Chapter 3
Sex Determination in the *Caenorhabditis elegans* Germline

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Abstract How is sex determined? In the animal kingdom, there are diverse sets of mechanisms for determining organismal sex, with the predominant ones being chromosomally based, either a dominant-acting sex chromosome or the ratio of the number of X chromosome to autosomes, which lead to oocyte-producing females and sperm-producing males. The resulting germline sexual phenotype is often the logical consequence of somatic sex determination. In this respect however, the *Caenorhabditis elegans* hermaphrodite is different from mammals and *Drosophila*. In fact in the *C. elegans* hermaphrodite germline, male gametes are transiently produced in a female body during larval development. To override chromosomal signals, sex determination of germ cells strongly depends on post-transcriptional regulation. A pivotal role for male gamete production (spermatogenesis) is played by the *fem-3* mRNA, which is controlled through FBF and other RNA-binding proteins or splicing factors. Thanks to its powerful genetics, transparent body, small size, and the ability to make sperm and oocytes within one individual, *C. elegans* represents an excellent system to investigate cellular differentiation and post-transcriptional control.

Keywords Germ cells • Sex determination • RNA processing • *C. elegans* • Gametogenesis

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3.1 Introduction

Reproduction is one of the life's essential features. While vegetative growth or fission generates clonal progeny, sexual reproduction enhances genetic variation and therefore offers an additional possibility for adaptation. Sexual reproduction uses gametes that are generally of two types: sperm and oocytes. These specialized cells are produced through meiosis, allowing two different haploid genomes to combine at fertilization. This chapter describes the processes of sex determination in the germline.

3.1.1 Sex Determination Among Species

Although sex determination leads to a clear-cut decision, male or female, the mechanisms that are behind this process vary widely. These are directed through chromosomal signals as well as environmental cues such as temperature, hormones, and nutrition. In this chapter we focus on the mechanisms in *Caenorhabditis elegans*. Of course, much is also known in other model organisms, such as the fruit fly *Drosophila melanogaster* and vertebrates. These will be shortly introduced to point at possible links with *C. elegans*. In this chapter we have not included *Danio rerio* (zebrafish), a well-studied vertebrate model organism. In fact, in contrast to other fish species with male (XY) or female (ZW) heterogamety, zebrafish has no sex chromosomes. Instead, its sex determination is influenced by environmental cues and the presence of the gonad, which is crucial for female sex (Slanchev et al. 2005).

3.1.1.1 Mammals

As in many cases, important findings have come from investigation of disorders in humans. The role of the Y chromosome in mammalian sex determination is a classical example. More than 50 years ago, Ford and colleagues identified an abnormal karyotype in patients with Turner's syndrome (Ford et al. 1959). Individuals affected by this syndrome lack parts of, or the entire Y heterosome. Moreover, all patients are phenotypically female, indicating that the Y chromosome is not required for female development. Nevertheless, such genotypic X0 females are sterile due to gonadal dysfunction (Ford et al. 1959). Additional evidence for Y chromosome function came from patients with Klinefelter syndrome (XXY karyotype; Jacobs and Strong 1959). In spite of an additional X chromosome, Klinefelter individuals develop as males. Both disorders led to the identification of the Y chromosome as a major determinant for male sex determination in mammals.

Thirty years later, the discovery of the *Sry* (*S*ex determining *r*egion on *Y*) gene further illuminated the dominantly acting function of the Y chromosome in sex determination (Sinclair et al. 1990; Gubbay et al. 1990). The role of the *Sry* gene as a major effector for sex determination was confirmed with transgenic XX mice

which developed into phenotypic males when bearing a copy of this gene (Koopman et al. 1991). SRY is a transcription factor of the SOX family (SRY-related HMG box). The HMG box is crucial for its action because mutations in this domain lead to XY females (reviewed in Harley and Goodfellow 1994).

The general process of sex determination in mammals features two main events. Primary sex determination is strictly chromosomal and establishes the sexual identity of the gonads. At a later step, the sexual phenotype is shaped through hormones secreted by the sexually determined gonad. This second event is referred to as secondary sex determination. Mammalian gonads first arise as bipotential organs which later develop into either ovaries or testes. In response to *Sry*, the bipotential gonad is switched to the male fate. The female fate instead does not require *Sry* and is therefore considered as a default state (Ford et al. 1959; Sinclair et al. 1990; Jacobs and Strong 1959). In males, *Sry* activation results in upregulation of *Sox9* (SRY box containing gene 9) and repression of *Wnt4*, which would otherwise activate the female sex-determining pathway in the genital ridge (Kent et al. 1996; Tamashiro et al. 2008; Vaiman and Pailhoux 2000). Therefore, different genes are expressed thus leading to development of testis instead of ovary (for a review see Kashimada and Koopman 2010). In mammals, secondary sex determination is triggered by sex-specific hormones secreted by the testis or the ovary. It leads to somatic male or female phenotypes (reviewed in Ostrer 2000).

In mammals, germ cells are indistinguishable prior to colonization of the genital ridges (McLaren 1984; Donovan et al. 1986). Germ cell sex is determined through the sexual phenotype of the somatic environment, rather than their own heterochromosomal content (for review, see Ewen and Koopman 2010).

3.1.1.2 *Drosophila melanogaster*

Almost 100 years ago, Calvin Bridges observed that genotypic XXY flies developed as females and X0 flies developed as somatic sterile males (Bridges 1916). He concluded that the Y chromosome was required for male fertility in *Drosophila*, but that it was not necessary for sex determination. Thus, contrary to mammals, the *Drosophila* Y chromosome does not function in sex determination. Rather, the ratio between the number of X chromosomes and sets of autosomes (X:A) proved to be the main determinant for *Drosophila* sex (Bridges 1916). It took 60 years to identify the *sex-lethal* gene as the "reader" of the X:A ratio (Cline 1978). The expression of *Sxl* depends on at least four X-linked genes (*scute*, *sisA*, *runt*, and *unpaired*) which encode transcription factors that activate the early promoter of *Sxl* (*SxlPe*). The current working model postulates that activation of *SxlPe* is initiated when a threshold concentration of X-linked signal elements (XSE) is reached (reviewed in Salz and Erickson 2010). Upon activation of *SxlPe*, the late promoter of *Sxl* (*SxlPm*) is activated in all somatic cells of both sexes (Gonzalez et al. 2008). The activation of this second promoter ensures the maintenance of *Sxl* action in females (Gonzalez et al. 2008). Furthermore, action of the Sxl protein is restricted to females through alternative splicing of its pre-mRNA: an autoregulatory feedback mechanism leads to skipping of the third exon, which contains a stop codon (Cline 1984; Bell et al. 1991).

Sxl protein binds near the 3′ splice site and modulates splicing efficiency of specific introns in other pre-mRNAs that code for downstream effectors. For example, Sxl causes exon skipping in the female *transformer* (*tra*) mRNA, which results in female-specific splicing of *doublesex* (Hoshijima et al. 1991). The *tra* genes regulate the alternative splicing of the switch gene *doublesex* (*dsx*), which produces either the male or female isoform of the Doublesex protein (Baker and Wolfner 1988). *dsx* is responsible for somatic sexual development in most cells, including the somatic gonad (Coschigano and Wensink 1993; Ryner et al. 1996).

In *Drosophila,* how somatic tissues influence the sexual fate of germ cells is not well understood. The Jak/Stat pathway appears to be involved, perhaps being largely responsible for establishing the male fate in male gonads. Signals emanating from the female soma remain to be identified (Wawersik et al. 2005). While the somatic gonad participates in determining germ cell sexual fate, germ cells also determine their fate autonomously. In this respect, *Sxl* and other genes such as *Ovo* and *Otu* (*ovarian tumor*) contribute directly to female identity in XX germ cells (reviewed in Casper and Van Doren 2009).

