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Mechanisms of Subcellular mRNA Localization

Review

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Localization of RNA is a widespread and efficient way to target gene products to a specific region of a cell or embryo. This strategy of posttranscriptional gene regulation utilizes a variety of distinct mechanisms to regulate the movement and anchoring of different transcripts.

Introduction

During the past fifteen years, subcellular localization of RNA has emerged as a key mechanism through which cells become polarized. The localization of transcripts is an extremely efficient way to target gene products to individual subcellular compartments or to specific regions of a cell or embryo, making it an important posttranscriptional level of gene regulation. RNA localization is now known to be a widespread phenomenon that occurs in unicellular organisms, in animal and plant tissues, and in developing embryos from a variety of animal phyla (reviewed in Bashirullah et al., 1998; Jansen, 2001; Palacios and St Johnston, 2001; Chartrand et al., 2001; Lipshitz and Smibert, 2000; Seydoux and Schedl, 2001; Kloc et al., 2001; see Figure 1).

RNAs are localized for a variety of reasons. One of the major reasons is the production of a localized high concentration of protein (Figure 2A). Therefore, moving fibroblasts can produce large amounts of β-actin protein at their leading edge by the localization of the β-actin mRNA to that region of the cell. Another important reason for localizing RNA is to produce a gradient of morphogen (Figure 2B). An example is the bicoid protein in *Drosophila* eggs. A third reason is to initiate cell lineages by sequestering localized RNAs within a specific blastomere or daughter cell (Figure 2C). The specification of the germ cell lineage in amphibians, nematodes, and insects, the specification of yeast mating type, and the differentiation of neuroblasts all occur through the localization of mRNAs to specific blastomeres or daughter cells. A fourth reason is to segregate specific RNAs to particular organelles or subcellular structures (Figure 2D). An example of this phenomenon is the targeting of cyclin B mRNA to the mitotic spindle (Groisman et al., 2000). RNAs are also localized to restrict translation to a specific site within a cell or embryo (Figure 2E). This usually involves coordinate repression and activation of translation within specific regions of the cell or embryo. Examples include the localized translation of *Drosophila nanos* and *oskar* mRNAs and various mRNAs in neurons.

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How Is RNA Localized?

Following transcription in the nucleus, most RNAs exit through the nuclear pores into the cytoplasm, where they are translated. Certain classes of RNAs, however, have different destinies and are targeted to specific regions within the cell or embryo and in many instances are not translated until they reach their final destination. What is it about this special class that earmarks them to follow this pathway? The initial steps in the localization process are determined by the *cis*-acting elements within the RNA. This series of signals that are usually, but not exclusively, located within the 3′ UTR direct the binding of *trans*-acting factors to the RNA. So far, all *trans*-acting factors have been identified as proteins; however, it is quite possible that other classes of molecules such as small regulatory RNAs may play a role in this process. Binding of the factors likely influences the folding of the RNA into a specific spatial configuration facilitating the association of a series of other auxiliary proteins. This produces a large ribonucleoprotein (RNP) transport particle. It is probably through the protein factors that the RNA will recognize and associate with the proper pathway or subcellular structure that will direct it to its proper destination. In the case of pathways that may utilize cytoskeletal machinery, there likely is an active transport mechanism involving a molecular motor that, along with adaptor proteins, will propel the mRNA. Once at its destination, it will anchor through a molecular anchor, which could be either protein or, in some instances, another RNA (Kloc and Etkin, 1994; Heasman et al., 2001).

Studies in diverse systems such as oocytes, embryos, and somatic cells have demonstrated the existence of several potential mechanisms by which RNAs can be localized. These include the active directional transport of RNA on cytoskeletal elements, general degradation and localized RNA stability, random cytoplasmic diffusion and trapping, and vectorial transport from the nucleus to a specific target. A combination of mechanisms may be used to localize different RNAs; however, the most convincing evidence exists for the mechanisms of active directional transport on cytoskeletal elements and degradation combined with localized stability. Therefore, these two will be discussed.

Directed Transport on Cytoskeletal Elements

The cytoskeleton has been shown to play a role in the transport of many different RNAs in a variety of systems including somatic cells, yeast, and *Drosophila* (reviewed in Bashirullah et al., 1998; Jansen, 2001; Palacios and St Johnston, 2001). There are two classes of cytoskeletal networks that have been implicated in the transport of RNA cargo. These are actin microfilaments and microtubules. In general, the actin networks are involved in short-distance transport, while microtubules are involved in long-distance transport. In each case, a variety of molecular motors have been implicated as functioning in the transport of the RNAs along the cytoskeletal tracks. These fall into the classes of kinesin, kinesin-like, and dynein molecular motors that direct trafficking on microtubules and myosin-based motors that direct

