

# The Mysteries of Sexual Identity: The Germ Cell's Perspective

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Animal germ cells differentiate as sperm or eggs, depending on their sex. Somatic signals tell germ cells whether they reside in a male or female body, but how do germ cells interpret those external cues to acquire their own sexual identity? A critical aspect of a germ cell's sexual puzzle is that the sperm/egg decision is closely linked to the cell-cycle decision between mitosis and meiosis. Molecular studies have begun to tease apart the regulators of both decisions, an essential step toward understanding the regulatory logic of this fundamental question of germ cell biology.

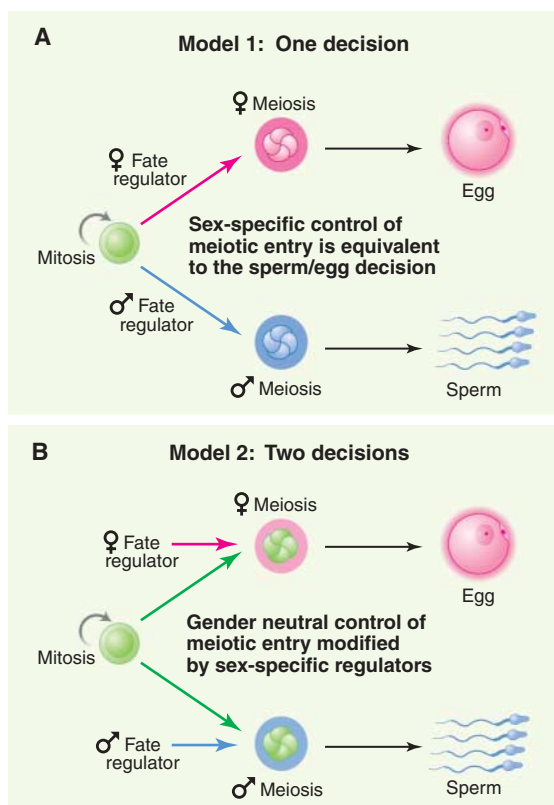
**G**erm cells confront two major cell-fate decisions as they move from an immature state into the world of sexuality.

One decision is entry into a germline-specific cell cycle, called meiosis, and the other is commitment to differentiate as sperm or egg. During embryonic development, germ cells increase their total cell number using the standard mitotic cell cycle, and they use that same cell cycle in adults for stem cell maintenance. Then, as germ cells mature, they embark on the meiotic cell cycle, which reduces the number of chromosomes from the typical two sets to a single set in both sperm and eggs. Upon fertilization, two sets of chromosomes are restored and the next generation can be launched. Of particular importance for this review, the sperm/egg and mitosis/meiosis decisions are closely coupled, which stands out as a fundamental aspect of germ cell regulation that is only beginning to be understood.

## Questions About Sexual Identity in Germ Cells

What directs a germ cell to differentiate as sperm or egg? One important and longstanding approach to this question has focused on how somatic tissues influence the sexual identity of germ cells. In mice, the question is usually rephrased to ask what somatic cues direct germ cells to transition from mitosis to meiosis; meiotic initiation is among the first signs of sexual dimorphism in the murine ovary (1). The molecular nature of somatic signaling depends on the organism: a

variant hedgehog pathway in *Caenorhabditis elegans* (2), the JAK-STAT (Janus kinase–signal transducer and activator of transcrip-



**Fig. 1.** Germ cell fate decisions. As germ cells mature, they enter meiosis and differentiate as sperm or egg. Germ cells are proposed to be uncommitted (green), female (fuschia), or male (blue).

tion) pathway in *Drosophila* testes (3), and retinoic acid signaling in mice (4, 5), for example. The common theme across these species is that the soma uses well-traveled molecular pathways to signal germ cells and influence their sexual identity.

