Chapter 17
Poly(ADP-Ribosyl)ation of Chromosomal Proteins, Epigenetic Regulation and Human Genomic Integrity in Health and Disease

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17.1 Introduction

The accurate hereditary transmission of genetic information, or "blue print", which makes up a human being, from generation to generation occurs with a high degree of fidelity. Molecular accuracy occurs not only due to the wealth of information stored in the long deoxynucleotide sequence of DNA of 6×10^9 billion units per diploid genome of somatic cells (primary structure). Instead, as it has become clear recently, the intrinsic ability of the DNA double helix (secondary structure) to interact with a multitude of proteins at the molecular level (epigenetic information) is pivotal for high binding specificity. According to the rapidly emerging field of epigenetics, the selectivity of DNA-protein interactions is occasionally dictated by electronegativity of DNA and intrinsic structural properties of the properly folded polypeptides, which may operate as single monomeric molecules, as in the case of histone H1 (Suganuma and Workman 2008; Izzo et al. 2008) or DNA metabolizing enzymes such as DNA polymerase β (Beard and Wilson 2006). More frequently however, DNA-protein complexes develop as highly organized oligomeric macromolecular scaffolds, including the nucleosomal histone octamers or "cores" (Tremethik 2007), which comprise 50% of the total nuclear protein, or as DNA replication (Tye and Swayer 2000) and transcription multi-subunit molecular machines (Malecka et al. 2009). More importantly in the context of this volume, these DNA-bound oligomeric protein structures are also found amongst post-translational modification enzymes, e.g. PARP-1 itself (Kawaichi et al. 1981; Mendoza-Alvarez and Alvarez-Gonzalez 1993). The organization of these mega-unit protein-nucleic acid complexes adopts very sophisticated, elaborate and highly regulated "biochemical engines" which orchestrate the individual molecular events that modulate chromosomal dynamics (Tremethik

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2007; Suganuma and Workman 2008). Indeed, the temporal and topographic distribution of these biochemical engines, ultimately determines the right patterns of gene expression. Not surprisingly, when epigenetic mechanisms are out of synchrony, cells and tissues may loose growth control, as it occurs in carcinogenesis (*vide infra*) or over time, it may also lead to altered genomic homeostatic systems that may result in the development of chronic disease.

17.2 Protein-Poly(ADP-Ribosyl)ation and Human Genomic Integrity

The proper maintenance of genomic integrity in individual human cells, and by inference, the proper balance between health and disease, requires flawless molecular integration between nucleosomal histone proteins (Althaus 1992) and complex oligomeric DNA metabolizing enzymes, e.g., PARP-1 (Mendoza-Alvarez and Alvarez-Gonzalez 1993), which epigenetically coordinate poly-functional proteins, such as the tumor suppressor protein known as p53 (for a recent review see Alvarez-Gonzalez 2007), a key transcription factor with DNA nick sensing ability. The dynamics of this elaborated genetic expression system seems to be orchestrated by these two "guardian angels" (Chatteerjee et al. 1999; Lane 1992; Tong et al. 2001) of genomic integrity which quickly detect, protect and facilitate the repair of nicks and double strand breaks on DNA (Althaus 1992) Interestingly, the molecular mechanism appears to involve the homodimerization of PARP-1 on a DNA nick (Mendoza-Alvarez and Alvarez-Gonzalez 1993), coupled with either the homodimerization and/or the tetramerization of sequence dependent-DNA binding of p53. Not surprisingly, the disruption of accurate protein-protein and protein-DNA interactions of the p53/PARP-1 macromolecular complex seems to either turn on (Wieler et al. 2003) or turn off (Conde et al. 2001) pivotal control genetic switches that dramatically increase tumor latency in murine experimental models (*vide infra*). In other words, PARP-1 and p53 molecular interplay on DNA appear to control the toggle switch between cell survival, following repair of genotoxic damage, or programmed cell death (apoptosis) after a threshold of un-repairable DNA-damage level has been reached (Kumari et al. 1998).

