Review

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Proteostasis and aging of stem cells

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The accumulation of misfolded or damaged proteins is an important determinant of the aging process. Mechanisms that promote the homeostasis of the proteome, or proteostasis, can slow aging and decrease the incidence of age-related diseases. Adult stem cell function declines during the aging process of an organism. This demise of somatic stem cell function could contribute to tissue degeneration and organismal aging. Accumulation of damaged proteins in embryonic stem cells (ESCs) may also have an impact on the aging process, because the passage of these proteins to progenitor cells during asymmetric division could compromise development and aging. Therefore, proteostasis maintenance in stem cells might have an important role in organismal aging. In this review, we discuss exciting new insights into stem cell aging and proteostasis and the questions raised by these findings.

Proteostasis maintenance during aging

The understanding of stem cell biology, differentiation and, cell reprogramming is currently one of the most intense and attractive fields in biology and medicine. Despite the insights gained into stem cell biology, the mechanisms that regulate stem cell identity and differentiation remain largely unknown. Pluripotent ESCs do not undergo replicative senescence and are considered to be immortal in culture [1,2]. Adult organisms have two types of stem cell: (i) adult somatic stem cells, which are found in several tissues and regenerate them; and (ii) germline stem cells (GSCs), which can generate gametes for reproduction [3]. GSCs are designed to maintain an unlimited proliferative capacity to fulfill their biological purpose: to be passed from one generation to the next. Adult somatic stem cells are critical for rejuvenating tissues and persist throughout the lifespan of the organism. However, adult somatic stem cell function declines during the aging process and this failure may contribute to age-related diseases [4,5] (Box 1).

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While genome stability is central for the survival of stem cells, proteome stability may play an equally important role in stem cell identity. Proteostasis is critical for organismal development and cell function [6,7]. The quality of the proteome is regulated by a complex network of cellular mechanisms that monitors the concentration, folding, cellular localization, and interactions of proteins from their synthesis through their degradation (Figure 1) [6–8]. Protein synthesis is controlled by translational rates, which are regulated by ribosome biogenesis, recruitment, and loading [9]. The binding of chaperones to nascent proteins assists their folding into the correct structure. Thermal or oxidative stress, aging, and misfolding-prone mutations challenge the structure of proteins. Chaperones assure the proper cellular localization and folding of proteins throughout their life cycle [10,11]. Misfolded, damaged, aggregated, or unnecessary proteins are degraded by the proteasome or through autophagy [12–15]. The accumulation of misfolded or damaged proteins has a deleterious effect on cell function and viability [6,16]. Damaged proteins can disrupt cellular membranes and form toxic aggregates, overwhelming the cellular machinery required for their degradation [17,18] and causing cell malfunction and death [19]. When the stability of the proteome is challenged, a series of cellular responses is activated to maintain the quality of the proteome [7,16] (Box 2).

Defects in proteostasis lead to many metabolic, oncological, cardiovascular, and neurodegenerative disorders [6,20]. The ability to maintain a functional proteome declines during the aging process [6,11,21,22]. In cells undergoing division, mother cells retain damaged proteins while generating daughter cells with pristine proteomes [23,24]. However, postmitotic cells hold a special distinction for their susceptibility to age-onset protein-aggregation diseases [20]. A decline in the capacity of the cell to protect its proteome has been correlated with multiple age-related diseases such as Alzheimer's [25], Parkinson's [26], and Huntington's [27] disease. Several signaling pathways, such as reduced insulin/insulin-like growth factor 1 (IGF-1) signaling (IIS) or dietary restriction (DR), can extend longevity [8]. Furthermore, longevity-promoting pathways modulate the proteostasis network, providing increased stability of the proteome and delaying aging and the onset of age-related diseases [8,28,29].

The immortality and biological purpose of ESCs and GSCs and the ability of adult somatic stem cells to persist throughout life and rejuvenate tissues suggest that these

Box 1. Adult somatic stem cell exhaustion: a hallmark of aging

Adult somatic stem cells are necessary for rejuvenating tissues and persist throughout the lifespan of the organism. However, adult somatic stem cell function declines during the aging process in tissues such as the brain, skin, blood, bone, and skeletal muscle [4,5]. Adult stem cell exhaustion is considered one of the tentative hallmarks of aging in organisms [4]. Stem cell decline with age may contribute to tissue dysfunction and age-associated diseases [4,5,118]. For instance, adult somatic stem cell failure may contribute to diseases such as frailty, atherosclerosis, and type 2 diabetes by reducing the regenerative potential of tissues [118]. Decreased hematopoiesis with age results in diminished generation of adaptive cells and in increased anemia and myeloid malignancies [119]. A decline in the proliferation of NSCs and neurogenesis produced by these cells with age [120–123] has been associated with progressive Parkinsonian disease and impairment of olfactory discrimination in mouse [123]. Besides adult somatic stem cells, specific progenitor and differentiated cells can persist throughout life in regenerative tissues and their decline with age may also contribute to age-related diseases such as type 2 diabetes and reduced immune function [5].

cells could have increased mechanisms to protect their proteome. Recently, new insights into proteostasis in stem cells have supported this hypothesis. Specifically, a role of protein degradation systems and proteotoxic stress responses has been shown. In addition, longevity mechanisms are important determinants of stem cell maintenance and function. Here we review these insights into proteostasis regulation and the role of longevity-promoting pathways in stem cells.

