# RNA sorting in *Xenopus* oocytes and embryos

**KIMBERLY L. MOWRY[1] AND COLETTE A. COTE**

Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, Rhode Island 02912, USA

ABSTRACT **Cytoplasmic localization of mRNA molecules has emerged as a powerful mechanism for generating spatially restricted gene expression. This process is an important contributor to cell polarity in both somatic cells and oocytes, and can provide the basis for patterning during embryonic development. In vertebrates, this phenomenon is perhaps best documented in the frog, *Xenopus laevis*, where polarity along the animal-vegetal axis coincides with the localization of numerous mRNA molecules. Research over the last several years has made exciting progress toward understanding the molecular mechanisms underlying cytoplasmic mRNA localization.—Mowry, K. L., Cote, C. A. RNA sorting in *Xenopus* oocytes and embryos. *FASEB J.* 13, 435–445 (1999)**

*Key Words: RNA localization · A-V axis · polarity · oogenesis · RNA binding protein*

## BACKGROUND

Localization of mRNA molecules can now be regarded as a widespread mechanism for generating cell polarity; localized mRNAs have been identified in a number of organisms, both in somatic cells and germ cells (reviewed in ref 1). Within somatic cells, localized mRNAs may encode proteins with roles in establishing or maintaining cell motility and morphology, and can act to provide regional functional specification. Examples of this include cell type-specific localization of actin mRNA isoforms (2–5) and an increasing number of RNA transcripts that are localized in neuronal cells (6).

Localized RNAs in eggs and oocytes have profound effects on patterning and development (reviewed in ref 7). For example, proper development of the *Drosophila melanogaster* embryo is dependent in part on the regionalization of several factors within the oocyte. Maternal mRNAs encoding the anterior and posterior determinants, *bicoid* and *nanos*, are localized to opposite poles of the oocyte (8, 9), and the transcript of the *gurken* gene is localized to the anterior/dorsal region of the oocyte (10). Determination of the *Drosophila* germline also relies on localization of mRNA; localization of *oskar* mRNA to the posterior pole initiates assembly of the germ plasm (11).

These examples illustrate the fundamental problems faced by the cell, whether it is a somatic cell or a germ cell. First, the RNAs destined for localization must be recognized from amid the vast array of RNAs within the cell. Second, the RNA must be transported or restricted to a specific region of the cell cytoplasm. Finally, the localized RNA molecules must remain at the correct cytoplasmic domain to ensure spatially appropriate expression. This review will focus on insights gained as to how this process is orchestrated by examining studies of RNA sorting in *Xenopus* eggs and oocytes.

## AXIS SPECIFICATION IN *XENOPUS*

Oocytes of this anuran are first polarized along the animal-vegetal (A-V)[2] axis, the single axis of developmental potential present prior to fertilization. The precise events specifying the A-V axis have yet to be fully elucidated; however, what may be considered the first cytological indicators of polarity are detectable by the earliest stages of oogenesis. Oocyte differentiation begins as mitotic divisions that produce sets of 16 cells connected by cytoplasmic bridges (12). One of the first indications of cellular polarity in the developing *Xenopus* oocyte is the arrangement of the cytoplasmic bridge, centriole pair, and chromosomes as the cells enter meiosis (13, 14). An axis is defined by the alignment of these features, and it has been suggested that it is this axis that becomes elaborated as the A-V axis during oogenesis (15). Externally, A-V polarity becomes most evident by mid- to late oogenesis (stages IV-VI) as pigment granules collect in the animal hemisphere. In addition, yolk platelets are asymmetrically distributed within the oocyte; the greatest concentration in both size and number is found within the vegetal hemisphere by the end of oogenesis (16).

[1] Correspondence: Brown University, 69 Brown St., Providence, RI 02912, USA. E-mail: kimberly_mowry@brown.edu

[2] Abbreviations: A-V, animal-vegetal; D-V, dorsal-ventral; ER, endoplasmic reticulum; FGF, fibroblast growth factor; GV, germinal vesicle; hnRNP, heterogeneous nuclear ribonucleoprotein; MC, mitochondrial cloud; Vg1RBP, Vg1 RNA binding protein; RNP, ribonucleoprotein; TGFβ, transforming growth factor β.

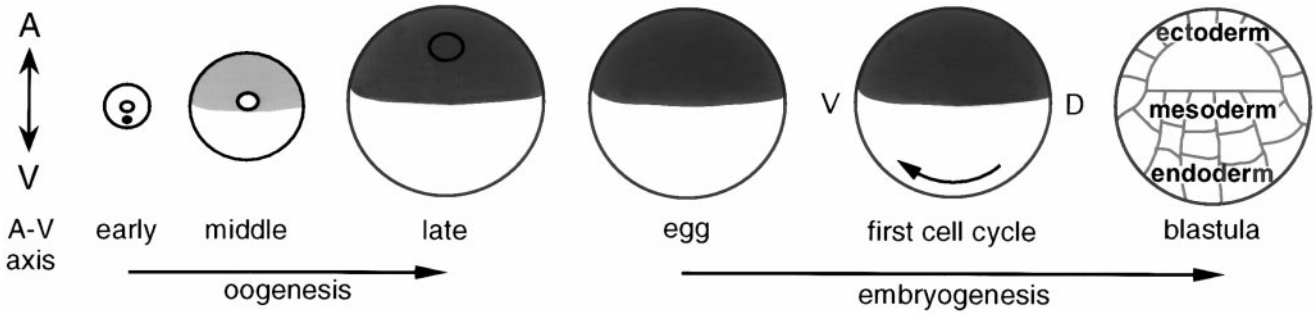


**Figure 1.** Axis specification during oogenesis and embryogenesis in *Xenopus*. Orientation of the oocytes and embryos are animal pole (A) at the top and vegetal pole (V) at the bottom, as indicated at the left. The early (stages I-II), middle (stages III-IV), and late (stages V-VI) stages of oogenesis are diagrammed at left. The oocyte nucleus (GV) is shown by an open circle, and the mitochondrial cloud is represented by a small black circle below the GV in the early stage oocyte. Selected stages during early embryogenesis are diagrammed on the right. An arrow shows the direction of vegetal yolk mass movement during cortical rotation, and the relationship to the future D-V axis is indicated. At the far right, a cross section of a blastula is depicted, indicating the specification of the three primary germ layers along the A-V axis.

Taken together, these features impart to the oocyte a characteristic two-toned appearance that allows for the simple identification of the animal and vegetal hemispheres. Dynamic reorganizations of cytoskeletal elements also occur throughout oogenesis (reviewed in refs 17, 18). These reorganizations can significantly influence the distribution of many organelles and molecules that are transported, anchored, segregated, or otherwise distributed along these elements, and may contribute to regional differences within the cortex and cytoplasm of both the animal and vegetal hemispheres.

Developmental potential along the A-V axis is coincident with the fate map of the three primary germ layers: the ectoderm, the mesoderm, and the endoderm (reviewed in ref 19; see **Fig. 1**). Animal hemisphere cells follow ectodermal fates, giving rise to the skin and nervous system, whereas vegetal hemisphere cells follow endodermal fates, primarily forming the gut; the mesoderm (ultimately muscle, blood, bone, etc.) is derived from cells in the marginal zone or equator (20). After fertilization, the radial symmetry about the A-V axis is broken during the first cell cycle by the process of cortical rotation whereby the egg cortex rotates 30° relative to the interior (21). The direction of rotation is coincident with the orientation of the future dorsal-ventral (D-V) axis (reviewed in ref 22). Cortical rotation may allow for a rearrangement of determinants within the egg, particularly within the vegetal cortex, which is the subcellular destination for a number of localized determinants, including maternally derived proteins and RNAs.

