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SYMPOSIUM REVIEW

# Proteostasis and ageing: insights from long-lived mutant mice

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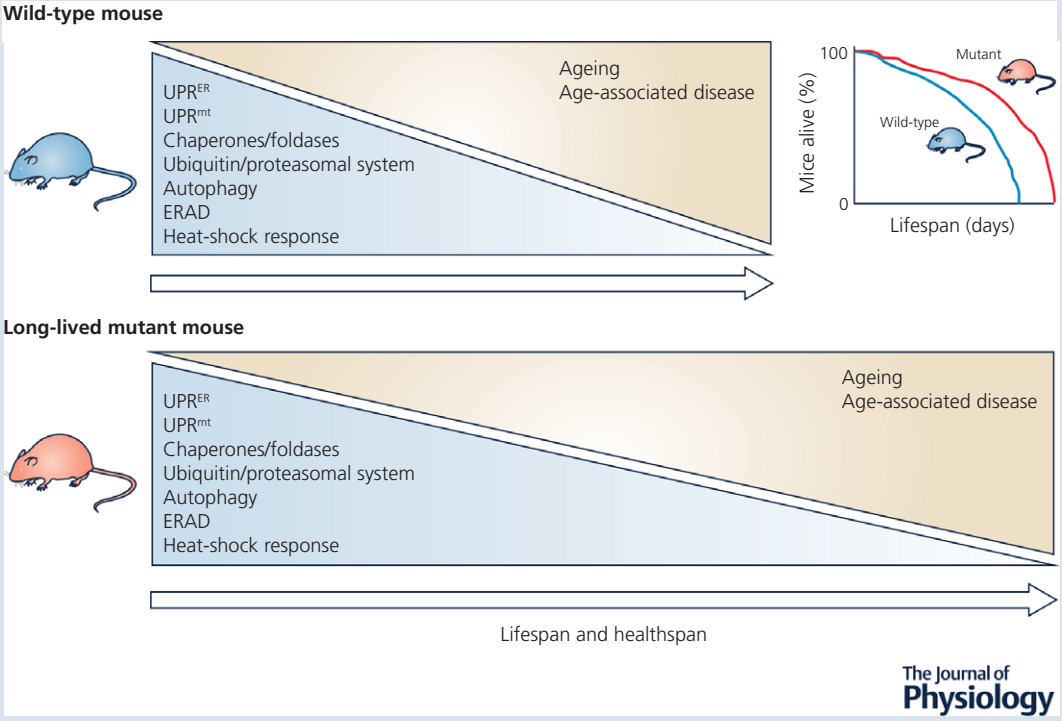


**Abstract** The global increase in life expectancy is creating significant medical, social and economic challenges to current and future generations. Consequently, there is a need to identify the fundamental mechanisms underlying the ageing process. This knowledge should help develop realistic interventions capable of combatting age-related disease, and thus improving

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late-life health and vitality. While several mechanisms have been proposed as conserved lifespan determinants, the loss of proteostasis – where proteostasis is defined here as the maintenance of the proteome – appears highly relevant to both ageing and disease. Several studies have shown that multiple proteostatic mechanisms, including the endoplasmic reticulum (ER)-induced unfolded protein response (UPR), the ubiquitin–proteasome system (UPS) and autophagy, appear indispensable for longevity in many long-lived invertebrate mutants. Similarly, inter-specific comparisons suggest that proteostasis may be an important lifespan determinant in vertebrates. Over the last 20 years a number of long-lived mouse mutants have been described, many of which carry single-gene mutations within the growth-hormone, insulin/IGF-1 or mTOR signalling pathways. However, we still do not know how these mutations act mechanistically to increase lifespan and healthspan, and accordingly whether mechanistic commonality occurs between different mutants. Recent evidence supports the premise that the successful maintenance of the proteome during ageing may be linked to the increased lifespan and healthspan of long-lived mouse mutants.

