Chapter 12
Protein Homeostasis and Ageing in *C. elegans*

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Abstract Understanding the molecular mechanism underlying ageing and age-related diseases is the best strategy to design therapies and interventions to effectively decrease ageing and age-related morbidity and mortality. A decline in proteome quality results in the accumulation of misfolded proteins that tend to aggregate in soluble or insoluble entities and has a negative impact on cell physiology. Protein aggregation has been considered a common hallmark of several neurodegenerative diseases and is also associated with normal ageing. Although it is still not clear how and why protein aggregation occurs, it seems that altered protein synthesis, folding, repair and degradation, commonly referred as protein homeostasis, play a central role in this process. As a consequence, modified proteins tend to form insoluble high molecular weight aggregates that actively influence cell metabolism, proteasomal activity and protein turnover. In some cases, protein aggregation may be beneficial by reducing proteotoxic effects of protein complexes. However, whether protein aggregates play a causal role in ageing phenotypes and lifespan remains to be determined, and this is one of the key goals of biomedical ageing research. *C. elegans* is proving to be a very useful model for studying the aggregation of human disease proteins. Although the significance of human protein aggregation in *C. elegans* as a model for protein homeostasis and disease is debatable, several potentially important models of proteotoxicity have been developed. In this chapter, I will describe the importance of studying normal *C. elegans* protein aggregation, and the relevance of worm models of conformational diseases to ageing and age-related disease research.

Keywords Ageing • Age-related diseases • *C. elegans* • Disease models • Protein aggregation • Protein homeostasis

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12.1 Introduction

The increasing average age of the global population is an issue that affects virtually every country around the world, because age is the single most important risk factor for the onset and progression of a group of human degenerative diseases that represent a huge social and economic burden. Therefore, unravelling the mechanisms underlying the ageing process in order to develop preventative therapies and interventions aimed at reducing or delaying age-related disease-associated morbidity should be a priority for biomedical research. It is not difficult to imagine the positive impact of such anti-ageing interventions in decreasing healthcare costs for the elderly, increasing the healthy years of life, and possibly extending lifespan.

In consequence, the growing interest in understanding the process of ageing is not surprising. In order to explore the basic mechanisms underlying ageing and age-related diseases, simple models that allow researchers to answer basic questions of why this process occurs, and to perform experiments related to this physiological process in short periods of time, are required. During the last 30 years, the roundworm *C. elegans* (*C. elegans*) has become a critical asset for ageing research. Amongst the many advantages of this nematode as a model system, the relatively short lifespan (around 3 weeks, depending on temperature) has made *C. elegans* particularly suitable for longitudinal studies on ageing and ageing-related diseases.

Through studies in *C. elegans*, as well as other invertebrate model organisms such as fruit flies (*Drosophila melanogaster*), budding yeast (*Saccharomyces cerevisiae*), a large number of genes has been shown to influence the lifespan. These genes encode a wide variety of proteins involved in the control of intracellular signalling processes, endocrine functions, metabolic functions, cell cycle checkpoint functions, cellular stress response and protein turnover, amongst others. Despite this wealth of information regarding mechanisms of ageing, the causes of ageing and the reasons why ageing is a risk factor for age-related disease are still not fully understood. This could be due to the multifactorial nature of ageing. Germline signalling, oxidative damage, mitochondrial function, inflammation, DNA damage, cell senescence, autophagy, and several other factors are thought to play a role in ageing (See Chaps. 4, 5, 6, 7, 10 and 11). Interestingly, all these physiological alterations are also related to the onset and/or progression of several diseases which suggests a mechanistic crosstalk between ageing and disease. Possibly one of the clearest examples of this kind of crosstalk is the breakdown of protein homeostasis which leads to significant alterations in protein synthesis, protein folding, protein repair and protein degradation. A failure of protein homeostasis leads to intra- and/or extracellular protein aggregation, a common feature of physiological ageing and of many diverse human diseases. For example, a common feature of ageing in many species is the accumulation of a particular kind of protein aggregates (sometimes referred to as lipofuscin) that are composed of fluorescent pigments, oxidized proteins, lipids, carbohydrates and metals [1, 2]. In addition, a broad range of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), frontotemporal dementia, and motor neuron disease are

characterized by neuronal damage that may be caused by aggregation and deposition of aberrant forms of particular proteins [3]. However, whether protein aggregates are a symptom or a cause of neurodegeneration and how they contribute to the impairment of neuronal function is still unclear. Additionally, abnormal forms of proteins are also associated with non-neurological systemic diseases like type II diabetes and several myopathies [4].

