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REVIEW

Caenorhabditis elegans Reproductive Aging: Regulation and Underlying Mechanisms

Shijing Luo and Coleen T. Murphy*

Department of Molecular Biology, Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jeresy

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Summary: Female reproductive decline is one of the first aging phenotypes in humans, manifested in increasing rates of infertility, miscarriage, and birth defects in children of mothers over 35. Recently, *Caenorhabditis elegans (C. elegans*) has been developed as a model to study reproductive aging, and several studies have advanced our knowledge of reproductive aging regulation in this organism. In this review, we describe our current understanding of reproductive cessation in *C. elegans*, including the relationship between oocyte quality, ovulation rate, progeny number, and reproductive span. We then discuss possible mechanisms of oocyte quality control, and provide an overview of the signaling pathways currently identified to be involved in reproductive span regulation in *C. elegans*. Finally, we extend the relevance of *C. elegans* reproductive aging studies to the issue of human female reproductive decline, and we discuss ideas concerning the relationship between reproductive aging and somatic longevity. genesis 49:53–65, 2011. © 2010 Wiley-Liss, Inc.

Key words: reproduction; aging; germ line; oocyte; *C. elegans*

INTRODUCTION

C. elegans as a Model for Longevity Studies

Over the last two decades, our understanding of longevity mechanisms has been dramatically expanded with breakthrough genetic studies using model organisms, in particular the nematode *Caenorhabditis elegans*. Many useful genetic manipulation tools and resources have been developed for *C. elegans* research, including whole-genome RNA interference libraries, chemical, UV, and transposon mutagenesis, and repositories for large numbers of mutant and transgenic strains. These tools and the fact that *C. elegans* has a short life span (2–3 weeks) have allowed rapid analysis of survival and aging phenotypes.

There is a strong conservation of longevity pathways from *C. elegans* to humans (Kenyon, 2005; Suh *et al*., 2008). Moreover, *C. elegans* is a multicellular eukaryotic organism with multiple tissues and complicated behaviors, making it a superb model organism to study the senescence of many biological functions and processes, including loss of mobility (Herndon *et al*., 2002; Huang *et al*., 2004; Iwasa *et al*., 2010), decline in muscle integrity (Herndon *et al*., 2002), increased cancer susceptibility (Pinkston *et al*., 2006; Pinkston-Gosse and Kenyon, 2007), and declines in chemotaxis, learning, and memory (Kauffman *et al*., 2010).

The *C. elegans* Reproductive System

The transparency of *C. elegans*' tissues and the spatio–temporal layout of its reproductive system (Fig. 1) have made it a powerful model to study the germ line and reproduction, including germ line stem cell biology, germ cell apoptosis, the transition from mitosis to meiosis, oocyte maturation, and fertilization (Hubbard and Greenstein, 2000; Kimble and Crittenden, 2007). The

Abbreviations: ARD, adult reproductive diapause; DTC, distal tip cell; IIS, insulin/IGF-1 signaling; MSP, major sperm protein; TGF-β, transforming growth factor.

*Correspondence to: Coleen T. Murphy, Department of Molecular Biology, Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, USA. E-mail: ctmurphy@princeton.edu

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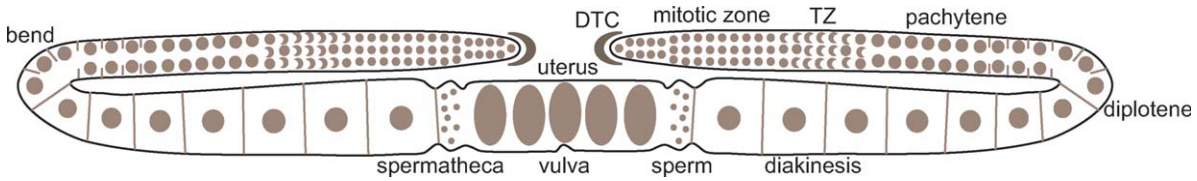

FIG. 1. The *C. elegans* gonad. The *C. elegans* hermaphrodite germ line contains two symmetric arms. Distal tip cells (DTC) send LIN-12/Notch signals to the distal germ line, maintaining the proliferative state of mitotic cell nuclei. Mitotic germ cell nuclei enter meiosis in the transition zone (TZ), marked by crescent-shaped nuclei. Germ cell nuclei exit the transition zone and enter the pachytene stage, complete the diplotene stage, and become arrested at diakinesis. Mature oocytes are fertilized by sperm in the spermatheca, and the fertilized embryo is stored in the uterus before finally being laid through the vulva.

C. elegans hermaphrodite germ line is a U-shaped tube structure containing two symmetric arms (Fig. 1). In each arm, the distal germ line receives the proproliferative LIN-12/Notch signal from the somatic distal tip cell (DTC) located at the tip of the gonad, whereas the first few rows of the syncytial distal germ line contain the stem cell nuclei that give rise to the rest of the proliferating germ cell nuclei in the mitotic region (Cinquin *et al*., 2010; Crittenden *et al*., 2006). In the transition zone, the germ cell nuclei begin meiosis and enter into the pachytene stage of meiotic prophase I. At the end of the pachytene stage, which occurs in the bend region, the syncytial germ cell nuclei begin to cellularize and grow larger. The bend is also the region where apoptosis takes place. The surviving germ cells then complete the diplotene stage and arrest at diakinesis. In the presence of sperm (either made prior to oogenesis or provided by males), the most proximal oocyte is activated by major sperm protein (MSP), completes meiotic maturation, and is ovulated into the spermatheca, where sperm are stored. The oocyte is fertilized in the spermatheca, and the fertilized embryo is pushed into the uterus and finally laid through the vulva.

