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Emerging Concepts Linking Mitochondrial Stress Signalling and Parkinson's Disease

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1. Introduction

Neurodegenerative diseases comprise of a large group of pathologies that affect a considerable number of individuals among the world's population with a higher incidence in elderly people. The most common neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), are characterised by the presence of protein aggregates in different regions of the brain. The causes of these diseases remain undetermined. Nevertheless, new information on this topic is continuously being brought to light.

Mitochondrial dysfunction has been implicated in the pathogenesis of PD. The first evidence for this came from studies using the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and the complex I inhibitor rotenone, which cause parkinsonism in animal models, making them a useful system for investigating the mechanisms underlying PD. The subsequent discovery of several gene mutations responsible for causing heritable forms of PD that are directly linked to mitochondria (either genes encoding proteins that are localised to mitochondria (PINK1) or have some role in mitochondrial function (parkin, DJ-1)) provided further insights into the importance of mitochondria in the pathogenesis of PD. More recently, some of the gene products of PARK loci have been found to participate in mitochondrial quality control systems. In this review, we describe, in detail the molecular mechanism of mitochondrial quality control as well as the importance of a mitochondrial unfolded protein response and its possible relevance to the symptoms of PD.

2. Mitochondria: Essential organelles of eukaryotic cells

Mitochondria are cellular organelles with a multitude of functions, ranging from the production of the majority of ATP in the cell, storage of calcium and buffering of its cytosolic concentration to participation in different pathways that influence cellular homeostasis and fate, including cell death cascades.

Mitochondria are two-membrane organelles with an outer membrane facing the cytosol and an inner membrane facing the mitochondrial matrix; the space between the membranes is referred to as the inter-membrane space (IMS). Both the outer and inner membranes are composed of phospholipids and proteins; however, they have different properties. The outer membrane is highly permeable to small molecules due to the presence of porins

within its structure, which allows the IMS to maintain approximately the same concentrations of ions and sugars as the cytosol. However, the inner membrane does not exhibit such permeability, thus the passage of molecules to the matrix is highly selective. Most IMS proteins have well-characterised roles in apoptosis following release from the mitochondria into the cytosol, such as cytochrome c, Smac/DIABLO, HtrA2/Omi, and endonuclease G (Endo G) (Radke *et al.*, 2008).

The inner mitochondrial membrane exhibits a particular organisation including several invaginations that form the mitochondrial cristae. This organisation makes the length of the membrane much greater and enables a higher efficiency of energy production. It is in the inner membrane of the mitochondria where the electron transport chain (ETC) is located. The ETC is comprised of five protein complexes and is responsible for the oxidative phosphorylation (OXPHOS) that results in the production of energy in the form of adenosine triphosphate (ATP), which is accompanied by consumption of oxygen and production of water. Complexes I, III and IV pump protons (H^+) into the IMS and create a gradient essential for the generation of ATP by complex V (ATP synthase). This proton gradient is crucial for the normal functioning of mitochondria and is known as the mitochondrial membrane potential ($\Delta\Psi m$). Reactive oxygen species (ROS) are also generated as a by-product of OXPHOS. ROS production can be partially modulated by two cofactors, coenzyme Q_{10} at complex III and cytochrome c at complex IV (Mattson *et al.*, 2008).

The mitochondrial matrix is a sub-compartment with a high protein content. It contains all of the enzymes required for the Krebs cycle, fatty acid oxidation and haeme synthesis and also incorporates several chaperones and proteases involved in the folding and degradation of proteins. In addition, the matrix contains mitochondrial DNA (mtDNA), together with the proteins required for its transcription and translation. Although most mitochondrial proteins (~1500) are encoded by nuclear DNA, thirteen proteins involved in the ETC are mitochondrially encoded (Ryan and Hoogenraad, 2007). This constitutes a challenge for the cell as both nuclear and mitochondrial transcription must be tightly coordinated. Due to its localisation near the ETC, mtDNA is susceptible to oxidative damage, which may result in mutations.

The number of mitochondria is variable according to the tissue and cell type, and it reflects the energetic needs of the cell. For example, neurons and muscle cells have a high mitochondrial content compared to other cell types. The number of mitochondria can be modulated, but these organelles cannot be generated de novo. Mitochondrial biogenesis consists of an increase in the translation of mitochondrial proteins (both nuclearly and mitochondrially encoded); recruitment of these proteins to the mitochondria, which results in mitochondrial enlargement; and finally, division and generation of additional mitochondrion units (Ryan and Hoogenraad, 2007). Within a cell, a mitochondrion cannot be considered as an individual and independent organelle. Mitochondria behave as a network exhibiting successive cycles of fusion (mitochondrion units combining with each other) and fission (separation of mitochondrion units). Mitochondrial dynamics make these organelles more capable of responding to different cellular demands. The proteins responsible for mitochondrial dynamics are relatively well characterised, although new proteins are constantly being identified that play roles in this active process.

Due to the importance of mitochondria for cellular homeostasis, it is obvious that the dysfunction of this organelle may result in severe consequences. Depending on the extent of damage, different signalling pathways will be activated. If the extent of damage is too severe, it might cause dissipation of the mitochondrial membrane potential and release of pro-apoptotic proteins (such as cytochrome c and HtrA2), resulting in cell death.

Mitochondrial dysfunction has long been associated with the natural chronic process of ageing as well as with neurodegenerative diseases, such as AD and PD, and metabolic diseases, such as diabetes. Neurons are particularly sensitive to mitochondrial dysfunction, which probably reflects their high energetic needs and their absolute dependence on mitochondria to obtain most of the ATP required for neuronal function (de Castro *et al.*, 2010). The presence of protein aggregates is also a characteristic of the neurodegenerative diseases PD and AD. The importance of proper protein folding has long been recognised, and the effects of un-/misfolding and aggregation have been linked to many pathological conditions. These aggregates may be cytosolic, such as Lewy bodies in PD and fibrillary tangles in AD, or extracellular, such as amyloid plaques in AD. However, little is known about the effects of unfolded proteins in the mitochondria.

