Chapter 16

Stress Responses During Ageing: Molecular Pathways Regulating Protein Homeostasis

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Abstract

The ageing process is characterized by deterioration of physiological function accompanied by frailty and ageing-associated diseases. The most broadly and well-studied pathways influencing ageing are the insulin/insulin-like growth factor 1 signaling pathway and the dietary restriction pathway. Recent studies in diverse organisms have also delineated emerging pathways, which collectively or independently contribute to ageing. Among them the proteostatic-stress-response networks, inextricably affect normal ageing by maintaining or restoring protein homeostasis to preserve proper cellular and organismal function. In this chapter, we survey the involvement of heat stress and endoplasmic reticulum stress responses in the regulation of longevity, placing emphasis on the cross talk between different response mechanisms and their systemic effects. We further discuss novel insights relevant to the molecular pathways mediating these stress responses that may facilitate the development of innovative interventions targeting age-related pathologies such as diabetes, cancer, cardiovascular and neurodegenerative diseases.

Key words Ageing, Heat shock, Immunity, Inflammasome, Proteostasis, Proteotoxic stress, Unfolded protein response

1 Introduction

Over the past years accumulating evidence suggest that stress-response and life-span regulation pathways share similar mechanisms [1, 2]. It is already known that accelerated ageing and ageing-associated diseases prevail when the organism loses the ability to adapt during stress caused by intrinsic or extrinsic burdens. Thus, the ability to cope with stress has a direct impact on physiological ageing. Impaired protein homeostasis and proteotoxic stress are considered a hallmark of ageing. Activation of stress-response pathways may ameliorate age-related proteotoxicity and induce life-span extension [3–5]. During ageing, the sophisticated mechanisms implicated in protein quality control, gradually deteriorate, leading to proteotoxicity and age-associated frailty. Such mechanisms include protein degradation-specific pathways, and networks for the proper folding and

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trafficking of nascent polypeptides. Adaptation of cellular proteostasis is mandatory in order to respond to loss of proteostatic control. Thus, identifying the players involved is essential towards developing strategies for efficiently tackling age-related pathologies. The heat shock response (HSR) that regulates the cytoplasmic proteostasis and the unfolded protein response (UPR) that regulates proteostasis during endoplasmic reticulum (ER) stress are two well-characterized pathways that have evolved independently to ensure proper protein folding. Key molecules implicated in proteotoxic stress response pathways are listed in Table 1. Perturbations in mitochondria may also initiate an UPR that activates transcription of nuclear-encoded mitochondrial chaperones for maintaining proper protein homeostasis [6]. The peroxisomal quality control system has also been implicated [7], expanding the list of organelle-specific proteotoxic stress-response pathways. In this review, we focus on key stress response pathways that preserve proteostasis in the cytoplasm and the ER, the systemic effects exerted by these pathways, and their role during ageing.

2 The Heat Shock Response

When organisms encounter unfavorable environmental or intrinsic conditions, such as heat stress, oxidative stress or overexpression of aggregation-prone proteins, cell defensive mechanisms become activated. Impairment of these mechanisms due to mutations or advanced ageing, may lead to neurodegenerative and protein conformational diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington disease) [8, 9]. The heat shock response (HSR) is activated within seconds after exposure to stress. The master regulators of the HSR are the heat shock transcription factor family of proteins. While there is only one heat shock transcription factor (HSF-1) in invertebrates, the mammalian genome encodes 4 (HSF-1-4) [10, 11]. In mammals HSF-1 regulates the HSR, whereas the other heat shock factors evolved to fulfil distinct functions throughout development, stress, and ageing [12–16]. HSF-1 becomes active upon exposure to elevated temperature and induces the expression of heat shock proteins (HSP) (chaperones). Five different HSP families are defined by their molecular weight: HSP100, HSP90, HSP70, HSP60, and small HSP (sHSP) [1].

