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Control of Oocyte Growth and Meiotic Maturation in *C. elegans*

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Abstract

In sexually reproducing animals, oocytes arrest at diplotene or diakinesis and resume meiosis (meiotic maturation) in response to hormones. Chromosome segregation errors in female meiosis I are the leading cause of human birth defects, and age-related changes in the hormonal environment of the ovary are a suggested cause. *C. elegans* is emerging as a genetic paradigm for studying hormonal control of meiotic maturation. The meiotic maturation processes in *C. elegans* and mammals share a number of biological and molecular similarities. Major sperm protein (MSP) and luteinizing hormone (LH), though unrelated in sequence, both trigger meiotic resumption using somatic G_s-adenylate cyclase pathways and soma-germline gap-junctional communication. At a molecular level, the oocyte responses apparently involve the control of conserved protein kinase pathways and post-transcriptional gene regulation in the oocyte. At a cellular level, the responses include cortical cytoskeletal rearrangement, nuclear envelope breakdown, assembly of the acentriolar meiotic spindle, chromosome segregation, and likely changes important for fertilization and the oocyte-to-embryo transition. This chapter focuses on signaling mechanisms required for oocyte growth and meiotic maturation in *C. elegans* and discusses how these mechanisms coordinate the completion of meiosis and the oocyte-to-embryo transition.

Keywords

Oogenesis; meiosis; meiotic maturation signaling; Major Sperm Protein; G-protein signaling; meiotic resumption; meiotic chromosome segregation; meiotic spindle

10. 1 Overview

10.1.1 Meiosis and the meiotic maturation divisions

Cells of the germline form gametes and establish an unbroken chain between generations. The physical links in this chain, forged by the union of gametes at fertilization, are dependent on the faithful execution of meiosis. Meiosis ensures the formation of euploid embryos by halving the number of chromosomes contributed by each gamete (see Chapter 6 by M. Colaiacovo). Despite this universal requirement in the sexual reproduction of eukaryotic organisms, meiosis is regulated differently in oocytes and spermatocytes. Whereas spermatocytes proceed through the meiotic divisions uninterrupted (see Chapter 7 by D. Chu and D. Shakes), oocytes almost invariably arrest once, and sometimes twice following premeiotic DNA replication and meiotic recombination, depending on the species. This unique characteristic of oocyte meiosis, as well as its close temporal association with fertilization, was recognized early by developmental biologists, who coined the term “meiotic maturation” for the nuclear and cytoplasmic changes occurring in oocytes just before zygote formation (Wilson 1925; Masui and Clarke 1979). Oocyte meiotic maturation is defined by the transition between diakinesis and metaphase of meiosis I and is

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accompanied by nuclear envelope breakdown, rearrangement of the cortical cytoskeleton, and meiotic spindle assembly (Fig. 10.1). The timing of the meiotic divisions with respect to fertilization varies among species (Fig. 10.1). Despite these differences in timing, molecular underpinnings of oocyte meiotic maturation are conserved among different animals. The discovery of Maturation Promoting Factor (MPF; Masui and Markert 1971; Masui 2001) in studies of meiotic maturation in amphibian oocytes provides a prime example. Genetic and biochemical analysis of the cell cycle, together with MPF purification, demonstrated that cyclin-dependent protein kinases are universal regulators of meiotic and mitotic cell cycle progression in eukaryotes (Morgan 2007).

This review, like its antecedent chapters, focuses on the regulation of key developmental events in the germ line, here oocyte growth and meiotic maturation, in the nematode *Caenorhabditis elegans*. As an experimental system, *C. elegans* is a relative newcomer to this area of reproductive and developmental biology, yet the worm offers a number of advantages, including the ability to observe the events of oocyte growth and meiotic maturation in intact living animals and the potential to perturb normal development by mutational analysis and RNA interference. Intercellular signaling between gametes, and between the soma and germ line, regulates oocyte meiotic maturation and is a focal point of current research in this field.

10.1.2 Maturation-promoting factor

In *C. elegans*, as for all examined species, MPF is a master regulator of cell cycle progression during oocyte meiotic maturation (Boxem et al. 1999; Burrows et al. 2006). Because studies in vertebrate systems established the foundation for the meiotic maturation field, these studies are briefly reviewed to provide context for discussion of the *C. elegans* system. Classic studies of amphibian oocyte meiotic maturation by Yoshio Masui first led to the discovery of MPF (Masui and Markert 1971; Tunquist and Maller 2003). MPF consists of the Cdk1 catalytic subunit and the cyclin B regulatory subunit (Dunphy et al. 1988; Gautier et al. 1988; Lohka et al. 1988; Gautier et al. 1990). The cyclin B/Cdk1 protein kinase is inactive in immature oocytes due to inhibitory CDK phosphorylations at Thr 14 and Tyr 15 catalyzed by the Wee1 or Myt1 kinases (Fig. 10.2; Kornbluth et al. 1994; Mueller et al. 1995). In *Xenopus*, these inhibitory phosphorylations are removed by the conserved Cdc25 phosphatase following stimulation with progesterone (Kumagai and Dunphy 1991), which leads to nuclear envelope breakdown. An initial signal for MPF activation is amplified by a positive feedback loop in which the active CDK promotes the inactivation of its inhibitors, Wee1 and Myt1 (Walter et al. 2000; Peter et al. 2002), and stimulates its activator, Cdc25 (Kumagai and Dunphy 1996). The Greatwall kinase is a new component this pathway for MPF activation (Yu et al. 2006; Zhao et al. 2008). Itself a MPF substrate, Greatwall inhibits protein phosphatase 2A (PP2A) activity, which in turn is an inhibitor of Cdc25. Greatwall exerts its effects by phosphorylating the PP2A inhibitor - endosulfine (Castilho et al. 2009; Mochida et al. 2010), which is required for meiotic maturation in *Drosophila* (Von Stetina et al. 2008). Since Greatwall is not found in *C. elegans*, but other pathway components are conserved (Fig. 10.2) another kinase might contribute to MPF activation loop in this organism. In fact, the *C. elegans*, polo-like kinase PLK-1 is required for timely germinal vesicle breakdown (GVBD) and the completion of meiosis (Chase et al. 2000). Active MPF phosphorylates substrates that function in key cellular processes of meiotic maturation including nuclear envelope breakdown, chromosome condensation, and spindle assembly. Subsequently, M-phase exit and anaphase chromosome segregation require the function of a multi-subunit E3 ubiquitin ligase called the anaphase promoting complex or cyclosome (APC/C; Peters 2002), which promotes cyclin B degradation and MPF inactivation.

10.1.3 Translational regulation and meiotic maturation

The regulation of translation is a critical aspect of meiotic maturation (Mendez and Richter 2001). The oocytes of most animals possess translationally repressed or “masked” mRNAs that are translated upon meiotic resumption or following fertilization. Progesterone-induced oocyte meiotic maturation in frogs requires new protein synthesis but not new transcription (Smith and Ecker 1969). Translation of several key regulatory proteins promotes meiotic progression, including the MPF subunit cyclin B (Hocheegger et al. 2001; Haccard and Jessus 2006), the novel cyclin Ringo/Speedy (Ferby et al. 1999; Lenormand et al. 1999), and Mos, which functions as a mitogen-activated protein kinase kinase kinase (MAPKKK; Sagata et al. 1988; Dupre et al. 2002; Haccard and Jessus 2006). In *Xenopus*, mitogen-activated protein kinase (MAPK) activates Cdc25 to promote meiotic maturation (Wang et al. 2007) and there is evidence for positive feedback, ensuring an all-or-none response (Liang et al. 2007). Members of the Aurora A family of serine/threonine protein kinases also play critical roles during oocyte maturation. The Eg2 aurora family kinase is phosphorylated and activated soon after progesterone stimulation in *Xenopus* oocytes (Andresson and Ruderman 1998). The Eg2 kinase phosphorylates the cytoplasmic polyadenylation element binding protein (CPEB) on Ser174 to promote polyadenylation and translation of *mos* mRNA (Mendez et al. 2000). Biochemical studies in the *Xenopus* system suggest that functionally redundant translational regulatory pathways control MPF activation in response to the meiotic maturation hormone (Haccard and Jessus 2006). The ability to conduct genetic analysis in the *C. elegans* system might prove helpful in teasing apart functional redundancies meiotic maturation regulatory pathways.

While the regulation of meiotic maturation in the *Xenopus* system is still being actively investigated, the current view is that cytoplasmic polyadenylation of mRNAs promotes translation by promoting a conformation in which the initiation factors eIF4E and eIF4G are productively engaged (Richter 2007). In mice, inactivation of CPEB using oocyte-specific RNAi reduced fertility and led to a variety of defects including premature meiotic maturation, parthenogenesis, and defective folliculogenesis (Racki and Richter 2006). It is clear that other translational control factors, besides CPEB, are critical for oocyte meiosis. For example, *Xenopus* Pumilio-2 is required for Ringo/Speedy translation in response to progesterone, which in turn results in the activation of CPEB and *mos* mRNA translation (Padmanabhan and Richter 2006).

In addition to the control of translation by the regulation of cytoplasmic polyadenylation, studies of mouse oocytes suggest the involvement of small RNA pathways in the completion of meiosis. An analysis of fertility defects of Dicer-deficient and Argonaute 2-deficient oocytes was reported in the mouse (Murchison et al. 2007; Tang et al. 2007; Kaneda et al. 2009). These studies suggest that translational regulation by microRNAs in the germline is important for normal oocyte meiotic maturation and zygotic development. While there has been considerable progress in elucidating the biochemistry of kinase cascades and translational control, it is less clear how hormonal signaling and soma-germline interactions tie into these intracellular processes to regulate meiotic maturation. The following section reviews studies that establish *C. elegans* as a genetic model system for studying the regulation of oocyte meiotic maturation by hormonal signaling.

10.2 Meiotic Maturation in *C. elegans*

10.2.1 Timing of events

Oocyte meiotic maturation can be viewed by time-lapse videomicroscopy of living animals (Ward and Carrel 1979). The timing of landmark events during meiotic maturation (McCarter et al. 1999) and the ultrastructure of the proximal gonad (Hall et al. 1999; Hall and Altun 2008) have been described. The nuclear envelope of the most proximal oocyte

breaks down ~5 min prior to ovulation as it enters meiotic M-phase from prophase (Fig. 10.3; McCarter et al. 1999). During maturation, the oocyte also undergoes a structural change termed cortical rearrangement (McCarter et al. 1999). These changes within the oocyte coincide with a reproducible sequence of somatic motor events mediated by the contractile proximal sheath cells and the distal spermatheca resulting in ovulation. As the nuclear envelope breaks down, microtubules gain access to the highly condensed bivalents and the acentriolar meiotic spindle begins to assemble (Yang et al. 2003). Fertilization appears to occur rapidly upon oocyte entry into the spermatheca (Ward and Carrel 1979; Samuel et al. 2001). The fertilized embryo enters the uterus approximately four minutes after ovulation and the meiotic divisions are completed there within approximately 30 minutes of nuclear envelope breakdown, with the MI division taking approximately 20 minutes and the MII division taking approximately 10 minutes (McCarter et al. 1999).