3.1.1.3   *C. elegans*

The primary signals for chromosomal sex determination are similar in *C. elegans* and in *Drosophila*. The balance between the number of X chromosomes and the number of sets of autosomes also determines the sexual phenotype in worms (Nigon 1952). Albeit essential for male fertility, the Y chromosome in *Drosophila* does not control sex determination (Bridges 1916). In *C. elegans* there is no Y chromosome. The "heterogametic" sex is therefore denoted as X0, as opposed to the XX "homogametic" sex. In *C. elegans*, low X dosage (X0) leads to activation of the male-specific switch gene *xol-1* (*X0 l*ethal) (Miller et al. 1988). *xol-1* is on top of the sex determination pathway and promotes male development. In contrast, *C. elegans* XX embryos undergo female development as a result of *xol-1* inactivation through the X-linked determinants *sex-1* and *fox-1* (Carmi et al. 1998; Hodgkin et al. 1994) and possibly others. It should be noted at this point that *C. elegans* females are truly protandric hermaphrodites because they transiently produce sperm (Miller et al. 1988).

The absence of *xol-1* in females allows *sdc-2* expression (*s*ex and *d*osage *c*ompensation) (Nusbaum and Meyer 1989). *sdc-2* functions together with *sdc-1* and *sdc-3* in hermaphrodites (DeLong et al. 1993; Villeneuve and Meyer 1987; Trent et al. 1991). In addition to their role in sex determination, *sdc* genes also function in dosage compensation (Lieb et al. 2000). The remaining part of the sex determination pathway consists of a cascade of negative regulatory interactions that alternately activate or repress male- or female-specific genes. More precisely, the SDC proteins repress *her-1* to prevent male development (Trent et al. 1991). In the absence of HER-1, TRA-2 blocks the action of the FEM proteins and, as a result, the active TRA-1 transcription factor determines the female fate. In contrast, X0 individuals have active HER-1, which represses TRA-2. Further downstream, the FEM proteins inactivate TRA-1 and therefore control male development by activating *fog-1* and *fog-3* (reviewed in Zarkower 2006). Activation of *fog-1* and *fog-3*
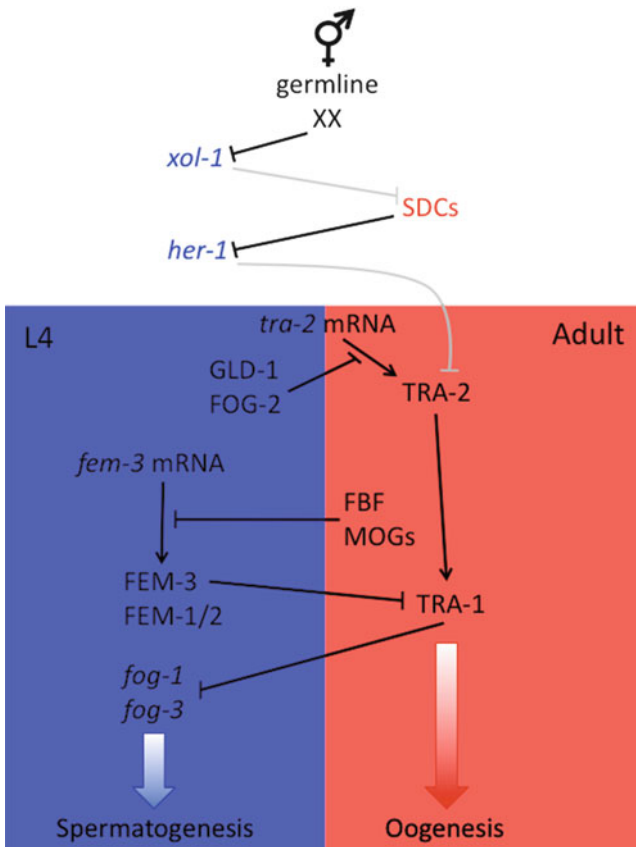
Fig. 3.1 Principal genes controlling sex determination in the *C. elegans* XX hermaphrodite germline. In response to a double dose of X signal elements, the *xol-1* gene is inactivated, thus allowing *sdc* to repress *her-1* and to promote dosage compensation. Post-transcriptional regulation for transient spermatogenesis and the sperm-oocyte switch characterize the *tra-2* and *fem-3* mRNAs. *tra-2* is repressed by GLD-1 and FOG-2, which are required for spermatogenesis. *fem-3* is repressed by the FBF and MOG proteins to allow the switch to oogenesis. While TRA-2 activates TRA-1, the FEM proteins repress its action. TRA-1 drives oogenesis and inhibits spermatogenesis by repressing *fog-1* and *fog-3*. Only the main interactions are shown

takes place either indirectly, through *tra-1*, or directly through the *fem* genes. In fact *fem-3;tra-1* double mutants produce oocytes and not sperm (Hodgkin 1986).

In *C. elegans*, the ratio of sex chromosomes to autosomes influences sex determination of the germ cells, as X0 animals exclusively make sperm and adult XX individuals continuously produce oocytes. Interestingly, the developmental balance is transiently shifted to the opposite sex during the "female" development, so that XX animals become self-fertile hermaphrodites. The determination of germ cell sex in the *C. elegans* hermaphrodite thus represents an extremely interesting system for the study of cell fate specification (Fig. 3.1).

3.1.2   Sexual Dimorphism in C. elegans

Sexual dimorphism in *C. elegans*, as in many other animals, is generated through the action of genes acting in the sex determination pathway. Germ cells in *C. elegans* X0 males are not responsible for their own sexual fate but depend on HER-1, which is mainly produced in the intestine and acts as a secreted ligand to shift the balance towards the male fate (Kuwabara 1996; Perry et al. 1993). HER-1 is only produced in X0 males. In the germline, HER-1 is responsible for the indirect activation of the *fem* genes, *fog-1*, and *fog-3,* which are needed for spermatogenesis (Hodgkin 1986; Ellis and Kimble 1995; Doniach and Hodgkin 1984; Barton and Kimble 1990). *tra-1* is the major effector of somatic sex determination in the hermaphrodite. It also promotes sexual dimorphism of the male germline. In fact, loss of *tra-1* results in transient spermatogenesis and continuous oogenesis in adult X0 individuals (Schedl et al. 1989; Hodgkin 1987). This indicates that *tra-1* is required for maintenance of spermatogenesis in males. Furthermore, partial absence of *tra-1* activity in males results in expression of *dmd-3* and *mab-3,* which contribute to the morphogenesis of the male copulatory organ (Mason et al. 2008). Recent studies have shown that in adults, male and hermaphrodite germlines not only differ in their content of mature gametes, but also in terms of the maintenance of germline stem cells (Morgan et al. 2010). Actually, the mitotic region in males is longer and thinner than in hermaphrodites. Moreover, the extent of the cell-cycle length appears to be shorter in males than in hermaphrodites (Morgan et al. 2010). This aspect of sexual dimorphism is caused by the influence of the somatic gonad on the distal germline and suggests that the mitotic region might play a role in early events of germline sex determination and commitment for meiosis (Morgan et al. 2010).

3.1.3   Cell Fate Decisions in the C. elegans Germline

Albeit it is interesting to study how the male fate is maintained throughout the development of X0 individuals, it is much more intriguing to understand how spermatogenesis is achieved in a somatic female environment of a hermaphrodite. Germ cells in *C. elegans* hermaphrodites develop either into male or female gametes, depending on the worm's developmental stage. The first three larval stages of *C. elegans* males and hermaphrodites are characterized by mitotic proliferation. During the L3 stage, germ cells that are located at the proximal end of the gonad begin to differentiate and thus enter the meiotic cell cycle. These cells continue differentiating during the fourth larval stage and are destined to undergo spermatogenesis in both males and hermaphrodites. In contrast, germ cells that enter meiosis in the L4 stage and beyond are destined to female development and differentiate as oocytes in hermaphrodites (reviewed in Schedl 1997 and Kimble and Crittenden

2007). Intriguingly, spermatozoans and oocytes are completely different in size and function, but develop from a common pool of undifferentiated cells.