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**Abstract figure legend** Primary cellular proteostatic mechanisms within mice. Multiple components of the proteostatic network are known to be deleteriously affected by ageing but such age-associated changes may be slowed and/or delayed in long-lived mice. Consequently, it has been suggested that the ability to better maintain proteostasis over the life-course underlies both the greater lifespan and healthspan of long-lived mutant mice. $UPR^{ER}$, endoplasmic reticulum stress unfolded protein response; $UPR^{mt}$, mitochondrial unfolded protein response; ERAD, endoplasmic reticulum associated protein degradation.

**Abbreviations** DR, dietary restriction; ERAD, endoplasmic reticulum-associated degradation; GH, growth hormone; IGF-1, insulin-like growth factor-1; IIS, insulin/IGF-1 signalling; mTOR, mechanistic (mammalian) target of rapamycin; $UPR^{ER}$, endoplasmic reticulum unfolded protein response; $UPR^{mt}$, mitochondrial unfolded protein response; UPS, ubiquitin-proteasome system.

## Introduction

It is now well established that the changes that occur during ageing at the level of the phenotype in multicellular organisms is highly conserved, and that ageing rate can be modulated through a number of environmental, genetic and pharmacological means (Gems & Partridge, 2013; Lamming *et al.* 2013; Selman, 2014). Over the last couple of decades a significant number of studies have demonstrated that disrupting signalling through the growth hormone (GH) (Brown-Borg *et al.* 1996; Coschigano *et al.* 2003), the insulin/IGF-1 (Selman *et al.* 2008) and the mechanistic target of rapamycin (mTOR) pathways (Selman *et al.* 2009; Arif *et al.* 2017) can extend lifespan in mice and improve late-life health. Excitingly, pharmacological manipulation of some of these pathways can also slow ageing in mice (Harrison *et al.* 2009), and polymorphisms in genes within these pathways are correlated with human longevity (Deelen *et al.* 2013; Passtoors *et al.* 2013). Without doubt one of the greatest challenges in mouse ageing research currently is to try and identify mechanistically how these interventions act to elicit their favourable effects, and to determine whether shared mechanisms drive longevity and healthspan across different mutants or whether such mechanisms are only specific to a particular mutant or specific signalling pathway. Such information is likely to be crucial if we are to ultimately design safe and effective interventions to extend late-life health and vitality in humans.

The successful maintenance of proteins within cells is termed proteostasis, which involves a number of cellular processes encompassing the initial synthesis of nascent proteins, through to the appropriate folding, transport and secretion of mature proteins, to the degradation (and recycling) of damaged and redundant proteins within the cell. Consequently, the inability of the cellular proteostatic machinery to maintain the proteome appropriately over the life-course has been implicated heavily in the ageing process and in underlying a number of age-associated pathologies (Vilchez *et al.* 2014; Labbadia & Morimoto, 2015). Cells contain a number of well-described mechanisms to help maintain proteostasis: the unfolded protein response (UPR), which is initiated following accumulation of unfolded/misfolded proteins within the endoplasmic reticulum ($UPR^{ER}$) or following mitonuclear protein imbalance and mitochondrial dysfunction ($UPR^{mt}$), the ubiquitin–proteasome system (UPS) and the lysosomal–autophagy pathway. In this brief review we will specifically focus on three components of

the cellular proteostatic machinery, namely the $UPR^{ER}$ and $UPR^{mt}$, and the UPS, and document the current evidence linking longevity in mutant mice to these proteostatic processes. Due to space issues we will not discuss the lysosomal–autophagy pathway, but direct the reader to excellent recent reviews on this subject (Lapierre *et al.* 2015; Carmona-Gutierrez *et al.* 2016).

## Endoplasmic reticulum (ER) stress and the unfolded protein response (UPR)

Proteostatic stress, through the build-up of aberrant (misfolded or unfolded) proteins can initiate a coordinated stress response within the ER – the $UPR^{ER}$ (Fig. 1). During ER stress, the UPR instigates a triad of adaptive cellular responses to reduce protein loading and initiate a return to proteostasis; (i) a general reduction in protein synthesis through translational repression,( ii) an upregulation in specific chaperones and foldases to increase protein folding capacity, and (iii) an enhancement in ER-associated degradation (ERAD) of aberrant proteins by the proteasome (Labbadia & Morimoto, 2015). The proximal sensors of the UPR are inositol requiring element-1 (IRE1), PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6). Upon ER stress, BiP/GRP78, a HSP70 family member, disassociates from these three sensors, thus triggering the UPR and enabling BiP/GRP78 to undertake chaperone activities in response to the accumulation of misfolded and unfolded proteins (Labbadia & Morimoto, 2015).

