Critical Reviews in Biochemistry and Molecular Biology, 43:135–162, 2008

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230801921338

informa
healthcare

Regulation of Maternal mRNAs in Early Development

Brian M. Farley and Sean P. Ryder
Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

Most sexually reproducing metazoans are anisogamous, meaning that the two gametes that combine during fertilization differ greatly in size. By convention, the larger gametes are considered female and are called ova, while the smaller gametes are male and are called sperm. In most cases, both gametes contribute similarly to the chromosomal content of the new organism. In contrast, the maternal gamete contributes nearly all of the cytoplasm. This cytoplasmic contribution is crucial to patterning early development; it contains the maternal proteins and transcripts that guide the early steps of development prior to the activation of zygotic transcription. This review compares and contrasts early development in common laboratory model organisms in order to highlight the similarities and differences in the regulation of maternal factors. We will focus on the production and reversible silencing of maternal mRNAs during oogenesis, their asymmetric activation after fertilization, and their subsequent clearance at the midblastula transition. Where possible, insights from mechanistic studies are presented.

Keywords embryogenesis, oogenesis, translational regulation, RNA-binding protein, RNA localization

1. INTRODUCTION

In the latter part of the 19th century, comparative embryology was a relatively young and vibrant field. Early embryologists, through a series of painstaking observations made using limiting and indefinite samples, described the basic steps that comprise the development of a single fertilized cell into a complex multicellular organism. The first comprehensive comparison of embryogenesis across the animal kingdom was published in a seminal two-volume series titled "A Treatise on Comparative Embryology" by Francis Balfour (Balfour, 1880; Balfour, 1881). In this work, Balfour declared that two major questions face comparative embryologists: (1) What is the origin and homology of germinal layers, tissues, and organs between species, and (2) How did the complicated organs of vertebrates evolve from their counterparts in simpler animals? A major goal of the early embryologists was to identify developmental themes that could unify the largely disparate observations made between species, mainly in an effort to reconcile their findings with Darwin's recently published principle of natural selection (Darwin, 1859). Now, nearly 130 years later, modern embryologists are addressing the same questions, bringing to bear the tools of molecular biology and genetics, the ability to generate transgenic organisms, and new approaches that enable genome-wide analysis of gene expression patterns. Similarities in the molecular processes underlying embryogenesis are strongly suggestive of origins through a common ancestor, but as yet do not explain the vast differences in morphology between metazoan species.

One theme that emerges from the study of embryogenesis is that post-transcriptional regulation of maternal mRNAs is critical to the developing zygote. As oocytes develop, the chromosomal content of the egg becomes locked in meiosis until the time of fertilization, precluding transcription of the mRNAs inherited by the new organism. Maternal transcripts are produced and reversibly silenced in the earlier stages of oogenesis, in some organisms requiring the support of "nurse" cells to provide mRNA to the maturing oocyte. Moreover, the embryos of most animals do not transcribe their DNA until the zygote has divided one or more times. In most cases, zygotic transcription does not begin until several cell divisions have occurred, after a number of patterning and cell fate specification events have taken place. Thus, activation of maternal transcripts by maternal regulatory factors provides the starting point for formation of the body plan.

In this review, we explore early development in common laboratory model organisms including *Drosophila melanogaster* (fruit fly), *Caenorhabditis elegans* (nematode worm), *Xenopus laevis* (frog), *Danio rerio* (zebrafish), and *Mus musculus*

Address correspondence to Sean P. Ryder, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605, USA. E-mail. Sean.Ryder@umassmed.edu

(mouse). Recent advances and mechanistic aspects of post-transcriptional regulation of maternal mRNAs are the focus of this work, beginning with gametogenesis and ending with the mid-blastula transition (MBT) when zygotic transcription takes over. This comparison not only reveals a number of similarities in the regulation of embryonic patterning, especially in germline specification, but also identifies several intriguing differences that provide a platform for speculation about the molecular basis of morphological heterogeneity. According to Balfour, the science of comparative embryology is "intimately bound up with comparative anatomy; without which it indeed becomes quite meaningless" (Balfour, 1880). Therefore, we begin by describing the anatomy of oocyte formation and early embryogenesis in each of these species.

2. EARLY DEVELOPMENT IN METAZOAN MODELS

At an anatomical level, the process of embryogenesis in laboratory model organisms appears to be incredibly divergent. Basic decisions in development are made at different times, and alternative strategies are used to coordinate formation of the body axes and to specify cell fate. For example, in some species, body axes are established prior to fertilization during oogenesis, while in others, the axes are defined by the penetration point of the spermatocyte. Moreover, some species proceed through the first rounds of division by duplicating nuclei without cytokinesis, leading to the formation of a syncytium where several nuclei share the same cytoplasm. This enables the establishment of morphogen gradients that translate the body axes into subsequent patterning of tissues and organs. Other species undergo incomplete cell division, where cytokinesis begins but does not finish, leading to a region of shared cytoplasm. Still others complete cytokinesis along with nuclear division, and as such must rely on inductive signaling to transduce the body axes to cell fate specification. The relationship of these development strategies transcends phylogenetic boundaries, as the species that make use of similar strategies are not always closely related to one another, suggesting that basic developmental processes have evolved multiple times over the course of evolution. Intriguingly, molecular analysis reveals a surprising degree of conservation in the pathways that regulate development, suggesting that even though early development is anatomically diverse, the gene regulatory networks are conserved.

D. melanogaster Early Development

D. melanogaster (fruit fly) is a gonochoristic species, meaning that there are two distinct sexes. Female flies produce oocytes from a niche of primordial germ cells located in the germarium at the tip of each ovariole (Figure 1A). As oocytes develop, they move through the ovariole into the ovary, where they await fertilization. At any given time, an ovariole contains a series of egg chambers that are progressively older as they become more distant from the germarium.

Each *Drosophila* primordial germ cell undergoes a series of organized cell divisions to produce a group of 16 cells that share cytoplasm via ring canals (reviewed in Mazumdar and Mazumdar, 2002). By the end of these divisions, only 2 cells have

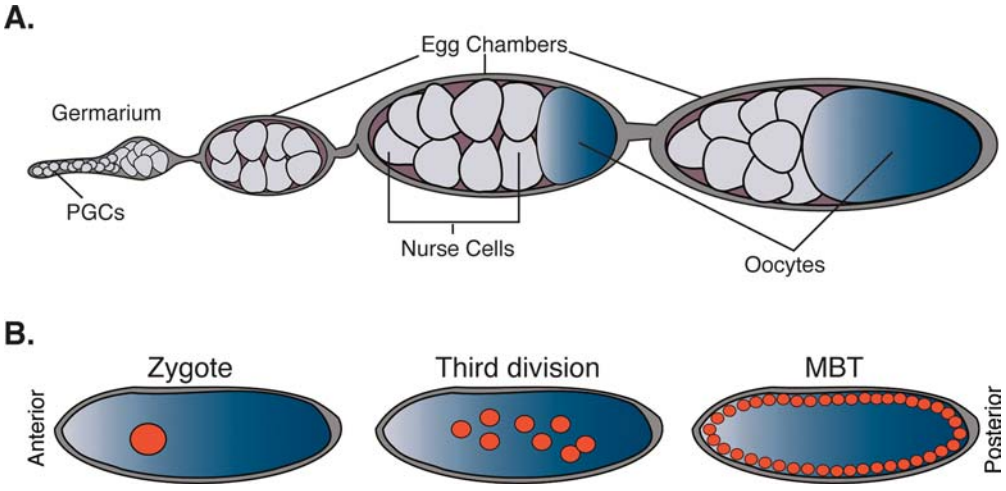

FIG. 1. (**A**) Schematic representation of *Drosophila* ovariole. Each ovariole contains a population of developing oocytes that age as they approach the ovary. Farthest away from the ovary is the germarium, which contains a population of primordial germ cells (PGC). Each PGC undergoes a precise pattern of divisions to produce an ordered cluster of cells. One of these cells (*blue*) becomes the oocyte, while the others become nurse cells. These cells supply mRNAs to the transcriptionally silent oocyte as it develops. (**B**) Schematic representation of *Drosophila* embryogenesis. After fertilization, the embryo (*blue*) undergoes a series of incomplete mitotic divisions where the nuclei (*red*) divide, but cytokinesis does not occur. This produces a syncytial embryo where the nuclei have a shared cytoplasm. As embryogenesis proceeds, the nuclei migrate to the periphery of the embryo, where they recellularize during the mid-blastula transition.

connections to all 4 of the cells that surround them. One of these cells becomes the oocyte, while the rest become nurse cells that support the oocyte. The nurse cells retain their cytoplasmic connection to the oocyte, which is transcriptionally dormant. All of the mRNA present in the oocyte is transcribed by the nurse cells and is transported through these channels. Transport depends on an ordered array of microtubules, the actin cytoskeleton, and bulk cytoplasmic streaming. Microtubule-dependent transport gives rise to a gradient of maternal mRNAs and proteins that establishes both the anterior-posterior and dorsal-ventral axes of the oocyte (reviewed in Bashirullah *et al*., 1999; Johnstone and Lasko, 2001). While the nurse cells are transporting maternal factors into the oocyte, it accumulates yolk protein from the hemolymph of the mother and rapidly grows in size. Late in oogenesis, the oocyte is surrounded in a layer of somatic cells called follicle cells. These cells secrete both the vitelline membrane and eggshell that surround the oocyte, and form a micropylar canal that permits sperm entry. Just prior to oocyte maturation, the nurse cells dump their cytoplasm into the oocyte and disappear, producing an oocyte ready for fertilization.

After fertilization, the embryo begins a series of rapid synchronous nuclear divisions without cytokinesis, resulting in a syncytial blastoderm (Figure 1B). At the ninth round of division, the nuclei migrate to the periphery of the embryo, and by the end of the fourteenth round, cellularization occurs around each nucleus. Transcription first initiates at the eleventh round, and increases rapidly until the thirteenth round, when the midblastula transition (MBT) takes place (Foe and Alberts, 1983; Edgar and Schubinger, 1986). At this time, the length of the cell cycle increases, and the cells begin to divide asynchronously.

C. elegans Early Development

C. elegans is a free-living soil nematode with two genders, self-fertile hermaphrodites and rare males. In contrast to *Drosophila*, both male and female gametes are produced in the same gonad from the same germline. In hermaphrodite worms, gametogenesis begins during the final larval stage, during which primordial germ cells differentiate into sperm. Between the transition from larvae to adult, sperm production ceases and oocyte production begins. All gametes derive from the same population of mitotically dividing primordial germ cells, which are located in the tip of the gonad farthest away from the vulva (Figure 2A). As these cells enter into meiosis, their plasma membranes break down, forming a syncytium. The germ cell nuclei arrest in diakinesis of meiosis I, and migrate to the walls of the gonad, forming a shared cytoplasmic cylinder ringed by nuclei.

As these nuclei proceed through the gonad, they recellularize to form spermatocytes during the L4 larval stage, then forming symmetrical oocytes after the transition into adulthood. The newly formed oocytes receive maturation cues as they approach the uterus, then pass through the spermatheca, an organ that contains the previously produced spermatocytes and/or spermatocytes introduced by copulation with males. Once fertilized, the oocyte completes meiosis and rapidly establishes an anterior-posterior body axis, with the point of sperm entry determining the posterior pole of the embryo (Goldstein and Herd, 1996; Wallenfang and Seydoux, 2000) (Figure 2B). The first embryonic cell division occurs along this axis, giving rise to a larger anterior blastomere, and a smaller posterior blastomere (reviewed by Hubbard and Greenstein, 2005).

The larger anterior blastomere is the first of six founder cells that form during the first cell divisions. Some founder cells have their fates specified autonomously, while the others require an inductive signal from a nearby cell. Each founder cell is committed to differentiating into a limited subset of tissue types prior to gastrulation (Figure 2C). In contrast, the smaller posterior daughter is the progenitor of the entire germ line (termed the P lineage). This pattern of division is repeated three more times, each round forming a larger anterior daughter and a smaller posterior daughter. In each of these divisions, the anterior daughter is a founder cell, while the posterior daughter retains the cytoplasmic determinants of germ cell fate, and thus remains committed to the germ lineage. After these rounds of asymmetric division, the germline blastomere undergoes one final symmetric division to produce two primordial germ cells, which remain transcriptionally quiescent until larval development. This is in contrast to somatic blastomeres, which initiate transcription as soon as the four-cell stage (Seydoux *et al*., 1996; Batchelder *et al*., 1999). Cells do not grow during early embryogenesis, so the volume of the embryo stays constant.

There are a number of key differences in early development between *C. elegans* and *Drosophila*. Oocytes in *C. elegans* are symmetrical and the body axes are not established until after fertilization, where their establishment is triggered by the sperm entry point. Embryonic divisions comprise both the nucleus and the cytoplasm, defining individual cells rather than a syncytium. Finally, transcription initiates much earlier in *C. elegans* embryos than it does in *Drosophila*.

X. laevis Early Development

A convenient model organism that has been extensively used to study vertebrate development is the African clawed frog *X. laevis*. Oocytes are large and easy to observe, and significant quantities can be obtained through routine surgery. The ovaries of *X. laevis* differ dramatically from those of mammals (Figure 3). Relative to the size of the organism, frog ovaries are much larger than those of mice or humans, and each ovary contains many more developing oocytes. These oocytes develop over a period of 3 years. In contrast to flies and worms, frog oocyte maturation does not occur in "assembly-line" fashion. Instead, maturation of oocytes appears to be a stochastic process. The oocytes derive from a population of mitotically dividing primordial germ cells (PGCs) that arrest in prophase of meiosis I. Each oocyte becomes ensheathed in follicle cells that excrete a vitelline membrane around the oocyte. During meiotic arrest, the developing oocyte is actively undergoing transcription in preparation for the rapid series of cellular divisions that occur after fertilization (Mitchell and

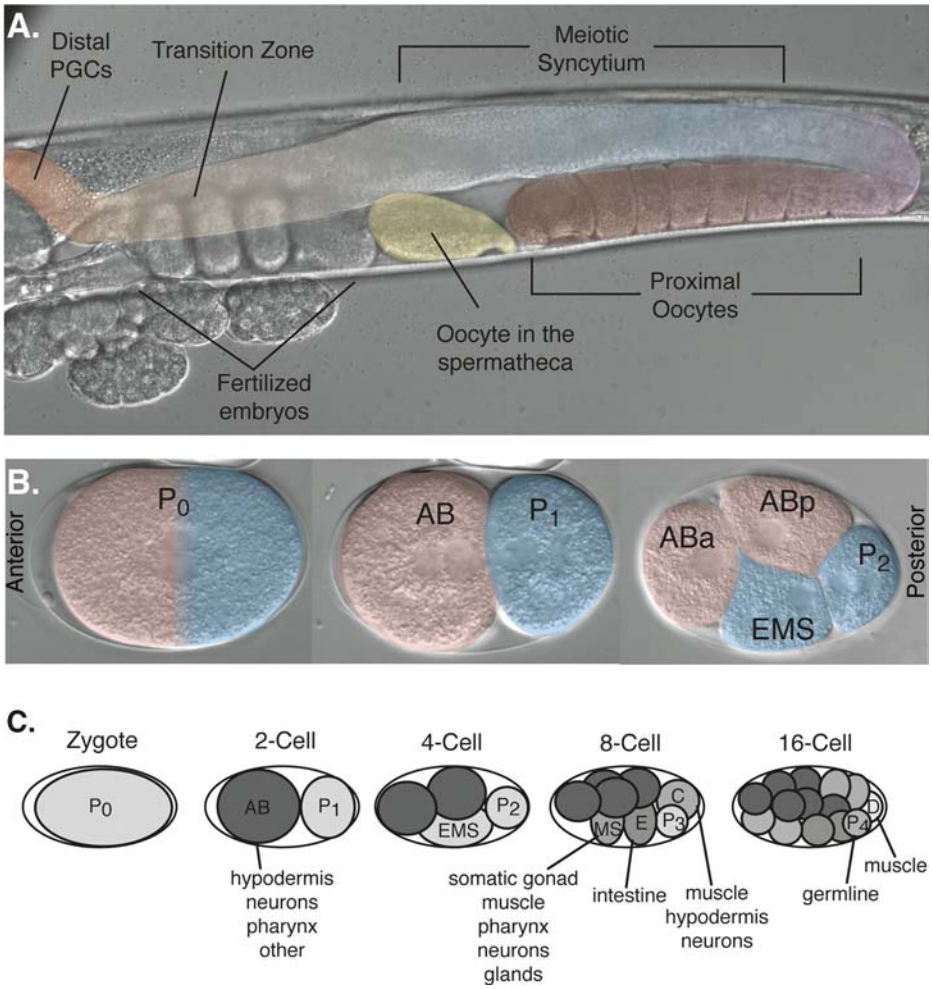

FIG. 2. (**A**) False-color image of *C. elegans* germline. Oogenesis begins at the distal tip of the gonad (*red*) where a mitotically dividing population of primordial germ cells resides. These cells transition into meiosis (*tan*), and the plasma membrane of each cell disappears, forming a syncytium of meiotically arrested nuclei (*blue*). These nuclei recellularize (pink), generating immature oocytes. The oocytes remain arrested in meiosis I until they approach the spermatheca (*yellow*), at which point they complete both meiotic divisions and enter the spermatheca, where fertilization occurs. Fertilized embryos emerge from the spermatheca and are retained in the uterus of the mother for multiple cell divisions. (**B**) False-color image of early *C. elegans* embryos. After fertilization, the male and female pronuclei fuse and migrate toward the posterior pole, demarcating the anterior (*pink*) and posterior (*blue*) halves of the embryo. The first cell (*P*0) divides asymmetrically to produce a larger anterior daughter (*AB*), and a smaller posterior daughter (*P*1). In turn, AB divides to produce two identical daughters (*ABa* and *ABp*), while P1 divides in a similar fashion to P0, producing a larger anterior daughter (*EMS*), and a smaller posterior daughter (P2). (**C**) Founder cells of the early *C. elegans* embryo. Each division of the P lineage produces a larger anterior daughter called a founder cell. Each of these cells is committed to producing a limited number of tissue types early in embryogenesis, as labeled.

Hill, 1986). In addition, the oocyte replicates its mitochondria many times, generating a dense region of mitochondria called the mitochondrial cloud, or Balbiani body (Billett and Adam, 1976).