In summary, the development of gametes in *C. elegans* hermaphrodites is the result of three fundamental decisions. First, during embryogenesis, cells are specified as germ cells rather than somatic blastomeres. Second, cells proliferate, not only during larval stages L2 and L3, but also throughout later developmental stages, when they keep dividing only in the distal portion of the germline. Third, when germ cells differentiate by entering the meiotic cell cycle, they can develop either as spermatids or as oocytes (Fig. 3.2).

3.2 The Players in *C. elegans* Sex Determination

3.2.1 *xol-1 Is a Master Switch Gene Controlling Sex Determination in C. elegans*

As mentioned above, the sex of *C. elegans* embryos is determined by the ratio between the number of X chromosomes and sets of autosomes (Nigon 1952). Studies by Madl and Herman determined that an X:A ratio of 0.67 leads to males, while a ratio higher than 0.75 results in female development (Madl and Herman 1979). The signals from the X chromosome correspond to at least *fox-1* and *sex-1* (Carmi et al. 1998; Hodgkin et al. 1994). Other signals originate from the autosomes (the *sea* genes (Powell et al. 2005; reviewed in Wolff and Zarkower 2008). An X:A ratio equal or below 0.75 results in the activation of *xol-1* and therefore leads to the male fate. Conversely*,* an XX genotype leads to inactivation of *xol-1* (Miller et al. 1988). Therefore, *xol-1* functions as a master switch gene that controls sex determination, analogous to *Sxl* in *Drosophila*. In addition to its role in sex determination, *xol-1* inhibits dosage compensation in males by repressing the *sdc* genes (Miller et al. 1988; Rhind et al. 1995; Nusbaum and Meyer 1989). *xol-1* is regulated both transcriptionally by the SEA proteins and SEX-1, and post-transcriptionally by the RNA-binding protein FOX-1 (Skipper et al. 1999; Carmi et al. 1998; Powell et al. 2005). Although the exact mechanism of *xol-1* regulation is not fully understood, two lines of evidence indicate that *xol-1* is the unique target of chromosomal signals. In fact, *xol-1* null mutations only affect male development, while hermaphrodites develop normally (Miller et al. 1988). Furthermore, XX animals that ectopically express *xol-1* develop as males, indicating that *xol-1* acts as an early switch factor for sex determination (Rhind et al. 1995). A recent study proposes that *xol-1* is also transcriptionally regulated by the feminizing protein TRA-1, which sits at the end of the sex determination cascade. Therefore, at least in somatic tissues, regulation of *xol-1* may include a feedback loop (Hargitai et al. 2009).
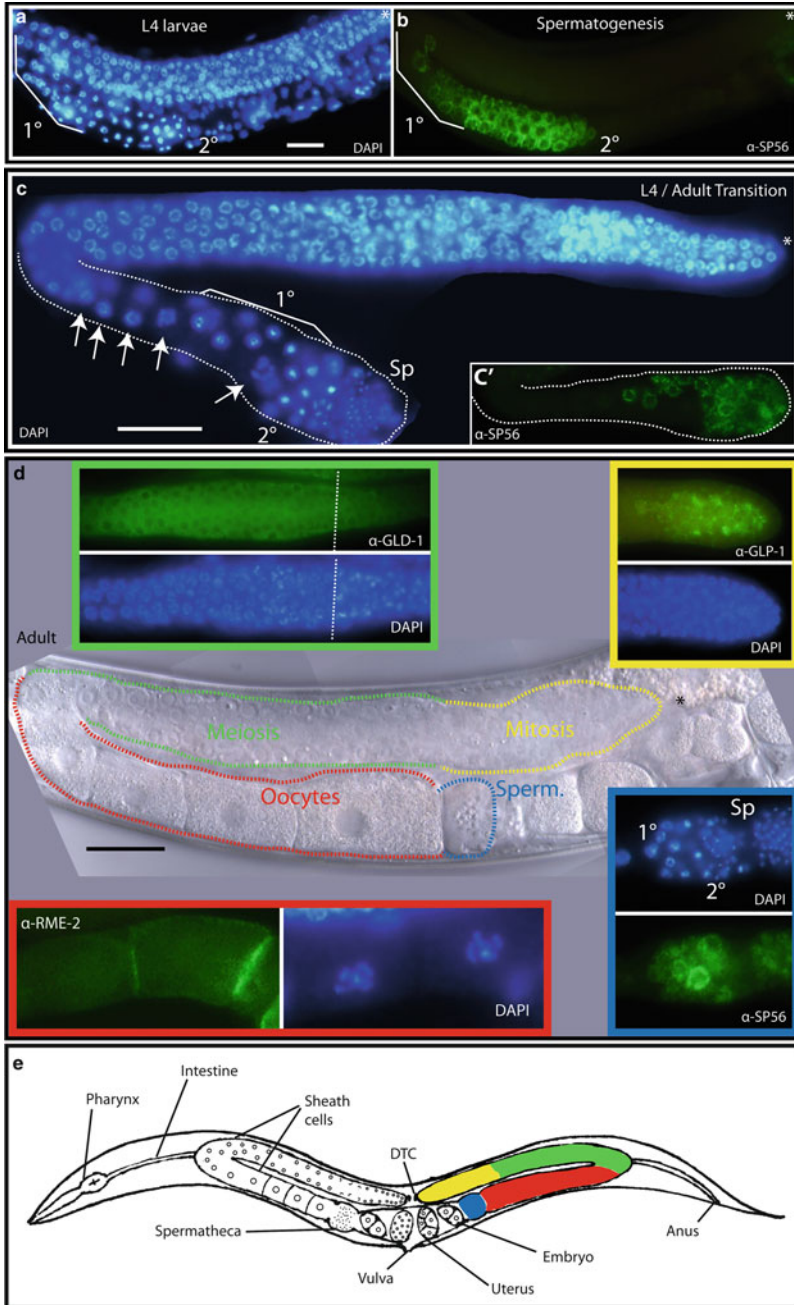
Fig. 3.2 Morphology and protein expression throughout hermaphrodite germline development. (**a**, **b**) germline arm of an L4 larva. Chromosomal staining with DAPI shows primary (1°) and secondary (2°) spermatocytes. No spermatids are visible at this step. Immunolocalization of sperm-specific poly-peptides in the cytoplasm using anti-SP56 antibodies (Ward et al. 1986). (**c**) Staining of a dissected gonad at the transition from L4 to adult. Spermatids and spermatozoans are shown (Sp).

3.2.2 Dosage Compensation and Somatic Sex Determination

Although sex determination in *C. elegans* is initiated by the dose of X chromosomes, normal development requires identical amounts of X-chromosomal gene products in both sexes. Dosage compensation in *C. elegans* is controlled by three *sdc* genes that act downstream of *xol-1* (Rhind et al. 1995). Among these, *sdc-2* plays a predominant role in dosage compensation, as well as promoting hermaphrodite sex determination (Chu et al. 2002; Dawes et al. 1999). SDC-2 belongs to a protein complex containing SDC-1, SDC-3, DPY-21, DPY-26, DPY-27, DPY-28, and DPY-30 (reviewed in Meyer 2005). This dosage compensation complex functions as repressive machinery that reduces by half the transcription on both X chromosomes in hermaphrodites (Meyer and Casson 1986). The mechanism that controls X0 specific *her-1* transcription and therefore sex determination requires the same complex, but lacks DPY-21. It also differs in other aspects. First SDC-2 recognizes elements on the X chromosome, while *her-1* is located on an autosome and is recognized by SDC-3. Second, in hermaphrodites, the transcription rate of *her-1* is reduced by 20-fold and not by 2-fold, as for X-linked genes (Yonker and Meyer 2003). By repressing *her-1*, *sdc* genes affect the activity of all genes that are further downstream in the sex determination pathway. They therefore play a dual role in dosage compensation and sex determination.

