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Pathway to Totipotency: Lessons from Germ Cells

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Oocytes and sperm are some of the most differentiated cells in our bodies, yet they generate all cell types after fertilization. Accumulating evidence suggests that this extraordinary potential is conferred to germ cells from the time of their formation during embryogenesis. In this Review, we describe common themes emerging from the study of germ cells in vertebrates and invertebrates. Transcriptional repression, chromatin remodeling, and an emphasis on posttranscriptional gene regulation preserve the totipotent genome of germ cells through generations.

Lewis Wolpert, in his classic text "The Triumph of the Embryo," remarked that gastrulation, not birth, death, or taxes, is the most important event in our lives (Wolpert, 1991). Gastrulation may be a milestone for our bodies, but it is gametogenesis that gives us an "afterlife," propelling our genome into future generations. The remarkable ability of germ cells to generate a complete organism has fascinated biologists for more than a century and remains a central question in research today. What are the molecular mechanisms that underlie totipotency (see Table 1)? Are these mechanisms unique to the germline or do they also exist in somatic cells? Does gametogenesis involve mechanisms fundamentally different from those driving somatic differentiation? After more than a century of studies examining germ cells in different organisms, general themes are starting to emerge. In this Review, we discuss characteristics that distinguish germ cells from somatic cells and speculate on the mechanisms that allow germ cells to develop, proliferate, and differentiate while avoiding the loss of totipotency that befalls somatic cells.

Table 1. Glossary of Terms

Term	Definition
Totipotency	The ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues. Strictly speaking, only zygotes, and in some organisms their immediate descendents, are totipotent. Here, we use the term totipotency to refer to the *potential* of a cell (or its descendents) to produce all types and argue that this potential is maintained in the female germline throughout development.
Pluripotency	The ability of a single cell to produce differentiated cell types representing all three germ layers. The pluripotency of a cell can be observed experimentally by following its descendents during normal growth, during tumor growth, or following injection into a host blastocyst. Embryonic stem cells are a type of pluripotent cell that can be grown and induced to differentiate into many cell types in culture.
Primordial germ cells (PGCs)	Primordial germ cells are the founder cells for the germline. They divide symmetrically and all their descendents are germ cells. In many organisms, primordial germ cells are motile and migrate to the somatic gonad.
Germline stem cells (GSCs)	Germline stem cells are PGC descendents that have acquired the ability to both self-renew AND generate daughters that begin gametogenesis.
Gametogenesis	The process by which germ cells differentiate into gametes (oocytes or sperm). In many animals, an important aspect of gametogenesis involves meiosis, the process by which germ cells undergo recombination and become haploid.
Germ granules	Large ribonucleoprotein complexes typically found around the nuclei of germ cells and predicted to function in posttranscriptional gene regulation. Here, we suggest that germ granules give germ cells the ability to differentiate while maintaining a totipotent genome.

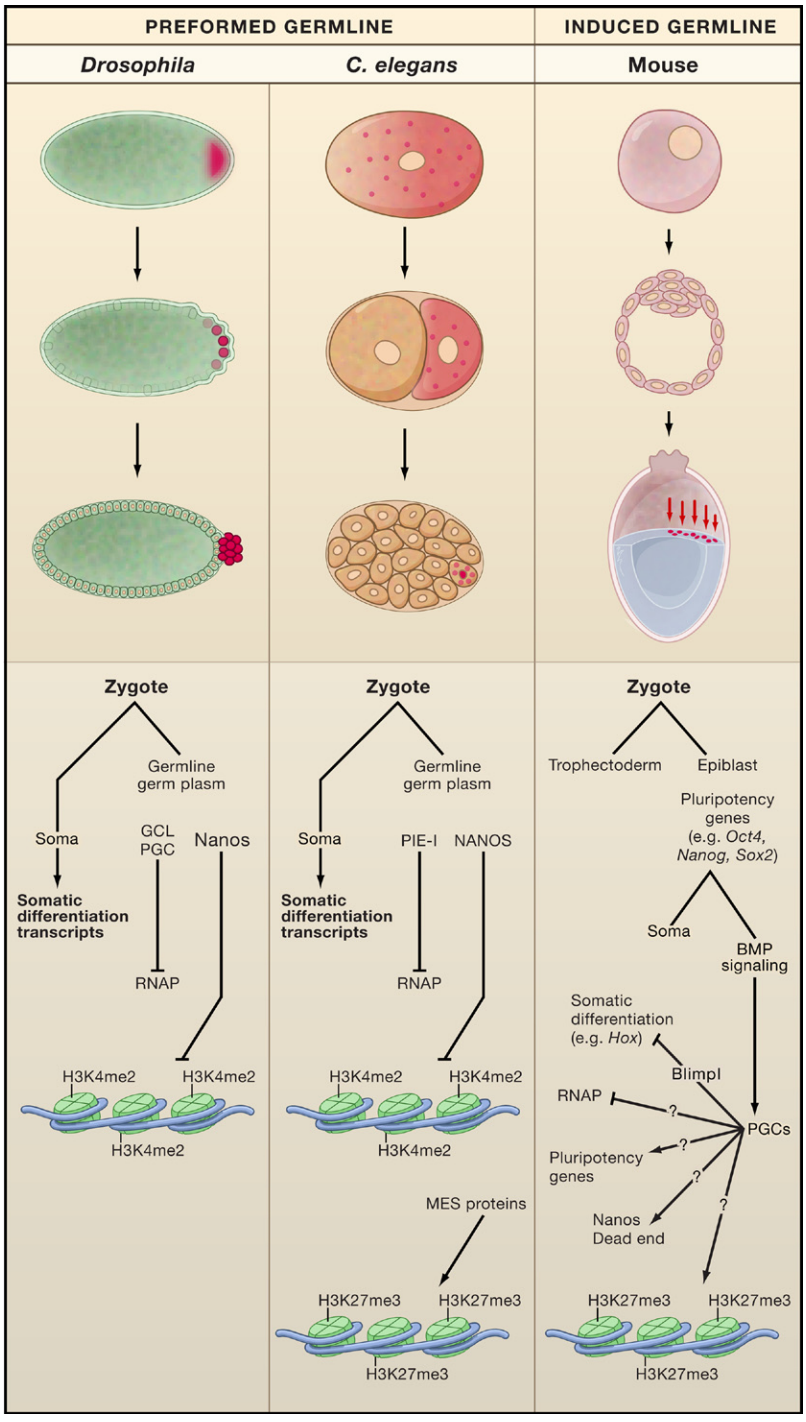

Figure 1. Mechanisms of Germline Formation

(Upper panels) In organisms where the germline determinants are preformed during oogenesis, germ plasm (red) is partitioned asymmetrically in the oocyte (*Drosophila*) or in the embryo (*C. elegans*). In mice, where the germline is induced, signals emanating from the extraembryonic ectoderm (such as Bmps, red arrows) induce uncommitted cells to become germline cells. (Lower panels) In both models, germline formation is driven by inhibiting transcription (for example, by GCL and PGC in *Drosophila* and PIE-1 in *C. elegans*) and by modifying chromatin (for example, by MES proteins in *C. elegans* and by Blimp1 in mice). Germline formation in mammals also requires the continued expression of key pluripotency genes (such as *Oct4*). RNAP, RNA polymerase.

