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Cellular stress response pathways and ageing: intricate molecular relationships

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Ageing is driven by the inexorable and stochastic accumulation of damage in biomolecules vital for proper cellular function. Although this process is fundamentally haphazard and uncontrollable, senescent decline and ageing is broadly influenced by genetic and extrinsic factors. Numerous gene mutations and treatments have been shown to extend the lifespan of diverse organisms ranging from the unicellular Saccharomyces cerevisiae to primates. It is becoming increasingly apparent that most such interventions ultimately interface with cellular stress response mechanisms, suggesting that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Here, we survey the molecular mechanisms that link ageing to main stress response pathways, and mediate age-related changes in the effectiveness of the response to stress. We also discuss how each pathway contributes to modulate the ageing process. A better understanding of the dynamics and reciprocal interplay between stress responses and ageing is critical for the development of novel therapeutic strategies that exploit endogenous stress combat pathways against age-associated pathologies.

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Introduction

Ageing is a complex process associated with progressive decay of physiological function and homeostasis. At the molecular level, ageing is characterized by the gradual accumulation of deleterious modifications in nucleic acids, proteins, lipids and carbohydrates. In humans, general age-related frailty is also associated with severe pathological conditions such as cancer and neurodegenerative diseases.

Over the past 20 years, ageing research has culminated in the identification of classical signalling pathways that influence ageing in a variety of species (Kenyon, 2010). Accumulating findings indicate that longevity depends on the ability of the organism to cope with extrinsic or intrinsic stressors (Kirkwood and Austad, 2000). Indeed, compromised stress responses are linked to the onset of many age-related diseases. ‘Stress’ is broadly defined as a noxious factor (physical, chemical or biological), which triggers a series of cellular and systemic events, resulting in restoration of cellular and organisinal homeostasis. To cope with conditions of stress, organisms have developed a wide range of sophisticated stress response mechanisms, acting at the cellular or organelle-specific level. Notably, exposure to mild stress activates cellular homeodynamic mechanisms, without mounting a comprehensive stress response, which better prepare the organism against stronger insults and promote long-term survival. This phenomenon is known as hormesis (Calabrese et al, 1987; Rattan, 2008).

Much of our understanding of the link between activation of stress response pathways and longevity, as well as, the impact of ageing on the effectiveness of stress response mechanisms derive from studies in model organisms including yeast, worms, flies and mice. Here, we review the main cellular stress response mechanisms, focusing on the effects of ageing on the capacity of the cell to mount a successful stress response. Furthermore, we discuss the influence of stress response on the ageing process. Maintaining efficient mechanisms for counterbalancing stress is emerging as a potential strategy towards ameliorating age-associated pathologies. To this end, we highlight several open questions that need to be addressed before manipulation of stress response pathways can be considered for therapeutic intervention.

The heat shock response

Exposure of cells and organisms to unfavourable conditions such as heat, oxidative and osmotic stress, heavy metals and proteasome inhibitors induces a highly conserved programme of gene expression leading to selective transcription and translation of heat shock proteins (HSPs) (Lindquist and Craig, 1988; Morimoto, 2008). Based on their molecular weight, HSPs are categorized into the HSP100, HSP90, HSP70, HSP60 and the small HSP (sHSP) families. The heat shock response is orchestrated by a set of heat shock transcription factors (HSFs). The mammalian HSF family consists of four members (HSF1-4), while Drosophila, Caenorhabditis elegans and yeast express only one (HSF1) (Morimoto and Santoro, 1998; Anckar and Sistonen, 2007; Akerfelt et al, 2010). Activation of heat shock response is a complex process that involves trimerization and translocation of HSF1 to the
nucleus, where it binds to heat shock elements within the promoters of heat shock genes (Figure 1). HSF1 undergoes multiple post-translational regulatory modifications such as phosphorylation (Sorger and Pelham, 1988; Knauf et al., 1996; Kline and Morimoto, 1997; Holmberg et al., 2001; Guettouche et al., 2005), sumoylation (Hietakangas et al., 2003; Anckar et al., 2006) and acetylation (Westerheide et al., 2009). Upon sufficient induction of the heat shock response pathway, HSF1 returns to monomeric form and interacts with HSP90, HSP70 and HSP40 chaperones (Abravaya et al., 1992; Shi et al., 1998; Zou et al., 1998). In this state, HSF1 remains inactive and the response is terminated. The tightly regulated initiation, execution and termination of the response, through complex post-translational modifications and protein interac-
tions, underscores the requirement for precise activation of the heat shock pathway upon stressful conditions.