Protein homeostasis is important to maintain cells in a healthy state; therefore, it is essential to prevent the accumulation of proteins in non-native forms. A recent study in the nematode *C. elegans* has shown that in this multicellular animal, a considerable number of proteins aggregate with age (David *et al.*, 2010). These aggregation-prone proteins may also be related to the deregulation of proteostasis in some neurodegenerative diseases. A better understanding of the mechanisms involved in protein misfolding and aggregation will certainly help to explain the causes and/or progression of certain diseases.

3. Concepts of mitochondrial quality control

As mentioned above, oxidative damage is likely to occur in mitochondria (the main ROS source), possibly affecting proteins, lipids and mtDNA. Multiple quality control systems exist to protect mitochondria and ultimately, the cell against damage (Figure 1). Primarily, as part of a molecular quality control system within the organelle, mitochondrial chaperones and proteases play roles in the processes of protein folding, the assembly of protein complexes and the degradation of misfolded or damaged proteins. Second, at the organellar level, there is another mechanism of quality control that depends on the dynamic nature of mitochondrial fusion and fission events (Tatsuta and Langer, 2008). When fusing with healthy mitochondria, damaged mitochondria can recover by sharing essential solutes, metabolites and other components from the healthy partner. However, if the damage is too severe, mitochondria will not undergo fusion but instead will lose their mitochondrial membrane potential and be eliminated by autophagy, a process also known as mitophagy. Finally, under sufficiently severe damage, cellular quality control mechanisms can be activated. In this case, upon the opening of the mitochondrial permeability transition pore (mPTP) and release of pro-apoptotic proteins from the IMS, the cell may undergo apoptosis.

The focus of this review will be on the molecular quality control of mitochondria because both the organellar and cellular aspects of mitochondrial quality control have been extensively reviewed (de Castro *et al.*, 2010, Knott and Bossy-Wetzel, 2008, Tatton *et al.*, 2003).

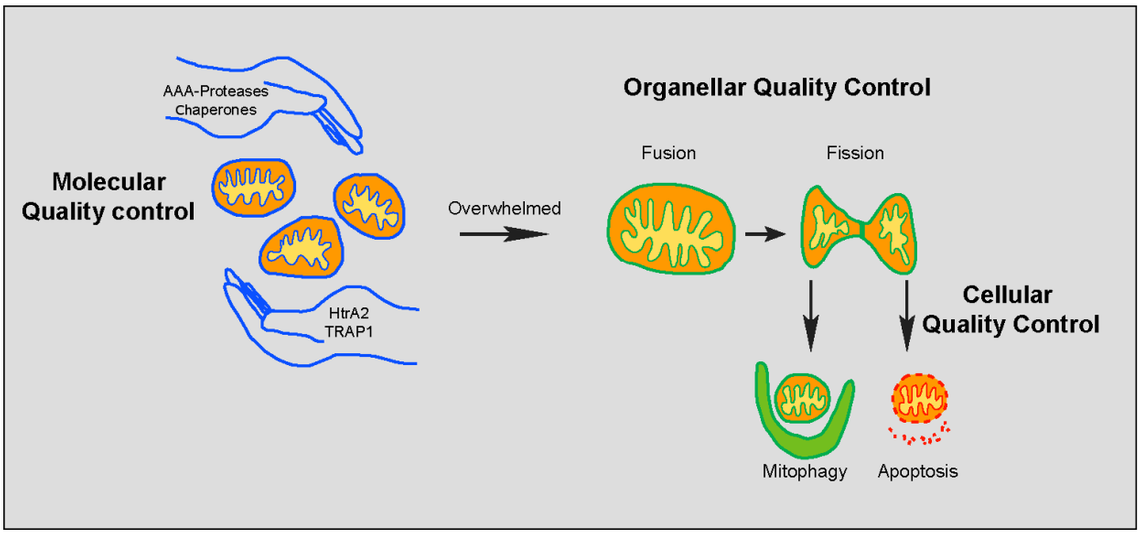

Fig. 1. Mitochondrial Quality Control is assured by different mechanisms and effectors; at the molecular level, it is assured through the protective action of chaperones and proteases, such as molecular chaperones in the mitochondrial matrix, AAA-proteases in the inner membrane, and HtrA2 and TRAP1 in the IMS (drawing highlighted in blue). When this mechanism of defence is not sufficient to maintain fully functional mitochondria, a second level of quality control exists at the organellar level. Fusion with healthy mitochondria might be sufficient to restore the functionality of a mitochondrion. Otherwise, fission might occur, producing small mitochondria that will not fuse again but will be degraded by autophagy (mitophagy) (highlighted in green). If the damage to the mitochondria is too severe, it might lead to the opening of the mitochondrial permeability transition pore (mPTP), releasing pro-apoptotic proteins into the cytosol, such as cytochrome c and HtrA2, resulting in the activation of apoptosis (drawing highlighted in red).

3.1 Effectors of molecular quality control: Chaperones and proteases

Molecular chaperones play critical roles in the maintenance of protein homeostasis by aiding in the folding of newly synthesised and/or imported proteins, the assembly of multimeric protein complexes, protein translocation across membranes and protein degradation. In addition, molecular chaperones are essential for cellular signalling. In eukaryotes, chaperones are segregated to specific places, such as the cytosol, endoplasmic reticulum (ER) and mitochondria. The mechanisms through which the levels of chaperones are modulated have not been well studied. Nevertheless, it is known that signalling pathways activating chaperone-encoding genes are repressed by free chaperones that are not engaged by client proteins (Yoneda *et al.*, 2004). In contrast, if the levels of client proteins are too high, the availability of free chaperones can be dramatically decreased, and the repressor effect will not occur, possibly resulting in increased transcription of chaperones.