Specification of the Germline by Repression of Somatic Programs

In most animals, the first germ cells (primordial germ cells, PGCs; see Table 1) are formed during embryogenesis before many adult tissues, often at peripheral sites in the embryo. Two general modes of PGC specification have been described (Figure 1). In the "preformation" mode, PGCs are specified by a specialized maternal cytoplasm (germ plasm) that is asymmetrically partitioned during oogenesis and/or after fertilization to specify just a few cells to enter the germ lineage. In the "induction" mode, PGCs arise later in embryogenesis from pluripotent progenitors induced to become germ cells by extracellular signals. Although preformation is common among the popular experimental model organisms (*Drosophila*, *Caenorhabditis elegans*, *Xenopus*, and zebrafish), PGC formation by induction—first demonstrated in the mouse (Tam and Zhou, 1996)—may be the most widespread (and ancestral) mode of germ cell specification in metazoans (Extavour and Akam, 2003). Despite these differences in PGC origin, a common theme has emerged from studies in *Drosophila*, *C. elegans*, and mice: PGC specification depends on mechanisms that inhibit the expression of somatic genes.

Inhibition of RNA Polymerase II in Drosophila *and* C. elegans *Early Germ Cells*

In *Drosophila* and *C. elegans* embryos, somatic cell nuclei initiate mRNA transcription soon (~1 hr) after fertilization, but PGCs and their precursors do not accumulate zygotic mRNAs until after the onset of gastrulation (~3 hr post fertilization) (Blackwell, 2004). During transcription, a repeat motif (YSPTSPS) in the carboxy-terminal tail of RNA polymerase II becomes phosphorylated on serine 5 (Ser5) during formation of the preinitiation complex at the promoter and on serine 2 (Ser2) during elongation. Although phosphorylated Ser5 and Ser2 (P-Ser5 and P-Ser2) are abundant in all somatic cell nuclei, P-Ser5 is reduced and P-Ser2 is undetectable in the germ lineages of *Drosophila* and *C. elegans*

before gastrulation (Blackwell, 2004). In *C. elegans*, residual P-Ser5 in the germline blastomeres requires the general transcription factor TFIIB, the mediator component RGR-1, and the CTD kinase CDK-7, suggesting that at least some components of the preinitiation complex are active in early germ cells (Blackwell, 2004). Although these experiments should be interpreted with caution as they rely on phospho-specific antibodies whose specificities can vary with antigen concentration (Palancade and Bensaude, 2003), collectively these data suggest that transcription is blocked at a step between initiation and elongation in early germ cells.

In *C. elegans*, the transcriptional block depends on PIE-1, a maternally inherited germ plasm component (Figure 1). In the absence of PIE-1, germline blastomeres become positive for P-Ser2 and P-Ser5 and begin to accumulate zygotic mRNAs at the same time as somatic blastomeres. Unlike other germ plasm components, which are predominantly cytoplasmic, PIE-1 also accumulates in nuclei, consistent with a direct role in transcriptional repression. Studies in cultured human cells indicate that PIE-1, a putative RNA-binding protein, can interact with, and inhibit, p-TEFb, the nuclear kinase complex responsible for Ser2 phosphorylation (Blackwell, 2004).

A direct sequence homolog of PIE-1 does not exist in *Drosophila*, but the germ plasm components *germ cell less* (*gcl*) and *polar granule component* (*pgc*) have been proposed to play similar roles. Like *pie-1* mutants, *gcl* and *pgc* mutants accumulate zygotic transcripts and P-Ser2 in early germ cells (Blackwell, 2004). When expressed ectopically in somatic cells, GCL and PGC inhibit P-Ser2 accumulation, suggesting that, like PIE-1, these proteins can interfere directly with RNA polymerase II activity (Leatherman and Jongens, 2003; and A. Nakamura, personal communication). However, the mechanisms involved are not yet known.

Chromatin-Based Mechanisms of Transcriptional Repression

Transcription in the embryonic germline appears also to be regulated at the level of chromatin modification. In *Drosophila* and *C. elegans*, early germ cells have reduced levels of H3-K4me2 (dimethylation of lysine 4 on histone H3), a methyl mark linked with transcription (Schaner and Kelly, 2006). In *Drosophila*, this reduction correlates with low P-Ser5 and P-Ser2. In *C. elegans*, H3-K4me2 levels drop only in later stages, after PIE-1 disappears from the germ lineage and PGCs become positive for P-Ser2 and P-Ser5. *C. elegans* PGCs also exhibit an elevated ratio of trimethylated to dimethylated H3-K27, a mark correlated with transcriptional repression. These observations suggest the existence of two independent modes of transcriptional repression in germ cells: an early mode involving direct inhibition of RNA polymerase II and a later, chromatin-based mode involving nucleosome modification (Schaner and Kelly, 2006).

Whereas *pie-1*, *gcl*, and *pgc* participate in the first mode, *nanos* homologs in *Drosophila* and *C. elegans* have been implicated in the second (Figure 1). Germ cells lacking *nanos* occasionally express zygotic transcripts prematurely in *Drosophila* (Blackwell, 2004) and exhibit elevated H3-K4me2 levels in both *Drosophila* and *C. elegans* (Schaner and Kelly, 2006). Nanos is a cytoplasmic protein thought to function primarily as a translational regulator, so its effects on transcription are likely to be indirect. In *C. elegans*, a more direct role may be played by MES-2, a histone H3-specific methyl transferase required for trimethylation of H3-K27 in PGCs. MES-2, with its partners MES-3 and MES-6, form the *C. elegans* Polycomb complex, which also functions in adult germ cells. In fact, in *C. elegans*, repressive, chromatin-based mechanisms are maintained in the germline throughout development, with a special emphasis on silencing the X chromosome. MES-dependent silencing is so extensive that genes with essential roles in the germline are mostly excluded from the X chromosome (Schaner and Kelly, 2006).

What happens if transcription is not repressed in early germ cells? If transcription is activated in PGC precursors, as in *pie-1* and *gcl* mutants, PGCs are not formed. If transcription is turned on after PGC formation, PGCs often exhibit migration defects and die (*nanos* and *pgc* mutants), or they reach the somatic gonad but their descendents degenerate (*mes* mutants) (Blackwell, 2004; Schaner and Kelly, 2006). Lineage analyses have shown that loss of PGCs in *C. elegans pie-1* mutants is due to transformation of the germ cell precursors to a somatic cell fate (Schaner and Kelly, 2006). Similarly, inhibition of the cell death program in *Drosophila Nanos* mutants revealed that the dying pole cells express somatic markers, consistent with a germline-to-soma transformation (Hayashi et al., 2004). Collectively, these observations suggest that repression of mRNA transcription is essential to inhibit somatic differentiation and promote germ cell fate and viability.

Mice Do It Too

Drosophila and *C. elegans* both belong to the "preformation" category of animals where PGCs are formed early and require inheritance of maternal germ plasm. Could similar repressive mechanisms also operate in animals where the germline is formed by inductive signals later in development? Surprisingly, the answer appears to be yes, at least in mice.

