

Neurodegenerative conditions associated with ageing: a molecular interplay?

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Abstract

The ageing process precipitates dramatic alterations in the physiology of all organisms, including reduced cellular function, compromised resistance to stress and pathological agents, and increased likelihood of developing age-related diseases. Among the most characteristic pathologies associated with old age are numerous late-onset neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases. In addition to stroke, which also inflicts loss of neuronal cells, these conditions account for ever-increasing debilitation among the elderly. Recent studies in model organisms such as the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*, which offer the prowess of sophisticated genetic approaches, have uncovered significant, novel aspects of the molecular mechanisms that underlie both neurodegeneration and the ageing process. These advances hold promise that the intimate link between the aged state and the manifestation of several neurodegenerative diseases will be deciphered. Here, we discuss the mechanisms by which ageing interfaces with, and influences, the progression of neurodegeneration.

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1. Introduction

Ageing represents the major risk factor for a plethora of age-related diseases (Squier, 2001). The ageing process is not a programmed process, in the sense that no genes are known to have evolved specifically to cause damage and senescence. Therefore, mechanisms of ageing might not be expected to be as highly conserved between distantly related organisms as are the mechanisms of development and metabolism. Given the complexity of the ageing process, several mechanisms may well contribute to senescent decline. However, mounting evidence from *Caenorhabditis elegans*, *Drosophila*, and mammalian systems suggests that modulators of the rate of ageing are conserved over large evolutionary distances (Longo and Finch, 2003; Partridge and Gems, 2002). What is particularly exciting is that individual genes such as the *age-1* gene of *C. elegans* can

have dramatic effects on lifespan and vitality (Friedman and Johnson, 1988a,b). Thus, experimental manipulations in genetic systems can be expected to provide significant insight into the biology of ageing. Elucidation of the basic molecular mechanisms underlying the progressive decline in cellular function that accompanies ageing and eventually leads to senescence will have an immediate impact on the design of novel interventions that could reduce or delay age-related deterioration in humans.

Age-related decline in health confers significant cost and burden on our society. Although marked advances in diagnosing and treating disorders associated with the elderly have been accomplished, we still understand very little about the basic molecular mechanisms of ageing and their contribution to the development and progression of age-related disorders. How does ageing trigger or modulate neurodegenerative conditions most prevalent in old individuals, such as Alzheimer's? Do the molecular mechanisms of ageing and those of neurodegenerative disorders intersect at some point(s)? What are the common denominators?

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Recent studies are beginning to uncover the intricate interplay between the ageing process and age-associated pathological conditions. Aggregation of modified proteins, disturbance of ion homeostasis, protein and DNA modification, oxidative stress, as well as impairment of energy production are some of the key mechanisms linking ageing to neurodegeneration. Nevertheless, many pieces of the puzzle are still missing. Hence, in-depth analysis of the basic biology of ageing is essential for the rational design of interventions aiming to improve human health at old age. In this review, we survey the link between ageing and the molecular mechanisms implicated in neurodegenerative conditions and review findings in simple, invertebrate model organisms such as *C. elegans* and *Drosophila*, that provide significant insights pertinent to this connection.

2. Common mechanisms

The physiological and cellular changes that accompany ageing may have pronounced effects on many neurodegenerative disorders, frequently accelerating their progression and exacerbating their symptoms. Below, we outline some of the pathways through which ageing feeds into and modulates the development of age-related neurodegenerative pathologies.

2.1. Protein aggregation

Several neurodegenerative conditions are often characterized by the formation of insoluble protein aggregates. For example, inclusions that contain proteins with expanded polyglutamine stretches in Huntington's disease and several spino-cerebellar ataxias; cytoplasmic Lewy bodies that contain α -synuclein in Parkinson's disease; intracellular neurofibrillary tangles that contain tau protein, and extracellular deposits of the amyloid peptide in the most common form of dementia, Alzheimer's disease (Kopito, 2000). Normally, irreversibly damaged proteins are recognized by chaperones, and targeted for degradation (Soti and Csermely, 2003). However, proteasome level and function decreases with ageing, and some oxidized, aggregated proteins exert a direct inhibition on proteasome activity (Carrard et al., 2002). Due to these degradation defects, damaged proteins accumulate in the cells of aged organisms, and by aggregating may cause a variety of problems (Soti and Csermely, 2002). Protein aggregates can be either structured (e.g. amyloid) or amorphous. In either case, they tend to be insoluble and metabolically stable under physiological conditions (Kopito, 2000). They are refractory to proteolysis and accumulate in inclusion bodies. Their accumulation—intracellular and extracellular—is tightly linked to neuronal degeneration of organ failure in many diseases, including ageing-related neurodegeneration and systemic amyloidosis (Soti and Csermely, 2002).

