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Gametogenesis: Exploring an Endogenous Rejuvenation Program to Understand Cellular Aging and Quality Control

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Abstract

Gametogenesis is a conserved developmental program whereby a diploid progenitor cell differentiates into haploid gametes, the precursors for sexually reproducing organisms. In addition to ploidy reduction and extensive organelle remodeling, gametogenesis naturally rejuvenates the ensuing gametes, leading to resetting of life span. Excitingly, ectopic expression of the gametogenesis-specific transcription factor Ndt80 is sufficient to extend life span in mitotically dividing budding yeast, suggesting that meiotic rejuvenation pathways can be repurposed outside of their natural context. In this review, we highlight recent studies of gametogenesis that provide emerging insight into natural quality control, organelle remodeling, and rejuvenation strategies that exist within a cell. These include selective inheritance, programmed degradation, and de novo synthesis, all of which are governed by the meiotic gene expression program entailing many forms of noncanonical gene regulation. Finally, we highlight critical questions that remain in the field and provide perspective on the implications of gametogenesis research on human health span.

1. INTRODUCTION

Homologous recombination: a process found in all forms of life that provides high-fidelity, template-dependent repair of DNA double-strand breaks

Transcription factor (TF): a protein that binds gene promoters to regulate RNA production

Senescence-associated factor: a molecule that accumulates inside of a cell as it ages

For a species to persist, organisms have a biological imperative to create new offspring. Sexual reproduction is the process by which genetic information is combined from two parental organisms. In metazoans, sexual reproduction relies on the formation of haploid gametes, egg and sperm, through a highly regulated developmental program called gametogenesis. Although somatic tissues deteriorate with age, the germline—the cell lineage from which gametes are derived—is pristinely passed from parents to progeny through successive generations (81). The list of fundamental pathways that ensure the maintenance of germline fitness amid cellular aging of the soma continues to grow, and how these events are coordinated remains under investigation.

Gametogenesis involves the coordinated execution of several key events. Pioneering studies in *Saccharomyces cerevisiae* have led to the discovery of conserved mechanisms that regulate homologous recombination and chromosome segregation during gametogenesis (termed sporulation in yeast) (reviewed in 41, 71); however, the distinct morphology of yeast gametes (also called spores) compared to those of the metazoan egg and sperm has led to the general assumption that evolutionarily conserved aspects of gametogenesis are restricted to the structural and dynamic changes that occur at chromosomes. Recent studies have challenged this viewpoint, revealing gametogenesis-specific organelle remodeling pathways that share similarities between yeast and metazoans, which suggests that the conserved aspects of the gametogenesis program extend beyond chromosome-specific processes (79, 118, 132, 137).

At the cellular level, aging manifests as an accumulation of various hallmarks, many of which are conserved from yeast to humans (reviewed in 76, 93). As we continue to learn about the molecular factors associated with aging, we are entering an exciting new era where strategies to prevent or reverse the hallmarks of aging are being developed (reviewed in 20, 96). However, verified strategies that target these age-related defects remain limited. Gametogenesis is a natural context in which age-associated damage is removed, creating gametes that are born young, regardless of the precursor's age. In budding yeast, a p53-related master transcription factor (TF), Ndt80, drives life span resetting and removal of conserved senescence-associated factors. Excitingly, ectopic expression of *NDT80* can activate its targets (30) and extend the life span of aging cells (152), demonstrating that aspects of meiotic rejuvenation can be repurposed to reverse the detrimental effects of age in a somatic context. This finding also suggests that one or more of the gene expression subprograms initiated by Ndt80 can counteract cellular damage to help reset the aging clock. In this review, we examine gametogenesis research that has provided insights into cellular quality control and rejuvenation. The life cycle of budding yeast provides a uniquely tractable platform for the investigation of these pathways and thus is the primary focus; however, relevant examples from metazoans are highlighted where possible.

2. AGING, GAMETOGENESIS, AND REJUVENATION IN BUDDING YEAST

Budding yeast is an excellent model for studying replicative aging and gametogenesis-based rejuvenation (**Figure 1**). In this section, we provide a brief overview of the yeast life cycle, highlighting relevant details for this review. Due to space limitations, we direct the reader to explore thorough reviews on yeast gametogenesis (112), meiotic chromosome dynamics (41, 71), and asymmetric division of organelles and age-associated damage in mitosis (35, 69, 156) for further insight into these topics.

During mitosis, a mother cell produces a bud that inherits a copy of the genome and a subset of organelles to become a daughter cell. Until a mother cell is extremely old, all its daughters are born young. This is because several mechanisms exist to prevent senescence-associated factors, damaged

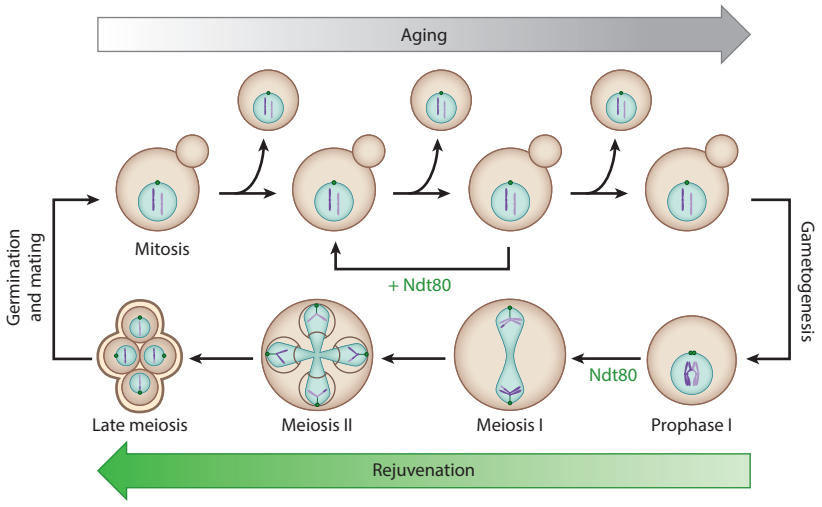


Figure 1

The *Saccharomyces cerevisiae* life cycle includes replicative aging and gametogenesis-based rejuvenation. (*Top*) During mitotic growth, a mother cell produces a bud that will grow and inherit a copy of the genome and organelles (not shown) to form a daughter cell. While the daughter cell is born young, the mother cell accumulates age-associated damage over time (see **Figure 2**), eventually causing senescence and death after approximately 25 divisions. (*Bottom*) Gametogenesis is induced by starvation conditions, which causes a diploid progenitor cell to undergo two meiotic divisions and differentiate into four haploid gametes. The meiotic transcription factor Ndt80 signals for the transition from prophase I to meiosis I as well as for the induction of meiotic rejuvenation pathways. Upon completion of gametogenesis, gamete life span is completely reset. When nutrients become available, gametes undergo germination, and mating returns the cell to a diploid state. Interestingly, ectopic expression of the Ndt80 transcription factor (+ Ndt80) has been shown to revert aging cells to a younger state and extend replicative life span. The centrosomes (termed spindle pole bodies in yeast) (*dark green circles*) facilitate spindle formation for chromosome segregation as well as gamete membrane formation.

proteins, and dysfunctional organelles in the mother cell from being passed on to the daughter cell (reviewed in 69). Each mother cell has a finite replicative life span and can only produce approximately 25 daughter cells before senescence and eventual death (107, 135). The contributions of genomic instability, senescence-associated factors (reviewed in 76), organelle dysfunction (91), and hypertrophy (77, 114) to aging, senescence, and cell death remain under investigation.

Each time a mother cell produces a daughter cell during mitosis, a chitinous ring called a bud scar is left behind on its surface (18). Experimentally, bud scars can be fluorescently labeled, and the number of divisions a mother has undergone, i.e., its replicative age, can be quantified (122). Although individual cells have a finite life span, mitotic budding yeast can grow indefinitely as a population since daughter cells continue to be produced. However, under nutrient-limiting conditions, diploid mother cells undergo gametogenesis, culminating in the resetting of their life span (152).

Entry into gametogenesis is mediated by the meiotic TF Ime1, whose activation is dependent on the integration of environmental (e.g., nutrient availability and pH) and intrinsic (e.g., ploidy and respiration competency) cues (reviewed in 112). Following Ime1 activation, meiotic cells undergo DNA replication concomitantly with the induction of programmed double-strand breaks (DSBs) and changes in chromosome structure. Repair of DSBs results in the activation of the Ndt80 TF, which induces the genes required for two rounds of meiotic divisions, meiosis I and

meiosis II, as well as for gamete maturation. During meiosis I, homologs separate from each other; then, in meiosis II, sister chromatids split. The onset of meiosis II also marks the modification of the centrosomes [called spindle pole bodies (SPBs) in yeast, henceforth referred to as centrosomes for simplicity] to become membrane-organizing centers in addition to their conventional role as microtubule-organizing centers. As meiosis II progresses, a double-membrane structure called the prospore membrane (henceforth referred to as the gamete membrane for simplicity) extends from the cytoplasmic face of each of the four modified centrosomes to encompass each nuclear lobe and proximal organelles (110). The growing gamete membrane serves as a border between cellular material that will or will not be inherited by the gametes. By late meiosis, the gamete membrane is closed, serving as a foundation for the formation of the spore wall (reviewed in 111). Each gamete now contains a single copy of the genome as well as a full complement of organelles (discussed in Sections 3 and 4).

Budding yeast gametes reside inside an ascus sac, which is formed from the plasma membrane and cell wall of the progenitor cell (reviewed in 111). Detection of nutrients initiates germination, and gametes of opposite mating types fuse together to produce a zygote (reviewed in 64). The resulting diploid cell starts the mitotic cell cycle with full replicative potential. Thus, budding yeast mitosis and meiosis provide natural contexts to study aging and rejuvenation at the cellular level.