Mouse PGCs arise in the epiblast during gastrulation, in a process dependent on signaling from extraembryonic tissues (Figure 1). At 7.25 days of embryogenesis, a cluster of approximately 40 PGCs is detected in the allantois, a structure located in the extraembryonic mesoderm at the posterior end of the primitive streak. Single-cell profiling experiments have begun to define the transcriptional program of these cells (Ohinata et al., 2006). Remarkably, a key feature is the downregulation of *Hox* transcripts, which are actively transcribed in neighboring somatic cells but turned off in PGCs. Several lines of evidence suggest that the downregulation of *Hox* genes is essential for PGC specification and depends

on the transcriptional repressor Blimp1. First, Blimp1 expression parallels PGC specification in the epiblast and precedes *Hox* gene downregulation. Lineage tracing experiments confirmed that cells expressing Blimp1 in the epiblast are committed to the germ cell lineage and may in fact represent the earliest committed pool of germline progenitors. Mutants lacking Blimp1 exhibit a reduced-size PGC cluster, which fails to proliferate and migrate normally. Finally, PGCs lacking Blimp1 fail to downregulate *Hox* transcripts. Together these results point to Blimp1 as a critical regulator of PGC fate and suggest that, as in *Drosophila* and *C. elegans*, specification of the mouse germline requires inhibition of somatic transcriptional programs (Ohinata et al., 2006).

Blimp1 is a predicted histone methyl transferase that is widely expressed in development and participates in the specification of somatic cells, including the terminal differentiation of B cells into plasma cells (Ohinata et al., 2006). How then is PGC fate specified upon Blimp1 induction in the epiblast? Cells that express Blimp1 lose *Hox* gene expression but maintain expression of other transcripts, including *Oct4*, *Nanog*, and *Sox2* (Yabuta et al., 2006). These transcription factors have been implicated at the core of the transcriptional network that maintains the pluripotency of embryonic stem cells (Boyer et al., 2005; Loh et al., 2006). Knockouts of each of these genes cause defects in the establishment of pluripotency in vivo, and, conversely, overexpression of these genes can convert differentiated cells into pluripotent stem cells in vitro (Silva et al., 2006; Takahashi and Yamanaka, 2006). Maintenance of *Oct4*, *Nanog*, and *Sox2* transcripts in PGCs suggests that PGC specification in mice involves a careful balance between inhibition of somatic differentiation genes and maintenance of a "pluripotency program" present in embryonic cells.

Following specification, starting at embryonic day 8, mouse PGCs undergo extensive epigenetic reprogramming, first losing H3-K9 dimethylation and DNA methylation (Ohinata et al., 2006). These changes may be essential to erase parental imprints and to eventually activate the expression of differentiation genes (Maatouk et al., 2006). PGCs also increase H3-K27 trimethylation, a modification also observed in *C. elegans* PGCs and correlated with X inactivation (Ohinata et al., 2006). Remarkably, during the period of chromatin reorganization, mouse PGCs become transiently negative for the P-Ser2 epitope of RNA polymerase II (Y. Seki and M. Saitou, personal communication), suggesting that as in *C. elegans* and *Drosophila*, PGCs in mice undergo a period of transcriptional inactivity early in development. H3-K4 dimethylation, however, remains relatively constant during PGC specification in mice (Ohinata et al., 2006). Although differences exist, the consensus that emerges from studies of invertebrates and vertebrates is that PGC specification involves inhibition of transcription and genome-wide chromatin remodeling.

How to Turn on Germline Genes?

Soon after their specification, PGCs activate the transcription of germline-specific genes. As early as embryonic day 7.25 in the mouse, PGCs turn on the transcription of the *Nanos3* and *Dead end* genes (Yabuta et al., 2006). In animals with germ plasm, these RNAs are inherited maternally in the germ plasm but are also transcribed in PGCs later in development. Transcription factors that activate the expression of these genes have not yet been identified in any organism. Elimination of Oct4 from PGCs leads to apoptosis (Kehler et al., 2004), but the critical targets of Oct4 in PGCs are not known. In fact, whether the transcription profile of PGCs is defined mostly by repressive mechanisms or also depends on PGC-specific transcriptional activators remains to be determined. A few germ cell-specific transcriptional activators have been described, but these function mostly in later stages to turn on the expression of the large groups of genes required for meiosis and gamete formation (see Dejong, 2006 for an excellent review of transcriptional regulation in gametes).

Although PGCs express germline-specific genes, several lines of evidence suggest that they retain the potential to convert back to a pluripotent state capable of generating somatic cell descendents (Figure 2). First as mentioned above, mouse PGCs continue to express the pluripotency genes *Oct4*, *Nanog*, and *Sox2* (Yabuta et al., 2006). Second, PGCs can give rise to teratocarcinomas, embryonic tumors containing differentiated somatic cell types representative of all three germ layers. Like embryonic stem cells, embryonal carcinoma cells derived from teratocarcinomas (Figure 2) can give rise to somatic cell types when injected into early preimplantation-stage mouse embryos (Brinster, 1974). In fact, the parallels between embryonal carcinoma and embryonic stem cells, and the ability of embryonic stem cells to make PGCs in vitro, have led to the speculation that embryonic stem cells may be derived from PGCs (Matsui and Okamura, 2005; Zwaka and Thomson, 2005), rather than from primitive embryonic (inner cell mass) cells as initially believed. Consistent with this view, PGCs can be induced in culture to form pluripotent embryonic germ cells, which share many of the properties of embryonic stem cells.

Germline Stem Cells: Balancing Renewal and Differentiation

After their initial specification, PGCs migrate to the gonad, proliferate, and eventually form stable progenitors (germline stem cells: GSCs, see Table 1) that continually regenerate as well as form differentiated descendents (gametes) into adulthood. Like PGCs, GSCs continue to express the key pluripotency genes *Oct4* and *Nanog* (Guan et al., 2006). However, during normal development, GSCs are limited to forming a single differentiated cell type, the gamete, one of the most specialized cells in the body. In fact, until recently, GSCs from adult mouse testes were believed to be restricted to differentiating only sperm. A remarkable new study now challenges that dogma by demonstrating that testes GSCs can give rise to embryonic stem-like cells in cul-

ture (Guan et al., 2006). GSCs cultured from adult testes can spontaneously differentiate into all three embryonic germ layers, contribute to multiple organs, and show germline transmission when injected back into blastocysts. Pluripotent cells have also been found recently in testes of early postnatal mice (Kanatsu-Shinohara et al., 2004). These new findings suggest that GSCs maintain, or can regain, the pluripotency of PGCs and can become as versatile as embryonic stem cells when cultured in vitro. The minor differences in extracellular signals—Glial cell line-derived neurotrophic factor (Gdnf) for GSCs in vivo versus leukemia inhibitory factor for embryonic stem cells and pluripotent GSC-derived cells in vitro—and the difference in expression of relatively few genes raise the possibility that only a few changes are needed to transform GSCs into embryonic stem-like cells. The remarkable developmental plasticity of adult GSCs clearly distinguishes them from all somatic adult stem cells and offers a potential alternative (testes!) to the use of embryos for regenerative medicine.

