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Physiological Control of Germline Development

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Abstract

The intersection between developmental programs and environmental conditions that alter physiology is a growing area of research interest. The *C. elegans* germ line is emerging as a particularly sensitive and powerful model for these studies. The germ line is subject to environmentally regulated diapause points that allow worms to withstand harsh conditions both prior to and after reproduction commences. It also responds to more subtle changes in physiological conditions. Recent studies demonstrate that different aspects of germ line development are sensitive to environmental and physiological changes and that conserved signaling pathways such as the AMPK, Insulin/IGF, TGF β , and TOR-S6K, and nuclear hormone receptor pathways mediate this sensitivity. Some of these pathways genetically interact with but appear distinct from previously characterized mechanisms of germline cell fate control such as Notch signaling. Here, we review several aspects of hermaphrodite germline development in the context of “feasting,” “food-limited,” and “fasting” conditions. We also consider connections between lifespan, metabolism and the germ line, and we comment on special considerations for examining germline development under altered environmental and physiological conditions. Finally, we summarize the major outstanding questions in the field.

Keywords

Stem cell; *C. elegans*; Nutrient; Diapause; Lifespan; TOR; TGF β ; Insulin; Notch; AMPK

5.1 Introduction

Natural selection favors species that evolve mechanisms to reproduce within the limits of available resources. It is not surprising, therefore, that reproduction, including the development and function of the germ line, might be intimately linked to aspects of physiology that are influenced by environmental conditions. Successful species might also

optimize reproduction when environmental resources are abundant and conserve reproductive energy expenditure when resources are scarce.

The nematode *C. elegans* likely represents an extreme case with respect to a boom-and-bust economy of resources and the evolution of developmental and reproductive plasticity to cope with these ecological challenges. One of the most striking examples of this plasticity is the multiple stages at which worms can undergo a reversible developmental arrest or diapause, allowing for dispersal while delaying or suspending reproduction. Prior to a commitment to reproduction, worms can enter a diapause at least two times during development. If conditions are extremely poor, they arrest in the first larval stage (L1) just after hatching (L1 diapause) (Johnson et al. 1984). If they pass this point, after an assessment of poor conditions in the late L1, they can enter an alternate L2 stage (L2d) that in the face of continued deprivation and high population density leads to dauer formation. Dauer (German for “enduring”) larvae are characterized by changes in the cuticle, pharynx, mouth, metabolism, and behavior that facilitate dispersal while delaying reproduction and aging (see Fielenbach and Antebi 2008; Bargmann 2006; Hu 2007, for reviews). Even after the commitment to and commencement of gametogenesis, starved animals can survive in a reversible “adult reproductive diapause” (ARD) state in which their germ line is severely diminished but remains poised to regenerate once conditions improve (Angelo and Van Gilst 2009; Seidel and Kimble 2011). Finally, as adults, reduced food abundance has several effects on reproduction. Not only has *C. elegans* evolved developmental and reproductive strategies to deal with boom-or-bust resource allocation, but recent findings suggest that animals also respond to intermediate conditions, tailoring the rate and progress of germline development to environmental and metabolic cues.

From the early days, the *C. elegans* field has considered the effects of unfavorable conditions (such as starvation, crowding, elevated temperature) versus favorable conditions on dauer formation (Cassada and Russell 1975; Klass and Hirsh 1976) and on lifespan (Klass 1977). However, the effects of physiological changes on reproduction in general and on different stages of germline development in particular have gained increasing attention only recently. Here we focus on the impact of environmental resources on the development of the germ line during larval stages and on the sustained “development” that is required for continuous gametogenesis in adulthood. For the purpose of comparing and contrasting the effects of different resource scenarios on the germ line, we refer to “feasting” boom conditions (abundant food, low population density), “food-limited” intermediate conditions of food availability, and “fasting” bust/starvation conditions.

C. elegans is a very powerful system to study the intersection between genetically encoded and environmentally regulated effects on germline development. Historically, these influences on germline development have been studied in relative isolation. Initially *C. elegans* reproduction, nutrition and ageing were examined simultaneously (Klass 1977). However, the study of germline development as a field developed separately from fields focused on sensory perception, feeding behavior, metabolism, and aging. Recently, these areas have converged. Because so much of *C. elegans* biology is conserved across evolution, this convergence offers a remarkable opportunity to investigate the mechanisms of physiological impact on the germ line in a whole-organism context.

Several themes are emerging that will likely engage the research community for many years to come. First, the germ line is highly sensitive: “feasting” worms quickly produce full self-progeny broods, while “food-limited” and “fasting” worms modulate reproduction to different degrees. This sensitivity likely reflects *C. elegans* evolutionary history and the fact that reproduction is not all-or-nothing. As a result, physiology can impact several points of germline development and function in different ways. Subtle changes in food abundance or

quality, for example, provoke a different response from all-out starvation. Second, the timing of physiological changes alters the reproductive response. In particular, mechanisms that enable worms to withstand poor conditions prior to the commitment to reproduction differ from those that are engaged after the commitment to reproduction. Third, cellular mechanisms vary depending on the specific changes and on the responding tissues. Finally, contrary to the view that the modulation of development and reproduction by environmental factors such as food availability is just a matter of sufficient material for cell division, growth and reproduction, there appear to be specific signaling pathways through which reproduction can be influenced by environmental conditions that alter physiology. These highly conserved signaling pathways, some of which have been studied in other phenotypic contexts in *C. elegans*, are important for similar responses in other organisms as well.

Here, we focus on aspects of hermaphrodite germline development that are altered by changes in food or feeding (feasting, food-limited and fasting) from hatching through larval life and adulthood. We also consider connections between lifespan and germline development. Finally, we comment on special considerations for examining germline development and on the major outstanding questions in the field. We refer the reader to Kim et al. (2012) (Chap. 10) for the influence of sperm on the germ line. Due to the broad scope of this subject we do not cover additional environmental influences such as physiological stress or pathogenesis, but we refer the reader to recent reviews (see, for example, Gartner et al. 2008; Darby 2005; Braeckman et al. 2009).

5.2 Feasting

For *C. elegans*, the true “feast” is likely an apple in an orchard in late autumn littered with dense patches of rotting fruit and a high bacterial load (Félix and Braendle 2010; Kiontke and Sudhaus 2006). The key feature of the feast experience is that good food is continuously present in excess given the population density of the worms. These conditions allow worms to avoid diapause and continue rapidly through four larval stages to the reproductive adult. Laboratory conditions under which three worms are typically transferred to a fresh bacterial lawn before the previous lawn is exhausted also represent feast-like conditions, even if laboratory strains of OP50 bacteria (at concentrations of $\sim 10^9$ or greater colony forming units per ml) are not necessarily the most preferred food source (Shtonda and Avery 2006; Kiontke and Sudhaus 2006). Thus, much of our current knowledge of germline development comes from analyses performed under what will be described here as “feasting” conditions.

5.2.1 Larval Germline Development While Feasting

Under feast conditions, worms hatch from their eggshell as L1 larvae and immediately feed. At hatching, the two somatic gonad precursor cells Z1 and Z4 flank the two germline precursors Z2 and Z3. These four cells are encased in a basement membrane and remain quiescent until midway through the first larval stage (L1) when, provided the worm feeds, they resume mitotic cell division cycles and continue development. Under these conditions, both germ cells and somatic gonad cells divide in the L1 with the latter cells arresting after the generation of ten cells, until the L3 stage when they resume divisions (Hirsh et al. 1976; Kimble and Hirsh 1979).

