sclerosing cholangitis and Crohn's disease--a comprehensive review. Clin Rev Allergy Immunol 48:182–191
22. Goto Y et al (2015) IL-10-producing CD4(+) T cells negatively regulate fucosylation of epithelial cells in the gut. Sci Rep 5:15918
23. Goto Y et al (2014) Innate lymphoid cells regulate intestinal epithelial cell glycosylation. Science 345:1254009

References for Section 14.6

24. Brown G (2006) Dectin-1: a signalling non-TLR pattern-recognition receptor. Nat Rev Immunol 6:33–38
25. Kerrigan AM, Brown GD (2011) Syk-coupled C-type lectins in immunity. Trends Immunol 32:151–156
26. Netea MG et al (2008) An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat Rev Microbiol 6:67–78
27. Legentil L et al (2015) Molecular interactions of β-(1→3)-glucans with their receptors. Molecules 20:9745–9766
28. Wesener DA et al (2017) Recognition of microbial glycans by soluble human lectins. Curr Opin Struct Biol 44:168–178
29. Marakalala MJ et al (2011) Dectin-1: a role in antifungal defence and consequences of genetic polymorphisms in humans. Mamm Genome 22:55–65

References for Section 14.7

30. Bork S et al (2004) Growth-inhibitory effect of heparin on *Babesia* parasites. Antimicrob Agents Chemother 48:236–241
31. Yokoyama N et al (2006) Erythrocyte invasion by *Babesia* parasites: current advances in the elucidation of the molecular interactions between the protozoan ligands and host receptors in the invasion stage. Vet Parasitol 138:22–32
32. Takabatake N et al (2007) Glycophorin A-knockout mice, which lost sialoglycoproteins from the red blood cell membrane, are resistant to lethal infection of *Babesia rodhaini*. Vet Parasitol 148:93–101
33. Kobayashi K et al (2013) Analyses of interactions between heparin and the apical surface proteins of *Plasmodium falciparum*. Sci Rep 3:3178
34. Inomata A et al (2015) Heparin interacts with elongation factor 1α of *Cryptosporidium parvum* and inhibits invasion. Sci Rep 5:11599

References for Section 14.8

35. Freeze HH et al (2017) Chapter 45: Genetic disorders of glycosylation. In: Varki A, Cummings RD, Esko JD, Stanley P, Hart GW, Aebi M, Darvill A, Kinoshita T, Packer NJ, Prestegard J, Schnaar R, Seeberger P (eds) Essentials of glycobiology, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
36. Takeda J et al (1993) Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. Cell 73:703–711
37. Hill A et al (2017) Paroxysmal nocturnal haemoglobinuria. Nat Rev Dis Primers 3:17028
38. Almeida A et al (2006) Hypomorphic promoter mutation in the mannosyltransferase-encoding PIG-M gene causes inherited glycosylphosphatidylinositol deficiency. Nat Med 12:846–851
39. Tanigawa J et al (2017) Phenotype-genotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties. Hum Mutat 38:805–815

References for Section 14.9

40. Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34:637–650
41. Caruso R et al (2014) NOD1 and NOD2: signaling, host defense, and inflammatory disease. Immunity 41:898–908
42. Johannssen T, Lepenies B (2017) Glycan-based cell targeting to modulate immune responses. Trends Biotechnol 35:334–346
43. Kimura Y et al (2016) The innate immune receptor Dectin-2 mediates the phagocytosis of cancer cells by Kupffer cells for the suppression of liver metastasis. Proc Natl Acad Sci U S A 113:14097–14102
44. Hansen JD et al (2011) Sensing disease and danger: a survey of vertebrate PRRs and their origins. Dev Comp Immunol 35:886–897

References for Section 14.10

45. Crocker PR et al (2007) Siglecs and their roles in the immune system. Nat Rev Immunol 7:255–266
46. Pillai S et al (2012) Siglecs and immune regulation. Annu Rev Immunol 30:357–392
47. Macauley MS et al (2014) Siglec-mediated regulation of immune cell function in disease. Nat Rev Immunol 14:653–666
48. Angata T et al (2015) Therapeutic targeting of Siglecs using antibody- and glycan-based approaches. Trends Pharmacol Sci 36:645–660

References for Section 14.11

49. Ohtani K et al (2012) Biological functions of the novel collectins CL-L1, CL-K1, and CL-P1. J Biomed Biotechnol 2012:493945
50. Medzhitov R, Janeway C Jr (2000) The toll receptor family and microbial recognition. Trends Microbiol 8:452–456
51. Rooryck C et al (2011) Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome. Nat Genet 43:197–203
52. Gorelik A et al (2017) Developmental activities of the complement pathway in migrating neurons. Nat Commun 8:15096
53. Huang S et al (2008) Genomic analysis of the immune gene repertoire of amphioxus reveals extraordinary innate complexity and diversity. Genome Res 18:1112–1126

References for Section 14.12

54. Arase H et al (2016) Rheumatoid rescue of misfolded cellular proteins by MHC class II molecules: a new hypothesis for autoimmune diseases. Adv Immunol 129:1–23
55. Kohyama M et al (2016) Monocyte infiltration into obese and fibrilized tissues is regulated by PILRα. Eur J Immunol 46:1214–1223
56. Kishida K et al (2015) Negative regulation of DSS-induced experimental colitis by PILRα. Int Immunol 27:307–314
57. Wang J et al (2012) Neutrophil infiltration during inflammation is regulated by PILRα via modulation of integrin activation. Nat Immunol 14:34–40
58. Satoh T et al (2008) PILRα is a herpes simplex virus-1 entry coreceptor that associates with glycoprotein B. Cell 132:935–944

Chapter 15
Next Generation Medical Care

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15.1 Roles of Glycoscience in Development of Personalized Medicine and Rational Medical Practices

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Keywords Progression of degeneration, Regeneration process, Atrophy, Scaring

1. Significance in the field of glycoscience and its current situation
   It is glycoscience for us to use the information of carbohydrate structures determined as knowledge to understand nature of life. Indeed, glycoscience has accelerated linking between etiology of a disease and the presence of unique carbohydrates. Based on this advancement, medical practices have come to use the information for biomarkers to develop personalized medicine.

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2. Impact on the other fields of research
   The determined carbohydrate structures allow understanding of the nature of life, especially promoting understanding the link with homeostasis, and the progression of degenerative diseases. A series of results from glycoscience will lead to novel concepts regarding the above diseases.
3. Significance as the fundamental research
   A prediction through the history of glycoscience is important. Concretely, basic research in glycoscience is expected to provide clues for understanding the functions and roles linked with the etiologies and pathogenesis of some diseases that have not been well established as to diagnosis and treatment.
4. Possible application for industry and medicine, if any
   Established biomarkers will promote the development of rational diagnostic approaches involving blood samples for the progression of some diseases. Personalized medicines will use this advantage for disease progression of degenerative diseases to define the progression [1–4].
5. Future perspectives
   Progression of degeneration, regeneration, atrophy, and scaring has not been parametrically determined. Established biomarkers will be applied to measure the progression to find pharmaceutical and medical practical targets.
6. Problems to be solved
   Information on glycan structures should be organized with pathological knowledge, from which pathophysiological understandings can be reconstructed along with facts in glycoscience. This will be established within 10 years (Fig. 15.1).

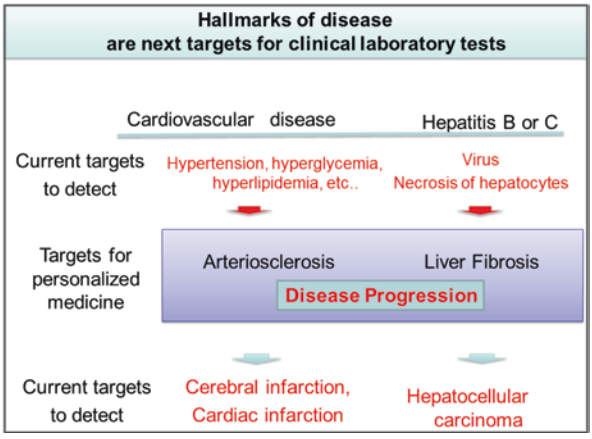

Fig. 15.1 Glycobiomarkers are expected to measure the progression of arteriosclerosis and liver fibrosis

15.2 Health Monitoring

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Keywords Aging, Biomarker, IgG glycosylation, Medical check

1. Significance in the field of glycoscience and its current situation
   Care for older people and preventive medicine are very important in twenty-first century medicine. Increased medical expense is a serious problem and medicine can not make humans happy in all cases. To prevent life-style associated disease such as hypertension and hyperlipidemia, health monitoring is very important. To know about human aging and/or a healthy status, establishment of biomarkers is required. It is a well-known fact that the oligosaccharide structures on glycoproteins change on birth, differentiation, carcinogenesis and aging. Among many serum glycoproteins, IgG oligosaccharides dramatically change on aging and inflammation [5, 6]. To apply changes in IgG glycosylation to medical checks, more convenient and simple assay system is required other than HPLC and mass spectrometry.
2. Impact on the other fields of research
   Health monitoring is associated with researche on aging, atherosclerosis, and cancer. It might also be linked to sports research [7, 8].
3. Significance as the fundamental research
   Health monitoring is not associated with basic research. However, it is important to investigate the underlying molecular mechanism that links a biomarker for health monitoring of aging and disease prevention.
4. Possible application for industry and medicine, if any
   Health monitoring could be applied to various fields such as medical checks, preventive medicine, and food manufacture.
5. Future perspectives
   When many Japanese people are rich and interested in their own health, research on health monitoring is interesting.
6. Problems to be solved
   Health monitoring research requires more objective and scientific estimation. It is very important to make a differential diagnosis of non-alcoholic steatohepatitis from fatty liver in a medical check [9]. This will take more than 10 years (Fig. 15.2).

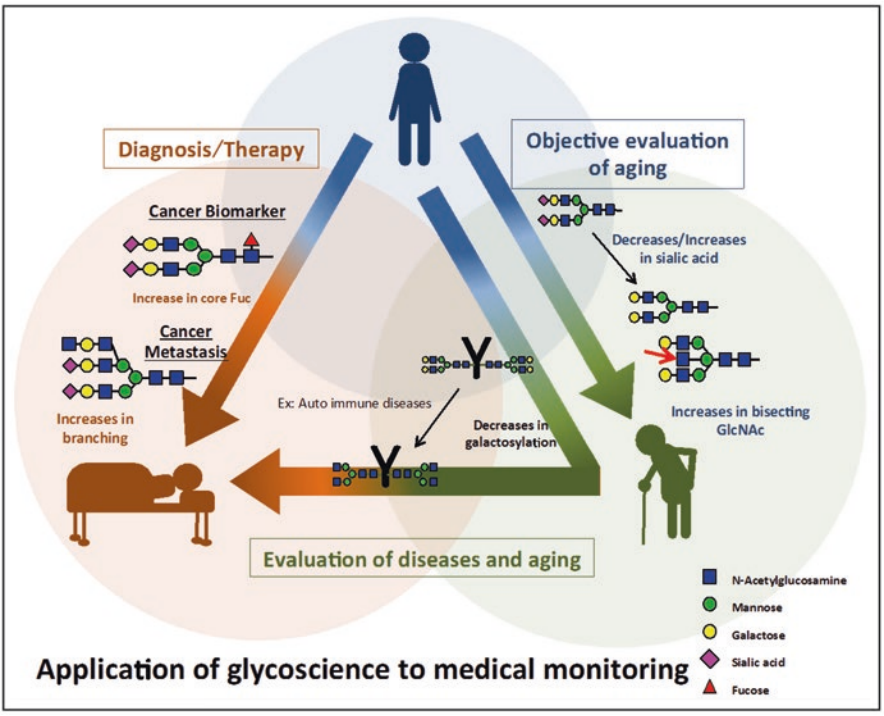

Fig. 15.2 The oligosaccharide structures on glycoproteins change with disease progression and aging. IgGs are the most abundant serum glycoproteins and their oligosaccharide structures have been investigated in terms of immune diseases and aging from long time ago. To be more specific for human diseases, the combination of IgG glycan changes and other glyco-biomarkers is required

15.3 Presymptomatic Diagnosis, Particularly for Dementia, Cancer and Hydrocephalous

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Keywords Presymptomatic phase, Cancer, Dementia, Biomarker

1. Significance in the field of glycoscience and its current situation
   It is undoubtedly important for dementia and cancer how early they are detected. To overcome these diseases, detection of the presymptomatic phase, which is prior to the early disease phase without any symptoms, is highly effective. A change in a specific glycan has been revealed to be a good marker for various diseases (particularly for cancer), and we already know that some glycan alterations indeed occur concomitantly with the development of dementia or cancer. In the future, use of those glycan changes as presymptomatic markers will be required. That such a nice glycan biomarker for presymptomatic detection has not been found so far this is a significant challenge to be tackled in the glycoscience field.
2. Impact on the other fields of research
   A presymptomatic marker has not been developed for not only glycans but also for other biological molecules, other than gene mutation [10, 11]. For multicomponent diseases like cancer and dementia, markers reflecting the state of organism are desirable. Discovery of presymptomatic marker glycans would accelerate development of further presymptomatic markers derived from other biological molecules.
3. Significance as the fundamental research
   The presymptomatic phase is the very initial step in the pathway to disease and a turning point as to whether the disease develops or not. For serious medical problems to be solved such as cancer and dementia, detection of the presymptomatic phase is the first step to completely overcome these diseases.
4. Possible application for industry and medicine, if any
   Identification of the glycan molecules specifically reflecting presymptomatic states would lead to the necessity of a way or kit to measure these molecules, and accelerate the development of drugs and technologies to recover the state to a healthy one or to stop further progress of the presymptomatic states.
5. Future perspectives
   If you can detect dementia and cancer in the presymptomatic phase and further reverse it, the appearance of symptoms can be significantly delayed. Even though aging of the population will proceed in future, extension of a healthy life span would result in a decrease in healthcare cost and maintenance of the working population.

6. Problems to be solved
   The most difficult challenge is to identify the molecules specifically reflecting the presymptomatic phases of dementia and cancer. To achieve this, we need to collect samples from healthy people without any symptoms over a long period of time, and analyze glycan changes. This will take more than 10 years (Fig. 15.3).

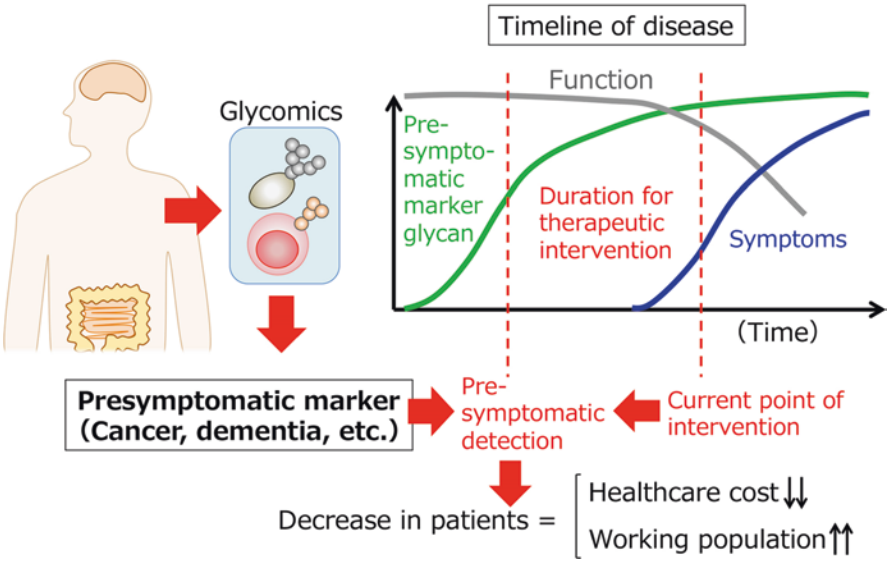

Fig. 15.3 Development of presymptomatic glycan markers would enable extension of the duration for therapeutic intervention, resulting in decreases in patients and healthcare cost, and an increase in the working population

15.4 Core Technologies for Elucidating the Role of Glycans in Disease

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Keywords Exome sequencing, Mutation, Phenotype, Loss of function, Structure-function relationship

1. Significance in the field of glycoscience and its current situation
   One of the most important issues in life science is to reveal the mechanisms of diseases. Information is accumulating on the relationship between disease (phenotype) and causative genes (mutations). The statistics in the OMIM database show that 5991 phenotypes are known for which the molecular basis is known, and 3743 genes are known with phenotype-causing mutations (as of May 24, 2017). Recent progress of exome sequencing and sharing of phenotype databases has greatly enhanced our understanding of genetic diseases. In the glycobiology field, nearly 70 inherited human glycosylation disorders have been identified (as of 2013), and it is estimated that 2% of the total genome encodes glycosylation pathways. This means that many more glycosylation disorders will be identified [12].
2. Impact on the other fields of research
   Glycosylation disorders cause abnormalities in nearly every organ system. This means that physicians from every specialty will likely encounter patients who have glycosylation defects [12]. It should be noted that addition of N-linked glycans can be a pathogenic mechanism. Up to 1.4% of known disease-causing mutations are predicted to give rise to gains-of-glycosylation [13].
3. Significance as the fundamental research
   It remains challenging in many cases to predict the effect of each mutation on the structure and function an individual protein [14]. The continuous progress of basic science including the structural biology field is important and it is essential to predict a structure-function relationship very accurately.
4. Possible application for industry and medicine, if any
   Gene therapy and personalized medicine will be realized in the near future through the mutual collaboration between basic science and clinical medicine.
5. Future perspectives
   Although the data is accumulating on the mechanisms of diseases, comprehensive databases connecting phenotypes with the causative genes are very few. It will be necessary to construct a public database to share such information.

6. Problems to be solved
   Even though we can reveal the mechanism of a disease with loss of function, in most cases we cannot find a way to treat it. Structural biology is expected to play a central role in such therapy, but the treatment need to be considered from various aspects [15, 16] (Fig. 15.4).

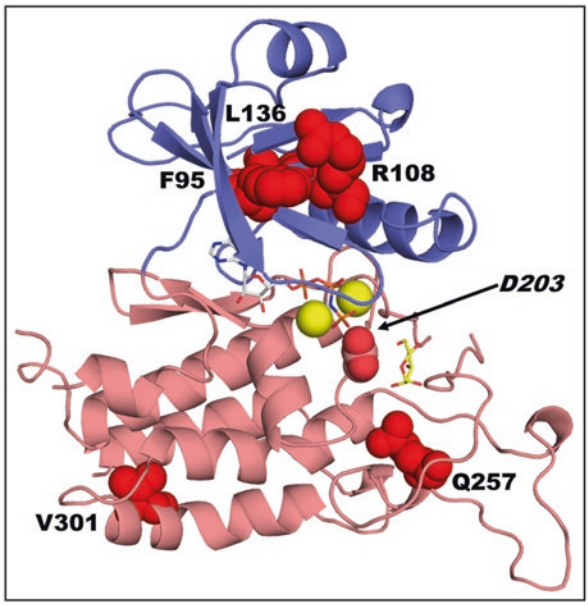

Fig. 15.4 Mutation mapping onto the 3D structure of protein *O*-mannosyl kinase POMK, a causative gene product of dystroglycanopathy. The side chains of disease mutations are highlighted in red. The side chain of the putative catalytic center D203 is also indicated

References

References for Section 15.1

1. Hirao Y et al (2014) Glycoproteomics approach for identifying glycobiomarker candidate molecules for tissue type classification of non-small cell lung carcinoma. J Proteome Res 13:4705–4716
2. Ocho M et al (2014) Application of a glycoproteomics-based biomarker development method: alteration in glycan structure on colony stimulating factor 1 receptor as a possible glycobiomarker candidate for evaluation of liver cirrhosis. J Proteome Res 13:1428–1437
3. Sogabe M et al (2014) A novel glyco-biomarker for ovarian cancer that detects clear cell carcinoma. J Proteome Res 13:1624–1635
4. Kuno A et al (2013) A serum "sweet-doughnut" protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. Sci Rep 3:1065

References for Section 15.2

5. Shinzaki S et al (2008) IgG oligosaccharide alterations are a novel diagnostic marker for disease activity and the clinical course of inflammatory bowel disease. Am J Gastroenterol 103:1173–1181
6. Azuma K et al (2014) Twin studies on the effect of genetic factors on serum agalactosyl immunoglobulin G levels. Biomed Rep 2:213–216
7. Sato M et al (2015) Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects. Liver Int 35:925–935
8. Oh S et al (2015) Moderate to vigorous physical activity volume is an important factor for managing nonalcoholic fatty liver disease: a retrospective study. Hepatology 61:1205–1215
9. Kamada Y et al (2015) A novel noninvasive diagnostic method for nonalcoholic steatohepatitis using two glycobiomarkers. Hepatology 62:1433–1443

References for Section 15.3

10. Sperling RA et al (2013) Preclinical Alzheimer disease-the challenges ahead. Nat Rev Neurol 9:54–58
11. Tian Q et al (2012) Systems cancer medicine: towards realization of predictive, preventive, personalized and participatory (P4) medicine. J Intern Med 271:111–121

References for Section 15.4

12. Freeze HH (2013) Understanding human glycosylation disorders: biochemistry leads the charge. J Biol Chem 288:6936–6945
13. Vogt G et al (2007) Gain-of-glycosylation mutations. Curr Opin Genet Dev 17:245–251
14. Nagae M et al (2017) 3D structural analysis of protein *O*-mannosyl kinase, POMK, a causative gene product of dystroglycanopathy. Genes Cells 22:348–359
15. Cohen FE, Kelly JW (2003) Therapeutic approaches to protein-misfolding diseases. Nature 426:905–909
16. Taniguchi-Ikeda M et al (2011) Pathogenic exon-trapping by SVA retrotransposon and rescue in Fukuyama muscular dystrophy. Nature 478:127–131

Chapter 16
Life-Style Related Disease and Aging

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16.1 Diabetes

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Keywords Diabetes, GnT-IVa, Pancreatic β cell, Insulin secretion, glycosylation

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1. Significance in the field of glycoscience and its current situation
   Current dramatic alterations on dietary habit and insufficient exercise have increased obese-related diabetic patients. More than 400 million people are patients and more than 20% of total medical budget costs for the treatment [1]. In Japan, about half of people are patients of life-related diseases, and more than 3.17 million people are diabetes. GnT-IVa expression is attenuated in pancreatic β cells of diabetic patients and high fat-diet administrated mice [2, 3]. Reduction of GnT-IVa impairs glucose sensor function and insulin secretion of β cells and evokes diabetes [3, 4]. Forced GnT-IVa expression in β cells prevents the high fat-diet induced diabetes that suggests the engineering of GnT-IVa has a potential to be a novel strategy for the prevention of diabetes [4].
2. Impact on the other fields of research
   GnT-IVa is abundantly expressed in various cancers and should be deeply involved in cancer progression, encompassing the glucose absorption for sustaining their unlimited proliferation.
3. Significance as the fundamental research
   It provides important information to elucidate the disease process of type 2 diabetes caused by high fat-diet [3, 4], and pathogenesis of various obesity-related diseases.
4. Possible application for industry and medicine
   If inhibitor compounds for GnT-IVa were obtained, they would likely have a potential to be a candidate for the prevention and treatment of type 2 diabetes [4].
5. Future perspectives
   It would lead to the development of novel drugs for treatment of the dietary habit related diabetes. It is also useful for other diseases associating with cellular glucose absorption, e.g. cancer.
6. Problems to be solved
   Explore of chemical compounds for controlling GnT-IVa expression and development of a system for specifically delivering drags to the target cells would be essential.

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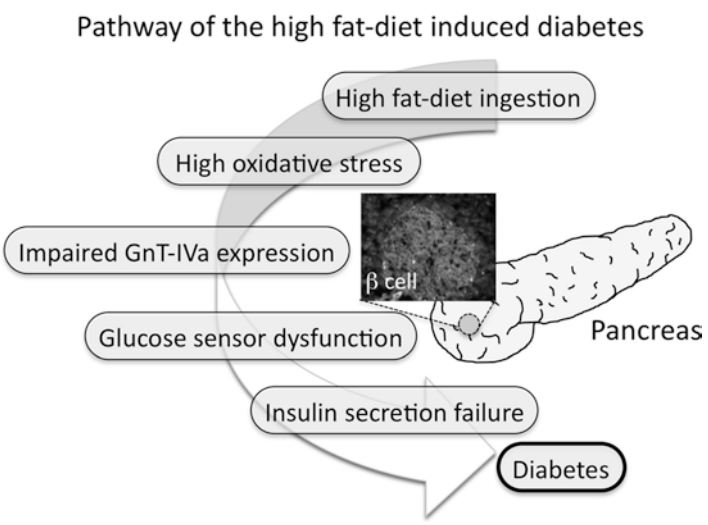

Fig. 16.1 Pathway of the high fat-diet induced diabetes. High fat-diet ingestion attenuates the GnT-IVa expression and consequently abolishes glucose sensor function of pancreatic β cells. This results in the failure of the glucose-stimulated insulin secretion and evokes diabetes

16.2 Obesity

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Keywords Adipocyte, Lifestyle disease, Obesity

1. Significance in the field of glycoscience and its current situation
   Obesity, a condition in which body fat is accumulated, is a risk factor for several diseases, including diabetes and hypertension. In obesity, adipocytes show aberrant hypertrophy, which causes dysfunction of the adipocytes. The roles of glycans in adipocytes are largely unclear with a limited number of studies showing the involvement of glycans such as α2,6-sialic acid [5], GM3 ganglioside [6], and O-GlcNAc [7, 8] in the growth or differentiation of adipocytes and obesity.
2. Impact on other fields of research
   The obese population is increasing in the world. According to a WHO report in 2016, approximately 641 million people are obese. Elucidation of the glycan functions in obesity would lead to lowering of the obesity rate and risk for lifestyle diseases.
3. Significance as fundamental research
   The mechanisms of obesity and related hypertrophy and differentiation of adipocytes are not fully understood. In particular, the involvement of glycans in obesity is largely unclear. Elucidation of the functions of glycans in adipose tissue will lead to understanding of the mechanisms of obesity and the functions of adipocytes.
4. Possible application for industry and medicine, if any
   As you can see from the large obese population and the involvement of obesity in various diseases, measures against obesity are strongly desired by the public. Clarification of the obesity mechanisms could lead to development of new anti-obesity drugs and foods, which could meet social demands.
5. Future perspectives
   Elucidation of the roles of glycans in obesity would lead to development of new compounds that suppress or augment expression of the target glycans.

6. Problems to be solved
   Current methods for glycan analysis are sufficient to analyze the structures and functions of glycans and related glyco-genes in adipocytes, but only if an appropriate model system is available. At present, model animals for obesity such as high-fat diet feeding or ob/ob mice have already been established, so we have few problems preparing samples. A major problem is the low number of scientists who deal with this issue.

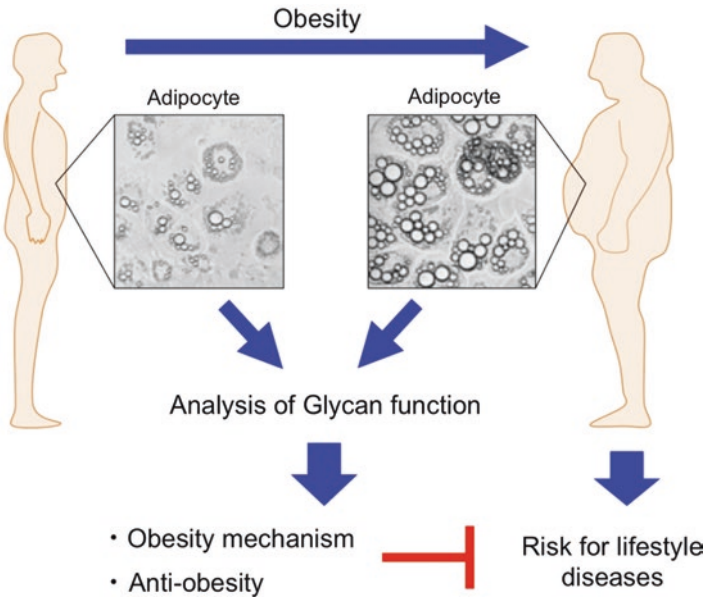

Fig. 16.2 Obesity. Adipocytes show hypertrophy in obesity. Elucidation of the glycan functions in adipocyte growth and differentiation will lead to both understanding of the mechanisms of obesity and development of new anti-obesity compounds. This will lead to lowered risks of life-style diseases

16.3 Chronic Obstructive Pulmonary Disease(COPD)

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Keywords COPD, Keratan sulfate, Smoking, Fut8, Glycotherapeutics

1. Significance in the field of glycoscience and its current situation
   Chronic obstructive pulmonary disease (COPD) consists of emphysema and chronic bronchitis, and is expected to become the 3rd leading cause of death worldwide. In Japan, more than five million people are potential patients and only 300 thousand people are now being treated. The risk factors are smoking and environmental pollutants. Bacterial or viral infections cause exacerbation. Fut8 KO mice exhibit emphysema [9], and heterozygous KO mice are sensitive to smoking and an excellent murine model for emphysema [10]. Reduced Fut 8 expression is observed in COPD patients [11]. Keratan sulfate (KS) decreased in the lungs of cigarette-smoke-exposed mice [12]. Administration of KS disaccharide inhibits exacerbation of inflammation in model mice [13] and the above Fut8 KO model mice and this effect is almost the same as that of dexamethasone. Currently. no effective treatment exists and problems with, steroid therapy have been pointed out, and thus a novel therapy is expected.
2. Impact on other fields of research
   The role of KS in the prevention of inflammation would be useful other diseases and the interaction of KS with other molecules such as C-type lectin would be interesting as to the protein-glycan interaction.
3. Significance as fundamental research
   It is useful for eluciation of the underlying mechanism by which COPD occurs using a murine model for emphysema [12] as well as for solving the problems of inflammation and exacerbation in COPD [13].
4. Possible application for industry and medicine, if any
   KS and KS disaccharides or related derivatives would be likely candidates for glycotherapeutics for the treatment of COPD and its exacerbation [12].
5. Future perspectives
   Research on COPD is also useful for other diseases involving inflammation including cancer.
   Hopefully such an approach will lead to the development of glycotherapeutics because currently no suitable treatment is available.
6. Problems to be solved
   Since there are no data regarding possible receptors in vivo for KS and its derivatives, identification of such receptor(s) in vivo such as C-type lectin would be essential (Box 16.1).