Location, Location, Location

If adult GSCs are pluripotent, what maintains them as a stem cell population and what restricts their differentiation to gametes in vivo? The signals that regulate the important decision to self-renew or to differentiate emanate from the niche that the GSCs occupy in the somatic gonad (Wong et al., 2005). In *Drosophila* and *C. elegans*, specific somatic gonadal cells have been identified as the source of extracellular signals (Unpaired and Dpp in *Drosophila*, LAG-2/DELTA in *C. elegans*) that maintain germ cells as stem cells. *Drosophila* GSCs adhere to the niche cells and divide asymmetrically: the daughter born away from the niche receives less signal and is free to differentiate. In *C. elegans*, a reservoir of mitotic germ cells is maintained close to the niche; germ cells born/pushed far enough away from the niche enter meiosis. A similar mechanism of population homeostasis may also operate in the mammalian testis. An obvious anatomical GSC niche has not yet been identified in the testis, but two signals from the somatic Sertoli cells, Gdnf and the Ets-related molecule (Erm), help regulate GSC self-renewal (Wong et al., 2005).

Notably, many of the genes that act downstream of the niche signal to regulate the balance between renewal and differentiation encode cytoplasmic proteins predicted to regulate mRNAs. GSC renewal in *Drosophila* requires Nanos and Pumilio, two translational repressors; Piwi, an Argonaute protein implicated in RNA regulation by small noncoding RNAs; Dicer-1, a double-stranded RNaseIII essential for microRNA (miRNA) biogenesis; and Pelota, a cytoplasmic protein with homology to translation release factor 1 (Hatfield et al., 2005; Wong et al., 2005; Xi et al., 2005). Similarly, the genetic hierarchy that regulates the balance between GSC renewal and meiotic entry in *C. elegans* consists almost entirely of RNA-binding proteins (Kimble and Crittenden, 2005).

Whether RNA-binding proteins also contribute to GSC maintenance and differentiation in mice is not yet known. Knockouts of the Nanos homologs *Nanos3* and *Nanos2* cause a dramatic loss of germ cells in embryos and testes, respectively, raising the possibility that the GSC maintenance function of these RNA regulators has been conserved from invertebrates to vertebrates (Tsuda et al., 2003). Two POK-domain-containing transcriptional repressors, Plzf and Bcl6b, are also required in GSCs for renewal (Oatley et al., 2006; Wong et al., 2005). In somatic cells, these transcription factors associate with a large transcriptional regulatory complex that includes Sin3 and the histone deacetylases Hdac1 and Hdac4. Plzf also interacts with Bmi1, a Polycomb group protein, which functions with Ezh2 (homolog of *C. elegans* MES-2). Whether these interactions also occur in GSCs is not yet known, but consistent with epigenetic regulation, GSCs have a unique distribution of methyl modifications on their histone tails (Payne and Braun, 2006). Interestingly chromatin remodeling has also been implicated in germline stem cell maintenance in *Drosophila* (Xi and Xie, 2005). Thus, downstream of the niche signals, the balance between GSC self-renewal and differentiation is regulated by mechanisms acting on chromatin in the nucleus and by mechanisms acting on RNA in the cytoplasm.

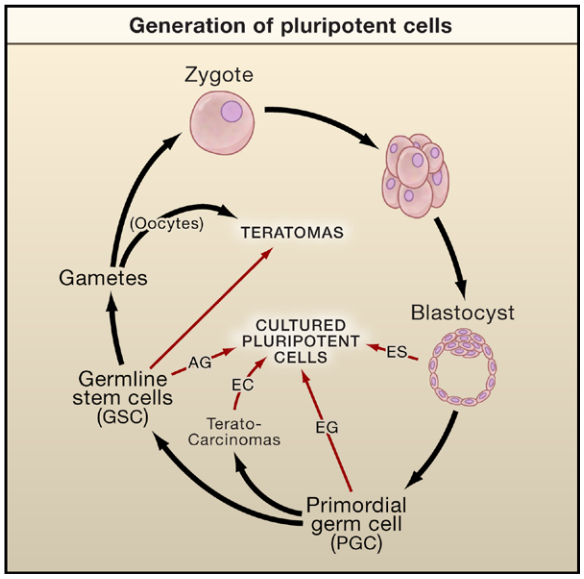

Figure 2. Pluripotent Cells Can Be Generated at Several Stages of Mammalian Germ Cell Development

Black arrows indicate events that can occur naturally in the embryo, whereas red arrows indicate experimental manipulations. For example, primordial germ cells (PGCs) can form teratomas during embryogenesis, and oocytes can form teratomas in the ovaries of adults. In contrast, adult germline (AG) stem cells do not normally give rise to teratomas but can do so when removed from the testes, cultured in vitro, and injected back into a mouse. As discussed in the text, it is uncertain whether embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst or from primordial germ cell precursors that form during blastocyst culturing. Embryonic germ (EG) cells are derived from PGCs, and embryonal carcinoma (EC) cells are derived from teratocarcinomas.

Commitment—To Be or Not to Be

A surprising property of GSCs is that their commitment to differentiation is reversible. Using conditional reversible ablation of STAT, a transcriptional target downstream of the unpaired signal in the *Drosophila* testes, the Matunis lab has shown that GSC daughters (gonioblasts) that have left the niche and initiated differentiation can revert to GSCs and reassociate with the niche after normal signaling is restored (Brawley and Matunis, 2004). This backtracking is remarkable especially because it can occur after the gonioblasts have begun to divide into cysts where daughter cells are linked by intercellular bridges (see below). A return to GSCs presumably means that the intercellular bridges can be closed and single cells formed again. This potential is not unique to the male germline: cystoblasts containing up to eight cells in *Drosophila* ovaries have also been observed to revert to GSCs when one of the signaling molecules in the niche, Dpp, is overexpressed (Kai and Spradling, 2004). Whether differentiating germ cells normally revert to GSCs in wild-type flies is not yet known.

In the mammalian testes, differentiating germ cells also form intercellular bridges, and an often-cited model implies that these incomplete divisions commit the cells to the pathway of transit amplification and differentiation. However, in agreement with the *Drosophila* findings, recent observations have begun to question the irreversibility of the fate of these transit-amplifying cells. Clones of two, four, and eight interconnected spermatogonia continue to express genes required for GSC self-renewal, and live imaging in the mouse has shown that these clones occasionally break down into single cells or shorter chains (S. Yoshida, personal communication). We do not yet know whether gradual commitment to differentiation is a property unique to GSCs or also a characteristic of somatic stem cells. In *C. elegans*, germ cells that have initiated meiotic pachytene (female germline), or even completed meiotic prophase (male germline), can revert back to mitosis and form tumors, suggesting that GSC differentiation remains reversible even after meiosis onset (Kimble and Crittenden, 2005). Whether this plasticity is a by-product of GSC pluripotency or is a property built in to replenish the GSC pool in response to environmental stresses remains to be determined.

