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Opinion

TGF-ß signaling as an organismal proteostasis regulator

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Various mechanisms act in a coordinated manner to maintain proteostasis in different cellular organelles. Nevertheless, with aging, certain proteins escape proteostasis surveillance, misfold, and aggregate. This process can lead to neurodegeneration. Despite the cellular nature of proteostasis, it is regulated by intertissue communication. How these intertissue signaling mechanisms coordinate proteostasis across the organism is largely obscure. Recent studies unveiled that the transforming growth factor (TGF)-β signaling cascade is an organismal proteostasis regulator. Here, we focus on the known roles of the TGF-β pathway as a coordinator of proteostasis and describe the messengers and biological activities that are controlled by this pathway. We also discuss open questions and highlight the potential clinical relevance of these discoveries.

Highlights

The proteostasis network (PN) is a nexus of mechanisms that maintain the integrity of the proteome in different cellular organelles. The competence of the PN declines with age, exposing the organism to proteinopathies.

The PN is regulated at the organismal level by intertissue communication.

The tumor growth factor (TGF)-β signaling cascade emerges as a proteostasis-regulating pathway that is activated by mitochondrial stress, exposure to bacterial metabolites, and knockdown of the Fibrillarin complex.

In the nematode *Caenorhabditis elegans*, ASI neurons have crucial roles in the regulation of organismal proteostasis by governing TGF-β signaling.

The integrity of the proteome is supervised and maintained by the proteostasis network

Cellular and organismal functionality is entirely dependent on the integrity of the proteome. To maintain accurate **protein homeostasis (proteostasis)** (see Glossary), various biological mechanisms act in a coordinated manner to assist and supervise proper maturation of nascent polypeptides, and preserve the integrity of mature proteins throughout their lifecycles [1]. Early in life, these specialized mechanisms, which are collectively known as the 'proteostasis network' (PN), competently maintain proteostasis. However, aging, mutations, and environmental stressors promote protein misfolding, overwhelming the PN and impairing its ability to clear aggregated proteins. This hazardous process jeopardizes proteostasis and, in some cases, is associated with the development of a group of maladies that are collectively known as '**proteinopathies**' [2]. While proteinopathies affect various organs, the most prevalent diseases of this group cause brain degeneration. This group of neurodegenerative disorders includes Alzheimer's disease (AD) [3], Huntington's disease (HD) [4], limbic-predominant age-related TDP-43 encephalopathy (LATE) [5], and amyotrophic lateral sclerosis (ALS) [6], all incurable, late-onset maladies that exert major burdens on patients, public health systems, and societies across the globe.

Although these illnesses are tightly linked with toxic protein aggregation **(proteotoxicity)**, they exhibit key mechanistic differences. AD is associated with the aggregation of β amyloid (Aβ) peptides, cleavage products of the amyloid precursor protein (APP), which create highly toxic oligomers [7]. Polyglutamine (polyQ) expansion diseases comprise a group of at least nine proteinopathies [8], which are related to the aggregation of proteins that bear abnormally long stretches of polyglutamine (polyQ) repeats [9]. This group of maladies includes HD, which is correlated with the aggregation of huntingtin [4], and Machado-Joseph disease (MJD), which is linked with the aggregation of Ataxin-3 [10].

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Here, we delineate the links between neurodegeneration-causing toxic protein aggregation and signaling mechanisms that coordinate proteostasis across tissues, focusing on **TGF-β**.

Organelle-specific proteostasis-restoring mechanisms

The PN not only functions to maintain the integrity of the proteome under unstressed conditions, but also has nodes that are activated upon exposure to acute stress. Metabolic and environmental insults, such as elevated temperature and exposure to toxic compounds, lead to massive protein aggregation, which challenges the PN and suppresses proteostasis. To cope with acute proteotoxic stress, cellular organelles have evolved specialized stress response mechanisms that recognize misfolded polypeptides and modulate gene expression to restore proteostasis.

One such stress response mechanism is the heat shock response (HSR) [11]. This mechanism is activated upon a rapid and significant increase in temperature, and the subsequent accumulation of aggregated proteins in the cytosol. The HSR modifies gene expression to elevate the levels of molecular chaperones, collectively known as **heat shock proteins (HSPs)**. This cascade of events is initiated by members of the HSP70 family, which recognize misfolded proteins and activate transcription factors, such as heat shock factor 1 (HSF-1). Upon exposure to heat, HSF-1 enters the nucleus, forms trimers, and induces the expression of various genes, including HSP-coding genes [12]. HSPs act in a coordinated manner to refold damaged proteins and reinstate cytosolic proteostasis [13] (Figure 1A).

