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# The *Xenopus* Maternal-to-Zygotic Transition from the Perspective of the Germline

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## Abstract

In *Xenopus*, the germline is specified by the inheritance of germ-plasm components synthesized at the beginning of oogenesis. Only the cells in the early embryo that receive germ plasm, the primordial germ cells (PGCs), are competent to give rise to the gametes. Thus, germ-plasm components continue the totipotent potential exhibited by the oocyte into the developing embryo at a time when most cells are preprogrammed for somatic differentiation as dictated by localized maternal determinants. When zygotic transcription begins at the mid-blastula transition, the maternally set program for somatic differentiation is realized. At this time, genetic control is ceded to the zygotic genome, and developmental potential gradually becomes more restricted within the primary germ layers. PGCs are a notable exception to this paradigm and remain transcriptionally silent until the late gastrula. How the germ-cell lineage retains full potential while somatic cells become fate restricted is a tale of translational repression, selective degradation of somatic maternal determinants, and delayed activation of zygotic transcription.

## 1. INTRODUCTION

Primordial germ cells (PGCs), sole precursors of the gametes in the adult animal, are able to differentiate into all cell lineages, including themselves. Thus, PGCs are considered the “stem cells” of the species. PGC specification is an early event in embryonic development and relies on a unique set of germ-cell determinants. In mammals, germ-cell determinants are induced within a small population of cells prior to gastrulation by BMP signaling (reviewed in Saitou & Yamaji, 2012). In species including worm, fly, fish, and frog, these germ-cell determinants are synthesized during early oogenesis and aggregate into germ plasm (reviewed in King, 2014; Seydoux & Braun, 2006). After fertilization, the germ plasm distributes to just a few blastomeres and those cells acquire the PGC fate. Although initiation of germline development can occur through these two distinct mechanisms, many mammalian PGC-specific genes are components of the maternally synthesized germ plasm in other species. Interestingly, a number of these genes encode RNA-binding proteins that function in regulating zygotic genome activity, totipotency, proliferation, differentiation, and

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migration of PGCs. They do so through controlling translation and mRNA stability as discussed in this chapter.

In classic studies, Nieuwkoop and Faber (1956) described “cytoplasmic inclusions” or germ plasm at the vegetal pole of eggs and early embryos of *Xenopus laevis* that segregated exclusively into the germline. Both loss- and gain-of-function experiments supported the conclusion that germ plasm is necessary for PGC formation (Nieuwkoop & Sutasurya, 1976; Smith, 1966; Wakahara, 1977, 1978). Recent compelling experiments have shown that germ plasm is both required and sufficient to specify the germline in *Xenopus*. Germ plasm introduced into animal pole blastomeres caused them to enter the germ-cell lineage and give rise to functioning gametes, albeit, in small numbers (Tada, Mochii, Orii, & Watanabe, 2012). The only requirement for proper migration was that these animal pole blastomeres be placed back into the endodermal region.

Most of oogenesis is spent in G2 of the first meiotic prophase. At this time, high rates of transcription from all four copies of each chromosome achieve maximum transcript production. The products of these maternal transcripts are sufficient to meet the requirements of the embryo through early development in the absence of zygotic transcription. Fully-grown oocytes are arrested at this meiotic stage and can remain quiescent for a long period of time. In response to the hormone progesterone, oocytes are released from G2 prophase arrest, undergo maturation events including germinal vesicle breakdown (GVBD), and the completion of meiosis I. Matured oocytes now arrest at the second meiotic metaphase and, after passage through the oviducts, are capable of being fertilized. Sperm entry triggers completion of meiosis II. The female pronucleus then fuses with the male pronucleus, leading to the formation of the zygote and initiation of embryonic development (reviewed in Ferrell, 1999; Heasman, 2006).

Although the diploid genome is reconstituted at fertilization, in *Xenopus laevis*, the major activation of the zygotic genome occurs ~6 h later, midway through the blastula stage. This critical time is known as the mid-blastula transition (MBT) and it is distinguished by a shift in the cell cycle as well as *de novo* RNA synthesis. For the 12 divisions prior to the MBT, the cell cycle is synchronous, and occurs fairly rapidly, every 25 min. To achieve such rates, the gap phases are skipped and the cell cycle goes from mitosis to DNA synthesis to mitosis. At the MBT, the embryo is comprised of some 4000 cells, cell divisions slow and the gap phases enter the cycle. All major developmental decisions up until this point have been made from maternal transcripts. These decisions include the specification of the three major axes (animal/vegetal, dorsal-anterior/ventral, right/left), the three primary germ layers (ectoderm, mesoderm, and endoderm), and the germ-cell lineage (reviewed in Heasman, 2006; King, 2014; White & Heasman, 2008). However, the transition from maternal to zygotic genetic control of development begins before the MBT, at fertilization, when degradation of maternal messages begins and ends during gastrulation when zygotic transcripts are required for further embryonic development. Thus the maternal-to-zygotic transition (MZT) encompasses a longer developmental time period than the MBT (Langley, Smith, Stemple, & Harvey, 2014; Tadros & Lipshitz, 2009; Fig. 1).

The cleavage stages before the MBT accomplish the important task of generating enough cells to begin the process of regional differentiation according to germ layer identity. Not surprisingly, these early-stage blastomeres are maintained in a pluripotent state by the expression of the maternal factors, Oct 60 and Oct 25, orthologs of mammalian Oct 3/4 (Cao et al., 2004; Cao, Siegel, & Knochel, 2006; Cao, Siegel, Oswald, & Knochel, 2008; Hinkley, Martin, Leibham, & Perry, 1992; Whitfield, Heasman, & Wylie, 1993). Post-MBT, these factors gradually decline and are replaced by lineage-specific transcription factors such as Xsox17, Bix4, GATAs, and Xnrs, many of which are activated by the maternal transcription factor, VegT, at the MBT (reviewed in Heasman, 2006; White & Heasman, 2008). Thus, as development proceeds and genetic control is ceded to the zygotic genome, developmental potential gradually becomes more restricted within the primary germ layers. The germline is a notable exception to this paradigm and raises the fundamental question as to how this lineage retains the potential for totipotency while the somatic cells surrounding them become fate restricted. There is strong evidence for overlapping “antidifferentiation” mechanisms involving repression at both transcriptional and translational levels that operate to preserve the germline through the MZT (Leatherman & Jongens, 2003; Venkatarama et al., 2010). In this chapter, we will examine the MZT within the context of germline specification.

## 2. GERM-PLASM RNAs AND CYTOSKELETAL DYNAMICS: STAGE VI OOCYTE

In Stage VI *Xenopus* oocytes, germ plasm is organized into numerous small islands. At the ultrastructural level, each of these germ-plasm islands contains mitochondria, endoplasmic reticulum, membraneless electron-dense materials and matrix. The electron-dense material or germinal granules, can be fibrillar or round-shaped and is a hallmark of germ plasm (Heasman, Quarmby, & Wylie, 1984). RNAs within the germ plasm are found with distinct localization patterns (Fig. 2). Germinal granules contain *nanos* RNA, while *Xpat* and *DeadSouth* are peripherally associated with granules. *Xdazl*, *Xlsirts*, *Xwnt11*, *DEADSouth*, and *fatvg* are found within the matrix as ribonucleoprotein particles (RNPs) (Kloc et al., 2002). The significance of this organization of germ-plasm mRNAs in relation to when they may be translated after fertilization is not known. In full-grown oocytes, the germ-plasm islands are finely dispersed in the subcortex at the vegetal pole, located between the cortical granules and yolk. This geographic location and the structural organization of the germ plasm are likely maintained by a highly organized cytoskeletal network, which is established during early stages of oogenesis (Gard, Cha, & King, 1997). At least, two types of intermediate filament are closely associated with germ plasm. These include a large amount of vimentin, which is colocalized with germ plasm (Godsave, Anderton, Heasman, & Wylie, 1984; Torpey, Heasman, & Wylie, 1990; Wylie, Brown, Godsave, Quarmby, & Heasman, 1985; Wylie, Heasman, Parke, Anderton, & Tang, 1986), and a well-developed network of cytokeratin filaments that can be easily detected in the vegetal cortex and subcortex (Gard, 1999; Kloc, Bilinski, & Dougherty, 2007; Kloc et al., 2005; Torpey, Heasman, & Wylie, 1992). Ultrastructural analysis reveals that some germ-plasm islands are surrounded and penetrated by the long cytokeratin filaments (Kloc et al., 2007). Although actin filaments (Gard, 1999) and a small number of microtubules (Gard, 1991, 1994; Pfeiffer & Gard, 1999)

are present in the vegetal subcortex region, it is unclear whether they play a direct role in maintaining germ-plasm islands there. Recently, it has been reported that depletion of Vangl2 and aPKC, which results in a reduction in the acetylated microtubule cytoskeleton, disrupts vegetal localization of *VegT* and *Wnt11* mRNAs (Cha, Tadjuidje, Wylie, & Heasman, 2011). These results suggest that the microtubule cytoskeleton plays a role in maintaining at least some RNA components at the vegetal pole.