Spindle pole body (SPB): the microtubule-organizing center embedded in the nuclear envelope in budding yeast

Extrachromosomal circular DNA (ecDNA): a circular molecule of DNA that is typically generated from a repetitive region of the genome and does not contain a centromere

Outer nuclear membrane (ONM): the nuclear envelope membrane that interfaces with the cytoplasm

Inner nuclear membrane (INM): the nuclear envelope membrane that interfaces with nucleoplasm and chromatin

Gametogenesis uninherited nuclear compartment (GUNC): a fifth membrane-bound compartment that forms during meiosis II and is destroyed upon programmed lysosomal permeabilization

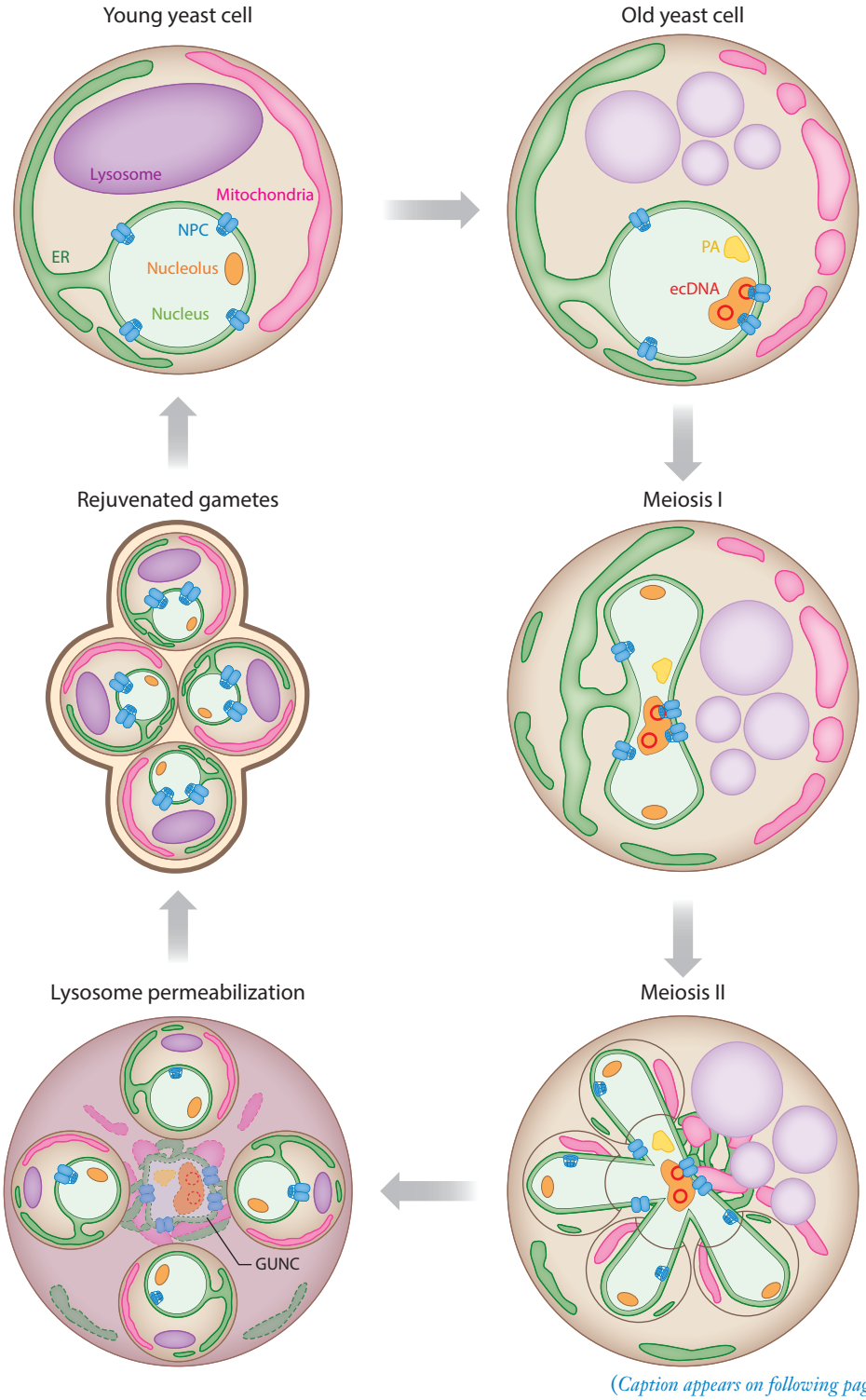
3. TARGETED DEGRADATION OF AGING BIOMARKERS IN THE NUCLEUS

The nucleus accumulates many forms of age-associated damage. In both yeast and metazoans, aging leads to genome instability, extrachromosomal circular DNA (ecDNA) formation (36, 86, 133, 157; reviewed in 3), nucleolar defects (17, 106, 134, 146), accumulation of protein aggregates (2, 19, 48, 128; reviewed in 155), decreased integrity of the nuclear envelope, and nucleocytoplasmic transport defects, which can contribute to age-associated diseases, including neurodegeneration, cancer, and progeria (28, 33, 47, 56, 63, 94, 125, 161). Thus, gametogenesis must ensure that aging biomarkers in the nucleus are not inherited by the developing gametes. In this section, we discuss what is known about the mechanisms that govern the removal of senescence-associated factors from nuclei.

3.1. Nuclear Remodeling, Degradation, and Formation of the Gametogenesis Uninherited Nuclear Compartment

The nucleus is encircled by a nuclear envelope, composed of an outer nuclear membrane (ONM) and an inner nuclear membrane (INM), that surrounds the chromatin and houses nuclear pore complexes (NPCs; see Section 3.4) that span both membranes (**Figure 2**). During mitosis and meiosis, the budding yeast nucleus undergoes closed divisions where the nuclear envelope remains contiguous (reviewed in 9). During meiosis II, the nucleus is reshaped into four connected lobes, each containing a single copy of the genome and a single centrosome. After the closure of gamete membranes, a portion of the nuclear envelope is left outside of the gametes (54, 79, 104). This gamete-excluded nuclear envelope–bound compartment is called the gametogenesis uninherited nuclear compartment (GUNC) and is eventually eliminated by mega-autophagy in late meiosis II following programmed destruction of the precursor cell's lysosome (42, 43; reviewed in 80) (**Figure 2**; see also Section 4.1). Thus, any protein or organelle that remains associated with the GUNC is eventually destined for degradation.

Prior to GUNC elimination by mega-autophagy, nucleophagy (also called nuclear autophagy), mediated by the autophagy receptor Atg39, has also been shown to be active during the meiotic



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

A model of yeast aging and gametogenesis that reflects our current knowledge of organelle segregation, organelle synthesis, and senescence-associated factor exclusion. As a young cell (*upper left*) undergoes successive cellular divisions, several age-associated morphologies accumulate. In the nucleus of an old cell (*upper right*), senescence-associated factors such as ecDNA (*red*) and PAs (*yellow*) are formed, the nucleolus (*orange*) becomes enlarged, and NPCs (*blue*) in the nuclear envelope cluster and lose permeability. Compared to the highly acidic young cell lysosome (called the vacuole in yeast; *purple*), the old cell lysosome loses acidity and fragments (*light purple*), the mitochondria (*pink*) depolarize and fragment, and the ER (*dark green*) accumulates misfolded proteins. During gametogenesis (following *arrows clockwise* starting at *upper right*), senescence-associated factors are sequestered away from the developing gametes into the GUNC, organelles are remodeled such that only a fraction are inherited, uninherited material is destroyed upon lysosomal permeabilization, and de novo synthesis of organelles occurs as gametes mature (*lower* to *middle left*). Together, these pathways converge to fully reset gamete life span. Abbreviations: ecDNA, extrachromosomal circular DNA; ER, endoplasmic reticulum; GUNC, gametogenesis uninherited nuclear compartment; NPC, nuclear pore complex; PA, protein aggregate.

program (102, 118). This suggests that selective autophagy may play a role in nuclear quality control prior to mega-autophagy. Atg39 has recently been shown to mediate nuclear envelope protrusion into the cytoplasm, through its associations with both the ONM and INM, culminating in the formation of nucleus-derived double membrane vesicles that are destined for lysosome-dependent degradation (103). Whether Atg39 plays an analogous role in gametogenesis is currently unknown. More generally, future studies aimed at understanding how GUNC formation is regulated, how macromolecules are targeted to the GUNC, and whether nucleophagy helps degrade portions of the GUNC will provide insights into what nuclear factors need to be eliminated to promote gamete health and development.

Septins: a conserved class of proteins that are found at the bud neck in mitosis or near the gamete membrane rim in meiosis

Diffusion barrier: located at the bud neck, a distinct domain that limits the lateral passage of cellular contents from mother to daughter cell, thus mediating asymmetric cell division

Ribosomal DNA (rDNA): a repetitive region of DNA required for ribosome biogenesis that can form extrachromosomal circular DNA when unstable

Nuclear pore complex core (NPC core): the portion of the nuclear pore complex that spans the outer and inner nuclear membranes of the nuclear envelope

3.2. Sequestration of Extrachromosomal Circular DNA and Protein Aggregates

In gametogenesis, nuclear aging factors, including protein aggregates (19, 128) (**Figure 2**) and ecDNA (36, 133) (**Figure 2**), are sequestered into the GUNC during meiosis II and are subsequently eliminated (79). Protein aggregate and ecDNA exclusion occurs at high fidelity (>99.5%), suggesting that robust mechanisms exist to ensure selective nuclear inheritance by gametes. It is unclear how ecDNA and protein aggregates are targeted to the GUNC, but elimination of these senescence-associated factors requires gamete membrane formation (79). Shaping of the gamete membrane requires the leading edge complex, which maintains the rim of the gamete membrane as it grows (83, 88, 105), and the removal of this complex at the end of meiosis II is needed for the closure of gamete membranes (38, 97, 120). In late meiosis II, the constrained opening of the rim is similar to the constrained bud neck in mitosis, which contains a septin-mediated diffusion barrier that prevents age-associated damage from being passed to the daughter cell (22, 31). However, removal of meiosis-specific septins Spr3 and Spr28 (34, 50, 55, 100, 119) or leading edge components has no effect on protein aggregate or NPC sequestration (79) (see Section 3.4) during gametogenesis. Therefore, it is unclear if the gamete membranes facilitate diffusion barriers and, if so, what the molecular nature of the barrier is.

Sequestration of ecDNA, including ribosomal DNA (rDNA) circles, suggests that the cell can distinguish these DNA species from the chromosomes. ecDNA does not contain a centromere and so will not be bound by spindle microtubules, but how ecDNA is specifically targeted to the GUNC remains unknown. One hypothesis is that ecDNA sequestration is mediated by NPCs. ecDNA has been shown to associate with the NPC core (discussed in Section 3.4) through the SAGA (Spt-Ada-Gcn5 acetyltransferase) complex in mitosis (36) and is sequestered to the GUNC at the same time as NPCs in meiosis. However, a direct role for SAGA in ecDNA sequestration during meiosis has not been observed (see "Author Response Letter" in 79). Thus, future studies

are required to determine whether NPC tethering through another mechanism plays a role in ecDNA sequestration to the GUNC.

3.3. Maintaining Nucleolar Homeostasis

The nucleolus is a membraneless organelle that mediates rRNA production, ribosome biogenesis, and cellular processes affected by aging, including pathways involved in maintaining the genome and proteostasis. Interestingly, nucleolar size is inversely correlated with longevity where nucleolar size increases with age (**Figure 2**) and is reduced in long-lived mutants (145, 146). In yeast, age-related nucleolar expansion and fragmentation arise due to upregulation of rRNA transcription as well as the accumulation of ecDNA (36, 133) and nucleolar proteins (106, 134, 152). Furthermore, many nucleolar proteins are highly prone to aggregation due to their intrinsically disordered regions (121).

During gametogenesis, a portion of the nucleolus is sequestered into the GUNC in meiosis II (79). The timing of nucleolar sequestration is the same as for sequestration of nuclear senescence-associated factors (see Section 3.2); however, nucleolar sequestration occurs regardless of the precursor cell's age. This suggests that, even in young cell gametogenesis, sequestration of a proportion of nucleolar proteins to the GUNC is programmed. In contrast to young cells, old cells exhibit enlarged, fragmented nucleoli, consistent with previous studies (106, 134). During gametogenesis, the amount of nucleolar material excluded to the GUNC correlates with replicative age of the old precursor cell such that the resulting gametes have similar nucleolar material to that of gametes produced by a young precursor (79). Taken together, this suggests that the cell can accurately regulate the amount of nucleolar material targeted to the GUNC, ensuring that the appropriate amount of nucleolar content is inherited by each gamete. Whether different ratios of nucleolar components are targeted to the GUNC to balance nucleolar composition, as in mitosis (59), will be important to determine in future studies.

