The Caenorhabditis elegans Gonad: A Test Tube for Cell and Developmental Biology

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ABSTRACT Sexual reproduction of multicellular organisms depends critically on the coordinate development of the germ line and somatic gonad, a process known as gonadogenesis. Together these tissues ensure the formation of functional gametes and, in the female of many species, create a context for production and further development of the zygote. Since the future of the species hangs in the balance, it is not surprising that gonadogenesis is a complex process involving conserved and multifaceted developmental mechanisms. Genetic, anatomical, cell biological, and molecular experiments have established the nematode Caenorhabditis elegans as a paradigm for studying gonadogenesis. Furthermore, these studies demonstrate the utility of C. elegans gonadogenesis for exploring broad issues in cell and developmental biology, such as cell fate specification, morphogenesis, cell signaling, cell cycle control, and programmed cell death. The synergy of molecular genetics and cell biology conducted at single-cell resolution in real time permits an extraordinary depth of analysis in this organism. In this review, we first describe the embryonic and post-embryonic development and morphology of the C. elegans gonad. Next we recount seminal experiments that established the field, highlight recent results that provide insight into conserved developmental mechanisms, and present future prospects for the field. Dev Dyn 2000;218:2–22. © 2000 Wiley-Liss, Inc.

Key words: germ line; meiosis; mitosis; cells signaling; Caenorhabditis elegans

GONAD ANATOMY AND DEVELOPMENT

This section will detail the major events that constitute gonadogenesis in C. elegans, but first a brief outline will serve to introduce the process. During early embryogenesis, the germ lineage is progressively set apart and specified. The gonad primordium in newly-hatched animals contains four cells (Z1, Z2, Z3, and Z4; Fig. 1) (Kimble and Hirsh, 1979; Sulston et al., 1983). During post-embryonic development, Z1 and Z4 give rise to the entire somatic gonad [in the hermaphrodite, these structures are distal tip cells (DTCs), sheath cells, spermathecae, and uterus] and Z2 and Z3 give rise to the germ line. In later larval stages, the germ line contains a stem-cell population that contributes cells to the meiotic pathway. Although germline nuclei reside in a syncytium at these stages, we refer to individual germline nuclei and their surrounding cytoplasm as a “germ cell.” Development of the soma and germ line in both sexes, hermaphrodites and males, is coordinated by intercellular signaling. The self-fertile hermaphrodites are essentially modified females that produce sperm for a short time early in gametogenesis and then produce exclusively oocytes as adults. Males produce only sperm and can mate with hermaphrodites to produce cross progeny. In this review, we have focused on gonadogenesis in the hermaphrodite. In addition to detailed lineage studies of the hermaphroditic and male somatic gonads (Kimble and Hirsh, 1979), there are several recent reviews on germline development (Schedl, 1997; Seydoux and Strome, 1999; Seydoux and Schedl, 2000). Several important topics not covered in this review are meiotic recombination (Albertson et al., 1997), spermatogenesis (L'Hernault, 1997), sex determination (Meyer, 1997; Hansen and Pilgrim, 1999; Kuwabara, 1999), and male development and mating behavior (Emmons and Sternberg, 1997).

Embryonic Development and Anatomy of the Somatic Gonad and Germ Line

Generation of gonad precursor cells. The germ line is a specialized cell lineage that gives rise to eggs and sperm. In C. elegans, as in many organisms, the
germ line is established and set apart from somatic lineages early during embryogenesis (Fig. 1A). Following formation of the zygote (P0), a series of asymmetric cell divisions results in the production of the primordial germ cell (PGC), P4. Germ cells are derived exclusively from P4 and all of the P4 descendants are germ cells. P4 enters the interior of the embryo during gastrulation (28 cell stage) and divides symmetrically at about the 100-cell stage to form the two germ line precursor cells, Z2 and Z3 (Sulston et al., 1983). Z2 and Z3 divide during post-embryonic development from within the gonad. Therefore, by analogy to other systems, P4 and its daughters Z2 and Z3 can all be referred to as primordial germ cells (PGCs). Like PGCs of other organisms, they are the sole progenitors of the germ line. All the somatic structures of the reproductively mature
adult gonad (described below) are derived from the mesodermal somatic gonadal founder cells, Z1 and Z4, which are descendants of the MS blastomere.

**Migration of gonad precursor cells.** Z1 and Z4 are born relatively late during embryogenesis and migrate extensively to associate with the germline precursor cells (Sulston et al., 1983). The mechanisms that guide the somatic gonadal founder cells during their extensive migration to the germ cells are unclear. However, Z2 and Z3 do not appear to be required for this migration since laser ablation of P4 results in the production of larvae with no germ line, but with a largely normal somatic gonad (Sulston et al., 1983). Electron micrographic studies indicate that Z2 and Z3 are joined and extend lobes into the intestine (Sulston et al., 1983). This attachment with the intestine is not maintained after hatching and thus may serve to nourish the germ cells during embryogenesis. Interestingly, primordial germ cells in the mouse and fruit fly *Drosophila melanogaster* also associate with endodermal cells during their migration to the genital ridges and gonadal mesoderm, respectively (Ginsberg et al., 1990; Jaglarz and Howard, 1995).

**Germ cell specification and P granules.** How is the germ cell fate established and differentiated from the soma? There have been several comprehensive reviews of this topic (Wylie, 1999; Seydoux and Strome, 1999). Here, we review some recent advances in this area, focusing on results that provide insight into differentiated properties of germ cells that are critical for gonadogenesis. During the early embryonic divisions, germline-specific ribonucleoprotein granules (P granules) are asymmetrically partitioned to germline blastomeres (Strome and Wood, 1982, 1983; Wolf et al., 1983; Seydoux and Fire, 1994). Germ cells across many phyla contain electron-dense organelles that are thought to contain critical molecules for germline development (Eddy, 1996). Thus, the mechanisms that are required for P-granule segregation to the germ line are of considerable interest. Maternal-effect mutations in the par genes (partitioning abnormal) alter the fates of early embryonic blastomeres and disrupt P-granule segregation (reviewed by Rose and Kemphues, 1998). The par genes function in a conserved pathway that establishes normal embryonic cell polarity. Hird et al., (1996) visualized the segregation of fluorescently-labeled P granules in real-time using laser scanning confocal microscopy. This important study established two modes of P-granule partitioning to the germ line: in early germline blastomeres (P0 and P1) P granules migrate through the cytoplasm in a microfilament-dependent manner; whereas in later germline blastomeres (P2 and P3), P-granule segregation involves association with the nucleus and is dependent on microfilaments and microtubules. Significantly, P granules that are mis-segregated by chance appear to be unstable in the somatic blastomeres (Hird et al., 1996). So far, mutations known to cause segregation of P granules into more than one blastomere (e.g., mes-I; Strome et al., 1995) compromise the identity of the potential germ lineage rather than the somatic lineage. In contrast, a *Drosophila* germ granule component, the product of the oskar gene, has been identified that can promote the formation of ectopic but functional germ cells when mislocalized (Ephrussi and Lehmann, 1992).

P granules are, nonetheless, important for germline development as revealed by several recent studies. The PIE-1 protein, which contains two CCCH-type zinc finger motifs, associates with P granules, though it is also found in the cytoplasm and nuclei of the germline blastomeres and Z2 and Z3 (Mello et al., 1996). *pie-1* is a maternal-effect gene required for generation of the germ line and for proper specification of blastomere identity (Mello et al., 1992). In *pie-1* mutant embryos (embryos produced by *pie-1* homozygous mutant mothers), the germline blastomere P0 develops like its sister cell EMS, and divides to produce an MS-like cell and a E-like cell (see Fig. 1). These abnormal P0 daughter cells in *pie-1* mutant embryos generate extra mesodermal and endodermal cells, respectively. Consequently, the germ line is not established. Interestingly, *pie-1* mutants inappropriate derepress the transcription of several genes in germline blastomeres (Seydoux et al., 1996), whereas transgenic nematodes engineered to ectopically express PIE-1 in somatic blastomeres exhibit a striking repression of mRNA accumulation. These results led to the hypothesis that germ cell fate determination relies on a PIE-1-dependent inhibitory mechanism in which zygotic gene expression is repressed in germline blastomeres (Seydoux et al., 1996). Consistent with this model, PIE-1 can function as a general transcriptional repressor in a heterologous system (Batchelder et al., 1999). Since PIE-1 is broadly expressed in the germ line, it is not clear whether PIE-1 must be associated with P granules to control blastomere identity. Two related CCCH-type zinc finger proteins, POS-1 and MEX-1, are also required for specification of germline blastomeres and are also components of P granules (Guedes and Preiss, 1997; Tabara et al., 1999a). Since members of this gene family are also present in vertebrates, it will be interesting to determine if related molecules are components of germ plasm in vertebrates (e.g., *Xenopus*). Currently there is no convincing evidence for the involvement of germ plasm in mammalian germ cell fate specification (see Wylie, 1999 for a review). Rather, allocation of epiblast cells to the germline lineage in the mouse depends on BMP4 signaling from the extra-embryonic ectoderm (Lawson et al., 1999).