In *C. elegans*, interactions between the soma and germ line occur at many levels to control reproduction. The somatic DTC maintains proliferation of stem cell nuclei in the distal germ line (Kimble and White, 1981). Somatic gonadal sheath cells and spermathecal lineage cells also play critical roles in germ cell nuclei mitotic proliferation, meiotic differentiation, oocyte growth, meiotic maturation, and ovulation (Govindan *et al*., 2009; Hall *et al.*, 1999; McCarter *et al*., 1997; Miller *et al*., 2003). A recent study found that the *C. elegans* early growth response factor family member *egrh-1* functions in both the intestine and somatic gonad to regulate oocyte development (Clary and Okkema, 2010).

C. elegans as a Model of Reproductive Aging

While many studies have addressed phenotypes of late aging, fewer have focused on early aging phenomena. In humans, female reproductive capacity declines dramatically after the mid-30s, and is marked by an increased risk of infertility, birth defects, and miscarriage, making it perhaps the earliest age-related decline that humans experience. These reproductive aging problems are thought to be due to declining oocyte quality rather than lack of oocytes because the problems arise a decade prior to menopause. As more and more women opt to have children later in life, addressing the issue of female reproductive aging has become increasingly important. As in humans, reproduction declines early in *C. elegans*, lasting only one-third of its life span. Recently, *C. elegans* has been developed as a model to study reproductive capacity decline with age (Andux and Ellis, 2008; Hughes *et al*., 2007; Luo *et al*., 2009, 2010). These studies established that (1) *C. elegans* reproductive aging is a genetically-regulated process; (2) *C. elegans* reproductive aging is limited by oocyte quality decline, as in humans; and (3) reproductive aging is normally coupled to, but also distinct from somatic aging.

MECHANISMS OF *C. ELEGANS* REPRODUCTIVE AGING REGULATION

Reproductive Aging Is Independent of Progeny Number

Four possible models could explain reproductive cessation in *C. elegans*: (1) *C. elegans* hermaphrodites produce only ~300 sperm, therefore exhaustion of self sperm could cause termination of reproduction; (2) *C. elegans* may only generate a limited number of oocytes; (3) *C. elegans* can generate an unlimited number of oocytes, but there is a limited number of high-quality oocytes; or (4) *C. elegans* oocyte quality declines with and is determined by maternal age, regardless of oocyte number. To rule out the first possibility, that of limitation by self-sperm, hermaphrodites (or spermless mutant hermaphrodites) were provided with sufficient sperm by mating with young wild-type males (hereafter referred to as "mated reproductive span"). When provided with excess sperm, reproduction still ceases early in adulthood (Hughes *et al*., 2007; Luo *et al*., 2009), suggesting that sperm number is not a limiting factor in *C. elegans* mated hermaphrodite reproductive aging.

Among the three remaining possibilities, the second and the third would suggest a usage-dependent mecha-

nism: in these two particular scenarios, the cessation of reproduction would be a consequence of exhaustion of good oocytes. If this were true, an extension of the reproductive period could be achieved through slower usage of oocytes in the early phase of reproduction. To test this hypothesis, Hughes and colleagues manipulated early reproduction by mating wild-type or spermless mutants (*spe-8* and *fog-2* mutants) with young wild-type males at different ages, and characterized their progeny production (Hughes *et al*., 2007). They found that the decline in late progeny production is independent of the number of progeny produced in the early phase of reproduction, showing that reproductive cessation in *C. elegans* is not due to a usage-dependent mechanism. Also, Andux and Ellis (2008) reported that mated older *fog-2* (spermless) mutant hermaphrodites produce more unhatched embryos and fewer fertilized embryos than younger *fog-2* mutant mothers. This suggests that (1) reproductive cessation is due to a decline in progeny quality, and (2) such decline is determined by maternal age rather than by the number of progeny produced. Furthermore, Luo *et al*. (2009) showed that neither slower usage of oocytes due to slowed ovulation rate nor a smaller brood size, as in the case of many small-body mutants, extends reproductive span (i.e., the reproductive period) (Luo *et al*., 2009), which again suggests that a usage-dependent mechanism does not underlie reproductive cessation. Additionally, ovulation rate and progeny number are not correlated with reproductive span (Luo *et al*., 2010). Together, these studies show that reproductive cessation in *C. elegans* is usage-independent, and that simply reducing the number of oocytes used or delaying progeny production does not extend reproductive span-that is, *C. elegans*, like humans, have a "use it or lose it" limitation of reproduction.

Oocyte Quality Decline Limits Reproductive Span

In humans, reproductive cessation occurs a decade before menopause, suggesting that declining oocyte quality, rather than quantity, is the major cause of maternal age-associated infertility and birth defects (ESHRE Capri Workshop Group, 2005). Mammalian oocytes exhibit increased abnormalities in fertilization, chromosome segregation, cleavage divisions, and stress response with age (Blondin *et al*., 1997; ESHRE Capri Workshop Group, 2005; Goud *et al*., 1999; Hiroshi Tamura *et al*., 2008; Jones, 2008; Tarin, 1996; te Velde and Pearson, 2002). Similarly, several *C. elegans* studies show that oocyte quality is the limiting factor for reproductive capacity decline (Andux and Ellis, 2008; Luo *et al*., 2010) (Fig. 2a). In mated hermaphrodites provided with sufficient sperm, every oocyte that is capable of being fertilized will acquire an egg shell and become a fertilized embryo that is easily distinguished from an unfertilized oocyte (Luo *et al*., 2010). Mated wild-type hermaphrodites produce only fertilized embryos in early reproduction, but start producing unfertilized oocytes as they age (Luo *et al*., 2010). Likewise, older worms are more likely to have a cluster of unfertilized oocytes in the uterus, where fertilized embryos are normally stored before being laid (Luo *et al*., 2010). These data indicate that the fertilizability of oocytes becomes compromised with age.