Generally, cytoplasmic inclusion bodies are pathognomonic features of cellular degeneration in most neurodegenerative diseases. These inclusion bodies are present singly or in low copy numbers and are nearly always accompanied by displaced and abnormally phosphorylated intermediate filaments (neurofilaments). Neurofilaments (type-IV intermediate filaments), which are the major cytoskeletal element in axons, frequently surround cytoplasmic inclusion bodies in many neurodegenerative diseases, including Lewy bodies in Parkinson's disease and hyaline inclusion bodies in amyotrophic lateral sclerosis (ALS). Intracellular inclusions in neurodegenerative diseases can occur within cell bodies or within cell processes (Kopito, 2000). Tests of neuronal function of aged cells, e.g. using electrophysiology, show that it is impaired, and the ultimate consequence of the cellular pathology is cell death. While the identification of the proteins involved in the aggregates and the cloning of genes implicated in familial neurodegenerative diseases has in recent years given clues to the mechanisms of these diseases, much remains uncertain. For example, are the protein aggregates a cause of the pathological state or simply a symptom? And how does impairment of neuronal function and ultimate cell death arise? These questions are not simply of academic importance, as the development of future therapies to prevent, delay, or lessen the symptoms of these diseases will be greatly aided by an understanding of their molecular nature (O'Kane, 2003).

2.2. Protein degradation

Protein synthesis and degradation are the two essential, interlinked cellular processes responsible for maintaining a functional protein content in every cell. A general decline in gene expression, translation and transcription has been observed to occur with increasing age in a wide variety of organisms and tissues, yet surprisingly, the levels of most enzymes and proteins remain relatively constant during normal ageing. Thus, one would predict that a decline in gene expression should be accompanied by an age-related decline in protein degradation. Indeed, this appears to be the case. Biochemical studies (Rattan and Clark, 1996), and micro-array expression assays (Lee et al., 1999), correlate lowered protein degradation capacities with senescent decline. Therefore, damaged proteins accumulate in the aged cell causing problems and dysfunction. Their accumulation is tightly linked to neuronal degeneration or organ failure in many 'protein-deposition' diseases (Kopito, 2000). For example, oxidatively damaged protein accumulation has been associated to age-related diseases such as Parkinson's and Alzheimer's diseases, amyotrophic lateral sclerosis and rheumatoid arthritis (Carrard et al., 2002).

2.2.1. Proteasome-mediated degradation

The age-related accumulation of oxidized and ubiquitinated proteins and the slowing down of protein turnover

raise the possibility that the proteasome degradation pathway becomes impaired with age. Indeed, oxidized proteins are preferentially degraded by the 20S proteasome in an ATP-independent fashion (Davies, 2001; Grune et al., 1997), while covalent attachment of ubiquitin marks proteins for ATP-dependent degradation by the 26S proteasome (Ciechanover et al., 2000; Hershko et al., 2000; Pickart, 2001; Pickart, 2000). The activities of the ubiquitinating enzymes E1, E2 and E3 do not show any consistent change with age, while accumulation of high molecular weight ubiquitin–protein conjugates is generally associated with ageing and certain age-related diseases (Ciechanover et al., 2000). Therefore, the observed accumulation of ubiquitin–protein conjugates most likely reflects a defect in the proteasomal system (Carrard et al., 2002).

Proteasome activity has been reported by different groups to decline with age in a variety of tissues and in cultured cells (Anselmi et al., 1998; Conconi et al., 1996; Keller et al., 2000a,b; Ponnappan et al., 1999; Shibatani et al., 1996), while other studies have shown that impairment of proteasome function may not be universal (Agarwal and Sohal, 1994; Keller et al., 2000b). For example, decreased proteasome CT-like activity has been reported in the heart, lung, kidney and liver when 12-, 24- and 28-month-old rats were compared with 3-week- or 3-month-old animals (Keller et al., 2000a). The spinal cord, hippocampus and cerebral cortex exhibited age-dependent decrease in proteasome activity while no change was observed in brain stem or cerebellum. In addition, impairment of proteasome function has been recently documented in Parkinson's and Alzheimer's diseases (Jenner, 2001; Keller et al., 2000b). This finding is particularly interesting in light of the numerous studies showing that proteasome inhibition is sufficient to induce neuronal cell death by triggering such events as caspase activation, cytochrome *c* release, elevated p53 expression, chromatin fragmentation and DNA laddering (Ding and Keller, 2001).

Age-related variations of gene expression patterns have been reported for both mitotic cells (human fibroblasts) and post-mitotic cells (rat skeletal myocytes), using the microarray technology (Lee et al., 1999; Ly et al., 2000). Less than 2% of the 6347 genes monitored were affected with age under either condition. In both situations transcription of several genes encoding the 20S or the 26S proteasome subunits were found to decline with age.

2.2.2. Lysosome-mediated degradation

Although the proteasome plays a central role in degradation of damaged and short-lived intracellular proteins, the lysosomal system is crucial for autophagic protein degradation, such as macroautophagy, microautophagy, and chaperone-mediated autophagy. Experimental evidence indicates that certain pathways of lysosomal protein degradation exhibit age-dependent decline in function. These losses in activity are likely a reflection of numerous factors. Macroautophagy and chaperone-

mediated autophagy are two cellular pathways by which proteins are targeted for degradation by lysosomes. Depressed rates of macroautophagy occur during ageing with decreases in autophagosome formation and delays in autophagosome fusion with lysosomes. In addition, age-dependent downregulation of a lysosomal receptor (LGP96) has been observed resulting in a decline of the chaperone-mediated autophagy rates. Furthermore, alterations in the activities of certain lysosomal hydrolases, including the cathepsins, have been reported to occur during ageing (Szweda et al., 2002).