The heat shock transcription factor family members share the same domain structure. The DNA binding domain (DBD) is located at the amino-terminus of the protein and comprises a helix-turn-helix motif. Under normal conditions, interaction with Hsp90 inhibits activation of HSFs [10, 17]. When cells experience heat stress, Hsp90 is recruited to unfolded and misfolded proteins, leaving HSF-1 monomers in an intermediate, activation-ready state. As part of the activation process, the hydrophobic heptad repeat domain HR-A/B interacts with the HR-C domain, the carboxy-terminal

Table 1
Key molecules involved in heat shock and ER stress responses

Name	Function	Reference
ATF6	ER-membrane-bound ER-stress-sensor. Translocates to the Golgi, where it gets cleaved, forming a transcription factor responsible for the upregulation of ER chaperones	[67, 68]
PERK	ER-membrane-bound ER-stress-sensor. Responsible for repressing global protein synthesis via phosphorylation of the α subunit of eIF2α	[65, 66]
IRE-1	ER-membrane-bound ER-stress-sensor. Mediates transcriptional regulation during ER stress through XBP-1	[65]
XBP-1	A bZIP, ER-stress-regulated transcription factor. Upon ER stress the *xbp-1* gene is alternatively spliced, generating the active transcription factor form	[63, 65]
eIF2α	mRNA translation initiation factor. Becomes phosphorylated during ER stress, attenuating protein synthesis	[65, 66]
GRP78	An ER chaperone and central regulator of ER stress. Interacts with and keeps the three ER-stress-sensors inactive during normal conditions. Dissociates upon ER stress, initiating UPR	[62–68]
CHOP	A stress-specific proapoptotic transcription factor. During ER stress it promotes apoptosis	[70, 71]
NLRP3	NLRP3 inflammasome composes a multiprotein complex capable of sensing intrinsic dangers such as ER stress. NLRP3 activation is responsible for cytokine secretion and inflammation	[90–93]
TXNIP	Links ER stress and inflammation via NLRP3 activation. During severe ER stress TXNIP promotes apoptosis	[92, 93]
miR-211, miR-30c-2-3p	miRNAs involved in ER stress adaptation	[77–82]
HSF-1	Master regulator of heat shock response gene transcription	[8, 10, 46]
HSP-90	Inhibits the function of HSF-1 under normal conditions	[10, 17]
HSP-70	Attenuates the heat shock response by binding to the active HSF-1	[29]
DAF-16/FoxO	Transcription factor mediating insulin/insulin-like growth factor signaling	[31, 38–40]
SIRT-1	Deacetylase, regulating the heat shock response through acetylation of HSF-1	[27]
HSR-1	Constitutively expressed noncoding RNA, implicated in activating the heat shock response	[47, 48]
PHA-4/FoxA	Regulates the expression of HSP-90 in a cell-non-autonomous manner	[104]

domain of the protein. The active HSF-1 transcription factor is a trimer, formed by interactions between the HR-A/B domains [18] which localize in nuclear stress bodies (NSBs) [19]. In this form, HSF-1 is capable of binding specific DNA sequences, the heat shock elements (HSEs), which are located in the promoter region of heat

shock genes. The transcription activation domain (AD) at the carboxy end of HSF-1, is no longer repressed by intramolecular interaction with the regulatory domain, and after proper posttranslational modifications, HSF-1 can activate expression of the heat shock genes [10]. Such posttranslational modifications include phosphorylation [20–24], sumoylation [25, 26], or acetylation [27]. While sumoylation and acetylation suppress the function of HSF-1, phosphorylation can exert a negative or positive impact on HSF-1 activity. Phosphorylation of S303, S307 and S308 represses HSF-1 activation, while phosphorylation of S230, S326 and S419 after exposure to stress triggers the formation of HSF-1 trimers. HSF-1 possesses a phosphorylation dependent sumoylation motif (PDSM), where sumoylation of lysine 298 cannot occur without concomitant phosphorylation of serine 303 and 307. Acetylation of HSF-1 at residue K80 is required for the attenuation of the HSR. This event inhibits the DNA binding ability of HSF-1. Therefore, the extent of HSR depends on an acetylation–deacetylation cycle. Importantly, caloric restriction and the heat shock response act synergistically, and this cross talk requires the deacetylase SIRT1 [27, 28].

Association of HSF-1 with the heat shock protein, HSP70, attenuates HSR [29]. This negative feedback loop maintains appropriate levels of heat shock proteins during the HSR. Two regulators of SIRT1, AROS and DBC1 also modulate HSR. AROS positively regulates the deacetlyase activity of SIRT1, while DBC1 suppresses it. These two regulators influence transcription of hsp70 genes through recruitment of HSF-1 to the hsp70 promoter, and altering the acetylation status of HSF-1. This regulation may occur in a SIRT1-independent fashion, perhaps via alternative deacetylases [30].