The developmental competence of oocytes also declines with age. Older hermaphrodites produce more unhatched embryos, a phenotype caused by developmental defects, such as chromosomal segregation errors (Andux and Ellis, 2008; Luo *et al*., 2010). While increased embryonic lethality can result from autosomal nondisjunction, increased male production indicates an increase in X chromosome segregation errors. In *C. elegans*, XO (male) progeny are produced from XX hermaphrodite mothers through meiotic X chromosome nondisjunction (Hodgkin *et al*., 1979). Nondisjunction occurs at a low frequency in young hermaphrodites and males are relatively rare in the population, but more male progeny are produced with increasing maternal age (Luo *et al*., 2010; Rose and Baillie, 1979; Tang *et al*., 2010). Together, the data suggest that chromosomal abnormalities increase with age. This is confirmed by the fact that the oocytes of older worms are more likely to contain an abnormal number of DAPI-stained bodies in their nuclei (Luo *et al*., 2010). Older embryos are also more susceptible to hypochlorite treatment (i.e., bleaching) and ionizing irradiation treatment, suggesting that their stress resistance declines with age (Luo *et al*., 2010).

In addition to the declines in their functional characteristics, oocyte morphology is also degraded in older mothers (Fig. 2b). Andux and Ellis reported that older virgin hermaphrodites have stacked oocytes in their proximal gonads, and embryos developed from stacked oocytes are less likely to hatch, suggesting that stacking at diakinesis of meiotic prophase I is correlated with defective oocytes (Andux and Ellis, 2008). Older wild-type animals produce more small embryos and tiny, embryo-like objects. This phenotype is elevated in apoptosis-defective mutants, so the increased frequency of smaller embryos is more likely to be the result of fertilization of smaller oocytes rather than due to the breakdown of larger oocytes. Consistent with these observations, Luo and colleagues found a small-oocyte phenotype in day-8 mated wild-type gonads, and showed that this morphology marker can be used as one of the predictors of the reproductive capacity of a worm (Luo *et al*., 2010). Small oocytes may lack sufficient resources required for proper embryogenesis. Since oocyte stacking is more frequently observed prior to small oocytes in the gonad (Luo and Murphy, unpublished data), it is possible that stacking is an

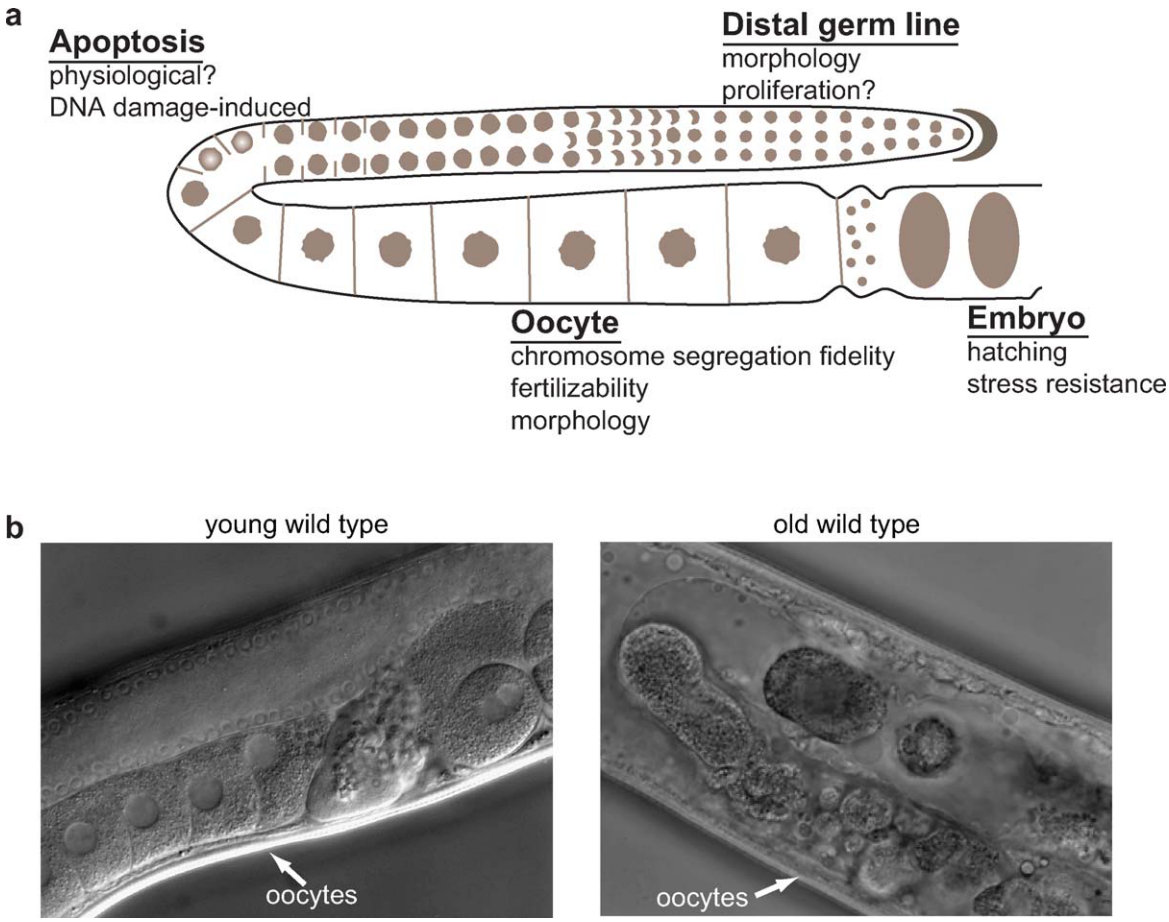

FIG. 2. Summary of cellular processes that are better maintained in mutants with extended reproductive spans. (**a**) Wild-type worms undergo declining reproductive ability with age, demonstrated through reduced embryo hatching and stress resistance; reduced oocyte fertilizability, chromosome segregation fidelity, and degraded oocyte morphology; and degraded distal germ line morphology and germ cell nuclei proliferation (Luo *et al*., 2010). DNA damage-induced apoptosis decreases significantly with age, independently of IIS and TGF-β regulation, while physiological apoptosis may also decrease in aged wild-type animals (Andux and Ellis, 2008). (**b**) Oocytes of young (day 1) and old (day 8) wild-type worms exhibit differences in morphology (Luo *et al*., 2010).

intermediate step in producing small oocytes. However, it is not yet known whether stacked oocytes and small oocytes reduce quality through the same mechanism or independently. Small oocyte size cannot be solely responsible for oocyte quality, since not all unhatched embryos are small (Andux and Ellis, 2008; Luo *et al*., 2010); so normal-sized old oocytes may also be insufficiently equipped or be defective in maturation and/or development.