Nonetheless, not all molecular details of the scenario described above have completely been elucidated. For example, even though both protein guardians of genomic integrity become covalently poly(ADP-ribosyl)ated in the process (Kumari et al. 1998; Mendoza-Alvarez and Alvarez-Gonzalez 2001; Simbulan-Rosenthal et al. 2001), we do not know whether PARP-1/p53 multimeric complexes involve either heterodimer formation or alternatively, a heterodimer of homodimers as a plausible protein-poly(ADP-ribosyl)ation enzymatic intermediate. Regardless of the complexity of p53/PARP-1 protein oligomers on DNA, one conclusion is undisputable: PARP-1 and p53 work in tandem, as DNA sensors and cell cycle checkpoints, respectively, and together facilitate either the repair of DNA-damage to promote cell survival (Bouchard et al. 2003), or launch the apoptotic program

(Scovassi and Poirier 1999; Alvarez-Gonzalez et al. 1999) that eliminates highly mutated cells, thus preventing the development of tissue-specific malignancies (Conde et al. 2001), or alternatively, the unstoppable process of chronic disease (*vide infra*).

17.3 Protein-Poly(ADP-Ribosyl)ation and Epigenetics

Of the epigenetic pathways listed above, reversible protein-poly(ADP-ribosyl)ation is known to be a transient enzymatic regulatory cycle initiated by a family of enzymes known as ADP-ribose polymerases, of which the best understood, and most abundant example, is poly(ADP-ribose) polymerase-1 or "PARP-1" for short (Alvarez-Gonzalez 2007). It has been reported that up to 2% of the total nuclear proteins of interphase chromatin may correspond to PARP-1 (Alvarez-Gonzalez 2007), a constitutive enzyme that is present throughout the cell cycle, in most somatic and germinal cells (Atorino et al. 2000), except mature erythrocytes. This fact alone implies that, leaving the histone proteins out, the next protein in line which structurally and functionally maintains the integrity of the human genome may very well be PARP-1 itself.

This chapter will focus on discussing how this ubiquitous eukaryotic enzyme and its partner catabolic protein, responsible for the hydrolytic degradation of protein-bound ADP-ribose polymers, namely poly(ADP-ribose) glycohydrolase (PARG) (Fig. 17.1), may directly (or indirectly) activate (or inactivate) the epigenetic balance between health and disease. Two of the main hallmarks of an epigenetic pathway are: (i) the information is not directly related to the primary structure of DNA, e.g., the overall genomic sequence; and (ii) the information is properly transmitted from generation to generation with a high degree of fidelity, just like the DNA replication pathway. Indeed, the covalent ADP-ribose polymerization of chromatin proteins (*vide supra*) fulfills both criteria and can thus be classified as an epigenetic pathway.

Another major characteristic of epigenetic mechanisms is the reversibility of the system. This implies the participation of two enzymatically catalyzed steps, one to transfer a given chemical moiety from a donor substrate onto a protein acceptor, and its counterpart, a different enzyme responsible for the removal of the protein-bound modifier as depicted on Fig. 17.1 for protein-poly(ADP-ribosyl)ation (*vide infra*). Thus, a classic biochemical cycle of post-translational protein functional regulation is typically characterized for having both an activating and a de-activating arm. Therefore, when this process occurs in the cell nucleus, and the protein subjected to specific chemical modification reactions is a DNA-binding protein, the biochemical cycle may also be classified as an epigenetic pathway.

As it might be expected, any malfunctions on either side of the epigenetic pathway may lead to loss of genomic integrity and cancer, or alterations of cell physiology via over- or under-activation of specific tissue- and cell-specific gene expression patterns, and therefore different kinds of genetic diseases and/or chronic diseases, including