The cytokeratin network at the vegetal pole has been studied extensively. Evidence supports the cytokeratin network as a scaffold, allowing some vegetally localized maternal RNAs to associate with it. These include the noncoding RNAs *Xlsirts* (Kloc & Etkin, 1994) and *VegT* mRNA, which encodes the master regulator for *Xenopus* germ layer specification (Zhang et al., 1998). Both *Xlsirts* and *VegT* RNPs in Stage VI oocytes are found associated with the cytokeratin network (Kloc et al., 2005). Strikingly, both *Xlsirts* and *VegT* mRNAs play important structural roles in maintaining the architecture of the cytokeratin network in the vegetal cortex. Depletion of these RNAs disrupts the network in a transcript-specific manner and impairs the proper formation of the germinal granules and subsequent development of the germline (Kloc et al., 2007, 2005). In the case of *VegT*, Heasman and colleagues have provided convincing evidence that it is the *VegT* mRNA, not the VegT protein, which is required for maintaining the structure of the cytokeratin network. In *Xlsirts*- or *VegT*-depleted oocytes, vegetal localization of a number of maternal RNAs is disrupted, likely due to the defective cytokeratin network (Heasman, Wessely, Langland, Craig, & Kessler, 2001; Kloc & Etkin, 1994; Kloc et al., 2005).

## 3. GERM PLASM AND CYTOSKELETAL DYNAMICS: OOCYTE MATURATION/FERTILIZATION

During oocyte maturation and subsequent fertilization, the cytoskeleton in the vegetal pole undergoes a series of dramatic changes. While vimentin remains colocalized with germ plasm throughout these transitions (Godsave et al., 1984), other cytoskeletal architectures are largely erased during the process of oocyte maturation. For example, the cytokeratin network is disrupted nearly completely after GVBD, with only some cytokeratin punctuate foci remaining within the vegetal cortex (Kloc et al., 2005). In addition, actin filaments and microtubules become barely detectable. It is likely that disassembly of the existing cytoarchitecture in the fully-grown quiescent oocyte prepares the oocyte for fertilization. The fertilized egg quickly builds a new cytoskeletal system, which facilitates the rapid coalescence of germ-plasm islands found widely distributed throughout the vegetal subcortex before egg activation (Ressom & Dixon, 1988).

In addition to cytoskeletal dynamics, a few other changes associated with oocyte maturation have been observed. For example, endogenous aPKC protein, which is homogenously distributed in the oocyte, is translocated to the animal hemisphere of the egg and can no longer be detected at the vegetal pole (Cha et al., 2011; Nakaya et al., 2000). Hermes protein, a germ-plasm RNA-binding protein highly expressed in oocytes, is markedly downregulated after GVBD, and cannot be detected during early stages of embryonic development (Song et al., 2007; Zearfoss, Chan, Wu, Kloc, & Etkin, 2004). Interestingly, Hermes protein colocalizes with Xvelo, the homologue of zebrafish Bucky ball, which

regulates assembly of the Balbiani body (Bb) in oocytes (Marlow & Mullins, 2008) and organizes germ plasm after fertilization (Bontems et al., 2009; Heim et al., 2014). Although the significance of these molecular events remains unclear, it is highly likely that some of these molecular changes trigger remodeling of the germ plasm that is required for the initiation of germline development after fertilization.

### 3.1 Postfertilization

To initiate PGC development, the germ-plasm islands (>500) that are widely dispersed in the oocyte's vegetal subcortex must coalesce into larger aggregates for segregation into a small number of blastomeres. This process begins at fertilization and with each division small aggregates are pushed into larger aggregates as the cleavage furrow advances. By the 32-cell stage, there are now on average 36 islands of germ plasm (Savage & Danilchik, 1993). During the first two *Xenopus* embryonic cell cycles, cleavage furrows are initiated in the animal hemisphere and move toward the vegetal pole. As a consequence of the first two embryonic cell divisions, germ-plasm aggregates become concentrated into the vegetal corner of each blastomere. In zebrafish embryos, germ-plasm RNPs first move toward the animal pole together with the cytoplasm during cytoplasmic streaming. Once streaming is completed, the blastoderm is created on top of the yolky mass. The large volume of yolk prevents the cleavage furrows from reaching the vegetal pole. For this reason, four large germ-plasm aggregates form at the periphery of the blastoderm as the zebrafish embryo divides. Interestingly, large germ-plasm aggregates can form in artificially activated eggs. Notably, it took 3–4 h for germ-plasm islands to coalesce in activated eggs, whereas the same process is largely completed by around 2 h postfertilization in normal embryos (Robb, Heasman, Raats, & Wylie, 1996). Considering that artificially activated eggs do not undergo cleavage, cleavage may not be absolutely required for coalescence of germ plasm.

A growing body of evidence suggests that formation of germ-plasm aggregates relies heavily on cytoskeletal dynamics after fertilization. Upon egg activation during fertilization, several cytoskeleton networks are established very rapidly in *Xenopus*. This includes a complex arrangement of cytokeratin network after egg activation (Kloc et al., 2005). Also, shortly after fertilization, a dense microtubule network is established in the entire fertilized egg. These microtubules, nucleated by the sperm centrosome and egg pronuclear-associated components, extend radially into the entire embryo. Treatments that block microtubule polymerization after egg activation (Ressom & Dixon, 1988; Savage & Danilchik, 1993), or that deplete the maternal kinesin-like protein, Xklp1, impair the aggregation process of the germ plasm. These studies have highlighted the importance of the microtubule network in germ-plasm aggregation.

It is worth mentioning that, shortly after fertilization, a unique microtubule network forms in the vegetal cortex. These microtubule arrays are organized into numerous parallel bundles and drive cortical rotation, a process that is essential for axis specification. This microtubule network exists only transiently and for a short period of time, and is disassembled prior to the beginning of the first embryonic division (Houliston & Elinson, 1991; Schroeder & Gard, 1992). In *Xenopus*, these microtubules are continuous with microtubules extending from the animal hemisphere. Polymerization of microtubules into the vegetal cortex is

regulated by vegetally localized maternal factors such as Trim36 (Cuykendall & Houston, 2009; Olson, Oh, & Houston, 2015) and Dead-end (Dnd1) (Mei et al., 2013), both of which are actually components of the germ plasm. There is no evidence to suggest that these vegetal cortical microtubules are essential for germ-plasm aggregation. Recently, the zebrafish maternal-effect mutant, *Hecate*, has been characterized, which carries mutations in the glutamate receptor-interacting protein 2a (grip2a) gene. *Hecate* embryos show defects in the alignment and bundling of microtubules at the vegetal cortex. Such bundling abnormalities result in defective initiation of Wnt signaling during cleavage and subsequent axis specification. In contrast, the aggregation of germ plasm occurs normally in *hecate* mutants at the 4-cell stage. Even in severely ventralized *hecate* embryos, the average number of PGCs is unaffected at 24 h postfertilization (Ge et al., 2014). It is also worth pointing out that the defects in *hecate* embryos, which are maternal-effect mutant, does not necessarily rule out the possibility that zygotic Grip2 may be involved in germ-cell development. In *Xenopus*, injection of morpholino antisense oligos that block Grip2.1 translation, or overexpression of a dominant negative Grip2.1, reduces average PGC number and impairs the proper anterior-posterior positioning of PGCs in embryos (Tarbashevich, Koebernick, & Pieler, 2007). Nonetheless, the observation that germ-plasm coalesces as normal into four large masses in *hecate* embryos, which are defective in vegetal cortical microtubules, suggests that these aligned vegetal cortical microtubule arrays are unlikely to be required for germ-plasm aggregation.

Our current knowledge regarding how cleavage coordinates germ-plasm aggregation comes largely from studies in zebrafish. In zebrafish, germ-plasm RNPs exist as single particles before fertilization. After fertilization, these particles begin to form aggregates. This process is accelerated as the first embryonic cell cycle begins. As the astral microtubules extend from the sperm aster toward the periphery of the blastodisc, germ-plasm RNPs and associated actin move outwardly. As a result, germ-plasm RNPs are displaced to the outer edge of the blastodisc and the center of the blastodisc becomes free from germ-plasm RNPs. During cytokinesis, through the action of astral microtubules of the bipolar spindle, some germ-plasm RNPs are recruited to the cleavage furrow, forming two rod-like structures. These rod-like structures are pushed in opposite directions as the cleavage furrow advances outwardly from the center of the blastodisc, leading to distal compaction of germ-plasm RNP aggregates at both ends of the mature cleavage furrow. This is reminiscent of astral microtubule-dependent germ-plasm transportation during *Drosophila* germ-cell development (Lerit & Gavis, 2011). Subsequently, in zebrafish, similar processes occur repeatedly during the second and third cytokinesis. Ultimately, the majority of germ-plasm RNPs is packaged into four compact masses located at the periphery of the embryo (Eno & Pelegri, 2013; Nair et al., 2013; Theusch, Brown, & Pelegri, 2006). These large germ plasm aggregates are inherited by a few blastomeres, leading to the specification of the germline.