Studies on the nematode *Caenorhabditis elegans* have contributed to a better understanding of the HSR, and its age-related decline. Recent studies have shown that proteostasis deterioration is coupled with the end of the reproductive period in *C. elegans*. Proteostasis collapses rapidly at this stage and declines gradually for the remaining duration of adult life. Overexpression of HSF-1 or DAF-16/FoxO reverses this rapid reduction, providing longer and healthier life span [31]. Maintenance of proteostasis has been linked to the germ line stem cells (GSC) and reproductive status. Sterile animals better preserve proteostasis in different somatic tissues, and this depends on several, nonredundant signaling pathways that involve HSF-1, DAF-16, DAF-12, DAF-9, DAF-36, NHR-80, and PHA-4 [32]. Therefore, without early GSC arrest, *C. elegans* cannot maintain the proper somatic proteostasis, leading to early death. Inhibition of oocyte production with the chemical 5-fluoro-2-deoxyuridine (FUdR) also improves the proteostasis and protects against stress. This effect is in part HSF-1, DAF-16, and DAF-12 independent [33].

Perturbation of HSF-1 function as well as the amount of chaperones found in the cell leads to changes in life span. Overexpression of HSF-1 or chaperone quantity elevation promotes long life [34–36], by better maintaining global proteostasis, while loss of HSF-1 results in premature ageing [37], and increased protein aggregate formation in cells. HSF-1 has also been implicated in life span extension by low insulin/IGF-1 signaling [38–40]. However, HSR and life span regulation do not always correlate. The *C. elegans gtr-1* (a G-protein coupled receptor) gene is required for the expression of HS genes, but it has no influence on life span [41]. Thus, although HSF-1 and proper HSR is essential for normal life span, there are exceptions where the HSR and the regulation of longevity pathways can be uncoupled.

Although HSF-1 is the master regulator of HSR, there are specific cases where other mediators are involved. In addition to HSF-1 (mammals) or HSF-3 (avians) activation during HSR, HSF-2 also becomes activated. Moreover, HSF-2 induces the expression of HS genes through increased activation of HSF-1 or HSF-3. Animals carrying HSF-2 mutations are more susceptible to mild heat shock, demonstrating the importance of HSF-2 in the regulation of proteostasis [42]. The primary hippocampal neurons of the neonatal rat embryos do not express HSF-1; therefore, they are incapable of responding to heat stress, while they express HSF-2 [43]. Mitotic cells are also hypersensitive to elevated temperature and proteotoxicity due to reduced binding and transactivating capacity of HSF-1 during the cell cycle. HSF-2 functions as an epigenetic regulator in mitotic cells. HSF-2 was shown to bind hundreds of loci or localize to condensed chromatin in mitotic and meiotic cells, respectively, driving transcription [44].

HSF-1 also plays an essential role in the proliferation of T cells. HSF-1 (−/−) T cells are unable to respond properly to immune system activating signals, and they exhibit cell cycle defects even at normal temperatures. In these cells the amount of cyclin E and cyclin A is reduced, without a large difference in their transcription [45]. Either HSF-1 is required for the transcription of regulatory genes which mediate translation of cyclin E and A proteins or HSF-1 itself is needed for the proper translation of these genes. Thus, investigating the tissue specific effects of HSF-1 and HSR could provide new insights into their regulation.

Although these findings provide a better understanding of the mechanisms and the functions of the HSR, several questions still remain. How stress stimuli are sensed, resulting in HSF-1 activation remains to be elucidated. Four models that are not mutually exclusive have been proposed, relevant to the triggering of the heat shock response [46]. The first model involves the HSP90 chaperone, as already described above. The second model suggests that a ribonucleoprotein complex consisting of the translation elongation factor eEF1A and a noncoding, constitutively expressed RNA molecule,