## LOCALIZED MATERNAL DETERMINANTS

Differences in developmental potential along the A-V axis have long been viewed as arising from cytoplasmic localization of maternal determinants (reviewed in ref 23). Thus, patterning in the embryo is dependent on the prelocalization of mRNAs and proteins within the oocyte. Within this context, specific localized mRNA molecules have been implicated in primary germ layer specification and localized RNAs have been identified as components of the germ plasm as well.

Localization of germ plasm, which specifies the germline (24), is a very early indicator of oocyte polarity. It is apparent that the source of germ plasm is the mitochondrial cloud (MC), which is an accumulation of granular-fibrillar material and mitochondria on the presumed vegetal side of the germinal vesicle (GV) in stage I oocytes (25). After the MC breaks down, during late stage I/early stage II of oogenesis, mitochondria and germinal granules are found in vegetal-cortical islands of germ plasm (25). In the egg, germ plasm persists as vegetal-cortical islands of yolk-free cytoplasm, containing germinal granules associated with large aggregations of mitochondria (26). During early cleavage, the islands of germ plasm coalesce into larger aggregates, which are eventually inherited by a subset of vegetal blastomeres, the primordial germ cells (27–29). Roles for localized RNAs in germ cell specification or determination have been suggested, as a distinct class of RNA molecules have been identified that are localized to the MC in early oocytes and segregate with the germ plasm during cleavage (30–34).

Mesoderm induction in *Xenopus* appears to rely at least in part on maternal determinants that are localized within the vegetal hemisphere (35, 36). Mesoderm is specified through an inductive event whereby a signal or combination of signals emanating from vegetal hemisphere cells induce a subset of overlying cells within the marginal zone of the embryo to become mesoderm (37). Studies of the molecular nature of the signal or signals secreted by

TABLE 1. *Localized RNAs in Xenopus*

| RNA | Pathway | Encoded protein | References |
|---|---|---|---|
| Localized to the vegetal hemisphere: | | | |
| βTrCP | | β-Transducin repeat containing protein | (63) |
| VegT | Late | T-box transcription factor | (46–49) |
| Vg1 | Late | TGFβ-like growth factor | (39, 55) |
| Xcat-2 | Early | *nanos*-like zinc finger protein | (56) |
| Xcat-3 | | Potential RNA helicase | (57) |
| Xdazl | Early | Related to DAZ (deleted in azoospermia) | (32) |
| Xlsirts | Early | Nontranslated RNA | (58) |
| Xpat | Early | Novel protein | (33) |
| Xwnt-11 | Early | wnt protein | (40) |
| Localized to the animal hemisphere: | | | |
| An1 | | Ubiquitin-like fusion protein | (55, 96) |
| An2 | | Mitochondrial ATPase subunit | (55, 97) |
| An3 | | DEAD box RNA helicase | (55, 98) |
| An4 | | Novel protein | (63) |
| βTrCP (2.5 kb) | | β-Transducin repeat containing protein | (63) |
| Oct-60 | | POU domain transcription factor | (59) |
| PABP | | Poly(A) binding protein | (62) |
| xlan4 | | Novel protein | (60) |
| x121 | | Novel protein | (61) |

the vegetal blastomeres have revealed the importance of a number of growth factors, including members of the fibroblast growth factor (FGF) and transforming growth factor β (TGFβ) superfamilies (reviewed in ref 38). Unlike the inducing activity of FGFs and TGFβ's, other factors such as noggin and members of the wnt proto-oncogene family act as modifiers (38). Independently, they are not capable of inducing mesoderm, but are able to modify the effects of other determinants to pattern a range of mesodermal cell types from dorsal (i.e., notochord and somites) to more ventral (i.e., blood, mesenchyme and muscle). Localized to the vegetal pole are mRNAs encoding members of the TGFβ and wnt families (39, 40), and a role in mesoderm induction for the TGFβ family member Vg1 has been supported experimentally (41–44). However, roles for localized mRNAs in mesoderm induction are not limited to growth factors. The recent results of Zhang et al. (45) have assigned a key role in patterning of the primary germ layers to VegT (46) [also known as Xombi (47), Antipodean (48), and Brat (49)], which encodes a member of the T-box family of transcription factors that is localized to the vegetal hemisphere. It is apparent that vegetally localized RNA molecules contribute to induction of mesoderm, but what about the endoderm, which arises from the vegetal cells themselves? A linkage between mesoderm induction and endoderm determination may be suggested by results implicating the localized mRNAs Veg T and Vg1 not only in mesoderm induction, but determination of endoderm as well (44, 45, 49, 50).

By definition, a localized determinant is a factor whose specific position and resulting activity impart a particular state of commitment to cells that selectively inherit it; as outlined above, determination of the germline, mesoderm, and endoderm may rely on localized RNA molecules. Thus, localization of RNAs along the A-V axis may be a key step in patterning the frog embryo.

## LOCALIZED RNAs IN *XENOPUS* OOCYTES

Unequal distribution of mRNA is apparent along the A-V axis of *Xenopus* eggs and oocytes (51–54), and studies investigating possible roles for localized RNA molecules in the determination of cell fates during *Xenopus* development have led to the discovery of maternal mRNAs that are differentially localized within the unfertilized egg (see **Table 1**). Initially, four localized transcripts were identified (55): An1, An2, and An3 mRNAs were preferentially retained within the animal hemisphere, and Vg1 mRNA was found exclusively within the vegetal hemisphere. Later investigations have identified additional RNAs that are localized to the animal or vegetal hemisphere during *Xenopus* oogenesis. Vegetally localized RNAs include Xcat-2 (56), VegT (46–49), Xcat-3 (57), Xdazl (32), Xlsirts (58), Xpat (33), and Xwnt11 (40). RNAs localized to the animal hemisphere include Oct-60 (59), xlan4 (60), x121 (61), and poly(A) binding protein (62), Recently, differential display polymerase chain reaction has revealed several localized variants of βTrCP and a novel transcript An4 (63), leaving little doubt that additional localized RNAs will be identified in *Xenopus* in years to come. However, a key question remains: How do these RNAs become localized such that their restricted expression patterns could specify distinct regions within the developing embryo?

## MODELS, MECHANISMS, AND QUESTIONS

Extensive studies in *Drosophila* and *Xenopus* have revealed a number of distinct RNA localization pathways (reviewed in ref 7). In *Xenopus*, RNAs are targeted to at least two destinations: An1, An2, An3, An4, βTrCP (2.5 kb), Oct-60, PABP, xlan4, and x121 are restricted to the animal hemisphere, whereas βTrCP (3.5 kb, 4.9 kb), TGFβ5, VegT, Vg1, Xcat-2, Xcat-3, Xdazl, Xlsirts, Xpat, and Xwnt-11 are localized to the vegetal hemisphere (see Table 1). Yet many, if not all, of these RNAs are present within the ooplasm concurrently, along with an overwhelming number of RNA molecules that are not localized. What mechanisms are available to distinguish a localized RNA from the myriad of nonlocalized RNAs or, for that matter, from one another? For RNAs destined for localization, how are the subcellular destinations determined such that RNAs can be restricted to either the animal or vegetal hemisphere? Are pathways for animal and vegetal localization distinct or overlapping? Within the animal and vegetal hemispheres, are there specific subdomains that are targeted by different sets of RNA molecules? While the regulatory mechanisms that govern the targeting of a specific RNA to its proper destination and restrict the timing of its localization have yet to be fully elucidated, considerable progress has been made in addressing these questions.