The hallmark of many age-related neurodegenerative dis-
eases, such as Alzheimer’s, Parkinson’s and Huntington’s disease is the formation of insoluble protein aggregates. Nevertheless, recent studies show that protein aggregation also occurs in a non-disease context during ageing (David et al., 2010). Global proteomics analysis revealed that several hundred proteins displayed increased aggregation propensity during normal ageing in C. elegans. Interestingly, mutations that reduce insulin/IGF-1 signalling prevent protein insolubi-
lity during ageing. Reduced insulin/IGF-1 signalling is also beneficial against pathology and disease caused by protein aggregation (Morley et al., 2002; Cohen et al., 2006). In the

Figure 1 General and organelle-specific stress response pathways influenced by the ageing process. Depending on the type of macromolecule and the site of damage, distinct stress response pathways, such as autophagy, heat shock response, UPRmt, UPRER, remodelled proteasome and the DNA damage response are initiated. Ageing broadly affects stress response pathways in multiple steps. For simplicity, only proteins with functions modulated by ageing are depicted. Double arrows denote bi-directional communication with the nucleus, which involves generation of stress signals in the stressed organelle or the cytoplasm, transduction of the signals to the nucleus and upregulation of stress-relieving proteins, which in turn function to ameliorate damage. Question marks denote lack of information about specific molecules mediating the effects of ageing. Although, a typical Golgi stress response pathway has not been described yet, several types of stress and also ageing may influence gene expression in the nucleus and cell homeostasis by impinging on Golgi function. BER, base-excision repair; BiP, Ig-binding protein; CHOP, C/EBP homologous protein; CMA, chaperone-mediated autophagy; DAF-16, abnormal dauer formation 16; DVE-1, defective proventriculus 1; GRP94, glucose-regulated protein 94; HSF1, heat shock factor 1; HSP, heat shock protein; HR, homologous recombination; IGF-1, insulin growth factor 1; LAMP-2A, lysosome-associated membrane protein 2A; NER, nucleotide-excision repair; NHEJ, non-homologous end joining; PERK, PKR-like ER kinase; UPR\textsuperscript{ER/mt}, unfolded protein response endoplasmic reticulum/mitochondrion; XBP-1, X-box-binding protein 1.
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The ubiquitin–proteasome system

Ageing is accompanied by accumulation of damaged and modified proteins. The build up of altered proteins is the result of a gradual deterioration of cellular quality control mechanisms, decreased protein degradation or a combination of both. The UPS is the main proteolytic mechanism, responsible for the degradation of damaged proteins and the turnover of most cytosolic and nuclear proteins. The process of protein degradation by the UPS involves two steps: tagging of the protein with a polyubiquitin chain and the degradation of the tagged protein by the proteasome (Ciechanover, 2005). Polyubiquitination is a complex reaction involving ubiquitin, a highly conserved 76 amino acids protein, and three different enzymes (E1–E3). The proteasome is a multicatalytic protease complex composed of one 20S catalytic core and two 19S regulatory caps (Jung et al., 2009).

Oxidative stress has important roles during the ageing process and age-related diseases. Oxidized proteins that escape the low-molecular weight and enzymatic anti-oxidative damage defences of the cell are recognized and degraded by the proteasome. In the presence of moderate oxidant concentrations, proteasomal degradation increases, whereas higher oxidation levels lead to proteolytic inhibition (Ding et al., 2006; Farout and Friguet, 2006; Breusing and Grune, 2008). Impairment of proteasomal activity induces a proteasome stress response that ultimately results in upregulation of proteasome subunit expression (Meiners et al., 2003; Ju et al., 2004). In addition, ubiquitin depletion triggers a ubiquitin stress response in yeast (Hanna et al., 2007). Under such conditions, loading of proteasomes with Ubp6, a deubiquitinating enzyme, is increased. In turn, this results in greater recycling of ubiquitin at the proteasome. It would be interesting to test whether this stress response is maintained during ageing, ensuring the availability of sufficient ubiquitin for degradation of accumulating damaged proteins.

Proteasome activity declines with age in a variety of tissues (Conconi et al., 1996; Shibatani et al., 1996; Anselmi et al., 1998; Ponnappan et al., 1999; Keller et al., 2000b). By contrast, the ubiquitination system does not appear as affected by age (Carrard et al., 2002). It should be noted that the decrease in proteasome activity likely has an important role in the physiological control of lifespan of specific cell types. For example, overexpression of the stress transcription factors HSF1 and DAF-16, suggesting that interventions designed to fortify cell proteostasis may successfully offset the consequences of ageing on protein aggregation pathologies.

