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Review

Protecting the future: balancing proteostasis for reproduction

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The proteostasis network (PN) regulates protein synthesis, folding, and degradation and is critical for the health and function of all cells. The PN has been extensively studied in the context of aging and age-related diseases, and loss of proteostasis is regarded as a major contributor to many age-associated disorders. In contrast to somatic tissues, an important feature of germ cells is their ability to maintain a healthy proteome across generations. Accumulating evidence has now revealed multiple layers of PN regulation that support germ cell function, determine reproductive capacity during aging, and prioritize reproduction at the expense of somatic health. Here, we review recent insights into these different modes of regulation and their implications for reproductive and somatic aging.

Highlights

The proteostasis network (PN) regulates protein synthesis, folding, and degradation to protect the integrity of the proteome.

The PN is essential during reproduction to support germ cell function and prevent the transmission of protein damage to the progeny.

PN dysregulation and accumulation of proteome damage in arrested oocytes may contribute to the age-associated decline of female reproductive capacity.

The reproductive system regulates proteostasis in somatic tissues via systemic signals that impact organismal health and longevity in model organisms.

Proteostasis regulation for reproduction

Maintaining a properly folded and functional proteome is essential for cellular function, organismal stress resilience, and longevity. This state of balance, known as proteostasis, relies on an extensive network of molecular chaperones and degradation machineries that influences the fate of proteins from their synthesis to their breakdown [1,2] (Box 1). The PN ensures that correctly folded proteins are generated and maintained throughout their lifetime, removes aberrant proteins, and monitors their levels to activate a series of regulatory cell stress responses when the integrity of the proteome is compromised [1,3]. Maintaining proteostasis is especially important in germ cells, which experience very active periods of protein biogenesis (Box 2) and must transmit a pristine proteome to the progeny to reset proteostasis at each new generation. Proteome integrity is constantly challenged by intrinsic and external stresses that promote the formation of misfolded and aggregated proteins, which can be toxic to cells [3]. For example, mutations and errors during transcription or translation can lead to the expression of unstable protein variants, and proteotoxic stress conditions, such as temperature or oxidative stress, cause a subset of proteins to misfold and aggregate [2]. Failure to maintain proteostasis in long-lived differentiated cells is a driver of cellular dysfunction in aging and is associated with many age-related diseases, including neurodegeneration and metabolic disorders [3]. By contrast, germ cells allow the transmission of genetic information to a potentially endless series of generations and thus are considered an immortal lineage. While the role of DNA stability in germ cells has been extensively studied, accumulating evidence now indicates that organisms have evolved specific PN mechanisms to maintain the integrity of the proteome during reproduction and that loss of proteostasis may contribute to the age-associated decline of female reproductive capacity [4–7]. Here, we discuss the role of proteostasis mechanisms in supporting germ cell function and how proteostasis failure may contribute to female reproductive aging, as well as the pathways that connect reproductive status to somatic proteostasis in invertebrates and their potential conservation.

The proteostasis of germ cells

Many aspects of proteostasis have been shown to be critical to support germ cell function and gamete production [8–11], which involves a high volume of protein synthesis and prolonged periods of limited transcriptional activity (Box 2). In addition, multiple levels of quality control

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Box 1. The PN

Molecular chaperones

Molecular chaperones are conserved throughout evolution and are expressed in all cellular subcompartments [106]. In metazoans, molecular chaperone genes expanded considerably to include paralogs that are constitutively expressed or stress inducible, localized to different cellular compartments, and expressed ubiquitously or in specific tissues [107]. The main chaperone families are HSP70, HSP40/DNAJ, HSP90, TRiC/CCT, and small HSPs (sHSPs). Chaperones typically recognize hydrophobic segments in their substrates to prevent non-native intra- and intermolecular interactions. This occurs through ATP-dependent or -independent cycles of protein binding and release, which in the case of HSP70 and HSP90 are regulated by key co-chaperones [106]. For example, the functional diversity of HSP70 chaperones is orchestrated by the expanded family of HSP40/DNAJ co-chaperones that dictate substrate specificity and recruit the HSP70 machinery to various subcellular sites [108]. Chaperones participate in a plethora of cellular functions through their essential role in *de novo* protein folding and trafficking, complex assembly, disaggregation and refolding of misfolded proteins, and degradation.

The UPS

Protein degradation by the UPS involves an enzymatic cascade that catalyzes the addition of polyubiquitin chains to the substrate, followed by the recognition and unfolding of the ubiquitinated substrate by the 19S regulatory proteasome subunit and degradation into peptides by the 20S proteolytic core [33]. In addition to important regulatory roles in cellular processes such as the cell cycle and signal transduction, the UPS mediates the degradation of misfolded and terminally damaged protein species as well as incomplete nascent chains on stalled ribosomes. Proteasome complexes localize to the cytosol and nucleus and are also a central component of ER quality control through the ER-associated degradation (ERAD) pathway [109].