Taken together, protein aggregation, as a consequence of defective protein homeostasis, is not only a hallmark of ageing but also accompanies a plethora of degenerative diseases. Therefore, studying mechanisms of protein homeostasis and aggregation in simple invertebrate models has the potential to provide us with valuable tools to control both the onset of ageing, as well as age-related diseases.

12.2 Protein Homeostasis and Protein Aggregation

Protein turnover means that proteins are continuously being synthesized, degraded and replaced with newly synthesized copies, at a rate that is specific for each protein. This process, referred to as protein homeostasis, is required to protect the functional integrity of the proteome by constantly supplying functional proteins, preventing potentially dangerous misfolded or damaged proteins from adversely affecting the cell. Interestingly, there are significant differences in the turnover of cellular proteins, arising from different proteins having different half-lives that range from minutes to the whole lifespan of a cell. This variability in protein half-life is probably due to the specific physiological function or the intracellular localization of each protein. For instance, proteins located inside membranous organelles like mitochondria or endoplasmic reticulum have very long half-lives [5]. As a consequence, intracellular proteins with a long half-life will be exposed for longer periods to extra or intracellular noxious effects that could alter their conformational structure and/or function. Furthermore, proteins that escape the surveillance of protein homeostasis mechanisms would remain in the cytosol with the consequent risk for the cell physiology. These kind of damaged proteins tend to accumulate and this could lead to the formation of different types of protein aggregates that, in turn, will play a critical role in ageing and age-related diseases.

Protein aggregation arises as a result of protein misfolding and alterations in primary structure in response to mutations, posttranslational modifications, local changes in pH or salt concentrations and during thermal or oxidative stress [6, 7]. In a very general sense, protein aggregates are oligomeric complexes of modified conformers, mainly produced by hydrophobic interactions but are often cross-linked, that turn into large, stable complexes [6–9] (Fig. 12.1). These aggregates have a poor solubility in water or detergent and do not exhibit the functions of their constituent proteins. In the literature, overlapping terms for protein aggregates can be found. Amongst others, the terms aggresomes, inclusion bodies, plaques, lipofuscin and ceroid are frequently used [10].

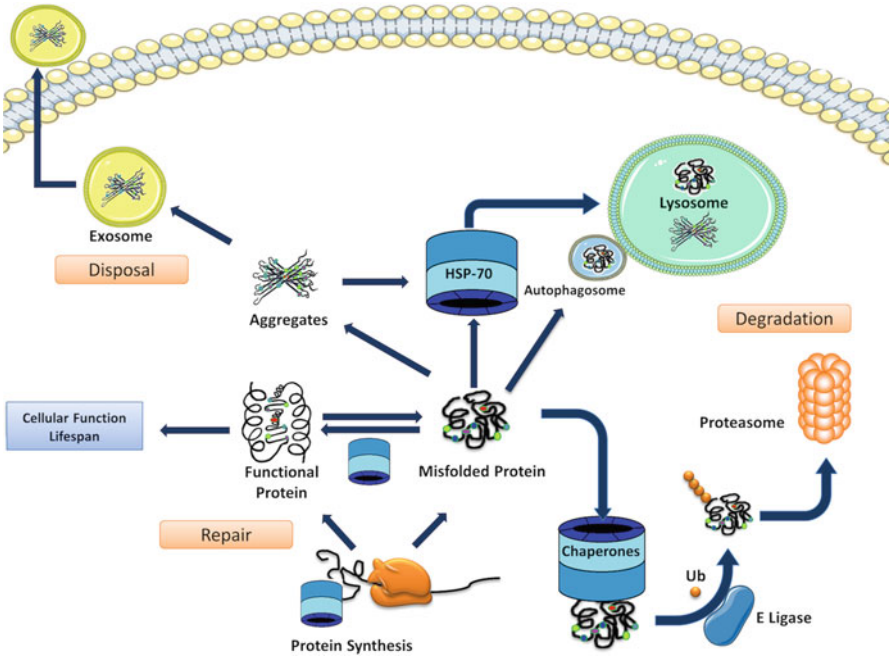

Fig. 12.1 Protein homeostasis. Basic mechanisms involved in the proteome preservation, the stress response, the ubiquitin/proteasome system and autophagy. Chaperones assist in the folding of new proteins and refold misfolded proteins while the two other proteolytic systems dispose damaged or misfolded proteins

It is possible that physiological changes elicited during the ageing process, such as a decrease in proteases and proteasome activity, accumulation of oxidative stress and interaction with heat-shock proteins, amongst others, could accelerate the progression and exacerbate the effects of protein aggregates. Mechanisms involved in the protein homeostasis response are critical for cellular defence and adaptation to stress during ageing. Therefore, the repair mechanism (heat shock response), the degradation system (the proteasome and the ubiquitin system) and the disposal systems (autophagy) should work in a concerted action to prevent damaged, obsolete or misfolded proteins to aggregate and avoid cytotoxic effects (Fig. 12.1), sometimes referred to as proteotoxicity.