Oocyte size is just one of the morphological markers of oocyte quality, however. In young worms, oocytes are packed closely together and make extensive contact with somatic tissues, while there are more cavities between neighboring oocytes and between oocytes and somatic gonad tissues in aging mothers (Luo *et al*., 2010). Thus, cellular contact may be crucial for normal signal transduction and uptake of important molecules, and contact failure may partially explain defective oocytes, regardless of their size.

Apoptosis and Germ Line Stem Cells in Oocyte Quality Control

At least half of the oogenic germ cell nuclei are removed by apoptosis in *C. elegans* (Gumienny *et al.*, 1999). In insects, such as the cockroach, ovarian apoptosis increases under starvation and reduces "female reproductive life span" (Edvardsson *et al*., 2009; Terashima and Bownes, 2004). In human oocytes, DNA fragmentation is associated with apoptosis (Wu *et al*., 2000). Thus, apoptosis may be a conserved mechanism involved in reproductive maintenance.

Several different types of apoptosis take place in the *C. elegans* germ line. "Physiological germ cell apoptosis" refers to the reduction of nuclei in the apoptotic zone prior to their cellularization into oocytes (Gumienny *et al*., 1999). Other forms of apoptosis are triggered by stress: "DNA-damage checkpoint-induced apoptosis" is caused by ionizing irradiation (Gartner

et al., 2000), while meiotic recombination and pairing checkpoints (Bhalla and Dernburg, 2005; Gartner *et al*., 2000), and environmental stresses, especially pathogen infections (Aballay and Ausubel, 2001; Salinas *et al*., 2006), can also induce apoptosis.

Two models could explain the role of apoptosis in oogenesis and oocyte quality maintenance with age: (1) physiological apoptosis may provide nurse cells for the remaining oocytes to ensure their proper growth and maturation (the "nurse cell model") and (2) DNA damage or other stress-induced apoptosis might eliminate defective germ cell nuclei, thereby maintaining genomic integrity of the germ line (the "elimination model") (Andux and Ellis, 2008; Gartner *et al*., 2008; Gumienny *et al*., 1999).

Andux and Ellis found that the apoptosis-defective *ced-3* and *ced-4* mutants produce more unhatched embryos, and that this effect on embryonic lethality is maternal and therefore reflects a decrease in oocyte quality. However, two other mutants that are defective only in DNA-damage-induced apoptosis but not physiological germ cell apoptosis, *egl-1* and *ced-9* mutants, do not seem to increase embryonic lethality, at least not to the same degree that *ced-3* and *ced-4* mutants do (Andux and Ellis, 2008). Wild-type animals produce more small embryos with age (Andux and Ellis, 2008; Luo *et al*., 2010), and this phenotype becomes more dramatic in *ced-3* and *ced-4* mutants (Andux and Ellis, 2008). Together, the data suggest the model that physiological apoptosis controls oocyte quality by reducing the number of germ cell nuclei to ensure proper resource allocation.

The levels of apoptosis after DNA damaging ionizing irradiation dramatically decrease in wild-type animals from day 2 to day 6 of adulthood (Luo *et al*., 2010), but the authors observed only a slight decrease in the level of physiological germ cell apoptosis during this interval (Luo *et al*., 2010) (Fig. 2a). Taken together, these data suggest that both the nurse cell model and the elimination model may play a role in regulating oocyte maintenance. It is possible that the DNA damage checkpoint may play a larger role when genotoxic stress accumulates and cells are more susceptible to damage, e.g., in late reproduction. Such a role would be magnified when there is excessive DNA damage triggered by ionizing irradiation, but may be unnoticed in untreated conditions early in reproduction because of lower amounts of DNA damage. In support of this notion, *ced-9* mutants exhibit increased embryonic lethality compared with wild type, but at a much later time than observed in *ced-3* and *ced-4* mutants, perhaps reflecting its increasing importance in late reproduction (Andux and Ellis, 2008). Physiological apoptosis, however, may play an important role early in reproduction, and a slight decrease in physiological apoptosis may be sufficient to make a difference in oocyte quality. While ovarian apoptosis may underlie fertility decline in insects (Edvardsson *et al*., 2009; Terashima and Bownes, 2004), it is required for oocyte quality maintenance in *C. elegans* (Andux and Ellis, 2008). Perhaps the correct level of apoptosis is required for optimal reproduction in each organism.

Another possible explanation for oocyte quality decline is a decrease in the quality of the germ cell nuclei prior to apoptosis (Fig. 2a). Garigan and colleagues reported that the distal germ lines of aged *C. elegans* are degraded (Garigan *et al*., 2002). The syncytial nuclei are often disrupted by cavities and grainy material in older animals, and frequently the nuclei become prematurely cellularized, resulting in shriveled gonads (Garigan *et al*., 2002). Luo *et al*. (2010) confirmed these aging signs in the distal germ line of older animals, and also found a positive correlation between these signs of distal germ line aging and the markers of oocyte morphology degradation in the same population of worms. Since the distal germ line is upstream of oocyte maturation, distal germ line integrity may influence oocyte quality. However, more direct evidence is needed to test whether there is a causal role, or whether the two tissues degrade simultaneously but mechanistically independently. The distal germ cell nuclei must be maintained at high quality so that these precursors can eventually develop into good oocytes. If they are degraded, since there is still an excess of precursor nuclei, the apoptosis machinery may then try to select the better nuclei through the elimination mechanism. Alternatively, the nurse cell model suggests that the reduced resources would be allocated to fewer surviving germ cell nuclei to ensure that they are still loaded with sufficient components (Fig. 2a). Defects in either of the two mechanisms would likely be detrimental to oocyte quality.

