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TRANSLATIONAL REGULATION AND RNA LOCALIZATION IN *DROSOPHILA* OOCYTES AND EMBRYOS

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■ **Abstract** Translational control is a prevalent means of gene regulation during *Drosophila* oogenesis and embryogenesis. Multiple maternal mRNAs are localized within the oocyte, and this localization is often coupled to their translational regulation. Subsequently, translational control allows maternally deposited mRNAs to direct the early stages of embryonic development. In this review we outline some general mechanisms of translational regulation and mRNA localization that have been uncovered in various model systems. Then we focus on the posttranscriptional regulation of four maternal transcripts in *Drosophila* that are localized during oogenesis and are critical for embryonic patterning: *bicoid* (*bcd*), *nanos* (*nos*), *oskar* (*osk*), and *gurken* (*grk*). *Cis*- and *trans*-acting factors required for the localization and translational control of these mRNAs are discussed along with potential mechanisms for their regulation.

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INTRODUCTION

There are several possible mechanisms for localizing specific proteins within a cell. Many proteins are directly targeted as such. However, proteins can be deployed at specific locations through mechanisms that operate on the mRNA that encodes them. For example, the mRNA can be localized in an untranslatable form and translated at the site where protein activity is required. Alternatively, protein localization can be accomplished strictly at the level of translational control, through the distribution or regulation of factors involved in either translational repression or activation. Proteolysis and posttranslational modification are further mechanisms by which protein activity can be spatially regulated.

Drosophila oogenesis and embryogenesis are valuable systems for studying protein localization since spatiotemporal organization of materials deposited in the egg is critical for establishing developmental decisions in the embryo. One idea that has emerged from work in *Drosophila* is that the mechanisms listed above are not mutually exclusive. Regulation of several transcripts involves an interplay between RNA localization and translational control. Many *Drosophila* mRNAs are specifically localized with the goal of producing a localized protein. Surprisingly, however, in several cases noted thus far, when mRNA localization is abrogated, protein localization and translation remain normal, implying an additional mechanism involving translational control.

Although this review focuses on the role of translational regulation in protein localization within the *Drosophila* oocyte and embryo, mechanisms uncovered from work on other systems are also discussed, since biochemistry has lagged behind genetics and cell biology in *Drosophila* until recently. First we outline some general models for translational regulation and RNA localization and then focus on the mechanisms that regulate the localized activities of four proteins: Bicoid (Bcd), and Nanos (Nos), which localize to the embryonic anterior and posterior, respectively; Oskar (Osk), which localizes to the oocyte posterior; and Gurken (Grk), which localizes to the anterodorsal corner of the oocyte. These factors represent some of the best-understood examples of protein localization occurring through a combination of mRNA localization and translational regulation. mRNA localization

(6, 112), translational control in development (259), and axis patterning in *Drosophila* (243) have recently been reviewed.

Drosophila Oogenesis and Syncytial Embryogenesis: A Synopsis

A *Drosophila* ovary is composed of a cluster of ovarioles, each of which consists of a string of egg chambers progressing through 14 morphologically defined developmental stages in an anterior to posterior direction (reviewed in 111, 215). A stem cell at the anterior end of each ovariole divides asymmetrically to generate a cystoblast, which divides four additional times with incomplete cytokinesis. A resultant 16-cell cyst contains cystocytes connected to each other by ring canals. One of the cystocytes will become the future oocyte while the other 15 will become nurse cells whose function during oogenesis is to synthesize and transport materials required for the growth and development of the oocyte.

As cysts move through the anterior region of the ovariole, the germarium, they become enclosed in a layer of somatic follicle cells. The oocyte acquires the most posterior position in the egg chamber, thus defining the anterior-posterior axis, and accumulates specific RNAs and proteins. In stage 8 the oocyte nucleus migrates to the anterodorsal corner of the oocyte, and asymmetric localization of several RNAs and proteins within the oocyte becomes apparent. Stages 8 to 10 are marked by rapid yolk uptake by the oocyte, resulting in substantial growth. From stages 10B to 12, the nurse cells transfer their cytoplasm into the oocyte through ring canals, and the mature egg is completed in stages 13 to 14.

Upon fertilization, 13 rapid synchronous mitotic divisions occur; these consist of DNA synthesis and mitosis but not cytokinesis, resulting in approximately 8000 nuclei sharing a common maternally inherited cytoplasm. Subsequently, these nuclei migrate peripherally within the embryo and are cellularized. This transition from the syncytial stage to the cellular blastoderm stage also corresponds to the transfer of developmental control from the maternal to the zygotic genome.

The oocyte and the syncytial embryo offer unique opportunities to study RNA and protein localization mechanisms within a single cell. In the germarium and during pre-vitellogenic stages of oogenesis, selected mRNAs such as *bcd*, *nos*, and *osk* are transcribed in the nurse cells and transported into the oocyte. *grk* is transcribed in the oocyte nucleus, although early in oogenesis it may also be transcribed in the nurse cells and transported into the oocyte. Later, a complex pattern of RNA and protein localization is established within the oocyte. *bcd* is anteriorly anchored by stage 8 of oogenesis and remains dormant until fertilization. *nos* achieves posterior localization within the oocyte by around stage 12 and is not translated until egg deposition. *osk* is posteriorly localized by stage 8, and translationally activated immediately upon localization. *grk* is posteriorly localized and translated within the oocyte prior to nuclear migration. Subsequently, *grk* mRNA relocates to the anterodorsal corner and is translated in this region.

MECHANISMS OF TRANSLATIONAL REGULATION

Translational repression is usually imposed at the step of initiation and generally involves binding of *trans*-acting factors to the noncoding region of a transcript. Binding can occur in the 5′ UTR, exemplified by the well-studied case of ferritin regulation (reviewed in 185). More frequently, however, repressors bind to the 3′ UTR and influence translation in a variety of ways. In this section, we first introduce the idea of mRNA circularization and then briefly discuss some models of translational repression and subsequent derepression/activation.

The Closed-Loop Model of Translation

mRNA molecules undergoing translation are thought to be circularized via interactions between *trans*-acting factors bound to the 5′ and 3′ ends (reviewed in 92, 187). This closed-loop model explains why a poly(A) tail on a transcript can stimulate translational efficiency, as was originally observed in reticulocyte extracts (44, 148) and also in vivo by analysis of poly(A) dynamics during development in various systems (4, 267). Circularization of mRNAs has been visualized in electron micrographs and by atomic force microscopy (26, 49, 87, 104, 252, 255). Linkage between 5′ and 3′ ends of a transcript is mediated at least in part through physical interaction between eIF4G, a component of the 5′ cap-binding complex eIF4F, and poly(A)-binding protein (PABP), which is bound in multiple copies to the poly(A) tail of a transcript (230). This interaction is believed to synergistically stimulate translational activation through altering either the mRNA affinity or the structural conformation of PABP or eIF4F subunits (reviewed in 187).

The mechanistic advantage of mRNA circularization to the process of translation remains unclear (reviewed in 74, 187). One idea is that it promotes reinitiation of ribosomes such that as they terminate translation at the 3′ end they can easily reinitiate on the same mRNA molecule by transferring to the 5′ end. Circularization also confers an advantage in terms of transcript stabilization because it protects both ends of the transcript from degradation. In addition, circularization could be a means to ensure that only full-length mRNAs, with a cap and poly(A) tail, will be translated, preventing translation of truncated transcripts. Developmentally regulated *trans*-acting factors that bind to the 3′ end of specific transcripts may influence translation at the level of initiation by affecting the ability of the mRNA to circularize (79).

General Translational Repression: mRNA Masking

mRNA masking refers to keeping mRNAs concealed in mRNP particles such that they are withheld from cellular processing events such as translation and degradation (reviewed in 214). Masking is thought to operate through proteins that bind to the mRNA and alter its conformation. At the correct time or place, the masking protein is influenced by a signal that alleviates its masking effect. Y-box proteins appear to be involved in mRNA masking (reviewed in 142); in *Xenopus*

oocytes, the Y-box protein FRGY2 (mRNP4) is an abundant component of masked mRNP particles (180, 229). Y-box proteins are also components of mRNPs in mammalian somatic cells (57). Mammalian Y-box proteins promote translation at low concentrations but inhibit translation at high concentrations (38, 56).

Surprisingly, in *Xenopus*, the *cis*-acting cytoplasmic polyadenylation element (CPE), located in the 3′ UTR and required for translational activation (see below), is also implicated in masking of *cyclin B1* mRNA (5, 39). In oocytes, CPE-binding protein (CPEB) exists in a complex with a protein called maskin and eIF4E (220). During oocyte maturation the interaction between maskin and eIF4E decreases, leading to the hypothesis that masking is achieved in oocytes by sequestering eIF4E in this complex. To derepress translation during oocyte maturation, eIF4E is released, allowing it to interact with eIF4G to form the cap-binding complex eIF4F, and promote initiation. This represents an example of translational repression occurring by preventing formation of the cap-binding complex.

Translational Repression by Deadenylation

Controlling the length of the poly(A) tail of specific mRNAs in the cytoplasm is a translational regulatory mechanism used during development in vertebrates and invertebrates (reviewed in 40, 178, 179). In general, extending the length of the poly(A) tail leads to translational activation whereas decreasing poly(A) tail length leads to translational repression. In *Xenopus*, no *cis*-acting elements have been identified as required for deadenylation in oocytes, but the presence of a CPE and AAUAAA hexanucleotide, both required for cytoplasmic polyadenylation (see below), prevents deadenylation, leading to the theory that all mRNAs lacking cytoplasmic polyadenylation signals are deadenylated in oocytes (62, 244). *Cis*-acting elements are required in embryos for deadenylation, and one element present in several RNAs is a 17-nt sequence called the embryonic deadenylation element, or EDEN (2, 15, 114, 115), which is sufficient for mediating deadenylation of a reporter RNA (162). Deadenylation of *Cdk2* mRNA depends on two unique elements that are distinct from EDEN (222). AU-rich elements termed AREs, usually containing the sequence AUUUA in tandem arrays, signal deadenylation in *Xenopus* embryos when present in the 3′ UTR of a chimeric mRNA (248). Two proteins are candidate *trans*-acting factors required for deadenylation: poly(A)-specific RNase (PARN) (100) and EDEN-BP (162), which binds to the EDEN sequence. An extract system has recently been developed from *Xenopus* oocytes that recapitulates ARE-mediated mRNA deadenylation (247). This work has led to the identification of a protein called embryonic poly(A)-binding protein (ePAB), which binds the ARE and is the major poly(A)-binding protein present in late oocytes and early embryos. Immunodepletion of oocyte extracts for ePAB enhances deadenylation, suggesting that ePAB has a role in stabilizing poly(A) tails and regulating deadenylation in early *Xenopus* development (247). Another example of regulation of a specific mRNA by deadenylation is the *hunchback* (*hb*) mRNA of *Drosophila*, which is deadenylated when Nos and

Pumilio (Pum) interact with 3′ UTR elements called Nos response elements (NREs) (258, 268).