12.2.1 Stress Response: The Repair System

Misfolded proteins tend to accumulate in the cytosol where they activate heat shock factor 1 (HSF-1), the master regulator of a particular class of proteins, the heat shock proteins (HSPs). HSPs are stress response factors that are rapidly induced in response to elevated temperatures and other stress stimuli by the activation, via

phosphorylation and deacetylation, of the master regulator of the heat shock response, HSF-1. Many HSPs act as molecular chaperones, i.e., they recognize partially denatured proteins and prevent protein misfolding and aggregation, and protect intracellular components during stress conditions (see also Chap. 9). During unstressed conditions, HSPs are constitutively expressed to facilitate protein folding and the assembly of oligomeric protein complexes (Fig. 12.1).

There is evidence suggesting that the capacity of HSPs to cope with stress decreases with ageing. For example, it has been reported that the induction of the most abundant stress-inducible HSP, HSP70, is decreased by ageing in rat hepatocytes [11], human mononuclear cells and lymphocytes [12]. Similar results can be found with other stress responsive HSPs, like HSP90, whose expression is reduced in fibroblasts from old rats [13]. Due to their chaperone-like activity, small HSPs could regulate protein aggregation, playing an important role in disorders characterized by an aberrant protein folding such as AD.

The first report relating HSPs and ageing was provided by Tatar's group in extra-copy HSP-70 *D. melanogaster* lines, where they found that higher levels of HSP-70 protein were associated with a decrease in mortality [14]. Similar results were found later in long-lived *C. elegans* strains carrying extra copies of an *hsp-70* family member [15] and in a number of studies, where overexpression of various HSP also extended lifespan in this nematode [16–18]. Since then, a number of laboratories have demonstrated links between stress response and lifespan, including the observation that overexpression of the gene encoding HSF-1 increases lifespan [19, 20]. Additional studies in *Drosophila* also showed that overexpression of various other HSP leads to lifespan extension [17, 18, 21, 22] suggesting that the role of HSP in stress response is evolutionarily conserved in similar way to influence lifespan. However, it is important to note that HSP overexpression could be deleterious when is expressed in certain tissues or combined with thermal stress [23]. Moreover, HSF-1 and several chaperones are required for the survival of cancer cells [24, 25].

Taken together, these data support the idea that stress response plays a determining role in ageing and disease maintaining protein homeostasis (Fig. 12.2).

12.2.2 Ubiquitin/Proteasome System: The Degradation System

Some damaged or obsolete proteins are marked for degradation by specific chaperones, but most of them are marked by the covalent attachment of several units of the small (8 kDa) protein ubiquitin (Ub) and thereby assigned to be degraded by a large, ATP-dependent, complex called the ubiquitin/proteasome (UPS). Oxidized proteins are preferentially degraded by the 20S proteasome [26], a multimer of 28 subunits arranged in 4 rings staked in a cylinder-like structure, while ubiquitinated proteins are marked for ATP-dependent degradation by the 26S proteasome, the result of the association of the 20S proteasome with the regulatory subunit 19S [27]. Protein ubiquitination starts when a protein substrate receives a covalent linkage of ubiquitin catalyzed by a group of enzymes commonly known as E ligases. The attachment