HSR-1 (heat shock RNA-1) catalyzes the HSF-1 trimer formation. Downregulation of HSR-1 by RNAi makes cells more susceptible to heat stress. Furthermore, ectopic expression of eEF1A and HSR-1 results in HSF-1 trimer formation [47]. In this case RNA molecules serve as sensors most probably via conformational changes [48]. A third model suggests that HSF-1 itself is capable of sensing changes in ambient temperature, transforming into the active form. This could explain the fact that in about 1 min after heat shock the hsp70 promoter is saturated with active HSF-1 trimers. A motif including disulfide bonds between two cysteine residues in the DBD domain and neighboring aromatic amino acids may serve as an intrinsic sensor on HSF-1 [49, 50]. The fourth model involves a nervous system controlled HSR. In *C. elegans* HSR is under the control of thermosensory neurons which regulate and coordinate the response in the whole organism (discussed below in more detail) [51]. Despite accumulating data, we are just starting to understand the tissue specific and cell-non-autonomous roles of HSR. Importantly, HSF-1 is not only active under stress conditions, but is an essential transcription factor also during development [52–57]. Moreover, HSF-1 is required for the survival of cancer cells [58]. This effect may be p53-dependent [59]. Therefore, HSF-1 may interact with p53 rendering the efficiency of cancer treatments dependent on the genetic background of cells [60, 61]. Thus, in addition to understanding fundamental cellular processes and stress response pathways, delineating the precise regulation of proteostasis through HSR could facilitate the development of new drugs against age-associated diseases.

3 Endoplasmic Reticulum Stress and the Unfolded Protein Response

The ER is a complex organelle performing various cellular functions. It is essential for the proper folding and post-translational modification of secreted and membrane-bound proteins and it also serves as a calcium storage organelle among other functions. Perturbations of ER homeostasis caused by physiological or pathological conditions result in ER stress, a condition characterized by overload of misfolded proteins. In response to ER stress, cells mount the unfolded protein response (UPR) to restore normal ER function.

3.1 Canonical and Noncanonical ER Stress-Induced Signaling Pathways

UPR is mediated by an elaborate signaling pathway that functions to ameliorate the accumulation of unfolded proteins in the ER. ER proteostasis is achieved either by proteasomal degradation of aberrant polypeptides in a process termed endoplasmic reticulum associated degradation (ERAD), by attenuating de novo protein synthesis or by inducing expression of chaperones, which are vital for proper protein folding [62–64]. The UPR is orchestrated by evolutionary conserved signaling events composing three consecutive phases

with different effector functions, namely, adaptation, alarm, and apoptosis. These phases are directed by three major ER stress sensors, the PKR-like ER kinase (PERK), the activating transcription factor 6 (ATF6) and the inositol requiring enzyme-1 (IRE-1). Accumulation of misfolded proteins in the ER triggers ER stress. As a consequence, the otherwise ER-stress-sensor-bound GRP78 chaperone dissociates from the three ER transmembrane receptors, launching UPR [64, 65]. During adaptation, the tripartite signaling cascade facilitates reestablishing normal proteostasis. Protein load in the ER is moderated by translation attenuation, effected by the PERK-mediated phosphorylation of eukaryotic initiation factor 2 (eIF2α) [66]. On the other UPR arm, ATF6 is subjected to proteolytic cleavage after translocation to the Golgi apparatus, forming a transcription factor responsible for the upregulation of ER chaperones such as GRP78 and GRP94 [67, 68]. Activation of IRE-1 facilitates XBP-1 activation, which serves as a transcription factor of genes involved in proteostasis [65]. In addition, the IRE-1 branch of the UPR may also induce apoptosis by causing endonucleolytic decay of ER-localized mRNAs during stress [69]. When adaptive mechanisms fail to compensate in the face of protracted or excessive ER stress, apoptosis is induced to protect the organisms by eliminating compromised cells. The proapoptotic transcription factor C/EBP homologous protein (CHOP), which blocks the expression of antiapoptotic protein BCL-2, plays a central role in these apoptotic mechanisms [70, 71]. ER stress and UPR pathways have also been implicated in the pathogenesis of diseases associated with stress responses. In addition to biochemical approaches [71, 72], novel tools for in vivo monitoring of ER stress uncover new aspects of the pathophysiology of ageing-associated diseases [73–76].

Apart from the extensively discussed canonical UPR pathways, accumulating evidence suggests that miRNAs are important determinants of ER stress responses [77, 78]. However, their role is only starting to be dissected. Intriguingly, UPR may induce or suppress miRNAs, some of which exerting pro-adaptive whereas others pro-apoptotic effects. miRNAs have been suggested to function as UPR rheostats, coupling different components of the response and regulating ER stress-induced apoptosis [79–82]. miR-211 has been identified as a prosurvival miRNA which serves as a switch between adaptation and the apoptotic phase of the UPR. Indeed, PERK-induced miRNA expression prolongs the adaptation phase by attenuating the expression of *chop*, thus delaying ER stress induced apoptosis [79]. A mechanism which converges two of the three UPR components has also been identified. This mechanism involves the PERK-mediated induction of a miRNA (miR-30c-2-3p), responsible for XBP-1 expression [80]. Nevertheless, the transition from adaptation towards apoptosis and the contribution of miRNAs to UPR dependent mechanisms are only now starting to be appreciated.