Translational Repression at the 60S Subunit Joining Step

Translation initiation requires recognition of the mRNA 5′ cap by eIF4F, and the recruitment of the 43S preinitiation complex containing the small ribosomal subunit (reviewed in 74, 173). Subsequently, this complex scans the mRNA until reaching a start codon, at which point the large 60S ribosomal subunit is recruited to form an active 80S ribosome (reviewed in 164). The majority of translational regulatory events are thought to act at the cap-binding step, thereby controlling recruitment of the 43S preinitiation complex. However, recent evidence implicates the 60S subunit joining step as also subject to regulation (158). In mammalian erythroid cells, a specific mRNP complex, containing hnRNP K and hnRNP E1, represses translation of the 15-lipoxygenase (LOX) mRNA through an interaction with a specific sequence in the 3′ UTR termed the differentiation control element (DICE) (159, 160). This block in translation occurs downstream of 43S recruitment and scanning, at the 60S subunit joining step. In support of this, when a reporter mRNA bearing the DICE from the LOX mRNA is subject to translational repression, toeprinting analysis indicates that the preinitiation complex is stalled at the start codon (158). The translation block probably targets one or both of the initiation factors required for the 60S subunit joining step: eIF5 and eIF5B (22, 165, 175, 176). The *Drosophila* homologue of eIF5B is dIF2, which interacts physically and genetically with the DEAD-box RNA helicase Vasa (Vas) (19, 119), which in turn has been implicated in translational activation of germline mRNAs (69, 141, 181, 225, 238, 239), suggesting that Vas might enable translation by alleviating this type of translational repression.

Mechanisms of Derepression/Activation

When repression is conferred by a specific repressor protein binding to a transcript, derepression is likely achieved through deactivation or displacement of the repressor protein, or disruption of a translational regulatory complex containing this protein. There may also be instances of competition between binding of repressor and activator proteins to the same transcript.

Cytoplasmic Polyadenylation

Cytoplasmic polyadenylation is necessary to activate dormant maternal mRNAs during development (reviewed in 40, 178, 179). As mentioned above, cytoplasmic polyadenylation in *Xenopus* oocytes depends on the presence of a CPE, UUUUUAU, in the 3′ UTR (61, 144). A second CPE specific for mRNAs that are activated during *Xenopus* embryogenesis consists of a minimum of 12 U residues (204–206). Characterization of oocyte CPEs led to the identification of CPEB, which contains a zinc finger domain and two RNA recognition motifs (RRMs) (80).

Immunodepletion of CPEB in extracts (80) and injection of CPEB antibody in vivo (221) both prevent polyadenylation and thus translational activation of mRNAs containing the CPE. In addition to CPEB, cleavage and polyadenylation specificity factor (CPSF), and poly(A) polymerase (PAP) are also required for cytoplasmic polyadenylation (11, 43).

In *Drosophila* development, cytoplasmic polyadenylation is required to activate translation of key embryonic axis patterning determinants such as *bcd*, *torso* (*tor*), and *Toll* (*Tl*) (190). Interestingly, the *cis*-acting sequences required for cytoplasmic polyadenylation in *Drosophila* are not the same as in vertebrates. Within the *Tl* 3′ UTR, a 192-nt segment contains sequences required for its cytoplasmic polyadenylation (192). No consensus motif that controls cytoplasmic polyadenylation has yet been identified in the different *Drosophila* targets. Lack of conservation in *cis*-acting sequences between vertebrates and invertebrates also implies that the *trans*-acting factors that bind to these sequences may not be conserved.

Nevertheless, the *Drosophila* homologue of CPEB, oo18 RNA-binding protein (Orb), is required for oogenesis (27, 108, 109). *orb* mutants demonstrate defects in oocyte determination, signaling between germline and soma, localizing mRNAs such as *Bicaudal-D* (*Bic-D*), *fs*(*1*)*K10* (*K10*), *osk*, and *grk* and in dorsal-ventral and anterior-posterior patterning of the oocyte and embryo (27, 89, 109). Recent work indicates that Orb may be involved in cytoplasmic polyadenylation and translational activation of *osk* (23). Interestingly, the Bruno (Bru) protein (253), required to repress *osk* translation, shows homology to EDEN-BP, which is thought to be required for deadenylation in *Xenopus* (162). However, in vitro translation experiments with *osk* argue against the modification of poly(A) length as being a factor in its translational regulation (20, 121). Therefore, although the CPEB homologue Orb is required for oogenesis in *Drosophila*, a definitive role for Orb in polyadenylation in this system remains to be demonstrated.

A genetic approach was undertaken in *Drosophila* to search for potential *trans*-acting factors generally required for cytoplasmic polyadenylation in vivo (122). Since this process is required for activating maternal mRNAs, mutations in some of the factors involved may cause female sterility. A screen of second chromosome female-sterile mutants for those that prevented translational activation of dormant maternal mRNAs led to the identification of *cortex* (*cort*) and *grauzone* (*grau*). Cytoplasmic polyadenylation and translational activation of *bcd* and *Tl* during embryogenesis, and of *tor* during oogenesis, are both disrupted in these mutants. Injection of a polyadenylated *bcd* transcript into *cort* mutants resulted in its translation, indicating that the defect is in the polyadenylation process or regulation of this process. Cloning of *grau* identified it as a transcription factor required to activate *cort* (25). Mutations in *grau* and *cort* exhibit aberrant chromosome segregation in meiosis I and cause an arrest in meiosis II (122, 161, 196). Thus these genes may have a role in linking the progression through meiosis with translational control during development (25). In addition, two potential *trans*-acting factors have been observed to cross-link to the 192-nt element within the *Tl* 3′ UTR, but the identity

of these proteins and their relevance to cytoplasmic polyadenylation remain to be investigated (192).

The mechanism by which polyadenylation and deadenylation influence translation initiation is not well understood (reviewed in 178). Increasing the length of the poly(A) tail is thought to increase the number of molecules of poly(A)-binding protein that can bind the transcript, and it has been proposed that this stimulates translational efficiency through the interaction between poly(A)-binding protein and eIF4G. This model has been questioned because of the low abundance of PABP1 in *Xenopus* oocytes (271), but the recent discovery of ePAB (247) suggests that several poly(A)-binding proteins may function at different times during development. In *Drosophila*, PABP also appears to be quite rare in ovaries (121). An alternate possibility by which cytoplasmic polyadenylation might activate translation of some transcripts is through ribose methylation of the cap (102, 103).

MECHANISMS OF LOCALIZATION OF SPECIFIC RNAs

Here we first discuss the idea that some shuttling proteins may be involved in both exporting selected transcripts out of the nucleus and mediating their subsequent localization within the cytoplasm. Next, focusing on the *Drosophila* egg chamber, we introduce the concept of transport of mRNP complexes from the nurse cells into the oocyte in particles called sponge bodies. Third, we briefly outline the role of the cytoskeleton in active localization of mRNAs during oogenesis. Within the oocyte, localization of some mRNAs is achieved by additional mechanisms such as selective degradation or passive diffusion, which are not discussed here (reviewed in 123).

Linking Nuclear Export to mRNA Localization

Recent data from several systems indicate that some localized mRNAs associate in the nucleus and are packaged with proteins involved in their cytoplasmic localization (reviewed in 193). hnRNPs (heterogeneous nuclear ribonucleoproteins) are proteins that associate with hnRNAs in the nucleus and are involved in mRNA packaging. Some hnRNPs are restricted to the nucleus, whereas others shuttle between the nucleus and the cytoplasm (167, 246). Several hnRNPs bind to mRNAs that are localized in the cytoplasm, implicating them in mRNA transport: hnRNP A2 binds to the 21-nt *cis*-acting sequence required for localizing the myelin basic protein mRNA in oligodendrocytes (86); the *Xenopus* hnRNP I homologue, VgRBP60, binds to the vegetally localized mRNA *Vg1* (33); and the *Drosophila* Squid (Sqd) hnRNP A/B homologue binds to the localized *grk* and *fushi tarazu* (*ftz*) transcripts (106, 156; and see below). Another *trans*-acting factor that binds to *Vg1* mRNA is the *Xenopus* homologue of the zipcode-binding protein (ZBP) called Vera (42), or Vg1 RBP (81). In chick embryo fibroblasts, ZBP binds to

the localized β-actin transcript (182). ZBP contains motifs required for nuclear localization and export, suggesting that, like the hnRNPs, its role in cytoplasmic localization of transcripts may begin in the nucleus. For the most part, the evidence implicating the proteins discussed above in cytoplasmic localization is indirect, with the exception of the role of Sqd in localization of the *ftz* transcript in *Drosophila* embryos, as is discussed below.

mRNP Transport from the Nurse Cells to the Oocyte in the *Drosophila* Ovary

Developing and mature germ cells in various animals are characterized by the presence of nuage particles, electron-dense structures that are associated with RNA, protein, and mitochondria (reviewed in 50, 188). In *Drosophila* oogenesis, nuage particles are present in the perinuclear region of nurse cells. These particles may be precursors to polar granules (133–135), which are morphologically similar structures found in the pole plasm, specialized cytoplasm in the posterior of the oocyte required for embryonic pole cell development. During oogenesis, nuage has been proposed to function in transport of RNAs and proteins destined for the pole plasm.

Surrounding the perinuclear nuage in nurse cells are structures referred to as sponge bodies (265). Like nuage, sponge bodies are electron-dense and contain RNA and mitochondria. Sponge bodies are distinguished from nuage by the presence of elongated bodies or vesicles and by the fact that some of their protein components differ from those of nuage particles. Sponge bodies are present in nurse cells, oocytes, and in the ring canals that link those cells, suggesting that like nuage, they also function as transport vehicles for mRNPs that move from the nurse cells into the oocyte. This hypothesis has been supported by the identification of some of the protein and RNA components of these particles. In *Xenopus*, a seemingly homologous structure to sponge bodies, called the mitochondrial cloud, migrates to the vegetal pole during development, where it is believed to form the germinal granules (83).