Response to proteostasis stress in stem cells

A series of cellular responses are activated to maintain the integrity of the proteome when damaged proteins accumulate (Box 2). The heat shock response (HSR) is an essential mechanism to assure proper cytosolic protein folding and ameliorate chronic and acute proteotoxic stress [16,22]. The endoplasmic reticulum (ER) also has a critical role in protein folding [30,31]. The ER uses complex surveillance mechanisms to promote proper protein folding and activates the unfolded protein response (UPR^{ER}) to prevent the accumulation of misfolded proteins that are targeted for degradation by ER-associated degradation (ERAD) or autophagy [30–32]. If protein misfolding overwhelms the cellular ability to maintain the quality of the proteome, the ER coordinates with mitochondria to activate apoptosis [32]. Mitochondrial activity is associated with cellular dysfunction and aging [33]. A surveillance mechanism formed by chaperones and proteases, known as the mitochondrial UPR (UPR^{mt}), maintains the quality of the proteome in mitochondria [34]. Activation of these pathways or increased levels of chaperones are associated with enhanced protection against proteotoxic stress [35].

Reactive oxygen species (ROS) generated by the mitochondrial respiration process are frequently responsible for DNA and protein damage. Both mouse ESCs (mESCs)

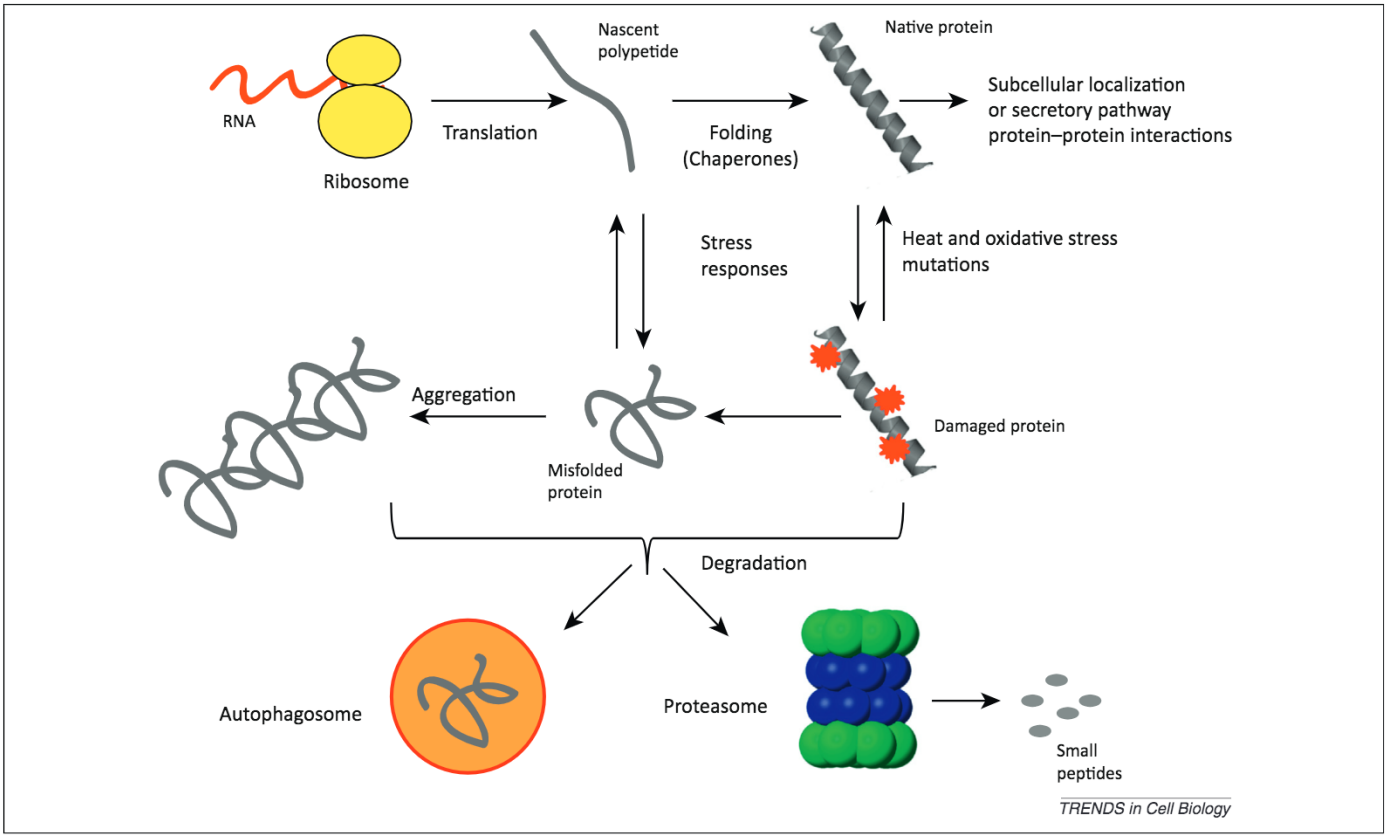

Figure 1. The proteostasis network. Protein synthesis is regulated by translational rates. Translation is controlled by ribosome biogenesis, recruitment, and loading. Chaperones assist the folding of nascent polypeptides into their correct structure. To achieve their function, native proteins are localized to their specific cellular compartment and the correct protein–protein interactions are established. Thermal or oxidative stress and misfolding-prone mutations damage and challenge the structure of proteins. When the stability of the proteome is challenged, a series of cellular stress responses are activated to maintain the quality of the proteome such as the heat-shock response or the unfolded-protein response. Misfolded, damaged, aggregated, or unnecessary proteins are degraded by the proteasome or through autophagy.