During ageing UPR components deteriorate, shifting the balance towards a more apoptotic pathway [83, 84]. In aged mouse livers, misfolded proteins accumulate as a consequence of decreased enzymatic activities of the ER chaperones PDI and GRP78 [85]. Additionally, PERK mRNA levels are significantly reduced in the hippocampus of aged rats compared to younger animals, yet GADD34 and CHOP expression levels are induced in the cortical tissue of aged mice and cells, indicating a shift from a protective adaptive response towards an apoptosis-competent response [83, 84, 86]. Hence, aged animal cells are more vulnerable to apoptotic cell death as a consequence of limited ER stress resistance.

Intriguingly, mild stress may exert beneficial effects, promoting longevity through adaptation, whereas severe ER stress may accelerate ageing and aggravate age-associated diseases. This phenomenon, termed hormesis, has attracted much attention, and UPR exhibits characteristics of a hormetic response. ER stress intensity may range between prolonged, damaging ER stress and mild, beneficial ER stress. Given that ER stress adaptation capacity declines during ageing, a slight induction of UPR may be an effective strategy to alleviate age-associated maladies and augment life span. This concept is supported by observations in β cells, which survive better and maintain physiological activity when UPR is restored or maintained at low levels, a mechanism that protects mice from type 1 diabetes [87].

3.2 Cross Talk Between ER Stress and Inflammation-Dependent Networks: From Adaptation to Death

As discussed earlier, UPR pathways sense different grades of proteotoxic stress and elicit different responses accordingly. However, the precise mechanisms leading to apoptotic cell death remain largely elusive. Noncanonical pathways mediated by miRNAs are partially responsible for the transition from the adaptation phase towards a pro-apoptotic phase. Additional noncanonical UPR pathways have been implicated in the regulation of UPR and the conversion of adaptation to terminal UPR. Some of these networks link stress signaling mechanisms to immune responses and inflammation. In *C. elegans* the apoptotic receptor CED-1 activates a network of PQN/ABU proteins which are involved in the noncanonical UPR pathway and immune response activation, enhancing animal survival [88]. This study provides evidence of how an apoptosis receptor (CED-1), evokes UPR during ER stress, preventing apoptosis. In another paradigm, neuronal expression of an octopamine G protein-coupled catecholamine receptor OCTR-1, limits innate immunity by downregulating PQN/ABU proteins and the p38 MAPK in non-neuronal cells [89]. These findings suggest that the nervous system regulates and links UPR with immune responses during exogenous threats in a systemic manner.

Inflammation, one of the first immune system responses to infection, has been linked to uncontrolled ER stress in inflammatory pathologies such as neurodegenerative diseases and type 2 diabetes.

The NLRP3 inflammasome has been implicated in sensing intrinsic ER stress, causing subsequent release of the highly pro-inflammatory cytokine IL-1β [90]. In astrocytes the link between ER stress and inflammation is attributed to the uncoupling protein 2 (UCP2). Loss of UCP2 induces ER stress and exacerbates NLRP3 inflammasome activation in astrocytes of the mouse midbrain [91]. Although the association of severe ER stress and inflammation has long been identified, the molecular connections between these two responses remain unknown. Thioredoxin-interacting protein (TXNIP) appears to tightly link irredeemable ER stress and NLRP3 inflammasome activation, leading to β cell death [92, 93]. Thus, TXNIP has been suggested to play an important role in switching from an adaptive response to the apoptotic response induced by severe ER stress. In conclusion, significant advances have been made in the past years towards clarifying the relationship between ER stress and inflammation and the transition between the different UPR phases. Further studies hold promise of identifying intervention targets to efficiently battle inflammatory diseases.

4 Systemic Effects in the Regulation of Stress Responses and Ageing

The ability of an organism to cope with endogenous and exogenous threats has a direct impact on physiology and healthy ageing. Organismal resistance against such hazards is achieved through physiological pathways that influence tissue communication, by coupling both cell-intrinsic and systemic events. To investigate the influence of such systemic effects on ageing and health span, whole animal studies are required. The genetic model organisms, *C. elegans* and *Drosophila*, have proven of great value and have contributed to shed ample light on cell-non-autonomous effects that regulate ageing. It is long known that dietary restriction and the insulin/insulin-like growth factor-like signaling pathway regulate organismal life span and mitigate or lessen age-related diseases. Nevertheless, the importance of hormonal and endocrine signals has not been fully appreciated.