3.2.3 Germline Sex Determination Genes

HER-1 acts as a secreted ligand for male sex determination by inhibiting the function of the transmembrane receptor TRA-2A (Perry et al. 1993; Hamaoka et al. 2004; Kuwabara 1996). The current model postulates that TRA-2A represses the FEM proteins (FEM-1, -2, -3) in XX animals, thereby leaving transcription factor TRA-1 active. TRA-1 is the terminal activator for female development in somatic tissues (for review, see Zarkower 2006). In X0 individuals, HER-1 binds and represses TRA-2A. As a consequence, the FEM proteins are active and repress TRA-1 to achieve male development (Hodgkin 1986). To do so, the FEM proteins, together with CUL-2, direct proteasome-mediated degradation of TRA-1 (Starostina et al. 2007).

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Fig. 3.2 (continued) The first oocytes are visible (*white arrows*). The distal end of the germline is indicated by an *asterisk*. (**d**) DIC image of an adult germline with the localization of markers for mitosis (GLP-1), meiosis (GLD-1), spermatogenesis (SP56), and oogenesis (RME-2). The *inset* (*bottom, right*) with spermatocytes and spermatids comes from a younger worm in which oocytes have not yet pushed all male germ cells into the spermatheca. The dotted line in the inset with GLD-1 expression indicates the transition zone between mitosis (*right*) and meiosis (*left*). (**e**) Drawing of an adult hermaphrodite. The germline has the same color code as in panel (**d**): *yellow* = mitosis; *green* = different stages of the meiotic sequence; *red* = mature and maturing oocytes; *blue* = sperm. The somatic gonad is made up of the distal tip cell (DTC), the sheath cells, spermatheca and uterus. Nuclear morphology is shown by DAPI staining. Bar: 10 μm

In the germline of hermaphrodites, which produce both spermatids and oocytes, sex determination is more complex because additional factors are required for this process. Nevertheless, *C. elegans* hermaphrodites offer the possibility to study regulation of spermatogenesis and oogenesis within the same individual. Moreover, both gametes are produced from a single pool of germline stem cells (for a review, see Schedl 1997). This additional regulation requires the control of *tra-2* and *fem-3* (for a review, see Puoti et al. 2001; Ellis 2010). As a first step, *tra-2* is repressed for the onset of spermatogenesis in the L3 larva (Doniach 1986). After a transient period of spermatogenesis, the onset of oogenesis coincides with the repression of spermatogenesis. *fem-3* regulation plays a pivotal role in the sperm-oocyte switch (Kimble et al. 1984; Ahringer and Kimble 1991; Barton et al. 1987). While the sex determination pathway culminates with the activation or repression of *tra-1* in somatic tissues, *fog-1* and *fog-3* are also required in the germline for spermatogenesis in both males and hermaphrodites (reviewed in Ellis and Schedl 2007). Moreover, *fbf* and the *mog* genes are required for oogenesis by repressing *fem-3*, but do not influence somatic sex determination (Gallegos et al. 1998; Graham and Kimble 1993; Graham et al. 1993; Zhang et al. 1997). The sexual fate of germ cells in the hermaphrodite therefore depends on a balance between *tra-2* and *fem-3* activity. In fact *tra-2(gf)* mutations result in feminized germlines, whereas *fem-3(gf)* alleles lead to masculinization. Furthermore, gain-of-function mutations in *tra-2* and *fem-3* suppress each other (Barton et al. 1987).

3.3 Regulation of Germline Sex Determination in *C. elegans*

3.3.1 Transcriptional Control of her-1

After *xol-1,* which is controlled by the X:A ratio, *her-1* is the next gene in line for male sex determination. Ectopic expression of *her-1* in XX animals results in partial masculinization (Trent et al. 1991). How does XOL-1 determine the activity of *her-1* in males? While the activation of *her-1* in X0 animals remains mysterious, there is evidence that *her-1* must be repressed in hermaphrodites. When comparing X0 and XX animals, *her-1* mRNA levels are clearly reduced in the latter, indicating that *her-1* is transcriptionally regulated (Trent et al. 1991). Furthermore, *her-1* gain-of-function alleles correspond to mutations in the *her-1* promoter region and lead to increased transcription (Trent et al. 1991; Perry et al. 1994). Finally, elevated levels of *her-1* RNA are also observed in *sdc-1* or *sdc-2* mutants, suggesting that the latter are involved in transcriptional repression of *her-1* in hermaphrodites (Trent et al. 1991). Together with SDC-1 and SDC-2, SDC-3 preferentially recognizes the *her-1* promoter region (Yonker and Meyer 2003). After recognition by SDC-3, the whole dosage compensation machinery, except DPY-21, is recruited by SDC-2 to the regulatory region of *her-1*. This results in *her-1* silencing (Yonker and Meyer 2003; Chu et al. 2002). In the male germline, *her-1* expression is constantly maintained to ensure continuous spermatogenesis (Hodgkin 1980).

3.3.2   Role of the Transcription Factor TRA-1 in the Germline

tra-1 loss-of-function (lf) XX mutant animals are somatically transformed into males (Hodgkin and Brenner 1977). *tra-1* is thus required for the female fate. It regulates, among other genes, two homologs of the *Drosophila melanogaster doublesex* gene: *mab-3* and *dmd-3* (Raymond et al. 1998; Mason et al. 2008). In the germline the situation is less clear since *tra-1 (lf)* XX or X0 gonads transiently produce sperm and then switch to oogenesis (Schedl et al. 1989). Therefore, XX *tra-1* null mutants have normal germlines making both sperm and oocytes. In contrast, *tra-1* X0 mutants produce ectopic oocytes, indicating that *tra-1* is required for continuous production of sperm in males (Hodgkin 1987). The *tra-1* gene encodes two mRNAs that are translated into two transcription factors containing either two or five zinc-finger motifs (Zarkower and Hodgkin 1992). The larger transcript is present at constant levels throughout development, while the shorter peaks during the second larval stage (Zarkower and Hodgkin 1992). Nevertheless, the levels of both *tra-1* mRNAs are similar in males and hermaphrodites, indicating that *tra-1* is regulated post-transcriptionally (Zarkower and Hodgkin 1992). All *tra-1* activity is attributed to the larger transcript that encodes the TRA-1A protein, while the shorter TRA-1B iso-form has no obvious function (Zarkower and Hodgkin 1992). TRA-1A, referred to as TRA-1 in this report, belongs to the GLI family of transcriptional repressors, but it also includes potential activators of transcription (Koebernick and Pieler 2002). TRA-1 is activated by TRA-2 and TRA-3, and repressed through CUL-2 and three FEM proteins, which target TRA-1 for proteasome-mediated degradation (Starostina et al. 2007; Schvarzstein and Spence 2006; Sokol and Kuwabara 2000). TRA-1 acti-vation by TRA-2 is achieved through the intracellular part of TRA-2, which binds to TRA-1 (Lum et al. 2000; Wang and Kimble 2001). In addition, TRA-3 can cleave the intracellular part of TRA-2 to generate an active peptide that binds and represses FEM-3, thus preventing degradation of TRA-1 (Starostina et al. 2007; Sokol and Kuwabara 2000). Nuclear TRA-1 represses the transcription of *fog-3*, a terminal regulator for spermatogenesis (Chen and Ellis 2000). Additional results show that TRA-1 is also involved in positive regulation of *fog-3*, possibly explaining the requirement of TRA-1 for continuous spermatogenesis in males (Chen and Ellis 2000). These results place TRA-1 as a regulator for both somatic and germline sex determination in males and hermaphrodites (Chen and Ellis 2000).