Exuperantia (Exu) protein is the most specific marker known for sponge bodies (265). Exu is required for the anterior accumulation of *bcd* and efficient posterior localization of *osk* within the oocyte, as well as for the apical localization of both *bcd* and *osk* mRNAs within the nurse cells (10, 218, 264). GFP-Exu is visualized in particles surrounding the nurse cell nuclei, distributed throughout the cytoplasm, and localized around the ring canals (138, 251). Time lapse laser scanning microscopy was used to track the movement of Exu-particles in living oocytes and to test the effect of microtubule or actin depolymerization on that movement (234). Within the nurse cells, random movement in the cytoplasm, perinuclear localization, and clustering at ring canals was found to require three seemingly non-overlapping populations of microtubules. The movement of these particles through ring canals and into the oocyte did not require the actin or microtubule cytoskeleton. This is in contrast to vesicle and mitochondrial transport

through ring canals, which does depend on the actin cytoskeleton (13, 14). Within the oocyte the accumulation of Exu at the anterior requires microtubules (234).

Immunoprecipitation with anti-Exu antisera was used to identify and analyze Exu-containing complexes, which presumably contain other sponge body components (264). This analysis uncovered an RNase-sensitive association between Exu and least six other proteins. *osk* mRNA was also identified as a component of this complex, consistent with the requirement for Exu in *osk* localization. A direct interaction was observed between Exu and the Y-box protein Ypsilon Schachtel (Yps) (236). Yps colocalizes with Exu throughout oogenesis (264) and binds to RNA (236). The presence of Yps in these particles supports the idea that they contain masked mRNAs, because this class of proteins is involved in translational regulation (see above). Also consistent with their proposed role in transporting dormant mRNAs is the observation that sponge bodies are not seen to associate with ribosomes (265). Another protein present in sponge bodies and that forms an RNase-sensitive association with Exu and Yps is the DEAD-box protein Maternal expression at 31B (Me31B) (41; A. Nakamura & S. Kobayashi, personal communication). A *Xenopus* homologue of Me31B, Xp54, is a component of dormant mRNPs in oocytes (105). Thus sponge bodies appear to be involved in transporting translationally dormant mRNPs including localized mRNAs from the nurse cells into the oocyte.

The Role of the Cytoskeleton in RNA Localization

RNAs and proteins are often localized by associating with molecular motors, factors that move directionally along cytoskeletal tracks in an energy dependent manner (reviewed in 60, 241). Two large families of motors that use the microtubule cytoskeleton are the minus end–directed dyneins and the plus end–directed kinesins. The family of myosins are plus end-directed and make use of the actin cytoskeleton.

During *Drosophila* oogenesis, some transcripts achieve their localization by transport along the microtubule cytoskeleton. Several lines of evidence support this idea. The first is the overall polarity of microtubules during oogenesis. During stages 1 to 7, a microtubule organizing center (MTOC) is situated at the posterior of the oocyte, focusing microtubule minus ends at the posterior, while plus ends extend to the anterior and into the nurse cells (Figure 1). This organization depends on the functions of *Bic-D* and *egalitarian* (233). Subsequently, in stages 7 to 8, there is a reversal of microtubule directionality due to relocation of the MTOC to the anterior of the oocyte (235). This event requires signaling pathways involving Notch/Delta, and protein kinase A (PKA) as well as the activity of *mago nashi* (*mago*) (107, 110, 146, 154, 186). This functional microtubule distribution has been supported by experiments showing that from stage 8 the minus end–directed kinesin-related protein, Nod-β-gal, localizes to the anterior while the plus end motor Khc-β-gal shows the opposite localization

(28, 29). Therefore, the early organization of the microtubule cytoskeleton may mediate the localization of RNAs and proteins from the nurse cells into the oocyte, whereas the later reorganization would then be required to localize a subset of these factors within the oocyte. In keeping with this idea, the requirement for Exu in localizing mRNAs into the oocyte occurs after the reorganization of the microtubule cytoskeleton, suggesting that sponge bodies may offer an alternative transport mechanism for selected transcripts entering the oocyte, after cytoskeletal reorganization (127).

The second observation to underscore the role of the microtubule cytoskeleton is that treatment with microtubule depolymerizing drugs disrupts localization of *bcd* and *osk* (28, 168, 223). Localization of these RNAs is also affected in genetic backgrounds where microtubule directionality is altered. For example, in mutants that have microtubule minus ends at both poles, *bcd* localizes to both ends, while *osk* accumulates near the center of the oocyte (28, 76, 78, 107, 183).

The third line of evidence directly links specific molecular motors to transcript localization within the oocyte. As discussed below, dynein is implicated in anterior localization of *bcd* RNA (194), and kinesin I is required for *osk* localization to the posterior (17). *osk* localization also requires the activities of several maternal effect genes that encode regulators of the actin and microtubule cytoskeletons, including *cappuccino* (*capu*), *spire* (*spir*), *chickadee* (*chic*), and *Tropomyosin II* (*TmII*). Tropomyosin binds filamentous actin and may act to stabilize microfilaments (3, 55). *capu* encodes a formin-homology-domain containing protein related to the product of the chicken *limb deformity* locus (52). Capu protein probably functions to link the microtubule cytoskeleton with the actin cytoskeleton, via an interaction with profilin, an actin-binding protein encoded by *chic* (32, 136). *spir* also encodes an actin-binding protein that interacts with Rho-family GTPases including RhoA, Rac1, and Cdc42 (254). These GTPases control the assembly and organization of the actin cytoskeleton, by responding to extracellular cues and interacting with downstream effectors (12). Recent work (202, 240) also implicates a putative kinase, PAR-1, as essential for directing *osk* to the posterior pole. Mammalian homologues of PAR-1 phosphorylate various microtubule-associated proteins (45), and in *Drosophila par-1* mutants the organization of the microtubule cytoskeleton within the oocyte is disrupted. In these oocytes, *bcd* localizes normally, but *osk* RNA is mislocalized to the center, and, consequently, abdominal patterning and pole cells are disrupted in embryos produced by *par-1* mothers. *par-1* is also required for oocyte differentiation and for microtubule organization in the early egg chamber (34, 90). In *C. elegans*, the *par-1* homologue is required maternally for the first embryonic cell division to be asymmetric (94). These results linking *osk* and *bcd* with molecular motors or molecules involved in cytoskeletal organization are consistent with the model that localization of these transcripts is mediated, at least in part, through the microtubule cytoskeleton. However, many details of this mechanism, including exactly how specific transcripts associate with motor proteins, remain to be established.

TRANSLATIONAL CONTROL AND RNA LOCALIZATION COOPERATE IN THE DEPLOYMENT OF SPECIFIC *DROSOPHILA* PROTEINS ESSENTIAL FOR EMBRYONIC AXIS DETERMINATION

Several translationally controlled RNAs have been intensively studied because of their importance in establishing the spatial axes of the embryo. Despite progress in identifying RNA-binding proteins that regulate localization and translation of these RNAs, in no case is there yet a complete understanding of the mechanism by which these specific regulators function. Localization and translational regulation of four transcripts is considered in the next sections.

bicoid

An anterior to posterior gradient of Bcd protein establishes head and thoracic development (reviewed in 219). During oogenesis the *bcd* transcript undergoes a multistep localization pathway, culminating in a strictly anterior concentration from which it will be translationally activated during embryogenesis, producing a steep concentration gradient (10, 218). *bcd* mRNA is transcribed in nurse cells and transported to the oocyte in two separable phases: event A, commencing in stages 4 to 5, and event B, commencing around stage 6. There is redundancy between events A and B in that either is thought to be able to promote later steps of localization (127).

CIS-ACTING ELEMENTS REQUIRED FOR LOCALIZATION OF *BCD* *bcd* mRNA localization depends on its 3′ UTR, which forms a complex secondary structure including multiple stem-loops (126, 131, 198) (Figure 2). Stem-loops IV and V together can mediate event A completely, and are sufficient for normal localization up until embryogenesis (127). Deletion analysis identified a 50-nt segment within stem-loop V, named the *bcd* localization element 1 (BLE1), which when present in two copies can confer normal localization up to stages 9 to 10 (129). A point mutant in BLE1 eliminates event A localization but maintains event B and all later stages (127). BLE1 is hypothesized to represent a binding site for a *trans*-acting factor that may mediate event A localization, and mutational analysis has identified RNA structural elements necessary for this event (128). *Cis*-acting elements necessary for event B and later localization events have not been mapped as precisely.

TRANS-ACTING FACTORS REQUIRED FOR LOCALIZATION OF *BCD* The functions of three maternal genes, *exu*, *swallow* (*swa*), and *staufen* (*stau*), are required for *bcd* mRNA concentration at the anterior of oocytes and embryos (10, 218, 223). Another protein related to Exu, Exu-like (Exl), which cross-links to the dimerized BLE1 region, may also have a role in *bcd* localization (130). As discussed above, the requirement for Exu is likely due to its association with sponge bodies, although

bcd mRNA has not yet been identified in those structures. Microtubules are also necessary for correct *bcd* localization (168). Microtubule depolymerization produces the same phenotype as seen in *swa* mutants, whereby *bcd* is evenly distributed within the oocyte instead of anteriorly anchored (10, 218). Commencing at stage 10, Swa protein colocalizes with *bcd* mRNA in a microtubule-dependent manner at the anterior of the oocyte (84, 194). In *grk* mutants, which have microtubule minus ends at each pole, *bcd* and Swa localize to both poles, suggesting that they are transported along the microtubule cytoskeleton. Furthermore, Swa contains a coiled-coil domain through which it interacts with Ddlc-1, a light chain of cytoplasmic dynein. When this interaction is disrupted in vivo, Swa is unlocalized, leading to the hypothesis that Swa is an adaptor protein within the oocyte, connecting *bcd* mRNA to the dynein motor and enabling the transcript to be transported directionally along the microtubule cytoskeleton (194). Swa contains a region with distant homology to an RNA–binding domain (24), but has not been demonstrated to bind to *bcd* directly.