3.4. Modular Segregation of Nuclear Pore Complexes

NPCs are conserved supramolecular structures that are embedded in the nuclear envelope and mediate nucleocytoplasmic transport. Composed of over 30 different subunits in multiple copies, NPCs contain multiple structural elements, including the NPC core, which spans the nuclear envelope, and the nuclear basket that interacts with chromatin (reviewed in 6). Some of these NPC subunits (also called nucleoporins) are among the most long-lived proteins in eukaryotic cells and are thereby subject to the gradual accumulation of age-associated damage (147). In yeast, worms, and mammals, aging causes NPCs to cluster together and become leaky as they fail to maintain a nuclear-cytoplasmic permeability barrier (33, 94, 125, 131, 147).

During meiosis II, NPCs are sequestered into the GUNC regardless of the age of the progenitor cell (**Figure 2**). Surprisingly, before gamete membrane closure, subunits of the nuclear basket return to the gametes while the subunits of the NPC core remain associated with the GUNC and are subsequently degraded (79). How and why the nuclear basket is inherited is unclear (reviewed in 80). It is possible that nuclear basket inheritance occurs as a result of chromatin organization and/or posttranslational modifications (reviewed in 124). The returning nuclear basket structures could facilitate the assembly of new NPCs in the gametes (101). Consistently, studies in human cells show that subassemblies of the NPC are retained in the endoplasmic reticulum (ER) during open mitosis for subsequent NPC reassembly. In late anaphase, the ER initiates nuclear envelope formation, and the NPC subassemblies become nucleation sites for new NPC formation (29). Thus, modular NPC segregation could play a role in NPC assembly and function in different contexts.

Nuclear basket: a dynamic subcomplex of the nuclear pore complex located on the nuclear side that functions in nuclear-cytoplasmic trafficking, chromatin regulation, mRNA biogenesis and mRNA export

Open mitosis: cell division involving coordinated disassembly and reassembly of the nuclear envelope

Interestingly, a similar sequestration and exclusion event occurs with NPCs during metazoan spermatogenesis. In insects and mice, NPC exclusion occurs coincident with the development of the acrosome, a structure external to the nucleus that may be acting analogously to the yeast gamete membranes (51, 70, 150). Therefore, the similarity in phenotype across such diverse evolutionary lineages suggests an ancestral need to reorganize the nucleus and NPCs during meiosis, perhaps for gamete rejuvenation.

4. ORGANELLE QUALITY CONTROL PATHWAYS

Gametes inherit a copy of the genome and a complete set of organelles that are required for gamete development. During both mitosis and meiosis, chromosome segregation is largely mediated by centrosomes and spindle microtubules; however, the mechanisms that govern organelle inheritance are rather divergent. In mitosis, cytoplasmic organelles are transported between the mother cell and the budding daughter cell by myosin motor proteins on polarized actin filaments (reviewed in 13, 69). By contrast, a polarized network of actin is not required in meiosis (143); thus, alternative mechanisms must exist to ensure that each gamete acquires the complete complement of cytoplasmic organelles from the precursor cell.

Gametes inherit approximately 30% of the progenitor cell's cytoplasm, including portions of most organelles (14, 79, 118, 132, 137). Since gamete membrane formation provides a proximity-based inheritance mechanism, relocalization of organelles to the developing gamete nuclei could be one potential mechanism promoting inheritance of select organelles. Conversely, localization of organelles to the cell periphery or toward the GUNC may act to promote their exclusion and degradation. In this section, we will discuss the nuances of global organelle remodeling that occurs during gametogenesis.

4.1. Lysosome Reacidification

The yeast lysosome-like vacuole (henceforth referred to as the lysosome for simplicity) facilitates the degradation and recycling of cellular materials. As cells age, the lysosome loses acidity and becomes fragmented, which decreases degradation efficiency, disrupts proteostasis, and diminishes the bioavailable pool of iron within the cell, leading to mitochondrial perturbations (65, 74, 75; reviewed in 5) (**Figure 2**). During gametogenesis, the gamete membrane acts as a barrier between cellular contents that will and will not be inherited by the developing gametes. After gamete membrane closure, all uninherited contents, including the GUNC, are degraded by mega-autophagy. This event is mediated by the release of hydrolases upon programmed permeabilization of the lysosome within the progenitor cell (called vacuolar lysis in yeast) (42, 43). Although the nature of the cue(s) that trigger lysosomal membrane permeabilization is unknown, the timing is precise and relies on the expression of *NDT80* (42). Furthermore, global degradation of uninherited material is critical for gamete formation, and the degraded products might provide building blocks for gametes as they continue to mature and eventually germinate.

Each gamete forms its lysosome de novo (42, 126, 137). Although this seems energetically unfavorable, de novo synthesis provides an opportunity to create a fully rejuvenated lysosome. In worms (*Caenorhabditis elegans*), the lysosome is not destroyed but instead is reacidified during oocyte maturation (10, 129). Signaling from proximal sperm cells triggers the expression and assembly of vacuolar-type ATPase (V-ATPase) protein pumps in the lysosome membrane of the maturing oocytes prior to fertilization. This in turn signals for protein aggregate clearance by microautophagy (10, 62). Thus, lysosome-mediated degradation of age-associated damage seems to be conserved, and gametogenesis reacidifies the lysosome by at least two known mechanisms: increased V-ATPase assembly and/or de novo synthesis.

4.2. Remodeling of the Mitochondrial Network

Mitochondria play a crucial role in a plethora of cellular processes, including the production of energy through central metabolism and the production of protein cofactors such as heme and iron–sulfur clusters. In budding yeast, mitochondria exist as an interconnected tubular network that contains approximately 10–20 mitochondrial genomes [mitochondrial DNA (mtDNA)] (95). In mitotically dividing cells, mitochondria are tethered to the cell periphery via the mitochondria-ER-cortex anchor (MECA) (82, 87) complex but also communicate with multiple organelles in the cell. For example, the ER–mitochondrial encounter structure (ERMES) complex is important for phospholipid transfer between the ER and mitochondria (reviewed in 85). Furthermore, a nuclear-mitochondrial tether called Cnm1 has recently been discovered and is important for phospholipid homeostasis (46).

Mitochondrial DNA (mtDNA): the genetic material inside mitochondria

Reactive oxygen species (ROS): highly reactive chemicals produced by the mitochondria

As yeast cells age, the mitochondrial network becomes fragmented with more oxidizing redox potential and higher superoxide levels compared to mitochondria in daughter cells (74, 89, 99). Elevated reactive oxygen species (ROS) could damage mtDNA, protein, and lipids, thereby contributing to cellular aging (reviewed in 11). During gametogenesis, only about half of the mitochondria from a young progenitor cell are inherited by the four haploid gametes (14). Efficient mitochondrial segregation partially relies on the meiosis-specific septins and the leading edge component Ady3; however, when *ADY3* and the septin-organizing gene *GIP1* are deleted, approximately 20% of gametes are still able to inherit mitochondria (137), suggesting that alternative mechanisms for mitochondrial segregation exist.

At the onset of meiosis II, meiotic kinase Ime2 promotes the degradation of MECA subunits, resulting in the detachment of mitochondria from the cell periphery and mitochondrial collapse toward the dividing nuclei (132) (**Figure 2**). As the gamete membrane forms, mitochondria that are associated with the developing gamete nuclei are enveloped and inherited by the developing gametes. In parallel, a subset of mitochondria is excluded from the developing gametes (132), presumably by interacting with the GUNC (79). This pool of mitochondria is eventually destroyed by mega-autophagy (42, 43). The molecular nature of tether(s) that mediate meiosis-specific localization of mitochondria (e.g., gamete nuclear envelope–associated versus GUNC-associated) is currently unknown, but Cnm1 (46) is an attractive candidate to investigate further.

The separation of mitochondria into inherited and discarded pools during meiosis raises the intriguing possibility that the cell can distinguish and sort dysfunctional and healthy mitochondria, similar to mitosis (68, 99). Consistent with this notion, several studies have identified measurable differences between inherited and discarded mitochondrial populations: (*a*) In mouse oogenesis, mitochondria containing mutations that disrupt oxidative phosphorylation are not inherited by the developing egg (49, 136); (*b*) in fly oogenesis, mitochondrial fragmentation isolates mitochondrial genomes and allows for mitochondrial selection based on adenosine triphosphate (ATP) production (92); and (*c*) in grasshopper (*Thermobia domestica*) oogenesis, either mitochondria with abnormal morphology are eliminated from the Balbiani body, which delivers organelles to germ cells that will develop into eggs, or germ cells that receive abnormal mitochondria are eliminated by apoptosis (151). Further studies are required to determine if membrane polarity differences, mtDNA mutation, morphological defects, and/or other cues are responsible for mitochondrial exclusion from developing gametes.

4.3. Compartmentalization and Targeted Degradation of the Cortical Endoplasmic Reticulum

The ER is a highly structured organelle that is dynamically remodeled in response to cellular demand and mediates a wide range of functions, including protein synthesis, lipid metabolism, and

interorganelle communication. As cells age, the ER gradually loses its ability to clear unfolded proteins, causing the accumulation of misfolded proteins (reviewed in 53). Historically, two regions of the ER have been distinguished: (*a*) the cortical ER, which is localized around the cell periphery, and (*b*) the perinuclear ER, which is indistinguishable from the nuclear envelope in yeast (123, 127). Quality control pathways involving the nuclear envelope are therefore applicable to the perinuclear ER and are discussed in Section 3.1.

Long-lived asymmetrically retained proteins (LARPs): full-length proteins or fragments that are unequally divided during mitosis, undergo slow turnover, and accumulate inside the mother cells during aging

Like the mitochondrial network, the cortical ER undergoes Ndt80-dependent remodeling during gametogenesis, resulting in the inheritance of only a fraction of the ER (118, 137) (**Figure 2**). The cortical ER forms cable-like structures prior to meiotic divisions. Then, in meiosis II, the majority of the cortical ER abruptly detaches from the cell periphery and collapses onto the dividing nuclei. As the gamete membrane matures, only the cortical ER that is proximal to the developing nuclei is expected to be inherited by gametes. Six plasma membrane–ER tethers are responsible for holding the cortical ER to the plasma membrane (98). Interestingly, Scs2 and Scs22 detach with the bulk of the cortical ER, while Tcb1, Tcb2, Tcb3, and Ist2 remain at the plasma membrane and continue to tether small compartments of the ER to the plasma membrane even after bulk cortical ER collapse (118). These gamete-excluded portions of the ER are eventually degraded by mega-autophagy (42, 43).

The cortical ER is also partially degraded by ER autophagy (ERphagy), which is mediated by the autophagy receptor Atg40, prior to gamete membrane closure and mega-autophagy (102, 118). Thus, it is also possible that selective ERphagy may play a role in eliminating damaged ER content. To date, gametogenesis-mediated turnover of the cortical ER has been observed in young progenitor cells (12, 118). In metazoans, ERphagy receptors preferentially degrade ER that contains misfolded protein aggregates (4, 32, 52); thus, future studies are required to investigate whether and how the ER turnover pathways prevent age-associated ER damage from being inherited by the gametes.