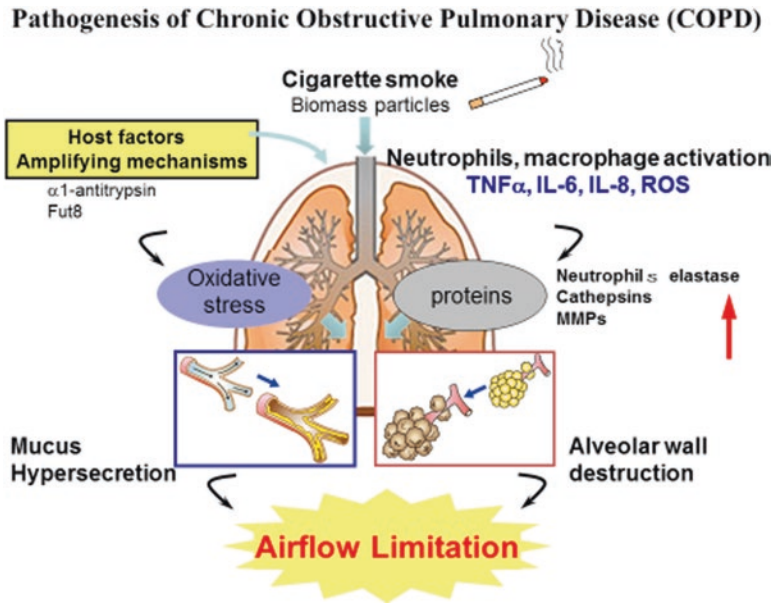

Fig. 16.3 Pathogenesis of Chronic Obstructive Lung Disease (COPD). Risk factors and related molecules are shown in the figure

Box 16.1: Interstitial Pneumonia

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Interstitial pneumonia is a lung disease characterized by interstitial inflammation and scar formation, which causes serious respiratory failure. As inflammation progresses, fibrous connective tissues accumulate (= fibrosis) in interstitial tissue of the lung, which results in the alveolus wall becoming thick and hard. Consequently, the efficiency of the gas exchange decreases. Although it is often asymptomatic, progressive shortness of breath and chronic dry cough are the typical symptoms of interstitial pneumonia. In some patients, the specific cause of interstitial pneumonia can be identified as chronic inhalation of mine dust or fungus (pneumoconiosis and hypersensitivity pneumonitis, respectively). Drug-induced interstitial pneumonia is also frequently observed in Japan, and infection or autoimmune diseases are other common causes. When the probable cause cannot be determined, the condition is called idiopathic pulmonary fibrosis.

KL-6 is a glycoprotein belonging to the MUC mucin family, whose molecular weight is over 5000 kDa. In interstitial pneumonia, large amounts of KL-6 are produced by regenerating lung epithelial cells, and leaked into the blood by the destruction of the epithelial barrier and increased vascular permeability. Therefore, KL-6 is believed to be a candidate biomarker for interstitial pneumonia.

16.4 Alzheimer's Disease

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Keywords Alzheimer's disease, Amyloid ß-protein, Ganglioside, amyloidosis, Microenvironment

1. Significance in the field of glycoscience and its current situation
   Along with the increasing aged population, Alzheimer's disease (AD) has become a serious global issue. The full picture of AD pathology remains to be clarified; however, it is generally accepted that assembly of amyloid ß-protein (Aß), a physiological cleavage product of amyloid precursor protein, occurs at the initial point of the pathological process of the disease. Thus, clarification of the molecular mechanism underlying the Aß assembly is one of the fundamental subjects of AD research. In this regard, it was previously reported that Aß binds to ganglioside (GM1) on the surface of neurons in the brain, leading to the formation of ganglioside-bound Aß (GAß) that works as a seed for pathological assembly of soluble Aß into amyloid fibrils [14–16]. Many studies are needed to validate the GAß hypothesis; however, it may provide new insights into the unique spatiotemporal aspects of AD pathology [17, 18].
2. Impact on the other fields of research
   There are more than 30 different types of amyloidosis, whose causes are basically unknown. The GAß hypothesis, which suggests a pathological role of interaction between amyloidogenic protein and ganglioside, may provide new insights into the pathogeneses of these amyloidoses.
3. Significance as the fundamental research
   This study is expected to contribute to understanding of the physiological and pathological significance of the microenvironment composed of membrane lipids from various cell biological aspects.
4. Possible application for industry and medicine, if any
   This study may have an impact on the development of novel diagnostic and therapeutic measures for various amyloidoses including Alzheimer's disease.
5. Future perspectives
   Clarification of the mechanism underlying the interaction between membrane lipids such as ganglioside and physiological proteins will likely facilitate development of biology and medical science in a broad range of fields.
6. Problems to be solved
   Analytic technology should be developed for determination of the structure of the microenvironment composed of membrane lipids and the structural alteration of protein in the microenvironment.

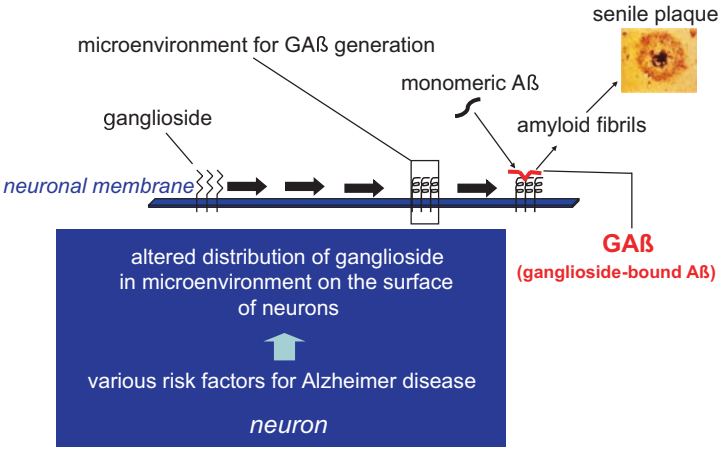

Fig. 16.4 Mechanism underlying Aß assembly in the brain (hypothetical model)
Distribution and expression of gangliosides in the membrane microenvironment is likely altered in the presence of various risk factors for Alzheimer's disease, leading to generation of ganglioside-bound Aß (GAß), which acts as a seed for Aß assembly in the brain

16.5 Alzheimer's Disease

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Keywords Tau, O-GlcNAcase, APP, BACE1, GnT-III

1. Significance in the field of glycoscience and its current situation:
   Currently, 46 million people live with dementia. There are no fundamental therapeutics for Alzheimer's disease (AD), the most common dementia. Two pathological hallmarks observed in AD brains, amyloid plaque (Aβ) and neurofibrillary tangle, are therapeutic targets. Neurofibrillary tangle consists of phosphorylated tau. Since Tau *O*-GlcNAcylation competes with phosphorylation for the same serine/threonine residues [19], *O*-GlcNAcase inhibitors are expected to suppress tau phosphorylation. Indeed, in tau model mice, *O*-GlcNAcase inhibitor prevented tau phosphorylation [20] and a phase 1 trial is ongoing. Aβ-cleaving enzyme, BACE1 is another therapeutic target, but the inhibitor could have serious side effects, since BACE1 has several physiological substrates [21]. Bisecting GlcNAc modification by glycosyltransferase GnT-III, found in BACE1 plays a critical role in blocking lysosomal BACE1 localization [22]. Since GnT-III knockdown specifically inhibits the APP cleavage by BACE1, GnT-III targeting could be novel way to inhibit BACE1 with less side effects. Since APP *O*-glycosylation was recently shown to be critical for Aβ production [23], blocking of APP *O*-glycosylation could be another AD therapeutic strategy.
2. Impact on other fields of research
   Glycobiology approach to understand Alzheimer's disease pathogenesis will be of valuable benefit to cell biology, neurobiology, and proteomics areas.
3. Significance as fundamental research
   Glycobiology approach focusing on key pathological molecules will have a novel and fruitful outcome for basic understanding of the functional role of protein glycosylation.
4. Possible application for industry and medicine, if any
   Japan is historically leading glycotechnology area in the world and therefore advantageous for the development of glyco-targeted drugs against dementia and neurodegenerative disease.
5. Future perspectives
   Development of glycotechnology-based drugs against dementia and neurodegenerative disease will generate novel types of therapeutic strategies.
6. Problems to be solved
   Validation of glycol-targeted therapeutics by basic study is necessary.

16.6 Parkinson Disease

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Keywords Parkinson disease, Synuclein, Gaucher disease, Glucocerebrosidase, Exosome

1. Significance in the field of glycoscience and its current situation.
   Parkinson disease (PD) is one of the common neurodegenerative diseases. Unfortunately, the cause of the disease is still unknown and the effect of currently available medicine is insufficient. PD is neuropathologically characterized by the intraneuronal formation of Lewy body, which is composed of assembled synuclein (SC). Given that patients with Gaucher disease (GD), which is the most common glycolipid storage disorder, frequently show PD symptoms [24, 25], implication of the causative genes of GD (glucocerebrosidase gene, GBA) for development of PD pathology has been actively studied. To date, although detailed molecular mechanisms are still unknown, it is suggested that GBA mutations induce intracellular accumulation and assembly of SC, leading to neurodegeneration [26, 27]. Alternatively, it is also suggested that gangliosides on the surface of exosomes, which are physiologically excreted from cells, facilitate pathological SC assembly in the brain [27].
2. Impact on the other fields of research
   Evidence is accumulating that lysosomal disorders such as Gaucher disease accelerate pathological process of neurodegenerative diseases. Studies in this research field may contribute to develop novel therapeutic strategies for the neurodegenerative diseases, including Parkinson disease.
3. Significance as the fundamental research
   On the basis that lysosomal dysfunction likely plays a signify role in the development of various neurodegenerative disease, studies in this research filed may impact on clarification of pathogenesis of various neurodegenerative diseases.
4. Possible application for industry and medicine, if any
5. Future perspectives
   Development of small-molecule chaperones may be further accelerated to treat various hereditary diseases.
6. Problems to be solved
   It should be clarified at molecular and cellular levels how lysosomal disorders induced by mutations of causative genes such as GBA are responsible for the development of neurodegenerative diseases such as PD.

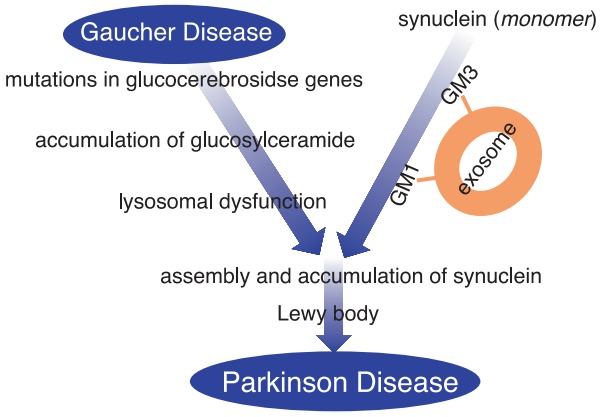

Fig. 16.5 Involvement of glycolipid in the development of Parkinson disease (hypothetical model) Accumulation of glycolipid in Gaucher disease likely impairs physiological degradation of synuclein, leading to its accumulation and assembly, resulting in neurodegeneration. Pathological assembly of synuclein is also facilitated through interaction with gangliosides (GM1and GM3) on the surface of exosomes

16.7 Rheumatoid Arthritis (RA)

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Keywords Rheumatoid arthritis, ACPA, N-glycan, Sialic acid, Galactose

1. Significance in the field of glycoscience and its current situation
   Measurement of serum antibodies reactive with citrullinated proteins (ACPA) is being used as a reliable diagnostic marker for RA. As for changes in N-glycans on serum IgG of RA patients, reduced galactosylation and/or sialylation were reported a while ago [28]. To analyze *N*-glycans on ACPA, ACPA was purified from patients' sera, showing that both galactosylation and sialylation were reduced. When similar analysis was performed in mouse disease models, reduced sialylation was detected. In these mouse models, the presence or absence of sialic acids on antigen-specific IgG determines whether IgGs play pathogenic roles or rather exhibit protective functions for RA [29, 30].
2. Impact on the other fields of research
   In a recent report, the roles of *N*-glycans, particularly of galactose and sialic acids, were clearly demonstrated, and their relevance to pathogenesis was comprehensively shown [31]. Therefore, it contains a lot of information providing insights into functional analysis of glycans on glycoproteins [31].
3. Significance as the fundamental research
   The fact that fine structures at the ends of *N*-glycans on functional proteins markedly influence the biological functions of the proteins has a huge impact because of clear demonstration of the importance of glycosylation.
4. Possible applications for industry and medicine, if any
   Modification of *N*-glycans on autoantibodies in autoimmune diseases or of IgG in IVIG therapy should lead to the development of novel ways controlling refractory diseases [32].
5. Future perspectives
   By isolation of autoantibodies and modification of *N*-glycans on IgGs, we can develop novel therapeutics. Furthermore, more efficient antibody functions can be developed in cancer immuneotherapy [32].
6. Problems to be solved
   Isolation of B cell clones that secrete autoantibodies in patients with RA and other autoimmune diseases. Establishment of systems for stable and massive production of IgGs with preferable forms of *N*-glycans. This will be carried out within 5 years (Fig. 16.6).

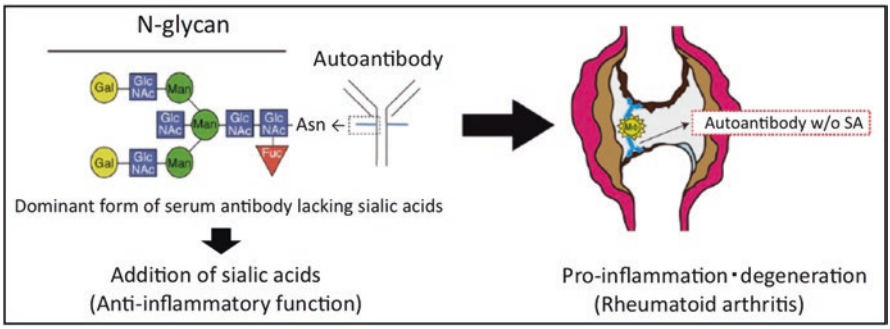

Fig. 16.6 By adding sialic acids to the ends of N-glycans on ACPA IgG, a change of inflammatory antibodies to anti-inflammatory ones can be achieved for application to the treatment for and protection from rheumatoid arthritis

16.8 *O*-GlcNAcylation in Neurodegenerative Diseases and Diabetes

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Keywords *O*-GlcNAcylation, Alzheimer's disease, diabetes, insulin resistance, *O*-GlcNAc transferase, *O*-GlcNAcase

1. Significance in the field of glycoscience and its current situation
   The *O*-linked β-N-acetylglucosamine modification (*O*-GlcNAcylation) of proteins is a dynamic posttranslational modification which occurs both in the nucleus and the cytoplasm [33, 34]. *O*-GlcNAc is unique in that it is not elongated or modified into more complex structures. *O*-GlcNAc transferase (OGT) adds acetylglucosamine to the hydroxyl group of serine or threonine residues on proteins. *O*-GlcNAcase (OGA) removes *O*-GlcNAc from proteins. *O*-GlcNAc regulates transcription, translation, epigenetics, signal transduction etc. Aberrations in *O*-GlcNAcylation are closely corelated with neurodegenerative diseases such as Alzheimer's disease and Lou Gehrig's disease (amyotrophic lateral sclerosis), type 2 diabetes, cardiovascular disease and cancer. The brains of Alzheimer's disease patients are more extensively phosphorylated and hypo-*O*-GlcNAcylated to tau and concentration of the β-amyloid precursor protein is higher than that in in the normal brain [35]. In type 2 diabetes, an increase in *O*-GlcNAcylation through the hexosamine biosynthetic pathway is associated with glucose toxicity, chronic hyperglycemia-induced insulin resistance and diabetic complications [36]. It was recently found that a single amino acid substitution in the tetratricopeptide repeat domain of OGT causes X-linked intellectual disability (XLID) [37].
2. Impact on the other fields of research
   Kinases and OGT can use the same serine and threonine residues. *O*-GlcNAc prevents phosphorylation. The finding of *O*-GlcNAcylation has stimulated research on phosphorylation.
3. Significance as the fundamental research
   The glycosylation of proteins after translation was long thought to occur only in the secretory pathway or at the cell surface. In 1983 it was found that an *O*-GlcNAc is added to intracellular cytoplasmic and nuclear proteins. Since then, numerous nuclear, cytoplasmic and mitochondria proteins, especially those that are abundant in the nucleus, have been shown to be *O*-GlcNAcylated.
4. Possible applications for industry and medicine, if any
   Inhibitors of OGA will be useful in therapeutic medicine for the treatment of Alzheimer's disease and amyotrophic lateral sclerosis by increasing O-GlcNAcylation and restraining phosphorylation. For diabetes, restraining or inhibiting the *O*-GlcNAcylation of proteins, inhibitors of OGT will be useful for

the prevention of and therapeutics for glucose toxicity, insulin resistance, vascular complications and erectile dysfunction.

5. Future perspectives
   More specific inhibitors for OGT and OGA and more specific monoclonal antibodies against *O*-GlcNAc should be developed. Using these inhibitors and antibodies, it will be possible to elucidate the role of *O*-GlcNAc in neurodegenerative diseases and diabetes and will become important areas of investigation in the future.
6. Problems to be solved
   Although phosphorylation involves the action of many kinds of kinases, *O*-GlcNAcylation is performed by only three OGT isoforms. The mechanism for the regulation of *O*-GlcNAcylation by OGT is unclear and needs to be solved. Since it is difficult to detect *O*-GlcNAc by physical techniques such as mass spectrometry, more improved methods will need to be developed.

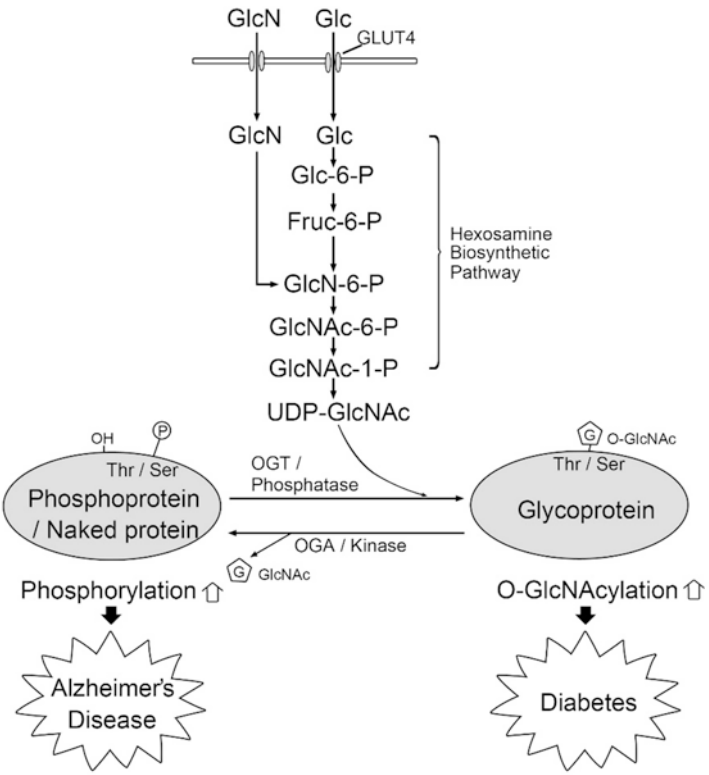

Fig. 16.7 Interplay between *O*-GlcNAcylation and phosphorylation. An elevated level of *O*-GlcNAcylation causes diabetes via an increased influx of glucose and glucosamine into the hexosamine biosynthetic pathway. About 2–5% the glucose that is taken up by cells goes through hexosamine biosynthetic pathway. Hyperphosphorylation and hypo-*O*-GlcNAcylation causes Alzheimer's disease

References

References for Section 16.1

1. Cho NH et al (2015) IDF diabetes atlas, 7th edn. International Diabetes Federation, Belgium
2. Gunton JE et al (2005) Loss of ARNT/HIF1 beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 122:337–349
3. Ohtsubo K et al (2005) Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes. Cell 123:1307–1321
4. Ohtsubo K et al (2011) Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. Nat Med 17:1067–1075

References for Section 16.2

5. Kaburagi T et al (2017) The inhibitory role of α2,6-Sialylation in Adipogenesis. J Biol Chem 292:2278–2286
6. Kabayama K et al (2007) Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proc Natl Acad Sci U S A 104:13678–13683
7. Vosseller K et al (2002) Elevated nucleocytoplasmic glycosylation by *O*-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. Proc Natl Acad Sci U S A 99:5313–5318
8. Ishihara K et al (2010) Characteristic increase in nucleocytoplasmic protein glycosylation by *O*-GlcNAc in 3T3-L1 adipocyte differentiation. Biochem Biophys Res Commun 398:489–494

References for Section 16.3

9. Wang X et al (2005) Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. Proc Natl Acad Sci U S A 102:15791–15796
10. Gao C et al (2012) Sensitivity of heterozygous alpha1,6-fucosyltransferase knock-out mice to cigarette smoke-induced emphysema: implication of aberrant transforming growth factor-beta signaling and matrix metalloproteinase gene expression. J Biol Chem 287:16699–16708
11. Kamio K et al (2012) alpha1,6-Fucosyltransferase (Fut8) is implicated in vulnerability to elastase-induced emphysema in mice and a possible non-invasive predictive marker for disease progression and exacerbations in chronic obstructive pulmonary disease (COPD). Biochem Biophys Res Commun 424:112–117
12. Gao C et al (2017) A keratan sulfate disaccharide prevents inflammation and the progression of emphysema in murine models. Am J Physiol Lung Cell Mol Physiol 312:L268–l276
13. Kobayashi S et al (2013) A single dose of lipopolysaccharide into mice with emphysema mimics human chronic obstructive pulmonary disease exacerbation as assessed by micro-computed tomography. Am J Respir Cell Mol Biol 49:971–977

References for Section 16.4

14. Yanagisawa K et al (1995) GM1 ganglioside-bound amyloid ß-protein (Aß): a possible form of preamyloid in Alzheimer's disease. Nat Med 1:1062–1066
15. Hayashi H et al (2004) A seed for Alzheimer amyloid in the brain. J Neurosci 24:4894–4902
16. Matsuzaki K et al (2010) Aß polymerization through interaction with membrane gangliosides. Biochim Biophys Acta 1801:868–877
17. Oikawa N et al (2015) Imbalance in fatty-acid-chain length of gangliosides triggers Alzheimer amyloid deposition in the precuneus. PLoS One 10:e0121356
18. Oikawa N et al (2014) Influence of APOE genotype and the presence of Alzheimer's pathology on synaptic membrane lipids of human brains. J Neurosci Res 92:641–650

References for Section 16.5

19. Liu F et al (2004) O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. Proc Natl Acad Sci U S A 101:10804–10809
20. Hastings NB et al (2017) Inhibition of O-GlcNAcase leads to elevation of O-GlcNAc tau and reduction of tauopathy and cerebrospinal fluid tau in rTg4510 mice. Mol Neurodegener 12:39
21. Kitazume S et al (2001) Alzheimer's beta-secretase, beta-site amyloid precursor protein-cleaving enzyme, is responsible for cleavage secretion of a Golgi-resident sialyltransferase. Proc Natl Acad Sci U S A 98:13554–13559
22. Kizuka Y et al (2015) An aberrant sugar modification of BACE1 blocks its lysosomal targeting in Alzheimer's disease. EMBO Mol Med 7:175–189
23. Kitazume S et al (2010) Brain endothelial cells produce amyloid from amyloid precursor protein 770 and preferentially secrete the *O*-glycosylated form. J Biol Chem 285:40097–40103

References for Section 16.6

24. Neudorfer O et al (1996) Occurrence of Parkinson's syndrome in type I Gaucher disease. QJM 89:691–694
25. Machaczka M et al (1999) Parkinson's syndrome preceding clinical manifestation of Gaucher's disease. Am J Hematol 61:216–217
26. Grey M et al (2015) Acceleration of α-synuclein aggregation by exosomes. J Biol Chem 290:2969–2982
27. Aflaki E et al (2016) A new glucocerebrosidase chaperone reduces α-Synuclein and glycolipid levels in iPSC-derived dopaminergic neurons from patients with Gaucher disease and parkinsonism. J Neurosci 36:7441–7452

Reference for Section 16.7

28. Parekh RB et al (1985) Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature 316:452–457
29. Kaneko Y et al (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science 313:670–673
30. Anthony RM et al (2012) Novel roles for the IgG Fc glycan. Ann N Y Acad Sci 1253:170–180
31. Ohmi Y et al (2016) Sialylation converts pathogenic anti-citrullinated protein IgG antibodies into effective inhibitors of murine arthritis. Nat Commun 7:11205
32. Li T et al (2017) Modulating IgG effector function by Fc glycan engineering. Proc Natl Acad Sci U S A 114:3485–3490

References for Section 16.8

33. Hart GW et al (2007) Cycling of *O*-linked β-N-acetylglucosamine on nucleocytoplasmic proteins. Nature 446:1017–1022
34. Natarsha N et al (2017) In: Varki A et al (eds) Chapter 19: The *O*-GlcNAc Modification, Essentials of Glycobiology, 3rd edn. Cold Spring Harbor Laboratory Press, New York
35. Lefebvre T et al (2003) Evidence of a balance between phosphorylation and *O*-GlcNAc glycosylation of tau proteins–a role in nuclear localization. Biochim Biophys Acta 1619:167–176
36. Akimoto Y et al (2011) Morphological changes in diabetic kidney are associated with increased *O*-GlcNAcylation of cytoskeletal proteins including α-actinin 4. Clin Proteomics 8:15
37. Vaidyanathan K et al (2017) Identification and characterization of a missense mutation in the *O*-linked β-N-acetylglucosamine (*O*-GlcNAc) transferase gene that segregates with X-linked intellectual disability. J Biol Chem 292:8948–8963

Chapter 17
Congenital Disorders of Glycosylation (CDG), Neuromuscular Related Diseases

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17.1 Congenital Disorders of Glycosylation (CDG): Screening

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Keywords CDG, Mass spectrometry, Rare diseases, Next-generation sequencing

1. Significance in the field of glycoscience and its current situation
   CDG represents a group of disorders affecting glycosylation and currently includes 70 diseases. Recently, an increasing number of diseases that had been classified into other groups turned out to be CDG [1]. The symptoms of CDG are quite diverse as the glycosylation defect occurs on many glycoproteins and leads to alterations in their quantity and function, making clinical diagnosis difficult. Effective screening for CDG patients with developmental delay of unknown origin is expected to extend our knowledge on the function of glycosylation as well as on the causes and the pathogenesis of new diseases [2, 3].

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2. Impact on other fields of research
   Identification of patients and new CDG is a core part of actions promoting rare diseases as a public health priority.
3. Significance as fundamental research
   The Diagnosis of various types of CDG will provide a basis for a strategy to reveal the biological functions of glycans in the context of disease pathogeneses.
4. Possible application for industry and medicine, if any
   With recent attention to rare diseases, the diagnosis of new diseases is promoting the development of related orphan drugs.
5. Future perspectives
   Molecular diagnosis/screening of CDG by means of mass spectrometry play pivotal roles as a means of pre-analysis before next-generation sequencing (NGS) or as an essential method for characterizing the molecular phenotypes after genetic diagnosis by NGS.
6. Problems to be solved
   Accumulation of a basic database enabling statistical analysis to determine decreases/increases of specific glycoforms is necessary to make glycan profiling by means of mass spectrometry reliable.

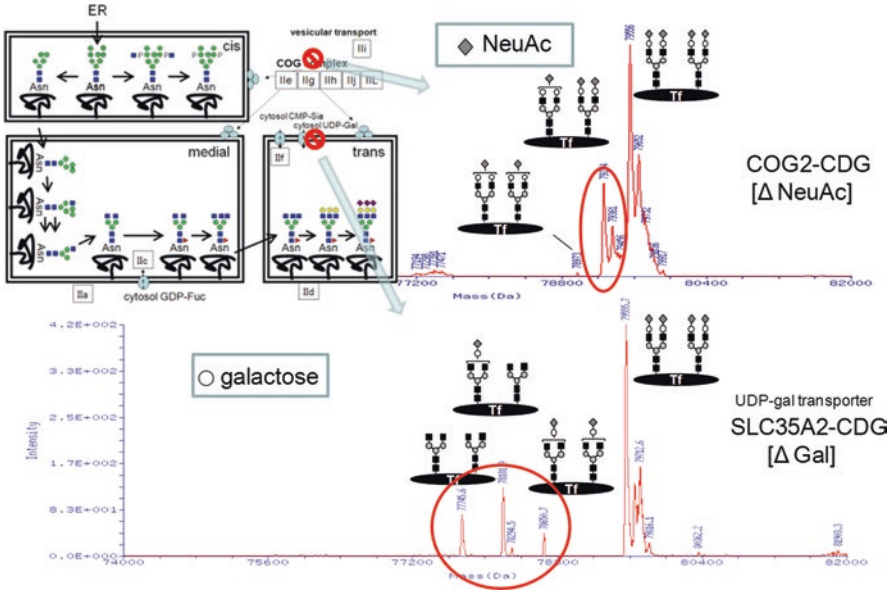

Fig. 17.1 Molecular diagnosis of COG2-CDG (upper) and SLC35A2-CDG (lower) by mass spectrometry of serum transferrin

17.2 *NGLY1*-Deficiency

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Keywords Genetic disorder, Modifier genes, *NGLY1*-deficiency, Non-lysosomal glycan catabolism, PNGase

1. Significance in the field of glycoscience and its current situation
   NGLY1 is a cytoplasmic deglycosylating enzyme for *N*-linked glycans [4]. It occurs widely in eukaryotes, and its activity was first described in 1993 by a Japanese group. NGLY1 is involved in non-lysosomal catabolism of *N*-glycoproteins in the cytosol [4]. Its functional importance in mammalian cells was first indicated by the discovery of *NGLY1*-deficiency, a human genetic disorder caused by mutations of the *NGLY1* gene [5]. So far the total number of confirmed patients is less than 50, and no Japanese patient has been reported. The symptoms of the patients includes global developmental delay, hypotonia, a movement disorder, seizures, abnormal electroencephalography etc. The detailed mechanism of the pathophysiology of *NGLY1*-deficiency has not been clarified in detail, and no effective therapeutic method has been developed.
2. Impact on other fields of research
   NGLY1-deficiency can be regarded as a specific form of "protein accumulation disease" [6]. More recent results also suggested that pathophysiology of this disease is also related with the defective activation of transcription factor(s) [7]. Clarification of the pathophysiology of *NGLY1*-deficiency may facilitate the understanding, as well as drug development, of other diseases such as other genetic disorders, life style-related diseases, and autoimmune diseases.
3. Significance as fundamental research
   NGLY1 is involved in the non-lysosomal degradation of *N*-glycoproteins, which has only partly been clarified. From basic science standpoints, clarification of such a basic biological process will be imperative to obtain a deeper insight into the functional importance of this novel process.
4. Possible application for industry and medicine, if any
   From our analysis of *Ngly1*-KO mice and others, it is expected that symptoms of *NGLY1*-deficiency could be largely dependent on the environmental factors such as food, as well as the genetic background [8]. Clarification of such factors, both environmental and genetic, will lead to the development of a therapy for this disease.

5. Future Perspectives
   The research on *NGLY1*-deficiency aims at not just finding a cure for this disease; currently there is an international team with different area of expertise are formed thanks to the efforts of research foundations established by patients' families.
6. Problems to be solved
   Currently ENGase, another cytosolic deglycosylating enzyme, is thought to be a promising drug target for *NGLY1*-deficiency. We should learn more of the environmental/genetic factors to obtain the whole picture of the pathophysiology of the disease.