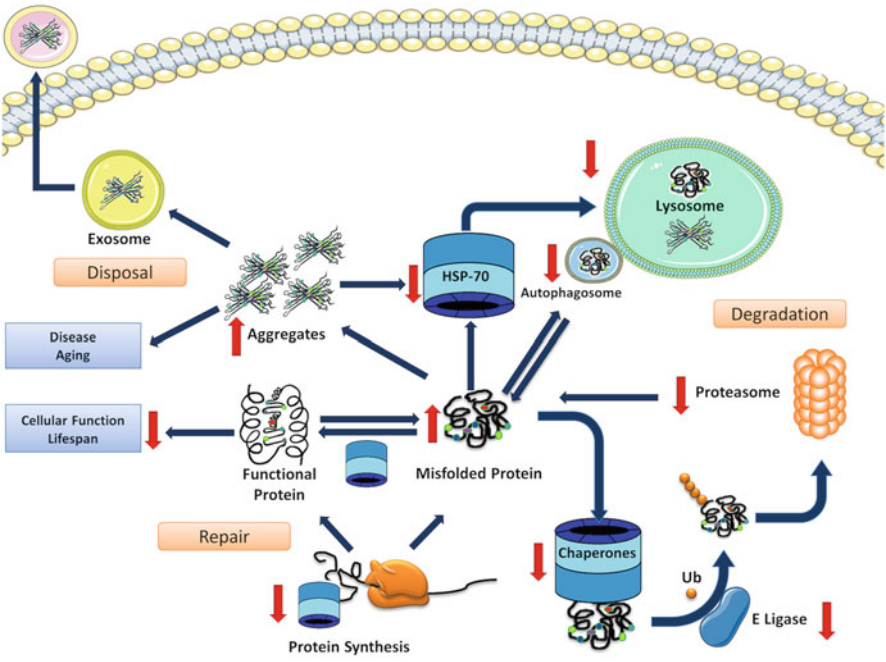

Fig. 12.2 Protein homeostasis alterations during ageing. Changes in the activity of the three main mechanisms involved in protein homeostasis. Light down arrows indicate decline in function or activity, changes in protein levels while up arrows indicate accumulation or increase in function

of Ub to a target protein involves its activation by an E1 (Ub-activating) enzyme and its subsequent transfer to an E2 (Ub-conjugating) enzyme. The E2 transfers Ub moieties to the substrate through its association with an E3 ligase [28]. These repeated cycles of ubiquitination generate the polyubiquitin chain that is recognized by the regulatory complex of the proteasome (Fig. 12.1).

While an age-dependent failure in proteasome function does not seem to be universal, a large body of evidence suggests that there is a decline in proteasome activity with age in several tissues and its impaired activity has been associated with several age-related diseases [29–31]. The reasons for this decline in proteasome activity are not fully understood but the reasons could be tissue-specific; e. g., changes in the stoichiometry of the catalytic units, down-regulation of the proteasome subunits and posttranslational modifications, or it could be in response to changes in extrinsic factors like age-related ATP depletion. In humans, several brain structures like the cerebral cortex, hippocampus and spinal cord show impaired proteasome activity with ageing [32]. Fibroblasts obtained from healthy centenarians have been reported to have a more active proteasome compared with those obtained from young donors [33]. A decrease in proteasome activity has also been reported during the progression of PD [34] and AD [35]. Proteasome inhibition is able to induce apoptotic-like cell death, and has been proposed as a novel therapeutic target

for some types of human cancer. Microarray experiments in human fibroblasts and rat skeletal myocytes have shown a decrease in the transcription of several genes encoding the 20S or the 26S proteasome subunits during cellular senescence [36]. A decrease in free ubiquitin, downregulation of some ubiquitin-conjugated enzymes and E3 ligase has been reported during ageing.

This decline in protein degradation during ageing leads to the formation of insoluble protein aggregates [37] and age-related decline in proteasome activity has been associated with the development of several conformational pathologies, particularly neurodegenerative diseases (Fig. 12.2). Therefore, it is highly possible that the modulation of proteasome activity plays an important role in controlling lifespan in different species.

12.2.3 Autophagy: The Disposal System

Lysosomes are organelles specialized in the degradation of damaged or dysfunctional intra- and extracellular components. Lysosomes can engulf and degrade even whole organelles. In this chapter, I will focus on the removal of protein aggregates through autophagy (see also Chap. 15). Three different types of autophagy have been described; microautophagy, chaperone-mediated autophagy and macroautophagy [38]. Microautophagy is a mechanism not well characterized in mammals, where the lysosomal membrane invaginates to engulf a portion of the cytosol. Chaperone-induced autophagy is a mechanism where a motif of five amino acids is recognized by a particular HSP-70 to translocate these misfolded or damaged proteins across the lysosome membrane. This mechanism is preferentially activated during stress response and is present in most cell types. Alterations in this mechanism lead to the aggregation of misfolded proteins and, in consequence, contribute to the progression of several neurodegenerative diseases. Macroautophagy is a process where a part of the cytosol containing misfolded proteins, protein aggregates and/or organelles are engulfed in a double membrane vesicle called autophagosome that will fuse with a lysosome to complete the degradation process. This is a complex process that requires protein-protein and protein-lipid recognition as well as an intricate kinase nucleation process to form the autophagosome. Interestingly, this mechanism is negatively regulated by the mTOR (mechanistic target of rapamycin) pathway that has been involved in the regulation of lifespan in invertebrates and mammals. Since macroautophagy is critical to maintain protein homeostasis and energetic balance, due to its ability to remove malfunctioning mitochondria, a decline in its activity has been related to multiple pathologies including cancer, neurodegenerative and metabolic diseases [39].