In light of the fact that P granules contain poly-A+ RNA (Seydoux and Fire, 1994), it is particularly exciting that several RNA-binding motif-containing proteins are associated with P granules. These include the KH domain proteins GLD-1 (Jones et al., 1996) and MEX-3 (Draper et al., 1996), the Vasa-related DEAD box proteins GLH-1 and GLH-2 (Gruidl et al., 1996), and the RGG box protein PGL-1 (Kawasaki et al., 1998). An attractive speculation is that P granule-as-
sociated RNA-binding proteins may function to regulate the translation of mRNAs (Kawasaki et al., 1998). P granule components may also function in assembly, structural maintenance, or segregation of P granules (Draper et al., 1996; Kawasaki et al., 1998). Thus, P-granule components seem to have several distinct roles in germline development as revealed by genetic and phenotypic analyses.

Recently, three *C. elegans* genes with similarity to the *Drosophila nanos* gene have been identified (Subramaniam and Seydoux, 1999; Kraemer et al., 1999), one of which (*nos-2*) produces an mRNA that is associated with P granules (Subramaniam and Seydoux, 1999). *Drosophila nanos* has multiple functions in germline development, being required for germ cell migration, maintenance of germ cell viability, and regulation of mitosis and transcription in the germ line (Wylie, 1999; Forbes and Lehmann, 1998; Deshpande et al., 1999). *nos-2* is the first mRNA component of P granules to be identified (see below for further discussion of *C. elegans nos*-related genes). As more P granule-associated RNA molecules are identified, it will be interesting to learn whether any represent direct targets for translational control by other P granule components. The resolution of the more general question of a causal relationship between P granule components and establishment of the germ lineage also awaits future results.

Post-Embryonic Development and Anatomy of the Somatic Gonad and Germ Line

**Gonadogenesis in the early larval stages (L1 and L2).** At hatching, the gonad is comprised of four cells: the two germline precursor cells, Z2 and Z3, and the somatic gonadal founder cells, Z1 and Z4. These four cells are separated from the non-gonadal soma by a gonadal basal lamina (Fig. 1B). Z1 and Z4 divide during post-embryonic development via a reproducible and largely invariant cell lineage to generate 143 cells in the hermaphrodite and 56 cells in the male (Kimble and Hirsh, 1979). In the hermaphrodite, Z1 and Z4 descendants form the somatic cells of the two U-shaped gonad arms (DTCs and gonadal sheath cells), the spermathecae, and the shared uterus (Fig. 1). In the male, Z1 and Z4 descendants form the somatic cells of a single J-shaped gonad arm, the vas deferens, and the seminal vesicle. In contrast to the somatic gonad lineage, the germ cell divisions appear to be variable with regard to timing and divisions planes (Kimble and Hirsh, 1979). However, the possibility that some lineal relationships within the germ line exist and may be important with regard to choice of sexual fate, meiotic entry, or gametogenesis has not been ruled out.

Z1 and Z4 divide in the middle of the L1 larval stage. Two Z1/Z4 granddaughter cells (Z1.aa and Z4.pp) play critical roles during germline development and are named the distal tip cells (DTCs) because they are located at the anterior and posterior tips of the gonad throughout development in hermaphrodites and at the distal tip of the gonad in males (Fig. 1). The gonad has a distal-proximal axis that corresponds to the development of the germ line from mitosis through meiosis and gametogenesis with the proximal-most point defined as the place where embryos (in the hermaphrodite) or gametes (in the male) exit the worm. Classic cell ablation experiments (Kimble and White, 1981) demonstrated two major roles for the DTCs in hermaphrodites: promoting germline mitosis (and/or inhibiting meiosis) and controlling gonadal outgrowth (so called “leader function”). One salient difference between males and hermaphrodites is that the male linker cell exerts the “leader function” necessary for gonadal outgrowth, whereas the two male DTCs are non-migratory and remain at the distal end to promote germline mitosis and/or inhibit meiosis. These roles of the DTCs are discussed in more detail in sections on the DTC-germline interaction and gonad morphogenesis (see below).

**Gonadogenesis in the later larval stages (Late L2–L4).** By the end of the L2 stage, the hermaphrodite somatic gonad consists of 12 Z1/Z4 descendants (Fig. 1B), whereas the germ line has proliferated to ~ 30 cells (Kimble and White, 1981). The DTCs are arranged at the distal tips of the elongating gonads. By contrast, the remaining ten Z1/Z4 descendants move to the center of the gonad at the L2/L3 molt to form the hermaphrodite somatic gonadal primordium (SPh, as distinct from the male somatic gonadal primordium, SPm; Fig. 1B; see Kimble and Hirsh, 1979). The SPh can form in two alternate arrangements, depending on the outcome of signaling between the cells Z1.aaa and Z4.aaa (Fig. 1B). One of these cells becomes the anchor cell (AC), while the other becomes a ventral uterine precursor cell (VU). The AC is a non-dividing cell that plays several major roles during gonadogenesis: vulval induction (reviewed by Kornfeld, 1997; Sundaram and Han, 1996), uterine cell fate specification, and organization of the uterine-vulval connection. The specification of the AC and its subsequent roles in gonadogenesis are described in more detail below.

As the somatic cells rearrange to form the SPh, the germ cells are displaced from the central region of the gonad and segregate into anterior and posterior populations which fill the two gonad arms (Fig. 1B). In the L3 stage, proximal germ cells enter meiotic prophase. As the gonad elongates, proximally positioned germ cells continue to enter the meiotic pathway. The mechanisms controlling germ cell entry into and progression through the meiotic pathway are discussed below.

Several important reproductive structures, made up of 140 cells, are generated from the L3 and L4 stage divisions of the three classes of blast cells found in the SPh. Four sheath/spermathecal (SS) precursor cells (two per gonad arm) generate the gonadal sheath and the spermathecae, while two dorsal uterine precursor cells (DU) and three VU cells generate the dorsal and ventral uterus, respectively. A number of useful cell-
type specific markers for differentiated cell fates within the somatic gonad have been described (Table 1).

The gonadal sheath. The gonadal sheath cells appear to play several roles important for the structure, integrity, and reproductive functions of the gonad (McCarter et al., 1997; Rose et al., 1997). The ten thin gonadal sheath cells can be subdivided into five pairs (1–5) with each pair having a distinct position along the proximal-distal axis of each gonad arm (Fig. 1C). These elongated myoepithelial cells lie between germ cells and the gonadal basal lamina (Hirsh et al., 1976; Kimble and Hirsh, 1979; Strome, 1986; Hall et al., 1999). The distal sheath cells (pair 1) have an unusual cellular structure with a flattened soma pressed into the gonad such that the cytoplasm is concentrated into a series of wedges that insert between the germ cells. Pair 1 distal sheath cells also extend finger-like filopodia between distal germ cells. The proximal sheath cells (pairs 3–5; see Fig. 2) contain thick and thin filaments and contract to drive ovulation (Strome, 1986; Myers et al., 1996; McCarter et al., 1997; Rose et al., 1997; Hall et al., 1999). The proximal sheath cells are positioned in an interdigitating pattern (Fig. 2), form gap junctions with one another, and are closely apposed to oocytes (Hall et al., 1999). On their basal surfaces, the proximal sheath cells attach to the gonadal basal lamina via hemi-adherens junctions, which also serve to anchor the actin cytoskeleton and the contractile apparatus within the sheath cells. At their apical face, the proximal sheath cells often form gap junctions with oocytes. Yolk particles synthesized by the intestine (Kimble and Sharrock, 1983) gain access to oocytes for receptor-mediated endocytosis (Grant and Hirsh, 1999) by first moving through the sheath pores (Hall et al., 1999). The most proximal sheath cells, pair 5, directly attach to the spermatheca.

The spermatheca and uterus. The spermatheca (1 per gonad arm) is a flexible accordion-like structure connected to the gonad arm distally and to the uterus proximally. The spermatheca expands greatly to accommodate oocytes, which are fertilized as they enter from the gonad arm during ovulation. The walls of the spermatheca are highly involuted, enabling expansion and providing an adherent surface for the spermatozoa awaiting an ovulated oocyte. Surface views of the spermatheca show a series of pleated ridges that may aid in the expansion of the spermatheca (Fig. 3). The uterus connects to the spermathecae and the vulva, functioning as a holding area for developing embryos prior to their expulsion through the vulva. The uterine lineages serve to place the cells in the correct position for morphogenesis (Newman et al., 1996). Following the formation of these structures by the larval-stage divisions, the gonad becomes sexually mature at the adult stage.