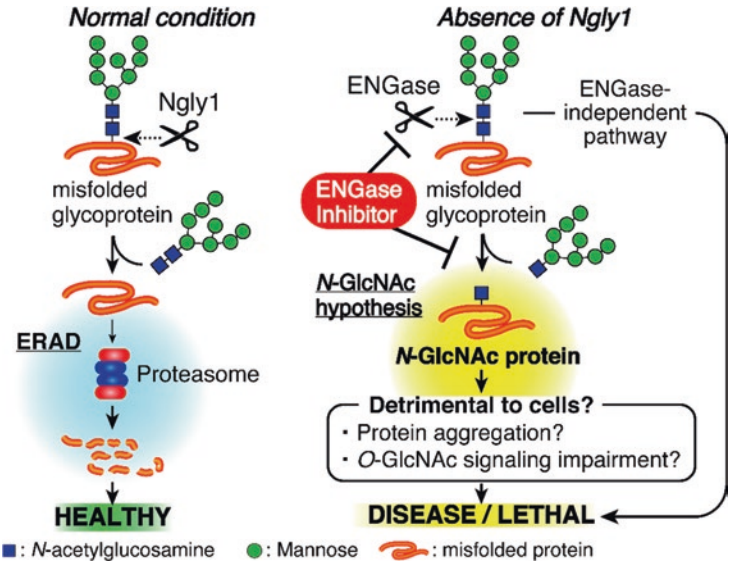

Fig. 17.2 Potential mechanism for the pathophysiology of *NGLY1*-deficiency. Under normal conditions, misfolded glycoproteins released into the cytosol are deglycosylated by Ngly1, while the deglycosylated proteins are degraded by the proteasome. With compromised Ngly1 activity, however, ENGase, another cytosolic deglycosylating enzyme, stochastically acts on misfolded glycoproteins, forming "*N*-GlcNAc proteins". Excess formation of *N*-GlcNAc proteins could somehow be detrimental to cells. An ENGase-independent mechanism for the pathological conditions of *NGLY1*-deficiency is also expected. (Adapted from Fujihira et al., PLOS Genetics 2017)

17.3 Dystroglycanopathy

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Keywords Dystroglycanopathy, Muscular dystrophy, O-mannosylglycan, Glycotherapeutics

1. Significance in the field of glycoscience and its current situation
   The elusive cause of dystroglycanopathies is the defective synthesis of *O*-mannose sugar chains on α-dystroglycan [9]. Dystroglycanopathies are represented by congenital muscular dystrophy, cobblestone lissencephaly, and eye anomalies including Fukuyama congenital muscular dystrophy found almost exclusively in Japan. The abnormal muscle and brain phenotypes of dystroglycanopathies can be explained by abnormal glycosylation of α-dystroglycan [10–12]. Recent glycan analysis of α-dystroglycan revealed that *O*-mannosylglycan contains ribitol-5-phosphate as a sugar component [13]. It should be noted that glycans containing ribitol-5-phosphate were first identified in mammals. So far, there is no known cure for dystroglycanopathies. The above findings will lead to new treatments with sugars for these diseases.
2. Impact on other fields of research
   Since the cause of dystroglycanopathies is the defective synthesis of *O*-mannose sugar chains on α-dystroglycan, the importance of protein glycosylation has been established. The findings affect the clinical fields of neurology, pediatrics, and ophthalmology, and also the biology of myology, neuroscience, developmental biology, etc.
3. Significance as fundamental research
   The discovery of *O*-mannosylglycan and its significance have contributed to the recognition of sugar chains as an important biological material. The discovery of a new sugar component, like ribitol-5-phosphate, is of interest as to the diversity of glycans and their functions.
4. Possible application for industry and medicine, if any
   Currently no effective treatment for dystroglycanopathies exists and novel therapies with sugars are expected.
5. Future perspectives
   Enhanced α-dystroglycan glycosylation may be a novel strategy for curing dystroglycanopathies in the future. The findings expand our knowledge on the glycosylation machinery and glycan functions in the human body.
6. Problems to be solved
   It is necessary to identify other proteins modified by *O*-mannosylglycan and ribitol-5-phosphate, the biosynthetic pathway for ribitol-5-phosphate, and the presence of other *O*-mannosylglycan structures in the future.

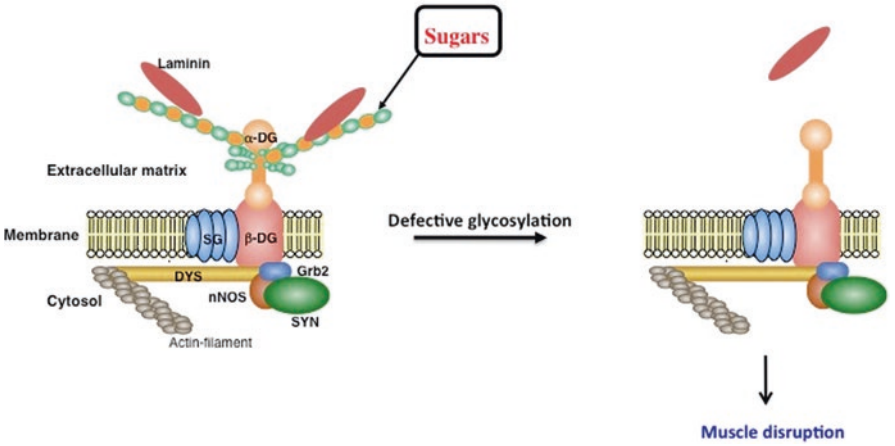

Fig. 17.3 Dystroglycanopathy induced by an O-mannosylation defect. The defective synthesis of O-mannose glycans on α-dystroglycan (α-DG) reduces the binding to laminin, which then induces the destruction and necrosis of muscle, and finally causes dystroglycanopathies

References

References Section for 17.1

1. Jaeken J (2011) Congenital disorders of glycosylation (CDG): it's (nearly) all in it! J Inherit Metab Dis 34:853–858
2. Hennet T (2012) Diseases of glycosylation beyond classical congenital disorders of glycosylation. Biochim Biophys Acta 1820:1306–1317
3. Wada Y (2016) Mass spectrometry of transferrin and apolipoprotein C-III for diagnosis and screening of congenital disorder of glycosylation. Glycoconj J 33:297–307

References Section for 17.2

4. Suzuki T (2015) The cytoplasmic peptide: *N*-glycanase (PNGase) – basic science encounters a human genetic disorder. J Biochem 157:23–34
5. Lam C et al (2017) Prospective phenotyping of *NGLY1*-CDDG, the first congenital disorder of deglycosylation. Genet Med 19:160–168
6. Huang C et al (2015) Endo-β-*N*-acetylglucosaminidase forms *N*-GlcNAc protein aggregates during ER-associated degradation in Ngly1-defective cells. Proc Natl Acad Sci U S A 112:1398–1403
7. Tomlin FM et al (2017) Inhibition of NGLY1 inactivates the transcription factor Nrf1 and potentiates proteasome inhibitor cytotoxicity. ACS Cent Sci 3:1143–1155
8. Fujihira H et al (2017) Lethality of *Ngly1*-knockout mice is partially rescued by the additional deletion of the *Engase* gene. PLoS Genet 13:e1006696

References Section for 17.3

9. Endo T (2015) Glycobiology of α-dystroglycan and muscular dystrophy. J Biochem 157:1–12
10. Chiba A et al (1997) Structures of sialylated *O*-linked oligosaccharides of bovine peripheral nerve α-dystroglycan. The role of a novel *O*-mannosyl-type oligosaccharide in the binding of α-dystroglycan with laminin. J Biol Chem 272:2156–2162
11. Yoshida A et al (2001) Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. Dev Cell 1:717–724
12. Yoshida-Moriguchi T et al (2010) *O*-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. Science 327, 88–92
13. Kanagawa M et al (2016) Identification of a post-translational modification with ribitol-phosphate and its defect in muscular dystrophy. Cell Rep 14:2209–2223

Chapter 18
Glycan Biomarkers for Cancer and Various Disease

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18.1 Liver Fibrosis

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Keywords Liver fibrosis, Glyco-biomarker, Glyco-diagnostics, Glycoprotein, Lectin

1. Significance in the field of glycoscience and its current situation
   Glycoproteins that exhibit disease-associated glyco-alteration and are secreted into the blood have the potential to act as biomarkers for the *in vitro* diagnostics (IVD) for a target disease. Numerous glycoproteins have been studied to date as candidate glyco-biomarkers accompanied by rapid advances in glycomics/

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glycoproteomics technologies and have attracted a great deal of attention in the "discovery phase" of clinical applications [1]. Among them, development of hepatic disease-associated glyco-markers has become the leading research area. Detection systems for them for clinical application have been continuously reported since the early 1990s [2], and some examples are applicable for IVD [3]. Recently, a glyco-diagnostic agent for estimation of the progression of liver fibrosis was developed [4, 5], and approved by PMDA. This is the first achievement of commercialization of glycomics/glycoproteomics research.

2. Impact on other fields of research
   The knowledge and technologies derived from a series of R&D projects will accelerate development of other disease-related glyco-biomarkers, resulting in further glyco-technology to meet clinical demands.
3. Significance as fundamental research
   In-depth glyco-analysis of the fibrosis-related glyco-biomarker will allow identification of expressing cells in the liver to reveal the biological functions of a glyco-biomarker (glycoprotein) in the context of fibrogenesis/fibrolysis.
4. Possible application for industry and medicine, if any
   A kit for IVD has been commercialized.
5. Future perspectives
   Elucidation of the molecular function/mechanism of a fibrosis marker will lead to the development of an alternative antifibrotic drug for chronic hepatitis.
6. Problems to be solved
   Improvement of the sensitivity of the IVD kit will be necessary to assess the early stage of fibrosis in non-alcoholic steatohepatitis.

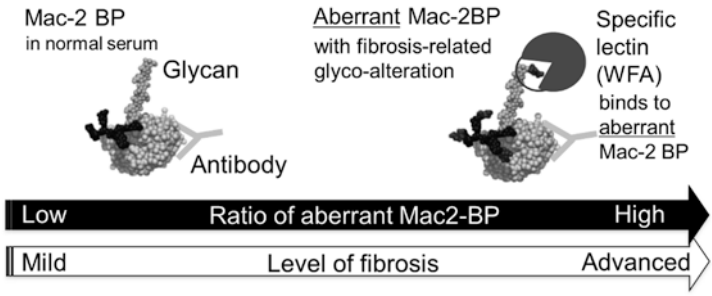

Fig. 18.1 Glycan-based immunoassay for estimating disease progression

18.2 Hepatocellular Carcinoma

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Keywords Cancer bio-marker, Fucosylation, NASH (Non-alcoholic steatohepatitis), HBV, Mac-2 binding protein (Mac-2bp)

1. Significance in the field of glycoscience and its current situation
   Hepatocellular Carcinoma is the fifth leading cause of cancer-related death in Japan. Recently, the patient number of HCC derived from non-alcoholic steatohepatitis has increased due to changes of life-style in Japan. Since most serum glycoproteins are produced by the liver, changes in glycosylation of those proteins could be biomarkers for liver diseases including HCC. While AFP (α-fetoprotein) and PIVKA-II are well-known biomarker for HCC, approximately 20–30% of HCC patients are negative for AFP and PIVKA-II. Fucosylated AFP (AFP-L3) is clinically used as a HCC marker with high specificity [6, 7]. Recently, M2BPGi (aberrant glycosylation of Mac-2 binding protein) is being clinically used as a liver fibrosis biomarker, especially in patients with chronic hepatitis C [8].
2. Impact on other fields of research
   Liver diseases are the best materials for glyco-biomarkers. Glycosylation and liver metabolism are promising candidates for next-generation of hepatic research.
3. Significance as fundamental research
   The liver is the biggest organ for metabolism. The expression profile of many glycosyltransferase genes in the liver is different from in other organs. Biochemistry is the strongest tool for hepatology. Hepatic dysfunction is related to the abnormality of other organs [9].
4. Possible application for industry and medicine, if any
   Development of novel biomarkers for NASH [10].
   Novel glyco-medicine for NASH.
   Development of glycan DDS.
5. Future perspectives
   Biomarker for HCC derived from NASH patients.
   Novel glyco-therapy for HBV.
   Novel glyco-therapy for liver fibrosis.
   Liver-senescence biomarker.
6. Problems to be solved
   Diagnosis system for NASH. Is a liver biopsy essential for NASH patients?
   Multiple organ-related liver researches.
   Within 10 years.

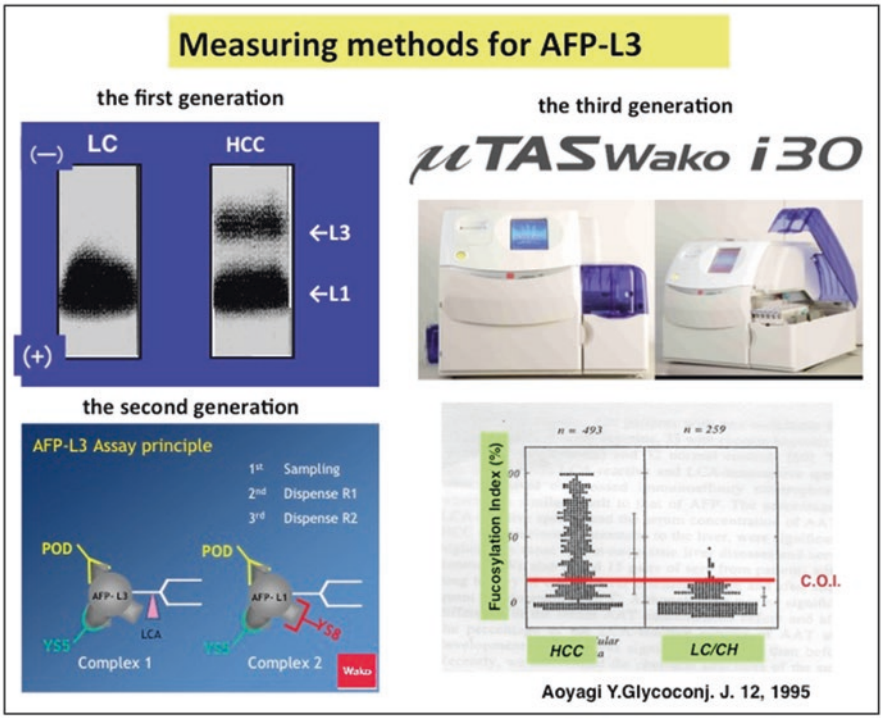

Fig. 18.2 There are three measurement methods for AFP-L3. The first generation type is lectin electrophoresis, the second one LiBASys and the third one μ-TAS, which can assay AFP-L3 within 15 min. All methods show high specificity for AFP-L3 as a HCC biomarker

18.3 Pancreatic Cancer

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Keywords Cancer biomarker, Porcine type glycan vaccination, Chronic pancreatitis, Cancer microenvironment, Early diagnosis

1. Significance in the field of glycoscience and its current situation
   Pancreatic cancer is the fourth leading case of cancer death in Japan. Since early diagnosis is very difficult, the 5-year survival rate is less than 10%. While CA19–9 is a well-known cancer biomarker for pancreatic cancer, there are the problems of pseudo-positive cases and negative results in Lewis-negative patients (10% in Japan). Fucosylated haptoglobin is a novel type of cancer biomarker for pancreatic cancer and the linkage of its fucosylation changes with the progression of pancreatic diseases [11, 12]. Recently, it was reported that vaccination with porcine-type glycan (α1–3 galactose epitope) is a promising therapy for pancreatic cancer [13].
2. Impact on other fields of research
   The microenvironment of pancreatic cancer is very unique. Found on gene-manipulation mouse model research, genetic abnormality is probably involved in the incidence of pancreatic cancer. To overcome pancreatic cancer, the combination of glyco-science with genomics and immunology would be essential.
3. Significance as fundamental research
   Once pancreatic cancer is diagnosed with CT/US, curative therapy in most cases is difficult. Basic researchers should elucidate the detailed mechanism of pancreatic carcinogenesis. An approach through genome research alone is limited.
4. Possible application for industry and medicine, if any
   The development of a curative therapy for pancreatic cancer is the most important issue in modern medicine. If a researcher finds a completely curative therapy for half patients with pancreatic cancer, its impact will be worthy of a Nobel prize.
5. Future perspectives
   A Glycan antibody for fucosylated haptoglobin and a more specific assay for CA19–9 seem to be next targets for pancreatic cancer [14]. NASH research could be applied to pancreatic cancer research.
6. Problems to be solved
   A molecular mechanism of pancreatic carcinogenesis. Early diagnosis with an imaging technique. Identification of high risk groups for pancreatic cancer [15].

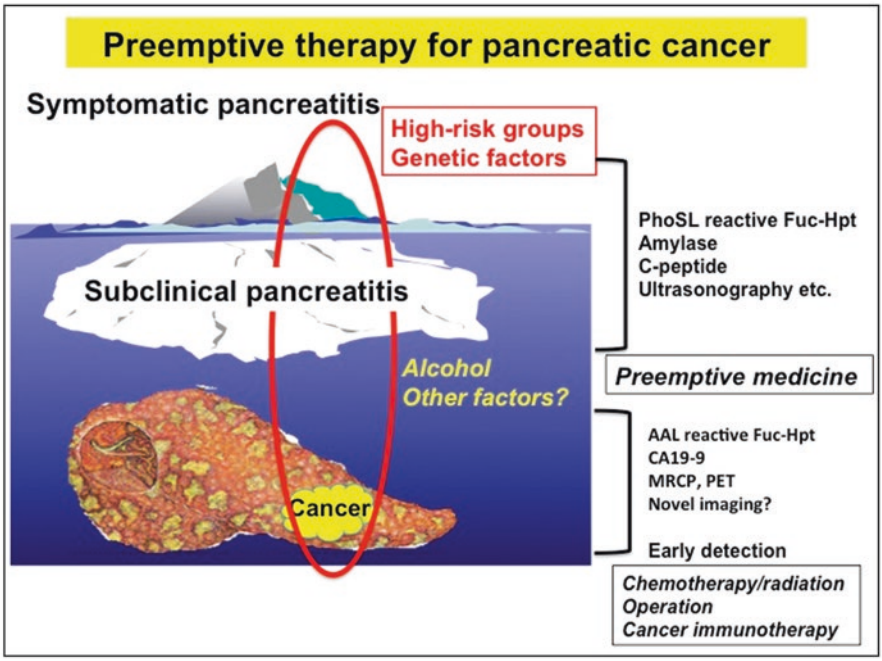

Fig. 18.3 To overcome pancreatic cancer, glyco-biologists should clarify pancreatic carcinogenesis, identify high-risk groups for pancreatic cancer, and develop preemptive therapy. An early diagnosis should be performed by means of image diagnosis and glyco-biomarkers, and preemptive medicine should be administrated via cancer immunotherapy

18.4 Gastric Cancer

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Keywords Gastric cancer, Gland mucin, *H. pylori*, Knockout mice, Cancer prevention

1. Significance in the field of glycoscience and its current situation
   Gastric cancer is the second leading cause of cancer-related death worldwide, and thus remains one of the most common malignant tumors. Gland mucins secreted from the lower part of the gastric mucosa contain *O*-glycans carrying terminal α1,4-linked GlcNAc (αGlcNAc) that is formed by α1,4-*N*-acetylglucosaminyltransferase (α4GnT) [16]. αGlcNAc acts as an antibiotic against *Helicobacter pylori* (*H. pylori*) by suppressing cholesterol α-glucosyltransferase (αCgT) on the microbe [17]. *A4gnt* KO mice exhibit a complete lack of αGlcNAc in the gland mucins, and spontaneously develop differentiated-type gastric adenocarcinomas through a hyperplasia-dysplasia-carcinoma sequence associated with tumor-promoting inflammation [18]. Overall, αGlcNAc plays important roles in preventing gastric cancer. Based on the results obtained by glycobiology research, the development of novel strategies for prevention of and therapies for gastric cancer is expected.
2. Impact on other fields of research
   αGlcNAc analogs and/or αCgT inhibitors could be utilized as antibiotics against *H. pylori*. αGlcNAc will possibly serve as a biomarker to predict the prognosis for gastric cancer, because αGlcNAc loss in differentiated-type gastric adenocarcinoma cells is associated with progression and poor outcome in the patients [19].
3. Significance as fundamental research
   A4gnt KO mice are a useful animal model for studying gastric cancer, because the histopathology of gastric cancer developed in *A4gnt* KO mice resembles that of differentiated-type gastric adenocarcinomas in humans.
4. Possible application for industry and medicine, if any
   Decreased expression of αGlcNAc and increased proliferative activity of gastric epithelial cells are associated with a well-known risk factor for gastric cancer, chronic atrophic gastritis [20]. Thus, it is possible to screen high-risk patients with chronic atrophic gastritis in pathological examination.

5. Future p erspectives
   Development of antibiotics for *H. pylori* and screening of high-risk patients with chronic atrophic gastritis could allow us to devise novel prevention strategies for gastric cancer. It will also be possible that understanding of the regulatory mechanism for gastric cancer development with αGlcNAc will lead to the development of new molecular targeting drugs for this disease.
6. Problems to be solved
   It is crucial to clarify the detailed molecular mechanism of gastric cancer development in *A4gnt* KO mice.

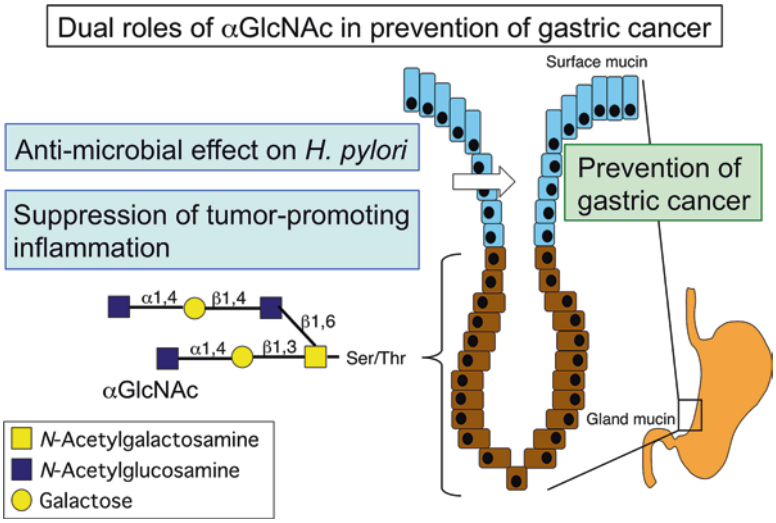

Fig. 18.4 αGlcNAc in the gland mucins prevents gastric cancer by having an anti-microbial effect on *H. pylori* and by suppressing tumor-promoting inflammation

18.5 Prostate Cancer

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Keywords Prostate cancer, Prostate specific antigen, Biomarker, Glycosyltransferase, Drug discovery

1. Significance in the field of glycoscience and its current situation
   Prostate cancer (PC) is the most common male malignancy worldwide including Japan. The prostate specific antigen (PSA) test has resulted in over-diagnosis and over-treatment due to its low specificity. The diagnostic and malignant potential estimation accuracies with glycan biomarkers such as PSA (Siaα2,3Gal- and LacdiNAc structure) [21, 22] are superior to the conventional PSA test. Moreover, upregulation of GnT-V [23] or GCNT1 [24], and downregulation of LARGE and/or β3GNT1 [25] are obvious in aggressive PC. Downregulation of GnT-V or GCNT1, and upregulation of LARGE or β3GNT1 in PC cells suppressed tumor formation and metastasis in a mouse xenograft model. These glycosyltransferase and their products are promising as biomarkers as well as targets for anti-cancer agents.
2. Impact on other fields of research
   Aberrant glycosylation of glycoproteins and cancer-associated changes of glycosyltransferases such as GnT-V, GCNT1, LARGE and β3GNT1 are promising targets for the development of biomarkers and anti-cancer agents for other cancers.
3. Significance as fundamental research
   Although the mechanism of malignant alternation of prostate cancer has been intensively investigated as to genetic mutations and proteins, further investigation on glycosyltransferase regulation and its glycan alternation is required.
4. Possible application for industry and medicine, if any
   Aberrant glycosylation PSA is a promising diagnostic and prognostic biomarker for PC. Inhibitors of GnT-V and GCNT1, and activators of LARGE and β3GnT1 are good candidates for drug discovery for PC.
5. Future perspectives
   Investigations on the etiology and development of diagnostic and therapeutic drugs using the glycobiology technique is a potential methodology for other malignancies such as breast cancer, lung cancer, colon cancer and so on .
6. Problems to be solved
   Since there are no data on possible transcription factors for glycosyltransferases and possible receptors for PC associated aberrant glycosylated molecules, identification of the transcription factor(s) or receptor(s) should be performed (Box 18.1).

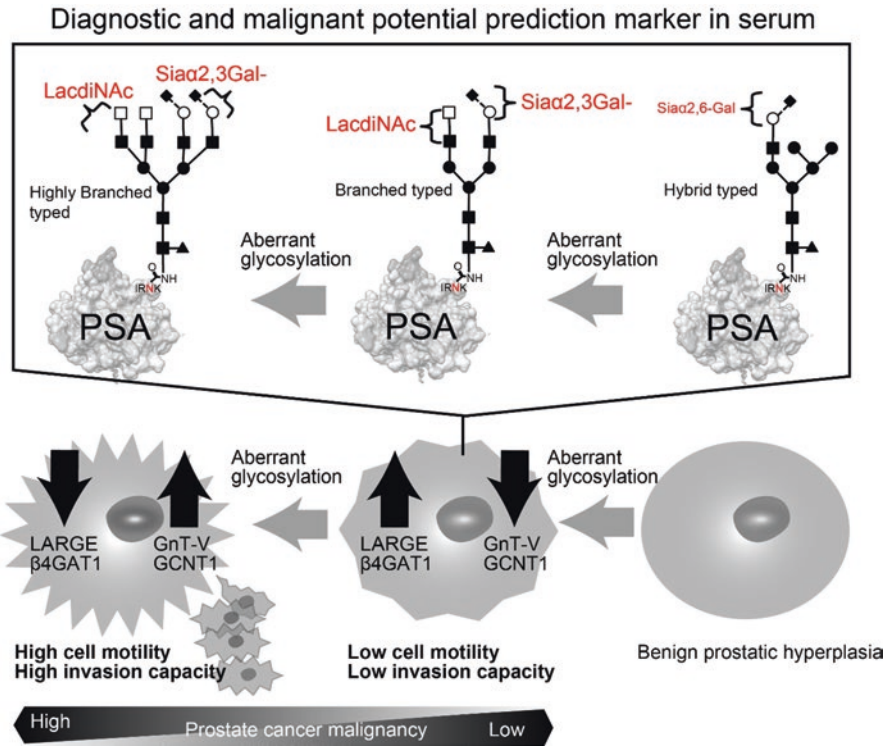

Fig. 18.5 Aberrant *N*-glycosylation change of PSA and relationship between prostate cancer malignancy and glycosyltransferase expression

Box 18.1: Blood Group Antigen and Glycans

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There are different types of human blood groups, harboring either protein antigens or carbohydrate antigens. While the ABO-blood group antigen is a representative carbohydrate antigen, others such as the Lewis-, I/i-, P-, and Sd^a-blood groups have also been identified as carbohydrate antigens. Many blood groups such as the Rh-, MN-, or Kell-blood groups, related to protein antigens, are expressed only on the surface of erythrocytes, whereas carbohydrate antigens as blood groups are also expressed in the tissues in which the expression of glycosyltransferases, involved in their biosynthesis, is observed. Polymorphisms (single-nucleotide polymorphisms, SNPs) of glycosyltransferase genes may alter the activity or substrate specificity of the enzymes, resulting in different blood groups. Therefore, many glycans and glycan-related substances may be the causal factors for blood group incompatibility during tissue transplantation or transfusion. These are considered as important research subjects.

References

References for Section 18.1

1. Taniguchi N et al (2009) The second golden age of glycomics: from functional glycomics to clinical applications. J Proteome Res 8:425–426.
2. Turner GA (1992) *N*-glycosylation of serum proteins in disease and its investigation using lectins. Clin Chim Acta 208:149–171.
3. Kagebayashi C et al (2009) Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. Anal Biochem 388:306–311.
4. Kuno A et al (2013) A serum "sweet-doughnut" protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. Sci Rep 3:1065.
5. Ito K et al (2017) Serum WFA^+-M2BP levels predict liver fibrosis, development of hepatocellular carcinoma, and overall survival: a meta-analysis. J Gastroenterol Hepatol 32:1922–1930.

References for Section 18.2

6. Taketa K (1990) Alpha-fetoprotein: reevaluation in hepatology. Hepatology 12:1420–1432.
7. Noda K et al (1998) Gene expression of alpha1-6 fucosyltransferase in human hepatoma tissues: a possible implication for increased fucosylation of alpha-fetoprotein. Hepatology 28:944–952.
8. Kuno A et al (2013) A serum "sweet-doughnut" protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. Sci Rep 3:1065.
9. Wang Y et al (2015) Loss of α1,6-fucosyltransferase suppressed liver regeneration: implication of core fucose in the regulation of growth factor receptor-mediated cellular signaling. Sci Rep 5:8264.
10. Kamada Y et al (2015) A novel noninvasive diagnostic method for nonalcoholic steatohepatitis using two glycobiomarkers. Hepatology 62:1433–1443.

References for Section 18.3

11. Okuyama N et al (2006) Fucosylated haptoglobin is a novel marker for pancreatic cancer: a detailed analysis of the oligosaccharide structure and a possible mechanism for fucosylation. Int J Cancer 118:2803–2808.
12. Nakano M et al (2008) Site-specific analysis of *N*-glycans on haptoglobin in sera of patients with pancreatic cancer: a novel approach for the development of tumor markers. Int J Cancer 122:2301–2309.
13. Deguchi T et al (2010) Increased immunogenicity of tumor-associated antigen, mucin 1, engineered to express alpha-gal epitopes: a novel approach to immunotherapy in pancreatic cancer. Cancer Res 70:5259–5269.

14. Uozumi N et al (2010) Identification of a novel type of CA19-9 carrier in human bile and sera of cancer patients: an implication of the involvement in nonsecretory exocytosis. J Proteome Res 9:6345–6353.
15. Miyoshi E, Kamada Y (2016) Application of glycoscience to the early detection of pancreatic cancer. Cancer Sci 107:1357–1362.

References for Section 18.4

16. Nakayama J et al (1999) Expression cloning of a human α1,4-*N*-acetylglucosaminyltransferase that forms GlcNAcα1→4Galβ→R, a glycan specifically expressed in the gastric gland mucous cell-type mucin. Proc Natl Acad Sci U S A 96:8991–8996.
17. Kawakubo M et al (2004) Natural antibiotic function of a human gastric mucin against *Helicobacter pylori* infection. Science 305:1003–1006.
18. Karasawa F et al (2012) Essential role of gastric gland mucin in preventing gastric cancer in mice. J Clin Invest 122:923–934.
19. Shiratsu K et al (2014) Loss of gastric gland mucin-specific *O*-glycan is significantly associated with progression of differentiated-type adenocarcinoma of the stomach. Cancer Sci 105:126–133.
20. Yamada S et al (2015) Reduced gland mucin-specific *O*-glycan in gastric atrophy: a possible risk factor for differentiated-type adenocarcinoma of the stomach. J Gastroenterol Hepatol 30:1478–1484.

References for Section 18.5

21. Ishikawa T et al (2017) An automated micro-total immunoassay system for measuring cancer-associated alpha2,3-linked Sialyl *N*-glycan-carrying prostate-specific antigen may improve the accuracy of prostate cancer diagnosis. Int J Mol Sci 18:pii: E470
22. Hagiwara K et al (2017) *Wisteria floribunda* agglutinin and its reactive-glycan-carrying prostate-specific antigen as a novel diagnostic and prognostic marker of prostate cancer. Int J Mol Sci 18:pii: E261
23. Tsui K-H et al (2008) Evaluating the function of matriptase and *N*-acetylglucosaminyltransferase V in prostate cancer metastasis. Anticancer Res 28:1993–1999
24. Kojima Y et al (2015) Detection of Core2 beta-1,6-*N*-Acetylglucosaminyltransferase in post-digital rectal examination urine is a reliable Indicator for extracapsular extension of prostate cancer. PLoS One 10:e0138520
25. Bao X et al (2009) Tumor suppressor function of laminin-binding alpha-dystroglycan requires a distinct beta3-*N*-acetylglucosaminyltransferase. Proc Natl Acad Sci U S A 106:12109–12114

Part IV
Food Implicated in Glycans and Its Function

Foreword

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Glycans play a pivotal role in the food industry and there have been many excellent studies reported on starch, regarding the structure and function of this important food. In Japan, starch, as well as other types of glycans are extensively used as food additives and related food materials. However, scientific evidence regarding the relationship between glycans and functional food is lacking. Glycans that are produced by microbiota and by plants and insects have received considerable attention in recent years, but numerous unresolved problems continue to exist.