Age-related changes in macroautophagy activity have been described in several tissues including liver and brain [40]. Additionally, it has been found that age-related changes in insulin levels downregulate the activity of this clearance system [41]. Interestingly, caloric restriction is able to maintain macroautophagy in old mice, probably by a decrease in mTOR signalling [42]. It is interesting to note that

chaperone-mediated autophagy decreases in almost all tissues of old rodents [43] suggesting the relevance of this system for ageing and disease. In line with this, rapamycin, a drug that increases worm's lifespan through a restoration of autophagy, increases lifespan in mice, probably by a mechanism involving autophagy [44].

12.3 Protein Homeostasis in *C. elegans*

Several studies in *C. elegans* have lead to a better understanding of the role of stress response in ageing and age-related diseases. There is ample evidence supporting that the overexpression of chaperones, HSP induction by thermal stress or activation of the transcriptional activator HSF-1 leads to a significant lifespan extension [16, 19, 20, 45]. In line with this, long-lived mutants present high levels of heat-shock proteins [46]. In a recent study, it has been shown that stress response deteriorates soon after the onset of the reproductive period in *C. elegans* [47]. The expression of human proteins involved in neurodegenerative diseases produces toxic effect and decreases lifespan (see below), suggesting that alterations of protein homeostasis have profound impact in regulating lifespan. Over the last few years, various screens of small molecules have been conducted to find long-sought interventions in ageing. Recently, a series of chemicals have been identified in *C. elegans* that stabilize the protein homeostasis network and extend lifespan [48].

In *C. elegans*, the 26S proteasome consists of a 20S protease core particle that is capped at one or both ends by the 19S regulatory particle, which has an approximate molecular mass of 700 kDa [49, 50]. A double stranded RNA interference (RNAi) study of the 26S proteasome subunits has shown that a knockdown of most of these genes produces embryonic and post-embryonic lethality, suggesting that proteasome activity is critically required for development [51]. The entire 26S proteasome core and, as expected, most of the regulatory subunits tested in that study were lethal. However, loss of some of the regulatory subunits (*rpt-9*, *rpt*-10 and *rpt*-12) has no effect on *C. elegans* survival. Recently, it has been shown that several components of an E3 ligase family (SCF CUL-1 complex) function in the postmitotic adult somatic tissues of *daf-2* mutants to promote longevity, suggesting a role for the proteasomal system in *C. elegans* lifespan [52]. In line with this, the overexpression of one 19S proteasome subunit, RPN-6, was shown to increase proteasome activity and extend lifespan at 25 °C but not 20 °C [53]. However, mutation of a gene encoding a different 19S subunit, *rpn-10*, causes reductions in the proteasome activity but increases stress resistance and lifespan at 25 °C [54]. Since mutants with both increased and decreased levels of proteasomal activity can have extended lifespans, it is not clear how the changes in ubiquitin proteasome activity that occur naturally with age might affect normal lifespan. In an elegant experiment, Holmberg's group used constitutively ubiquitinated fluorescent proteins as reporters for proteasome activity and found a tissue-specific decline in proteasome activity, with neurons being more affected than muscle [55]. However, an in vitro study found an upregulation of the proteasome subunits 19S and 20S in lysates from aged

worms [56] suggesting that the observed decrease in proteasomal activity is not due to decreased proteasome levels. This could be explained by a tissue-specific decline in the half-life of the proteasomal subunits with age. Other degradative processes, like lysosomal (autophagic) and proteasomal degradation, as well as the activity of cytosolic and mitochondrial proteases, are closely related to the proteasome to maintain the continuous turnover of damaged and obsolete biomolecules and organelles [57]. It has been proposed that the ageing process involves a decrease in protein degradation [58, 59], leading to the accumulation of damaged or obsolete proteins and lipofuscin, as well as mitochondrial failure. In line with this, hundreds of proteins with diverse functions were found in detergent-insoluble extracts from old but not young *C. elegans* worms [2, 60]. Moreover, reduction of the expression of many genes encoding proteins that become insoluble during ageing results in extended lifespan consistent with a connection between the aggregation process and ageing [2, 60].

Taken together, studies in *C. elegans* have demonstrated that protein homeostasis collapses during ageing, leading to an accumulation of protein aggregates as well as to significant changes in cell physiology. Some of these changes are similar to those observed in human neurodegenerative diseases suggesting that *C. elegans* models protein aggregation diseases could shed light on the mechanisms controlling ageing and disease.