Similar mechanisms respond to the accumulation of misfolded proteins in other cellular organelles, including the endoplasmic reticulum (ER) and mitochondria [14]. The ER stress response mechanism, known as the ER **unfolded protein response (UPR)**^{ER}, has at least four branches. Three of these share common principles. The HSP70 family member BiP/Grp78 senses the misfolded proteins accruing within the ER lumen and activates ATF6, IRE1, and PERK, which in turn, trigger the translocation of transcription factors (ATF6, XBP1, and ATF4, respectively) into the nucleus. Here, these factors enhance the expression of genes that encode ER chaperones, which increase the protein-folding capacity within the lumen of this organelle. They also enhance the levels of factors that elevate **ER-associated protein degradation (ERAD)**. Activation of the stress sensor PERK also triggers phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2α), which reduces protein synthesis to lessen the burden on the protein-folding machinery [15] (Figure 1B).

The fourth UPR^{ER} pathway was discovered in a *Caenorhabditis elegans* strain that carries mutated *xbp-1* and, thus, is incapable of activating the IRE-1 and XBP-1 signaling pathway. Exposure of these animals to ER stress resulted in elevated expression of at least 34 genes. Nine of these genes share sequence similarities and resemble a mammalian cell surface scavenger receptor that directs damaged extracellular proteins to lysosomal degradation. This family of nine genes were termed 'activated in blocked UPR' (*abu) genes* [16].

Mitochondria also respond to protein misfolding by activating gene expression programs in the nucleus and mitochondrial genome [17]. This mechanism, termed the 'mitochondrial unfolded protein response' (UPR^{mt}), [18,19], was first described in mammalian cells [20], and later in worms [21]. It shares similar principles with the HSR and UPR^{ER}. Proteostasis imbalance within the mitochondria is sensed by the HSP70 family member HSP6, which elicits mitochondria-to-nucleus signaling. This activates two transcription modulators, ATFS-1 and DVE-1, which translocate into the nucleus and induce the expression of various gene networks, including genes that encode mitochondrial chaperones and proteases [22,23]. The UPR^{mt}-induced gene products are shuttled to the mitochondria, where they act to restore proteostasis [19,24] (Figure 1C).

Glossary

ER-associated protein degradation (ERAD): specialized mechanism that retrotranslocates misfolded proteins from the ER lumen to the cytosol, where they are degraded by the ubiquitin–proteasome system.
Heat shock proteins (HSPs): set of chaperones that are activated when cells are exposed to elevated temperatures. These chaperones are also involved in the supervision and promotion of proteostasis under different proteotoxic conditions and in unstressed state.
Protein homeostasis (proteostasis): describes the nexus of molecular mechanisms that act in concert to maintain the integrity of the proteome. These mechanisms supervise protein synthesis, folding, post-translational modifications, and intermolecular interactions, control protein aggregation, and direct terminally misfolded proteins for degradation.
Proteinopathies: groups of disorders that emanate from the accumulation of toxic protein aggregates in cells and tissues. Neurodegenerative disorders, such as Alzheimer's disease and Huntington's disease, comprise a subgroup of proteinopathies.
Proteotoxicity: general term that describes various toxic effects that stem from protein misfolding and aggregation. In many cases, proteotoxicity underlies the development of late-onset disorders.
Transforming growth factor (TGF)-β: signaling pathway with key roles in the development and functionality of mammals and nematodes. Recently, it was also found to be a coordinator of organismal proteostasis.
Unfolded protein response (UPR): common name of several mechanisms that are activated in various cellular organelles when misfolded proteins challenge organellar proteostasis. Upon the accumulation of misfolded proteins, a chaperone member of the HSP70 family initiates a cascade of events that culminates in activation of a transcription factor and modulation of gene expression that assists the restoration or proteostasis in the affected organelle. UPR mechanisms function in the cytosol (HSR), endoplasmic reticulum (UPR^{ER}), and mitochondria (UPR^{mt}) and probably in additional organelles.