Several elements of the UPR machinery show a general decline in activity with advancing age (Naidoo, 2009), with various chaperones showing significant age-associated reductions at both the mRNA and protein level in mice (Nuss *et al.* 2008). In *C. elegans,* the induction of a number of components of the $UPR^{ER}$ following tunicamycin treatment decreased with advancing age, including transcript levels of spliced *xbp-1* and $UPR^{ER}$ target genes (Taylor & Dillin, 2013). Constitutive activation of *xbp-1* was sufficient to increase late-life resistance to ER stress but did not affect worm lifespan (Taylor & Dillin, 2013). Interestingly, these same authors then went on to show that both neuronal- and intestinal-specific activation of *xbp-1* increased lifespan in *C. elegans*, and that neuronal activation both induced the $UPR^{ER}$ within intestinal cells and rescued ER stress resistance during ageing (Taylor & Dillin, 2013). Inactivation of specific UPR genes shortened the lifespan of long-lived mutant worms (Henis-Korenblit *et al.* 2010; Shore *et al.* 2012), and intestinal IRE1 appears necessary for lifespan extension under dietary restriction (DR) in *Drosophila* (Luis *et al.* 2016). In addition, DR appears to afford protection against age-associated declines in components of the proteostatic network in mouse liver (Mitchell *et al.* 2016). Several comparative studies have investigated components of the $UPR^{ER}$, with longer-lived species tending to have greater constitutive levels of chaperones (e.g. HSP60, HSP70, HSP90, GRP78) compared to shorter-lived species (Salway *et al.* 2011*a*; Pride *et al.* 2015). However, in long-lived Snell and $Ghr^{-/-}$ mice, mRNA transcript levels of various chaperones did