5. GLOBAL DEGRADATION AND RESYNTHESIS OF PROTEINS

Genome-wide studies have identified a subset of proteins that are asymmetrically retained in the mother cell, suggesting that a specific balance of proteins must be inherited by the daughter cell to reestablish proteostasis (159). Interestingly, ~135 of these proteins have been found to be long-lived asymmetrically retained proteins (LARPs) (138, 144), which have limited turnover and accumulate inside of the mother cell. It is unclear if the accumulation of LARPs or other subsets of asymmetrically inherited proteins contributes to the aging phenotypes; however, their exclusion from the newly forming daughter cell suggests that some may reduce life span. Indeed, many LARPs seem to have evidence of damage such as fragmentation or modification when analyzed by mass spectrometry (144).

Like mitosis, gametogenesis must ensure that damaged or excess proteins that accumulate in response to age-associated stress (e.g., excess nucleolar proteins) are not inherited by the gametes. After gamete membrane closure, lysosome permeabilization induces a mega-autophagy event that degrades all material that was not inherited by the gametes, including the GUNC (42, 43, 79) (see Section 4.1). However, upregulation of both autophagy and proteasome genes is observed prior to lysosomal membrane permeabilization, suggesting that protein turnover occurs in early gametogenesis while gametes are still being formed (12). Evidence of ERphagy has been shown (118) (see Section 4.3) and several autophagy genes are expressed during gametogenesis, including the nucleophagy-specific receptor *ATG39* (see Section 3.1) and the mitophagy-specific receptor *ATG32* (12). Additionally, ribosome degradation occurs in late gametogenesis, with resynthesis occurring following gamete membrane closure (45). Ribosomes are essential for synthesizing new

proteins; therefore, this mass turnover of ribosomes may provide an opportunity to create new ribosomes that are devoid of modifications or damage. Since autophagy is critical for murine oogenesis (57), categorizing the proteins that are degraded by selective macroautophagy will give more clues into which proteins are excluded from the developing gametes.

In parallel with macroautophagy, proteasome expression is also upregulated in gametogenesis and has been linked with the elimination of amyloid-like assemblies that develop in the cytoplasm. Prior to meiotic divisions, the RNA-binding protein Rim4 forms amyloid-like structures to repress translation of its bound targets (7). During the transition from meiosis I to meiosis II, Rim4-mediated translational repression is rapidly reversed via Ime2-dependent phosphorylation, which targets Rim4 for proteasomal degradation (21). Although aggregation of Rim4 is not an age-dependent phenomenon, these studies demonstrate that gametogenesis can eliminate such amyloid-like protein assemblies. Further studies are required to determine whether other types of amyloid-like assemblies can be cleared in this manner during gametogenesis. Additionally, the proteasome has been shown to play a role in rDNA stability and prevent ecDNA formation (66). Thus, identification of the proteins degraded by the proteasome during gametogenesis could provide additional insight into mechanisms of life span resetting.

Open reading frame (ORF): a DNA sequence between a start codon and stop codon that can be transcribed into messenger RNA that can be translated into a protein

6. COMPLEX GENE REGULATION PATHWAYS

Genome-wide data sets have revealed that gametogenesis induces the expression of many genes in a highly regulated manner, including alternative transcript and protein isoforms, which are not expressed during mitosis (12, 25, 30, 40, 78). Since life span resetting is an intrinsic feature of gametogenesis in budding yeast, understanding how the meiotic gene expression program contributes to quality control and cellular remodeling will provide critical insights into how the rejuvenation pathways are regulated. In this section, we explore different categories of alternative gene regulation that are prevalent in gametogenesis and discuss how a specific TF, Ndt80, drives the expression of canonical and alternative isoforms, thereby providing insight into how this transcriptional regulator may reprogram a progenitor cell to a fully rejuvenated state.

6.1. Noncanonical Gene Regulation

Over 90% of the yeast genome is actively translated during the meiotic program in a temporally regulated fashion (12). Additionally, several surprising gene expression modalities are present in gametogenesis (**Figure 3*a–f***), suggesting pervasive noncanonical gene regulation. In comparison to mitosis, net translational levels are lower in meiosis, but globally, translational regulation appears to be widespread. Furthermore, translation initiation at non-AUG codons, especially to initiate translation upstream of annotated open reading frames (ORFs), is seen for approximately half of the transcripts expressed in meiosis but is rare in mitotic cells (12).

One source of alternative translation arises from previously unannotated AUG-initiated short ORFs (**Figure 3*b***). When the *S. cerevisiae* genome was first sequenced, ORFs were defined as genetic units that contained a canonical start and stop codon and were at least 100 codons long (39, 60). Due to this cutoff, these short ORFs were not annotated and thus have not been included in genome-wide analyses. In human cells, a subset of short ORFs have been shown to produce microproteins that are critical for proliferation (23). To date, the expression of over 2,000 short ORFs has been detected in yeast gametogenesis using translational profiling (12); however, their biological significance remains to be determined.

During gametogenesis, hundreds of alternative 5′-extended messenger RNA (mRNA) isoforms are expressed in a temporally regulated manner and contain AUG-initiated upstream ORFs (uORFs) that dampen translation of the downstream ORF (12, 25, 148). Mechanistic

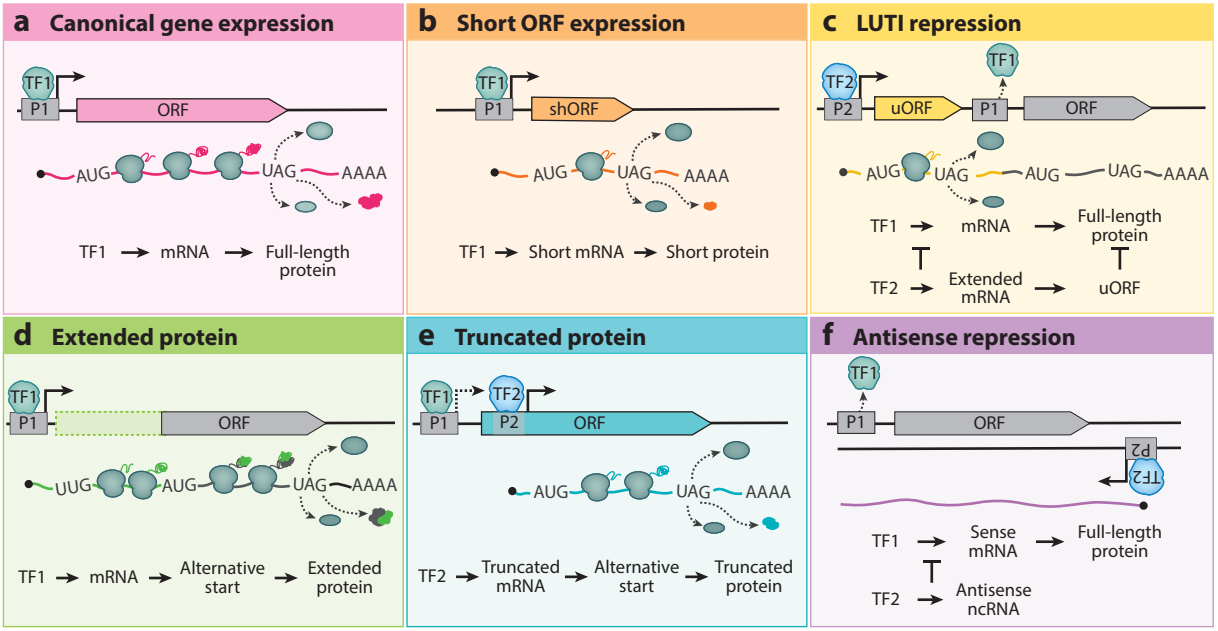


Figure 3

Complex gene regulation pathways in gametogenesis. (*a*) In canonical gene expression regulation, TF1 binds to a proximal P1 to facilitate transcription of an ORF-carrying transcript. This canonical transcript is processed and exported to the cytoplasm where it is translated by ribosomes, producing a protein product. (*b*) shORFs behave like the ORF in panel *a*, except the protein product is less than 100 amino acids in length. (*c*) Expression of a LUTI from an upstream P2 represses transcription from the proximal P1. The resulting LUTI transcript is translationally inert as ribosomes are captured by the uORF(s) in the LUTI 5′ leader, resulting in reduced or no protein expression from the ORF. (*d*) Translation of extended proteins can be initiated from an alternative near-cognate start site that resides upstream of the conventional AUG. (*e*) Truncated proteins form when TF2 binds to an alternative P2 located inside the conventional ORF, inducing expression of an alternative truncated transcript. This may result in ribosomes translating from an in-frame downstream start site, leading to the formation of a shorter protein. (*f*) Antisense repression of an ORF can occur when transcription induced by TF2 from P2 on the opposite strand prevents transcription from canonical P1 by TF1. Abbreviations: LUTI, long undecoded transcript isoform; mRNA, messenger RNA; ncRNA, noncoding RNA; ORF, open reading frame; P, promoter; shORF, short ORF; TF, transcription factor; uORF, upstream open reading frame.

insights into the function of these extended mRNAs, termed long undecoded transcript isoforms (LUTIs), came from studying an essential kinetochore gene *NDC80*, whose regulation is critical for gamete fitness and viability (24, 27). The LUTI-based mechanism repurposes gene-activating TFs to function as repressors (**Figure 3*c***; discussed in Section 6.2). Central to this regulation is the TF-dependent induction of the LUTI, which downregulates protein synthesis in a tunable manner through the combined act of transcriptional and translational interference (reviewed in 149). For genes that are regulated by LUTIs, protein synthesis is primarily determined by the TF-dependent toggling between the LUTI and the protein-coding mRNA isoforms. It is worth noting that while the uORF-based translational repression appears to be ubiquitous among transcripts categorized as LUTIs, transcriptional interference occurs in approximately 50% of LUTI-carrying genes (148). Histone 3 lysine 36 trimethylation (H3K36me3) and changes in nucleosome position are among the strongest predictors of LUTI-based repression. This finding suggests that physiologically impactful LUTI-based regulation involves the coupling of upstream transcriptional start-site selection to downstream chromatin remodeling. Thus, studies aimed at understanding how LUTIs are involved in meiotic cellular quality control and rejuvenation are important areas of future investigation.

Long undecoded transcript isoform (LUTI): an extended transcript containing upstream ORFs that may downregulate mRNA and protein production from the canonical ORF

Genome-wide studies also provide evidence for the production of hundreds of other understudied alternative transcripts and proteins (reviewed in 67). First, there are many extended proteins that typically utilize non-AUG start codons, which are upstream of the conventional start site. Use of these non-AUG start codons is in part a result of lower eIF5A levels in meiotic cells (44) (**Figure 3*d***). Second, truncated proteins (12) (**Figure 3*e***) also occur due to alternative transcription start-site usage, as revealed by focused studies of Ndt80 targets (162) and genome-wide transcription start-site and end-site sequencing data sets (26). To date, an Ndt80-mediated N-terminal truncation of Mrk1, which originates from a truncated transcript, has been shown to be important for efficient gametogenesis progression. Lastly, transcriptional interference by noncoding transcripts also occurs during gametogenesis. Transcriptional interference can occur on the antisense strand (**Figure 3*f***), as in the case of Ime4 (72), or sense strand, as in the case of Ime1 (154). Biological roles for gametogenesis-specific transcript and protein isoforms remain an open area of investigation.