Box 2. Cellular stress responses

Regulation of protein synthesis represents a major component of cellular stress responses. Proteotoxic stress induces global attenuation of protein synthesis by inhibiting translation initiation [124] or pausing translation elongation [73–75]. Ribosome-associated chaperones such as HSP70 or nascent polypeptide-associated complex (NAC) play a critical role in promoting polypeptide elongation [73–75]. Under proteotoxic stress, these chaperones relocalize from ribosomes to protein aggregates resulting in diminished translational capacity and pausing of polypeptide elongation [73–75].

In addition, when the stability of the proteome is challenged a series of cellular responses such as the HSR or the UPR is activated to maintain the quality of the proteome, increasing the levels of chaperones and the degradation of misfolded proteins [6,16]. Three branches operate in parallel in the UPR^{ER}: activating transcription factor 6 (ATF6); double-stranded RNA-activated protein kinase (PRK)-like ER kinase (PERK); and inositol-requiring enzyme 1 (IRE1) [31]. On accumulation of unfolded proteins, ATF6 is delivered to the Golgi where proteases liberate its N-terminal cytosolic fragment enabling it to activate UPR genes in the nucleus. After sensing a stress, PERK oligomerizes and phosphorylates itself and eIF2α. This inhibits eIF2α and mRNA translation, thus reducing the flux of proteins in the ER. Yet, ATF4 mRNA, which has a short open reading frame in the 5′ untranslated region, is particularly translated when eIF2α is limiting. ATF4 is a transcription factor that induces CHOP, chaperones, GADD34, and various genes implicated in the antioxidant response, redox enzymes, and cell-death pathways. IRE1 transmits the UPR via unconventional XBP1 mRNA splicing. When spliced, XBP1 is translated and induces the expression of chaperones and ERAD proteins.

Under normal conditions, heat shock factor 1 (HSF-1) is negatively regulated by HSP-70/90 in eukaryotes. On non-permissive heat, HSPs such as HSP-70 restore proper folding of destabilized proteins. Inhibitory binding of HSF-1 by HSPs is released in times of protein misfolding stress, which enables HSF-1 trimerization, translocation to the nucleus, and the activation of genes required to maintain proteostasis, especially HSPs. When proteostasis is restored, HSPs negatively regulate HSF-1 and abolish the transcriptional stress response [35].

Likewise, in mammalian cells when unfolded proteins accumulate in the mitochondrial matrix, CHOP is transcriptionally upregulated via JNK2 and c-Jun. CHOP induces the transcription of the protease ClpP and the chaperonin HSP60. When unfolded proteins accumulate in the intermembrane space, AKT kinase is activated and the estrogen receptor is alpha phosphorylated, resulting in the induction of Htra2, an IMS protease, and the transcription of NRF1 [125].

and human ESCs (hESCs) generate fewer ROS than their differentiated counterparts [36,37]. In parallel, ESCs exhibit higher antioxidant defense potential that diminishes during differentiation. For instance, the glutathione/thioredoxin system enzymes (Tgr, Gpx2/3/4, Gsta3, Prdx2, Pdh2) are highly expressed in ESCs compared with their differentiated counterparts. These enzymes prevent ROS accumulation and promote a redox environment compatible with proper tertiary conformation of proteins [38]. An increase in the levels of ROS limits the lifespan of adult somatic stem cells such as hematopoietic stem cells (HSCs) and neural stem cells (NSCs) [39–42]. Notably, ROS levels serve as signals for differentiation or self-renewal in mouse adult somatic stem cells. HSCs retain self-renewal capacity under reduced conditions [43], whereas increased oxidative status promotes the stem cell activity of neuroepithelial stem cells in the central nervous system [44].

Notably, ESCs have increased levels of heat-shock proteins (HSPs). For instance, mESCs exhibit increased levels of HSPA1a, HSPA1b, HSPA9 (also known as mortalin), and HSPB1 [36] compared with their differentiated counterparts. Likewise, hESCs also have increased levels of HSPA1b [37]. However, hESCs do not show increased levels of HSPB1 [37]. Both HSP27 and HSPA9 levels decrease during mESC differentiation into neurogenic embryoid bodies [45]. Taken together, these data suggest that ESCs might have a greater ability to respond to protein misfolding. However, these increased levels of HSPs might not be conserved in all adult somatic stem cell types. Consistent with findings in ESCs, the HSR is attenuated on differentiation of neural progenitor cells [46]. HSP25 is excluded from neural precursors and other differentiating cells. However, the levels of HSPB1, HSPB5, HSPB6, and HSP60 decrease when human adipose-derived adult stem cells differentiate [47].