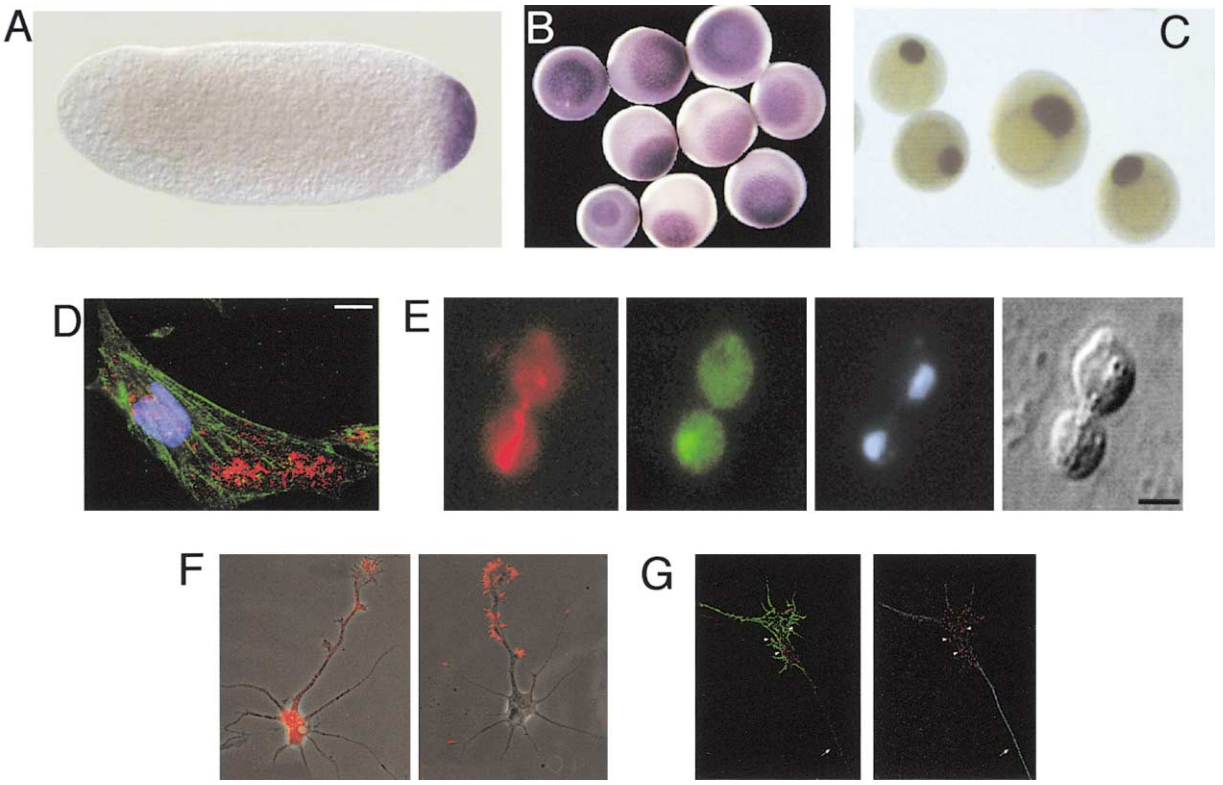

Figure 1. Examples of Localized RNAs in Different Organisms and Cell Types

(A) The localization of maternal *nanos* mRNA at the posterior of an activated, unfertilized *Drosophila* egg is the result of two different mechanisms: generalized degradation and local protection. Whole mount in situ hybridization. (Courtesy Dr. Howard Lipshitz).

(B) fatVg mRNA localization in the vegetal cortex of stage IV–VI *Xenopus* oocytes, whole mount in situ hybridization.

(C) Xpat mRNA localized in the mitochondrial clouds of stage I *Xenopus* oocytes, whole mount in situ hybridization. Xpat mRNA is associated with the germ plasm and is localized through the METRO pathway.

(D) Colocalization of β-actin mRNA (red) in the leading lamellae of chicken fibroblast with phosphorylated myosin (green immunofluorescence). Nucleus stained blue with DAPI (Courtesy R. Singer).

(E) Ash1 mRNA (red) localized with Ash1 p-myc protein (green) in budding yeast. Cell nuclei stained blue with DAPI. Last image shows the same cells in Nomarski (Courtesy of R. Singer).

(F) β-actin mRNA localization in the neurite and growth con (left) and β-actin protein highly enriched in growth cone and filopodia (right) (Courtesy G. Bassell).

(G) Localization of ZBP1 (Zipcode binding protein) (red) along with F-actin (green, left) and microtubules (blue, right) in neurite and growth cone (Courtesy G. Bassell).

trafficking on actin microfilaments. It should be emphasized that despite many reports of the role of the cytoskeleton in RNA transport, there is very little data that demonstrates the mechanism of how such a system operates. Therefore, a great deal of analysis remains to be carried out in this area. Despite these caveats, there are still several good examples that support the role of the cytoskeleton in RNA transport.

The first example of potential cytoskeletal involvement in RNA localization was in the frog *Xenopus laevis* (Yisraeli et al., 1990; Yaniv and Yisraeli, 2001). In *Xenopus*, there are two major pathways through which RNAs are localized to the vegetal cortex during oogenesis (Kloc et al., 2001; King et al., 1999). The pathway that localizes RNAs during the earliest stages of oogenesis (stages I and II) is called the METRO or early pathway and utilizes a structure called the mitochondrial cloud as a vehicle to transport RNAs. The force that drives the cloud and its associated RNAs to the cortex is not known but is of considerable interest.

The second pathway, which functions during stages III–V, is called the late pathway and localizes mRNAs such as Vg1 and FatVg to the vegetal pole of the oocyte in a microtubule-dependent manner (Yisraeli et al., 1990; Kloc et al., 2001). An intriguing discovery was that during late stage II and stage III of oogenesis, the late-pathway RNAs are found in a wedge-shaped pattern between the germinal vesicle (nucleus, abbreviated GV) and the vegetal cortex (Kloc et al., 2001). This structure also consists of a subdomain of the endoplasmic reticulum (ER; Deschler et al., 1997). Importantly, this ER-containing wedge-shaped structure became visible as the cloud migrated to the vegetal pole. As this membranous ER fraction is forming behind the migrating cloud, the uniformly distributed Vg1 mRNA associates with the ER. There is evidence that the Vg1 mRNA colocalizes with vesicle-like structures of ER when transiting to the vegetal cortex (Kloc et al., 2001). Thus, a model emerges in which the late-pathway function depends on the establishment of the late-pathway machinery through the

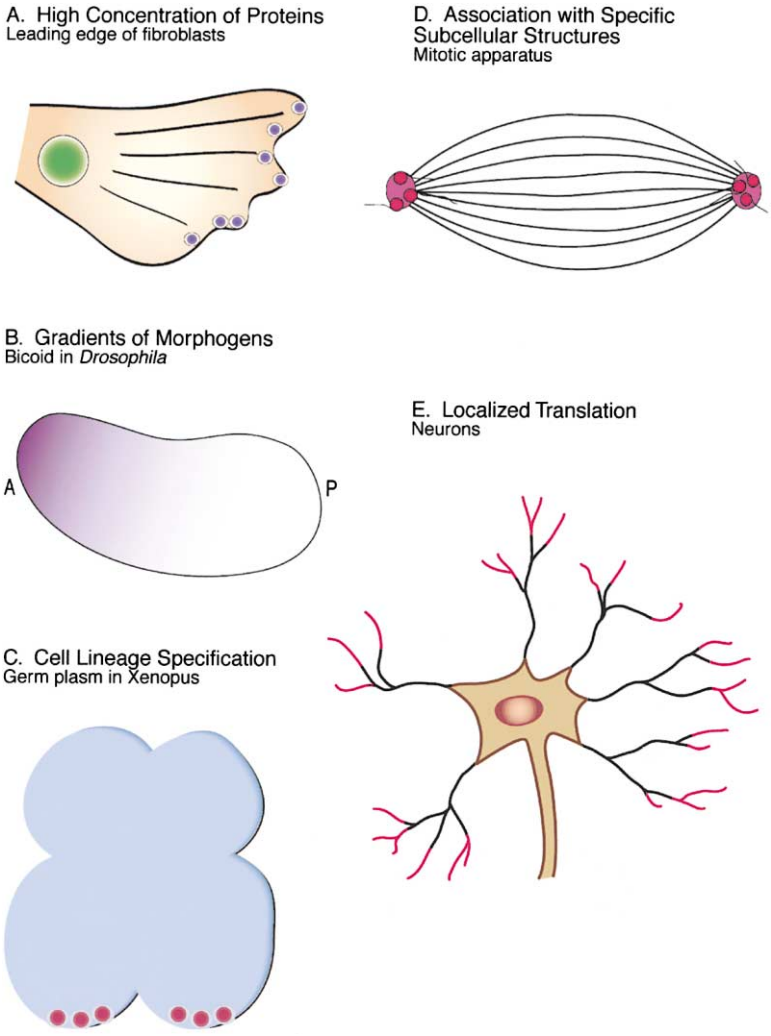

Figure 2. Examples of the Roles of Localized RNAs in Different Systems

(A) The production of high levels of protein in specific regions of cells as represented by the accumulation of localized β-actin mRNA and protein at the leading edge of a fibroblast.