Open Borders: Germ Cells Share Cytoplasm

In the gonad of many organisms, germ cells are connected to each other by cytoplasmic bridges (Pepling et al., 1999). Typically, the bridges are formed through incomplete cytokinesis and link small clusters of differentiating germ cells ("cysts" in *Drosophila* and mice). In some species, such as *C. elegans*, the bridges connect both mitotic and differentiating germ cells to a common cytoplasm.

Several hypotheses have been put forth to explain the function of intercellular bridges (Pepling et al., 1999). The assumption in all cases is that intercellular bridges permit sharing of cytoplasmic constituents between cells. In females, whose gametes typically are large, sharing allows multiple germ cells to contribute to the cytoplasm of a single cell selected to become an oocyte. In the *Drosophila* ovary, each cyst contains a vesicular network of endoplasmic reticulum (fusome) that snakes its way through the intercellular bridges and helps translocate RNAs and proteins to the future oocyte. At the end of oogenesis, the 15 "nurse" cells dump nearly all their contents into the single oocyte. Similarly, in the *C. elegans* ovary, all germ cells contribute to a common cytoplasm, but only a subset of germ cells inherit this cytoplasm and grow into oocytes, whereas others are eliminated by apoptosis (Gumienny et al., 1999). Inter-germ cell connections have also been observed in the mammalian ovary (Pepling et al., 1999), but whether they serve a similar function is not known, especially since they are present only during a relatively short period in fetal development.

In males, all differentiating germ cells become gametes, yet spermatogonia are still connected by bridges. In mouse *Tex14* mutants (Greenbaum et al., 2006), bridges do not form and germ cells die during meiosis I, so bridges are important, but why?

A common hypothesis is that intercellular bridges help synchronize the differentiation of gametes. In the mammalian testes, however, synchrony extends across independent syncytia. Additionally, in *C. elegans*, mitotic and meiotic germ cells are connected to the same common cytoplasm. Thus, cytoplasmic bridges are not necessarily linked to synchronous development.

Another hypothesis is that, after the meiotic divisions, bridges between haploid gametes allow for sharing of sex chromosome transcripts and help neutralize the impact of null alleles in animals heterozygous for mutations in essential spermatogenic genes. Despite the strong evidence for transcript sharing in mammalian spermatids, equilibrating haploid messages is unlikely to be the universal reason for cytoplasmic sharing. Not all organisms have haploid gene expression (for example *Drosophila* males), and in some cases males are the homogametic sex.

Another possible reason for cytoplasm and transcript sharing is suggested by recent studies in somatic cells showing that enhancers can act stochastically. Single-cell analyses have clearly shown that enhancers can work in a probabilistic fashion to affect transcription initiation (Fiering et al., 2000). Given the repressive mechanisms and extensive epigenetic reprogramming that occur in PGCs and their descendents, germ cells may be particularly prone to stochastic gene expression and its potential harmful consequences. Cytoplasm sharing would act to buffer against transcript imbalances and help neutralize differences between neighboring cells (Guo and Zheng, 2004). Further investigation is clearly needed in this unique and important area of gamete formation.

Germ Granules: The RNA World of Germ Cells

Another characteristic of germ cells is the presence in their cytoplasm of large (micron-range), nonmembranous, RNA-rich organelles. "Germ granules" (see Tables 1 and 2)

Table 2. Germ Granules: Conserved Components

	Function	Organism: Protein Name and Germ Granule Type	References
Proteins			
Vasa and related DEAD box RNA helicases	RNA unwinding	*Drosophila*: Vasa in nuage and polar granules; *C. elegans*: GLH-1-4 in P granules; *Xenopus*: XVLG1, perinuclear in PGCs; Zebrafish: Vasa, perinuclear in PGCs; Mouse: Mvh in chromatoid body; Humans: Vasa, perinuclear in fetal oocytes	(Linder and Lasko, 2006; Raz, 2000)
Argonaute-related	RNA regulation by microRNAs	*Drosophila*: Aubergine in nuage, PIWI in polar granules; Mouse: Miwi, Mili, Ago2, Ago3 in chromatoid body	(Harris and Macdonald, 2001; Megosh et al., 2006; Kotaja et al., 2006)
Maelstrom (HMG Box)	Chromatin regulation, RNA-mediated interference	*Drosophila*: Maelstrom in nuage; Mouse: Maelstrom in chromatoid body	(Findley et al., 2003; Costa et al., 2006)
Tudor domain proteins (methyl-binding module)	Binding to Sm proteins, recruitment of mitochondrial ribosomal RNA to polar granules in *Drosophila*	*Drosophila*: Tudor in polar granules; Mouse: Mtr and Rnf17 in chromatoid body	(Thomson and Lasko, 2005)
Sm proteins	Splicing in the spliceosome, P granule localization and germ cell fate in *C. elegans*	*C. elegans*: Sm in P granules; *Xenopus*: Sm in nuage in oocytes; Mouse: Sm in chromatoid body	(Barbee et al., 2002; Bilinski et al., 2004; Moussa et al., 1994; Chuma et al., 2003)
Dcp1	Decapping of mRNAs in P bodies, localization of Oskar RNA to the germ plasm in *Drosophila*	*Drosophila*: dDcp1 in cytoplasmic mRNPs in oogenesis; Mouse: Dcp1a in chromatoid body	(Lin et al., 2006; Kotaja et al., 2006)
Dcp2	Decapping of mRNAs in P bodies	*Drosophila*: dDcp2 in cytoplasmic mRNPs in oogenesis; *C. elegans*: DCP-2 in P granules	(Lin et al., 2006; Lall et al., 2005)
Dhh1p/Rck (DEAD box RNA helicase)	Repression of translation (also in P bodies)	*Drosophila*: Me31b in cytoplasmic mRNPs in oogenesis; *C. elegans*: CGH-1 in P granules	(Nakamura et al., 2001; Navarro et al., 2001)
Scd6p/Rap55 (Sm-like domain)	Repression of translation (also in P bodies)	*Drosophila*: Trailer Hitch in cytoplasmic mRNPs associated with ER in oogenesis; *C. elegans*: CAR-1 in P granules	(Wilhelm et al., 2005; Boag et al., 2005; Squirrell et al., 2006; Audhya et al., 2005)
RNAs			
Nanos (mRNA)	Repression of translation	*Drosophila*: *Nanos* RNA enriched in germ plasm; *C. elegans*: *nos-2* RNA enriched in P granules; *Xenopus*: *Xcat2* RNA enriched in vegetal cortex; Zebrafish: *nos1* RNA enriched in germ plasm; Pea Aphid: nanos RNA in nuage-like structure in oocytes	(Forrest and Gavis, 2003; Subramaniam and Seydoux, 1999; Mosquera et al., 1993; Koprunner et al., 2001; Chang et al., 2006)
Mitochondrial large and small ribosomal RNAs (noncoding RNAs)	Translation of *germ cell less* (*gcl*) in *Drosophila*	*Drosophila*: in polar granules; *Xenopus*: in germinal granules	(Kobayashi et al., 2005)

Only components shown to localize to germ granules in two or more species are listed. Many others have been described in only one organism thus far but potentially are also conserved components (see for example Thomson and Lasko, 2005).

have been described in over 80 species, ranging 8 phyla from rotifers to mammals (Eddy, 1975). In animals where the germline is preformed, germ granules are present continuously in germ cells (with the exception of mature sperm) and are inherited maternally with the germ plasm. In mammals, germ granules appear in PGCs and remain in their descendents through gametogenesis. In the mouse, germ granules are thought to be absent from mature gametes, zygotes, and early embryos (Eddy, 1975).