Several zebrafish maternal-effect mutants show defects in the aggregation of germ-plasm RNPs. These include *cellular island*, which carries mutation in Aurora B Kinase that is critical for cleavage furrow formation during cytokinesis (Yabe et al., 2009), and *motley*, a maternal-effect mutant deficient in the chromosomal passenger protein Birc5b/Survivin. Birc5b/Survivin regulates chromosomal passenger complex function, astral microtubule

remodeling in the initiation of cytokinesis furrow ingression, and cortical microfilament dynamics (Nair et al., 2013). In addition, overexpression of *Bucky ball*, which encodes a RNA-binding protein important for Bb formation (Marlow & Mullins, 2008), induces abnormal germ-plasm aggregation, and generates ectopic PGCs in the zebrafish embryo (Bontems et al., 2009).

After coalescence of the germ plasm is completed, the location of zebrafish germ plasm remains unchanged in the embryo until morphogenesis begins. In *Xenopus*, until the 16-cell stage, germ plasm aggregates are located in the subcortex at the vegetal pole. As cleavage proceed, germ plasm changes its location from the vegetal subcortical regions to deep within the yolky endodermal mass (Ressom & Dixon, 1988; Savage & Danilchik, 1993). Interestingly, this movement can occur in artificially activated eggs and is independent of microtubules, microfilaments, or protein synthesis, although it takes longer in activated eggs as compared to fertilized embryos. This again suggests that cleavage may not be absolutely required for the germ-plasm dynamics in *Xenopus* embryos. In activated eggs, it seems that germ plasm is carried by cytoplasmic flow from the region of the vegetal pole toward the interior (Ressom & Dixon, 1988). By the blastula stage, four to five PGCs with concentrated germ plasm can be found in the endodermal mass. There, PGCs later divide a few times and eventually migrate out of the endoderm. At the tadpole stage, they migrate into the gonad where they proliferate and differentiate into gametes.

## 4. MATERNAL DETERMINANTS FOR TWO LINEAGES INHERITED BY THE SAME BLASTOMERES: A DILEMMA

While the overwhelming majority of mRNAs are distributed uniformly throughout the *Xenopus* oocyte, several hundred are found concentrated specifically at the vegetal pole. As alluded to earlier, many of these localized maternal RNAs are highly enriched in a cytokeratin-containing fraction, a characteristic that allowed their isolation (Mosquera, Forristall, Zhou, & King, 1993; Pondel & King, 1988). RNAs appear to localize using two different pathways during oogenesis while some use both pathways. In general, germ-plasm RNAs accumulate within the Bb along with mitochondria during the earliest oocyte stages. All RNAs identified thus far that localize into the Bb have functions in the germline (reviewed in King, Messitt, & Mowry, 2005). The Bb was shown to expand toward the vegetal pole, pushing germ plasm into the subcortex in Stage I to II oocytes (Wilk, Bilinski, Dougherty, & Kloc, 2005). RNAs encoding factors that function only in the soma such as Vg1 and VegT, localize within large particles transported along microtubules (reviewed in Pratt & Mowry, 2013). Localization of these RNAs occurs after germ-plasm RNAs have arrived at the vegetal cortex and for this reason, has been called the late pathway. However, not all RNAs that become restricted to PGCs during development are localized within the Bb germ plasm. A set of RNAs with dual functions in both the oocyte and PGCs (Dead-end) or in PGCs and the soma (Fatvg) is localized during the late pathway. In summary, by the end of oogenesis, maternal RNAs required to specify both germline and somatic lineages become localized to the oocyte vegetal cortex. The latter include VegT and Vg1 responsible for endoderm/mesoderm identity (Birsoy, Kofron, Schaible, Wylie, & Heasman, 2006;

Zhang & King, 1996) and Xwnt11 and Trim36 for dorsal/ventral patterning (Cuykendall & Houston, 2009; Tao et al., 2005).

Such colocalization of determinants for different lineages presents a dilemma that must be resolved before the MBT. As the fertilized egg divides, germ plasm is asymmetrically segregated into only a few cells, while localized somatic RNAs are activated for translation and are passively transmitted into all vegetal cells, including PGCs (King et al., 2005; Lai, Singh, & King, 2012; Venkatarama et al., 2010). Thus, well before zygotic gene transcription begins 12 divisions later at the MBT (~4000 cells), determinants, including VegT, are available to initiate somatic gene-expression programs in PGCs (Fig. 3; Venkatarama et al., 2010). *VegT* RNA is actually translated earlier, after maturation, so its protein is available from the earliest stages of development. It is likely preloaded into nuclei and ready to initiate somatic gene programs at the MBT (Stennard, Zorn, Ryan, Garrett, & Gurdon, 1999). In spite of the presence of VegT, however, PGCs do not activate the endoderm gene-expression program, which includes the transcription factors, *Xsox17α* and *Bix4* (Casey et al., 1999; Hudson, Clements, Friday, Stott, & Woodland, 1997). Instead, they remain competent to form all cell types. How are PGCs protected from somatic fates at this time? What prevents VegT protein from activating the endoderm genetic program in PGCs at the MBT?

## 5. MECHANISMS FOR PROTECTING THE GERMLINE DURING THE MZT

Two critical activities appear to be required in *Xenopus* PGCs to protect them from VegT activity and other somatic differentiation signals: (1) translational repression of VegT by Pumilio and Nanos, and (2) transient genome-wide suppression of mRNA transcription at the MBT. The latter activity ensures that somatic differentiation programs remain inactive when zygotic transcription is initiated in the rest of the embryo (Fig. 3). Nanos-family members are distinguished throughout the animal kingdom by a conserved CCHC Zinc-finger motif that is required for RNA binding (Hashimoto et al., 2010). Alone, Nanos has little RNA-sequence-specific binding activity, but in all species studied it acts as a translational repressor (reviewed in Lai & King, 2013; Lai, Zhou, Luo, Fox, & King, 2011). Like Nanos, Pumilio (PUM) is also a member of a conserved family of proteins found in all eukaryotes. The conserved C-terminal region contains eight tandem repeats that defines its RNA-binding domain and confers specificity in RNA recognition for binding. PUM specifically binds an 8-nt canonical sequence within the 3′UTRs (**UGUA**NAUA) of targeted RNAs for repression (Jaruzelska et al., 2003; Lai et al., 2012; Wang, Opperman, Wickens, & Hall, 2009; Wharton, Sonoda, Lee, Patterson, & Murata, 1998; Zamore, Williamson, & Lehmann, 1997; Zhang et al., 1997); hence, it is called the Pumilio Binding Element (PBE; formerly Nanos Response Element). The first four nucleotides (UGUA) are absolutely required and N could be A, U, or C. One change in the last three nucleotides (AUA) is also tolerated and those PBEs are referred to as noncanonical (ncPBE). The last three nucleotides most common in noncanonical PBEs are AUG and AAA (Table 1).

Although Nanos activity mediates repression and PUM selects the RNA target, there are reports that other regions of PUM can mediate repression alone (Padmanabhan & Richter, 2006; Weidmann & Goldstrohm, 2012). Two Pumilio family proteins have been

characterized in *Xenopus* oocytes that differ significantly in their N-terminal regions, while their RNA-binding domains are virtually identical and are expected to bind the same set of RNAs (Ota, Kotani, & Yamashita, 2011). PUM has many targets, associates with proteins other than Nanos, including Dazl (Fox, Urano, & Reijo Pera, 2005; Padmanabhan & Richter, 2006), and is part of complexes in both somatic and germ cells. Thus, the challenge is to identify PGC RNAs that bind PUM, but which require either Nanos for repression or Dazl for activation. Recently, a set of RNAs involved in meiosis and repressed by Nanos2 has been identified in mouse male gonads by immunoprecipitation with Nanos antibody (Suzuki, Igarashi, Aisaki, Kanno, & Saga, 2010; Suzuki & Saga, 2008). While *Xenopus* has only one *nanos* gene, the mouse has three. Interestingly, the Nanos2-interacting proteins identified in this study include the CCR4-NOT deadenylation complex. These findings suggest that Nanos represses expression of these RNAs and leads to their degradation.