In *C. elegans*, increased neuronal activity induced by dietary restriction contributes to life span extension. Alterations of nutritional status induced by caloric restriction activate *skn-1* in a pair of neurons (ASI neurons) in the head of the animal. This in turn leads to induced metabolic activity of non-neuronal body tissue and ultimately promotes longevity in an endocrine fashion [94]. Similarly, excision of insulin-like-peptide-producing cells from the *Drosophila* brain not only increases glucose levels, resembling effects in diabetic patients, but also induces stress resistance and life span extension due to systemic effects [95].

Similar genetic studies revealed that alterations in organismal life span and stress resistance driven by the germ lineage are also

due to endocrine effects [32, 96, 97]. Indeed, genotoxic stress in germ cells enhances systemic proteotoxic stress resistance. Specific extrinsic and intrinsic insults targeting the DNA of the germ cells may transiently activate innate immune responses, which in return trigger the ubiquitin–proteasome system (UPS) in somatic tissues. Somatic stress resistance and proteostasis are enhanced by immunity-related peptides secreted upon ERK MAP kinase activation in compromised germ cells [97]. Thus, protein homeostasis responses may be mediated by systemic effects. Similarly, ER and mitochondrial UPR likely exert systemic effects that have direct impact on longevity and age-related maladies. Although the consequences of mitochondrial function in longevity are long known [98, 99], the systemic effects exerted by mitochondria remain largely enigmatic. Neuronal or muscle specific ablation of *cco*-1 induces mitochondrial UPR in the intestine by a so far unknown mechanism, influencing the survival of the animal [100]. Systemic effects exerted by UPR have also been recently demonstrated, further to UPR initiated by cell non-autonomous signals [101, 102]. OCTR-1 expressing neurons modulate ER protein homeostasis in the gut during adulthood via regulation of the IRE-1/XBP-1 arm of the tripartite UPR signaling cascade [102]. In addition, neurotransmitters released upon ER UPR initiated by ER stress in a cell-autonomous fashion activate ER UPR in distal cells [101]. This activation confers protection against ER stress and ultimately promotes organismal longevity. Collectively, these findings highlight the importance of UPR coordination between distal cells towards maintaining protein homeostasis and prolonging ageing.

Analogous mechanisms also coordinate HSR pathways. Two recent studies in *C. elegans* demonstrate cell-non-autonomous control of the HSR. A genome-wide RNAi screen identified 7 positive and 59 negative novel modifiers of the HSR. These modifiers act at particular steps during the HSR. Interestingly, although negative regulators show tissue specificity, positive regulators are expressed throughout the animal [103]. More precisely, overexpression of *hsp-90* in the muscle leads to expression of the protein in tissues that normally do not express *hsp-90*, such as the intestine. Moreover, elevated expression of *hsp-90* in the intestine or neurons reduces the severity of muscle degeneration in *unc-54* mutants. These effects are regulated by the PHA-4 FoxA transcription factor [104]. FoxA-mediated expression of HSP90 maintains organismal proteostasis in a neuronal-independent cell-non-autonomous fashion providing, a global response towards preventing impairment of organismal health, and augmenting survival.

In *C. elegans*, the AFD thermosensory neurons coordinate organismal HSR induction. Regulation of HSR is due to neuroendocrine effects, since mutations affecting AFD neurons inhibit HSR in distal tissues [51]. AFD deficient animals are still capable of moderating protein aggregation by HSF-1-derived chaperone

expression, indicating that genes not involved in ambient temperature sensation are required for the expression of heat shock genes [105]. One candidate for this role is *gtr-1*, a G-protein coupled receptor not expressed in AFD neurons, but in other neurons necessary for the HSR [41]. Therefore, upregulation of HSF-1-regulated genes is also possible in a thermosensory neuron-independent manner.

These findings indicate that neuroendocrine signals mediate proteotoxic stress defense at the level of the whole organism. Indeed, recent studies have revealed that hypothalamic mitofusin 2 (MFN2) regulates whole body energy balance, by modulating ER/mitochondrial homeostasis and function in pro-opiomelanocortin (POMC) neurons [106]. Therefore, the nervous system rapidly responds to a variety of stimuli and releases warning signals to sensitize distal cells and tissues against threatening events.