10.2.2 *C. elegans* sperm use MSP to promote meiotic maturation

In a landmark study, McCarter et al. (1999) showed that a sperm-associated signal promotes oocyte meiotic maturation and contraction of the follicle-like gonadal sheath cells, prior to and independent of fertilization. In females (genetically altered XX animals that produce no sperm), oocytes mature and are ovulated at low rates (<0.1 maturations per gonad arm per hr). Mating of spermless females to wild-type males, or fertilization incompetent sperm-defective (*spe*) mutant males, restores a normal rate of oocyte maturation (~2.5 maturations per gonad arm per hr; McCarter et al. 1999). Oocytes also appear to exhibit an increase in metabolic activity in the presence of sperm (Yang et al. 2010). A gonad arm in a female produces ~15 diakinesis arrested oocytes. Thus, sperm signaling is not required for germline development up the diakinesis stage. However, in the absence of sperm, the assembly-line production of oocytes halts. The continued presence of sperm is required for continued oogenesis. As explained below, in addition to promoting meiotic maturation, sperm stimulate the cytoplasmic flows that drive oocyte growth.

In nematodes, the ancestral mating system appears to be male–female, whereas hermaphroditism and parthenogenesis are derived alternative reproductive strategies (Cho et al. 2004; Kiontke et al. 2004; Kiontke and Fitch 2005; Woodruff et al. 2010). The regulation of meiotic maturation by sperm in many nematodes might function to preserve germline resources, for if oocytes undergo meiotic maturation in the absence of sperm, they rapidly lose competence for fertilization, become endomitotic, and are expelled from the animal through the vulva. The stimulation of meiotic resumption by sperm provides an efficient means for uniting developmentally fertilization-competent sperm and oocyte.

The sperm signal for oocyte meiotic maturation was identified biochemically (Miller et al. 2001). The surprising result was that the major sperm proteins (MSPs), central cytoskeletal elements required for the actin-independent motility of nematode spermatozoa (Bottino et al. 2002), have a dual role in *C. elegans* reproduction, functioning as hormones for oocyte meiotic maturation and gonadal sheath cell contraction (Miller et al. 2001). An *in vivo* bioassay was used in which sperm-conditioned medium or sperm lysates were injected into the uterus of unmated *fog-2* female animals, and oocyte meiotic maturation and gonadal sheath cell contraction were monitored by time-lapse videomicroscopy. Using meiotic maturation as a functional readout, the bioactive factors were purified to homogeneity with reversed-phase high-performance liquid chromatography and shown to contain only the MSPs by mass spectrometry. Injection of anti-MSP antibodies into the uterus of hermaphrodites results in a reduction in ovulation rates (Miller et al. 2001), consistent with the hypothesis that MSPs function as endogenous signals. A multigene family of twenty-eight genes encodes MSP proteins that share approximately 97–100% identity (Burke and Ward 1983; Klass et al. 1984; Ward et al. 1988). Several MSP isoforms, including MSP-38, MSP-77, and MSP-142, have been expressed in bacteria, either with or without N-terminal

6His-Tags, and shown to induce meiotic maturation and sheath cell contraction when injected into the uterus at a range of concentrations (25–100 nM) (Miller et al. 2001; Govindan et al. 2009). The actual concentration range for signaling is likely considerably lower because the initial volume of injected MSP solution (~50 pl) subsequently diffuses within the uterus, spermatheca, and gonad arm.

The identification of a signaling role for MSP raised the question of how spermatozoa, devoid of ribosomes, endoplasmic reticulum (ER), and Golgi, release a cytoskeletal protein lacking a signal sequence. Non-motile spermatids and motile spermatozoa appear to bud MSP-containing vesicles to signal sheath cells and oocytes (Kosinski et al. 2005). MSP vesicles appear to be labile structures, which provides a potential basis for release of MSP in an extracellular form that exhibits a graded distribution in the gonad.

MSP is a bipartite signal for meiotic maturation and sheath cell contraction because a synthetic peptide consisting of evolutionarily conserved C-terminal 21 amino acids stimulates sheath cell contraction at normal levels, but not meiotic maturation (Miller et al. 2001; Yin et al. 2004). By contrast, an MSP deletion derivative lacking this C-terminal region can promote meiotic maturation, but is less efficient in its ability to promote sheath cell contraction (Miller et al. 2001). This result suggests that MSP possesses two separable signaling functions and therefore likely activates distinct signal transduction pathways for meiotic maturation and sheath contraction (Miller et al. 2001). Consistent with this prediction and as described below, meiotic maturation requires G_s -adenylate cyclase-protein kinase A (PKA) signaling in the gonadal sheath cells, whereas sheath cell contraction requires EGL-30/ G_q signaling (Govindan et al. 2009). Divergent MSP-related proteins, called VAPs (for VAMP-associated protein) share only ~20% amino acid identity with nematode MSP yet fold into similar seven-stranded immunoglobulin sandwich-like structures (Kaiser et al. 2005). Human, *Drosophila* and *C. elegans* VAPs are able to signal meiotic maturation and sheath contraction using the *C. elegans* bioassay (Tsuda et al. 2008). A phylogenetic analysis of MSP domain-containing proteins indicates that the VAP clade is widely distributed among plants, animals, and protists, but that three additional clades are largely nematode specific (Tarr and Scott 2005). Recent results in *Drosophila*, *C. elegans*, and mammals suggest that some VAP family members can function as extracellular signals (Tsuda et al. 2008).

10.2.3 Molecular readouts of MSP signaling

MSP promotes meiotic maturation and sheath cell contraction and activates signaling pathways that are important for both processes. These readouts include activation of MAPK in the germline, reorganization of oocyte microtubules, localization of the AIR-2 Aurora B kinase to chromatin, reorganization of ribonucleoprotein complexes in oocytes, and stimulation of actomyosin-based motility. Each of these readouts of MSP signaling is now discussed.

10.2.3.1 Activation of MPK-1 MAPK in the germline—In *C. elegans*, the MPK-1 MAPK pathway functions in multiple developmental processes in the germline, including the specification of the male germline fate in males and hermaphrodites, progression through pachytene, the negative regulation of physiological apoptosis in the germline, and the control of oocyte growth and meiotic maturation (Lee et al. 2007). MPK-1 appears to have multiple targets that function in diverse cellular processes in the germline, including morphogenesis and cellular organization of the gonad, oocyte growth control, and oocyte organization and differentiation (Lee et al. 2007; Arur et al. 2009). Proximal oocytes exhibit MAPK activation in the presence of sperm, as detected using antibodies that specifically recognize the diphosphorylated-activated form of MPK-1 MAPK (dpMPK-1; Miller et al.

2001; Page et al. 2001; Lee et al. 2007). Injection of MSP into the uterus of unmated females is sufficient to generate dpMPK-1 in oocytes within 40 minutes of injection (Miller et al. 2001; Jud et al. 2008). MAPK activation plays critical roles in regulating cytoplasmic and nuclear events of meiotic maturation in invertebrates and vertebrates (Ferrell 1999a, b; Haccard and Jessus 2006; Liang et al. 2007), presumably through phosphorylation of specific targets. While the exact functions of MAPK in meiotic maturation are not fully defined at a mechanistic level, the identification of MAPK substrates will provide multiple handles on this problem (Arur et al. 2009, 2011).

10.2.3.2 Microtubule reorganization prior to fertilization—The meiotic spindles of most animal oocytes are distinctive from mitotic spindles of somatic cells in terms of their mechanism of assembly, their function, and their modes of regulation. The female meiotic spindles of many species are both acentriolar and anastral (Albertson and Thomson 1993). Instead of relying on centrosomes for assembly of a bipolar spindle, the meiotic chromatin functions to nucleate microtubules, which self-organize through incompletely understood mechanisms that involve sorting by microtubule motors and microtubule dynamics (Heald et al. 1996; Matthies et al. 1996; Walczak et al. 1998; Skold et al. 2005). Assembly of a bipolar meiotic spindle through chromatin-dependent mechanisms requires that the cytoplasmic microtubules gain access to the nuclear environment, which occurs upon germinal vesicle breakdown (GVBD) during the meiotic maturation process. MSP causes GVBD, which as suggested by studies in other systems (Kalab et al. 2011), might expose spindle assembly proteins to a RanGTP gradient, thereby driving spindle assembly.

Interestingly, MSP might promote meiotic spindle assembly through effects on microtubule dynamics that occur prior to GVBD. MSP is sufficient to trigger the reorganization of cytoplasmic microtubules in the oocyte prior to GVBD and fertilization (Harris et al. 2006). When MSP is absent, as in females or older hermaphrodites, microtubules are enriched at the proximal and distal cortices of oocytes. In mated females or younger hermaphrodites, microtubules are dispersed evenly in a net-like fashion throughout the cytoplasm of proximal oocytes. A quantitative assay for oocyte microtubule reorganization was used to show that purified MSP is sufficient to direct cytoskeletal remodeling in the oocyte. The presence of MSP affects the localization and density of growing plus ends, as well as their directionality of movement.

10.2.3.3 Localization of the AIR-2 Aurora B kinase to oocyte chromatin—The establishment, maintenance, and stepwise loss of sister chromatid cohesion are essential for faithful meiotic chromosome segregation. The AIR-2 Aurora B kinase provides a potential link between the sister chromatid cohesion maintenance apparatus and MSP signaling. Chromatin localization of the AIR-2 to the meiotic chromosomes of proximal oocytes depends on the presence of sperm (Schumacher et al. 1998) and MSP is sufficient to promote AIR-2 chromatin localization (Govindan et al. 2009). During anaphase I, sister chromatid cohesion at the short arm of the bivalent, which mediates interhomolog association, is selectively removed to allow separation of homologs. By contrast, sister chromatid cohesion at the long arm of the bivalent must be maintained until anaphase II. *C. elegans* has holocentric chromosomes and microtubules appear to attach to both lateral and poleward ends of meiotic chromosomes (Albertson and Thomson 1993; Howe et al. 2001; Wignall and Villeneuve 2009). The structure and function of holocentric *C. elegans* chromosomes depends on both conserved and novel factors (Maddox et al. 2004; Zetka 2009). A novel protein, LAB-1 is required to protect sister chromatid cohesion during meiosis I (de Carvalho et al. 2008). LAB-1 binds to the long arm of the bivalent and excludes the AIR-2 Aurora B kinase. AIR-2 localizes to the interface between homologs where it phosphorylates the REC-8 meiosis-specific cohesin kleisin subunit promoting its cleavage by separase at anaphase I (Kaitna et al. 2000; Rogers et al. 2002). *C. elegans* has

three meiosis-specific kleisin paralogs, *rec-8*, *coh-3*, and *coh-4*, which are required for sister chromatid cohesion during meiosis yet carry out specific functions (Severson et al. 2009). Among these, REC-8 is uniquely required for maintaining sister chromatid cohesion after meiosis I (Severson et al. 2009). Thus, when sperm are present for fertilization, oocytes prepare in advance to initiate chromosome segregation. MSP signaling might be integrated with oocyte intrinsic mechanisms to maintain a high fidelity of chromosome segregation.