TRA-1 is also regulated at the level of its protein, which is cleaved differently in males and in hermaphrodites (Schvarzstein and Spence 2006). First, the TRA-1 levels are higher in hermaphrodites as compared to males. Second, a shorter prod-uct, TRA-1[100] accumulates in adult hermaphrodites, while in masculinized animals, which produce only sperm, the larger isoform is predominant (Schvarzstein and Spence 2006). Taken together, full-length TRA-1 promotes spermatogenesis in adult males, and the shorter TRA-1[100] is required for oogenesis (Schvarzstein and Spence 2006, reviewed in Ellis 2008). Finally, in feminized *fem-1* and *fem-3* mutants both isoforms of TRA-1 accumulate, indicating that the FEM complex controls TRA-1 degradation (Schvarzstein and Spence 2006).

3.3.3 Post-transcriptional Regulation: 3'UTR-Mediated Control in the Germline

Many germline genes encode RNA-binding proteins, and most of them are required for germline development (reviewed in Lee and Schedl 2006; Nousch and Eckmann 2012, Chap. 8). In addition, the 3′untranslated regions (UTR) are primary regulatory elements for the expression of many germline mRNAs (Merritt et al. 2008). In fact, based on GFP reporter genes flanked by specific 3′UTRs, most germline genes are faithfully expressed, therefore suggesting that post-transcriptional mechanisms play a major role in the germline. This does not, however, apply to genes that are specifically expressed in sperm (Merritt et al. 2008). One of the best characterized translational regulators is the STAR/KH domain protein GLD-1, which has three distinct functions in the germline: initiation of meiosis, progression through the meiotic prophase in oocytes, and spermatogenesis in hermaphrodites (Kadyk and Kimble 1998; Jones et al. 1996; Francis et al. 1995a,b). The GLD-1 protein physically interacts with several mRNAs, including the *tra-2* mRNA (Jan et al. 1999; Jungkamp et al. 2011; Lee and Schedl 2001, 2004).

3.3.4 Transitory Spermatogenesis and Translational Control of tra-2 by GLD-1 and FOG-2

tra-2 promotes female fates in both germline and somatic tissues (Hodgkin and Brenner 1977). The discovery of *tra-2* gain-of-function *(gf)* mutant alleles led to the identification of *cis*-acting negative regulatory elements in its 3′UTR. These elements (TGE, Tra and GLI element; also known as DRE, direct repeat element) correspond to two 28-nucleotide tandem repeats (Goodwin et al. 1993). Dominant mutations in the TGEs transform XX animals, but not X0 males, into females producing only oocytes (Doniach 1986). *tra-2(gf)* mutations do not affect steady-state levels of *tra-2* transcripts suggesting that the TGEs neither control transcription nor stability of their mRNA. Rather, *tra-2(gf)* mRNAs are preferentially associated with polyribosomes indicating that gain-of-function mutated *tra-2* mRNAs are more actively translated than the wild-type transcripts (Goodwin et al. 1993). To allow transient spermatogenesis during the fourth larval stage, the TGEs are bound and repressed by FOG-2 and a dimer of GLD-1 (Jan et al. 1999; Clifford et al. 2000; Ryder et al. 2004). Repression of *tra-2* and transient spermatogenesis is abrogated in both *tra-2(gf)* and *fog-2(lf)* mutants, indicating that the binding of GLD-1/FOG-2 to the TGEs is abolished (Schedl and Kimble 1988; Jan et al. 1999; Clifford et al. 2000). Since HER-1 is not transcribed in hermaphrodites, *tra-2* expression must be post-transcriptionally repressed in hermaphrodites for spermatogenesis. In males, *her-1* is active and represses *tra-2*, allowing continuous spermatogenesis (Kuwabara 1996). Therefore post-transcriptional regulation of *tra-2* is critical for transient spermatogenesis in hermaphrodites.

3.3.5 The Sperm-Oocyte Switch and Repression of fem-3 by FBF, MOGs, DAZ-1

The role of *fem-3* in the germline of XX animals is particularly intriguing. On the one hand, in the absence of *fem-3*, XX animals develop as fertile females (Hodgkin 1986). On the other hand, *fem-3* gain-of-function alleles lead to masculinized germlines that are genotypically XX (Barton et al. 1987; Hodgkin 1986). Again, *fem-3* is regulated post-transcriptionally through its 3′UTR. More specifically, a five-nucleotide element (Point Mutation Element, PME) acts *in cis* to achieve repression of *fem-3* and therefore the switch from spermatogenesis to oogenesis (Ahringer and Kimble 1991). The PME is bound by the conserved RNA-binding proteins FBF-1 and FBF-2 (Zhang et al. 1997). *fem-3(gf)* mutations abolish FBF-binding and could therefore abrogate repression of *fem-3* (Zhang et al. 1997; Zanetti et al. 2012). The FBF proteins belong to the Puf family and include members in all eukaryotes. The most prominent example is *Drosophila* Pumilio, which prevents the translation of the *hunchback* mRNA (Murata and Wharton 1995; Wharton et al. 1998). Additional factors that are involved in regulation of *fem-3* are the translational activator DAZ-1 and the MOG proteins (Otori et al. 2006; Graham et al. 1993). DAZ-1 (*Deleted in Azoospermia*) binds and increases FBF translation and is therefore required for oogenesis.

Six *mog* genes (masculinization of the germline) are also strictly implicated in the post-transcriptional regulation of the *fem-3* mRNA via its 3′UTR. *mog* loss-of-function mutations lead to masculinized germlines, but do not affect the XX soma (Graham and Kimble 1993; Graham et al. 1993). X0 *mog* mutant males are largely normal in that they produce sperm, but have occasional somatic defects. Two observations suggest that the *mog* genes are possible regulators of *fem-3*. *fem-3* is epistatic to *mog* because *mog(lf); fem-3(lf)* double mutants produce oocytes and *fem-3(gf)* and *mog(lf)* have masculinized germlines (Graham and Kimble 1993). Therefore, *mog* genes are not essential for oogenesis, but rather for the switch from spermatogenesis to oogenesis. Additional evidence comes from somatic expression of a reporter transgene flanked by the *fem-3* 3′UTR, which is derepressed in the absence of *mog* (Gallegos et al. 1998).

3.4 fem-3: A Paradigm to Study RNA Regulation

3.4.1 The RNA-Binding Protein FBF

FBF-1 and FBF-2 specifically bind to the wild-type *fem-3* 3′UTR (Zhang et al. 1997). Both proteins are highly similar and contain a conserved RNA-binding motif composed of eight tandem repeats that are found throughout the Puf family members (Zhang et al. 1997; Zamore et al. 1997). *Drosophila* Pumilio plays an essential role in establishing an anterior–posterior gradient of *hunchback* mRNA throughout the syncytium of the embryo (Murata and Wharton 1995). It binds to the Nanos