The mitochondrial chaperones and proteases are essential components of the mitochondrial quality control mechanism because they selectively remove unfolded or misfolded proteins, avoiding their deleterious accumulation. A failure in molecular quality control is emerging as a feature of neurodegenerative diseases, such as PD.

3.1.1 AAA protease chaperones

ATP-dependent proteases are present in the mitochondrial matrix as well as in the inner membrane. The AAA+ (ATPases associated with various cellular activities) superfamily of peptidases is able to eliminate misfolded proteins from the mitochondria and plays a regulatory role during mitochondrial biogenesis by processing proteins. Mammalian inner mitochondrial membranes possess hetero- and homo-oligomeric *m*-AAA and homo-oligomeric *i*-AAA protease complexes. The *m*-AAA protease presents its catalytic site on the mitochondrial matrix, while the *i*-AAA protease catalytic site is directed to the IMS. The homo-oligomeric complexes are composed of AFG3L2 subunits, while the hetero-oligomeric are composed of paraplegin and AFG3L2 subunits. Mutations in these proteases have been implicated in neurodegenerative conditions; mutations in the paraplegin subunit cause hereditary spastic paraplegia which is characterised by cell-specific axonal degeneration, and mutations in *AFG3L2* lead to a dominant heritable form of spinocerebellar ataxia (Lee *et al.*, 2011). Furthermore, a mutation in the mitochondrial chaperone Hsp60 has also been identified in hereditary spastic paraplegia. The mitochondrial matrix chaperone Hsp60 mediates ATP-dependent folding of a wide variety of proteins, making it an important player in the molecular mitochondrial quality control system.

3.1.2 PINK1, HtrA2 and TRAP1

The mitochondrial protein PTEN-induced putative kinase 1 (PINK1) plays important roles in mitochondrial quality control. It participates in molecular quality control through interactions with high-temperature-requirement protein A2 (HtrA2) and possibly TNF receptor–associated protein 1 (TRAP1) and at the organellar level via its activity in a signalling pathway involving parkin and mitophagy. The *PINK1* and *PARK13* (HtrA2) genes have been found to be associated with PD. Mutations in *PINK1* cause an autosomal recessive form of the disease, which can be characterised by early onset (Valente *et al.*, 2004). Additionally, mutations in the HtrA2 gene have been found in sporadic PD patients, and HtrA2 protein has been detected in Lewy bodies (Strauss *et al.*, 2005). Furthermore, an increase in phosphorylated HtrA2 in brain tissues of sporadic PD patients and, conversely, reduced phosphorylation in PD patients with *PINK1* mutations have also been reported (Plun-Favreau *et al.*, 2007).

3.1.2.1 HtrA2

HtrA2 (also known as Omi) was first identified as a mammalian homologue of the bacterial endoprotease HtrA (high temperature requirement A). In bacteria, HtrA acts as a chaperone at normal temperatures and as a protease at high temperatures and removes damaged or denatured proteins from the periplasm (Faccio *et al.*, 2000). HtrA2 is a serine protease with an amino-terminal inhibitor of apoptosis (IAP) domain identical to those in the *Drosophila* protein Reaper. It is localised in the IMS, and following certain stimuli, it is released into the cytosol, where it binds to IAPs, inhibiting their effects and promoting apoptosis. In addition to its caspase-dependent action, HtrA2 promotes cell death in a caspase-independent manner through increased proteolytic activity (Martins *et al.*, 2003). HtrA2 was initially thought to be a pro-apoptotic protein homologous to Reaper. However, the observation that HtrA2-mutant mice present an extremely shortened lifespan (~30 days) and a neurological phenotype with parkinsonian features has promoted a different view of the role of this protein (Martins *et al.*, 2004).

The phosphorylation state of HtrA2 influences its proteolytic activity and is dependent on PINK1 (Plun-Favreau *et al.*, 2007). PINK1 and HtrA2 interact with each other, although it is not clear whether PINK1 phosphorylates HtrA2. Nevertheless, phosphorylation of HtrA2 occurs upon p38 MAPK activation and requires PINK1. It appears that p38, PINK1 and HtrA2 are components of a stress-sensing pathway and that phosphorylation of HtrA2 increases its proteolytic activity and enhances its cell-protective effects. The observation that higher levels of phosphorylated HtrA2 are present in the brains of sporadic PD patients has led to the hypothesis that this stress-sensing pathway is activated in PD. However, the precise stimulus that originates the stress is not known. Interestingly, in brains from PD patients with *PINK1* mutations, the levels of phosphorylated HtrA2 are reduced, emphasising the importance of PINK1 for HtrA2 phosphorylation. This reduced HtrA2 phosphorylation might account for the pathogenic effects of PD-linked *PINK1* mutations. Thus, HtrA2 appears to be part of a quality control pathway, acting downstream of PINK1.

HtrA2 knockout (KO) mice show accumulation of unfolded proteins in the mitochondria as well as a defect in respiration. Loss of HtrA2 augments ROS levels, and treatment with anti-oxidants is neuroprotective in this condition. The up-regulation of the transcription factor C/EBP homology protein (CHOP) specifically in the brain of the HtrA2 KO mice indicates activation of the integrated stress response. Enhanced levels of CHOP have also been detected in brain tissue of idiopathic PD patients (Moisoi *et al.*, 2009). This increased level of CHOP specifically in the brain may account for the neuronal loss observed in this mouse model. However, the observation that CHOP is also up-regulated in regions of the brain that do not undergo neuronal death indicates that CHOP up-regulation is an early event that precedes neuronal loss.

HtrA2 also interacts with both intracellular amyloid β (Aβ) and the C-terminal tail of presenilins, although the functional significance of these interactions is obscure (Gupta *et al.*, 2004, Park *et al.*, 2004). More recently, HtrA2 has been linked to amyloid precursor protein (APP) metabolism (Huttunen *et al.*, 2007). A small percentage of HtrA2 localises alongside ER membranes and binds to immature APP *in vitro*. Finally, in HtrA2$^{-/-}$ cells, APP is stabilised, and Aβ production is increased. These observations further suggest that HtrA2 has a protective effect and functions in protein homeostasis.