Developing oocytes pass through six stages. Stage I oocytes are small and transparent, and the Balbiani body is clearly visible. Stage II oocytes are slightly larger, and are unpigmented and opaque. During the third year of oogenesis, the oocyte first becomes pigmented (Stage III), and then begins accumulating yolk proteins from the bloodstream of the mother and rapidly grows in size (Stage IV). During this time, a number of asymmetries arise in the oocyte. The nucleus migrates to one pole of the oocyte, called the animal pole, while most of the yolk proteins migrate to the opposite pole, called the vegetal pole (Figure 4A). In addition, the oocyte becomes asymmetrically pigmented, with the animal pole becoming darker than the vegetal pole. During

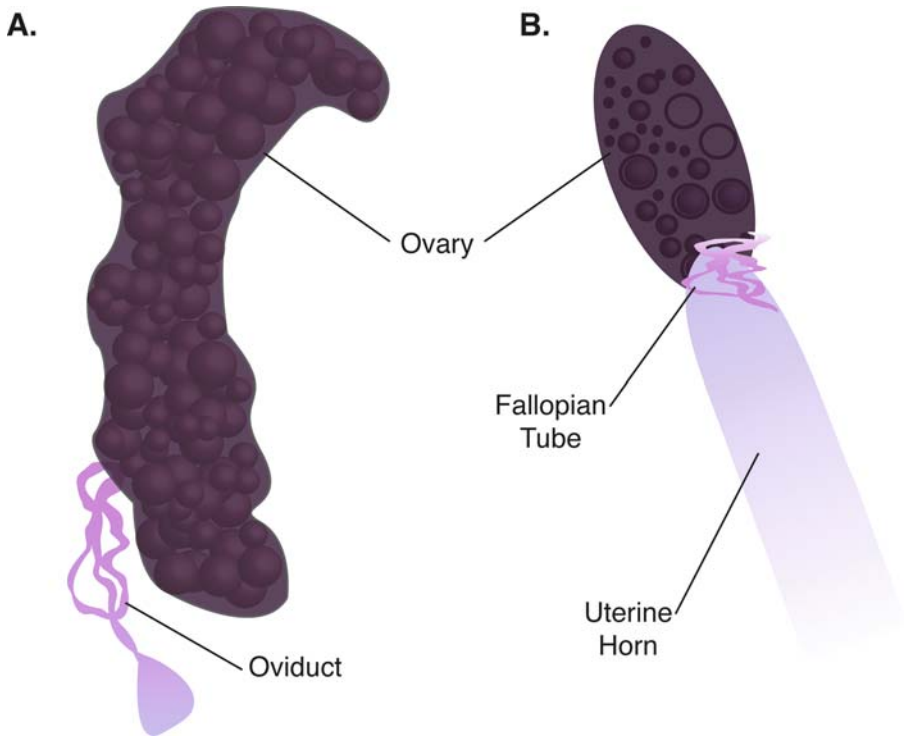

FIG. 3. Comparison of the ovaries of *X. laevis* and *M. musculus* (**A**) Xenopus female reproductive system. The ovary is shown in dark purple. Oocytes in various stages of development can be found throughout the ovary. Mature oocytes are released from the ovary and move through the oviduct to the cloaca. (**B**) Female mouse reproductive system. The mouse ovary is much smaller than the frog ovary. As with frogs, oocytes in several stages of development are present throughout the ovary. Oocytes are released from follicular cysts into the fallopian tube, where they are fertilized. Embryos then pass into the uterine horn, where they implant.

stage V of oogenesis, yolk accumulation begins to slow down, and the difference in pigmentation between the two poles of the embryo becomes more pronounced. Stage VI oocytes have ceased accumulating yolk, and are sensitive to the hormone progesterone, which triggers oocyte maturation (Smith *et al*., 1968). This causes the nuclear envelope to break down, and permits the oocyte to progress to metaphase of meiosis II, where it again arrests.

Mature oocytes are radially symmetrical, but this symmetry changes dramatically after fertilization. The outer, microfilament-rich layer of the embryo, called the cortex, rotates so that the vegetal pole of the cortex is opposite the point of sperm entry. This establishes the dorsal-ventral axis of the embryo. After cortical rotation, a series of rapid, synchronous divisions begin that continue until there are approximately 4000 cells in the embryo. During these divisions, the volume of the embryo does not increase, and each cell cycle lacks the associated G phases. At the 4000-cell stage, the mid-blastula transition occurs, the cell divisions become asynchronous and zygotic transcription begins.

D. rerio Early Development

D. rerio (zebrafish) is a second convenient vertebrate model organism. Like worms, the embryos are transparent, allowing for easy visualization of developmental processes such as organ formation and angiogenesis. Oogenesis occurs in the ovary of adult females (described in Selman *et al*., 1993). Immature oocytes are located within a group of primary oogonia. In early oogenesis, they are surrounded by a layer of somatic follicle cells. Immature oocytes proceed through pachytene of meiosis prophase I, at which point they arrest. Arrested oocytes begin accumulating cortical granules and yolk, and it is at this stage where the animal-vegetal pole of the oocyte manifests. The germinal vesicle migrates toward the animal pole, and follicular cells near this pole specialize to generate the micropylar canal, through which fertilization takes place. At the same time, numerous RNAs localize to both poles and to the cortex of the oocyte (Howley and Ho, 2000). Upon hormone induced maturation, the oocyte completes meiosis I and arrests in metaphase of meiosis II. At this stage, it is ovulated into the ovarian canal, where the change in environment triggers activation.

Fertilization occurs when a sperm enters the egg through the micropylar canal at the animal pole (Wolenksy and Hart, 1987; reviewed in Kimmel *et al*., 1995). Like *Xenopus* oocytes, zebrafish oocytes are radially symmetric about the animal-vegetal axis (Figure 4B). Unlike *Xenopus* oocytes, the point of sperm entry does not directly induce asymmetry, as the point of sperm entry is predetermined at the animal pole. Once the egg is fertilized,

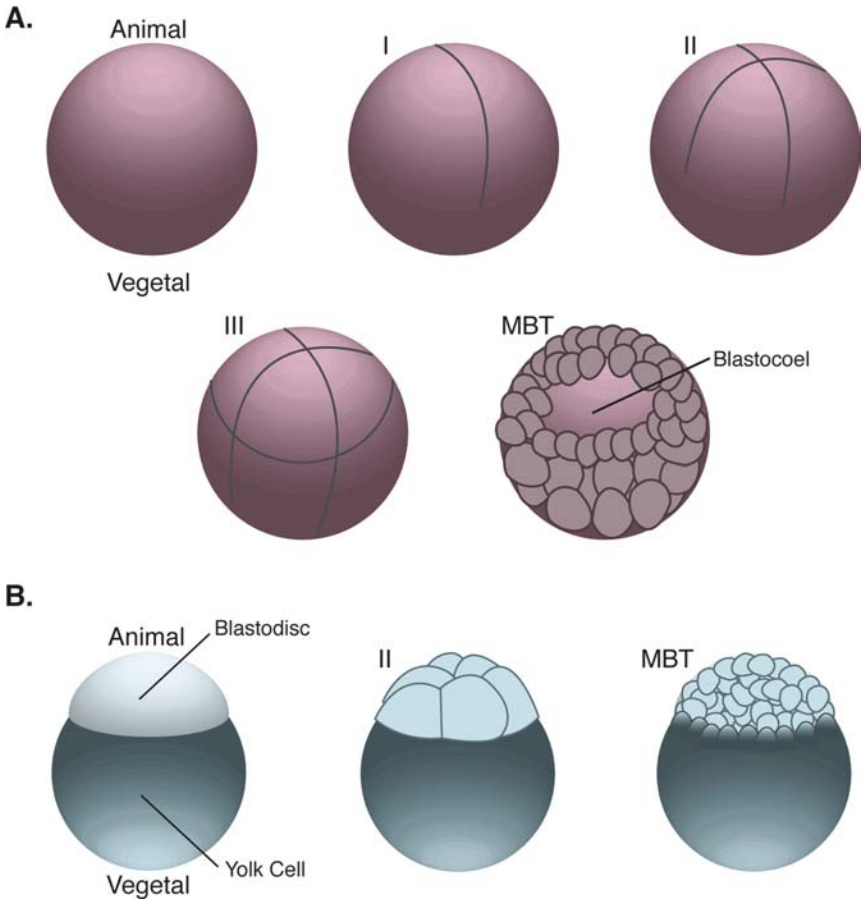

FIG. 4. (**A**) Early*Xenopus* embryogenesis. After fertilization, the embryo divides along the longitudinal axis to produce two indistinguishable daughters. This division begins in the animal pole of the embryo and continues through the vegetal pole. Prior to the completion of the first division, the second longitudinal division occurs on a plane perpendicular to the first. The embryo continues dividing synchronously, alternating between meridional and longitudinal divisions. After several rounds of division, a hollow cavity called the blastocoel is formed in the animal half of the embryo. Concurrent with the formation of the blastocoel, the midblastula transition occurs, and cell divisions become asynchronous. The animal half contains more numerous and smaller cells than the vegetal half. (**B**) Early zebrafish embryogenesis. Immediately following fertilization, the cytoplasm of the embryo redistributes, forming a clear blastodisc above an opaque yolk. The first cell division is symmetric, and only divides the blastodisc, forming two equally sized cells. Further divisions are symmetric and synchronous, resulting in a mass of equally sized cells above the yolk mass. The layer of cells adjacent to the yolk (called the yolk syncytial layer) have cytoplasmic connections to the yolk.

it completes meiosis II and the male and female pronuclei fuse. Concurrently, the ooplasm begins a rapid redistribution toward the animal pole of the fertilized egg, forming a transparent, yolk-free bulge called the blastodisc. The blastodisc eventually develops into the entire animal, while the yolk remains largely intact throughout embryogenesis, providing nutrients to the developing embryo until it is eventually depleted. Ooplasm redistribution also causes the relocalization of a number of maternal mRNAs (Howley and Ho, 2000). Once the formation of the blastodisc is complete, a series of rapid (∼15 minutes each) synchronous cell divisions that only divide the blastodisc begin. After each round of synchronous divisions, the cells do not increase in volume, so the embryo remains the same size as the mature egg. This type of cell division continues until approximately the 512-cell stage, at which the ratio of cytoplasmic to nuclear volumes triggers the midblastula transition (MBT), as well as the onset of zygotic transcription (Kane and Kimmel, 1993). During the MBT, cell divisions in the embryo become asynchronous, and the formation of a rough body plan begins.

M. musculus Early Development

Oogenesis in mice (*M. musculus)* proceeds within the ovary of adult females. As with frogs and fish, oocyte development is a stochastic process that begins when a primordial germ cell enters meiosis. Each oocyte becomes surrounded in a layer of follicle cells that secrete signaling factors that cause the oocyte to arrest in prophase of meiosis I. Oocytes advance to metaphase of meiosis II on exposure to progesterone. Mature oocytes are

ovulated, then fertilized in the fallopian tube. Fertilization enables the embryo to complete meiosis and commence mitotic divisions, which average about 12 hours each. The first three divisions are symmetric, and produce a loose ball of cells. Toward the end of the eight-cell stage, the cells of the embryo undergo compaction, and form a tight sphere of cells, which are joined together by gap junctions that permit the transfer of small molecules between cells. Zygotic transcription initiates at the two-cell stage in mouse embryos (reviewed in Schultz, 1993), far sooner than in other model organisms.

Common Themes in Early Development

Despite the significant differences in the morphology of adults in the species discussed here, there are a number of common themes underlying their early development. In each species, there is a rapid response to a biological cue that induces the oocytes to progress through meiosis and thus become incapable of transcription. Concomitant with the progression through meiosis, the genetic program of the oocyte changes dramatically in preparation for fertilization. Post-transcriptional regulation of maternal mRNAs plays a crucial role in this reprogramming.

With the exception of mice, newly fertilized embryos of all of these species undergo a series of rapid mitotic divisions without either of the G phases present in a normal cell cycle. This produces a blastula of cells that comprises roughly the same volume as the oocyte and does not resemble the eventual shape of the adult. Despite the "ball of cells" morphology in the blastula stage, a number of cell specification events have already taken place that profoundly influence subsequent differentiation and organ formation. Because the zygotic genome is transcriptionally quiescent for most of the blastula stage, the defining moments in the life of an organism occur without the benefit of transcription. Therefore, regulation of maternal mRNAs by maternal proteins plays a central role in the determination of cell fates and the establishment of embryonic axes.

3. POST-TRANSCRIPTIONAL REGULATION OF MATERNAL mRNA IN OOGENESIS

Regulation of Maternal mRNA During Oocyte Differentiation

The gametes of most species derive from a small population of mitotically dividing primordial germ cells (PGCs). While PGCs are typically capable of producing either sperm or eggs, each individual produces only one type exclusively, with the exception of hermaphroditic species like *C. elegans*. Thus, two important regulatory events must occur prior to gametogenesis: daughters of PGC division must enter meiosis, and then they must commit to differentiation into haploid gametes. In *C. elegans,* both processes are regulated at the post-transcriptional level, which we discuss here.

Regulation of Oogenesis in C. elegans

Hermaphrodite *C. elegans* produce both sperm and eggs from the same niche of primordial germ cells (Figure 2A). Both the order in which sperm and eggs are produced and the timing of the transition from sperm production to egg production are tightly regulated. Spermatocytes are produced during larval stages of development, while oocytes are produced in adults. Interestingly, the switch from spermatogenesis to oogenesis depends largely on the post-transcriptional regulation of a few key transcripts in the germline. One of these is *fem-3* (Rosenquist and Kimble, 1988), a transcript produced during the larval stages of germline development that encodes a novel protein that promotes spermatogenesis and inhibits oogenesis (*fem* mutants lead to feminization of the germline) (Hodgkin, 1986). Thus, oocyte production requires the silencing of *fem-3* transcripts (Barton *et al*., 1987; Ahringer and Kimble, 1991). Repression of *fem-3* translation depends on a five-nucleotide sequence present in its 3′-UTR named the point mutation element (PME) (Ahringer and Kimble, 1991) (Figure 5A). Mutations within this element result in worms that produce sperm exclusively (Barton *et al*., 1987). Moreover, the *fem-3* 3′-UTR is sufficient to confer a *fem-3*-like expression pattern to a LacZ reporter, suggesting that the *fem-3* 3′ UTR is both necessary and sufficient for maintaining the correct expression pattern of FEM-3 (Gallegos *et al*., 1998). Mutations within the PME activate translation in oocytes, demonstrating that the PME is the primary *cis*-element required for repression (Gallegos *et al*., 1998).

Using a yeast-three hybrid assay, Wickens and coworkers identified two nearly identical proteins, named FBF-1 and FBF-2 (collectively referred to as FBF – *fem-3* binding factor) that bind to the PME (Zhang *et al*., 1997). Inactivation of both genes by RNAi results in the same phenotype as *fem-3* alleles with disruptions in the PME, suggesting that FBF acts directly through the PME to repress *fem-3* translation and to enable the onset of oogenesis (Zhang *et al*., 1997). FBF-mediated repression of *fem-3* is antagonized by GLD-3, a Bicaudal-C-like RNA binding protein (Eckmann *et al*., 2002). FBF cannot bind to PME-containing sequences in the presence of GLD-3, and GLD-3 binds directly to FBF. Therefore, GLD-3 binding to FBF is proposed to inhibit the association of FBF with RNA (Eckmann *et al*., 2002), providing a plausible mechanism for reversible silencing of *fem-3* translation. In this example, the antagonistic action of specific RNA-binding proteins mediates the onset of oogenesis by regulating the expression of key genes at the post-transcriptional level.

From Mitosis to Meiosis in C. elegans

The transition from mitosis to meiosis occurs in the vicinity of a somatic gonad cell called the distal tip cell (DTC). As the name suggests, this cell is located at the distal tip of the gonad, and it secretes LAG-2, a Delta-like ligand that promotes mitosis (Henderson *et al*., 1994). As primordial germ cells move away from the distal tip cell, they are exposed to diminishing

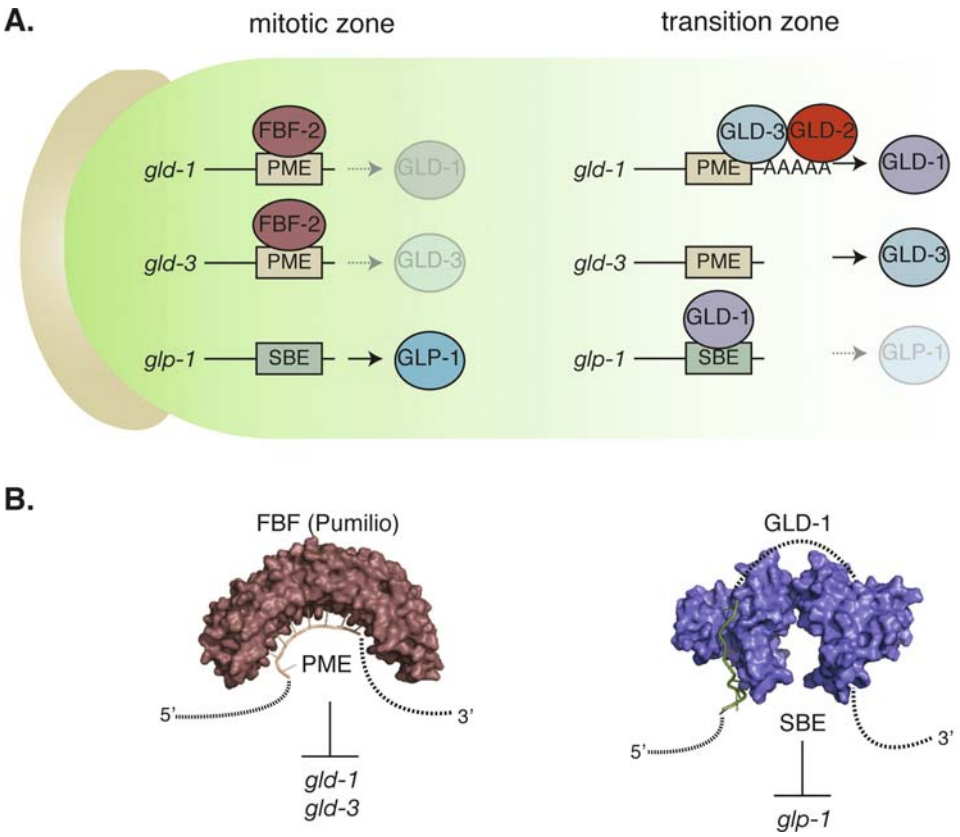

FIG. 5. Regulation of developmental events by FBF in the *C. elegans* distal gonad. (**A**) FBF represses translation from *gld-1* and *gld-3* transcripts in the mitotic region of the gonad by binding a point mutation element (PME) in 3′ UTR of each transcript. In the transition zone, FBF expression is reduced, which permits the translation of GLD-1 and GLD-3. The poly(A) tail of *gld-1* transcripts is lengthened by GLD-2/GLD-3, which promotes the translation of GLD-1. GLP-1 translation is repressed by GLD-1 in the transition zone of the gonad through the STAR binding element (SBE). The gradient of LAG-2 is denoted in green. (**B**) Structural models of FBF bound to the PME, and GLD-1 bound to the SBE. FBF structure modeled on the crystal structure of Human Pumilio1 bound to RNA (Wang *et al*., 2002b), while the GLD-1 structure is modeled on the NMR structure of SF-1 (Liu *et al*., 2001).

concentrations of LAG-2 and begin to transition into meiosis. LAG-2 promotes mitosis by interacting with one of the *C. elegans* Notch receptors, GLP-1. Null mutations of *glp-1* result in worms that lack a germline entirely, underscoring the importance of Notch signaling in maintaining a mitotically dividing pool of germ cell precursors (Austin and Kimble, 1987).

FBF and GLD-3 also participate in regulating the mitosis to meiosis switch (reviewed by Kimble and Crittenden, 2007) (Figure 5). Double mutants of *fbf-1* and *fbf-2* produce spermatocytes exclusively (Zhang *et al*., 1997), but they also prematurely initiate meiosis in the larval germline, leading to a depletion of mitotically dividing germ cell precursors (Crittenden *et al*., 2002), and the eventual disappearance of the germline. Surprisingly, FBF-1 and FBF-2 play different roles in regulating the entry into meiosis. Single mutants of *fbf-1* have fewer mitotically dividing germ cells than wild-type worms (Crittenden *et al*., 2002), while mutants of *fbf-2* have more mitotically dividing germ cells compared to wild-type organisms (Lamont *et al*., 2004). Moreover, FBF-1 and FBF-2 have remarkably different patterns of expression; FBF-1 protein is expressed primarily in the proximal three-fourths of the gonad, while FBF-2 is expressed only at the distal tip of gonads (Lamont *et al*., 2004). This suggests that these factors, though seemingly redundant, play different biological roles.