Interestingly, in the recently discovered starvation-induced "adult reproductive diapause" (ARD) state, the germ line shrinks significantly, with only a small population of germ line stem cell nuclei remaining (Angelo and Van Gilst, 2009). Once the ARD state is released by feeding, the surviving germ line stem cell nuclei regenerate a new germ line and fully recover its function. This suggests that, at least in starvation-induced ARD, maintenance of germ line stem cell nuclei proliferation is essential to the maintenance of reproductive capacity. This may be also true in germ line aging. Garigan *et al*. (2002) observed that older gonads frequently contain relatively few nuclei, and there are fewer mitotic region cells in older animals (Killian and Hubbard, 2005). Similarly, Luo *et al*. (2010) found that the number of mitotic germ cell nuclei in this region decreases significantly with age. Thus, a decrease in mitotic germ cell nuclei proliferation may contribute to the aging of the germ line (Fig. 2a). However, we should note that Crittenden and colleagues reported that although the mitotic region shortens with age, the number of mitotic germ

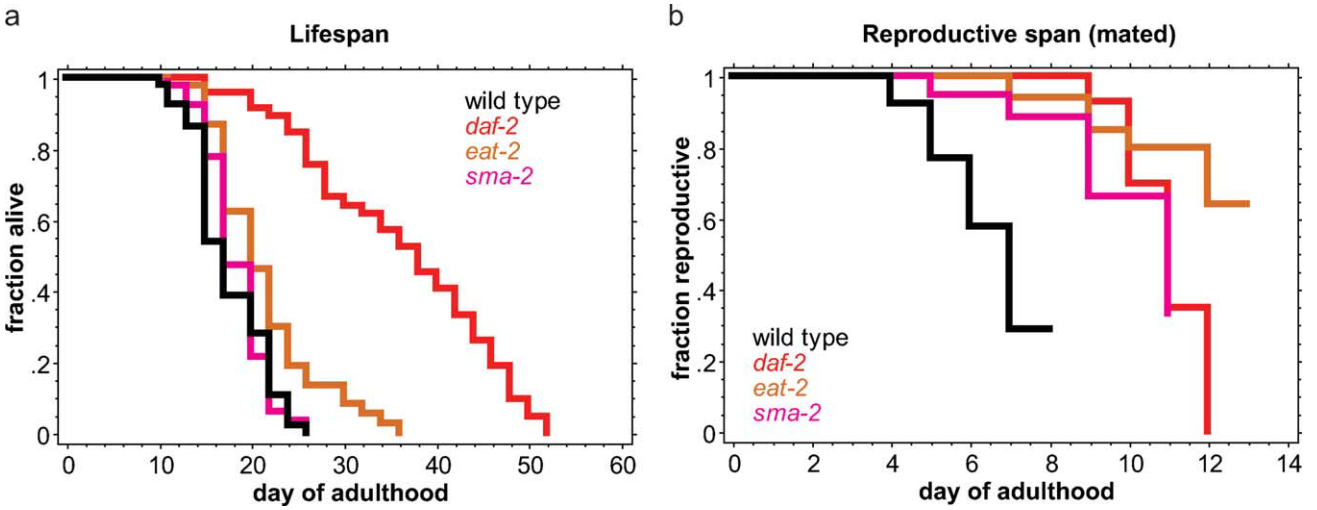

FIG. 3. Life spans and reproductive spans of wild-type, IIS, dietary restriction, and TGF-β mutant worms. (**a**) The insulin/IGF-1 signaling mutant *daf-2(e1370)* and the dietary restriction mutant *eat-2(ad465)* both significantly extend life span compared to wild type, while the TGF-β Sma/Mab signaling mutant *sma-2(e502)* does not (Luo *et al*., 2009). (**b**) *daf-2(e1370)*, *eat-2(ad465)*, and *sma-2(e502)* mutants all significantly extend mated reproductive span (Luo *et al*., 2009).

cell nuclei remains constant from day 1 to day 6 of adulthood (Crittenden *et al*., 2006). Also, there is a vast excess of germ cell nuclei (>5,000) compared with the maximum number of oocytes made (Crittenden *et al*., 2006). Thus, further analysis is required to more precisely dissect the relationship between germ line stem cell nuclei proliferation and germ line and oocyte aging.

TRANSCRIPTIONAL CHANGES IN AGED OOCYTES

In addition to the gross morphological and functional changes in aging oocytes, transcriptional comparisons between young and old oocytes reveal striking changes and indicate molecular processes that may be particularly susceptible to maternal age (Luo *et al*., 2010). Old oocytes exhibit declines in the expression of many genes that are likely to maintain oocyte function, including chromosome segregation, cell cycle, DNA damage response and repair, and proteolytic pathway genes (Luo *et al*., 2010). Functional analyses of these genes reveal that some gene classes are likely generally required for oocyte development (e.g., chromosome segregation fidelity and cell cycle regulation genes), while others may become increasingly important with age (e.g., DNA damage response/repair genes) (Luo *et al*., 2010). For example, proteasome activity is required to eliminate oxidatively-damaged proteins at the time of oocyte maturation (Goudeau and Aguilaniu, 2010), suggesting that proteolysis genes are important in aged worms that have accumulated carbonylated proteins in the germ line. Strikingly, many of the genes that decline with age in *C. elegans* oocytes also decline in aging mammalian oocytes, including the SMC condensins, cyclins, DNA mismatch repair components, and proteolytic pathway members, suggesting that molecular processes crucial for oocyte quality maintenance are conserved between worms and mammals (Hamatani *et al*., 2004; Luo *et al*., 2010; Steuerwald *et al*., 2007). In addition, the *C. elegans* oocyte expression analysis identified several unknown genes that are downregulated in older oocytes and are also required for successful reproduction, revealing possible functions for those uncharacterized genes (Luo *et al*., 2010).

