

GETTING THE MESSAGE ACROSS: The Intracellular Localization of mRNAs in Higher Eukaryotes

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■ **Abstract** The intracellular localization of mRNA, a common mechanism for targeting proteins to specific regions of the cell, probably occurs in most if not all polarized cell types. Many of the best characterized localized mRNAs are found in oocytes and early embryos, where they function as localized determinants that control axis formation and the development of the germline. However, mRNA localization has also been shown to play an important role in somatic cells, such as neurons, where it may be involved in learning and memory. mRNAs can be localized by a variety of mechanisms including local protection from degradation, diffusion to a localized anchor, and active transport, and we consider the evidence for each of these processes, before discussing the *cis*-acting elements that direct the localization of specific mRNAs and the *trans*-acting factors that bind them.

CONTENTS

INTRODUCTION	570
THE BIOLOGICAL ROLES OF LOCALIZED mRNAs	571
Asymmetric Divisions	571
Localized Cytoplasmic Determinants in <i>Drosophila</i>	572
Localized Cytoplasmic Determinants in Other Organisms	574
Control of the Direction of Cell Signaling	576
Functions of Localized mRNA in Cell Polarity	577
Excluding Proteins from Regions of a Cell	578
Role of Localized mRNAs in Synaptic Plasticity	579
Translational Control and mRNA Localization	580
MECHANISMS FOR THE LOCALIZATION OF mRNAs	581
Diffusion and Local Anchoring	581
Localized Degradation	581
Localized Synthesis	582
Active Transport	582

Actin-Dependent Transport	588
Multistep Localization	588
COUPLING mRNAs TO THE TRANSPORT MACHINERY	589
<i>Cis</i> -Acting Elements	589
<i>Trans</i> -Acting Factors	596
PROSPECTS	600

INTRODUCTION

The first localized mRNA was discovered almost 20 years ago with the demonstration that β -actin mRNA accumulates in the myoplasm of Ascidian eggs (Jeffery et al. 1983). Since then, dramatic improvements in the resolution and sensitivity of in situ hybridization techniques have led to the identification of a large number of localized mRNAs in many different cell types throughout the animal kingdom, and even in yeast and plant cells (Bouget et al. 1996, Long et al. 1997, Takizawa et al. 1997). Indeed, several cells have now been shown to localize multiple mRNAs to different positions. At least 20 mRNAs accumulate at either the animal or vegetal pole of the *Xenopus* egg, whereas *bicoid*, *oskar*, and *gurken* mRNAs localize to three distinct positions within the *Drosophila* oocyte (St Johnston 1993a, Mowry & Cote 1999). Furthermore, about 10% of randomly chosen cDNAs recognize transcripts that localize within *Drosophila* germline cysts (Dubowy & Macdonald 1998). Although it is still not possible to estimate what proportion of mRNAs are localized in any particular cell type, it is now clear that the intracellular localization of specific mRNAs occurs in most if not all polarized cells and provides an important mechanism for targeting proteins to the cellular regions where they are required.

While the inventory of localized transcripts increases year on year, progress in understanding the mechanisms of mRNA transport has been comparatively slow, largely because this is a difficult process to analyze. Unlike other intracellular trafficking events, such as nucleo-cytoplasmic transport or protein secretion, the cytoplasmic localization of mRNAs does not involve movement between membrane-bound compartments that can be purified by fractionation, and the specific mRNAs that localize to a particular site represent only a small fraction of the total mRNA in the cell. Although these two factors have hindered biochemical analysis, recent work has identified several of the key RNA-binding proteins that interact with localized mRNAs and has begun to reveal the cell-biological mechanisms that target these transcripts to their correct locations. In parallel, genetic screens in both yeast and *Drosophila* have identified *trans*-acting factors required for the localization of mRNAs with essential developmental functions and have contributed significantly to our understanding of how these mRNAs reach their destinations. In this review, we first discuss the biological roles played by localized mRNAs before concentrating on recent advances in elucidating the mechanisms of mRNA localization.

THE BIOLOGICAL ROLES OF LOCALIZED mRNAs

The primary purpose of mRNA localization is to target the expression of the protein encoded by a transcript to a particular region of a cell, and in parallel, to prevent its expression elsewhere. The importance of this mechanism for protein localization depends on whether there is an alternative pathway for localizing the protein to the regions where it is required, and mRNA localization is often redundant with protein targeting signals that direct the polypeptide to the same destination. For example, vimentin, desmin, and vinculin mRNAs localize to the costamere that surrounds a developing chick myofibril, but the corresponding proteins localize to the same sites earlier in muscle development and must therefore be directed there by an independent protein sorting pathway (Fulton & L'Ecuyer 1993). Similarly, *inscuteable* mRNA localizes to the apical side of *Drosophila* embryonic neuroblasts, but the protein still localizes normally when the localization signals are removed from the mRNA (Knoblich et al. 1999). A particularly clear example of redundancy between protein and mRNA localization is provided by Prospero, a homeodomain transcription factor that segregates into the smaller of the two daughter cells produced by the asymmetric divisions of these neuroblasts, where it is required for the proper specification of ganglion mother cell (GMC) fate (Hirata et al. 1995, Knoblich et al. 1995). Both Prospero protein and mRNA localize to the apical side of the cell during interphase and move in metaphase to form a basal crescent that then segregates into the GMC, which does not transcribe this gene itself. Because Prospero protein contains a targeting signal that directs it to the basal crescent independently of its RNA, the GMC still develops normally in *staufen* mutants, which disrupt the localization of *prospero* mRNA (Broadus et al. 1998). However, these mutants strongly enhance the defects caused by hypomorphic *prospero* mutants because the localization of the mRNA reinforces the protein localization by supplying a source of Prospero to the GMC. In all of these cases, mRNA localization is not essential and seems to provide a back-up mechanism for protein localization.