FBF-2 is an important post-transcriptional regulator of the mitosis to meiosis transition. FBF-2 production requires functional *glp-1* signaling in the distal gonad, as FBF-2 expression is sharply reduced at the restrictive temperature of a *glp-1(ts)* strain. In addition, the *fbf-2* promoter has a binding site for LAG-1, a transcriptional effector of the *glp-1* signaling pathway (Lamont *et al*., 2004), suggesting that LAG-2 induces *fbf-2* transcription. If so, it is possible that FBF-2 mediates the *glp-1* signaling response at the distal tip of the gonad.

How do FBF-1 and FBF-2 coordinate entry into meiosis? There is no definitive answer, but the transcripts encoding two additional RNA-binding proteins required for germline

development (*gld-1* and *gld-3*) are regulated by FBF-2 (Crittenden *et al*., 2002; Eckmann *et al*., 2004) (Figure 5). GLD-1 is a STAR-domain RNA-binding protein (Lee and Schedl, 2001) that represses translation of *glp-1* mRNA in meiotically dividing cells (Marin and Evans, 2003) by recognizing a sequence termed the STAR-binding element (SBE, Ryder *et al*., 2004). GLD-3 encodes a KH-domain RNA-binding protein that, in addition to its role in deactivating FBF-1, is proposed to be a specificity adapter for an atypical cytoplasmic poly(A) polymerase GLD-2 (Wang *et al*., 2002a). The GLD-2/GLD-3 poly(A) polymerase complex acts directly on *gld-1* mRNA, extending its poly(A) tail and promoting its translation (Suh *et al*., 2006). Thus, GLD-1 represses genes that promote mitosis, while GLD-3, in concert with GLD-2, activates genes that promote meiosis. FBF-2 represses the translation from both of these mRNAs, and thus inhibits meiosis, though little is known about the biochemical mechanism.

FBF is a founding member of the Pumilio and FBF (PUF) family of proteins, which function to repress mRNA translation or promote mRNA turnover in several species (Zhang *et al*. 1997; Curtis *et al*., 1997; Zamore *et al*., 1997; Wharton *et al*., 1998; Olivas and Parker, 2000; reviewed in Wickens *et al*., 2002). *Drosophila* Pumilio is required for the deadenylation and subsequent translational repression of both *hunchback* and *bicoid* mRNAs during early development, but the identity of the associated deadenylase involved in regulation is currently not known (Gamberi *et al*., 2002; Wreden *et al*., 1997). Yeast Puf3p promotes cytoplasmic deadenylation and subsequent turnover of specific transcripts by recruiting the Ccr4p/Caf1p exonuclease complex (Olivas and Parker, 2000; Goldstrohm *et al*., 2007). It is reasonable to assume that FBF promotes deadenylation and/or blocks some other stage of translation, but this has not yet been explicitly demonstrated.

While possible regulatory targets for FBF-2 in the mitosis to meiosis transition have been identified, the role of FBF-1 is still unknown. As mentioned above, *fbf-1* mutants have fewer mitotically proliferating cells in the distal region of the gonad, but FBF-1 is only weakly expressed in the mitotic region of the gonad in comparison to its stronger proximal expression. FBF-1 may influence mitotic proliferation by repressing mitosis-promoting transcripts in the proximal germline.

Regulation of Maternal mRNAs During Oocyte Maturation

Oocyte maturation is marked by dramatic changes in the genetic program of the egg as it prepares for the rounds of rapid division that follow fertilization. Some of the maternal transcripts produced in the immature oocyte are translationally repressed until maturation. These maternal mRNAs are crucial to the developing zygote; in addition to driving the cell cycle, they guide axis patterning and cell fate specification until the onset of zygotic transcription.

Immature oocytes typically arrest in pachytene phase of meiosis prophase I. This arrest must be relieved before fertilization and the first mitotic divisions can occur. In the following section, we present specific examples of maternal transcript regulation as it relates to oocyte maturation in several organisms. Intriguingly, although the biology of oogenesis varies dramatically from species to species, mechanistic themes emerge from the comparison of maternal mRNA regulatory pathways.

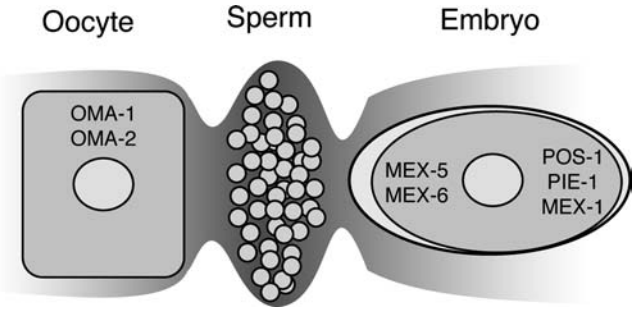

FIG. 6. The oocyte-to-embryo transition in *C. elegans*. Symmetrical oocytes are fertilized when they pass through the spermatheca. Fertilization induces rapid polarization of the embryonic axes, leading to posterior accumulation of the germline-specific proteins PIE-1 and MEX-1. OMA-1 and OMA-2, redundant factors present in the oocyte, are required to prevent the premature entry into embryonic fate. Rapid degradation of OMA-1 and OMA-2 is required for axis polarization. PIE-1, MEX-1, OMA-1 and OMA-2 are CCCH-type tandem zinc finger proteins related to mammalian TTP, an RNA-binding protein that regulates mRNA stability.

Oocyte Maturation in C. elegans

In worms, oocyte maturation occurs just prior to fertilization and is triggered by major sperm protein (MSP) released by the spermatheca (Miller *et al*., 2001). Only the most proximal oocyte undergoes maturation, which is punctuated by the breakdown of the nuclear envelope and the completion of both meiotic divisions, producing a mature oocyte ready for fertilization (Figure 2A, Figure 6).

Completion of oocyte maturation depends on OMA-1 and OMA-2, redundant CCCH-type zinc finger proteins expressed in the oocytes (Detwiler *et al*., 2001). Knocking down both proteins with RNAi causes premature initiation and subsequent failure of the oocyte maturation process. OMA-1 and OMA-2 are related to the mammalian factor tristetraprolin (TTP), an RNA-binding protein that is implicated in the destabilization of tumor necrosis factor alpha mRNA (Lai *et al*., 1999). By analogy, OMA-1 and OMA-2 may promote the targeted destruction of specific maternal transcripts at the oocyte-to-embryo transition. It is possible that these proteins directly activate or indirectly regulate genes required for completing meiosis at the RNA level. However, the ability of OMA-1 and OMA-2 to interact with RNA has not yet been

demonstrated, and as such the nucleotide sequence specificity and the mRNA target specificity has not been explored.

The levels of OMA-1 and OMA-2 increase throughout oogenesis, reaching a maximum just prior to fertilization, and then their concentration rapidly decreases after fertilization through the first mitotic division (Figure 6). The timely degradation of OMA-1 and OMA-2 is essential, as mutations that stabilize OMA-1 and OMA-2 in embryos cause embryonic lethality (Lin, 2003). The terminally differentiated embryos from these mutant mothers have an excess of pharyngeal and intestinal tissue (Lin, 2003) and mislocalized P granules (Shimada *et al*., 2006), RNA-rich bodies that normally accumulate in the embryonic lineage that specify the germline (Strome and Wood, 1982). OMA-1 and OMA-2 degradation occurs in an asymmetric manner, showing a slight bias for the posterior daughter of the first mitotic division. By the four-cell stage, bulk cytoplasmic OMA-1 and OMA-2 is degraded. Residual protein is only found in association with P granules (Lin, 2003).

OMA-1 degradation requires well timed, sequential phosphorylation at two key threonine residues. It is first phosphorylated by MBK-2 at threonine 239 immediately after fertilization, and then by GSK-3 at threonine 339 (Nishi and Lin, 2005). Phosphorylation by GSK-3 both *in vitro* and *in vivo* is sensitive to the phosphorylation state of T239, as mutations that prevent phosphorylation at T239 also prevent phosphorylation at T339 by GSK-3 (Nishi and Lin, 2005). This supports the hypothesis that phosphorylation by MBK-2 primes OMA-1 for rapid GSK-3 dependent degradation. Phosphorylation of OMA-1 is also indirectly dependent on the cell cycle regulating kinase CDK-1 and its binding partner, CKS-1 (Shirayama *et al*., 2006). Additionally, depleting embryos of Cyclin B3 also stabilizes OMA-1 (Shirayama *et al*., 2006), suggesting that its function is intimately tied to the progression of the first mitotic cycle. Stabilization of OMA-1 inhibits proper localization of two additional TTP-like zinc finger proteins required to pattern the early embryo (MEX-1 and PIE-1, Figure 6) (Shirayama *et al*., 2006; Shimada *et al*., 2006). As a result, Mello and colleagues postulated that OMA-1 and OMA-2 act as a lynchpin that prevents premature patterning prior to fertilization (Shirayama *et al*., 2006). The mechanism of this block is not known.

Progesterone Induced Oocyte Maturation in Xenopus laevis

Xenopus oocytes rapidly activate silenced mRNAs upon exposure to a hormonal cue that promotes maturation. Immature *Xenopus* oocytes are arrested in pachytene of meiosis prophase I. Upon exposure to progesterone (or insulin), the nuclear envelope breaks down and meiosis resumes, eventually arresting once more at metaphase of meiosis II. Exposure to progesterone causes the cytoplasmic polyadenylation and subsequent translational activation of a number of maternal transcripts, including the cell cycle regulators mos and cyclin B1 (Frank-Vaillant *et al*., 1999, Sarkissian *et al*., 2004). These mRNAs are thought to be polyadenylated by the pre-mRNA processing pathway in the nucleus, and their poly(A) tails are shortened upon nuclear export, thus repressing their translation. Progesterone exposure causes the poly(A) tails of these mRNAs to be lengthened once again, permitting their translation (reviewed in Richter, 2007). It is not entirely clear how progesterone triggers these events, but it is known that the atypical cyclin-dependent kinase activator RINGO/Spy is near the beginning of the pathway (Padmanabhan and Richter, 2006).

RINGO/Spy is necessary and sufficient for advancing arrested *Xenopus* oocytes to metaphase II of meiosis during oocyte maturation. Over expression of RINGO/Spy protein or premature expression in immature oocytes causes the accumulation of maturation promoting factor (MPF) and premature progression through meiosis I (Ferby *et al*., 1999). Conversely, inhibition of RINGO/Spy protein with morpholino oligonucleotides blocks the accumulation of MPF and thus the maturation of oocytes (Ferby *et al*., 1999). Thus, tight regulation of RINGO/Spy expression is necessary for temporal control of oocyte maturation.

RINGO/Spy mRNA is present throughout the late oocyte, but it is translationally repressed by the *Xenopus* Puf family member Pumilio-2 until progesterone stimulation (Padmanabhan and Richter, 2006). Translational repression of RINGO/Spy mRNA is mediated through Pumilio-2 binding sites present in its 3′-UTR. Pumilio-2 binds to the 3′-UTR of RINGO/Spy both *in vitro* and *in vivo*. Deletion of the Pumilio-2 binding sites abrogates binding and permits the premature translation of a GFP reporter mRNA, demonstrating that translational repression of RINGO/Spy is mediated by the direct association of Pumilio-2. After exposure to progesterone, Pumilio-2 can no longer associate with the 3′-UTR of RINGO/Spy, which permits translation. It is not known how progesterone stimulation causes the dissociation of Pumilio-2, but it may lead to a post-translational modification of Pumilio-2 that renders it incapable of binding to RINGO/Spy mRNA.

Cytoplasmic polyadenylation of maternal transcripts requires RINGO/Spy protein, but how RINGO/Spy activates this pathway is unknown. Phosphorylation of the RNA-binding protein cytoplasmic polyadenylation element binding protein (CPEB) by Aurora A kinase depends on the translation of RINGO/Spy protein (Padmanabhan and Richter, 2006). Thus, RINGO/Spy may permit the activation of Aurora A or some other factor upstream in the pathway.

Phosphorylation of CPEB permits the translation of a number of maternal mRNAs silenced prior to oocyte maturation, including the mRNA encoding Cyclin B1, a component of MPF (Gautier *et al*., 1990). Over expression of Cyclin B1 in immature oocytes causes the resumption of meiosis, so the precise temporal control of its expression is crucial to developmental timing (Hartley *et al*., 1996). Temporal control of Cyclin B1 expression is mediated by its 3′-UTR, which contains multiple $U_{4-6}A_{1-2}U$ sequence motifs, called cytoplasmic polyadenylation elements (CPEs). These elements are required for the polyadenylation and subsequent translation of Cyclin B1 mRNA during oocyte maturation (de Moor and Richter, 1999; Hake and Richter, 1994).

These elements are specifically recognized *in vivo* by CPEB, and this specific interaction is required for the translational derepression of Cyclin B1 mRNA (Stebbins-Boaz *et al*., 1996). In addition to CPEB, the RNA-binding protein Musashi plays a role in the rapid polyadenylation of specific transcripts, though the precise mechanism of Musashi function and its integration with the CPEB pathway remain unclear (Charlesworth *et al*., 2006).

Cyclin B1 mRNA is regulated in three separate ways during oogenesis: (1) it is deadenylated upon nuclear export, (2) it is translationally repressed prior to oocyte maturation, and (3) it is readenylated during oocyte maturation. CPEB plays a critical role in all three processes (Figure 7). CPEB recruits poly(A) ribonuclease (PARN) to CPE containing transcripts in immature oocytes (Kim and Richter, 2006). PARN catalyzes cytoplasmic deadenylation of Cyclin B1 mRNA in immature oocytes (Copeland and Wormington, 2001), and inactivation of PARN by injection of an anti-PARN antibody causes premature readenylation (Kim and Richter, 2006), suggesting that PARN antagonizes the activity of a cytoplasmic poly(A) polymerase. The association of PARN with Cyclin B1 mRNA is stage specific, as it co-immunoprecipitates with CPEB in immature oocyte extracts, but not in mature oocyte extracts. The dissociation of PARN from CPEB is caused by the phosphorylation of CPEB. During oocyte maturation, serine 174 of CPEB is phosphorylated by Aurora A kinase (Mendez *et al*., 2000a), which blocks its interaction with PARN *in vitro* and *in vivo* (Kim and Richter, 2006), thus permitting cytoplasmic polyadenylation of Cyclin B1 mRNA.

In addition to persistent deadenylation, premature translation of Cyclin B1 mRNA is blocked by specific inhibition of translation initiation (Barkoff *et al*., 2000). CPEB directly interacts with maskin, an eIF4E-interacting protein (Stebbins-Boaz *et al*., 1999). Maskin inhibits the formation of translation initiation complexes by competing with eIF4G for binding to eIF4E, thus preventing formation of the loop between the poly(A) tail and the cap complex required for efficient translation. Upon oocyte maturation, maskin dissociates from CPEB in a cytoplasmic polyadenylation-dependent manner (Cao and Richter, 2002), as the injection of cordycepin, an adenosine triphosophate analog that prevents elongation of poly(A) tails, into maturing oocytes inhibits the dissociation of maskin from CPEB. In addition, injection of poly(A) RNA, but not poly(C) RNA, prevents the dissociation of maskin from Cyclin B1 mRNA. If poly(A) binding protein (PABP) is co-injected with poly(A) RNA, the ability of maskin to dissociate from CPEB at oocyte maturation is restored, suggesting that binding of PABP to Cyclin B1 mRNA mediates dissociation of maskin (Cao and Richter, 2002). A plausible hypothesis is that a complex of PABP/eIF4G directly competes with maskin for binding to eIF4E. Consistent with this idea, the yeast homolog of PABP (Pap1) binds directly with eIF4G, promoting the formation of a closed, circular complex of mRNA and translation initiation factors (Wells *et al*., 1998). Thus, the extension of Cyclin B1 mRNA poly(A) tails would lead to an increase in local PABP concentration that is ultimately responsible for the derepression of Cyclin B1 translation.

In early oocytes, where Maskin is not expressed, cyclin B1 translation is repressed by an alternate mechanism. Instead, CPEB recruits eIF4E1b to its mRNA targets. eIF4E1b has a reduced affinity for the 5′-cap structure relative to eIF4E, and it is also incapable of binding to eIF4G (Minshall *et al*., 2007). eIF4E1b binds to 4E-T, a homolog of *Drosophila* Cup. Inhibition of eIF4E1b in early oocytes by the injection of an anti-eIF4E1b antibody causes premature maturation, demonstrating that eIF4E1b represses translation of proteins required for maturation (Minshall *et al*., 2007).

Cytoplasmic polyadenylation of Cyclin B1 mRNA requires a heteromeric complex of proteins called cleavage and polyadenylation specificity factor (CPSF), which is activated upon phosphorylation of CPEB at serine 174 by Aurora A kinase (Mendez *et al*., 2000b). Active polyadenylation of Cyclin B1 mRNA begins at the same time that PARN dissociates, demonstrating that even immature oocytes have cytoplasmic polyadenylation activity. In addition to CPSF, polyadenylation also requires the recruitment of symplekin and an atypical cytoplasmic poly(A) polymerase (xGLD-2) related to nematode GLD-2 (Barnard *et al*., 2004). Like its worm counterpart, xGLD-2 lacks apparent RNA binding domains, and presumably must rely on association with a specificity determinant such as CPEB to be recruited to specific mRNAs.

During the maturation of mouse oocytes, cyclin B1 mRNA is regulated by a similar process. The level of cyclin B1 protein is initially low in immature oocytes, and gradually increases until metaphase of meiosis I, when it is maximally expressed. During this time, the level of cyclin B1 mRNA in the oocyte does not significantly change. Injection of cyclin B1 mRNA into immature mouse oocytes accelerates maturation, suggesting that the repression machinery can be overwhelmed by a high concentration of mRNA (Polanski *et al*., 1998). During meiotic maturation, the poly(A) tail of cyclin B1 mRNA is lengthened, and this is required for translation (Tay *et al*., 2000). Contained within the 3'UTR of cyclin B1 mRNA are three CPEs, which are required for both translational repression in immature oocytes, as well as translational activation in maturing oocytes (Tay *et al*., 2000). Much like in *Xenopus* oocytes, these elements are bound by CPEB, and the phosphorylation of CPEB by an Aurora A kinase (IAK1/Eg2 in mice) is required to relieve translational repression during oocyte maturation (Tay *et al*., 2000; Hodgman *et al*., 2001). The conservation of this pathway across vertebrate species suggests that it plays a central role in oocyte maturation.