(B) The production of gradients of morphogens in oocytes and embryos as in the case of *bicoid* mRNA localization in *Drosophila* producing a gradient of Bicoid protein.

(C) Cell lineage specification. Localized RNAs are used in a variety of systems in which they are partitioned unequally into daughter cells or into blastomeres of embryos to determine a cell lineage. The case depicted is the localized RNAs involved in specifying the germ cell lineage in amphibians.

(D) Association of RNA with different organelles or cell structures. This example is of the localization of cyclin B mRNA at the poles of the mitotic spindle.

(E) Some RNAs are localized to a specific region of a cell or embryo to allow for localized translation such as RNAs at the synapses of neurons (red) .

movement of the mitochondrial cloud and its associated RNAs to the vegetal cortex. The Vg1 mRNA may associate with ER membrane vesicles that transit along the microtubules and anchor at the vegetal cortex. However, the identity of the molecular motors or adaptor proteins operating within the late pathway is not known. Based on the use of a microtubule cytoskeletal network, the motors are likely to be members of either the kinesin or dynein families. This suggests that there may be a functional association between the localizing RNA, the membrane vesicles of the ER, and the microtubule cytoskeleton.

Another example of membrane and microtubule cytoskeletal involvement in RNA localization is at the posterior pole of *Drosophila* oocytes. The posterior pole membrane contains a posterior plasma membrane domain (PMD) that requires Rab11-dependent endocytic trafficking for its formation. Mutation of the Rab11 gene results in ectopic localization of *oskar* mRNA, whereas localization of *gurken* and *bicoid* mRNAs and signaling between the oocyte and the somatic follicle cells are unaffected (Jankovics et al., 2001). The ectopic *oskar* mRNA localization in the Rab11 mutants is a result of an abnormally polarized oocyte microtubule cytoskeleton. This is probably caused by a defect in the attachment of microtubule plus ends to PMD and the inability of *oskar* mRNA and the components of its translational machinery to move to the proper posterior location (Dollar et al., 2002). These results suggest that membranous structures within the oocyte play an important role in microtubule organization and in *oskar* mRNA localization (Jankovics et al., 2001). This highlights the importance of membranes in *oskar* mRNA localization and is consistent with the coordinate function of the ER and microtubules in the localization of late-pathway RNAs in *Xenopus*. Thus, the use of membrane structures and microtubule cytoskeletal networks in the process of RNA localization appears to be utilized in diverse species.

In *Caenorhabditis elegans*, the PAR-1 kinase is localized to the posterior of the zygote and is required for anterior-posterior axis formation. The *Drosophila* PAR-1 homolog also localizes to the posterior of the oocyte along with *oskar* mRNA. Shulman et al. (2000) found that in par-1 mutants, *bicoid* mRNA accumulates normally at the anterior, but *oskar* mRNA is mislocalized to the center of the oocyte. In addition, they found that the oocyte microtubule cytoskeleton was disorganized. Thus, it is likely that

one function of *Drosophila* PAR-1 may involve organizing the oocyte microtubule network to direct *oskar* mRNA to the posterior. This result also demonstrates the utilization of common components in anterior-posterior polarization in *Drosophila* and *C. elegans* and the importance of the microtubule cytoskeleton in RNA transport. It also suggests that the same molecules and machinery can be coopted for use in establishment of polarity as well as in localization of mRNAs in different systems (Tomancak et al., 2000).

Microtubules have also been implicated in RNA localization in somatic cells such as growing neurons. These cells have to localize various molecules over very long distances from the cell body to the filapodia of growth cones, a distance of 1 m or more in large animals. The β-actin mRNA and *trans*-acting Zipcode binding protein (ZBP1) are localized in transport granules that translocate along the neural processes via a microtubule-dependent pathway (Zhang et al., 2001).

An important question concerns how an RNA may recognize the proper cytoskeletal transport system. Is it the presence of specific factors that bind to each RNA, directing it to the proper target? For *bicoid* mRNA, it appears that association with factors in the nurse cells is important for its proper microtubule-dependent localization (Cha et al., 2001). During oogenesis, *bicoid* mRNA is synthesized in the nurse cells and is transported to the oocyte where microtubules and Exuperantia protein mediate localization to the anterior pole. Injected *bicoid* mRNA exhibits a nonpolar microtubule-dependent transport to the closest cortical surface of the oocyte. However, *bicoid* mRNA, when first exposed to nurse cell cytoplasm, shows microtubule-dependent transport to the anterior cortex. This interesting result suggests that it is Exuperantia-*bicoid* mRNA complex formation in the nurse cell cytoplasm, not the polarity of the microtubules, that allows anterior-specific microtubule-dependent transport in the oocyte. Thus, the directed transport of specific mRNAs to various compartments of the cell or oocyte is likely to be dependent upon the association with *trans*-acting factors.

One of the strongest cases for the role of molecular motors in RNA localization is the use of dynein motors in the localization of transcripts of the pair-rule and wingless (wg) segmentation genes. These transcripts are localized apically of the layer of peripheral nuclei during zygotic development in the syncytial blastoderm stage embryo. Localization of these transcripts is mediated by signals within their 3′ UTRs and is driven on microtubules by the minus end-directed molecular motor dynein (Wilkie and Davis, 2001).

Recently, it was shown that egalitarian (Egl) and bicaudal-D (BicD) are in a complex that associates selectively with transcripts that localize apically in *Drosophila* blastoderm embryos (Bullock and Ish-Horowicz, 2001). The authors propose that the Egl/BicD complex links specific RNAs to dynein and the microtubule cytoskeleton. The other important finding was that this machinery is present in both embryos and oocytes, and some maternal RNAs possess common signals that function both in the oocyte and in apical localization in blastoderm embryos. Thus, Egl and BicD are possible components of dynein motor complex that propels localization of various transcripts along the microtubule tracks during *Drosophila* oogenesis and embryogenesis. The fact that Egl and BicD orthologs are also present in other organisms suggests that they may be components of evolutionarily conserved RNA localization machinery.

The best-studied example of the role of actin microfilaments in RNA transport is in yeast. Actin microfilaments are involved in translocating the Ash1 mRNA to the daughter bud tip, which is crucial for mating-type switching (reviewed by Chartrand et al., 2001; Jansen, 2001). The Ash1-containing complex is linked by an adaptor protein, She3p, to the myosin motor Myo4p. This then moves the transport complex along actin cables (Bohl et al., 2000; Takizawa and Vale, 2000; Long et al., 2000). In general, most other eukaryotic organisms rely extensively on microtubule-based transport instead of actin-based transport of RNA. However, there are several examples of actin cables involved in RNA transport in other eukaryotic organisms, including the movement of *prospero* mRNA from the apical to basal side of *Drosophila* neuroblasts (Roegiers and Jan, 2000; Bassell and Singer 2001; Broadus and Doe, 1997) and the movement of actin mRNA to the leading edge of fibroblasts (Hill and Gunning 1993, Kislauskis et al. 1993). Actin microfilaments have also been shown to function in the anchoring of transcripts such as Vg1 at the vegetal cortex in *Xenopus* oocytes (Yisraeli et al., 1990; Kloc et al., 2001).