2.3. Calcium homeostasis

Calcium ions participate in a large number of cellular processes. Resting cells have a cytoplasmic Ca^{2+} concentration of about 100 nM, which may rise up to 1000 nM upon stimulation. Intra-cellular calcium homeostasis is regulated by a variety of channels and ion pumps, which function to either transport calcium into the cytoplasm or to remove it (Fig. 1). The first category includes molecules of the plasma membrane, such as voltage-, receptor- and store-operated channels, that respond to different stimuli. A different set of channels are found on the ER membrane. Among the latter are the inositol triphosphate receptor channels (InsP3Rs) and the ryanodine receptor channels (RYRs), as well as channels opened by NAADP and S1P. Moreover, a mitochondrial uniporter also shows conductivity to calcium ions. The removal of calcium from the cytoplasm is carried out by several pumps and exchangers: the Ca^{2+} -ATPase pumps and $\text{Na}^+/\text{Ca}^{2+}$ exchangers at the plasma membrane and the sarco-ER ATPase (SERCA) at the endoplasmic reticulum, and the $\text{Na}^+/\text{Ca}^{2+}$ exchangers and permeability transition pores in mitochondria (Berridge et al., 2000).

When calcium levels exceed their normal spatial and temporal boundaries, they may induce apoptosis and/or necrosis. Calcium ions contribute to the disruption of mitochondrial membrane, which is followed by cytochrome *c* release and down-regulation of the apoptotic inhibitor Bcl2. Excessive calcium influx induces cell swelling and reduction of plasma membrane integrity, resulting in necrotic cell death (Maccioni et al., 2001; Syntichaki and Tavernarakis, 2003). The importance of calcium homeostasis in neurodegenerative cell death has been demonstrated by studies with *C. elegans*. Experiments have revealed at least four proteins of the endoplasmic reticulum that regulate intracellular calcium levels and are required for necrotic cell death. These are calreticulin and calnexin, which are calcium-binding chaperones, InsP3R and RyR. In addition, gain-of-function mutations in DEG-3, an acetylcholine receptor subunit, which forms a calcium channel together with DES-2, induce necrosis in nematodes (Treinin et al., 1998; Yassin et al., 2001). The mechanisms by which calcium induces neurodegeneration are under investigation. A major pathway involves the activation of the calcium-dependent proteases calpains (Syntichaki et al., 2002).