12.4 *C. elegans* as a Model for Human Disease

C. elegans has been instrumental in identifying molecular pathways underlying the ageing process, such as DAF-16/FOXO and TOR signalling. However, there are several caveats that are important to consider. Although *C. elegans* and *D. melanogaster* genomes are highly homologous to the human genome (40 % and 60 %, respectively), both of them belong to a phylum with a significant divergence from the common ancestor with humans, leading to a lack of genes that could be critical for human physiology and, in consequence, for critical mechanisms regulating ageing. Additionally, these two species are able to enter a long-lived stage of developmental arrest in response to harsh conditions (*dauer* for nematodes and diapause for flies), which is a lifespan-extending process that is clearly not shared with mammals. One of the main limitations of these models is that their somatic adult tissues have limited regenerative capabilities associated with a very low or nonexistent cell proliferation. As a consequence, these models fail to adequately represent the cellular and molecular mechanism involving tissue-specific ageing, and the role of stem cells in mammalian ageing. This is particularly important when it comes to complex diseases associated with ageing, such as cancer or type II diabetes [61, 62].

Even though *C. elegans* is an invertebrate model with so many experimental benefits, including the study of protein homeostasis and protein aggregation, it is not obvious how good of a model they are in the study of human diseases.

12.4.1 Worm Models of Neurodegenerative Diseases

Basic neuronal functions are conserved between vertebrates and invertebrates, including *C. elegans*, but it is not clear whether the specific mechanisms altered in human neurodegenerative diseases are also conserved in *C. elegans*. To address this question is not simple, since the molecular mechanism responsible for the development of neurodegenerative diseases remain controversial and the results obtained in worms should be confirmed in mammals. Nevertheless, it is interesting to note that the expression of aggregation-prone proteins associated with neurodegenerative process, such as, ***α***-synuclein, ß-amyloid, or tau produces cytotoxic effects in *C. elegans*. One of the main caveats of this model is the lack of a complex well-structured brain, with the corresponding absence of important structures involved in neurodegenerative diseases, like cerebral cortex and substantia nigra. In addition, nematode axons lack shields of myelin, and most worm neurons are often too small for electrophysiological studies. However, the nervous system of this nematode has been studied in great detail. An adult hermaphrodite has just 302 neurons and the pattern of synaptic processes is fully described. The position and the neurotransmitters used by every single neuron have also been mapped (publicly available in the worm atlas, http://www.wormatlas.org). A virtue of this model is its transparency, which is particularly useful to visualize GFP (Green Fluorescent proteins)-tagged proteins during the whole life cycle in vivo and facilitates the observation of degeneration and cell death by simple optical methods. An additional advantage of this model is the ease with which RNA interference (RNAi) and transgenic over-expression can be carried out, allowing the assessment of gene knockdowns and protein over-expression on a particular worm model of interest.

As mentioned above, a broad range of neurodegenerative diseases such as AD, PD, Huntington's disease (HD), frontotemporal dementia, and motor neuron disease are characterized by neuronal damage that may be caused by aggregation and deposition of abnormal proteins. In order to create a worm model for any of these diseases, the proteins that are thought to aggregate and thereby induce neurodegenerative diseases have to be expressed in worms with or without a reporter, which could be a fluorescent protein tag or an epitope. Pan-neuronal promoters are frequently used for proteins expressed in several human tissues, but sometimes the tissue-specific toxicity of the proteins could be lethal for the transgenic worms, in which case promoters specific to individual neurons are used. Using this approach, several worm models of neurodegenerative diseases have been generated. In the following sections, I will describe the most relevant models and discuss their implications.

12.4.2 ß-Amyloid and Tau Aggregation: Alzheimer's Disease and Tauopathies

Alzheimer's disease (AD) is a dementia produced by the degeneration of neurons and neuronal processes in the cerebral cortex and several subcortical structures. In the brain of patients affected by this disease, it is common to find ß-amyloid plaques and neurofibrillary tangles of tau protein [63].

The first ß-amyloid worm model was made by Christopher Link, by expressing a human ß-amyloid peptide n under the control of the muscle specific promoter *unc-54* [64]. With age, this strain shows ß-amyloid aggregates, similar to those observed in AD patients, in muscle, and the worms become paralyzed. This model has been widely used and has proven particularly useful to determine the anti-aggregation properties of the small chaperone HSP-16.2 [65] and other HSP induced by a moderate heat-shock, pointing out the relevance of endogenous chaperones in the modulation of protein aggregates [66]. A decrease in the main pathway controlling lifespan from yeast to mammals, the insulin-like growth factor pathway [67] and caloric restriction [68] also decrease ß-amyloid aggregation in this strain suggesting a relationship between protein aggregation and ageing. By using this strain, it was possible to show that soluble oligomeric forms of ß-amyloid are more toxic than the high molecular weight aggregated forms. An interesting variant of this strain was created to express ß-amyloid in muscle [69] or in neurons [70], upon temperature upshift. This is a temperature inducible strain that starts the expression of ß-amyloid when shifted from the permissive temperature (16 °C) to the non-permissive (23 °C). This shift of temperature results in paralysis in approximately 24 h. Interestingly, this strain has been instrumental to study the effects on protein homeostasis of compounds known to increase lifespan. Thioflavin T, an amyloid-binding dye, prevents the aggregation observed in this model, as well as increases lifespan in a HSF-1 and SKN-2 depending way [71]. Similar effects have been observed feeding this strain with reserpine [72], tetracycline [73], coffee extract [74], 5-fluorodeoxyuridine [75], ethanolic extract of Liuwei Dihuang [76], copper [77], curcumin [71], amongst others, demonstrating the ability of these compounds to modulate protein aggregation, and highlighting the possibility that some of these compounds might one day be used to prevent or treat AD.