Responsive Element (NRE) in the *hunchback* 3′UTR and represses its translation via deadenylation in the presence of the Nanos protein, which is restricted to the posterior of the embryo (Wreden et al. 1997; Wharton et al. 1998; Sonoda and Wharton 1999). Another deadenylation-independent mechanism has been proposed but also leads to translational repression of the *hunchback* mRNA (Chagnovich and Lehmann 2001; Wreden et al. 1997). To date, FBF has been found to be a broad-spectrum gene regulator potentially targeting 7 % of all protein coding genes in *C. elegans* (Kershner and Kimble 2010). In addition to its role in RNA binding, FBF interacts with poly(A) polymerase GLD-2 and with deadenlyase CCF-1/Pop2p, therefore suggesting two different roles of FBF in mRNA regulation: activation through poly(A) tail extension and repression through deadenylation (Suh et al. 2009). At least in vitro, the action of FBF on the *gld-1* RNA is predicted to occur through deadenylation and translational silencing (Suh et al. 2009; see also Nousch and Eckmann 2012, Chap. 8). FBF binds to the *fem-3* 3′UTR to achieve the switch from spermatogenesis to oogenesis in hermaphrodites (Zhang et al. 1997). This switch requires, in addition, the binding of NOS-3, a worm homolog of *Drosophila* Nanos (Kraemer et al. 1999). Therefore, parts of the mechanism that governs embryonic polarity in *Drosophila* and germ cell sex determination in *C. elegans* have been conserved throughout evolution (Kraemer et al. 1999; Zhang et al. 1997). Even if the mechanism of FBF action is not fully understood, it is hypothesized that FBF reduces the expression of *fem-3* via repression of translation, as shown for Pumilio and Nanos on *hunchback* mRNA (Crittenden et al. 2002; Hansen et al. 2004b; Wharton et al. 1998; Wreden et al. 1997).

To date, two additional mechanisms of *fem-3* regulation have been proposed. FEM-1 and FEM-3 might be subjected to degradation by the proteasome when targeted by the F-box protein SEL-10 (Jager et al. 2004). Furthermore, *sel-10* functions upstream of *fem-3* and loss-of-function mutations for *sel-10* cause weak masculinization of hermaphrodites. Therefore SEL-10 might be required for female fates via degradation of FEM-3, at least in somatic tissues (Jager et al. 2004). Another interesting possibility is that *fem-3* might be regulated through the stabilization of its mRNA (Zanin et al. 2010; Zanetti et al. 2012). In fact, *fem-3* mRNA levels are increased in the absence of *larp-1*, suggesting that *larp-1* controls the switch from spermatogenesis to oogenesis by decreasing *fem-3* mRNA levels (Zanin et al. 2010). *larp-1* codes for a protein with an ancient La RNA-binding motif, and functions in oogenesis by lowering Ras-MAPK signaling (Nykamp et al. 2008). Masculinization of the germline is more pronounced in *larp-1(lf);fem-3(q22)* double mutants than in *fem-3(q22)* animals (Zanin et al. 2010). In addition, in masculinized *fem-3(gf)/+* heterozygotes *fem-3(gf)* mRNA is more abundant than its wild-type counterpart indicating that the former is stabilized (Zanetti et al. 2012).

3.4.2   Do Splicing Factors Regulate *fem-3*?

As mentioned above, the MOG proteins are implicated in the post-transcriptional regulation of *fem-3* (Graham and Kimble 1993; Graham et al. 1993; Gallegos et al. 1998). Many MOG proteins are homologs of well-known splicing factors:

MOG-1, -4, and -5 are, for example, the worm homologs of yeast PRP16, PRP2, and PRP22, respectively (Puoti and Kimble 1999; Puoti et al. 2001). This finding suggests that MOG might regulate *fem-3* through the processing of *fem-3* itself, or via other transcripts, which in turn are required for *fem-3* repression (Kasturi et al. 2010; Belfiore et al. 2002; Puoti and Kimble 1999). General splicing, however, and in particular the splicing of *fem-3,* is normal in most *mog* null mutants (Kasturi et al. 2010). The first direct evidence for *mog* action in splicing came with *mog-2,* which encodes the worm homolog of the well-conserved U2A′ protein (Zanetti et al. 2011). The U2A′ protein is a component of the spliceosomal U2snRNP complex, which includes the protein U2B′′ and the U2snRNA (Mattaj et al. 1986; Scherly et al. 1990; Sillekens et al. 1989). *C. elegans mog-2* mutants are not defective in general splicing, but are less efficient in processing cryptic splice sites (Zanetti et al. 2011). Nonetheless, *fem-3* mRNA splicing is unaffected in *mog-2* animals. It is therefore possible that the Mog phenotype is caused by splicing detects in mRNAs that code for repressors of *fem-3*. Although the splicing targets of *mog* remain to be found, it is tempting to compare MOG-2 with *Drosophila* SNF (*San*s-*f*ille), the unique fly homolog of both U1A and U2B′′ (Nagengast and Salz 2001). SNF functions as an accessory factor in somatic and germ cell sex determination (Oliver et al. 1988; Steinmann-Zwicky 1988).

Finally, an RNAi-based survey identified homologs of splicing factors that control germline proliferation and sex determination in *C. elegans* (Kerins et al. 2010). For example, *teg-4*, *ddx-23* and *prp-17* mutants are defective in germline proliferation and sex determination, indicating that reduced splicing efficiency may affect germ cell development, with few consequences on somatic development (Kerins et al. 2010; Zanetti et al. 2011; Konishi et al. 2008; Mantina et al. 2009).

3.5 Making Appropriate Amounts of Sperm: Fine-Tuned Control of the Sperm-Oocyte Switch

In order to produce the appropriate number of sperm per gonadal arm, *fem-3* has to be exclusively active at a given time, and in the correct set of cells. GLD-3 is another RNA-binding protein regulator of spermatogenesis (Zhang et al. 1997; Eckmann et al. 2002). *gld-3* encodes a homolog of *Bicaudal-C* family of RNA-Binding proteins with two KH domains (Eckmann et al. 2002). A closer look at the phenotype of *gld-3* mutants indicates that GLD-3 is required for continuous spermatogenesis and repression of oogenesis. In hermaphrodites the number of sperms produced is strongly reduced (Eckmann et al. 2002). The possibility of GLD-3 regulating *fem-3* in this process has been explored. GLD-3 binds to FBF and functions upstream of FBF (Eckmann et al. 2002). Importantly, this interaction antagonizes the binding of FBF to the *fem-3* mRNA. Therefore, it has been proposed that a competition between GLD-3 and FBF promotes spermatogenesis by release of the *fem-3* mRNA from FBF (Eckmann et al. 2002). However, additional studies have shown that GLD-3 does not only bind to FBF-1 and FBF-2. In fact GLD-3 has also been found to associate with

GLD-2 to form an active cytoplasmic poly(A) polymerase (Wang et al. 2002). This finding leads to an alternative hypothesis for GLD-3: it could antagonize the repressing activity of FBF by promoting polyadenylation of target mRNAs, perhaps of *fem-3*, and thus favor spermatogenesis (Suh et al. 2009; Wang et al. 2002).

In addition to binding to GLD-3, GLD-2 associates with RNP-8, another RNA-binding protein, and forms a different cytoplasmic poly(A) polymerase that functions in oogenesis (Kim et al. 2009). *rnp-8* mutants produce more sperm than normal because the switch from spermatogenesis to oogenesis is delayed (Kim et al. 2009). Depending on its partner, GLD-2 either promotes spermatogenesis with GLD-3 or favors oogenesis when bound to RNP-8 (Kim et al. 2009; Suh et al. 2009). Moreover, GLD-2 also functions in sex determination by binding to either FBF-1 or FBF-2 (Suh et al. 2009). In fact, *gld-2; fbf-1* mutants are masculinized, while *gld-2; fbf-2* animals are feminized in their germlines (Kim et al. 2009). Putative target mRNAs of the GLD-2/RNP-8 poly(A) polymerase were obtained by co-immunoprecipitation (Kim et al. 2010). These targets include many maternal mRNAs that function in oogenesis of which the most prominent are *tra-2*, *gld-1*, *gld-3*, *gld-2*, and *rnp-8*. Remarkably, masculinizing mRNAs such as *fem-3, gld-3,* and *fog-1* were also identified as possible GLD-2/RNP-8 targets, suggesting that polyadenylation is not the only driving force for transient spermatogenesis in hermaphrodites (Kim et al. 2010).