During the late L2 stage, the somatic gonad cells reorganize, forming a central primordium flanked by two gonad “arms,” each of which is each capped by a single distal tip cell (DTC). The DTCs guide gonad migration and express ligands that activate the germline-expressed GLP-1/Notch receptor (Kimble and Hirsh 1979; Kimble and White 1981; Austin and Kimble 1987, see also Hansen and Schedl 2012, Chap.4). Notch pathway activity maintains the distal germ cells in a proliferative (versus differentiated) fate. Complete removal of GLP-1/Notch signaling at any stage causes all germ cells to differentiate (Austin and Kimble

1987), while reducing GLP-1/Notch signaling changes the relative balance of proliferative and differentiated germ cells without reducing the cell cycle rate of the remaining proliferative cells (Michaelson et al. 2010). Hyperactive Notch signaling prevents specification of the differentiated fate, causes continuous specification of the proliferative fate, and can elevate cell cycle (Berry et al. 1997; Maciejowski et al. 2006). Additional signals promote robust larval proliferation to expand the larval germline progenitor pool (Korta and Hubbard 2010, and references therein).

Several critical events in hermaphrodite germline development take place in the L3 stage. First, two separate mechanisms move the anterior and posterior distal tip cells toward the head and tail of the animal, respectively. These mechanisms are (1) a centrifugal DTC migration program (dependent on *gon-1* and *mig-24/hlh-12*; Tamai and Nishiwaki 2007; Brelloch et al. 1999) and (2) robust germline proliferation (McGovern et al. 2009). This latter mechanism is impaired in suboptimal environmental conditions, as will be discussed below. In feasting conditions with proper DTC migration and robust larval germline proliferation, the proximal germ cells in the mid-L3 stage take on a nuclear morphology and expression of markers characteristic of early meiotic prophase when they reach a distance of 13 cell diameters from the distal end (Hansen et al. 2004). Once this initial differentiation event occurs, the proliferative zone of the germ line is demarcated as the area distal to the mitosis/meiosis border. Proximal to this border, all germ cells are differentiated (in prophase of meiosis I and undergoing gametogenesis). Importantly, while the worms are in feasting conditions, the proliferative zone continues to expand rapidly during the L3 and L4 stages, reaching a total of ~200 cells per gonad arm and stretching over ~20 cell diameters by the late L4 stage (Hansen et al. 2004; Killian and Hubbard 2005).

If the germline stem/progenitor pool does not rapidly expand in the L3 and L4 stages, the number of cells in the adult germline proliferative zone is reduced, affecting fecundity. Recent studies indicate that many factors—genetic, anatomical, and physiological—can influence this vital expansion of the larval proliferative zone. For example, mutations in *pro-1* (Killian and Hubbard 2004), ablation of the distalmost pair of sheath cells (Killian and Hubbard 2005), or reduced food abundance (Korta et al. 2012) all interfere with the accumulation of larval proliferative germ cells. This physiologically sensitive progenitor cell accumulation thus serves as a good model for understanding developmental and physiological control of cell proliferation. Using somewhat counterintuitive genetic screening assays, genes were identified that are required for expansion of the larval proliferative germ cell population under well-fed conditions (Michaelson et al. 2010; Hubbard 2011, Dalfó and Hubbard, unpublished). These screens took advantage of an inappropriate cell-cell interaction that occurs when larval germ cells maintain an undifferentiated state but do not proliferate properly (see Hubbard 2011, for further explanation). These and related approaches identified genes required for robust larval germline proliferation—that is, key genes that mediate developmental or physiological responses—while avoiding genes required for more general aspects of cell proliferation. Among these, the effects of the insulin/IGF pathway on this accumulation will be discussed here in the context of “feasting,” since the sensitivity of this pathway in the germ line to dietary changes not been documented directly. Two other pathways, TGF β and TOR, will be discussed below under “food-limited” since they have been associated with responses to food limitation.

5.2.2 The Insulin/IGF-Like Signaling Pathway Promotes the Larval Germline Cell Division Cycle

The many roles of the sole insulin/IGF-like receptor (IIR) DAF-2 in *C. elegans* have been reviewed elsewhere (Kenyon 2010; Kleemann and Murphy 2009; Landis and Murphy 2010; Fielenbach and Antebi 2008; Taguchi and White 2008; Hubbard 2011). Briefly, the *daf-2*

gene was initially discovered and named for its role in the dauer decision. Certain reduction-of-function *daf-2* mutations cause worms to enter the dauer pathway constitutively, regardless of the environmental conditions, suggesting that the activity of this pathway signals an environment sufficient to support reproductive development. Insulin/IGF-like signaling (IIS) modulates metabolism, response to toxins, hypoxia, immunity, post-reproductive lifespan, and reproductive timing. IIS acts through a highly conserved PI3K-dependent pathway and, in most contexts, negatively regulates the activity of the FOXO transcription factor DAF-16. Two exceptions are the control of germline cell cycle arrest in both the L1 and in dauer diapause that depend on *daf-18*/PTEN but not *daf-16*/FOXO (see Sect. 5.4, below).

The accumulation of proliferative germ cells during the L3 and L4 stages under “feast” conditions is sensitive to *daf-2*/IIR activity in a manner dependent on *daf-18*/PTEN and *daf-16*/FOXO (Michaelson et al. 2010). Of the 40 putative insulin-like ligand genes in the *C. elegans* genome, two were identified for their marked role in larval germline expansion: *ins-3* and *ins-33*. Additional ligands are likely also involved, since the *ins-3* and *ins-33* double mutant phenotype is not as strong as that of the *daf-2* mutant. Nevertheless, the activities of these ligands display several interesting characteristics. First, although the germline proliferation defect resulting from reducing *ins-3* or *ins-33* activity can be completely suppressed by loss of *daf-16*/FOXO, reduction of these ligand-encoding genes does not cause nuclear localization of intestinal DAF-16::GFP, nor does it activate target gene expression in the intestine. In other words, a reduction of *daf-2*, *ins-3*, or *ins-33* exerts similar phenotypic effects on the germ line, but different effects on the intestine. Somehow, the animal distinguishes between activities of these ligands on different target tissues. Second, reporters for *ins-3* and *ins-33* are expressed in head neurons, and head and uterine cells, respectively, and they are required in the soma for their role in larval germline cell cycle progression. Third, the reduction of activity of each ligand gene alone yields the same phenotype as reducing both together. This intriguing result is hard to reconcile with a simple ligand–receptor interaction model, and future studies will be required to determine precisely how these ligands are working. One hypothesis is that a physical interaction occurs between them; an alternative hypothesis is that a ligand–receptor relay exists, possibly including an alternate receptor.

One model to explain the observation that *daf-2* mutants fail to properly expand the larval germ line is that they retain dauer-like characteristics that prevent germline proliferation (see below “food-limited”). However, the data do not support this hypothesis and are consistent with a general model in which the DAF-2/IIR signaling cascade is reused after the window of opportunity for the dauer decision has passed (Michaelson et al. 2010). This conclusion is based on several observations. First, in the role of DAF-2 signaling in expanding the larval germ line, *daf-16* activity is required primarily within the germ line and expression of *daf-16* in neurons (a predominant site of action for the dauer role) has no effect. Second, temperature-shift and timed RNAi experiments demonstrated a post-dauer-decision period of sensitivity. Third, reducing *ins-3* and *ins-33* activity does not induce dauer formation, even at high temperatures, nor does it extend the time of reproduction (the time over which progeny are produced in adulthood) (Michaelson et al. 2010), as is observed upon reduction in *daf-2* activity (Dillin et al. 2002).

While this influence of IIS on larval germline progenitor cell accumulation has yet to be directly linked to environmental changes, the expression of *ins-3* in neurons suggests a possible physiological connection similar to the well-documented role for insulin signaling in *Drosophila* to promote germline cell cycle in response to diet (Drummond-Barbosa and Spradling 2001; LaFever and Drummond-Barbosa 2005; Hsu et al. 2008). This is an area for future investigation. In addition, it will be of interest to identify the targets for DAF-16

activity in the germ line that are important for promoting robust larval germline proliferation and to determine how they influence the cell cycle.