While marine products, such as fucoidan, and its use in industrial applications are interesting issues, the chemical structure of such materials and their functional roles still need to be solved. Oligosaccharides and related glycans that are found in milk have been extensively studied worldwide.

Non-enzymatic glycosylation, a process that is referred to as glycation (Maillard reaction or aminocarbonyl reaction) has been extensively studied in relation to browning reactions that occur in various food products such as miso, soy sauce and coffee and, more importantly, hemoglobin, which results in the formation of hemoglobin A1c, which is now used as a biomarker for diabetes. Moreover, structure-function relationships of AGEs (Advanced glycation end-products) and their receptors have been extensively studied and considerable progress has been made into the roles that such substances play in relation to aging, inflammation and various diseases. It is noteworthy that numerous studies regarding various aspects of glycation have been reported in international journals related to the field of glycobiology or glycoscience.

Chapter 19
Food Implicated in Glycans and its Function

Masaaki Tokuda, Sayuri Akuzawa, Tadasu Urashima, Yoshinobu Kimura, and Teruko Konishi

19.1 Rare Sugars

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Keywords Rare sugar, Psicose, Allulose, Allose, Monosaccharide

1. Significance in the field of glycoscience and its current situation
   Rare sugars are monosaccharides that rarely exist in nature and we do not have enough knowledge on them. Development of the production of rare sugars will allow researchers to elucidate their functions [1]. For example, D-psicose (D-allulose) has anti-diabetic and anti-obesity functions [2, 3], and D-allose has

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an anti-oxidative function [4]. While D-tagatose is good for dental health. There functions could be utilized for foods and medicine for prevention of and improvement in of life-style related diseases. It has been realized that monosaccharides have to be considered as "biologically active substances" after various functions of rare sugars are found and reported. The further study of rare sugars will open up a new field of glycoscience and the great possibility of innovative use of these sugars.

2. Impact on the other fields of research
   The rare sugar research will have a great impact on those who are researching polysaccharides and oligo-saccharides that have been believed to have functions. It is also important to have combined research activities including sugar-chain and glycosylation research.
3. Significance as the fundamental research
   There are more than 50 kinds of monosaccharides including rare sugars. It will become possible to make a systematic data base of monosaccharides. A new research field of monosaccharide glycoscience will be established.
4. Possible application for industry and medicine, if any
   Rare sugars can be used to make functional foods and medicine [5]. D-psicose (D-allulose) has already been used for innovation.
5. Future perspectives
   Besides D-psicose (D-allulose), D-allose and D-tagatose are expected to be used for foods and medicine. Extensive study of more than 50 kinds of rare sugars may have a great impact in research and innovation.
6. Problems to be solved
   Mass production of all rare sugars must be established and then research to reveal functions will have to be accomplished. There is a need for more number of researchers in multidisciplinary research fields and financial support.

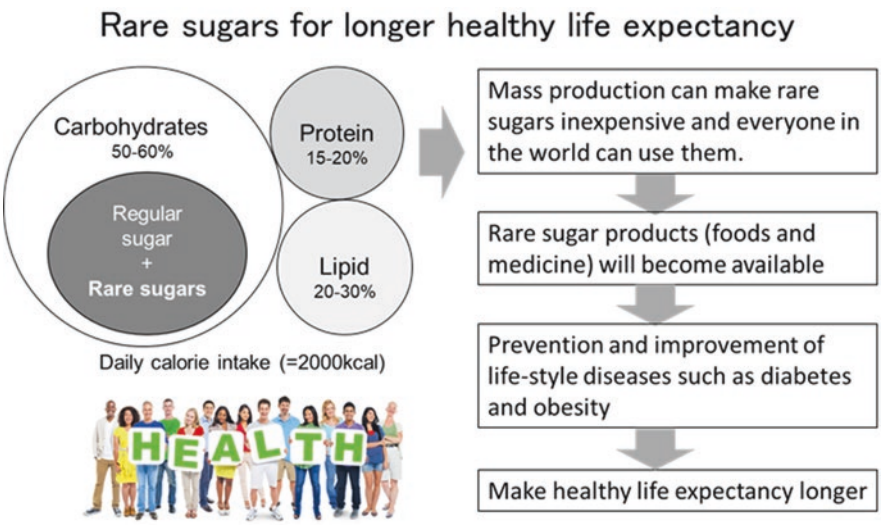

Fig. 19.1 Mass production of various kinds of rare sugars that have functions will make rare sugar-containing foods and medicine available all over the world. Prevention and improvement of life-style related diseases by rare sugars will lead to longer healthy life expectancy for people

19.2 Control of Physical Properties of Starches and Their Texture

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Keywords Starch, Depressiveness, Modified structure, Rheology, Texture

1. Significance in the field of glycoscience and its current situation
   Cereals are essential to maintain life, considering that approximately 59.3% of the energy intake is from carbohydrates, and also that as to energy consumption ratio by food group, cereals account for 41.5% and potatoes 2% [6]. Cooked and processed foods make use of the characteristics of starches and the development of various textures [7]. The textures depend on the molecular structure and dispersed state of the starches, which are the main constituents of the foods [8]. By controlling these factors, it is possible to guess the physical properties of the foods and design foods with the desired texture [9]. Presently, there are methods involving application of high pressure, chemical modification, and enzymatic modification to native starches to change their structure and physical properties [10]. It is essential to develop new methods of measurement and analysis to study the relationship between the physical properties of starches and the texture of cooked or processed foods.
2. Impact on the other fields of research
   Showing the correlation between the structure of starches, and the rheological properties of cooked or processed foods and their textures would also be useful for describing the functions of the human body. It is necessary to understand the mechanisms of human sensory function and physiological responses to clarify what the textures are.
3. Significance as fundamental research
   On the assumptions that starch is an ultra-macromolecule and that its structure is a repetition of a microstructure of a certain fraction, it is possible to explain the structure and the mechanism of bulk behavior of starches, and to obtain an understanding of the three-dimensional conformation based on a conventional two-dimensional microstructure.

4. Future perspectives
   Understanding the relationship between the microstructure of starches and their bulk behavior would give a practical choice to the Food Industry Association. Additionally, considering not only the physical properties of starches and its control but also spaciousness as a factor, it might be possible to understand the texture in the oral cavity in a more practical way.
5. Problems to be solved
   Currently, it is necessary to limit the dispersion conditions, statistically process the measured values and investigate the rule because the relation between the collapse of the crystallinity of a starch and its viscoelastic behavior is unknown.

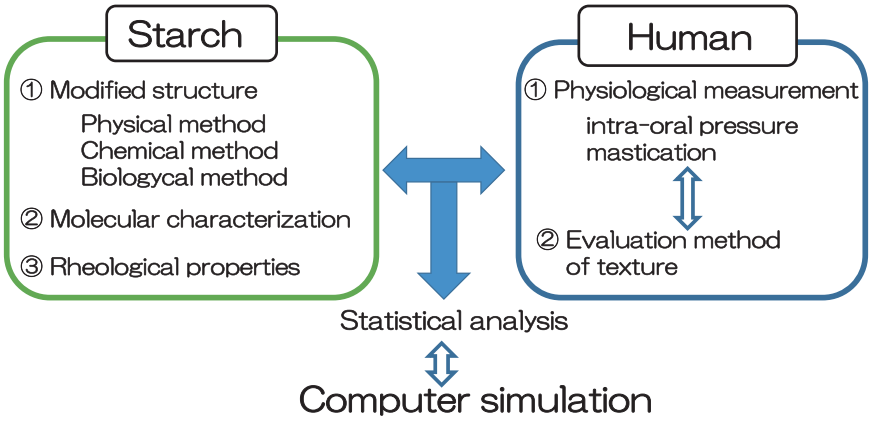

Fig. 19.2 Road map of the study for understanding the relationship between the structure, rheological properties, and texture of starches

19.3 Health Promotion of Newborns Through Artificial Production of Milk Oligosaccharides

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Keywords Milk oligosaccharides, Human milk oligosaccharides, Anti -infection, Immune-modulation, Prebiotics

1. Significance in the field of glycoscience and its current situation
   Human milk contains around 250 different oligosaccharides, totalling 12 ~ 13 g/L, of which about 160 have been structurally characterized [11]. Of these oligosaccharides most are not digested or absorbed within the infant's small intestine and thus reach the colon, acting as prebiotics that promote the growth of beneficial colonic bacteria, as decoy receptors that inhibit the adhesion of pathogenic microorganisms to the epithelium, and as immune modulators that control the secretion of inflammatory cytokines [12]. On the other hand, bovine mature milk, which is used in the production of infant milk replacers, contains only low concentrations of oligosaccharides, whose structures are mostly different from those of human milk oligosaccharides [13]. Recently, it was shown that some human milk oligosaccharides including 2′-fucosyllactose (2'-FL), lacto-N-neotetraose (LN*n*T), lacto-N-tetraose (LNT), and 3′-sialyllactose (3'-SL) can be produced on a large scale, and clinical trials have begun in which milk replacers incorporating 2'-FL and LN*n*T are bottle fed to infants [14]. The results of *in vivo* experiments suggest that these oligosaccharides will be utilizable as anti-infection agents for *Campylobacter jejuni*, rotavirus, etc., as immune modulating agents [15], and also as food additives to be incorporated into functional foods, including infant milk replacers.
2. Impact on other fields of research
   Milk oligosaccharides, especially human ones, which vary in chemical structure, should have the potential to be utilized as materials for studies that aim to determine the sugar specificities of lectins including galectins, selectins and siglecs. It has been hypothesized that these lectins are significant for the interaction between pathogens and host receptors, and intercellular interaction between endothelial cells and platelets or lymphocytes; it can therefore be expected that studies on the sugar specificities of these lectins will provide information that will promote the development of useful antipathogenic medical agents.
3. Significance as fundamental research
   It is hoped to clarify the sugar receptors for several pathogens including rotavirus, norovirus, *Campylobacter jejuni*, enteropathogenic *E. coli*, and uropathogenic *E. coli*, which can invade host epithelial cells. It is also hoped to clarify the lectins and sugar epitope units that are related to intercellular interaction between endothelial cells, platelets, etc.

4. Possible applicant for industry and medicine, if any
   The best candidates for anti-pathogenic agents for *Campylobacter jejuni*, rotavirus, norovirus, *Streptocuccus B* pathogenic bacteria, enteropathogenic *E. coli* and uropathogenic *E. coli* appear to be 2′FL, 3′-SL, LN*n*T, LNT, etc. The best candidates among milk oligosaccharides for immune modulating agents that control the secretion of inflammatory cytokines remain to be determined.
5. Future perspectives
   Incorporation of human milk oligosaccharides into infant formula. The development of anti-inflammatory and anti-infection agents [16].
6. Problems to be solved
   The development and improvement of industrial scale production of human milk oligosaccharides. In vivo and clinical studies on anti-infection and immune-modulation effects of milk oligosaccharides. The development of methods for the industrial scale separation of oligosaccharides from the milk of dairy farm animals.

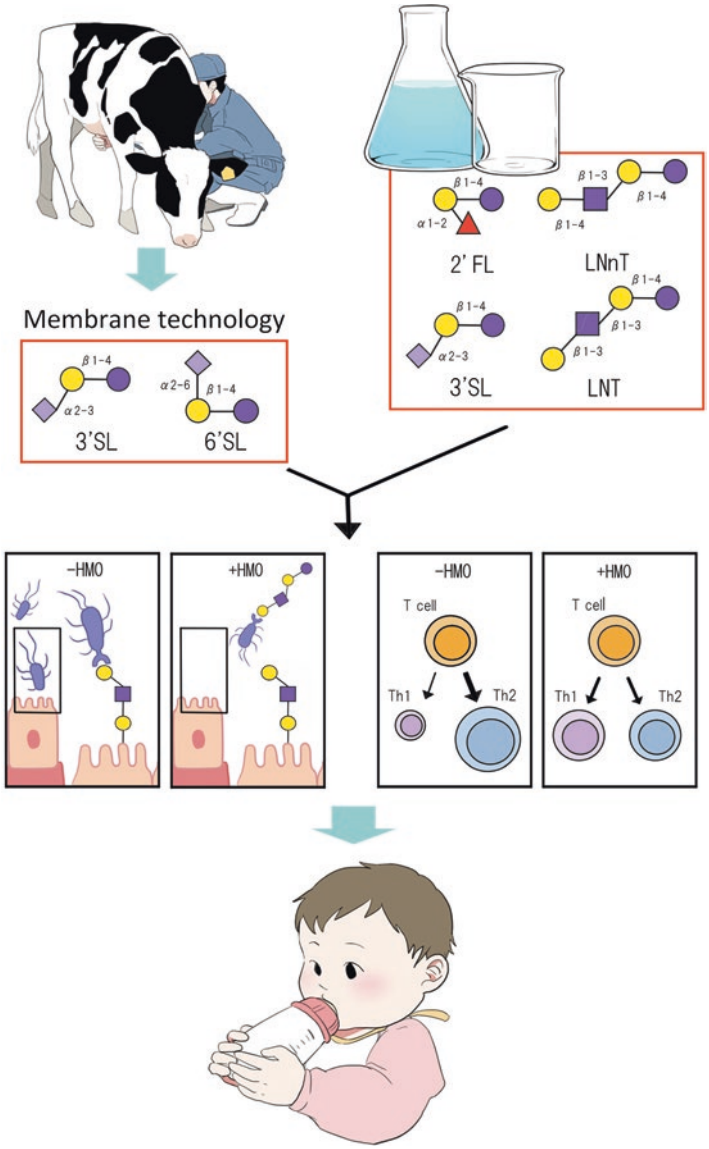

Fig. 19.3 Health promotion of newborns through artificial production of milk oligosaccharides. (Adapted from Fig. 5 of Ref. [13])

19.4 Application of Bioactive Oligosaccharides to Development of Functional Foods

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Keywords Plant glycans, Insect glycans, Antigenic oligosaccharides, Functional foods

1. Significance in the field of glycoscience and its current situation
   Oligosaccharides are essential materials for food industries, their diversities of nutrient factors (primary function) or rheological properties (secondary function) having made a considerable contribution to food industries. Recently, various functions of oligosaccharides in food materials, which are involved in biological regulations, have been unveiled, and applied studies of these bioactive oligosaccharides for development of functional foods have become an active area of glycoscience or glycotechnology. In particular, oligosaccharides found in plant food materials often exhibit various immunological activities or regulative activities toward cell proliferations [17–21]. Therefore, elucidation of the physiological functions of these bioactive oligosaccharides will open the way for new glyco-food industries by allowing preparation of neoglycoconjugates consisting of bioactive oligosaccharides and existing food polymers or edible materials.
2. Impact on the other fields of research
   Given the structural diversity and immuno-activity of plant or insect oligosaccharides, these oligosaccharides possibly have many physiological functions to be unveiled and have potential as pharmaceutical materials in addition to functional food materials.
3. Significance as fundamental research
   Oligosaccharides found in plant or animal food materials have high potential as to unidentified biological activities, and some of them have recently been postulated to have a chaperon-like activity. Therefore, unveiling and elucidation of the physiological functions of these oligosaccharides should provide important information for understanding of the cellular immune system or protein quality control system.
4. Possible application for industry and medicine, if any
   N-Glycans or other oligosaccharides from plant, animal, and insect food materials exhibit immunological activity, therefore, these oligosaccharides can be used as resources for pharmaceuticals or functional foods.

5. Problems to be solved

Although various biofunctions of animal, plant and insect oligosaccharides have been reported and they have been used as raw materials for functional foods, some of them have fully characterized from the viewpoint of biochemistry or molecular biology. Therefore, biochemical and/or molecular biological studies to obtain solid evidence of their functions, especially those involved in immune responses, are prerequisite.

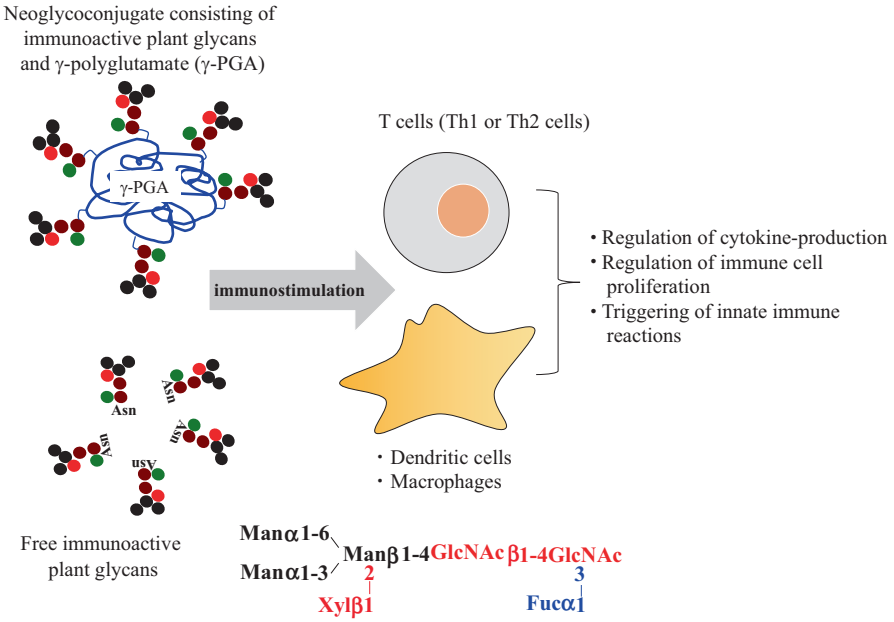

Fig. 19.4 Development of food-derived glyco-materials that regulate the activities of immune cells

19.5 Structure and Bioactivities of Fucoidan as a Functional Algal Polysaccharide

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Keywords Fucoidan, Algal polysaccharides, Sulfated polysaccharides, Functional polysaccharides, Oligosaccharides

1. Significance in the field of glycoscience and its current situation
   Fucoidan is a sulfated polysaccharide contained in brown algae such as *Laminaria japonica* and shows a great deal of bioactivity, including anti-tumor activity [22, 23]. This well-known group of functional polysaccharides has attracted considerable research attention and may have potential as carcinostatic agents. Depending on the algae from which they are derived, the structure and bioactivity of fucoidan vary [24]. Moreover, the bioactivity is affected by the fucoidan molecular weight and particularly by polysaccharide structure [23–25]. However, which part of the structure of fucoidan is responsible for bioactivity and how the bioactivity functions and is recognized intracellularly have not been elucidated. Although research concerning oligosaccharides could be useful in this endeavor and in analyzing structural details, the fact that enzymes that can produce fucoidan oligosaccharides have not been commercialized may pose a challenge.
2. Impact on the other fields of research
   Fucoidan demonstrates a great deal of functional bioactivity. A deeper understanding of the functional mechanism of polysaccharides in general could help identify not only how fucoidan is recognized intracellularly but also how it interacts with other proteins.
3. Significance as the fundamental research
   Drawing on the fucoidan research that identified which structure in the molecular chain is recognized by saccharide receptors or is responsible for polysaccharide bioactivity, we can generally clarify the *in vivo* recognition mechanisms for functional polysaccharides.
4. Possible application for industry and medicine, if any
   The oligosaccharides that possess the specific structure for recognition in the cell or a recognition site for a receptor could be developed into carcinostatic agents with minimal side effects. Moreover, it is possible that oligosaccharides will demonstrate a new bioactivity not exhibited by polysaccharides.

5. Future perspectives
   Fucoidan oligosaccharide research will clarify *in vivo* recognition mechanisms for fucoidan, thereby enabling identification of the intracellular recognition mechanism of polysaccharides in general. Furthermore, fucoidan oligosaccharides could serve as new drug agents derived from functional polysaccharides.
6. Problems to be solved
   The first step in this endeavor should be to clarify the relationship between the polysaccharide structure and their bioactivity. Specifically, preparing fucoidan-decomposing enzymes, which produce fucoidan oligosaccharides, should be an easy first step. Pinpointing the part of the fucoidan molecule responsible for bioactivity may be necessary to advance this research.

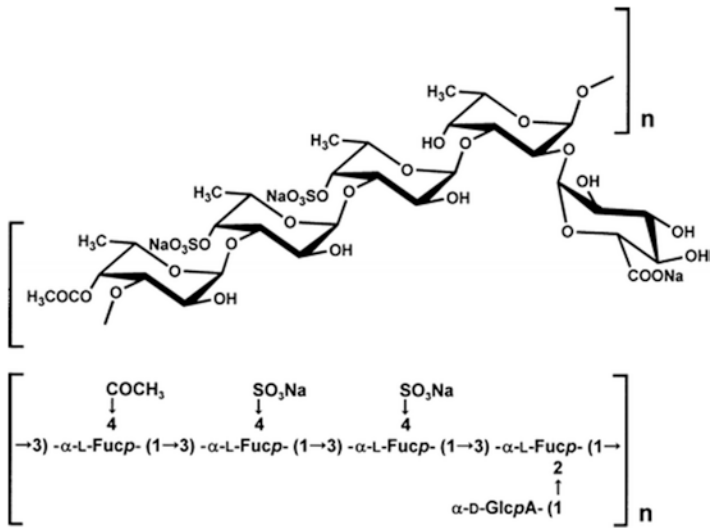

Fig. 19.5 Structure of fucoidan from *Cladosiphon okamuranus* [25] Fucoidan is α-(1,3) linked fucose as a backbone with sulfate groups substituted at a position C-4 of the fucose

References

References Section for 19.1

1. Izumori K (2002) Bioproduction strategies of rare sugars. Naturwissenschaften 89:120–124.
2. Hossain A et al (2015) Rare sugar D-allulose: potential role and therapeutic monitoring in maintaining obesity and type 2 diabetes mellitus. Pharmacol Ther 155:49–59
3. Itoh K et al (2015) Beneficial effects of supplementation of the rare sugar "D-allulose" against hepatic steatosis and severe obesity in Lep(ob)/Lep(ob) mice. J Food Sci 80:H1619–H1626
4. Shinohara N et al (2016) D-Allose attenuates overexpression of inflammatory cytokines after cerebral ischemia/reperfusion injury in gerbil. J Stroke Cerebrovasc Dis 25:2184–2188
5. Hayashi N et al (2010) Study on the postprandial blood glucose suppression effect of D-psicose in borderline diabetes and the safety of long-term ingestion by normal human subjects. Biosci Biotechnol Biochem 74:510–519

References Section for 19.2

6. National Institute of Health and Nutrition (eds) (2016) National Institutes of Biomedical Innovation, Health and Nutrition. DAI-ICHI SHUPPAN Co. Ltd., Tokyo, p 35, 200
7. Akuzawa S, Kawabata A (2003) Relationship among starches from different origins classified according to their physicochemical properties. J Appl Glycosci 50:121–126
8. Jane JL (2006) Current understanding on starch granule structures. J Appl Glycosci 53:205–213
9. Murakami S et al (2015) Effect of strain behavior property on baking productivity of rice batter. Nihon Rheorogy Gakkaishi 43:145–149
10. Chiu CW, Solarek D (2009) In: BeMiller J, Whister R (eds) Modification of starches: starch chemistry and technology, 3rd edn. Academic, New York, pp 629–656

References Section for 19.3

11. Kobata A (2016) In: McGuire M, McGuire M, Bode L (eds) Structures, classification, and biosynthesis of human milk oligosaccharides: prebiotics and probiotics in human milk. Academic, London, pp 17–44
12. Urashima T, Messer M, Oftedal OT (2016) In: McGuire Mi, McGuire Ma, Bode L (eds) Oligosaccharides in the milk of other mammals: prebiotics and probiotics in human milk. Academic, London, pp 45–139
13. Bode L (2012) Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology 22:1147–1162
14. Puccio G et al (2017) Effects of infant formula with human milk oligosaccharides on growth and morbidity: a randomized multicenter trial. J Pediatr Gastroenterol Nutr 64:624–631
15. Goehring KC et al (2016) Similar to those who are breast-fed, infants fed a formula containing 2′-fucosyllactose have lower inflammatory cytokines in a randomized controlled trial. J Nutr 146:2559–2566
16. Lin AL et al (2017) Human milk oligosaccharides inhibit growth of group B streptococcus. J Biol Chem 292:11243–11249.

References Section for 19.4

17. Wilson IBH et al (2001) Analysis of Asn-linked glycans from vegetable foodstuffs: widespread occurrence of Lewis a, core α1-3-linked fucose and xylose substitutions. Glycobiology 11:261–274
18. Okano M et al (2004) Role of pollen-derived oligosaccharides in human IgE and T cell responses in Japanese cedar pollinosis. Clin Exp Allergy 34:770–778
19. Kimura M et al (2011) *N*-Glycans linked to glycoproteins in edible beans (Zatsu-mame): natural resources for bioactive oligosaccharides. Biosci Biotechnol Biochem 74:155–158
20. Itano S et al (2016) Immunomodulatory activity of glycopolymers bearing highly clustered *N*-glycans for Th1 and Th2 immune response. Proc Jpn Soc Immunol 45:166
21. Osada T, Maeda M, Tanabe C, Furuta K, Vavricka CJ, Sasaki E, Okano M, Kimura Y (2017) Glycoform of a newly identified pollen allergen, Cha o3, from Chamaecyparis obtuse (Japanese cypress, Hinoki). Carbohydr Res 448:18–23

References Section for 19.5

22. Lei W et al (2016) A review about the development of fucoidan in antitumor activity: a review about the development of fucoidan in antitumor activity: progress and challenges. Carbohydr Polym 154:96–111
23. Satoru K et al (2003) Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. Biochem Pharmacol 65:173–179
24. Kyung-Tae K et al (2015) Molecular weight and sulfate content modulate the inhibition of α-amylase by fucoidan relevant for type 2 diabetes management. PharmaNutrition 3:108–114
25. Takeshi T et al (2009) Structural characteristics and in vitro macrophage activation of acetyl fucoidan from *Cladosiphon okamuranus*. Glycoconj J 26:1019–1028

Part V
Glycan-Related Materials and Their Use for Biomaterials

Foreword

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Polysaccharides, which are renewable materials abundantly present in living organisms, have been variously used as bio resources. For example, cellulose-modified fiber materials, in particular acetylcellulose, have come to be used as highly functional materials such as films for the polarizing plates of liquid crystal displays (LCDs). In recent years, the manufacturing technology for cellulose nanofibers and chitin nanofibers has progressed, and they are attracting attention as light and strong new materials. It is expected that the use as next-generation high-performance materials will continue in the future as various base materials such as column carriers showing high resolution performance, highly functional gel materials, and biomedical applications such as drug delivery systems and regenerative medicine. In addition, necklace-like polymers and supramolecular gels made from cyclic sugars such as cyclodextrin are attracting attention as, for example, tough polymers that do not easily break, and material design based on a new concept in Japan is developing. We added Boxe(s) for readers to understand their significance in related to Glycoscience and its application which we could not include. Please see page 331 Box 20.1: Triacetyl Cellulose (TAC) Used for Liquid Crystal Display Thin Film.

Chapter 20
Glycan-related Materials and their use for Biomaterials

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20.1 Cellulose Nanofiber Materials

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Keywords Cellulose, Nanofibers, Bio-based materials, Sustainable resources, Carbon-neutral

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1. Significance in the field of glycoscience and its current situation
   Cellulose nanofibers (CNFs) are semi-crystalline nanofibers with a width of 4 to 20 nm, and are 1/5 as light as steel while exhibiting 5 times its strength. Their coefficient of thermal expansion is 1/50 that of glass, which is comparable to that of quartz. Half of the composition of wood consists of cellulose nanofibers. The pace of initiatives aimed at extracting CNFs for use as materials is increasing worldwide. Research on the manufacture, functionalization, structuring and utilization of cellulose nanofibers is being actively conducted. Various applications that make use of the high specific surface area, edibility, lightness, high strength, low thermal expansion, biodegradability, biocompatibility and other characteristics of cellulose nanofibers are being developed in the field of automotive components, electronic devices, energy devices, medical applications, and cosmetics and food [1–4]. The 2014 revision to the Japan Revitalization Strategy approved by Cabinet Decision clearly mentioned the promotion of initiatives aimed at accelerating the research and development on cellulose nanofibers (a mention retained in the 2015, 2016 and 2017 revisions).
2. Impact on the other fields of research
   To utilize CNFs as large scale materials, vertical interdisciplinary research and development involving forestry and forest products science, pulp and paper science, chemistry, polymer science, plastics processing science, mechanical/electronic engineering for automotive and electronic devices and medical science is inevitable (Box 20.1).
3. Significance as the fundamental research
   Cellulose nanofibers are a fundamental element of plant cell walls. Research on the manufacture, functionalization, structuring and utilization of cellulose nanofibers is inevitable for our future society based on sustainable resources.
4. Possible application for industry and medicine, if any
   Various applications that make use of the high specific surface area, edibility, lightness, high strength, low thermal expansion, biodegradability, biocompatibility and other characteristics of cellulose nanofibers are being developed in the fields of automotive, electronic devices, medical materials, food and cosmetics.

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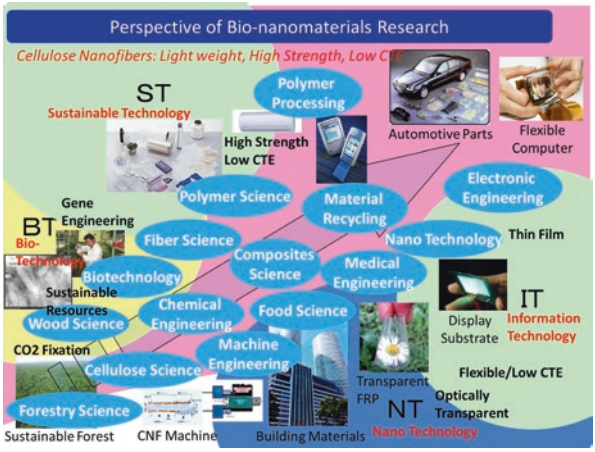

Fig. 20.1 Perspective of bio-nanomaterials research based on cellulose nanofibers

Box 20.1: Triacetyl Cellulose (TAC) Used for Liquid Crystal Display Thin Film

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Triacetylcellulose in which all the hydroxyl groups of cellulose are substituted with acetyl groups is commonly called TAC (tack). It is practically used as a polarizing plate protective film or antireflection film used on the surface of liquid crystal displays by Fujifilm or Konica Minolta. It is a highly functional material boasting a high share in the world. TAC has a smooth surface, uniform thickness, high transmittance throughout the visible region, and is suitable as a support for high performance films. In addition, even if a colorless and transparent film is optically nonuniform, uneven light leak called moiré pattern can be seen when passing through a polarizing plate, but TAC has no optical anisotropy and can not see a moire pattern, It has excellent characteristics as a polarizing plate protective film.

A transparent TAC film without adequate birefringence and distortion was developed based on many basic studies for precisely controlling the molecular structure and orientation of cellulose derivatives that have been developed in Japan for many years.