6.2. The Versatility of Ndt80

Ndt80 is a master regulator of gametogenesis. In addition to driving the start of chromosome segregation, Ndt80 is critical for initiating several meiotic pathways, including organelle remodeling (118, 132) (Sections 4.2 and 4.3) and GUNC formation (79) (Section 3.1). Ectopically expressed Ndt80 is sufficient to extend life span, suggesting that some of the quality control and/or rejuvenation pathways can be turned on to counteract cellular aging (152). Thus, identifying which Ndt80 targets are important for gamete health may be therapeutically relevant.

Among the potential Ndt80 targets quantified at the protein level in a recent study, 3 different modes of gene regulation emerge (25). First, 166 genes show an expected pattern: Ndt80 activates mRNA synthesis, which corresponds to elevated ribosome footprints over the ORF (representing translation) and a subsequent increase in protein levels (**Figure 3*a***). Second, 24 genes show Ndt80-mediated induction of LUTI expression, leading to a 5′-extended version of the transcript that decreases protein levels (**Figure 3*c***). Finally, 8 genes show Ndt80-dependent promoter switching from an upstream start site that was previously driving a LUTI to a downstream start site, resulting in the induction of a canonical transcript that is more efficiently translated. In this scenario, Ndt80 expression overcomes prior silencing of these genes, ultimately leading to upregulation of protein expression.

Studies of Ndt80 exemplify how a single TF can elicit multiple effects on gene expression during gametogenesis and give insight into how a small number of TFs can coordinate waves of gene upregulation and downregulation that modulate cellular rejuvenation. To date, 179 TFs have been identified in budding yeast, and 142 are expressed during gametogenesis (12). With the abundance of gene regulation mechanisms that exist in gametogenesis, understanding how a small number of TFs can facilitate such dynamic changes can provide insight into how gametes are reprogrammed to a youthful state. It is possible that other TFs, in addition to Ndt80, can extend life span in budding yeast, so a complete understanding of which TFs can facilitate cellular rejuvenation will be important for developing gametogenesis-based strategies for counteracting cellular aging.

7. PERSPECTIVES

In this review, we have highlighted gametogenesis-specific quality control and rejuvenation pathways that converge to reset gamete life span, many of which are conserved from yeast to metazoans (also reviewed in 61, 80). Because gametes are necessary for producing brand-new offspring,

a gamete represents the cellular state of a young cell, at the levels of both organelle health and gene expression. Currently, our understanding of gametogenesis reveals pathways that ensure that (*a*) senescence-associated factors and damaged proteins are excluded and/or eliminated from the developing gametes, (*b*) organelles are remodeled to facilitate inheritance, and (*c*) de novo organelle biogenesis pathways are activated to complement inherited organelles for gamete maturation. If any of these requirements are not met, gametes can be rendered unfit, inviable, or incompetent for zygote formation; alternatively, the resulting progeny could suffer from developmental issues or organelle-related defects.

7.1. Future Directions

Budding yeast has played a pivotal role in elucidating the mechanisms in gametogenesis that drive rejuvenation and quality control, but several areas remain unexplored. First, there are still many organelles and macromolecules that have not been carefully monitored during gametogenesis, which could reveal more quality control and rejuvenation strategies. For example, Golgi structures, which appear as scattered foci in budding yeast, seem to coalesce at centrosomes in early meiosis II before a subset of mitochondria and the ER localize to the gamete nuclei in young cells (137). The Golgi network is important for maintaining proteostasis, so it will be interesting to test how aging affects the Golgi network and whether this causes a subset of the damaged Golgi apparatus to be targeted for degradation. As a more complete understanding of individual organelle quality control pathways emerges, a whole-cell view of rejuvenation can be developed.

Second, many organelles and proteins appear to localize into distinct populations that facilitate inheritance or gamete exclusion coupled with subsequent degradation. This raises the intriguing hypothesis that the cell can differentiate and sort healthy and dysfunctional organelles and proteins during gametogenesis. Identification of organelle quality markers that allow for such sorting would be useful for two reasons: (*a*) From an experimental standpoint, being able to compare the composition and function of healthy versus unhealthy organelles could provide insight into age-associated organellar decline, and (*b*) from a therapeutic standpoint, organelle-specific aging biomarkers could be useful in targeting their removal in somatic contexts. For example, portions of the cortical ER that are not inherited by the daughter cell in mitosis (117, 138, 142) or by the gametes in meiosis (118) are tethered to the plasma membrane by a common set of tethers (Tcb1, Tcb2, Tcb3, and Ist2). It is possible that tethering to the plasma membrane is one mechanism for marking regions of the ER containing misfolded proteins, so determining whether the uninherited, tethered ER contains more misfolded proteins could be one way to address this outstanding question.

Third, to complement genome-wide, temporal measurements of gene and protein expression, more systematic assessments of gametogenesis are needed to identify the important players required to remove all hallmarks of aging to fully reset life span (reviewed in 93). For example, gametogenesis entails changes in chromatin states (16, 73, 84), but whether chromatin-based events, such as epigenetic changes, can help restore the rDNA locus, exclude ecDNA, or sequester nucleolar proteins remains to be determined. Furthermore, an in-depth assessment of the metabolome during gametogenesis could provide insight into how metabolic changes could contribute to rejuvenation.

Finally, determining which Ndt80 targets prevent, eliminate, or help the cells tolerate age-associated damage will be important in future studies. Hundreds of transcripts are expressed following Ndt80 activation. These transcripts fall into distinct expression clusters, indicating that Ndt80 triggers a cascade of gene expression waves by inducing additional TFs. Even among the well-established transcriptional targets of Ndt80, ~10% appear to be LUTIs (25). Therefore, Ndt80 and/or its downstream TFs can directly induce LUTI-based repression, in addition to

their known function in gene activation. Interestingly, several TFs associated with stress response pathways are transiently induced during gametogenesis, downstream of Ndt80 activation (12). This includes Hac1, the homolog of human Xbp1, which is involved in the ER unfolded protein response and induces LUTIs (153). The expression of these conserved stress response pathways during gametogenesis is somewhat surprising since their activation often drives pathogenic states in aging. However, exposure to modest levels of stress leads to a phenomenon known as hormesis, which is associated with longevity and cellular protection, although the molecular mechanisms by which this is achieved are not fully understood (reviewed in 58). Gametogenesis might be a physiological manifestation of hormesis; therefore, determining what targets are expressed during the transient activation of stress response pathways and determining how each pathway contributes to gametogenesis-specific rejuvenation are exciting questions for future studies.

Hormesis: a dose–response phenomenon characterized by a low-dose response that is opposite in effect to that seen at high doses (e.g., low levels of stress promoting longevity, but high levels being detrimental to an organism)

7.2. Application

The knowledge gained by the study of gametogenesis, for which natural strategies have evolved to maintain an immortal germline, may enable strategies to combat age-related damage in other cell types. In budding yeast, the meiotic TF Ndt80 is required for meiotic progression as well as for many of the rejuvenation events discussed in this review. Ndt80 shares homology with two p53-related germline proteins in metazoans (90): TAp63α, which is important for oocyte quality control in mice (139), and CEP-1, which is highly expressed in the worm (*C. elegans*) germline (37), suggesting that the function of Ndt80 in gametogenesis may be conserved. As our knowledge of Ndt80-mediated life span extension grows, determining what aspects are conserved in metazoans will be critical. Moreover, two other life span–extending strategies, dietary restriction and spermidine treatment, induce expression of two meiotic TFs, Ime1 and Ndt80 (158), so future studies on Ndt80 could also give insight into how these treatments promote longevity.

In a broader sense, development of gametogenesis-based rejuvenation strategies is reminiscent of partial reprogramming to the pluripotent state where transient expression of the Yamanaka factors, Oct3/4, Sox2, Klf4, and c-Myc (OSKM), has been shown to revert mouse and human cells to a younger state (see the sidebar titled Partial Reprogramming to the Pluripotent State). *NDT80* expression is sufficient to rejuvenate the nucleolus and extend life span (152); however, it

PARTIAL REPROGRAMMING TO THE PLURIPOTENT STATE

Expression of the Yamanaka factors, Oct3/4, Sox2, Klf4, and c-Myc (OSKM), reprograms differentiated human and mouse cells back to an induced pluripotent stem (iPS) cell state (109, 140, 141, 160). In mouse models, persistent expression of Yamanaka factors causes teratoma formation (1, 108, 116); however, applying cycles of short inductions of OSKM in progeroid mice (*Lmna* mutation G609G) ameliorates age-associated damage and extends both health span and life span without teratoma or cancer formation (termed partial reprogramming) (115). Interestingly, applying OSKM to middle-aged mice also improves glucose tolerance and tissue regeneration, but whether transient OSKM can extend life span in naturally aging mice remains to be seen.

Excitingly, transient expression of OSKM, in combination with expression of LIN28 and NANOG, is sufficient to eliminate various types of age-associated damage while maintaining the identity of each cell type in human cells. For example, the epigenetic clock is reset, protein degradation pathways are restored, mitochondrial function is improved, inflammation is reduced, and regenerative capacity is restored (130). Thus, understanding how partial reprogramming to a pluripotent state reverts differentiated cells to a younger state holds tremendous potential for developing rejuvenation strategies in somatic cells.

is also possible that other gametogenesis genes elicit quality control and/or rejuvenation properties independently of *NDT80*. Thus, systematic identification of which meiotic genes are capable of reprogramming cells from an older state to a younger state could lead to new therapies to counteract cellular aging.

The development of gametogenesis-based rejuvenation strategies in aging cells has the potential to directly impact specific disease treatments as well. First, gametogenesis can clear amyloid-like assemblies through a process that requires hyperphosphorylation (21). Tau amyloids and TDP-43 aggregates, which have been observed in neurodegenerative patients, are hyperphosphorylated but not targeted for disaggregation and degradation (reviewed in 8, 113). Thus, gametogenesis may provide new insight into what factors are required for clearing these misfolded protein assemblies. Second, NPC function progressively declines with age, and loss of nucleocytoplasmic transport is seen in amyotrophic lateral sclerosis (ALS) and Huntington's disease (28, 56, 63, 161). Thus, understanding the mechanisms underlying NPC turnover during gametogenesis could provide novel strategies for reestablishing nucleocytoplasmic transport in somatic cells. Finally, hundreds of meiotic genes are aberrantly induced across various cancer types and promote tumor proliferation and metastasis (15). Thus, understanding the functions of meiotic gene expression programs could help inform targets for cancer therapies.

8. CONCLUSION

Gametogenesis provides a natural context to study the pathways that converge to maintain an immortal germline. Technical advances that allow for live-cell fluorescence imaging and multiomic dissection of gametogenesis have begun to reveal the organelle quality control and gene regulation pathways that facilitate life span resetting; however, further research is required to understand gametogenesis-mediated rejuvenation strategies at the whole-cell level. Studies in budding yeast reveal that gametogenesis pathways can be leveraged outside their normal context to extend life span. Therefore, gametogenesis holds tremendous potential for creating a road map for cellular rejuvenation and developing rational strategies to combat age-associated damage in the metazoan soma.