In addition to RNP-8 and GLD-3, which compete for GLD-2 binding (Kim et al. 2009), GLS-1 (germline sterile) antagonizes FBF for GLD-3 binding (Rybarska et al. 2009). At least in vitro, the novel protein GLS-1 recruits GLD-3 from the FBF/GLD-3 duplex (Rybarska et al. 2009). Moreover, *gls-1* functions in oogenesis and is epistatic to *gld-3* and other genes that are required for spermatogenesis (Rybarska et al. 2009). Taken together, GLS-1 is on the top of a pathway that regulates GLD-3, which in turn counters FBF-directed repression of *fem-3* mRNA for the control of appropriate amounts of sperm in hermaphrodites (Rybarska et al. 2009).

3.5.1 FOG-1 and FOG-3 Are Terminal Regulators for Spermatogenesis

fog-1 function is restricted to the germline as it causes transformation of all germ cells into oocytes in both XX and X0 animals, without affecting the somatic phenotype (Barton and Kimble 1990). Additional phenotypic characterization revealed a dose-dependent function of *fog-1* and genetic interaction with *fbf* (Thompson et al. 2005; Barton and Kimble 1990; Ellis and Kimble 1995). In fact, *fog-1* is epistatic to *fbf* and the FBF proteins repress *fog-1* mRNA by binding to regulatory elements in its 3′UTR to promote oogenesis (Thompson et al. 2005). Therefore, *fog-1* is a target of FBF. High doses of FOG-1 promote spermatogenesis, whereas low FOG-1 levels are required for germ cell proliferation (Thompson et al. 2005). In fact *fog-1;fbf-1fbf-2* triple mutants are feminized but only make very few oocyte-like cells. *fbf-1;fbf-2* double mutants are masculinized and also largely underproliferative (Crittenden et al. 2002). However, with one dose of *fog-1, fog-1/+;fbf-1 fbf-2*

germlines are masculinized but show much more proliferation than *fbf-1 fbf-2* or *fog-1; fbf-1 fbf-2* germlines (Thompson et al. 2005). FOG-1 is a member of the CPEB family of RNA-binding proteins and might therefore control translation by regulating the poly(A) tails of target mRNAs (Luitjens et al. 2000; Mendez and Richter 2001). FOG-1 is found in germ cells that are committed to spermatogenesis, but not in spermatocytes and spermatids, indicating that high doses of FOG-1 are required for the onset on spermatogenesis (Lamont and Kimble 2007). A model for *fog-1* action suggests that low doses of FOG-1, which are necessary for germ cell proliferation, are maintained in the distal region of the germline by the combined action of *fbf* and *glp-1/Notch* signaling. Moving proximally, the level of FOG-1 increases in germ cells destined to spermatogenesis (Thompson et al. 2005). The different germ cell decisions thus appear closely related and the amount of sperm produced is under the control of FOG-1 (Lamont and Kimble 2007). In addition to being post-transcriptionally regulated by FBF, *fog-1* is also transcriptionally repressed by TRA-1 (Chen and Ellis 2000). Nevertheless, FOG-1 targets that function in spermatogenesis have not been identified to date.

Additional *fog* genes also direct spermatogenesis. *fog-3* codes for a putative deadenylase and functions in parallel with *fog-1* at the end of the sex determination cascade (Chen and Ellis 2000; Ellis and Kimble 1995). FOG-2 is located more upstream as it cooperates with GLD-1 for *tra-2* mRNA-binding and repression for the onset of spermatogenesis in hermaphrodites (Table 3.1) (Jan et al. 1999; Schedl and Kimble 1988; Clifford et al. 2000).

3.6 Sex Determination and Germline Proliferation: Same Players, Similar Mechanisms, but Different Outcomes

The previous section pointed out an additional role of *fog-1* in germ cell proliferation (Thompson et al. 2005). This applies to many other genes involved in germline sex determination (also see Hansen and Schedl 2012, Chap. 4). Proliferation of germ cells and maintenance of germline stem cells are primarily controlled by *glp-1/Notch* signaling in *C. elegans* (Austin and Kimble 1987; Morgan et al. 2010). *glp-1(0)* germlines produce only 8–16 spermatids, indicating that proliferating germ cells enter meiosis instead of expanding through mitotic divisions (Austin and Kimble 1987). Conversely, *glp-1* gain-of-function germlines are tumorous: they produce neither spermatids nor oocytes but contain thousands of mitotic nuclei (Berry et al. 1997). Tumorous germlines are also found in *gld-1(lf) gld-2(lf)* double and *glp-1(0); gld-1(lf) gld-2(lf)* triple mutants indicating that *glp-1* functions upstream and represses both *gld-1* and *gld-2* (Kadyk and Kimble 1998). *gld-1* alleles cause a variety of mutant phenotypes, such as masculinization, feminization, and germline tumors (Francis et al. 1995b). Its intriguing genetics therefore indicate multiple functions. For example, the tumorous phenotype of *gld-1* mutants is strongly enhanced in the absence of *gld-2* (Kadyk and Kimble 1998). Moreover, *gld-1* is also synthetically required for meiosis with *gld-3*, another sex determination gene (Kadyk and Kimble

Table 3.1 Comprehensive list of genes involved in germline sex determination. Genes are listed alphabetically. The relative molecular identity and role in sex determination are based on the reference allele(s) and principal publications. Some of the genes listed are not described in this review

Gene	Alleles	Protein function/identity	Role in sex determination	References
atx-2	tm3397	Translational regulator	Acts downstream of FOG-2; promotes female fate	Maine et al. (2004), Ciosk et al. (2004)
cpb-1	tm2821	Cytoplasmic polyadenylation element binding (CPEB) protein	Spermatogenesis	Luitjens et al. (2000)
cpb-2	ok1772	CPEB protein	Spermatogenesis	Luitjens et al. (2000)
cpb-3	tm1746	CPEB protein	Spermatogenesis	Luitjens et al. (2000)
cul-2	t1664	Ubiquitin ligase	Targets TRA-1 for degradation	Starostina et al. (2007)
ddx-23	tj21	Splicing factor PRP28	Sperm-oocyte switch	Konishi et al. (2008)
fbf-1	ok91	PUF RNA-binding protein, homolog of Pumilio	fem-3 repression for oogenesis	Zhang et al. (1997), Kim et al. (2009)
fbf-2	q704	PUF RNA-binding protein, homolog of Pumilio	Redundant with FBF-1; spermatogenesis	Zhang et al. (1997), Kim et al. (2009)
fem-1	e1965	Ankyrin repeat-containing protein	Spermatogenesis	Doniach and Hodgkin (1984)
fem-2	e2105	Protein phosphatase	Spermatogenesis	Chin-Sang and Spence (1996)
fem-3	e1996lf; q20gf	Novel Protein	Spermatogenesis	Hodgkin (1986), Barton et al. (1987)
fog-1	e2121	CPEB protein	Spermatogenesis	Schedl and Kimble (1988)
fog-2	q71	N-terminal F-box and C-terminal FOG-2 homology domain	Spermatogenesis	Schedl and Kimble (1988)
fog-3	tm4376	N-terminal similar to vertebrate Tob	Spermatogenesis	Ellis and Kimble (1994)
daz-1	tj3	RNA recognition motif	fbf translational activator and oogenesis	Karashima et al. (2000), Otori et al. (2006)
gld-1	q485	KH RNA-binding protein	Maintenance of meiotic prophase in oocytes	Ellis and Kimble (1994), Francis et al. (1995a,b)
gld-2	q497	Cytoplasmic poly(A) polymerase subunit	Promotes spermatogenesis and/or oogenesis	Wang et al. (2002), Kim et al. (2009)
gld-3	q730	KH RNA-binding protein; PAP subunit with GLD-2	Promotes spermatogenesis by antagonizing FBF	Eckmann et al. (2002)
gls-1	ef8	Cytoplasmic RNA regulatory protein	Represses GLD-3, promotes oogenesis	Rybarska et al. (2009)