10.2.3.4 Reorganization of ribonucleoprotein particles—Cortically-localized aggregates of ribonucleoprotein particles (RNPs) form in the oocytes of unmated females or in older adult hermaphrodites that have depleted their self sperm (Schisa et al. 2001; Jud et al. 2007, 2008; Noble et al. 2008; Patterson et al. 2011). The large RNPs that accumulate in arrested oocytes might function to translationally repress and preserve mRNAs that are needed for meiotic maturation and early embryo development. MSP was shown to be sufficient to promote the dissolution of large RNP foci in oocytes (Jud et al. 2008).

10.2.4 The gonadal sheath cells regulate oocyte meiotic maturation

The gonadal sheath cells function as the major determinant of all described germline responses to the MSP hormone (Miller et al. 2003; Govindan et al. 2006, 2009; Harris et al. 2006; Jud et al. 2008; Nadarajan et al. 2009). A role for the gonadal sheath cells in regulating meiotic maturation was suggested from an analysis of the POU-homeobox gene *ceh-18*, which is expressed in the gonadal sheath cells and required for their proper differentiation and function (Greenstein et al. 1994; McCarter et al. 1997; Rose et al. 1997). Oocytes in *ceh-18* mutant females exhibit a defect in meiotic arrest and undergo MAPK activation, meiotic maturation and ovulation despite the absence of sperm (Greenstein et al. 1994; Miller et al. 2003; Govindan et al. 2006; Suzuki and Han 2006).

10.2.4.1 Antagonistic G_{α_s} and $G_{\alpha_{o/i}}$ pathways regulate meiotic maturation—

Besides their essential mechanical role in the process of ovulation (see below), the gonadal sheath cells regulate meiotic maturation through both inhibitory and stimulatory pathways. In the absence of sperm, the sheath cells are critical for inhibiting meiotic maturation, thereby preventing maturation, ovulation, and ultimately wastage of metabolically costly oocytes. An RNAi screen in a *fog-2* female background was used to define negative regulatory pathways that inhibit meiotic maturation in the absence of sperm (Govindan et al. 2006). This screen identified *goa-1*, which encodes $G_{\alpha_{o/i}}$, as a strong negative regulator of meiotic maturation. *goa-1* is expressed in the germline and somatic gonad, but the use of somatic RNAi-deficient strains indicates that *goa-1* functions in the somatic gonad to inhibit meiotic maturation in the absence of sperm (Govindan et al. 2006).

The gonadal sheath cells also have an essential function in promoting meiotic maturation when sperm are present. This activation function is mediated by components of the G_{α_s} -adenylate cyclase-protein kinase A pathway (Govindan et al. 2006, 2009). RNAi to the stimulatory G_{α_s} protein, encoded by *gsa-1*, prevents oocytes from undergoing meiotic maturation despite the presence of sperm (Govindan et al. 2006). *gsa-1* is an essential gene; *gsa-1* null mutants die as L1 stage larvae (Korswagen et al. 1997). Genetic mosaic analysis using a *gsa-1* null mutant demonstrated that *gsa-1* is required in somatic cells of the gonad for meiotic maturation (Govindan et al. 2009). Importantly, *gsa-1* was shown to be dispensable in germ cells for meiotic maturation and there was no evidence for maternal contribution of *gsa-1* to early development. *acy-4*, which encodes one of four *C. elegans* adenylate cyclases, is required for meiotic maturation (Fig. 10.4). Genetic mosaic analysis established that *acy-4* functions in the gonadal sheath cells to promote meiotic maturation (Govindan et al. 2009). Likewise, genetic mosaic analysis revealed that *kin-1*, which encodes the catalytic subunit of cAMP-dependent PKA, is required in the gonadal sheath

cells for meiotic maturation (S. Kim, J. A. Govindan, and D. Greenstein, unpublished results). As expected, *acy-4* acts by regulating PKA activity because a mutation in *kin-2*, which encodes the cAMP-binding regulatory subunit of PKA, suppresses the sterility of *acy-4* null mutants (Govindan et al. 2009).

In female animals, PKA activity must be kept off to prevent meiotic maturation. Constitutively-activated *gsa-1* alleles, *acy-4* over-expression, or inactivation of *kin-2* all derepress meiotic maturation in female backgrounds (Govindan et al. 2006, 2009). In the regulation of meiotic maturation, GOA-1/G_{o/i} appears to function as an inhibitory G protein because mutations in *gsa-1* and *acy-4* are epistatic to *goa-1* (Govindan et al. 2009). That *goa-1* can regulate meiotic maturation in the hermaphrodite background is established from experiments in which expression of a constitutively-activated G_{o/i} protein under control of its endogenous promoter in a multi-copy array significantly reduced meiotic maturation rates (Govindan et al. 2006). Thus, the antagonistic interaction between G_{o/i} and G_s might serve to couple meiotic maturation rates to sperm availability. *gsa-1* and *acy-4* are not required for sheath cell contractions, however EGL-30/G_q was shown to be necessary and sufficient for sheath contractions (Govindan et al. 2009). Thus, multiple sheath cell G protein pathways appear to be required for germline meiotic maturation responses to sperm (Fig. 10.4).

Two models were considered to explain the requirement for G_s-adenylate cyclase-PKA signaling in the gonadal sheath cells for MSP responses in the germline (Govindan et al. 2009). G_s-adenylate cyclase-PKA signaling might affect the competence of oocytes to respond to MSP. In this scenario, the G_s pathway would not directly sense the MSP gradient, but would enable oocytes or sheath cells to respond via other receptor pathways. This model is difficult to reconcile with multiple lines of experimental evidence. Most importantly, activation of G_s-adenylate cyclase-PKA signaling in the sheath cells by multiple means is sufficient to drive meiotic maturation in the absence of MSP (Govindan et al. 2009). The possibility that G_s-adenylate cyclase signaling has an earlier developmental role in the gonadal sheath cell lineages was excluded by the finding that phosphodiesterase inhibitors suppress the sterility of *acy-4* mutant adults. The possibility that MSP is unavailable to bind MSP receptors or that the receptors themselves are not expressed was excluded by MSP binding and localization studies. Thus, the model currently favored is one in which unidentified MSP receptors on gonadal sheath cells are G protein-coupled receptors (GPCRs). In this scenario, G_s-coupled receptors would trigger meiotic maturation, G_{o/i}-coupled receptors would inhibit meiotic maturation in the absence of MSP, and G_q-coupled receptors would promote sheath cell contraction (Fig. 10.4). The identification of the sheath cell MSP receptors will represent a critical test of this model.

10.2.4.2 Gap-junctional communication and the control of meiotic maturation

—The conclusion that the gonadal sheath cells function as the major initial MSP sensors raises the question of how this information is communicated to the germline. Transmission electron microscopy and freeze-fracture analysis revealed that sheath cells form gap junctions with oocytes (Hall et al. 1999). Gap junctions were observed at regions of extensive contact between sheath cell somata and oocytes. In addition, gap junctions were seen at sites where finger-like sheath cell processes extended between oocytes. Proximal sheath cells are also connected to one another via gap junctions (Hall et al. 1999).

inx-14 and *inx-22*, which encode innexin/pannexin gap junction proteins, negatively regulate meiotic maturation, oocyte MAPK activation, oocyte microtubule reorganization, and the localization of AIR-2 to chromatin in the absence of MSP (Govindan et al. 2006, 2009; Harris et al. 2006; Whitten and Miller 2007). *inx-14* also regulates the assembly of RNP granules in arrested oocytes (J. Schisa, unpublished results). INX-14 and INX-22 are

expressed in the germline and co-localize at plaque-like structures at the interface between oocytes and sheath cells (Govindan et al. 2009), consistent with the possibility that they are components of sheath-oocyte gap junctions. Recently, sheath cell components of sheath-oocyte gap junctions have been identified as *inx-8* and *inx-9* (T. Starich and D. Greenstein, unpublished results). *inx-8* and *inx-9* promoter fusion constructs showed expression in the gonadal sheath cells (Starich et al. 2001; Altun et al. 2009), and this observation has been confirmed using specific antibodies and rescuing GFP protein fusions (T. Starich and D. Greenstein, unpublished results). INX-8 and INX-9 expression is also observed in the somatic gonad progenitors Z1 and Z4, the DTCs, and cells of the somatic gonadal primordium. *inx-8* and *inx-9* are found in the same operon and share approximately 87% identity at the protein sequence level. Deletion of either *inx-8* or *inx-9* produces no apparent mutant phenotype, however inactivation of both genes causes sterility in both hermaphrodites and males. In the double mutant, few or no Z2/Z3 germ cell descendents are observed, which might reflect a failure of germ cells to proliferate or survive (T. Starich and D. Greenstein, unpublished results). A similar sterile phenotype is also observed in *inx-14* deletion alleles in both sexes (Govindan et al. 2009).

The observation that a germline innexin, *inx-14*, and the somatic gonadal innexins, *inx-8*/*inx-9*, share a common sterile phenotype suggests that soma-germline gap junctions may play a more global role in the gonad beyond mediating communication between oocytes and gonadal sheath cells. Thus, there appears to be an early role for soma-germline gap junctions that is needed for germ cell survival and a later role that functions in the regulation of meiotic maturation. Apparently, *inx-22* is not required for the early germ cell proliferation or survival role as a deletion allele is fertile (Whitten and Miller 2007). However, *inx-22* is found in an operon with *inx-21*, which is also expressed in the germline as detected using specific antibodies, and *inx-21(RNAi)* in an *inx-22* mutant, but not a wild-type background, results in a sterile phenotype (T. Starich and D. Greenstein, unpublished results). It will be important to address whether specific small molecules move through these gap junctions to control meiotic maturation and germ cell survival.

Genetic epistasis analysis was used to examine the relationship between *inx-22*, which is a negative regulator of meiotic maturation and *gsa-1* and *acy-4*, which are positive regulators. Genetic mosaic analysis was used to reduce *gsa-1(+)* function in the somatic gonad in an *inx-22* background (Govindan et al. 2009). The experimental observation was that an *inx-22* mutation suppresses the sterility defect caused by loss of *gsa-1(+)* function in the somatic gonad, suggesting that gap-junction proteins function downstream of G_s signaling. Neither *inx-22* nor *inx-14* and *inx-22* depletion suppresses *acy-4* null sterility. Possibly, unidentified targets of G_s-ACY-4 signaling might therefore regulate meiotic maturation in parallel with gap junction proteins. Alternatively, it might not be possible to eliminate gap-junctional communication between oocytes and sheath cells with available genetic tools on account of the germline survival function of the gonadal innexins.