Compared to control *ornithine decarboxylases1* (*ODC*), both maternal *VegT* and *survivin* (*Xsurv*) RNA levels at Stage 8 are significantly lower in PGCs than in somatic cells and they are not detected by Stage 10 (Venkatarama et al., 2010). Maternally expressed Xsurv is a positive regulator of cell-cycle progression localized to the mitotic spindle (Murphy, Sabel, Sandler, & Dagle, 2002; Yan et al., 2005). Loss of Xsurv may reflect the difference in cell-cycle regulation in PGCs (Zust & Dixon, 1975). These differences in RNA levels cannot be accounted for by zygotic expression of *VegT* RNA in the embryo sample. Specific antisense depletion of maternal VegT shows that most of VegT at Stage 10 is maternal RNA (Zhang et al., 1998). Therefore, it seems that *VegT* and *Xsurv* are regulated differently (less stable) in PGCs than in somatic cells. PGCs depleted of Nanos activity misexpress the downstream targets of VegT, Xsox17, and Bix4 (Lai et al., 2012). The phenotype can be rescued by injection of *Nanos* RNA. These results suggest that Nanos normally represses translation of the maternal *VegT* mRNA within the germline. Indeed, the 3′UTR of *VegT* contains one canonical PBE that binds PUM and is required for VegT repression (Table 1; Lai et al., 2012). Further, sometime between MBT and gastrulation, *VegT* is dramatically reduced in PGCs relative to the whole embryo, but not in *nanos-depleted* embryos (Lai et al., 2012). The elimination of somatic determinants from PGCs is part of the MZT for the germline. Thus, RNA degradation does indeed play a role in Nanos/PUM translational repression in *Xenopus* early embryos. Nanos functions to repress RNAs that normally specify endoderm and promote apoptosis, both of which are critical for preserving the germline.

What other germline RNAs may be regulated in this manner? Cyclin B1, a central regulator of the cell-cycle, is a prime example also targeted for repression by PUM/Nanos in other species (Lai et al., 2011). Pumilio also binds and represses *Xwnt11* but in this case does not require Nanos (Lai & King, 2013). Scores of other RNAs found within the germ plasm contain PBEs, but without direct testing for repressive activity, no conclusions can be drawn (see candidates in Table 1). Of interest is *Vg1* RNA, a somatic determinant involved in endoderm and mesoderm patterning (Birsoy et al., 2006). *Vg1* RNA contains 1 PBE and 2 ncPBEs and may be regulated as VegT is regulated. PUM mRNA itself has the highest number with 1 PBE and 6 ncPBEs, suggesting that a negative autoregulatory loop may operate in the germline. That is, PUM may be allowed to only reach a certain concentration

within PGCs before its mRNA is repressed and degraded. Transcripts encoding Nanos, Dead-end, and all helicases except DeadSouth, do not have PBEs within their 3′UTRs.

## 6. PGCs ARE TRANSCRIPTIONALLY REPRESSED AT THE MBT

The second level of protection for PGCs during the MZT acts to prevent translated somatic determinants from causing inappropriate transcription. While somatic cells enter the MBT, PGCs are transcriptionally repressed and therefore unable to respond to maternally inherited signals like VegT. Similar to the *Drosophila* and *Caenorhabditis elegans* germline, the absence of new transcription at the MBT correlates with the unphosphorylated state of the carboxy-terminal domain in RNA polymerase II that must be phosphorylated for transcriptional elongation steps to occur. Zygotic transcription in PGCs does not occur until late gastrula stages, well after somatic endoderm cells have become fate restricted (Venkatarama et al., 2010; Wylie, Snape, Heasman, & Smith, 1987). What accounts for this delay? In *nanos* loss-of-function mutants in *Drosophila*, *C. elegans*, and *Xenopus*, the delay in transcription is lost and PGCs are transcriptionally active at the same time as somatic cells are at the MBT (Deshpande, Calhoun, Jinks, Polydorides, & Schedl, 2005; Lai et al., 2012; Schaner, Deshpande, Schedl, & Kelly, 2003). How does Nanos' function as a translational repressor in PGCs play a role in the transient genome-wide suppression of mRNA transcription at the MBT?

## 7. HOW DOES THE GERMLINE ESCAPE THE MBT?

It seems unlikely that PGCs have a different timing mechanism from somatic cells for initiating zygotic transcription. Global regulation of transcription must follow very conserved rules. It seems more likely that the mechanism governing the delay in transcription for both the soma and PGCs will be related. Studies on the regulation of MBT onset suggest that the first step is that genomic DNA is released from a repressed state. This event is correlated with the nuclear-to-cytoplasmic (N/C) ratio and depletion of a putative repressor (reviewed in Langley et al., 2014). Cell cycle regulation, therefore, is one component, driving changes in the N/C ratio although nuclear size also plays a role (Jevtic & Levy, 2015). As mentioned, a target of Nanos/PUM repression, apparently conserved across species, is cyclin B1. Cyclin B1 regulates the cell cycle in a concentration-dependent fashion (Kotani, Yasuda, Ota, & Yamashita, 2013; Mendez & Richter, 2001). By repressing the cyclin B1 mRNA, the levels of Cyclin B1 protein would be expected to decline in PGCs. Consistent with this observation, whereas endodermal cells can divide every 40–75 min, PGCs undergo cell divisions less frequently, dividing only during three discrete time periods by the time they exit the endoderm at tadpole stages. A second requirement for correct initiation of zygotic transcription depends on *de novo* translation and accumulation of key maternal transcription factors, which are in limited supply. In *Xenopus*, VegT was identified as being one of these critical factors (Skirkanich, Luxardi, Yang, Kodjabachian, & Klein, 2011). Translational repression of VegT, cyclinB1, and other maternal factors in PGCs by Nanos/PUM may contribute to the observed delay in transcription; sufficient levels of core factors are not achieved. In PGCs, VegT is translationally repressed early on and degraded by gastrulation (Lai et al., 2012). In zebrafish, the pluripotency factors, Oct 91 and Sox, were also found to be key maternal factors important in this regard (Harvey et al., 2013; Lee

et al., 2013). Interestingly, maternal Sox7 is found in the germ plasm, like VegT. It, too, is a potential target for Nanos/PUM repression as the Sox7 3′UTR contains no less than 7 PBEs (Table 1).

PGCs have an open chromatin structure early that becomes condensed later. Global silencing of mRNA transcription could be explained at the level of chromatin structure. Dimethylated lysine 4 of histone H3 (H3K4) or hyperacetylated Histone H4 (Penta) are markers of transcriptionally active chromatin; methylation of lysine 9 of histone H3 (H3K9) marks inactive chromatin. When PGCs and somatic cells were isolated from embryos at MZT stages (Fig. 1) and compared for these histone modifications, no significant differences were found (Venkatarama et al., 2010). All cells stained positively for H4Penta and H3meK4, indicating an active chromatin structure. Interestingly, H1c linker protein, which restricts chromatin remodeling, is not expressed in PGCs while it is in the endoderm. PGCs may retain the more permissive oocyte linker variant, B4 (Venkatarama et al., 2010). For *Xenopus* PGCs, global transcriptional repression does not involve a dramatic alteration of chromatin architecture. Indeed, the mechanism involved prevents transcription in spite of “permissive” chromatin.

## 8. RNA DEGRADATION DURING THE MZT

Two important events occur during the MZT: turnover of maternal gene products and activation of the zygotic genome. In some species, for example mammals, these two events are accomplished in a very narrow time window shortly after fertilization. In mice, interfering with either degradation of maternal gene products or zygotic genome activation (ZGA) leads to developmental arrest around the 2-cell stage, suggesting that these two events are tightly coupled. Interestingly, degradation of maternal gene products and activation of the zygotic genome are not tightly coupled in many other species. In *Drosophila*, zebrafish, and *Xenopus*, degradation of maternal mRNAs lasts a long time. While some RNAs are degraded relatively rapidly, a large proportion of maternal mRNAs remains in the embryo even by the end of gastrulation (Bashirullah et al., 1999; Ferg et al., 2007; Mathavan et al., 2005). In these species, high-level ZGA occurs abruptly at the MBT, which corresponds to the 14th, 12th, and 10th embryonic cell division in *Drosophila*, *Xenopus*, and zebrafish, respectively. In *Drosophila*, turnover of maternal mRNAs is essential for ZGA. Mutation of Smaug, an RNA-binding protein essential for the destabilization of a large subset of the maternal mRNAs, causes a significant reduction in zygotic transcription at 2–3-h postfertilization (Benoit et al., 2009). In zebrafish, turnover of maternal RNAs does not seem to be necessary for ZGA. It has been reported that maternal-zygotic Dicer-mutant zebrafish embryos, which do not process precursor miRNAs into mature miRNAs, display mild defects during gastrulation, brain formation, somitogenesis, and heart development (Giraldez et al., 2005). Considering that inhibition of ZGA leads to the death of the embryo during gastrulation in both zebrafish and *Xenopus* (Kane et al., 1996; Sible, Anderson, Lewellyn, & Maller, 1997; Stack & Newport, 1997), the finding that maternal-zygotic Dicer mutants can survive for a number of days argues that interfering with maternal RNA degradation has no, or only a minimal, effect on ZGA in zebrafish. Thus, whether degradation of maternal gene products is a prerequisite for ZGA may be species specific.