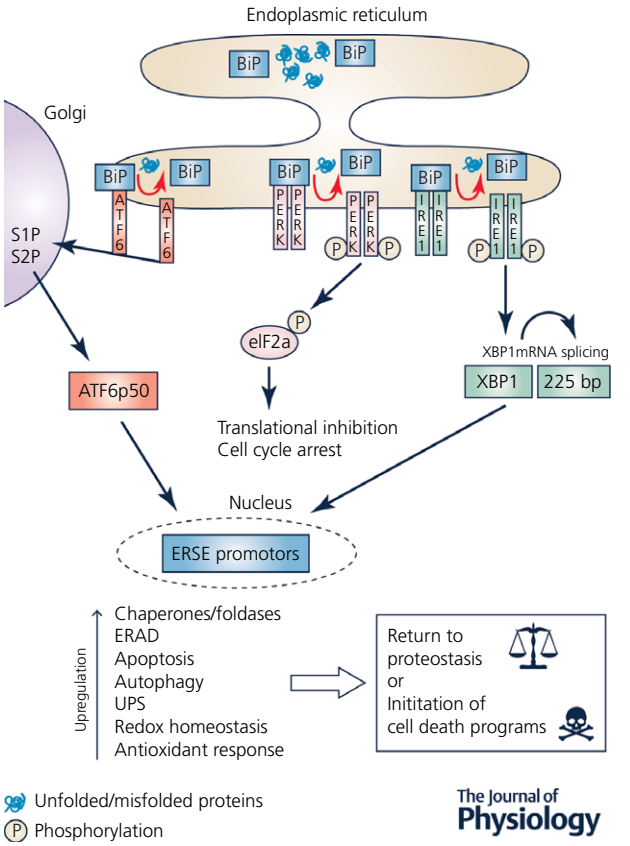


**Figure 1. Main components of the endoplasmic reticulum (ER) unfolded protein response (UPR) in mammals**
The intracellular build-up of unfolded/misfolded proteins results in ER stress that initiates an adaptive response – the UPR – which invokes a cellular cascade in an attempt to return the cell back to proteostasis. The proximal UPR sensors are PKR-like ER kinase (PERK), inositol requiring element-1 (IRE1), and activating transcription factor 6 (ATF6). Following ER stress, BiP/GRP78 disassociates from PERK, IRE1 and ATF6, resulting in the initiation of the UPR, and also allows *BiP* itsel to undertake various chaperone activities. Activated PERK results in the phosphorylation of eukaryotic initiation factor 2$\alpha$ (eIF2$\alpha$) that leads to inhibition of translation and cell cycle arrest, thus reducing protein loading within the cell. IRE1 splices a 225 base pair intron from its substrate XBP1, thus activating XBP1, which translocates to the nucleus and binds to specific endoplasmic reticulum stress elements (ERSE) within the nucleus resulting in the upregulation in expression of multiple genes, including cellular chaperones and foldases. Upon disassociation from Bip, ATF6 is cleaved by site-1 protease (S1P) and site-2 protease (S2P) within the Golgi apparatus to an active form ATF6 p50, which then translocates to the nucleus and induces endoplasmic reticulum associated protein degradation (ERAD) by the ubiquitin–proteasome system (UPS). If the ER stress is prolonged or severe and the UPR cannot return the cell to proteostasis, then cell death programmes, including the apoptosis cascade, will be initiated in order to remove the damaged cells.

not show a consistent increase in expression across a range of tissues (Swindell *et al.* 2009).

Almost without exception, the little research examining proteostasis in long-lived mutant mice has been undertaken in long-lived GH-deficient Snell dwarf mice. Primary skin fibroblasts from Snell dwarfs have been shown to be hypersensitive to the ER stressors thapsigargin and tunicamycin compared to cells derived from control mice (Salmon *et al.* 2008). In agreement, fibroblasts derived from long-lived naked mole rats (Salmon *et al.* 2008) and long-lived IIS mutant mice (M. M. Page, D. J. Withers and C. Selman, unpublished observations) are similarly significantly more sensitive to ER stress than cells derived from wild-type mice. The ER-stress sensitive phenotype of Snell dwarf fibroblasts was associated with lower *Ire1a* expression, a reduced ratio of spliced:unspliced *Xbp1* and a lower expression of various *Xbp1*-associated target genes (Sadighi Akha *et al.* 2011). While ER stress did not differentially affect several chaperones (BiP, PDI, ERp72, GRP94) in Snell dwarf fibroblasts compared to control fibroblasts, various pro-apoptotic markers (e.g. CHOP, caspase-12 levels, caspase-3 activity, c-JUN phosphorylation) were all significantly increased in Snell dwarf fibroblasts following ER stress (Sadighi Akha *et al.* 2011). These findings led the authors to speculate that the ER stress sensitive phenotype of Snell dwarf fibroblasts enabled a more rapid induction of apoptosis and subsequent removal of damaged cells, leading to a quicker return to proteostasis. Interestingly, IRE1 activation in human brain correlates with Alzheimer's disease (AD) pathology, and neuronal-specific deletion of *Ire1* in a mouse model of AD reduced amyloid precursor protein expression and rescued learning and memory capacity (Duran-Aniotz *et al.* 2017). The transcription factor ATF4, a central component of the cellular stress response, and several of its downstream targets are also elevated in fibroblasts from both long-lived Snell and PAPP-A knockout mice following tunicamycin exposure (Li & Miller, 2015). Hepatic ATF4 protein levels are also significantly increased in a number of different long-lived mouse models (Li *et al.* 2014). While clearly more research needs to be done in other mutant models, it has been shown that the addition of IGF1 to murine NIH/3T3 cells previously exposed to ER stress causes attenuation of apoptosis and an induction in *BiP/Grp78* expression (Novosyadlyy *et al.* 2008). Reducing IIS pharmacologically in cells through NT219 treatment, which impairs IGF1R kinase activity and causes degradation of insulin receptor substrates 1 and 2, activated various chaperones, leading to the accrual of prion protein within intracellular aggresomes, although interestingly NT219 reduced both proteasomal and autophagy activity (Moll *et al.* 2016).