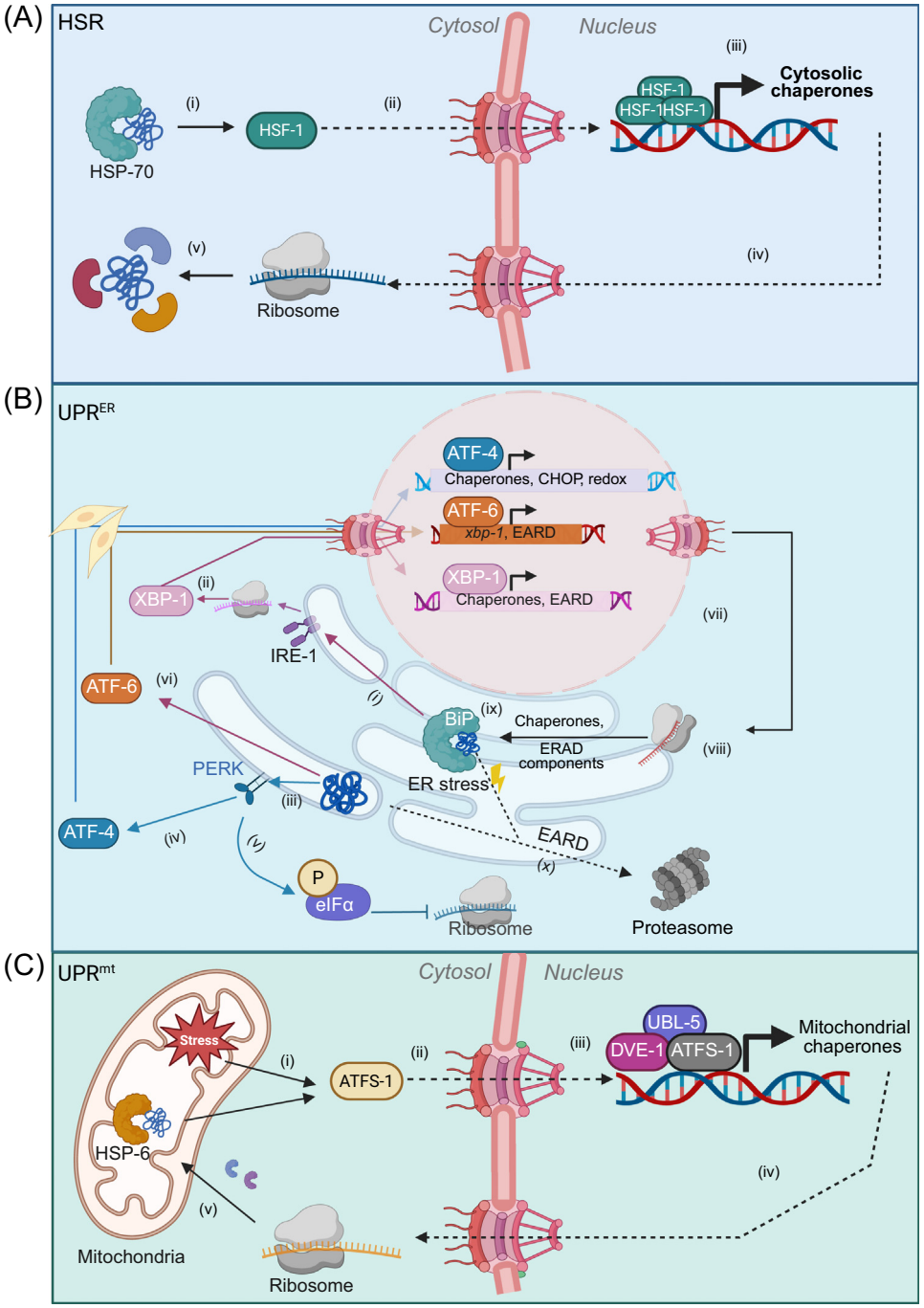

Figure 1. Cellular stress response mechanisms. Cellular organelles respond to protein aggregation by activating stress response mechanisms that modulate gene expression to promote protective activities and restore proteostasis. (A) Elevated temperature impairs proteostasis in the cytosol and activates the heat shock response (HSR). (i) A cytosolic heat shock protein (HSP)-70, recognizes protein aggregates and (ii) promotes the translocation of heat shock factor (HSF)-1 into the nucleus, where (iii) it regulates its target genes, including chaperone-coding genes. (iv) The resulting transcripts are translated and the proteins help restore proteostasis. (B) Three canonical endoplasmic reticulum (ER) unfolded protein

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The analogous mammalian UPR^{mt} mechanism supervises mitochondrial integrity in cells by initiating a signaling cascade, which activates the ATFS-1-related transcription factor, ATF5 [25].

Various studies nominate the nucleus as an additional key protein quality control organelle. First, nuclear and cytosolic aggregated proteins have been shown to be deposited in an intranuclear quality (INQ) compartment, which is adjacent to the nucleolus [26], indicating that misfolded cytosolic proteins are sequestered by a nuclear quality control mechanism. This has been further supported by the observation that defective ribosomal products are shuttled from the cytosol of mammalian cells to the nucleolus, where they are degraded by the ubiquitin–proteasome system (UPS) [27] (Figure 2). In *C. elegans*, the linker of nucleoskeleton and cytoskeleton (LINC) complex, which mediates transcriptomic modulations in response to mechanosensory stimuli, is involved in the promotion of Aβ-mediated proteotoxicity [28]. Similarly, components of the LINC complex regulate proteostasis in *Drosophila* [29], and govern cell senescence in mammalian systems [30].

These stress response mechanisms communicate [31] to enhance the organismal adaptability to ever-changing environmental conditions, and enable the maintenance of proteostasis and preservation of organismal functionality. The HSR and UPR mechanisms appear to function in an intracellular fashion, sending signals from the challenged organelle to the nucleus to modulate gene expression. However, mounting evidence indicates that intertissue communication orchestrates proteostasis at the organismal level.