5.2.3 The Adult Germ Line After a Life of Feasting

In feasting conditions, before the adult molt, spermatogenesis ends and subsequent gametes that form are oocytes. In adult hermaphrodites, oocytes mature and are ovulated approximately every 23 min per gonad arm in an assembly-line fashion (McCarter et al. 1999, see also Kim et al. 2012, Chap. 10). In the spermatheca, they are fertilized either by stored self-sperm or by sperm introduced from a male by copulation. Embryos develop in the uterus to the ~30-cell stage when eggs are laid through the vulva out onto the substrate (Fig. 5.1; also see Pazdernik and Schedl 2012, Chap. 1).

This pace of reproduction in feasting conditions requires tremendous resources to generate the estimated 200-fold volumetric increase from germ cell to oocyte at a pace that produces 3–6 embryos per hour (Hirsh et al. 1976; McCarter et al. 1999). Germ cells retain an opening to a cytoplasmic core prior to spermatogenesis or to late stages of oocyte development (Hirsh et al. 1976). Therefore many cells contribute to material that flows through this core and is loaded into oocytes (Wolke et al. 2007). Germ cell nucleoli are relatively large and germ cytoplasm is densely packed with ribosomes, suggesting high translational capacity (Hirsh et al. 1976). In addition, raw material for oocyte growth likely comes from the contents of female germ cells that undergo programmed cell death during the pachytene of prophase of meiosis I. Estimates of as few as 2 (Gumienny et al. 1999) and as many as 30 (Jaramillo-Lambert et al. 2007) germ cells may die per oocyte generated. Interestingly, the rate of programmed cell death appears to include a physiological component since stressors can elevate it (Gartner et al. 2008). In addition, energy must be directed to the synthesis of lipid-rich yolk and other lipids that are transferred from the intestine to oocytes (Kimble and Sharrock 1983; Grant and Hirsh 1999).

5.3 Food-Limited Conditions

There are myriad ways that food limitation can be imposed on worms either in the wild or in the laboratory. Laboratory methods vary from generally reducing food abundance, altering the overall quality of food, or restricting food intake, to changing specific diet components or reducing uptake of specific nutrients, altering key metabolic enzyme activities, or genetic changes within specific cell types such that they can no longer respond to nutrients or nutrient signaling (see below). In addition to well-characterized environmental sensory triggers for dauer diapause that couple physiology to a developmental program, many different adult laboratory restrictions and feeding regimes have been described in the context of studies on lifespan extension due to “dietary restriction” or “caloric restriction” (see below). Generally, these restrictions are defined as reducing caloric intake without compromising nutrition. For the purposes of this discussion, the more general “food-limited” term is used to encompass a broad range of conditions that reduce food availability (including regimes that extend lifespan) and that lie as intermediate between starvation and feasting.

For details of intermediary metabolism in the worm, see (Braeckman et al. 2009). One of the interesting results of metabolic studies is that unlike changes that occur during dauer, restricting food does not appear to cause a general slowing of metabolism but rather changes flux through different metabolic pathways (Houthoofd et al. 2002b; Houthoofd and Vanfleteren 2006, and references therein). Future studies are required to link these changes to specific germline responses.

5.3.1 Larval Germline Development Under Conditions of Food Limitation

Worms possess robust and complex sensory and metabolic systems to monitor their environment. That these systems may be deployed in circumstances other than the few known diapause stages is a relatively new and interesting general area of investigation (see Edison 2009; Macosko et al. 2009). Germline development appears to be particularly sensitive to environmental changes. The potential for gamete production extends far into the lifetime of the animal (Mendenhall et al. 2011; Angelo and Van Gilst 2009), and, though energetically expensive, it is vital for reproductive success. Therefore, it “makes sense,” anthropomorphically speaking, for the germ line to be both sensitive and responsive to environmental changes to balance reproduction with available resources. Altering the sensory environment and/or metabolic status of the animal can influence the germ line at several points during larval germline development, prior to commitment to gametogenesis.

5.3.1.1 Dauer, a Special Case—If worms hatch into suboptimal (but not total starvation) conditions, development of the first larval stage begins. Late in the L1 stage, however, reassessment of environmental conditions determines whether animals proceed immediately into a reproductive mode or enter the nonfeeding dauer diapause stage. Here, we consider dauer as a special case since compared with other diapause states, it involves radical changes, both morphological and physiological, that only occur in response to prolonged poor conditions. While a detailed discussion of dauer regulation is beyond the scope of this review (the reader is directed to Fielenbach and Antebi 2008; Bargmann 2006; Hu 2007), a few salient features are mentioned here that are relevant to germline development in dauer and to the reuse of dauer-decision pathways in post-dauer-decision stages of germline development.

The combined assessment of cues reporting ambient worm population density, food, and temperature (Golden and Riddle 1984; Bargmann and Horvitz 1991) determines whether an animal will enter dauer or will proceed to the L3 and L4 stages toward becoming reproductive adults. The dauer larva is a remarkable adaptation: during dauer entry, animals secrete a desiccation-resistant cuticle and store fat to sustain them as nonfeeding dauer larvae which exhibit motile behaviors to facilitate dispersal. Dauer larvae can thereby suspend development and extend lifespan for months. When conditions improve, they undergo a special molt and reenter a reproductive developmental pathway.

“Dauer pheromone,” a main cue for population density, was identified ~30 years ago as a crude preparation that could induce dauer entry and inhibit dauer exit (Golden and Riddle 1982). Worms are now known to secrete at least ten different ascarosides (derivatives of the dideoxy sugar ascarylose) (Edison 2009) many of which are active in nondauer contexts such as mating or social behavior (Jeong et al. 2005; Srinivasan et al. 2008; Butcher et al. 2007, 2008, 2009; Pungaliya et al. 2009; Macosko et al. 2009). The precise identities and concentrations of ascarosides in the immediate environment likely convey a tremendous amount of information since ascarosides are often most potent in mixtures (Srinivasan et al. 2008; Butcher et al. 2007, 2008). Moreover, in addition to different baseline potencies observed in different assays, distinct combinations can be more potent in one assay over another. Ascarosides may also provide information about the composition and condition of the population since the ascarosides secreted by worms differ with stage and culture conditions (Kaplan et al. 2011). Interestingly, some ascarosides (ascr#1/C7 and ascr#3/C9) peak in L3-L4 stages (Kaplan et al. 2011), a time when the germline proliferative zone is expanding. Because ascaroside abundance is altered when worms are partially starved, “food-limited” conditions likely produce both sensory and metabolic responses.

The decision to enter and exit dauer involves no fewer than four highly conserved signaling pathways, including GPCR/cGMP signaling thought to act upstream of parallel Insulin/IGF

and TGF β pathways (Fig. 5.2), both of which converge on the activity of a nuclear hormone receptor (related to LXR and Vitamin D receptor) DAF-12 (see Fielenbach and Antebi 2008; Hu 2007).

The relationship between the dauer decision, specific neurons and sensory-responsive signaling is well understood for the paired sensory ASI neurons and TGF β . Two largely nonoverlapping TGF β pathways in *C. elegans* correspond to TGF β -like and BMP-like signaling and affect dauer and body size, respectively. The DAF-7/TGF β pathway regulates dauer formation (Savage-Dunn 2005). ASI-specific expression of a *daf-7*/TGF β reporter is elevated under nondauer or dauer exit conditions (Schackwitz et al. 1996; Ren et al. 1996) in response to DAF-11/PKG signaling (Murakami et al. 2001). Recently, two ASI-specific, redundantly acting, GPCRs were identified as specific receptors for one of three potent dauer-inducing ascarosides (McGrath et al. 2011).