One caution is that while localization processes among organisms such as yeast, *Drosophila*, *Xenopus*, and somatic cells appear to have similar attributes, the molecules used are probably different. For example, yeast rely on specialized actin-myosin adaptor molecules for localization. To date, homologs for some of these adapters have not been found in other organisms. Therefore, one must be cautious in making generalizations regarding mechanisms and molecules between different systems. Additionally, the use of common components in the localization process does not necessarily prove that mechanisms are conserved between different systems. We really know very little about how the cytoskeleton and molecular motors are used in the different systems, and it is quite possible that these basic cell polarity components are coopted in different (or a number of) ways for use in the RNA localization process.

Localization via General Degradation and Localized Protein Stability

Differential stability is another mechanism used to produce a localized concentration of RNA. In *Drosophila*, Ding et al. (1993) demonstrated that for Hsp83, mRNA degradation played an important role in its localization to the posterior pole in embryos. Now it is known that the posteriorly localized Hsp83 transcripts are protected from degradation (Bashirullah et al., 1999). This mechanism also plays a role in localization of *nanos* and *Pgc* RNAs. The regulation of the degradation pathway is quite interesting and involves the use of *cis*-acting instability elements that direct degradation and protection elements that protect the transcripts from degradation at their final destinations. While currently only a limited number of examples exist for this mechanism, the high degree of conservation of the degradation machinery between *Drosophila* and *Xenopus* indicates that this level of regulation of transcript localization may be common. The degradation machinery appears to be conserved in *Xenopus* oocytes based on the ability of *cis*-

acting *Drosophila* degradation elements of Hsp83 to function in *Xenopus* oocytes (Bashirullah et al., 1999). For *nanos* and *Hsp83* mRNAs, these elements are located in the 3′ UTR (Bashirullah et al., 1999).

What Are the Localization Signals on the RNAs and Factors that Bind Them?

The transport of localized RNAs to their final destination requires the presence of *cis*-acting sequences and *trans*-acting proteins that interact with them. The nature of such localizing signals is still rather elusive, and the common rules that govern whether or not a sequence will function as a localization element are unknown. One of the major limitations in determining the rules is the technology used to assay their function as well as the difficulty in accurately predicting RNA secondary structure. However, despite these limitations, there are several principles that are emerging. One is that localization elements are rarely found as single sequences but rather consist of multiple components. In some cases there is redundancy among the elements such that each can function alone but all are needed for maximal localization efficiency. A second principle is that there are general localization elements that direct the association of a transcript with the localization machinery, and there are specific elements that direct the targeted RNA to a particular subcellular structure. Third, the localization machinery may be conserved between different systems as demonstrated by the identification of common strategies and components of the machinery. Finally, localization elements may be nearby or overlap sequences involved in the regulation of other processes such as translation (see discussion in section on translational regulation).

Redundancy of Elements and Conservation of trans*-Acting Factors*

The analysis of the *cis* elements and *trans*-acting factors involved in the localization of Vg1 mRNA to the vegetal pole of *Xenopus* oocytes provides a very important paradigm. A 360 nt region of the Vg1 3′ UTR, called the Vg1 localization element (VgLE), possesses a number of different subelements that function redundantly in its localization (Mowry and Cote, 1999; Yaniv and Yisraeli, 2001; Deshler et al., 1998). Fine structural mapping of the subelements within the VgLE has resulted in a rather complex picture of the critical localization elements that still need to be sorted out in order to fully understand the precise roles of each (see Mowry and Cote, 1999, and Yaniv and Yisraeli, 2001, for discussion). Regardless, a general rule is emerging that the partially redundant elements alone produce weak localization, but together they function efficiently to produce robust localization of the transcripts.

Trans-acting factors that bind to the subelements include Vg1RBP (Yaniv and Yisraeli, 2001) and the VgRBPs p78, p69, p60, p40, p36, and p33 (Mowry and Cote, 1999). A novel 75 kDa protein, Vera, that interacts specifically with the VgLE was isolated in another search for *trans*-acting factors. Upon cloning of Vg1RBP and Vera, the two proteins were found to be the same (Deshler et al., 1998; Havin et al., 1998). In addition, Vg1RBP and Vera were found to be the homolog of the mammalian Zipcode binding protein that is associated with localizing β-actin mRNA in fibroblasts (Ross et al., 1997). This finding demonstrates the conservation of *trans*-acting factors across species and cell types.

An 85 nt region within the Vg1 3′ UTR contains a hexanucleotide UUUCUA called the Vg1 motif 1 (VM1) (Mowry and Cote, 1999). This element plays an important role in Vg1 mRNA localization. It was found that the VgRBP60 protein binds to the VM1 element and that VgRBP60 is a homolog of the human hnRNP protein, hnRNP1 (Cote et al., 1999). This finding was important, since another hnRNP protein, hnRNP A2, was found to bind to the myelin basic protein (MBP) mRNA localization element (Kwon et al., 1999), and the *Drosophila* homolog of hnRNP A1 (Squid) was shown to be required for localization of *gurken* mRNA in the oocyte (Norvell et al., 1999). These findings highlighted the importance of hnRNPs in the process of RNA localization.

Since hnRNPs first form in the nucleus, their association with *cis*-acting elements of localized RNAs suggests that the first step in producing a transport particle occurs in the nucleus. This finding could provide important insights into the mechanism that distinguishes localized transcripts from those that do not localize, since it suggests that the nuclear history and assembly into a primary transport particle may be important in this distinction.

Nanos mRNA in *Drosophila* is another example of RNAs utilizing multiple partially redundant localization elements. *Nanos* has four different regions within its 3′ UTR that function to localize the mRNA to the pole plasm at the posterior pole of the oocyte. These function weakly on their own but most efficiently when all are present (Bergsten and Gavis, 1999; Crucs et al., 2000). A 75 kDa protein has been shown to bind to a 41 nt region, and binding of this protein may be important for localization (see section on structure of elements below).

Another example of an mRNA with multiple redundant localization elements is Fatvg mRNA that is localized to the vegetal cortex of *Xenopus* oocytes (Chan et al., 1999). This mRNA possesses multiple elements that function in localization through the late pathway; however, for most efficient localization of the RNA, all of these must be present. Interestingly, Fatvg localizes through both the METRO and late pathways, so in addition to the late localizing element already identified, it is likely that it possesses additional elements for localization through the METRO pathway (Chan et al., 1999).