These mechanistic studies of oocyte maturation highlight several key themes in the regulation of maternal mRNAs during early development. Oocyte maturation is a rapid process, presumably because many of the mRNAs encoding regulatory machinery have already been transcribed and processed into mature mRNAs. This is exemplified by the translation of RINGO/Spy, which can be activated by the dissociation of a regulatory factor from its 3′-UTR. Thus, activation of reversibly repressed

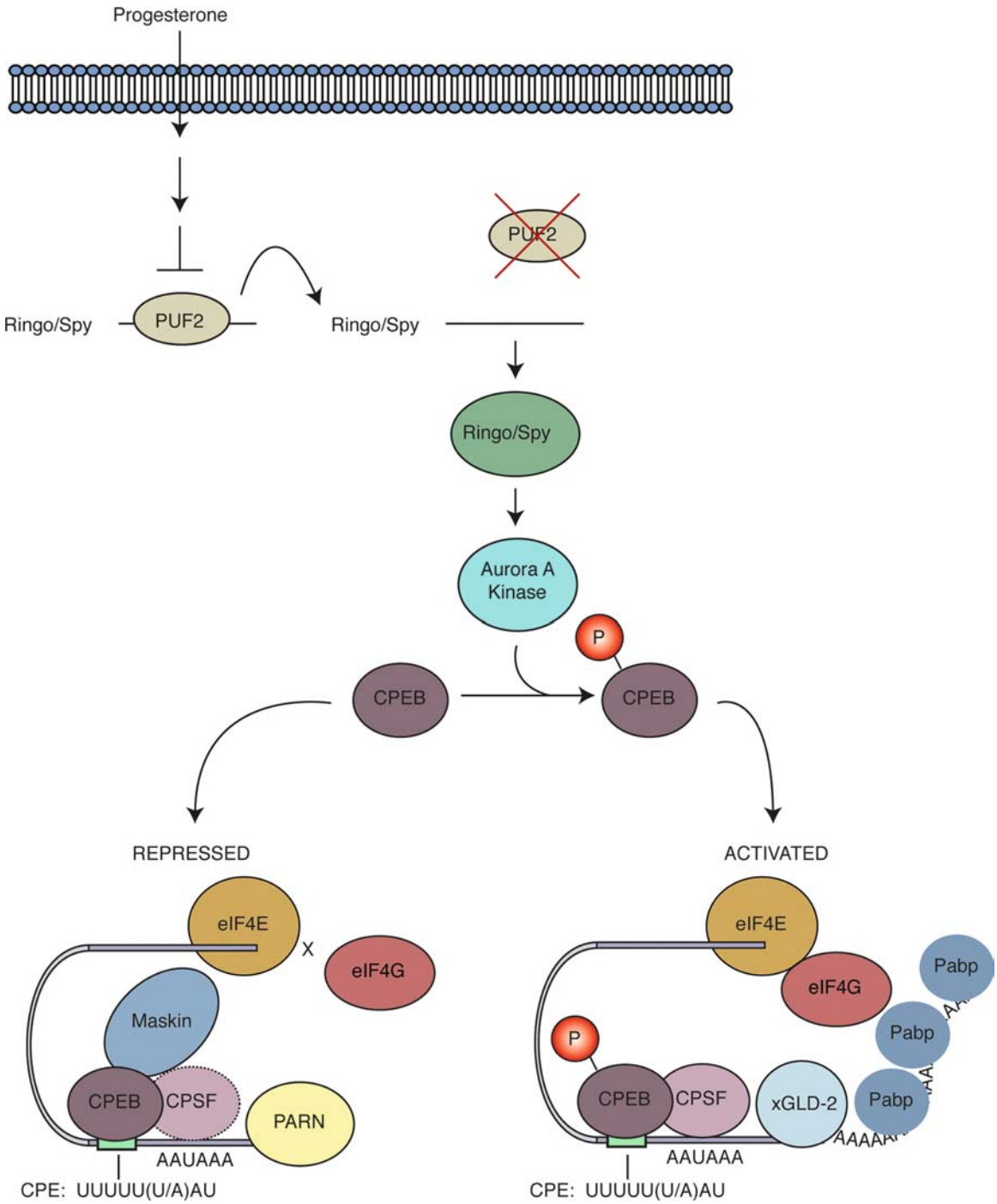

FIG. 7. Schematic representation of progesterone-mediated activation of Cyclin B1 translation in maturing *Xenopus* oocytes. Before exposure to progesterone, translation of Cyclin B1 mRNA is repressed by Maskin and PARN. Maskin competes with eIF4G for binding to eIF4E, preventing translation initiation, while PARN is a ribonuclease that prevents the extension of the poly(A) tail. Exposure to progesterone indirectly relieves Puf2-mediated repression of Ringo/Spy, which in turn indirectly activates Aurora A kinase which drives phosphorylation of CPEB. As a result, PARN no longer binds to the 3′-UTR and CPSF is activated. The dashed line around CPSF indicates that its presence in repressed complexes is controversial. xGLD-2 extends the poly(A) tail, producing binding sites for Pabp. Pabp binds to both eIF4G and eIF4E, allowing it to compete with Maskin and thus drive initiate of translation.

mRNAs prepares the oocyte to quickly react to an environmental stimulus. The regulation of cytoplasmic polyadenylation also highlights that post-transcriptional regulation in oocyte maturation occurs in cascades, where the regulation of one mRNA eventually leads to the downstream regulation of more mRNAs. This may afford the opportunity for regulation at multiple points in a pathway by competing stimuli, and amplification of regulatory signals. Another concept demonstrated by the regulation of Cyclin B1 mRNA is that critical targets are regulated via mutliple mechanisms. The translation of Cyclin B1 mRNA is regulated by at least three processes, which may act to ensure Cyclin B1 repression if a random mutation weakens the activity of any one regulatory pathway.

4. CONTRIBUTION OF MATERNAL mRNA TO EMBRYONIC PATTERNING

RNA Localization in Early Development

A common regulatory theme in the early development of both invertebrates and vertebrates is the specific localization of maternal mRNAs. Beginning in oogenesis, mRNAs encoding genes required for the specification of fundamental body axes as well as cell fates become enriched in specific regions of the oocyte. The resulting gradients of maternal mRNAs as well as the proteins they encode is crucial to patterning of the developing organism during embryogenesis. Here, we discuss how RNA localization in early development influences the specification of primordial germ cells, as well as somatic tissue lineages.

Specification of Primordial Germ Cells by Germ Plasm

The specification of cells destined to produce the entire germ line, called primordial germ cells (PGCs), is one of the first decisions made in the early embryos of many species. In all commonly studied model organsisms, with the exception of mice, PGCs are specified by a dense collection of cytoplasmic granules called germ plasm. These granules are rich in both maternal mRNAs as well as RNA-binding proteins, though the complete composition for any species has yet to be determined. In most species, germ plasm is asymmetrically localized during oogenesis, and continues to be asymmetrically distributed to specific cells during embryogenesis. Eventually, the cells that contain germ plasm stop dividing, and remain both transcriptionally silenced and quiescent until development of the germline occurs in juveniles. These cells are the primordial germ cells; below we present how germ plasm contributes to their specification.

In both *D. rerio* and *X. laevis*, germ plasm is a region rich in mitochondria and electron dense germinal granules that begin to accumulate near the nucleus during the early stages of oogenesis. As oogenesis proceeds, the germ plasm migrates to the vegetal cortex of oocytes, where it remains until fertilization. Upon fertilization of *X. laevis* oocytes, the germ plasm initially rotates with the cortex, but then detaches and concentrates once more in the vegetal pole of the embryo. In contrast, zebrafish germ plasm migrates to the base of the blastodisc, and accumulates at the distal ends of the first two cleavage furrows. In both species, the germ plasm is partitioned to only one daughter of each cell division, which ensures that only a small number of cells (approximately 5 to 10) in the blastula will become PGCs. Upon gastrulation, these cells migrate to the future site of the gonad.

Drosophila germ plasm (called pole plasm) also forms during oogenesis. The ovarian nurse cells first begin secreting proteins required to tether pole plasm components to the posterior pole of the developing oocytes, followed by the maternal mRNAs required for PGC fate specification (reviewed in Williamson and Lehmann, 1996; Ephrussi and Lehmann, 1992). The pole plasm remains at the posterior pole of the oocyte during fertilization, and is incorporated into a small number of cells at the posterior pole of the embryo during blastomere recellularization. The cells that inherit the pole plasm become the PGCs, and similar to their counterparts in *Xenopus* and zebrafish, migrate to the future site of the gonad during gastrulation.

In contrast, germ granules in *C. elegans* (called P granules) persist in the germline throughout the entire life cycle (Strome and Wood, 1982). P granules are distributed homogenously throughout the syncytial gonad as well as in developing oocytes. Upon fertilization, P granules rapidly segregate to the posterior half of the embryo, and are only distributed to the posterior daughter of the first cell division (Strome and Wood, 1982). This pattern of asymmetric P granule distribution is repeated three more times to produce a single cell containing P granules, called P4. Prior to gastrulation, this cell undergoes one final symmetric division, giving birth to two P granule containing primordial germ cells. Similar to *X. laevis, D. rerio*, and *D. melanogaster*, these cells migrate to the future location of the gonad during gastrulation.

In contrast to the species described above, mice lack germ plasm in both oocytes and embryos, and instead rely on an inductive mechanism to specify germ cells (reviewed in Hayashi *et al*., 2007). Specification begins approximately 6 days after fertilization, after the embryo is implanted in the uterine wall. A small cluster of cells in the proximal epiblast is induced by the immediately adjacent extraembryonic tissue to express *fragilis*, an interferon-inducible transmembrane protein (Saitou *et al*., 2002). Of the cells expressing *fragilis*, only a fraction begin expressing *stella*, the first marker of germ cell fate (Saitou *et al*., 2002). By an unknown mechanism, these cells begin expressing the transcriptional repressor *Blimp1* at 7.5 days post-fertilization. *Blimp1* serves to restrict the fate of germ cells by repressing key elements of the somatic transcription program, such as the *homeobox* genes (Ohinata *et al*., 2005; Saitou *et al*., 2005). Inductive specification of germ cell fate is not unique to mice, as a number of urodele amphibian species, such as the axolotl, specify their germ cells in a conceptually similar manner (Johnson *et al*., 2003).

The specification of primordial germ cells by germ plasm is a common feature of both the invertebrate and vertebrate model

organisms described here, excluding mice, but a number of questions still remain about the process. The mechanism by which germ cell fate is specified by components of the germ plasm is not understood, and the molecular mechanisms that guide germ plasm localization in oocytes and embryos remain an area of active investigation. In contrast, it is well known that localization of several conserved maternal mRNAs to the germ plasm during oogenesis is crucial to germline specification.

Nanos Contributes to Germ Cell Specification in Many Species

One maternal transcript found in the germ plasm of multiple species encodes Nanos, a CCHC zinc finger RNA binding protein (Curtis *et al*., 1997). *Nanos* was first discovered in *Drosophila*, where it was shown to play a role in the specification of abdomen in developing embryos. (Nusslein-Volhard *et al*., 1987; Wang and Lehmann, 1991; Gavis and Lehmann 1992). *Nanos* mRNA localizes to the pole plasm in the posterior of developing oocytes. This localization depends on sequence elements present in its 3′-UTR (Gavis *et al*., 1996a), and on components of pole plasm including *oskar, tudor*, and *vasa* (Wang *et al*., 1994). Over-expression of Oskar expands the zone of *nanos* transcript accumulation in the developing oocyte and increases the relative concentration of *nanos* mRNA localized to the posterior pole (Smith *et al*., 1992). These results suggest that *nanos* mRNA interacts with structural components of pole plasm.

Work by the Gavis lab identified a 41-nucleotide fragment of the *nanos* 3′-UTR that confers weak posterior localization to an mRNA reporter in oocytes. Three copies of this element are sufficient to confer *nanos*-like localization to the reporter (Bergsten and Gavis, 1999; Bergsten *et al*., 2001). p75, a cytoplasmic protein expressed during early embryogenesis, specifically interacts with this element as determined by UV crosslinking and competition assays (Bergsten *et al*., 2001). Mutations within the minimized localization element disrupt *in vitro* binding of p75 and also disrupt *in vivo* localization of the reporter mRNA construct, suggesting that *nanos* localization is dependent on a specific interaction with p75. However, the gene encoding p75 has not been determined, nor is it known how or if p75 interacts with pole plasm components to tether *nanos* mRNA to the posterior pole of oocytes.

Unlike most transcripts localized during *Drosophila* oogenesis, *nanos* mRNA is not actively localized by microtubule-associated motor proteins (Forrest and Gavis, 2003). Instead, its localization appears to involve a random diffusion and entrapment mechanism. Real-time confocal imaging of live egg chambers with GFP tagged *nanos* mRNA demonstrates that this transcript localizes to puncta at the posterior of the oocyte. Surprisingly, treatment of live egg chambers with microtubule destabilizing drugs does not disrupt localization of the labeled *nanos* mRNA, suggesting that neither microtubule directed transport nor ooplasmic streaming is required for successful localization. In contrast, an intact actin cytoskeleton is required to anchor transported *nanos* mRNA to the posterior pole of the oocyte. Treatment of egg chambers with Latrunculin A or Cytochalasin D, pharmacological inhibitors of actin polymerization, perturbs the accumulation of *nanos* transcripts at the posterior pole.

The localization of *nanos* mRNA in *Drosophila* oocytes is inefficient. Only 4% of *nanos* mRNA is localized to the pole plasm (Bergsten and Gavis, 1999). However, *nanos* protein is translated only in the posterior of the oocyte. As a result, *nanos* mRNA must also be translationally repressed outside of the posterior pole. The mechanism of *nanos* translational regulation has been studied extensively, and will be discussed later in this review.

The mechanism of *nanos* transcript localization appears to be well conserved from invertebrates to vertebrates. Xcat2, a *Xenopus* gene with a region of homology to *nanos*, localizes to the germ plasm in a similar manner (Mosquera *et al*., 1993). During early oogenesis, Xcat2 mRNA is detected in the mitochondrial cloud adjacent to the nucleus (Kloc and Etkin, 1995). As oogenesis proceeds, Xcat2 mRNA condenses into the germinal granules, and then migrates with the mitochondrial cloud to the vegetal cortex of the oocyte. Unlike other mRNAs present in the mitochondrial cloud, such as Xwnt11 and Xlsirts, Xcat2 localization is highly specific to germinal granules (Kloc *et al*. 1998).

Each distinct step in the localization of Xcat2 mRNA requires a specific *cis*-acting element in the 3′-UTR. Initial localization to the mitochondrial cloud requires a 250-nucleotide region that is both necessary and sufficient to localize Xcat2 mRNA to the mitochondrial cloud (Zhou and King, 1996). The subsequent localization of Xcat2 mRNA to the germinal granules requires a second *cis*-acting element in the 3′-UTR called the germinal granule localization element (Kloc *et al*., 2000). Disruption of this element prevents the localization of mitochondrial cloud mRNA to the germinal granules. Moreover, insertion of the element into the 3′-UTR of Xlsirt drives aberrant accumulation of this transcript into germinal granules. However, the *trans*-acting factors required for germinal granule localization have not been identified.

Within the Xcat2 mitochondrial cloud localization element (MCLE) are six UGCAC repeats. A reporter mRNA carrying the Xcat2 MCLE with mutations in all six repeats fails to localize to the mitochondrial cloud during oogenesis. Thus, UGCAC motifs are required for Xcat2 localization during oogenesis. Surprisingly, other maternal mRNAs localized to both the mitochondrial cloud and the vegetal cortex during oogenesis have a statistically significant increase of CAC repeats in their 3′-UTRs, suggesting that the CAC motifs are a *cis*-acting element required for localization in oocytes (Betley *et al*., 2002). The 75-kD KH domain RNA binding protein Vg1RBP/Vera recognizes the sequence UUCAC (Deshler *et al*., 1998), and has been implicated in the localization of mRNAs to both the mitochondrial cloud as well as the vegetal cortex (Deshler *et al*., 1997; Kwon *et al*., 2002; Choo *et al* 2005). Deletions of the UGCAC motifs in the Xcat2 MCLE disrupt Vg1RBP/Vera binding *in vitro*, suggesting

that the interaction of Vg1RBP/Vera with CAC motifs is required for localization *in vivo* (Choo *et al*., 2005). This suggests that localization of Xcat2 mRNA requires the association of a specific RNA binding protein with a region of its 3′-UTR.

Similar to *Drosophila nanos* transcripts, Xcat2 mRNA localizes to the mitochondrial cloud by a microtubule-independent diffusion and entrapment mechanism (Chang *et al*., 2004). Fluorescently labeled Xcat2 mRNAs introduced into early oocytes initially diffuse throughout the entire cytoplasm and subsequently accumulate into the mitochondrial cloud (Chang *et al*., 2004). After photobleaching of a region of the cytoplasm, fluorescence intensity returns at a rate similar to that of diffusion of large biomolecules in the cytoplasm of cultured mammalian cells, suggesting that Xcat2 mRNA transport is largely due to diffusion (Chang *et al*., 2004). After cold treatment to depolymerize microtubules and drug treatment to prevent their repolymerization, Xcat2 mRNA is still capable of localizing to the mitochondrial cloud. The similarities between the localization of *Drosophila nanos* and *Xenopus* Xcat2 suggest that the two mechanisms derive from a common ancestor, and that the localization of *nanos*-like transcripts is a well conserved process across multiple species.

Consistent with this hypothesis, *nanos1* is an essential component of germ plasm necessary for specifying primordial germ cell fate in zebrafish (Koprunner *et al*., 2001). As with *Drosophila*, active localization of *nanos1* mRNA is inefficient (Koprunner *et al*., 2001). Unlike in flies, delocalized zebrafish *nanos*1 mRNA is rapidly degraded in the cytoplasm, and its 3′-UTR is necessary and sufficient to guide both localization and turnover activities (Koprunner *et al*., 2001). Surprisingly, GFP reporter mRNA with the *Xenopus* MCLE inserted into its 3′-UTR is capable of localizing to the mitochondrial cloud in zebrafish oocytes. Further, deletion of the UGCAC elements in the MCLE abolishes this localization, implying that at least a portion of the *nanos* mRNA localization pathway is conserved between zebrafish and *Xenopus* (Kosaka *et al*., 2007). Intriguingly, the zebrafish *nanos1* 3′-UTR is capable of directing the localization of an injected GFP reporter mRNA in a wide range of fish species (Saito *et al*., 2006), further suggesting that the localization of *nanos* mRNA is an evolutionarily conserved process.

The functional similarity of *nanos* proteins between *Drosophila* and zebrafish in specifying primordial germ cell fate suggests that *nanos* activity first arose in a distant metazoan ancestor. In addition, the similarity of the mechanism of *nanos* mRNA localization between *Xenopus* and *Drosophila*, as well as the ability of *Xenopus* localization elements to direct localization in zebrafish, argues that localization of this transcript to germ plasm is a well conserved process. However, the *trans*-acting factors required to localize *nanos* mRNA to germ plasm have not been defined in any species, nor is it known how *nanos* mRNA remains anchored to specific regions of the oocyte or embryo after it has been localized.

Localization of maternal mRNAs to germ plasm demonstrates a passive mechanism of localization. Presumably, each mRNA contains *cis*-localization elements that are specifically recognized by RNA-binding proteins throughout the cytoplasm. These proteins may directly interact with structural components of germ plasm, or they may recruit other factors for this interaction. As the protein-mRNA complexes diffuse throughout the cytosol, those that come in contact with germ plasm remain bound, causing the enrichment of specific mRNAs in germ plasm. This enrichment may also require specific proteins already present in germ plasm.

RNA Localization Plays a Role in Patterning Somatic Lineages

In addition to the specification of primordial germ cells, RNA localization is also crucial for the specification of somatic lineages in many species. In *Drosophila* oocytes, localization of *bicoid* and *nanos* mRNAs to opposite poles is crucial for establishing the anterior-posterior axis, which in turn is required for the determination of the overall body plan (Gavis and Lehmann, 1992; Irion *et al*., 2006; Irion and St. Johnson, 2007). Because this has been reviewed extensively elsewhere, we will not discuss it here (Johnstone and Lasko, 2001). Instead, we will focus on the role of mRNA localization in establishing the animal-vegetal axis in *Xenopus* oocytes.