Late larval and adult gonadogenesis. The two equivalent gonad arms of the adult hermaphrodite gonad have been described at an ultrastructural level (Hirsh et al., 1976; Hall et al., 1999; see Fig. 1C). The distal portion of the gonad contains syncytial germline nuclei surrounded by incomplete membranes. The germ cells are connected to a core cytoplasm, also called the rachis. The stem-cell population is restricted to the distal-most part of the germ line; germ cells enter meiosis as they move proximally (the regulation of meiotic entry is discussed below). In hermaphro-

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**TABLE 1. Somatic Gonad Markers**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Cells</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>anti-LIN-26</strong></td>
<td>Cells of the early somatic gonad. All cells of the SPh, not the DTC. Uterine cells (L4 and adult).</td>
<td>den Boer et al., 1998</td>
</tr>
<tr>
<td><strong>lag-2::gfp</strong></td>
<td>DTC, AC</td>
<td>Hendersen et al., 1994; Wilkinson et al., 1994; Fitzgerald and Greenwald, 1995</td>
</tr>
<tr>
<td><strong>lin-3::lacZ</strong></td>
<td>AC</td>
<td>Hill and Sternberg, 1992</td>
</tr>
<tr>
<td><strong>cdh-3::gfp</strong></td>
<td>AC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pettitt et al., 1996</td>
</tr>
<tr>
<td><strong>anti-CEH-18</strong></td>
<td>DTC, sheath 1–5</td>
<td>Greenstein et al., 1994</td>
</tr>
<tr>
<td><strong>unc-73::gfp</strong></td>
<td>DTC, sheath</td>
<td>Steven et al., 1998</td>
</tr>
<tr>
<td><strong>lim-7::gfp</strong></td>
<td>Sheath 1–4</td>
<td>Hall et al., 1999</td>
</tr>
<tr>
<td><strong>anti-MHCA, anti-MHCB&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>Sheath 3–5</td>
<td>Rose et al., 1997</td>
</tr>
<tr>
<td><strong>MH27</strong></td>
<td>Apical cell junctions, spermatheca and uterus</td>
<td>Newman et al., 1996</td>
</tr>
<tr>
<td><strong>UL8::lacZ</strong></td>
<td>Spermathecal cells, utse</td>
<td>den Boer et al., 1998</td>
</tr>
<tr>
<td><strong>lin-11::lacZ, lin-11::gfp</strong></td>
<td>π cells</td>
<td>den Boer et al., 1998; Hanna-Rose and Han, 1999; Newman et al., 1999</td>
</tr>
<tr>
<td><strong>cog-2::gfp</strong></td>
<td>π cells</td>
<td>Hanna-Rose and Han, 1999</td>
</tr>
</tbody>
</table>

<sup>a</sup>A list of markers used in several recent publications for assessing somatic gonadal differentiation. See original sources for details of the expression pattern.

<sup>b</sup>Useful for analyzing AC/utse fusion (see Hanna-Rose and Han, 1999; Newman et al., 1999).

<sup>c</sup>See Figure 2.
dites, the first ~40 germ cells to enter meiotic prophase in each gonad arm differentiate as spermatocytes, which complete meiosis to form approximately 160 sperm during the fourth larval stage of development. Upon progression to the adult stage, the germ cells differentiate exclusively as oocytes. Oocytes undergo ovulation and enter the spermatheca where they are fertilized (see below). Early embryonic development takes place in the uterus (Albertson, 1984; Albertson and Thomson, 1993; McCarter et al., 1999).

DEVELOPMENTAL GENETICS OF GONAD MORPHOGENESIS: MODELS FOR CELL FATE SPECIFICATION AND ORGANOGENESIS

AC/VU Signaling

Intercellular signaling is involved in the formation and configuration of the SPh (Fig. 1B). The SPh can form in two alternative configurations depending on the outcome of an intercellular signaling event. The fates of two cells of the SPh, Z1.ppp and Z4.aaa, are naturally variable: in half the worms Z1.ppp becomes the anchor cell (AC) and Z4.aaa becomes a ventral uterine precursor cell (VU), and in the other half Z4.aaa becomes the AC and Z1.ppp becomes a VU (Fig 1B). Therefore, both Z1.ppp and Z4.aaa have the potential to adopt the AC fate or VU fate, but in the wild type, only one AC forms (Kimble and Hirsh, 1979). A combination of cell ablation studies and elegant genetic studies revealed that LIN-12, a founding member of the LIN-12/Notch family of receptors, mediates the interaction between these two cells and ensures that the two will adopt different fates (Kimble, 1981; Greenwald et al., 1983; Seydoux and Greenwald, 1989). In the absence of lin-12 function, both Z1.ppp and Z4.aaa adopt the AC fate, whereas in animals with hypermorphic lin-12 mutations no AC forms and both Z1.ppp and Z4.aaa become VUs. The ligand for this interaction, LAG-2, is similar to the Notch ligands Delta and Serate (Henderson et al., 1993; Tax et al., 1994; Wilkinson et al., 1994). The data support a model in which an initially small difference in receptor and/or ligand activity is amplified by a mechanism that involves transcriptional feedback (Seydoux and Greenwald, 1989; Wilkinson et al., 1994). These studies have given molecular insight into how cells of equal potential adopt
two different fates. (LIN-12/Notch mediated signaling is discussed in more detail below).

The Uterus and the Uterine-Vulval Connection

_Uterine morphogenesis_. Several recent studies have established the _C. elegans_ uterus as a tractable experimental system for studying organogenesis. The uterus, where embryos are housed until egg-laying (~128 cell stage), derives from descendants of the VU cells (Z1.ppa, Z4.aap, and Z1.ppp or Z4.aaa) and the DU cells, Z1.pap and Z4.apa. Ultimately, 28 cells comprise the dorsal uterus and 32 cells form the ventral uterus (Kimble and Hirsh, 1979). A major step toward understanding uterine morphogenesis was the reconstruction of the uterus from electron micrographs of serial sections (Newman et al., 1996). The uterine lumen consists of eight large multinucleate toroidal epithelial cells (ut toroids; Fig. 4) formed by the fusion of certain DU and VU descendants. Cell fusions play diverse roles in animal development and are relatively frequent during _C. elegans_ development (see accompanying Reviews article in this issue by B. Podbilewicz). The uterine system may prove valuable for studying cell fusions since there is relatively little cell migration involved in the formation of the ut toroids: the cell lineage positions the cells in the correct position for the appropriate fusions (Kimble and Hirsh, 1979; Newman et al., 1996).

In addition to forming a cohesive structure containing a lumen properly connected to the spermathecal lumen, the uterine cells (VU descendants) also form the connection between the uterus and the vulva through which eggs must pass to exit the worm during egg-laying. The process by which an internal epithelium becomes connected to an external epithelium is a fundamental problem in developmental biology, to which recent work has provided several insights. Establishment of the uterine-vulval connection requires the proper differentiation of: 1) the uterine seam cells (utse); 2) the uv1 cells (uterine-vulval cell 1; see Fig. 4); and 3) 1° vulval cell progeny (Newman et al., 1996; the 1° vulval progeny, vulF, and vulE cells are depicted in Fig. 4). The utse is H-shaped, with component nuclei on both sides connected by a thin laminar cytoplasmic bridge that separates the forming uterine and vulval lumens (Fig. 4). The utse cells may also function to anchor the uterus within the body cavity, since these cells form attachments with epidermal cells of the lateral hypodermis (seam cells). The uv1 cells form apical cell junctions with the vulF cells and the utse (Newman et al., 1996; see Fig. 4).
**Uterine developmental genetics.** Several recent studies have identified cell-cell interactions and conserved transcription factors that regulate uterine morphogenesis. The LIN-12 receptor (the same receptor that acts in the AC/VU decision described above) is necessary for certain VU descendants to adopt the π fate as opposed to the ρ fate (Newman et al., 1995; Wilkinson and Greenwald 1995; reviewed by Newman and Sternberg, 1996). The π cells are six VU granddaughters that give rise to utse cells and uv1 cells (π progeny, discussed earlier). The ρ cells are six VU granddaughters that give rise to uterine toroidal epithelial cells (ut1-4) and the spermathecal-uterine valve (sujn; Newman et al., 1996, Fig. 4). The LIM domain transcription factor LIN-11 is subsequently expressed in the π cells and their progeny (Newman et al., 1999). In the wild type, the AC fuses with the utse (Newman et al., 1996). However, in lin-11 mutants the utse cell differentiates abnormally and AC/utse fusion fails (Newman et al., 1999). Mutations in cog-2, (for connection of gonad defective) also interfere with the fusion of the AC and utse (Hanna-Rose and Han, 1999). An egg-laying defective phenotype results as a consequence of the AC/utse fusion defects in lin-11 and cog-2 mutants, apparently caused by the blockage of the uterine and vulval lumens by the AC (Hanna-Rose and Han, 1999; Newman et al., 1999). The cog-2 gene encodes a SOX domain transcription factor that is expressed in the π cells. Lineage analysis of π precursors suggests they adopt a normal fate in cog-2 mutants. Thus, cog-2 mutations may cause a very specific defect by either affecting the ability of the utse to fuse with the AC or to induce the fusion event (Hanna-Rose and Han, 1999). Although LIN-11 and COG-2 transcription factors are both expressed in π cells, they are not dependent upon each other for expression (Newman et al., 1999). These studies provide genetic and molecular evidence for the importance of the utse in uterine morphogenesis.