Xcat2, MBP, and Gurken Possess Both General and Specific Localization Elements

Xenopus Xcat2 mRNA is localized early during oogenesis through the METRO or early pathway (Kloc et al., 2001, 2002; King et al., 1999). The mRNA is found in the germ plasm associated with the electron dense germinal granules (Kloc et al., 2001). Within the Xcat2 3′ UTR are sequences, such as the mitochondrial cloud localization element (MCLE), that serve a general localization function by directing the mRNA to the mitochondrial cloud, the main structural component of the early or METRO localization pathway (Zhou and King, 1996; Kloc et al., 2000b). In addition, the Xcat2 3′ UTR possesses a second region, the germinal granule localization element (GGLE), that directs the mRNA to the germinal granules within the germ plasm-containing region of the mito-

chondrial cloud (Kloc et al., 2000b). The two signals are both needed for proper localization of Xcat2 mRNA, since the MCLE can only direct the Xcat2 mRNA to the cloud but not to the germinal granules, while the GGLE is needed for association with the germinal granules. By itself the GGLE will not localize an RNA to the mitochondrial cloud. Interestingly, the GGLE added to another METRO-localizing RNA, Xlsirts, which is normally not found on the germinal granules, will direct this RNA to the granules. This demonstrates the utilization of compound signals within the RNAs, some of which function as general localization signals and others that have a more specific function. Other examples of this type of organization of *cis* elements are found in the mammalian myelin basic protein (MBP) and the *Drosophila gurken* mRNAs. MBP mRNA contains signals directing the transcript from the cell body to the process and another signal directing it to the myelin compartment (Ainger et al., 1997), while *gurken* mRNA possesses signals within the 5′ and 3′ UTRs as well as the coding region that direct the mRNA to different regions (Saunders and Cohen, 1999; Thio et al., 2000).

The Role of trans*-Acting Factors in mRNA Localization, Translation, and Anchoring*

Specific RNA binding proteins recognize the *cis*-acting sequences, bind to RNA, and form particles that are transported to specific sites within the cell. The best-characterized RNA binding protein that is required for localization of several different mRNAs in diverse cell types is Staufen. During *Drosophila* oogenesis, Staufen colocalizes with *oskar* mRNA and is involved with its localization, anchoring, and translation at the posterior pole. Also, Staufen protein plays a role in anchoring the anterior determinant *bicoid* at the anterior pole of the egg. Staufen also is present in somatic cells where it associates with *prospero* mRNA during the asymmetric divisions of embryonic neuroblasts and plays a role in its localization. Staufen also plays an important role in the localization of RNAs in vertebrate neurons (reviewed in Palacios and St Johnston, 2001; Lipshitz and Smibert, 2000; Kiebler and DesGroseillers, 2000; Roegiers and Jan, 2000).

Staufen performs its multitude of functions through judicious use of multiple RNA binding domains. The Staufen protein consists of five double-stranded RNA binding domains (dsRBDs) and a short insertion that splits dsRBD2 into two halves (Micklem et al., 2000). Full-length Staufen protein lacking this insertion is able to associate with *oskar* mRNA and activate its translation, but fails to localize the RNA to the posterior. In contrast, Staufen lacking dsRBD5 localizes *oskar* mRNA normally but does not activate its translation. Thus, dsRBD2 is required for the microtubule-dependent localization of *oskar* mRNA and dsRBD5 for the derepression of *oskar* mRNA translation, once localized. Additionally, the dsRBD5 domain directs the actin-dependent localization of *prospero* mRNA, indicating that distinct domains of Staufen mediate microtubule- and actin-based mRNA transport.

Deletion of dsRBD5 inhibits the localization of Staufen protein and *prospero* mRNA in neuroblasts. This domain binds to Miranda protein, which colocalizes with Staufen and *prospero* mRNA at the basal side of the neuroblast and is required for the localization of both Staufen protein and *prospero* mRNA. While there is no direct evidence, it is possible that Miranda functions as an adaptor protein linking the Staufen complex to the actin microfilament localization machinery (Palacios and St Johnston, 2001). Therefore, Staufen protein is the quintessential *trans*-acting factor that is involved in anchoring, transport, and translational regulation.

The anchoring of Vg1 mRNA to the vegetal cortex in *Xenopus* oocytes is dependent upon actin microfilaments (Yisraeli et al., 1990). A *trans*-acting factor, Prrp (proline-rich RNA binding protein), was found to bind to the VgLE (Zhao et al., 2001). Prrp also associates with other late-pathway mRNAs but not with RNAs such as Xcat2 or Xwnt11 that use the METRO pathway and anchor in a microfilament-independent manner. The proline-rich domain of Prrp interacts with profilin, which promotes actin polymerization, and Mena, another actin binding protein. This strengthens the conclusion that anchoring of late-pathway-localizing RNAs involves actin microfilaments. It will be interesting to determine how Xlsirts, a noncoding RNA, and the mRNA-encoding VegT, a transcription factor involved in germ layer specification, both of which are also involved in anchoring Vg1 mRNA (Kloc and Etkin, 1994; Heasman et al., 2001), fit into this cytoskeletal organization. As progress in this area continues and more factors are identified and characterized, it will become clearer as to whether or not other *trans*-acting factors are multifunctional or if the general rule is to have unique factors that have evolved for the transport of individual transcripts.

Sequence or Structure of cis*-Acting Elements?*

The analysis of *cis*-acting localization elements has been rather unsatisfying from the standpoint of identification of common sequences that direct localization of different RNAs. What is apparent is that both primary sequence and secondary structure within the localization elements are important for their function. The data suggests that within localization elements are regions that form stem-loop structures that are critical for proper localization of the mRNA (Yaniv and Yisraeli, 2001; Chartrand et al., 2001). One example is the bicoid localization element (BLE) within the 3′ UTR of the *Drosophila bicoid* mRNA, which forms specific stem-loop structures that are conserved among different *Drosophila* species (Macdonald and Kerr, 1998). Mutational analysis demonstrated that these structures must be maintained for proper localization of the *bicoid* mRNA. In addition, proper stem-loop structures also are required for binding of Staufen, which functions in anchoring the *bicoid* mRNA at the anterior pole. An important finding was that compensatory mutations that changed the primary sequence but maintained the proper stem-loop structure were sufficient for Staufen binding, transport particle formation, and possibly anchoring (Ferrandon et al., 1997). This was critical evidence for the importance of the secondary structure (instead of the primary sequence) of a particular localization element for proper function.