Importantly, during the protracted dauer entry process, metabolism shifts to storage in preparation for starvation which occurs during the nonfeeding dauer stage. In contrast to more sudden starvation responses, in anticipation of initiating the dauer pathway, the pre-dauer second larval stage is extended (L2d), allowing the animal to prepare for future nutrient deprivation by slowing development and metabolism, while storing energy (see Riddle and Albert 1997, and references therein). Dauer-stage animals display metabolic (Wadsworth and Riddle 1989; Burnell et al. 2005) and gene-expression changes (Jones et al. 2001; Holt and Riddle 2003; Wang and Kim 2003) that favor utilization of stored fats, suggesting that fat reservoirs function as a nutrient source for the dauer stage. These alterations (see Braeckman et al. 2009, for additional primary references) are primarily a shift away from the direct use of biosynthetic precursors such as amino acids and nucleic acids obtained from dietary sources, to the generation of these precursors from fat (via fatty acid β oxidation, gluconeogenesis, and the glyoxylate cycle). Energy (ATP) generation from acetyl CoA, via the citric acid cycle and oxidative phosphorylation is also altered as acetyl CoA production is shifted away from glycolysis with more reliance on fatty acid β oxidation. Moreover, the proper rationing and utilization of stored material is important for dauer survival. Dauer survival is greatly reduced in the absence of AMPK signaling, a defect that has been linked to abnormally rapid use of stored lipid reserves in the hypodermis (Narbonne and Roy 2009).

5.3.1.2 The Germ Line During Dauer—Dauer formation leads to a progressive establishment of cell cycle arrest, including the germ line. Dauer precedes substantial growth of the germline stem cell pool, and dauer larvae ultimately arrest germline proliferation, likely in the G2 stage (Narbonne and Roy 2006). Importantly, since the meiotic fate is not yet specified prior to or during dauer, elevated Notch activity, which causes a failure in specification of the differentiated germ cell fate, would not be expected to drive hyperproliferation during dauer. Interestingly, like the inhibition of germline proliferation in the L1 diapause (see below) the inhibition of germline proliferation in dauer can be genetically separated from the general somatic cell cycle inhibition. To identify the genetic mechanism by which the germ line limits its proliferative capacity during dauer, Narbonne and Roy (2006) screened for mutations that would permit germline proliferation during *daf-2*-mutation-induced dauer conditions. Interestingly, they found that germline proliferation is kept in check by the activity of the AMPK orthologs *aak-1* and *aak-2* (Narbonne and Roy 2006). AMPK activity is exquisitely sensitive to levels and ratios of ATP, ADP, and AMP, being most active under elevated AMP conditions that signal energy deficit (Hardie 2011). Therefore, from a purely energetic standpoint, one interpretation of these results is that if AMPK levels are low, regardless of the actual cellular levels of AMP and ATP, the germ cells interpret the energy climate as “good” and will proceed to inappropriately proliferate the germ line. These results might also suggest that the ATP/

AMP ratio (as opposed to other metabolic signals) may be paramount for the control of germline proliferation in response to dauer-promoting signals. Alternatively, the phenotype may be related to the role of AMPK activity in lipid rationing (Narbonne and Roy 2009). For the dauer-induced germline proliferation arrest, AMPK and its upstream regulator PAR-4/LKB1 coordinate proliferation with somatic development during dauer, in response to input from both the TGF β and insulin-like pathways (Fig. 5.2).

In terms of the metabolic and germline responses to the environment, dauer differs from “food-limited” conditions defined by a chronic low-food condition or a qualitative change in food intake (see below). The differences likely reflect the special preparation for dauer and its timing prior to reproductive commitment. How these different sensory, physiological, and metabolic conditions and the specific dynamics of their onset affect germline development remains to be determined.

5.3.2 Sensory and Nutritional Control of Post-Dauer-Decision Germline Expansion

Once animals bypass the developmental window during which they might enter dauer, they are committed to “reproductive” development. Under replete conditions, the timing of this commitment is accompanied by rapid accumulation of proliferative germ cells in the L3 and L4 stages that eventually constitute the adult germline stem cell pool. This accumulation requires the *daf-2*/Insulin-IGF-like (IIS) pathway, as described above. Although IIS control of larval germline cell cycle has not yet been linked directly to nutritional or metabolic cues as in other organisms, two additional highly conserved pathways have been found recently to link physiology and larval germline expansion in response to sensory and metabolic cues: TGF β and TOR-S6K.

5.3.2.1 Post-Dauer-Decision Expansion of the Germline Progenitor Pool: A Sensory Link via TGF β

—The same TGF β pathway ligand and receptors that are required for the dauer decision (*daf-7/TGF β* , *daf-1/TGF β RI* and *daf-4/TGF β RII*) were identified in large-scale RNAi screens for genes that affect larval germline development (Dalfó et al. 2012; Dalfó and Hubbard, unpublished). Depletion of these gene activities by RNAi or mutation, or depletion of the relevant R-Smads, causes a reduction of both the number of cells in the adult progenitor pool and brood size by up to ~50 %, similar to what is observed with IIS pathway mutants (Table 5.1). Since IIS acts in parallel with the TGF β pathway for the dauer decision (Fig. 5.2), converging on DAF-12, a plausible hypothesis was that these pathways might constitute a parallel cassette that acts similarly for dauer and for reproductive germline proliferation control. However, this hypothesis proved incorrect: while IIS is important germline-autonomously to promote robust larval cell cycle progression, the TGF β receptor pathway is required germline-nonautonomously in the DTC and has no effect on cell cycle (Dalfó et al. 2012). As in the dauer pathway (Vowels and Thomas 1992), the DAF-3/co-Smad and DAF-5/Sno-Ski are downstream negatively regulated targets of TGF β signaling in the control of germline progenitor expansion, and they, too, are required in the DTC for this role. Moreover, although germline proliferation is greatly reduced during dauer, TGF β pathway activity for expansion of the larval proliferative zone does not require the activity of the nuclear hormone receptor DAF-12, as it does for dauer. That is, the *daf-7* mutant dauer-constitutive phenotype (25°) is suppressed by loss of *daf-12*, while the larval germline defect persists. These results, and others, support the hypothesis that the TGF β roles in L1/L2 regulation of dauer entry and in L3/L4 germline progenitor accumulation are separate (Dalfó et al. 2012).

The effects of reduced TGF β signaling share some similarities with reduced *glp-1*/Notch activity: TGF β mutants display neither inappropriate cell death in the distal germ line nor any deviation from wild-type cell cycle parameters (L4 mitotic- and S-phase indexes) (Dalfó

et al. 2012; Michaelson et al. 2010). In addition, reducing TGF β signaling causes a further severe reduction in proliferative germ cells in the background of reduced (*rf*) *glp-1/Notch* activity. Depletion of *daf-3/CoSmad* or *daf-5/Sno-Ski* suppresses this enhancement back to *glp-1(rf)* levels. Thus the TGF β pathway acts differently from the IIS pathway and more similarly to GLP-1/Notch in that it promotes the proliferative fate and/or interferes with differentiation, without influencing cell cycle (Table 5.1). Given this Notch-like role and the requirement for TGF β receptor (TGF β R) signaling in the DTC, TGF β signaling might be postulated to affect the production or activity of the Notch ligands LAG-2 and APX-1 produced in the DTC (Henderson et al. 1994; Nadarajan et al. 2009). However, no change in the levels of ligand reporters or transcripts were observed, and reduced TGF β signaling still lowered proliferative germ cell numbers in the absence of GLP-1 (in a triple null mutant *gld-2 gld-1; glp-1* strain that produces proliferative germ cells despite the absence of *glp-1*). These results suggest that the TGF β pathway acts in parallel with Notch signaling to inhibit differentiation (Dalfó et al. 2012).

DAF-7/TGF β itself is expressed by the paired ASI ciliated chemosensory neurons (Ren et al. 1996; Schackwitz et al. 1996). Surprisingly, these neurons and the environmental cues they mediate also influence the expansion of the L3-L4 larval germline progenitor pool (as measured by early adult total germline progenitors) in a *daf-3* and *daf-5*-dependent manner (Dalfó et al. 2012). Using conditions or timing that circumvented dauer formation, genetic ablation of cilia, physical ablation of ASI neurons, application of crude dauer pheromone, or reduced food (5×10^8 colony forming units/ml) were found to reduce proliferative germline cell numbers in a *daf-5*-dependent manner. These results suggest that, similar to the cues that alter *daf-7/TGF β* reporter expression during the dauer decision, sensing of low pheromone levels and high food concentration during L3-L4 stages is required to expand the progenitor cell pool, and that this information is passed from cilia in ASI to the DTC germline stem cell niche via TGF β (Dalfó et al. 2012).