The mitochondrial unfolded protein response ($UPR^{mt}$) is a highly conserved signalling response, which is induced following mitonuclear protein imbalance and dysfunction, leading to a cytoprotective transcriptional response that maintains proteostasis and has been reported to correlate with lifespan (Jovaisaite & Auwerx, 2015). A number of studies have identified associations between the induction of the $UPR^{mt}$ and longevity in worms (Rauthan *et al.* 2015; Merkwirth *et al.* 2016), flies (Owusu-Ansah *et al.* 2013) and mice (Houtkooper *et al.* 2013; Merkwirth *et al.* 2016), and $UPR^{mt}$ may help explain the longevity of mitochondrial mutants (Lapointe & Hekimi, 2008; Ristow & Schmeisser, 2011). However, it should also be noted that other studies have reported no such association (Bennett *et al.* 2014; Mulvey *et al.* 2016), and indeed $UPR^{mt}$ induction may be detrimental under certain conditions (Lin *et al.* 2016). Surprisingly little research on the $UPR^{mt}$ has been undertaken in long-lived mutant mice, although markers of the $UPR^{mt}$ were recently shown to be elevated in cells and tissues from long-lived Surf1 knockout mice (Pharaoh *et al.* 2016).

## The ubiquitin–proteasome system

The ubiquitin–proteasome system (UPS) plays a critical role in maintaining proteostasis within cells through the recognition and subsequent degradation of misfolded and damaged proteins, and by maintaining quality control of newly synthesised proteins. The UPS is a highly conserved proteolytic pathway that consists of a core 20S catalytic particle, whose activity is subsequently modulated by a number of regulatory subunits including the 11S (PA28) and 19S (PA700) subunits (Jung & Grune, 2012). Interaction of the 19S regulator with two $\alpha$-rings on the 20S core, leads to the formation of a larger 26S proteasome complex that accounts for the majority of the proteolytic activity within cells under steady-state conditions (Jung & Grune, 2012). An inducible form of the 20S proteasome (i20S), induced by cytokines such as interferon gamma (IFN$\gamma$) and tumour necrosis factor alpha (TNF$\alpha$), also exists, which is thought to be key to antigen presentation by the major histocompatibility complex (Johnston-Carey *et al.* 2015). In common with the other proteostatic pathways, multiple components of the UPS are known to be negatively affected by ageing (Grune *et al.* 2001; Vilchez *et al.* 2014). For example, activity of the 26S proteasome declines in human peripheral blood lymphocytes with age, and this was correlated with an increased number of post-translational modifications on proteasomal subunits (Carrard *et al.* 2003). Similar declines in the activity of both the 20S and 26S proteasome have been described during ageing and senescence in a wide-range of species, tissues and cells (Vilchez *et al.* 2014; Raynes *et al.* 2017).

Elevated UPS activity has been correlated with longevity in model organisms (Tonoki *et al.* 2009; Kruegel *et al.* 2011; Rana *et al.* 2013; Chondrogianni *et al.* 2015), and appears essential to the longevity of IIS mutant worms (Ghazi *et al.* 2007; Matilainen *et al.* 2013).