Orchestration of proteostasis across the organism by neurons and glia

When *C. elegans*, a useful model organism for the study of aging and proteostasis [32], is exposed to a gradient of temperatures, it migrates to the area where the temperature is similar to its cultivation temperature. This thermotactic behavior depends on the activity of several sets of neurons, including the AFD thermosensory neurons and their postsynaptic partners, the AIY interneurons [33]. Surprisingly, loss-of-function mutations in *gcy-8*, which lead to inactivation of AFD neurons, prevented heat-stressed animals from activating the HSR in distal tissues. Analogously, mutations that cause *ttx-3* inactivation, thereby preventing AIY neurons from completing their development [34], also lead to the inability of remote tissues to activate the HSR when the worm is exposed to heat. This organismal HSR regulation is executed, at least partially, by serotonin [35]. The HSR is also regulated across the organism by glial cells, independently of the thermosensory neuronal circuit [36]. This regulation was observed when *hsf-1* was overexpressed in astrocyte-like cephalic sheath glial cells. This led to the induction of a noncell-autonomous HSR in peripheral tissues, prolonged lifespan, and elevated resistance to heat stress

response pathways (UPR^{ER}) are activated when aggregated proteins accrue within the lumen. (i) The chaperone BiP recognizes protein aggregates within the ER and activates IRE-1, which (ii) promotes the splicing and translation of the transcription factor XBP-1. (iii) XBP-1 enters the nucleus and regulates the expression of ER chaperones and components of the ER-associated protein degradation (ERAD) pathway. The kinase PERK is also activated when ER proteostasis is impaired. (iv) PERK leads to the migration of the transcription factor ATF-4 into the nucleus and (v) phosphorylates the elongation factor eIF2α, thereby inhibiting the translation of chaperone-client proteins. (vi) The third UPR^{ER} mechanism activates the transcription factor ATF-6, which is shuttled to the nucleus, where it elevates the expression of xbp-1 and of genes that encode ERAD components. (vii) The transcripts that result from the activities of UPR^{ER}-regulated factors are exported to the cytosol and (viii) translated. The resulting proteins are transported into the ER and help restore proteostasis either by (ix) refolding aggregated proteins or (x) directing them for degradation. (C) The mitochondrial unfolded protein response (UPR^{mt}) functions in the mitochondria and is activated by metabolic impairments, such as oxidative stress or the accumulation of protein aggregates. (i) Aggregates are recognized by the chaperone HSP-6, which (ii) directs the transcription factor ATFS-1 into the nucleus, where (iii) it teams up with UBL-5 and DVE-1 to modulate gene expression. (iv) The resulting transcripts are translated in the cytosol and (v) transported into mitochondria to restore proteostasis. Figure was created using BioRender.

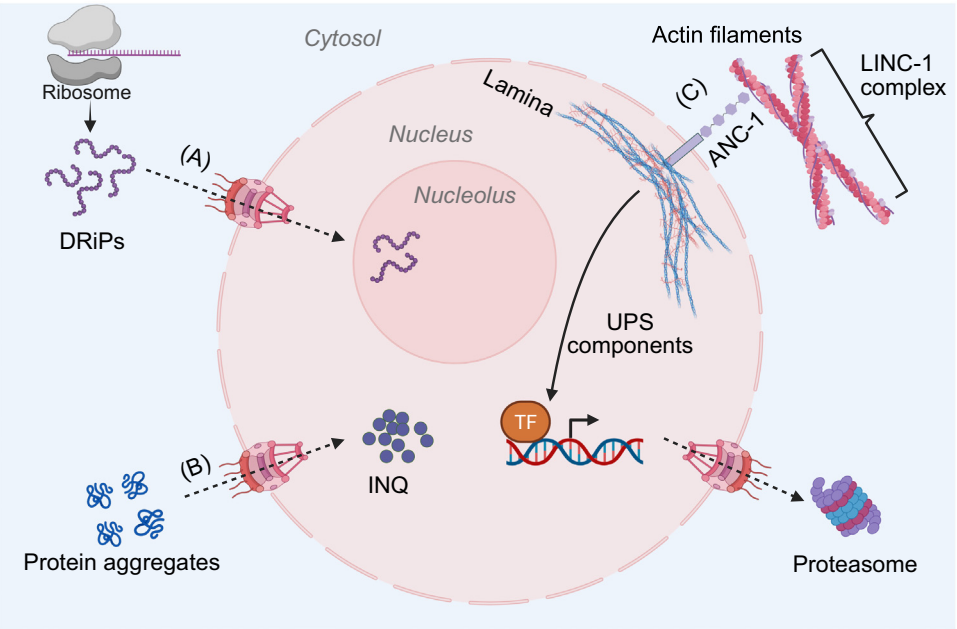

Figure 2. Roles of the nucleus in proteostasis. (A) Excessive defective ribosomal products (DRiPs) are shuttled to the nucleolus, where they undergo ubiquitin–proteasome system (UPS)-mediated degradation. (B) Certain cytosolic aggregated proteins are convoyed and deposited in the nucleoplasm at deposition sites known as the intranuclear quality compartment (INQ). (C) The linker of nucleoskeleton and cytoskeleton (LINC) complex modulates gene expression to enhance UPS activity and promote proteostasis. Figure was created using BioRender.

and pathogenic bacteria. These observations established a regulatory link between neurons, glia, and the soma [37]. Similarly, the knockdown of the neuronal genes *gtr-1* or of *nhl-1*, both are expressed in chemosensory neurons, averted HSR induction in remote cells [38,39].