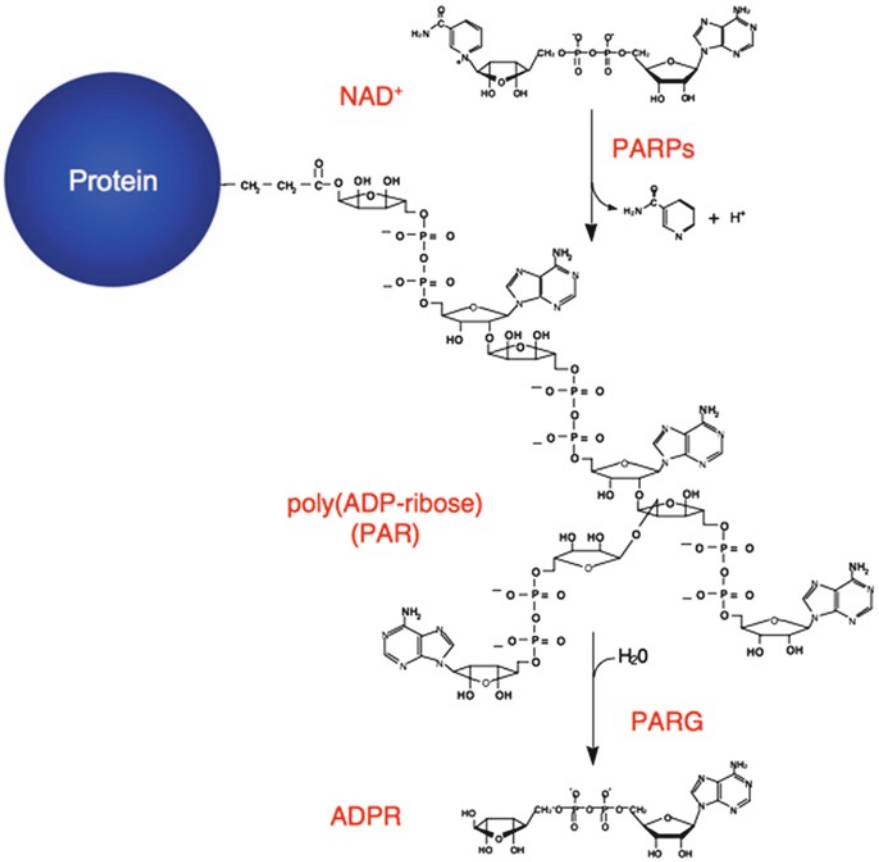

Fig. 17.1 Enzymatic cycle of protein-poly(ADP-ribosyl)ation. A family of post-translational modification enzymes known as ADP-ribose polymerases, e.g., PARP-1 and PARP-2 (PARPs) utilize the oxidized form of β-nicotinamide adenine dinucleotide (NAD^+) as an ADP-ribose donor (Loetscher et al. 1987) to assemble covalently bound polymers of ADP-ribose (PAR) of more than 240 ADP-ribose units onto DNA-binding proteins, including PARP-1 and PARP-2 themselves. PARPs efficiently catalyze four kinetically distinct chemical reactions: (i) ADP-ribose chain initiation (Mendoza-Alvarez and Alvarez-Gonzalez 1999); (ii) ADP-ribose chain elongation (Alvarez-Gonzalez 1988); (iii) ADP-ribose polymer branching (Alvarez-Gonzalez and Jacobson 1987); and (iv) chain termination. The enzyme responsible for the degradation of these chromatin-bound polymers is poly(ADP-ribose) glycohydrolase(s) (PARG) which generates large amounts of free ADP-ribose or "ADPR" (Pacheco-Rodriguez and Alvarez-Gonzalez 1999)

aging. This is the main reason why, there has been a recent expansion of research efforts to better understand epigenetic pathways, with the resulting explosion of literature reports in the field of epigenetics in the last few years, including three chapters in this volume, one on histone changes and gene expression (Bernstein and co-workers, Chap. 15), another one on histone modifications and DNA repair (Gospodinov and Herceg, Chap. 16), and this one on protein-poly(ADP-ribosyl)ation.

Obviously, due to the multi-factorial origin of many human pathological conditions, a common molecular origin for the substantially different patterns

of gene expression observed when comparing normal cells and tissues with those typical of chronic disease, an epigenetic abnormality may provide a target for effective pharmacological intervention. Therefore, a thorough understanding of the protein molecules, their chemical modifications and the enzymes involved should provide with a more transparent window of observation into human health and disease. However, connecting the dots between the development of chronic diseases over time and key molecular events that actually tilt the homeostatic balance between health and disease seems to be rather elusive at best.

In the following segments of this review, a small but significant number of human ailments that have been reported to be related to protein-poly(ADP-ribosyl)ation abnormalities will be briefly reviewed. It is also important to say that in an attempt to keep them all somewhat related, the topics selected for discussion have a common point of relationship, and that is that they all involve a step of specific tissue or organ inflammation, early in the process of pathological development.

17.4 Protein-Poly(ADP-Ribosyl)ation Abnormalities and Chronic Disease

All diseases listed below will connect the epigenetic pathway of protein-poly(ADP-ribosyl)ation with: (i) acute phase responses and inflammation, at the molecular level; and (ii) the ultimate outcome of chronic disease as an imbalance between cell survival and cell death (apoptosis and/or necrosis).