Supporting evidence suggests that HSPs may play a role in stemness and differentiation. Inhibition of HSP90 leads to mESC differentiation whereas overexpression of HSP90β partially rescues this phenotype [48]. HSP90 associates with Oct-4 and Nanog, protecting them from degradation by the ubiquitin–proteasome system (UPS) [48]. HSPs might also be significant determinants for the genesis of several tissues. HSPB5 overexpression modulates the activity of MyoD, the master regulator of myogenesis, by reducing its synthesis and increasing its degradation, therefore retarding differentiation [49]. HSPA8 (a non-inducible HSP) negatively influences the stability of proapoptotic Bim mRNA, increasing HSC survival and preventing their differentiation [50]. Bim mRNA is required for apoptosis during hematopoiesis and leukemogenesis. HSP70 indirectly triggers erythropoiesis by preventing caspase-3-mediated cleavage of GATA-1 [51], an essential transcriptional factor for maturation and differentiation within the erythroid lineage. Several components of the UPR^{ER} have an important role during differentiation. For example, IRE1 increases lymphopoiesis of B cells [52], XBP1 induces osteogenic and plasma differentiations [53], and CHOP promotes differentiation of B cells, erythrocytes, osteocytes, and chondrocytes [54–57]. Furthermore, the UPR^{ER}, as a stress-coordinated pathway, has an important role in the regulation of differentiation of the mouse intestinal epithelial stem cell [58]. The transition from stem cell to transit-amplifying cells of the intestine is accompanied by induced ER stress and activity of the UPR^{ER}. ER stress induction by Perk–eIF2α can promote loss of stemness. In organoid cultures of primary intestinal epithelium, when Perk–eIF2α is inhibited, stem cells accumulate. Taken together, these observations make it difficult clearly to correlate high levels of HSPs or cytotoxic protection with adult somatic stem cell differentiation and further insights into the impact of these mechanisms on stem cell function are needed. In addition, it will be fascinating to define whether the UPR^{mt} is enhanced in stem cells or whether it has a role in stem cell function.

Protein degradation systems as a determinant of stem cell function

When damaged or misfolded proteins cannot be 'rescued' by chaperones and the UPRs, they are degraded through the proteasome or autophagy. The proteasome is a complex

proteolytic machine formed by the assembly of several subunits that mostly degrades proteins that have been modified by the attachment of ubiquitin [12]. The UPS is critical for maintaining the proper concentration of many regulatory proteins involved in the cell cycle, apoptosis, inflammation, signal transduction, and other biological processes [12,59]. In addition, the UPS is a key component of the protein quality-control system to terminate damaged proteins [14]. The proteasome exists in several forms but its major assembly is formed by the core particle (20S), which contains the proteolytic active sites, and the regulatory particle (19S), which regulates the activity of the holocomplex (26S, single capped, and 30S, double capped) [12]. Although 20S particles can exist in a free form, they are inactive and unable to degrade proteins [60]. 19S recognizes polyubiquitylated proteins and unfolds and translocates these proteins to 20S for degradation [12,59].

The UPS has been shown to regulate ESC pluripotency and cellular reprogramming [61–63]. hESCs exhibit high proteasome activity compared with their differentiated counterparts such as neurons, fibroblasts, or trophoblasts [63]. This increased proteasome activity is correlated with increased levels of the 19S proteasome subunit PSMD11/RPN-6 [61,63,64], which is an essential subunit for the activity of the 26S/30S proteasome that stabilizes the otherwise weak interaction between the 20S core and the 19S cap [63,65]. GSCs can acquire *in vitro* properties similar to those of ESCs such as pluripotency [66]. GSCs generate the gametes that will produce embryos after reproduction. ESCs and oocytes share a common transcriptome signature [64] and hESCs provide an *in vitro* system to study oocyte development [67]. Similar to hESCs, human oocytes have increased expression levels of PSMD11 [64]. Notably, oocytes and gonads of *Drosophila melanogaster* have increased 26S proteasome activity and accumulate fewer damaged proteins than aging somatic tissues [68,69]. Although proteasome activity declines in somatic tissues during the aging process, maturating oocytes maintain their high activity [68]. Whether adult somatic stem cells also have enhanced proteasome activity remains to be elucidated, but the maintenance of this activity may critically impact organismal aging. Increased proteasome activity was found to be necessary for maintaining hESC pluripotency [63]. Additionally, other components of the UPS regulate pluripotency in mESCs such as the deubiquitinating enzyme Psmd14 and the E3 ligase Fbxw7 [61]. Psmd14 is part of the 19S proteasome subunit and its deubiquitinating activity is essential for mESC pluripotency [61]. These findings raise the intriguing question of why these cells need enhanced activity of the proteasome. ESCs show a remarkable capacity to replicate continuously in the absence of senescence. Therefore, increased proteostasis ability in ESCs could be required to avoid senescence and maintain an intact proteome either for self-renewal or for the generation of an intact cell lineage. Notably, degradation of damaged proteins is triggered on the first signs of mESC differentiation [70,71]. Induction of the proteasome activator PA28, normally associated with the immunoproteasome, is required for degradation of these damaged proteins during the first signs of cell fate specification [71]. Increased proteasome activity could also be critical for maintaining the proper concentration of many regulatory proteins at specific times, such as transcription factors involved in either pluripotency maintenance or the differentiation process. Interestingly, proteolytic degradation by the proteasome has a role in controlling transcription factor and Pol II binding to regulatory regions of cell type-specific gene domains in ESCs, thereby restricting permissive transcriptional activity and keeping genes in a potentiated state, ready for activation at specific stages [72]. Another possibility is that increased proteasome activity may be coupled to an intrinsic challenge to hESCs such as increased translation, which could be associated with translation errors. However, protein expression has not been examined in ESCs. In this context, it will be fascinating to analyze the role of ribosome-associated chaperones (Box 2) [73–75] in translational rates in ESCs.