5.3.2.2 Post-Dauer-Decision Expansion of the Germline Progenitor Pool: A Nutrition Link via TOR and S6K—

Restricting food either by reducing the bacterial concentration (to 1×10^8 colony forming units/ml) or by protein deprivation (by loss of *pept-1*) severely inhibits the accumulation of germline progenitors in larval stages (Korta et al. 2012). Importantly, this effect involves cell cycle control and occurs independently of continued signaling by *glp-1/Notch*. For example, reducing bacterial concentration causes a ~65–75 % decrease in the number of young-adult proliferative germ cells in wild type (from ~200 to 50 cells) despite the presence of GLP-1/Notch signaling. Even when *glp-1* activity is reduced (e.g., in *glp-1(rf)* mutants, ~100 proliferative cells are retained), the proliferative germ cell pool is further reduced by ~65 % to ~35 cells under the same food restriction (Korta et al. 2012).

The conserved TOR (Target of Rapamycin) pathway links nutrition to cell growth and aspects of cell division in organisms from yeast to mammals. In mammals, the TOR/RAPTOR complex (TORC1) responds to PI3K-mediated growth factor signaling as well as nutrient and energy load, and much of this response is mediated by the conserved substrate S6K (p70 ribosomal S6 kinase) (for a general review, see Wullschleger et al. 2006). Recent work indicates that TOR and S6K are important in *C. elegans* for accumulation of larval germline progenitors and for the control of this accumulation by diet (Korta et al. 2012). Interfering with TOR pathway activity causes numerous defects (Long et al. 2002). Most strikingly, mutations in the *C. elegans* TORC1 complex components TOR or RAPTOR (*let-363* and *daf-15*, respectively) cause an L3-stage larval arrest phenotype that is similar to but distinct from dauer (Long et al. 2002; Jia et al. 2004). TOR and S6K also influence the accumulation of larval germline progenitors, and the somatic effects of TOR and S6K can be separated from germline-autonomous roles of this pathway (Korta et al. 2012). Among

the major findings are that *rsks-1/S6K*, the sole S6K-encoding gene in *C. elegans*, acts germline-autonomously to influence the expansion of the larval germline progenitor pool by both promoting cell cycle progression and inhibiting differentiation. Similar to reduced *daf-2*-mediated IIS, loss of *rsks-1* slows the germline cell cycle in larvae but not adults (Korta et al. 2012). Also similar to IIS (Pinkston et al. 2006), reducing S6K markedly reduces the size (cell number) of germline tumors (Korta et al. 2012). However, unlike the effects of reducing IIS, loss of S6K does not specifically slow the G2 phase of the cell cycle and its germline phenotype does not depend on the activity of *daf-16/FOXO*. Also, in contrast to reduced IIS, loss of *rsks-1* potentially enhances the Glp-1 meiotic entry phenotype (total loss of proliferative germ cells) of *glp-1(rf)* mutants and suppresses *glp-1(gf)* phenotypes. In addition, germline reduction of TOR or RAPTOR causes a dramatic reduction in the number of germline progenitors, a phenotype that is partially mediated by S6K and eIF4E. Finally, the larval germline role of *rsks-1/S6K* appears distinct from its role in longevity since the germline phenotype is not suppressed by the same genetic alterations that suppress the longevity phenotype. These results suggest that regulation of larval germline progenitors and lifespan by S6K occurs via distinct S6K targets (Korta et al. 2012).

Interestingly, S6K is also required for the effects of certain aspects of diet on the growth of the larval germline progenitor pool. The same food-limiting regimes (reduced bacterial concentration, pharyngeal pumping or amino acid uptake) that cause a severe decrease in the number of wild-type or *glp-1* germline progenitors amassed by the early adult cause only a mild further decrease in the absence of *rsks-1*. These results suggest that the S6K (and likely TOR) is a key mediator of the effect of nutrition on establishment of the germline progenitor pool (Korta et al. 2012). It will be of interest to determine the targets of S6K in this role and the precise molecular pathways that influence germline proliferation and differentiation in response to dietary restriction.

5.3.3 Reproduction Under Conditions of Food Limitation: It remains to be determined whether dietary restriction affects additional aspects of adult germline development. Because reducing food intake or altering food composition of adult worms (and other organisms) has a marked effect on longevity (Fontana et al. 2010), multiple methods of dietary restriction (DR) have been developed in *C. elegans* for analysis of the effects of caloric restriction on lifespan (compared in Greer and Brunet 2009). Not surprisingly, the vast majority of manipulations that restrict food intake also reduce fecundity of unmated adult hermaphrodites. Fecundity or brood size is the culmination of multiple aspects of reproductive biology including earlier cumulative effects on germline development including the proliferation and maintenance of germline stem cells, meiosis, cell death, sex determination, gametogenesis, and fertilization. In addition, studies of brood size can be confounded by defects in egg-laying. Therefore, detailed analyses of the effects of altering diet on specific aspects of germline development will advance the field.

Specific DR protocols that extend lifespan and negatively influence brood size include (1) genetic methods such as mutations in *eat-2* that reduce pharyngeal pumping rate (Avery 1993; Lakowski and Hekimi 1998) or *pept-1* that reduces amino acid absorption (Meissner et al. 2004), (2) diluted bacteria in liquid culture (Klass 1977; Houthoofd 2003; Panowski et al. 2007), (3) chemically defined liquid media (Houthoofd et al. 2002a; Szewczyk et al. 2006), (4) serial dilution of bacteria on solid media (Greer et al. 2007), (5) total absence of bacteria on plates (Kaeberlein et al. 2006; Lee et al. 2006) (that is, fasting, where brood size is also limited by retention of embryos and subsequent matricide), and (6) metformin treatment (Onken and Driscoll 2010), which may mimic DR. Two methods of DR that influence lifespan but for which brood size has not been reported are intermittent fasting (Honjoh et al. 2009) and treatment with the glucose analog 2DG (Schulz et al. 2007). Interestingly, two treatments that extend lifespan but do not reduce brood size are reduced

peptone in agar plates (Hosono et al. 1989) and treatment with resveratrol (Wood et al. 2004). Therefore, these two aspects of life history are not always linked.

What is, perhaps, surprising from the analyses of dietary restriction in the context of lifespan extension is that while pathways that mediate these effects overlap and interact, specific methods to restrict caloric intake can be associated primarily with the activity of specific pathways (Greer and Brunet 2009). That is, in many cases, the activity of a single effector can dictate whether the dietary restriction regimen extends lifespan. For example loss of *pha-4* suppresses lifespan extension caused by DR imposed by reduced pumping in the *eat-2* mutant background (Panowski et al. 2007), but not as imposed by reducing food on solid media (Greer and Brunet 2009), while *aak-2* affects the latter (Greer et al. 2007) and not the former (Curtis et al. 2006). This is very exciting since it suggests that a limited number of specific pathways may also link dietary restriction to germline development and that they mediate different aspects of the restriction or the response. A reduction in reproductive capacity could be simply a secondary effect of a simple lack of critical building blocks necessary for basic cellular processes such as germ cell proliferation or oocyte growth. As with lifespan, to argue a pathway-specific response, as opposed to a simple lack of “stuff,” the genetic “suppressibility” of nutritional manipulations will be important to establish. In cases where a suppressor is not yet identified, the effect of a pathway in the presence or absence of pathway components might suggest a dependency relationship (e.g., Korta et al. 2012).