Various studies have indicated that the HSR is not the sole stress response mechanism that is regulated across tissues by signaling mechanisms. To test whether UPR^{ER} activity is coordinated at the organismal level, worms that express a constantly active XBP-1 in their neurons (XBP-1s) were created. This expression was capable of initiating the UPR^{ER} in distal tissues, thereby enhancing proteostasis and prolonging lifespan via cell non-autonomous signaling mechanisms [40]. Furthermore, the expression of XBP-1s in targeted glial cells or specifically in RIM and RIC interneurons, triggers the UPR^{ER} within intestinal cells, thereby augmenting stress resistance and promoting longevity through neuropeptide signaling [41,42]. In addition, the ASI-RIM/RIC neuronal circuit has a significant role in the activation of UPR^{ER} subsequent to odorant exposure [41]. Dopaminergic neurons were also found to participate in the organismal orchestration of UPR^{ER} [43]. These findings underscore the roles of various neurons, interneurons, and glial cells as facilitators of organismal stress responses and longevity via intertissue communication across the nematode.

Similarly, neurons regulate the activity of the UPR^{mt} cell non-autonomously [44]. This involves ASI and RIM neurons, which govern this mechanism through TGF-β signaling pathways. DAF-7, a TGF-β morphogen mainly produced by ASI sensory neurons [45], interacts with DAF-1 receptor in RIM interneurons to synchronize the intestinal UPR^{mt} during neuronal mitochondrial stress. Moreover, the induction of mitochondrial stress within ASI neurons has been shown to precipitate intestinal UPR^{mt}, extend lifespan, enhance resistance to pathogens, and modify metabolism.

Additional signaling mechanisms that originate from neurons, facilitated by retromer-dependent Wnt signaling [46], and the secretion of serotonin and neuropeptides [47–50], were also found to regulate UPR^{mt} across tissues [51]. Glia cells are vital contributors to the coordination of UPR^{mt} at the organismal level, because the activation of this stress response mechanism in astrocyte-like glial cells conveys signals to neurons, which subsequently transmit the signal to peripheral regions [52].

These studies substantiate the regulatory roles of sensory neuronal circuits, glial cells, and the germline as coordinators of stress responses, which sense proteotoxic threats and activate neuroendocrine mechanisms to communicate the danger to other tissues [53]. They also predict that the activation of stress response mechanisms will promote protection from chronic proteotoxicity. Nevertheless, a loss-of-function mutation in *gcy-8* mitigated proteotoxicity in model *C. elegans* that express polyQ-YFP stretches in their muscles [54]. Analogously, the knockdown of *gtr-1* or of *nhl-1* alleviated the toxicity of Aβ that was expressed in muscles [38,39]. The surprising opposing effects of sensory neuron deactivation on acute proteostasis impairments, such as heat shock, and chronic stresses, like the expression of aggregation-prone proteins, may be explained by a dual functional role of these neurons. These cells not only activate stress response mechanisms upon exposure to hazardous conditions, but also actively suppress the induction of these programs when *C. elegans* is unstressed [55]. Accordingly, inactivation of these sensory circuits alleviates the negative regulation, enabling *C. elegans* to efficiently respond to chronic stress [54]. These insights raise the questions of which signaling mechanisms communicate sensory information to distal tissues and whether they are linked with the aging process.

Aging is a highly regulated process that governs proteostasis

What underlies the progression of aging and why the integrity of molecules, cells, and tissues deteriorate over time, are key questions that have puzzled scientists for decades. While aging was thought to be an utterly stochastic process [56], it became clear that this process is partially regulated, and governed by several signaling pathways [57]. Two prominent aging-regulating pathways are the insulin/IGF signaling (IIS) [58] and the TGF-β cascade [59]. Reducing IIS activity extends lifespans of different organisms, including *C. elegans*, *Drosophila* [60,61], mice [62], and probably humans [63]. The genome of *C. elegans* encodes a single insulin/IGF receptor, *daf-2* [64]. DAF-2 negatively regulates the activities of several transcription factors by controlling their cellular localization. DAF-16/FOXO [65] and SKN-1/NRF [66] are IIS-regulated transcription factors that are retained in the cytosol by IIS-mediated phosphorylation. Similarly, the cellular localization of HSF-1, a transcription factor that is vital for the lifespan-regulating functions of the IIS [67], is indirectly controlled by IIS-governed phosphorylation [68]. Accordingly, knocking down the expression of *daf-2* hyperactivates its downstream transcription factors, modifies gene expression, and promotes longevity [58,66,67].

A mechanistic link between aging and proteostasis has been demonstrated by several lines of research. For example, IIS reduction mitigates the toxicity of myriad aggregation-prone, neurodegeneration-causing proteins. Aβ-mediated toxicity is alleviated by the knockdown of *daf-2* in *C. elegans* [69] and reducing IGF1 signaling alleviates AD-associated phenotypes in model mice [70,71]. Similarly, IIS reduction by genetic [72] or chemical [73] means protects *C. elegans* from polyQ-promoted toxicity, as well as from the toxicity of additional disease-causing aggregative proteins [74]. This counter-proteotoxic axis involves all the aforementioned IIS-regulated transcription factors, which are also crucial for proteostasis maintenance [69,75,76]. Similarly, dietary restriction, which also slows the pace of aging [77], mitigates the toxicity of proteotoxic proteins in *C. elegans* models [78].