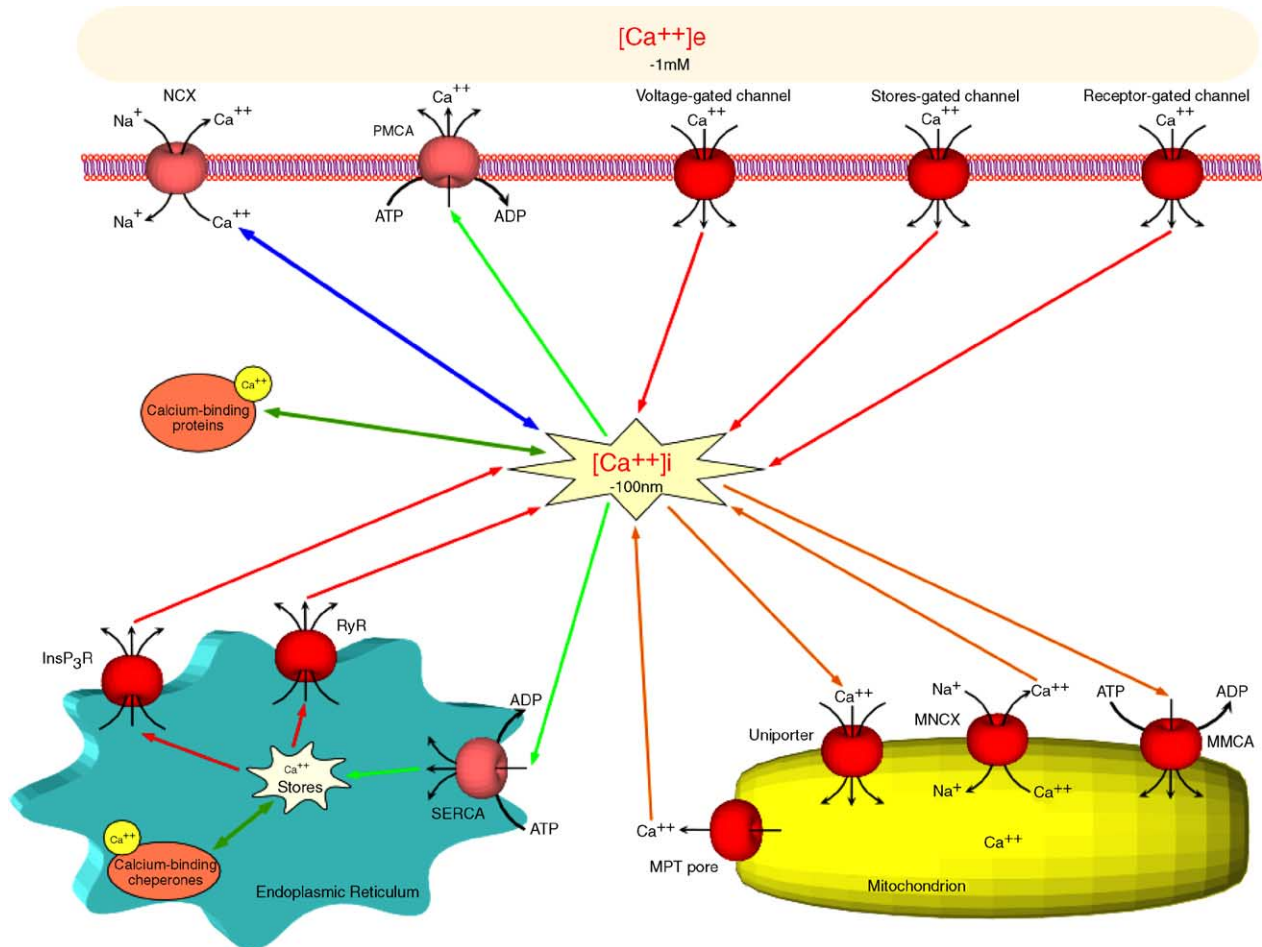


Fig. 1. Calcium homeostasis. Intracellular calcium concentration ($[Ca^{2+}]_i$) is tightly regulated within narrow limits. Under pathological conditions however, regulatory mechanisms are overwhelmed and intracellular calcium concentration increases via two main routes (red arrows). First, by calcium influx from extracellular pools ($[Ca^{2+}]_e$) through various channels (voltage, receptor, or concentration-gated channels) and, under extreme circumstances, through the sodium/calcium exchanger (NCX). Under normal conditions NCX is the major pathway for calcium efflux, but it can also contribute to Ca^{2+} influx (reverse mode exchange) especially during strong depolarization, and with increased intracellular sodium (blue arrow; [Kristian and Siesjo, 1998](#)). Second, by release from endoplasmic reticulum stores, through the ryanodine (RyR), and 1,4,5-inositol triphosphate receptors (IP₃R). Counterbalancing mechanisms fight to halt calcium concentration increase in the cytoplasm (green arrows). The plasma membrane calcium pump (PMCA), and NCX, together with SERCA function to restore normal calcium levels. Increased intracellular calcium concentration drives calcium overload at mitochondria (brown arrows), through the mitochondrial membrane calcium pump (PMCA), and relaxed specificity channels (uniporter). In turn, calcium overload triggers secondary release of calcium from mitochondrial stores, through the mitochondrial NCX (MNCX) and mitochondrial pores opened during mitochondrial permeability transition (MPT; [Halestrap et al., 1998](#); [Lemasters et al., 1998](#); [Nicholls et al., 1999](#); [Zhu et al., 2000](#)). Calcium-binding proteins in the cytoplasm and in the endoplasmic reticulum offer additional calcium buffering capacity (dark green arrows).

In mammals, excess release of the excitatory neurotransmitter glutamate inflicts excitotoxic death, which resembles necrosis. Glutamate binds to and opens specific kainate, AMPA and NMDA, ionotropic receptor channels on postsynaptic neurons. Gating of these channels provokes a cataclysmic influx of calcium ions inside the cell, either directly (through NMDA receptors that conduct both calcium and sodium) or indirectly, via the secondary activation of voltage-gated calcium channels ([Fern and Moller, 2000](#)). The sharp net increase of intracellular calcium concentration is a major pleiotropic death-signaling event which interfaces with both necrotic and apoptotic mechanisms ([Fig. 2](#)). The contribution of each type of death to excitotoxicity correlates with the severity and the

abruptness of the intracellular calcium concentration rise ([Sattler and Tymianski, 2000](#)). More dramatic changes precipitate necrosis, while relatively mild increases preferentially induce apoptosis ([Martin et al., 1998](#); [Mattson, 2000](#)). In stroke, the area immediately affected by blood shortage, called the infarct, is usually the focal point of necrosis ([Lee et al., 1999](#)). However, both necrotic and apoptotic types of cell death are evident within the surrounding tissue, called the ischemic penumbra, which suffers less oxygen and nutrient deprivation ([Lipton, 1999](#)). Excitotoxicity also incurs the production of reactive oxygen species. Similar phenomena transpire in Alzheimer's disease, where both necrosis and apoptosis appear to contribute to neuronal loss. Indeed, A β deposits in senile