An example that suggests both primary sequence and secondary structure are important is the short localization element (TLS) in the *Drosophila* K10 mRNA. Predictions indicate that this localization element also contains a region of stem-loop secondary structure. Mutations in the stem inactivated the signal; however, compensatory

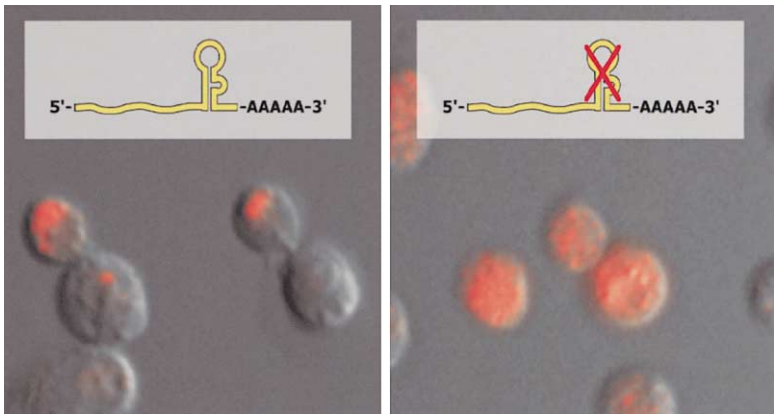

Figure 3. The Role of Secondary Structure in the Localization of Ash1 mRNA in Yeast

(Left) The normal distribution of *ash1* mRNA to the daughter cell in yeast.
(Right) The aberrant distribution of the RNA when mutations are made that effect the stem-loop structure. (Courtesy of P. Chartrand and R. Singer.)

mutations restoring base pairing reactivated localization function (Serano and Cohen, 1995). Importantly, the restored signal is only partially effective in localization, indicating that primary sequence may also be important for optimal functioning. The ability to precisely quantitate the localization potential of mutant RNAs will be important for interpretation of future experiments in this area.

cis-acting localization elements in the yeast Ash1 mRNA were found both in the 3′ UTR and in the coding region (Chartrand et al., 1999; Gonzalez et al., 1999). Analysis of these elements showed that their function was dependent upon the formation of a proper stem-loop structure and that this stem-loop was responsible for both localization and formation of the transport RNP particle (Figure 3).

There also is evidence that long-range interactions between elements involving *trans*-acting factors are important for proper localization. The *nanos* localization element consists of multiple partially redundant subelements within the 3′ UTR (Bergsten et al., 2001). Each subelement on its own can localize inefficiently; however, for proper localization the adjacent elements must collaborate.

The future challenge will be to understand the rules involved in creating a functional localization element. This will encompass a combination of structural predictions along with functional assays. One goal will be to produce artificial localization signals from the predictions. In addition to primary sequence information and secondary structure, it will be important to consider the role of tertiary structure in the function of the localization signals. Finally, in order to fully understand the nature of the localization process, it will be necessary to determine how interactions with *trans*-acting factors influence the structure and function of the elements.

What Is the Relationship between Localization and Translation?

A large amount of evidence has emerged linking the processes of RNA localization and translational regulation. In theory, this regulation could take the form of temporal control, so that the localized RNA is translated at the proper developmental stage, not necessarily when it becomes localized. It could also take the form of spatial control, if translation is able to occur only when the RNA becomes localized. In reality, translational regulation of localized RNAs appears to have characteristics of each of these modes of control, and for each localized RNA, the relative contribution of each mode seems to differ. Several molecular mechanisms through which translational control of localized mRNAs may be brought about are diagrammed in Figure 4.

Drosophila

Much of the evidence linking RNA localization and translational regulation has come from studies of axis determination in *Drosophila*. Anterioposterior axis determination in the *Drosophila* embryo depends on the proper localization of the *bicoid* mRNA to the anterior and *nanos* mRNA at the posterior of the oocyte. Localization of *oskar* mRNA is important since it is an upstream regulator of Nanos and also organizes the germ plasm at the posterior pole. Recent work has focused on identifying the molecules that regulate the translation of these mRNAs. Several of these molecules regulate the translation of more than one mRNA, resulting in the coordinated control of translation of a number of different mRNAs.

The *bicoid* mRNA is localized to the anterior of the oocyte. It is translationally regulated in a polyadenylation-dependent fashion (Salles et al., 1994). Regulation of the *bicoid* mRNA is atypical in that it is controlled temporally rather than spatially, as revealed by mutants that do not localize *bicoid* properly but still translationally regulate Bicoid protein production at the correct time. *Oskar* and *nanos* mRNAs are localized to the posterior and are also translationally regulated. However, they differ from bicoid in being regulated in a localization-dependent manner. Translation of unlocalized *oskar* mRNA is repressed by the Bruno protein (Kim-Ha et al., 1995) and its interacting partner Apontic (Lie and Macdonald, 1999b) along with Bic-C (Mahone et al., 1995; Saffman et al., 1998). A *cis* sequence element in the *oskar* 3′ UTR has been identified that mediates this repression and has been termed the Bruno response element (BRE). Evidence suggests that translational repression of *oskar* mRNA is specifically derepressed by a factor localized to the posterior of the oocyte and may require an interaction between the 5′ region of the mRNA and the BRE in the 3′ UTR (Gunkel et al., 1998).

Factors thought to be required for translation of *oskar* mRNA are Oskar protein itself, Vasa, Staufen, Aubergine, and Orb (Lipshitz and Smibert, 2000; Palacios and St Johnston, 2001). Whether or not translation of *oskar* is dependent on the polyadenylation status of the mRNA is