Staufen (Stau) is required for the final stage of *bcd* localization, anterior anchoring of the transcript (218). In the egg, Stau is highly concentrated at the posterior, but also shows a weak anterior accumulation, dependent on *bcd* (58, 216). RNA injection experiments demonstrated that nt 181–660 of the *bcd* 3′ UTR (consisting of stem-loops III, IV, and V) could recruit endogenous Stau into particles that migrate along microtubules (58). Dimerization or multimerization of this region appears to be required for particle formation (59). Thus Stau may bind *bcd* directly and anchor it at the anterior in a microtubule-dependent manner.

Stau contains five double-stranded RNA-binding domains (dsRBDs). dsRBD1, 3, and 4 can bind to dsRNA in vitro (145, 217), and a transgene bearing mutations within dsRBD3 that disrupt RNA binding cannot rescue mislocalization of *osk* or *bcd*, indicating that the function of Stau depends on its RNA-binding activity (171). dsRBD5 and a large insertion within dsRBD2 of Stau are both required for *bcd* localization (145). However, transgenes bearing deletions of either of these regions are still capable of rescuing head defects of *stau* mutants, indicating the presence of Bcd activity. Thus, in addition to mediating its localization, Stau is hypothesized to be involved in either prevention of degradation or translational activation of the *bcd* mRNA.

BCD IS TRANSLATIONALLY ACTIVATED THROUGH CYTOPLASMIC POLYADENYLATION

bcd is dormant throughout oogenesis. Poly(A) test (PAT) analysis has revealed that at this time *bcd* has a poly(A) tail of ∼70 nt (190). During embryogenesis, the lengthening and shortening of the *bcd* poly(A) tail correlates with its translational status, reaching ∼140 nt by 1 to 1.5 h, when *bcd* translation is active, and shortening again later. Transgenic experiments demonstrated the requirement of the poly(A) tail for *bcd* translation. The maternal-effect bcd^{E1} mutation results in embryos that exhibit defective anterior development due to a lack of functional Bcd, which can be rescued by injecting wild-type *bcd* mRNA into the anterior of the embryo (46, 48). Injection of a transcript lacking 537 nt of the 3′ UTR has no rescuing

ability, but the addition of 150–200 A residues to the 3′ end in vitro allowed this RNA to partially rescue the morphogenetic defect, whereas addition of only 40–60 A residues did not, suggesting that an extended poly(A) tail is required for the translational activation and function of *bcd* (190). Cytoplasmic polyadenylation and translational activation of *bcd* were uncoupled by the insertion into the *bcd* 5′ UTR of a 63-nt sequence complementary to a segment of the coding region (*bcd*-AS) (245). *bcd*-AS RNA was polyadenylated normally when injected into the anterior of wild-type embryos, but could not be translated in vivo, indicating that although the process of polyadenylation is necessary, it is insufficient for *bcd* translation. The structural modification caused by this mutation likely disrupted interaction between the 5′ and 3′ UTRs of the transcript (245).

BCD IS TRANSLATIONALLY REPRESSED BY NOS When *nos* is ectopically expressed at the anterior, translation of *bcd* is repressed, and the RNA has a shorter poly(A) tail (67, 257). Through PAT assays and injection experiments, Nos has been shown to influence *bcd* polyadenylation (268). Injection of *bcd* into the anterior but not the posterior of wild-type embryos results in its polyadenylation, but polyadenylation and translational activation do occur upon injection into the posterior of *nos* mutants, indicating that *bcd* is not being regulated correctly. Even when *bcd* is polyadenylated in vitro and then injected into the wild-type posterior, it is not translated, suggesting that it is deadenylated by Nos, leading to translational repression, as is the case for *hb* (see below). Both the *hb* and *bcd* 3′ UTRs contain NREs (258), which confer translational repression by Nos. Thus Nos appears to be capable of regulating *bcd* translation, suggesting that during embryogenesis, any *bcd* mRNA that is not restricted to the anterior can be repressed translationally, possibly via Nos-dependent deadenylation. However, the significance of this regulation in vivo is not clear since *bcd* mRNA is strictly anteriorly localized, and Nos activity is posteriorly localized.

nanos

A posterior to anterior gradient of Nos protein acts to specify posterior development. Mutants lacking Nos activity do not form an abdomen (118), and mutants with ectopic Nos activity at the anterior form a mirror image abdomen at the expense of head and thoracic development (67, 257). *nos* mRNA, like *bcd*, is concentrated by the end of oogenesis at the site at which it will be translated during embryogenesis (250). However, unlike *bcd*, which exhibits nearly complete anterior localization, and which is translated even when unlocalized (47), *nos* mRNA is found throughout the embryo; only 4% is estimated to be posteriorly localized (8). As only the localized fraction of the transcript gets translated (68), restriction of Nos activity to the posterior depends primarily on translational regulation.

During stages 1 to 7 of oogenesis, *nos* is expressed in the nurse cells and accumulates in the oocyte. Like several other oocyte-localized mRNAs, it concentrates at the anterior during stages 7 to 8 (249). As for *bcd*, the early oocyte localization

of *nos* mRNA is unnecessary for its subsequent targeting (8). By stage 10, high levels of *nos* are transcribed in the nurse cells and subsequently transferred into the oocyte. Posterior concentration within the oocyte begins around stage 12, and shortly after egg deposition, translation of the posteriorly localized transcript produces a gradient of Nos protein (249).

CIS-ACTING ELEMENTS REQUIRED FOR TRANSLATIONAL REPRESSION OF *NOS* 3′ UTR elements are required both for translational repression of *nos* in the bulk cytoplasm and translational activation in the posterior (37, 68, 69, 209). Fusion of the first 184 nt of the *nos* 3′ UTR to the *tor* reading frame conferred the translational regulation profile of wild-type *nos*, consisting of translational repression outside the posterior, concentration of the transcript in the posterior, and pole plasm–dependent translational activation; thus this region was termed the translational control element (TCE) (37). However, there is redundancy between the two halves of this element such that the region encompassing nt 6–96 alone is capable of mediating strong translational repression and producing wild-type development, and so was also named the TCE, a designation used here (37, 69, 209).

Computer modeling of the TCE predicts formation of two extended stem-loops (II and III) (35) (Figure 3), mutations which abolish translational repression. The loop portion of stem-loop II corresponds to the binding site for the translational repressor Smaug (Smg) (discussed below) (36, 37, 208, 209). At least one of the mutations in stem III that abolishes TCE-mediated translational repression does not act by affecting the ability of Smg to bind to stem-loop II, implying that stem-loop III may regulate the binding of a distinct factor also required for translational repression (35).

CIS-ACTING ELEMENTS REQUIRED FOR LOCALIZATION AND TRANSLATIONAL ACTIVATION OF *NOS* As the nurse cells are rapidly transferring their cytoplasm to the oocyte during the period when *nos* concentrates in the pole plasm, *nos* accumulation might not involve active localization but only specific anchoring (8). Furthermore, translationally repressed *nos* outside the pole plasm is unstable and degraded (7, 37, 209), thus degradation of *nos* mRNA may help restrict Nos protein to the posterior. Regardless of the mechanism by which *nos* localizes, transcripts that reach the posterior are translationally activated. A 547-nt segment of the 3′ UTR consisting of partially redundant elements is required to direct all stages of *nos* localization throughout oogenesis (66). Although this segment overlaps with the TCE, the TCE itself is not essential for localization. Mutational analysis within the TCE, including separation of stem-loops II and III from each other, has revealed that the requirements for this region in localization and translational regulation can be uncoupled. In addition, the TCE from *D. virilis* can substitute for that of *D. melanogaster* for translational repression but not localization, implying a conservation in recognition sequences for one event but not the other (35).

A 41-nt element downstream of the TCE, and highly conserved in *D. virilis*, can influence localization and translational repression, and was named the minimal

element (ME) (9). Normally this region acts synergistically with the TCE to confer localization. Three mutations within the ME eliminated both its own function and that of the linked TCE with respect to localization, indicating that the ME has a long-range effect on the TCE. Such a long-range effect is not present for the translational regulatory function of the TCE. As for the TCE, the repressor and localization functions of the ME can be uncoupled.

TRANS-ACTING FACTORS REQUIRED FOR TRANSLATIONAL REPRESSION OF *NOS* Smg was identified as a protein that binds specifically to the *nos* 3′ UTR (36, 209). Mutational analysis indicated that the Smg binding site, referred to as the Smg recognition element (SRE1), was within a predicted stem-loop (stem-loop II described above) (37, 209). A second SRE (SRE2) is located within nucleotides 97–185. Cloning of *smg* (36, 208) revealed a predicted protein lacking any previously identified RNA-binding motifs, but containing a Sterile Alpha Mating (SAM) domain, a motif implicated in protein binding (reviewed in 195).

Several lines of evidence indicate that Smg represses translation of unlocalized *nos*. Mutational analysis revealed a correlation between the ability of Smg to bind to *nos in vitro*, and translational repression in vivo (209). A wild-type *nos* transgene bearing mutations in the SREs produces ectopic *nos* activity, suggesting defective repression, whereas the addition of three stem-loops containing SREs (3xSRE) to a *nos*/*bcd* transgene, consisting of the *nos* open reading frame fused to the *bcd* 3′ UTR, can confer translational repression. Regulation of *nos* translation by Smg has also been demonstrated through an in vitro translation system derived from embryonic extracts (208). A reporter transcript fused to three functional SREs is translated less efficiently than one bearing point mutations in these domains. Translational efficiency is equalized when Smg is immunodepleted from embryonic extracts or when late-stage embryos, where Smg is less abundant, are used, suggesting that Smg is the repressor mediating this effect. A probable null allele for *smg* shows ectopic Nos activity, and overexpression of Smg in sensitized genetic backgrounds leads to enhanced repression of *nos in vivo* (36).

TRANS-ACTING FACTORS REQUIRED FOR LOCALIZATION AND TRANSLATIONAL ACTIVATION OF *NOS* *nos* mRNA does not localize to the posterior if the pole plasm has not assembled there. Thus all genes required for pole plasm assembly are directly or indirectly required for *nos* localization and activation. Ectopic localization of *osk* results in ectopic Nos activity, dependent on the functions of *vas* and *tudor* (*tud*) (54, 210). When either the TCE or the entire 3′ UTR of *nos* is present, no Nos activity is ever detected in *vas* mutants, although low levels are present in *osk* mutants (69). However, when the *nos* 3′ UTR is present but the TCE is deleted, translation no longer depends on *vas*, as assessed by the production of posterior embryonic segments, implying that the role of Vas in activating *nos* translation involves overcoming repression by the TCE (37). Vas is a DEAD-box RNA helicase (119), and therefore could be directly involved in *nos* translational activation.