3.1.2.2 TRAP1

Another piece of evidence relating PINK1 to mitochondrial molecular quality control comes from an *in vitro* study that revealed a physical interaction between PINK1 and the mitochondrial chaperone TRAP1 (Hsp75) (Pridgeon *et al.*, 2007). PINK1 phosphorylates TRAP1 (also located in the IMS), and this protects the cell against apoptosis induced by H_2O_2 treatment. Notably, the PINK1-dependent phosphorylation of TRAP1 inhibits the release of cytochrome c from mitochondria and thereby increases cell survival under certain stress conditions (Pridgeon *et al.*, 2007). TRAP1 may therefore be another downstream effector of PINK1 involved in the molecular mitochondrial quality control pathway. TRAP1 is structurally related to the HSP90 family, possesses an ATP-binding domain and shows ATPase activity *in vitro*. TRAP1 activity can be inhibited by the Hsp90 inhibitors radicicol and geldanamycin. Nevertheless, TRAP1 does not form complexes with the classic co-chaperones of Hsp90 and might have distinct functions from the other members of the HSP90 family (Felts *et al.*, 2000). In addition, TRAP1 exerts a protective effect against

apoptosis when cells are subjected to various apoptotic stimuli (Hua *et al.*, 2007, Masuda *et al.*, 2004). Moreover, high levels of TRAP1 protein may be involved in drug resistance in certain cancer treatments (Costantino *et al.*, 2009, Landriscina *et al.*, 2010). More detailed studies are necessary to better understand the roles of TRAP1 in mitochondrial quality control and possibly in other signalling pathways.

It is clear that maintenance of proteostasis is crucial for the health of the cell, and it is logical to hypothesise that regulation of quality control mechanisms must be of great importance when an insult occurs. As mentioned above, the various sub-compartments of the cell exhibit different subsets of chaperones and proteases that have location-specific functions. Accordingly, depending on the insult and the organelles affected, different stress responses might be activated.

3.2 A mitochondrial unfolded protein response (UPR^{mt}) with its origin in the mitochondrial matrix

The concept of anterograde communication between the nucleus and mitochondria is well established; however, the communication process from the mitochondria to the cytosol and nucleus, so-called retrograde signalling, remains unclear. The main problem in understanding this process lies in identification of the molecules responsible for the initiation of the signal in the mitochondria and determination of how this signal is transmitted to the nucleus to alter nuclear gene expression. The mitochondrial retrograde signalling pathway is associated with both normal and pathophysiological conditions. This retrograde signalling pathway exists in simple organisms, such as yeast, where it has primarily been studied, and in multicellular organisms. Despite the fact that this signalling pathway is conserved, the proteins and molecular mechanisms involved do not seem to be.

Compartment specificity in signalling by chaperones has been demonstrated for the ER, the cytoplasm and the mitochondrial matrix. These compartment-specific signals are responses to the presence of unfolded proteins in each of these compartments. Following the accumulation of unfolded proteins in the cytosol, heat shock–sensitive transcription factors (HSFs) are activated and increase the transcription of proteins involved in adaptation to this stress, a process known as the heat-shock response (HSR). The signal derived from an excess of unfolded proteins in the ER, on the other hand, is sensed by the lumenal domains of transmembrane proteins and is propagated through their cytoplasmic domains, ultimately leading to the transcription of nuclear genes encoding proteins that alleviate the stress in the ER (e.g., the ER chaperone BiP). This process is known as the unfolded protein response of the ER (UPR^{ER}) (Benedetti *et al.*, 2006).

The stress responses can also be induced by drug treatments. For example, treatment of cells with arsenite, which results in the accumulation of unfolded proteins in the cytoplasm, selectively activates cytoplasmic chaperone gene expression. On the other hand, treatment of cells with tunicamycin, which blocks ER-specific N-linked glycosylation, activates the UPR^{ER}, resulting in increased expression of ER-localised chaperones (Yoneda *et al.*, 2004).

Remarkably, little is known about the specific stress response resulting from the accumulation of unfolded proteins in the mitochondria, despite the fact that many different model systems have been used to study it. Here, we describe recent findings achieved in *C. elegans* and, to a lesser extent, mammalian model systems in detail.

3.2.1 *C. elegans*

The nematode *C. elegans* has been extensively used to study the UPR^{mt}. The ability to use sensitive reporter genes as well as the ease of genetically manipulating these worms makes them convenient models to address this issue. The existence of a UPR^{mt} in *C. elegans* became evident after it was observed that provoking stress in the mitochondrial matrix, either by reduction of mtDNA through treatment with ethidium bromide or down-regulation of mitochondrial chaperones or proteases (*spg-7*), resulted in transcriptional up-regulation of the mitochondrial chaperones *hsp-6* (the *C. elegans* orthologue of human mtHsp70) and *hsp-60* (Yoneda *et al.*, 2004). Up-regulation is a specific response for these stress modulator molecules, as the cytosolic or ER chaperones are not up-regulated nor are other mitochondrial enzymes. Further studies using RNAi library screening led to the discovery of several genes required for UPR^{mt} signalling (Figure 2).