5 Conclusions

Protein homeostasis is a fundamental prerequisite for cell survival. Subcellular compartment-specific stress responses are important determinants of cell proteostasis and crucial for organismal survival, health span, and life span (Fig. 1). Various age-associated diseases are caused by the deregulation of proteostasis [4, 15]. Protein aggregate deposition has been implicated in neurodegenerative disorders. Alzheimer's disease, Parkinson's disease, and Huntington's disease are some of the late-onset pathologies associated with protein malformation and impaired protein aggregate clearance mechanisms [107].

Numerous studies in cell cultures and animal models indicate that the ability of cells to respond efficiently to detrimental environmental effects declines with age [108]. In vitro [109, 110] and in vivo [111–114] findings have revealed that the amount of HSP70 proteins in response to heat shock decrease during ageing. However, HSF-1 protein levels remain constant throughout life span. Instead, the DNA-binding ability of HSF-1 is impaired in aged rat tissues, compared to young controls [115, 116]. Overexpression of DNAJ chaperones suppresses the cytotoxic effects exerted by mutant huntingtin aggregates in cells [117] and flies [118], and improves mental skills of mouse models of Huntington's disease [119]. By contrast, HSR is blocked by accumulation of polyglutamine-expanded huntingtin protein [120, 121]. Surprisingly, the most affected HSF-1 target genes are involved in cytoskeletal binding, focal adhesion and GTPase activity, rather than in proteostasis [121]. Overexpression of the HSP70 interacting protein (Hip) increases the efficiency of HSP70 binding to its substrates leading to reduced accumulation of the polyglutamine-expanded androgen receptor, improving the