5. Future perspectives
   Based on the high mechanical performance and sustainability of cellulose nanofibers the time will come when a variety of materials for automotive, electronic devices, medical materials can be created from a sustainable plant biomass.
6. Problems to be solved
   Functionalization of cellulose nanofibers designed for various applications and their production cost (Within 3–5 years).

20.2 Chitin and Chitosan Nanofiber Materials

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Keywords Chitin, Nanofiber, Biological property, Physical property

1. Significance in the field of glycoscience and its current situation
   Chitin is the main component of crab shells and is extracted as a nanofiber structure. Chitin nanofibers (CNF) can be homogeneously dispersed in water and exhibit higher processing ability for commercial utilization than conventional chitin [5]. CNF exhibit efficient mechanical properties, low thermal expansion, high viscosity, and a unique thixotropic nature. These properties allows applications as reinforcement fillers and food additives to improve the physical properties of plastics and foods, respectively. Moreover, CNF have a variety of biological properties [6], for example, (1) preventive effects after oral administration on acute ulcerative colitis, plasma metabolites and gut microorganisms, (2) effects after skin care on wound healing, atopic dermatitis-like skin lesions, and anti-aging, and (3) effects after plant administration on growth and elicitor activity.
2. Impact on other fields of research
   CNF are deeply related to cellulose nanofibers. A variety of biological properties make chitin nanofibers advantageous over conventional cellulose nanofibers.
3. Significance as fundamental research
   Since conventional chitin is insoluble in water, it was difficult to develop potential functions of chitin. On the other hand, CNF can be dispersed homogeneously in water. Thus, we can characterize CNF easily [7],
4. Possible application for industry and medicine, if any
   CNF exhibit efficient physical properties and unique biological properties. CNF will be commercially applied after considering these properties.
5. Future perspectives
   A venture company has been launched to provide CNF. When CNF are commercially applied as novel functional materials, the chitin industry will be developed.
6. Problems to be solved
   We must search for potential functions of CNF. Then, based on these functions, we must develop an exit strategy for commercial application of CNF to respond effectively to social needs.

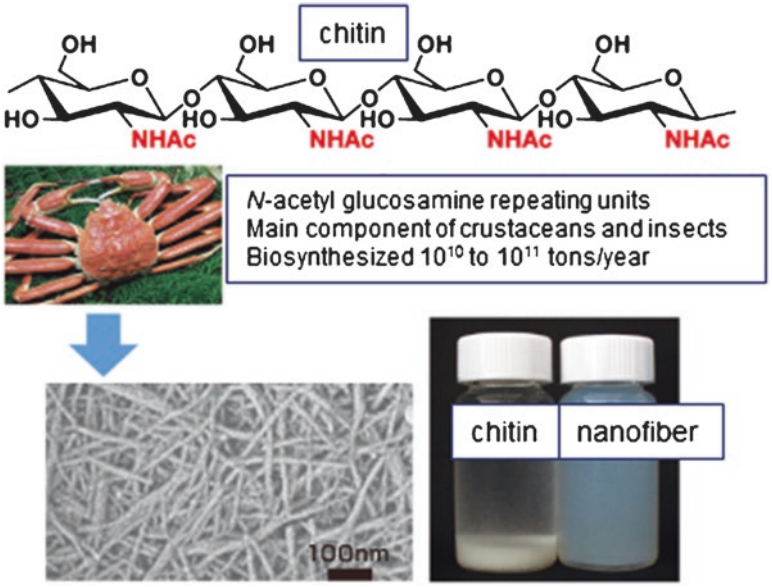

Fig. 20.2 Chitin nanofibers extracted from crab shells. They show high formability, efficient mechanical properties and several biological functions

20.3 Amylosic Composite Materials

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Keywords Helical polymer, Supramolecule, Hierarchical structure, Enzymatic polymerization, Smart polysaccharide

1. Significance in the field of glycoscience and its current situation
   Amylose is a food polysaccharide, that is, as a component of starch. It can be expected to be a functional material owing to its regularly controlled helical structure as well as non-toxic and biodegradability [8]. Because of the difficulty in complete separation of amylose from starch, phosphorylase-catalyzed enzymatic polymerization is considered to be a powerful tool to obtain pure amylose [9, 10]. Maltooligosaccharide is required in the polymerization system and the reaction progresses at the non-reducing end of the primer. Therefore, primers immobilized with functional substrates at the reducing end can be employed in the enzymatic polymerization to produce amylosic composite materials with well-defined structures [11]. Furthermore, the products construct higher-ordered structures through helical formation of amylose chains. Practical applications of enzymatically prepared amylosic composite materials are expected.
2. Impact on other fields of research
   In addition to the non-toxic and biodegradable properties of amylose, the synergistic effect on combination with functional substrates will lead to applications as environmentally benign- and biomaterials [12].
3. Significance as fundamental research
   By means of the formation of a double helix and helical inclusion complex with functional molecules, amylosic composite materials can be used in research on supramolecular and hierarchically structural composite materials.
4. Possible application for industry and medicine, if any
   The synergistic effect of amylose characteristics and combination with various functional substrates will lead to application as functional materials such as biomaterial [9].
5. Future perspectives
   The precision enzymatic synthesis of composite materials will lead to application of amylose as a medical smart polysaccharide besides food polysaccharides.
6. Problems to be solved
   To develop new applications of amylose, additional characteristic properties besides being a helical polymer are demanded.

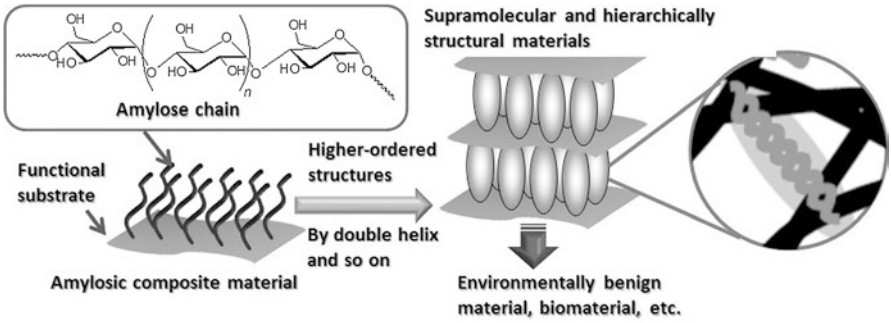

Fig. 20.3 Construction of supramolecular and hierarchically structural composite materials through formation of a higher-ordered structure from amylose

20.4 Supramolecular Gel Materials Formed by Cyclodextrins

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Keywords Cyclodextrin, Self-healing, Polymer gel, Tough polymer

1. Significance in the field of glycoscience and its current situation
   Cyclodextrins (CDs) are cyclic oligomers consisting of 6–8 glucose units. CDs form inclusion complexes selectively with various guest molecules. Therefore, they have been used for medical application and drug delivery systems. Since then, they have been studied for applications to foods and materials for controlled release. CDs are now commercially available at low cost, so they are used as new materials for various applications in daily use. CDs have been investigated for the construction of new supramolecular gels and materials, because they can be used as self-healing materials and adhesives using their specific property of non-covalent reversible host-guest interactions [13].
2. Impact on other fields of research
   Although cyclodextrins have been used for drug delivery systems and foods so far, they are now being investigated as self-healing and stimuli-responsive materials using their host-guest interactions. They are now also being studied as a component of artificial muscle and a part of new actuators [14].
3. Significance as fundamental research
   Cyclodextrins have unique inclusion properties. Supramolecular materials formed through host-guest interactions show drastic changes in physical properties compared with materials formed only through covalent bonds due to their selective reversible bonds.
4. Possible application for industry and medicine, if any
   As supramolecular materials formed by host-guest interactions show unique physical properties due to their non-covalent nature [15], they have potential use for drug delivery systems and medical applications [16].
5. Future perspectives
   Since supramolecular materials containing CDs have potential for enhancing the physical properties of polymer materials only via the addition of small amount. As supramolecular gels containing CDs show high toughness, they might be used for medical purposes [17].
6. Problems to be solved
   Mass production of CD at low cost (5 years).

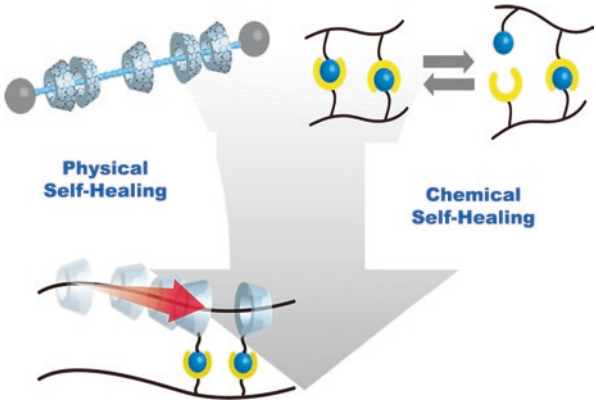

Fig. 20.4 Chemical and physical self-healing

20.5 Recyclability of Glycans

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Keywords Polysaccharide, Glyco-Chemistry-Cycle, Green chemistry, Biocatalyst, Polysaccharide biomass

1. Significance in the field of glycoscience and its current situation
   Almost all naturally occurring carbohydrates exist as glycosidic compounds; they include oligo- or poly-saccharides, glycolipids, glycoproteins, and nucleosides. For the biosynthesis of these compounds, it is necessary to activate the anomeric center of the saccharide unit by introducing a phosphate ester leaving group so that the anomeric carbon atom is attacked by a hydroxyl group of the aglycon part. Catalysts responsible for the glycosylation of these activated saccharides are synthases. After being utilized, these glycosidic compounds are finally converted to carbon dioxide and water via combustion or degradation catalyzed by glycosidases from bacteria [18]. It is, therefore, obvious that two kinds of enzymes, synthases and glycosidases, are involved in the processes of glycosylation and deglycosylation in nature, constructing a large carbon cycle system. A new carbon cyclic system that involves the production of functional glycosidic compounds based on the concept of "Glyco-Chemistry Cycle System" is proposed [19–21].
2. Impact on other fields of research
   This is a low environment-loading and highly economical process because the characteristic feature of inexpensive glycosidases is that they catalyze both glycosylation and deglycosylation reactions due to the complementarity of glycosidases and, therefore, the resulting products can be converted to the starting materials enzymatically without using any drastic reaction conditions.
3. Significance as fundamental research
   In this cyclic system, one glycosidase plays two roles: glycosidic bond formation and glycosidic bond cleavage. This concept consists of the transformation of a polysaccharide biomass to refined raw materials, the anomeric activation of the raw materials, polymerization (glycosylation) catalyzed by a glycosidase to give functionalized oligosaccharides, and the degradation of the products by the glycosidase. Basic research is inevitable to achieve this cyclic system.
4. Possible application for industry and medicine, if any
   Global strategy based on new biobased materials: Human milk oligosaccharides as nutrient additives (support for developing countries), glycoproteins (biomedicine), elicitors (oligosaccharides save the world), desert greening (environment), and cellulose nanofiber vehicle (energy saving).

5. Future perspectives
   All carbohydrate materials produced by enzymes of natural origin can have biodegradability that is tailored by the designer even if they have an artificial structure. Enzymatic glycosylation [22] as well as conventional chemical glycosylation will synergistically contribute not only to the creation of new glycotechnology, but also to the progress of the Green Chemistry in the future.
6. Problems to be solved
   (a) Constant supply of polysaccharide biomass source
   (b) Use of artificial intelligence for elucidation of structures and functions of biocatalysts
   (c) Protection-free activation of sugars
   (d) Scale preparation of biocatalysts through gene technology.

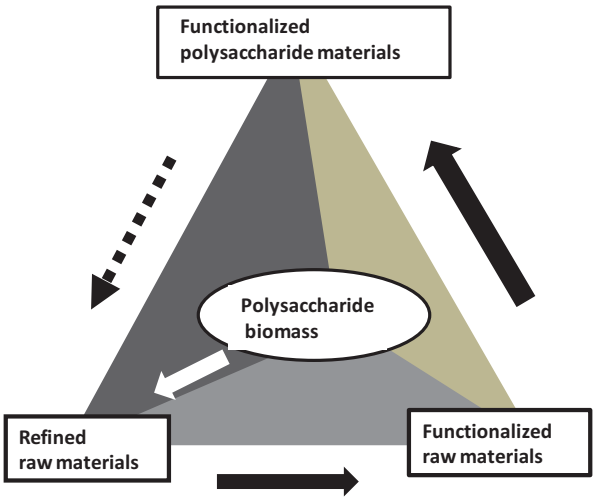

Fig. 20.5 Glyco-Chemistry Cycle System based on glycosidases and synthesis of functional polysaccharide materials [20, 21]

20.6 Polysaccharide Nanogel Engineering for Biomaterials

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Keywords Nanogel, Self-assembly, Biomaterials, Drug delivery system, Regenerative medicine

1. Significance in the field of glycoscience and its current situation
   Polysaccharides derived from living organisms have been used for a long time as biomaterials such as drug delivery systems (DDS) and regenerative medicine. Associating polymers in which a hydrophobic group is partly introduced into a water-soluble polysaccharide form a nano-sized self-assembled gel (nanogel). Polysaccharide nanogels have attracted attention as new carriers for biopharmaceuticals such as proteins and nucleic acids [23, 24]. Various polysaccharide-based microspheres, porous gels, fibers, and sheets have been constructed by using reactive polysaccharide nanogels as a building block or a cross-linker in a bottom up manner [25]. These materials have a novel gel structure with nanogels as nanomatrices and are used as a functional extracellular matrix in regenerative medicine, for example, as an excellent scaffold material for bone regeneration [26].
2. Impact on other fields of research
   It has a ripple effect on other research areas such as hybrid materials of polysaccharide nanogels with other biomolecules such as proteins, nucleic acids and exosomes, and also organic-inorganic materials of nanogels with apatite, quantum dots and magnetic particles [27].
3. Significance as fundamental research
   With the development of science of self-organization using polysaccharides as functional components, it will become possible to develop new integrated polysaccharide-based materials with new functions.
4. Possible application for industry and medicine, if any
   Nanogels of hydrophobized pullulan show excellent antitumor activity as therapeutic cancer vaccines through complexing with oncogenic antigen proteins, and clinical trials are being conducted [24]. In addition, cationic hydrophobized pullulan nanogel has been demonstrated to be superior as a nasal vaccine carrier for the prevention of pneumococci, and efforts aimed at clinical trials are proceeding.

5. Future perspectives
   Polysaccharide-based materials have high potential as highly functional materials for nanomedicines such as drug delivery systems and regenerative medicines due being highly biodegradable and environmentally friendly materials.
6. Problems to be solved
   Tailor-made synthesis of polysaccharides, establishment of chemical modification methods.

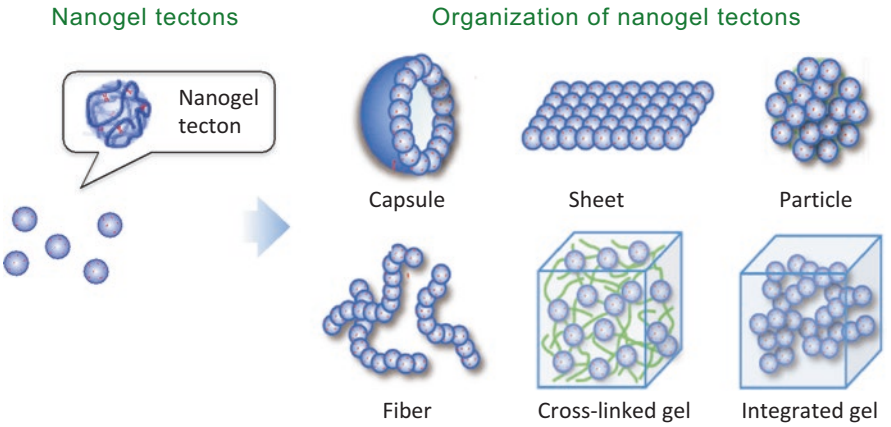

Fig. 20.6 Development of polysaccharide-based materials

20.7 Glycopolymer Materials for Nanomedicine Application

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Keywords Glycopolymer, Multivalency, Biomaterials, Molecular Recognition, Signal Transduction

1. Significance in the field of glycoscience and its current situation
   Saccharides are ligands of biology, and recognized by proteins and cells. The saccharide-protein interactions are weak in the monomeric state, but amplified in the assembling structure based on the multivalent interaction. Glycopolymers are biofunctional polymers that have pendant saccharides and exhibit large multivalent effects [29]. Glycopolymers are utilized as biomaterials that are potential materials for drug delivery systems and scaffolds for tissue engineering due to their specific interaction with protein and cells. Glycopolymers are also applicable to the molecular recognition units for biosensors, proteome and cell engineering. The problems for the practical use are the preparation method and control of the polymer properties.
2. Impact on other fields of research
   Since glycopolymers can control multivalency, glycopolymer study on molecular recognition contribute to the basic biology. Glycopolymers are known as molecular recognition polymers and contribute to the biomaterials and synthetic polymer areas [30].
3. Significance as fundamental research
   Glycopolymers are utilized for molecular recognition in proteins, viruses, bacteria and cells *in vivo* and *in vitro*. Glycopolymers are prepared with various valencies and practical oligosaccharides, and investigated to clarify the molecular recognition including thermodynamics.
4. Possible application for industry and medicine, if any
   Glycopolymers have already been applied to the analysis of sugar recognition proteins, and separation of proteins with glycopolymer immobilized gels and substrates [31]. Drug delivery systems and cell scaffolds for specific organs are strongly expected as an application of glycopolymers.
5. Future perspectives
   The cell analysis method with glycopolymers is expected to be standardized with glycopolymers for basic biology. At the same time, the supply of glycopolymers is expected to be improved based on the polymer technology.

6. Problems to be solved
   Facile and practical approaches for glycopolymers are needed. A stable supply of oligosaccharides is also a key for the practical realization of glycopolymers. The molecular structure of glycopolymers are better to be standardized because current glycopolymer studies are performed by various kinds of polymers (5 years).

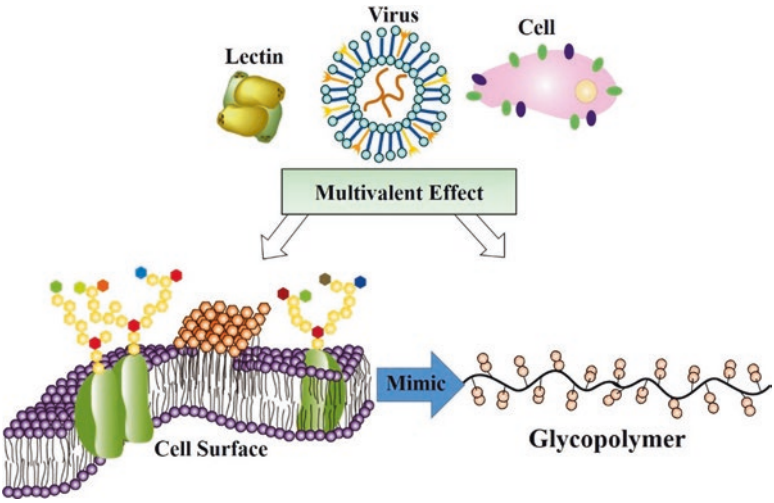

Fig. 20.7 Glycopolymers mimic the multivalent structure of sugar on the cell surfaces. Due to the multivalent structure of sugars, the interactions between proteins, viruses and cells are amplified

20.8 Optical Resolving Agents

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Keywords Enantioseparation, Chiral stationary phase, HPLC, Phenylcarbamate, Enantiomer

1. Significance in the field of glycoscience and its current situation
   Many drugs are chiral and their pharmaceutical activities often differ between enantiomers. Therefore, the development of enantiomerically pure drugs has been highly recommended [33]. For such development, efficient preparative and analytical methods for enantiomers are strongly required. Enantioseparation (optical resolution) by HPLC and SFC, which can be used on both preparative and analytical scales, has attracted much attention [34]. The key to these approaches is the chiral stationary phases (CSPs) with high ability, and many CSPs derived from small chiral molecules and polymers have already been reported [35]. Among these CSPs, phenylcarbamate and benzoate derivatives of cellulose and amylose are most frequently used for the enantioseparation of a wide range of chiral compounds today [35–37]. The chiral separation abilities of the polysaccharide derivatives seem much better than those of other CSPs [35].
2. Impact on other fields of research
   In fields dealing with chiral compounds, preparation of pure enantiomers and precise speedy determination of their purity with trace amounts of samples are highly required. The separation and analysis of enantiomers by chiral HPLC and SFC are extremely valuable as the methods suitable for these purposes [34].
3. Significance as fundamental research
   This separation method is based on the chiral recognition ability of polysaccharide derivatives as to chiral compounds, and the steric structure of polysaccharide derivatives plays an essential role [36]. Study on this separation method is attractive and important as basic research to unravel the relationship between polymer structure and molecular recognition.
4. Possible application for industry and medicine, if any
   The chiral stationary phases (CSPs) for HPLC derived from polysaccharide derivatives are widely used for the development of chiral drugs [37], and if we can develop even better CSPs, they will be more used by pharmaceutical companies.

5. Future perspectives
   If polysaccharide-based CSPs with higher ability are developed, it is expected that the CSPs can be more frequently used in the production of more chiral compounds by combination with asymmetric synthesis.
6. Problems to be solved
   It is required to elucidate the chiral recognition mechanism of polysaccharide-based CSPs at the molecular level and to find a method for variously changing their abilities (10 years and more).

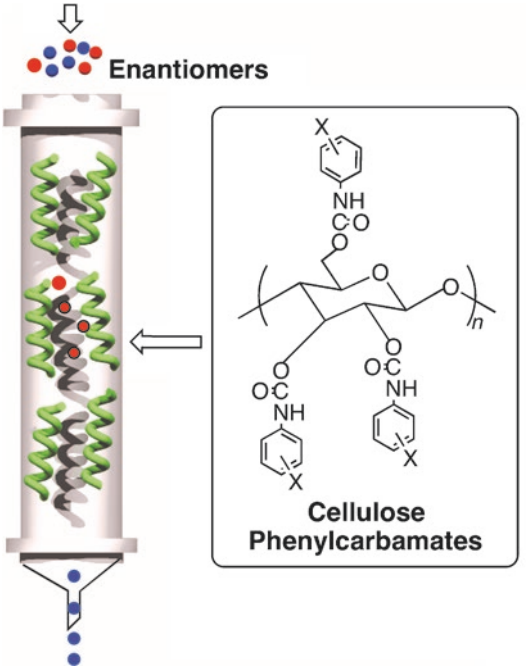

Fig. 20.8 Separation of enantiomers by HPLC using cellulose phenylcarbamate derivatives as chiral stationary phases

20.9 Polysaccharides for Regenerative Medicine

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Keywords Chitin, Chitosan, Alginic acid, Hyaluronic acid, Tissue engineering

1. Significance in the field of glycoscience and its current situation
   Four major polysaccharides used in regenerative medicine, chitin [38], chitosan [38], alginic acid [39] and hyarulonic [40] acid are industrially mass-produced as biomaterials. They are used to prepare hydrogels, films and sponges utilized for medical devices and scaffold of tissue engineering in regenerative medicine. Another category of polysaccharides include various glycosaminoglycans and proteoglycans of biological activity for cell adhesion and for enhancement of growth factors [41, 42]. We mainly focus on four polysaccharides in this text. Chitin and chitosan are linear glycoside polymer of *N*-acethylglucosamine and glucosamine, respectively, and are extracted from crab shell. Alginic acid consists of linier glycoside polymer of α-*L*-gluronic acid and β-*D*-mannuronic acid, and are extracted from brown algae. Hyaluronic acid is a linear glycoside polymer of *N*-acethylglucosamine and glucuronic acid, extracted from chicken cockscomb and also produced by bacterial fermentation
2. Impact on other fields of research
   Chitosan is soluble in an acidic aqueous solution and easy to fabricate, while chitin is insoluble and difficult to fabricate. Both of them are biocompatible and are used for wound dressings (films or meshes), tissue engineering scaffolds (films, hydrogels, and sponges), and nerve conduits for regeneration after peripheral nerve injury [38].
3. Significance as fundamental research
   Calcium ion added to an alginate solution can crosslink gluronic acids in alginate molecules and form a calcium alginate hydrogel. Calcium alginate gels are biocompatible but less cell-adhesive. They are used for by entrapment of β-islets as immunologically-isolating membranes, and as a scaffold for chondrocytes in cartilage tissue engineering. Recently, they are be used as fast-gelling materials in microfluidics-based fabrication and as an ink for 3D bio-printing [39].
4. Possible application for industry and medicine, if any
   Hyaluronic acid is abundant in the extracellular matrices dermis. It forms a transparent soft hydrogel with a swelling property and moisturizing effects. The hydrogel does not have enough mechanical strength. Hyaluronic acid can be crosslinked and mixed with other materials to yield composites. They are used for wound dressings and medical devices in plastic surgery, orthopedics, and ophthalmology, and also for scaffold in skin tissue engineering [40].

5. Future perspectives
   Chitin, chitosan, and alginic acid can be produced from a plentiful supply of raw material, crab shells and brown algae (seaweed). Hyaluronic acid is extracted from chicken cockscombs. Recently, bacterial fermentation is replacing extraction for mass-production. These polymers are used as food additives, supplements, components for cosmetics, etc. Then mass-production of these polymers are established.
6. Problems to be solved
   On the other hand, mass-production of other polysaccharide, especially glycosaminoglycans and proteoglycans with various interesting biological activities, has not been established yet, but is promising for future application, parallel with the progress of stem cell biology [41, 42]. Utilization of bioactive polysaccharides will expand from the present utilization as structural biomaterials if the mass-production of glycosaminoglycans is established in the near future (within 10 years).

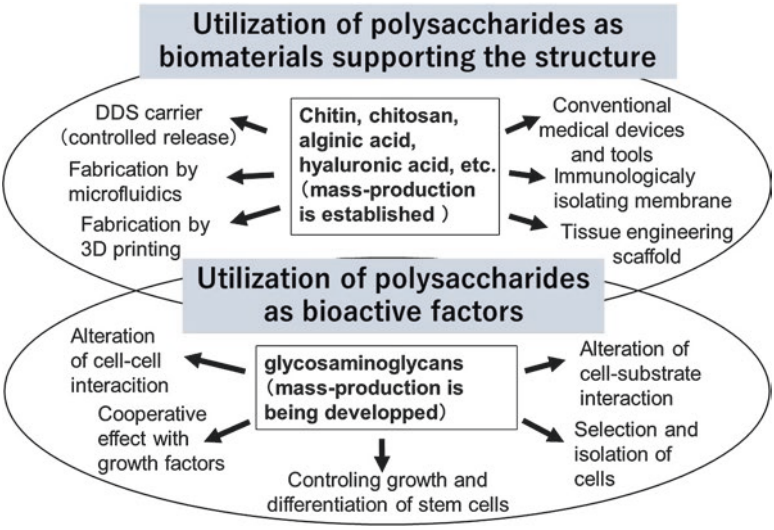

Fig. 20.9 Utilization of bioactive polysaccharides will expand from the present utilization as structural biomaterials if the mass-production of glycosaminoglycans is established in the near future

References

References Section for 20.1

1. Eichhorn SJ et al (2010) Review: current international research into cellulose nanofibers and nanocomposites. J Mater Sci 45:1–33
2. Nakagaito AN et al (2010) Displays from transparent films of natural nanofibers. MRS Bull 35:214–218
3. Moon RJ et al (2011) Cellulose nanomaterials review: structure, properties and nanocomposites. Chem Soc Rev 40:3941–3994
4. Lindströma T, Aulin C (2014) Market and technical challenges and opportunities in the area of innovative new materials andcomposites based on nanocellulosics. Scandinavian J Forest Res 29:345–351

References Section for 20.2

5. Ifuku S, Saimoto H (2012) Chitin Nanofibers: preparations, modifications, and applications. Nanoscale 4:3308–3318
6. Azuma K et al (2014) Preparation and biomedical applications of chitin and chitosan nanofibers. J Biomed Nanotechnol 10:2891–2920
7. Ifuku S (2014) Chitin and chitosan nanofibers: preparations and chemical modifications. Molecules 19:18367–18380

References Section for 20.3

8. Egashira N et al (2017) Enzymatic grafting of amylose on chitin nanofibers for hierarchically construction of controlled microstructures. Polym Chem 8:3279–3285
9. Nishimura T, Akiyoshi K (2016) Amylose engineering: phosphorylase-catalyzed polymerization of functional saccharide primers for glycobiomaterials. Wiley Interdiscip Rev Nanomed Nanobiotechnol 9:e1423
10. Shoda S et al (2016) Enzymes as green catalysts for precision macromolecular synthesis. Chem Rev 116:2307–2413
11. Kadokawa J et al (2015) Preparation of multiformable supramolecular gels through helical complexation by amylose in vine-twining polymerization. Polym Chem **6**:6402–6408
12. Nishimura T et al (2015) Glyco star polymers as helical multivalent host and biofunctional nano-platform. ACS Macro Lett 4:367–371

References Section for 20.4

13. Harada AE (2012) Supramolecular polymer chemistry. Wiley VCH, Weinheim
14. Iwaso K et al (2016) Fast responsive dry-type artificial molecular muscles with {c2}daisy chains. Nat Chem 8:625–632

15. Okumra Y et al (1998) Inclusion-dissociation transition in the complex formation between molecular nanotubes and linear polymer chains in solutions. Phys Rev Lett 80:5003–5006
16. Harada A et al (1992) Molecular necklace: rotaxane containing many threaded α-cyclodextrins. Nature 356:325–327
17. Nakahata M et al (2011) Redox-responsive self-healing materials formed from host-guest polymers. Nat Commun 2:511

References Section for 20.5

18. Coelho MAA, Ribeiro BD (2016) White biotechnology for sustainable chemistry. Royal Society of Chemistry, Cambridge
19. Taniguchi N et al (2008) Experimental glycoscience. Springer, Tokyo
20. Shoda S et al (2016) Enzymes as green catalysts for precision macromolecular synthesis. Chem Rev 116:2307–2413
21. Shoda S et al (2003) Green process in Glycotechnology. Bull Chem Soc Jpn 76:1–13
22. Fraser-Reid B et al (2001) Glycoscience II, chemistry and chemical biology. Springer, Berlin

References Section for 20.6

23. Sasaki Y, Akiyoshi K (2010) Nanogel engineering for new nanobiomaterials: from chaperoning engineering to biomedical applications. Chem Rec 10:366–376
24. Tahara Y, Akiyoshi K (2015) Current advances in self-assembled nanogel delivery systems for immunotherapy. Adv Drug Deliv Rev 95:65–76
25. Tahara Y et al (2015) Nanocarrier-integrated microspheres: nanogel tectonic engineering for advanced drug delivery systems. Adv Mater 27:5080–5088
26. Hashimoto Y et al (2018) Nanogel tectonics for tissue engineering: protein delivery systems with nanogel chaperones. Adv Healthcare Mater 23:1800729
27. Kawasaki R et al (2016) Magnetically guided protein transduction by hybrid of nanogel chaperone with iron oxide nanoparticles. Angew Chem Int Ed 55:11377–11381

References Section for 20.7

28. Miura Y et al (2016) Glycopolymer Nanobiotechnology. Chem Rev 116:1673–1692
29. Zhang Q et al (2013) Sequence-controlled multi-block Glycopolymers to inhibit DC-SIGN-gp120 binding. Angew Chem 125:4531–4535
30. Paszek MJ et al (2014) The Cancer Glycocalyx mechanically primes integrin-mediated growth and survival. Nature 511:319–325
31. Oh YI et al (2013) Tailored Glycopolymers as anticoagulant heparin Mimetics. Angew Chem 125:12012–12015
32. Kiessling LL, Grim JC (2013) Glycopolymer probes of signal transduction. Chem Soc Rev 42:4476–4491

References Section for 20.8

33. Subramanian G (ed) (2007) Chiral separation techniques: a practical approach, 3rd edn. Wiley-VCH, Weinheim
34. Carreira E, Yamamoto H (eds) (2012) Comprehensive chirality, vol. 8, separations and analysis. Elsevier, Amsterdam
35. Okamoto Y, Yashima E (1998) Polysaccharide derivatives for chromatographic separation of enantiomers. Angew Chem Int Ed 37:1020–1043
36. Ikai T, Okamoto Y (2009) Structure control of polysaccharide derivatives for efficient separation of enantiomers by chromatography. Chem Rev 109:6077–6101
37. Shen J, Okamoto Y (2016) Efficient separation of enantiomers using Stereoregular chiral polymers. Chem Rev 116:1094–1138

References Section for 20.9

38. Anitha A et al (2014) Chitin and chitosan in selected biomedical applications. Prog Polym Sci 39:1644–1667
39. Sun J, Tan H (2013) Alginate-based biomaterials for regenerative medicine applications. Materials 6:1285–1309
40. Kim H et al (2017) Hyaluronate and its derivatives for customized biomedical applications. Biomaterials 123:155–171
41. Okolicsanyi RK et al (2014) Mesenchymal stem cells, neural lineage potential, heparin sulfate proteoglycans and the matrix. Dev Biol 388:1–10
42. Furukawa J et al (2016) Glyconomics of human embryonic stem cells and human induced pluripotent stem cells. Glycoconj J 33:707–715

Part VI
Educational Materials and Training for Glycosciences

Foreword

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To promote glycoscience research, it is essential to educate and train young researchers. In Japan, several educational and training activities have been taking place. For example, the GlycoForum website has been referred to by students and researchers around the world; it has even been presented in Nature Reviews. Glycoscience researchers in Japan have voluntarily written and submitted articles, making them available on GlycoForum. Also, an online e-learning web site for studying the basics of glycoscience has also been developed. This site, called ”E-learning for glycomics”, is a system offering a fun way for high-school students to self-study and provides quizzes based on the content presented. In recent years, domestic training for researchers has also been taking place, providing opportunities to gain training experience in lectin and glycomics analytics technologies. Currently, web resources on the Internet are a necessity to deepen understanding of the glycosciences, and in fact several glycan-related databases have been constructed. To allow computers to be able to handle these glycan data stored in databases, chemoinformatics is important. Such technologies will also be introduced.