Gene	Allele(s)	Protein	Function	References
her-1	e1518, n695	Secreted cystein-rich protein	Determines male fate in the germline and in the soma	Hodgkin (1980)
larp-1	q783	La-related RNA-binding protein	Promotes oogenesis by repressing fem-3	Zanin et al. (2010)
lin-45	sy96, dx19	RAF protein homolog	Spermatogenesis	Hsu et al. (2002), Lee et al. (2007)
mag-1	tm645	Exon–exon junction complex, homolog of Mago nashi	Oogenesis	Kawano et al. (2004)
mek-2	n2516, q425	MAP kinase kinase	Spermatogenesis	Hsu et al. (2002), Lee et al. (2007)
mep-1	q660	Zinc-finger DNA binding protein	Sperm/oocyte switch; soma-germline distinction	Belfiore et al. (2002), Unhavaithaya et al. (2002)
mog-1	q223	Splicing factor PRP16	Sperm/oocyte switch; oogenesis	Graham and Kimble (1993), Puoti and Kimble (1999)
mog-2	q75	Spliceosomal protein U2A'	Sperm/oocyte switch; oogenesis	Zanetti et al. (2011)
mog-3	q74	Splicing factor sharing homologies with Cwf25 and Cwc25	Sperm/oocyte switch; oogenesis	Kasturi et al. (2010)
mog-4	q233	Splicing factor PRP2	Sperm/oocyte switch; oogenesis	Puoti and Kimble (2000)
mog-5	q449	Splicing factor PRP22	Sperm/oocyte switch; oogenesis	Puoti and Kimble (2000)
mog-6	q465	Nuclear cyclophilin-like protein	Sperm/oocyte switch; oogenesis	Belfiore et al. (2004)
mpk-1	ga117	Mitogen-activated protein kinase	Spermatogenesis	Hsu et al. (2002), Lee et al. (2007)
nos-3	oz231	Homolog of *Drosophila* NANOS	fem-3 repression; oogenesis	Kraemer et al. (1999)
prp-17	oz273	Splicing factor PRP17/CDC40	Oogenesis	Kerins et al. (2010)
puf-8	q725	RNA-binding protein related to Pumilio	Switch to oogenesis	Bachorik and Kimble (2005)
rnp-8	tm2435	RRM RNA-binding protein; PAP subunit with GLD-2	Determines the oocyte fate	Kim et al. (2009)
rnp-4	ok622	Exon–exon junction complex, hortholog of Y14	Oogenesis	Kawano et al. (2004)
rpn-10	tm1349	Proteosomal ubiquitin receptor	Prevents spermatogenesis	Shimada et al. (2006)
sel-10	ar41	F-Box protein	Targets FEM proteins for degradation; oogenesis	Jager et al. (2004)

Table 3.1 (continued)

Gene	Alleles	Protein function/identity	Role in sex determination	References
sdc-1	n485	Zinc-finger protein	Hermaphrodite and female development	Villeneuve and Meyer (1987)
sdc-2	y55	Novel protein	Hermaphrodite and female development	Nusbaum and Meyer (1989)
sdc-3	y132	Zinc-finger protein	Hermaphrodite and female development	DeLong et al. (1993)
teg-4	oz210	Splicing factor SAP130	Oogenesis	Mantina et al. (2009)
tra-1	e1099, e1575gf	GLI transcription factor protein	Female fates; continuous spermatogenesis in males	Hodgkin and Brenner (1977), Hodgkin (1987)
tra-2	e1095lf, e2046gf	Transmembrane receptor	Oogenesis	Hodgkin and Brenner (1977), Doniach (1986)
tra-3	e1107	Calpain regulatory protease	Cleavage of TRA-2; oogenesis	Hodgkin and Brenner (1977), Sokol and Kuwabara (2000)
xol-1	y9	GHMP kinase	Male development; inhibits dosage compensation	Miller et al. (1988)

1998; Hansen et al. 2004a,b; Eckmann et al. 2004). Although not as strong as in *glp-1(gf)* or *gld-1 gld-2* mutants, germline tumors have also been observed as proximally proliferating nuclei in *gld-3 nos-3* double mutants (Hansen et al. 2004b). Similarly, *mog; gld-3* double mutants are also overproliferative, indicating an additional role of *mog* in the decision between mitosis and meiosis (Belfiore et al. 2004; Kasturi et al. 2010; Zanetti et al. 2011). As mentioned above, both *fbf* genes are required for robust germline proliferation, and genetically interact with *fog-1* in this process. Therefore, germline proliferation and sex determination are tightly linked to each other. Both processes follow each other and share many common players.

As in sex determination, many regulatory events controlling mitosis or meiosis rely upon post-transcriptional mechanisms (Marin and Evans 2003; Crittenden et al. 2003; Ogura et al. 2003; Puoti et al. 2001; Clifford et al. 2000). Finally, since meiosis is different in oocytes and sperms, the sex of germ cells must be determined before meiosis has started. Even more dramatically, hermaphrodite and male germlines are sexually dimorphic in their distal region indicating that germ cells are sexually determined at a time when they still divide mitotically (Morgan et al. 2010). Nevertheless, their sexual fate remains labile since it can be redirected to oogenesis in *fog, her-1*, or *fem* mutants or to spermatogenesis in *mog(0) or fem-3(gf)* mutants (Barton et al. 1987; Chen and Ellis 2000; Graham and Kimble 1993).

3.7 The Role of Maternal RNAs, New Regulatory Mechanisms

In *C. elegans*, some of the genes that control sexual fate maternally supply mRNA and/or protein through the oocyte for zygotic sex determination (Ahringer et al. 1992; Doniach 1986; Doniach and Hodgkin 1984; Graham et al. 1993; Rosenquist and Kimble 1988). In some cases, maternal mRNAs are translated upon fertilization and permit protein synthesis before zygotic transcription sets in. A new role for maternal RNA has now been proposed for *fem-1*, which is required for spermatogenesis and male development. Heterozygous *fem-1(0)/+* progeny can be obtained by mating a wild-type male with a feminized *fem-1* null mutant. Because *fem-1* null alleles are recessive, such progenies are expected to be phenotypically wild type (Doniach and Hodgkin 1984). Andrew Spence's group has found that progeny descended from females that produce no *fem-1* RNA, and therefore are totally lacking maternal *fem-1* RNA, are unable to use the paternal wild-type copy of *fem-1*. As a consequence, their germlines are feminized even if one wild-type copy of *fem-1* is available (Johnson and Spence 2011). Intriguingly, the open reading frame of the *fem-1* RNA does not need to be intact, indicating that the maternal effect is caused by the *fem-1* RNA and not its protein product. Moreover, the effect is heritable thus suggesting that epigenetic control licenses the expression of zygotic *fem-1* (Johnson and Spence 2011). This mechanism uses sense RNA and is independent of *rde-1*, indicating that it is distinct from RNA interference (Tabara et al. 1999; Fire et al. 1998). Similarly to what has been proposed for RNA interference and epigenetic control, we notice again that the germline makes great efforts to avoid expressing foreign gene products (Kelly and Fire 1998).

3.8   Summary Paragraph

Sex determination has been studied in numerous species. Among these, the nematode *C. elegans* has a prominent role because it led to numerous genes that function in various aspects of gamete formation. Here we describe most of these genes and put them into their regulatory and functional contexts.

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