During most of development, germ granules localize around the nucleus. In oocytes with germ plasm, germ granules are also found away from nuclei, segregating with the germ plasm to embryonic sites where the germline will form. Historically, germ granules have been called by a variety of names reflecting their different morphology at different developmental stages and in different organisms (nuage for perinuclear granules, polar granules in the *Drosophila* germ plasm, P granules in *C. elegans*, mitochondrial cloud in *Xenopus* oocytes, chromatoid body in mammalian spermatocytes; Kloc et al., 2004).

In this Review, we use the generic term "germ granules" to refer to all these structures, with the understanding that germ granules most likely are a heterogeneous collection of related, but not necessarily identical, cytoplasmic ribonucleoprotein (RNP) complexes. Germ granules components fall into three general classes: proteins, mRNAs, and noncoding RNAs (Table 2). Many of these associate with germ granules only during specific developmental stages. This observation is consistent with the view that germ granules are dynamic structures, whose compositions change as germ cells mature and differentiate.

Germ Granules as Hubs for Posttranscriptional Regulation of Gene Expression

What is the function of germ granules? In 1968, Mahowald reported that germ granules associate transiently with ribosomes in the germ plasm of *Drosophila* embryos and proposed that germ granules store and regulate the translation of mRNAs necessary for germline development (Mahowald, 1968). Consistent with this hypothesis, mRNAs coding for essential germline factors (e.g., Nanos and PGC) have been identified in germ granules, and many of the protein components of germ granules are implicated in various aspects of mRNA regulation (Table 2). The specific mechanisms involved, however, remain poorly understood and are likely to be complex. For example, the DEAD box helicase Vasa, a widely conserved component of germ granules, is required for the expression of several proteins in *Drosophila*, including Gurken in oocytes and Nanos in the germ plasm of embryos. Vasa regulation of Gurken depends on an interaction with the translation factor eIF5B, suggesting that Vasa functions directly in the translational activation of Gurken RNA (Johnstone and Lasko, 2004). Vasa regulation of Nanos, however, does not require binding to eIF5B (Johnstone and Lasko, 2004) but requires recruitment of other germ granule components to the germ plasm, including Tudor.

Tudor is another evolutionarily conserved component of germ granules (Thomson and Lasko, 2005). Tudor localizes to both germ granules and mitochondria and has been proposed to promote the translation of germ granule RNAs by recruiting mitochondrial large ribosomal RNA (mtlrRNA) and nucleating the formation of mitochondrial-like ribosomes near germ granules (Kobayashi et al., 2005). Consistent with this hypothesis, inhibitors of prokaryotic translation, predicted to block mitochondrial but not cytoplasmic translation, interfere with the translation of at least one germ granule RNA in *Drosophila* (*gcl*, Kobayashi et al., 2005). These inhibitors, however, do not affect the translation of *nanos*, so not all germ granules RNAs are translated by mitochondrial-like ribosomes. An intimate connection between mitochondria and germ granules has been observed in several organisms and has led some to propose that mitochondria contribute many components to germ granules (Reunov et al., 2000). In eggs with germ plasma, an interaction between mitochondria and germ granules may also serve to target selected mitochondria to PGCs (Cox and Spradling, 2003).

Tudor contains 11 copies of a module called the Tudor domain (Thomson and Lasko, 2005). These domains are required for germ granule integrity in *Drosophila* germ plasm (Arkov et al., 2006) and are predicted to bind to methylated proteins, in particular SM proteins (Thomson and Lasko, 2005). SM proteins are best known for their role as core components of the spliceosome but surprisingly also localize to germ granules in mouse spermatocytes (Chuma et al., 2003; Moussa et al., 1994), *Xenopus* oocytes (Bilinski et al., 2004), and *C. elegans* embryos (Barbee et al., 2002). Several lines of evidence support a connection between Tudor and SM proteins in germ granules. In mice, the Tudor-domain protein Mtr-1 coprecipitates and colocalizes with SM proteins in chromatoid bodies (Chuma et al., 2003). During the assembly of small nuclear ribonuclear protein (snRNP) complexes, SM proteins are methylated in the cytoplasm by the methyltransferase PMRT5 and its partner MEP50. Remarkably, *Drosophila* Tudor interacts with Valois, a MEP50 homolog, and mutations in Valois/MEP50 and dart5/PMRT5 interfere with Tudor localization (Anne and Mechler, 2005; Gonsalvez et al., 2006). These mutations also interfere with germ cell specification, as does direct depletion of SM proteins in *C. elegans* (Barbee and Evans, 2006). Together these observations suggest that an interaction between Tudor and SM proteins contributes to germ granule function, perhaps by promoting the assembly of RNP complexes.

Germ Granules Have Somatic Cousins?

An important advance in our understanding of germ granules has come with the discovery that germ granules share components with the processing bodies (P bodies) of somatic cells. P bodies are cytoplasmic foci where untranslated mRNAs accumulate, awaiting degradation or translational reactivation (Anderson and Kedersha, 2006). In yeast and mammalian cells, P bod-

ies contain the 5′ to 3′ mRNA degradation machinery (including the decapping proteins DCP1 and DCP2) and proteins implicated in translational repression (Dhh1p/Rck and Scd6p/Rap55). In mammalian cells, P bodies also contain components of the RNA-dependent silencing machinery (Argonaute and microRNAs). Remarkably, all of these components have recently been reported in germ granules. In mouse round spermatids, Dcp1, the Argonaute homologs Miwi, Ago2, and Ago3, and the microRNAs let-7, miR-21, and miR-122a colocalize with Vasa in the chromatoid body (the germ granule of spermatids) (Kotaja et al., 2006). In *C. elegans* embryos, the decapping subunit DCP-2, the Dhh1p homolog CGH-1, and the Scd6p homolog CAR-1 colocalize with the germ granule component PGL-1 on germ granules (Audhya et al., 2005; Boag et al., 2005; Lall et al., 2005; Navarro et al., 2001; Squirrell et al., 2006). In *Drosophila* oocytes, these same components (dDcp1, dDcp2, Dhh1p/Me31b, and Scd6p/Trailer Hitch) coassemble in RNP particles that transport translationally repressed Oskar RNA to the germ plasm (Lin et al., 2006; Nakamura et al., 2001; Wilhelm et al., 2005). The *Drosophila* Argonaute homolog Piwi interacts with Vasa and is a transient component of polar granules in early embryos (Megosh et al., 2006).