A paradoxical trait of several neurodegenerative disorders is that, although the relevant mutant protein implicated in the pathogenesis of the disease is expressed in a wide range of neurons, only specific neuronal subtypes are prone to degeneration. Interestingly, even though the components of the heat shock response are present in all cells, different tissues show differential chaperoning capacity and induction of the pathway (Kern et al., 2010). This disparity, combined with tissue-specific alterations in the ubiquitin–proteasome system (UPS) activity during ageing (Holmberg et al., 2001; Tonoki et al., 2009), may explain the increased vulnerability of certain cell types. However, whether activation of the heat shock response or other proteostatic mechanisms is atypical in degenerating neuronal types remains to be investigated.
proteasome capacity dramatically decreases during plasma cell differentiation (Cenci et al., 2006). This is paradoxical given that plasma cells are highly active in antibody production. Nevertheless, the decrease in abundance and activity of proteasome predisposes plasma cells to apoptosis protecting from excessive humoral response.

Multiple mechanisms have been implicated in the age-dependent decline of the proteasome. Decreased expression of proteasomal subunits has been reported in several experimental setups (Ly et al., 2000; Bulteau et al., 2002). In addition, changes in proteasomal enzymatic activities are caused by increased oxidative stress during ageing (Carrard et al., 2002). Moreover, oxidized and damaged proteins can directly inhibit the proteasome leading to depletion of active proteolytic units (Terman and Brunk, 2004). However, the impact of each of these factors on age-related loss of proteasome function is not yet fully characterized. The threshold of age-related modifications above which the proteasome is overwhelmed and becomes dysfunctional, needs to be determined. Intriguingly, many of the genes mutated in age-related diseases such as Alzheimer’s and Parkinson’s disease, have a role in UPS (Keller et al., 2000a; Jenner, 2001). Maintenance or enhancement of the proteolytic activity of the proteasome during ageing might provide protection against neuronal cell death documented in these diseases. It is important to note that accumulation of unfolded and damaged proteins, Following proteasome inhibition, leads to activation of the heat shock and the endoplasmic reticulum (ER) stress response (Kisselev and Goldberg, 2001). Thus, the ubiquitin–proteasome system is tightly integrated into a wider and complex network of cellular proteostasis-preserving mechanisms.

**Organelle-specific stress response pathways**

**Endoplasmic reticulum**

The ER is the organelle where newly synthesized proteins destined for secretion, integration into the plasma membrane or distribution to various organelles, are folded and post-translationally modified. The environment within the ER is highly crowded with chaperones, processing enzymes and client proteins (Stevens and Argon, 1999). In this cluttered and aggregation-prone environment, complex ER quality control mechanisms ensure the proper translation and folding of nascent proteins as well as the degradation of improperly folded polypeptides (Ellgaard and Helenius, 2003). Key chaperones and folding sensors reside in the ER, including the Ig-binding protein (BiP)/glucose-regulated protein 78 (GRP78), GRP94, calnexin, calreticulin and protein disulfide isomerase (PDI) (Naidoo, 2009). Several of these vital chaperones and enzymes show decreased mRNA, protein levels and/or enzymatic activity in various tissues during ageing (Erickson et al., 2006; Paz Gavilan et al., 2006; Hussain and Ramaiah, 2007; Naidoo et al., 2008; Nuss et al., 2008). Consequently, age-dependent decline of protein folding efficiency creates an unstable ER environment, not capable of sustaining homeostasis under steady-state or elevated stress conditions.

Conditions that elicit increased load of misfolded proteins within the ER trigger the ER stress or unfolded protein response (UPRER) (Schroder and Kaufman, 2005; Ron and Walter, 2007). UPRER helps restore the normal function of ER through upregulation of ER chaperones, halting protein translation and stimulating the degradation of misfolded proteins (Prostko et al., 1993; Kaufman, 1999; Hampton, 2000). In situations of persistent stress, failure of UPRER to restore ER homeostasis results in apoptosis (Szegedi et al., 2006). Apart from its role in the relief from stress induced by various triggers, UPR is important for the differentiation and proper function of professional secretory cells, which have increased protein folding demands in the ER, such as antibody-secreting plasma cells, pancreatic β cells, hepatocytes and osteoblasts (Wu and Kaufman, 2006). Downstream signalling during the UPRER response is mediated by three transmembrane sensors: the inositol requiring element-1 (IRE-1), the PKR-like ER kinase (PERK) and the activating transcription factor 6 (ATF6). The molecular chaperone BiP/GRP78 retains these transmembrane receptor proteins in an inactive state. When critical level of unfolded proteins is exceeded, BiP/GRP78 dissociates from IRE-1, PERK and ATF6 to facilitate protection against the overwhelming load of misfolded proteins (Zhang and Kaufman, 2006). Activation of UPRER through titration of BiP/GRP78 away from the three sensors of ER stress is reminiscent of the mechanism by which HSF is mobilized. Activation of ER stress mechanism via direct recognition of unfolded proteins by stress transducers, as well as, a hybrid recognition model, involving both mechanisms has been proposed (Ron and Walter, 2007).