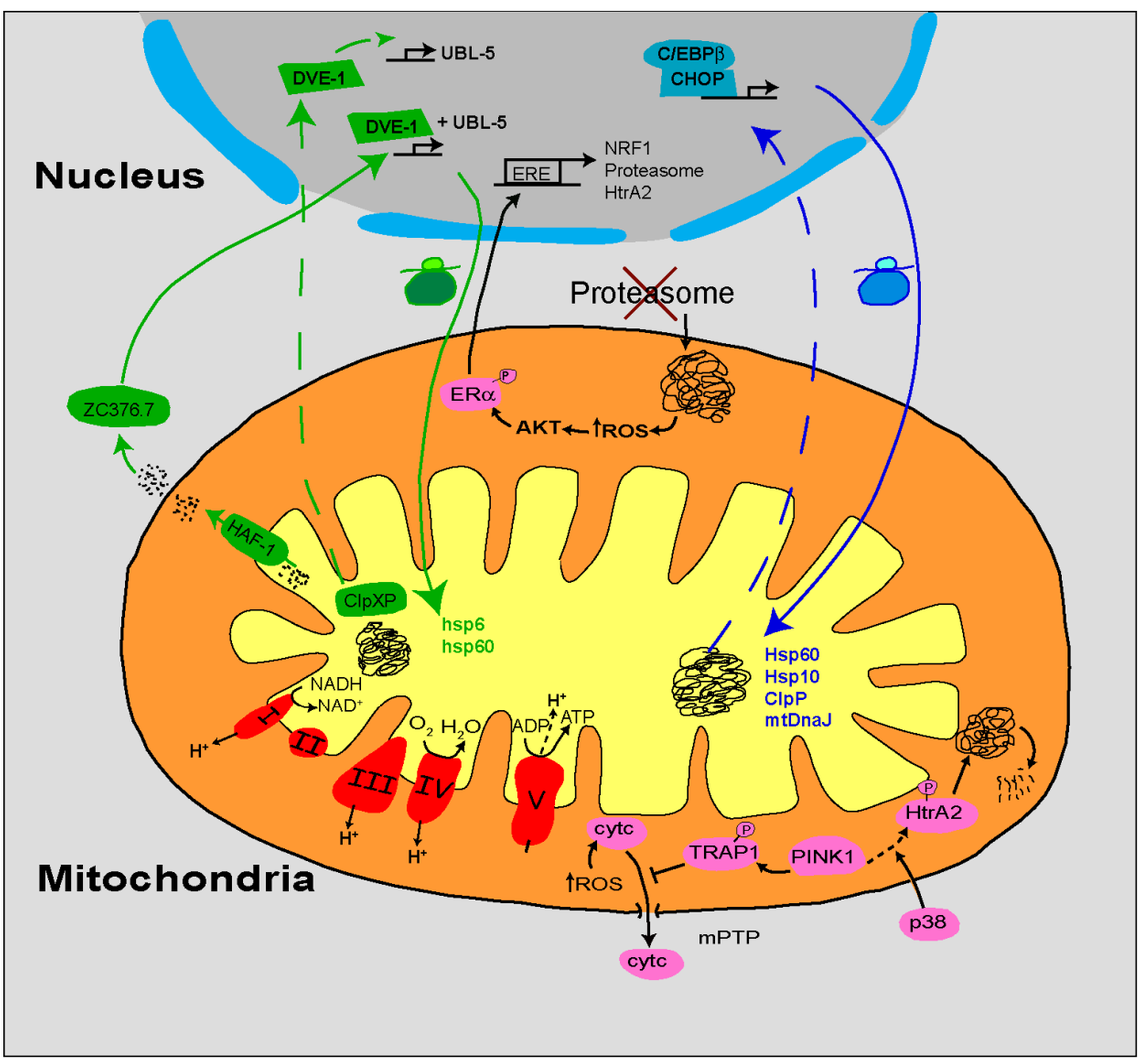

Fig. 2. Mitochondrial stress signalling

Key components of the molecular quality control machinery and mitochondrial unfolded protein response (UPR^{mt}) are represented here. Communication between mitochondria and the nucleus is tightly regulated. Retrograde signalling transmits information from the mitochondria to the nucleus (dashed lines), whereas anterograde signalling transmits information in the opposite direction (solid lines). The mechanism of UPR^{mt} activation in the nematode *C. elegans* is highlighted in green on the left. In *C. elegans*, the accumulation of unfolded proteins in the mitochondrial matrix may be sensed by the ClpXP complex, which might generate a signal leading to certain nuclear events. The nature of this signal remains elusive, although ClpXP activity is known to result in the formation of small peptides that are actively extruded to the IMS through the peptide transporter HAF-1. Once in the IMS, the peptides can be released to the cytosol and activate a receptor or other effector molecules. The bZIP transcription factor ZC3376.7 is activated by this peptide efflux from mitochondria and translocates to the nucleus, where it up-regulates UPR^{mt}-responsive genes. Nuclear events occur on UPR^{mt} include the transcriptional up-regulation of ubl-5 and redistribution of DVE-1 in the nucleus. While both events are downstream of ClpXP, only *ubl-5* transcription activation is downstream of HAF-1. This UPR^{mt} activation results in increased levels of both hsp60 and hsp6 mitochondrial chaperones. The mechanism of UPR^{mt} activation in mammalian cells is highlighted in blue on the right. The presence of excess unfolded proteins in the mitochondrial matrix promotes transcriptional up-regulation of Chop and formation of a complex between the transcription factor CHOP and C/EBPβ in the nucleus, resulting in transcriptional activation of mitochondrial stress responsive genes, such as Hsp60, Hsp10, ClpP and mtDnaJ and increased levels of their encoded proteins in the mitochondria. HtrA2 is located in the IMS and is part of the protein quality control machinery. The proteolytic activity of HtrA2 is phosphorylation-dependent, requires the presence of PINK1 and is mediated through p38 pathway activation. The molecular chaperone TRAP1 is also located in the IMS and might be responsible for the proper folding and assembly of other proteins or complexes. TRAP1 also has a cytoprotective effect in inhibiting the release of cytochrome c when there is an increase level of ROS in mitochondrial. The unfolded protein response of the IMS is represented in the upper middle part of the mitochondria in the figure. This stress response can be activated on accumulation of aggregates of misfolded proteins in the IMS, which can be a result of failure of the proteasome, or due to failure of the molecular quality control in this compartment. The presence of protein aggregates leads to overproduction of ROS, activation of AKT and phosphorylation of the Estrogen Receptor α (ERα). Activation of ERα receptor results in transcription of genes, such as transcription factor NRF1, proteasome and HtrA2.