SIGNALING PATHWAYS THAT REGULATE *C. ELEGANS* REPRODUCTIVE AGING

Insulin/IGF-1 Signaling

In *C. elegans*, the insulin/IGF-1 signaling (IIS) pathway is best known for its role in longevity and dauer state regulation (Kenyon *et al*., 1993; Kimura *et al*., 1997; Lin *et al*., 1997; Ogg *et al*., 1997; Riddle *et al*., 1981). There are about 40 insulin-like molecules in the *C. elegans* genome (Gregoire *et al*., 1998; Hua *et al*., 2003; Kawano *et al*., 2006; Li *et al*., 2003; Murphy *et al*., 2007; Pierce *et al*., 2001), but only one insulin-like receptor, DAF-2. Mutations in *daf-2* can more than double the worm's life span (Kenyon *et al*., 1993) (Fig. 3a). Insulin-like agonist binding activates DAF-2, triggering a kinase cascade that ultimately phosphorylates the FoxO transcription factor DAF-16, excluding it from the nucleus and preventing its transcriptional activity (Hertweck *et al*., 2004; Kimura *et al*., 1997; Lin *et al*., 1997; Morris *et al*., 1996; Ogg *et al*., 1997; Paradis *et al*., 1999; Paradis and Ruvkun, 1998). In loss-of-func-

Table 1
Summary of Age-Affected Cellular Processes in daf-2 and sma-2 Mutants

	Embryo	Oocyte			Proximal germ line, apoptotic zone			Distal germ line	
	Viability *(mbk-2, gei-4)*[a]	Chromosome segregation *(smc-4, klp-7)*	Fertilizability *(mlh-1, F47G4.4)*	Morphology *(cyb-3, E03H4.8)*	Physiological apoptosis *(ced-1)*	DNA damage-induced apoptosis	DNA damage repair *(clk-2, uev-2)*	Morphology *(cyb-3, E03H4.8)*	Proliferation *(cdc-25.2, epi-1)*
daf-2	++[b]	+++	+++	+++	+	−[c]	+++	+	+
sma-2	++	+++	+++	+++	+	−	+++	+	+

[a]Example genes upregulated in *sma-2* mutant oocytes affecting the processes (Luo *et al*., 2010).
[b]Cellular processes that are maintained with age in *daf-2* and *sma-2* mutants. More + indicates better maintenance.
[c]Cellular processes that are not maintained with age in *daf-2* and *sma-2* mutants.

tion *daf-2* mutants, however, DAF-16 is constitutively translocated into the nucleus and its transcriptional program is activated. In addition to its effect on life span, mutations in *daf-2* significantly extend mated reproductive span (Hughes *et al*., 2007; Luo *et al*., 2009) (Fig. 3b). *daf-2* mutations extend reproductive span by improving oocyte and germ line maintenance (Luo *et al*., 2010) (Table 1). Old *daf-2* mutants produce fewer unfertilized oocytes, unhatched embryos, and males than wild-type animals of the same age. In addition, the oocytes and distal germ lines of *daf-2* mutants degrade more slowly than those of wild type. Although *daf-2* mutants have fewer proliferating germ cell nuclei, both in larvae and adults (Luo *et al*., 2010; Michaelson *et al*., 2010), there is a smaller decrease in mitotic cell number with age in *daf-2* mutants compared with wild type (Luo *et al*., 2010).

The reproductive span extension of *daf-2* mutants is genetically dependent upon *daf-16* (Hughes *et al*., 2007; Luo *et al*., 2009). Intestinal DAF-16 activity plays a major role, and neuronal DAF-16 makes a small contribution in *daf-2's* regulation of longevity, while muscular DAF-16 is not required (Libina *et al*., 2003). By contrast, both intestinal and muscle DAF-16 are required for *daf-2's* reproductive span extension, while neuronal DAF-16 has no effect (Luo, et al. 2010). IIS regulates both life span and reproductive span in adulthood, but IIS activity late in development also affects progeny production and reproductive span (Dillin *et al*., 2002; Luo *et al*., 2010). Thus, while IIS regulates both reproductive span and life span, the sites and timing of IIS activity are slightly different for the two processes.

Dietary Restriction

Another manipulation known to extend both life span and reproductive span is dietary restriction (DR). In fact, life span extension and slowing of reproductive activity are the hallmarks of dietary restriction. DR reduces progeny number and extends and delays the reproductive period of *C. elegans* hermaphrodites (Huang *et al*., 2004; Hughes *et al*., 2007), female *Drosophila* (Chapman and Partridge, 1996), and female rodents (Holehan and Merry, 1985; McShane and Wise, 1996; Selesniemi *et al*., 2008). *eat-2*, which encodes a mutation in the acetylcholine receptor, is a genetic model of dietary restriction in *C. elegans* due to its reduced ability to ingest food (Lakowski and Hekimi, 1998) (Fig. 3b). Like *daf-2*, the role of *eat-2* in reproductive span regulation was originally identified through its longevity phenotype (Huang *et al*., 2004; Hughes *et al*., 2007; Luo *et al*., 2009) (Fig. 3a,b), and both its life span and reproductive span are genetically dependent on the FoxA transcription factor PHA-4 (Luo *et al*., 2009; Panowski *et al*., 2007).

In the extreme case of starvation, adult worms can also undergo a long period of reproductive diapause until food becomes available again (Angelo and Van Gilst, 2009). Furthermore, food availability also affects *C. elegans*'s reproductive strategy, since worms modify their production of males and outcrossing frequency after starvation to increase genetic variation (Morran *et al*., 2009). Therefore, it is possible that modifying the reproductive schedule and preserving reproductive capacity, awaiting an improved environment, is an evolved strategy that worms and other species use to adapt to suboptimal conditions.