Many maternal mRNAs are synthesized very early during oogenesis. In fact, in most species, more than 50% of protein-coding genes are expressed in the oocyte. In fully-grown oocytes, these maternal RNAs are very stable. After fertilization, a significant fraction of the maternal RNA pool is actively degraded. Existing evidence in the literature suggests that a significant amount of maternal mRNA turnover events relies on zygotic transcription, especially zygotically transcribed miRNAs (Bushati, Stark, Brennecke, & Cohen, 2008; Giraldez et al., 2006; Lund, Liu, Hartley, Sheets, & Dahlberg, 2009). The pioneering work of Newport and Kirschner elegantly demonstrated that, prior to the MBT, the zygotic genome is globally silenced. Large-scale zygotic transcription occurs at the MBT. Intriguingly, even though the major ZGA occurs at the MBT, the transcriptional machinery is present in pre-MBT stage embryos (Newport & Kirschner, 1982; Prioleau, Huet, Sentenac, & Mechali, 1994). In at least two independent studies in *Xenopus*, phosphorylated Ser2 in the CTD of RNA polymerase II was detected during early cleavage stages and indicated that Pol II-dependent transcription elongation could occur (Blythe, Cha, Tadjuidje, Heasman, & Klein, 2010; Collart, Ramis, Down, & Smith, 2009). These results suggested that at least a low level of RNA polymerase II-dependent transcription may occur pre-MBT. Indeed, it has been observed that a small number of zygotic genes are transcribed during pre-MBT stages when the zygotic genome is globally silenced in both *Drosophila* (Ali-Murthy, Lott, Eisen, & Kornberg, 2013; Edgar & Schubiger, 1986; Harrison, Botchan, & Cline, 2010; Harrison, Li, Kaplan, Botchan, & Eisen, 2011; Karr, Weir, Ali, & Kornberg, 1989; Liang et al., 2008; ten Bosch, Benavides, & Cline, 2006) and *Xenopus* (Blythe et al., 2010; Nakakura, Miura, Yamana, Ito, & Shiokawa, 1987; Shiokawa et al., 1989; Skirkanich et al., 2011; Suzuki, Ueno, & Hemmati-Brivanlou, 1997; Tan et al., 2013; Yang, Tan, Darken, Wilson, & Klein, 2002). The pre-MBT zygotic transcription is unlikely to be "transcriptional noise." Among 151 genes that are transcribed prior to the *Xenopus* MBT, many are associated with cellular events such as cell division, apoptosis, signal transduction, and phosphorylation. The pluripotency-associated gene, Oct 25, is also on the list of pre-MBT genes (Tan et al., 2013). Interestingly, some of the pre-MBT transcriptional events are precisely regulated by signaling pathways that are essential for early embryonic patterning. For example, at least a subset of pre-MBT transcription in *Xenopus* embryos relies on Wnt/β-catenin signaling (Blythe et al., 2010; Skirkanich et al., 2011; Yang et al., 2002). Inhibition of β-catenin-dependent pre-MBT transcription impairs axis specification, leading to ventralized embryos (Yang et al., 2002). Interestingly, we recently found that transcription of *Xenopus* miR-427, the homologue of miR-430, which plays key roles during the zebrafish MZT, can be detected as early as the 16-cell stage. Given that miR-427 is essential for deadenylation of maternal mRNAs during the *Xenopus* MZT (Lund et al., 2009), it seems likely that the low level of transcription occurring prior to the MBT may be important to initiate and coordinate the turnover of maternal RNAs. Later on the degradation machinery is accelerated by the major ZGA, leading to a rapid clearance of maternal products during gastrulation (Fig. 4).

## 9. STABILITY OF GERMLINE RNAs AND PROTEINS

Like all other maternal RNAs, germ-plasm RNAs are degraded during embryonic development. Unfortunately, in *Xenopus*, it is largely unclear when germ-plasm RNAs are

degraded and how their turnover is regulated. So far, only a few studies have addressed these important questions. These studies suggest that germ-plasm RNAs are degraded through at least two different mechanisms. The first wave of germ-plasm RNA turnover occurs during gastrulation, and is most likely focused on the clearance of germ-plasm RNAs from the soma. Within PGCs, these maternal germ-plasm RNAs are protected and turnover is very slow. Little is known about the temporal control of germ-plasm RNAs in terms of translation and decay. However, based upon limited experimental evidence in the literature, we suggest a few general themes and highlight important questions that remain to be addressed.

The stability of germ-plasm RNAs must be regulated differently in the soma than in the germline as germ-plasm components can be detrimental to establishing somatic cell fates, but essential for PGC development. A good example is Nanos whose expression in the soma leads to abnormal development but its loss from germ plasm results in the failure of the germline while the soma is unaffected (Lai et al., 2012; Luo, Nerlick, An, & King, 2011). During early cleavage stages in zebrafish, nanos and other germ-plasm RNAs are also present in the soma and must be degraded there (Kedde et al., 2007). However, it is not clear whether that situation holds for *Xenopus*. The early history of germ-plasm RNAs is well established from whole mount *in situ* hybridization (WISH) analyses, but precise quantitation of these RNAs within germ plasm or within the ooplasm or soma has not been determined as this would require a separation of the two. For example, we know that in the oocyte, germ-plasm RNAs are either sequestered in the germinal granules or associated with the germ-plasm matrix. As described above, by the 8-cell stage, they coalesce into a few large aggregates that are inherited later by a few blastomeres, which consequently become PGCs. During these processes, germ-plasm RNAs are transported into the PGCs along with other components of the germ plasm. Once these processes are complete, highly concentrated germ-plasm RNAs, including *nanos1* (Forristall, Pondel, Chen, & King, 1995), *Xpat* (Hudson & Woodland, 1998), *Xdazl* (Houston, Zhang, Maines, Wasserman, & King, 1998), *DEADSouth* (MacArthur, Houston, Bubunenko, Mosquera, & King, 2000), and *dnd1* (Horvay, Claussen, Katzer, Landgrebe, & Pieler, 2006), can be detected in a few blastomeres by WISH. However, detection of highly concentrated germ-plasm RNAs in PGCs by WISH does not rule out the possibility that a certain amount of these RNAs fails to be transported into PGCs and is left in somatic cells. How efficient the process is at shuttling germline RNAs exclusively into PGCs is not known. Studies in *C. elegans* have revealed that, during segregation of the germline from soma, a certain amount of germ plasm is indeed left in the somatic cells and these RNAs are rapidly degraded (Zhang et al., 2009; Zhao, Tian, & Zhang, 2009). In *Xenopus* embryos, mechanisms capable of actively degrading germ-plasm RNAs in the soma have recently been identified (see below for details). Future work will have to establish in a more quantitative way the distribution and turnover of germ-plasm RNAs. Only then will it be possible to establish whether different mechanisms operate to control the stability of germ-plasm RNAs in the soma versus the germline.

Although only a few studies address how germ-plasm RNAs are degraded, the temporal and spatial expression patterns of many germ-plasm RNAs have been studied. RNA-seq analysis

of RNAs isolated at different stages from *Xenopus tropicalis*, offers some insights (Collart et al., 2014). In this study, polyadenylated and nonpolyadenylated RNAs were quantitated. If we look at germ-plasm RNAs with *VegT* as a comparison, germline RNAs appear polyadenylated soon after fertilization and remain stable through the MBT (Fig. 4A). A general picture that emerges from these studies is that germ-plasm RNAs are degraded dramatically during gastrulation. For example, degradation of *Xpat* (Hudson & Woodland, 1998), *Xdazl* (Houston et al., 1998), *DEADSouth* (MacArthur et al., 2000), *dnd1* (Horvay et al., 2006), and *nanos1* (Lai et al., 2011), was detected easily by Northern Blot or RT-PCR analysis of whole gastrulae RNA. Interestingly, persistent expression of these germ-plasm RNAs can be detected in PGCs by WISH even as late as the tadpole stages (Kataoka et al., 2006). The significance of this degradation event during gastrulation is not fully understood. In our opinion, it is critically important to clarify in which cell lineages this RNA turnover event occurs. If degradation of germ-plasm RNAs during gastrulation occurs mainly in PGCs, it would suggest that this turnover event is a critical step during the MZT within the germline. If it happens in somatic cells, it would suggest that this turnover event is more likely responsible for removal of germ-plasm components from the somatic tissues, which ensures proper segregation of the germline from soma. In principle, these two possibilities are not mutually exclusive.