Tauopathies are a group of neurodegenerative diseases where the protein tau is aberrantly hyperphosphorylated, dissociates from the microtubules and gradually aggregates into neurofibrillary tangles. AD, Prick's disease and frontotemporal lobar degeneration (FTLD) are the main diseases associated with this condition.

A worm model of tauopathy was established by expressing wildtype and mutant tau from FTLD under the control of the pan-neuronal promoter *aex-3* [78]. The resultant strains are uncoordinated due to a neurodegeneration process associated with insoluble tau aggregation, but mutant tau is significantly more toxic. This strain was used to search for genes that could modulate the uncoordinated phenotype by RNAi screening of the whole genome, and 60 genes were found that enhance this phenotype [79]. Amongst those genes are kinases, phosphatases, chaperones and

proteases suggesting a critical role of protein homeostasis in the control of tauopathies. Since the hyperphosphorylated form of tau has the highest tendency to aggregate, a transgenic worm expressing pseudohyperphosphorylated tau driven by the pan-neuronal promoter *rgef-1* was generated. Resultant transgenic worms present developmental defects in motorneurons, and local broadening of the axons suggesting that this could be a better model to study tauopathies in *C. elegans*.

Interestingly, several pharmacological intervention studies have been performed using this model. For example, the inhibition of tau aggregation by cmp16, an aminothienopyridazine-like compound, reduces the neuronal damage and significantly increases lifespan of tau mutants [80]. Tau models have also been used to screen libraries of compounds in order to identify drugs that could ameliorate tau neurotoxicity [81]. The main hit of this screen was the antipsychotic azaperone that inhibits the D2 dopamine receptor to decrease tau aggregation and increase lifespan in this model.

12.4.3 Polyglutamine Repeat Disease; Huntington's Disease

Huntington's disease (HD) is a neurodegenerative disorder that is the result of a CAG (glutamine, Q) triplet expansion in the N-terminal of huntingtin, a protein important for development but of unclear function. Once huntingtin is attached to an expanded track of glutamines (polyQ), it is prone to misfolding and aggregation and becomes toxic for neurons. The first HD worm model was provided by the Hart lab by expressing a huntingtin fragment containing a 150 repeat polyQ (Ht-Q150) driven by the promoter *osm-10* in the sensory neurons [82]. This construct leads to a selective death of chemosensory neurons mediated by caspase 3 [82]. This model was used to identify genes whose loss of function enhances the Ht-Q150 toxicity leading to the identification of the polyQ enhancer-1 gene (*pqe-1*). PQE-1 encodes a nuclear protein that contains a glutamine/proline rich domain suggesting that this protein exerts its protective effect by competing for proteins sequestered by Ht-Q150. This model has been used to unravel the role of specific histone deacetylases, such as *sir2.1*, in the control of Ht-Q150 toxicity. A similar model was developed by expressing an N-terminal 57 residue fragment of huntingtin fused to GFP and driven by the *mec-3* promoter (Ht-polyQ::GFP) in ten non-essential neurons, including six touch receptor cells [83]. As expected, the more polyQ repeats were in the transgene, the bigger was the deficit in touch responsivity of the transgenic worms. Similarly to the previously described model, the overexpression of *sir 2.1* histone deacetylase induced by resveratrol provided protection against Ht-polyQ::GFP [84].

Probably one of the most popular models to study Huntington's disease in *C. elegans* was developed by the Morimoto group by directly fusing different lengths (Q19 and Q82) of polyQ repeats to YFP and directing the expression to the muscle [85]. By fusing different polyQ lengths to YFP, this group was able to show that polyQ of a length ranging from 35 to 40 repeats is able to induce stress response and

toxicity in this worm [86] and that polyQ aggregates affect protein homeostasis [87]. Another model, where polyQ expression is directed to the muscle by the *unc-54* promoter, increases mitochondria degradation [88] and this effect is suppressed by the co-expression of ubiquilin [89] suggesting the relevance of protein homeostasis, particularly the proteasome, in mitochondria degradation.