TGF-β Sma/Mab Signaling

The Sma/Mab pathway is one of the two canonical TGF-β signaling pathways in *C. elegans*. Mutants of the TGF-β Sma/Mab pathway are small and have defective male tails (Savage *et al*., 1996). DBL-1 is the TGF-β-related ligand (Morita *et al*., 1999; Suzuki *et al*., 1999), and SMA-6 and DAF-4 are type I and type II receptors. The signal transducers include the R-Smads SMA-2 and SMA-3 and the Co-Smad SMA-4, and SMA-9 is a transcription cofactor (Liang *et al*., 2003; Savage *et al*., 1996; Savage-Dunn, 2005; Savage-Dunn *et al*., 2003). Upon binding of the DBL-1 ligand, the type I and type II receptors assemble and phosphorylate the Smad signal transducers, which then enter the nucleus, bind to DNA, and recruit transcription cofactors and factors to activate and suppress target gene expression (Massagué, 2000). The ligand DBL-1 is mainly expressed in neurons, while the receptor and Smad proteins are expressed in multiple tissues, including intestine, pharynx, hypodermis, muscle, and the somatic reproductive system.

In addition to its other roles, the TGF-β Sma/Mab pathway also regulates reproductive aging: mutants of the pathway extend reproduction, in some cases doubling the reproductive span (Luo *et al*., 2009) (Fig. 3b). However, the pathway has an insignificant effect on life span (Fig. 3a); thus, it is the first reported pathway that regulates reproductive span independently of somatic longevity (Luo *et al*., 2009). TGF-β Sma/Mab mutants extend reproductive span independently of the two major somatic aging regulators, *daf-16* (IIS pathway transcription factor) and *pha-4* (dietary restriction pathway transcription factor) (Luo *et al*., 2009). Similar to reduced IIS, reduced TGF-β Sma/Mab signaling delays reproductive aging by better maintaining fertilizability, chromosome segregation fidelity, stress resistance, oocyte morphology, distal germ line morphology, and distal germ line proliferation (Luo *et al*., 2010) (Table 1). Mosaic analysis and tissue-specificity studies reveal that signals from the soma regulate aging of the reproductive system: TGF-β Sma/Mab signaling acts in the hypodermis to promote aging of the germ line and oocytes, while IIS acts in the intestine and muscle (Luo *et al*., 2010). The fact that TGF-β Sma/Mab signaling acts in hypodermis to regulate reproductive aging suggests that a signal is sensed in the soma and is transmitted to the germ line; this signal has not yet been identified (Luo *et al*., 2010).

TGF-β Sma/Mab signaling in the hypodermis is necessary and sufficient for body size regulation (Wang *et al*., 2002; Yoshida *et al*., 2001). Interestingly, hypodermal SMA-3 activity is also necessary and sufficient to restore the long reproductive span of *sma-3* mutants to normal (short) reproductive span (Luo *et al*., 2010). Therefore, the pathway regulates two different biological processes, growth and reproduction, through its activity in the same tissue. However, the fact that reproductive span regulation is independent of body size is supported by several lines of evidence. First, none of the non-TGF-β small mutants that have been tested extend reproductive span (Luo *et al*., 2009). Secondly, while the activity of the SMA-9 transcription cofactor is required during early larval development to regulate body size growth (Liang *et al*., 2003), it acts in adulthood to regulate reproductive span (Luo *et al*., 2010). Last but not least, the transcriptional targets regulated by TGF-β Sma/Mab signaling in oocytes are distinct from those involved in body size regulation (Liang *et al*., 2007; Luo *et al*., 2010).

COORDINATION OF SOMATIC AGING AND REPRODUCTIVE AGING

While *daf-2* and *eat-2* mutants have both long life spans and long reproductive spans, TGF-β Sma/Mab mutants slow the rate of reproductive aging without concomitantly slowing somatic aging (Luo *et al*., 2009). Reduced TGF-β Sma/Mab signaling has very little effect on longevity, and matricide (mortality induced by progeny hatching within the mother) increases at the same rate as in wild-type animals. While matricide stops in wild type around day 7–8, in concert with reproductive cessation, it continues in TGF-β Sma/Mab mutants due to their extended reproduction. Matricide is caused by defects in egg-laying, likely due to declining muscle integrity. Thus increasing matricide with age suggests that the rate of somatic decline is similar in wild type and TGF-β Sma/Mab mutants. The *daf-2* and *eat-2* longevity mutants experience less age-related matricide than TGF-β Sma/Mab mutants at the same age, likely due to their improved somatic (muscle) health. Thus, aging of the reproductive system is normally coupled with aging of the soma in wild-type animals as well as *daf-2* and *eat-2* mutants. TGF-β Sma/Mab signaling may normally mediate soma-to-germ line communication to adjust the reproductive rate, but such coupling is broken in the TGF-β Sma/Mab mutants (Luo *et al*., 2009).

The increased rate of matricide with age indicates that reproduction itself requires the soma to be in peak physical condition, otherwise reproduction may be detrimental to the mother and to unproduced progeny, as in the case of old TGF-β Sma/Mab mutants. The soma can merely survive postreproductively well below the peak level of function. In fact, in both worms and humans, many biological functions, including motility, pathogen resistance, learning, and memory, etc., all peak during the reproductive period, but begin to decline soon afterwards, offering one model to explain the long post-reproductive life span of both worms and humans.