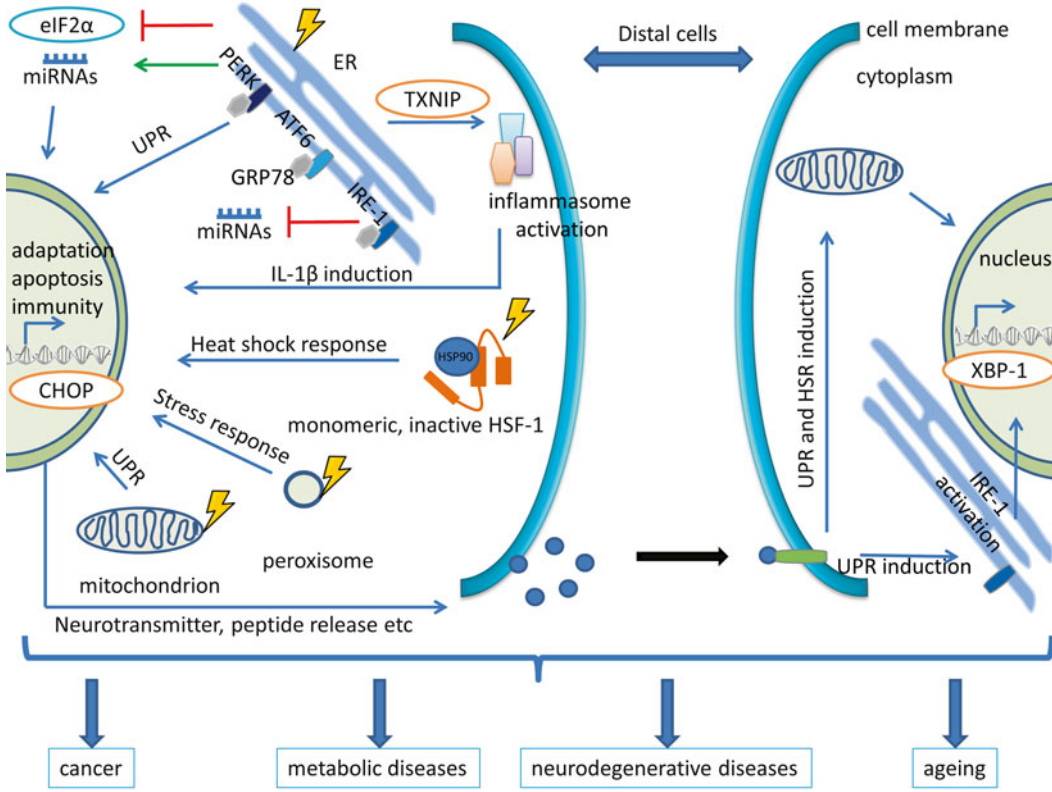

Fig. 1 Proteotoxic stress response mechanisms. Cell-autonomous and cell-non-autonomous stress response pathways triggered under conditions of cellular stress. On the upper left part, signals from the stressed ER are depicted, promoting cellular adaptation through eIF-2α phosphorylation and regulation of miRNAs. Switching from adaptation to apoptosis is part of the noncanonical pathways, involving inflammasome activation and IL-1β secretion. Additional organelle-specific response pathways important in maintaining proteostasis, including the heat shock response and the mitochondrial and peroxisomal UPR are shown. Collectively, these stress response pathways have been implicated in the regulation of longevity, and in the pathogenesis of ageing-related disorders via endocrine effects exerted mainly by neuron-released peptides

symptoms of spinobulbar muscular atrophy [122]. Another activator of HSP70, ML346, a barbituric acid scaffold acts through HSF-1, FOXO, and Nrf-2 to induce chaperone expression and proper protein folding in conformational diseases [123]. These new regulatory molecules comprise attractive intervention targets against diseases associated with aberrant HSR.

Additionally, the cross talk between ER stress and inflammation has been implicated in obesity and metabolic dysfunction [124]. Metabolic diseases such as diabetes and obesity are associated with proteotoxic stress and more specifically with the UPR network [125]. For example, the insulin secreting β-cells are more susceptible to ER stress induced apoptosis when the PERK component of the UPR is compromised, resulting in the manifestation of diabetes [126, 127]. Interestingly, administration of

tauroursodeoxycholic acid, an ER stress mitigator, confers protection against type 1 diabetes, through UPR regulation and β-cell preservation [87]. In metazoans, compounds that bind and stain amyloid-β deposits and enhance proteostasis also promote longevity [128]. Such small compounds, which specifically regulate UPR pathways, may be effective in interventions against diseases associated with aberrant HSR.

Stress responses and inflammation also play crucial roles in the development of tumors. Cancer cells are characterized by alterations in metabolic activity some of which resemble the metabolic response of non-transformed cells [129]. This metabolic activity directly depends on the tissue microenvironment; however, the role of paracrine and endocrine signals is not well understood. Given that proliferating tumor cells require increased protein folding, manipulation of proteostasis and stress-response pathways may provide a promising therapeutic strategy against cancer. Apoptosis could be triggered in cancer cells by inducing severe stress. Alternatively cancer cells could be mitigated by completely abrogating and limiting stress responses, impairing adaptation to stressful conditions. To this end inhibitors or small molecules targeting UPR pathways have been developed to ameliorate protein misfolding diseases or as potential anticancer drugs with some promising results [130–134].

Stem cells exhibit high proteasome activity allowing them to cope with proteotoxic stress and avoid replicative senescence. Recent studies have revealed novel players of proteostasis in human embryonic stem cells (hESCs) that link longevity and stress resistance. PSMD1 has been shown to efficiently promote proteostasis in hESCs [135]. α-Synuclein also appears to play an important role during pluripotent stem cell (iPS) differentiation, involving ER stress response pathways [136]. Furthermore, ER stress plays important role in epithelial stemness, through UPR, in a PERK dependent manner [137]. HIF-2α also contributes to the maintenance of human hematopoietic stem/progenitor cell (HSPCs) and in the survival of human acute myeloid leukemia cells by protecting against ER stress-induced apoptosis [138]. Given that stem cells are required for tissue regeneration, and ageing is associated with decay in regeneration potential, proteostasis may promote longevity by maintaining the normal function of stem cells.

The signaling pathways described here collectively restore or maintain normal protein homeostasis levels through reducing demand and limiting protein aggregation, by enhancing proper folding and therefore safeguarding against proteostasis-related diseases. Mild stressors could potentially be used to precondition cells to more effectively respond to metabolic stress and ageing. Therefore, preadaptation against upcoming stress insults could provide an efficient strategy for an organism to better cope against life-threatening hazards. To this end it is crucial to further

understand the molecular networks involved and identify thresholds below which a stressor exerts beneficial effects, thus minimizing deleterious consequences. In addition, cellular therapies against degenerative and age-associated diseases with a prominent cell non-autonomous component may prove beneficial. However, major advances are still required in order to understand how these organelle-specific pathways may impinge on whole organismal survival in an autocrine, paracrine or endocrine fashion.

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