SUMMARY POINTS

1. Gametogenesis is a natural developmental program that prevents age-associated damage from being passed on to progeny. Studies in budding yeast have given insight into the quality control and rejuvenation pathways that converge to reset the life span of gametes.
2. A portion of the nucleus is excluded from the developing gametes and forms the gametogenesis uninherited nuclear compartment (GUNC), which contains extrachromosomal circular DNA (ecDNA), protein aggregates, excess nucleolar material, and nuclear pore complexes (NPCs) and is eventually eliminated.
3. The mitochondria and endoplasmic reticulum undergo extensive remodeling to facilitate a subset of each organelle to be inherited and the remaining fraction to be excluded from the developing gametes.
4. The lysosomal membrane of the precursor cell undergoes programmed permeabilization during late gametogenesis, and all cellular material that was excluded from the gametes is destroyed by mega-autophagy.

5. During gametogenesis, over 90% of the yeast genome is actively translated. This includes genes involved in selective autophagy and proteasome-mediated degradation, and transcription factors involved in stress responses. In many of these cases, alternative gene and protein isoforms are also seen.
6. Extensive research on the master transcription factor Ndt80 reveals how a single transcription factor can trigger upregulation and downregulation of gene expression by targeting noncanonical gene regulatory pathways.

FUTURE ISSUES

1. Continued systematic dissection of gametogenesis in young precursor cells is required for a whole-cell understanding of cellular rejuvenation. This includes studying epigenetic, metabolomic, and lipid changes during gametogenesis.
2. Comparing how quality control and rejuvenation pathways facilitate the removal of age-associated damage is still needed for some organelles (e.g., the endoplasmic reticulum and Golgi apparatus).
3. Ectopic expression of the meiotic transcription factor Ndt80 is sufficient to extend replicative life span in budding yeast, suggesting that gametogenic rejuvenation pathways can be repurposed outside their normal context. To date, Ndt80 has been shown to rejuvenate the nucleolus; however, whether Ndt80 rejuvenates other aspects of the cell has not yet been determined. Furthermore, identifying the complete set of Ndt80 targets that elicit rejuvenation in aging cells will give insight into mitigating age-associated damage in a cell.
4. As our understanding of gametogenesis-based rejuvenation pathways continues to grow, determining which aspects are conserved and capable of removing age-associated damage in metazoans will provide new therapeutic targets for preventing or treating age-associated disease.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Abad M, Mosteiro L, Pantoja C, Cañamero M, Rayon T, et al. 2013. Reprogramming *in vivo* produces teratomas and iPS cells with totipotency features. *Nature* 502(7471):340–45
2. Aguilaniu H, Gustafsson L, Rigoulet M, Nyström T. 2003. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299(5613):1751–53
3. Ain Q, Schmeer C, Wengerodt D, Witte OW, Kretz A. 2020. Extrachromosomal circular DNA: current knowledge and implications for CNS aging and neurodegeneration. *Int. J. Mol. Sci.* 21(7):2477
4. An H, Ordureau A, Paulo JA, Shoemaker CJ, Denic V, Harper JW. 2019. TEX264 is an endoplasmic reticulum-resident ATG8-interacting protein critical for ER remodeling during nutrient stress. *Mol. Cell* 74(5):891–908.e10
5. Aufschnaiter A, Büttner S. 2019. The vacuolar shapes of ageing: from function to morphology. *Biochim. Biophys. Acta Mol. Cell Res.* 1866(5):957–70
6. Beck M, Hurt E. 2017. The nuclear pore complex: understanding its function through structural insight. *Nat. Rev. Mol. Cell Biol.* 18(2):73–89
7. Berchowitz LE, Kabachinski G, Walker MR, Carlile TM, Gilbert WV, et al. 2015. Regulated formation of an amyloid-like translational repressor governs gametogenesis. *Cell* 163(2):406–18
8. Billingsley ML, Kincaid RL. 1997. Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. *Biochem. J.* 323(Part 3):577–91
9. Boettcher B, Barral Y. 2013. The cell biology of open and closed mitosis. *Nucleus* 4(3):160–65
10. Bohnert KA, Kenyon C. 2017. A lysosomal switch triggers proteostasis renewal in the immortal *C. elegans* germ lineage. *Nature* 551(7682):629–33
11. Brandon M, Baldi P, Wallace DC. 2006. Mitochondrial mutations in cancer. *Oncogene* 25(34):4647–62
12. Brar GA, Yassour M, Friedman N, Regev A, Ingolia NT, Weissman JS. 2012. High-resolution view of the yeast meiotic program revealed by ribosome profiling. *Science* 335(6068):552–57
13. Bretscher A. 2003. Polarized growth and organelle segregation in yeast. *J. Cell Biol.* 160(6):811–16
14. Brewer BJ, Fangman WL. 1980. Preferential inclusion of extrachromosomal genetic elements in yeast meiotic spores. *PNAS* 77(9):5380–84
15. Bruggeman JW, Koster J, Lodder P, Repping S, Hamer G. 2018. Massive expression of germ cell-specific genes is a hallmark of cancer and a potential target for novel treatment development. *Oncogene* 37(42):5694–700
16. Bryant JM, Govin J, Zhang L, Donahue G, Pugh BF, Berger SL. 2012. The linker histone plays a dual role during gametogenesis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 32(14):2771–83
17. Buchwalter A, Hetzer MW. 2017. Nucleolar expansion and elevated protein translation in premature aging. *Nat. Commun.* 8(1):328
18. Cabib E, Bowers B. 1971. Chitin and yeast budding: localization of chitin in yeast bud scars. *J. Biol. Chem.* 246(1):152–59
19. Cabrera M, Novarina D, Rempel IL, Veenhoff LM, Chang M. 2017. A simple microfluidic platform to study age-dependent protein abundance and localization changes in *Saccharomyces cerevisiae*. *Microb. Cell* 4(5):169–74
20. Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. 2019. From discoveries in ageing research to therapeutics for healthy ageing. *Nature* 571(7764):183–92
21. Carpenter K, Bell RB, Yunus J, Amon A, Berchowitz LE. 2018. Phosphorylation-mediated clearance of amyloid-like assemblies in meiosis. *Dev. Cell* 45(3):392–405.e6
22. Chao JT, Wong AKO, Tavassoli S, Young BP, Chruscicki A, et al. 2014. Polarization of the endoplasmic reticulum by ER-septin tethering. *Cell* 158(3):620–32
23. Chen J, Brunner A-D, Cogan JZ, Nuñez JK, Fields AP, et al. 2020. Pervasive functional translation of noncanonical human open reading frames. *Science* 367(6482):1140–46
24. Chen J, Tresenrider A, Chia M, McSwiggen DT, Spedale G, et al. 2017. Kinetochore inactivation by expression of a repressive mRNA. *eLife* 6:e27417
25. Cheng Z, Otto GM, Powers EN, Keskin A, Mertins P, et al. 2018. Pervasive, coordinated protein-level changes driven by transcript isoform switching during meiosis. *Cell* 172(5):910–14.e16

26. Chia M, Li C, Marques S, Pelechano V, Luscombe NM, van Werven FJ. 2021. High-resolution analysis of cell-state transitions in yeast suggests widespread transcriptional tuning by alternative starts. *Genome Biol.* 22(1):34
27. Chia M, Tresenrider A, Chen J, Spedale G, Jorgensen V, et al. 2017. Transcription of a 5′ extended mRNA isoform directs dynamic chromatin changes and interference of a downstream promoter. *eLife* 6:e27420
28. Chou C-C, Zhang Y, Umoh ME, Vaughan SW, Lorenzini I, et al. 2018. TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. *Nat. Neurosci.* 21(2):228–39
29. Chou Y-Y, Upadhyayula S, Houser J, He K, Skillern W, et al. 2021. Inherited nuclear pore substructures template post-mitotic pore assembly. *Dev. Cell* 56(12):1786–1803.e9
30. Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, et al. 1998. The transcriptional program of sporulation in budding yeast. *Science* 282(5389):699–705
31. Clay L, Caudron F, Denoth-Lippuner A, Boettcher B, Buvelot Frei S, et al. 2014. A sphingolipid-dependent diffusion barrier confines ER stress to the yeast mother cell. *eLife* 3(3):e01883
32. Cunningham CN, Williams JM, Knupp J, Arunagiri A, Arvan P, Tsai B. 2019. Cells deploy a two-pronged strategy to rectify misfolded proinsulin aggregates. *Mol. Cell* 75(3):442–56.e4
33. D'Angelo MA, Raices M, Panowski SH, Hetzer MW. 2009. Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. *Cell* 136(2):284–95
34. De Virgilio C, DeMarini DJ, Pringle JR. 1996. *SPR28*, a sixth member of the septin gene family in *Saccharomyces cerevisiae* that is expressed specifically in sporulating cells. *Microbiology* 142(10):2897–905
35. Denoth-Lippuner A, Julou T, Barral Y. 2014. Budding yeast as a model organism to study the effects of age. *FEMS Microbiol. Rev.* 38(2):300–25
36. Denoth-Lippuner A, Krzyzanowski MK, Stober C, Barral Y. 2014. Role of SAGA in the asymmetric segregation of DNA circles during yeast ageing. *eLife* 3(3):e03790
37. Derry WB, Putzke AP, Rothman JH. 2001. *Caenorhabditis elegans* p53: role in apoptosis, meiosis, and stress resistance. *Science* 294(5542):591–95
38. Diamond AE, Park J-S, Inoue I, Tachikawa H, Neiman AM. 2009. The anaphase promoting complex targeting subunit Ama1 links meiotic exit to cytokinesis during sporulation in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 20(1):134–45
39. Dujon B, Alexandraki D, André B, Ansorge W, Baladron V, et al. 1994. Complete DNA sequence of yeast chromosome XI. *Nature* 369(6479):371–78
40. Duncan CDS, Mata J. 2014. The translational landscape of fission-yeast meiosis and sporulation. *Nat. Struct. Mol. Biol.* 21(7):641–47
41. Duro E, Marston AL. 2015. From equator to pole: splitting chromosomes in mitosis and meiosis. *Genes Dev.* 29(2):109–22
42. Eastwood MD, Cheung SWT, Lee KY, Moffat J, Meneghini MD. 2012. Developmentally programmed nuclear destruction during yeast gametogenesis. *Dev. Cell* 23(1):35–44
43. Eastwood MD, Meneghini MD. 2015. Developmental coordination of gamete differentiation with programmed cell death in sporulating yeast. *Eukaryot. Cell* 14(9):858–67
44. Eisenberg AR, Higdon AL, Hollerer I, Fields AP, Jungreis I, et al. 2020. Translation initiation site profiling reveals widespread synthesis of non-AUG-initiated protein isoforms in yeast. *Cell Syst.* 11(2):145–60.e5
45. Eisenberg AR, Higdon A, Keskin A, Hodapp S, Jovanovic M, Brar GA. 2018. Precise post-translational tuning occurs for most protein complex components during meiosis. *Cell Rep.* 25(13):3603–17.e2
46. Eisenberg-Bord M, Zung N, Collado J, Drwesh L, Fenech EJ, et al. 2021. Cnm1 mediates nucleus–mitochondria contact site formation in response to phospholipid levels. *J. Cell Biol.* 220(11):e202104100
47. Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, et al. 2003. Recurrent *de novo* point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 423(6937):293–98
48. Erjavec N, Larsson L, Grantham J, Nyström T. 2007. Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. *Genes Dev.* 21(19):2410–21
49. Fan W, Waymire KG, Narula N, Li P, Rocher C, et al. 2008. A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. *Science* 319(5865):958–62