In this review, we leave the soma and focus on what lies downstream of somatic signals to control germ cell sexual identity. A priori, the

sperm/egg decision might be considered as distinct from the cell cycle decision between mitosis and meiosis, which takes place in both sexes. However, the timing of the mitosis/meiosis decision and features of meiosis itself (e.g., recombination and symmetry of divisions) are often sex-specific, suggesting a close relationship between the mitosis/meiosis and sperm/egg decisions. One must therefore ask three questions: (i) What molecular machinery inside germ cells directs their differentiation as sperm or egg? (ii) What molecular machinery governs the transition from mitosis to meiosis? and (iii) What is the relationship between regulators of the sperm/egg and mitosis/meiosis decisions? We consider two models. One idea is that the sperm/egg and mitosis/meiosis decisions are really one and the same (Fig. 1A). According to this model, a sex-specific regulator induces a sex-specific meiotic cell cycle and at the same time commits the germ

cell to differentiation as sperm or egg. The other idea is that the two decisions are governed by distinct regulatory inputs (Fig. 1B). By this model, a gender-neutral mechanism governs entry into meiosis, but sex-specific regulators work in parallel to induce sex-specific aspects of meiosis and differentiation as sperm or egg. To distinguish between these two ideas, the molecular mechanisms controlling both mitosis/meiosis and sperm/egg decisions must be determined. That ultimate goal has not yet been reached for any organism, but progress has been made, and a molecular solution to this puzzle is on the horizon.

## Molecular Regulators of Germ Cell Fates

Our understanding of the mitosis/meiosis and sperm/egg decisions comes from studies in three model organisms—the nematode *C. elegans*, the fruit fly *Drosophila melanogaster*, and the mouse *Mus musculus*. We take a quick look at some of the key molecular regulators in the following order: regulators of mitosis, of entry into meiosis, and finally of differentiation as sperm or egg.

Germ cells are maintained in a state of undifferentiated mitotic divisions by sequence-specific RNA-binding proteins of the widely conserved PUF (Pumilio and FBF) family in both worms and flies (6–8). In mice, two PUF proteins, called Pum1 and Pum2, have been implicated in maintenance of germline stem cells, but that role has not yet been confirmed (9). In *C. elegans*, FBF keeps germ cells undifferentiated and dividing mitotically by the direct repression of specific mRNAs that encode regulators of both entry into the meiotic cell cycle and sexual identity (8, 10). In flies, the mechanism is largely unknown.

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Regulators that direct germ cells into the meiotic cell cycle have been found definitively in mice and nematodes. A novel cytoplasmic protein, called STRA8 (stimulated by retinoic acid-8), governs initiation of meiosis in embryonic mouse ovaries (11). In *C. elegans*, three broadly conserved cytoplasmic proteins accomplish this feat—two RNA-binding proteins (GLD-1/quaking and GLD-3/Bicaudal-C) and the catalytic subunit of an enzyme that adds adenosine residues to the 3' end of mRNAs, a poly(A) polymerase called GLD-2 (12). A possible common theme is that the regulators are cytoplasmic, but without a better understanding of how STRA8 functions, it is premature to conclude a conserved mechanism.

Regulators of the sperm/egg decision have been identified in *C. elegans*. Most notable are FOG-1 (feminization of germ line) and FOG-3, which both reside at the end of a complex regulatory pathway controlling germ cell sex (13). FOG-1 is a homolog of the vertebrate CPEB (cytoplasmic polyadenylation element binding) translational regulator, and therefore is likely to control gene expression at a post-transcriptional level (13, 14). FOG-3, on the other hand, bears an N-terminal motif typical of the poorly understood vertebrate Tob/BTG proteins; its molecular role in controlling germ cell sex remains uncharted territory (15). In animals lacking either FOG-1 or FOG-3, germ cells that normally differentiate as sperm are sexually transformed into oocytes. Therefore, both FOG-1 and FOG-3 promote the sperm fate at the expense of oogenesis.