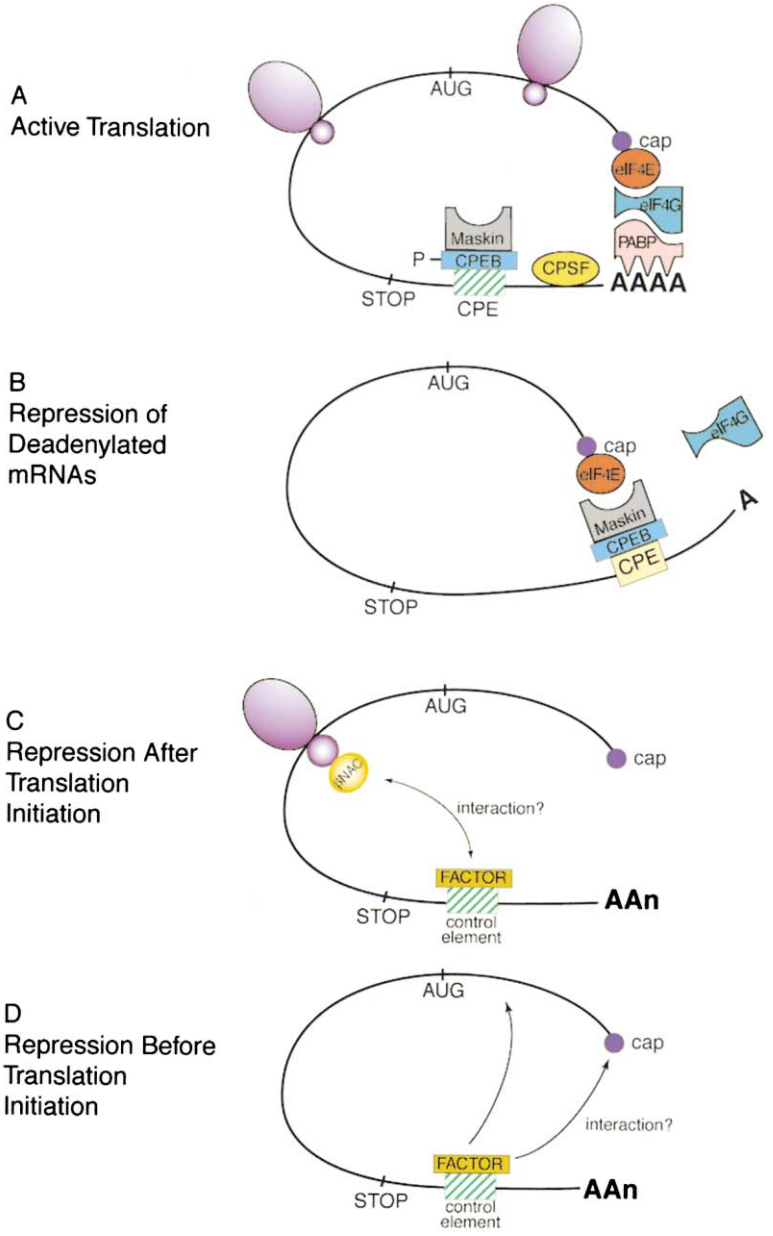

Figure 4. Mechanisms of Translational Control of Localized mRNAs

Localized mRNAs are frequently translationally repressed throughout the bulk of the cytoplasm and are translationally active only at their site of localization. Several different mechanisms of translational regulation appear to act on these mRNAs.

(A) Active translation. In general, the mRNA is polyadenylated and is bound by poly(A) binding protein (PABP). PABP also interacts with eIF4G which, in complex eIF4E, allows for ribosomal recruitment. For cytoplasmic polyaydenylation element (CPE)-containing mRNAs such as *c-mos*, the CPE is bound by phosphorylated CPE binding protein (CPEB). Phosphorylated CPEB recruits factors required for polyadenylation, such as cleavage and polyadenylation specificity factor (CPSF), to the mRNA.

(B) Polyadenylation-dependent repression, e.g., *c-mos*. CPEB is not phosphorylated. Maskin, which has a binding site for eIF4E similar to that of eIF4G, binds eIF4E, excluding eIF4G from the complex and preventing ribosomal recruitment.

(C) Repression after translation initiation, e.g., *nanos*. Repression is mediated by a specific *cis* sequence that is usually in the 3′ UTR, which for *nanos* is the TCE that is bound by a *trans*-acting factor, Smaug. Repression of *nanos* also requires βNAC, a protein associated with the ribosomal machinery. This raises the possibility of an interaction between the 3′ UTR and the translating ribosome.

(D) Repression before translation initiation. Like repression after translation initiation, a specific *cis* sequence in the 3′ UTR is bound by a *trans*-acting factor required for repression. This *trans* factor then somehow prevents ribosomal recruitment. Molecular details of this mode of repression are not clear. Figure modified from Mendez and Richter (2001) and Macdonald (2001). Details of polyadenylation-mediated regulation can be found in Mendez and Richter (2001).

unclear due to the presence of seemingly contradictory evidence from in vivo and in vitro analyses. *Drosophila* Orb has similarity to the *Xenopus* cytoplasmic polyadenylation element binding protein (CPEB). In vivo studies show that in *orb* mutants, *oskar* mRNA has a reduced poly(A) tail.This suggests that translational regulation of *oskar* mRNA may be polyadenylation dependent (Chang et al., 1999). In contrast, evidence from in vitro studies in *Drosophila* oocyte extracts indicates that translation of *oskar* mRNA is independent of cap recognition and the presence of a poly(A) tail (Castagnetti et al., 2000; Lie and Macdonald, 1999a). While differences in the assay systems used may help explain the results, the presence of data detecting both polyadenylation-dependent and -independent mechanisms of translation raises the possibility that more than one mechanism of translational regulation may operate on this mRNA. Further studies are needed to resolve this issue.

The requirement of Vasa for translation sheds light on how translational regulation of *oskar* mRNA may occur at a molecular level. Recently, Vasa has been shown to interact with dIF2, the *Drosophila* homolog of yeast IF2, which is thought to facilitate the interaction between the initiator methionine tRNA and the small subunit of the ribosome (Carrera et al., 2000). The observation of this interaction links translation of *oskar* mRNA at the posterior with events of translation initiation, suggesting that translation of *oskar* mRNA may be regulated at this level.

Like *oskar* mRNA, *nanos* mRNA is translated specifically at the posterior pole. While a proportion of *nanos* mRNA is localized to the pole, up to 96% of the mRNA remains unlocalized (Bergsten and Gavis, 1999). Therefore, the functional significance of repressing the translation of the unlocalized *nanos* mRNA is evident. The *cis* element responsible for repression has been termed the translation control element (TCE), and the factor that

interacts with this element is called Smaug (Dahanukar et al., 1999; Gavis et al., 1996; Smibert et al., 1999). A region of the TCE has been identified that is required for repression but is not bound by Smaug, indicating that an additional factor may be involved in repression (Crucs et al., 2000). A detailed study of the *nanos* TCE has revealed that the secondary structures required for repression of translation overlap but are functionally distinct from the sequences necessary for localization (Crucs et al., 2000). Thus, the interaction of the localization machinery and the translational repressors may be mutually exclusive, ensuring that localization and repression are separate events. Localization itself is not entirely sufficient for translation of *nanos* mRNA; Oskar protein is also required. Biochemical evidence of an interaction between Oskar and Smaug suggests that an interaction between the two may allow translation of the *nanos* mRNA (Dahanukar et al., 1999).

How is *nanos* mRNA repressed prior to localization? Two pieces of evidence suggest that translational repression of *nanos* occurs on the ribosome. First, fractionation experiments revealed that much of the *nanos* mRNA is present in ribosomal fractions despite its translational inactivity (Clark et al., 2000). Second, the *bicaudal* gene, which is required for *nanos* translational repression, encodes the *Drosophila* homolog of the β subunit of nascent polypeptide-associated complex (βNAC), which is associated with the ribosome and binds peptides just after synthesis (Markesich et al., 2000). This suggests that the repressing factors bound to *nanos* mRNA function by preventing the passage of the mRNA through the ribosome, not by preventing initiation of translation. The *cis* elements responsible for repression lie in the 3′ UTR; therefore, factors bound to this region must somehow interact with and affect the ribosome or the coding region prior to translation. Further studies are needed to illuminate the mechanism by which this translational regulation occurs on a molecular level.

Like the anterioposterior axis, establishment of the dorsoventral axis of *Drosophila* also depends on RNA localization and translational regulation. The *gurken* mRNA is localized to the dorsoanterior corner of the *Drosophila* oocyte at stage 9 of oogenesis and is required for formation of dorsal structures of the embryo. Gurken protein appears during stages 10–12. K10, Squid, and Bruno (Norvell et al., 1999; Saunders and Cohen, 1999) have been suggested to be involved in the translational repression of Gurken prior to this stage. Supporting a role for Bruno in *gurken* translation, the 3′ UTR of *gurken* mRNA contains elements identical to the Bruno response elements found in *oskar* mRNA (Kim-Ha et al., 1995). As with other localized RNAs, translation also requires the function of specific factors. Several of these factors are required for translation of *oskar* mRNA at the posterior and are also required for translation of *gurken* mRNA at the dorsoanterior corner (Cooperstock and Lipshitz, 2001). Thus, translational regulation of localized mRNAs is coordinately controlled, linking the establishment of anterioposterior and dorsoventral axes through these factors.