A 75-kDa protein (p75) that binds the ME of the *nos* 3′ UTR may mediate localization or anchoring (9). Mutations that prevent binding of p75 abolish both the localization and translational repressive properties conferred by the ME. p75 is predicted to function in localization or anchoring, and not in translational control, since it does not bind to the TCE region, and since the translational repression defect in the ME mutants could be explained by the fact that they may also disrupt formation of the Smg binding site stem-loop. Furthermore, other mutations within the ME that do not disrupt p75 binding have stronger effects on translational repression, one of which alters the SRE2 sequence.

POTENTIAL MECHANISMS FOR LOCALIZATION AND TRANSLATIONAL ACTIVATION OF *NOS* Repression of *nos* in the bulk cytoplasm by Smg, and potentially other repressors, must be overcome in the posterior cytoplasm. Since Smg and Osk interact directly (36), a complex of pole plasm components including Osk and Vas may deactivate Smg on *nos* transcripts that enter the posterior region, leading to derepression of translation. Alternatively, mutual exclusion between the binding of translational repressors and localization factors may mediate localization of a portion of the transcript to the posterior, in keeping with data suggesting overlapping binding sites for repressors and localization factors, and the discovery of the p75 protein (8, 9, 35). Cloning and identification of p75 and/or other potential localization factors will shed more light on this function.

The different behavior of multiple TCEs in *cis* and in *trans* has been interpreted in several ways. When wild-type *nos* is overexpressed at the posterior, its translation is increased (67), and overexpressing Smg in a wild-type background has no phenotype (36). By contrast, when multiple copies of the 6–185-nt region of the *nos* 3′ UTR are added to the same transcript, localization is unaffected but posterior development is reduced, implying reduced translation (8). The presence of multiple copies of this region in *cis* might permit the simultaneous binding of translational repressors and localization factors, allowing the RNA to be both localized and repressed, and may imply that in the wild-type scenario these are mutually exclusive (8). However, multiple TCEs in *cis* may simply be more efficient at recruiting Smg than multiple copies of the same element in *trans* (36), enabling the increased Smg-bound *nos* to titrate out a derepressor in the posterior, possibly Osk, as Osk and Smg interact directly.

To investigate the mechanism of *nos* translational repression, the distribution of *nos* in polyribosomal profiles was analyzed (30). Transcripts that are being actively translated are expected to cosediment with polysomes, whereas transcripts that are blocked at initiation would be predicted to sediment with initiation complexes. Surprisingly, although only 4% of *nos* is localized and thus translated in the early embryo, over 50% of the transcript showed an EDTA- and puromycin-sensitive association with polysomes, suggesting that the translational block for the unlocalized *nos* occurs after initiation. This association is maintained in *vas* and *osk* mutants in which little or none of the *nos* transcript is translated. Substitution of the *nos* 3′ UTR had no effect on polysomal association, supporting the idea that

repression does not act at this step. Substantial translational runoff following cycloheximide treatment was observed for *nos* mRNA in preblastoderm embryonic extracts, arguing against a block at either elongation or termination. These results suggest an alternative mechanism of regulation, such as premature translational termination or degradation of the nascent polypeptide after its translation (30).

Potentially relevant to this hypothesis is the *bicaudal* (*bic*) gene, which encodes the *Drosophila* beta NAC homologue (139). Some embryos from *bic* mutant females have a bicaudal phenotype that results from ectopic Nos activity, but, unlike other mutants that cause this phenotype, in *bic* mutants ectopic Nos is produced without ectopic *osk* expression. Beta NAC is a subunit of the Nascent polypeptide Associated Complex (NAC), which associates with the ribosome where it binds to emerging polypeptides (262). Thus beta NAC could play a role in regulating the translation of polypeptides on the ribosome, and the ectopic Nos in *bic* mutants may result from loss of this function. Since *bic* is a hypomorphic allele, it may particularly affect genes whose translation is most sensitive to this regulation (139).

NOS IS A TRANSLATIONAL REPRESSOR The role of Nos in abdominal specification is achieved through translational repression of the maternal *hb* transcript, contributing to an anterior to posterior gradient of the Hb transcription factor (88, 91, 224, 231). Nos forms a translational regulatory complex with Pum, and the NRE sequences within the *hb* 3′ UTR (149, 212, 258). Like *bcd*, the poly(A) profile of *hb* correlates with its translational status, and the *hb* 3′ UTR is polyadenylated when injected into the posterior of *nos* or *pum* mutant embryos, but not the wild-type posterior (268). Injection of an in vitro polyadenylated *hb* 3′ UTR into the wild-type posterior but not the anterior results in complete deadenylation. Thus Nos and Pum are thought to promote the deadenylation of *hb*, leading to its translational repression. This repression does not act through the cap-binding step of translation initiation, since Nos and Pum can also repress a transcript that is translated from an internal ribosomal entry site (IRES) in a cap-independent manner (256).

The complex of Nos and Pum, bound to the *hb* NRE, recruits the NHL protein Brain Tumor (Brat) (213). Specific mutations in *pum*, *nos*, or *brat* that prevent Brat recruitment abolish regulation of *hb*, and maternal effect *brat* mutants demonstrate the same posterior segmentation defects as *nos* and *pum* mutants (117, 118, 213, 256). The NHL domain is sufficient to mediate the recruitment and function of Brat for *hb* repression. This domain has not been assigned a molecular function, but it is present in several proteins implicated in RNA processing (64, 65, 207).

Nos and Pum have also been implicated in repression of *Cyclin B* translation in pole cells (1), and Pum binds to NRE sequences in the maternal *Cyclin B* 3′ UTR (150, 213). This function of Nos and Pum, however, is not mediated through Brat, indicating that the ternary complex of Nos, Pum, and NRE sequences can interact with different cofactors. In *Xenopus*, a homologue of Pum (Xpum) interacts with a Nos homologue (Xcat-2) and with CPEB, and XPum binds to the 3′ UTR of *cyclin B1* mRNA, suggesting conservation in the translational regulatory properties of

these factors (150). Similarly, in *C. elegans* a Pum homologue (FBF) can interact with homologues of both Nos (NOS-3) and CPEB (CPB-1), and FBF binds to the 3′ UTR of at least one target transcript, *fem-3*, to mediate translational repression (101, 124, 272). In *S. cerevisiae*, two Pum-like proteins have been demonstrated to bind to 3′ UTRs of selected transcripts and mediate posttranscriptional regulation (157, 228). At least one of these functions through deadenylation, suggesting that it may operate through a mechanism similar to Pum.

oskar

Embryos lacking maternal *osk* activity do not form pole cells or develop posterior structures (116). The primary role of Osk in both these processes is demonstrated by experiments in which *osk* is ectopically expressed at the anterior through substitution of the 3′ UTR, which leads to ectopic abdominal development and pole cell formation (54). Like Nos, restriction of Osk activity to the posterior is critical, and this is achieved through a combination of mRNA localization and translational regulation. *osk* mRNA is synthesized in the nurse cells and, throughout early oogenesis, becomes concentrated in the oocyte (53, 97). Within the oocyte, a transient anterior concentration of *osk* is evident at stages 7–8, followed by posterior localization in stages 8–9 that is maintained until early embryogenesis. High levels of *osk* are synthesized in the nurse cells in stage 10 and transferred into the oocyte. Osk protein first appears in stage 8, in a restricted pattern at the posterior pole (96, 141, 181). Two isoforms of Osk, Long Osk and Short Osk, are produced from two in-frame start codons. Short Osk alone can confer wild-type development.

CIS-ACTING SEQUENCES REQUIRED FOR REGULATION OF *OSK* As for *nos*, sequences within the *osk* 3′ UTR are necessary for its localization: Early concentration in the oocyte depends on nt 532–791; release of *osk* from the anterior is mediated by two elements in different regions of the 3′ UTR (nt 242–363 and 791–846); and posterior localization of *osk* depends on the first 242 nt (98). Translational repression of *osk* in the bulk cytoplasm also depends on sequences within the 3′ UTR and on Bru (see below), a protein that binds to three regions of the *osk* 3′ UTR (A, B, and C) (96, 181, 253). A consensus sequence of 7–9 nt is found six times within these regions, and mutating these sequences reduces binding of Bru, leading to their designation as Bru response elements (BREs). Flies bearing an *osk* transgene mutated for the BREs (*oskBRE*$^{-}$), in an *osk* mutant background, produce Osk precociously during stages 7–8. Conversely, addition of BRE sequences to the *exu* 3′ UTR leads to a reduction in translation of this transcript, indicating that these sequences can mediate translational repression (96). Unlike *nos*, where the 3′ UTR is sufficient to confer translational activation, the *osk* 3′ UTR can mediate posterior localization of a heterologous transcript, but not translational activation (181, 200). An element between the first and second start codons (m1 and m2) is necessary for translational activation. This active region in the 5′ end is considered

a derepressor element because it is only necessary for translational activation when translation is being repressed through the BREs (79).

TRANS-ACTING FACTORS REQUIRED FOR TRANSLATIONAL REPRESSION OF *OSK* Four proteins and/or genes have been implicated thus far in translational repression of *osk*: Bru, Apontic (Apt), p50, and *Bicaudal-C* (*Bic-C*) (Figure 4). Bru, an RRM-type RNA–binding protein, was identified by virtue of its specific binding to the *osk* 3′ UTR (96, 253) and is the product of the *arrest* (*aret*) gene (21, 197). Bru accumulates in the oocyte and colocalizes with *osk*. A role for Bru in *osk* regulation in vivo was demonstrated through the use of a transgene that localizes *osk* to the anterior and that contains the BREs. Ectopic *osk* expression from this transgene leads to embryonic head defects, and this phenotype is enhanced in the background of a heterozygous *aret* mutation (253). Experiments in *Drosophila* cell-free translation systems have supported the conclusion that Bru is involved in *osk* repression (20, 121). Apt interacts with Bru both in the two-hybrid system and biochemically (120). Progeny from females *trans*-heterozygous for combinations of *aret* and *apt* mutations exhibit defects in anterior patterning. This phenotype can be suppressed by reduction of *nos*, suggesting that it is caused by ectopic Osk, and thereby implying that Apt, like Bru, has a role in regulating *osk* translation.

p50 cross-links to both the 5′ and 3′ ends of the *osk* mRNA (79). Within the 3′ UTR, p50 binds to the AB region. Reduction in p50 binding enhances Bru binding, suggesting that there may be competition between p50 and Bru for binding to the same site. However, immunoprecipitation experiments showed that the two proteins are capable of binding to the same repressor element simultaneously. Transgenes carrying a mutation that specifically reduces p50 binding show precocious *osk* translation at stage 7, suggesting that p50 is also required for translational repression of *osk*.