Activated PERK phosphorylates the translation initiation factor eIF2α, preventing protein synthesis from further overwhelming the ER (Figure 1). Activated ATF6 translocates to the Golgi, where it is cleaved by proteases to form an active 50 kDa transcription factor, which enters the nucleus and upregulates the transcription of genes encoding ER chaperone proteins such as BiP, PDI and GRP94 (Yoshida et al., 1998). IRE-1 activation results in X-box-binding protein 1 (XBP-1) splicing and activation. The activated spliced form of XBP-1 (XBP-1s) acts as a transcription factor, which enhances the expression of genes involved in ER homeostasis as well as in export and degradation of misfolded proteins (Yoshida et al., 2001, 2003; Calfon et al., 2002; Lee et al., 2003).

PERK mRNA expression is lower in aged rats compared with young animals (Paz Gavilan et al., 2006). Interestingly, in aged mice subjected to acute sleep deprivation, activation of PERK and the subsequent inhibition of mRNA translation are impaired (Naidoo et al., 2008). Such alleviation of the translation block initiates a vicious circle, where new protein synthesis further aggravates ER stress. CHOP, a transcription factor of the C/EBP family, is expressed at low levels under normal conditions and it is markedly induced upon sustained ER stress (Zinszner et al., 1998). CHOP also mediates apoptosis under conditions of extreme ER stress (McCullough et al., 2001). Contrary to PERK, CHOP levels are higher in various tissues of aged rodents (Paz Gavilan et al., 2006; Hussain and Ramalha, 2007; Naidoo et al., 2008). Exposure of aged animals to stressors further increases the levels of CHOP, whereas young animals show no increase of the protein under similar conditions. It appears that aged animals fail to mount a timely ER stress response due to alterations in the expression of key components of the response. Moreover, aged cells display increased levels of CHOP, which further facilitates apoptosis, reducing the threshold for initiation of cell death.

Recent studies in *C. elegans* show that XBP-1s synergizes with DAF-16 to activate genes, which lead to enhanced ER
stress resistance and also promote the longevity of mutants with reduced insulin/IGF-1 signalling (Henis-Korenblit et al., 2010). It remains to be determined whether the IRE-1/XBP-1 axis is modified during ageing, which would impede coordination with other stress response factors and consequently impair the ER stress response. ER stress activates both the ubiquitin–proteasome and the macroautophagy–lysosome proteolytic system (Yorimitsu et al., 2006; Ding and Yin, 2008). Whether ageing hinders the activation of these degradation systems, leading to overflow of damaged proteins remains to be seen. Interestingly, defective ER stress response has been associated with age-related pathologies such as diabetes, heart disease and neurodegenerative disorders (Lindholm et al., 2006; Yoshida, 2007; Lin et al., 2008). Whether ageing precipitates common alterations of the UPR<sub>ER</sub> components that underlie these diverse diseases is unclear.

**Mitochondria**

Mitochondrial dysfunction has been associated with oxidative stress, accelerated ageing, apoptosis, neurodegenerative disorders and other pathological conditions. The matrix of mitochondria contains a specific set of chaperones involved in importing, refolding and preventing aggregation of proteins encoded both by the nuclear genome and by mtDNA. The main mitochondrial stress proteins are HSP60, mtHSP70, HSP10, mtGrpE and mtDnaJ. Perturbation of the folding environment in mitochondria elicits the mitochondrial unfolded response (UPR<sub>mt</sub>) by inducing expression of nuclear genes that encode mitochondrial chaperons (Zhao et al., 2002; Kuzmin et al., 2004; Yoneda et al., 2004). The mitochondrial stress response involves the transcription factors CHOP and C/EBPβ (Zhao et al., 2002). Several downstream components of the UPR<sub>mt</sub> have also been identified. Upon proteotoxic conditions, CLPP-1, a proteolytic subunit of the mitochondrial Clp protease, generates peptides that are transported to the cytosol by the ABC transporter HAF-1. Activation of UPR<sub>mt</sub> correlates with the formation of a complex between UBL-5 (a ubiquitin-like protein) and DVE-1 (a homeobox containing transcription factor) and the subsequent relocation of the complex to the nucleus. UPR<sub>mt</sub> also triggers the relocation of the bZip transcription factor ZC376.7 to the nucleus (Benedetti et al., 2006; Haynes et al., 2007, 2010).