Macroautophagy (hereafter referred to as autophagy) is a self-catabolic mechanism through which dysfunctional and unnecessary components of the cell such as organelles and proteins are degraded. In addition, autophagy provides a means to keep energy and nutrients to levels compatible with survival under starvation and stress. These components are engulfed in a double membrane, the autophagosome, which is subsequently fused with lysosomes. Lysosomal enzymes degrade the contents of autophagosomes, producing amino acids and fatty acids that are recycled in the cytoplasm [13]. A multitude of stressors such as ROS, starvation, DNA damage and ER stress activates autophagy in terminally differentiated cells [13].

Both mESCs and hESCs exhibit higher autophagy activity on early differentiation [76]. Induced pluripotent stem cells generated from patients with Parkinson's disease show more autophagic vacuoles when differentiated into dopaminergic neurons [77], suggesting an active rejuvenation step to generate a pool of 'healthy' cells. Experiments with adult somatic stem cells cultured *in vitro* such as human mesenchymal stem cells (hMSCs) [78], HSCs, dermal stem cells (DSCs), and epidermal stem cells [79] suggest that autophagy activity is increased in these cells compared with their differentiated counterparts. It is noteworthy that experimental conditions might be unfavorable and lead to higher autophagy levels in adult somatic stem cells. Developing conditions that would mimic the stem cell niche are necessary for a better understanding of autophagy regulation in these cells. In fetal and postnatal mHSCs, a deficiency in essential autophagy genes such as FIP200 or Atg7 deregulates proliferation, suggesting that autophagy is required for stemness in fetal and postnatal mHSCs [80–83]. FoxO3, a forkhead transcription factor linked to stem cell maintenance and longevity [84], maintains the expression of proautophagy genes in adult mHSCs to allow a quick autophagic response on stress [85]. Notably, old mHSCs have higher basal levels of autophagy activity, a characteristic required for their cloning efficiency, and are able to induce autophagy much like young HSCs. However, autophagy activity in young HSCs is not required for their cloning efficiency [85]. This observation is controversial because previous data showed the opposite effect [81–83,86]. The difference might be that one study [85] used a drug to block autophagy in normally

developed adult mHSCs whereas the latter study used a genetic model to block autophagy that causes severe defects early in life leading to death and thus looked at fetal and early stages. The higher levels of autophagy activity in old adult mHSCs were due to attenuated nutrient (2-NBD glucose) uptake [85]. That old adult mHSCs maintain an autophagy potential similar to young mHSCs and exhibit higher levels of autophagy for their survival confronts the prevailing, traditional view where compromised autophagy is seen as a determinant of aging [13].

In NSCs or cardiac stem cells (CSCs), autophagy activity increases on their differentiation [87–89]. This enhanced autophagy might be due to a specific increase in the requirements of their differentiated counterparts, such as neurons, to recycle their cellular components. During the initial period of neuronal differentiation (E15.5 mouse embryos), expression of the autophagy genes *Atg7*, *Becn1*, *Ambra1*, and *LC3* are increased *in vivo* in the mouse embryonic olfactory bulb (OB) [87]. *In vitro* neuronal differentiation of OB-derived stem cells is accompanied by increased autophagy flux and LC3 lipidation in Tuj1-positive cells [87]. Blocking autophagy chemically or genetically can impair NSC and CSC differentiation [87–89]. Inhibition of autophagy by 3-MA or wortmannin decreases neurogenesis of OB-derived stem cells. In addition, *Ambra1* loss-of-function mice show decreased neural markers in the E13.5 OB [87]. However, FIP200 is required for NSC proliferation [90]. Knock down of *Becn1* or *Atg7* suppresses the expression of cardiomyocyte markers such as α-actin and smooth muscle α-actin in embryoid bodies of mESCs [88,89]. Moreover, treatment of embryoid bodies with the autophagy inhibitor NH_4Cl or bafilomycin A1 decreases the number of beating foci whereas activation with rapamycin increases their number [88,89]. Similarly, *ex vivo* treatment of E8.5 mouse embryos with rapamycin increases the expression of cardiomyocyte markers in the second heart field.

Overall, these observations suggest a higher degree of protection, at least, to cytotoxic stresses in adult somatic stem cells. Consistent with this idea, impairment of autophagy in epidermal stem cells, DSCs, and HSCs leads to increased susceptibility to cytotoxic stress such as etoposide, doxorubicid, or UV [79]. In addition, autophagy might be an efficient mechanism to replace transcription factors and associated proteins of stemness and initiate more rapid differentiation, especially in ESCs.