In frogs, maternal mRNAs begin to localize to both the animal and vegetal poles in late oogenesis. These mRNAs encode factors required for the specification of tissue types, and upon fertilization, they contribute to the determination the dorsal-ventral axis of the embryo. Very little is known about the mechanism of mRNA localization to the animal pole, but vegetal pole localization is a well studied phenomenon.

Vegetal localization of maternal mRNAs occurs after the germ plasm accumulates in the vegetal cortex of the oocyte, and proceeds in two distinct phases (Figure 8). First, mRNAs localize to a wedge-shaped network of microtubules and endoplasmic reticulum located between the nucleus and the vegetal cortex. From here, they are transported along the microtubules to the vegetal cortex, where they associate with the actin cytoskeleton (Yisraeli *et al*., 1990). One of the best-studied vegetally localizing mRNAs is Vg1, which encodes a transforming growth factor beta (TGF-β) protein (Weeks and Melton, 1987). Vg1 induces the specification of both endoderm and mesoderm in *Xenopus* embryos, and ectopic expression of recombinant Vg1 causes the formation of extra mesoderm (Thomsen and Melton, 1993). Thus, restriction of Vg1 expression to the vegetal hemisphere of the embryo is crucial for *Xenopus* development.

Localization of Vg1 mRNA is mediated by a 340-nucleotide element in its 3′-UTR, called the Vg1 localization element (VgLE) (Mowry and Melton, 1992). This element is both necessary and sufficient to localize mRNAs to the vegetal cortex. Contained within the VgLE are multiple copies of two short sequence motifs: the VM1 (UUUCUA) motif, and the E2 (UUCAC) motif, both of which are necessary, but not sufficient, for localization (Lewis *et al*., 2004).

A different RNA binding protein binds specifically to the VM1 and E2 motifs, and this specific recognition is required for

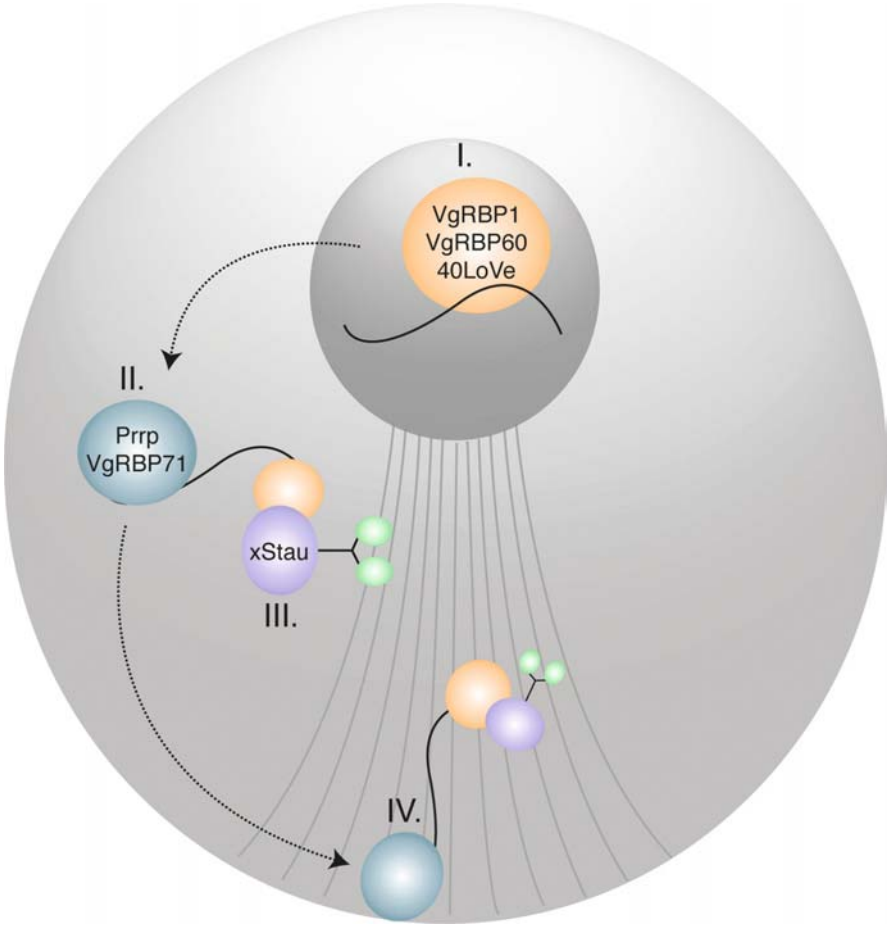

FIG. 8. Localization of Vg1 mRNA in *Xenopus* oocytes. VgRBP1/Vera, VgRBP60/hnRNP I, and 40LoVe associate with the 3′-UTR of Vg1 mRNA in the nucleus (*I*) forming a core RNP. Upon nuclear export (*II*), the RNA binding proteins Prrp and VgRBP71 bind to a distinct region of the Vg1 3′-UTR. Concurrently, a complex of xStau and kinesin bind to the core RNP (*III*), localizing Vg1 mRNA to a wedge of microtubules in the vegetal half of the oocyte. The Vg1 RNP is then transported from the nucleus by kinesin, and is anchored to the vegetal cortex by Prrp (*IV*).

localization. The VM1 motif is recognized by VgRBP60/hnRNP I, a member of the hnRNP family of nuclear RNA-binding proteins (Cote *et al*. 1999), while the E2 motif is recognized by a homolog of the yeast zipcode binding protein (ZBP) Vg1RBP/Vera (Deshler *et al*., 1997; Deshler *et al*., 1998). Point mutations that disrupt either of the motifs abolish specific binding *in vitro* and perturb localization of reporter mRNAs *in vivo* (Cote *et al*., 1999; Deshler *et al*., 1998). Together, the data strongly suggest that each protein has a function that is both unique and necessary.

Another protein required for localization is termed 40 kDa localization vegetal (40LoVe). This protein requires both VM1 and E2 motifs in order to bind to the VgLE. 40LoVe is an hnRNP D family RNA binding protein, and it has been shown to directly interact with the VgLE by UV crosslinking in oocyte extracts. However, it is not known if this interaction is independent or if it requires prior association of VgRBP60/hnRNP I or VgRBP1/Vera. Mutation of either the VM1 or E2 motifs in the VgLE disrupts 40LoVe binding as well as localization of a reporter mRNA, and depletion of 40LoVe in oocytes by the addition of a 40LoVe-specific antibody also inhibits the localization of a fluorescently labeled reporter mRNA containing a wild-type VgLE. (Czaplinski *et al*., 2005). Both VgRBP1/Vera and VgRBP60/hnRNP I can be co-immunoprecipitated with 40LoVe, further supporting the idea that the three proteins are all components of a vegetally localizing ribonucleoprotein complex (RNP) (Czaplinksi *et al*., 2006). It is not known if 40LoVe association depends on the association of VgRBP60/hnRNP I or VgRBP1/Vera, nor is the function of this RNP complex well understood.

An adapter protein is required to tether Vg1 mRNA to these structures. In *Xenopus*, a homolog of the *Drosophila* Staufen protein (XStau) is responsible for this activity (Yoon and Mowry, 2004). Like Staufen, XStau contains multiple double-stranded RNA binding domains. Unlike Staufen, it also contains a tubulin-binding domain. XStau associates with the microtubule transport protein kinesin and with Vg1 mRNA, and both functions

are required simultaneously. Injection of a truncated XStau that contains only the dsRNA binding domains inhibits localization of VgLE containing reporter mRNAs. Thus, XStau serves as a bridge between the Vg1 3′-UTR and the microtubule transport machinery. However, it is not known if XStau interacts directly with Vg1 mRNA, or if it requires other proteins such as VgRBP1/Vera.

The assembly of the Vg1 microtubule-transport RNP occurs in a sequential manner, where VgRBP60/hnRNP I, VgRBP1/Vera, and 40LoVe first associate with Vg1 mRNA in the nucleus and XStau joins the RNP in the cytoplasm (Kress *et al*., 2004). This may be entirely accounted for by the fact that VgRBP60/hnRNP I, VgRBP1/Vera, and 40LoVe are all expressed in both the nucleus and the cytoplasm, while XStau is expressed only in the cytoplasm (Kress *et al*., 2004; Yoon and Mowry, 2004; Czaplinski *et al*., 2005), or it may be possible that XStau association requires the prior association of the other three proteins. The biological significance of this stepwise RNP assembly pathway is not clear, but it may exist to ensure that XStau binds only to mRNAs destined to be localized to the vegetal pole. It has yet to be demonstrated that XStau has the ability to bind to RNA in a specific, high-affinity manner, and it may depend on cooperative interactions with other RNA binding proteins to be targeted to specific mRNAs.

In addition to a microtubule-dependent transport RNP, a separate RNP required for tethering to the actin cytoskeleton may form on Vg1 mRNA. Two more RNA binding proteins, proline rich RNA binding protein (Prrp) and VgRBP71 also bind specifically and directly to the VgLE (Zhao *et al*., 2001; Kroll *et al*., 2002), as well as directly to one another (Kroll *et al*., 2002). Prrp also interacts with profilin, a protein that is thought to be involved in the polymerization of actin microfilaments (Zhao *et al*., 2001). Thus, Prrp and VgRBP71 may form a complex on Vg1 mRNA that tethers it to the vegetal cortex. Interestingly, Prrp and VgRBP71 also interact with a number of mRNAs that localize to the animal pole of the oocyte, and may serve as a general method of tethering mRNAs to the oocyte cortex (Kroll *et al*., 2002). Thus, there may be substantial overlap between the vegetal and animal mRNA localization pathways.

Vegetal localization of maternal mRNAs in *Xenopus* oocytes highlights a number of key features in the regulation of maternal mRNAs during early development. First, the sequential assembly of proteins on the 3′-UTR of Vg1 demonstrates that a complex composed of multiple specific RNA binding proteins is capable of recruiting regulatory factors to an mRNA. This ensures that regulatory complexes are only recruited to specific mRNAs, and suggests that recognition of maternal transcripts is a combinatorial process. Localization of Vg1 mRNA also demonstrates the complex nature of *cis*-acting regulatory elements in the 3′-UTRs of mRNAs. Although the precise sequences have not yet been identified, the VgLE contains elements required for targeting the mRNA to microtubules as well as tethering it to the actin cytoskeleton. This suggests that there are at least two discrete elements in the 3′-UTR, and both are required for proper regulation of Vg1 mRNA. Also, Vg1 localization demonstrates the critical role of higher order RNP complex formation in maternal mRNA regulation. Instead of a single, multi-functional RNA-binding protein, appropriate localization of Vg1 mRNA requires no less than six proteins arranged into two distinct complexes.

The mechanism of vegetal localization of Vg1 mRNA also contrasts with the mechanism of Xcat-2 mRNA localization to germ plasm. Vg1 localization relies on directed transport, while Xcat-2 mRNA is localized by a passive diffusion/entrapment mechanism. Despite these differences, the end result is the same: RNA is enriched in a specific region of the oocyte.

Regulation of Maternal mRNAs by Localized Proteins

During early embryogenesis, a series of decisions are made that ultimately determine the morphology and body plan of a developing embryo. One of the earliest decisions is the establishment of a body axis along which the embryo is polarized. In model invertebrates, this axis is usually the anterior-posterior axis, which is already present in *Drosophila* oocytes and is rapidly determined after fertilization in *C. elegans*. In model vertebrates, the axis is the animal-vegetal axis. In both *Xenopus* and zebrafish, this axis is determined during oogenesis and is carried over into the embryo. In all four organisms, the primary body axis is specified by the asymmetric distribution of maternal proteins and mRNAs. In addition to the RNA localization mechanisms described above, this asymmetric distribution can also arise from differential translation or stability of maternal transcripts in different regions of the embryo. Here, we discuss two examples: regiospecific translational repression of *nanos* mRNA in *Drosophila*, and the establishment of opposing gradients of tandem CCCH-type zinc finger RNA binding proteins during early *C. elegans* embryogenesis.

Translational Control of Nanos

As described earlier, the accumulation of *nanos* protein at the posterior pole of Drosophila oocytes is required for the proper expression of abdominal segments, as well as posterior structures in the embryo. Localization of *nanos* transcripts to the posterior pole in part drives this accumulation, but because localization of *nanos* transcripts is inefficient, proper spatial expression requires an additional translational silencing mechanism (Figure 9). Like localization, translational control of *nanos* depends upon its 3′-UTR (Dahanukar and Wharton, 1996). A distinct 90-nucleotide translation control element (TCE) is required for translational repression of unlocalized *nanos* mRNA in oocytes (Dahanukar and Wharton, 1996). Translationally repressed *nanos* mRNA is eventually degraded in oocytes, and this activity also depends on the TCE (Dahanukar and Wharton, 1996).

Multiple, redundant stem loop structures present in the TCE are required for the appropriate expression of *nanos* protein (Smibert *et al*., 1996). They are recognized by the sterile alpha motif (SAM) RNA binding protein Smaug (Smibert *et al*.,

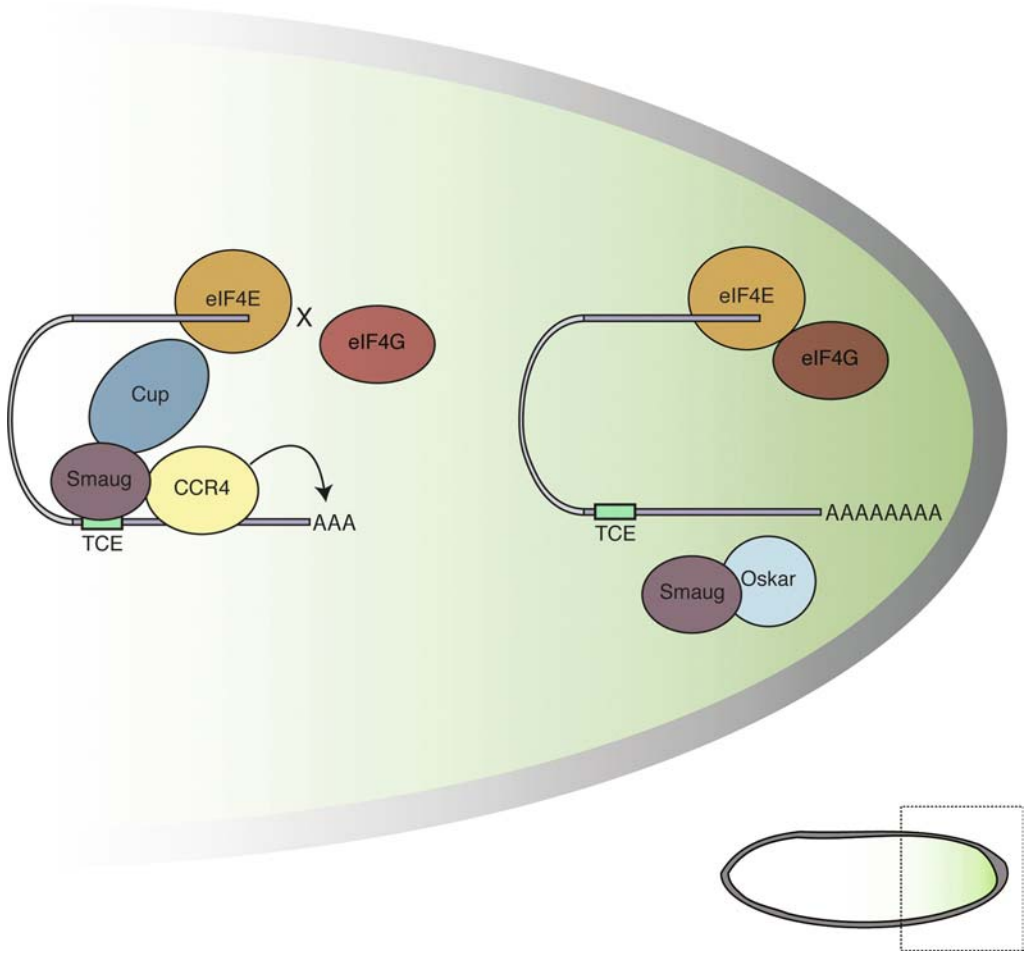

FIG. 9. Translational regulation of *nanos* in early *Drosophila* embryos. The translation of Nanos protein (*green*) is restricted to the posterior pole of the early embryo (*inset*) by Smaug, which recruits Cup and CCR4 to the 3′ UTR of *nanos* mRNA. Cup competes with eIF4E for binding to eIF4G, preventing translation initiation. CCR4 is the deadenylase that removes the poly(A) tail from *nanos* transcripts. Smaug is prevented from associating with *nanos* mRNA in the pole plasm by Oskar, which permits translation of Nanos.

1999). Mutations in the TCE that disrupt Smaug binding also cause the ectopic expression of *nanos* protein in the anterior of embryos, suggesting that direct association of Smaug with *nanos* mRNA mediates translational regulation of Nanos (Dahanukar and Wharton, 1996; Gavis *et al*., 1996b; Smibert *et al*., 1996; Smibert *et al*., 1999).

Smaug regulates Nanos expression both by inhibiting translation of *nanos* mRNA, as well as recruiting deadenylating factors to *nanos* mRNA. Smaug interacts directly with the eIF4E-binding protein Cup (Nelson *et al*., 2004). Like Maskin in *Xenopus*, Cup competes with eIF4G for binding to eIF4E, inhibiting the formation of translation initiation complexes. Translational repression of *nanos* mRNA is dependent on Cup, as mothers heterozygous for both Cup and Smaug only weakly repress the translation of a luciferase reporter mRNA carrying Smaug binding sites (Nelson *et al*., 2004).

In addition to translational repression, Smaug also causes the deadenylation of *nanos* mRNA by the recruitment of the CCR4 deadenylase complex (Semotok *et al*., 2005). Smaug's role in deadenylation was first discovered in the regulation of *Hsp83* mRNA, another maternal transcript crucial for early development in flies. The expression pattern of *Hsp83* mRNA is similar to that of *nanos* mRNA; it is enriched in the pole plasm of late stage oocytes but a substantial portion of the mRNA remains delocalized in the bulk cytoplasm (Semotok *et al*., 2005). Enrichment depends on Smaug, as embryos lacking Smaug do not accumulate *Hsp83* mRNA at the posterior pole. *Hsp83* transcripts are deadenylated after fertilization in a Smaug- and CCR4 dependent fashion, as *Hsp83* poly(A) tails are not shortened in Smaug or CCR4 mutants. Moreover, CCR4 complexes co-immunoprecipitate with Smaug in the absence of RNA,

indicating that CCR4–Smaug interaction is not mediated by RNA (Semotok *et al*., 2005).

Smaug protein is present throughout early embryos, yet *nanos* mRNA that localizes to the pole plasm is translationally active. Thus, this pool of *nanos* mRNA must somehow escape Smaug-mediated translational silencing. As with *Hsp83*, *nanos* mRNA is deadenylated after fertilization in a Smaug- and CCR4-dependent manner. When the pole plasm component Oskar is over expressed, *nanos* mRNA is not deadenylated and persists throughout the entire embryo (Zaessinger *et al*., 2006). Oskar is a protein of unknown molecular structure that accumulates in the pole plasm prior to *nanos* mRNA localization. Oskar has been shown to associate with the RNA-binding domain of Smaug *in vitro*, (Dahanukar *et al*., 1999) suggesting that Oskar relieves Smaug-mediated translational repression of *nanos* mRNA by preventing its association with *nanos* mRNA. This permits *nanos* mRNA to be specifically translated at the posterior pole of the embryo.