Are germ granules the germline equivalent of P bodies? P body components are also present in two other classes of somatic RNPs: stress granules and neuronal granules (Anderson and Kedersha, 2006). Unlike P bodies, stress and neuronal granules contain ribosomal subunits. Stress granules abort the translation of many RNAs in stressed cells and contain stalled 48S preinitiation complexes. Neuronal granules deliver mRNAs and inactive ribosomes to specific translation sites in dendrites (Anderson and Kedersha, 2006). Polysomes have been detected at the periphery of germ granules in the *Drosophila* germ plasm (Mahowald, 1968) and in the rat chromatoid body (Parvinen, 2005), but whether inactive ribosomal subunits are present in the germ granules themselves is not known. P bodies, stress granules, and neuronal granules all contain mRNAs that are not translated, so a core function for these cytoplasmic RNP granules may be to keep mRNAs out of polysomes (Anderson and Kedersha, 2006). Germ granules likely share this function, as mRNAs in germ granules are often kept translationally silenced before activation. Bruno, a component of the *Drosophila* nuage (Snee and Macdonald, 2004), inhibits Oskar translation by promoting the oligomerization of Oskar mRNA into large (50–80S) RNP complexes that exclude ribosomes, suggesting a closer relationship between these particles and P bodies (Chekulaeva et al., 2006). Live imaging of *Drosophila* ovaries, however, has revealed that germ granule components associate with several types of RNP granules, each with distinct origins, localizations (perinuclear or cytoplasmic), and dynamic properties (static or moving) (Snee and Macdonald, 2004). Germ granules may thus be as diverse as their somatic cousins, with functions ranging from RNA localization/decay to translational activation/repression. A complete component list is not yet available for any granule but may be necessary before we can determine how many classes of germ granules exist and draw direct parallels to somatic granules (Anderson and Kedersha, 2006).

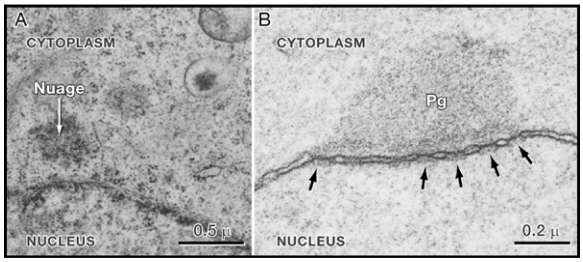

Figure 3. Perinuclear Germ Granules
Perinuclear germ granules in (A) the primordial germ cell of a rat at embryonic day 10 (nuage) and (B) in an adult *C. elegans* germ cell (Pg). (A) is reprinted from Eddy (1974) with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc. (B) is reprinted from Pitt et al. (2000) with permission from Elsevier.

The Work Horses of Germ Cells

During most of germ cell development, germ granules are perinuclear (nuage) and proximal to nuclear pores (Figure 3). Ultrastructural studies have revealed that virtually all germ granules in adult *C. elegans* associate with pore-rich regions of the nuclear membrane (except for the more dispersed granules of oocytes), and that 75% of nuclear pores in germ cells are in direct contact with germ granules (Pitt et al., 2000). This distribution suggests that most mRNAs synthesized in germ cells have a high likelihood of encountering a germ granule immediately upon exiting the nucleus. A survey of five *C. elegans* mRNAs encoding developmentally regulated proteins revealed that all were enriched in germ granules, in contrast to ubiquitously expressed actin and tubulin mRNAs, which were uniformly distributed through the cytoplasm (Schisa et al., 2001). Thus, germ granules could potentially participate in the majority of gene regulation events that occur in germ cells. As discussed above, relatively few transcriptional activators have been identified in germ cells, with the exception of those used during gametogenesis. In contrast, RNA-binding proteins have been implicated in the regulation of most aspects of germ cell development, from PGC migration and stem cell maintenance to sex determination and cell-cycle regulation (Leatherman and Jongens, 2003; Vasudevan et al., 2006). Perhaps the increased emphasis on posttranscriptional regulation in germ cells accounts for the relative prominence and unique distribution of germ granules compared to somatic granules: germ granules manage a larger and more diverse work load than their somatic cousins. Interestingly, two studies in *C. elegans* have reported that somatic cells develop perinuclear, germ granule-like structures under conditions when transcription is globally misregulated (Unhavaithaya et al., 2002; Wang et al., 2005). Increased numbers of RNP granules may be a universal cellular

response to cope with permissive transcription. However, the extent to which germ granules participate in all posttranscriptional events in germ cells remains to be determined. An alternative is that perinuclear germ granules serve primarily to sort newly transcribed mRNAs, which then facilitates posttranscriptional events that occur in the cytoplasm (Snee and Macdonald, 2004). Perinuclear sorting and trapping of mRNAs may be particularly important in germ cells that share cytoplasm through intracellular bridges (described above). Chromatoid bodies have been observed in the cytoplasmic bridges that connect rat spermatids (Parvinen, 2005), consistent with a potential function in regulating transcript sharing.

A Counterbalance to Totipotency?

A central role for germ granules in maintaining proper gene expression in germ cells is supported by an exciting recent study reporting the first teratoma in an invertebrate (Ciosk et al., 2006). GLD-1 and MEX-3 are two translational regulators expressed in complementary patterns in the *C. elegans* gonad. Loss of either of these two proteins gives rise to distinct sterile phenotypes, but loss of both simultaneously causes germ cells to "transdifferentiate" into somatic cells. Several fully differentiated somatic cell types are formed in *gld-1*;*mex-3* double mutants, including muscle, neurons, and intestine. The cells form a disorganized mass in the ovary, reminiscent of the teratomas of mammals. Remarkably, "transdifferentiation" in *gld-1*;*mex-3* mutants requires both female development (oogenesis) and meiotic entry and is preceded by a period where germ cells that have begun meiosis (pachytene stage) are present but lack germ granules (Ciosk et al., 2006). Although a causal effect between loss of germ granules and transdifferentiation has yet to be demonstrated, it is tempting to speculate that loss of germ granules could lead to premature translation of maternal RNAs normally activated only at the start of embryogenesis (Ciosk et al., 2006). That germ cells that have initiated meiotic prophase can be induced to form somatic cell types indicates that germ cells, at least in the female line, remain pluripotent even during differentiation.

Are RNA regulators also implicated in teratoma formation in mammals? Until recently the molecular lesions leading to teratoma formation were not known. The identification of mouse strains with high incidence of teratomas made it possible to genetically map loci that contribute to teratomas such as the *Ter* locus. In the 129 inbred strain background, *Ter* causes a dramatic increase in testicular teratomas. The *Ter* locus was recently shown to encode the mouse homolog of Dead end (Youngren et al., 2005), a predicted RNA-binding protein essential for PGC viability in zebrafish (Weidinger et al., 2003). Interestingly, when not in the 129 strain background, the *Ter* mutation also results in a PGC-loss phenotype. These results suggest that, as in *C. elegans*, loss of an RNA-binding protein essential for germ cell development combined with additional mutation(s) can cause germ cells to transdifferentiate into somatic cells. Although the mechanisms that lead to transdifferention remain mysterious, these studies strongly suggest that posttranscriptional mechanisms, perhaps housed in germ granules, are essential to keep-in-check the totipotency of germ cells and delay somatic differentiation until fertilization. In this regard it is interesting to note that neoblasts, the totipotent cells that give planaria their extraordinary regenerative capacity, contain a chromatoid body-like structure in their cytoplasm, which disappears upon differentiation (Shibata et al., 1999).