Remarkably, the meiotic maturation process in *C. elegans* and mammals share a number of molecular and biological similarities (see Govindan et al. 2009 for an in-depth discussion and complete references). For example, MSP and LH, though unrelated in sequence, both trigger meiotic resumption using somatic G_s-adenylate cyclase-PKA pathways and soma-to-germline gap-junctional communication. Mural granulosa cells on the periphery of the follicle express the LH receptor, which is a GPCR. Cumulus granulosa cells form gap junctions with the oocyte using specialized extensions, called transzonal projections, which penetrate the zona pellucida and reach the oocyte cell surface. In both systems, interfering with the function of soma-to-germline gap junctions permits meiotic maturation in the absence of the maturation hormone. At a molecular level, the oocyte responses apparently involve the control of conserved protein kinase pathways and post-transcriptional gene

regulation in the oocyte. At a cellular level, the responses include nuclear envelope breakdown, cortical cytoskeletal rearrangement, assembly of the acentriolar meiotic spindle, chromosome segregation, and likely changes important for fertilization and the oocyte-to-embryo transition. A major difference between the systems is that G_s -adenylate cyclase-PKA signaling also has a function within the oocyte to maintain meiotic arrest in vertebrates and mammals. In *C. elegans*, G_s -ACY-4 signaling functions exclusively in the gonadal sheath cells to promote meiotic maturation, as established by genetic mosaic analysis.

10.2.4.3 Regulation of meiotic maturation by VAB-1 MSP/Eph receptor

signaling—Whereas the sheath cell MSP receptors, proposed to be GPCRs (Govindan et al. 2009, see below), have as of yet eluded detection, prior work identified the VAB-1 Eph receptor as an oocyte MSP receptor (Miller et al. 2003; Corrigan et al. 2005; Govindan et al. 2006; Cheng et al. 2008). Adult hermaphrodite *vab-1* null mutant animals are fertile (George et al. 1998), exhibit normal rates of meiotic maturation, and respond to MSP (Miller et al. 2003). However, unmated *vab-1* null mutant females modestly derepress meiotic maturation (Miller et al. 2003; Corrigan et al. 2005; Govindan et al. 2006; Cheng et al. 2008). The regulated endocytic trafficking of the VAB-1 MSP/Eph receptor appears to be a factor in the regulation of oocyte meiotic maturation. In the absence of the MSP ligand the VAB-1 Eph receptor inhibits meiotic maturation while either in or in transit to the endocytic recycling compartment (ERC; Cheng et al. 2008). The localization of VAB-1::GFP in oocytes to the RAB-11-positive ERC was shown to be antagonized by MSP signaling. Interestingly, G_s -adenylate cyclase-PKA signaling in the gonadal sheath cells was shown to be required for the trafficking of VAB-1::GFP to the oocyte plasma membrane from the ERC when MSP is present. Thus, the VAB-1 MSP/Eph receptor appears to play a non-essential modulatory role, in contrast to the G_s -adenylate cyclase-PKA pathway, which is required for meiotic maturation. The VAB-1 receptor pathways might contribute to the robustness of the response to sperm.

10.2.5 Control of meiotic maturation and the regulation of translation

The TIS11-type CCCH zinc finger domain-containing proteins OMA-1 and OMA-2, hereafter referred to as OMA proteins, are redundantly required for oocyte meiotic maturation and ovulation (Detwiler et al. 2001; Shimada et al. 2002). In *oma-1; oma-2* double mutants, MAPK activation is not sustained, nuclear envelope breakdown does not occur properly, and AIR-2 fails to localize to oocyte chromatin. Although the mechanism by which the OMA proteins promote meiotic maturation remains to be determined, they function upstream of the conserved cell cycle regulators WEE-1.3 and CDK-1 (Detwiler et al. 2001). *wee-1.3(RNAi)* in *oma-1; oma-2* double mutants can drive oocytes into M-phase, however fertilization does not occur (Detwiler et al. 2001; Burrows et al. 2006). OMA proteins have been shown to repress the translation of *nos-2* and *zif-1* in oocytes (Jadhav et al. 2008; Guven-Ozkan et al. 2010). OMA proteins bind to the 3'-UTRs of *nos-2* and *zif-1* (Jadhav et al. 2008; Guven-Ozkan et al. 2010). Repression of *zif-1* in oocytes also requires the eIF4E-binding protein SPN-2 (Guven-Ozkan et al. 2010). Neither *nos-2* nor *zif-1* is required for meiotic maturation, yet their regulation might indicate a general function for OMA proteins in regulating translation in oocytes.

OMA proteins are multifunctional—they interact with TAF4, a subunit of TFIID, to repress RNA polymerase II-mediated transcription in the zygote and the germline blastomere P1 (Guven-Ozkan et al. 2008). This transcriptional repression function of OMA proteins is likely not relevant for the regulation of meiotic maturation because this activity only manifests upon phosphorylation by the dual-specificity tyrosine-phosphorylation-regulated protein kinase MBK-2. MBK-2 only becomes active in oocytes upon meiotic maturation (Stitzel et al. 2006; Cheng et al. 2009). In fact, phosphorylation of OMA-1 by MBK-2 was

shown to displace SPN-2 from the *zif-1* 3-UTR, thereby alleviating translational repression (Güven-Ozkan et al. 2010). In *C. elegans*, as in many species, fully grown oocytes appear to be transcriptionally inactive (Starck 1977; Gibert et al. 1984; Schisa et al. 2001; Walker et al. 2007). Thus, translational control by OMA proteins might play an important role in meiotic maturation. Because the *C. elegans* germline develops as a syncytium, it is difficult to assess directly whether meiotic maturation requires translation in oocytes. That translational regulation is critical for oogenesis is clear. For example, the translational regulators GLD-1 and GLD-2, which function downstream in the GLP-1 signaling pathway in the stem cell vs. meiotic development decision (see Chapter 4 by D. Hansen and T. Schedl) are each individually required for the formation of normal oocytes (Francis et al. 1995; Kadyk and Kimble 1998). Further, IFE-1, one of three eIF4E isoforms, promotes the accumulation of *oma-1* mRNA on polysomes in developing oocytes (Henderson et al. 2009). Further analysis of the OMA proteins will likely continue to provide important insights into the role of translational control during meiotic maturation.

10.2.6 Regulation of ovulation

C. elegans ovulation is an attractive physiological model for how intercellular signaling influences the behavior of smooth muscle. In worms, myoepithelial cells form a smooth-muscle structure known as the gonadal sheath, and their coordinated function is needed for ovulation. The ability to observe the function of the gonadal sheath cells and their dynamic interaction with cells of an epithelial tube, the spermatheca, provides an ideal format for analyzing the interplay between cell structure and intercellular communication. Nonetheless, the study of ovulation is complicated due to the involvement of many genes and multiple tissues (Iwasaki et al. 1996; Kostic et al. 2003; Aono et al. 2004; Gissendanner et al. 2008; Pilipiuk et al. 2009).

During ovulation, the proximal gonadal sheath cells contract rapidly, the distal constriction of the spermatheca dilates, and sheath cells pull the distal spermatheca over the mature oocyte. The maturing oocyte signals its own ovulation in two ways: it modulates sheath contractions, which includes an increase in contraction rate and intensity during ovulation, and it induces spermathecal dilation during ovulation (Iwasaki et al. 1996; McCarter et al. 1999). Mutations that lead to defective ovulation cause an endomitotic oocyte (Emo) phenotype (Iwasaki et al. 1996). When oocytes are retained in the gonad arm due to defective ovulation, they undergo multiple rounds of nuclear envelope breakdown (M-phase entry) and S-phase, and become highly polyploid.

10.2.6.1 EGF receptor and IP₃ signaling for ovulation—The signal from the maturing oocyte that controls dilation of the distal spermatheca is thought to be LIN-3/EGF, which triggers LET-23/EGF receptor signaling in the distal spermatheca and possibly the sheath cells (Clandinin et al. 1998; McCarter 1998). LIN-3/LET-23 signaling in the gonadal sheath cells also promotes ovulatory contractions (Yin et al. 2004). The *let-23* pathway required for spermathecal dilation during ovulation is *let-60/ras*-independent and involves a downstream IP₃-mediated pathway (Clandinin et al. 1998). Mutations in two genes, *lfe-1/itr-1* and *lfe-2*, respectively gain-of-function and loss-of-function, were isolated in a genetic screen for suppressors of *let-23* sterility. *lfe-1/itr-1* and *lfe-2* encode an inositol (1, 4, 5) triphosphate receptor and an inositol (1, 4, 5) triphosphate-3-kinase, respectively (Clandinin et al. 1998). These results suggest that spermathecal dilation is likely to be dependent on calcium release regulated by IP₃. Consistent with this possibility, spermathecal dilation requires the function of a Ca²⁺ release-activated Ca²⁺ channel expressed in sheath and spermathecal cells (Yan et al. 2006; Lorin-Nebel et al. 2007). Further, a mutant allele of *ipp-5*, which encodes an inositol 5-phosphatase, predicted to lower IP₃ levels, exhibits an

unusual ovulation phenotype in which the spermatheca overextends, thereby ovulating two oocytes per cycle (Bui and Sternberg 2002).

IP₃ signaling also plays an important role in sheath cells. A reduction-of-function mutation in *itr-1* disrupts both basal sheath cell contractions in response to a synthetic MSP C-terminal peptide and ovulatory contractions (Yin et al. 2004). Phospholipase C (PLC)-mediated hydrolysis of the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP₂) generates IP₃. Both *plc-1* and *plc-3* are required for ovulation (Kariya et al. 2004; Yin et al. 2004; Vazquez-Manrique et al. 2008), as is phosphatidylinositol-4-phosphate 5 kinase, encoded by *ppk-1*, which is needed for the synthesis of PIP₂ (Xu et al. 2007). GFP reporter constructs indicate that PLC-3 and PPK-1 are expressed in sheath and spermathecal cells (Yin et al. 2004; Xu et al. 2007), whereas PLC-1 is expressed only in the spermatheca (Kariya et al. 2004; Yin et al. 2004). PLC-3 promotes both the basal and ovulatory sheath cell contractions (Yin et al. 2004). By contrast, PLC-1 is not required for basal or ovulatory sheath cell contractions, consistent with a role in the spermatheca. Indeed, mutations in *plc-1* or *plc-1(RNAi)* cause spermathecal entry and exit defects (Kariya et al. 2004; Yin et al. 2004). PLC-1 expression in the spermatheca requires the FOS-1/JUN-1 heterodimeric transcriptional activator (Hiatt et al. 2009). RNAi of *fos-1* or *jun-1* disrupts ovulation and this defect is rescued by expression of PLC-1 in the spermatheca (Hiatt et al. 2009).