Nanos mRNA may also be regulated by a Smaug-independent pathway, as approximately half of the mRNA co-sediments with polysomes in sucrose gradient centrifugation of embryo lysates (Clark *et al*., 2000). This suggests that multiple ribosomes are present on these transcripts, consistent with successful translation initiation. Thus, failure to accumulate translated Nanos must be due to a block in translation elongation or to co-translational degradation. This interpretation is bolstered because treatment with puromycin, a chain-terminating ribosome inhibitor, causes *nanos* mRNA to shift out of the polysomal fractions (Clark *et al*., 2000). This experiment is crucial, because it has recently been shown that translational silencing by *Drosophila* Bruno, which also requires Cup, causes silenced transcripts to condense into large ribonucleoprotein complexes that lack ribosomes but co-sediment with polysomes in a sucrose gradient (Chekulaeva *et al*., 2006). Thus, the co-sedimentation of a transcript with the polysome fraction does not necessarily indicate that it is associated with translating ribosomes.

Nanos mRNA is initially transcribed during oogenesis, but Smaug is not expressed until early embryogenesis (Dahanukar *et al*., 1999). Thus, another repressor of *nanos* translation must function during oogenesis. Glorund, a homolog of hnRNP F/H, binds directly to the TCE of *nanos* mRNA and is expressed throughout oocytes and early embryos. Mutants of *glorund* ectopically express *nanos* in both oocytes and early embryos, demonstrating that Glorund represses *nanos* translation prior to the expression of Smaug (Kalifa *et al*., 2006). The mechanism of Glorund-mediated repression is unknown, and it may differ substantially from the mechanism of Smaug-mediated repression. While it is intriguing to speculate that Glorund may be involved in post-initiation repression of *nanos* translation, there is no evidence as of yet to support this hypothesis. The coordinated temporal repression of *nanos* translation throughout early *Drosophila* development underscores the concept of multiple pathways regulating the same critical target.

RNA-Binding Protein Gradients in C. elegans *Early Embryos*

In contrast to flies, fish, and frogs, *C. elegans* oocytes remain symmetrical until fertilization. However, *C. elegans* embryos rapidly polarize prior to the first cell division. The point of sperm entry breaks the symmetry and eventually determines the posterior pole. The cortex of the oocyte contains a dynamic actinomyosin network that continuously contracts and expands. Prior to fertilization, these contraction and expansion events are balanced throughout the oocyte so that no net movement of the cortex occurs (Munro *et al*. 2004). Upon fertilization, the actinomyosin network is destabilized in the vicinity of the sperm entry point, which causes a dramatic rearrangement of the cortex (Figure 10). The network contracts towards the anterior pole of the embryo, producing two distinct regions of the cortex that are nearly equal in size (Munro *et al*., 2004). This cortical rearrangement causes the asymmetric distribution of several *par* proteins (*par* stands for cytoplasmic partitioning defective), which are an ensemble of cortically associated kinases and regulatory proteins crucial for early development. PAR-3 and PAR-6, novel PDZ-domain containing proteins (Etemad-Moghadam *et al*., 1995; Hung and Kemphues 1999), and the atypical protein kinase C (PKC-2) (Tabuse *et al*., 1998) are delocalized throughout the cortex prior to fertilization. Together, they prevent PAR-1, a predicted Ser/Thr kinase (Guo and Kemphues 1995), and PAR-2, a RING-finger protein (Levitan *et al*., 1994), from associating with the cortex. During fertilization, cortical rearrangement causes the rapid localization of PAR-3/PAR-6/PKC-2 to the anterior cortex, which permits PAR-1 and PAR-2 to associate with the posterior cortex (Munro *et al*., 2004) (Figure 10A). Thus, shortly after fertilization, the anterior-posterior axis is formed by rapid cortical rearrangements of several crucial maternal proteins.

The localized PAR proteins ultimately cause the asymmetric localization of at least five different CCCH-type tandem zinc finger proteins (MEX-1, MEX-5, MEX-6, PIE-1, and POS-1, Figure 10B). Tristetraprolin (TTP), the first identified member of this family, mediates the destabilization of tumor necrosis factor alpha (TNF-α) transcripts in mice (Lai *et al*., 1999). TTP is a sequence specific RNA-binding protein; it binds with high affinity to the sequence UAUUUAUU (Brewer *et al*., 2004). If the *C. elegans* CCCH-type tandem zinc finger proteins also regulate mRNA stability or translation, then redistribution of these proteins could provide the first direct step in patterning the expression of maternal transcripts, thus establishing the fates of early blastomeres.

PAR-1 and PAR-2 restrict the expression of the partially redundant MEX-5 and MEX-6 proteins to the anterior of the embryo by an unknown mechanism (Schubert *et al*., 2000). MEX-5 and MEX-6 then promote the restriction of PIE-1, MEX-1, and POS-1 to the posterior of the embryo. In *mex-5;mex-6* double mutants, PIE-1, MEX-1, and POS-1 expression is delocalized throughout the embryo. This causes the mis-specification of most cell types in the early embryo and consequently, embryonic

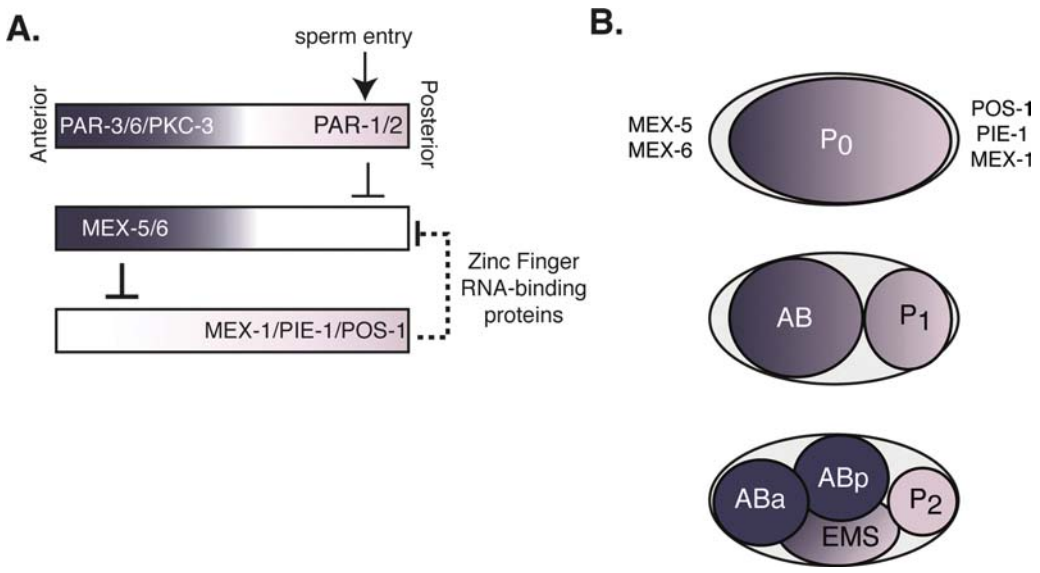

FIG. 10. (**A**) Axis polarization in *C. elegans*. The entry point of the sperm provides the first polarization cue. Microtubules polymerize from the sperm pronucleus asters, driving asymmetric accumulation of several PAR proteins within the cortical cytoplasm. (**B**) This in turn establishes opposing gradients of several CCCH-type tandem zinc finger proteins (MEX-5 and MEX-6, represented in dark purple, and MEX-1, PIE-1, and POS-1 (*pink*). These proteins are critical for early development, mutation of all but MEX-6 leads to embryonic lethality including misspecification of embryonic cell fates.

lethality (Schubert *et al*., 2000). PIE-1, MEX-1, and POS-1 each play a different but crucial role in *C. elegans* development. PIE-1 is required to maintain the transcriptional quiescence of the germline lineage (Mello *et al*., 1996), MEX-1 is required to restrict muscle fate to one blastomere at the eight-cell stage (Schnabel *et al*., 1996), and POS-1 is required to specify pharyngeal, intestinal, and germline fates (Tabara *et al*., 1999). Thus, tandem zinc finger proteins play a number of essential yet diverse roles in the development of the early embryo.

Despite the central roles that tandem zinc finger proteins play in *C. elegans* development, very little is known about their binding specificities or regulatory targets. Of the five tandem zinc finger proteins mentioned above, only the RNA-binding activities of MEX-5 and MEX-6 have been studied in detail (Pagano *et al*., 2007). MEX-5 and MEX-6 bind to a specificity determinant that is significantly more degenerate than the sequence recognized by TTP. Each recognizes six to eight uridines within an eight 8-nucleotide window. Because the 3′-UTRs of annotated *C. elegans* transcripts are remarkably rich in uridines, nearly all maternal transcripts are candidate binding partners for these proteins. Strikingly, the differences in binding specificity between MEX-5, MEX-6, and TTP can be almost entirely accounted for by a difference in one amino acid present in each zinc finger. Changing these residues in MEX-5 to its equivalent in TTP confers TTP-like specificity to MEX-5 (Pagano *et al*., 2007). Thus, the identities of these two residues are believed to play a substantial role in determining the RNA binding specificity of other tandem zinc finger proteins. MEX-1, PIE-1 and POS-1 harbor a unique pair of residues at these positions, so it is predicted that they will each bind to RNA with different specificity.

POS-1 interacts with and represses translation from *glp-1* mRNA in early embryogenesis (Ogura *et al*., 2003). As described above, *glp-1* is required for maintaining the population of germ cell precursors in the gonad. In addition, it is also required for establishing the fates of anterior blastomeres in the early embryo. At the two-cell stage, *glp-1* mRNA is expressed ubiquitously throughout the embryo, while GLP-1 protein is expressed only on the surface of the anterior blastomere (Evans *et al*., 1994). The translational repression of *glp-1* in early embryos is mediated by two elements in its 3′-UTR: the temporal control region (TCR) and the spatial control region (SCR), both of which are required for proper regulation of GLP-1 expression in the early embryo (Evans *et al*., 1994) (Figure 11). Deletion of either the SCR or TCR results in ectopic expression of GLP-1 throughout the early embryo. POS-1 has been shown to bind to both the TCR and SCR by yeast three hybrid (Ogura *et al*., 2003), and GLP-1 is also expressed ectopically throughout the early embryo in *pos-1* mutants (Ogura *et al*., 2003), demonstrating that POS-1 is a translational repressor of *glp-1*. However, it is unlikely that the *pos-1* mutant phenotype is solely the result of the misregulation of *glp-1*, as the fates of cells that do not depend on *glp-1* for their specification are also perturbed in a *pos-1* mutant. Thus, POS-1 probably regulates several other maternal mRNAs, as well.

POS-1 is just one of many RNA binding proteins that regulate *glp-1* during early embryogenesis. SPN-4, an RNA recognition

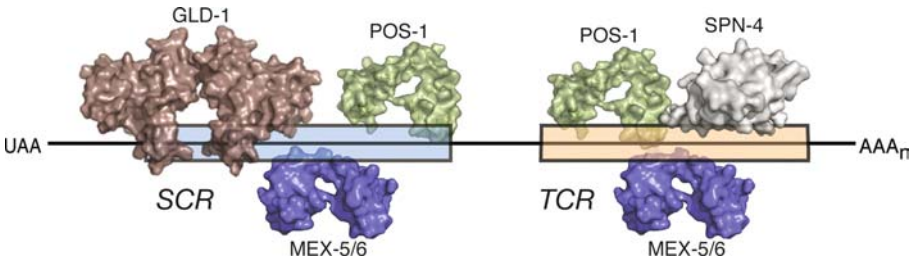

FIG. 11. Regulation of *glp-1* translation in *C. elegans*. Two elements present in the 3′-untranslated region of *glp-1* mRNA are required for appropriate spatial (SCR) and temporal (TCR) translation of GLP-1 protein in the distal germline and in the early embryo. No less than eight proteins mediate regulation through these elements (GLD-1, POS-1, MEX-5, MEX-6, SPN-4, PUF-5, PUF-6, and PUF-7). GLD-1 and POS-1 interact with the SCR, while POS-1 and SPN-4 interact with the TCR. MEX-5 and MEX-6 bind with relaxed specificity to both elements. The binding sites of PUF-5, PUF-6, and PUF-7 have not yet been mapped. The structure of GLD-1 is modeled on the NMR structure of SF-1 bound to RNA (Liu *et al*., 2001), the structures of MEX-5 and POS-1 are modeled on the NMR structure of Tis11d bound to RNA (Hudson et al., 2004), the structure of SPN-4 is modeled on the NMR structure of FOX-1 bound to RNA (Auweter *et al*., 2006).

motif (RRM) protein, interacts with both the TCR of *glp-1* mRNA as well as POS-1 protein, but opposite to *pos-1* mutants, *spn-4* mutants do not express GLP-1 at all in the early embryo. This suggests that POS-1 and SPN-4 may antagonize each other in regulating *glp-1* (Ogura *et al*., 2003). The STAR-domain RNA-binding protein GLD-1 also binds to the SCR of *glp-1*, which contains a consensus GLD-1 binding site (Marin and Evans, 2003; Ryder *et al*., 2004). Embryos depleted for GLD-1 ectopically express GLP-1 throughout the early embryo, similar to *pos-1* mutants. Thus, POS-1 and GLD-1 may coordinately regulate the translation of *glp-1* mRNA in early embryogenesis.

MEX-5 and MEX-6 also regulate *glp-1* expression during early embryogenesis. As mentioned earlier, MEX-5 and MEX-6 are weakly specific RNA binding proteins that recognize a highly degenerate uridine-rich consensus sequence (Pagano *et al*., 2007). Both the SCR and TCR of *glp-1* contain candidate MEX-5/6 binding sites, and GLP-1 is ectopically expressed throughout the embryo in *mex-5;mex-6* double knockdowns (Schubert *et al*., 2000). It is not clear if *glp-1* is directly controlled by MEX-5/6, or if deregulation is mediated by the ectopic expression of other RNA binding proteins that regulate *glp-1* directly.

GLP-1 protein is only expressed at the distal tip of the gonad and in the anterior of early embryos, even though its mRNA is present from throughout the gonad and in early embryos until the four-cell stage (Evans *et al*., 1994). Thus, *glp-1* mRNA must be translationally repressed throughout the meiotic germline. Recent evidence indicates that silencing is in part mediated by PUF-5 and PUF-6/7, members of the Puf family that includes FBF in *C. elegans* and Pumilio in flies. Triple knockdown of these factors leads to ectopic expression of GLP-1 in the proximal gonad and numerous oogenesis defects. These proteins are thought to bind directly to the TCR of *glp-1* transcripts, though their binding affinity and specificity are not known (Lublin and Evans, 2007).

glp-1 mRNA is both spatially and temporally regulated during early *C. elegans* development a complex process that proceeds through three separate developmental periods and requires the direct interaction of at least eight different RNA binding proteins. Although it has not yet been demonstrated, it is likely that *glp-1* is regulated both cooperatively and antagonistically at different times throughout development. Thus, the regulation of *glp-1* serves as an example of the potential for complex mechanisms of regulation of maternal mRNAs during early development.

5. THE MATERNAL TO ZYGOTIC TRANSITION

The final regulatory event in the life of maternal mRNAs is their degradation. Prior to the activation of zygotic transcription, maternally supplied mRNAs are degraded so that control over development is transferred to the zygotic genome. The timing and mechanism of this transition vary between model organisms. In *Drosophila, Xenopus,* and zebrafish, the transition from maternal to zygotic control occurs concurrently with the MBT, where synchronous cell cycles end and the individual blastomeres begin to grow. In contrast, the murine transition from maternal to zygotic control begins at fertilization with the clearance of most maternally supplied transcripts, and ends at the two-cell stage with the onset of zygotic transcription. Still different is the transition from maternal to zygotic control in *C. elegans*. Zygotic transcription is spatially as well as temporally regulated, as transcription is reinitiated in the somatic blastomeres from the four-cell stage, but remains repressed in the germline blastomere until gametogenesis begins in larval stages. Despite the early activation of somatic transcription in *C. elegans*, zygotic control of development does not dominate until the onset of gastrulation (Baugh *et al*., 2003).

Correctly timed clearance of maternal transcripts is critical for embryogenesis, as many maternal mRNAs encode proteins required for the establishment of early embryonic polarity, and their ectopic expression can interfere with later events in embryogenesis. One strategy employed to selectively degrade maternal mRNAs is the use of a zygotically transcribed selectivity factor that recognizes specific maternal mRNAs and recruits degradation machinery to them. This way, the degradation of maternal mRNAs begins as soon as zygotic transcription initiates.

MicroRNA-Mediated Clearance of Maternal Transcripts in D. rerio

In zebrafish, the key selectivity factor is the micro-RNA miR-430 (Giraldez *et al*., 2006). Expression of miR-430 begins shortly after the MBT, and remains high through gastrulation (Giraldez *et al*., 2005). A miR-430 binding site is sufficient to promote the decay of a reporter mRNA, and this decay is dependent on *Dicer* activity or co-injection of mature miR-430 (Giraldez *et al*., 2006). The ability to process miRNAs is necessary for the degradation of at least 750 transcripts at the MBT as determined by microarray analysis of wild-type fish compared to fish that lack both maternal and zygotic *Dicer* activity (Giraldez *et al*., 2006). Among these, 328 had annotated 3′-UTRs, all of which are enriched in miR-430 binding sites but not in binding sites for other miRNAs. Of the 3′-UTRs tested, four out of five confer miR-430-dependent degradation to a GFP reporter mRNA chimera. Maternal mRNAs in particular are enriched for miR-430 binding sites, implicating miR-430 as a major factor in maternal mRNA turnover (Giraldez *et al*., 2006). Surprisingly, miR-430 is only capable of degrading mRNAs in the somatic cells, as a GFP reporter containing the *nanos1* germ plasm localization element and multiple miR-430 binding sites is protected from degradation (Mishima *et al*., 2006). Thus, miR-430 appears to act specifically on maternal mRNAs outside of the germ plasm.

It is not known exactly how zygotic transcription is initiated in zebrafish, but the zebrafish TATA-binding protein (TBP), a core transcription factor required for Pol II transcription, has been implicated in the process. Embryos depleted for TBP fail to transcribe miR-430 and therefore fail to degrade most maternal transcripts at the MBT (Ferg *et al*., 2007). Thus, the initiation of zygotic transcription may be mediated by changes in the expression pattern of core transcriptional machinery. How the expression of transcriptional machinery is modulated in the embryo is not yet clear.

Maternal mRNA Clearance in Other Species

In addition to zygotic pathways of maternal mRNA degradation, pathways that use maternally supplied machinery are also known. In *Drosophila*, oocyte maturation triggers the degradation of at least 1069 maternal transcripts, representing 21% of all transcripts detectable in oocytes (Tadros *et al*., 2007). Of these, the degradation of 712 depends on the RNA binding protein Smaug, which is translated at oocyte maturation and requires the activity of PAN GU kinase (Tadros *et al*., 2007). However, it is not known if Smaug interacts directly with destabilized transcripts, or if it indirectly upregulates a more general destabilizing factor.

In Xenopus, approximately two thousand mRNAs are expressed in oocytes and early embryos. Of these transcripts, 24% have their polyadenylation state changed during oocyte maturation, fertilization, or both (Graindorge *et al*., 2006). Among these transcripts are a number of mRNAs encoding proteins involved in oocyte maturation, which are specifically deadenylated (and thus, translationally repressed) soon after fertilization (reviewed in Paillard and Osborne, 2003). This deadenylation is dependent on an approximately thirty five nucleotide sequence called the embryonic deadenylation (EDEN) sequence (Paillard *et al*., 1998), which is specifically recognized by the *elav*-like RNA-binding protein EDEN-BP (Paillard *et al*., 1998). The precise nucleotide specificity, and thus, target specificity of EDEN-BP is not clear. It is possible that EDEN-BP regulates the bulk deadenylation of many oocyte-specific maternal transcripts.