Gametes: Unleashing Totipotency at Fertilization

Gametes are highly differentiated cells with unique specializations dedicated to the exceptional tasks that these cells must complete (such as meiosis and fertilization). Yet the union of two gametes creates a totipotent zygote capable of generating all cell types. How do gametes shed their specializations and regain the ability to express somatic genes? What does each gamete bring to the zygote to endow it with such magnificent potential?

Oocytes: Carrying the Burden

Several lines of evidence suggest that the potential for totipotency resides primarily in oocytes. Unlike spermatozoa, oocytes retain the potential to form teratomas (Stevens and Varnum, 1974) and express the pluripotency factors Oct4 and Sox2. In parthenogenic animals, oocytes can initiate embryonic development entirely on their own. In fact, even in sexually reproducing animals, oocytes can initiate development without sperm, if they are provided with an artificial stimulus for activation and a reprogrammable diploid nucleus following somatic cell nuclear transfer.

What molecular events are required to unleash totipotency following fertilization? Two global changes must occur: (1) establishment of a new zygotic transcriptional network and (2) degradation of maternal mRNAs and proteins.

The mechanisms that initiate zygotic transcription are poorly understood but are, by definition, dependent on the collection of transcription factors present in the egg at the time of fertilization. In mammals, unique patterns of histone modifications have been reported on male and female pronuclei following fertilization (Santos et al., 2002). Brg1, a component of the mammalian ATP-dependent SWI/SNF-related chromatin remodeling complex, is present in oocytes and is required for zygotic gene activation (Bultman et al., 2006). Loss of Brg1 reduces the transcription of 30% of genes expressed in zygotes and leads to arrest in the two-cell stage.

The inefficiency of somatic cell nuclear transfer suggests that, in addition to reprogramming factors in the oocyte cytoplasm, oocyte and sperm nuclei must also contain modifications that facilitate reprogramming. In mammals both global and imprint-specific DNA methylation patterns are established during gametogenesis and in early embryogenesis (Morgan et al., 2005). During spermatogenesis, most, but not all, of the histone-containing nucleosomes are replaced with highly basic arginine-rich protamines. Chromatin extraction experiments have suggested that sperm DNA is packaged in an orderly and reproducible pattern (Ward et al., 2000).

Whether these modifications facilitate totipotent reprogramming remains to be determined.

In many organisms, mRNAs present in oocytes are rapidly degraded at the onset of embryogenesis. Presumably, degradation of maternal mRNAs allows the zygotic genome to take over and introduce spatial and temporal differences in mRNA distribution. In *Drosophila*, maternal mRNA degradation depends on recruitment to 3′UTRs of the CCR4/POP2/NOT deadenylase (Semotok et al., 2005). In zebrafish, a single miRNA, Mir-430, expressed at the onset of zygotic transcription, accelerates the deadenylation and clearance of several hundred maternal mRNAs (Giraldez et al., 2006). Instances of protein degradation have also been reported (Bowerman and Kurz, 2006). For example, in *C. elegans*, the microtubule-severing complex katanin must be rapidly degraded after the last meiotic division to avoid interfering with the formation of the first mitotic spindle. In animals with germ plasm, germ plasm RNAs and proteins must be eliminated from somatic lineages (Bowerman and Kurz, 2006). Surprisingly, many of these degradation events appear independent of fertilization and are carried out by machinery present in the oocyte itself and activated by egg activation (*Drosophila*) or by the meiotic cell cycle (*C. elegans*) (Bowerman and Kurz, 2006; Tadros and Lipshitz, 2005). So in addition to the ability to reprogram nuclei, oocytes also have the machinery to reset their own cytoplasm, effectively erasing the "gamete program" to make way for somatic development.

Why We Also Need Sperm

In most animals, the drive for sexual reproduction has imposed limits on the totipotency of oocytes by requiring sperm factors to initiate development. For example, in many animals, completion of meiosis and translation of certain maternal mRNAs depend on calcium oscillations triggered by factors brought in by the sperm at fertilization (Schultz, 2005). Paternal effect mutations in *Drosophila* and *C. elegans* have also identified sperm proteins necessary for embryonic development (Fitch et al., 1998). In addition, recent studies in mammals have demonstrated that sperm also transmit RNAs that can affect the phenotype of the embryo. Male mice heterozygous for the Kit^{tm1Alf} allele give rise to offspring that, even if genotypically wild-type, exhibit the same white-spotted phenotype as mice homozygous for the Kit^{tm1Alf} allele. This dominant paternal effect appears to be due to transmission of RNAs, presumably miRNAs, generated from the Kit^{tm1Alf} allele during spermatogenesis, which decrease the levels of wild-type *Kit* mRNA in the progeny (Rassoulzadegan et al., 2006). What other RNAs are transmitted in sperm? The highly abundant, sperm-specific Piwi small RNAs (piRNAs) (Kim, 2006) are possible candidates, as are other less abundant mRNAs (Miller et al., 2005), but this possibility remains controversial.

Conclusions and a Hypothesis

The remarkable plasticity and regenerative abilities of germ cells suggests that germ cells maintain a totipotent genome through most of development. In the male, totipotency is retained through the adult germline stem cells and then likely is lost during sperm differentiation. Nucleosome divesting, chromatin repackaging, and epigenetic modifications prepare the male genome for rapid reprogramming by the egg cytoplasm following fertilization. In the female germline, totipotency may be maintained throughout development, as evidenced by the ability of female germ cells to form teratomas even after the onset of oogenesis. We suggest that this remarkable ability rests primarily on two mechanisms: (1) establishment of a unique chromatin in germ cell nuclei and (2) transfer of most the control of gene expression during germ cell development to the cytoplasm.

Transcriptional repression and chromatin remodeling block PGCs from following somatic differentiation programs and may be key to initiating a lasting transcriptional profile compatible with totipotency, i.e., one where the transcriptional status of each gene remains flexible. We propose that, during germ cell development, the requirements to maintain a totipotent genome make it difficult for germ cells to rely on a "DNA-centric" program of gene regulation, as is typical in most somatic cells. Germ cells instead rely on an "RNA-centric" program of posttranscriptional regulation. By transferring most of the burden of gene regulation to the cytoplasm, germ cells ensure that their genome remains plastic. We suggest that germ granules are the physical manifestation of this increased emphasis on posttranscriptional mechanisms. By acting as regulatory hubs for mRNAs, germ granules allow germ cells to differentiate into gametes while retaining a genome that can produce somatic cell types.

Many questions remain. How is the transcriptional profile of germ cells specified and what properties make it so responsive to reprogramming? In *C. elegans* and *Drosophila*, are there pluripotency factors similar to Oct4? Just how much of gene regulation in germ cells depend on posttranscriptional mechanisms? How do germ granules function and what is their relationship to somatic RNA granules? In mammals, where the germline is not preformed, how are germ granules induced in PGCs? How do oocytes maintain totipotency while undergoing meiosis? How is totipotency unleashed at fertilization? These questions and many others will continue to keep germ cell biologists busy for years to come. The good news is that, as more scientists working on different organisms join the search, fundamental properties of germ cells are becoming easier to discern.

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