During the last few years, a dizzying wave of literature reports connecting the field of protein-poly(ADP-ribosyl)ation to a large number of chronic human diseases others than cancer, has steadily been accumulating in the literature (for a recent example see Pacher and Szabo 2008). However, due to the plethora of molecular pathways that appear to be regulated by this reversible protein modification cycle, and the fact that most reports have just touched upon the surface of significance PAR metabolism, and a cohesive and detailed metabolic integration of the depth and breath of ailments connected to this unique and transient biochemical pathway is quickly becoming a web that is quite complex and thus very difficult to untangle.

As a result, in this review, an attempt is made to strictly focus on the significance of the molecular and epigenetic properties of PARP-1 and its protein partners in a group of selected chronic diseases, including arthritis, cancer, chronic heart failure and myocardial infarction, diabetes, and stroke, from the protein chemistry point of view.

17.5 Protein-Poly(ADP-Ribosyl)ation and Aging

The time-dependent accumulation of genotoxic damage, and of the molecular wear and tear of particularly proteins and enzymes in most human tissues, typically manifests as a combination of chronic diseases. In a given individual, these clinical

ailments together with a decreased efficiency of specific biochemical pathways resulting from genetic mutations or not, are generally associated with the process of aging. While it may sound too pretentious, to even relate any specific biochemical pathway to aging, one must ideally focus on a few potentially important molecular events as key contributors to this rather complex phenomenon.

As it might also be expected, the observation of unusual levels of protein-poly(ADP-ribosyl)ation activity, as a function of aging, in peripheral blood samples from animal species with a characteristic lifespan (Grube and Burkle 1992) has been reported. Interestingly, in these studies, it was clear that the steady state levels of PARP activity correlated with the life span of each species tested with rodents at the lower end of the spectrum and humans at the opposite end (Grube and Burkle 1992). In fact, Homo sapiens displayed a fivefold higher level of PARP activity when compared to rats. Even though several explanations have been proposed to explain this difference (Beneke and Burkle 2007), a full mechanistic explanation for this dramatic difference remains to be established.

While it is recognized that the process of aging is obviously very complex, a discussion to understand the physiology and environmental factors that contribute to the unavoidable time-dependent reduction of physiological efficiency in mammals is well beyond the scope of this chapter. Nonetheless, it must be noted that it has recently been reported that tissue inflammation and endothelial dysfunction may also significantly contribute to the acceleration of aging manifestations (Csiszar et al. 2008). Thus, identifying the molecular connection of these interesting observations to protein-poly(ADP-ribose) metabolism and genetic integrity (El-Domyati et al. 2008) is very attractive. Furthermore, unraveling how PARP-activity and its role in the maintenance of genomic integrity (Alvarez-Gonzalez 2007) contributes to the genetic decision making process between cell survival (Perkin and Gilmore 2006) and p53-dependent apoptosis (Pietsch et al. 2008) may shed light on the molecular steps of the aging process.

17.6 Protein-Poly(ADP-Ribosyl)ation and Arthritis

Amongst the most common examples of chronic diseases, that may be associated with protein-poly(ADP-ribosyl)ation, enzymatic anomalies, and tissue inflammation, we may identify several autoimmune diseases (Negri et al. 1990), including systemic lupus erythematosus (SLE) (Jeoung et al. 2004), glomerulonephritis (Messmer et al. 2000) and Sjogren's syndrome (Rosen and Casciola-Rosen 2004). These arthritic or rheumatoid diseases are characterized for the accumulation of autoantigens, autoantibodies, or a high concentration of antigen-antibody immunocomplexes, within the tissue involved. Indeed, one of the most frequent antigenic culprits observed in autoimmune diseases is either PARP-1 or PAR [polymers of (ADP-ribose)] (Fig. 17.1), or sometimes, both.

It must be noted however, that these do not represent the only protein complexes detected, and thus, may or may not represent the main immune elicitors. Interestingly,

little has been done to properly distinguish between two distinct (ADP-ribosyl)ation antigenic scenarios with regards to the presence of a high antibody titer to PAR in these patients. It is not clear whether these antigens represent protein-free PAR molecules or protein-bound ADP-ribose chains.