egl-38 is required for the normal uv1 cell fate (Chamberlin et al., 1997). In egl-38 mutants, uv1 cells behave like utse cells (Chamberlin et al., 1997). Furthermore, specification of the uv1 fate is dependent on LIN-3/EGF signaling from the vulF cells (Chang et al., 1999). egl-38 is required for lin-3 expression in vulF cells (Chang et al., 1999) and encodes a PAX protein similar to the mammalian PAX2, PAX5, and PAX8 proteins (Chamberlin et al., 1997). Since PAX2 mutants affect morphogenesis of the mammalian urogenital system (Torres et al., 1995), studies of egl-38 function in *C. elegans* may contribute to an understanding of general aspects of organ morphogenesis.

A major goal of future work will be to elucidate further the molecular underpinnings of uterine morphogenesis. Genetic approaches will continue to be informative for assembling genetic pathways for uterine morphogenesis and for deducing functional relationships between the components. Several screening strategies have proven fruitful but are not yet saturated. For example, many defects in uterine morphogenesis can disrupt egg-laying, an easily scored phenotype. This approach identified new egg-laying defective mutants affecting the uterine vulval connection (Hanna-Rose and Han, 1999). Another approach takes advantage of another easily scored phenotype, an everted vulva (Ev). Mutants with this phenotype often lack a uterus or have an abnormal uterus (Seydoux et al., 1993). Since this phenotypic class is also not saturated, it represents a promising avenue for future genetic studies.

One potential limitation of genetic screens for uterine defects is that uterine morphogenesis occurs late in development and may require essential genes. One way around this limitation is the use of a screening strategy that allows the identification of conditional mutations. Another way is a tissue-specific gene knockout strategy as introduced for studying the role of lin-26 in the formation of the somatic gonad epithelium (den Boer et al., 1998). lin-26 is required for cell fate specification of non-neuronal epithelial cells and confers an embryonic lethal mutant phenotype (Labouesse et al., 1994, 1996). To study later roles for lin-26, den Boer et al., (1998) generated transgenic worms containing lin-26 promoter deletions such that lin-26 was expressed embryonically, but not post-embryonically in the somatic gonad. This analysis revealed multiple roles for lin-26 in development of the somatic gonad, including an essential role in generating the uterine epithelium.

**Gonad Arm Elongation**

Epithelial tube formation, bud extension, and guided cell migration are critical morphogenetic processes for shaping complex organs and tissues (Hogan, 1999). During gonad arm elongation in the *C. elegans* hermaphrodite, the DTCs first migrate in the anterior and posterior directions, respectively, then move dorsally (L3/L4 molt), then finally “about face” (L4 stage), moving centrally along the dorsal surface to generate a U-shape (see Fig. 1). How are these complex morphogenetic processes orchestrated? Important clues have come from the genetic and molecular analysis of gon-1, conserved pathfinding genes (unc-5, unc-6, and unc-40), and genes originally identified based on their function during programmed cell death (ced-2, ced-5, and ced-10).

gon-1 is required for the dual processes of bud extension and epithelialization during gonadogenesis (Blelloch and Kimble, 1999; Blelloch et al., 1999). The migration of “leader” cells (DTCs in the hermaphrodite and the linker cell in the male) provides a simple model for bud extension since a single cell is required for extension of a gonad arm (Kimble and White, 1981). In gon-1 mutants, the leader cells are correctly generated and specified but do not extend cellular processes and fail to migrate. In addition, cell ablation and expression studies were used to demonstrate a role for gon-1 in uterine morphogenesis separable from its role in gonad extension (Blelloch and Kimble, 1999; Blelloch et al., 1999). gon-1 encodes a secreted metalloproteinase, sug-
suggesting that remodelling of the basal lamina may be critical for gonad extension. During gonad extension, the gonadal basal lamina must be remodelled as the leader cells migrate along the basal laminae of the body wall muscles and the epidermis. Analysis of low power EM views of gon-1 mutants revealed DTC abnormalities including prominent plasma membrane invaginations and the accumulation of membranous material. The cellular basis for these abnormalities is unclear but might be related to a primary defect in remodelling the basal lamina. For instance, the DTC may attempt to extend cellular processes at its leading edge but remain trapped within an extracellular matrix (Blelloch and Kimble, 1999; Blelloch et al., 1999). Further EM work and real-time analysis of DTC migration will be very informative. Matrix metalloproteinases play critical roles in mammalian reproductive function and during tumor formation and metastasis (see Hulboy et al., 1997; Matrisian, 1999 for reviews). Thus, cell biological principles gleaned from a study of gon-1 function are likely to be relevant to a wide array of biological processes.

The unc-5, unc-6, and unc-40 genes are part of a global system that controls the directionality of cell migration and axonal pathfinding along the dorsal-ventral axis (Hedgecock et al., 1987, 1990). These genes were identified by virtue of the fact that in unc-5, unc-6, and unc-40 mutants, the DTCs frequently fail to migrate dorsally (Hedgecock et al., 1987, 1990). Null alleles are incompletely penetrant for the DTC migration defects, suggesting that overlapping genetic pathways are involved. unc-6 encodes the likely ortholog of mammalian netrin, required for commissural axon pathfinding (Serafini et al., 1994, 1996). Both UNC-6 and netrin also share sequence similarity with the B2 subunit of laminin and are likely to be associated with the basal lamina (Wadsworth et al., 1996). Molecular and genetic results suggest that unc-5 and unc-40 encode UNC-6 receptors and are expressed by DTCs (Hedgecock et al., 1990; Leung-Hagesteijn et al., 1992;
Chan et al., 1996; Su et al., 2000). Several recent studies have provided insights into the regulation of the *unc-5*/*unc-6*/*unc-40* pathway and its coordination with larval developmental stage progression (Antebi et al., 1998; Su et al., 2000). In *daf-12* and *mig-8* mutants, dorsal migration of the DTCs is delayed or absent (Hedgecock et al., 1987; Antebi et al., 1998). *daf-12* encodes a member of the nuclear hormone receptor superfamily, which is important for regulating larval developmental stage progressions (Yeh, 1991). Thus, the dorsal phase of DTC migration may involve the regulation of the *unc-5*/*unc-6*/*unc-40* pathway by endocrine factors (Antebi et al., 1998). Consistent with this model, the UNC-5 netrin receptor is transcriptionally upregulated in the DTCs late in the L3 stage, at which dorsal migration occurs (Su et al., 2000).

Transcriptional upregulation of *unc-5* is dependent on *daf-12* and *mig-8* function (Su et al., 2000).

Another interesting group of genes affecting the migration of the DTCs is *ced-2*, *ced-5*, and *ced-10* (Ellis et al., 1991). In these mutants, the DTCs often abort migration prematurely or execute supernumerary turns that result in abnormal gonad shapes (Wu and Horvitz, 1998). Ultrastructural and genetic studies revealed that these genes are likely to function in a cellular process shared by cell migration and engulfment and/or phagocytosis after apoptosis (Hedgecock et al., 1983; Ellis et al., 1991; Wu and Horvitz, 1998).

*ced-5* is required in the engulfing cell (Wu and Horvitz, 1998) and encodes a protein with sequence similarity to human DOCK180 (Hasegawa et al., 1996) and *Drosophila* Myoblast City (Erickson et al., 1997). Myoblast City is required for processes that involve cytoskeletal rearrangements, including myoblast fusion, dorsal closure, and epithelial cell migrations (Erickson et al., 1997). Interestingly, DOCK180 interacts with the cytoskeleton-associated protein CRK, which has been implicated in cell migration and integrin-mediated signaling events (reviewed by Giancotti and Ruoslahti, 1999).

DTC migration is likely to be dependent on integrin function since mutations in the *ina-1* α-integrin gene cause many cell migration defects, including DTC migration defects (Baum and Garriga, 1997). Thus, DTC migration may provide a general model for the regulation and function of the cytoskeleton in an array of processes in a multicellular organism.