50. Fares H, Goetsch L, Pringle JR. 1996. Identification of a developmentally regulated septin and involvement of the septins in spore formation in *Saccharomyces cerevisiae*. *J. Cell Biol.* 132(3):399–411
51. Fawcett DW, Chemes HE. 1979. Changes in distribution of nuclear pores during differentiation of the male germ cells. *Tissue Cell* 11(1):147–62
52. Forrester A, De Leonibus C, Grumati P, Fasana E, Piemontese M, et al. 2019. A selective ER-phagy exerts procollagen quality control via a Calnexin-FAM134B complex. *EMBO J.* 38(2):e99847
53. Frakes AE, Dillin A. 2017. The UPR^{ER}: sensor and coordinator of organismal homeostasis. *Mol. Cell* 66(6):761–71
54. Fuchs J, Loidl J. 2004. Behaviour of nucleolus organizing regions (NORs) and nucleoli during mitotic and meiotic divisions in budding yeast. *Chromosome Res.* 12(5):427–38
55. Garcia G, Finnigan GC, Heasley LR, Sterling SM, Aggarwal A, et al. 2016. Assembly, molecular organization, and membrane-binding properties of development-specific septins. *J. Cell Biol.* 212(5):515–29
56. Gasset-Rosa F, Chillon-Marinas C, Goginashvili A, Atwal RS, Artates JW, et al. 2017. Polyglutamine-expanded huntingtin exacerbates age-related disruption of nuclear integrity and nucleocytoplasmic transport. *Neuron* 94(1):48–57.e4
57. Gawriluk TR, Hale AN, Flaws JA, Dillon CP, Green DR, Rucker EB. 2011. Autophagy is a cell survival program for female germ cells in the murine ovary. *Reproduction* 141(6):759–65
58. Gems D, Partridge L. 2008. Stress-response hormesis and aging: "That which does not kill us makes us stronger." *Cell Metab.* 7(3):200–3
59. Girke P, Seufert W. 2019. Compositional reorganization of the nucleolus in budding yeast mitosis. *Mol. Biol. Cell* 30(5):591–606
60. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, et al. 1996. Life with 6000 genes. *Science* 274(5287):546–67
61. Goodman JS, King GA, Ünal E. 2020. Cellular quality control during gametogenesis. *Exp. Cell Res.* 396(1):112247
62. Goudeau J, Aguilaniu H. 2010. Carbonylated proteins are eliminated during reproduction in *C. elegans*. *Aging Cell* 9(6):991–1003
63. Grima JC, Daigle JG, Arbez N, Cunningham KC, Zhang K, et al. 2017. Mutant huntingtin disrupts the nuclear pore complex. *Neuron* 94(1):93–107.e6
64. Haber JE. 2012. Mating-type genes and *MAT* switching in *Saccharomyces cerevisiae*. *Genetics* 191(1):33–64
65. Henderson KA, Hughes AL, Gottschling DE. 2014. Mother-daughter asymmetry of pH underlies aging and rejuvenation in yeast. *eLife* 3:e03504
66. Hendrickson DG, Soifer I, Wranik BJ, Kim G, Robles M, et al. 2018. A new experimental platform facilitates assessment of the transcriptional and chromatin landscapes of aging yeast. *eLife* 7:e9911
67. Higdon AL, Brar GA. 2021. Rules are made to be broken: a "simple" model organism reveals the complexity of gene regulation. *Curr. Genet.* 67(1):49–56
68. Higuchi R, Vevea JD, Swayne TC, Chojnowski R, Hill V, et al. 2013. Actin dynamics affect mitochondrial quality control and aging in budding yeast. *Curr. Biol.* 23(23):2417–22
69. Higuchi-Sanabria R, Pernice WMA, Vevea JD, Alessi Wolken DM, Boldogh IR, Pon LA. 2014. Role of asymmetric cell division in lifespan control in *Saccharomyces cerevisiae*. *FEMS Yeast Res.* 14(8):1133–46
70. Ho H-C. 2010. Redistribution of nuclear pores during formation of the redundant nuclear envelope in mouse spermatids. *J. Anat.* 216(4):525–32
71. Hong S, Joo JH, Yun H, Kim K. 2019. The nature of meiotic chromosome dynamics and recombination in budding yeast. *J. Microbiol.* 57(4):221–31
72. Hongay CF, Grisafi PL, Galitski T, Fink GR. 2006. Antisense transcription controls cell fate in *Saccharomyces cerevisiae*. *Cell* 127(4):735–45
73. Hu J, Donahue G, Dorsey J, Govin J, Yuan Z, et al. 2015. H4K44 acetylation facilitates chromatin accessibility during meiosis. *Cell Rep.* 13(9):1772–80
74. Hughes AL, Gottschling DE. 2012. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. *Nature* 492(7428):261–65
75. Hughes CE, Coody TK, Jeong M-Y, Berg JA, Winge DR, Hughes AL. 2020. Cysteine toxicity drives age-related mitochondrial decline by altering iron homeostasis. *Cell* 180(2):296–310.e18

76. Janssens GE, Veenhoff LM. 2016. Evidence for the hallmarks of human aging in replicatively aging yeast. *Microb. Cell* 3(7):263–74
77. Janssens GE, Veenhoff LM. 2016. The natural variation in lifespans of single yeast cells is related to variation in cell size, ribosomal protein, and division time. *PLOS ONE* 11(12):e0167394
78. Kim Guisbert KS, Zhang Y, Flatow J, Hurtado S, Staley JP, et al. 2012. Meiosis-induced alterations in transcript architecture and noncoding RNA expression in *S. cerevisiae*. *RNA* 18(6):1142–53
79. King GA, Goodman JS, Schick JG, Chetlapalli K, Jorgens DM, et al. 2019. Meiotic cellular rejuvenation is coupled to nuclear remodeling in budding yeast. *eLife* 8:e47156
80. King GA, Ünal E. 2020. The dynamic nuclear periphery as a facilitator of gamete health and rejuvenation. *Curr. Genet.* 66(3):487–93
81. Kirkwood TBL. 1987. Immortality of the germ-line versus disposability of the soma. In *Evolution of Longevity in Animals*, ed. AD Woodhead, KH Thompson, pp. 209–18. Boston, MA: Springer
82. Klecker T, Scholz D, Fortsch J, Westermann B. 2013. The yeast cell cortical protein Num1 integrates mitochondrial dynamics into cellular architecture. *J. Cell Sci.* 126(13):2924–30
83. Knop M, Strasser K. 2000. Role of the spindle pole body of yeast in mediating assembly of the prospore membrane during meiosis. *EMBO J.* 19(14):3657–67
84. Krishnamoorthy T, Chen X, Govin J, Cheung WL, Dorsey J, et al. 2006. Phosphorylation of histone H4 Ser1 regulates sporulation in yeast and is conserved in fly and mouse spermatogenesis. *Genes Dev.* 20(18):2580–92
85. Kundu D, Pasrija R. 2020. The ERMES (Endoplasmic Reticulum and Mitochondria Encounter Structures) mediated functions in fungi. *Mitochondrion* 52:89–99
86. Kunisada T, Yamagishi H, Ogita Z, Kirakawa T, Mitsui Y. 1985. Appearance of extrachromosomal circular DNAs during *in vivo* and *in vitro* ageing of mammalian cells. *Mech. Ageing Dev.* 29(1):89–99
87. Lackner LL, Ping H, Graef M, Murley A, Nunnari J. 2013. Endoplasmic reticulum-associated mitochondria–cortex tether functions in the distribution and inheritance of mitochondria. *PNAS* 110(6):E458–67
88. Lam C, Santore E, Lavoie E, Needleman L, Fiacco N, et al. 2014. A visual screen of protein localization during sporulation identifies new components of prospore membrane-associated complexes in budding yeast. *Eukaryot. Cell* 13(3):383–91
89. Lam YT, Aung-Htut MT, Lim YL, Yang H, Dawes IW. 2011. Changes in reactive oxygen species begin early during replicative aging of *Saccharomyces cerevisiae* cells. *Free Radic. Biol. Med.* 50(8):963–70
90. Lamoureux JS, Stuart D, Tsang R, Wu C, Glover JNM. 2002. Structure of the sporulation-specific transcription factor Ndt80 bound to DNA. *EMBO J.* 21(21):5721–32
91. Li Y, Jiang Y, Paxman J, O'Laughlin R, Klepin S, et al. 2020. A programmable fate decision landscape underlies single-cell aging in yeast. *Science* 369(6501):325–29
92. Lieber T, Jeedigunta SP, Palozzi JM, Lehmann R, Hurd TR. 2019. Mitochondrial fragmentation drives selective removal of deleterious mtDNA in the germline. *Nature* 570(7761):380–84
93. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. *Cell* 153(6):1194–217
94. Lord CL, Timney BL, Rout MP, Wente SR. 2015. Altering nuclear pore complex function impacts longevity and mitochondrial function in *S. cerevisiae*. *J. Cell Biol.* 208(6):729–44
95. MacAlpine DM, Perlman PS, Butow RA. 2000. The numbers of individual mitochondrial DNA molecules and mitochondrial DNA nucleoids in yeast are co-regulated by the general amino acid control pathway. *EMBO J.* 19(4):767–75
96. Mahmoudi S, Xu L, Brunet A. 2019. Turning back time with emerging rejuvenation strategies. *Nat. Cell Biol.* 21(1):32–43
97. Maier P, Rathfelder N, Finkbeiner MG, Taxis C, Mazza M, et al. 2007. Cytokinesis in yeast meiosis depends on the regulated removal of Ssp1p from the prospore membrane. *EMBO J.* 26(7):1843–52
98. Manford AG, Stefan CJ, Yuan HL, MacGurn JA, Emr SD. 2012. ER-to-plasma membrane tethering proteins regulate cell signaling and ER morphology. *Dev. Cell* 23:1129–40
99. McFaline-Figueroa JR, Vevea J, Swayne TC, Zhou C, Liu C, et al. 2011. Mitochondrial quality control during inheritance is associated with lifespan and mother-daughter age asymmetry in budding yeast. *Aging Cell* 10(5):885–95