Another aging-regulating signaling pathway is the TGF-β signaling cascade, which has various functions in development, tissue homeostasis, control of chromatin modeling, and transcriptomic landscape in different cell types [79]. In mammals, this pathway has two main downstream branches; one is the TGF-β/activin pathway, which primarily signals through SMAD2/3 proteins, and regulates different processes, including cell proliferation and differentiation. The second branch is the bone morphogenetic protein (BMP) pathway, which signals through the SMAD1/5/8 proteins, and, among other functions, governs development and tissue regeneration [80]. These pathways are conserved in *C. elegans* and, among the five *C. elegans* genes that encode TGF-β ligands, *daf-7* is a regulator of lifespan [59]. This effect of TGF-β on aging is mediated through the transcriptional regulator DAF-3, which functions in association with DAF-5, and is controlled by the TGF-β/DAF-7 pathway [81]. Under unstressed conditions, TGF-β signaling enhances the DAF-8/DAF-14/SMAD pathway activity, and suppresses the DAF-3/DAF-5 branch to support reproductive development.

Since the roles of the IIS as a coordinator of proteostasis have been comprehensively reviewed elsewhere [82,83], we focus here on the emerging roles of TGF-β as a coordinator of organismal proteostasis and its links with the neuronal and reproductive systems.

TGF-β regulates unfolded protein response mechanisms and proteostasis across the organism

While TGF-β signaling is known to regulate various biological processes [84], recent studies highlight its pivotal role in proteostasis. In *C. elegans*, TGF-β/DAF-7, which is mainly secreted by ASI sensory neurons [45], orchestrates cell non-autonomous activation of the UPR^{mt} in the intestine during neuronal mitochondrial stress. This requires RIM interneurons and components of the TGF-β pathway, including the receptors DAF-1 and DAF-4. Strikingly, ASI-specific mitochondrial stress alone is sufficient to induce UPR^{mt} activation in the intestine, leading to extended lifespan and increased pathogen resistance, which are dependent on TGF-β signaling [85]. Similarly, chemosensory neurons of *C. elegans* detect and enable the avoidance of pathogenic bacteria via TGF-β-dependent mechanisms [86]. Upon exposure to metabolites of hazardous bacteria, *C. elegans* activates the UPR^{ER} as a defense mechanism. Similar to the activation of UPR^{mt}, this stimulation is governed by the RIM neurons and entirely dependent on DAF-7 and its receptor DAF-1 [87] (Figure 3).

Collectively, these studies define a neuronal axis that regulates the UPR mechanisms of the mitochondria and ER across *C. elegans* tissues by modulating TGF-β signaling. However, is this regulatory axis conserved from worms to mammals?

Numerous indications suggest that TGF-β signaling is also a cellular proteostasis regulator in different mammalian tissues. For instance, activation of TGF-β signaling promotes muscle wasting and aging by directing muscle proteins to proteasomal degradation [88]. Moreover, this pathway has bidirectional relations with autophagy, given that, on the one hand, it induces protein digestion by autophagy in human hepatocellular carcinoma cell lines [89] and, on the other hand, its activity is governed by autophagy in human myofibroblasts [90]. TGF-β signaling also promotes autophagy in worms, because DAF-7 acts as a systemic factor that activates autophagy in distal cells in response to cuticle damage [91]. Reduced activity of TGF-β signaling was also reported to lower the rates of necrosis and inflammation, and promote proteostasis in the ER and mitochondria of rats [92]. The links between TGF-β and the integrity of mitochondria in mammals have been further supported by the finding that, in human fibroblasts, TMEM2-induced extracellular matrix (ECM) remodeling drives mitochondrial fragmentation and activates the UPR^{mt}. This requires TGF-β signaling to promote mitochondrial fission [93].