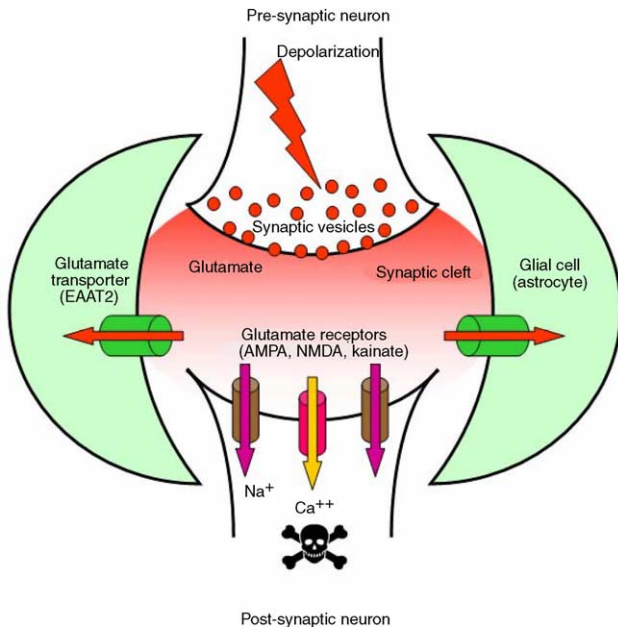


Fig. 2. Excitotoxic cell death. Many deleterious conditions can converge to induce unrestrained glutamate release at synapses, initiating a cascade of events that eventuates in the death of post synaptic cells (Choi, 1992). For example catastrophic depolarization occurs during hypoxia or hypoglycemia, which compromise energy production and thus, the ability of the cell to maintain membrane potential (Grabb et al., 2002). Conditions of hypoxia or hypoglycemia develop in stroke due to ischemia caused by occlusion of blood vessels, blocking circulation in areas of the brain. Also, over stimulation of neurons during seizure is known to have the same effect on glutamate release (Holmes, 2002).

plaques facilitate free radical production by the Fenton reaction, or through interactions with type 2-scavenger receptors and receptors for advanced glycation end products of the plasma membrane. Free radicals oxidize plasma membrane lipids, thus possibly leading to massive calcium entry. $A\beta$ has been implicated in massive calcium entry due to over-stimulation of NMDA receptors. Indeed, $A\beta$ contributes to a decline in astroglial clearance capacity of glutamate that is released from presynaptic terminals, possibly via peroxidation of critical groups of the transporter or other proteins (Hynd et al., 2004; Molnar et al., 2004).

Disturbance of calcium homeostasis has been directly related to the ageing process. Studies of gene expression in the hypothalamus and cortex of young and adult mice, using high-density oligonucleotide arrays have revealed that expression of Na^+/K^+ -, Ca^{2+} - and H^+ -ATPases, which are all implicated in regulation of intracellular calcium concentration, was reduced in both hypothalamus and cortex, (Jiang et al., 2001). The Ca^{2+} -ATPase, is also affected by reactive oxygen species produced during ageing.

2.4. Oxidative stress

One of the clear themes that has emerged from several lines of ageing research is that oxidative stress is a major factor in ageing and in cellular senescence (Carrard et al.,

2002; Finkel and Holbrook, 2000). Mitochondrial and cytoplasmic production of hydrogen peroxide, superoxide free radicals and hydroxyl free radicals can cause substantial modifications of DNA, lipids and protein (Stadtman, 1992). The notion that these alterations result in a cumulative macromolecular damage, which contributes to senescent decline is the foundation of the free radical theory of ageing originally outlined by Harman in 1957 (Harman, 1988). Key findings in support of this hypothesis include that generation of reactive oxygen species (ROS) is highly correlated with longevity in many species (Ku et al., 1993), that transgenic lines with increased expression of anti-oxidant proteins such as glutathione reductase, Cu^{2+} - Zn^{2+} SOD and catalase can have extended lifespan (Mockett et al., 1999; Orr and Sohal, 1994; Parkes et al., 1998; Sun and Tower, 1999), and that an SOD/catalase mimetic compound can extend lifespan in the nematode *C. elegans* (Melov et al., 2000). Further support is provided from studies of the nematode *C. elegans* dauer larvae and *age-1* mutant strains, both of which show increased lifespan and have increased levels of SOD that could protect from macromolecular damage, thus contributing to lifespan extension (Larsen, 1993; Vanfleteren and De Vreese, 1995).

Oxidative stress also plays an important role in neurodegenerative disorders: the concept that oxidative stress occurs in Parkinson's disease derives from the fact that the metabolism of dopamine can generate free radicals and other ROS. ROS can be generated as a consequence of auto-oxidation of dopamine. Oxidative stress may be initiated by a decline in the anti-oxidative defense system or a decrease of antioxidant concentration caused by other factors. Glutathione (GSH) is an important intracellular antioxidant; the most robust and significant alteration in antioxidant defense in Parkinson's disease is a decrease in GSH concentration. Another consistent finding in Parkinson's disease patients is a defect in oxidative phosphorylation due to a decrease in the electron transport chain complex I activity in the substantia nigra. It remains controversial whether a decrease in GSH concentrations precedes the defect of oxidative phosphorylation or vice versa (Schulz et al., 2000). 4-Hydroxy-2-nonenal (HNE) is a marker of lipid peroxidation that may react with proteins to form stable adducts; it has been found that the 58% of the nigral neurons of Parkinson's disease patients, and only the 9% of the nigral neurons in a control group, contains HNE-modified proteins (Jenner, 2003; Kruman et al., 1997). Evaluation of DNA modifications in peripheral blood leukocytes of untreated Parkinson's disease patients revealed extensive oxidative chromosomal damage at the peripheral level (Migliore et al., 2002).