The knowledge of molecules governing the sperm/egg decision in nematodes makes it possible to ask whether germ cells are irreversibly committed to their sexual identity. That question has been addressed by turning key regulators on or off at will, which is done using genetic tricks (e.g., RNA-mediated interference). The answer is that sexual identity is labile: adult females making eggs can be switched into spermatogenesis (16), and adult males producing sperm can be switched into oogenesis (15). Notably, germ cells adopt their sexual fate at about the same time they leave the mitotic cell cycle and enter meiosis (17). That temporal coincidence underscores the connection between the two decisions but says little about the underlying mechanism or logic of their relationship.

A prominent theme emerging from model organisms is the use of RNA regulatory proteins to control germ cell fates. Why RNA regulation is used for fate regulation in germ cells remains a matter for speculation. One idea is that differentiation of germ cells as sperm or egg is actually a transient phenomenon that must be reversed after fertilization. Regulation at the level of mRNA stability or translation might facilitate that reversal. Indeed, RNA regulators have also turned out to be critical for maintaining germ

cell totipotency—the capacity to support differentiation of all cell fates in the next generation (18). How these RNA regulators interface with epigenetic regulation at the DNA level remains an open question and an interesting challenge for future studies.

### A Molecular Link Between the Mitosis/Meiosis and Sperm/Egg Decisions

With specific regulators of the mitosis/meiosis and sperm/egg decisions in hand, one can begin to explore their molecular relationship. The initial identification of certain regulators of the mitosis/meiosis decision (e.g., GLD proteins) and others of the sperm/egg decision (e.g., FOG-1) lent support to model 2 (10–13). However, more in-depth studies revealed that the GLD and FOG regulators actually influence both decisions, revealing multiple molecular links between them (13). Most notable is FOG-1, which acts at the end of the pathway and is therefore likely to be one of its most important regulators. FOG-1 turns out to affect the two decisions in a dose-dependent manner, promoting mitosis at low levels but driving germ cells into the sperm fate at high levels (10). Is this FOG-1 duality a bizarre solution specific to nematodes, or the harbinger of a more universal phenomenon? In *Xenopus*, the FOG-1 homolog, called CPEB, promotes mitosis when present at a low level and progression through meiosis when more abundant (19). In *Drosophila*, the FOG-1 homolog, called Orb, has been implicated in controls of both entry into meiosis and germline sex determination (20). It is too early to conclude that this dual mechanism has been conserved, but the similarities are striking. Indeed, a molecular strategy in which a germline sexual regulator also promotes mitosis may explain the unusual tumors typical of putative germline sex determination genes in *Drosophila* (21), as well as having important implications for testicular cancers in mammals.

So, can we distinguish between the opposing models set forth earlier, one arguing that the mitosis/meiosis and sperm/egg decisions are the same and the other that they are different? In nematodes, the two decisions turn out to be governed by many of the same regulators, which could be argued to support model 1. But FOG-1 influences the two decisions by a dose-dependent mechanism, which could be interpreted as supporting model 2. Low FOG-1 and high FOG-1 might affect distinct mRNAs because of differences in binding affinity, or the two FOG-1 levels might affect the same mRNAs in different ways, for example, promoting translation at one level but inhibiting it at the other. A real understanding of the relationship between the two decisions requires identification of the targets of both FOG-1 and FOG-3 and a

molecular explanation of how these two key regulators control their targets.

### Remaining Questions and Puzzles

The intrinsic regulators of germ cell sex have long been a mystery, but insights drawn from work in model organisms have now opened a tantalizing crack in that large black box. Our first glimpse inside reveals some unexpected answers and a host of new questions. Will RNA regulation dominate the molecular strategy used to control germ cell fates? Or will transcriptional regulators, which have so far been elusive, prove to be the real key? Are PUF proteins universal regulators of germline stem cells? What about other germ cell fate regulators like the FOG and GLD proteins? Do their vertebrate homologs also control germ cell fates, or have they been co-opted for this task in nematodes by some quirk of evolution? And how do human germ cells fit into the picture? The answers to these basic questions of germ cell biology are now within experimental reach.

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10.1126/science.1142109