The ALP

Autophagy directs misfolded proteins and large aggregates to the lysosome, an acidic membrane-bound organelle containing hydrolytic enzymes for the breakdown of a wide range of macromolecules, including proteins [44]. The targeting of cellular content for lysosomal degradation by autophagy can occur according to various modes of cargo delivery. Macroautophagy, the main and most-studied pathway, involves the sequestration of substrates into double-membrane structures known as autophagosomes, followed by fusion with lysosomes to form autolysosomes [110]. By contrast, microautophagy occurs by the invagination of cytoplasmic contents at the lysosomal membrane [111], and chaperone-mediated autophagy (CMA) is initiated by recognition of the KFERQ motif on protein substrates by HSC70 and targeting to the LAMP-2A receptor at the lysosomal membrane [112].

exist to prevent the transmission of cellular damage to the progeny, and studies in model organisms suggest that degradation pathways are important to eliminate aberrant protein species in oocytes prior to fertilization [4,12,13]. In this section, we discuss recent insights into the role of the PN in supporting germ cell function and maintaining proteome health during reproduction (Figure 1).

Cytosolic chaperones and heat shock factors (HSFs) in germ cells

Molecular chaperones assist protein folding and prevent aggregation and therefore are essential for the expression and maintenance of a functional proteome in all cells (Box 1). A comprehensive analysis of gene expression across 32 human tissues revealed that among the chaperone genes showing a tissue-specific expression pattern (31 of 324 genes), about half are restricted to the testis [1,14]. Among all tissues, the largest number of tissue-specific genes was also found in testis [14], suggesting that a highly specialized proteome requires the expression of a specific chaperone network in this tissue. By contrast, chaperone genes do not appear to be selectively expressed in ovaries [14]. Perhaps this observation could be related to tissue physiology, as the testes are located outside the body cavity and maintained at 2–4°C lower than the core body temperature, and may require the expression of a different set of chaperone machineries optimal for protein folding and maintenance under these conditions. Notably, among the five J-domain proteins specifically expressed in testes, DNAJB8 has been shown to be particularly effective to prevent the aggregation and toxicity of aggregation-prone polyglutamine proteins in cellular models of protein conformational diseases [15]. The testis-enriched HSP70-family chaperone HSPA2 has been extensively studied in the context of spermatogenesis, where it was shown to be involved at various steps. In mice, HSPA2 is essential for the completion of meiosis I, as

Box 2. Overview of gametogenesis in humans

Oogenesis

Oocyte development begins in the fetal ovary where germ cells enter meiosis I and arrest at prophase I, and supporting somatic cells are recruited to the developing oocyte to form ovarian follicles [113]. At the time of birth, all future eggs are present in the ovaries in a dormant state that will persist until growth is reinitiated prior to ovulation. Following puberty, primary oocytes are sequentially recruited to the developing follicle pool, where they undergo a growth phase before resuming meiosis [114]. During this phase, the oocyte increases in volume and is highly transcriptionally and translationally active to accumulate the RNAs and proteins necessary to support meiosis, fertilization, and early embryogenesis. Once meiotic arrest is released, oocytes complete meiosis I, an uneven division that results in the formation of the secondary oocyte, containing most of the cellular content, and a polar body (see Figure 1 in main text). The secondary oocyte arrested at metaphase II is released during ovulation, and following fertilization will complete meiosis II and produce a second polar body and a fertilized egg. Transcriptional activity is halted prior to meiotic resumption, so that the transcripts present in the oocyte at the end of the growth phase control development until transcription of the zygotic genome is activated in early embryonic development. Therefore, precise regulation of protein biogenesis and proteome maintenance is essential for oocyte growth and maturation.

Spermatogenesis

In contrast to oogenesis, the production of sperm in testis is initiated throughout adult life and occurs in massive quantities [115]. Spermatogenesis involves the differentiation of spermatogonium, or spermatogonial stem cells (SSCs), into spermatozoa through three different phases: mitosis, meiosis, and spermiogenesis. SSC divide mitotically to replenish the stem cell pool and produce primary spermatocytes. The primary spermatocyte undergoes the first meiotic division to produce two secondary spermatocytes, which divide into two spermatids each following meiosis II [115] (see Figure 1 in main text). The development of spermatids into mature spermatozoa, known as spermiogenesis, involves the acquisition of the specific morphology and structures essential for motility and interaction with the female oocyte. During this process, the Golgi apparatus forms the acrosome over the anterior half of the sperm cell's head, the flagellum forms, the nucleus condenses, the cytoplasm is jettisoned, and mitochondria form a ring around the base of the flagellum. Further functional maturation of spermatozoa occurs during transit through the epididymis and the female reproductive tract. The extreme level of condensation of DNA in the haploid nucleus and the absence of cytoplasmic content means that sperm cells are essentially transcriptionally and translationally inactive and must rely on the integrity of their proteome in post-testicular maturation and for fertilization.

the chaperone is required for the formation of the CDC2/cyclin B1 complex [16]. HSPA2 is also important for chromatin reorganization in post-meiotic spermatids and for sperm–oocyte recognition [17,18]. Oxidative stress can lead to proteolytic degradation of HSPA2 [19] and downregulation of HSPA2 levels by conserved circular RNAs protects sperm against heat stress [20]. These results suggest that fine-tuned HSPA2 levels may be critical for sperm function. Ubiquitously expressed chaperones from the CCT/TRiC, HSP70, and HSP90 families are also involved in spermatogenesis and sperm–egg recognition, and dysregulation of chaperone expression in male infertility may contribute to reduced sperm function [6]. In mouse oocytes, Hsp90α is expressed at high levels and appears to play a key role in meiosis, as inhibition of Hsp90 leads to the disruption of key meiotic kinases and results in severe meiotic defects [10].