In addition to the intrinsically stressful cellular context accompanying ageing, organisms have to also cope with external environmental challenges. Mitochondria isolated from the liver of old rats display increased susceptibility to hyperthermic conditions compared with young animals. Moreover, activation of the mitochondrial stress response is compromised in old animals, which fail to properly upregulate the mitochondrial stress proteins HSP60 and HSP10 (Haak et al., 2009). Finally, the processes of protein import and damaged protein degradation, which are mediated by mitochondrial stress proteins and are vital for mitochondrial homeostasis, become inefficient during ageing (Craig and Hood, 1997; Bulteau et al., 2006). However, the molecular mechanisms that bring about this decline are not understood.

**The lysosome**

Autophagy is one of the main processes mediating both bulk and specific degradation of cellular components, including whole organelles and protein aggregates. Cargo destined for degradation are delivered to lysosomes, where they are recycled. Three main types of autophagy have been defined on the basis of lysosomal delivery mechanisms: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) (Cuervo, 2004; Mizushima et al., 2008). Macroautophagy entails the sequestration of portions of the cytoplasm within a double-membrane autophagic vacuole, called autophagosome. The autophagosomes fuses with secondary lysosomes to form an autolysosome, where hydrolases degrade the sequestered material (Yorimitsu and Klionsky, 2005). In microautophagy, which is less well characterized, the lysosomal membrane itself invaginates to engulf cytosolic components (Marzella et al., 1981). CMA is a highly selective form of autophagy that requires unfolding of the protein before internalization into the lysosome for degradation (Dice, 2007; Cuervo, 2010). In addition to turnover of cellular material, autophagy is involved in development, differentiation and tissue remodelling. Although a basal level of macroautophagy and CMA is observed in various cell types, these pathways are maximally activated under conditions of stress (Figure 1). Analysis of mice harbouring tissue-specific, conditional knockout alleles of autophagy genes demonstrates that the capacity to modulate the rate of intracellular content degradation in response to stress or a nutrient-depleted environment is vital both for cell and organismal survival (Komatsu et al., 2005, 2006; Haral et al., 2006; Nakai et al., 2007). The decreased lysosomal-mediated degradation observed in rodent livers during ageing is attributed to defects both in the clearance of autophagic vacuoles and in the hormonal regulation of macroautophagy (Terman, 1995; Vittorini et al., 1999; Donati et al., 2001, 2008; Brunk and Terman, 2002). Lysosomes isolated from livers of old rats show lower rates of CMA compared with young animals (Cuervo and Dice, 2000). This decline in the efficiency of CMA is the result of altered dynamics and stability of LAMP-2A, the lysosomal receptor that recognizes substrates targeted for CMA (Kiffin et al., 2007).

Autophagy is a complex process with multiple steps that could potentially be altered by ageing. However, age-induced modifications of specific components of macroautophagy have yet to be studied systematically. Is it possible to restore the function of the entire pathway by manipulating the levels of one key protein? Interestingly, recent findings show that modulation of the amount of LAMP-2A in a transgenic mouse model is sufficient to maintain CMA activity until advanced age (Zhang and Cuervo, 2008). Livers from these transgenic animals display improved cellular homeostasis and resistance to toxic compounds. Furthermore, restoration of normal levels of Aig8, whose expression is impaired during ageing, extends lifespan (Simonsen et al., 2008). In addition, autophagy is activated when proteasome capacity is exceeded, in an effort to compensate during excessive demands for cellular proteolysis (Ding et al., 2007; Ding and Yin, 2008). It would be interesting to investigate whether activation of this backup proteolytic mechanism is impaired during ageing, leading to accumulation of misfolded and/or damaged proteins.