Other factors involved in gonad morphogenesis and DTC migration are rapidly coming to the fore. For example, *gon-2* is required for the initiation and continuation of post-embryonic somatic gonadal divisions (Sun and Lambie, 1997). In addition, several transcription factors are expressed by DTCs and are required for normal migrations, including the *pax-6*-related gene *vab-3* (Chisholm and Horvitz, 1995; Zhang and Emmons, 1995; Zhang et al., 1998; Nishiwaki, 1999) and the POU-gene *ceh-18* (Greenstein et al., 1994). Further analysis of mutants obtained in large-scale genetic screens may elucidate general molecular mechanisms utilized during DTC migration (Hedgecock et al., 1990; Nishiwaki, 1999).

**CELL CYCLE CONTROL IN THE GERM LINE**

Germline Mitosis and Meiosis

*C. elegans* germ cells, like those in many organisms, undergo a pre-meiotic period of proliferation followed by a post-meiotic period (beginning late in the L3 stage) during which some germ cells enter meiosis while others remain mitotic. The mitotic germ cell population that is present in the post-meiotic period can be regarded as a germline stem-cell population since it persists for the life of the organism, is self-renewing, and produces cells that can differentiate as gametes. In *C. elegans*, several aspects of germ line proliferation are controlled by soma-to-germ line signaling.

**Control of early germline proliferation.** Once the gonad primordium is established, the proliferation of the germline precursors, *Z2* and *Z3*, is dependent on first, the presence of food, and second, the presence of the precursors to the somatic gonad, *Z1* and *Z4*. If *L1* larvae hatch in the absence of food, many blast cells (including *Z2* and *Z3*) fail to divide until food is present. Larvae can survive for several days under these starvation conditions (Ambros, 1997). Recently, Subramaniam and Seydoux (1999) observed that two of the three *C. elegans* nanos-related genes and several *pumilio*-related genes are required for *Z2* and *Z3* to arrest in the absence of food. For example, in larvae devoid of *nos-1* and *nos-2* function, *Z2* and *Z3* often divide inappropriately in the absence of food (Subramaniam and Seydoux, 1999). The nature of the signal(s) that indicate the presence (or absence) of food has not been defined. The proliferation of *Z2* and *Z3* also requires a signal from the somatic gonad blast cells *Z1* and *Z4*. If *Z1* and *Z4* are ablated by a laser microbeam, *Z2* and *Z3* neither divide nor enter meiosis (Kimble and White, 1981). Indeed, the ablation of embryonic precursors to *Z1* and *Z4* results in the death of *Z2* and *Z3* (Kimble and White, 1981). The molecular basis of these signaling events is unknown but is amenable to genetic analysis. In contrast to later post-embryonic germline proliferation, these early proliferative signaling events do not appear to be mediated by the GLP-1 receptor (see below for discussion of GLP-1).

Mutations in several additional loci cause severe early germline proliferation defects. Animals bearing mutations in either *glp-3* (Kadyk et al., 1997) or *glp-4* (Beanan and Strome, 1992) produce approximately 10–20 germ cells instead of the usual total (~1,500). In *glp-3* mutants, germ cells apparently arrest in prophase (or late G2) of the mitotic cell cycle (Kadyk et al., 1997). Germ cells in *glp-4*(*bn2ts*) mutants arrest in prophase of the mitotic cell cycle at the non-permissive temperature (Beanan and Strome, 1992). Interestingly, animals homozygous for non-conditional *glp-4* alleles display only modest defects in germline proliferation and, instead, exhibit major defects in meiotic prophase progression (Qiao et al., 1995). Thus, *glp-3*
and glp-4 may define genes with important cell cycle functions in the germ line. Cell ablation studies (Mc
carter et al., 1997) demonstrate a role for the sheath/
spermathecal lineages in promoting germline prolifer-
tion, though an understanding of the basis for this
effect will require further work.

Mutations in genes that encode cell-cycle compo-
nents such as ncc-1, a C. elegans Cdk1 homolog (Boxem
et al., 1999), or emb-30, a C. elegans APC4 homolog
(Furuta et al., 2000), affect general aspects of post-
embryonic cell division and also interfere with early
germine proliferation. Mutations in other genes such
as emb-5 appear to adversely affect the efficiency of
mitosis without interfering with meiosis (Hubbard et
al., 1996). Future work will define more precisely the
roles of these and other genes in germline develop-
ment.

GLP-1-mediated germline proliferation. Signal-
ing between the somatically derived DTC and the germ
line in C. elegans is a classic example of induction. In
seminal experiments, Kimble and White (1981) showed
that ablation of one DTC causes all germ cells in the
same gonad arm to enter meiosis. Signaling between
the DTC and the germ line is mediated by GLP-1, a
LIN-12/Notch receptor family member, and utilizes a
core signaling pathway that is conserved among meta-
zoans. Activity of GLP-1 in the germ line promotes
mitosis and/or inhibits meiosis. In the absence of glp-1
function, ~4–8 germ cells are produced that enter
meiosis early and differentiate as sperm (Austin and
Kimble, 1987). Signal-independent activation of the
GLP-1 receptor caused by the dominant glp-1(oz112)
mutation results in a tumorous phenotype in which
germin cells divide continuously and never enter meiosis
(Berry et al., 1997; see Fig. 5).

Genetic and biochemical studies have converged on a
general mechanism for LIN-12/Notch-mediated signal
transduction. Briefly, ligand binding results in a series
of proteolytic events that gives the intracellular do-
main of the receptor access to the nucleus where, in
concert with nuclear factors, it brings about changes in
gene expression (see Greenwald, 1998; Kimble et al.,
1998; Artavanis-Tsakonas et al., 1999 for review). In
the C. elegans germine mitosis/meiosis decision, the
DTC-produced ligand LAG-2 [a member of the DSL
(Delta, Serrate, LAG-2) family of ligands] interacts
with the germline-produced receptor GLP-1 (Lambie
and Kimble, 1991; Crittenden et al., 1994; Henderson
et al., 1994; Tax et al., 1994). A positive downstream
effector of this signaling in the germ line is LAG-1, a

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Fig. 5. Tumorous phenotype of glp-1(oz112gf) germ line. DAPI-
stained dissected gonads showing germline nuclear morphology. A:
Wild-type adult hermaphrodite gonad. B: Homozygous glp-
1(oz112 gf) adult hermaphrodite gonad (25°C). Arrows indicate mitotic
nuclei. Scale bars = 10 μm. Courtesy of Laura Wilson Berry and Tim
Schedl, modified from Berry et al., 1997. Used with permission of the
Company of Biologists Ltd.
have identified the phenotypic effects of mutations in the LIN-12–mediated signaling. Large-scale genetic screens have exploited to identify loci encoding modifiers of GLP-1 and gld-1 (ego) andSel-12 (Sel-1) family of transcriptional regulators (Christensen et al., 1996; Berry et al., 1997). Mutations that disrupt the function of any of these core components, putative activity of the GLP-1 receptor (Berry et al., 1997). Furthermore, this gld-2 gld-1 double mutant phenotype is independent of glp-1 activity (Kadyk and Kimble, 1998). Distinct from their redundant role in meiotic entry, gld-1 (see below for further discussion of gld-1) and gld-2 play non-redundant roles in gametogenesis (Francis et al., 1995a,b; Kadyk and Kimble, 1998).

The genetic tractability of C. elegans and its distinctive gonadal and germ line phenotypes have been exploited to identify loci encoding modifiers of GLP-1 and LIN-12–mediated signaling. Large-scale genetic screens for second-site mutations that suppress and/or enhance the phenotypic effects of mutations in glp-1 or lin-12 have identified sog (suppressor of glp-1), ego (enhancer of glp-1), and sel (suppressor and/or enhancer of lin-12) genes (Maine and Kimble, 1993; Qin et al. 1995; Sundaram and Greenwald, 1993; Tax et al., 1997; Levitan and Greenwald, 1995). The fact that genetic interactions are observed between sel genes and both lin-12 and glp-1 is not surprising since the LIN-12 and GLP-1 proteins are partially redundant (Lambie and Kimble, 1991) and functionally interchangeable (Fitzgerald et al., 1993).

The suppressor/enhancer approach affords a powerful and relatively unbiased means to identify components and modifiers of LIN-12/Notch signaling. In addition to the conserved core signaling components lag-1 and lag-2 (Qiao et al., 1995; Tax et al., 1997), loci encoding other important factors have been defined by suppressor/enhancer alleles. These genes encode products acting at many different steps of the signaling pathway. Proteins involved in receptor proteolysis, processing and trafficking have been identified such as SEL-12 (Levitan and Greenwald, 1995), SEL-1 (Grant and Greenwald, 1996, 1997), SEL-9 (Wen and Greenwald, 1999), and SUP-17 (Tax et al., 1997; Wen et al., 1997). Activity of the intracellular domain of the receptor is influenced by SEL-10, which contains an F-box and likely functions in ubiquitin-mediated protein degradation (Hubbard et al., 1997). Further experiments will clarify the roles of these and other more recently molecularly characterized genes such as sel-5 (Fares and Greenwald, 1999), and ego-1 (Smardon et al., 2000; see below), as will molecular analysis of additional sog, ego and sel genes.