The precise connection between LET-23 activation, likely in sheath and spermathecal cells, and IP₃ generation remains to be determined. Signaling effectors likely functioning upstream of PLC-3 and PLC-1 activation include the Rho/Rac-family guanine nucleotide exchange factor VAV-1 (Norman et al. 2005), RHO-1 GTPase (McMullan and Nurrish 2011), and the ARK-1 tyrosine kinase (Hopper et al. 2000). How the sheath and spermathecal cells coordinate their behaviors during ovulation is unclear. Not only must mature oocytes enter the spermatheca at ovulation, but fertilized embryos must exit the spermatheca in a timely fashion. Specific disruptions in IP₃ signaling and the actin cytoskeleton reduce fertility by interfering with exit of the fertilized embryo from the spermatheca, thereby disrupting the reproductive assembly line (Kariya et al. 2004; Kovacevic and Cram 2010).

10.2.6.2 Yolk lipoprotein metabolism and ovulation—Several intersecting lines of evidence provide an indication that lipid signaling might play a role in promoting ovulation. Growing oocytes take up yolk lipoprotein particles produced by the intestine (Kimble and Sharrock 1983) by a process of receptor-mediated endocytosis (Grant and Hirsh 1999). Intestinal cells secrete yolk lipoprotein particles into the pseudocoelom which then pass through the gonadal basal lamina and pores in the gonadal sheath cells to gain access to the oocyte surface (Hall et al. 1999). *rme-2* encodes a member of the LDL receptor superfamily and is the oocyte yolk receptor (Grant and Hirsh 1999). *rme-2* is required for yolk uptake and transport of cholesterol into oocytes (Grant and Hirsh 1999; Matyash et al. 2001). Interestingly, *rme-2* mutants display ovulation defects (Grant and Hirsh 1999), though the basis for these defects has been mysterious. The heterodimeric E2F EFL-1/DPL-1 transcription factor is required for the expression of *rme-2* in the germline (Chi and Reinke 2006) and expression of *rme-2* using the germline-specific *pie-1* promoter partially rescues the ovulation defects of strong loss-of-function *dpl-1* mutants (Chi and Reinke 2009). Oocytes utilize yolk lipoprotein particles to generate polyunsaturated fatty acid derivatives, including F-series prostaglandins, to generate signals that promote sperm guidance to the spermatheca (Kubagawa et al. 2006; Edmonds et al. 2010). Possibly, lipid signals dependent on *rme-2* might also function to promote ovulation. Such a possibility might explain the finding that the EGRH-1 transcription factor is required in the intestine for normal ovulation (Clary and Okkema 2010). *egrh-1* mutants might affect the quantity or quality of yolk lipoprotein particles received by oocytes, thereby affecting ovulation. Alternatively, the

disruption of a major pathway for endocytosis and lipid transport in *rme-2* mutants might have deleterious side effects on membrane trafficking in oocytes. Since *rme-2* mutant oocytes are small and misshapen, the effects could even be more indirect. Further work will be needed to explain fully the role of *rme-2* in ovulation.

10.2.6.3 Smooth muscle structure and function and ovulation—The myoepithelial gonadal sheath cells provide a valuable system for studying the cell biology and function of a smooth muscle cell type (Strome 1986; McCarter et al. 1997; Rose et al. 1997; Hall et al. 1999; Ono et al. 2007). Mutations that perturb actomyosin contractility in sheath cells can cause an Emo phenotype and infertility (Myers et al. 1996; Wissmann et al. 1999; Ono and Ono 2004; Ono et al. 2008). However, some mutations that cause hypercontractility of the sheath cells also result in infertility. For instance, worms double mutant for *tni-1* and *unc-27*, two Troponin I isoforms expressed in the sheath cells, display defects in oocyte production, meiotic maturation, and ovulation (Obinata et al. 2010). In the future, studies of smooth muscle physiology in the context of ovulation will no doubt benefit from continued improvements in methods for measuring calcium concentrations and for recording ion channel activity in the gonad (Rutledge et al. 2001; Samuel et al. 2001).

10.3 Control of Oocyte Growth and Coordination with Meiotic Maturation

Fertility depends on germline stem cell proliferation, meiosis and gametogenesis, yet how these key transitions are coordinated is unclear. Recent data suggest that the continued presence of sperm maintains the adult hermaphrodite gonad in an active reproductive mode. The MSP hormone promotes the production and growth of oocytes and works in concert with the GLP-1/Notch pathway to regulate an optimal allocation of germline stem cells into oocytes. This section focuses on the cellular processes of pachytene progression, oocyte growth, and their regulation by MSP signaling.

10.3.1 Pachytene progression and oogenesis

Once germ cells have entered meiosis, progression through the pachytene stage in both sexes requires the germline function of genes of the MAP kinase signaling pathway, including *let-60/ras*, *ksr-2*, *lin-45/raf*, *mek-2/mapkk*, and *mpk-1/mapk* (Church et al. 1995; Ohmachi et al. 2002; Lee et al. 2007). An increase in dpMPK-1 levels is observed midway through the pachytene stage, consistent with the conclusion that activated MPK-1 is needed for germ cells to progress from an early (or distal) to a late (or proximal) pachytene stage (Lee et al. 2007). The continued presence of sperm in the gonad is needed for MPK-1 activation in this region of the gonad. Young adult *fog-2(oz40)* females (8 hr after the L4 to adult molt) exhibit dpMPK-1 in the proximal pachytene region and a single distal oocyte at the -6 position (Lee et al. 2007). This distal activation of MPK-1 occurs independently of the presence of sperm. By contrast, older *fog-2(oz40)* females (20 hr after the L4 to adult molt) display undetectable dpMPK-1 levels in the germline (Lee et al. 2007). This result fits well with the observation that the flux of germ cells through pachytene is significantly reduced in unmated adult females (Jaramillo-Lambert et al. 2007). *acy-4* mutant hermaphrodites produce fewer oocytes than the wild type and exhibit dpMPK-1 in the pachytene region in the early adult stage but not later (Govindan et al. 2009), suggesting a role for the sheath cells in maintaining MAPK activation in the pachytene region. Cell ablation studies are also consistent with the possibility that cells in the sheath/spermathecal lineages promote progression through pachytene (McCarter et al. 1997). One model is that the distal sheath cells (pairs 1 and 2) might produce a secondary signal to trigger MPK-1 activation in the pachytene region, thereby promoting meiotic prophase progression. In this model, the production of this hypothesized secondary signal would be dependent on the

continued presence of MSP in the gonad, as sensed by the proximal sheath cells via the G_s-adenylate cyclase pathway.

10.3.2 Oocyte growth

Prior to cellularization at late stages of gametogenesis, *C. elegans* germ cells maintain a connection to the cytoplasmic core of the gonad (rachis), the contents of which are shared with developing oocytes (Hirsh et al. 1976; Wolke et al. 2007). In adult hermaphrodites, germ cells that exit pachytene either differentiate as oocytes or undergo apoptosis (Gumienny et al. 1999). Female meiotic germ cells destined for apoptosis might function as nurse cells (Gumienny et al. 1999; Jaramillo-Lambert et al. 2007) by contributing mRNA, protein, and cellular organelles to growing oocytes, which are transcriptionally quiescent themselves (see Chapter 9 on Apoptosis by A. Gartner). Oocytes in the loop region grow primarily by receiving actomyosin-dependent flow from the core cytoplasm (Wolke et al. 2007), and yolk uptake in the most proximal oocytes also contributes to their growth (Grant and Hirsh 1999; Wolke et al. 2007).

The pathways that control incomplete cytokinesis in the distal germline, and that promote cellularization of developing oocytes in the proximal germline, are incompletely understood. Several genes with important functions in cytokinesis are required for oocyte cellularization, including *mlc-4*, *mel-11* and *cyk-1*, which encode the regulatory light chain of non-muscle myosin, a myosin phosphatase regulatory subunit and an actin regulator, respectively (Swan et al. 1998; Shelton et al. 1999; Piekny and Mains 2002). The anillin ANI-2 is required for developing oocytes to maintain their connection to the core cytoplasm (Maddox et al. 2005). Thus, *C. elegans* oogenesis appears to provide a useful experimental system for addressing the cell biological mechanisms by which interconnected cysts form and breakdown, as occurs in mammalian female germ cell development (Pepling and Spradling 1998). It will be essential to determine the basic cell biological principles underlying incomplete cytokinesis and oocyte cellularization before the field can achieve a relatively complete understanding of the regulatory mechanisms. Nonetheless, several studies have begun to address how intercellular signaling regulates oocyte growth and cellularization. The PTP-2 protein tyrosine phosphatase functions in the germline as a negative regulator of oocyte growth; *ptp-2* mutant oocytes grow abnormally large in the presence of sperm (Gutch et al. 1998; Yang et al. 2010). PTP-2 is required for the MPK-1 MAPK activation in the germline, which might explain the growth defect because oocytes also grow abnormally large when a temperature-sensitive *mpk-1* mutant is upshifted (Lee et al. 2007). The small oocyte phenotype observed in the constitutively-activated *let-60/ras(ga89)* allele depends on the presence of sperm in the gonad (Lee et al. 2007). Two identified MPK-1 substrates function to restrict oocyte growth, whereas eight identified substrates promote growth (Arur et al. 2009). Analysis of the growth-promoting substrates is complicated by effects on dpMPK-1 levels.

10.3.2.1 Actomyosin-dependent cytoplasmic streaming—Oocytes in the loop region of the gonad grow by receiving flow from the cytoplasmic core (Wolke et al. 2007). This flow was shown to be dependent on the actomyosin cytoskeleton, but independent of microtubules. Cytoplasmic streaming requires the continued presence of sperm in the gonad but does not depend on meiotic maturation (Wolke et al. 2007). In young adult females observed right after the L4 to adult molt, flows were observed and were therefore independent of sperm. By contrast, adult females observed on the second day of adulthood did not exhibit flows, however mating restored the flows. Thus, initial growth of oocytes in the young adult stage is independent of sperm, but sperm needs to be continually available for additional oocytes to form. This result provides an explanation for the original observation that sperm promote oocyte production (Ward and Carrel 1979).

A series of elegant oil injection studies suggested that the oocytes generate the forces driving cytoplasmic streaming (Fig. 10.5). Neither germ cell apoptosis nor sheath cell contraction is required for the cytoplasmic flow. While the mechanism of force generation is unclear, several testable models were proposed (Fig. 10.5; Wolke et al. 2007).