In building models for RNA localization, at least three steps can be hypothesized: *1*) Recognition: only a subset of molecules in the cell are localized; these molecules must be recognized. *2*) Regionalization: the RNA molecules must be transported or otherwise sequestered in defined regions of the cytoplasm. *3*) Maintenance: the localized molecules must remain restricted to the correct cytoplasmic domain to ensure localized expression. Recognition of localized RNAs could be RNA based, where the RNA itself carries a *cis*-acting localization sequence, or could require recognition of a nascent polypeptide product produced during translation of the RNA. At present, however, evidence exists to support only recognition of RNA sequences during localization, and progress has been made in defining the recognition sequences (see below). Regionalization can result from a number of mechanisms. Transport of RNA on cytoskeletal elements, perhaps by motor proteins, has been implicated in vegetal localization of Vg1 RNA (64). Regionalization may also occur by local protection from degradation, as has been shown for Hsp83 mRNA in *Drosophila* (65). In contrast, vectorial nuclear export may provide a means to target RNA molecules to distinct cytoplasmic regions, as has been suggested for several zygotic transcripts in *Drosophila* (66). RNAs may also accumulate at a specific site by entrapment, due to the presence of localized binding factors. Such a model has been suggested for localization of Xcat-2 RNA (67), and perhaps many of the RNAs localized to the animal hemisphere as well. Maintenance of localization requires mechanisms that will restrict diffusion of the RNA, once localized. For RNAs localized to the vegetal hemisphere, this appears to be mediated through binding or attachment to the cortical cytoskeleton (30, 31, 64). RNAs localized to the animal hemisphere, by contrast, are not associated with the cortex (30). Other as yet undetermined factors may restrict these RNAs within the animal hemisphere; however, it is notable that RNAs localized to the animal hemisphere are present in a graded distribution along the A-V axis, whereas those localized to the vegetal hemisphere are tightly restricted to the vegetal cortical region (see, for example, ref 68).

## TWO VEGETAL LOCALIZATION PATHWAYS

At least two pathways operate in *Xenopus* oocytes to localize RNAs to the vegetal cortex (30, 31; see **Fig. 2**). mRNAs localized by the early pathway include Xcat 2, Xdazl, Xpat, Xwnt-11, and the Xlsirts (30–33). Transcripts localized by the early pathway first associate with the MC during stages I-II, and from late stage II to early stage III are localized to the vegetal cortex in a process described in detail below. The paradigm of the other pathway that acts to localize mRNAs to the vegetal pole is Vg1 RNA, which is localized to the vegetal cortex during late stage III to early stage IV (69).

## THE EARLY PATHWAY

The early pathway (see Fig. 2) is comprised of at least three major steps (30, 31, 70). Translocation of specific RNAs from their site of synthesis to the mitochondrial aggregates that surround the GV comprises the first step of the pathway (70). During the prestage I period of oogenesis, mitochondria are arranged in a fine ring around the GV and interspersed within this ring are a number of mitochondrial aggregates or precloud structures (25). Upon synthesis and transport out of the GV, RNAs that use this pathway first move freely about the cytoplasm before translocating as discrete particles to the aggregates (70, 71). The MC proper, containing mitochondria, electron-dense granulofibrillar material (presumptive germ plasm), and smooth endoplasmic reticulum (ER) (25), forms by the apparent condensation of several of these aggregates into an initial cap-like structure on one side of the GV. The second step in the early vegetal RNA localization pathway occurs during early stage I, when these RNAs appear to coalesce to one dominant aggregate,

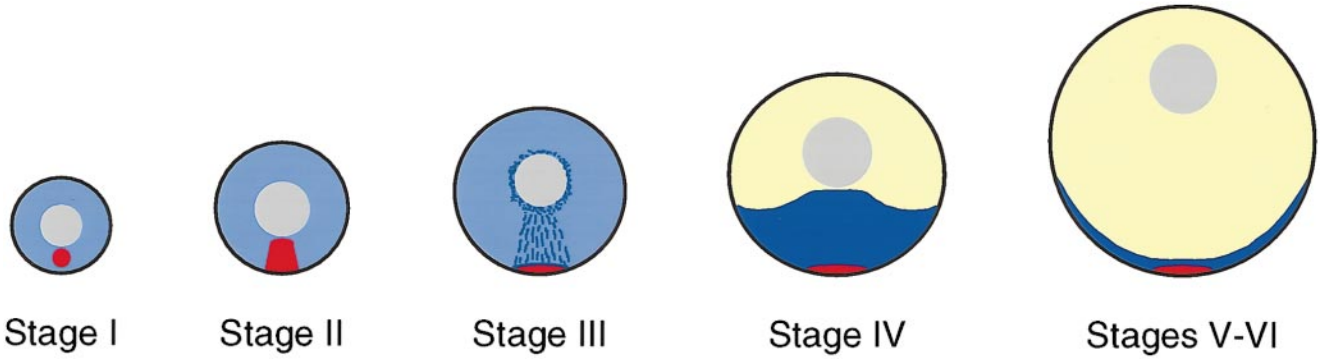


**Figure 2.** Two distinct pathways for RNA localization to the vegetal cortex of *Xenopus* oocytes. Early in oogenesis (stage I), early pathway RNAs (e.g., Xlsirt, Xcat-2, and Xwnt11 RNAs; shown in red) accumulate in the mitochondrial cloud (MC, red circle). During stage II, as the MC breaks down, these RNAs are translocated to the vegetal cortex. By stage III they are anchored within a discrete region (denoted by a red disk), where they remain through the remainder of oogenesis. By contrast, during the earliest stages of oogenesis, Vg1 RNA (blue) is found uniformly distributed within the stage I-II oocytes. Vg1 RNA begins to translocate to the vegetal pole by early stage III (dark blue), initially to an area that overlaps the position of the early RNAs (red). During stage IV, Vg1 RNA is cleared from the animal hemisphere cytoplasm; by stages V-VI, RNA is anchored to the vegetal cortex. In contrast to the restricted cortical position occupied by the early RNAs (red), Vg1 RNA (blue) extends along the vegetal cortex from the apex of the vegetal pole toward the marginal zone.

the MC (70). Again, the location of the MC relative to the GV in these previtellogenic oocytes is one of the first indicators of polarity as the MC is thought to reside on the vegetal side of the GV (25). By early stage II, these RNAs appear to sort to specific regions within the MC: the Xlsirt RNAs appear evenly distributed along the surface whereas Xcat-2 mRNA forms a ring around the outside and Xwnt11 mRNA becomes situated toward the center (31). By late stage II-early stage III of oogenesis, the MC breaks down (25) and appears as a wedge-like shape with its apex near the GV and its base expanding toward the vegetal cortex (31). Translocation from the dispersed MC and the anchoring of these RNAs within the vegetal cortex form the third step of the pathway (30, 31). Once anchored, the RNAs are confined within a relatively restricted distribution pattern where they remain throughout the remainder of oogenesis (30–33).

After fertilization the RNAs are distributed in the vegetal cortex in islands, reminiscent of germ plasm (30, 32, 33, 72). During cleavage, the RNAs are inherited with a subset of vegetal blastomeres, which suggests these RNAs are components of the germ plasm (30, 32, 33, 72). Further evidence that the RNAs localized through the early pathway are components of the germ plasm comes from ultrastructural analysis, in which Xcat-2 was detected on germinal granules in oocytes; Xlsirts and Xwnt11, by contrast, were associated with the fibrillar network of the germ plasm (34). It has been proposed that localization through the early pathway may serve to distribute these RNAs into the germ cell lineages (34).