Clearly, since protein-free PAR's have not been detected in healthy cells and tissues, one may argue that ADP-ribose chains must be exclusively present as protein-bound polymers, e.g., poly(ADP-ribosyl)ated-chromatin adducts. However, given the wide spectrum of proteins that can be subjected to this post-translational covalent modification reaction, we still do not know the oligomeric structure of most chromatin protein targets, and more importantly, how chromatin protein adducts play an immunogenic role in tissue inflammation at the molecular level. A very likely possibility is that abundant polypeptides produced by immune cell rupture and release of nucleoplasmic components during the inflammatory response results in the release of chromatin components, especially the most abundant proteins in chromatin, the histone proteins (Kouzarides 2007; Tremethik 2007), which become effective immunogens. This becomes even more relevant as lymphocytes and macrophages recruited to swollen tissues via diapedesis, rupture as a result of triggering the apoptotic program (Kumari et al. 1998; Alvarez-Gonzalez et al. 1999; Messmer et al. 2000).

Once immune cells have fulfilled their physiological function as acute phase response recruiters and the immunological defense system of the injured individual mounts an attack on micro-environmental microbial opportunists, an autoimmune reaction against self nucleoplasmic components, e.g., chromatin molecular components, including PARP-1 and protein-bound PAR's, also concomitantly ensues, leading to arthritic pain and associated symptoms.

17.7 Protein-Poly(ADP-Ribosyl)ation and Cancer

It has become evident that the complex process of carcinogenesis and the protein-poly(ADP-ribosyl)ation cycles were definitely related at the molecular level (for a recent review on this topic see Alvarez-Gonzalez 2007). Ever since it was reported in the early 1980s that PARP is activated by strand breaks on DNA (Benjamin and Gill 1980), which when occurring in intact cells and tissues, as a result of carcinogen exposure, causes a dramatic increase in the levels of chromatin-bound PAR's (Juarez-Salinas et al. 1979).

Over the last 30 years, literally thousands of reports have scrutinized the relationship between DNA-damage, DNA repair and poly(ADP-ribose) metabolism from most biomedical angles, from the molecular and genetic level to organ physiology, cancer biology and clinical chemotherapy regimens. Since 1980, protein-poly(ADP-ribosyl)ation has undoubtedly been a very active area of research as far as cancer is concerned. The efforts in this area have been quite substantial, and literature reports are too numerous to list here. For readers wishing to obtain further information on this topic, a reference is listed below (Alvarez-Gonzalez 1999) to a

book published just before the end of the twentieth century. Now, in the dawn of the twenty-first century, as a result of the tireless efforts of dozens of protein-poly(ADP-ribosyl)ation experts, whom have published world-class experimental work performed during the last 45 years, in leading Universities and medical research-intensive institutions across the globe, from Canada and the USA to Japan and also to France, Germany, Italy, Spain, Switzerland, UK, etc., we have apparently reached a climax of unparalleled success with the recent application of highly specific competitive inhibitors of PARP-1 and PARP-2 to enhance the efficacy of standard cancer chemotherapy regimens in patients with different kinds of tumors (Donawho et al. 2007; Plummer et al. 2008). Nonetheless, in spite of the promise and excitement in this arena, this is still the early phase of the currently ongoing clinical trials with these cancer chemotherapeutic agents, which rely mostly on their potency as PARP inhibitors in vitro, and it is still possible that they may affect other enzymes with similar biochemical activity, e.g., using NAD^+ as a substrate, and thus, may affect other physiological phenomena with serious side effects.

Obviously, we must continue our experimental efforts with diligence until we identify all proteins, genetic markers and all molecular details of epigenetic control, to fully elucidate the various poly(ADP-ribosyl)ation epigenetic cycles and completely unravel how a given protein-poly(ADP-ribosyl)ation cycle participates in the different phases of cancer initiation, promotion, progression, en even metastasis. One outcome is almost certain, we should witness the unveiling of key poly(ADP-ribosyl)ated proteins as important players in the maintenance of genomic integrity and cancer, such as p53 (Kumari et al. 1998; Mendoza-Alvarez and Alvarez-Gonzalez 2001; Alvarez-Gonzalez 2007).

Again, in order to keep this chapter focused as it pertains to the role of protein-bound PAR metabolism in human health and disease, the brief discussion in this section will center on the relationship of this post-translational modification reaction with cancer via the epigenetic regulation of transcription (p53) and tissue inflammation via NF-kappa B regulation of gene expression (see below).