5.4 Fasting

Severe food deprivation has many consequences. Depending on the stage at which animals experience starvation, the response of the germ line and, indeed, the animal's overall response differs. While most of these consequences have been investigated at diapause points, it bears mention that worms are exquisitely sensitive to and respond rapidly to such changes throughout larval life (Liu et al. 1997). Substantial changes in the transcription levels of key metabolic genes have been observed within 1 h of food removal in the L4 stage. Also, both differential stage-dependent and shared transcriptional responses occur (Van Gilst et al. 2005). Like mammals, both larval and adult worms quickly mobilize fat upon starvation (McKay et al. 2003). One highly conserved and critical response to fasting that facilitates this mobilization is downregulation of the sterol regulatory element binding protein (SREBP) transcription factor by a sirtuin, SIR-2.1 (Walker et al. 2010).

5.4.1 Larval and Adult Starvation—Total starvation of hatchlings causes L1 arrest, whereas near-starvation of hatchlings (e.g., axenic culture conditions—see Johnson et al. 1984) permits delayed development. Both L1 arrest and delay are reversible upon refeeding. L1 larvae that hatch in the absence of food arrest both somatic and germline cell cycle progression and development in a *daf-18/PTEN*-dependent manner (Baugh and Sternberg 2006; Fukuyama et al. 2006). Somatic cell arrest occurs in the G1 and is dependent on the FOXO transcription factor DAF-16 that is antagonized by PI3K signaling through the IIS and DAF-2. Gene expression profiling during L1 arrest and recovery indicates that RNAPol II is poised on the promoters of development and growth genes during L1 starvation-induced arrest, allowing for rapid recovery once conditions improve (Baugh et al. 2009). Interestingly, germline cell cycle arrest has somewhat different control, reminiscent of the arrest at dauer: it occurs in the G2 and is dependent on *daf-18/PTEN*, but not *daf-16/FOXO* (Fukuyama et al. 2006).

From the sensory standpoint, L1 starvation involves neuronal signaling in response to a subset of amino acids. Neuronally required metabotropic G protein-coupled glutamate receptors respond to an amino acid signal, particularly leucine. These responses then

modulate the activity of specific neurons (AIY and AIB) to inhibit and promote the starvation response, respectively (Kang and Avery 2009). The same receptors were found to modulate fat accumulation in the worm (Greer et al. 2008). This type of global starvation-signaling system is likely important in ensuring an appropriate whole-organism response to changing environmental conditions.

How different tissues such as the L1 germ line integrate and respond to specific starvation cues—both sensory and metabolic—to control arrest and recovery is an area for future study. Very recently, the activities of intestinal microRNAs were implicated in survival during L1 arrest. In addition, *miR-71* was identified as important for recovery from L1 arrest, affecting only a subset of tissues (including the intestine) and mediating both DAF-16-dependent and -independent processes (Zhang et al. 2011).

Total food deprivation of L4-stage hermaphrodites results in different outcomes: L4 or adult arrest, adult matricide (“bagging”), or a distinct survival-enhancing “adult reproductive diapause” (ARD) (Angelo and Van Gilst 2009). The relative proportion of these outcomes varies depending on the exact time of starvation (Angelo and Van Gilst 2009; Seidel and Kimble 2011). The matricide phenotype among comparative older animals (those already competent to produce embryos when starvation occurs) is not unexpected: worms removed from food will immediately reduce the rate of egg-laying causing progeny to develop internally where they hatch and devour their mother if they cannot escape to the outside (Trent et al. 1983). This control of egg-laying is mediated by neuropeptides and cGMP signaling and TGF β pathway components (see Schafer 2005, for a review). The ARD outcome, however, was unanticipated. Strikingly, ARD is characterized by germline atrophy that occurs over the first 10 days of starvation, ultimately leveling off with ~35 germ cells per gonad arm. Remarkably, even after 30 days of starvation, ARD animals survive and can reestablish the germ line with its full distal-proximal developmental pattern over the subsequent 72 h once returned to food. These starved and recovered animals are capable of producing progeny either from surviving self-sperm or when mated (Angelo and Van Gilst 2009). The germline atrophy is partially dependent on the programmed cell death pathway (Angelo and Van Gilst 2009), and appears to be associated with oogenesis (Seidel and Kimble 2011). Further, when the matricide phenotype is prevented by interfering with embryonic development, all animals are capable of ARD (Seidel and Kimble 2011).

Interestingly, oocytes are continuously produced in starved ARD animals, albeit at a much-reduced rate of one per 8 h (in contrast to ~3 per hour per gonad arm in fed adults) (Seidel and Kimble 2011). This ability to make one oocyte at a time likely reflects an adaptation to preferentially direct dwindling energetic resources to the continued production of progeny, regardless of how few (see below, Sect. 5.5 for further discussion). Other aspects of growth conditions (amount or quality of food, degree of crowding) may or may not influence ARD (Seidel and Kimble 2011), but the extent to which these possible influences can be attributed to differences in worm or bacteria strains or differences in growth conditions remains to be determined. Finally, the proportion of worms that exhibit ARD after starvation is partially dependent on the activity of a nuclear hormone receptor, NHR-49, an HNF-4a ortholog that, in mammals, has been implicated in promoting fatty acid oxidation and gluconeogenesis in response to food deprivation (Angelo and Van Gilst 2009). It will be important to establish the mechanism by which the distal-most cells are protected from degradation, the proliferation status of the distal-most cells under ARD, and the cellular and molecular mechanisms underlying germ cell renewal.

The unexpected germline atrophy and capacity for regrowth that are characteristic of ARD are very exciting to the field. ARD demonstrates a remarkable plasticity of germ line in response to the environment and the renewal of the germ line in *C. elegans* from a greatly

diminished pool of distal germ cells. The results of Angelo and Van Gilst (2009) demonstrated that ARD is indeed a reproductive diapause state (MacRae 2010) in that ARD reversibly alters gametogenesis and reproductive capacity in response to environmental adversity. In this case, it also leads to an extension of reproductive competence and lifespan (Angelo and Van Gilst 2009). Additional insight into mechanisms underlying ARD and the environmentally regulated plasticity of the germ line that it reveals is indeed an attractive area for further investigation.

5.5 Fat Metabolism, Reproduction, and Aging

5.5.1 Two Examples Connecting Fat, Reproduction, and/or Aging—Lipid

biology is essential to many aspects of cellular function and both cell-cell communication and global organismal signaling, including the germ line. Two examples here illustrate the utility of *C. elegans* to understand these conserved metabolic processes and their impact at the cellular and organismal levels.

One example is the effect of dietary supplementation of specific polyunsaturated fatty acids (PUFAs) on the germ line (Brock et al. 2006). A particularly interesting class is the C20 PUFAs since they are precursors to eicosanoids and other bioactive moieties, and they play a prominent role in membrane biology. Dietary supplementation and subsequent uptake of dihomo- γ -linolenic acid (DGLA), but not other related fatty acids, causes dramatic dose-dependent sterility. Supplementation in the L2/L3 stages causes larval germline degradation and elevated levels of cell death in the adult germ line. Both genetic and biochemical results argue for the specificity and sensitivity of the effect and support the hypothesis that exogenous DGLA may interfere with signaling critical for germline development. Alternatively a byproduct of DGLA metabolism could be cytotoxic. The precise nature of the germline loss and the relationship between this effect and endogenous fatty acid function will be interesting to determine.