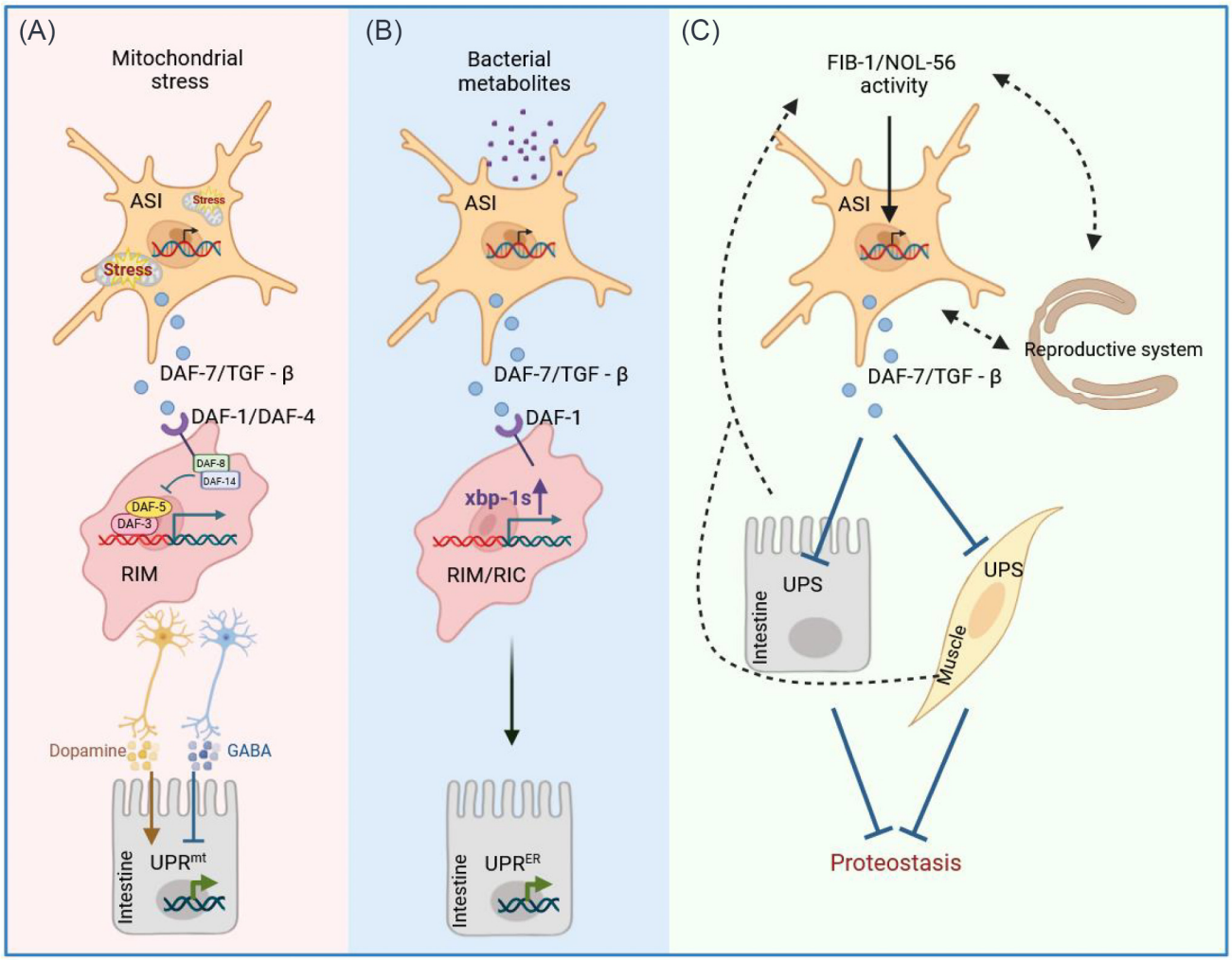

Figure 3. The regulation of stress response mechanisms and proteostasis signaling in *Caenorhabditis elegans* by transforming growth factor (TGF)-β. (A) Mitochondrial stress and (B) exposure to bacterial metabolites are sensed by ASI neurons. This leads to modification of gene expression and secretion of TGF-β (DAF-7), which binds the receptor DAF-1/4 and activates the transcription factors DAF-3 and DAF-5. (A) The signal induced by mitochondrial stress leads to the secretion of dopamine and/or GABA, which activate or repress the mitochondrial unfolded protein response (UPR^{mt}) respectively, in the intestine. (B) Bacterial metabolite-induced signaling enhances the endoplasmic reticulum unfolded protein response (UPR^{ER}) in intestinal cells. (C) The activity of the FIB-1-NOL-56 complex in somatic cells is sensed by ASI neurons. Although this involves the reproductive system, it remains unclear whether this communication is dependent on neurons or whether muscle and intestinal cells directly communicate with the reproductive system. The signaling induced by the activity of the FIB-1-NOL-56 complex suppresses ubiquitin–proteasome system (UPS) activity, thereby impairing proteostasis. Figure was created using BioRender.

The conserved roles of TGF-β signaling in the regulation of acute proteotoxic conditions raise the question of whether this signaling pathway also controls the PN responses to chronic proteotoxicity and, if so, which organelles are involved in this regulation.