Zygotic transcription in mouse embryos begins at the mid-one-cell stage, and destabilization of maternal transcripts begins during oocyte maturation. By the middle of the two-cell stage, 90% of most maternal mRNA species have been degraded (Alizadeh *et al*., 2005). However, it is not known what machinery is responsible for the degradation of maternal mRNAs, nor is it known how the degradation of most maternal mRNAs is coordinated.

Given the large proportion of maternal mRNAs that are deadenylated or degraded over the course of development in *C. elegans* (Baugh *et al*., 2003), *Drosophila* (Tadros *et al*., 2007) and Xenopus (Graindorge *et al*., 2006), it is likely that a destabilization factor similar to zebrafish miR-430 exists in these species as well. The factor could plausibly be either an RNA-binding protein or a zygotically transcribed microRNA, though it is unlikely that a microRNA serves this function in *C. elegans*, as the first regulatory microRNAs do not become expressed until the larval stages (Abbot *et al*., 2005).

Bulk maternal mRNA clearance plays an important role in the regulation of early development, as it permits a rapid modification of the genetic program of an oocyte or embryo in response to an environmental cue. There are three points in the development of an organism where a drastic change in genetic program is necessary: at oocyte activation, when the oocyte prepares for the rapid cell divisions that occur after fertilization, at fertilization, and at the mid-blastula transition, when cell divisions begin to slow down and desynchronize. At each of these transitions, the oocyte or embryo is entering into a dramatically different period than the one it is currently in, and a substantial reprogramming of the oocyte or embryo is necessary.

CONCLUDING REMARKS

The field of comparative embryogenesis has progressed substantially since the observations made at the turn of the nineteenth century. The sophistication of modern experimental techniques has increased to the point where the mechanism of early development can be studied in great detail. It is now clear that post-transcriptional regulation of maternal mRNAs is responsible for many of the events that occur during the process of early development, ranging from control over the meiotic cell cycle in gametogenesis to the specification of totipotent germ cell precursors in early embryogenesis. Such post-transcriptional regulation reflects several basic biological pathways, including regulation of mRNA maturation and nuclear export, alternative sub-cellular localization, regulation of the rate of translation, and control of the rate of mRNA decay.

As with transcription, specific factors modulate expression through cis-acting signals found within the noncoding regions of the mRNA. However, it remains difficult to *ab initio* locate *cis*-acting regulatory elements within RNA sequences. This is due to the complexity of RNA signals, which can contain elements of secondary and tertiary structure and can be located within distant regions of a sequence. Mechanistic characterization of post-transcriptional regulation is further complicated in that few RNA-binding proteins recognize their cognate RNA targets with high affinity or exquisite sequence specificity. Most recognize multiple RNA targets, often as components of multi-factor complexes (Tenenbaum *et al*., 2000). In this case, sequence specificity and high affinity are thought to derive from the sum of relatively weak contributions made by each protein of the complex (Keene and Tenenbaum, 2002). Because multi-component systems can display combinatorial behavior, it is also difficult to map the network of RNA targets recognized by a single protein. Yet, without knowing the rules for RNA recognition by specific proteins, we cannot understand the molecular basis for mRNA discrimination by these regulatory factors. Rigorous determination of the nucleotide sequence specificity and mRNA target specificity of the crucial RNA-binding proteins involved in early development will enable the *a priori* identification of new regulatory targets and provide some insight on the mechanism of their regulation.

The examples presented here focus on a few critical maternal mRNAs, but recent genome-wide studies in *Drosophila*, *C. elegans*, and zebrafish demonstrate that approximately half of the genome is expressed in oocytes and early embryos (Tadros *et al*., 2007; Baugh *et al*., 2003; Giraldez *et al*., 2006). Moreover, the expression levels of nearly half of these mRNAs change independently of zygotic transcription. Thus, our current understanding represents a tiny fraction of all post-transcriptionally regulated maternal mRNAs. The application of genome-wide approaches will enable the extensive comparison of post-transcriptional regulatory networks between species.

As quantitative and genome-wide analyses are applied to the field of comparative embryology, it will be intriguing to learn if the regulatory pathways that guide early development display a greater degree of conservation than the highly divergent morphology suggests. Because post-transcriptional regulation is crucial to early patterning, one can imagine that small differences in these pathways could lead to extensive and profound differences in body plan. If so, over an evolutionary time scale, it is possible that the diversity of metazoan morphology reflects an accumulation of discrete changes in these conserved pathways, rather than wholesale evolution of novel pathways.

ACKNOWLEDGMENTS

Dedicated to the memory of Dr. Joshua J. Warren, 1974–2007. The authors thank Dr. Ruth Zearfoss for helpful comments concerning this review. S.P.R. is supported by a Scholar Award from the Worcester Foundation for Biomedical Research, a pilot award from the National Multiple Sclerosis Society (PP1418), and by grant number NS059380 from the National Institute of Neurological Disorders and Stroke.

REFERENCES

Ahringer, J., and Kimble, J. 1991. Control of the sperm-oocyte switch in Caenorhabditis elegans hermaphrodites by the fem-3 3′ untranslated region. *Nature* 349:346–348.

Austin, J., and Kimble, J. 1987. glp-1 is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. *Cell* 51:589–599.

Auweter, S.D., Fasan, R., Reymond, L., Underwood, J.G., Black, D.L., Pitsch, S., and Allain, F.H. 2006. Molecular basis of RNA recognition by the human alternative splicing factor Fox-1. *EMBO J* 25:163–173.

Balfour, F.M. 1880. *A Treatise on Comparative Embryology*, Vol. 1. London: Macmillan and Co.

Balfour, F.M. 1881. *A Treatise on Comparative Embryology*, Vol. 2. London: Macmillan and Co.

Barkoff, A.F., Dickson, K.S., Gray, N.K., and Wickens, M. 2000. Translational control of cyclin B1 mRNA during meiotic maturation: coordinated repression and cytoplasmic polyadenylation. *Dev Biol* 220:97–109.

Barnard, D.C., Ryan, K., Manley, J.L., and Richter, J.D. 2004. Symplekin and xGLD-2 are required for CPEB-mediated cytoplasmic polyadenylation. *Cell* 119:641–651.

Barton, M.K., Schedl, T.B., and Kimble, J. 1987. Gain-of-function mutations of fem-3, a sex-determination gene in Caenorhabditis elegans. *Genetics* 115:107–119.

Bashirullah, A., Cooperstock, R.L., and Lipshitz, H.D. 1998. RNA localization in development. *Annu Rev Biochem* 67:335-94.

Batchelder, C., Dunn, M.A., Choy, B., Suh, Y., Cassie, C., Shim, E.Y., Shin, T.H., Mello, C., Seydoux, G., and Blackwell, T.K. 1999. Transcriptional repression by the Caenorhabditis elegans germ-line protein PIE-1. *Genes Dev* 13:202–212.

Baugh, L.R., Hill, A.A., Slonim, D.K., Brown, E.L., and Hunter, C.P. 2003. Composition and dynamics of the Caenorhabditis elegans early embryonic transcriptome. *Development* 130:889–900.

Bergsten, S.E., and Gavis, E.R. 1999. Role for mRNA localization in translational activation but not spatial restriction of nanos RNA. *Development* 126:659–669.

Bergsten, S.E., Huang, T., Chatterjee, S., and Gavis, E.R. 2001. Recognition and long-range interactions of a minimal nanos RNA localization signal element. *Development* 128:427–435.

Betley, J.N., Frith, M.C., Graber, J.H., Choo, S., and Deshler, J.O. 2002. A ubiquitous and conserved signal for RNA localization in chordates. *Curr Biol* 12:1756–1761.

Billett, F.S., and Adam, E. 1976. The structure of the mitochondrial cloud of Xenopus laevis oocytes. *J Embryol Exp Morphol* 36:697–710.

Brewer, B.Y., Malicka, J., Blackshear, P.J., and Wilson, G.M. 2004. RNA sequence elements required for high affinity binding by the zinc finger domain of tristetraprolin: conformational changes coupled to the bipartite nature of Au-rich MRNA-destabilizing motifs. *J Biol Chem* 279:27870–27877.

Cao, Q., and Richter, J.D. 2002. Dissolution of the maskin-eIF4E complex by cytoplasmic polyadenylation and poly(A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation. *EMBO J* 21:3852–3862.

Chang, P., Torres, J., Lewis, R.A., Mowry, K.L., Houliston, E., and King, M.L. 2004. Localization of RNAs to the mitochondrial cloud in Xenopus oocytes through entrapment and association with endoplasmic reticulum. *Mol Biol Cell* 15:4669–4681.

Charlesworth, A. Wilczynska, A., Thampi, P., Cox, L.L., and MacNicol, A.M. 2006. Musashi regulates the temporal order of mRNA translation during Xenopus oocyte maturation. *EMBO J* 25:2792–2801.

Chekulaeva, M., Hentze, M.W., and Ephrussi, A. 2006. Bruno acts as a dual repressor of oskar translation, promoting mRNA oligomerization and formation of silencing particles. *Cell* 124:521–533.

Choo, S., Heinrich, B., Betley, J.N., Chen, Z., and Deshler, J.O. 2005. Evidence for common machinery utilized by the early and late RNA localization pathways in Xenopus oocytes. *Dev Biol* 278:103–117.

Clark, I.E., Wyckoff, D., and Gavis, E.R. 2000. Synthesis of the posterior determinant Nanos is spatially restricted by a novel cotranslational regulatory mechanism. *Curr Biol* 10:1311–1314.

Copeland, P.R., and Wormington, M. 2001. The mechanism and regulation of deadenylation: identification and characterization of Xenopus PARN. *RNA* 7:875–886.

Cote, C.A., Gautreau, D., Denegre, J.M., Kress, T.L., Terry, N.A., and Mowry, K.L. 1999. A Xenopus protein related to hnRNP I has a role in cytoplasmic RNA localization. *Mol Cell* 4:431–437.

Crittenden, S.L., Bernstein, D.S., Bachorik, J.L., Thompson, B.E., Gallegos, M., Petcherski, A.G., Moulder, G., Barstead, R., Wickens, M., and Kimble, J. 2002. A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. *Nature* 417:660–663.

Crittenden, S.L., Eckmann, C.R., Wang, L., Bernstein, D.S., Wickens, M., and Kimble, J. 2003. Regulation of the mitosis/meiosis decision in the Caenorhabditis elegans germline. *Philos Trans R Soc Lond B Biol Sci* 358:1359–1362.

Curtis, D., Treiber, D.K., Tao, F., Zamore, P.D., Williamson, J.R., and Lehmann, R. 1997. A CCHC metal-binding domain in Nanos is essential for translational regulation. *EMBO J* 16:834–843.

Czaplinski, K., Kocher, T., Schelder, M., Segref, A., Wilm, M., and Mattaj, I.W. 2005. Identification of 40LoVe, a Xenopus hnRNP D family protein involved in localizing a TGF-beta-related mRNA during oogenesis. *Dev Cell* 8:505–515.

Czaplinski, K., and Mattaj, I.W. 2006. 40LoVe interacts with Vg1RBP/Vera and hnRNP I in binding the Vg1-localization element. *RNA* 12:213–222.

Dahanukar, A., Walker, J.A., and Wharton, R.P. 1999. Smaug, a novel RNA-binding protein that operates a translational switch in Drosophila. *Mol Cell* 4:209–218.

Dahanukar, A., and Wharton, R.P. 1996. The Nanos gradient in Drosophila embryos is generated by translational regulation. *Genes Dev* 10:2610–2620.

Darwin, C. 1859. *The Origin of Species*, London: John Murray.

de Moor, C.H., and Richter, J.D. 1999. Cytoplasmic polyadenylation elements mediate masking and unmasking of cyclin B1 mRNA. *EMBO J* 18:2294–2303.

Deshler, J.O., Highett, M.I., Abramson, T., and Schnapp, B.J. 1998. A highly conserved RNA-binding protein for cytoplasmic mRNA localization in vertebrates. *Curr Biol* 8:489–496.

Deshler, J.O., Highett, M.I., and Schnapp, B.J. 1997. Localization of Xenopus Vg1 mRNA by Vera protein and the endoplasmic reticulum. *Science* 276:1128–1131.

Detwiler, M.R., Reuben, M., Li, X., Rogers, E., and Lin, R. 2001. Two zinc finger proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in C. elegans. *Dev Cell* 1:187–199.

Eckmann, C.R., Crittenden, S.L., Suh, N., and Kimble, J. 2004. GLD-3 and control of the mitosis/meiosis decision in the germline of Caenorhabditis elegans. *Genetics* 168:147–160.

Eckmann, C.R., Kraemer, B., Wickens, M., and Kimble, J. 2002. GLD-3, a bicaudal-C homolog that inhibits FBF to control germline sex determination in C. elegans. *Dev Cell* 3:697–710.

Edgar, B.A., and Schubiger, G. 1986. Parameters controlling transcriptional activation during early Drosophila development. *Cell* 44:871–877.

Ephrussi, A., and Lehmann, R. 1992. Induction of germ cell formation by oskar. *Nature* 358:387–392.

Etemad-Moghadam, B., Guo, S, and Kemphues, K.J. 1995. Asymmetrically distributed PAR-3 protein contributes to cell polarity and spindle alignment in early C. elegans embryos. *Cell* 83:743–752.

Evans, T.C., Crittenden, S.L., Kodoyianni, V., and Kimble, J. 1994. Translational control of maternal glp-1 mRNA establishes an asymmetry in the C. elegans embryo. *Cell* 77:183–194.

Foe, V.E., and Alberts, B.M. 1983. Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in Drosophila embryogenesis. *J Cell Sci* 61:31–70.

Forrest, K.M., and Gavis, E.R. 2003. Live imaging of endogenous RNA reveals a diffusion and entrapment mechanism for nanos mRNA localization in Drosophila. *Curr Biol* 13:1159–1168.

Frank-Vaillant, M., Jessus, C., Ozon, R., Maller, J.L., and Haccard, O. 1999. Two distinct mechanisms control the accumulation of cyclin B1 and Mos in Xenopus oocytes in response to progesterone. *Mol Biol Cell* 10:3279–3288.

Gallegos, M., Ahringer, J., Crittenden, S., and Kimble, J. 1998. Repression by the 3′ UTR of fem-3, a sex-determining gene, relies on a ubiquitous mog-dependent control in Caenorhabditis elegans. *EMBO J* 17:6337–6347.

Gamberi, C., Peterson, D.S., He, L., and Gottlieb, E. 2002. An anterior function for the Drosophila posterior determinant Pumilio. *Development* 129:2699–2710.

Gautier, J. Minshull, J., Lohka, M., Glotzer, M., Hunt, T., and Maller, J.L. 1990. Cyclin is a component of maturation-promoting factor from Xenopus. *Cell*. 60:487–494.

Gavis, E.R., Curtis, D., and Lehmann, R. 1996. Identification of cis-acting sequences that control nanos RNA localization. *Dev Biol* 176:36–50.

Gavis, E.R., and Lehmann, R. 1992. Localization of nanos RNA controls embryonic polarity. *Cell* 71:301–313.

Gavis, E.R., Lunsford, L., Bergsten, S.E., and Lehmann, R. 1996. A conserved 90 nucleotide element mediates translational repression of nanos RNA. *Development* 122:2791–2800.

Giraldez, A.J., Mishima, Y., Rihel, J., Grocock, R.J., Van Dongen, S., Inoue, K., Enright, A.J., and Schier, A.F. 2006. Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 312:75–79.

Goldstein, B., and Hird, S.N. 1996. Specification of the anteroposterior axis in Caenorhabditis elegans. *Development* 122:1467–1474.

Goldstrohm, A.C., Seay, D.J., Hook, B.A., and Wickens, M. 2007. PUF protein-mediated deadenylation is catalyzed by Ccr4p. *J Biol Chem* 282:109–114.

Gonsalves, F.C., and Weisblat, D.A. 2007. MAPK regulation of maternal and zygotic Notch transcript stability in early development. *Proc Natl Acad Sci U S A* 104:531–536.

Graindorge, A., Thuret, R., Pollet, N., Osborne, H.B., and Audic, Y. 2006. Identification of post-transcriptionally regulated Xenopus tropicalis maternal mRNAs by microarray. *Nucleic Acids Res* 34:986–995.

Guo, S., and Kemphues, K.J. 1995. par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* 81:611–620.

Hake, L.E., and Richter, J.D. 1994. CPEB is a specificity factor that mediates cytoplasmic polyadenylation during Xenopus oocyte maturation. *Cell* 79:617–627.

Hayashi, K., de Sousa Lopes, S.M., and Surani, M.A., 2007. Germ cell specification in mice. *Science* 316:394–396.

Henderson, S.T., Gao, D., Lambie, E.J., and Kimble, J. 1994. lag-2 may encode a signaling ligand for the GLP-1 and LIN-12 receptors of C. elegans. *Development* 120:2913–2924.

Hodgman, R., Tay, J., Mendez, R., and Richter, J.D. 2001. CPEB phosphorylation and cytoplasmic polyadenylation are catalyzed by the kinase IAK1/Eg2 in maturing mouse oocytes. *Development* 128:2815–2822.

Howley, C., and Ho, R.K. 2000. mRNA localization patterns in zebrafish oocytes. *Mech Dev* 92:305–309.

Hubbard, E.J.A., and Greenstein, D. 2005. Introduction to the germ line (September 1, 2005). In: *Wormbook*, The C. elegans research community, http://www.wormbook.org.

Hudson, B.P., Martinez-Yamout, M.A., Dyson, H.J., and Wright, P.E. 2004. Recognition of the mRNA AU-rich element by the zinc finger domain of TIS11d. *Nat Struct Mol Biol* 11:257–264.

Hung, T.J., and Kemphues, K.J. 1999. PAR-6 is a conserved PDZ domain-containing protein that colocalizes with PAR-3 in Caenorhabditis elegans embryos. *Development* 126:127–135.

Irion, U., Adams, J., Chang, C.W., and St Johnston, D. 2006. Miranda couples oskar mRNA/Staufen complexes to the bicoid mRNA localization pathway. *Dev Biol* 297:522–533.

Irion, U., and St Johnston, D. 2007. bicoid RNA localization requires specific binding of an endosomal sorting complex. *Nature* 445:554–558.

Johnson, A.D., Crother, B., White, M.E., Patient, R., Bachvarova, R.F., Drum, M., and Masi, T. 2003. Regulative germ cell specification in axolotl embryos: a primitive trait conserved in the mammalian lineage. *Philos Trans R Soc Lond B Biol Sci* 358:1371–1379.

Johnstone, O., and Lasko, P. 2001. Translational regulation and RNA localization in Drosophila oocytes and embryos. *Annu Rev Genet* 35:365–406.

Joseph, E.M., and Melton, D.A. 1998. Mutant Vg1 ligands disrupt endoderm and mesoderm formation in Xenopus embryos. *Development* 125:2677–2685.

Kalifa, Y., Huang, T., Rosen, L.N., Chatterjee, S., and Gavis, E.R. 2006. Glorund, a Drosophila hnRNP F/H homolog, is an ovarian repressor of nanos translation. *Dev Cell* 10:291–301.

Kane, D.A., and Kimmel, C.B. 1993. The zebrafish midblastula transition. *Development* 119:447–456.

Keene, J.D., and Tenenbaum, S.A. 2002. Eukaryotic mRNPs may represent posttranscriptional operons. *Mol Cell* 9:1161–1167.