**Patterning of Germline Development and Entry into Meiosis**

In the late-larval and adult germ line, mitotic cells occupy the distal-most region. The proximal border of this mitotic zone is quite distinct; yet it is not apparent what mechanisms delineate it so clearly. The distribution of metaphase nuclei can be observed in fixed gonad preparations: they are more frequently observed in the central part of the mitotic zone, though occasionally they can be observed as far away as 25 cell diameters from the distal tip (Crittenden et al., 1994; Hall et al., 1999). The DTC extends tentacle-like processes or cytonemes that extend on average approximately eight cell diameters from the distal tip, corresponding to the average position of mitotic nuclei, though occasionally these processes can be seen to extend to the maximal extent of the mitotic zone (Fitzgerald and Greenwald, 1995; Hall et al., 1999). Therefore, one possible mechanism for placement of the proximal border of the mitotic zone is that the ligand in the DTC plasma membrane contacts cells directly in order to activate GLP-1 and maintain proliferation of nearby cells. Consistent with this possibility, the transmembrane domain of the LAG-2 is critical for proper localized function (Fitzgerald and Greenwald, 1995; Henderson et al., 1997). In addition, it is possible that there is some germline-mediated transduction of the signal to more proximal cells. Expression studies indicate a high level of plasma membrane-associated GLP-1 protein is restricted to the distal mitotic zone of the adult hermaphrodite gonad (Crittenden et al., 1994). Since glp-1 RNA is found throughout the germline, translational control is likely to play a significant role (Crittenden et al., 1994). Consistent with this hypothesis, lacZ reporter mRNAs bearing the glp-1 3’-UTR are translated only in the distal germ line (Evans et al., 1994). Interestingly, GLP-1 protein is present at high levels throughout the gonad in dominant glp-1(oz112) mutants that exhibit a tumorous germline phenotype (Berry et al., 1997). Based on this result, a positive feedback mechanism between GLP-1 signaling and glp-1 expression has been proposed (Berry et al., 1997). Further studies will be needed to elucidate the mechanisms underlying this feedback control. Finally, it is also possible that multiple mechanisms, both glp-1-dependent and glp-1-independent, contribute to the precise positioning of the border. For example, the distal sheath filopodia extend to the proximal border of the mitotic zone (Hall et al.,
1999), but the functional significance of the sheath filopodia vis-à-vis mitosis is not known.

The nature of the germline stem-cell population itself has not been well defined. Clearly all of the germ cells have the potential to enter meiosis if GLP-1-mediated signaling is removed, but the question remains as to whether the stem-cell population is uniform in its potential across the ~20 cell diameter region in which meiosis can occur. That is, do certain distal-most cells exhibit classic stem-cell behavior in which asymmetrical division results in a stem cell and a daughter cell that moves proximally (itself undergoing additional divisions)? Or is the process more random within the population?

Another intriguing question is how the distal-to-proximal pattern of meiosis and mitosis is established and maintained during earlier stages of germline development. How is the initiation and maintenance of meiosis spatially restricted to the proximal-most region of the developing germ line? One hypothesis is that the proximal-most germ cells enter meiosis by default due to their increased distance from the DTC as the germ line grows. Recent analyses point to the likely existence of additional mechanisms that refine spatial and temporal aspects of germline patterning. Several genetic and anatomical manipulations (including perturbations of the proximal somatic gonad) can interfere with the spatial patterning of meiosis and mitosis to cause a proximal proliferation (Pro) phenotype in which ectopic mitotic germ cells are found proximal to meiotic cells and gametes (Seydoux et al., 1990; Henderson et al., 1997; Kadyk and Kimble, 1998; Schedl, 1997; Westlund et al., 1997; E.J.A.H., unpublished results). This phenotype results in sterility since the gametes never gain access to the proximal part of the gonad. Molecular analysis of genes that mutate to this phenotype will provide additional insight into the spatial and temporal regulation of the mitosis/meiosis decision during germline development.

**Control of Meiotic Progression**

As germline nuclei progress proximally out of the distal mitotic zone, they enter meiosis (Fig. 1C). The region where germ cells undergo the transition from the mitotic cell cycle to meiotic prophase is called the transition zone (Crittenden et al., 1994). Transition zone nuclei often have a characteristic crescent-shaped cytological appearance. This morphology likely corresponds to leptotene and zygotene stages of prophase of meiosis I. Cytologically recognizable nuclei in the pachytene stage of meiotic prophase I are clearly visible before the loop. The germline nuclei complete the process of cellularization within the proximal region of the gonad (Fig 1C). The exact point at which germline nuclei no longer have access to syncitial cytoplasm, such that they are fully cellularized, has not yet been determined.

**Early prophase of meiosis I: ego-1.** Mutations in ego-1 were originally identified as enhancers of a temperature sensitive partial loss-of-function allele of glp-1. On their own, ego-1 mutants display defects in many steps in germline development including mitosis, meiosis, and gametogenesis, but they exhibit a distinctive phenotype in meiotic progression (Smardon et al., 2000). At any time in post-meiotic development of the germ line, the transition zone is proportionally larger in ego-1 mutant animals than in the wild type, suggesting a role in the execution of early stages of meiosis. The ego-1 locus encodes a protein with sequence similarity to tomato RNA-directed RNA polymerase and Neurospora crassa QDE-1, both of which have been implicated in post-transcriptional gene silencing. Indeed, ego-1 may be involved in mediating some aspects of RNA-mediated interference in the germ line (Smardon et al., 2000; see below).

**Meiotic progression: gld-1.** A striking phenotype is observed in gld-1(null) mutants: germ cells in the female mode (i.e., those destined to become oocytes) enter meiosis normally from the distal stem-cell population, proceed to pachytene, but then exit meiosis and re-enter the mitotic cell cycle (Francis et al., 1995a). Thus, in gld-1 mutant hermaphrodites mitotic germ cells replace oogenesis and a germline tumor forms. The GLD-1 protein contains a KH domain, an RNA-binding motif (Jones and Schedl, 1995). gld-1 plays additional non-essential roles in germline development including a positive role in promoting spermatogenesis in hermaphrodites and a negative role in regulating mitotic proliferation in both sexes (Francis et al., 1995a,b). One hypothesis is that gld-1 prevents inappropriate production of proteins that normally act in later meiotic divisions in the germ line or the mitotic divisions in the early embryo (Francis et al., 1995a; Jones et al., 1996; Schedl, 1997). Consistent with this hypothesis, GLD-1 functions as a translational regulator of the tra-2 mRNA, which encodes a key sex determination protein (Jan et al., 1999). The identification of additional targets of GLD-1 will be critical for understanding the role of gld-1 in meiotic progression and oogenesis.

**Pachytene exit: the RAS/MAP kinase pathway.** Once germ cells have entered meiosis, progression beyond the pachytene stage of meiosis I requires the MAP kinase signaling pathway in the germ line (Church et al., 1995). Mutations in let-60 (RAS), mpk-1/sur-1 (MAP kinase), and mek-2 (MAP kinase kinase) cause a similar phenotype in which exit from the pachytene stage of prophase of meiosis I is blocked. Mutations in the RAS/MAP kinase pathway genes also result in gonad disorganization (Church et al., 1995). Cell ablation studies suggest that cells in the sheath/spermathecal lineages also promote pachytene exit (McCarter et al., 1997). The distal sheath cells (pair 1 and 2) could promote the exit of germ cells from the pachytene stage by triggering RAS/MAP kinase pathway activation in the germ line. Strong support for this model would come from the identification of genes required in the somatic gonad for pachytene exit. In contrast to mutations in RAS/MAP kinase pathway genes, sheath cell
ablations do not cause gonad disorganization (McCarter et al., 1997).

**Meiotic Maturation, Ovulation, and Completion of the Meiotic Divisions**

Fully grown oocytes remain in the diakinesis stage of prophase I prior to undergoing meiotic maturation, ovulation, and fertilization. The nuclear envelope of the most proximal oocyte breaks down ~5 min prior to ovulation as it enters meiotic M-phase from prophase (Ward and Carrel, 1979; 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 proximal sheath cells and the distal spermatheca resulting in ovulation. Laser ablation studies demonstrate a critical role for the proximal sheath and the distal spermatheca in ovulation (McCarter et al., 1999). During ovulation the mature oocyte enters the spermatheca and is fertilized. The fertilized oocyte then passes into the uterus where both meiotic divisions are completed and embryogenesis begins (Albertson, 1984; Albertson and Thomson, 1993; McCarter et al., 1999).