There is mounting evidence that oxidative stress is also involved in the pathogenesis of Alzheimer's disease. Even though the total brain levels of GSH appeared to be unaffected in Alzheimer's disease, the levels of glutathione transferase, a protective enzyme against HNE are decreased in the brain and ventricular CSF of autopsied Alzheimer's

disease subjects, and HNE, is elevated in Alzheimer's disease brain and CSF (Lovell et al., 1998). A significant increase of 8-hydroxyguanosine, and an oxidized amino acid (nitrotyrosine) has been detected in neurons of patients with Alzheimer's disease, moreover the increased oxidative damage is an early event in Alzheimer's disease that decreases with the progression of the disease (Nunomura et al., 2001). Patients with probable Alzheimer's disease show increased metabolism of arachidonic acid-derived products, a sign of substantial oxidative stress (Tuppo et al., 2001). It is now clear that oxidative stress may play an important role in the pathogenesis of ALS: increased levels of markers for oxidative damage to proteins and DNA have been measured in the motor cortex of sporadic ALS patients; HNE levels were increased in CSF of ALS patients, and increased modification of proteins by HNE was found in the lumbar spinal cord of ALS patients. However, the role of altered glutathione metabolism founded in ALS is ambiguous. (Migliore and Coppede, 2002).

Ubiquitin–protein conjugates accumulate with age in different tissues. This accumulation is also observed in certain pathological situations such as Parkinson's and Alzheimer's diseases. However, it seems that only few specific proteins are accumulating as ubiquitinated forms in these pathologies. On the contrary, oxidation affects a wider range of proteins although some proteins as aconitase and adenine nucleotide translocase have been shown to be more sensitive to oxidation and to preferentially accumulate in ageing flies (Carrard et al., 2002). Oxidative stress is also a potent inducer of apoptosis in a wide variety of cell types. For example, Jurkat T lymphocytes undergo apoptosis when treated with moderate concentrations of H₂O₂. Oxidative stress also enhances or accompanies apoptosis triggered by non-oxidative stimuli. For example, severe oxidative stress occurs in apoptosis induced by tumor necrosis factor (TNF) and the Fas-ligand. Moreover, apoptosis induced by TNF and Fas-ligand can be inhibited by antioxidants such as *N*-acetylcysteine (Zhang and Herman, 2002). Ageing is also associated with an increase in oxidative stress via accumulation of reactive oxygen species (ROS) and H₂O₂ in cells and tissues. Such age-related accumulation of oxidants is likely to accelerate the progression of neurodegeneration.

2.5. Mitochondrial function

A significant decline in respiratory chain activity/efficiency is an integral part of the ageing process in many human tissues. For example, a major decrease in respiratory chain complex I and IV activity is associated with old age (Byrne, 2002). Mitochondrial respiratory chain defects have also been reported in most of the major neurodegenerative diseases including Parkinson's disease, Alzheimer's dementia, amyotrophic lateral sclerosis and Huntington's disease. Among the abnormalities reported is a pronounced decline in respiratory complex I and IV activity, in both cerebral and

non-cerebral tissues. In addition, a number of mitochondrial DNA mutations have been identified in aged tissues, including, most significantly, the 4977 base pair deletion initially identified in CREO syndromes and referred to as the common deletion with a striking age-dependent increase in the striatum and substantia nigra (Dujon et al., 1976). Generally, respiratory chain function at a given age depends partly on the efficiency of the inherited genome and partly on the extent of accumulation of acquired mitochondrial pathology. A low level of mtDNA mutation accumulation is an integral part of the ageing process. These mutations are unevenly distributed, at least in the case of large deletions resulting in a patchy bioenergetic inefficiency associated with the ageing process. Qualitatively, this resembles the situation with mtDNA-related mitochondria cytopathies. Such changes are likely to cause cell loss or neuronal dysfunction and reduce the safety margin that must be breached by other pathoetiological processes, thus allowing symptoms to develop earlier or in a more severe form (Byrne, 2002).

Mitochondria have a central role in the apoptotic process (Hengartner, 2000). Disruption of the mitochondrial transmembrane potential results in the release of various pro-apoptotic factors into the cytosol, most notably cytochrome *c*, as well as the significant reduction of energy in the cell. Apoptosis is clearly an important mechanism of neuronal loss in age-associated neurodegenerative disease, and a search for anti-apoptotic factors is currently important in experimental neuropharmacology (Byrne, 2002). Much work is centered on the mechanisms whereby cytochrome *c* and pro-apoptotic factors are released from mitochondria. A crucial aspect involves the permeability transition pore that mediates the permeabilization of the mitochondrial inner membrane to molecules up to 1.5 kDa, following calcium overload (Syntichaki and Tavernarakis, 2003). This pore may contribute to delayed cell death of a necrotic type through calcium efflux from mitochondria into the cytosol, and to apoptosis through release of cytochrome *c* and other pro-apoptotic factors (Byrne, 2002).

3. Genetic dissection of ageing in *C. elegans*

C. elegans is a particularly powerful organism for experimental investigation of the ageing process. This small (approximately 1 mm) free-living hermaphroditic nematode feeds on an *E. coli* diet in the laboratory, completes a reproductive life cycle in 2.5 days at 25 °C, progressing from a fertilized embryo through four larval stages to become an egg-laying adult, and lives for about 2 weeks (Larsen et al., 1995). Since *C. elegans* can reproduce by self-fertilization it is possible to raise genetically identical populations that do not undergo inbreeding depression. Nematodes exhibit visible changes in behavior and appearance over the course of their lives. As they age, nematodes feed (as can be measured by pharyngeal pumping), move and defecate more

slowly than their younger counterparts (Bolanowski et al., 1981; Duhon and Johnson, 1995; Klass, 1977). Ageing worms appear rough and lumpy, with a generally distorted morphology. Death is usually assayed by a failure to respond to touch with an eyelash hair, failure to move, and/or failure to pump in food. Similarly, old animals are more sensitive to thermal stress (Lithgow et al., 1995). Analysis of ageing tissues is simplified in that all somatic cells are post-mitotic and there is no tissue regeneration.