Some recent studies have demonstrated that miR-427 and miR-18 play important roles in controlling the turnover of *DeadSouth* and *dnd1*, respectively. *Xenopus* DEADSouth, a homologue of mammalian DDX25, is a DEAD-box RNA helicase that is specifically localized in the germ plasm. Its function during PGC development can be substituted by VASA/ DDX4 (Yamaguchi, Taguchi, Watanabe, & Orii, 2013). The 3′UTR of *DEADSouth* mRNA contains three miR-427 targeting sequences (Yamaguchi, Kataoka, Watanabe, & Orii, 2014). When the 3′UTR of *DEADSouth* is fused to a fluorescent protein, Venus, the reporter construct undergoes rapid turnover during gastrulation. By the tadpole stage, the reporter RNA is degraded completely in somatic tissues and Venus can be detected only in PGCs (Kataoka et al., 2006). Mutation in these miR-427 targeting sequences or knockdown of miR-427 interferes with clearance of the reporter RNA in somatic cells (Yamaguchi et al., 2014). Interestingly, the expression of miR-427 could not be detected in isolated PGCs (Yamaguchi et al., 2014). This raises the intriguing possibility that the miR-427-dependent turnover, which begins during gastrulation, does not occur in the PGCs. It is solely responsible for removal of *DEADSouth* mRNA from the somatic tissues. Therefore, it is unlikely that miR-427-dependent turnover plays a role during the MZT within the germline. In the case of *dnd1*, a regulatory element within the 3′UTR, which regulates vegetal localization of *dnd1* in the oocyte, plays an essential role in somatic *dnd1* mRNA clearance. This *dnd1* mRNA turnover event is mediated by miR-18, which binds to the GCACUU(U) sequence in this regulatory element of the *dnd1* 3′UTR. Interfering with the binding of miR-18 to *dnd1* significantly increases the level of endogenous *dnd1 RNA* in somatic tissues at the tadpole stage (Koebernick, Loeber, Arthur, Tarbashevich, & Pieler, 2010). As expected, when the *dnd1* 3′UTR is fused to GFP, it is competent to direct PGC-specific GFP expression. However, the PGC-specific GFP expression can be observed only when this miR-18-dependent somatic clearance event is complete (Dzementsei, Schneider, Janshoff, & Pieler, 2013; Koebernick et al., 2010). Taken together, these two lines of evidence support

the idea that the dramatic decrease in the level of germ-plasm RNAs during gastrulation is a consequence of somatic RNA clearance.

The important question remains, how are maternal germ-plasm RNAs degraded within PGCs during germline development? Taking advantage of the *X. laevis* and *borealis* hybrid embryos, it was found that after the dramatic RNA degradation events during gastrulation, *nanos1* RNA, presumably located in PGCs, remained stable at least until the end of the tailbud stage. Zygotic transcription of *nanos1* does not occur in early-stage embryos (Lai et al., 2011). In the case of *DEADSouth*, maternal messages are removed nearly completely by the tailbud stage. Around the same time, embryos start to express zygotic *DEADSouth* (Yamaguchi et al., 2014). Other than these two RNAs, we do not know how other maternal germ-plasm RNAs are regulated during PGC development. Another issue is the half-life of the proteins encoded by germ-plasm RNAs. Nanos is present in germ plasm until PGCs leave the endoderm at Stage 39, well past when its RNA is detected. Xdazl protein has been detected even as late as tadpole Stage 52 and within germ cells of the developing gonads (Kataoka et al., 2006). Clearly, more analysis needs to be done to assess the gene-expression profiles within PGCs during germline development. Such information would lay the foundation to study how ZGA and removal of maternal germ-plasm RNAs are coordinated during germline development.

## 10. THE PGC LATE-GASTRULA TRANSITION

Germ plasm can be thought of as a mechanism to continue the potential for totipotency exhibited by the oocyte, forward into development. Germ plasm then, functions as an “escape hatch” for totipotency from the oocyte. Interestingly, some of the germ-plasm components that are passed on to PGCs as untranslated RNAs, are translated in the oocyte. These include the RNA-binding proteins Dead-end, DeadSouth, Xdazl, and RINGO/Spy. These proteins play a role in translational regulation or in regulating meiotic events. After maturation and fertilization, are these proteins degraded while being preserved as RNAs within the germ plasm? Is this one mechanism for carrying gametic identity forward through embryogenesis? What is the fate of oocyte proteins in the soma? We simply do not know the answers to these questions in *Xenopus*.

Segregation of the germline from the somatic endoderm occurs at gastrulation when the germ plasm moves to a perinuclear location; subsequent divisions result in both daughter cells receiving germ plasm. Before complete separation from the soma, PGCs are presumptive PGCs or pPGCs. Only after separation and during gastrulation do PGCs initiate their zygotic transcription program, or the PGC Late-Gastrula Transition (Fig. 3). Which maternal factor(s) activate the PGC program remains unknown. Importantly, *Xenopus* Oct 25, Oct 60, and Oct 91 are in the same POU subclass as the human pluripotency factor, Oct 3/4 (Hinkley et al., 1992), and Oct 3/4 acts as a functional homolog for Oct 25 or Oct 60 in rescue experiments (Cao et al., 2006). Maternal Oct 60 is expressed in isolated pPGCs prior to gastrulation and Oct 91 is expressed *de novo* in PGCs after they segregate from the endoderm (Hinkley et al., 1992; Venkatarama et al., 2010). The RNA-seq results from *X. tropicalis* for the Oct and Sox pluripotent factors show they generally decline after the MBT

except for Oct 91, which is newly transcribed in PGCs (Fig. 4B; Collart et al., 2014; Tan et al., 2013).

*Xenopus* Oct 60 plays a key role in maintaining pluripotency by suppressing signaling in differentiation pathways including Activin/Nodal, BMP, and WNT (Cao et al., 2004, 2006, 2008; Whitfield et al., 1993). Oct 25 is not expressed in PGCs, while Oct 60 expression in pPGCs persists until gastrulation. Importantly, zygotic Oct 91 is subsequently expressed *de novo* in PGCs (Venkatarama et al., 2010). Oct 60 expression and function have primarily been investigated in somatic cells and its role in PGCs remains unknown. The maternally expressed transcription factor, Sox7, localizes to the vegetal pole and may have a role in PGC fate in addition to its known role in somatic cells (Zhang, Basta, Fawcett, & Klymkowsky, 2005; Zhang, Basta, & Klymkowsky, 2005). Interestingly, maternal expression of Oct 60 depends on an Octamer-Sox-binding motif in its promoter region and Oct 60 has been shown to bind this motif (Morichika, Sugimoto, Yasuda, & Kinoshita, 2014). Perhaps, Oct 60 and Sox proteins promote PGC transcription during the PCG-LGT. Consistent with this proposal, TRANSFAC promoter analysis identifies putative Oct and Sox binding motifs within the Oct 91 promoter region.

What gene programs are likely to be initiated at the PGC-LGT? Beyond keeping competency for totipotency, a zygotic differentiation program must be activated that confers the ability to migrate. Directed migration is one characteristic that PGCs acquire, which is missing from somatic endoderm. Because PGCs originate outside the gonads within embryonic endoderm, PGCs must migrate through somatic tissues and reach the developing gonads. Once inside the presumptive gonad, the PGCs divide, enter meiosis, and differentiate into definitive gametes.

Mutational analysis has identified three different signaling systems that affect PGC migration: CXCR4/SDF1, Notch/Delta2 (Morichika et al., 2010), and KIF13B-PIP3-GRIP2.1. PGCs begin their migration out of the endoderm and toward the presumptive gonads as early as tailbud (Stage 24), 10–12 h after PGC transcription begins (Terayama et al., 2013; Venkatarama et al., 2010). At this time, they are found clustered at the midline in the posterior endoderm. Early midline clustering is likely a passive step as CXCR4 is not expressed (RNA or protein) at this stage but it is during lateral and dorsal movements (Nishiumi, Komiya, & Ikenishi, 2005). Six hours later, PGCs are found spread more laterally and express CXCR4 on their surface (Takeuchi, Tanigawa, Minamide, Ikenishi, & Komiya, 2010) while its ligand, SDF-1 is expressed in the dorsal mesentery (Braun et al., 2002). Also around this time, a decreased attachment to ECM components like fibronectin, reduced adhesion to somatic endodermal cells, and downregulation of E-cadherin occurs in isolated PGCs but not in endodermal cells (Dzementsei et al., 2013).