10.3.2.2 MSP signaling, the sheath cells, and the control of cytoplasmic streaming—The MSP hormone is sufficient to promote the sustained actomyosin-dependent cytoplasmic streaming that drives oocyte growth (Govindan et al. 2009). Injection of MSP into unmated females that do not exhibit flows (e.g., at 24 hours post L4) caused the flows to resume. Efficient oocyte production and cytoplasmic streaming require G_s -adenylate cyclase signaling in the gonadal sheath cells. Thus the gonadal sheath cells coordinate oocyte growth and meiotic maturation with sperm availability. Phosphorylation of the regulatory myosin light chain (rMLC) increases the ATPase activity of nonmuscle myosin and is required for myosin motor function, which is needed for the actomyosin-dependent cytoplasmic streaming that drives oocyte growth (Fig. 10.3; Wolke et al. 2007). Phosphorylated rMLC (p-rMLC) was detected in the germline and gonadal sheath cells of hermaphrodites and mated females, but not two-day old unmated adult females (Nadarajan et al., 2009). MSP injection was shown to induce p-rMLC formation rapidly (within 15 minutes) throughout the germline and in the gonadal sheath cells (Nadarajan et al. 2009). Sustained gonadal flows and p-rMLC formation also require the function of G_s -adenylate cyclase signaling in the gonadal sheath cells. This activity of MSP appears to be important for promoting oocyte growth (see below; Nadarajan et al. 2009). Thus, MSP appears to promote oocyte meiotic maturation in part through coordination of several of its component processes. Genetic evidence suggests that MSP and G_s -adenylate cyclase signaling regulate oocyte growth and meiotic maturation in part by antagonizing gap-junctional communication between sheath cells and oocytes.

10.3.2.3 GLP-1/Notch signaling and the control of sperm-dependent oocyte growth—Surprisingly, a genetic screen for mutations that cause oocytes to grow abnormally large in the presence of sperm recovered reduction-of-function alleles of *glp-1/Notch* (Nadarajan et al. 2009). GLP-1/Notch signaling restricts the growth of oocytes to the proper size in response to the MSP signal. Germline GLP-1 activity negatively regulates MSP-dependent cytoplasmic streaming, whereas it promotes oocyte cellularization. Several observations suggest that GLP-1 functions in the distal germline in response to DTC signaling to regulate oocyte growth. Laser ablation of the DTC and co-depletion of the DTC-expressed GLP-1 ligands LAG-2 and APX-1 cause a large oocyte phenotype. Oil injection studies, similar to those first performed by Wolke et al. (2007), showed that oocytes grow abnormally large when the proliferative zone is occluded by oil injection (Nadarajan et al. 2009). Distal oil injection not only suppressed the small oocyte phenotype of the *glp-1(ar202)* gain-of-function mutant, but phenocopied a reduction in *glp-1* function. These results suggest that GLP-1/Notch functions in the proliferative zone to regulate MSP-dependent oocyte growth. Consistent with this possibility, normal oocyte growth was shown to require the function of several mediators of GLP-1 signaling, including the LAG-1 transcription factor and the FBF-1/2 RNA-binding proteins.

Several lines of evidence suggested that the role of *glp-1* in oocyte growth is separable from the proliferation versus meiotic entry decision (Nadarajan et al. 2009). One notable finding was that blocking apoptosis strongly suppressed the *glp-1* oocyte growth defect without affecting the premature meiotic entry defect. It is not clear whether the *ced-3* and *ced-4* requirement for the *glp-1* large oocyte phenotype involves their apoptotic function, or perhaps a cell death-independent function. The results of Nadarajan et al. (2009) suggest a model in which two major signaling centers in the adult hermaphrodite gonad, distal GLP-1 signaling and proximal MSP signaling, work in opposition to regulate the differentiation of

germ cells into functional oocytes (Fig. 10.3). In this model, the adult hermaphrodite gonad is rapidly switched into a reproductive mode by the MSP hormone. MSP signaling provides the impetus for oocyte growth, differentiation, and the completion of meiosis, and GLP-1 signaling both provides the raw material for gametogenesis and modulates additional processes that restrict oocyte growth, including cytoplasmic streaming and oocyte cellularization.

10.4 Meiotic Maturation and Regulation of the Oocyte-to-Embryo Transition

In many animals, fertilization triggers egg activation (the process whereby the oocyte completes the meiotic divisions), the blocks to polyspermy, and activation of the embryonic program (Runft et al. 2002; Horner and Wolfner 2008). Fertilization is extensively discussed in the Chapter 11 by M. R. Marcello, G. Singaravelu, and A. Singson. Recent findings provide views into how signaling mechanisms initiated during meiotic maturation and extending through fertilization control the completion of meiosis and prepare the zygote for embryonic patterning. In *C. elegans*, fertilization (Goldstein and Hird 1996) and the downstream PAR proteins (Kemphues et al. 1988) are required for the establishment of embryonic polarity. Polarity establishment in the *C. elegans* embryo occurs via a cue associated with the sperm centrosome complex and microtubules (St Johnston and Ahringer 2010; Nance and Zallen 2011). Chapter 12, by S. M. Robertson and R. Lin, discusses the function of the OMA proteins in the oocyte-to-embryo transition and the role of MBK-2 in controlling the degradation of maternal proteins after completion of meiosis. Here, we focus on the role of meiotic maturation and fertilization in promoting the completion of meiosis.

10.4.1 Function and regulation of the oocyte meiotic spindle

In many animal oocytes, the microtubule arrays of the meiotic spindle form independently of a centriole-containing centrosome. The short barrel-shaped meiotic spindles of *C. elegans* oocytes form by microtubule nucleation around meiotic chromatin and are both acentriolar and anastral (Fig. 10.6; Albertson and Thomson 1993; Howe et al. 2001; Yang et al. 2003). Time-lapse videomicroscopic observations of meiotic chromosomes and spindles indicate that meiosis I spindle assembly initiates prior to ovulation when the oocyte is in the gonad arm (Yang et al. 2003). Both meiotic divisions are then completed in the uterus following fertilization (Albertson and Thomson 1993; McCarter et al. 1999; Yang et al. 2003).

The *C. elegans* oocyte is emerging as a powerful model for studying the assembly, regulation, and function of the meiotic spindle (Muller-Reichert et al. 2010; Fabritius et al. 2011). One salient feature of this system is that meiotic spindle assembly is a microtubule driven process and F-actin is not required for translocation of the spindle to the cortex (Yang et al. 2003). This contrasts with the situation in mouse oocytes in which cortical movement of the meiotic spindle is actin-based (Na and Zernicka-Goetz 2006; Dumont et al. 2007; Li et al. 2008; Schuh and Ellenberg 2008). This property appears to provide some experimental flexibility for the separate dissection of meiotic spindle assembly and cytokinesis. At the same time, it cautions against generalizing results to all instances of acentrosomal spindle assembly.

During oogenesis, the maternal centrioles are eliminated, likely disappearing before the gonad loop region (Kim and Roy 2006; Zhou et al. 2009), and embryogenesis thus depends on the sperm-supplied centriole pair (Wolf et al. 1978; O'Connell et al. 2001). An early study using co-suppression methodology suggested that centrosome elimination during oogenesis requires the function of the *cki-2* cyclin-dependent protein kinase inhibitor (Kim and Roy 2006). However, subsequent analysis of a *cki-2* null mutant found no evidence of a centrosome elimination defect during oogenesis (Buck et al. 2009). The mechanism of centrosome elimination thus remains an exciting mystery for future investigations.

10.4.1.1 Meiotic spindle positioning—The meiotic spindle forms in close association with the cortex to facilitate the extrusion of small polar bodies thereby preserving oocyte cytoplasm. The meiotic spindle assembles parallel to the cortex and then rotates to be perpendicular (Fig. 10.6; Albertson and Thomson 1993). Two mechanisms appear to be particularly important for the cortical localization of the meiotic spindle, the first occurring before nuclear envelope breakdown and the second occurring at anaphase (Fabritius et al. 2011). Early translocation involves the distal (away from the spermatheca) migration of the oocyte nucleus prior to nuclear envelope breakdown (McCarter et al. 1999; McNally et al. 2010). Oocyte nuclear migration can occur in the absence of sperm (McCarter et al. 1999) and requires the function of *mpk-1* (Lee et al. 2007) and kinesin-1 (McNally et al. 2010). Kinesin-1 activity requires the UNC-116 kinesin-1 heavy chain, the KLC-1 kinesin-1 light chain, and the KCA-1 kinesin-1 binding protein (Yang et al. 2005). An attractive possibility is that kinesin-1 function in nuclear migration might involve the function of the ZYG-12 KASH domain protein (Zhou et al. 2009) because kinesin-1 functions together with the UNC-83 KASH domain protein to position nuclei in somatic cells (Meyerzon et al. 2009). Interestingly, the ZYG-12 interacting partner, SUN-1 is phosphorylated in diakinesis oocytes (Penkner et al. 2009).

The late spindle translocation process requires the APC/C (Yang et al. 2005), cytoplasmic dynein (Ellefson and McNally 2009; van der Voet et al. 2009), and a protein complex containing the NUMA-related LIN-5, abnormal spindle-like, microcephaly-associated ASPM-1, and calmodulin CMD-1 (van der Voet et al. 2009). Late translocation involves a 90° rotation of the meiotic spindle (Albertson and Thomson 1993; Yang et al. 2005; Ellefson and McNally 2009; van der Voet et al. 2009). Kinesin-1 has also been proposed to anchor the meiotic spindle to the cortex until completion of MII (Yang et al. 2005; McNally et al. 2010). Recent data indicate that cyclin B/Cdk1 inhibits meiotic spindle rotation and anaphase spindle shortening. By promoting cyclin B degradation, the APC/C couples spindle rotation and chromosome segregation (Fig. 10.6; Ellefson and McNally 2011).