Chapter 21
Educational Materials and Training for Glycosciences

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21.1 Educational Materials and Training for Glycosciences

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Keywords GlycoForum, Glycoscience, Glycomics, database, Training, E-learning

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1. Significance in the field of glycoscience and its current situation
   The establishment of structural analytical methods for glycans prepared from glycoproteins, glycosaminoglycans, and glycolipids; the detection of glycans using lectins or antibodies; information regarding gene expression of glycosyltransferases, etc.; the accumulation and organization of glycan-related information and searches using this information; and a system to make queries about the function and related information of characterized structures; these can all be considered "Glycomics".
   Although each of these areas of glycomics needs to be established and developed, it is necessary to share the obtained results with other researchers to promote understanding of the glycosciences.
   Despite the fact that there have been fragmented activities, such as the introduction of database development based on experimental results obtained by various research groups including AIST, formalized training courses have not been made available to researchers.
   On the other hand, with the purpose of contributing to glycoscience, Seikagaku Corporation developed the website "GlycoForum" in 1997 (Fig. 21.1) [1]. Its main content comprises explanation of fundamental terms (GlycoWords) required to understand glycoscience as well as various series focused on specialized genres (e.g., hyaluronan, glycogenes and glycomicrobiology). GlycoWord serves as a handbook for glycoscience by providing explanations of the meaning and background of terms, covering seven genres and 187 terms, and is all written by experts in the field. The specialized series, especially the hyaluronan series written by 40 world leaders, has become an excellent site to introduce the field. GlycoForum has been maintained and updated for many years through the continuous operation by Seikagaku Corporation and the cooperation of a wide range of researchers, contributing to the improvement of understanding of glycoscience. Since 2000, it has been introduced as a recommended website by Nature Reviews [2].
   In addition, an e-learning site, "e-learning for glycomics", has been developed for users to study glycans on the Internet [3]. This site was developed to allow users to gain basic knowledge required to be able to use the computer tools for analyzing glycans provided by RINGS (Resource for Informatics of Glycomes at Soka). It consists of four chapters: "Overview of glycoscience", "Basics of glycobiology", "To use RINGS tools", and "The RINGS Tools Collection". Each chapter provides an online quiz which displays evaluation results to allow users to review the content.
   In regards to study groups and research meetings, the Forum of Young Glycoscientists and the Glycoinformatics Young Scientists Community have held study meetings and seminars every year. Many young researchers from around the country gather to share their latest research results, or to attend "glycohackathons" where participants can focus on programming in a week-long camp-style event.
   Moreover, the Nanotech Career-up Alliance (Nanotech CUPAL) was established based on subsidized projects for fostering science and technology personnel called "The construction project for the consortium of the fostering of science and technology personnel" in FY2014, where the TIA (Tsukuba

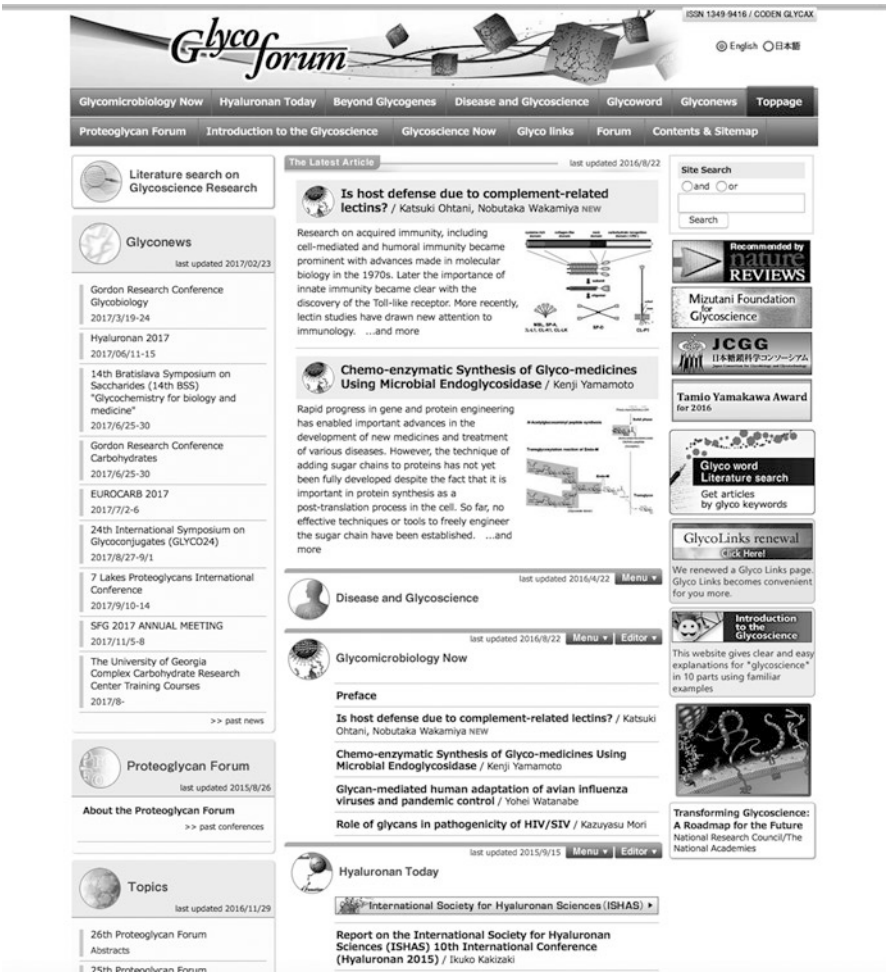

Fig. 21.1 GlycoForum website

Innovation Arena) and Kyoto University's Nanotechnology Hub, play a central role in training (1) Nanotech Research Professionals (N.R.P.) and (2) Nanotech Innovation Professionals (N.I.P.) [4]. Since FY2016, KEK and Tokyo University joined as new TIA members in addition to AIST, Tsukuba University and NIMS, and now the research areas are expanded not only to nanotechnology but also to bioscience and computer science, etc. In FY2016, the Lectin Application Technology Working Group was selected as one of 39 projects named Kakehashi (meaning "linking bridge"): TIA collaborative research program [5].

With this collaborative framework a line of education programs was started based on the CUPAL system, which include workshops (held three times in

FY2016 with three more planned for 2017), a summer school, and CUPAL NIP-to experience glycan profiling technology (Fig. 21.2).

2. Impact on other fields of research
   Although it is known that over 50% of proteins are glycosylated, analytical methods to easily analyze glycosylation are not available. If this situation is improved, it is certain that it would greatly affect many research areas.
   GlycoForum and "e-learning for glycomics" serve as handbooks for beginners to the glycosciences and researchers from other research areas. GlycoForum especially uses many figures to allow users to gain a deeper understanding of the text. Additionally, materials from these sites can be used for educational/research purposes as well as presentations as a convenience to users.

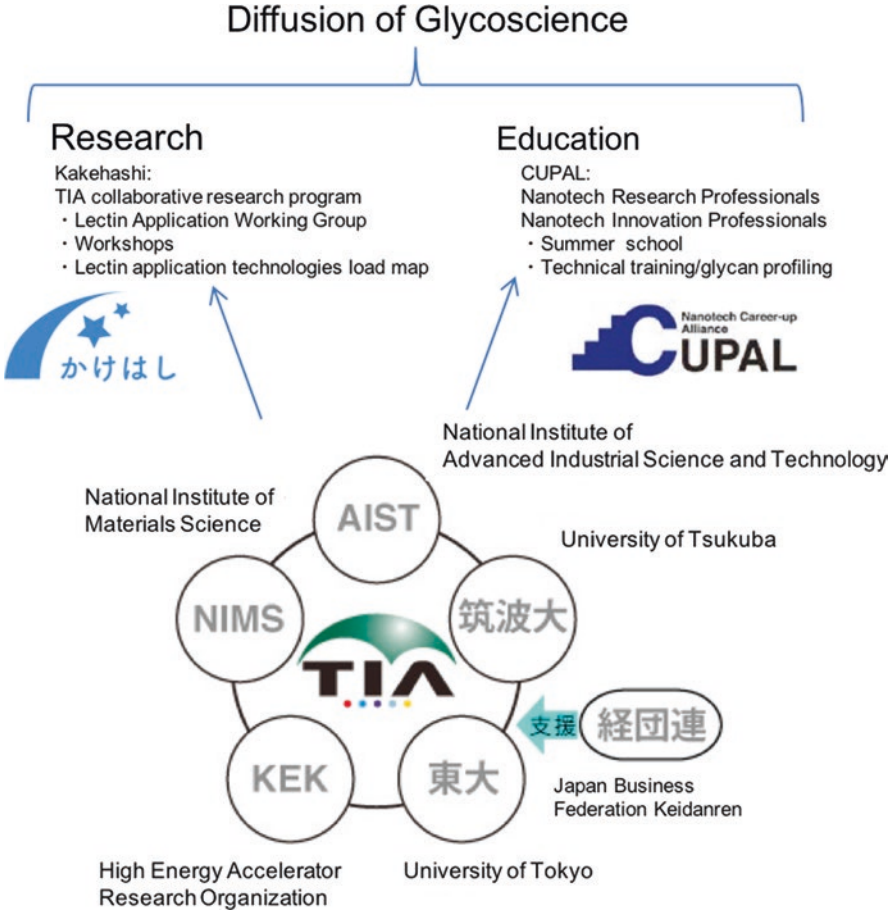

Fig. 21.2 In the framework of the TIA Kakehashi program, five institutes consisting of AIST, the University of Tsukuba, NIMS, KEKE, and the University of Tokyo, established a Lectin Application Working Group in 2016, each holding a half-day workshop. In addition, for educational and diffusive purposes, a summer school for students as well as two technical training sessions for industries were planned for 2017

The most serious problems of glycoscience are the lack of both opportunities of education to learn basic knowledge of glycans and to gain experience in glycan analysis. By providing such opportunities in the framework of TIA (around Tsukuba), it will generate greater opportunities to propagate glycoscience to other fields.

3. Significance as fundamental research
   The glycosciences must directly face the issues of evolution, species diversity, and polymorphisms within species among others; issues that are not very problematic in other disciplines. Until answers to these problems are obtained, it will remain a basic science. Until then, activities to publish and publicize the results of glycomics for other researchers in other fields must continue.
   On the other hand, many scientists now acknowledge the essential importance of glycoscience, while it is true that there are few opportunities to learn basic knowledge and experience the basic procedures of glycan analysis. Once these hurdles are overcome, many inventions will be made in various fields including the life sciences.
   GlycoForum and other websites play an important role in disseminating information about the glycosciences. For example, the number of hits to GlycoForum was approximately 960,000 in 2016, consisting of accesses from many research institutions, educational institutions, and industry, thus helping to promote fundamental research on glycans. Moreover, there are many uses of databases available on the Internet, such as those introduced in the next section, so it can be thought that these resources can contribute to fusion research in all areas of life science research in the future.
4. Possible application for industry and medicine, if any
   In the medical field, glycosciences have overcome several challenges and produced results. In material science as well, we can expect much progress to be made in the future. It is important to teach and pass on analytical techniques, to publish, and to expand the use of integrated knowledge of glycomics so that they can be used for individual problems.
   Considering the extreme difficulty of both understanding and applying glycoscience, it is possible in the future that a new service industry supporting glyco-education and glyco-research will develop.
5. Future perspectives
   In the future, the glycosciences can greatly contribute to the medical field and material science. Moreover, all-Japan team construction is necessary with TIA as an FS trial toward glycoscience education and propagation to increase the population of glycoscience researchers, which will further expand to a worldwide network.
6. Problems to be solved
   Within 5 years:
   The establishment and improvement of glycomics is necessary, as is the steady continuation of its propagation.

On the other hand, accesses to the GlycoForum web site have increased, and in order to maintain it as a necessary and trusted information resource for glycoscience research, its information must be renewed. To that end, collaboration and maintenance with many experts are required, and networks based on experts' initiatives are necessary. Focusing on the genres and areas provided until now, in the future, it is important to further enhance and update this site. It will be necessary to develop schemes to accumulate more terms for GlycoWord on a global scale, increasing the number of terms that can be edited annually.

Within 10 years:

First of all, it is necessary to show the importance of glycoscience by more and more successful investigations and industrial applications, and in the next step it is necessary to systemize the facts for the purposes of both education and propagation.

21.2 Glycan-Related Databases and the Life Science Database Integration Program (JST)

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Keywords Glycan structure, Repository, Database integration

1. Significance in the field of glycoscience and its current situation
   Databases of research results are accumulations of research results, and by making them into databases, it becomes possible not only to record and provide research results, but also to link with data of various databases. Therefore, databases are indispensable for carrying out research. Several glycan-related databases have been developed by domestic research institutions and organizations, including KEGG GLYCAN [6]. With the support of the Ministry of Education, Culture, Sports, Science and Technology and JST/NBDC, the Database Integration Coordination Program (DICP) was started (Fig. 21.3), and the Japanese Consortium for Glycobiology and Glycotechnology Database (JCGGDB) was constructed (Fig. 21.4) as a database of the Japanese Consortium for Glycobiology and Glycotechnology (JCGG) [8]. In addition, the international glycan structure repository GlyTouCan was developed under this program (Fig. 21.5), starting the development of a fundamental infrastructure for storing glycan information, which is indispensable for glycan research [9, 10]. GlyTouCan is an international repository analogous to GenBank and PDB. These repositories assign an arbitrary ID (accession number) to a gene sequence or protein structure, respectively, and they are used by researchers all over the world because it is necessary to refer to that ID when writing manuscripts. Although various glycan-related databases have been developed in recent years, there was no repository for registering glycan structures (sequences), and there was an increasing necessity for an international repository for unifying glycan structures (sequences). Therefore, GlyTouCan (https://glytoucan.org) was developed as a repository that assigns arbitrary IDs to glycan structures (sequences). Links from one glycan structure to other entries in glycan-related databases are also provided through a user friendly Web interface. Moreover, since FY2017, construction of a glycoscience portal, GlyCosmos (https://glycosmos.org), began under a new JST project. With the development of GlyCosmos, information from various fields such as proteomics, lipidomics and genomics will be integrated through glycans.

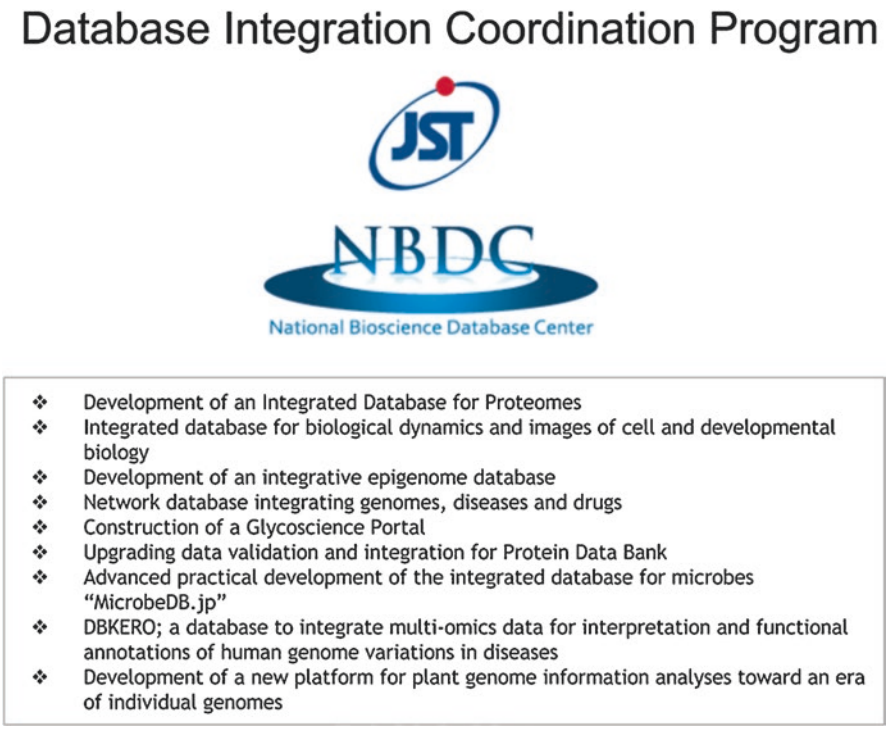

Fig. 21.3 The Life Science databases being supported by JST/NBDC

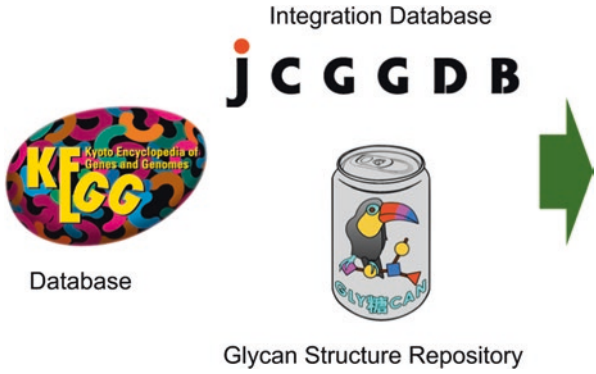

Fig. 21.4 Glycan-related databases began with their construction by each organization, but then integration started, and the glycan structure repository serving as their foundation was constructed. In the future, as further promotion of integration and cooperation with other research fields progresses, it will continue to develop

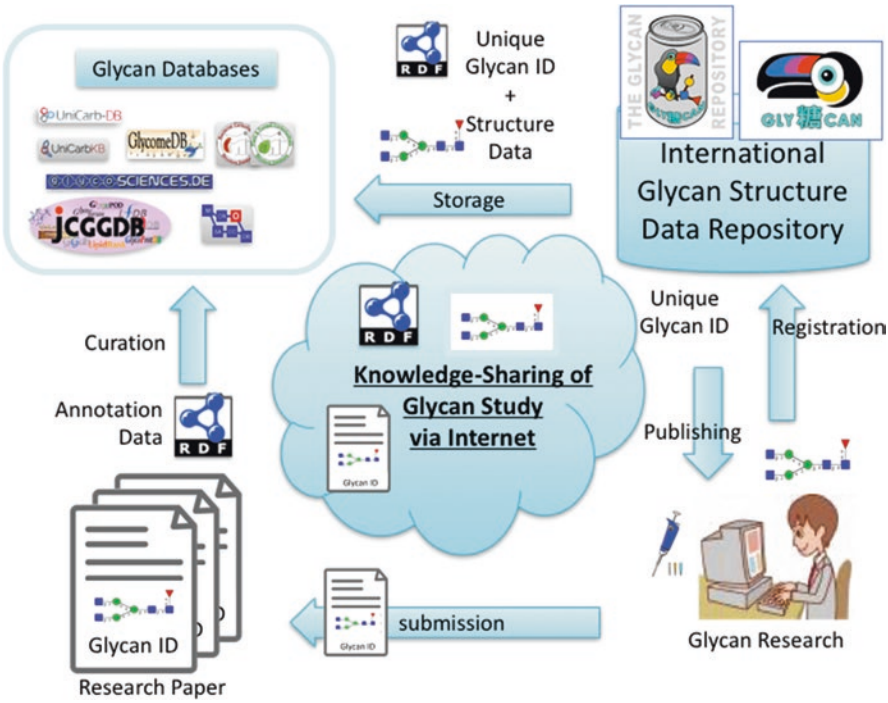

Fig. 21.5 Scientists can obtain glycan IDs by registering glycan structures. These IDs can be cited in their manuscripts, which are then taken up by curated databases along with their metadata such as organism and experimental conditions. Curated databases would also use glycan IDs, by which the relationship to other databases, provided by GlyTouCan, can also be viewed

2. Impact on other fields of research

   The role of databases is to record various research results, to construct a foundation to effectively use them as data, and to provide the information to other researchers. Collaboration and integration with databases of other research areas will lead to a deepening of the understanding of the importance of glycans.

   KEGG GLYCAN has been developed for over 10 years. It allows users to consistently browse pathways, genes, and compound information related to basic glycans, and cooperation with omics fields other than glycomics continues. On the other hand, JCGGDB integrates domestic glycan-related databases, making cross-searching possible. JCGGDB data has also been reconstructed using Semantic Web technology and is provided as the ACGG-DB (Asian Community of Glycoscience and Glycotechnology Database).

   Simultaneously with the development of ACGG-DB, GlyTouCan was developed, and as the basis for uniquely representing glycans, WURCS was developed as a new notation for carbohydrate structures [11]. WURCS makes it possible to arbitrarily describe ambiguous glycan structures. Since GlyTouCan also uses Semantic Web technology, it has become possible to dynamically link

from one accession number to external glycan-related databases, including not only JCGGDB and KEGG GLYCAN, but also chemical compound and protein databases such as the database of chemical compounds PubChem, and the protein structure repository PDB (Protein Data Bank).
Furthermore, among the aims of the GlyCosmos project, a glycoconjugate repository will be developed to complement the protein sequence repository UniProt, lipid databases, and GlyTouCan. The plan is also to accumulate data on glycan-related metabolism and signaling pathways, which will include incorporation of glyco-gene data of other species. On the other hand, WURCS will also be extended to accommodate this data expansion. Moreover, information on the three-dimensional structures of glycans is increasingly being accumulated, and so data necessary for three-dimensional structure analysis of glycoconjugates, which is considered to be important for recognition, will also be collected. Therefore, it will become easier to obtain data on various research areas that have been difficult to obtain in the past. Information in glycan-related databases will also become more easily accessible to researchers in other research fields and it is expected that it will be possible to more easily conduct research that makes use of glycan information.

3. Significance as fundamental research
Databases have an important role to record and provide research results, and they are important as an essential foundation for research. Basic research on glycan informatics to realize this is important.
Conventionally, glycans were expressed in a character string format such as IUPAC, and sometimes it is difficult to read when the notation becomes complicated. By referring to a glycan structure using its GlyTouCan ID, it is possible to refer to the glycan structure simply by using its ID such as in a paper. In addition, data across databases are easier to link together by comparing glycans using just their IDs. Thus databases of various fields have become easy to integrate. Databases that store research results are the foundation for research. Therefore, it is essential to enrich these databases, and to maintain and sustain them for fundamental scientific research.
4. Possible application for industry and medicine, if any
Utilization of research data accumulated in databases is important, as it can be directly and indirectly used for various applications. Databases pertaining to medicine and the environment, in particular, will aid elucidation of the functions of glycans. By incorporating glycan function in these databases, contributions can be made to glycan biomarker and biofuel research.
5. Future perspectives
Research data are produced on a daily basis, and it is of utmost importance to figure out how to store these research results into a database that is easily applicable to research. Integration and collaboration between databases are being promoted, and it is becoming possible for users to obtain the information they want, which contributes to shortening of research time and improvement of research quality.

Glycan information can now be easily accessed by life science researchers, and it has become easier to integrate with other omics fields such as proteomics and lipidomics. At present, database integration and cooperation in the life science field including glycosciences are being actively implemented, and realization of these are expected to provide new knowledge in the fields of medicine and industry.

6. Problems to be solved

Within 5 years

Currently the WURCS format being used in GlyTouCan is limited, and the unique representation of complex and more ambiguous glycans is not possible. Thus the development of WURCS is a difficulty that will need to be addressed within the next 5 years in order to fully integrate glycan-related databases. Moreover, it is necessary to promote GlyToucan and make it even more easily accessible to users.

Within 10 years

It is not always possible to use newly produced research data in existing systems. Also, it may be difficult to claim that cooperation between databases is sufficient. Furthermore, the development of easy-to-use interfaces should also be performed on an ongoing basis.

It is necessary to update existing databases so that they can be integrated with others. For this to be possible, the maintenance and expansion of ontologies, etc. are insufficient. To implement database integration for practical use by researchers, it is necessary for researchers to cooperate not only in Japan but internationally to solve these problems.

21.3 Glyco-Chemoinformatics

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Keywords Molecular simulation, Molecular dynamics, Molecular orbital method, Computational chemistry, Standardization

1. Significance in the field of glycoscience and its current situation
   The characteristics of glycan structures are diversity and flexibility, and it is possible for computational science and molecular simulation to provide molecular information, etc. for systems for which experiments are difficult. Therefore, their importance will increase in the future. On the other hand, in order to accurately record various research results, it is essential to clearly represent the glycan and related molecules involved. Therefore, WURCS was developed as a unique glycan structure notation (Fig. 21.6) [11]. In addition, by utilizing the fragment molecular orbital method and the replica exchange molecular dynamics method, it is possible to calculate additional information about glycans, such as their conformation and energy, which was traditionally difficult to compute, thus producing a variety of new knowledge [12, 13].
2. Impact on other fields of research
   Computational science and molecular simulation are used not only for glycans but in various fields, and it is expected that researchers in other areas and boundary areas will obtain interesting results by conducting research including glycans. In addition, WURCS, which can clearly express glycan structures, is useful for cooperation with other areas, and future development can be expected. GlyTouCan adopts WURCS as its basic glycan structure notation, allowing ambiguous glycan structures to be distinguished. Moreover, it became possible to cooperate with external glycan-related databases, and to link with databases of compounds and proteins such as PubChem [14], PDB [15] and so on.
3. Significance as fundamental research
   It is very important to accurately describe structural information on glycans in order to ensure accuracy in recording glycan research data. It is also important for the development of glycoscience research to conduct research using computational science on systems that can not be experimentally examined and to complement the interpretation of experimental results with computational science.
4. Possible application for industry and medicine, if any
   Molecular simulations are currently being applied to drug discovery, but in many cases glycans are not considered. In the future, as technology advances and data development progresses, it is expected that molecular simulation of glycans and related molecules can be readily applied to medical and industrial materials development. In particular, research and development is underway so that molecular simulation of glycoproteins can be easily carried out, contributing to applications in drug discovery, etc.

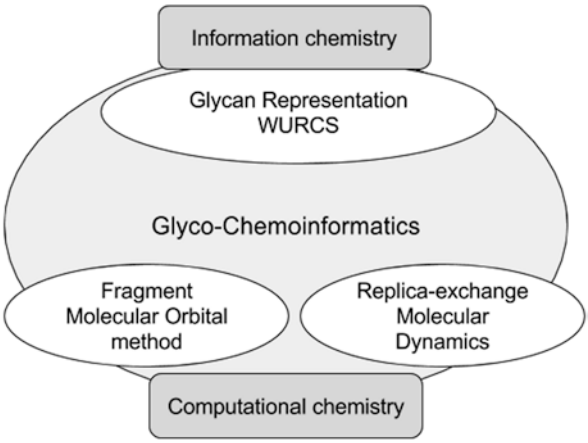

Fig. 21.6 Glyco-chemoinformatics overview

5. Future perspectives
   With standardization, progress in computational science and increasing speed of computer calculations, it is possible to perform highly accurate calculations that were difficult up to now, and it is expected that more accurate information will be produced.
6. Problems to be solved
   Within 10 years:

   - Standardization of glycan structures
   - Improvement of parameters of molecular dynamics simulations
   - Refinement of molecular orbital calculations

References

References Section for 21.1

1. GlycoForum: http://www.GlycoForum.gr.jp/index.html
2. Gagescu R (2000) Sweet talk. Nat Rev Mol Cell Biol 1:164
3. RINGS e-learning website: http://www.rings.t.soka.ac.jp/e-learning/
4. Nanotech Carreer-up Alliance.: https://nanotechcupal.jp/
5. TIA Kakehashi Project: https://www.tia-nano.jp/kakehashi/events.html

References Section for 21.2

6. Aoki-Kinoshita KF, Kanehisa M (2015) Glycomics analysis using KEGG GLYCAN. Methods Mol Biol 1273:97–107
7. Maeda M et al (2015) JCGGDB: Japan consortium for Glycobiology and Glycotechnology database. Methods Mol Biol 1273:161–179
8. Aoki-Kinoshita K et al (2016) GlyTouCan 1.0 – the international glycan structure repository. Nucleic Acids Res 44:D1237–D1242
9. Aoki-Kinoshita KF et al (2013) The fifth ACGG-DB meeting report: towards an international glycan structure repository. Glycobiology 23:1422–1423
10. Tanaka K et al (2014) WURCS: the Web3 unique representation of carbohydrate structures. J Chem Inf Model 54:1558–1566

References Section for 21.3

11. Matsubara M et al (2017) WURCS 2.0 update to encapsulate ambiguous carbohydrate structures. J Chem Inf Model 57:632–637
12. Re S et al (2012) Conformational flexibility of N-glycans in solution studied by REMD simulations. Biophys Rev 4:179–187
13. Sawada T et al (2008) Ab initio fragment molecular orbital studies of influenza virus hemagglutinin-sialosaccharide complexes toward chemical clarification about the virus host range determination. Glycoconj J 25:805–815
14. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE (2019) PubChem 2019 update: improved access to chemical data. Nucleic Acids Res 47(D1):D1102–D1109
15. Berman H, Henrick K, Nakamura H (2003) Announcing the worldwide protein data bank. Nat Struct Biol 10:980

Summary and Future Perspectives for Glycoscience

At this time, we obtained information concerning more than 150 items from researchers in each specialied area by using a questionnaire method to publish this book. In each item, you will find important research subjects listed and issues to be addressed in the coming 5 or 10 years. The items can be grouped into 6 caegories: Future technological advances to elucidate the structures and functions of glycans, Glycans and biopharmaceuticals, Sugar chains (glycans) involved in medical science and medical care, Food implicated in glycans and its function, Glycan-related materials and their use for biomaterials, and Educational materials and training for glycosciences. The six parts are sumarried below, and finally the future perspectives will be discussed.