Vegetal transport of the early RNAs does not appear to rely on any cytoskeletal components, since the association of the localized RNAs with the MC is not disrupted by treatment with either nocodazole or cytochalasin B, microtubule and microfilament depolymerizing agents, respectively (31, 70). Moreover, integrity of the MC is unaffected by these drugs (25). Zhou and King (67) have suggested that localization of Xcat-2 RNA to the MC may occur by selective entrapment rather than active transport. However, anchoring the RNAs to the cortex appears to be microfilament dependent, as treatment with cytochalasin B released the respective RNAs from their cortical positions (31). Rather than freely diffusing throughout the cytoplasm after drug treatment, the RNAs remained as discrete particles, perhaps suggesting an interaction with intermediate filament components of the cortex (31). As we shall see, the early pathway is distinct from the late or Vg-like pathway by virtue not only of its timing (stages I-II), but also its reliance on transport through the MC and a lack of dependence on microtubules.

## THE LATE PATHWAY

Vg1 RNA exemplifies localization by the late pathway, and has been the focus of experiments to elucidate the pathway. However, this pathway is not limited to Vg1 RNA, as localization of VegT RNA proceeds via the late pathway (46) and other, perhaps as yet undiscovered, RNAs are likely to use this mechanism as well. Hallmarks of the late pathway include a reliance on the cytoskeleton and vegetal translocation of RNA during stages III-IV (see Fig. 2).

Vg1 mRNA is found uniformly distributed within the oocyte during stages I-II of oogenesis (69) and is excluded from the MC (72). By stage III of oogene-

sis, a portion of Vg1 mRNA is detected in the vegetal cortex in a region that appears to overlap the positions of the early-localized RNAs (31). During mid-oogenesis (stages III-IV), Vg1 RNA is translocated to the vegetal hemisphere, with a concomitant 'clearing' of Vg1 mRNA from the animal hemisphere cytoplasm (69). Analysis of sectioned stage III oocytes revealed an apparent channeling of Vg1 transcripts toward the position of the early-localized RNAs, particularly to the site occupied by the Xlsirt RNAs within the vegetal cortex (31). By stages V-VI, Vg1 mRNA occupies a tight cortical shell from the apex of the vegetal pole to the future marginal zone (69), where it remains until maturation, when Vg1 mRNA is released from the cortex and diffuses toward the equatorial region (39). Vg1 mRNA along with its protein product is inherited by the vegetal blastomeres during cleavage (73, 74). Mis-expression of either Vg1 or VegT in the animal hemisphere leads to expression of mesoderm (and endoderm in some cases) in cells that would normally form ectoderm (41, 42, 46–50); depletion of maternal VegT mRNA results in dramatic repatterning of the germ layers (45), underscoring the importance of regulating vegetal localization of these RNAs.

## THE ROLE OF THE CYTOSKELETON IN Vg1 RNA LOCALIZATION

In contrast to what is observed for early-localized RNAs, Vg1 mRNA localization by the late pathway depends on cytoskeletal elements. Biochemical experiments examining the association of Vg1 mRNA with cytoskeletal components revealed a 35- to 50-fold enrichment of Vg1 mRNA in the detergent-insoluble fraction of stage VI oocytes (64, 75) Upon maturation, Vg1 mRNA was released into the soluble fraction, coinciding with the breakdown of cortical cytokeratin filaments (64, 75, 76). Moreover, Vg1 RNA is also selectively associated with isolated vegetal, but not animal, cortices from stage VI oocytes, which were shown to contain microtubule, microfilament, and cytokeratin networks (57). Yisraeli et al. (64) assayed the effects of cytoskeletal inhibitors on the distribution of Vg1 mRNA during the periods of active localization. Disruption of the microtubule network by depolymerizing agents such as nocodazole led to a dispersal of Vg1 mRNA within the cytoplasm of late stage III oocytes, suggesting that the translocation process is microtubule dependent (64). Similar treatment of stage VI oocytes had no effect on the anchoring of Vg1 to the vegetal cortex, but treatment of late stage oocytes with cytochalasin B to disrupt microfilaments led to a release (although incomplete) of Vg1 mRNA from the cortex (64). By contrast, cytochalasin B treatment had no effect on Vg1 translocation in stage III oocytes (64). Consequently, association of Vg1 mRNA with the vegetal cortex is dependent at least in part on the integrity of the microfilament network within the oocyte cortex. Based on these results, Yisraeli et al. (64) proposed a two-step model for Vg1 RNA localization in which microtubule-mediated translocation is followed by microfilament-dependent anchoring at the vegetal cortex. As a corollary to this, many have wondered whether translocation of Vg1 RNA might rely on microtubule-dependent motor proteins. However, an interaction between a localized RNA and any motor protein has yet to be identified.

## POTENTIAL CROSS-TALK BETWEEN THE EARLY AND LATE PATHWAYS

It is clear that there are two spatially and temporally distinct vegetal localization pathways in *Xenopus* oocytes, which differ further in a requirement for cytoskeletal elements. However, several lines of evidence suggest there is significant cross-talk between the two pathways. The first evidence for interactions between the pathways came from experiments suggesting a link between localization of Xlsirt RNAs and Vg1 mRNA (77). The inability of the Xlsirt RNAs to be translated, combined with their localization by the early pathway (31), led to suggestions that they may act either as a structural component of the cortex or be involved in the localization of other RNAs. Evidence for the latter was provided by a study in which the injection of antisense Xlsirt oligodeoxynucleotides into stage IV oocytes led to a destruction of Xlsirt RNA (77). Concomitant with this was a release into the cytoplasm of Vg1, but not Xcat-2, mRNA from its original cortical position (77). Although no direct Xlsirt RNA-Vg1 RNA link has been established, these results illustrate a requirement for Xlsirt RNA in the anchoring of Vg1.

Further support for interactions between the two pathways was obtained by refined temporal and spatial analysis of the pathways. By late stage II of oogenesis, while the majority of Vg1 mRNA is uniformly distributed within the oocyte, a subset of the RNA appears to occupy a region of the cortex occupied by the early RNAs (31). During early stage III, the RNA assumes a wedge-like distribution in the vegetal hemisphere, with apparent channeling of the RNA toward the region of the cortex containing the early RNAs (31). This wedge region may comprise a subdomain of ER, as suggested through experiments that revealed an apparent overlap in the distribution pattern of TRAPα, an integral ER protein (78), or GRP78, a protein found in the lumen of the ER (72), with the wedge-shaped pattern of Vg1 mRNA in stage II/III oocytes. The interaction between Vg1 RNA

and the wedge domain is microtubule independent and may represent an early step in the late pathway (72). Based on these observations, Kloc and Etkin (72) have proposed a model in which the movement of the MC during these early oogenic stages somehow determines or orients the correct cytoskeletal tracks required for the localization of RNAs using the late pathway. It is intriguing that the MC itself contains a γ-tubulin-positive structure that may function as a microtubule organizing center with the capacity to establish these microtubule tracks (72). By this model, establishment of the late pathway is dependent on the early pathway.