Longevity-promoting pathways regulate stem cell function

A series of signaling pathways promote longevity and provide increased stability to the proteome, delaying the onset of age-related diseases [8,28,91]. Reduced IIS extends lifespan in both invertebrates and vertebrates [84,92,93] and correlates with increased longevity of humans [92,94]. The insulin/IGF-1 receptor activates a conserved phosphatidylinositol (PI) 3-kinase/PDK/AKT signaling cascade that phosphorylates FOXO transcription factors, thereby preventing their nuclear localization. When IIS signaling is reduced, FOXO accumulates in the nucleus and regulates downstream genes that extend lifespan and increase stress resistance in worms, flies, and mice [84,93]. Delayed aging by IIS reduction protects worms and mice from protein-aggregation toxicity [8,28]. Notably, FOXO transcription factors are important regulators of the proliferation and self-renewal of NSCs and HSCs in mice. A combined deficiency of FoxO1, FoxO3, and FoxO4 depletes the NSC and HSC pools in mice [41,42]. FOXO transcription factors protect from oxidative stress and promote the expression of antioxidant enzymes [95]. Combined deficiency of FoxO1, FoxO3, and FoxO4 increases ROS levels in NSCs and HSCs [41,42], which may increase protein misfolding. FoxO3 is essential for regulating this process in mice, because FoxO3 deficiency alone increases ROS levels and depletes the pool of NSCs and HSCs [96,97].

Among invertebrates, birds, and mammals, experimental paradigms that limit reproductive investment also cause lifespan extension [98]. Hypothetically, the need for repairing and preventing damage to the germline dominates resource allocation strategies, while the somatic tissues age and deteriorate [99]. In support of such theories, modulations of reproduction that eliminate germ cells in *Caenorhabditis elegans* and *D. melanogaster* provide effective mechanisms for extending lifespan [98,100]: phenotypes that may be caused by heightened resource availability and proteome stability within the postmitotic soma [29,101]. Similar to hESCs, proteasome activity and RPN-6 levels are increased in these germline-lacking worms [29]. Furthermore, increased proteasome activity, *rpn-6* expression, and longevity are modulated by DAF-16, the worm FOXO transcription factor [29]. Notably, FOXO4 is necessary for increased proteasome activity in hESCs and reduces the potential of these cells to differentiate into neural lineages [63,102]. In addition, hESC pluripotency requires FOXO1 [103]. Therefore, FOXOs cross evolutionary boundaries and link hESC function to invertebrate longevity modulation. FOXO4 is specifically critical for the differentiation of hESCs into neural cells and it will be fascinating to understand how this regulation is achieved. However, the loss of FOXO3 in mouse causes increased neurogenesis during development followed by NSC depletion in adulthood [96]. Interestingly, it was recently found that FOXO3-bound genes thoroughly overlap with those bound by the proneuronal bHLH transcription factor ASCL/MASH1 in cultured neural progenitor cells [104]. FOXO3 represses the expression of specific ASCL1 neurogenic targets and restrains neurogenesis. Therefore, FOXO3 may help maintain the NSC pool by negatively regulating neurogenesis. These results suggest that FOXO4 and FOXO3 might have opposing effects in hESCs versus mouse NSCs. Different hypotheses could explain these opposing effects: different cell-type requirements (hESCs versus NSCs); the different models and species used for these assays (*in vitro* cultured hESCs versus mouse models); the differentiation stage at the time point chosen; and that different FOXO isoforms may act in different pathways during cellular commitment. It is intriguing to speculate that FOXO4 may be required for the differentiation of hESCs into neural cells and that FOXO3 is later required for maintaining the pool of adult NSCs and avoiding a premature burst of neurogenesis. Accordingly, FOXO4 levels decrease during neural differentiation of hESCs whereas FOXO3 levels increase [63,102].

Reduced food intake without malnutrition, or DR, also extends lifespan in multiple species and delays the onset of diverse pathologies related to age [8,105]. DR decreases protein synthesis by modulating translational rates [106,107], which can improve proteostasis maintenance. The decrease in the load of nascent polypeptides to the proteostasis machinery may allow more efficient protein folding and degradation and, therefore, decrease the accumulation of misfolded and damaged proteins. The protein mammalian target of rapamycin (mTOR) plays a pivotal role in the modulation of translational rates induced by DR [106,107]. mTOR associates with other proteins to form two different complexes: mTORC1 and mTORC2. mTORC1 activity is inhibited by DR, resulting in lifespan extension and delayed onset of protein aggregation [108]. Recent studies suggest a role of DR in stem cell proliferation. Stem cell function of mouse intestinal stem cells (ISCs) was found to be increased by DR via a non-cell-autonomous mechanism acting through adjacent Paneth cells present in the ISC niche. DR downregulated mTORC1 activity in Paneth cells but not in the ISCs, creating an environment where ISC function is enhanced [109]. Decreased activity of mTORC1 in Paneth cells upregulates levels of bst1, a protein that promotes cell proliferation in bone marrow. Regulation of bst1 levels by mTORC1 is essential for the improved ISC function on DR. Similarly, the regenerative potential of muscle satellite cells increased on DR in young and old mice [110]. The number of satellite cells per fibers is increased after 3 months of DR. DR also increases neurogenesis [111], but the effects are incompatible with *in vivo* application because a high deprivation of intake is required. These data suggest a beneficial role in various tissues mediated by DR that can help us understand its pro-longevity role by enhanced stem cell function.