Another example involves one of the earliest known lifespan-altering genes, *clk-1*, which encodes an enzyme required for the ubiquinone biosynthesis (Lakowski and Hekimi 1996). Loss of *clk-1* is highly pleiotropic and causes a delay in germline development relative to somatic development (Shibata et al. 2003). A mutation in *dsc-4* suppresses the germline delay, but not the rate of overall postembryonic development. DSC-4 encodes a protein related to the large subunit of the microsomal triglyceride transfer protein that is required for the secretion of LDLs in mammals. In worms, it is expressed in the intestine, the site of yolk production, and *dsc-4* appears to act in the same pathway as a subset of the vitellogenins. Consistent with the notion that cholesterol reduction would reduce LDL levels in worms, as in mammals, and that LDL production is an important aspect of the *clk-1* germline phenotype, depletion of cholesterol also suppresses the germline delay in *clk-1* mutants with less effect on other phenotypes. In addition, consistent with the hypothesis that the reduction of reactive oxygen species (ROS) production is also involved, elevating ROS by mutation of a superoxide dismutase, *sod-1*, also suppresses the *clk-1* mutant delay in egg production. These observations prompted a model in which the level of oxidized LDL-like lipoproteins correlates with the rate of germline development relative to somatic development. Whether this effect is due to a specific metabolic product or is due to more general cellular responses remains to be determined. The ARK-1 kinase was identified as a possible germline-autonomous mediator since it similarly suppressed *clk-1* mutant defects (Shibata et al. 2003). It will be of interest to determine the origin of the “delay” in germline development that is associated with *clk-1*. In any case, these results underscore additional interesting connections between germline development and lipid biology.

5.5.2 The Germ Line Limits Lifespan—Two observations related to lifespan and the germ line have sparked much debate in the context of evolutionary theory: loss of the germ line extends lifespan and animals outlive their reproductive period (see, for example Mukhopadhyay and Tissenbaum 2007; Hughes et al. 2007). Following on these considerations, intriguing connections have been made between *C. elegans* lifespan, fat metabolism and the germ line (see recent reviews by Branicky et al. 2010; Watts 2009).

The 1999 report that ablation of the germ line with an intact somatic gonad extends lifespan in *C. elegans* (Hsin and Kenyon 1999) opened a new area of investigation into how the presence of the germ line limits lifespan. This effect depends on DAF-16/FOXO, the nuclear hormone receptor DAF-12, the ankyrin-repeat containing protein KRI-1, the lipase LIPL-4, and the FOXA transcription factor PHA-4 (Hsin and Kenyon 1999; Arantes-Oliveira et al. 2002; Berman and Kenyon 2006; Wang et al. 2008; Lapierre et al. 2011). Germline proliferation in both larval and adult stages appears to influence lifespan (Arantes-Oliveira et al. 2002). In the wake of these results, *glp-1* temperature sensitive (ts) loss-of-function alleles have become a proxy for germline ablation. Results obtained with these alleles alone must be interpreted with caution, however, since *glp-1* may have activities in other tissues such as the nervous system (Singh et al. 2011) that may influence lifespan, feeding and processing of environmental cues.

5.5.3 Taking Ecology and Evolution into Consideration: A Unifying Hypothesis for Fat, Reproduction, and Aging

—So what is the connection between fat, reproduction, and aging? While a detailed discussion of these connections is beyond the scope of this review, some speculation regarding these connections can be made from the vantage point of the germ line and *C. elegans* ecology. Returning to the themes stated in the introduction, we speculate that the successful evolution of *C. elegans* as a rapidly-reproducing, highly fecund species in environments with patchy resources required exquisite co-evolution of sensory, metabolic, and reproductive strategies. This notion could help explain why the germ line exhibits tremendous responsiveness and plasticity with respect to environmental challenges. One question that emerges, and that will be helpful to consider as the field grapples with the organismal integration of these components, is: to what extent did the demands of reproductive success drive *C. elegans* evolution versus the demands of metabolism? We favor the hypothesis that the physiological responses of the worm to its environment have been honed to maximize the production of at least some offspring. Indeed, larger self-progeny broods produced by strains with elevated sperm production extend generation time, suggesting a possible disadvantage (Hodgkin and Barnes 1991). For a short-lived organism, it may be more important to produce a small number of offspring under as many conditions as possible than to produce the large numbers of progeny seen in the “feasting” laboratory conditions. Even extreme brood-limiting outcomes such as the matricide caused by reduced egg-laying in response to low food (Trent et al. 1983) may ultimately benefit the species, since the surviving progeny can undergo larval arrest and disperse to more favorable environments.

Taking these ecological and evolutionary points into consideration, any possible advantage of extending lifespan after loss of the germ line is difficult to reconcile. Loss of the germ line is a highly abnormal and reproductively (hence evolutionary) dead-end scenario. By contrast, rapid and wild fluctuations in resources were likely common challenges to this species over the course of evolution. Thus, one could speculate that the extension of lifespan that occurs with adult dietary restriction may well enable an aging hermaphrodite to encounter a male and resume reproduction later in life.

How might lipid metabolism figure into these considerations? Under “feast” conditions, major fat stores are released from the intestine and are taken up by developing oocytes

(Kimble and Sharrock 1983; Grant and Hirsh 1999). In the abnormal scenario in which oocytes are not produced, lipid-rich material accumulates in the pseudocoelomic space. This observation suggests that the intestine does not receive a “don’t dump” signal from the “non-oogenic” germ line. Indeed, germline-less worms accumulate certain lipids and lifespan extension in *glp-1* mutants depends on the targets of several transcription factors that alter fat metabolism. For example, lifespan extension in *glp-1* requires the DAF-16/FOXO-regulated lipase, LIPL-4 (Wang et al. 2008; O’Rourke et al. 2009; Lapierre et al. 2011) as well as the NHR-80/HNF4-regulated stearoyl-CoA desaturase FAT-6, which converts stearic acid to oleic acid (Goudeau et al. 2011). Indeed, exogenous oleic acid can restore longevity to *glp-1* mutants lacking stearoyl-CoA desaturase activity (Goudeau et al. 2011).

One perhaps naïve hypothesis to unify these observations and considerations is that the failure to utilize fats and lipids in the production of new germ cells (e.g., as occurs in the absence of the germ line) may cause the buildup of unusual quantities of particular substrates for substrate-dependent metabolic reactions, and hence shift metabolic flux toward conditions reminiscent of age-defying stages such as dauer where lipids are stored and utilized in a controlled manner. If this were the case, the somatic gonad must act as a sensor or responder—likely by way of DAF-12 (Yamawaki et al. 2010).

5.6 Prospects, Challenges, and Open Questions

Physiology impacts many aspects of germline development and function—and hence reproduction—in all organisms. Because physiology is a whole-organism phenomenon that engages many historically separate fields of biological inquiry (including neurobiology, metabolism, growth, and cellular stress), the possibility of understanding and linking them is both fascinating and daunting. The relative anatomical simplicity, experimental accessibility, and depth of knowledge accrued by the community of *C. elegans* researchers makes *C. elegans* an outstanding model organism for these studies. The boom-and-bust aspects of this organism’s ecological resources as well as its possible evolutionary prioritization of reproduction make it a particularly sensitive and therefore attractive model.

The very same whole-organism view that makes these connections so interesting also introduces challenges, both conceptual and experimental. Conceptually, dietary changes affect many aspects of worm biology, and conversely, germline defects can influence whole-worm biology. In addition, the question of whether a given dietary manipulation is altering a developmental process due to a simple reduction of a rate-limiting building block or whether it is triggering a more specific change in a signaling cascade needs to be addressed. Given available genetics and genomics tools, finding genetic changes or network fluxes that reverse the effects of specific physiological manipulations should be feasible. This combination of facile environmental manipulation, cell biology and genetics bodes well for the power of this model system to uncover important mechanisms that link different aspects of physiology, germline development, and fertility.