Bic-C is a KH domain protein capable of RNA binding in vitro (189). Embryos from females heterozygous for *Bic-C* alleles demonstrate patterning defects including a bicaudal phenotype (132, 147), which would be consistent with ectopic Osk activity. In homozygous *Bic-C* mutants Osk does not localize to the posterior and *osk* is precociously translated in stages 7–8, suggesting that *Bic-C* may play a role in translational regulation of *osk* (189).

TRANS-ACTING FACTORS REQUIRED FOR LOCALIZATION AND TRANSLATIONAL ACTIVATION OF *OSK* Stau is required to localize *osk* to the posterior pole of the oocyte (53, 97). Stau and *osk* colocalize within the oocyte (216) and show coincident mislocalization in mutants that disrupt oocyte microtubule polarity, suggesting that they are transported together along microtubules (76, 183). Stau is required for translational activation of *osk*, and even for *oskBRE*$^{-}$, indicating that Stau does not operate simply as a derepressor (96). However, the expression of Short Osk from a transgene can induce endogenous *osk* translation in *stau* mutants, indicating that the requirement of Stau for *osk* translation can be bypassed (140), as is also evident when *osk* gene dosage is increased (210). Both localization and translational

regulation of *osk* depend on the region of Stau containing the dsRBDs (145). The dsRBD2 insertion is required for *osk* localization, whereas dsRBD5 is required for its translational activation. Since Stau lacking dsRBD5 can activate translation of *oskBRE*$^{-}$, but not wild-type *osk*, dsRBD5 may have a derepressor function. Components of the pole plasm are also required for translational activation of *osk. vas* and *tud* mutants show a reduction in levels of Short Osk. Osk also regulates its own expression (141, 181). Short Osk is present in phosphorylated and unphosphorylated forms, and its phosphorylation depends on *vas* but not *tud* (140, 141). Several *osk* alleles that disrupt Osk interaction with Vas (16) also prevent Osk phosphorylation.

Orb directly binds to *osk* mRNA, and *orb* mutants show defects in localization of *osk* mRNA to the posterior of the oocyte and a failure of *osk* translation (23, 27, 141). This could indicate a direct role for *orb* in localization or anchoring of *osk* mRNA. Alternatively, since Osk is required to maintain its own transcript at the posterior, the *orb* mutant phenotype could be due to a defect in translational activation leading to a defect in anchoring. This is supported by the observation that *osk* poly(A) tails are reduced in length in *orb* mutants (23; and see below).

Aubergine (Aub) is required for accumulation of both Osk isoforms (266). In *aub* mutants, *osk* localizes normally but fails to remain anchored at the posterior, producing low levels of protein in a broad posterior region. Ectopically expressed *osk* at the anterior, achieved through substitution of the 3′ UTR, can be robustly translated in *aub* mutants, suggesting that Aub does not control Osk stability, and that Aub is only required for translational activation of *osk* in the presence of the endogenous *osk* 3′ UTR. Aub is still required for translation of *oskBRE*$^{-}$, suggesting a requirement in translational activation rather than derepression.

Two additional proteins, p68 and p50, both cross-link to a region of 130 nt, between m1 and m2 of *osk* (79). Large deletions that abolish derepression also reduce or eliminate the binding site of these proteins. Introduction of a transgene carrying an inversion that abrogates binding of p68 and p50 results in translational repression even when the transcript is localized. However, the presence of this derepressor element alone cannot translationally activate a transcript which contains the BREs fused to the *bcd* 3′ UTR. Similar constructs lacking BREs are translated (54, 181), suggesting that factors in the posterior pole are required in addition to the derepressor element to overcome BRE-mediated repression. The binding of p50 to both the 5′ and 3′ ends of *osk* mRNA may provide a means for circularizing the transcript and may allow *trans*-acting factors bound to the 3′ UTR to influence translation initiation at the 5′ end (79).

POTENTIAL MECHANISMS FOR TRANSLATIONAL CONTROL OF *OSK* Cell-free translation systems using *Drosophila* ovarian extracts have recently been used to study *osk* translational regulation (20, 121). Such experiments demonstrated that reporter transcripts containing the *osk* 3′ UTR were regulated in the same way whether or not they contained a poly(A) tail, indicating that in vitro, regulation is independent of cytoplasmic polyadenylation or deadenylation. These results are contrary to in vivo studies in *orb* mutants (23) but are supported by temporal analysis of *osk*

poly(A) length in vivo, which does not appear to change at the onset of translational activation (253). However, poly(A) length changes may be difficult to monitor *in vivo* because of the relatively small portion of the *osk* transcript that becomes translationally active (8). Extract systems have also been used to test whether Bru repression depends on the 5′ cap structure. The addition of free cap analogue to ovarian extracts was not found to disrupt BRE-mediated *osk* regulation of reporter transcripts, indicating that Bru inhibition also does not act through the cap-binding step of translation initiation (121).

gurken

Grk, a member of the TGF-α family of proteins, plays an integral role in two signaling processes responsible for establishing both the anterior-posterior and dorsal-ventral axes of the egg and embryo (reviewed in 155). Grk first accumulates at the posterior of the oocyte and signals to the neighboring follicle cells, specifying their fate as posterior (76, 183). This leads to the reorganization of the microtubule network within the oocyte, and the migration of the nucleus, and *grk*, to the future anterodorsal region of the oocyte. Here Grk signaling specifies adjacent follicle cells as dorsal (151, 174). As for *bcd*, *nos*, and *osk*, spatio-temporal restriction of Grk activity depends on a combination of mRNA localization and translational regulation.

grk mRNA concentrates at the posterior of the oocyte during early oogenesis and then exhibits a diffuse distribution during oocyte nuclear migration (151). Some *grk* mRNA may migrate with the nucleus, and this may occur in association with the endoplasmic reticulum (ER), since Grk is a secreted protein, and double-labeling experiments indicate that *grk* and the ER colocalize within the oocyte (191). Following this migration, *grk* exhibits an anterior ring of expression, which by stage 9 becomes restricted to the anterodorsal corner where it is maintained until at least stage 10B (151). The distribution of Grk protein is similar to that of *grk* mRNA, accumulating in the oocyte in early stages and later confined to the anterodorsal corner (153, 183).

CIS-ACTING ELEMENTS REQUIRED FOR REGULATION OF *GRK* Sequences within the 5′ and 3′ UTRs, as well as within the coding region, have been implicated in directing *grk* localization. Early accumulation of the transcript within the oocyte requires elements within the *grk* promoter or 5′ UTR (237). Deletion analysis of a reporter construct bearing the *grk* 5′ UTR suggested that nt 1–35 may be important for stable *grk* localization during early to mid-oogenesis, so this region was named the *grk* localization element 1 (GLE1) (191). An element in the 5′ end of the coding region, called the anterior cortical ring (ACR) element, is required for transcript stability and/or localization during mid-oogenesis in order to achieve the anterior ring of expression (237). Sequences within the 3′ UTR are required for the final stage of *grk* localization, whereby the transcript becomes focused in the anterodorsal corner. The 3′ UTR has also been implicated in translational

repression of *grk*, because a fusion construct lacking the 3′ UTR is translated across the anterior cortex at a time when a similar construct bearing the 3′ UTR shows the same mRNA distribution but is only translated in the anterodorsal corner (191). An element consisting of nt 96–155 of the *grk* 5′ UTR, called GLE2, has also been implicated in *grk* localization and translational activation; however, this element is also required for stability of *grk* mRNA after stage 8, so its other effects may be indirect.

TRANS-ACTING FACTORS REQUIRED FOR LOCALIZATION OF *GRK* Several factors such as *capu*, *spir*, and *maelstrom* (*mael*) are required indirectly for *grk* mRNA localization through their role in cytoskeletal assembly (discussed above) (31, 52, 137, 151). Also, in *orb* mutants *grk* mRNA is distributed broadly across the anterior of the oocyte, rather than focused in the anterodorsal corner (27, 184). In these ovaries, Grk levels are reduced and the protein is mislocalized around the oocyte cortex (153), resulting in dorsal-ventral patterning defects (27, 184). It is not known whether Orb functions directly in *grk* localization or is involved in translational activation, as is predicted by its homology to CPEB.

Female sterile mutations in *sqd* and *K10* prevent *grk* mRNA from localizing to the anterodorsal corner. Instead, the anterior ring of *grk* mRNA persists and is actively translated, leading to a dorsalization phenotype (93, 151, 263). Sqd, or Hrp40 (93, 143), is a member of the hnRNP family of proteins (see above), some of which shuttle between the nucleus and the cytoplasm (86, 95, 113, 167, 246). Three isoforms of Sqd, termed A, B, and S, are generated through alternative splicing, and these isoforms differ in their intracellular localization and function (156). SqdA and SqdS each have distinct roles in *grk* regulation and together can fulfill this requirement for *sqd*. SqdS, which is nuclear, appears to function in *grk* mRNA localization, while SqdA, which is thought to be cytoplasmic, is implicated in *grk* translational regulation. Sqd can be cross-linked to the *grk* 3′ UTR (106, 156), and *sqd* mutants exhibit the same pattern of *grk* mRNA mislocalization as *grk* transcripts lacking the 3′ UTR (237), implying that the binding of Sqd to the *grk* 3′ UTR is required to achieve the final localization pattern of the transcript. K10 interacts directly in vitro with all three isoforms of Sqd (156). In wild-type, K10 is localized to the oocyte nucleus (170, 200) as is one of the Sqd isoforms; however, in *K10* mutants, Sqd is no longer present in the oocyte nucleus (156). Given the similar mutant phenotypes, these results suggest that K10 may function upstream of Sqd in *grk* regulation.