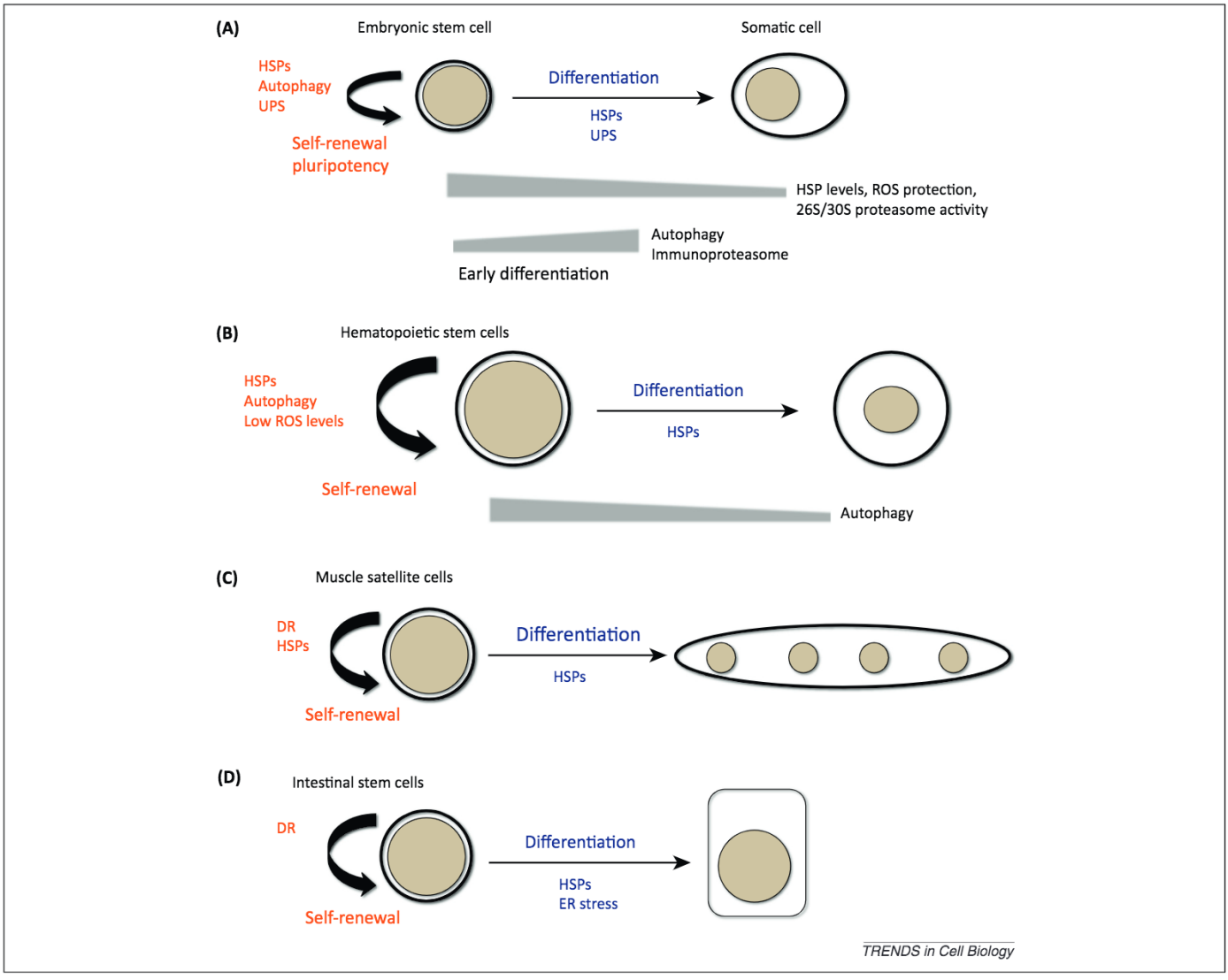

Figure 2. Proteostasis in stem cells. **(A)** Embryonic stem cells (ESCs) exhibit increased levels of heat-shock protein (HSPs) and 26S/30S proteasome activity and are more protected from reactive oxygen species (ROS) than their differentiated counterparts. In an active rejuvenation step, both autophagy and the immunoproteasome activities increase during the first days of differentiation. HSPs, autophagy, and the ubiquitin–proteasome system (UPS) are required to maintain ESC features such as self-renewal and pluripotency. HSPs and the UPS are required for differentiation of ESCs into specific cellular lineages. **(B)** Hematopoietic stem cells (HSCs) exhibit increased levels of autophagy activity compared with their differentiated counterparts. Low levels of ROS and increased levels of HSPs and autophagy activity are required to maintain HSC self-renewal. HSPs are required for differentiation of HSCs. **(C)** Dietary restriction (DR) improves muscle satellite cell self-renewal. HSPs are required for differentiation of muscle satellite cells. **(D)** DR and increased levels of HSPs improve intestinal stem cell (ISC) self-renewal. Endoplasmic reticulum (ER) stress and HSPs affect differentiation of ISCs.