Activation of macroautophagy or CMA through pharmacological interventions is potentially an effective approach to maintain efficient clearance mechanisms in a damage-prone environment. Indeed, pharmacological upregulation
of macroautophagy slows down the progression of disease in fly and mouse models of neurodegeneration (Ravikumar et al, 2004). The importance of maintaining an efficient autophagic response is also demonstrated by the fact that all long-lived C. elegans mutants display increased macroautophagy (Melendez et al, 2003; Hars et al, 2007; Hansen et al, 2008; Toth et al, 2008). Nonetheless, excessive activation of autophagy may lead to depletion of the essential autophagic components and failure of proper response to stress. Consistent with this notion, premature ageing in a mouse model of progeria is accompanied with extensive basal activation of autophagy (Marino et al, 2008). Moreover, examination of Alzheimer’s disease patient brains revealed increased autophagy (Lipinski et al, 2005; Imam et al, 2006; Wilson and Bohr, 2007). In addition, treatment of healthy cells with Aβ both increased initiation of autophagy and decreased the rate of autophagosome clearance due to reduced lysosomal function. Therefore, autophagy upregulation may have adverse effects if initiated when cellular degradation mechanisms are already overwhelmed. Activation of autophagy above a crucial threshold, may also lead to cell demise due to interference with pro-survival mechanisms and digestion of anti-apoptotic molecules (Kourtis and Tavernarakis, 2009). Evaluation of the degradation capacity of cells through lifespan, and genetic manipulation of autophagic processes in model organisms during ageing will provide significant insights into the role of autophagy in senescent decline, and may contribute to the development of intervention strategies targeting age-associated neurodegenerative disorders.

**The nuclear DNA damage response**

In contrast to most biomolecules such as proteins and lipids, which can be recycled several times over the lifespan of a cell, DNA cannot be resynthesized afresh to eliminate damage. Instead, cells maintain elaborate genome maintenance machinery to mend their genetic material. DNA damage can be induced by exogenous hazards, such as UV radiation, by endogenous toxic by-products of cellular metabolism, such as reactive oxygen species (ROS) or by spontaneous chemical reactions, such as hydrolysis (Lindahl, 1993; De Bont and van Larebeke, 2004; Hoeijmakers, 2009). Defects in genome maintenance mechanisms underlie the pathology of the vast majority of progeroid syndromes, suggesting that DNA damage, which also accumulates during normal ageing, contributes to age-related deterioration (Dolle et al, 1997; Sedelnikova et al, 2004; Garnis et al, 2008). In addition, DNA repair mechanisms are subject to modifications throughout the lifespan of a cell, leading to gradual loss of repair accuracy and efficiency.

The genome maintenance apparatus of the cell consists of multiple complex repair pathways, each targeting a specific category of DNA lesion (Hoeijmakers, 2009). Base-excision repair (BER) removes subtle lesions of DNA that affect only one DNA strand, such as oxidized bases (Barnes and Lindahl, 2004; Caldecott, 2008). The complementary strand is used as a template for repair. Ageing has a negative impact on BER mechanisms (Figure 1). Both a drop in the activity of BER enzymes and a reduction in the inducibility of the pathway has been observed in aged mice (Cabelo et al, 2002, 2006; Chen et al, 2002; Intano et al, 2003; Lu et al, 2004; Krishna et al, 2005; Imam et al, 2006; Wilson and Bohr, 2007).

Nucleotide-excision repair (NER) is a multistep process involving numerous proteins that target helix-distorting lesions, resulting from UV exposure and carcinogenic compounds (Hanawalt, 2002). NER comprises two subpathways: the global-genome NER (GG-NER), which scans the genome for helix distortions (Gillet and Scharer, 2006; Sugasawa, 2006), and the transcription-coupled NER (TC-NER), which removes lesions that block transcription elongation (Fousteri and Mullenders, 2008). Studies of NER both in vitro and in young versus old animals show that NER efficiency declines with age (Vijg et al, 1985; Wei et al, 1993; Goukassian et al, 2000, 2002; Xu et al, 2000; Yamada et al, 2006). It is not clear whether this decrease is caused by diminished activity of NER enzymes in old animals or by defects in the induction of the DNA damage response.

Double-strand breaks (DSBs) are the most severe form of DNA damage. DSBs are repaired through non-homologous end joining (NHEJ), which merely joins two loose DNA ends with a risk of mutagenesis and information loss, or through homologous recombination, which uses the intact sister chromatid as a template to copy the missing information and seal the broken ends in an error-free manner. The main detrimental outcome of age-related alterations in DSB repair is the increase in cancer incidence, accompanied by genome rearrangements and loss of heterozygosity, which are characteristics of erroneous NHEJ (Fuscoe et al, 1994; DePinho, 2000). Notably, the availability of the Ku protein, which recognizes and binds DSBs is reduced with age (Frasca et al, 1999; Um et al, 2003; Doria et al, 2004; Ju et al, 2006; Seluanov et al, 2007).