TRANSLATIONAL REPRESSION OF *GRK* Grk is localized to the anterodorsal corner earlier than *grk* mRNA (191), implying that translational repression prevents protein synthesis from the unlocalized transcript. In support of this idea, *grk* is translated across the anterior margin in stage 8, when overexpressed, suggesting that high levels of the mRNA can titrate out a translational repressor (152). K10 has been suggested to function in translational repression of *grk* because in *K10* mutants, translation occurs from an anterior ring of *grk* mRNA, at a time when

the transcript shows an anterior ring distribution in wild-type but is translationally silent (191). Since the *grk* 3′ UTR is required for its translational repression, a repressor protein is predicted to bind to this region of the transcript. Bru is a possible candidate since the *grk* 3′ UTR contains consensus BREs, and Bru can be cross-linked to *grk* mRNA (96). In addition, Bru shows a colocalization with *grk* mRNA in the anterodorsal region during stage 10, the only divergence it shows from colocalization with *osk* in the oocyte (253). In vitro, Bru interacts directly with Sqd, suggesting that Sqd may shuttle *grk* out of the nucleus and link it to Bru to ensure correct regulation (156). However, a requirement for Bru in *grk* translational regulation has not yet been reported.

TRANS-ACTING FACTORS REQUIRED FOR TRANSLATIONAL ACTIVATION OF *GRK* Alleles of the *spindle* (*spn*) *genes*, *spnA*, *spnB*, *spnC*, *spnD*, and *spnE* (*homeless*) (232), demonstrate mislocalization of *grk* mRNA such that the anterior ring of expression persists until around stage 10 (71, 73, 77). This mislocalization produces ventralization, a phenotype opposite to that produced by a similar *grk* mRNA mislocalization in *K10* mutants, due to the fact that Grk protein levels are severely reduced at mid-oogenesis in *spn* mutants (71, 77), whereas in *K10* mutants, *grk* is actively translated from mRNA distributed across the anterior cortex (153, 201). Mutations in *okra* (*okr*) (197) show similar phenotypes to *spn* mutants but demonstrate a reduction in Grk levels in the early and middle stages of oogenesis (71). Double mutants for *K10* and alleles of *okr*, *spnB*, and *spnD* show a range of phenotypes, precluding a straightforward epistatic designation. In *okr*, *spnB*, and *spnD* mutants, levels of K10 protein in the oocyte nucleus are reduced, which may explain their *grk* mRNA mislocalization phenotype (71).

Mutations in the *spn* genes and *okr* also exhibit defects in karyosome formation (71, 77). Cloning of *okr* and *spnB* revealed homology to factors required in yeast for DNA double-strand break (DSB) repair (71). During mitosis, repair of DSBs is necessary to protect cells from damage to their DNA, whereas in meiosis, repair of DSBs is required for the processes of crossing over and recombination (166, 177). *okr* appears to be required for DSB repair in both mitosis and meiosis, whereas the function of the *spn* genes is restricted to meiosis (71).

Vas has multiple functions during oogenesis, including a role in karyosome formation resembling that of the *spindle* genes. The small fraction of oocytes that reach late stages of oogenesis in the absence of Vas activity display duplicated anterior eggshell structures at the posterior and a ventralization phenotype (225, 238, 239). In a *vas* null mutant there is a mild *grk* mRNA mislocalization phenotype but an extreme reduction in levels of Grk protein, and in a variety of *vas* allelic combinations, levels of Grk protein can be correlated with the degree of defects in axis patterning of the allele.

Vas may provide the link between the role of the *spn* genes in DSB repair and their effect on *grk* translation. In yeast, prevention of DSB repair during meiosis activates a checkpoint that leads to meiotic arrest, dependent on the gene MEC1 (125). The *Drosophila* homologue of this gene, *mei-41* (199, 203), when

doubly mutant with alleles of *okr*, *spnB*, and *spnD*, can restore correct dorsoventral patterning, suggesting that these genes act through a meiotic checkpoint. *mei-41* mutations cannot suppress mutations in *vas*, potentially placing *vas* downstream of this checkpoint. In keeping with this idea, in *spnB* mutants, Vas mobility is shifted on SDS-PAGE gels, suggesting that a posttranslational modification occurs in the absence of *spnB* function. This modification is eliminated in double mutants for *spnB* and *mei-41*. These observations have led to a model whereby the detection of DSBs activates a meiotic checkpoint resulting in posttranslational modification of Vas, which reduces its ability to activate *grk* translation (72).

Mutations in *aub* and *encore* (*enc*) also cause severe reductions in Grk protein and ventralization phenotypes (82, 197, 266). In addition, both are required for karyosome formation (71, 242). Little is known about the function of *aub* in *grk* regulation. Enc colocalizes with *grk* mRNA at the posterior of the oocyte and in the anterodorsal region (242). In *enc* mutants, there is a mild *grk* mRNA mislocalization phenotype but a strong reduction in Grk levels (82). *enc* is unlikely to be involved in the meiotic checkpoint pathway, as *enc* mutations cannot be suppressed by *mei-41* mutations and do not induce modification of Vas (242).

LINKING *GRK* EXPORT, LOCALIZATION, AND REGULATION *grk* represents one of the few transcripts whose localization has been proposed to involve vectorial export from the nucleus. Vectorial export, as opposed to transport in the cytoplasm, is envisaged as localization of a transcript in the cytoplasm by controlling the direction of its nuclear export (reviewed in 123). This model was primarily based on observations of apical localization of pair-rule transcripts in the embryonic blastoderm (63), but doubts about the validity of this general mechanism for mRNA localization have recently arisen due to experiments with the pair-rule transcript *ftz* (106). When the *ftz* transcript alone was injected into blastoderm embryos, it was not apically localized, demonstrating that the localization mechanism cannot be entirely cytoplasmic. However, preincubation of the transcript with nuclear extracts from *Drosophila* or human cells prior to injection allowed it to be apically localized in a microtubule-dependent manner. Specifically, preincubation with the *Drosophila* Sqd protein or the human proteins hnRNP A1, A2, and B was sufficient to mediate apical localization, dependent on the presence of the *ftz* 3′ UTR.

UV cross-linking demonstrated that Sqd can bind to the 3′ UTRs of both *ftz* and *grk* (156, 106). Since *grk* also requires Sqd for its localization, its localization may be cytoplasmically controlled, as for the injected *ftz* transcript. However, this has not been proven for *grk*, so it remains possible that this transcript exhibits vectorial nuclear export. The direct interaction between Sqd and Bru potentially links nuclear export, cytoplasmic localization, and translational repression of *grk* (156). Also important is the fact that Bru, Vas, Orb, and Aub are all implicated in the translational regulation of both *osk* and *grk* transcripts, indicating potential overlap in the mechanism of translational regulation of these two mRNAs. However, despite these hypotheses, both the significance of translational regulation for

localizing Grk activity and the mechanism by which *grk* may be translationally regulated remain unknown.

FUTURE DIRECTIONS

Rapid progress has been made in recent years both in understanding the link between RNA localization and translational control, and in the identification of *cis*- and *trans*-acting factors required to mediate these processes for specific transcripts. Thus this field has advanced to a stage where questions about the mechanisms by which these regulatory factors influence translation can be addressed. Much remains unclear in the general models of translational regulation. Work on the *Drosophila* transcripts described here as well as on many others will contribute to refining these models.

Cytoplasmic polyadenylation is a well-established method of translational regulation during development, but the mechanism by which it influences translation initiation is not understood. In *Drosophila* it is still an open question whether cytoplasmic polyadenylation is restricted to embryogenesis or whether it is also a means of achieving translational activation during oogenesis. Also unclear is whether the differences in *cis*-acting sequences involved in cytoplasmic polyadenylation between *Drosophila* and vertebrate model systems implies differences in the underlying mechanism.

Recent work on *nos* and *osk* has demonstrated that their translational regulation does not involve cap-dependent repression (30, 121). The association of untranslated *nos* with polysomes might imply a new mechanism for translational repression (30). It is not yet known at which step *osk* or *grk* translation is regulated, but this work will be aided by the newly established in vitro translation systems from *Drosophila* ovaries and embryos (20, 70, 121). It will be of interest to discover whether the 60S ribosomal subunit joining step of translation initiation, implicated in the translation block of the mammalian LOX transcript (158), will also be important for translational control of developmentally regulated transcripts in *Drosophila*. The interaction between *Drosophila* eIF5B (dIF2) (19), one of the translation initiation factors required for this step (165), and Vas, implicated in translational activation of several germline transcripts (69, 141, 181, 225, 238, 239), could indicate that these factors act to regulate a translational block at this step of initiation.

Several of the proteins described in this review are posttranslationally modified by phosphorylation. The relevance of phosphorylation to their activity is not well understood. The kinases involved may have many targets, making it more difficult to identify their specific roles in translational control and axis patterning. PAR-1, recently characterized due to its role in *osk* mRNA localization (202, 240), is predicted to be a kinase, raising the possibility that it may phosphorylate components of the pole plasm such as Osk. It is not yet known what function of Osk is regulated by phosphorylation. Posttranslational modification of Vas, presumably by

phosphorylation, may be signaled by nuclear events, and appears to be an intermediate step in *grk* translational regulation (72). It will be interesting to determine both the mechanism controlling this sequence of events and whether other functions of Vas depend on its phosphorylation. In addition, phosphorylation is involved in regulation of masking and cytoplasmic polyadenylation in several systems, but this remains to be investigated in *Drosophila*.

Many of the potential *trans*-acting factors discussed in this review cross-link to target mRNAs, defining them functionally as RNA-binding proteins. In several instances such as Smg (36, 208) and Apt (120), these proteins lack any previously characterized RNA-binding domains. Thus investigation of proteins such as these is likely to lead to new designations of motifs involved in RNA binding. A good example of this is Pum, which binds to NRE sequences in several *Drosophila* targets, and in combination with Nos mediates translational repression (reviewed in 163). The region of Pum required for RNA binding (256, 270) has been named a Puf domain and has been identified in other proteins in many different species (270, 272). This domain shows no similarity to other domains involved in RNA binding, and thus represents a new RNA-binding motif (51, 269). The polar granule component, Tud (75), contains at least 10 copies of a motif designated the tudor domain, which has subsequently been identified in several nucleic-acid binding proteins (reviewed in 169). As yet there is no direct evidence that this domain is involved in nucleic-acid binding.