Given its nuclear proteostasis-regulating roles, and that reducing the activity of the nucleolar FIB-1 complex extends lifespan of nematodes [94], we asked whether this complex also involved in the coordination of proteostasis in the face of chronic proteotoxicity? Knocking down the expression of the methyltransferase coding gene *fib-1,* or of its interacting, 2′-*O*-methylation-performing partner *nol-56* [95] in *C. elegans that express Aβ or polyQ35-YFP in their muscles* greatly

mitigated proteotoxicity of these disease-causing proteins. This protection was dependent on TGF-β signaling and its downstream transcriptional regulator DAF-3. Since TGF-β signaling negatively regulates the activity of DAF-3 [96], it is conceivable that knockdown of *nol-56* reduces TGF-β activity, thereby hyperactivating DAF-3 to promote proteostasis. HSF-1 is also needed for *nol-56* RNAi-mediated protection from proteotoxicity. This observation is consistent with the role of HSF-1 as a negative regulator of *daf-7* [97]. Given that HSF-1 is negatively regulated by the IIS [68], and that this transcription factor interwinds the IIS and TGF-β signaling pathways [97], these results suggest that these two mechanisms also functionally interact as regulators of organismal proteostasis through HSF-1. ASI neurons, which govern UPR mechanisms and regulate lifespan [85,87], also coordinate proteostasis across the organism in a TGF-β-dependent manner. This orchestration of proteostasis is achieved, at least partially, by enhancement of UPS activity [98].

Taken together, these studies clearly show that TGF-β signaling is a regulator of organismal proteostasis, and propose that this pathway functions in ASI and RIM neurons to regulate the activities of multiple proteostasis-promoting mechanisms, in addition to the UPS. They also raise the question of which messengers carry the proteostasis-promoting TGF-β-regulated signals from neurons to distal cells.

Outstanding questions

How do somatic tissues inform neurons which proteotoxic protein is challenging their proteostasis?

What neuronal components receive and integrate the signals?

Which messengers carry the proteostasis-promoting signal to the target tissues and which receptors receive it?

What are the roles of the reproductive system in the orchestration of proteostasis across the organism?

How does the TGF-β pathway respond to dissimilar proteotoxic challenges in remote tissues?

Neuron to soma messengers that carry the TGF-β proteostasis-promoting signal

The identification of messengers that coordinate proteostasis across cells and tissues is vital because these molecules may be used as components of future proteostasis-enhancing cocktails for the treatment of neurodegenerative disorders. Although little is known about these molecules, several studies point at molecules that carry proteostasis-promoting signals between tissues.

Since neurotransmitters [35,99] and neuropeptides [42,100] are crucial intermediaries of signaling pathways that sustain proteostasis, Wang *et al.* tested whether neurotransmitter and/or neuropeptide release is involved in UPR^{mt} activation across tissues. Using *C. elegans* mutants in which key components of neurotransmission, including small clear vesicles (SCVs; via *unc-13*), dense core vesicles (DCVs; via *unc-31*), and neuropeptide maturation (via *egl-21*) were disrupted, Wang *et al.* discovered that neurotransmitter release is essential for cell non-autonomous UPR^{mt} activation during ASI neuron-specific mitochondrial stress. Strikingly, systematic analysis of neurotransmitter-deficient strains demonstrated that ASI neurons bidirectionally regulate intestinal UPR^{mt}: whereas dopamine signaling promotes activation and GABA signaling suppresses it [85]. Interestingly, TPH-1, which encodes tryptophan hydroxylase, the rate-limiting enzyme vital for the biosynthesis of serotonin, is required for the expression of *daf-7* [101], suggesting that serotonin is also involved in proteostasis regulation by TGF-β signaling.

Although neuropeptides were also found to be important carriers of proteostasis-conferring signals [42,100], it is currently unclear whether neuropeptides are also regulated by the TGF-β cascade to preserve the integrity of the proteome in distal tissues. A possible hint to the involvement of neuropeptides is the large number of neuropeptide-coding genes that exhibit modulated expression levels upon knockdown of *nol-56* [98]. These include genes that encode neuropeptides that were reported to be proteostasis regulators, such as *nlp-13* and *nlp-18* [100]. Nevertheless, systematic studies are needed to further explore the possible roles of neurotransmitters, neuropeptides, and perhaps other molecules as carriers of proteostasis-promoting signals downstream of TGF-β signaling (Figure 3).

Concluding remarks

While the mechanistic links between aging and the onset of proteotoxic diseases has long been established, the IIS was at the center of scientific efforts to understand these links in detail.

Nonetheless, TGF-β signaling emerges as an additional key regulator of proteostasis across tissues and a modulator of proteostasis-promoting mechanisms. While, in some cases, TGF-β signaling promotes proteostasis, in other cases it aggravates proteotoxicity. Thus, it is possible that, as shown in the context of cancer [102], this pathway has opposing roles in the face of different proteotoxic challenges. While this research direction is in its infancy, we foresee future efforts to explore the mechanisms that are governed by TGF-β and regulate proteostasis, especially in mammalian systems. Understanding in detail which neurons in the mammalian brain receive and integrate proteotoxic cues to modulate TGF-β activity, which molecules carry the signals to other tissues, and which mechanisms are activated in the target cells upon receiving these signals, will open new research directions for the development of novel therapies. Among other questions, it will be important to examine whether the activities of folding chaperones, protein degradation mechanisms, and the attachment of proteostasis-controlling post-translational modifications, are affected by TGF-β signaling. TGF-β modulators are likely to be combined with IIS inhibitors and other molecules to create future therapeutic cocktails for the treatment of neurodegenerative disorders (see Outstanding questions).

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Declaration of interests

The authors declare no competing interests.

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