The cellular movements that underpin these migration steps—blebbing and persistent blebbing at the leading edge—and which are required for directional migration, all occur in isolated PGCs cultured in a serum-free medium with a fibronectin substrate. As expected, these cellular behaviors require F-actin, myosin II activity, and RhoA/Rho-associated protein kinase (ROCK) signaling. ROCK was identified as an early zygotic transcript in PGCs (Venkatarama et al., 2010). Antisense depletion of either maternal *Xdazl* or *Dead-end*

RNA prevents this step from occurring and PGCs are lost soon afterward (Horvay et al., 2006; Houston & King, 2000). Both of these RNA-binding proteins play a role in regulating translation, suggesting that maternal control of translation may extend to zygotically synthesized transcripts in the PGCs.

Finally, PGCs will give rise to the gametes and only they are competent to execute the reductional divisions during meiosis. How is this ability carried forward in PGCs through the MZT, but not in the soma? What maternal and/or zygotic factors are required to maintain competence for meiosis? These are questions that remain to be answered.

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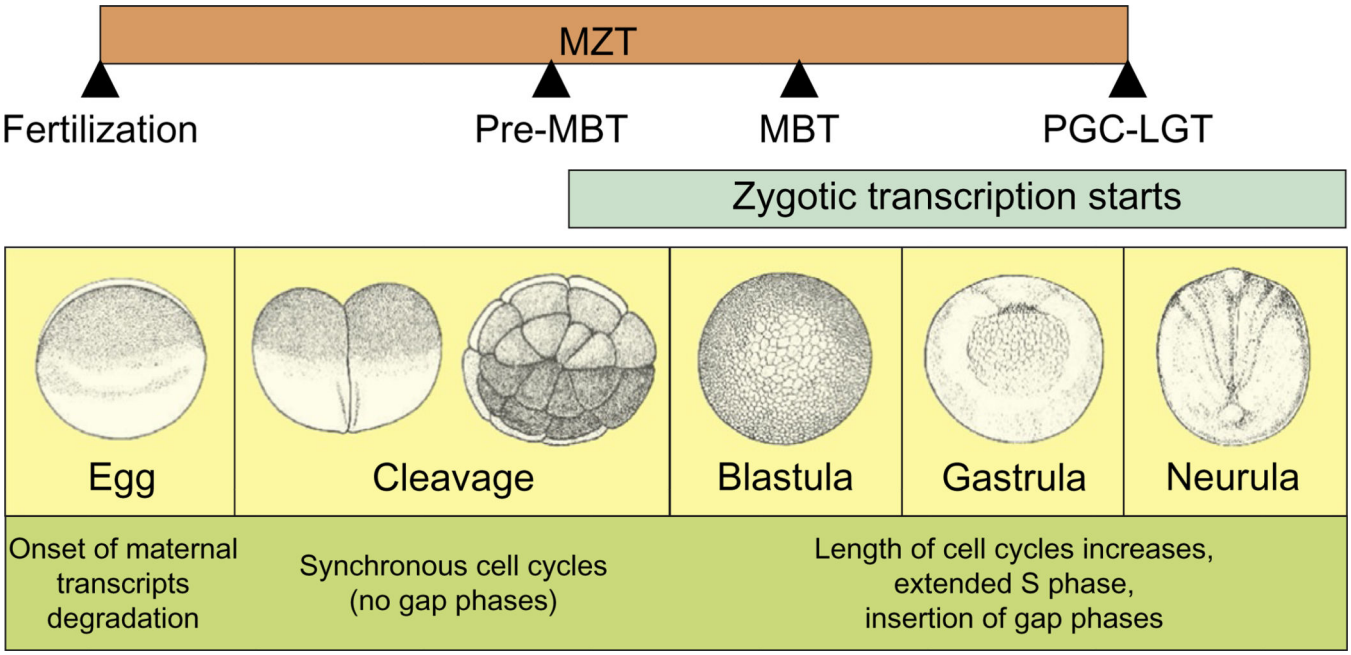


**Figure 1.**

The maternal-to-zygotic transition (MZT). MZT is a period that occurs very early during embryonic development, when degradation of maternal transcripts is initiated and is complete with the first morphological change caused by zygotic transcription, gastrulation. After the 12 mitotic divisions, the synchronous cell cycles are followed by asynchronous cleavages, with the introduction of gap phases during a developmental event called the "mid-blastula transition" (MBT). Most zygotic genes are silent until large-scale zygotic gene activation (ZGA) that, in *Xenopus*, coincides with the MBT. Several genes required for embryo patterning are transcribed before the MBT (pre-MBT) starting at the 32-cell stage. PGC transcription begins later, during the transition between gastrula and neurula (*p*rimordial germ cell *l*ate-gastrula transition, PGC-LGT). Images of *Xenopus* embryos are shown to highlight the different developmental stages.

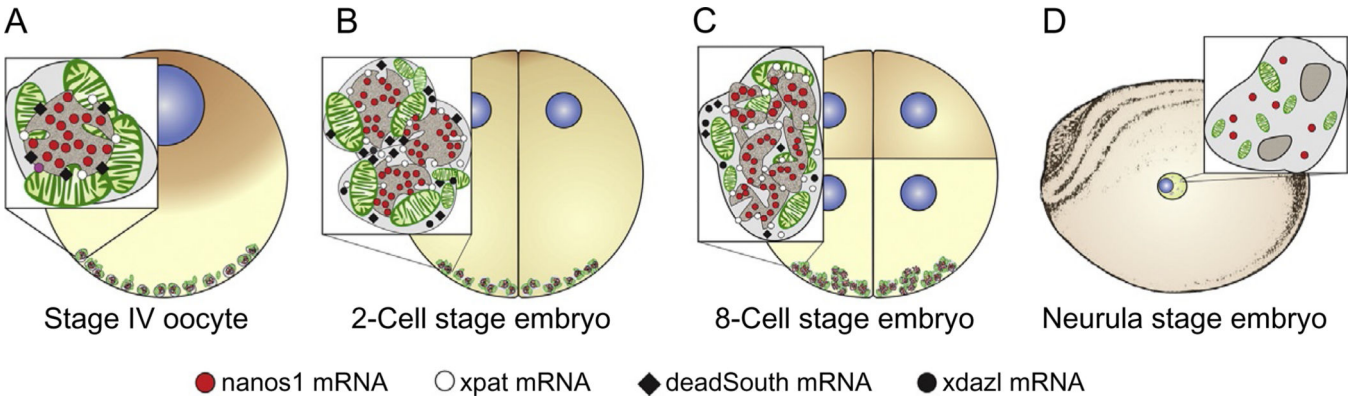


**Figure 2.**

Schemes of germ-plasm structure during *Xenopus laevis* embryogenesis. At oocyte Stage VI, germ-plasm islands are associated with the vegetal cortex (A). Between fertilization and the 8-cell stage, germinal granules (GGs) undergo ultrastructural changes thought to represent a permissive state for translation. After fertilization, the germ plasm contains a large number of small (250 nm) spherical GGs (B). During early cleavage, GGs coalesce into progressively more complex aggregates at the apex of vegetal pole blastomeres (C). Germ plasm at the 8-cell stage contains a few irregularly shaped large GG(about 2000 nm) that, in cross section, show complex and variable morphology (C). Between the gastrula and neurula stages, large GGs disappear and primordial germ cells from the neurula stage contain only a few small (300 nm) GGs (D).