The heat shock response (HSR), mediated by the transcription factor HSF1 and related HSFs, leads to the rapid induction of heat shock genes in response to cytosolic proteotoxic stress, many of which correspond to molecular chaperones initially discovered as heat shock proteins (HSPs) [21]. HSFs have been shown to be involved in both oogenesis and spermatogenesis via noncanonical mechanisms, distinct from the classical HSR [22]. Initial evidence that the activity of HSFs is important for gametogenesis was provided by studies in *Drosophila*, which demonstrated that HSF1 is essential for oogenesis [23]. In mice, HSF1 is a maternal gene required for embryo development and its knockout results in female infertility [24,25]. Depletion of HSF1, which is highly expressed in oocytes, results in severe meiotic defects during oogenesis [10]. HSF1 activates the expression of several molecular chaperones in non-stressed oocytes and the HSF1-mediated expression of Hsp90α is essential for normal meiotic progression [10]. In addition, HSF1 regulates the expression of meiotic genes, including components of the cohesin complex, specifically in female germ cells [26]. By contrast, HSF1 is not strictly required for sperm production in mice but has an unusual role in quality control in response to stress. Male germ cells lack a typical

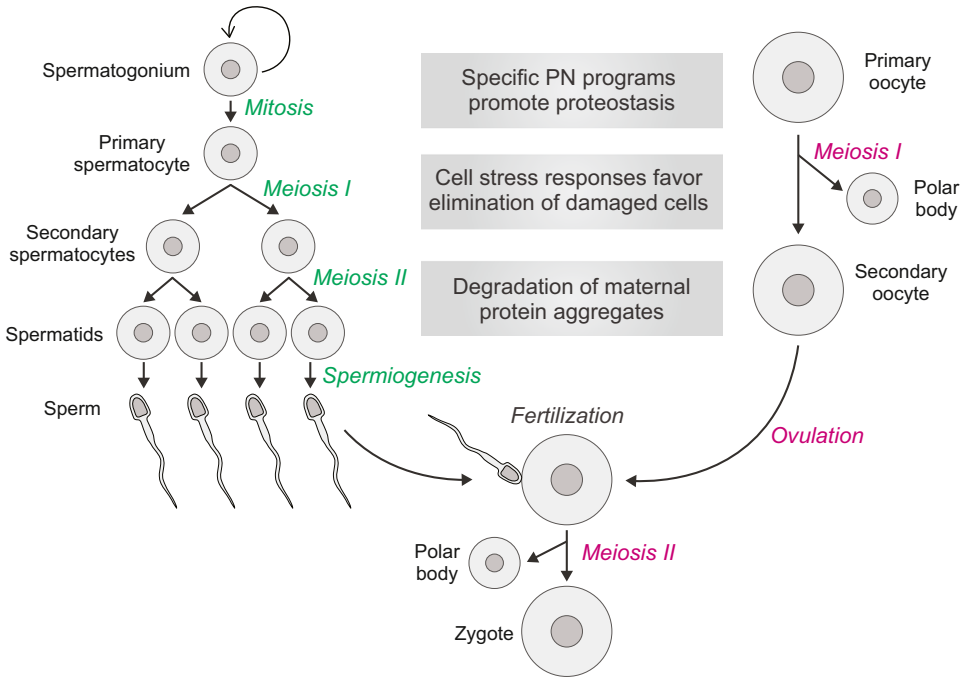

Figure 1. Mechanisms that promote proteostasis in germ cells. The different steps of male (left) and female (right) gametogenesis and subsequent fertilization are represented. Several mechanisms to maintain proteostasis during reproduction have been identified and can be broadly classified as: (i) specific proteostasis network (PN) programs, including elevated expression of ubiquitous components and the expression of specialized components, to maintain the proteome of germ cells and prevent protein damage; (ii) the elimination of damaged cells via apoptosis, which can be triggered by the activation of cell stress responses; and (iii) the removal of misfolded and aggregated proteins by degradation pathways before or following fertilization to reset proteostasis.

HSR and are hypersensitive to elevated temperatures [27]. HSF1 does not induce the expression of heat shock genes in testes during heat stress, but protects immature germ cells from cell death and promotes the apoptosis of pachytene spermatocytes [27]. In agreement, overexpression of constitutively active HSF1 leads to the apoptotic death of spermatocytes resulting in male infertility [28]. During heat stress, HSF1 activates the expression of the proapoptotic T cell death-associated gene (*Tdag51*) [29], suggesting that HSF1 acts as part of a quality control program that triggers the elimination of spermatocytes that have experienced excessive stress. While *Hsf1* knockout has limited effects on male fertility in mice, double disruption of *Hsf1* and *Hsf2* has an additive phenotype and results in complete arrest of spermatogenesis [30]. Both HSF1 and HSF2 regulate several multicopy genes located on sex chromosomes that are required for proper chromatin organization in post-meiotic spermatids [31,32]. This suggests that HSF1 and HSF2 have overlapping functions in spermatogenesis that are essential for male fertility. It will be important to determine how HSF1 is recruited to different genomic loci in oocytes and sperm and how its activity is regulated to promote or prevent apoptosis during stress at the different stages of spermatogenesis. The identification of HSF1 targets in male and female germ cells, under physiological and stress conditions, will shed light on the specific transcriptional programs that define HSF1 function in reproduction.