17.8 Protein-Poly(ADP-Ribosyl)ation in Heart Failure and Myocardial Infarction

The primordial role of cell death and apoptosis in heart failure has also gained a lot of attention in the last 12 years (Bromme and Holtz 1996). The hypoxic or ischemic shock caused by a sudden decrease in blood flow, and thus oxygen homeostasis, through the heart, may trigger localized vascular inflammation to avoid significant damage. Alternatively, massive hypoxic conditions may elicit the endothelial and myocyte cell death programs (de Boer et al. 2000). Thus, the epigenetic role of protein-poly(ADP-ribosyl)ation in modulating the various phases of the apoptotic response (Alvarez-Gonzalez et al. 1999) makes PARP-1 a potential target for cardio pharmacological modulators that may help avoid the dramatic consequences of tissue damage during chronic, or even acute, for that matter, myocardial infarction.

In the year 2000, a solid attempt was made to find the connection between the biochemical activation of PARP-1 and the epigenetic inactivation of this pivotal pathway in homeostatic heart physiology during chronic heart failure (de Boer et al. 2000). In that report, it was clear that there was a molecular mechanistic connection between protein-poly(ADP-ribosyl)ation and myocardial infarction, although the link was more towards the end of the pathway leading to massive heart damage (Pillai et al. 2005).

More recently, most industrialized nations have reported cardiovascular and heart disease as the number one cause of unexpected deaths. Because of this, there is currently a lot of interest worldwide to develop preventive and therapeutic measures to turn the tide in favor of human health. As a result, many investigators studying various genetic and epigenetic factors that contribute to heart disease are actively pursuing all kinds of molecular leads to ameliorate the population morbidity and mortality due to this particular problem. Obviously, protein-poly(ADP-ribosyl)ation is not the exception to this rule (Molnar et al. 2006).

A sudden burst in the investigation of PARP inhibitors as potential effective drugs in treating heart disease has been sparked (Booz 2007; Bartha et al. 2008) in the last 2 years. Nonetheless, in spite of the undeniable potential of PARP inhibitors as efficient therapeutic agents to reduce the consequences of heart failure, one should keep in mind that not all details of the exact molecular mechanism have been elucidated. Thus, it is anticipated that a significant body of experimental evidence describing the exact genetic and epigenetic role of protein-poly(ADP-ribosyl)ation pathway in heart disease will become available within the next 10 years.

17.9 Protein-Poly(ADP-Ribosyl)ation and Diabetes

An indirect connection between the covalent poly(ADP-ribosyl)ation or chromatin proteins and the pathogenesis of diabetes has been considered since the 1960s (Lindall and Lazarow, 1964) via NAD metabolism and function, as a prosthetic or co-enzyme factor, that facilitates the balance between oxidative catabolism (NAD/NADP) and reductive anabolism (NADH/NADPH).

Based on this information, as well as key data obtained with the application of powerful affinity chromatography purification techniques (Alvarez-Gonzalez et al. 1983) to study NAD^+ metabolism and protein-poly(ADP-ribosyl)ation together, it was concluded that there is actually a molecular signaling pathway that takes information from bio-energetic pathways in the NAD/NADH-dependent mitochondrial synthesis of ATP in the mitochondrion and the epigenetic poly(ADP-ribosyl)ation of chromatin proteins in the nucleoplasm (Loetscher et al. 1987). Interestingly, this link does not only involve the epigenetic regulation of key proteins involved in endocrine transcription (Valdor et al. 2008), but also the PARP-1 gene itself (Masutani et al. 1999).

To further illustrate the complexity of the direct and indirect metabolic links between protein-poly(ADP-ribosyl)ation, pyridine nucleotide metabolism, and diabetes in pancreatic tissue, the Japanese group of Okamoto and collaborators reported in 1999 that the CD38-cyclic ADP-ribose signaling system plays a pivotal

role in insulin secretion (Okamoto 1999) and thus channels overall NAD consumption to the cytoplasm, away from the nucleoplasm. Therefore, even though this pathway does not represent post-translational modification of proteins directly, it undermines the efficiency of protein-poly(ADP-ribosyl)ation in the nucleoplasm by depleting the intracellular NAD+pool as well.