Last, some early RNAs are capable of using either pathway, as has been demonstrated for Xcat-2 and Xpat (33, 71). Whereas the endogenous RNAs are localized by the early pathway (30, 31, 33), exogenous Xcat-2 or Xpat mRNAs can localize independently of the MC and exploit the late pathway when injected into stage IV oocytes (33, 71). This ability to use either pathway raises questions about selectivity and specificity of the localization pathways. These include how the 'correct' pathway is chosen and what additional factors are involved in both the selection and use of this correct pathway during a defined period of oogenesis. Some insight into this is provided by experiments to render Vg1 capable of using the early pathway (72). Endogenous Vg1 RNA is uniformly distributed within a stage I oocyte (69), and is conspicuously absent from the MC during this stage (31). However, Vg1 sequences can be targeted to the MC, as shown recently by Kloc and Etkin (72) using an injected chimeric Xlsirt-Vg1 mRNA. These experiments demonstrate that given the right signal—in this case, Xlsirt sequences—Vg1 can associate with the MC and potentially use the early pathway. Moreover, endogenous Vg1 RNA must lack a signal recognizable by the early pathway, whereas Xcat-2 and Xpat RNAs apparently contain signals recognizable by both pathways.

## RNA RECOGNITION: THE ROLE OF *CIS*-ACTING ELEMENTS

What are the specific signals that mediate RNA localization? It is increasingly clear that recognition of RNAs for localization relies on *cis*-acting sequences residing in the 3′ UTRs of the localized messages (reviewed in ref 1). For some localized RNAs, detailed mapping studies have revealed discrete sequence elements within the 3′ UTRs that are sufficient to direct proper localization (see ref 1 and text below). In most cases these sequence elements are relatively large, and this has complicated efforts to understand how such sequences can act as signals to promote localization.

One view of RNA recognition is that all localized RNAs should inherently contain relatively conserved sequence elements that are recognized by the existing localization machinery to promote localization. Such an element may be extensive and consist of a stretch of conserved primary sequence elements. Conversely, recognition of an element by the localization machinery may depend predominantly on the secondary or tertiary structures adopted by the RNA (79). Such structures include stem loops, bulges, tetraloops, double-stranded regions, and pseudoknots, any of which may provide recognition and/or binding sites for *trans*-acting localization factors. In contrast to an extensive albeit generalized secondary structure, a localization element may contain significantly smaller, discrete subelements with local domains of secondary structure. These subelements may independently direct individual steps of a localization pathway or could act in concert with other elements to coordinate the entire pathway. In such a model, it seems reasonable that a set of RNAs (for instance, Xcat-2 and Xlsirts) that use the same localization pathway also contain shared recognition elements. Likewise, in multistep localization pathways, RNAs that share only a subset of steps may contain both similar and modified or additional signals. However, at present, the solution to the recognition problem does not appear to be a simple one.

Analysis of the 3′ UTR sequence element requirements for Xcat-2 mRNA localization has identified at least two localization signals. The first is a 250 nt region within the 5′ end of the 3′ UTR that is required for translocation by the early pathway (67). The Xlsirt RNAs, which are also localized by the early pathway, are short interspersed repeat transcripts containing repeat units of ~80 nt flanked by unique sequences (31, 80). Analysis of sequence requirements for Xlsirt localization revealed that the unique sequences did not support localization and that an array of three repeat sequences were able to confer localization on a nonlocalized reporter transcript (58). Unfortunately, comparison of these two early localization signals reveals no immediately apparent similarities or homologies (although shared structural homologies will undoubtedly be difficult to discern). The second localization signal found in the 3′ UTR of Xcat-2 directs localization along the late pathway (71). The signal is bipartite consisting of a 150 nt region (which is contained within the 250 nt early localization signal) at the 5′ end of the 3′ UTR, along with the last 120 nt of the 3′ UTR (71). The recognition of these signals, particularly from one another, is a critical first step in the localization process, as the recognition event may ultimately determine the RNA's final subcellular destination.

Localization of Vg1 mRNA is directed by a 340 nt

sequence element contained within the 5′ half of its 3′ UTR (81). This signal is bipartite as well: two partially redundant subelements of 85 nt and 140 nt at the 5′ and 3′ ends of the element are sufficient for localization (82). Redundancy within the element is also indicated, as much of the sequence can be deleted, 15 nt at a time, and still retain function (82). Remarkably, duplication of the first 85 nt of the element can alone direct all steps in the Vg1 localization pathway (82), suggesting that the Vg1 localization element may be composed of relatively conserved and potentially redundant sequence motifs that are reiterated within the element. Indeed, five such reiterated motifs have been identified (78, 82): E1 (UAUUUCUA, two copies), E2 (UUCAC, five copies), E3 (UGCACAGAG, two copies) E4 (CUGUUA, three copies), and VM1 (UUUCUA, three copies). Two of these motifs, E2 and VM1, have been shown to be critical for localization (78, 82). Deletion of all copies of E2 within the 340 nt Vg1 localization element abolished localization, whereas similar deletion of E1, E3, or E4 sequences impaired but did not destroy localization (78). For VM1, introduction of base changes within the motifs abolished localization (82), as did 20 nt substitutions, which removed all copies of the motif (83). Thus, critical sequence motifs within the Vg1 localization element may at least in part be represented by reiterated sequence motifs as small as 5 to 6 nt, and these motifs have been shown to provide binding sites for *trans*-acting localization factors (78, 82–84; unpublished results[3]).

## RNA RECOGNITION: *TRANS*-ACTING FACTORS

Distinct from the identification of the RNA and its destination are the mechanism and machinery that carry out the localization process. Presumably, localized RNAs interact through their localization elements (acting as recognition and/or binding sites) with *trans*-acting factors. The complement of these interactions may form a specific ribonucleoprotein (RNP) complex that is responsible for directing localization. Until recently, little has been known about the characteristics of specific *trans*-acting factors that interact with a given localized RNA. Moreover, understanding regarding the precise molecular interactions between these *cis*- and *trans*-acting factors has been lacking.

[3] Cote, C. A., Gautreau, D., Terry, N. A., and Mowry, K. L. A *Xenopus* protein related to hnRNP I has an essential role in cytoplasmic RNA localization. Manuscript submitted for publication.

## INTERACTIONS BETWEEN THE Vg1 LOCALIZATION ELEMENT AND PROTEIN FACTORS

Biochemical approaches aimed at identifying proteins that interact directly with localized RNAs are now providing insight into the RNA–protein interactions that underlie localization. Through UV-crosslinking analysis, a set of oocyte proteins that bind in a sequence-specific manner to the Vg1 localization element were identified as candidate localization factors (78, 85, 86). Some or all of these proteins could play important roles, as localization was suggested to proceed through formation of an RNP complex containing the Vg1 localization element and multiple proteins (86). The different proteins within an RNP complex may perform distinct roles in the localization process. For example, a 69 kDa Vg1 RNA binding protein (Vg1RBP) that binds *in vitro* to the Vg1 localization element (85) has been reported to be a microtubule-associated protein that may mediate an interaction between Vg1 RNA and microtubules (87). In addition to the 69 kDa Vg1RBP, five other proteins (VgRBP78, VgRBP60, VgRBP40, VgRBP36, VgRBP33) were identified and the binding activities of all but VgRBP78 were found to be enriched during stages III and IV of oogenesis, the period of active localization (86). Finally, a 75 kDa protein (Vera) was identified by its ability to bind to the Vg1 mRNA localization element and to colocalize with components of the ER (84). Thus far, three of the Vg1 RNA binding proteins (Vg1RBP, VgRBP60, and Vera) have been implicated as important players in the localization process.