Ubiquitin-like protein 5 (UBL-5) is essential for UPR^{mt} activation, although its down-regulation does not result in the accumulation of misfolded proteins in the mitochondria, suggesting that it must act downstream of this event. Moreover, upon mitochondrial stress, *ubl-5* is transcriptionally up-regulated, and its protein levels are increased in the nucleus (Benedetti *et al.*, 2006). This might be a feed-forward mechanism to amplify the stress response. It is of note that UBL-5 mRNA levels are reasonably high in mitochondrion-rich human tissues, such as heart, skeletal muscle, liver, and kidney, and that polymorphisms in UBL-5 have been linked to obesity and diabetes (Benedetti *et al.*, 2006).

dve-1 is another gene involved in the UPR^{mt} (Haynes *et al.*, 2007). The DVE-1 protein is a putative transcription factor that is localised to the nucleus and possesses a predicted

homeobox-related DNA-binding domain. Under mitochondrial stress, DVE-1 exhibits an altered nuclear distribution (punctate) and binds to the promoters of the mitochondrial chaperone genes *hsp-6* and *hsp-60*. Furthermore, in stressed worms, DVE-1 forms a complex with UBL-5. Interestingly, UBL-5 is also able to form a complex with the mammalian orthologue of DVE-1, SATB2, but the relevance of the formation of this complex in the UPR^{mt} in mammalian cells is unknown (Haynes *et al.*, 2007).

CLPP-1 is also essential for UPR^{mt} signalling. CLPP-1, the *C. elegans* orthologue of the bacterial ClpP protease, is localised to the mitochondrial matrix. ClpP is the ATP-dependent proteolytic subunit (with a cylindrical shape) of a heteromeric complex formed with a partner AAA+ ATPase (Ortega *et al.*, 2004). Bacterial ClpP functions in conjunction with two partner chaperones, ClpX and ClpA, associated with a wide range of substrates. Moreover, an increase in the levels of misfolded proteins enhances the degradation of these substrates. ClpX is the only ClpP partner known in eukaryotes, and to date, the substrates of mitochondrial ClpP remain unknown. In *C. elegans*, CLPP-1 is necessary for DVE-1 activation. Silencing of CLPP-1 by RNAi prevents the redistribution of DVE-1 in the nucleus and the transcriptional up-regulation of UBL-5, suggesting an upstream action of CLPP-1 in the activation of the UPR^{mt}. Once CLPP-1 is localised to the mitochondria, it is likely that it senses the mitochondrial perturbation and mediates the proteolysis signal that is sent to the nucleus. Interestingly, CLPP-1–dependent proteolysis is required for the UPR^{mt} as an inhibitor of CLPP-1 proteolytic activity abolishes its effect in activating the stress response (Haynes *et al.*, 2007). ClpX is also relevant for UPR^{mt} signalling in *C. elegans*. There are two isoforms of *ClpX* genes in *C. elegans*, both of which encode proteins homologous to bacterial ClpX, and when both genes are silenced, the UPR^{mt} is attenuated. It appears that the function of ClpX in the worm is comparable to that in bacteria and mammals (Haynes *et al.*, 2010). In bacteria, ClpP breaks down proteins into small peptides. Furthermore, studies in yeast have shown that mitochondrial peptides can be extruded to the IMS and subsequently to the cytosol.

The mitochondrial transporter responsible for the extrusion of peptides from the mitochondrial matrix in yeast is Mdl1p. Interestingly, there are multiple orthologues of Mdl1p in *C. elegans*, and the most evolutionarily conserved of these, HAF-1, plays a role in UPR^{mt} signalling as deletion mutations in the *haf-1* gene cause an impairment of this response. Additionally, HAF-1 seems to be located in the inner mitochondrial membrane, which is consistent with its function in transporting cargo from the matrix to the IMS (Haynes *et al.*, 2010). Epistasis analysis has placed HAF-1 upstream of *ubl-5* transcriptional induction, but not of DVE-1 redistribution in the nucleus, which suggests the existence of other transcription factors involved in the UPR^{mt}.

Subsequently, the protein ZC376.7, which includes a C-terminal leucine zipper domain, a predicted nuclear localisation sequence, and a nuclear export sequence, was identified. In unstressed worms, ZC376.7 is localised diffusely in the cytosol, but following mitochondrial stress, it is translocated to the nucleus. ZC376.7 appears to act downstream of HAF-1 and CLPP-1 because the nuclear localisation of this transcription factor in stressed worms is attenuated by down-regulation of both HAF-1 and CLPP-1 (Haynes *et al.*, 2010). Further studies will be required to better understand this signalling mechanism. It is still unclear how small peptides can activate UPR^{mt} signalling and influence the subcellular distribution of ZC376.7. Is there a cytoplasmic receptor for these small peptides? Is the nature of the

peptides important, or rather, is the rate of efflux the most important feature? It is also of particular interest to determine the downstream effectors that influence DVE-1 function. Many questions remain unanswered, although knowledge on this topic is progressively increasing.

Interestingly, a recent study using *C. elegans* as a model system demonstrated that the UPR^{mt} can be activated in a cell-non-autonomous manner (Durieux *et al.*, 2011). Mitochondrial stress caused by down-regulation of a component of the ETC in the nervous system results in activation of the UPR^{mt} in the intestine of the animal. However, it appears that UBL-5 is not required for UPR^{mt} activation in this cell-non-autonomous manner. It is still not known which signal is transmitted from the nervous system to the periphery, and additional research will be required to address these questions further (Figure 3).