Future Technological Advances to Elucidate the Structures and Functions of Glycans

Improvements in and innovation of various glycan-based technologies such as structural analysis, synthesis, imaging, etc., are critical issues. The terms “smart”, “automation”, and “easy-to-use” are most important for the use of glycan technology and its popularization. Further progress in analytical techniques for examining complex sugar chains such as glyco-proteomics and glyco-lipidomics can clearly lead to a more efficient development of biomarkers for various diseases and more useful drug discovery support technologies.

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Glycans and Biopharmaceuticals

Biopharmaceutical development has entered into a new era from the age of traditional approaches: performance-oriented type pharmaceutical development with high quality and high function. Sugar chains are added to most biopharmaceuticals including antibodies, since they affect drug efficacy and product stability in blood as well as target specificity. Techniques for preparing uniform sugar chains on biopharmaceuticals are also being established. It is important to identify sugar chains that contribute to drug efficacy and stability and to develop biopharmaceuticals that enhance these properties.

In addition, new drugs that mimic sugar chains are now attracting attention as new potential drug targets. In addition to the sialidase inhibitor Relenza and Tamiflu (a medicine for the influenza virus), the glucosylceramide synthase inhibitor Erygistat (a medicine for Gaucher disease) and glucose sodium cotransporter inhibitor Canagliflozin (a medicine for type 2 diabetes) have been approved in recent years. Rivipansel (a drug for sickle cell disease), a pan selectin inhibitor, is in a phase III clinical trial.

A major turning point for vaccine development has also arrived. Generally, when a sugar chain having low antigenicity is used as a vaccine, the use of an adjuvant that enhances its immunogenicity is essential.

Sugar Chains (Glycans) Involved in Medical Science and Medical Care

Since many of membrane proteins and secreted proteins are modified by glycans, glycans are involved in almost all medical fields such as development, regeneration, nervous system, immunity, infections, genetic diseases, cancer, and adult diseases. To thoroughly elucidate the roles of sugar chains that spread over a wide range of medical fields, we should first analyze the comprehensive regulatory mechanism responsible for glycogene expression and the control of glycan expression. A genome-wide analysis for the control by epigenome and miRNA are also required. The construction of a database for these data will allow us to obtain novel information that is not currently available by data mining.

Failures of sugar chains and glycogenes involved in the normal development and maintenance of homeostasis causes various diseases such as congenital carbohydrate disorder (CGD), *NGLY1* deficiency, lysosomal disease (LSD), etc. These are all genetic diseases that are caused by a deletion or a mutation (including SNP) of a gene such as an enzyme that is involved in the synthesis of sugar chains, degradation, metabolism and related issues. They also account for many rare diseases. Due to the progress made in genome-wide analysis technology, their number is expected to increase in the future.

Glycans on the cell surface change and function in various ways, reflecting the states of cells such as the development of cancer. Identifying glycans that reflect cancer and become therapeutic targets derived therefrom, and drug discovery are issues that need to be addressed and achieved in the future. Especially, refractory cancers, pancreatic cancer and brain tumors, are important.

Furthermore, rare diseases, adult diseases, and mental diseases are also important new targets. It is also important to identify mechanisms responsible for infections that can occur via glycans of viruses, bacteria, and protozoa and to develop drugs and vaccines based on our understanding of these mechanisms. Lastly, when considering next-generation medical care, the development of biomarkers that can be used to evaluate health, aging, and disease-free status will be important aspects associated with medical care.

Food Implicated in Glycans and Its Function

Although there are some nutrient polysaccharides in nature, such as milk oligosaccharides and fucoidans, which are said to have physiological activities, their physiological functions have not yet been completely verified and elucidating their structural functions is indispensable. Furthermore, in order to supply them as a food, it will be necessary to establish a method for preparing them on a mass-scale.

Glycan-related Materials and Their Use in Biomaterials

Polysaccharides are widely used as bio-resource materials. Cellulose nanofibers and chitin nanofibers are currently attracting attention, since they can be economically produced and can be further functionalized. They are also expected to be used in drug delivery systems and in recyclable medical materials.

Educational Materials and Training for Glycosciences

From the viewpoint of open science, it is very important to make it possible for anyone to freely and efficiently use the abundant information generated from comprehensive functional analyses and structural analyses of glycans. In combination with other omics data, the possibility of applying glycans to real-world situations is virtually unlimited. For this purpose, it is essential not only to improve the current databases but also to promote further education in the field of glycoscience.

Future Challenges: Organizations and Systems to Promote the Comprehensive Acquisition and Effective Utilization of Glycan Related Information

Unlike other life science fields, it is clear that our current understanding of glycans is insufficient. The main reason for this is that basic technologies related to the analysis of glycan structures and their synthesis are in an immature state. This is particularly true for the developent on automation systems. If these problems related to fundamental technologies could be solved in 5 or 10 years, it will be possible to comprehensively acquire glycan related information in various phases of development and to create viable and important databases. By using such comprehensive information in conjunction with other biological information, we will be able to develop a clue to solving problems and enigmas that are currently unsolved.

For example, such system could be applied to undiagnosed diseases and rare diseases whose causes are unknown. As mentioned above, glycans are involved in various biological phenomena; therefore, it is predicted that not only CGD and LSD but also a considerable part of undiagnosed diseases and rare diseases are likely caused by an abnormality associated with glycan structure or composition. Though a correlation analysis of SNP related to sugar metabolism based on genomic information, it will be possible to elucidate the cause of many rare diseases. In the super aging society that is to come, not only cancer and adult diseases but also rare diseases might manifest themselves in the future. To address these issues, advance countermeasures will be needed and a more complete understanding of the functions of glycans will contribute to this.

Furthermore, considering infectious diseases, glycans on a cell surface must be the receptors for other various pathogens as well as influenza, the malaria parasite, and *Helicobacter pylori,* and likely play a major role when an infection is formed. However, glycans are not subjects of major concern to researchers and doctors in the cases of infections. It is necessary to further promote awareness in this area and to spread the field of glycoscience beyond academic societies. Accomplishing this will allow glycan information to be more easily accessible and system in support of collaborative research will arise. The northern limit of pathogens is currently increasing due to global warming; studies of tropical pathogens using glycans as infectious receptors is now an urgent issue. Such information is to be made available to the scientific public.

Although only a few examples are given here, glycans and polysaccharides are not limited to basic research in life science and material science; their application range has now spread to drug discovery, regenerative medicine, deseases such as cancer, adult diseases, and rare diseases, as well as food, environment, and energy. For glycans to permiate into these areas and for them to be effectively utilized, it will be necessary to comprehensively acquire glycan-related information, and to then make it possible to effectively utilize it, and to prepare the environment as an open area of science. Many people from other fields participate in glycoscience research and this has permitted the field to fuse with the glycan field, thus permitting currently unresolved academic issues to be discussed and elucidated. Beyond that, various innovations will surely be created.

Glossary

α-synuclein α-synuclein was reported as a component other than β amyloid in Alzheimer's disease but was later found to be in the family with Parkinson disease and has attracted a great deal of interest.

Acquired immunity Also known the adaptive immune system, composed of highly specialized, systemic cells and processes equipped in vertebrates. Distinct from innate immunity, in adaptive immunity, a relatively long time is needed to create a series of immunoglobins (antibodies) and immunological memory, by which an enhanced response to subsequent encounters with pathogens and foreign molecules derived from them is substantiated. As in the innate immune system, the adaptive system includes humoral immunity components such as complements and antibodies in addition to cell-mediated immunity components. Both antibody responses and cell mediated immune responses are carried by two different lymphocytes, i.e., B cells and T cells. In antibody responses, B cells are activated to secrete antibodies that are specific to antigens, a mechanism that enables vaccinations. A hierarchal adaptive immune system is explained by an immune network theory based of the concept clonal selection.

Adjuvant An adjuvant is a substance or molecule that is administered together with an antigen for increasing the potency of a vaccine. Freund's complete adjuvant (FCA), in which membranes derived from M. tuberculosis are suspended in mineral oil, and Incomplete Freund's adjuvant (IFA), in which tubercle bacillus components have been removed from FCA, are generally used. However, the mechanisms of their actions are varied and not completely known. It is considered that antigen presenting cells such as macrophages and dendritic cells are stimulated by mixing microorganisms other than the target and degradation products thereof when the antigen alone is not sufficient to obtain a high immune response.

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AGE (Advanced Glycation End products) The Mallard reaction also designated as glycation, non-enzymatic glycosylation browning reactions and aminocarbonyl reactions, produces early stage products but AGE formation occurs at the last stage of the process. AGE formation is considered to be one of the causes of diabetes.

Allose and Psycose These sugars are classified as rare sugars because very few are naturally occurring in nature. These sugars contain very little calories if any and are reported to be effective for the prevention of diabetes and obesity.

Amyloidosis Amyloidosis is a rare disease in which abnormal proteins are produced in bone marrow and then accumulate in the heart, kidney, spleen, nervous tissue and the gastrointestinal tract. Men mainly suffer form amyloidosis. There are several types such as a familiar type in which immunoglobulin accumulates, and a type that occurs due to long term dialysis as well as types due to rheumatoid arthritis and inflammatory disease of the intestine.

Antibody-dependent cellular cytotoxicity (ADCC) Antibody-dependent cellular cytotoxicity (ADCC) is an immune defense mechanism; Fc receptor-bearing effector cells, such as natural killer cells and macrophages, recognize and kill antibody-coated target cells.

Antigenic glycans Antigenic glycans are sugar chains that are attached to the cell surface of parasites or protozoa such as malaria, African Trypanosoma, ameba, Leishmanial and Schistosoma, all of which are antigenic and are glycan candidates for vaccine production.

Apoptosis Apoptosis is a type of death of cells that constitute the body of an organism. It is the active death of controlled/regulated cells, namely programmed cell death. Unlike necrosis, where external factors are induced, inflammatory reactions are not involved in this process.

Artificial intelligence (AI) An attempt to realize intelligence by means of a machine, that is similar or even better to the intelligence of a human. AI also implies a series of related techniques for attaining this purpose.

Autism spectrum disorder (ASD) Autism spectrum disorder (ASD) is classified as a neurodevelopment disorder in the diagnosis and statistical manual of mental disorders of the American Psychiatric Association 5th Edition (DSM-5). It has two characteristics, namely, “difficulty in social communication” and “limited interest”. It is a congenital brain dysfunction including so-called autism and Asperger’s syndrome.

Autoimmune disease Autoimmune disease is a collective term for diseases caused by immune cells attacking their own substances. In “systemic autoimmune disease” such as rheumatoid arthritis and collagen diseases typified by systemic lupus ery-

thematosus (SLE), inflammation occurs in tissues in the entire body. In "organ-specific autoimmune diseases" such as glomerulonephritis, autoimmune hemolytic anemia, and Hashimoto disease, organ function is degraded by antibodies that attack specific organs.

Automated synthesis of glycans Preparing glycan not manually but by an automatic procedure. Including the development of an automated synthesizer.

β-elimination A chemical method used to release *O*-glycans from glycoproteins. Under alkaline conditions with heating, an *O*-glycan is released from a glycoprotein at the β position. In the presence of a reducing agent, the reducing terminal end of the released *O*-glycan is converted to sugar alcohol (e.g. *N*-acetylgalactosamine is converted into *N*-acetylgalactosaminitol). *O*-glycosylated Ser or Thr residues are converted to Ala or α-aminobutyric acid residues at the same time.

β-glucans A general name for polysaccharides composed of β-linked glucoside linkages, which are extensively found in algae, fungi and plants as β1-3 glucans such as laminarin and curdlan, and β1-4 glucans (cellulose), while they are only exceptionally found in animals in the case of tunicate cellulose. Usually, β-glucans are referred to as fungal β1-3 glucans, which are immunogenic to animals, and thus, not to cellulose which is basically an insoluble fiber. β-glucan structures, which are substantially diverse, consist of a β-3 backbone and β1-6 branches with a varying frequency, length and modifications by other monosaccharides. They are thought to activate an innate immune system in animals via endogenous lectins such as dectin and Mincle, while many of these substances remain to be elucidated.

B cell B cells are one of a class of lymphocytes and produce an antibody.

Binding analysis (Intermolecular interaction) A method for determining the binding affinity in a quantitative manner for protein-protein, protein-small chemical, and protein-glycan interactions.

Biobetters Different from biosimilar, the term biobetter refers to a recombinant biopharmaceutical protein drug that has been intentionally improved over the original drug (innovator). However, both biosimilars and biobetters build on the success of previously existing, approved biological drugs, possibly providing less commercial risk than developing new innovator biological drugs. On the other hand, there are many but unestablished approaches for this improvement. However, one such focuses is glycans, because there are a few examples where the function of a pharmaceutical drug has been improved by glycan engineering: they include ADCC-activity reinforced IgG by eliminating core fucose of the *N*-glycan attached to Fc region and elongated in vivo stability in erythropoietin by introducing additional *N*-gycosylation sites.

Biofuel Instead of natural resources such as petroleum and coal fuels, crops such as corn and sugarcane can be fermented to produce ethanol which can be used as a transportation fuel. It is a renewable energy source based on the carbon cycle.

Biomarker A biomarker is the molecule that can be used for the diagnosis of various diseases and for monitoring the progress of a disease. It is typically measured in serum and urine etc. In the case of biomarker for cancer, it is referred to as a tumor marker.

Biopharmaceuticals Also known as biological medical products, biologicals, or biologics, biopharmaceuticals are pharmaceutical drug products manufactured from biological sources, such as antibodies, hormones, nucleic acids, the production of which involve multiple processes, with a much higher cost compared with totally synthesized small pharmaceuticals. One of the greatest concerns is post-translational modifications including glycosylation, which not only greatly increases molecular diversity and complexity but also often affects protein solubility, stability and functional aspects.

Biosimilars Also known as a follow-on biologic or subsequent entry biologic. Different from conventional drugs, which are generally low molecular weight chemical compounds, it is substantially difficult to make a copy product of a biologic medical product, such as antibodies and hormones, most of which are glycoproteins. Biosimilars are officially approved versions of original "innovator" products, provided that their full comparability is demonstrated. They can be manufactured by any company when the original product's patent expires. Biosimilar issues arise from the structural complexity of these drugs concerning post-translational modifications; e.g., heterogeneity of glycosylation, deformation of protein structure, and aggregation.

Bipolar disorder Bipolar disorder is a mental disorder in which a manic state and a depressed state repeat one another and is generally referred to as manic depression. There are two types; one is bipolar type I with a severe manic state and depression and the other is bipolar type II with a light manic state and depressed state.

Bone morphogenetic protein (BMP) The bone morphogenetic protein (BMP) is a member of the TGF-β superfamily. It binds to the dimer of type I and type II receptors and sends a signal to the nucleus by phosphorylating the transcription factor SMAD. BMP contributes to the dorsoventral axis determination in an embryo, the maintenance of stem cells, the induction of nascent tissue and organs, pattern formation, the induction of cell death, the control of cell differentiation, and so on.

Caenorhabditis elegans *Caenorhabditis elegans* is a model organism that is widely used in genomic and post-genomic studies. *C. elegans* consists of ca 1000 somatic cells and the length of the body is about 1 mm. All of its the developmental processes and neural network system have been elucidated.

Canagliflozin Canagliflozin has been developed as an inhibitor of sodium/glucose cotransporter SGLT-2, and has been approved for the treatment of type 2 diabetes. The compound apparently mimics a natural *O*-glucoside, phlorhizin, while a C-glycoside is introduced in canagliflozin.

Carbohydrate-recognition domain (CRD) Technical terms for classifying animal lectins based on structural domains were proposed by Kurt Drickamer in 1988. In those days, there were two known types of animal lectins: one being calcium-dependent type lectins and the other being soluble β-galactoside-binding lectins (later named galectins), both of which have characteristic amino acid sequences to constitute respective CRDs, i.e., C-type CRD and S-type (SH-requiring) CRD, while the latter naming was found to be irrelevant and the galectins were renamed. However, the concept proposed is widely applicable to other lectin families not only in animals but also in other organisms including plants and bacteria, and the number of CRDs, on the basis of protein family, now makes up more than 50. A related term, CBM (carbohydrate-binding module) is also used in regard to glycohydrolases.

Carbohydrate-related enzymes A generic name of enzymes that break down or synthesize carbohydrates.

CD1d CD1d is an antigen-presenting molecule which presents the self- or non-self-glycolipid with a Galα1-linkage to the T cell receptor on natural killer cells (NKT). Although the most well-known glycolipid ligand is α-galactosylceramide (α-GalCer), it is still unclear which glycolipid is the in vivo ligand.

Cellular immunity Cellular immunity is an immune reaction, in which immune cells such as macrophages, cytotoxic T cells, and natural killer cells directly act on the antigen. Th1 cells, a type of helper T cells, recognizes antigens presented by dendritic cells causing them to produce cytokines such as IL-2, and activate these immune cells.

Chemical biology Application of small molecules produced through synthetic chemistry to studies of biological systems including enzyme reactions, biomolecular dynamics and regulation of biological pathways within cells and cell-cell interaction.

Chemical synthesis of glycans Glycan synthesis based on a chemical method. It is easy to prepare a compound on a large scale, but sometimes it is necessary to overcome problems associated with stereo-selectivity, yield etc.

Chondroitin sulfate (CS) CS is a glycosaminoglycan that is and present in bone, cartilage and skin and is composed of two repeating sugars such as glucuronic acid and *N*-acetylglucosamine. It is one of the glycosaminoglycans that makes up two sugar repeating such as glucosamine and uronic acid. The hydroxyl group or the

amino group of glucosamine is typically sulfated and shows structural heterogeneity and regulates a variety of physiological active molecules.

Chronic pancreatitis In chronic pancreatitis, the fibrosis of pancreatic tissue occurs and this inhibits pancreatic function. This is an irreversible and progressive disease. Major risk factors are alcohol consumption, gall bladder stones, autoimmune diseases.

Click chemistry Click chemistry is a term that was introduced by K. B. Sharpless. Click reactions usually join a biomolecule and a reporter molecule, for example a copper-catalyzed azide-alkyne cycloaddition can be achieved in a one pot reaction. Click Chemistry is often used in conjunction with complex cell lysates, and has been further adapted for use in living cells and animals to monitor the behavior of glycoconjugates such as glycoproteins/glycolipids.

Collison cross section (CCS) CCS is a molecular property that is calculated from the experimental value for ion mobility. It correlates with the shape of a molecule and it is dependent on the drift time and mass-to-charge ratio (*m/z*). Importantly, CCS can be used as an additional parameter for identifying the molecule.

Complement Complements belong to a group of blood proteins responsible for immune responses. They are activated by classical, alternative, and lectin pathways. They function to kill bacteria by attacking membranes, the opsonization of antigens, and the chemotactic stimulation of macrophages.

Core fucose Core fucose is a product of Fut8, an α 1,6 fucose glycan. Core fucose added to glycoproteins can serve as a biomarker for hepatocarcinoma, pancreatic cancer and non small cell lung cancer. Mice with Fut8 knocked out develop emphysema.

Crohn's disease Crohn's disease is an inflammatory bowel disease of unknown origin which causes discontinuous chronic granulomatous inflammations in the entire digestive tract from the oral cavity to the anus.

Cryo-electron microscopy (Cryo-EM) A technique used to obtain a three-dimensional image of a biomacromolecule with a near-atomic resolution without the need for a crystallization step. Atomic-scale structures of complex macromolecule, such as large membrane proteins and spliceosomes, have been cetermined. The Novel Prize in Chemistry in 2017 was awarded for this cryo-EM technique.

Dementia Dementia is a type of cognitive disorder, in which the normally developed intelligence is irreversibly reduced by an acquired brain disorder. Half of dementia patients have Alzheimer type dementia. In addition to Alzheimer type, Lewy body dementia and vascular dementia are included in the three major classes

of dementia that account for about 85% of the total patient population. There is currently no treatment available for this disorder.

Dermatan sulfate (DS) DS is a special type of Chondroitin sulfate and is present in skin and vascular vessels. It consists of two repeating units composed of glucuronic acid and *N*-acetylglucosamine.

Diabetic complication There are several complications associated with diabetes which include diabetic comas and infections. In chronic stages, microvascular disorders (peripheral nerve disturbance, disorders result in nerve disorder, auto-nervous disorders facial nerve palsy, muscle atrophy etc.) Diabetic retinopathy (half of blind adults suffer from disorder) diabetic nephropathy (this disease causes kidney insufficiency and results in the need for blood dialysis).

DNA methylation DNA methylation occurs mainly in cytosine (C) and is involved in epigenetics. The CpG island in the gene promoter region is not extensively methylated at the early stage of development. It undergoes methylation upon differentiation, resulting in the suppression of gene expression. In cancer cells, the expression of tumor suppressor genes is suppressed by abnormal methylation.

Drosophila *Drosophila (Drosophila melanogaster)* is a small fly, about 2–4 mm in length. The entire genome of *D. melanogaster* has been determined, and this insect is extensively used as a model organism in many scientific fields.

Embryonic development The process of the zygote becoming an embryo.

Endoplasmic reticulum (ER) The endoplasmic reticulum (ER) is a eukaryotic organelle, and is divided into two types, the rough ER with many ribosomes attached to its surface and the smooth ER, which lacks ribosomes. The rough ER is a site of protein synthesis, and the synthesized protein enters the lumen of the ER through a translocon, where glycans are added to proteins and three-dimensional structures of proteins are formed. Only proteins with the correct conformation are transported to the cis-Golgi apparatus.

Enzymatic synthesis of glycans Glycan synthesis based on an enzymatic method using a series of glycosyltransferases and glycosylhydrolases. It is easy to prepare a compound stereo-selectively by taking advantage of the specificity of enzymes. However, problems sometimes arise in terms of obtaining stable enzymes or expensive donor substrates (sugar-nucleotides) etc.

Enzyme replacement therapy Enzyme replacement therapy is a medical treatment for replacing an enzyme that is not produced in the body. It is currently available for some lysosomal storage diseases by providing an enzyme via intravenous infusion. This therapy is currently used in the treatment of in seven lysosome diseases such as gauche disease.

Exosome Exosomes are secreted from almost all cells. It is a membranous small vesicle with a diameter of around 50–150 nm. In the body, it is found in saliva, blood, urine and amniotic fluid, malignant ascites and other fluid. Exosomes are also secreted from cultured cells. Exosomes contain various proteins, lipids and RNA. MicroRNA has been of interest regarding its use as a biomarker for various diseases, including cancer.

Extracellular matrix (ECM) Extracellular matrix is a fibrous or reticular structure that is located on the outside of somatic cells constituting the living body. In the case of an animal, it is composed of cell adhesion molecules such as collagen, proteoglycans, hyaluronic acid, fibronectin and so on. It plays an important role in supporting tissues and controlling cell proliferation and differentiation.

Fibroblast growth factor (FGF) The fibroblast growth factor (FGF) is involved in angiogenesis, wound healing, and embryogenesis. It plays an important role in proliferation and differentiation in a wide range of cells and tissues. FGF is a heparin binding protein and an interaction between FGF and heparan sulfate is necessary for the signal transduction of FGF.

Fixation of glycans The fixation of various glycans on a surface such as glass by covalent bonds. The procedure is usually used in preparing a glycan-array for use in searching for glycan-binding proteins.

Fluorescence resonance energy transfer (FRET) Energy transfer between a donor chromophore and an acceptor chromophore. Initially the donor chromophore is excited, and the resulting energy is then transferred to the adjacent acceptor chromophore. The efficiency of FRET is inversely proportional to the sixth power of the distance between the donor and acceptor.

Fluorescent labeling A molecule such as a protein or glycan can be chemically labeled with stable fluorescent derivatives, either directly or indirectly. This method permits the highly sensitive detection of molecules and real-time measurements of biomolecular interactions.

Follow-on biologics/subsequent entry biologics Biopharmaceuticals, for which the patent has expired, are referred to as innovator drugs. The issue of whether further improvement can be made for these biopharmaceuticals from either aspects of safety, stability and efficacy, such follow-on biologics have options; i.e., “biosimilars” and “biobetters”.

Fucosylation Fucosylation is catalyzed by the action of several fucosyltransferase enzymes. GDP-fucose is the donor substrate and acceptor substrate(s) are glycoproteins, glycolipids and oligosaccharides. The enzyme transfers a fucose unit to a glycan. GDP-fucose is produced via two pathways, one from extracellular fucose

designated as the Salvage pathway and the other via a De novo pathway in which GDP-fucose is produced from GDP-mannose. This is important for cancer biomarkers adhesion molecules, CGD (congenital disorders of glycosylation) and antibody therapy.

Functional polysaccharides Functional polysaccharides are similar to free HMOs (Human Milk Oligosaccharides), and are structurally related, glycan epitopes of human-milk glycoproteins, such as mucins, function as prebiotic factors in the formation and stabilization of favorable intestinal microbiota and as effective decoy receptors for pathogenic gastrointestinal bacteria, viruses, and yeasts. Multiple glycosylation sites at single protein macromolecules lead to an increase in the potency of these effects, however, there are other alternatives.

Fut8 Fut8 is one of the glycosyltransferases designated as α 1, 6 fucosyltransferase and its gene. The enzyme adds an α 1, 6 fucose (core fucose) unit to the inner most *N*-acetylglucosamine of *N*-acetylglucosamine. Since the deletion of core fucose from IgG enhances the activation of ADCC by 50–100 fold (antibody dependent cellular cytotoxicity) this technique is applicable for use as antibody therapy against cancer. Therefore Fut8 is one of the fucosylation enzymes.

Ganglioside Gangliosides are defined as sialic acid-containing glycoshingolipids. They are most abundant neuronal tissues. It has been known that gangliosides are involved in various biological events, including development, differentiation, and cancer etc.

GCNT1 GCNT1 is an abbreviation for Core 2 β1, GlcNAc transferase and it is expressed at high levels in prostate cancer tissues.

Glycan diagnosis reagents This reagent focuses on changes in the glycan structure of glycoproteins that are located on the cell surface or in the blood and are used for biomarkers for various diseases. Measurements of most of biomarkers involves determining protein levels whereas this biomarker is indicative of a change in glycan structure.

Glycan metabolism A systemic study of the synthesis and degradation of glycans that are attached to glycoproteins, glycolipids, and proteoglycans. Free glycans derived from such glycoconjugates are also included.

Glycan modification of glycoconjugates Glycan moieties of glycoproteins and glycolipids can be modified by chemical or enzymatic treatment. It is possible to produce glycoconjugates having different glycan structures via the modulation of the cellular glycan processing machinery (glycosyltransferase, glycohydrolase, sugar-nucleotide transporter, etc).

Glycan-protein complex (Glycan-protein interaction) Glycans bind to a specific protein and form glycan-protein complex. Glycans often express their physiological functions through interactions with biomacromolecules such as lectins and anti-glycan antibodies. It is of vital importance to investigate glycan-protein interaction modes for understanding glycan functions.

Glyceroglycolipid Glyceroglycolipid is a generic name for a glycolipid-containing glycerol containing fatty acid(s) or alkyl chain(s).

Glycobiologics Glycosylated medical products that are produced by biological processes. See Biopharmaceuticals.

Glycoconjugate A glycoconjugate is the complex carbohydrate in which proteins and lipids are bound to sugars.

Glycomics Systematic analyses of all glycan structures of a given cell type, tissue, or organism.

Glycomimetics Chemically synthesized pharmaceuticals that target glycan recognition molecules involved in glycan synthesis (e.g., glycosyltransferases), recognition (e.g., endogenous lectins), degradation (e.g., glycosidases) and transport (e.g., sugar transporter). Such representatives include the well-known Relenza and Tamiflu, inhibitors of neuraminidases produced by the influenza virus. More recently, canagliflozin has been approved for the cure of diabetes as an inhibitor to sodium/glucose cotransporter SGLT-2, while eliglustat is a curative medicine for Gaucher's disease by inhibiting glucosylceramide synthase. Though still in a trial phase, pan-selectin inhibitor Rivipansel and galectin inhibitor TDG139 have been developed for curing sickle cell disease and pulmonary fibrosis, respectively.

Glycoproteins bearing homogenous glycans Glycoproteins are biosynthesized by the action of a series of glycosyltransferases and glycohydrolases in a non–template-directed manner, giving rise to heterogeneous glycoforms. It is difficult therefore to elucidate the functional properties of individual glycan structures and site-specific glycosylation. Under these circumstances, the development methodology for the chemical synthesis of glycoproteins bearing homogeneous glycans may lead the elucidation of the functional properties of a specific glycan.

Glycoproteomics Systematic analyses of all glycan structures, glycan attachment positions, and core proteins of glycoproteins.

Glycosaminoglycans (GAG) GAG previously known as mucopolysacchrides are long unbranched polysaccharides consisting of repeating disaccharide units of an amino sugar and a hexose derivative such as a uronic acid and galactose. Included among them are heparin, heparin sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate and hyaronan. Glycosaminoglycans are present as a complex with core proteins. Except for hyaluronan, all glycosaminoglycans are sulfated.

Glycosidase Glycosidases with various specificities have been identified from various organisms and are divided into an exo-type and an eno-type. Some of them are useful tools for the structural analysis of sugar chains. Endo-β *N*-glycosidase (ENGase) and peptide-*N*-glycanase (PNGase) are involved in the quality control of eukaryotic glycoproteins, and their association with NGly 1 disease, a rare disease, is accompanied by the failure of glycoprotein metabolism. In addition, attempts have been made to equalize sugar structures of glycoproteins by utilizing the glycosyltransfer activity of fungal-derived ENGase.

Glycosphingolipids(GSLs) A glycosphingolipid is an amphipathic functional glycoconjugate that contains a sugar chain as a hydrophilic group and a ceramide as a hydrophobic group. In cell membranes, it forms microdomains with cholesterol, GPI-anchored glycoproteins, and phosphorylating enzymes such as Src, and then controls signal transduction, cell adhesion, proliferation, and differentiation.

Glycosyl reaction A reaction in which a sugar or sugars are chemically or enzymatically attached to high molecular weight molecules like proteins and lipids.

Glycotherapeutics Most common member of this group is heparin, an anti-blood coagulant. Erythropoietin, Pneumococcus vaccine, and the influenza vaccine also belong to the same category. A selectin antagonist has recently been developed and is of interest because it prevents severe pain due to Sickle cell anemia and acute leukemia.

GM1 GM1 is a ganglioside that contains one molecule of sialic acid and is located in the mucous membrane of the small intestine. It binds specifically to the B subunit of the cholera toxin.

GM3-deficient diseases Diseases induced by a deficiency in the enzyme responsible for synthesizing ganglioside GM3.

GnT-V GnT-V is one of the enzymes among β1, 6 GlcNAc transferases which adds an α 1, 6 linked GlcNAc to the biantennary sugar chain of *N*-glycans of glycoproteins. It is implicated in cancer metastasis, the regulation of the T cell receptor or EGF. Once this enzyme acts to produce β1, 6 branched chain, structural elongation occurs leading to products such as polylactosamine. It's gene is designated as Mgat5.