Vg1RBP and Vera have both been purified and cloned recently (83, 84), with a surprising result. The cDNA sequences for Vg1RBP and Vera are identical (83, 84) as well as highly homologous to ZBP-1, an RNA binding protein that functions to localize β-actin mRNA in chick fibroblasts (88, 89). Vg1RBP/Vera is likely to play a significant role in the localization process as disruption in Vg1RBP and Vera binding activities correlated with defects in Vg1 mRNA localization (78, 83). However, there is disagreement as to the RNA binding site for Vg1RBP/Vera. Havin et al. (83) have reported that Vg1RBP interacts specifically with two discrete regions (VM1 motifs) of the localization element, which overlap two previously identified protein binding sites (86). In the analysis of Vg1RBP binding sites, 20 nt substitutions were shown to interfere with both Vg1RBP binding *in vitro* and localization *in vivo* (83). By contrast, Deshler et al. (78, 84) have reported that Vera interacts with sequences (E2 motifs) that lie outside these regions. Deletion of all E2 motifs within the Vg1 localization element was shown to disrupt both Vera binding *in vitro* and localization *in vivo* (78). Moreover, using a transcript containing

multiple copies of the E2 motif, Vera was shown to bind directly to E2 (84). Thus, Vera is reported to bind to E2 motifs, whereas Vg1RBP is reported to recognize VM1 motifs. Based on the cDNA sequences, the predicted molecular mass for Vg1RBP/Vera is 69 kDa (83, 84). The difference in the published molecular masses (69 kDa for Vg1RBP and 75 kDa for Vera) may reflect processing events that could influence both the binding specificity and the activity of a protein. Future experiments to further refine the binding specificity of Vg1RBP/Vera will undoubtedly resolve this issue.

In a separate analysis of the VM1 sequence, point mutations within the motif revealed the 60 kDa Vg1 RNA binding protein, VgRBP60, to have a critical role in localization (82; see footnote 3). Base changes within VM1 were shown to eliminate binding of VgRBP60 alone and abolish localization *in vivo* (82; see footnote 3). In these experiments, binding of Vg1RBP/Vera was apparently unaffected. Purification and cloning of VgRBP60 revealed this protein to be highly homologous to a human hnRNP protein, hnRNP I (see footnote 3). In human cells, hnRNP I and PTB, which are alternatively processed isoforms of one another (90–92), have been implicated in diverse aspects of RNA biogenesis, including control of alternative splicing and nuclear RNA transport (reviewed in ref 93). hnRNP I/PTB has also been reported to shuttle between the nucleus and cytoplasm (94), presenting intriguing mechanistic possibilities. For example, could the association of a specific complement of RNA binding proteins with an RNA in the nucleus act to target that RNA molecule for cytoplasmic localization? On this note, it is interesting that the Vg1RBP/Vera sequence contains both a nuclear export signal and a nuclear localization signal (83, 84). In addition, another hnRNP molecule, hnRNP A2, has been shown to bind specifically to the RNA signal for transport of myelin basic protein mRNA in oligodendrocytes (95). Thus, a model may be implicated in which the earliest steps in the RNA localization pathway occur in the nucleus rather than in the cytoplasm.

## CONCLUDING REMARKS

Not surprisingly, a review of RNA localization in *Xenopus* has raised more questions than answers. However, several generalities can be drawn. For instance, all *cis*-acting elements identified thus far map within the 3′ UTRs of the respective RNAs. In the past, *cis* element identification had been focused primarily over a broad range, identifying relatively expansive regions of the 3′ UTR that are sufficient to direct localization. Only within the past few years have extensive studies been undertaken to further refine the precise RNA–protein interactions necessary for the localization of a given RNA. Work toward elucidation of the mechanisms directing the localization of RNAs in *Xenopus* oocytes is at an exciting stage. Future results promise to be most intriguing. FJ

We thank Jim Denegre for comments on the manuscript. Our work on RNA localization in *Xenopus* is supported by a grant (R01 HD30699) from the National Institutes of Health.

## REFERENCES

1. St. Johnston, D. (1995) The intracellular localization of messenger RNAs. Cell 81, 161–170
2. Cheng, H., and Bjerknes, M. (1989) Asymmetric distribution of actin mRNA and cytoskeletal pattern generation in polarized epithelial cells. J. Mol. Biol. 210, 541–549
3. Hoock, T. C., Newcomb, P. M., and Herman, I. H. (1991) β Actin and its mRNA are localized at the plasma membrane and the regions of moving cytoplasm during the cellular response to injury. J. Cell Biol. 112, 653–664
4. Hill, M. A., and Gunning, P. G. (1993) Beta and gamma actin mRNAs are differentially located within myoblasts. J. Cell Biol. 122, 825–832
5. Kislauskis, E. H., Li, Z., Singer, R. H., and Taneja, K. L. (1993) Isoform-specific 3′-untranslated sequences sort α-cardiac β-cytoplasmic actin messenger RNAs to different cytoplasmic compartments. J. Cell Biol. 123, 165–172
6. Steward, O. (1997) mRNA localization in neurons: a multipurpose mechanism? Neuron 18, 9–12
7. Gavis, E. R. (1997) Expeditions to the pole: RNA localization in *Xenopus* and *Drosophila*. Trends Cell Biol. 7, 485–492
8. Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M., and Nüsslein-Volhard, C. (1988) The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. EMBO J. 7, 1749–1756
9. Wang, C., and Lehmann, R. (1991) *Nanos* is the localized posterior determinant in *Drosophila*. Cell 66, 637–647
10. Neuman-Silberberg, F. S., and Schüpbach, T. (1993) The *Drosophila* dorsoventral patterning gene *gurken* produces a dorsally localized RNA and encodes a TGFα-like protein. Cell 75, 165–174
11. Ephrussi, A., and Lehmann, R. (1992) Induction of germ cell formation by *oskar*. Nature (London) 358, 387–392
12. Coggins, L. W. (1973) An ultrastructural and radioautographic study of early oogenesis in the toad *Xenopus laevis*. J. Cell Sci. 12, 71–93
13. Al-Muktar, K. A. K., and Webb, A. C. (1971) An ultrastructural study of primordial germ cells, oogonia and early oocytes in *Xenopus laevis*. J. Embryol. Exp. Morphol. 26, 195–217
14. Kalt, M. R. (1973) Ultrastructural observations on the germ line of *Xenopus laevis*. Zeit. Zellforsch. 138, 41–62
15. Gerhart, J., Black, S., Scharf, S., Gimlich, R., Vincent, J.-P., Danilchik, M., Rowning, B., and Roberts, J. (1986) Amphibian early development. BioScience 36, 541–549
16. Danilchik, M. V., and Gerhart, J. (1987) Differentiation of the animal-vegetal axis in *Xenopus laevis* oocytes. I. Polarized translocation of platelets establishes the yolk gradient. Dev. Biol. 122, 101–112
17. Klymkowsky, M. W., and Karnovsky, A. K. (1994) Morphogenesis and the cytoskeleton: studies of the *Xenopus* embryo. Dev. Biol. 165, 372–384
18. Gard, D. (1995) Axis formation during amphibian oogenesis: reevaluating the role of the cytoskeleton. Curr. Top. Dev. Biol. 30, 215–252
19. Heasman, J. (1997) Patterning the *Xenopus* blastula. Development 124, 4179–4191
20. Dale, L., and Slack, J. M. W. (1987) Fate map of the 32 cell stage of *Xenopus laevis*. Development 99, 527–551
21. Vincent, J.-P., and Gerhart, J. C. (1987) Subcortical rotation in *Xenopus* eggs: An early step in embryonic axis specification. Dev. Biol. 123, 526–539

22. Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rwoning, B., and Stewart, R. (1989) Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of embryonic dorsal development. Development Suppl., 37–51
23. Wilson, E. B. (1925) *The Cell in Development and Heredity*, The MacMillian Co., New York
24. Eddy, E. M. (1975) Germ plasm and the differentiation of the germ cell line. Int. Rev. Cytol. 43, 229–281
25. Heasman, J., Quarmby, J., and Wylie, C. C. (1984) The mitochondrial cloud of *Xenopus* oocytes: The source of germinal granule material. Dev. Biol. 105, 458–469
26. Czolowska, R. (1969) Observations on the origin of the 'germinal cytoplasm' in *Xenopus laevis*. J. Embryol. Exp. Morphol. 22, 229–251
27. Blackler, A. W. (1958) Contribution to the study of germ-cells in the Anuran. J. Embryol. Exp. Morphol. 6, 491–503
28. Ressom, R. E., and Dixon, K. E. (1988) Relocation and reorganization of germ plasm in *Xenopus* embryos after fertilization. Development 103, 507–518
29. Savage, R. M., and Danilchik, M. V. (1993) Dynamics of germ plasm localization and its inhibition by ultraviolet irradiation in early cleavage *Xenopus* embryos. Dev. Biol. 157, 371–382
30. Forristall, C., Pondel, M., Chen, L., and King, M. L. (1995) Patterns of localization and cytoskeletal association of two vegetally localized RNAs, Vg1 and Xcat-2. Development 121, 201–208
31. Kloc, M., and Etkin, L. (1995) Two distinct pathways for the localization of RNAs at the vegetal cortex in *Xenopus* oocytes. Development 121, 287–297
32. Houston, D. W., Zhang, J., Maines, J. Z., Wasserman, S. A., and King, M. L. (1998) A *Xenopus* DAZ-like gene encodes an RNA component of germ plasm and is a functional homologue of *Drosophila boule*. Development 125, 171–180
33. Hudson, C., and Woodland, H. R. (1998) *Xpat*, a gene expressed specifically in germ plasm and primordial germ cells of *Xenopus laevis*. Mech. Dev. 73, 190–198
34. Kloc, M., Larabell, C., Chan, A. P.-Y., and Etkin, L. D. (1998) Contribution of METRO pathway localized molecules to the organization of the germ cell lineage. Mech. Dev. 75, 81–93
35. Gurdon, J. B., Mohun, T. J., Fairman, S., and Brennan, S. (1985) All components required for eventual activation of muscle-specific actin are localized in the sub-equitorial region of an uncleaved amphibian egg. Proc. Natl. Acad. Sci. USA 82, 139–143
36. Lemaire, P., and Gurdon, J. B. (1994) A role for cytoplasmic determinants in mesoderm patterning: cell-autonomous activation of the goosecoid and Xwnt-8 genes along the dorsoventral axis of early *Xenopus* embryos. Development 120, 1191–1199
37. Nieuwkoop, P. D. (1969) The formation of mesoderm in urodelean amphibians. I. Induction by the endoderm. Roux's Arch. EntwMech. Org. 162, 341–373
38. Kessler, D. S., and Melton, D. A. (1994) Vertebrate embryonic induction: Mesodermal and neural patterning. Science 266, 596–604
39. 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-β. Cell 51, 861–867
40. Ku, M., and Melton, D. A. (1993) Xwnt-11: a novel maternally expressed *Xenopus* wnt gene. Development 119, 1161–1173
41. Dale, L., Matthews, G., and Colman, A. (1993) Secretion and mesoderm-inducing activity of the TGF-β-related domain of *Xenopus* Vg1. EMBO J. 12, 4471–4480
42. Thomsen, G. H., and Melton, D. A. (1993) Processed Vg1 protein is an axial mesoderm inducer in *Xenopus*. Cell 74, 433–441
43. Kessler, D. S., and Melton, D. A. (1995) Induction of dorsal mesoderm by soluble, mature Vg1 protein. Development 121, 2155–2164
44. Joseph, E. M., and Melton, D. M. (1998) Mutant Vg1 ligands disrupt endoderm and mesoderm formation in *Xenopus* embryos. Development 125, 2677–2685
45. Zhang, J., Houston, D. W., King, M. L., Payne, C., Wylie, C., and Heasman, J. (1998) The role of maternal VegT in establishing the primary germ layers in *Xenopus* embryos. Cell 94, 515–524
46. Zhang, J., and King, M. L. (1996) *Xenopus* VegT RNA is localized to the vegetal cortex during oogenesis and encodes a novel T-box transcription factor involved in mesodermal patterning. Development 122, 4119–4129
47. Lustig, K. D., Kroll, K. L., Sun, E. E., and Kirschner, M. W. (1996) Expression cloning of a *Xenopus* T-related gene (*Xombi*) involved in mesodermal patterning and blastopore lip formation. Development 122, 4001–4012
48. Stennard, F., Carnac, G., and Gurdon, J. B. (1996) The *Xenopus* T-box gene, Antipodean, encodes a vegetally localized maternal mRNA and can trigger mesoderm formation. Development 122, 4179–4188
49. Horb, M. E., and Thomsen, G. H. (1997) A vegetally localized T-box transcription factor in *Xenopus* eggs specifies mesoderm and endoderm and is essential for embryonic mesoderm formation. Development 124, 1689–1698
50. Henry, G. L., Brivanlou, I. H., Kessler, D. S., Hemmati-Brivanlou, A., and Melton, D. A. (1996) TGFβ signals and a pre-pattern in *Xenopus laevis* endodermal development. Development 122, 1007–1015
51. Sagata, N., Okuyama, K., and Yamana, K. (1981) Localization and segregation of maternal RNAs during early cleavage of *Xenopus laevis* embryos. Dev. Growth Differ. 23, 23–32
52. Carpenter, C. D., and Klein, W. H. (1982) A gradient of poly(A)$^+$ sequences in *Xenopus laevis* eggs and embryos. Dev. Biol. 91, 43–49
53. Phillips, C. R. (1982) The regional distribution of poly(A) and total RNA concentrations during early *Xenopus* development. J. Exp. Zool. 223, 265–275
54. King, M. L., and Barklis, E. (1985) Regional distribution of maternal messenger RNA in the amphibian oocyte. Dev. Biol. 112, 203–212
55. Rebagliati, M. R., Weeks, D. L., Harvey, R. P., and Melton, D. A. (1985) Identification and cloning of localized maternal mRNAs from *Xenopus* eggs. Cell 42, 769–777
56. 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. Development 117, 377–386
57. Elinson, R. P., King, M. L., and Forristall, C. (1993) Isolated vegetal cortex from *Xenopus* oocytes selectively retains localized mRNAs. Dev. Biol. 160, 554–562
58. Kloc, M., Spohr, G., and Etkin, L. D. (1993) Translocation of repetitive RNA sequences with the germ plasm in *Xenopus* oocytes. Science 262, 1712–1714
59. Hinkley, C. S., Martin, J. F., Leibham, D., and Perry, M. (1992) Sequential expression of multiple POU proteins during amphibian early development. Mol. Cell. Biol. 12, 638–649
60. Reddy, B. A., Kloc, M., and Etkin, L. D. (1992) The cloning and characterization of a localized maternal transcript in *Xenopus laevis* whose zygotic counterpart is detected in the CNS. Mech. Dev. 39, 143–150
61. Kloc, M., Reddy, B. A., Miller, M., Eastman, E., and Etkin, L. D. (1991) x121: A localized maternal transcript in *Xenopus laevis*. Mol. Reprod. Dev. 28, 341–345
62. Schroeder, K. E., and Yost, H. J. (1996) *Xenopus* poly(A) binding protein maternal mRNA is localized during oogenesis and associated with large complexes in blastula. Dev. Genet. 19, 268–276
63. Hudson, J. W., Alarcón, V. B., and Elinson, R. P. (1996) Identification of new localized RNAs in the *Xenopus* oocyte by differential display PCR. Dev. Genet. 19, 190–198
64. Yisraeli, J., 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 RNA. Development 108, 289–298
65. Ding, D., Parkhurst, S. M., Halsell, S. R., and Lipshitz, H. D. (1993) Dynamic Hsp83 RNA localization during *Drosophila* oogenesis and embryogenesis. Mol. Cell. Biol. 13, 3773–3781
66. Davis, I., and Ish-Horowicz, D. (1991) Apical localization of pair-rule transcripts requires 3? sequences and limits protein diffusion in the *Drosophila* blastoderm embryo. Cell 67, 927–940
67. Zhou, Y., and King, M. L. (1996) Localization of *Xcat-2* RNA, a putative germ plasm component, to the mitochondrial cloud of *Xenopus* stage I oocytes. Development 122, 2947–2953
68. Perry-O'Keefe, H., Kintner, C. R., Yisraeli, J., and Melton, D. A. (1990) The use of in situ hybridisation to study the localisation of maternal mRNAs during *Xenopus* oogenesis. In: In situ Hybridisation: Application to Developmental Biology and Med-