The ubiquitin-proteasome system (UPS) in germ cells

The UPS is the primary mechanism for the selective degradation of proteins and is important for many regulatory aspects of germ cell biology (Box 1) [33]. Proteasome-mediated proteolysis has an evolutionarily conserved role in meiosis, where chromosome-localized 26S proteasomes

mediate the correct pairing of homologous chromosomes and crossover recombination during the first meiotic division [8,34]. In oocytes, the ubiquitin ligase CRL4 is essential for meiotic cell cycle progression by targeting protein phosphatase 2A (PP2A) for proteasomal degradation [35]. In mammalian testes, specialized proteasome complexes are predominant, which contain the testis-specific 20S subunit α4s/PSMA8 and the regulatory particle PA200 [36,37]. This 'spermatoproteasome' mediates the degradation of acetylated histones in response to meiotic DNA damage and is essential for meiosis progression and fertility [36–38].

The proteasome also removes damaged proteins to maintain proteostasis in germ cells. *Drosophila* oocytes possess an elevated capacity for 26S proteasome-dependent degradation, which, in contrast to somatic tissues, is maintained during aging [39,40]. This correlates with the limited accumulation of damaged and aggregated proteins in aged oocytes compared with somatic tissues [39]. In *Caenorhabditis elegans*, carbonylated proteins, a product of oxidative damage, accumulate in the female germline but are eliminated in oocytes prior to fertilization [13]. This process relies on a functional proteasome [13], indicating that proteasomal degradation of oxidatively damaged proteins contributes to the resetting of proteostasis during reproduction. Proteasome activity declines in aging mouse oocytes, which correlates with increased accumulation of oxidatively damaged proteins and may contribute to the age-related decline in oocyte quality [41] (see later). This suggests that UPS-mediated protein quality control is important to maintain proteostasis in young mouse oocytes and support female reproductive function. Mouse embryonic stem cells were shown to eliminate damaged proteins upon differentiation, which correlates with elevated 20S proteasome activity [42]. This raises the possibility that, in addition to its role in degrading maternal proteins to facilitate the maternal-to-zygotic transition and allow the expression of embryonic proteins [43], UPS-mediated degradation of damaged maternal proteins may participate in a rejuvenation process to reset proteostasis in early mammalian embryonic development.

The autophagy–lysosome pathway (ALP) in germ cells

In addition to proteasomal degradation, cells engage the ALP to eliminate terminally damaged proteins and organelles (Box 1) [44]. Macroautophagy is important for female reproduction, as knockdown of genes essential for autophagosome formation results in a substantial decrease in oocyte numbers and reduced litter size in mice [9,45]. As for the UPS, insights into the role of the ALP in protein quality control during gametogenesis and reproduction have been provided by studies in model organisms. In yeast, protein aggregates and other age-induced damaged material is sequestered away from chromosomes, excluded from gametes, and eliminated by lysosome-like vacuolar lysis during meiosis [46]. Protein aggregates that accumulate in the developing *C. elegans* oocyte are cleared in a process coupled to oocyte maturation, which involves the acidification of lysosomes and subsequent degradation of aggregates via microautophagy [4,12]. In *C. elegans*, oocyte maturation is initiated prior to fertilization by actin-like major sperm proteins (MSPs) secreted by sperm. This pathway has been proposed to also trigger the elimination of protein aggregates [4]. Lysosome acidification was also detected upon oocyte maturation in *Xenopus*, suggesting that this mechanism may be conserved [4]. Interestingly, autophagy is activated following fertilization in mice, a process required for the degradation of maternal proteins to allow the production of new proteins encoded by the zygotic genome [47]. This is likely to also contribute to the ALP-mediated degradation of sperm structures following fertilization, including paternal mitochondria (see later). Whether the ALP is also engaged to eliminate damaged maternal proteins and reset embryonic proteostasis during mammalian reproduction remains to be addressed.

Mitochondrial quality control in germ cells

Abnormalities in the quantity, quality, and function of mitochondria are associated with poor fertility in both male and female gametes [48]. The mitochondrial unfolded protein response

(UPR^{mt}) is a conserved transcriptional program activated in response to multiple forms of mitochondrial stress, including the accumulation of unfolded proteins in the mitochondrial matrix and mutations of mitochondrial DNA [49]. The mitochondrial caseinolytic protease proteolytic subunit (CLPP), a key activator of the UPR^{mt} [50], is required for oogenesis, spermatogenesis, and embryo development in mice [11]. In the absence of CLPP, mitochondrial function and dynamics are profoundly altered in mouse oocytes, suggesting an important role for this enzyme in the regulation of mitochondrial proteostasis during oogenesis [11]. In addition, recessive mutations in *Clpp* have been identified as a cause of the human Perrault syndrome characterized by hearing loss and ovarian failure [51]. Further studies are warranted to explore the role of the UPR^{mt} in male and female gametogenesis and its activation in response to stress in germ cells.

Another aspect of mitochondrial quality control is mitophagy, whereby damaged mitochondria are engulfed and degraded by macroautophagy. In metazoans, the progeny receives maternal mitochondria only, and paternal mitochondria are degraded through the ALP following fertilization [52–54]. During oogenesis, damaged mitochondria are selectively eliminated and healthy mitochondria preferentially inherited [55]. This was recently shown to rely on fragmentation of the mitochondrial network, followed by the mitophagy of low-ATP-producing mitochondria during *Drosophila* oogenesis [56]. Therefore, mitophagy is a central process for mitochondrial inheritance and quality control during reproduction.