An interesting finding was that both mitochondrial small and large ribosomal RNAs (mtsrRNA and mtlrRNA) were required for pole cell formation in *Drosophila*. Recent evidence suggests that these RNAs are localized to the polar granules and organized into polysomes on the granule surface. This result suggests that a special class of mRNAs may be translated on the polar granules on these mitochondrial-type polysomes (Amikura et al., 2001). At the present time, the significance of this observation is not fully appreciated, and the nature of the RNAs that are translated on these structures is unknown. It was also reported that mtsrRNA and mtlrRNA were located on germinal granules in *Xenopus* oocytes (Kobayashi et al., 1998); however, recent reports indicate that they are not directly associated with the germinal granules (Kloc et al., 2000a). So, while it is clear that the mtsr and mtlrRNAs are important in germline formation in *Drosophila*, understanding the significance of their function in translational regulation within the polar granules will have to await further analysis.

Xenopus

Many localized mRNAs have been identified in *Xenopus*, and recent evidence suggests that some of these mRNAs are translationally regulated. A translational control element was identified in the Vg1 3′ UTR that is responsible for repression during the early stages of oogenesis (Otero et al., 2001; Wilhelm et al., 2000). This element is distinct from the Vg1 localization element and is able to confer repression on a reporter in stage III–IV oocytes in a polyadenylation-independent and cap-dependent manner. A second mRNA encoding Xcat2, which has similarity to *Drosophila* Nanos, is translationally silent throughout oogenesis but is translated later during the blastula stages of embryogenesis (MacArthur et al., 1999). Interestingly, Xcat2 mRNA is associated with the germinal granules during its period of translational repression and is released from the granules when translationally active (Kloc et al., 2000a, 2002). This suggests that association with the germinal granules may play a role in translational regulation.

Translational Control of Localized mRNAs in Synaptic Plasticity

Results from studies of mRNAs localized to dendritic processes of neurons have revealed that translational regulation of localized mRNAs is important for the synaptic plasticity responsible for learning and memory (Richter, 2001; Steward and Schuman, 2001). While the majority of the mRNAs present in neurons are located in the cell body, a number are specifically localized to the dendrites. One of these mRNAs is produced from the immediate early gene of unknown function, *Arc*. The *Arc* mRNA is localized to the synapse in response to synaptic activation through the N-methyl-D-aspartate receptor (NMDAR; Steward and Worley, 2001a). This is accompanied by an increase in newly translated Arc protein (Steward et al., 1998). Interestingly, Arc protein itself has been found associated with the NMDAR complex at postsynaptic sites (Husi et al., 2000). Steward and Worley (2001a, 2001b) have suggested that components of the NMDAR complex are assembled cotranslationally, necessitating the localization of the mRNA. Thus far, the *Arc* mRNA is the only RNA for which localization is linked to an event at the cell surface. It will be interesting to determine the extent of this phenomenon in the localization of other RNAs in neurons as well as other cell types.

A second mRNA localized specifically to the postsyn-

aptic regions of dendrites encodes the α subunit of calcium-calmodulin-dependent kinase II (CaMKII-α), which is required for learning and memory (Mayford et al., 1996). Synthesis of CaMKII-α protein is stimulated by the brain-derived neurotrophic factor (BDNF), known to function in synaptic plasticity (Aakalu et al., 2001; Kang and Schuman, 1996). Studies of CaMKII-α have provided important information about the mechanisms by which mRNAs are translationally regulated in neurons. The CaMKII-α mRNA contains two cytoplasmic polyadenylation elements (CPE) similar to those that bind CPEB and mediate translation during *Xenopus* oocyte maturation (Wu et al., 1998). Because CPEB is present in postsynaptic regions of the brain, translational regulation by CPEB that occurs in the brain may be similar to that occurring during *Xenopus* oocyte maturation (Wu et al., 1998). Consistent with this, comparison of rats reared in the dark with rats exposed to light revealed that light exposure had increased CaMKII-α polyadenylation and translation in the region of the brain responsive to visual stimuli (Wu et al., 1998). Thus, polyadenylation-dependent regulation of translation, a mechanism responsible for translational control of localized mRNAs in other systems, appears to be conserved in neurons and is functionally important for synaptic plasticity. One of the important issues to be addressed is whether or not localization to dendrites is required for translation.

Polyadenylation is not likely to account for all the translational regulation of localized RNAs that occurs in neurons, however. A recent study of five dendritically localized mRNAs, including both CaMKII-α and Arc, identified both cap-dependent and -independent components to the translational regulation of the mRNAs (Pinkstaff et al., 2001). The current evidence suggests that polyadenylation-dependent regulation is cap dependent due to the involvement of eIF4E, the cap binding protein. In studies using dicistronic messages, Pinkstaff et al. (2001) have identified internal ribosome entry sites (IRESs) in each of these mRNAs and have shown that, for one of the mRNAs, the IRES is more active in dendrites than elsewhere in the cell. Therefore, it is possible that factors are present in dendrites preferentially activating translation through the IRESs, adding another level of regulation to the mRNAs. An important point revealed by these studies is that multiple mechanisms of translational regulation appear to be operating on these localized mRNAs.

Future Perspectives

Recent work in the field of RNA localization has focused on identification and characterization of localized transcripts and initial attempts at understanding the mechanisms by which the RNAs are localized. The power of mutant analysis in *Drosophila* and yeast along with the elegant cell biological and biochemical analyses in somatic cells and *Xenopus* oocytes have provided important tools. We are now on the brink of a new era in this area of research that will rely on a combination of genetics, reverse genetics, cell biology, and biochemical approaches to dissect the intricacies of the machinery and mechanisms involved in localization. Some of the important issues that need to be addressed include the following. (1) The identity and composition of RNA transport particles, including how and where they are first assembled and whether there are common protein components for each localized transcript. (2) The mechanism by which the localization machinery is assembled, and the role of the cytoskeleton in transport. (3) The molecular basis by which RNAs are attached to the transport apparatus, and the molecular motors that drive the localization process. (4) The identity and structural basis of *cis*-acting localization elements. (5) The mechanisms by which the transcripts anchored at their final destination. (6) The role of the signals and signal transduction pathways that initiate the localization process. (7) The scope of translational regulation of localized transcripts, and the mechanisms by which translational regulation is linked to RNA localization. (8) The function of localized RNAs in normal biological processes and disease. And finally, (9) the development of new technologies to analyze the process. These might include sophisticated high-resolution imaging to allow close examination of the assembly of the localization machinery in living cells and embryos and the nature of the association between the transported RNAs and the cytoskeleton.

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