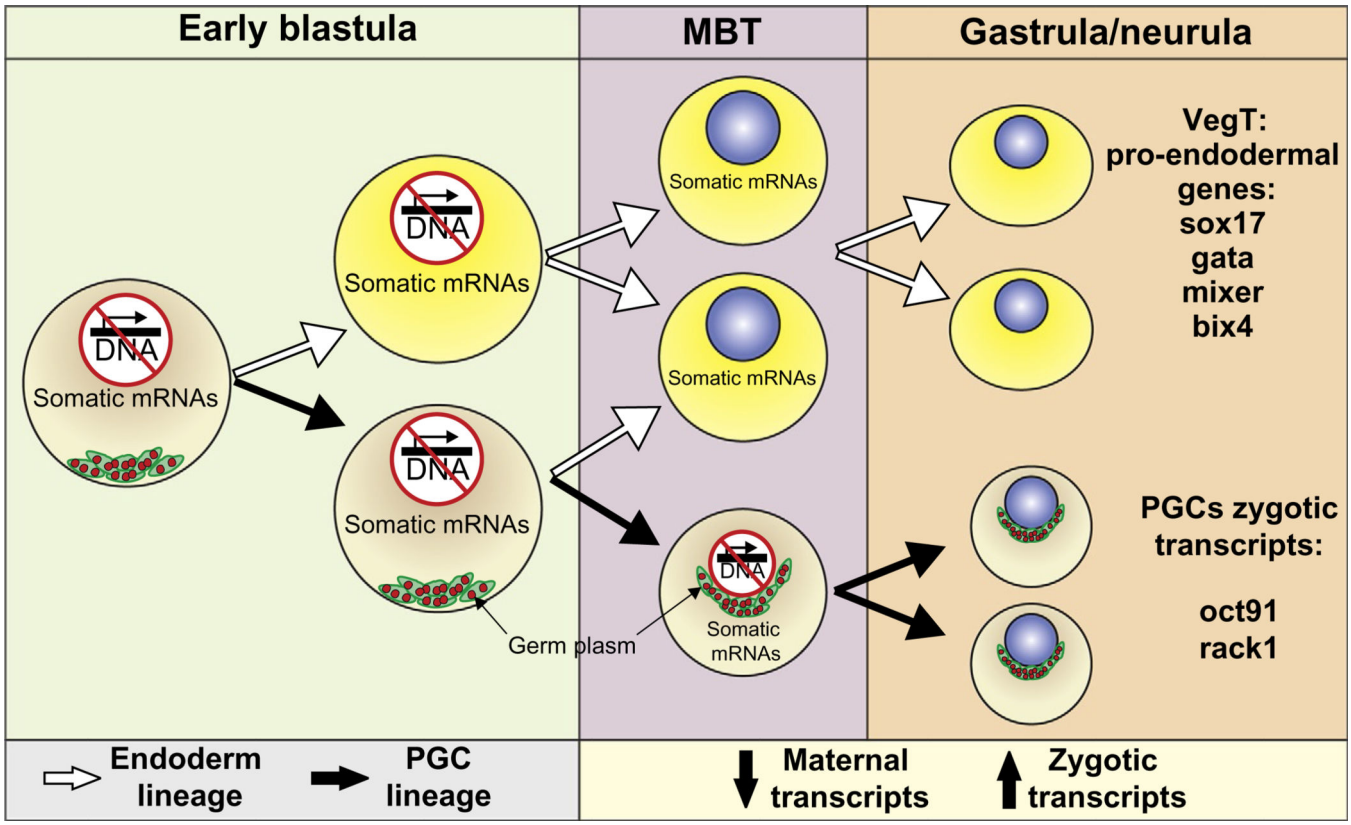


**Figure 3.**

Scheme of primordial germ cell (PGC) segregation from soma. In *Xenopus*, the germline is specified through the inheritance of germ plasm formed during oogenesis and asymmetrically segregated into the future germ-cell lineage (black arrows). Endodermal somatic fate is established by the inheritance of maternal somatic transcripts, including VegT (white arrows). Germ cells repress somatic maternal mRNAs. PGCs delay starting mRNA transcription to ensure that somatic differentiation programs remain inactive at the time that zygotic transcription is initiated in the rest of the embryo.

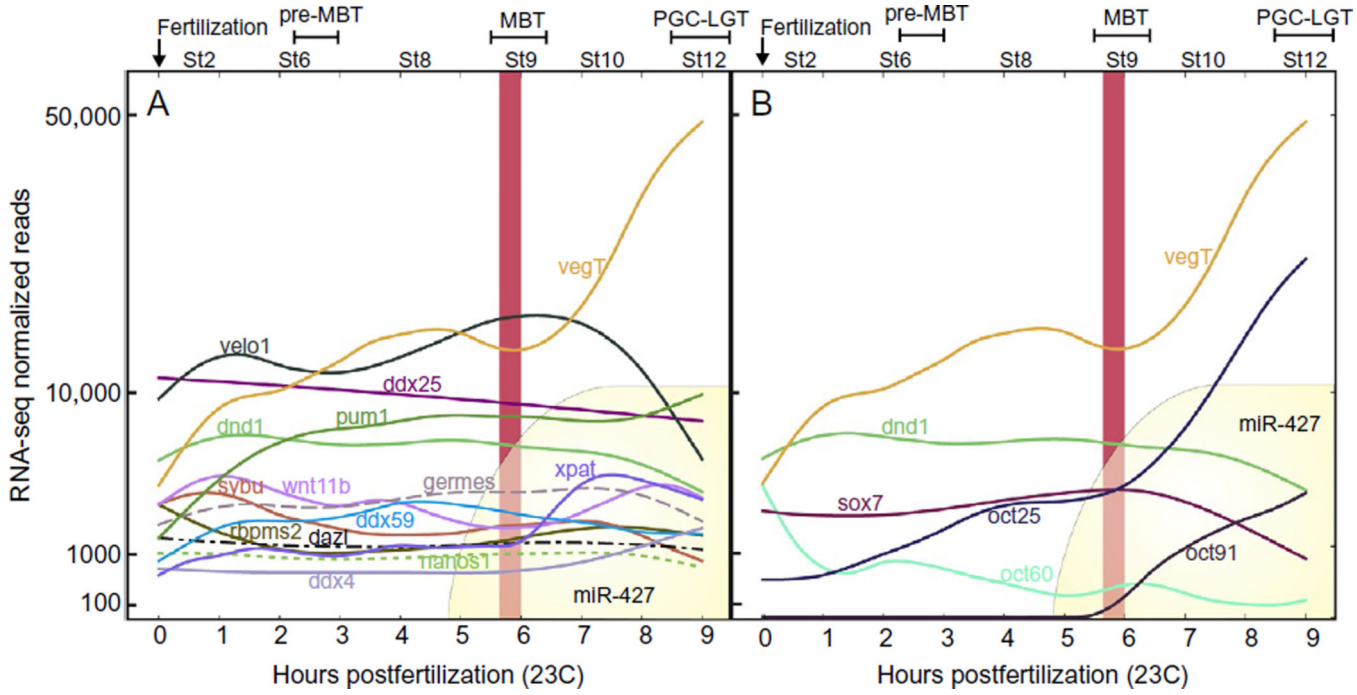


**Figure 4.**

Transcriptional profiling in *Xenopus* early development. Representation of gene-expression patterns of (A) PGC-related genes and (B) pluripotency factors between fertilization and the onset of gastrulation. Before the MBT, positive/negative changes in reads are because of polyadenylation and deadenylation rather than degradation while, after the MBT, miR-427-dependent degradation occurs (Collart et al., 2014). Polyadenylation of maternally deposited transcripts occurs immediately after fertilization and is required for zygotic transcription. Two waves of zygotic genome activation occur: At the 32/64-cell stage (pre-MBT), ~150 new transcripts are synthesized while the bulk of transcription occurs hours later, at the MBT (Tan et al., 2013). The maternal determinant *VegT* is degraded in PGCs while it is polyadenlyated and translated in the soma and zygotically transcribed at the MBT (included in both graphics as a reference). miR-427 is essential for deadenylation of maternal mRNAs during the MZT (yellow curves in A and B). (A) Germline RNAs appear to be polyadenylated soon after fertilization and remain stable through the MBT. (B) Pluripotency factors generally decline after the MBT except for Oct 91, which is newly transcribed in the PGCs. *Modified from* Collart et al. (2014).

## Table 1

Candidate RNAs for Pumilio/Nanos Repression

| PGC Transcripts | Canonical PBE: UGUANAUA | Noncanonical PBE: UGUANAUA[a] | Function |
|---|---|---|---|
| ddx25 (DeadSouth) | 0 | 2 | RNA helicase |
| Cdk9A | 3 | 3 | CTD-kinase, RNA synthesis |
| Cyclin B1[b] | 1 | 0 | Cell cycle regulator |
| Vg1 | 1 | 2 | Growth factor, endoderm patterning, mesoderm induction |
| otx1 | 1 | 4 | Transcription factor |
| pumilio1 | 1 | 7 | Translation |
| rbpms2 (hermes) | 0 | 2 | Translation |
| sox7 | 2 | 5 | Transcription factor |
| VegT[b] | 1 | 2 | Transcription factor, endoderm determinant |
| sybu | 0 | 5 | D/V axis |
| trim36 | 1 | 1 | D/V axis |
| wnt11b[b] | 1 | 5 | D/V axis |
| xdazl | 0 | 4 | Translation (activator) |
| xpat | 0 | 2 | Germ-plasm development |
| xvelo | 2 | 2 | Cell polarity |

[a] Noncanonical PBE: One change occurs in the last three nucleotides (AUA), most frequently AAA or AUG.

[b] Validated *in vivo* as repressed by Pumilio/Nanos or Pumilio.