Endoplasmic reticulum (ER) quality control in germ cells

Accumulation of unfolded proteins in the ER lumen activates a series of signaling pathways, collectively known as the unfolded protein response of the ER (UPR^{ER}), that induce the expression of numerous genes to restore homeostasis in the ER or initiate apoptosis if ER stress cannot be resolved [57]. During oogenesis, the UPR^{ER} is activated in granulosa cells (GCs) – the somatic cells that support oocyte development in ovarian follicles – in the later stages of oocyte growth, and higher levels of UPR^{ER} activation are positively correlated with the fertilization capacity of human oocytes [58]. However, excessive ER stress in GC has also been suggested to be involved in follicular selection, a phenomenon known as atresia that is initiated by the apoptosis of GCs [59]. Exposure to heat stress leads to ER stress-mediated induction of apoptosis by activation of the IRE1 and PERK pathways in spermatocytes, supporting a role for the UPR^{ER} in the elimination of damaged cells during spermatogenesis [60]. These observations suggest that levels of ER stress may play a role in the selection of germ cells during gametogenesis in both male and female.

Proteostasis in reproductive aging

The decline of female reproductive capacity is one of the first manifestations of human aging [61]. Oocytes enter meiosis during embryogenesis and remain arrested in meiotic prophase I for decades and therefore are highly susceptible to age-related dysfunction (Box 2). By contrast, spermatogenesis occurs throughout adult life, and although sperm quality and quantity progressively decline in aging, men remain fertile and can continue to reproduce until late in life. The ability of oocytes to support meiotic divisions, fertilization, and embryonic development, referred to as oocyte quality, declines abruptly during aging, as early as the third decade of life [62]. For example, aneuploidy rates increase dramatically in maternal aging and 35% of pregnancies are trisomic in women aged 40 years, against under 5% in women in their 20s [63]. While the underlying molecular basis for the precipitous age-related decline in oocyte quality is poorly understood, numerous cellular processes have been implicated, including defects in chromosome segregation due to compromised meiotic spindle assembly or loss of sister chromatid cohesion, telomere shortening, and mitochondrial dysfunction [48,64,65]. Proteins critical for chromosome segregation, such as cohesin and centromere-specific histones, are extremely long lived with limited capacity for renewal [66,67], suggesting that their deterioration with age could contribute to

defects in older oocytes. Findings that the expression of certain PN components declines during aging in oocytes [68] provide further support that proteostasis imbalance – a hallmark of aging in somatic tissues – may also be a contributing factor in female reproductive aging (Figure 2).

Cellular stress and protein damage in aging oocytes

Protein misfolding occurs continuously and leads to protein species that are not functional and are at risk for the formation of toxic protein aggregates. The prolonged arrest of oocytes before ovulation provides a large window of time during which cellular damage resulting from both endogenous and exogenous insults can accumulate. Oocytes are exposed to conditions that challenge protein conformation throughout their life, including thermal stress, oxidative stress, and toxic pollutants such as cadmium. In addition to direct protein modifications, damage to DNA and the gene expression machinery may result in the production of metastable protein variants and increase the burden on the PN. Among these, oxidative damage has been studied extensively and is regarded as a major factor contributing to age-associated defects in oocytes [69]. While reactive oxygen species (ROS) play an important role in meiosis and ovulation at basal levels, age-related increases in ROS levels and the reduction of antioxidant mechanisms disrupt homeostasis and lead to a state of chronic oxidative stress [70,71]. Elevated oxidative stress has been implicated in many of the defects associated with oocyte aging, including errors