Proteasomal function is also preserved in cells derived from human centenarians (Chondrogianni *et al.* 2000), and intranasal administration of human HSP70 to aged mice increased proteasome activity within the cerebral cortex, improved cognitive function and extended lifespan relative to untreated mice (Bobkova *et al.* 2015) In contrast, impairing the UPS shortens lifespan and increases age-associated pathology in flies (Liu & Pfleger, 2013) and mice (Min *et al.* 2008), and UPS dysfunction is associated with a number of human diseases (McKinnon & Tabrizi, 2014). Using an orthologue mapping approach it has also been shown that proteins associated with the UPS are candidate targets of selection in mammalian lineages associated with longevity (Li & de Magalhaes, 2013). Additional studies have reported that UPS activity tends to be enhanced within cells and tissues of long-lived organisms such as naked mole rats (Pride *et al.* 2015; Rodriguez *et al.* 2016), although these findings are not consistent across all studies (Salway *et al.* 2011*b*). Recently, it was reported that lifespan in primates was positively correlated with activity of the 20S, but not the 26S, proteasome within primary fibroblasts (Pickering *et al.* 2015). Further examination linked this association specifically to the immunoproteasome, with protein and mRNA levels of the immunoproteasome subunit PSMB8 in primary fibroblasts correlating with longevity across different primate species. Hepatic PSMB8 protein levels were similarly elevated in several long-lived mouse models, including Snell dwarf mice and rapamycin fed mice (Pickering *et al.* 2015). However, proteasomal activity was not higher in liver (Pickering *et al.* 2015), heart or brain of Snell dwarf mice relative to controls (Salway *et al.* 2011*b*), and cardiac levels of both the 20S proteasome and the immunoproteasome were reduced in mice treated intraperitoneally with rapamycin for 7 days (Zhang *et al.* 2015). As mentioned previously, reduced insulin/IGF-1 signalling (IIS) correlates with longevity across model organisms and also in humans (Gems & Partridge, 2013). A novel insight into the dynamic relationship between IIS and proteostasis in the context of ageing was recently provided by Tawo and colleagues (Tawo *et al.* 2017), who showed that the E3 ubiquitin ligase CHIP, a critical regulator of proteostasis, is involved in proteolysis of the insulin receptor, and that this function may promote longevity in worms and flies. Importantly, under conditions of proteostatic stress and during ageing, CHIP is preferentially directed towards the disposal of aberrant proteins and away from targeted degradation of the insulin receptor (Tawo *et al.* 2017).

## Concluding remarks

Despite the relatively large number of long-lived mutant mice being described over the last couple of decades, there is a clear absence of information regarding whether the underlying mechanism driving these longevity phenotypes show commonality across different mutants or are simply specific to a particular mutant. Such conservation is important in our quest to understand the mechanistic nature of ageing in model organisms and relate this ultimately to human ageing. For example, it is evident that cellular resistance to oxidative stress is not conserved across all long-lived mutant mice (Page *et al.* 2014; Hofmann *et al.* 2015). It is well established that organisms are endowed with a large number of tools to maintain proteostasis within their cells, and it is unequivocal that the effectiveness of this tool-kit declines over the life-course of an organism and that this decline is pervasively implicated in both ageing and age-related pathology. Emerging evidence is now revealing that various components of the proteostastic network are essential for longevity in some mutant worms and flies, and that the maintenance of these networks during ageing may also underlie lifespan differences across different vertebrates. However, there undoubtedly needs to be an improved appreciation of whether proteostatic mechanisms differ across particular long-lived mutant mice relative to control mice under both basal and stressed (including 'natural' ageing) conditions. Virtually all of what we currently understand about cellular stress phenotypes in long-lived mutants has been gleaned from studies in primary fibroblasts. There is clearly a need for a greater investigation of cellular stress phenotypes across different cell and tissue types, and whether sex-specific effects exist, particular relevant to those mutants and interventions that show clear sex-specific differences in longevity. In addition, a relatively straightforward comparative approach examining proteostasis in different mouse strains that are known to vary in 'natural' longevity may also provide additional insights. Given that significant interplay exists between different components of the proteostatic network, with compensation existing between proteasomal function and autophagy for example (Ding *et al.* 2007), it is certainly feasible that different mouse mutants may employ subtlety different ways in which to maintain their proteome, although the ultimate goal of preserving an optimal proteome is shared. A number of conditional mutants of the original long-lived mutants now exist and it will be interesting in the future to also investigate, as has been done in invertebrate model organisms, proteostatic mechanisms in the context of cell autonomous *versus* non-autonomous actions (see O'Brien & van Oosten-Hawle, 2016). As many of the genetic pathways known to extend lifespan in mice are pharmacologically amenable and given that several components of the proteostatic network are also druggable, specific targeting of specific pathways and proteostatic components should help to further drive forward our understanding of the relevance of proteostasis in mammalian longevity and healthspan, and thus help

focus research on realistic point of intervention ultimately capable of slowing human ageing and improving late-life health and wellbeing.

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## Additional information

### Competing interests

The authors declare no conflicts of interests.

### Author contributions

All authors were involved in the writing of the manuscript and approved the final version of the manuscript. All individuals designated as authors qualify for authorship, and all individuals who qualify for authorship are listed

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