The late stages of oocyte development (including meiotic maturation, ovulation, and the completion of the meiotic divisions following fertilization) provide a robust experimental system for studying the regulation of meiotic cell cycle progression by extracellular signals. Several experimental advantages have made this stage of development particularly amenable to molecular, genetic, and cell biological analyses: the oocyte is large, the bivalents are cytologically observable, the cell cycle events are well described, and maturation/ovulation can be observed directly by time-lapse videomicroscopy (Ward and Carrel, 1979; Albertson, 1984; Albertson and Thomson, 1993; McCarter et al., 1997; Rose et al., 1997; Hall et al., 1999). Mutations that lead to defective ovulation cause an endomitotic oocyte (Emo) phenotype (Iwasaki et al., 1996). The Emo phenotype results when oocytes undergo multiple rounds of nuclear envelope breakdown (M-phase entry) and S-phase, without cytokinesis or karyokinesis, and become highly polyploid. A large class of genes can mutate to an Emo phenotype and these include emo-1 (Iwasaki et al., 1996), mup-2 (Myers et al., 1996), ceh-18 (Greenstein et al., 1994; Rose et al., 1997), and rme-2 (Grant and Hirsh, 1999). 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). rme-2 encodes a member of the LDL receptor superfamily and is the likely oocyte yolk receptor (Grant and Hirsh, 1999). Interestingly, rme-2 mutants display ovulation defects (Grant and Hirsh, 1999), consistent with the idea that the maturing oocyte actively participates in its own ovulation (Iwasaki et al., 1996). During meiotic maturation, nuclear envelope breakdown is promoted by the CDK1-related kinase NCC-1 (Boxem et al., 1999). Thus, CDK-1 activation is likely to be downstream in a signal transduction pathway triggered by the presence of spermatids or spermatozoa (see below). The signal transduction pathway is likely to be branched since animals in which ncc-1 is not functional are defective in nuclear envelope breakdown, but not cortical rearrangement or ovulation (Boxem et al., 1999).

Four cell-cell interactions that regulate oocyte meiotic maturation and ovulation have been defined by McCarter et al. (1999) in a landmark study. First, a sperm-associated signal(s) promotes oocyte meiotic maturation independent of fertilization. In females (genetically altered XX animals that make only oocytes), oocytes mature and are ovulated at an abnormally low rate. Mating to wild-type males, or fertilization incompetent sperm defective (sper) mutants, restores the normal rate of oocyte maturation. The nature of this signal is unknown but may be amenable to biochemical analysis since methods for artificial insemination using sperm transfer are available (LaMunyon and Ward, 1994). Second, a sperm-associated signal stimulates the basal contractile activity of the proximal sheath cells. It is not known whether the activity affecting the oocyte is the same as that affecting the proximal sheath cells. The presence of an oocyte is not required for the sheath contractile promoting activity, since XX animals with a masculinized germline exhibit frequent sheath cell contractions. Third, the maturing oocyte modulates sheath contractions at ovulation, which includes an increase in contraction rate and intensity during ovulation. Fourth, the maturing oocyte induces spermathecal dilation during ovulation.

Spermathecal dilation is likely to involve a LIN-3/EGF/LET-23(EGF-receptor) signal transduction pathway mediating the signal from oocyte to the distal spermatheca (J. McCarter, B. Bartlett, T. Dang, R. Hill, M. Lee, and T. Schedl, unpublished results). Unlike the let-23 signal transduction pathway required for vulval development (see Kornfeld, 1997; Sundaram and Han, 1996 for a review), the pathway required for spermathecal dilation during ovulation is let-60(ras)-independent and involves a downstream IP₃-mediated pathway. Mutations in two genes, lfe-1/itr-1 and lfe-2 (likely gain-of-function and loss-of-function, respectively), were isolated in a screen for gonad-specific suppressors of a let-23 mutant that causes sterility and vulva defects. Mutations in these genes restore fertility but retain vulval defects. lfe-1/itr-1 and lfe-2 genes encode an inositol (1, 4, 5) triphosphate receptor and an inositol (1, 4, 5) triphosphate-3-kinase, respectively (Cladinin et al., 1998). This result suggests that spermathecal dilation is likely to be dependent on calcium release regulated by IP₃. Spermathecal dilation at ovulation may also require the function of a myosin phosphatase regulatory subunit encoded by the mel-11 gene (Wissmann et al., 1999).
The oocyte is normally fertilized as it enters the spermatheca during ovulation. The meiotic divisions are completed after transfer to the uterus where embryogenesis ensues (Albertson, 1984; Albertson and Thomson, 1993). In the absence of fertilization, ovulated oocytes do not complete the meiotic divisions (Ward and Carrel, 1979). Rather, they undergo endomitotic cycling and become highly polyploid. There is an experimentally unresolved issue concerning the meiotic status of endomitotic oocytes. Ward and Carrel (1979) reported that endomitotic oocytes complete meiosis I (MI), but not meiosis II (MII), prior to becoming endomitotic. However, in a re-evaluation, polar bodies were not observed by DAPI staining of ovulated but unfertilized oocytes (D. Greenstein, unpublished results). However, the possibility that they rapidly fuse with the endomitotic chromatin was not excluded. Therefore, further studies, including real-time analyses, will be needed to resolve this issue. Ovulated but unfertilized oocytes appear to set up a meiotic spindle, indicating they can progress as far as metaphase of MI (Furuta et al., 2000). Thus, one view is that fertilization triggers the ability of the oocyte to complete both meiotic divisions and suppresses endomitotic cycling. Other known activities of the sperm are providing the centrosome (Albertson, 1984), activating embryogenesis (see Browning and Strome, 1996), inducing the A/P axis (Goldstein and Hird, 1996), and providing the paternal genome (Sadler and Shakes, 2000).

GERMLINE SURVIVAL AND AGING

Germline Survival

Specialized cellular functions are needed for germ cells to complete their unique and complex developmental program. Efforts to identify these functions have relied on large-scale genetic screens for maternal-effect sterile (mes) mutations. These screens have identified a set of genes (mes-2, mes-3, mes-4, and mes-6) that are maternally necessary and sufficient for the survival of germ cells (Capowski et al., 1991; Paulsen et al., 1995; Garvin et al., 1998). The progeny of homozygous mes mutant mothers exhibit a sterile phenotype. Although embryonic development of the germ line appears normal in progeny of mes mutant mothers, germ-line proliferation is reduced and gametes do not form. Phenotypic analysis indicates that germ cells undergo degeneration and ultimately die in these mes mutants. Germ cell death in mes mutants appears to be necrotic or degenerative, by morphological and genetic criteria (Garvin et al., 1998). Interestingly, mes mutant males, though affected, exhibit milder germline phenotypes and can be fertile. A convincing series of genetic tests established that this difference relates to X chromosome dosage rather than sexual phenotype per se (Garvin et al., 1998).

Molecular analysis of several mes genes has shed considerable light on the role of this important gene class. MES-2 contains a SET domain and a CXC domain and is similar to Drosophila Enhancer of zeste, a member of the Polycomb group (PcG) of transcriptional regulators (Holdeman et al., 1998). Similarly, MES-6 is related to the Drosophila PcG member Extra sex combs and contains seven WD repeats (Korf et al., 1998). By contrast, mes-3 encodes a pioneer protein (Paulsen et al., 1995). Both MES-2 and MES-6 are nuclear proteins and are found in germ cells, as well as many other cells. Interestingly, MES-2 and MES-6 are mutually dependent for proper localization as well as dependent on mes-3 (Holdeman et al., 1998; Korf et al., 1998). The current model is that these mes genes are required for controlling gene expression in the germ line and that proper gene expression is required for germ line proliferation and survival. Identification of mes targets in the germ line would provide a major advance, and may be aided by genome-wide expression analyses (see below).

Several C. elegans nanos-related genes are required redundantly for germ cell survival beyond the L3 stage. Germ cell death in animals with reduced nos-1 nos-2 function appears to be independent of the apoptotic pathway since this germ cell death still occurs in ced-3 and ced-4 mutant backgrounds (Subramaniam and Seydoux, 1999; Kraemer et al., 1999; see below). This cell death does, however, resemble apoptosis morphologically in that dying cells adopt the characteristic “button-like” shape in a ced-4-dependent manner (Subramaniam and Seydoux, 1999). Future experiments will likely define other components of the nos-dependent pathway required for germ cell survival.