GERM LINE REGULATION OF SOMATIC AGING

In addition to the somatic regulation of reproductive aging, signals from the germ line regulate somatic aging. Removal of the germ line extends longevity, an effect that depends on DAF-16 nuclear localization in the intestine and that requires the presence of the somatic gonad and an ankyrin-repeat protein, KRI-1 (Berman and Kenyon, 2006; Hsin and Kenyon, 1999; Libina *et al*., 2003; Lin *et al*., 2001). The *daf-12* dafachronic acid pathway is required both for DAF-16 nuclear localization and the somatic gonad effect on longevity (Berman and Kenyon, 2006; Gerisch *et al*., 2007; Yamawaki *et al*., 2010). Recently, a histone H3K4 methyltransferase/demethylase complex was found to act in the germ line to regulate life span (Greer *et al*., 2010). Thus, a bidirectional flow of information between somatic and reproductive tissues may coordinate their rates of aging, possibly to optimize reproductive success and adaptation to adverse conditions.

CONSERVATION WITH HUMAN OOCYTE AGING AND IMPLICATIONS FOR HUMAN REPRODUCTION

Despite their vastly different life histories, chronological time frames, and reproductive strategies, the cellular and molecular regulation of *C. elegans* and human reproductive aging are strikingly similar. Although oocytes are continually produced in worms, while humans' total oocyte supply is produced at birth, both human and *C. elegans* females have long post-reproductive life spans, and undergo significant reproductive aging on proportional time scales (Cant and Johnstone, 2008; Luo *et al*., 2009). Both human and *C. elegans* oocytes are cell-cycle arrested at meiotic prophase I, and their release from arrest by hormone is coordinated with oocyte maturation (Greenstein, 2005; Mehlmann, 2005). The mechanisms underlying oocyte maturation are highly conserved between the two organisms. Most importantly, in both humans and worms, oocyte quality, rather than quantity, is the major cause of reproductive cessation. In particular, fertilizability, chromosome segregation fidelity, stress resistance, and morphology are compromised with age in both organisms (Blondin *et al*., 1997; ESHRE Capri Workshop Group, 2005; Goud *et al*., 1999; Hiroshi Tamura *et al*., 2008; Jones, 2008; Luo *et al*., 2010; Magli *et al*., 2007; Rose and Baillie, 1979; Rubio *et al*., 2003; Tang *et al*., 2010; Tarin, 1996; te Velde and Pearson, 2002). *C. elegans* reproductive aging is not only limited by oocyte quality, as it is in humans, but similar underlying molecular factors also contribute to oocyte quality maintenance in the two organisms.

IIS and dietary restriction regulate longevity from worms to mammals (Kenyon *et al*., 1993; Suh *et al*., 2008; Tu *et al*., 2002; Willcox *et al*., 2006, 2008). These pathways have been implicated in the regulation of mammalian reproductive aging (Castrillon *et al*., 2003; Holehan and Merry, 1985; Klein *et al*., 2000; McShane and Wise, 1996; Selesniemi *et al*., 2008) and *C. elegans* reproductive aging (Huang *et al*., 2004; Hughes *et al*., 2007; Luo *et al*., 2009, 2010). A recent genome-wide association study also identified ARHGEF7, a gene that interacts with FOXO3a, the human homolog of DAF-16/FOXO, as a candidate gene associated with age at menopause (Ong *et al*., 2009). Additionally, Foxo3a knockout mice exhibit a defect in follicular activation (Castrillon *et al*., 2003), again linking IIS and FoxO to fertility and aging.

TGF-β superfamily ligands influence mammalian reproduction through the regulation of follicle development (Knight and Glister, 2006; Trombly *et al*., 2009). Additionally, TGF-β signaling has been implicated in reproductive aging, as TGF-β members are upregulated in aged mouse oocytes (Hamatani *et al*., 2004). Our *C. elegans* transcriptional analyses show that many genes upregulated in TGF-β Sma/Mab mutant oocytes are similar to human and mouse oocyte genes that decline with age, including the condensin genes required for proper chromosome segregation, cell cycle genes, DNA mismatch repair genes, proteolytic pathway genes, and many others (Hamatani *et al*., 2004; Luo *et al*., 2010; Steuerwald *et al*., 2007). *lin-28*, which is upregulated in TGF-β Sma/Mab mutant oocytes, is also associated with human reproductive development and cessation (He *et al*., 2009; Ong *et al*., 2009; Perry *et al*., 2009; Sulem *et al*., 2009). Transcriptional studies suggest that many of the molecular mechanisms underlying oocyte quality maintenance are likely shared between *C. elegans* and humans through TGF-β signaling. Therefore, while mammals have a more complex and diverse TGF-β family that carries out many different functions, it is likely that some branch of TGF-β signaling may be involved in the regulation of reproductive cessation. If so, modulation of TGF-β signaling may offer new avenues to delay human reproductive aging, and our studies in *C. elegans* may provide insights into potential therapies for maternal age-associated infertility and birth defects.

CONCLUSIONS AND FUTURE DIRECTIONS

While human postreproductive life span has been greatly extended through improvements in medicine, nutrition, hygiene, and environment (Centers for Disease Control and Prevention, 1999; Finch and Crimmins, 2004), these factors have not influenced female reproductive span. Here, we have described recent studies on reproductive aging mechanisms in *C. elegans* and our current understanding of its regulation. These studies were the first efforts to establish *C. elegans* as a model to study reproductive capacity decline, and have laid the foundation for future research in the field. While several reproductive aging signaling pathways have been identified, many questions still remain. One immediate challenge is to identify soma-to-germ line signals that mediate the regulation of germ line and oocyte quality by TGF-β Sma/Mab and IIS. Direct experimental evidence is also required to address the relationship between germ cell nuclei proliferation, germ line integrity, and oocyte quality. In addition, the mechanism of reproductive span extension by dietary restriction must also be dissected. Finally, forward genetic screens will facilitate the discovery of additional novel regulators of reproductive span. Such studies will help us better understand how aging of the reproductive system is regulated in *C. elegans*, shed light on the relationship between reproductive and somatic aging, and further elucidate the implications for human reproductive aging.

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