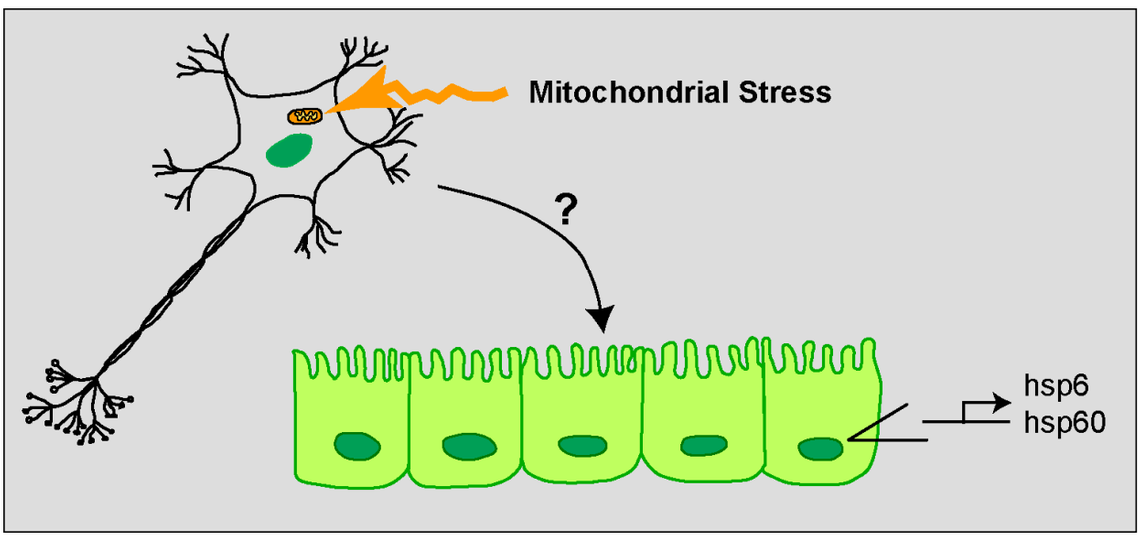

Fig. 3. Cell-non-autonomous activation of the UPR^{mt} in *C. elegans*.

Generation of mitochondrial stress in the central nervous system by depletion of an ETC protein subunit results in the activation of the UPR^{mt} in distant tissues, such as the worm intestine (transcriptional up-regulation of *hsp6* and *hsp60* in intestinal cells). The nature of the signal transmitted from the brain to the intestine is presently unknown.

3.2.2 Mammalian cells

Work by Zhao and colleagues brought to light the existence of an UPR^{mt} (Zhao *et al.*, 2002). The expression of a truncated form of the mitochondrial matrix protein ornithine transcarbamylase (ΔOTC), which accumulates in an insoluble state in the matrix, results in the activation of nuclear genes encoding mitochondrial stress proteins. Upon accumulation of ΔOTC in mitochondria, the mitochondrial chaperones Hsp60, Hsp10, and mtDnaJ as well as the protease ClpP are up-regulated. In mammalian cells, mtHsp70 is not up-regulated by ΔOTC accumulation, which is consistent with its function as a protein transporter across membranes, rather than a stress-sensing molecule. The transcription factors CHOP and CCAAT/enhancer-binding protein β (C/EBPβ) form a hetero-dimer and participate in this mitochondrial stress response by binding to the promoters of UPR^{mt}-responsive genes.

Furthermore, CHOP protein levels are increased by ΔOTC accumulation, indicating that *Chop* itself is a UPR^{mt}-responsive gene.

Subsequently, an AP-1 element, which is essential for CHOP function in the UPR^{mt}, was identified within the *Chop* promoter. Interestingly, C/EBPβ (but not C/EBPα) also contains an AP-1 site. Because c-Jun binds to AP-1 sites, the effect of a MEK inhibitor on the phosphorylation state of JNK upon UPR^{mt} induction was tested, and the results suggest that the MEK/JNK2 pathway could play a role in UPR^{mt} signalling (Horibe and Hoogenraad, 2007).

Six novel UPR^{mt}-responsive genes, encoding the mitochondrial proteases YME1L1 and MPPβ, the import component Tim17A, and the enzymes NDUFB2, Endo G and thioredoxin 2, have recently been identified (Aldridge *et al.*, 2007). In addition to the CHOP recognition element, these genes contain two other conserved elements in their promoters: mitochondrial unfolded protein response element (MURE) 1 and 2, which are present in nine out of the ten genes found to be up-regulated specifically in the UPR^{mt}, with the exception of the Hsp60/10 bidirectional promoter. The transcription factors that bind to the CHOP element are known (for example, CHOP and C/EBPβ), but the transcription factors that bind to the MURE elements remain elusive.

3.3 A distinct signalling pathway originating in the mitochondrial inter-membrane space

The ubiquitin–proteasome pathway probably represents the most common process of protein quality control within the cell. Conjugation of ubiquitin chains with a substrate is important for various physiological functions, from cell signalling to targeting substrates for degradation. The proteasome exists in both the nucleus and the cytoplasm and is responsible for protein turnover in these cellular compartments. Interestingly, the proteasome is also responsible for the degradation of ER proteins because a fraction of the proteasome is associated with ER membranes (Kalies *et al.*, 2005). In the ER, misfolded or damaged proteins are recognised by chaperones, such as BiP, and transported to the cytosol, where the proteasome degrades them. Inhibition of proteasome activity results in accumulation of misfolded proteins in the ER.

Similarly, inhibition of the proteasome also results in accumulation of unfolded proteins in the IMS (Radke *et al.*, 2008). Inhibition of either Hsp90 or the proteasome causes proteins to accumulate in the mitochondria (Margineantu *et al.*, 2007).

Endo G is an IMS protein that accumulates in the IMS following proteasome inhibition (Radke *et al.*, 2008). Overexpression experiments using either wild-type or a mutant form of Endo G have shown that both forms are substrates for ubiquitination, although the mutant form is ubiquitinated at a much higher level. The mitochondrial targeting signal is not required for conjugation with ubiquitin. These observations are consistent with the existence of a protein quality control system in the cytoplasm prior to the import of proteins to the mitochondria. Further analyses have shown that upon proteasome inhibition, wild-type Endo G is cleaved by HtrA2, while the mutant forms of the protein are not. Elimination of mutant Endo G appears to depend on the proteasome pathway.