Another area that warrants further investigation is how mRNP particles are loaded onto the cytoskeletal transport machinery. For both *bcd* and *osk*, Stau is thought to play a role in connecting them to the microtubule cytoskeleton. However, in the *Drosophila* nervous system, Stau appears to mediate mRNA transport via the actin cytoskeleton (18), and it is not known how this factor interacts with either cytoskeletal transport system. Mammalian Stau homologues are implicated in microtubule-dependent transport in neurons (99) and bind to tubulin via a domain that is not present in the *Drosophila* protein (261). Another protein whose requirement for mRNA localization and oocyte determination is thought to involve links with the microtubule cytoskeleton is Bic-D (172, 226, 223). *Bic-D* genetically interacts with the *Drosophila* homologue of *Lissencephaly-1* (*DLis-1*), required for localization of dynein heavy chain (Dhc) in the oocyte (227). *Bic-D*, *DLis-1*, and *Dhc* are thought to be involved in establishing a microtubule transport system early in oogenesis, necessary for growth of the oocyte. Within the oocyte, Swa appears to mediate association of *bcd* with the light chain subunit of dynein (Ddlc-1) (194). Although kinesin I is required for *osk* localization within the oocyte (17), it is not known how *osk* is linked to this motor protein. As yet no *Drosophila* transcripts have been purified in association with complexes containing molecular motors.

A great deal of attention has been given to regulatory mechanisms that involve transcriptional control of specific genes. Outside of a few specific systems, comparatively little work has been done on translational control of specific mRNAs, yet this mechanism is also fundamental to essential developmental processes. Further research seems certain to identify many other mRNAs with highly regulated

translation, and other developmental processes that rely on translational control. Work in model organisms such as *Drosophila* will most likely be instrumental in extending our understanding of this field.

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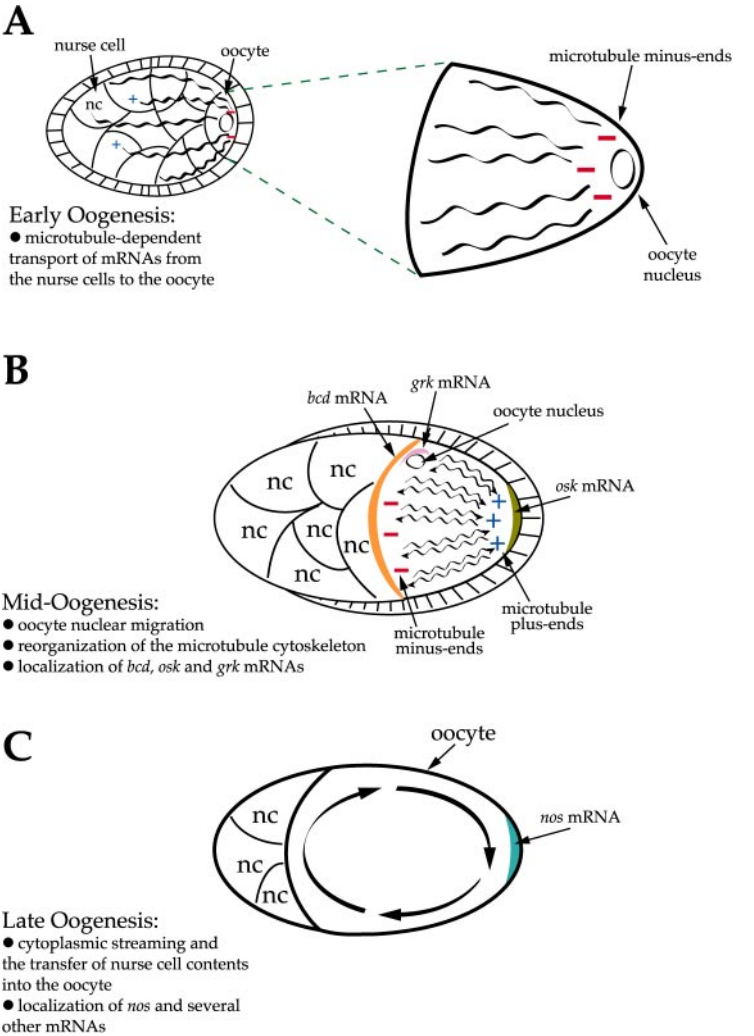

Figure 1 Organization of the microtubule cytoskeleton and mRNA localization. (*A*) During the early stages of oogenesis, microtubule minus-ends are concentrated at the posterior of the oocyte while plus-ends extend toward the anterior and into the nurse cells. (*B*) Subsequently a reorganization of the microtubule cytoskeleton occurs within the oocyte such that the microtubule minus-ends are anteriorly anchored and the plus-ends are located at the posterior. The oocyte nucleus migrates to the anterodorsal corner of the oocyte, and several mRNAs localize to specific positions within the oocyte. *bcd* mRNA is shown in orange, *grk* mRNA in pink, and *osk* mRNA in olive. (*C*) In the late stages of oogenesis, the nurse cells transfer their contents into the oocyte wherein rapid cytoplasmic streaming and localization of some specific factors such as *nos* mRNA, shown in blue, takes place (adapted from 60 and 109a).

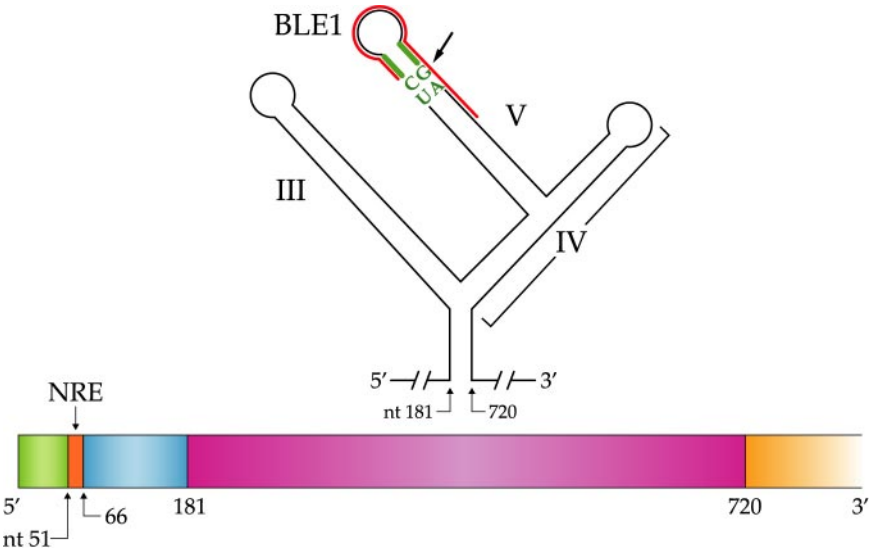

Figure 2 Schematic diagram of the *bcd* 3′ UTR showing a portion of the predicted secondary structure (126, 131, 198). The location of the consensus Nos response element (NRE; 258) is indicated in dark orange. The region of stem-loop V shown in red represents the *bcd* localization element (BLE1; 129), and the region shown in green represents a stretch of nucleotides which must be double-stranded for event A localization to occur (128). At least some of the individual nucleotides shown in green are also required for this event. Substitution of the G residue, indicated by the arrow, eliminates event A localization but maintains event B and later stages of localization (127).

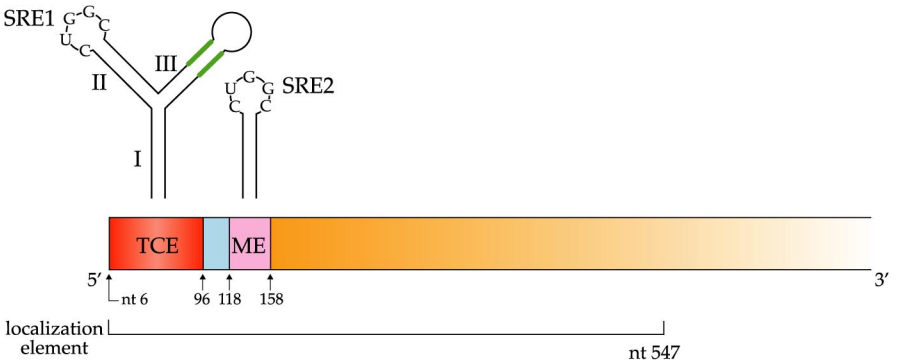

Figure 3 Schematic diagram of the *nos* 3′ UTR showing the predicted secondary structure. The translational control element (TCE; 69) is shown in red, and the minimal element (ME; 9) is shown in pink. The predicted secondary structure of the TCE includes two stem-loops (35). Stem-loop II contains the Smg recognition element (SRE1; 209). The region of stem-loop III shown in green represents a UA-rich stretch of nucleotides required for translational repression (35). A second SRE (SRE2) is located within a predicted stem-loop in the ME region (209).

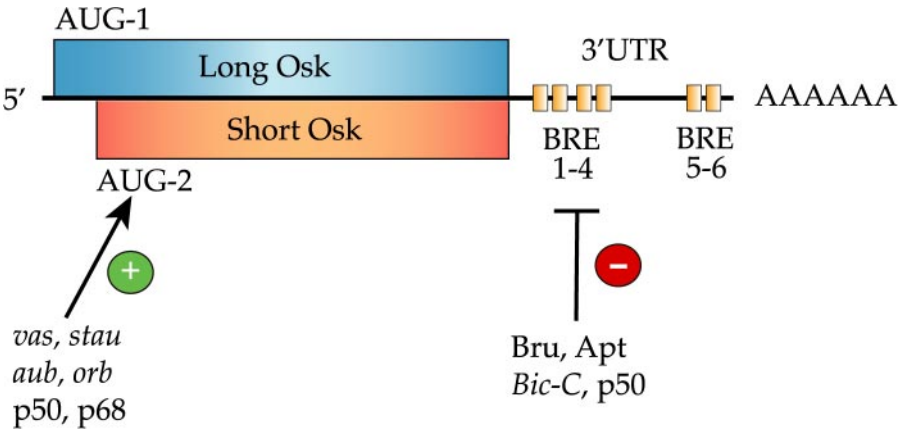

Figure 4 Schematic diagram of *osk* mRNA. Two isoforms of Osk protein, Long Osk and Short Osk, are produced from the *osk* transcript (141, 181). Genes and proteins implicated in translational regulation of *osk* are indicated. The *osk* 3′ UTR contains Bru response elements (BREs), shown in yellow, which confer translational repression through the binding of Bru (96, 253).