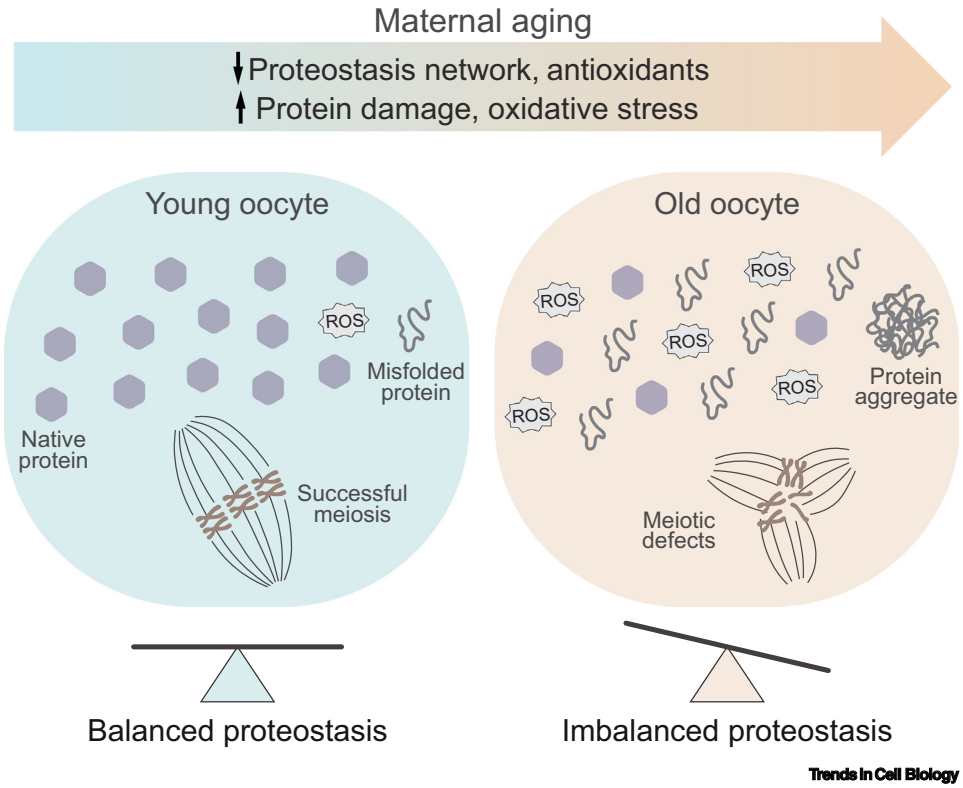

Figure 2. Age-dependent loss of proteostasis in oocytes. Loss of proteostasis during aging in arrested oocytes contributes to the age-related decline in oocyte quality. In young oocytes, a functional proteostasis network (PN) and antioxidant mechanisms limit protein misfolding and aggregation, thereby maintaining a healthy proteome that supports oocyte meiosis and function. During aging, a functional decline in molecular chaperones, degradation pathways, and antioxidant responses leads to the accumulation of misfolded and aggregated proteins and results in a state of chronic proteome imbalance. This compromises the ability to successfully complete meiosis and contributes to the decline of oocyte quality. Abbreviation: ROS, reactive oxygen species.

in meiotic spindle assembly and aneuploidy [72–74]. Recent studies have identified the production of reactive lipid aldehydes as a mechanism that leads to such damage. These species can irreversibly modify proteins and cause them to misfold and aggregate [75]. Among the proteins subject to oxidative damage in mouse oocytes are α-, β-, and γ-tubulin, which are key to the nucleation and assembly of the meiotic spindle [73]. Oxidative damage in the form of protein carbonylation also accumulates in aged *Drosophila* oocytes, which is associated with impaired development [39]. The major carbonylated targets include α-tubulin, β-tubulin, and the constitutively expressed HSP70-family chaperone Hsc70 [39]. Therefore, age-related damage in oocytes is likely to result in the accumulation of protein species that challenge the PN and contribute to the proteostasis imbalance in these cells (Figure 2).

Loss of proteostasis during maternal aging

The age-dependent decline in the ability of somatic cells to maintain a functional proteome is a major driver of cellular dysfunction during aging. Studies in model organisms have revealed extensive accumulation of protein aggregates in older animals, indicating a generalized imbalance of the proteome [76,77]. This is accompanied by reduced functionality of the PN, which is regarded as an underlying cause of age-related proteome imbalance [78,79]. Transcriptomic analyses of follicles from young and old mice have revealed that processes related to protein quality control are among the major classes of genes downregulated during aging [68,80,81]. Oocytes from older mice exhibit a marked decrease in the expression of several molecular chaperone genes belonging to the HSP70, HSP40, HSP90, and CCT/TRiC families, as well as components of the 20S and 19S proteasome, ubiquitination enzymes, and ubiquitin itself. Older oocytes also exhibit expanded nucleoli with high fibrillarin expression and as a result have increased levels of ribosomes, suggesting dysregulation of protein biogenesis [68]. Proteasome activity was also shown to decline in murine oocytes during maternal aging, which correlates with the accumulation of oxidative protein damage [41]. Inhibition of the proteasome in young oocytes leads to increased damage to proteins, including α-tubulin, thereby contributing to meiotic defects. Several proteasomal subunits were also found to be targets of oxidative damage, which could underlie the destabilization of proteasome complexes and subsequent loss of activity in old oocytes [41]. Overall, the profound age-dependent remodeling of protein quality control mechanisms would be expected to impair the ability of oocytes to manage the deleterious effects of proteotoxic stress, leading to a state of chronic proteome imbalance. Such loss of oocyte proteostasis may compromise the ability to successfully complete meiosis and contribute to the decline of cellular integrity during maternal aging (Figure 2).

Regulation of systemic proteostasis by germline tissues

Studies using model organisms have revealed that the reproductive system regulates the proteostasis capacity of somatic tissues, with important consequences for organismal health and longevity. These findings are consistent with the 'disposable soma' theory of aging, which states that reproduction and somatic maintenance compete for limited amounts of resources and that pathways preferentially allocating resources to the germline at the expense of somatic tissues may confer an evolutionary advantage [82]. However, somatic resilience and reproduction can be uncoupled, and specific molecular pathways, rather than mere energetic trade-offs, appear to govern the regulation of proteostasis mechanisms by the reproductive system. While most of the evidence that somatic proteostasis is modulated by reproductive tissues comes from studies in invertebrates, many of the key regulators involved are conserved from nematodes to humans. Notably, altered endocrine function during female reproductive aging in humans is associated with increased risk of cardiovascular disease and neurodegeneration [83], and menopause was shown to accelerate biological aging [84]. In this section, we discuss the different pathways known to connect reproductive tissues to somatic proteostasis regulation in nematodes (Figure 3) and their potential conservation in other species.