GPI (glycosylphosphatidylinositol)-anchor GPI-anchored proteins are generated in the endoplasmic reticulum by the covalent modification of the carboxyl-terminus of a protein with a preassembled GPI-anchor, a complex glycolipid. Many GPI-anchor proteins, including enzymes, receptors, etc. are known. Also, a number of GPI-anchor deficient diseases have now been identified.

Gray matter In the nerve tissue of the central nervous system, the site where neuronal cell bodies are present is gray matter. On the other hand, the site where no neuronal cell body and myelinated nerve fibers occupy is referred to as white matter.

Hedgehog Hedgehog is an evolutionarily conserved factor from *Drosophila* to humans. In mammals, there are three kins of Hedgehog, namely, Sonic Hedgehog, Indian Hedgehog, and Desert Hedgehog. Hedgehog signalling is involved in embryogenesis, somatic stem cell regulation, and tumor progression.

Helicobacter pylori *Helicobacter pylori* is a Gram-negative, helical shaped bacteria having several flagella and is found in the stomach. This bacteria produces urease and degrades urea to produce ammonia and CO_2 and neutralizes gastric acid. This is one of the causes of chronic gastritis, gastric ulcer, gastric cancer, and malignant lymphoma. From the surface of bacteria, a carcinogen, Gag A is produced and then secreted into the inside of gastric cells.

Hepatitis B virus. This virus is one of the several hepatitis viruses and is mainly transmitted through the mother to children.

Heterogeneity of glycans Generally, a glycoprotein contains various glycans, even though it is a single protein. In addition, the same amino acid in a protein at the same position on the molecule can have a variety of glycan structures attached to it.

High mannose type sugar chain (Oligomannose) The high mannose type sugar chain (Oligomannose) is a type of *N*-linked sugar chain, and all non-reducing terminal side residues are composed of mannose.

Highly pathogenic avian influenza virus The avian influenza virus uses wild water fowl as a natural host, grows in its intestinal tract, and infects other organisms through feces. However, it does not show pathogenicity in water fowl. The avian influenza virus that infects poultry to become a virulent strain is called as a highly pathogenic avian influenza virus.

High-throughput analysis The synthesis, analysis, and evaluation of glycans within a short period.

Hippocampus The hippocampus is a cortical part and is related to memory and spatial learning ability. It is located in the lower part of the lateral ventricle at the inner part of the temporal lobe of the cerebrum. The entorhinal cortex, the parasubiculum, the presubiculum, the subiculum, the hippocampus, and the dentate gyrus are located in the temporal lobe cortex, which is involved in memory.

Host A host is an organism that is susceptible to attack by parasites such as viruses and bacteria.

Human disease model Animal models of certain diseases show characteristics that are similar to various human diseases, especially genetic diseases. The use of such models is very useful in terms of revealing the pathomechanism of diseases and to develop new drugs and therapeutics for the treatment of such diseases.

Humoral immunity Humoral immunity is an immune reaction by antibodies. B cells are stimulated by cytokines produced by Th2 cells, a type of helper T cell, to differentiate into plasma cells that produce a large amount of antibodies.

Identification of self/non-self MHCI is expressed on autologous somatic cells, and cytotoxic T cells recognize MHCI and self peptides that are present on it. When normal MHCI and normal peptides on the somatic membrane are recognized as self, the cells are not attacked.

Immune checkpoint An immunity checkpoint is a mechanism that suppresses excessive immune reactions to decrease the occurrence of autoimmune diseases. Some cancers utilize it to escape immunity. Antibodies against CTLA-4 and PD-1 on T cells and PD-L1, which is a ligand for PD-1 of cancer cells, have been developed. Clinical applications of these antibodies have been started for use as a drug for releasing T cell immunosuppression.

Inhibitor A reaction inhibitor is a compound that prevents a chemical reaction or decreases its rate. An enzyme inhibitor is a compound that binds to an enzyme and decreases its activity. It is possible to elucidate aspects of glycan biosynthesis pathways and their function by using such enzyme inhibitor(s). Glycoproteins bearing sets of glycans that are different from native ones can be obtained by using these inhibitor(s) and it may lead to the elucidation of the functional properties of a specific glycan.

Innate immunity Innate immunity is a mechanism that rapidly detects invading pathogens and abnormal autologous cells via a receptor and eliminate them. It is conferred via the Toll-like receptor, Dectin, and Mincle etc. that recognize structures that are characteristically exposed in pathogenic surface layer. Although they have low specificity as compared with receptor molecules and antibodies in acquired immunity, they have the ability to respond to many types of foreign substances and surface molecules of pathogens. Therefore, it can be said that innate immunity is located at the forefront of biological defense.

Insect glycans Recombinant protein expression systems using the baculovirus-infected insect cells or silk worms are often used but the problem is that the glycan structures of insect cells are different from those of humans and generally contain short glycan chains and in vivo, these glycans have antigenicity and can cause allergy reactions. Therefore those glycan need to be changed to human types.

Interleukin-1 (IL-1) Interleukin-1 (IL-1) is a 17 kDa glycoprotein that is produced mainly by macrophages. Its most important function is inducing IL-2 production in helper T cells to promote T cell differentiation and proliferation *via* IL-2. IL-1 is also an important inflammatory cytokine. Large amounts of IL-1 are expressed in articular synovial cells in patients with rheumatoid arthritis.

Intestinal immunity The intestinal immune system is the largest immune system in the body, and is composed of Peyer's patches, mucosal epithelial cells, and immunocompetent cells, all of which exist therein. It induces the activation of immunity as a defense against infections and also oral tolerance to food.

Ion mobility spectrometry (IMS) An analytical method that permits ionized molecules in the gas phase to be separated and can be interpreted as gas-phase electrophoresis. Collision cross section (CCS), which is correlated with the shape of the ion, is calculated by observing the drift time. IMS is often combined with mass spectrometry (IM-MS) and has emerged as a promising tool for use in glycan analysis.

Isomer An isomer is defined as molecule that has the same molecular formula but a different structure. A structural isomer indicates a different binding relation between molecules such as fructose and glucose. Stereoisomers are made of the same atoms connected in the same sequence, but the atoms are positioned differently in space. The difference between stereoisomers can only be seen when the three-dimensional arrangement of the molecules can be observed.

Keratan sulfate (KS) KS is a glycosaminoglycan found in the cornea, cartilage and brain. In the cornea, keratan sulfate plays a role in water retention and in the brain it contributes to nerve plasticity.

Knockout (mouse) Gene knockout is a method for the complete inactivation of a gene function. Knockout mice are useful for elucidating unknown or know functions of glycogenes or specific glycans. Tissue-specific knockout mice can also be generated using the Cre/loxP system.

Lectins Lectins are a group of proteins that are not enzymes or antibodies that have the ability to bind to certain carbohydrate structures. Historically, the first lectin ricin was discovered in 1888 and, since then, many lectins have been identified with a variety of molecular scaffolds distinct from antibodies. More recently, many functional lectin domains such as those hidden as supporting elements to enzymes have been accidently found with no previous record of lectins. Representative animal lectins include dectins associated with innate immunity, calcium-dependent (C-type) lectins having diverse structures and functions, and β-galactoside-specific galectins that are involved in development and immunity. Galectins and selectins (belonging to C-type lectins) are also target for glycomimetics pharmaceuticals.

Leukemia inhibitory factor (LIF) Leukemia inhibitory factor (LIF) is a cytokine that belongs to the IL-6 family and has been identified as a factor that inhibits the proliferation of leukemia cells and induces differentiation into macrophages. It activates the Jak-Stat pathway, the PI3 kinase-Akt pathway, and the Grb2-MAP kinase pathway *via* LIFR/gp130. It is involved in neurogenesis, embryogenesis, bone metabolism, inflammation, and the maintenance of embryonic stem cells.

Lewy body Lewy bodies are is found inside neural tissue and are an abnormally round shaped inclusion body. Lewy bodies aremainly composed of synuclein and it has been suggested that it is correlated with the development of Parkinson's disease and Lewy body dementia.

Life-style disease Life-style diseases are related to diseases caused by the patients' life-style. Based on the definition by the ministry of Health and Welfare, a group of diseases whose onset and progression are implicated to the life-style regarding the intake of food, exercise, rest, smoking and drinking. Life-style diseases frequently result in the development of type II diabetes, obesity, COPD, hyperlipidemia, myocardial infarction and osteoporosis.

Lipid rafts Specialized membrane domains containing high concentrations of cholesterol and glycosphingolipids. A variety of proteins, especially those in cell signaling pathways and receptors, are enriched in lipid rafts. These specialized membranes are also referred to as microdomains.

Liquid biopsy Liquid biopsy is a technique for predicting diagnosis and treatments where a body fluid sample such as blood is collected, instead of the conventional biopsy that mainly collects tumor tissue by using an endoscope or a needle in the area of cancer research. Recently, it is developed and advanced as a method that is less burdensome for patients and leads to appropriate treatment based on tumor genome information.

Liver fibrosis Liver fibrosis is one of the precancerous stage for hepatocarcinoma and is usually observed in cirrhosis of the liver, including NASH.

Lysosomal diseases Lysosomal diseases occur due to a gene mutation or the mutation of a cofactor, resulting in decreased enzyme activities or their complete absence. These enzyme substrates are not degraded and accumulate in the brain, liver, kidney, skeletal muscle, heart and result in the development of a number of disorders. In humans, over 40 kinds of diseases are known.

Lysosomal enzymes Lysosomes are organelles found in eukaryotes such as humans and yeast. Lysosomal enzymes are produced in lysosomes and are largely hydrolases in which the optimal pH is acidic. There are many hydrolases that act on nucleic acids, glycans and lipids and over 70 species exist.

Mac binding protein 2 (MacBP2) MacBP2 was developed as an excellent biomarker for the diagnosis of liver fibrosis.

Machine learning An artificial intelligence method that uses computers to learn using a series of related data. Thus, the performance of a specific task will be greatly improved without the need for programming in advance.

Mannan This is a collective term for polysaccharides having mannose as a main component.

Mannose kinase ATP: D-mannose 6-phosphotransferase.

Mass spectrometry Mass spectrometry is a reliable method for measuring molecular mass by using very tiny amount of target molecules and provides structural information. The main steps in the measurements are ionization of the sample under vacuum in appropriate ways, separation of ions with different masses (m/z) and detection of the number of ions in each mass. Target molecules are low molecular compounds, nucleotides, carbohydrates, lipids peptides and proteins. Currently mass spectrometry plays a major role in proteome or metabolome research.

Medaka (Japanese rice fish) Medaka (*Oryzias latipes*) is a freshwater fish and is a model vertebrate organism similar to the zebrafish. It has been a popular pet since the seventeenth century in Japan. Whole genomic information is complete. Many well-characterized mutant and transgenic strains are available in Japan and these have been used in research in the field of developmental biology including glycobiology.

Metabolic labeling Metabolic labeling is used to probe the biochemical pathways that occur in a cell. It is accomplished by using chemical analogs such as amino acids and sugar precursors that mimic the structure of natural biomolecules like proteins and glycans.

Microbiota Aggregates of microorganisms that reside within a number of different tissues including the skin, lung, oral mucosa, and gastrointestinal tracts, etc. Microbiota have been found to be crucial for the hormonal and metabolic homeostasis status of their host and are related to the life-style of an individual, immunological reaction and the aging process etc. Metagenomics is used extensively for studying microbial communities.

Microenvironment The area surrounding tissues when angiogenesis is incomplete is an environment that is susceptible to the development of hypoxic conditions and the depletion of glucose, situations that are not observed in normal tissues. This appears to be one of the causes of malignant transformation and drug resistance.

Middle molecular pharmaceuticals An emerging type of pharmaceutical drugs, in which the size is in intermediate between small molecular chemical compound drugs like aspirin (180) and large molecular biological pharmaceuticals like antibodies (150,000), special emphasis being made that they can be chemically synthesized like the former and have a relatively high specificity and thus low side effects, similar to the latter. Combining the merits of the two types of pharmaceuticals, middle molecular pharmaceuticals are expected to play a role in the future as drugs, since they have a high cost-performance based on advanced biotechnologies including peptide and nucleic acid science.

Modification of *N*-glycans Modification of the glycosylated site(s) of *N*-glycans in glycoproteins results in the activation of glycoprotein functions such as an extension of its half-life in blood and activation of antibody reactivity .

Monosaccharide A monosaccharide is the minimum and basic unit of sugar and a sugar with three carbons is designated as a triose. Those with five and six carbons are designated as pentose and hexose, respectively. A monosaccharide with ketose group at C2 is designated as ketose.

Mucin-type glycan (*O*-linked glycan) The glycan added to the hydroxyl group of the side chain in serine or threonine residue of a protein is called an *O*-type glycan; one in which the first monosaccharide is *N*-acetylgalactosamine is called a mucin type glycan. There are 8 core structures of mucin type glycans, ranging from core 1 to core 8.

Mucopolysaccharidosis Mucopolysaccharidoses are a group of metabolic disorders caused by the absence or malfunctioning of lysosomal enzymes that are needed to degrade glycosaminoglycans (formerly called mucopolysaccharides). Most mucopolysaccharidoses are autosomal recessive disorders. Glycosaminoglycans accumulate in the cell, blood, and connective tissues. The result is permanent, progressive cellular damage which affects appearance, physical abilities, organs and system functioning, as well as mental development.

Mucous gland The mucous gland is a type of gland that produces a thick (mucopolysaccharide rich) whereas a serous gland produces a serous secretion.

Muscular dystrophy Muscular dystrophy is defined as a group of diseases that cause progressive necrosis and the regeneration of bone muscle, eventually resulting in weakness and loss of muscle mass. There are over 30 such diseases that are caused by gene mutations in gene coding proteins that are essential for muscle function. The Duchenne type, the most common variant, is caused by a mutation in the gene that codes for dystrophin. The Fukuyama type, which is the only type found in Japan, is the most common type of this disease.

Natural compounds Compounds obtained from bacteria, fungi, plants, and sea products. They include many bioactive molecules including anti-biotics, toxins, inhibitors, etc.

Neural plasticity Neural plasticity is the ability of the nervous system to change. The nervous system can be changed, even in adulthood. For example, glutamate receptors accumulate in the post-synaptic area which, in turn, induces swelling of this area. This functional and morphological change is called synaptic plasticity and is one of the fundamental mechanisms of learning and memory.

Neurodegeneration Neurodegeneration is defined as the progressive degeneration or loss of neurons. Because neurons are post-mitotic cells, they cannot reproduce. Therefore, neurodegeneration causes many neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's diseases. They are manifested as ataxia or dementia, and show similarities at the molecular level, i.e., aggregate formation of misfolded proteins.

Next generation high-throughput speed sequencer The next generation high – throughput speed sequencer is an apparatus that can could read DNA and RNA sequences much more quickly and cheaply than the previously used sequencing method by Sanger and can provide useful information for the diagnosis and therapy of diseases.

***N*-glycolyl-neuraminic acid (Neu5Gc)** Neu5Gc constitutes a representative xeno-antigenic glycan epitope in humans, while Neu5Gc-containing glycolipid has been long known as the Hanganutziu-Deicher (H-D) antigen. This happens as the result of the inactivation of a gene responsible for CMP-Neu5Ac hydroxylase (CMAH) in a common ancestor to human and old-world monkeys. Thus, neither of them can convert Neu5Ac to Neu5Gc, and all sialic acids remain the *N*-acetyl type.

NMR (nuclear magnetic resonance) spectroscopy NMR spectroscopy is an analytical method that is used to measure frequency of NMR active nuclei under an external magnetic field. Like many other spectroscopic methods, an NMR spectrum is obtained by electromagnetic radiation at the proper frequency. NMR spectroscopy is now a widely used and established essential tool for structural analysis ranging from small molecules to biomacromolecules such as proteins.

Non-alcoholic steatohepatitis (NASH) NASH is a disease that has attracted considerable interest and is a form of hepatitis caused by the accumulation of fat in the liver. Among non-alcoholic fatty liver disease, NASH is the most extreme phenotype and is considered to be an important cause of liver cirrhosis with an unknown etiology and sometimes develops into a primary hepatoma.

Non-lysosomal metabolism This type of metabolism does not occur in lysosomes but, rather, in proteasomes in the cytosol and produces free glycans.

Norovirus The norovirus is a virus denoted by the genus name of small round-structured virus or Norwalk-like virus in morphological classification by electron microscopy. It causes acute gastroenteritis symptoms such as vomiting and diarrhea due to oral infections. However, spontaneous recovery usually occurs within a few days.

Notch The Notch signaling pathway is evolutionarily conserved in multicellular organisms. It regulates cell fate in developmental processes and stem cells. It is initiated by the binding of the receptor (Notch) to the ligand (Delta or Jagged) which is expressed on the surface of a neighboring cell, resulting in the activation of the Notch signaling pathway in Notch expressing cells.

***O*-GlcNAc. O-GlcNAcylation** *O*-GlcNAc is linked to the hyroxyl group of serine or Thr residues on proteins and shows various types of activity. The *O*-GlcNAc structure is found in cytoplasmic or nuclear proteins and partly competes with phosphorylation. *O*-GlcNAc regulates numerous actions, including diabetes and Alzheimer's disease.

***O*-glucose, *O*-fucose** These sugars bind to serine or threonine in a protein. These glycans are implicated in Notch signaling as ligand molecules. *O*-fucose binds to either a domain of EGFR or thrombospondin.

Oligonucleotide therapeutics Among middle-molecular pharmaceuticals, DNA, RNA and their derivatives are expected to make up a novel class of biopharmaceuticals. They can target even mRNA and miRNA, which neither conventional small molecular nor large antibody drugs can accomplish, and thus, are applicable to the treatment of genetic disorders, cancer and virus infections. While oligonucleotide drugs can be produced more easily with much lower costs than antibody drugs and have higher target specificity, it should be pointed that there are some concerns regarding their in vivo stability, possible side-effects and difficulty in delivering the drug technology to make them successful. Representative oligonucleotide drugs include antisense, RNA interference (RNAi), aptamer and decoy.

Opsonization Opsonization is a phenomenon in which an antibody or complement binds to an antigen and the antigen is then readily taken up by phagocytic cells. In primary infections, complement works mainly in opsonization, whereas IgG functions mainly in secondary infections when antibodies are already made.

Organoid Organoid is a three-dimensional organ that is created in a test tube and has an anatomical structure similar to a real organ, but it is small and simple. They are formed from tissue stem cells, ES cells, and iPS cells and are self-assembled in three-dimensional cultures.

Osteoblast Osteoblast are located on the surface of bone tissue and play a role in bone formation. It secretes type I collagen and osteocalcin, bother of which are

substrates of bone. Hydroxyapatite is deposited on them to form bone tissue. Along with osteoclasts, which absorb old bones, they play an important role in bone metabolism.

Osteoclast differentiation Monocyte-macrophage precursor cells derived from bone marrow differentiate and fuse into osteoclasts that specialize in bone resorption. They are large, polynuclear dendritic motile cells.

Osteoporosis In osteoporosis, the amount of bone (bone mass) is reduced, and the affected bones then become weak and fragile. It is due to a collapse in the balance between bone formation and resorption, and it increases with aging. In postmenopausal women, osteoporosis is associated with reduced levels of female hormone production and aging.

Paired immunoglobulin-like type 2 receptor (PILR) Paired immunoglobulin-like type 2 receptors (PILR) are found in various immune cells, mainly in innate immune cells. The genes that code for inhibitory receptors and those of activating receptors are adjacent to each other on the genome. PILRα has an immunoreceptor tyrosine-based inhibition motif (ITIM) and suppresses signalling, whereas PILRβ has an immunoreceptor tyrosine-based activation motif (ITAM) and causes the activates activation of the signal.

Pandemic influenza virus A pandemic influenza virus is different from the seasonal influenza virus that is prevalent among humans each year. It is a novel type if influenza virus that occurs worldwide, and is broadly and rapidly spreading from human to human in the world.

Pattern recognition Lectins and antibodies recognize antigen epitope that consist of a couple of amino acid residues or carbohydrate residues that bind to antigen-binding site. In the case of pattern recognition, they recognize three-dimensional structures consisting of a repeating domain of a specific glycan component on a cell surface.

Plant allergen Plant components that induce allergenic reactions in humans. Hay fever and food allergies are well known and allergens are plant specific proteins and also contain glycan structures.

Plant cell wall Plant cell walls are mainly composed of cellulose, hemicellulose, and pectin. The most common hemicellulose is xyloglucan.

Plant glycans *N*-linked glycans of plants are similar to those of animal glycans. But in the case of *O*-glycans the first addition of a sugar to an amino acid residue are greatly different. In eukaryotes, GalNAc is attached to serine or threonine residues, whereas in plant, L-Arabinose is typically attached to hydroxyproline or D-Gal to hydroxyproline or serine.

Plant-made pharmaceuticals The production of pharmaceuticals, for example antibodies, in plants. It is expected that pharmaceuticals can be produced using this route with lower cost than by using mammalian cells. However, since the glycan machinery of plants is different from mammals, the glycan structures are different from those produced by mammalians. Glycan modification technology from a plant type to a human type will be needed if such materials are to be used therapeutically.

Pluripotent stem cells Pluripotent stem cells are stem cells that can differentiate into all types of cells belonging to the three germ layers, endoderm, mesoderm, and ectoderm.However, they do not differentiate into extraembryonic tissues such as placenta. They include embryonic stem cells (ES cell), epiblast stem cells, induced pluripotent stem cells (iPS cell), etc.

Polygenic disease Genetic disorders may also be complex, multifactorial, or polygenic, meaning they are likely associated with the effects of multiple genes.

Polysaccharide Polysaccharides are an important class of biological polymers and are composed by monosaccharide or disaccharides repeating units. Their structure or storage-related function is generally known.

Post-translational modification of histones Acetylation and methylation of the histone tail, which is in the *N*-terminal region of histones, induces changes in chromatin structure and regulates transcription. On modification by acetylation, the binding between chromatin and DNA is unclenched, and transcription is then promoted. The effect of methylation on transcription is divided into two areas, promotion and suppression, depending on the type and position of amino acids.

Prebiotics Prebiotics are not degraded or absorbed in the upper gastrointestinal tract and become a selective nutrient for bacteria that are co-localized in the upper gastrointestinal tract, resulting in an enhanced bacterial growth. Therefore prebiotics maintain a healthy balance for bacteria flora in the large intestine.

Prostate cancer The frequency of prostate cancer is increasing. A biomarker for prostate cancer is PSA (prostate specific antigen) which is a glycoprotein and some researchers have investigated changes in the PSA glycan in prostate cancer. Hopefully the application of this approach for the routine screening will be successful.

Rare disease The definition of a rare disease differs, depending on the country of origin. The number of patients in the US are below 200,000 and in the EC, the number is one out of 20,000 residents. Approximately 80% of rare diseases are now identified as a genetic defect due to the next generation high speed sequencer technology. However most are not curable due to the lack of therapeutics.

Receptor-like protein tyrosine phosphatase (RPTP) There are over 100 species of human tyrosine phosphatases (PTP), and they are classified into cytoplasmic PTP and receptor-like protein tyrosine phosphatase (RPTP). RPTP is classified into eight subtypes based on the structure of the extracellular region. PTP, which is responsible for dephosphorylation, functions in signal transduction and is important in cranial nerve, immunity, cancer and diabetes.

Regulatory gene A regulatory gene is located in DNA and enhances or inhibits gene transcription. Regulatory proteins that are formed via a regulatory gene, controls gene expression (initiation of transcription) by binding to a regulatory site.

Relenza/Tamiflu Representative molecular targeted drugs, which inhibit the neuraminidase (NA) function of influenza virus by mimicking a transition state of the substrate sialic acid. The first such drug that was introduced zanamivir (trade name, Relenza) is used for only oral inhalation, but oseltamivir (Tamiflu) was later developed as a prodrug that can be taken orally. Some of the other improved drugs that have been developed include laninamivir (Inavir), an inhalent and (Rapiacta), taken as an infusion. These compounds share a common substituent, namely, a guanidium group, at the C4-position of *N*-acetylneuraminic acid.

Rickettsia Rickettsia is an obligate intracellular parasite that can not grow extracellularly. Ticks and other arthropods serve as mediators. It causes typhus and rickettsial disease including tsutsugamushi disease.

Seaweed polysaccharide Seaweed polysaccharides are glycans derived from sea weeds such as alginic acid, karagenan and fucoidan,

Single gene disorder A single-gene disorder is the result of a single mutated gene.

Single nucleotide polymorphism (SNP) Single nucleotide polymorphism (SNP) is one type of genetic polymorphism. If genetic diversity, in which a single base is mutated in a genom of a certain species of organisms, is detected and at the same time the mutation is found at a frequency of 1% or more within a population, it is referred to as a SNP.

Single-molecule imaging A technique for visualizing biomolecules (e.g. protein, lipid) at a single-molecule level. It is used for monitoring the dynamic behavior of the molecule in real time.

Single-particle analysis One of the three-dimensional cryo-electron microscopy (Cryo-EM) methods that are used to obtain detailed structural information on biomacromolecules by observing and processing the images of many uniform molecules. A single-particle analysis can enhance the resolution compared with a single

image analysis. A three-dimensional structural image can be constructed using a set of images obtained from different angles. This analysis is applied for cryo-EM and other imaging methodologies. Hence atomic-scale resolution has been achieved in the structural analysis of biological macromolecules.

Solid-phase synthesis One of the chemical synthetic methods for preparing biomolecules including glycans. Solid-phase synthesis is a method in which molecules are bound on a bead and synthesized step-by-step. It is easier to remove excess reactants or byproducts. Although it is a powerful method for preparing many compounds in short periods of time, it is sometimes difficult to accomplish this on a large scale.

Spinal cord injury Spinal cord injury is a pathological condition in which the spinal cord has been damaged by a sufficiently strong external force to cause damage to the spinal cord. Similar disorders also occur due to internal causes such as spinal cord tumors and hernias. Different from the peripheral nerve, the central nervous system including the spinal cord is difficult to repair and regenerate once it is damaged.

Stem cell niche A stem cell niche is the microenvironment necessary for stem cells such as tissue stem cells to maintain their properties *in vivo*.

Stereo selectivity Chemical synthesis of glycosidic linkages by controlling the linkage-position and the anomeric configuration (α or β).

Structural analysis of glycans Determination of sequence, linkage, and anomeric configuration (α or β) of glycoconjugates (glycoproteins, glycolipids, proteoglycans, and polysaccharides).

Sugar analog Sugar analogs that mimic the structure of a natural sugar can be used to study the metabolic pathways and functions of glycans in cells. It is one of the very useful techniques for analyzing biological phenomena.

Sugar-cluster effect When lectins and anti-glycan antibodies recognize a glycan epitope on a cell surface, an increase in the apparent affinity is observed if it is present in the form of a multiple molecular form rather than a single molecule.

Sulfated glycan Glycans containing sulfate residues. The addition of a sulfate group catalyzed by various sulfotransferases. Sulfated glycans have been found in backbone glycans such an *N*-linked or *O*-linked glycans, glycosaminoglycans, and glycolipids.

Sulfated sugars Among glycoaminoglycans, except for hyarulonan, all compounds are sulfated. Sulfation is caused by the action of sulfotransferases and exhibit multiple structures and functions.

Supercomputer A supercomputer is distinct from a general personal computer in terms of its high performance. It plays a significant role in the field of computational science and it is now widely used in various fields, including for simulations of biological macromolecules.

Synthesis of glycopeptide Chemical synthesis of a glycopeptide (glycan and peptide that are attached to one another by a covalent linkage).

Toll-like receptors Receptor proteins expressed on animal cell surfaces, function to activate innate immunity by responding to a variety of exogenous substances including those of pathogens. In humans, 10 toll-like receptors (TLR-1-10) have been identified, each of which recognizes either epitoes of triacyl-lipoprotein, lipoteichoic acid, fungal polysaccharides, viral double-stranded RNA, Gram-negative bacterial lipopolysaccharides or Gram-positive bacterial peptidoglycans. In contrast to vertebrates which have evolved with acquired immunity, a defense system coordinated by innate immunity with toll-like receptors is necessary in invertebrates.

Transgenic Gene transgenic is a method for the specific gene expression in cells or animals, such in mice. Transgenic animals (mouse, zebra fish etc.) are useful to elucidate unknown or known functions of glycogenes or specific glycans in the body.

Tumor-associated carbohydrate antigens (TACA) Tumor-associated carbohydrate antigens are glycan epitopes that are specifically expressed in tumorigenic cells and tissues. While they are closely associated with known tumor markers such as sialyl Lewis X ($SiaLe^X$), sialyl Lewis a ($SiaLe^a$) and sialyl Tn (STn), their antigenicity is relatively low, and therefore, the combined detection of TACA and their carrier proteins is a keen issue of development. A classic representative of this includes the L3 fraction of α-fetoprotein (AFT-L3) for the detection of hepatocarcinoma with a plant lectin LCA that is specific for α1-6 core fucose. More recently, M2GPgi (Mac2-binding protein glycosylation isomer) has been approved as a diagnostic glycoprotein marker for hepatic fibrosis. To use TACA as a vaccine, the antigenicity of which is not high sufficient, it is necessary to combine the vaccine with an adjuvant to enhance the vaccination effect.

Vaccine According to the World Health Organization (WHO), a vaccine is a biological preparation that improves immunity to a particular disease. A vaccine is usually made from weakened or killed forms of pathogenic microorganisms, such as bacteria and viruses, which stimulates the immune system of the vaccinator. Edward Jenner found that patients who had suffered from cowpox in the past, were now more tolerant of smallpox. Based on this observation, he developed a vaccine for the smallpox virus. Vaccination is effective for diseases caused by both bacteria and viruses, while it's effect is limited in the case of retroviruses, such as the AIDS influenza viruses, because their surface antigen structures frequently change.

Wnt Wnt is a secreted glycoprotein that functions as a morphogen and is involved in the determination of cell fate, proliferation, cell death and so on. It binds to seven-transmembrane receptors Frizzled and co-receptors, LRP 5/6, Ror, and RYK, and then activates the β-catenin pathway, cellular polarity pathway, and a calcium pathway.

Xenoantigen Xenoantigenic glycan epitopes, that are not present in humans but are present in other organisms. It is long known that the αGal epitope is a major cause for hyper-acute rejection upon xeno-transplantation using the porcine heart. Similar to the case of the ABO blood group, to which antibodies are already present without transfusion, it is assumed that natural antibody which interacts with α-Gal epitope is generated by exposing various bacteria that are present as intestinal flora which produce glycan structures resembling this epitope. It is known that the α1,3-galactosyltransferase gene responsible for the biosynthesis of the αGal epitope is inactivated (pseudogene) in humans and old-world monkeys by frame-shift mutation in two parts.

X-ray crystallographic analysis X-ray crystallographic analysis is a method used to determine the atomic structure of a biological molecule such as a protein. Atoms in the crystal cause specific X-ray diffractions with different intensities. By analyzing the angles and intensities of these diffracted beams, it is possible to construct an atomic-scale three-dimensional structure of the specimen.

Yeast Yeast can be used to produce a large amount of protein. However, glycoproteins that are produced in yeast are structurally different from glycans that are produced by mammals. Therefore, in order use yeast to produce glycoproteins for therapeutics, their glycans will need to be modified to the mammalian type. Yeast are used as a model organism to elucidate glycan biosynthesis pathways.

Zebrafish The zebrafish (with *Danio retio* being the most common one) is a freshwater fish and is an important and widely used vertebrate model organism. Many well-characterized mutant and transgenic strains are available worldwide and show advances in the fields of developmental biology including glycobiology.

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