Numerous *C. elegans* genes, when mutated, can extend lifespan. Some of these genes regulate a key developmental switch, which directs young worms that find themselves in poor environments to adopt the long-lived stress-resistant form (the dauer larva). Under adverse conditions such as starvation, over-crowding or high temperature, larvae can enter an alternative life stage called the dauer (enduring) larva, during which animals move but do not feed. The dauer larva is a 'non-ageing' organism that survives for weeks or even months (Klass and Hirsh, 1976). When a dauer larva encounters favorable environmental conditions, it re-enters the life cycle at the fourth larval stage, progresses into adulthood to reproduce and then completes the final week or so of its lifespan.

Other genes control core processes, such as the overall rate of metabolism (Kirkwood and Finch, 2002). The best studied are the *agedaf* genes that function in an insulin-like signaling pathway required for dauer formation (Friedman and Johnson, 1988a,b; Kenyon et al., 1993), the 'clock' (*clk*) mutants in which development and rhythmic behaviors of the nematode are slowed (Hekimi et al., 1998; Lakowski and Hekimi, 1998), mutants with defects in sensory perception (Apfeld and Kenyon, 1999), and the eat mutants defective in pharyngeal pumping thought to experience dietary restriction effects (Guarente and Kenyon, 2000; Hekimi et al., 1998). Dietary restriction administered by *E. coli* rationing or by axenic medium, can also markedly extend *C. elegans* lifespan (Vanfleteren and Braeckman, 1999). Interestingly, almost all tested long-lived mutations of *C. elegans* appear to confer resistance to environmental stress, including oxidative stress, high temperature, and exposure to ultraviolet radiation (Van Voorhies and Ward, 2000; Vanfleteren and Braeckman, 1999). Another feature that seems common to some tested long-lived mutants to date is that metabolic rates of such *C. elegans* mutants are reduced compared with that of wild-type nematodes (Van Voorhies and Ward, 1999).

4. Cell death in *C. elegans*

C. elegans is also a well-suited organism for the study of normal and aberrant cell death at the cellular, genetic and molecular levels. The worm is essentially transparent throughout its life cycle and individual nuclei can be readily visualized using differential interference contrast optics. Elucidation of the lineage map has revealed that in

certain lineages, particular divisions generate cells which die at specific times and locations and that the identities of these ill-fated cells is invariant from one animal to another (Sulston and Horvitz, 1977; Sulston et al., 1983). The ability to easily recognize dying cells within a living animal has allowed easy identification of mutants with aberrant patterns of both apoptotic and necrotic cell death.

C. elegans development is characterized by the programmed death of 131 cells (Sulston and Horvitz, 1977; Sulston et al., 1983). Several genes participate in the process (Hengartner, 2000). The *ced-3* encodes a cysteine protease (caspase) that is essential for death execution. The *ced-4* gene product activates CED-3 activity and is also required for all programmed cell deaths. In cells fated to live, the death program is held in check by negative regulator CED-9, which can be antagonized by EGL-1. Cell corpses are removed by a group of genes that act in two parallel pathways (one includes *ced-1*, *ced-6*, and *ced-7*; another includes *ced-2*, *ced-5* and *ced-10*, and *ced-12*). These 'undertaker' genes are required for phagocytosis and degradation of dead cells. Analysis of *C. elegans* programmed cell death has had an important influence in advancing our understanding of mammalian apoptotic death mechanisms since regulator, executor and undertaker genes are functionally conserved from nematodes to humans (Hengartner, 2000; Leist and Jaattela, 2001a,b).

Similar to apoptosis, genetic studies of neurodegeneration in *C. elegans* have greatly facilitated the elucidation of the mechanisms involved (Syntichaki and Tavernarakis, 2003). Gain-of-function mutations in several *C. elegans* ion channel genes induce necrotic-like deaths of the neurons that express these channel genes. For example, dominant mutations in *deg-1* (degenerin; *deg-1* (d)) induce death of a group of interneurons of the nematode posterior touch sensory circuit (Chalfie and Wolinsky, 1990). Similarly, dominant mutations in the *mec-4* gene (mechanosensory; *mec-4* (d)) induce degeneration of six touch receptor neurons required for the sensation of gentle touch to the body (Driscoll and Chalfie, 1991). The *deg-1* and *mec-4* encode proteins that are very similar in sequence and are the founding members of the *C. elegans* degenerin family of ion channels, which can mutate to forms that induce cell degeneration of the cells in which they are expressed (Chalfie et al., 1993; Tavernarakis and Driscoll, 2001). *C. elegans* degenerins share sequence similarity with subunits of the vertebrate amiloride-sensitive epithelial Na⁺ channel (ENaC) that mediates Na⁺ absorption in epithelia of the distal part of the kidney tubule, the urinary bladder, the distal colon and the lung.