Several recent studies converge to indicate that weakened DNA surveillance mechanisms are responsible for increased frequency of DNA damage in old animals. For example, point mutations and genomic rearrangements accumulate with age in mice (Curtis and Crowley, 1963; Martin et al, 1985; Dolle et al, 1997, 2000; Tucker et al, 1999; Stuart et al, 2000; Vijg and Dolle, 2002). However, the net impact of age-related alterations of DNA repair mechanisms on the overall genome integrity and organism survival is not clear. Moreover, the mechanisms by which ageing impinges on the DNA damage repair pathways are not fully understood. An additional caveat is that excessive DNA damage response triggers apoptosis, which may cause harmful loss of functional cells in the context of ageing tissues, where self-renewal is limited.

**Hormesis: an anti-ageing strategy?**

The term hormesis describes the beneficial effects, resulting from the exposure of an organism to a low intensity stressor (Calabrese et al, 1987; Calabrese, 2004). The positive effect of hormesis is attributed to the stimulation and priming of stress response pathways by the stressor. Hormetic manipulations such as repeated mild heat shock result in increased stress-tolerance and extension in lifespan in various models (e.g. yeast, Drosophila, nematodes, rodents and human cells) (Minois, 2000; Cypser and Johnson, 2003; Rattan, 2004; Rattan et al, 2004). The molecular mechanisms by which exposure to low levels of stress confers hormetic resistance and adaptability to adverse conditions are not fully understood. Additional questions remain to be addressed before hormetic interventions and stress response mimetics can be used as an approach to influence ageing and delay or ameliorate age-related pathologies. What is the lower stress threshold for activating a hormetic response? Is there a collateral cost of
Repeated exposure to low stress? Which stress response pathways mediate the protective effects of hormesis?

**Crosstalk between stress response pathways: a domino effect?**

Accumulating findings indicate that distinct stress response pathways do not function in isolation but rather, are parts of a wider stress network with multiple hubs that serve as coordinators of various modules. The kinase target of rapamycin (TOR) is part of an evolutionary conserved signalling pathway that links extracellular stimuli with intracellular processes such as cellular growth, metabolism, translational control and proliferation. TOR also inhibits autophagy through phosphorylation of the ATG1 protein kinase (Wullschleger et al., 2006; Chan, 2009). The TOR kinase senses chaperone availability and responds differentially to mild and severe depletion of different chaperones (Qian *et al.*, 2010). This mechanism allows continuous integration of extracellular nutrient levels and intracellular protein homeostasis. Age-dependent alterations of the heat shock response pathway and consequently, fluctuations of chaperone levels might influence other stress response pathways such as autophagy through TOR (Figure 2). Thus, TOR signalling stands at the crossroads of metabolism, protein homeostasis and ageing.

Sestrins are a family of highly conserved cytoplasmic proteins, whose expression is induced by stress (Budanov *et al.*, 2004). In mammals, sestrins 1 and 2 block mammalian target of rapamycin (mTOR) signalling in response to genotoxic stress through a pathway that involves p53, adenosine monophosphate-activated protein kinase (AMPK) and tuberous sclerosis complex 2 (TSC2) (Budanov and Karin, 2008). In *Drosophila*, sestrin prevents excessive activation of TOR via a negative feedback mechanism and suppresses age-related pathologies (Lee *et al.*, 2010). Therefore, sestrins and TOR act as central nodes in the crosstalk between genotoxic stress and metabolic activity controlled by lipid/protein synthesis and autophagy (Figure 2). Another key regulator of autophagy is Beclin 1, which interacts with numerous proteins such as: Atg14L, UVRAG, Bif-1, Rubicon, Bcl-2, Ambra1, HMGB1, pNIP, VMP1, SLAM, IP(3)R, PINK and survivin (Kroemer *et al.*, 2010). Therefore, Beclin 1 integrates signals from diverse pathways, which are themselves subjected to age-dependent fluctuations.

Both the ER and mitochondrial UPR result in elevated expression of the CHOP gene encoding a bZIP transcription factor CHOP (C/EBP Homology Protein). Although CHOP is shared by the two responses, induction of the UPR<sup>ER</sup> does not cause upregulation of stress-inducible chaperones from non-mitochondrial compartments. Identification of the additional factors that provide specificity will further illuminate the interorganelle communication of stress response pathways with the nucleus.