A defect in proteasome activity may cause the accumulation of mitochondrial proteins and deregulate their activity. HtrA2 seems to be part of a second checkpoint following the action

of the proteasome in the cytosol (Radke *et al.*, 2008). Consistent with this, mutations in HtrA2 may impair its function and lead to unwanted accumulation of its putative substrates. Furthermore, mutant forms of IMS proteins are resistant to HtrA2 degradation, and this could augment their deleterious effect and result in mitochondrial collapse.

The consequences of unfolded protein accumulation in the IMS have not been explored, although a recent study by Papa and Germain showed that upon IMS stress, a distinct protective pathway is activated (Papa and Germain, 2011). Overexpression of a mutant form of the protein Endo G results in the formation of aggregates in the IMS and mitochondrial stress. Under these conditions, HtrA2 and proteasome components increase in an oestrogen receptor α (ERα)–dependent manner. Accumulation of mutant Endo G does not result in the up-regulation of CHOP or Endo G, in contrast to what is observed during the UPR^{mt}. Endo G accumulation also does not cause increased expression of BiP or BIM, which is a characteristic feature of the UPR^{ER}. Using a luciferase reporter with oestrogen-responsive elements (EREs), it has been observed that the accumulation of various IMS proteins activates ERE-containing genes. Additionally, the activation of EREs depends on ERα phosphorylation and not ERβ. ERα appears to be phosphorylated by AKT, which in turn is activated by ROS. ROS overproduction has been reported following mutant Endo G overexpression and is inhibited by the anti-oxidant *N*-acetylcysteine (NAC), suppressing the activation of the ERE reporter. Nuclear respiratory factor 1 (NRF1) is a transcription factor that activates the expression of genes involved in mitochondrial respiration and can be induced by ER activation. IMS stress results in enhanced *NRF1* transcription. Furthermore, increased levels of HtrA2 have been detected following IMS stress induction, as have increases in proteasome activity.

4. Conclusions

Neurodegenerative diseases include a vast number of pathologies, all of which involve the progressive degeneration of neurons. Many of these diseases are directly linked to the ageing process, such as AD and PD (meaning that age is the main, but not the sole, risk factor). The underlying mechanisms associated with the neurodegeneration remain undetermined. A better understanding of the causes and mechanisms involved in these diseases is essential to develop efficient treatments that not only attenuate their symptoms but also alter and possibly reverse disease progression.

Both mitochondrial dysfunction and protein aggregation have been implicated in PD. However, little is known about protein aggregation in the mitochondria and its possible links to PD. Mitochondria are essential organelles in the eukaryotic cell with a variety of functions, including energy production in the form of ATP. Maintenance of a functional ETC in the inner mitochondrial membrane is of extreme importance because dysfunction could considerably increase the generation of ROS, causing damage to different components of the organelle and subsequently the cell.

The majority of mitochondrial proteins are produced in the cytosol and imported to the organelle as precursors. To cross the mitochondrial membranes, these precursors need to be in an unfolded state and need to bind to membrane transporters to reach the mitochondrial matrix. Mitochondrial chaperones are therefore extremely important in assisting in the folding of the newly imported proteins as well as that of newly synthesised proteins in the matrix.

Through evolution, mitochondria have gained defence mechanisms against damage. Different lines of defence, termed molecular, organellar and cellular quality control, can be successively activated to respond to such damage. In this review, we focused on the molecular mechanisms of mitochondrial quality control, which rely on the actions of chaperones and proteases to maintain a healthy pool of mitochondria. Failure of this molecular quality control has been linked to neurodegeneration; it appears that many of the proteins involved in this aspect of mitochondrial quality control are associated with PD. For example, both PINK1 and HtrA2 have been linked to PD. PINK1 and HtrA2 are part of a stress-sensing pathway in which PINK1 facilitates HtrA2 phosphorylation, increasing its proteolytic activity and subsequently its protective effect (through a mechanism that is not clearly understood). Additionally, PINK1 acts upstream of the chaperone TRAP1, which might also be involved in mitochondrial quality control as its protective effect against various apoptotic stimuli has been well documented.

The discovery of an UPR^{mt} is also extremely interesting, and revealing the components and mechanisms involved in this stress response may shed light on new strategies for disease treatments.

Up-regulation of chaperones has been proposed for the treatment of a number of pathologies, but the results have not always been satisfactory. For example, up-regulation of Hsp70 can ameliorate some conditions and increase lifespan in animal models, but it also increases tumour development. Understanding the roles of mitochondrial stress modulators is therefore essential for the identification of putative targets for drug treatments.

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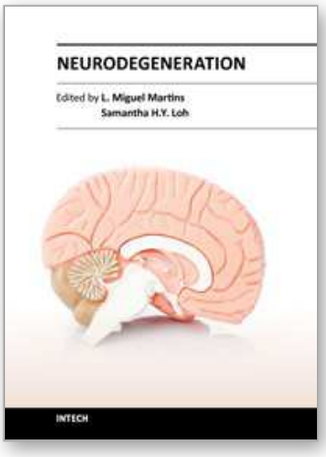

Neurodegeneration

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Currently, the human population is on a collision course for a social and economic burden. As a consequence of changing demographics and an increase in human individuals over the age of 60, age-related neurodegenerative disorders are likely to become more prevalent. It is therefore essential to increase our understanding of such neurodegenerative disorders in order to be more pro-active in managing these diseases processes. The focus of this book is to provide a snapshot of recent advancements in the understanding of basic biological processes that modulate the onset and progression of neurodegenerative processes. This is tackled at the molecular, cellular and whole organism level. We hope that some of the recent discoveries outlined in this book will help to better define the basic biological mechanisms behind neurodegenerative processes and, in the long term, help in the development of novel therapeutic approaches.

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