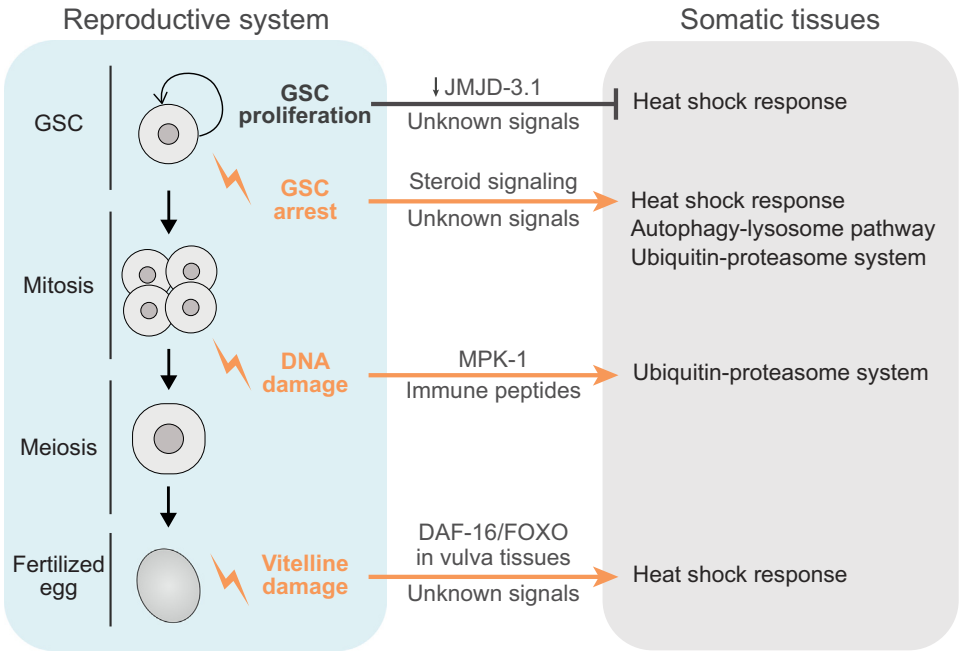

Figure 3. Regulation of somatic proteostasis by the reproductive system in *Caenorhabditis elegans*. Studies in *C. elegans* have revealed multiple modes of regulation of somatic proteostasis by the reproductive system. During normal development, signals from germline stem cells (GSCs) repress the heat shock response (HSR) at reproductive maturity as a result of reduced expression of the demethylase JMJD-3.1. In addition, various perturbations of the germline activate proteostasis-promoting pathways in somatic tissues. Mutations that induce GSC arrest enhance the HSR, the autophagy–lysosome pathway, and the ubiquitin-proteasome system (UPS). DNA damage to germ cells activates an MPK-1-dependent innate immune response that elevates the activity of the UPS in somatic tissues. Damage to the vitelline layer of the fertilized egg in the uterus activates a DAF-16/FOXO response specifically in vulval tissues and restores the HSR in the somatic tissues of adult animals.

The ability to maintain proteostasis declines with aging, increasing the risk of age-related protein misfolding diseases [78,79]. In *C. elegans*, proteostasis decline occurs abruptly in early adulthood at a timepoint that corresponds to the onset of reproduction [5,85]. This is associated with reduced inducibility of multiple cell stress responses, thus compromising stress resilience in adulthood [5]. At the molecular level, repression of the HSR in *C. elegans* adults results from increased trimethylation of histone H3 lysine 27 (H3K27me3) marks at stress gene loci, which interferes with HSF-1 binding and suppresses transcription initiation in response to stress [5]. In young animals, H3K27me3 is counteracted by the JMJD-3.1 demethylase, but the levels of *jmjd-3.1* mRNA decrease in somatic tissues as animals become reproductively mature. Mutations that arrest germline stem cells (GSCs), resulting in germline-less animals, prevent the decrease in *jmjd-3.1* and the repression of the HSR, indicating that signals from the germline are responsible for the programmed repression of the HSR in the soma at reproductive maturity (Figure 3) [5]. Decreased levels of H3K27me3 repressive marks have also been linked to longevity and stress resilience in *Drosophila* [86] and accumulation of H3K27me3 was detected in the killifish aging brain [87]. Age-related impairment of stress responses has also been observed in rats [88] and in human senescent cells [89,90]. However, an analysis of chromatin marks at proteostasis genes in senescent cells failed to detect any significant change in H3K27me3 [91]. Instead, reduced occupancy of the histone chaperone HIRA was detected at proteostasis gene loci in senescent cells, which could result in a less dynamic chromatin state less permissive for rapid gene activation in response to stress [91]. It remains to be addressed whether the chromatin landscape and cell stress

responses of somatic tissues can be influenced by the reproductive system in species other than *C. elegans*.

In *C. elegans*, GSC arrest also prevents protein aggregation and restores proteostasis in multiple somatic tissues of adult animals [5,85]. Removal of GSCs had previously been shown to extend lifespan in *C. elegans* and *Drosophila* [92,93] and factors required for this longevity phenotype are also important for somatic proteostasis modulation by the germline in *C. elegans* [85]. This includes DAF-16/FOXO, the transcription factor downstream of the insulin/IGF-1 signaling pathway involved in longevity and stress resistance, and the autophagy regulators PHA-4/FoxA and HLH-30/TFEB [85]. Germline-less animals exhibit increased proteasome activity, which results from the upregulation of the 19S proteasome subunit gene *rpn-6* by DAF-16 [94]. The expression of multiple ALP genes is also induced by PHA-4 and HLH-30 transcription factors in GSC-arrested animals [95,96]. Steroid hormone signaling is required for the longevity and increased proteostasis during GSCs arrest, although not all phenotypes are affected, suggesting additional signals (Figure 3) [85,97]. Intriguingly, these pathways are specific to GSC removal and are not initiated in other sterile mutants [5,85,92]. Together, these findings indicate that signals from GSCs have profound deleterious effects on the proteostasis of somatic tissues and determine organismal health in reproductive *C. elegans*. Although key regulators of these pathways such as HSF1, FOXO, and TFEB are conserved in mammals, females appear to lack adult GSCs [98,99], as the pool of primary oocytes is generated during development (Box 1). Thus, if regulation of proteostasis by the reproductive system occurs in female mammals, it is likely to originate from other cell types and to involve distinct signaling mechanisms. By contrast, GSCs are present in adult males and are required for the continuous production of gametes in mammals (Box 1). Interestingly, castration appears to increase male lifespan in rats and humans, suggesting that the interplay between reproductive function and longevity may be conserved [100,101].