Interestingly, several compounds that increase lifespan in *C. elegans* have also been shown to decrease polyQ aggregation through the activation of different mechanisms. For example, trehalose (a disaccharide) and celecoxib (a non-steroidal anti-inflammatory) both prevent polyQ toxicity and increase lifespan through a mechanism that involves decreased insulin-like growth factor signalling activity and increased stress resistance [90, 91]

12.4.4 α-Synuclein Toxicity: Parkinson's Disease

PD is a major neurodegenerative disease, but its prevalence is difficult to estimate because it can only by diagnosed when it is already in an advanced stage. PD is characterized by the loss of dopaminergic neurons in the substantia nigra resulting in involuntary movements. A pathological hallmark of this disease is the formation of a particular kind of aggregates known as Lewis bodies containing α-synuclein, neurofilaments and ubiquitin. α-synuclein is a small (approximately 14 kDa) and abundant protein in the human brain, but is also present in muscles, hearth and several other tissues in small quantities. In the brain, it is found in the presynaptic endings, and although its function is unclear, it seems to be important for the development of cognitive functions and neuronal plasticity. The toxicity of human α-synuclein expression in *C. elegans* varies depending on the promoter used to direct the expression of this protein. For example, the use of two different pan-neuronal promoters produces movement deficits (*aex-3*) or no effect at all (*unc-51*) [92, 93]. Interestingly, when α-synuclein is driven by the dopaminergic neuron-specific promoter *dat-1*, loss of dopaminergic neurons and dendrite degeneration has been reported [92–94] suggesting that α-synuclein is toxic for dopaminergic neurons. Torsin A, an abundant protein in the brain that is often present in the Lewis bodies, and Rab-1, a GTPase involved in the protein transport between the endoplasmic reticulum and the Golgi, provide protection against this neurodegeneration [94, 95]. A microarray analysis of worms expressing *dat-1*-controlled human α-synuclein showed an upregulation of genes involved in the ubiquitin proteasome and mitochondrial function, while several histones were downregulated. This suggests that α-synuclein could produce neurodegeneration not just by forming aggregates but through affecting protein homeostasis, mitochondrial activity and the control of protein expression [96]. α-synuclein::YFP and α-synuclein::GFP expressed in muscles have also been used to determine which genes modulate the aggregation pattern. Twenty genes, most of them involved in autophagy, out of 868 were found to increase α-synuclein aggregation when their expression was downregulated by RNAi [97]. A

similar approach identified 80 genes involved in lipid metabolism, vesicular transport, as well as some modulators of lifespan [98].

12.5 Conclusions and Perspectives

The devastating effects of neurodegenerative diseases and ageing are well documented. The economic burden of treating disease symptoms, as well as the psychosocial aspects of disease and ageing are a huge problem for modern societies. In consequence, unravelling the mechanisms underlying ageing and neurodegenerative diseases is imperative to delineate effective interventions and therapies to eventually prevent or cure neurodegenerative diseases, and improve healthspan and longevity.

Protein aggregation is not just a hallmark of conformational diseases but also plays a critical role in ageing. In this sense, *C. elegans* has proved to be an excellent model for the study of ageing. However, regarding neurodegenerative diseases, it is possible that *C. elegans* does not closely reflect the physiopathology of human neurodegenerative diseases. Worm models of high prevalence NDs have been developed and have helped unravel mechanisms controlling these diseases. The use of reverse and forward genetics on *C. elegans* models of disease has the potential to uncover mechanisms of regulation of neurotoxicity that can potentially be confirmed in mammals and extrapolated to humans. Some results obtained using worm models of neurodegenerative diseases await validation in mammalian systems, and it is important to keep in mind that several of these results could be due to the particular physiology of this nematode. It is possible that new models of neurodegenerative diseases will be generated in the near future to broaden the research in this kind of human diseases.

Additionally, these worm models of disease are an excellent platform to rapidly test a number of compounds, thanks to the clear phenotypes that are associated with protein aggregates (paralysis, loss of coordination, fluorescent aggregates, etc.). Although any identified compounds will need validation in mammalian systems, this approach greatly accelerates discovery of compounds with the potential to prevent or even cure neurodegenerative diseases.

Despite the caveat and limitations of *C. elegans* as a system for modelling human neurodegenerative disease, the results so far are encouraging, and it is highly possible that new proteins and mechanisms, as well as compounds mimicking those processes, will be identified using worm models of disease. It is also possible that new models of other conformational diseases will be developed in the near future, opening new avenues for the knowledge of shared mechanisms between ageing and disease.

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