Kim, J.H., and Richter, J.D. 2006. Opposing polymerase-deadenylase activities regulate Cytoplasmic polyadenylation. *Mol Cell* 24:173–183.

Kimble, J., and Crittenden, S.L. 2007. Control of germline stem cells, entry into meiosis, and the sperm/oocyte decision in C. elegans. *Annu Rev Cell Dev Biol* 23:405–433.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., and Schilling, T.F. 1995. Stages of embryonic development of the zebrafish. *Dev Dyn* 203:253–310.

Kloc, M., Bilinski, S., Pui-Yee Chan, A., and Etkin, L.D. 2000. The targeting of Xcat2 mRNA to the germinal granules depends on a cis-acting germinal granule localization element within the 3'UTR. *Dev Biol* 217:221–229.

Kloc, M., and Etkin, L.D. 1995. Two distinct pathways for the localization of RNAs at the vegetal cortex in Xenopus oocytes. *Development* 121:287–297.

Kloc, M., Larabell, C., Chan, A.P., and Etkin, L.D. 1998. Contribution of METRO pathway localized molecules to the organization of the germ cell lineage. *Mech Dev* 75:81–93.

Koprunner, M., Thisse, C., Thisse, B., and Raz, E. 2001. A zebrafish nanos-related gene is essential for the development of primordial germ cells. *Genes Dev* 15:2877–2885.

Kosaka, K., Kawakami, K., Sakamoto, H., and Inoue, K. 2007. Spatiotemporal localization of germ plasm RNAs during zebrafish oogenesis. *Mech Dev* 124:279–289.

Kress, T.L., Yoon, Y.J., and Mowry, K.L. 2004. Nuclear RNP complex assembly initiates cytoplasmic RNA localization. *J Cell Biol* 165:203–211.

Kroll, T.T., Zhao, W.M., Jiang, C., and Huber, P.W. 2002. A homolog of FBP2/KSRP binds to localized mRNAs in Xenopus oocytes. *Development* 129:5609–5619.

Kwon, S., Abramson, T., Munro, T.P., John, C.M., Kohrmann, M., and Schnapp, B.J. 2002. UUCAC- and vera-dependent localization of VegT RNA in Xenopus oocytes. *Curr Biol* 12:558–564.

Lai, W.S., Carballo, E., Strum, J.R., Kennington, E.A., Phillips, R.S., and Blackshear, P.J. 1999. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol Cell Biol* 19:4311–4323.

Lamont, L.B., Crittenden, S.L., Bernstein, D., Wickens, M., and Kimble, J. 2004. FBF-1 and FBF-2 regulate the size of the mitotic region in the C. elegans germline. *Dev Cell* 7:697–707.

Lee, M.H., and Schedl, T. 2001. Identification of in vivo mRNA targets of GLD-1, a maxi-KH motif containing protein required for C. elegans germ cell development. *Genes Dev* 15:2408–2420.

Levitan, D.J., Boyd, L., Mello, C.C., Kemphues, K.J., and Stinchcomb, D.T. 1994. par-2, a gene required for blastomere asymmetry in Caenorhabditis elegans, encodes zinc-finger and ATP-binding motifs. *Proc Natl Acad Sci U S A* 91:6108–6112.

Lewis, R.A., Kress, T.L., Cote, C.A., Gautreau, D., Rokop, M.E., and Mowry, K.L. 2004. Conserved and clustered RNA recognition sequences are a critical feature of signals directing RNA localization in Xenopus oocytes. *Mech Dev* 121:101–109.

Liu, Z., Luyten, I., Bottomley, M.J., Messias, A.C., Houngninou-Molango, S., Sprangers, R., Zanier, K., Kramer, A., and Sattler, M. 2001. Structural basis for recognition of the intron branch site RNA by splicing factor 1. *Science* 294:1098–1102.

Lublin, A.L., and Evans, T.C. 2007. The RNA-binding proteins PUF-5, PUF-6, and PUF-7 reveal multiple systems for maternal mRNA regulation during C. elegans oogenesis. *Dev Biol* 303:635–649.

Marin, V.A., and Evans, T.C. 2003. Translational repression of a C. elegans Notch mRNA by the STAR/KH domain protein GLD-1. *Development* 130:2623–2632.

Mazumdar, A., and Mazumdar, M. 2002. How one becomes many: blastoderm cellularization in Drosophila melanogaster. *Bioessays* 24:1012–1022.

Mello, C.C., Schubert, C., Draper, B., Zhang, W., Lobel, R., and Priess, J.R. 1996. The PIE-1 protein and germline specification in C. elegans embryos. *Nature* 382:710–712.

Mendez, R., Hake, L.E., Andresson, T., Littlepage, L.E., Ruderman, J.V., and Richter, J.D. 2000. Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. *Nature* 404:302–307.

Mendez, R., Murthy, K.G., Ryan, K., Manley, J.L., and Richter, J.D. 2000. Phosphorylation of CPEB by Eg2 mediates the recruitment of CPSF into an active cytoplasmic polyadenylation complex. *Mol Cell* 6:1253–1259.

Miller, M.A., Nguyen, V.Q., Lee, M.H., Kosinski, M., Schedl, T., Caprioli, R.M., and Greenstein, D. 2001. A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. *Science* 291:2144–2147.

Minshall, N., Reiter, M.H., Weil, D., and Standart, N. 2007. CPEB interacts with an ovary-specific eIF4E and 4E-T in early Xenopus oocytes. *J Biol Chem* 282:37389–37401.

Mishima, Y., Giraldez, A.J., Takeda, Y., Fujiwara, T., Sakamoto, H., Schier, A.F., and Inoue, K. 2006. Differential regulation of germline mRNAs in soma and germ cells by zebrafish miR-430. *Curr Biol* 16:2135–2142.

Mitchell, E.L., and Hill, R.S. 1986. The occurrence of lampbrush chromosomes in early diplotene oocytes of Xenopus laevis. *J Cell Sci* 83:213–221.

Mosquera, L., Forristall, C., Zhou, Y., and King, M.L. 1993. A mRNA localized to the vegetal cortex of Xenopus oocytes encodes a protein with a nanos-like zinc finger domain. *Development* 117:377–386.

Mowry, K.L., and Melton, D.A. 1992. Vegetal messenger RNA localization directed by a 340-nt RNA sequence element in Xenopus oocytes. *Science* 255:991–994.

Munro, E., Nance, J., and Priess, J.R. 2004. Cortical flows powered by asymmetrical contraction transport PAR proteins to establish and maintain anterior-posterior polarity in the early C. elegans embryo. *Dev Cell* 7:413–424.

Nelson, M.R., Leidal, A.M., and Smibert, C.A. 2004. Drosophila Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression. *EMBO J* 23:150–159.

Nishi, Y., and Lin, R. 2005. DYRK2 and GSK-3 phosphorylate and promote the timely degradation of OMA-1, a key regulator of the oocyte-to-embryo transition in C. elegans. *Dev Biol* 288:139–149.

Nüsslein-Volhard, C., Frohnhofer, H.G., and Lehmann, R. 1987. Determination of Anteroposterior Polarity in Drosophila. *Science* 238:1675–1681.

Ogura, K., Kishimoto, N., Mitani, S., Gengyo-Ando, K., and Kohara, Y. 2003. Translational control of maternal glp-1 mRNA by POS-1 and its interacting protein SPN-4 in Caenorhabditis elegans. *Development* 130:2495–2503.

Ohinata, Y., Payer, B., O'Carroll, D., Ancelin, K., Ono, Y., Sano, M., Barton, S.C., Obukhanych, T., Nussenzweig, M., Tarakhovsky, A., Saitou, M., and Surani, M.A. 2005. Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature* 436:207–213.

Olesnicky, E.C., and Desplan, C. 2007. Distinct mechanisms for mRNA localization during embryonic axis specification in the wasp Nasonia. *Dev Biol* 306:134–342.

Olivas, W., and Parker, R. 2000. The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast. *EMBO J* 19:6602–6611.

Pagano, J.M., Farley, B.M., McCoig, L.M., and Ryder, S.P. 2007. Molecular basis of RNA recognition by the embryonic polarity determinant MEX-5. *J Biol Chem* 282:8883–8894.

Paillard, L., Omilli, F., Legagneux, V., Bassez, T., Maniey, D., and Osborne, H.B. 1998. EDEN and EDEN-BP, a cis element and an associated factor that mediate sequence-specific mRNA deadenylation in Xenopus embryos. *EMBO J* 17:278–287.

Paillard, L., and Osborne, H.B. 2003. East of EDEN was a poly(A) tail. *Biol Cell* 95:211–219.

Pellettieri, J., Reinke, V., Kim, S.K., and Seydoux, G. 2003. Coordinate activation of maternal protein degradation during the egg-to-embryo transition in C. elegans. *Dev Cell* 5:451–462.

Polanski, Z., Ledan, E., Brunet, S., Louvet, S., Verlhac, M.H., Kubiak, J.Z., and Maro, B. 1998. Cyclin synthesis controls the progression of meiotic maturation in mouse oocytes. *Development* 125:4989–4997.

Richter, J.D. 2007. CPEB: a life in translation. *Trends Biochem Sci* 32:279–285.

Rosenquist, T.A., and Kimble, J. 1988. Molecular cloning and transcript analysis of fem-3, a sex-determination gene in Caenorhabditis elegans. *Genes Dev* 2:606–616.

Ryder, S.P., Frater, L.A., Abramovitz, D.L., Goodwin, E.B., and Williamson, J.R. 2004. RNA target specificity of the STAR/GSG domain post-transcriptional regulatory protein GLD-1. *Nat Struct Mol Biol* 11:20–28.

Saito, T., Fujimoto, T., Maegawa, S., Inoue, K., Tanaka, M., Arai, K., and Yamaha, E. 2006. Visualization of primordial germ cells in vivo using GFP-nos1 3'UTR mRNA. *Int J Dev Biol* 50:691–699.

Saitou, M., Barton, S.C., and Surani, M.A. 2002. A molecular programme for the specification of germ cell fate in mice. *Nature* 418:293–300.

Saitou, M., Payer, B., O'Carroll, D., Ohinata, Y., and Surani, M.A. 2005. Blimp1 and the emergence of the germ line during development in the mouse. *Cell Cycle*, 4:1736–1740.

Sakurai, T., Sato, M., and Kimura, M. 2005. Diverse patterns of poly(A) tail elongation and shortening of murine maternal mRNAs from fully grown oocyte to 2-cell embryo stages. *Biochem Biophys Res Commun* 336:1181–1189.

Sarkissian, M., Mendez, R., and Richter, J.D. 2004. Progesterone and insulin stimulation of CPEB-dependent polyadenylation is regulated

by Aurora A and glycogen synthase kinase-3. *Genes Dev* 18:48–61.

Schnabel, R., Weigner, C., Hutter, H., Feichtinger, R., and Schnabel, H. 1996. mex-1 and the general partitioning of cell fate in the early C. elegans embryo. *Mech Dev* 54:133–147.

Schubert, C.M., Lin, R., de Vries, C.J., Plasterk, R.H., and Priess, J.R. 2000. MEX-5 and MEX-6 function to establish soma/germline asymmetry in early C. elegans embryos. *Mol Cell* 5:671–682.

Schultz, R.M. 1993. Regulation of zygotic gene activation in the mouse. *Bioessays* 15:531–538.

Selman, K., Wallace, R.A., Sarka, A., and Qi, X. 1993. Stages of oocyte development in the zebrafish, Brachydanio rerio. *J Morphology* 203–224.

Semotok, J.L., Cooperstock, R.L., Pinder, B.D., Vari, H.K., Lipshitz, H.D., and Smibert, C.A. 2005. Smaug recruits the CCR4/POP2/NOT deadenylase complex to trigger maternal transcript localization in the early Drosophila embryo. *Curr Biol* 15:284–294.

Seydoux, G., Mello, C.C., Pettitt, J., Wood, W.B., Priess, J.R., and Fire, A. 1996. Repression of gene expression in the embryonic germ lineage of C. elegans. *Nature* 382:713–716.

Shimada, M., Yokosawa, H., and Kawahara, H. 2006. OMA-1 is a P granules-associated protein that is required for germline specification in Caenorhabditis elegans embryos. *Genes Cells* 11:383–396.

Shirayama, M., Soto, M.C., Ishidate, T., Kim, S., Nakamura, K., Bei, Y., van den Heuvel, S., and Mello, C.C. 2006. The Conserved Kinases CDK-1, GSK-3, KIN-19, and MBK-2 Promote OMA-1 Destruction to Regulate the Oocyte-to-Embryo Transition in C. elegans. *Curr Biol* 16:47–55.

Smibert, C.A., Lie, Y.S., Shillinglaw, W., Henzel, W.J., and Macdonald, P.M. 1999. Smaug, a novel and conserved protein, contributes to repression of nanos mRNA translation in vitro. *RNA* 5:1535–1547.

Smibert, C.A., Wilson, J.E., Kerr, K., and Macdonald, P.M. 1996. smaug protein represses translation of unlocalized nanos mRNA in the Drosophila embryo. *Genes Dev* 10:2600–2609.

Smith, J.L., Wilson, J.E., and Macdonald, P.M. 1992. Overexpression of oskar directs ectopic activation of nanos and presumptive pole cell formation in Drosophila embryos. *Cell* 70:849–859.

Smith, L.D., Ecker, R.E., and Subtelny, S. 1968. In vitro induction of physiological maturation in Rana pipiens oocytes removed from their ovarian follicles. *Dev Biol* 17:627–643.

Stebbins-Boaz, B., Cao, Q., de Moor, C.H., Mendez, R., and Richter, J.D. 1999. Maskin is a CPEB-associated factor that transiently interacts with elF-4E. *Mol Cell* 4:1017-1027.

Stebbins-Boaz, B., Hake, L.E., and Richter, J.D. 1996. CPEB controls the cytoplasmic polyadenylation of cyclin, Cdk2 and c-mos mRNAs and is necessary for oocyte maturation in Xenopus. *EMBO J* 15:2582–2592.

Strome, S., and Wood, W.B. 1982. Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of Caenorhabditis elegans. *Proc Natl Acad Sci U S A* 79:1558–1562.

Suh, N., Jedamzik, B., Eckmann, C.R., Wickens, M., and Kimble, J. 2006. The GLD-2 poly(A) polymerase activates gld-1 mRNA in the Caenorhabditis elegans germ line. *Proc Natl Acad Sci U S A* 103:15108–15112.

Tabara, H., Hill, R.J., Mello, C.C., Priess, J.R., and Kohara, Y. 1999. pos-1 encodes a cytoplasmic zinc-finger protein essential for germline specification in C. elegans. *Development* 126:1–11.

Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K.J., Miwa, J., and Ohno, S. 1998. Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in Caenorhabditis elegans. *Development* 125:3607–3614.

Tadros, W., Goldman, A.L., Babak, T., Menzies, F., Vardy, L., Orr-Weaver, T., Hughes, T.R., Westwood, J.T., Smibert, C.A., and Lipshitz, H.D. 2007. SMAUG is a major regulator of maternal mRNA destabilization in Drosophila and its translation is activated by the PAN GU kinase. *Dev Cell* 12:143–155.

Tay, J., Hodgman, R., and Richter, J.D. 2000. The control of cyclin B1 mRNA translation during mouse oocyte maturation. *Dev Biol* 221:1–9.

Tenenbaum, S.A., Carson, C.C., Lager, P.J., and Keene, J.D. 2000. Identifying mRNA subsets in messenger ribonucleoprotein complexes by using cDNA arrays. *Proc Natl Acad Sci U S A* 97:14085–14090.

Thomsen, G.H., and Melton, D.A. 1993. Processed Vg1 protein is an axial mesoderm inducer in Xenopus. *Cell* 74:433–441.

Vardy, L., and Orr-Weaver, T.L. 2007. The Drosophila PNG kinase complex regulates the translation of cyclin B. *Dev Cell* 12:157–166.

Wallenfang, M.R., and Seydoux, G. 2000. Polarization of the anterior-posterior axis of C. elegans is a microtubule-directed process. *Nature* 408:89–92.

Wang, C., Dickinson, L.K., and Lehmann, R. 1994. Genetics of nanos localization in Drosophila. *Dev Dyn* 199:103–115.

Wang, C., and Lehmann, R. 1991. Nanos is the localized posterior determinant in Drosophila *Cell* 66:637–647.

Wang, L., Eckmann, C.R., Kadyk, L.C., Wickens, M., and Kimble, J. 2002. A regulatory cytoplasmic poly(A) polymerase in Caenorhabditis elegans. *Nature* 419:312–316.

Wang, X., McLachlan, J., Zamore, P.D., and Hall, T.M. 2002. Modular recognition of RNA by a human pumilio-homology domain. *Cell* 110:501–512.

Weeks, D.L., and Melton, D.A. 1987. A maternal mRNA localized to the vegetal hemisphere in Xenopus eggs codes for a growth factor related to TGF-beta. *Cell* 51:861–867.

Wells, S.E., Hillner, P.E., Vale, R.D., and Sachs, A.B. 1998. Circularization of mRNA by eukaryotic translation initiation factors. *Mol Cell* 2:135–140.

Wharton, R.P., Sonoda, J., Lee, T., Patterson, M., and Murata, Y. 1998. The Pumilio RNA-binding domain is also a translational regulator. *Mol Cell* 1:863–872.

Wickens, M., Bernstein, D.S., Kimble, J., and Parker, R. 2002. A PUF family portrait: 3'UTR regulation as a way of life. *Trends Genet* 18:150–157.

Williamson, A., and Lehmann, R. 1996. Germ cell development in Drosophila. *Annu Rev Cell Dev Biol* 12:365–391.

Wolenski, J.S., and Hart, N.H. 1987. Scanning electron microscope studies of sperm incorporation into the zebrafish (Brachydanio) egg. *J Exp Zool* 243:259–273.

Wreden, C., Verrotti, A.C., Schisa, J.A., Lieberfarb, M.E., and Strickland, S. 1997. Nanos and pumilio establish embryonic polarity in Drosophila by promoting posterior deadenylation of hunchback mRNA. *Development* 124:3015–3023.

Yisraeli, J.K., Sokol, S., and Melton, D.A. 1990. A two-step model for the localization of maternal mRNA in Xenopus oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. *Development* 108:289–298.

Yoon, Y.J., and Mowry, K.L. 2004. Xenopus Staufen is a component of a ribonucleoprotein complex containing Vg1 RNA and kinesin. *Development* 131:3035–3045.

Zaessinger, S., Busseau, I., and Simonelig, M. 2006. Oskar allows nanos mRNA translation in Drosophila embryos by preventing its deadenylation by Smaug/CCR4. *Development* 133:4573–4583.

Zamore, P.D., Williamson, J.R., and Lehmann, R. 1997. The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. *RNA* 3:1421–1433.

Zhang, B., Gallegos, M., Puoti, A., Durkin, E., Fields, S., Kimble, J., and Wickens, M.P. 1997. A conserved RNA-binding protein that regulates sexual fates in the C. elegans hermaphrodite germ line. *Nature* 390:477–484.

Zhao, W.M., Jiang, C., Kroll, T.T., and Huber, P.W. 2001. A proline-rich protein binds to the localization element of Xenopus Vg1 mRNA and to ligands involved in actin polymerization. *EMBO J* 20:2315–2325.

Zhou, Y., and King, M.L. 1996. Localization of Xcat-2 RNA, a putative germ plasm component, to the mitochondrial cloud in Xenopus stage I oocytes. *Development* 122:2947–2953.

Editor: Marv Wickens