icine (Harris, N., and Wilkinson, D. G., eds) pp. 115–130, Cambridge University Press, Cambridge
69. Melton, D. A. (1987) Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes. Nature (London) 328, 80–82
70. Kloc, M., Larabell, C., and Etkin, L. D. (1996) Elaboration of the messenger transport organizer pathway for localization of RNA to the vegetal cortex of *Xenopus* oocytes. Dev. Biol. 180, 119–130
71. Zhou, Y., and King, M. L. (1996) RNA transport to the vegetal cortex of *Xenopus* oocytes. Dev. Biol. 179, 173–183
72. Kloc, M., and Etkin, L. D. (1998) Apparent continuity between the messenger transport organizer and late RNA localization pathways during oogenesis in *Xenopus*. Mech. Dev. 73, 95–106
73. Dale, L., Matthews, G., Tabe, L., and Colman, A. (1989) Developmental expression of the protein product of Vg1, a localized maternal mRNA in the frog *Xenopus laevis*. EMBO J. 8, 1057–1065
74. Tannahill, D., and Melton, D. A. (1989) Localized synthesis of the Vg1 protein during early *Xenopus* development. Development 106, 775–785
75. Pondel, M. D., and King, M. L. (1988) Localized maternal mRNA related to transforming growth factor mRNA is concentrated in a cytokeratin-enriched fraction from *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA 85, 7612–7616
76. Klymkowsky, M. W., Maynell, L. A., and Nislow, C. (1991) Cytokeratin phosphorylation, cytokeratin filament severing and the solubilization of the maternal mRNA Vg1. J. Cell Biol. 114, 787–797
77. Kloc, M., and Etkin, L. D. (1994) Delocalization of Vg1 mRNA from the vegetal cortex in *Xenopus* oocytes after destruction of xlsirt RNA. Science 265, 1101–1103
78. 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
79. Wyatt, J. R., and Tinoco, I. (1993) RNA structural elements and RNA function. In: The RNA World (Gesteland, R. F., and Atkins, J. F., eds) pp. 465–498, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
80. Spohr, G., Reith, W., and Sures, I. (1981) Organization and sequence analysis of a cluster of repetitive DNA elements from *Xenopus laevis*. J. Mol. Biol. 151, 573–592
81. Mowry, K. L., and Melton, D. A. (1992) Vegetal messenger RNA localization directed by a 340-nt sequence element in *Xenopus* oocytes. Science 255, 991–994
82. Gautreau, D., Cote, C. A., and Mowry, K. L. (1997) Two copies of a subelement from the Vg1 RNA localization sequence are sufficient to direct vegetal localization in *Xenopus* oocytes. Development 124, 5014–5020
83. Havin, L., Git, A., Elisha, Z., Oberman, F., Yaniv, K., Schwartz, S. P., Standart, N., and Yisraeli, J. K. (1998) RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization. Genes Dev. 12, 1593–1598
84. 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
85. Schwartz, S. P., Aisenthal, L., Elisha, Z., Oberman, F., and Yisraeli, J. K. (1992) A 69-kDa RNA-binding protein from *Xenopus* oocytes recognizes a common motif in two vegetally localized maternal mRNAs. Proc. Natl. Acad. Sci. USA 89, 11895–11899
86. Mowry, K. L. (1996) Complex formation between stage-specific oocyte factors and a *Xenopus* mRNA localization element. Proc. Natl. Acad. Sci. USA 93, 14908–14613
87. Elisha, Z., Havin, L., Ringel, I., and Yisraeli, J. K. (1995) Vg1 RNA binding protein mediates the association of Vg1 RNA with microtubules in *Xenopus* oocytes. EMBO J. 14, 5109–5114
88. Kislauskis, E. H., Zhu, X., and Singer, R. H. (1994) Sequences responsible for intracellular localization of β-actin messenger RNA also affect cell phenotype. J. Cell Biol. 127, 441–451
89. Ross, A. F., Oleynikov, Y., Kislauskis, E. H., Taneja, K., and Singer, R. H. (1997) Characterization of a β-actin mRNA zipcode-binding protein. Mol. Cell. Biol. 17, 2158–2165
90. Gil, A., Sharp, P. A., Jamison, S. F., and Garcia-Blanco, M. A. (1991) Characterization of cDNAs encoding the polypyrimidine tract-binding protein. Genes Dev. 5, 1224–1236
91. Patton, J. G., Mayer, S. A., Tempst, P., and Nadal-Ginard, B. (1991) Characterization and molecular cloning of polypyrimidine tract-binding protein: a component of a complex necessary for pre-mRNA splicing. Genes Dev. 5, 1237–1251
92. Ghetti, A., Pi;atnol-Roma, S., Michael, W. M., Morandi, C., and Dreyfuss, G. (1992) hnRNP I, the polypyrimidine tract-binding protein: distinct nuclear localization and association with hnRNAs. Nucl. Acids Res. 20, 3671–3678
93. Valcarcel, J., and Gebauer, F. (1997) Post-transcriptional regulation: the dawn of PTB. Curr. Biol. 7, 705–708
94. Michael, W. M., Siomi, H., Choi, M., Pi;atnol-Roma, S., Nakielny, S., Liu, Q., and Dreyfuss, G. (1995) Signal sequences that target nuclear import and nuclear export of pre-mRNA-binding proteins. Cold Spring Harbor Symp. Quant. Biol. 60, 663–668
95. Hoek, K. S., Kidd, G. J., Carson, J. H., and Smith, R. (1998) hnRNP A2 selectively binds the cytoplasmic transport sequence of myelin basic protein mRNA. Biochemistry 37, 1243–1371
96. Linnen, J. M., Bailey, C. P., and Weeks, D. L. (1993) Two related localized mRNAs from *Xenopus laevis* encode ubiquitin-like fusion proteins. Gene 128, 181–188
97. Weeks, D. L., and Melton, D. A. (1987) A maternal mRNA localized to the animal pole of *Xenopus* eggs encodes a subunit of mitochondrial ATPase. Proc. Natl. Acad. Sci. USA 84, 2798–2802
98. Gururajan, R., Mathews, L., Longo, F. J., and Weeks, D. L. (1994) An3 mRNA encodes an RNA helicase that co-localizes with nucleoli in *Xenopus* oocytes in a stage specific manner. Proc. Natl. Acad. Sci. USA 91, 2056–2060