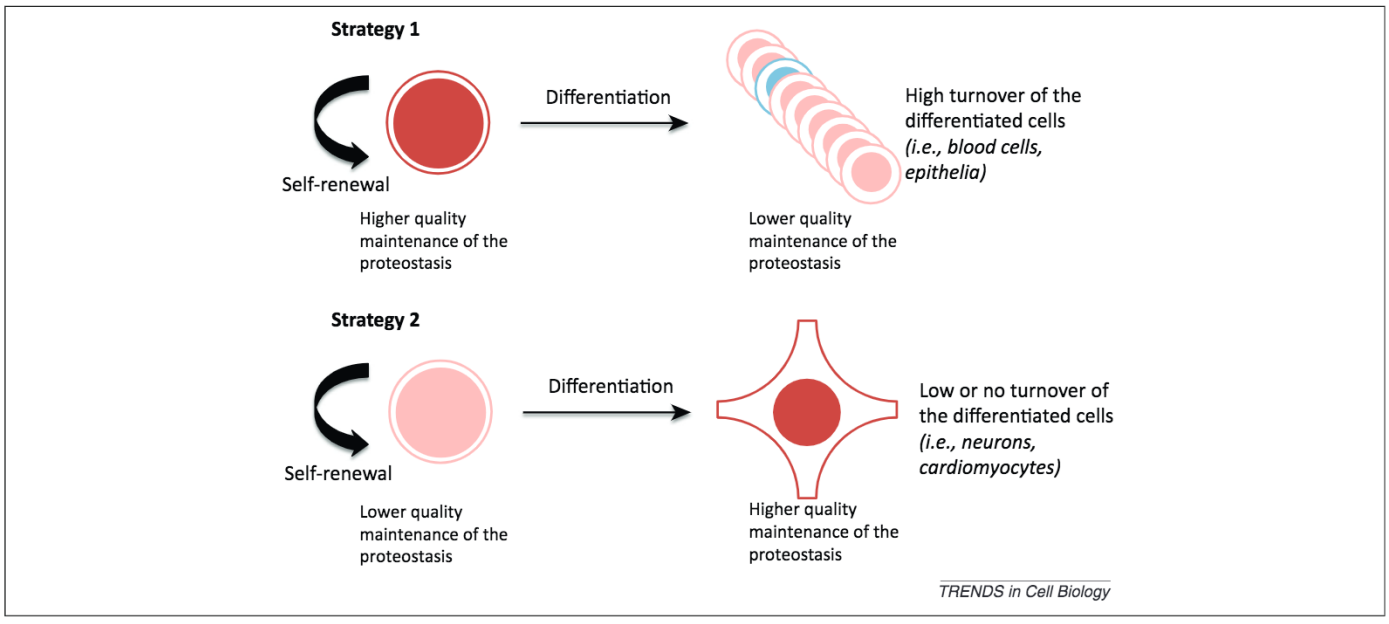

Figure 3. Proteostasis quality-maintenance strategies in stem cells. The model proposes two strategies: stem cells maintain high-quality proteostasis (darker red) and differentiated counterparts acquire higher-quality proteostasis (darker red) compared with the stem cell they are derived from (lighter red). We hypothesize that this might be important in the long-term versus short-term life of differentiated cells and the proliferation rate of stem cells. A compromised short-term differentiated cell (blue) will be diluted and turned over rapidly with limited consequences, whereas this might be more damaging with long-term and slowly regenerated differentiated cells.

Concluding remarks

Insights into the epigenome and transcriptome of stem cells have helped to define the mechanisms that regulate pluripotency or multipotency, differentiation, and cell reprogramming. Likewise, a better understanding of how stem cells regulate their proteostasis network will shed new light on stem cell biology and identity. In addition, it could have a great impact on cell therapy and organism health during the aging process.

ESCs exhibit higher levels of chaperones and higher antioxidant defense potential that could prevent the accumulation of misfolded proteins. ESCs also have increased proteasome activity. Whether this enhanced activity is necessary to potentiate the termination of damaged proteins or specific regulatory proteins remains unknown. Activation of the immunoproteasome and autophagy occurs during the early stages of ESC differentiation, providing a means to degrade damaged proteins and avoid passage to their differentiated counterparts. In adult somatic stem cells, the level of chaperones and autophagy activity depends on the stem cell type. Regardless of these differences, the proteostasis network critically impacts adult somatic stem cell function (Figure 2).

The differences in proteostasis pattern observed between stem cell types suggest distinctive mechanisms to ensure the functionality of their differentiated pools (Figure 2). Two strategies can be proposed (Figure 3): first, the stem cell maintains high-quality proteostasis; and second, differentiated counterparts acquire increased proteome surveillance compared with the stem cell they are derived from. This can be relevant regarding the number of divisions that stem cells undergo. Indeed, long-term stem cells that give rise to differentiated cells with a high turnover, such as blood cells and epithelia, seem to follow the first strategy. Having a high-quality pool of long-term and highly proliferative stem cells might be beneficial; if there is any impairment in the differentiated cells, it will be diluted and disappear rapidly, going unnoticed. The second strategy might be advantageous for differentiated cells such as neurons or cardiomyocytes that persist longer in the organism. Here, defects would have more severe consequences for the tissue and the organism.

Furthermore, a better knowledge of how stem cells maintain proteostasis may help us to understand how cancer stem cells are generated in an organism and to find specific treatments against these cells. The autophagy rate in breast cancer stem cells is higher than in parental cells [112]. When the autophagy gene Atg7 or Beclin1 is knocked down, self-renewal is impaired and its tumorigenicity reduced. By modulating ATP levels and the organization of subcellular structures, autophagy was shown to be important for glioblastoma stem cell migration and invasion [113]. Likewise, higher HSP levels have been reported in cancer stem cells [114]. HSP27 contributes to the maintenance of breast cancer stem cells [115,116] and DNAJB8 controls the early phase of renal cancer stem cell onset [117]. Because both stem cells and cancer stem cells rely on similar protective mechanisms, specifically targeting these pathways in cancer cells may be difficult. Therefore, deciphering the differences in proteostasis regulation between stem cells and cancer stem cells will be needed for efficient treatment implementation.

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