In addition, proteasome activity is important for DNA repair processes at various levels (Mieczkowski *et al.*, 2000; Luo *et al.*, 2001). Stress-induced activation of the proteasome in the nucleus declines during replicative senescence of human fibroblasts. This decline is due to ageing-dependent decrease of the expression and activity of poly-(ADP-ribose) polymerase (PARP) and other proteasome activators.

![Figure 2](image.png)
polymerase 1 (PARP-1), which stimulates proteasome (Bakondi et al., 2011).

Sirtuins link metabolic status to the regulation of longevity. Interestingly, several transcription factors involved in cellular stress responses, including FOXO3, p53, NF-κB and HSF1, are regulated by SIRT1 (Vaziri et al., 2001; Brunet et al., 2004; Yeung et al., 2004; Westerheide et al., 2009). It appears that SIRT1 may function to orchestrate different stress response pathways during ageing. Indeed, the beneficial effects of low caloric intake are mediated by members of the siru family. Given that SIRT1 directly deacylates HSF1 and therefore regulates the heat shock response, it is possible that the positive effect of sirtuin on lifespan might be mediated through a dynamic preservation of proteostasis. It remains to be determined whether SIRT1 activity is altered in vivo during ageing and whether this coincides with the age-related decline of various stress responses. Recently, parkin has been implicated in coordinating both ER and mitochondrial stress mechanisms (Bouman et al., 2010). This is particularly interesting given the fact that a disease-associated protein is upregulated in response to distinct organelle stress. An additional important question is how the status of the response to stress in a specific organelle influences its fate. Defects in UPRER or UPRMEM might signal the demise of the corresponding organelle through macroautophagy, in an effort to contain homeostasis imbalance. Accumulation of damaged organelles during ageing may stem from failure to emit or respond to such ‘eat-me’ signals. Along these lines, it is important to understand how a stress response initiated in one organelle is sensed by the cell’s homeostatic network and whether compensatory mechanisms are triggered to avoid a general collapse of cellular homeostasis.

Conclusions and perspectives

The process of ageing both influences and is influenced by cellular stress responses. Studies in different organisms converge to illustrate the multifaceted nature of this bi-directional crosstalk. Both the general heat shock response pathway in the cytosol and organelle-specific stress responses (UPRER, UPRMEM) are stepwise procedures depending on the detection of the damage, transmission of the stress signal to the nucleus, upregulation of stress combating proteins and translocation of these proteins to the site of damage. Ageing impinges on multiple points in this cascade to compromise the response to stress. Identification of these interference nodes will lead to a better understanding of how ageing influences stress resistance and undermines survival.

By segregating chaperones to specific organelles, cells have developed efficient strategies to monitor the folding environment, prevent and neutralize damage. While considerable progress has been made in recent years towards the characterization of cellular and organelle-specific stress response pathways, the relevant molecular mechanisms at operation in some organelles remain elusive. For example, although Golgi dysfunction has been implicated in several age-related diseases (Lane et al., 2002; Chiu et al., 2002a; Balyoyannis et al., 2004; Fujita et al., 2006; Hu et al., 2007). Whether a bona fide Golgi stress response pathway exists and is modified during ageing remains to be elucidated.

Uncontrolled activation of stress response pathways may have undesirable effects. Tumour cells, which have lost the ability to effectively control growth, express higher levels of chaperones (Jaatela, 1999; Calderwood et al., 2006). Increased resistance to stress via enhancement of the cellular stress response pathways may promote cancer development by helping cancer cells to cope with unfavourable conditions (Dai et al., 2007). In addition, although HSPs have beneficial effects on the preservation of homeostasis, their overexpression above a certain threshold may dampen heat shock response via negative feedback on HSF activation. Moreover, given that several stress response pathways are linked to cell death, their tight regulation is imperative, since excessive activation may lead to overall loss of functional cells. Hyperactivation of stress response pathways may also lead to depletion of critical cellular resources, aggravating adverse conditions or insults.

Although the gradual deterioration of stress response mechanisms is a general feature of ageing in diverse organisms, it remains unclear whether this decline is simply a corollary of the ageing process or a significant causative factor, contributing to senescence. Studies in simple model organisms, where stress response pathways can be genetically manipulated are poised to provide significant new insights into this issue. Importantly, such studies should also yield useful information about potential targets for pharmaceutical interventions aiming to augment cellular stress response and defence mechanisms during ageing in an effort to combat age-related health hazards.

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Conflict of interest

The authors declare that they have no conflict of interest.

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