10.4.1.2 MEI-1 and meiotic spindle assembly—The assembly of a bipolar female meiotic spindle requires the function of two interacting genes, *mei-1* and *mei-2* (Mains et al. 1990; Clandinin and Mains 1993; Clark-Maguire and Mains 1994a, b; Srayko et al. 2000). *mei-1* and *mei-2*, respectively, encode the p60 and p80 subunits of katanin (Clark-Maguire and Mains 1994a; Srayko et al. 2000), a dimeric microtubule severing AAA-ATPase first purified from sea urchin eggs (McNally and Vale 1993). Katanin function also controls meiotic spindle length and spindle shortening (McNally et al. 2006). Although *mei-1* and *mei-2* are required for the normal segregation of chromosomes during female meiosis, they are dispensable during male meiosis. Electron microscopy (EM) with tomography and 3-D modeling were used to compare microtubule organization in the oocyte meiotic spindle in the wild type and a *mei-1* null mutant (Fig. 10.6; Srayko et al. 2006). In the wild type, microtubule ends were distributed throughout the spindle, not just exclusively at the poles. Thus, the meiotic spindle appears to assemble as a network of interdigitating microtubules. In the *mei-1* mutant, fewer microtubules surrounded the chromatin. These microtubules appeared disorganized and did not sort into a bipolar spindle. The chromatin-associated microtubules in the *mei-1* mutant were also longer than in the wild type. These findings suggest that katanin functions to increase microtubule number and density in the vicinity of meiotic chromatin. Consistent with this model, meiotic spindles in a reduction-of-function *mei-2* mutant are significantly longer than in the wild type (McNally et al. 2006). In the EM tomography study, structural evidence for microtubule severing was observed, leading to a model in which katanin promotes bipolar meiotic spindle assembly by increasing the local density of microtubule polymers (Srayko et al. 2006). An exciting recent finding is that the viable and fertile MEI-1(A338S) mutant, which is defective in microtubule severing when expressed in *Xenopus* cells, is proficient in bipolar meiotic spindle assembly, spindle

rotation, and post-rotation spindle shortening in the worm (McNally and McNally 2011). MEI-1(A338S) meiotic spindles, however, were markedly longer than the wild type (McNally and McNally 2011). A model was proposed in which katanin contributes to the assembly of the bipolar meiotic spindle through a combination of microtubule binding and bundling activities, in combination with microtubule severing (McNally and McNally 2011).

10.4.1.3 Chromosome alignment and segregation on the meiotic spindle—In *C. elegans* oocytes, kinetochore proteins localize to cup-like structures, which encase the meiotic chromosomes, except for the mid-region, which is enriched for components of the chromosomal passenger complex, including AIR-2 (Fig. 10.6; Howe et al. 2001; Rogers et al. 2002; Monen et al. 2005; Dumont et al. 2010). Ultrastructural analyses of *C. elegans* oocyte meiotic spindles have not detected exclusive end-on attachments between kinetochores and microtubules, raising questions about the basis for chromosomal attachment, alignment and segregation (Howe et al. 2001; Srayko et al. 2006). Interestingly, the chromokinesin, KLP-19, which generates a polar ejection force promoting chromosome congression on the metaphase plate during mitosis, localizes to the mid-bivalent region during meiosis (Powers et al. 2004; Wignall and Villeneuve 2009; Dumont et al. 2010). Time-lapse imaging of KLP-19-depleted embryos revealed a spatial dispersion of meiotic chromosomes at anaphase I and an instability in the late anaphase spindle (Dumont et al. 2010). KLP-19 first becomes concentrated on meiotic chromosomes in the most proximal oocytes, but the sperm dependence of this localization has not been investigated. The BimC-related kinesin BMK-1 localizes to meiotic spindles in an AIR-2-dependent fashion. A *bmk-1* deletion allele and *bmk-1(RNAi)* do not disrupt meiosis and mitosis (Bishop et al. 2005; Saunders et al. 2007), a finding that contrasts with the essential requirement for BimC kinesins for maintenance of bipolar spindle structure and spindle pole separation in other systems (Kashina et al. 1997). By contrast, the Klp2-related kinesin KLP-18 is required for meiotic spindle bipolarity (Segbert et al. 2003; McNally et al. 2006; Wignall and Villeneuve 2009).

Recently it was observed that lateral microtubule spindles ensheath meiotic chromosomes and appear to promote their biorientation on the meiotic spindle (Wignall and Villeneuve 2009). This new model envisages a major role for lateral microtubule bundles and localized microtubule motors, such as KLP-19, in promoting chromosome congression and biorientation (Wignall and Villeneuve 2009). There is strong evidence that meiotic kinetochore proteins do play a role in orienting meiotic chromosomes, however (Dumont et al. 2010). The early function of kinetochores in aligning meiotic chromosomes on the metaphase plate (Dumont et al. 2010) might facilitate lateral attachments by microtubule bundles (Wignall and Villeneuve 2009). Interestingly, meiotic chromosomes separate at anaphase in the absence of kinetochore function; microtubules forming between the meiotic chromosomes appear to push them apart (Dumont et al. 2010). Current models for meiotic spindle assembly and function appear to rely on the meiotic spindle having microtubules of a defined polarity (Fig. 10.6), which has not been determined. In *Drosophila*, the female meiosis I spindle contains microtubules of mixed polarity (Liang et al. 2009). It will be crucial to address the polarity of microtubules in the oocyte before, during, and after meiotic maturation.

10.4.2 Fertilization and the completion of meiosis

In *C. elegans*, fertilization is required for the proper completion of the two successive meiotic divisions (Ward and Carrel 1979; McNally and McNally 2005). When the oocyte undergoes meiotic maturation but is not fertilized, as happens in fertilization-defective mutants, meiotic spindle assembly and anaphase I occurs normally and on schedule; however, the spindle midzone persists, the first polar body does not form, and the meiosis II

spindle fails to assemble (McNally and McNally 2005). Instead, these unfertilized oocytes undergo multiple cycles of nuclear reformation, S-phase, and nuclear envelope breakdown, becoming highly polyploid (Ward and Carrel 1979). No cell division is apparently possible in the absence of the sperm-contributed centrioles. The degradation of cyclin B that accompanies M-phase exit in many systems (Murray 2004) is incomplete if fertilization is blocked (McNally and McNally 2005). Fertilization might therefore be required for activation of the ZYG-11/CUL-2 E3 ubiquitin ligase, which is required for progression through meiosis II and the degradation of cyclins B1 and B3 after their functions have been completed (Liu et al. 2004; Sonnevile and Gonczy 2004; Deyter et al. 2010).

As in many animals (Horner and Wolfner 2008), an increase in cytosolic calcium levels accompanies fertilization in *C. elegans* (Samuel et al. 2001). A calcium signal at fertilization triggers APC/C and exit from meiosis II in *Xenopus* (Tunquist and Maller 2003; Liu and Maller 2005; Rauh et al. 2005). In *C. elegans*, the APC/C and separase are required for the completion of meiosis I (Furuta et al. 2000; Golden et al. 2000; Siomos et al. 2001; Davis et al. 2002; Shakes et al. 2011). Because the APC/C is required for meiosis I, it has been difficult to determine whether it also functions in meiosis II or polarity establishment. An analysis of conditional alleles at semi-permissive temperatures (Shakes et al. 2003) showed that the embryonic polarity defects of hypomorphic APC/C alleles (Rappleye et al. 2002) are likely due to effects on cell cycle progression during meiosis I (Shakes et al. 2003).

Activation of the APC/C is independent of fertilization in *C. elegans* because oocytes that undergo meiotic maturation and ovulation but not fertilization complete the metaphase-to-anaphase transition in meiosis I (McNally and McNally 2005). The APC/C and separase appear to have distinct roles in promoting cortical granule exocytosis required for formation of the eggshell (Sato et al. 2006; Bembenek et al. 2007). Yet, formation of the eggshell requires fertilization. Eggshell formation requires components of a cortical complex including three protein tyrosine phosphatase-like proteins (EGG-3, EGG-4, and EGG-5), and chitin synthetase (CHS-1) (Johnston et al. 2006; Maruyama et al. 2007; Parry et al. 2009). EGG-3, EGG-4, EGG-5, and CHS-1 are also required for the completion of meiosis, polar body formation, and the block to polyspermy (Johnston et al. 2006, 2010; Maruyama et al. 2007; Parry et al. 2009).

SPE-11 is a key sperm-derived factor needed for the completion of meiosis and eggshell formation (Hill et al. 1989; Browning and Strome 1996; McNally and McNally 2005). Fertilization with sperm from homozygous *spe-11* mutant males causes paternal-effect lethality due to a failure to produce polar bodies and defective cytokinesis in meiosis I and II (McNally and McNally 2005). The mutant embryos also exhibit defects in embryonic polarity (Hill et al. 1989). *spe-11* mutants can assemble meiosis I and II spindles, however, the anaphase I chromosomes appear to collapse. Because *spe-11* mutants assemble a meiosis II spindle, but unfertilized oocytes do not, the sperm must make unidentified contributions to meiosis II spindle assembly (McNally and McNally 2005). *spe-11* mutants were also observed to exhibit an incompletely penetrant polyspermy phenotype (Johnston et al. 2010), perhaps owing to the defect in eggshell synthesis. SPE-11 does not appear to exhibit conservation at the primary amino acid sequence level and its biochemical activity is unknown, though it exhibits a perinuclear localization pattern in sperm (Browning and Strome 1996). Because treatments that interfere with actin polymerization, such as Latrunculin A application or profilin (RNAi), phenocopy aspects of *spe-11* mutants (Yang et al. 2003), SPE-11 might regulate actin dynamics. Meiotic cytokinesis appears to involve a novel structural mechanism involving the ANI-2 anillin to ensure the formation of small polar bodies (Dorn et al. 2010). Future studies of SPE-11 and actin regulators may provide insights into the general cell biology of meiotic cytokinesis.

10.5 Conclusions and Future Directions

10.5.1 Control of oogenesis in the wild type

A decade ago, only five paragraphs were devoted to the control of oocyte growth and meiotic maturation in a review of *C. elegans* gonadogenesis (Hubbard and Greenstein 2000). It is clear that there has been immense progress in this field since then. As the field endeavors to obtain a complete understanding of oogenesis in the wild type, it is clear that studies utilizing forward and reverse genetics and genomics will continue to play a prominent role. A central question concerns the connection between the sheath cell signaling pathway (Fig. 10.4) and activation of protein kinase pathways in the germline that are critical for meiotic maturation (e.g., the MPK-1 and CDK-1 pathways). Whether translational regulation in the germline is a key target of meiotic maturation signaling is as of yet unclear. A major goal for the field is to connect phenotypes with cell biological and biochemical mechanisms. Yet, there are vast areas of the cell biology of oogenesis of which we have only limited knowledge. Models for how oocytes grow and cellularize are quite incomplete at cell biological and biochemical levels. Likewise, it is unclear how oocyte meiotic maturation is spatially restricted to the most proximal oocyte. A challenge for the field will be to achieve integration between the component parts of oogenesis. It is now clear that there is a connection between the presence of sperm in the gonad, oocyte growth, and downstream responses of germline stem cells to DTC signaling. It will be crucial to elucidate the dependencies between germline events and to understand the mechanisms and checkpoints by which they are controlled. Forward genetic screens and RNAi approaches (e.g., Green et al. 2011) will continue to provide entry points for unraveling the mechanisms of oogenesis.