Apoptosis in the Germ Line

Recently, it has been recognized that apoptosis occurs during the normal process of oogenesis in C. elegans (Gumienny et al., 1999), as in other organisms (McCall and Steller, 1998; see also Morita and Tilly, 1999 for a review). It has been estimated that roughly one half of all female germ cells die in the adult hermaphrodite gonad (Gumienny et al., 1999). Cell death occurs exclusively during the adult stage and primarily in the loop region containing pachytene stage meiotic germ cells. Pachytene stage cells are transcriptionally active (Starck, 1977; Gibert et al., 1984) and may serve as a nurse cell population, which is culled by programmed cell death (Gumienny et al., 1999). Germ cell corpses are rapidly engulfed by gonadal sheath cells. Programmed cell death in the germ line requires the core apoptotic machinery: the caspase CED-3, the Apaf-1 homolog CED-4, and the Bcl-2 family member CED-9 (reviewed by Cryns and Yuan, 1998; Metzstein et al., 1998). The current model is that the BH3-domain protein EGL-1 is a cell death activator that binds CED-9, thereby releasing CED-4, resulting in CED-3 activation (Conradt and Horvitz, 1998). There are several major differences between the germline and somatic cell death pathways. First, the gene egl-1 is required for many or all somatic cell deaths (Conradt and Horvitz, 1998) but is not required for germline deaths (Gumienny et al., 1999). Second, the dominant muta-
tion ced-9(n1950) blocks programmed cell death in the soma (Hengartner et al., 1992) but not in the germ line (Gumienny et al., 1999). Finally, programmed cell death in the germ line occurs in a syncytium and therefore requires mechanisms to restrict CED-3 activation to the dying germ cell. Clearly then, cell death in the germ line must be regulated by other means. Indeed, the RAS/MAP kinase pathway is required for germline programmed cell death. Since these genes are also required for exit from the pachytene stage of the meiotic cell cycle (see above), it is unclear whether this requirement reflects a cell cycle stage dependence of the apoptotic process or a distinct function (Gumienny et al., 1999).

Control of Aging by the Germ Line and Somatic Gonad

In an elegant set of experiments designed to test the hypothesis that a trade-off exists between longevity and reproduction, Hsin and Kenyon (1999) observed that the germ line in C. elegans exerts a negative influence on lifespan. Previous experiments had shown that lifespan is not extended when all four cells of the gonad primordium (Z1, Z2, Z3, and Z4) are ablated with a laser microbeam (Kenyon et al., 1993). The recent study demonstrates that if Z1 and Z4 (the somatic gonad precursors) are left intact, ablation of the germ line precursors (Z2 and Z3) extends lifespan. Taken together, the results imply a role for the germ line in lifespan-shortening and a role for the somatic gonad in lifespan-extension. Since ablation of Z1 and Z4 prevents the development of Z2 and Z3, it is not possible to test the role of Z1 and Z4 directly. A lifespan-extending activity of the somatic gonad becomes apparent, however, with the results of ablations performed in various daf (dauer formation defective) mutant backgrounds known to affect lifespan. Lifespan in C. elegans is regulated hormonally and involves the function of an insulin/IGF-1 receptor (Kimura et al., 1997; Apfeld and Kenyon, 1998). The lifespan-extending activity of the somatic gonad is likely to involve the insulin/IGF-1 receptor pathway and the activity of neurons that sense the environment (Hsin and Kenyon, 1999; Apfeld and Kenyon, 1999). The exact mechanism by which the somatic gonad and the germ line influence aging will be the focus of future work.

PERSPECTIVES AND FUTURE DIRECTIONS

Forward Genetics

Traditional forward genetic approaches will continue to provide a promising starting point for elucidating molecular mechanisms underlying gonadogenesis. The availability of an essentially complete genomic sequence (C. elegans Sequencing Consortium, 1999) and a marker-dense physical map (Coulson et al., 1995) facilitates these studies as does the increasing availability of markers for germline and somatic cell fates. A number of factors can be taken into account when designing genetic strategies for identifying gonad-defective mutants. First, maternal contributions to development of the germ line and somatic gonad can be quite extensive. Second, many important genes for gonadal development also have essential embryonic functions, and thus have maternal and/or zygotic embryonic lethal mutant phenotypes. In this regard, analysis of conditional mutations is a promising approach. Another potentially powerful approach is provided by genetic mosaic screens (Bucher and Greenwald, 1991) for essential zygotic genes that function in the somatic gonad (MS-derived) or the germ line (P4-derived). Anatomical studies such as EM ultrastructural studies of the wild type and mutants will also continue to be critical for understanding the cellular events underlying gonadogenesis.

Functional Genomics

Several recent discoveries have made reverse genetic approaches particularly attractive. Double stranded (ds) RNA-mediated interference (RNAi) is particularly effective in the germ line (Fire et al., 1998), apparently acting at a post-transcriptional level to reduce gene expression (Montgomery et al., 1998; Bosher et al., 1999). Phenotypes that mimic loss-of-function mutations in the targeted gene can be produced by injecting, feeding, or soaking animals with dsRNA (Fire et al., 1998; Tabara et al., 1998; Timmons and Fire, 1998). In addition, PCR-based gene knock-out methods facilitate the isolation of mutant alleles for predicted genes (Jansen et al., 1997; Dernburg et al., 1998). The large-scale application of this knock-out technology by the C. elegans Gene Knockout Consortium, together with systematic expression screens (Y. Kohara, personal communication), will undoubtedly provide valuable reagents for studying gonadogenesis. Also, germline development is likely to be amenable to genome-wide expression analyses using microarray technology (DeRisi et al., 1997). Finally, studies of gonadogenesis will also benefit from high through-put genome-wide approaches such as protein interaction mapping (Wahlout et al., 2000).

Reverse genetic advances are crucial since they provide a means to obtain functional read-outs for genes solely identified by genomic, molecular, or biochemical methods. For example, in one study, a modified version of the two-hybrid system was used to identify two proteins (FBF-1 and FBF-2) that bind the 3’-UTR of the germline sex determination gene fem-3 and are related to Drosophila Pumilio (Zhang et al., 1997). RNAi established that fbf-1 and fbf-2 are essential for the switch from spermatogenesis to oogenesis in hermaphrodites (Zhang et al., 1997). Additional pumilio-related genes in C. elegans and other organisms were then identified by database searches, defining the Puf superfamily (Zhang et al., 1997). Finally, some additional functional features of C. elegans Puf family members were deduced by RNAi experiments (Subramaniam and Seydoux, 1999).
RNAi is a boon for the molecular identification of genes encoding products that act in the germ line. Transformation rescue is the most common method used in the cloning of *C. elegans* genes identified by loss-of-function mutations. However, rescue of germ-line mutant phenotypes is problematic because transgenes, often carried on repetitive extrachromosomal arrays in worms, are generally not expressed in the germ line. Therefore, RNAi can be used as a means of testing candidate genes for “phenocopy” effects once the germ-line-acting gene has been mapped to a small region of the genome. Although this germ-line transcriptional silencing has hampered rescue experiments (and reporter-gene expression and development of enhancer-trapping technology), investigation of this phenomenon has led to interesting insights into germ-line-specific transcriptional effects. In addition, it was this technical limitation that ultimately led to the discovery of RNAi from attempts to use antisense technology as an alternate means of cloning (Guo and Kempheus, 1995; Fire et al., 1998). The transcriptional silencing of repetitive extrachromosomal arrays has been proposed to reflect their possible heterochromatin-like structure (Kelly et al., 1997). Consistent with this hypothesis, increasing the complexity of the injected DNA by diluting with random DNA can result in germ-line expression (Kelly et al., 1997). Moreover, the germ-line silencing mechanism depends on the maternal effect sterile genes *mes-2, mes-3, mes-4,* and *mes-6* (Kelly and Fire, 1998). *mes-2* and *mes-6* encode Polycomb group proteins, suggesting that they may repress expression from extrachromosomal arrays by altering chromatin structure (Holdeman et al., 1998; Korf et al., 1998). Suppression of certain aspects of transcription may be a general feature of germ line specification (see Saffman and Lasko, 1999 for review).

Recently, a connection between transcriptional gene silencing of repetitive arrays, repression of germ-line mobility of transposons, and RNAi has been provided by the discovery of mutants that are defective in all three processes (Tabara et al., 1999b; Ketting et al., 1999). Similarly, EGO-1 is required for normal germ-line development as well as RNAi in the germ line (Smardon et al., 2000). A better understanding of the molecular mechanisms underlying these related phenomena will undoubtedly facilitate technological advances for studying the germ line.

**Prospects for the Field**

Over the last several years, advances in this field have been impressive. In some ways however, these studies have only begun to scratch the surface. The underlying biological complexity is being revealed by the powerful approaches currently available for studying gonadogenesis in *C. elegans*. The relative anatomical simplicity and molecular genetic tractability of the worm provides a means for unearthing the molecular foundations of this process with undoubted relevance to other systems, including mammals. In addition, the *C. elegans* gonad provides fertile ground for probing fundamental issues in cell and developmental biology.

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