In addition to GSCs arrest, targeted perturbations of the fertilized embryo in the uterus can also promote somatic proteostasis in *C. elegans* [7]. Knockdown of genes involved in the extracellular vitelline layer of the eggshell activates a transcellular pathway that restores the ability to induce the HSR and prevent protein aggregation in the somatic tissues of reproductive mothers. This pathway relies on HSF-1 in somatic tissues and a DAF-16-mediated response in vulva tissues, suggesting that the integrity of the embryo is monitored by the egg-laying apparatus to detect damage and initiate an organismal protective response (Figure 3) [7]. Such embryo-to-mother communication may serve to reassess the commitment to reproduction and promote the survival of the parent when reproduction is compromised. The presence of an extracellular matrix coating the fertilized egg to provide physical protection to the developing embryo is common to all metazoans. Whether insults to the fertilized embryo's extracellular coat can influence maternal somatic resilience in other species remains to be established.

Another type of insult to the reproductive system, DNA damage, was also shown to increase somatic stress resistance in *C. elegans*. DNA damage to germ cells caused by either exogenous radiations or mutations initiates an innate immune response that is mediated by the MAP kinase MPK-1 and results in elevated organismal resistance to heat and oxidative stress [102]. In somatic tissues, this pathway activates the expression of multiple subunits of the proteasome, resulting in increased UPS activity (Figure 3). Following DNA damage, progeny production was paused but later resumed to generate a similar number of total offspring [102], suggesting that elevated proteostasis may promote somatic endurance to allow survival of the animal until more favorable conditions resume. Germ cells are particularly susceptible to DNA damage, a critical issue for cancer patients receiving radiation therapy or chemotherapy that often results in premature ovarian failure [103]. Innate immune responses to DNA damage have also been

described in humans [104] and it will be interesting to determine whether this also occurs upon DNA damage specifically in germ cells and whether it affects proteostasis and stress resilience in other tissues.

Concluding remarks

Germ cells have the remarkable ability to maintain a healthy proteome across generations, and several lines of evidence converge on proteostasis regulation being critical for reproduction. In this review, we present studies that have revealed a diversity of mechanisms that promote proteostasis in germ cells to support their function and protect progeny from inheriting proteome damage. The importance of proteostasis for successful reproduction has also been highlighted by findings suggesting that reduced PN functionality and the accumulation of protein damage in long-lived oocytes contribute to the age-related decline of reproductive capacity. The discovery that the reproductive system regulates the proteostasis of somatic tissues in nematodes suggests that organismal strategies have evolved to prioritize reproduction over somatic maintenance.

Future efforts to determine the composition and function of the PN in male and female gametogenesis will provide insights into key components that support reproductive function (see Outstanding questions). The use of model organisms will be instrumental for monitoring PN functionality in real time during reproduction using fluorescent reporters of PN activities. An important question to address is how maternal damaged proteins are eliminated during reproduction and it will be interesting to determine whether findings that lysosome acidification drives this process in *C. elegans* oocytes translate to other species [4,12]. Furthermore, understanding how the proteome is altered in older oocytes by assessing the solubility of individual proteins using proteomics approaches will be important to evaluate the effects of proteostasis imbalance in reproductive aging. It will be of particular interest to examine the role of the PN in maintaining the protein machineries that orchestrate chromosome segregation during meiosis, how it may impact rates of aneuploidy, and whether interventions to increase proteostasis capacity can improve oocyte quality during maternal aging. The systemic regulation of somatic proteostasis and longevity by the reproductive system in invertebrate model organisms [7,85,92,93] raises the possibility that such a mode of regulation could be conserved. A study in zebrafish recently showed that sterile males are more resistant to stress, suggesting that trade-offs between germline and somatic maintenance are conserved in vertebrates [105]. Performing similar studies in mice will reveal whether the reproductive system also impacts somatic proteostasis and organismal longevity in mammals. Elucidating the molecular pathways that act within and from germline tissues to regulate the PN could lead to new strategies to combat the age-related proteostasis decline and improve human reproductive-span and healthspan.

Outstanding questions

What is the composition of the PN in gametes and what is the role of cell stress responses during gametogenesis in normal physiology and during stress?

What is the basis for PN dysregulation in aging oocytes and how does it impact oocyte function? Which proteins are susceptible to age-related misfolding and aggregation during oocyte aging?

Do degradation pathways selectively remove damaged and aggregated proteins during mammalian reproduction to reset proteostasis?

Can interventions to increase proteostasis capacity in oocytes improve oocyte quality during maternal aging?

What is the nature of the signals from the reproductive system that modulate somatic proteostasis? Is this mode of regulation conserved and can it be targeted to enhance proteostasis in somatic tissues during aging?

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Declaration of interests

The authors declare no interests.

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