10.5.2 Oogenesis and reproductive aging

The control of oogenesis in *C. elegans* and mammals share a number of biological and mechanistic similarities. Yet, there are also fundamental differences. A central issue is the extent to which female reproductive aging—the decline in fertility as a function of time—can be modeled in the worm. Chromosome missegregation in female meiosis I is the leading cause of miscarriage and congenital birth defects in humans, such as Down syndrome (Hassold and Hunt 2001). This “maternal-age effect” is a major barrier to human reproduction. In human females, meiotic recombination occurs exclusively in the embryo and there is no generally accepted evidence for the existence of actual stem cells in the adult mammalian ovary (Zuckerman 1951; Peters et al. 1962; Telfer et al. 2005; Bristol-Gould et al. 2006; Eggan et al. 2006). This absence of stem cells in the adult human ovary is a major difference between the mammalian and *C. elegans* systems (see Chapter 4 by D. Hansen and T. Schedl); this difference is important for considering the origins of reproductive aging. Additionally, mammalian oocytes arrest in diplotene for years, but these oocytes are transcriptionally active; the importance of transcription in the oocyte is illustrated by mutant phenotypes obtained from generating conditional gene knockouts using the oocyte-specific Zp3 driver (Sun et al. 2008). By contrast, in *C. elegans* oocytes are transcriptionally inactive and arrest for days. In mammals, arrested oocytes are recruited each cycle, which then undergo considerable growth and development—folliculogenesis and imprinting occur in the time window before meiotic maturation. In *C. elegans* females, arrested oocytes are poised to mature as soon as the sperm signal appears, though mating stimulates oocyte production and growth, which is appropriate for the worm’s assembly-line reproductive strategy. There may be key differences in how aging impinges on these biological processes.

Reproductive aging is a new frontier in the *C. elegans* germline field. In considering how aging might impact the fidelity of the oocyte meiotic maturation divisions in the worm, it is necessary to address the complication that defects could occur in earlier events, extending

back to the behavior of the germline stem cells themselves. In female mammals by contrast, chiasmata established in utero link duplicated homologs by virtue of sister chromatid cohesion. Sister chromatid cohesion must be maintained through the entirety of reproductive life. Premature loss of sister-chromatid cohesion is a likely cause of chromosome segregation errors during meiosis I in human females (Wolstenholme and Angell 2000) and this view is supported by an analysis of mouse models (Revenkova et al. 2004, 2010; Hodges et al. 2005; Chiang et al. 2010; Lister et al. 2010).

Several pioneering studies have begun to address reproductive aging in the *C. elegans* female germline. For example, Hughes et al. (2007) found that dietary restriction and reduced insulin/IGF-1 signaling delayed reproductive aging. Interestingly, the age-related decline in the reproductive system was observed to be independent of progeny production. In these studies, feminization of the germline was not observed to delay reproductive aging, once sperm were supplied by mating at later times. It is important to point out, however, that the germline and somatic gonad actively function to inhibit meiotic maturation when sperm are absent. The energetics of this inhibition in comparison to fertility is unknown, but one would imagine it might be less costly, especially since oocyte production stops in the absence of sperm.

Several observations suggest that nutrition impacts reproductive aging and oocyte quality. Andux and Ellis (2008) reported that oocyte quality declines as worms age and that this decline affects both prophase-arrested oocytes and newly forming oocytes in older animals as well. An especially interesting finding was that germline apoptosis functions to preserve oocyte quality in aging worms. One might imagine that germline apoptosis might preserve oocyte quality by culling defective oocytes. However, mutations that specifically block DNA-damage-induced apoptosis do not lower oocyte quality. Therefore, a model was presented in which apoptosis in the germline optimizes the allocation of resources between developing oocytes.

In addition to insulin/IGF-1 signaling, the TGF β Sma/Mab signaling was observed to affect reproductive aging and oocyte quality (Luo et al. 2009, 2010). Mutations in the TGF β Sma/Mab were observed to delay reproductive aging and preserve oocyte quality. Since the TGF β Sma/Mab pathway affects somatic cell growth, one of several possibilities is that this pathway might affect the relative allocation of nutritional resources between the germline and soma. Heretofore, most genes affecting reproductive aging have been identified using a candidate gene approach. A recent advance is the use of forward genetics in combination with quantitative screening strategies to identify mutations affecting reproductive aging (Hughes et al. 2011).

An exciting recent finding was the observation that starvation of L4-stage hermaphrodites can bring about a state of “adult reproductive diapause” in which the entire germline, except the germline stem cells, undergoes resorption in a process involving apoptosis (Angelo and Van Gilst 2009). The state of adult reproductive diapause results from a starvation response of oogenic germ cells (Seidel and Kimble 2011). Repopulation of the germline and fertility was observed after refeeding (Angelo and Van Gilst 2009; Seidel and Kimble 2011). This work provides a new experimental model for investigating the impacts of aging and nutritive status on reproduction.

C. elegans is also emerging as a model for studying the reproductive impact of environmental toxicants. In mice, there is evidence that endocrine disrupters and environmental toxicants, such as bisphenol, can perturb the fidelity of meiotic chromosome segregation and that the final stages of oocyte growth and meiotic maturation are particularly sensitive (Hunt et al. 2003). However, bisphenol's effects are not specific for

late events of oogenesis because fetal exposure also disrupts synaptonemal complex assembly (Susiarjo et al. 2007). In *C. elegans*, bisphenol perturbs early events in meiosis, including double strand break repair, as well as the kinetics of remodeling the oocyte bivalents (Allard and Colaiacovo 2010). These recent findings highlight the potential of the *C. elegans* system for addressing the origin of aneuploidies and miscarriage in humans. For this potential to be realized, a comprehensive understanding of the normal mechanisms controlling oocyte growth and meiotic maturation and their integration into the overall germline program is essential.

As this new field develops, it is clear that standardized assays and biomarkers for reproductive aging and oocyte quality would facilitate the comparison of data between labs. Normal germline development and function in young animals is complex and involves soma-germline interactions and coordination between multiple cellular processes. The study of reproductive aging is expected to be at least as complicated. Normal *C. elegans* development is remarkable for its reproducibility. By contrast, extensive phenotypic and functional variance in reproduction is observed in populations of aging worms. In the future, we anticipate exciting progress in the study of reproductive aging through the interaction of *C. elegans* researchers studying germline development and aging.

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Fig. 10.1.

Oocyte meiotic maturation and egg activation. The oocytes of most animals arrest in meiotic prophase I (primary arrest), and resume meiosis (meiotic maturation) in response to hormonal stimulation (starfish, 1-methyladenine; *Xenopus*, progesterone; *C. elegans*, MSP; humans, LH). Meiotic maturation is accompanied by GVBD, cortical cytoskeletal reorganization, and meiotic spindle assembly (blue circles are polar bodies). The point of fertilization is species-dependent—in *C. elegans*, fertilization happens when the mature oocyte enters the spermatheca at ovulation. Oocytes of many species undergo secondary arrests before fertilization, which triggers egg activation, as indicated.

Fig. 10.2.

Pathways regulating MPF activity. Activation loops resulting in amplified MPF activation and meiotic maturation are in red. Regulators inhibiting MPF activation, and inhibited by the MPF-dependent feed-forward activation loops, are in black. The Greatwall kinase is not conserved in *C. elegans*, but other components are conserved: the PP2A catalytic subunit is LET-92; the PP2A structural subunit is PAA-1; there are several PP2A regulatory subunits (*pptr-1*, *pptr-2*, *rsa-1*, *sur-6*, C06G1.5, F47B8.3, T22D1.5); and α -endosulfine is K10C3.2.

Fig. 10.3.

A model for the control of germline proliferation, oocyte growth, and meiotic maturation by GLP-1/Notch and MSP signaling. One of the two adult hermaphrodite gonad arms is depicted (indicated are: DTC, distal tip cell; TZ, transition zone; and proximal oocytes, -1 to -3). p-rMLC is shown in brackets to indicate that MSP signaling is sufficient to promote rMLC phosphorylation in the germline, whereas the *glp-1* pathway appears dispensable.

Fig. 10.4.

A model for the regulation of meiotic maturation by G protein signaling. Somatic G_s-adenylate cyclase-protein kinase A signaling is required for oocyte meiotic maturation. EGL-30/G_q promotes sheath cell contraction. G_{o/i} and sheath-oocyte gap junctions function as inhibitors of meiotic maturation. Gray lines are the lipid bilayers of the sheath cell and oocyte.

Fig. 10.5.

Cytoplasmic streaming drives oocyte growth. Oil injection studies informative for probing the mechanism of oocyte growth (a–d). Injection in the pachytene region (a) or just prior to the loop (b) does not interfere with flow within the proximal gonad arm; damaging an oocyte by oil injection disrupts flow to that oocyte and flows are rerouted to neighboring oocytes (c). These results suggest that oocytes generate the force for flow (Wolke et al. 2007). (d) Distal oil injection causes a large oocyte phenotype and suppresses the small oocyte phenotype of *glp-1(gt)* mutants, suggesting that DTC signaling via GLP-1 in the distal arm regulates MSP-dependent oocyte growth (Nadarajan et al., 2009). (e–h) Models proposed by Wolke et al. (2007) for the generation of gonadal flow by the oocyte actomyosin cytoskeleton. Simplified oocytes receiving flow (blue arrows) from the cytoplasmic core of the *C. elegans* germ line are diagrammed. Cortical flow in the opposite direction draws material into the oocyte (e), similar to a model of cytoplasmic streaming proposed for early *C. elegans*

embryos. Oriented actin cables bring material into oocytes (f). Expansion of the oocyte cortex draws material into the oocyte (g). A stable actomyosin network in the oocyte (black lines) is coupled to dynamic actomyosin network (red lines and arrows) to bring material into oocytes.

Fig. 10.6.

Assembly and function of the oocyte meiotic spindle. (a) Time-lapse series of MI visualized with GFP:: α -tubulin and mCherry::Histone fusions (unpublished data generously provided by Valerie Osterberg, Sara Christensen, and Bruce Bowerman). (b, c) The meiotic spindle in wild-type (b) and *mei-1(null)* (c) embryos, detected using antibodies to tubulin (red) and DAPI (blue) to stain DNA. (d, e) Three-dimensional reconstruction of portions of the wild-type (d) and *mei-1(null)* (e) meiotic spindle assembled from tomographic data sets. Microtubules are shown in red, with their pole-proximal ends marked by white spheres and their pole-distal ends marked with blue spheres; chromatin is in green. Note, in the wild type, many microtubules terminated before reaching the pole (p) or the chromatin. By contrast, the *mei-1(null)* mutant spindle contains a disorganized array of longer microtubules. Panels (b–e) are from Srayko et al. (2006) and are used with permission. (f) Models for chromosome alignment and segregation. Chromosomes orient and align using kinetochore-dependent (Dumont et al. 2010) and lateral attachments (Wignall and Villeneuve 2009). The cup-like kinetochores are shown in blue and the mid-region enriched for components of the chromosomal passenger complex is shown in red. (g) A pathway for the control of anaphase spindle rotation and shortening (Ellefson and McNally 2011).