Experimentally, there are additional considerations. Physiological manipulations require extremely tight controls to reduce variability caused by small changes in conditions (e.g., temperature, differences in plates, presence of contamination) or in synchronization of worm populations. These subtle differences in the handling of worms may account for differences in results between laboratories, so in addition to being carefully controlled internally, they must be well documented to facilitate comparisons between laboratories. Other perhaps less obvious considerations and their consequences are, first, that “food-limited” worms often grow more slowly than well-fed worms. Therefore, the relative effects of any dietary alteration on developmental timing, both in the soma and germ line must be examined to

ensure proper interpretation of germline phenotypes. A second (and related) consideration is that many germline phenotypes occur as a secondary consequence of earlier and/or more distal defects and therefore must be interpreted with care. For example, a reduction in germline proliferation in the early L3 stage can delay initial meiotic entry later in the L3 stage and also delay the sperm-oocyte switch in the L4/adult (Killian and Hubbard 2004; Korta et al. 2012). In addition, proper oogenesis requires input from more distal parts of the germ line (Nadarajan et al. 2009; Kim et al. 2012, Chap. 10). Third, different forms of dietary restriction may affect different aspects of germline development. This possibility is likely since limiting factors required to promote cellular processes in mitosis versus gametogenesis, for example, will likely differ. Fourth, changes in the nutritional environment of the larva may confer effects that are latent and only revealed in later stages such as oogenesis. Similarly, aging and prior reproductive status must be taken into account. For example, in a study of late-life fertility after mating, Mendenhall et al. (2011) observed that among animals that are aged and then mated, “food-limited” *eat-2* mutant animals produce progeny significantly later than wild-type under similar mating conditions. However, long-lived *age-1* and *daf-2* mutants do not show a similar phenotype, suggesting that this effect cannot be attributed solely to aging. Fifth, feeding is itself a highly regulated aspect of *C. elegans* biology. For example, pumping rate and feeding behaviors are influenced by previous dietary experience, quality of the diet, starvation, and satiety signals. These controls utilize some of the same signaling pathways implicated in the dauer decision and in the control of larval germline expansion (You et al. 2006, 2008). Combinations of food quality and genetics will be revealing. For example, Lemire and colleagues (Reinke et al. 2010) analyzed the phenotypic effects of two strains of *E. coli*, OP50 and HT115, on wild-type worms and worms carrying a mutation in *nuo-1*, which encodes a subunit of mitochondrial complex I that is required for oxidative phosphorylation. While OP50 and HT115 produce similar broods in wild-type animals, *nuo-1* broods are reduced on HT115. Pathogenic *Bacillus* strains also reduce *C. elegans* fecundity compared OP50 (Rae et al. 2010). Thus, it is clear that not only the amount of food, but also the quality of food affects *C. elegans* reproduction. It will be important to determine the underlying causes of brood size differences. Finally, the potential for intergenerational effects of growth conditions and diet (see, for example, Grishok et al. 2000; Greer et al. 2011; Rechavi et al. 2011) on the germ line must be considered.

Many aspects of the germline response to physiology are completely open areas for investigation. In addition to food and pheromone signals, additional physiological changes that were not featured here influence germline development and reproductive success. These include the presence of sperm, various types of stress, and aging (see Rae et al. 2010; Luo et al. 2010; Hughes et al. 2007, 2011; Kim et al. 2012; Chap. 10). The range of genetic pathways known to interact with physiological sensors and effectors to alter the germ line and reproductive program is not nearly saturated. The mode and anatomical focus of action of these pathways that mediate these effects will be important to help understand how the germ line responds to physiological conditions and how these responses are coordinated with other tissues. For example, the *C. elegans* genome encodes many orphan receptors including large families of nuclear hormone receptors and G-protein-coupled receptors. In addition to reporting environmental conditions, these receptors likely facilitate systemic responses to pheromones, hormones, and other bioactive molecules such as biogenic amines, all of which may influence the germ line. Males and hermaphrodites also likely differ in their reproductive responses as they respond differently to certain physiological changes (see for example Tan et al. 2011; LeBoeuf et al. 2011). In addition, the contribution of cellular processes such as autophagy and intra-germline signaling is open. Understanding the mechanisms of germline sensitivity and plasticity, both at the level of physiological conditions and germline responses are very exciting areas for future studies.

5.7 Broader Implications

Many of the processes and pathways important for the germ line to interpret and respond to physiological changes, including metabolic pathways and signal transduction pathways, are highly conserved between worms, flies, and mammals, suggesting that findings in worms will be broadly applicable. For example, in both worms and flies, insulin/IGF signaling affects the cycling of germline stem cells—specifically in the G2, while TOR and S6K signaling affect both cell cycle and differentiation (Drummond-Barbosa 2008; Lafever et al. 2010; Michaelson et al. 2010; Korta et al. 2012). In addition, these investigations may be relevant to our understanding of stem cell regulation and cancer. For example, in the first half of the twentieth century, many studies explored the phenomenological impact of diet on tumor prevalence and growth (see, e.g., Rous 1914; Tannenbaum and Silverstone 1953), but only recently have organismal dietary changes in mammals been revisited and linked to signal transduction pathways known to promote cancer in humans (Kalaany and Sabatini 2009). The worm offers a simple system to explore these effects in mechanistic depth, and offers the benefit of unbiased function-based gene discovery. We look forward to learning more about the intersection of physiology and the germ line as this exciting field expands.

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Fig. 5.1.

The *C. elegans* lifecycle, diapause, and germline development. Figure adapted from (Wood et al. 1980) and modified from (Korta and Hubbard 2010). Time (hours at 25°), hatching and molts are indicated. Physiologically sensitive diapause and larval germline expansion are indicated. Circular arrow indicates renewal potential of adult germ cells

Fig. 5.2.
Signaling for developmental and germline response to poor conditions by PI3K, TGF β , AMPK, and S6K pathways. See text for details and references

Table 5.1

Genetic and nutritional control of the accumulation of the larval germline progenitor cells

Pathway or condition disrupted	Adult proliferative number ^a	Cellular mechanism(s) of defect	Additional defect on low food? ^b	Tissue-autonomy	Enhances Glp-1 phenotype?	Refs
none (wild type)	~200 n/a	—	major ^c	—	—	Michaelson et al. (2010), Korta et al. (2012), Dalfó et al. (2012)
GLP-1 (Notch)	~100 0	↓Prolif/↑diff	major ^c	germ line	—	Michaelson et al. (2010), Korta et al. (2012)
DAF-2 (Insulin/IGF)	~130 ~70	↓Cell cycle	ND	IIR pathway germ line	no	Michaelson et al. (2010)
DAF-1 (TGF b)	~125 ~90	↓Prolif/↑diff	none ^d	TGF b R pathway DTC	yes ^e	Dalfó et al. (2012)
RSKS-1 (TOR-S6K)	~100 n/a	↓Cell cycle ↓Prolif/↑diff	minor ^c	germ line	yes ^f	Korta et al. (2012)
PRO-1 (rRNA processing)	~70 n/a	ND	ND	distal sheath	yes	Killian and Hubbard (2004), Voutev et al. (2006)
Food (bacterial) Concentration	~50 ^c n/a	↓Cell cycle	—	—	yes ^c	Korta et al. (2012)

^aTop number: approximate number of proliferative zone nuclei in early adult hermaphrodite germ lines in the following mutant or food conditions, as assayed at 20°: *glp-1(e2141)*, *daf-2(e1370)*, *daf-1(m40)*, *rsks-1(sv31)*, *pro-1(na48)*, OP50 bacteria on solid media plated at 1×10^8 colony forming units. These same conditions were used, where relevant, to assess enhancement of *glp-1(rf)* as indicated in the 6th column Bottom number: indicates the number of proliferative zone nuclei under more stringent conditions: *glp-1(null)*, *daf-2(e1370)* after shift to 25°, and *daf-7(e1372)*; *daf-12(m20)* at 25°. “n/a” indicates “not applicable”; “ND” indicates “not determined”

^bReduced bacteria concentration on solid media

^cBacteria concentration 1×10^8 on solid media

^dBacteria concentration 5×10^8 on solid media

^eEarly adult phenotype is a further-reduced number of proliferative zone cells, but not an “all-meiotic” total loss of proliferative zone as seen in the most severe loss of *glp-1*

^fEarly adult enhancement phenotype includes elevated penetrance of “all-meiotic” phenotype