Other genetic alterations can also induce neurodegeneration in the nematode. Gain-of-function mutations in the *deg-3* gene, which encodes a protein related to the vertebrate nicotinic acetylcholine receptor, a channel highly permeable to Ca²⁺ cause vacuolar degeneration of *C. elegans* neurons (Treinin et al., 1998). In addition, expression of constitutively active, GTPase-defective, heterotrimeric G protein G

alpha_s (either from *C. elegans* or from rat) causes swelling and degeneration of many (but not all) cells in which the mutant gene is expressed (Berger et al., 1998; Korswagen et al., 1997). Ectopic expression of the toxic human beta amyloid peptide 1–42, derived from the APP precursor protein and implicated in the pathogenesis of Alzheimer's disease, in *C. elegans* body wall muscles causes animals to become progressively paralyzed as they develop and induces necrotic-like death of some cells around the nerve ring (Link, 2001). Although these genes normally are involved in distinct processes, it remains possible that they share a common death-activating mechanism: alteration of channel activity. Consistent with this possibility, G proteins are known to modulate channel activity. Likewise, some studies have linked beta-amyloid toxicity with altered channel function.

Calpain activation is required for the execution of neurodegenerative cell death in *C. elegans*. Reduction of calpain function by using the specific inhibitor MDL-28170, suppressed necrosis. RNA interference (RNAi) experiments and observations with double mutant strains showed that *clp-1* and *tra-3*, which encode two different calpain proteases of the nematode, are mainly involved in cell death process (Sokol and Kuwabara, 2000; Syntichaki et al., 2002).

A requirement of aspartyl proteases in both nematode and primate neurodegeneration has also been demonstrated (Syntichaki and Tavernarakis, 2003; Yamashima, 2000). Reduction of aspartyl protease activity by specific mutations, chemicals or starvation caused reduction of necrosis in *C. elegans* motor neurons (Syntichaki et al., 2002). At least six aspartyl proteases (ASP-1 to ASP-6) are encoded in the *C. elegans* genome (Tcherepanova et al., 2000). Complementation assays and RNAi experiments revealed that necrotic cell death is mediated by the enzymes ASP-3 and ASP-4, which can induce necrosis when over expressed (Syntichaki et al., 2002).

5. Concluding remarks

The key questions with regard to all age-related changes is whether they have a role in predisposing to age-related neurodegenerative diseases or modulating their severity and progression. Conversely, loss of proper regulation of cell death mechanisms may have a great impact on life span and normal ageing. Cancer incidence and the onset of many neurodegenerative disorders are closely associated with ageing. However, the enigma of how old age contributes to the manifestation of these pathologies remains largely unsolved. Studies in simple invertebrate model organisms should help address this question. For example, *Drosophila* homologs have been identified for the human genes encoding the β -amyloid precursor protein (APP) and the Cu²⁺/Zn²⁺ superoxide dismutase, which have been implicated in Alzheimer's disease and in a familial form of ALS, respectively. Mutations in the respective fly genes were shown to confer striking neuropathology in *Drosophila*

(Mutsuddi and Nambu, 1998). Similar neuropathology is observed by the expression of either wild-type or mutant forms of human α -synuclein in *Drosophila*. This replicates all of the key features of the Parkinson's disease pathology: adult-onset loss of dopaminergic neurons, the formation of filamentous intraneuronal inclusions containing α -synuclein and dysfunction in locomotion (Feany and Bender, 2000). Recent studies have also demonstrated that flies expressing wild-type or mutant human tau undergo brain neuron degeneration that reproduces a number of features of the human disorder, including adult onset, progressive necrosis, accumulation of abnormal tau and relative anatomic selectivity. Overproduction of the molecular chaperone Hsp70, which was found to colocalize with polyglutamine protein aggregates, suppresses polyglutamine toxicity and cell death (Bonini, 2001). Elevated Hsp70 expression also ameliorates α -synuclein toxicity in *Drosophila* dopaminergic neurons (Auluck et al., 2002). Interestingly, several studies implicate the heat-shock response system in *Drosophila* longevity (Kurapati et al., 2000; Landis et al., 2004; Morrow and Tanguay, 2003; Tower, 2000).

Deleterious modifications to proteins, nucleic acids and lipids accumulate as a consequence of normal metabolism and the action of free oxygen radicals, which in turn disrupt cellular and tissue function. The process of protein turnover (protein synthesis and degradation) is responsible for maintaining a functional protein content in every cell, as it constantly replaces damaged/non-functional proteins with new, functional ones. Moreover, regulated protein turnover has been correlated with, and could be instrumental in, the beneficial effects of caloric restriction. Changes in the efficiency of protein turnover, and also in the rate of protein modification, are hypothesized to result in the accumulation of damaged proteins that contribute to progressive decline of cellular function. However, a direct molecular link between ageing and regulation of protein turnover has not been established. Given newly developed experimental approaches and well-defined genetic systems in which ageing has been studied, work in model organisms such as nematodes and flies should now enable this link to be addressed in new experimental detail. One of the major powers of *C. elegans* as a model organism to study the interplay of ageing and neurodegeneration is the ability to perform unbiased genetic screenings for mutants showing specific phenotypes. This approach, coupled with the exceptionally detailed characterization of the nematode development and anatomy has already provided significant new insights into issues of both ageing and neurodegeneration and holds considerable potential for major advance in the future.

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