Interestingly, in spite of the obvious link between protein-poly(ADP-ribosyl)ation and the development of diabetes as one of the culprits (the other one is heart disease, *vide supra*) of what is now called metabolic syndrome, we still lack fundamental information about the molecular role that the biochemical pathway of chromatin poly(ADP-ribosyl)ation plays in pancreatic physiology, especially as a "stepping stone" into the genetic and epigenetic development of this increasingly more prevalent chronic disease.

17.10 Protein-Poly(ADP-Ribosyl)ation and Stroke

The localized and many times irreversible brain cell damage that occurs as a result of ischemia and hypoxic shock within minutes of blood vessel clogging is a well recognized clinical manifestation of a stroke episode. Also, as it was mentioned above, there seems to be a direct connection between constitutive PARP enzymatic activity and structural integrity of this protein to insure cell survival (Alvarez-Gonzalez 2001; Bouchard et al. 2003), when possible. However, massive ischemia may rapidly induce the cell death program or apoptosis (Scovassi and Poirier 1999) which results in the neuronal proteolytic degradation of PARP-1 (Joashi et al. 1999). Not surprisingly, about a decade ago, a search for a direct connection between the protein structure and function of PARP-1 and stroke was launched (Choi 1997; Endres et al. 1997).

After 12 years of research into stroke, hypotoxic neuronal damage and apoptosis, over 100 literature reports that dig deeper into our understanding of how exactly PARP may be used as a chemotherapeutic target to reduce the irreversible damage of brain function after a stroke, at least with experimental animal models (Haddad et al. 2008), indicates that prevention of PARP-1 enzymatic activation with competitive inhibitors, and its apparently consequential susceptibility to proteolytic degradation by caspases, probably represents a very interesting therapeutic possibility. Even though, we still lack a lot of information regarding the biochemical and epigenetic role of PARP-1 in neuronal homeostasis and health, the potential benefits of PARP-1 specific inhibition were elegantly summarized (Moroni and Chiarugi 2009) as this chapter was being written.

17.11 Concluding Remarks

Undoubtedly, the notion that protein-poly(ADP-ribosyl)ation cycles play a pivotal role in the developing stages of various chronic diseases of drastic pathological, physiological and anatomical differences is a very difficult biomedical concept to

accept. However, it is likely that all mechanisms of disease that operate at the genetic, biochemical and molecular levels coalesce at a central pivotal point, such as the concept of epigenetic control of tissue-specific gene expression, both temporally and topographically, within specific chromosomal domains at different stages of cell homeostasis and differentiation. Such a point may be at the level of the decision making process between cell survival and cell death (Alvarez-Gonzalez 2001; Bouchard et al. 2003). Alternatively, the important bridge in this cause effect relationship may be at the level of either maintenance of structural chromosomal and genomic integrity (Althaus 1992; Chatteerjee et al. 1999; Conde et al. 2001; Tong et al. 2001; Alvarez-Gonzalez 2007) or the ubiquitously characteristic inflammatory responses of tissues to all kinds of environmental insults, including microbial infections, via NF-kappa B dependent regulation of gene expression (Oliver et al. 1999; Chang and Alvarez-Gonzalez 2001; Hassa et al. 2003) or both.

Finally the undisputable link between chromatin protein-poly(ADP-ribosyl) ation with either the genetics of tissue inflammation via NF-kappa B-dependent gene expression and the four different stages of the apoptotic (Alvarez-Gonzalez et al. 1999) cell-death program (condemnation, commitment, execution and demolition) strongly suggests that other chronic ailments such as pulmonary and neurodegenerative diseases may also represent important pathological conditions arising over time as a result of abnormal ADP-ribose polymer metabolism. Further research into the molecular and epigenetic mechanisms of these diseases will either confirm or refute such an interesting possibility.

In closing, it can be concluded that, after almost 50 years since the discovery of chromatin-bound ADP-ribose polymers (Chambon et al. 1963), our scientific journey into either the biochemical, genetic, molecular, protein chemistry or epigenetic realms of chromosomal-poly(ADP-ribosyl)ation cycles, should provide us with thorough mechanistic details about the physiological function(s) of PARP enzymes, and their catalytic products, in balancing human health and disease. More importantly, the knowledge generated in this highly sophisticated, but very interesting field of protein structure and function, should helps us develop more effective, and disease-specific, therapeutic approaches to alleviate most chronic ailments in the near future.

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