100. McMurray MA, Thorner J. 2008. Septin stability and recycling during dynamic structural transitions in cell division and development. *Curr. Biol.* 18(16):1203–8
101. Mészáros N, Cibulka J, Mendiburo MJ, Romanauska A, Schneider M, Köhler A. 2015. Nuclear pore basket proteins are tethered to the nuclear envelope and can regulate membrane curvature. *Dev. Cell* 33(3):285–98
102. Mochida K, Oikawa Y, Kimura Y, Kirisako H, Hirano H, et al. 2015. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature* 522(7556):359–62
103. Mochida K, Otani T, Katsumata Y, Kirisako H, Kakuta C, et al. 2022. Atg39 links and deforms the outer and inner nuclear membranes in selective autophagy of the nucleus. *J. Cell Biol.* 221(2):e202103178
104. Moens PB. 1971. Fine structure of ascospore development in the yeast *Saccharomyces cerevisiae*. *Can. J. Microbiol.* 17(4):507–10
105. Moreno-Borchart AC, Strasser K, Finkbeiner MG, Shevchenko A, Shevchenko A, Knop M. 2001. Prospore membrane formation linked to the leading edge protein (LEP) coat assembly. *EMBO J.* 20(24):6946–57
106. Morlot S, Song J, Léger-Silvestre I, Matifas A, Gadal O, Charvin G. 2019. Excessive rDNA transcription drives the disruption in nuclear homeostasis during entry into senescence in budding yeast. *Cell Rep.* 28(2):408–22.e4
107. Mortimer RK, Johnston JR. 1959. Life span of individual yeast cells. *Nature* 183(4677):1751–52
108. Mosteiro L, Pantoja C, Alcazar N, Marión RM, Chondronasiou D, et al. 2016. Tissue damage and senescence provide critical signals for cellular reprogramming in vivo. *Science* 354(6315):aaf4445
109. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, et al. 2008. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* 26(1):101–6
110. Neiman AM. 1998. Prospore membrane formation defines a developmentally regulated branch of the secretory pathway in yeast. *J. Cell Biol.* 140(1):29–37
111. Neiman AM. 2005. Ascospore formation in the yeast *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 69(4):565–84
112. Neiman AM. 2011. Sporulation in the budding yeast *Saccharomyces cerevisiae*. *Genetics* 189(3):737–65
113. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, et al. 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314(5796):130–33
114. Neurohr GE, Terry RL, Lengefeld J, Bonney M, Brittingham GP, et al. 2019. Excessive cell growth causes cytoplasm dilution and contributes to senescence. *Cell* 176(5):1083–97.e18
115. Ocampo A, Reddy P, Martinez-Redondo P, Platero-Luengo A, Hatanaka F, et al. 2016. In vivo amelioration of age-associated hallmarks by partial reprogramming. *Cell* 167(7):1719–33.e12
116. Ohnishi K, Semi K, Yamamoto T, Shimizu M, Tanaka A, et al. 2014. Premature termination of reprogramming in vivo leads to cancer development through altered epigenetic regulation. *Cell* 156(4):663–77
117. Okada M, Kusunoki S, Ishibashi Y, Kito K. 2017. Proteomics analysis for asymmetric inheritance of preexisting proteins between mother and daughter cells in budding yeast. *Genes Cells* 22(6):591–601
118. Otto GM, Cheunkarndee T, Leslie JM, Brar GA. 2021. Programmed cortical ER collapse drives selective ER degradation and inheritance in yeast meiosis. *J. Cell Biol.* 220(12):e202108105
119. Ozsarac N, Bhattacharyya M, Dawes IW, Clancy MJ. 1995. The *SPR3* gene encodes a sporulation-specific homologue of the yeast *CDC3/10/11/12* family of bud neck microfilaments and is regulated by ABFI. *Gene* 164(1):157–62
120. Paulissen SM, Slubowski CJ, Roesner JM, Huang LS. 2016. Timely closure of the prospore membrane requires SPS1 and SPO77 in *Saccharomyces cerevisiae*. *Genetics* 203(3):1203–16
121. Paxman J, Zhou Z, Li Y, Tian W, Su H, et al. 2021. Age-dependent aggregation of ribosomal RNA-binding proteins links deterioration in chromatin stability with loss of proteostasis. bioRxiv 2021.12.06.471495. **https://doi.org/10.1101/2021.12.06.471495**
122. Powell CD, Quain DE, Smart KA. 2003. Chitin scar breaks in aged *Saccharomyces cerevisiae*. *Microbiology* 149(Part 11):3129–37
123. Preuss D, Mulholland J, Kaiser CA, Orlean P, Albright C, et al. 1991. Structure of the yeast endoplasmic reticulum: localization of ER proteins using immunofluorescence and immunoelectron microscopy. *Yeast* 7(9):891–911

124. Ptak C, Aitchison JD, Wozniak RW. 2014. The multifunctional nuclear pore complex: a platform for controlling gene expression. *Curr. Opin. Cell Biol.* 28(1):46–53
125. Rempel IL, Crane MM, Thaller DJ, Mishra A, Jansen DPM, et al. 2019. Age-dependent deterioration of nuclear pore assembly in mitotic cells decreases transport dynamics. *eLife* 8:e48186
126. Roeder AD, Shaw JM. 1996. Vacuole partitioning during meiotic division in yeast. *Genetics* 144(2):445–58
127. Rose MD, Misra LM, Vogel JP. 1989. *KAR2*, a karyogamy gene, is the yeast homolog of the mammalian BiP/GRP78 gene. *Cell* 57(7):1211–21
128. Saarikangas J, Caudron F, Prasad R, Moreno DF, Bolognesi A, et al. 2017. Compartmentalization of ER-bound chaperone confines protein deposit formation to the aging yeast cell. *Curr. Biol.* 27(6):773–83
129. Samaddar M, Goudeau J, Sanchez M, Hall DH, Bohnert KA, et al. 2021. A genetic screen identifies new steps in oocyte maturation that enhance proteostasis in the immortal germ lineage. *eLife* 10:e62653
130. Sarkar TJ, Quarta M, Mukherjee S, Colville A, Paine P, et al. 2020. Transient non-integrative expression of nuclear reprogramming factors promotes multifaceted amelioration of aging in human cells. *Nat. Commun.* 11(1):1545
131. Savas JN, Toyama BH, Xu T, Yates JR, Hetzer MW. 2012. Extremely long-lived nuclear pore proteins in the rat brain. *Science* 335(6071):942
132. Sawyer EM, Joshi PR, Jorgensen V, Yunus J, Berchowitz LE, Ünal E. 2019. Developmental regulation of an organelle tether coordinates mitochondrial remodeling in meiosis. *J. Cell Biol.* 218(2):559–79
133. Sinclair DA, Guarente L. 1997. Extrachromosomal rDNA circles—a cause of aging in yeast. *Cell* 91(7):1033–42
134. Sinclair DA, Mills K, Guarente L. 1997. Accelerated aging and nucleolar fragmentation in yeast *sgs1* mutants. *Science* 277(5330):1313–16
135. Sinclair DA, Mills K, Guarente L. 1998. Aging in *Saccharomyces cerevisiae*. *Annu. Rev. Microbiol.* 52:533–60
136. Stewart JB, Freyer C, Elson JL, Wredenberg A, Cansu Z, et al. 2008. Strong purifying selection in transmission of mammalian mitochondrial DNA. *PLOS Biol.* 6(1):e10
137. Suda Y, Nakanishi H, Mathieson EM, Neiman AM. 2007. Alternative modes of organellar segregation during sporulation in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 6(11):2009–17
138. Sugiyama S, Tanaka M. 2019. Distinct segregation patterns of yeast cell-peripheral proteins uncovered by a method for protein segregatome analysis. *PNAS* 116(18):8909–18
139. Suh E-K, Yang A, Kettenbach A, Bamberger C, Michaelis AH, et al. 2006. p63 protects the female germ line during meiotic arrest. *Nature* 444(7119):624–28
140. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5):861–72
141. Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4):663–76
142. Takizawa PA, DeRisi JL, Wilhelm JE, Vale RD. 2000. Plasma membrane compartmentalization in yeast by messenger RNA transport and a septin diffusion barrier. *Science* 290(5490):341–44
143. Taxis C, Maeder C, Reber S, Rathfelder N, Miura K, et al. 2006. Dynamic organization of the actin cytoskeleton during meiosis and spore formation in budding yeast. *Traffic* 7(12):1628–42
144. Thayer NH, Leverich CK, Fitzgibbon MP, Nelson ZW, Henderson KA, et al. 2014. Identification of long-lived proteins retained in cells undergoing repeated asymmetric divisions. *PNAS* 111(39):14019–26
145. Tiku V, Antebi A. 2018. Nucleolar function in lifespan regulation. *Trends Cell Biol.* 28(8):662–72
146. Tiku V, Jain C, Raz Y, Nakamura S, Heestand B, et al. 2017. Small nucleoli are a cellular hallmark of longevity. *Nat. Commun.* 8(6895):16083
147. Toyama BH, Savas JN, Park SK, Harris MS, Ingolia NT, et al. 2013. Identification of long-lived proteins reveals exceptional stability of essential cellular structures. *Cell* 154(5):971–82
148. Tresenrider A, Morse K, Jorgensen V, Chia M, Liao H, et al. 2021. Integrated genomic analysis reveals key features of long undecoded transcript isoform-based gene repression. *Mol. Cell* 81(10):2231–45.e11
149. Tresenrider A, Ünal E. 2018. One-two punch mechanism of gene repression: a fresh perspective on gene regulation. *Curr. Genet.* 64(3):581–88
150. Troyer D, Schwager P. 1982. Evidence for nuclear membrane fluidity: proacrosome migration and nuclear pore redistribution during grasshopper spermiogenesis. *Cell Motil.* 2(4):355–67

151. Tworzydlo W, Kisiel E, Jankowska W, Witwicka A, Bilinski SM. 2016. Exclusion of dysfunctional mitochondria from Balbiani body during early oogenesis of *Thermobia*. *Cell Tissue Res*. 366(1):191–201
152. Ünal E, Kinde B, Amon A. 2011. Gametogenesis eliminates age-induced cellular damage and resets life span in yeast. *Science* 332(6037):1554–57
153. Van Dalfsen KM, Hodapp S, Keskin A, Otto GM, Berdan CA, et al. 2018. Global proteome remodeling during ER stress involves Hac1-driven expression of long undecoded transcript isoforms. *Dev. Cell* 46(2):219–35.e8
154. van Werven FJ, Neuert G, Hendrick N, Lardenois A, Buratowski S, et al. 2012. Transcription of two long noncoding RNAs mediates mating-type control of gametogenesis in budding yeast. *Cell* 150(6):1170–81
155. von Mikecz A. 2009. Protein aggregation in the cell nucleus: structure, function and topology. *Open Biol. J.* 2(1):193–99
156. Wasko BM, Kaeberlein M. 2014. Yeast replicative aging: a paradigm for defining conserved longevity interventions. *FEMS Yeast Res*. 14(1):148–59
157. Wu S, Turner KM, Nguyen N, Raviram R, Erb M, et al. 2019. Circular ecDNA promotes accessible chromatin and high oncogene expression. *Nature* 575(7784):699–703
158. Wuttke D, Connor R, Vora C, Craig T, Li Y, et al. 2012. Dissecting the gene network of dietary restriction to identify evolutionarily conserved pathways and new functional genes. *PLOS Genet*. 8(8):e1002834
159. Yang J, McCormick MA, Zheng J, Xie Z, Tsuchiya M, et al. 2015. Systematic analysis of asymmetric partitioning of yeast proteome between mother and daughter cells reveals "aging factors" and mechanism of lifespan asymmetry. *PNAS* 112(38):11977–82
160. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, et al. 2007. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318(5858):1917–20
161. Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, et al. 2015. The *C9orf72* repeat expansion disrupts nucleocytoplasmic transport. *Nature* 525(7567):56–61
162. Zhou S, Sternglanz R, Neiman AM. 2017. Developmentally regulated internal transcription initiation during meiosis in budding yeast. *PLOS ONE* 12(11):e0188001