In contrast to the examples above, there are many cases where protein localization is achieved exclusively through the localization of the corresponding mRNA. Several situations in which mRNA localization is essential for important biological functions are described below, and this non-redundant mRNA localization can fulfil a number of important biological functions.

Asymmetric Divisions

Asymmetric divisions give rise to two daughter cells with different cell fates, and one way that this can be accomplished is by the partitioning of the mRNA for a cell fate determinant into only one daughter cell at mitosis. The best characterized example of this phenomenon comes from the study of mating type switching in the budding yeast, *Saccharomyces cerevisiae* (reviewed by Chartrand et al. 2001).

The asymmetric divisions of *Drosophila* neurogliaoblasts provide a second example where mRNA localization is responsible for determining different sibling

cell fates (Akiyama-Oda et al. 1999, Bernardoni et al. 1999). During these divisions, *gcm* mRNA localizes to one side of the cell at metaphase, where it is inherited by the daughter cell that gives rise to glia, whereas the other daughter produces only neurons. Both cells enter the neural lineage in *gcm* mutants, and ectopic expression of *gcm* produces extra glia, indicating the Gcm protein is required to specify glial identity. Unlike the asymmetric divisions of the neuroblasts, where both Prospero protein and mRNA are localized, Gcm protein does not localize, strongly suggesting that the difference between the two cell lineages is controlled by the asymmetric localization of *gcm* mRNA.

Localized Cytoplasmic Determinants in *Drosophila*

In many invertebrates and some vertebrates, the primary axis of the organism is already defined in the unfertilized egg through the localization of determinants that control the development of the regions of the embryo that inherit them after fertilization, and these determinants are often localized in the form of maternal mRNAs. Most evidence for the function of such maternal mRNAs comes from the *Drosophila* egg, which contains at least six localized transcripts that act in different ways to pattern the anterior-posterior axis of the embryo.

The anterior determinant in *Drosophila* is encoded by *bicoid* mRNA, which localizes to the anterior pole (Figure 1C) where it is both necessary and sufficient to define where anterior structures develop (Berleth et al. 1988, Driever 1993). Thus mutants that disrupt this localization cause loss of anterior pattern elements, whereas the injection of the mRNA at the posterior gives rise to embryos that develop a second head and thorax instead of the abdomen and telson (Schüpbach & Wieschaus 1986, Frohnhöfer & Nüsslein-Volhard 1987, St Johnston et al. 1989, Driever et al. 1990). *bicoid* RNA is translated after fertilization to produce a homeodomain transcription factor, which diffuses posteriorly to form a morphogen gradient that patterns the anterior of the embryo in two ways. First, Bicoid protein activates the transcription of various zygotic gap genes at different threshold concentrations to define different domains of the head and thorax of the embryo. Second, Bicoid binds directly to *caudal* mRNA to repress its translation, and the resulting posterior to anterior gradient of Caudal protein is also essential for the correct spatial regulation of gap gene expression (Rivera-Pomar et al. 1996).

Although the anterior cytoplasm contains only a single maternal mRNA determinant, a specialized region at the posterior of the egg, called the pole plasm, contains a number of mRNAs that play essential roles in the determination of the abdomen and the pole cells, the founders of germline lineage (St Johnston 1993b). The first mRNA to reach the posterior of the oocyte is *oskar* mRNA (Figure 1C), and mutants that abolish this localization disrupt all subsequent steps in the formation of the pole plasm, resulting in the development of embryos that lack both a germline and an abdomen (Lehmann & Nüsslein-Volhard 1986, Ephrussi et al. 1991, Kim-Ha et al. 1991). Furthermore, the localization of *oskar* is sufficient to

define the site of pole plasm formation. When *oskar* mRNA is mislocalized to the anterior of the oocyte by fusing the RNA to the *bicoid* localization signals, it directs the ectopic assembly of pole plasm, resulting in the formation of a second set of pole cells and an abdomen in place of the head and thorax (Ephrussi & Lehmann 1992). Unlike Bicoid, however, Oskar protein does not seem to play a direct role in determining cell fates in the embryo and acts instead to nucleate the polar granules of the pole plasm by recruiting other mRNAs and proteins to the posterior pole (Breitweiser et al. 1996).

One of the most important mRNAs recruited to the posterior of the egg downstream of *oskar* mRNA is *nanos* mRNA, which encodes the posterior determinant itself (Wang & Lehmann 1991). Nanos protein is translated after fertilization and diffuses to form a gradient that directs the formation of the abdomen indirectly by repressing the translation of maternal *hunchback* mRNA (Hülkamp et al. 1989, Struhl 1989a). This regulation is mediated through two *nanos* response elements (NREs) in its 3'UTR, and a similar NRE is also found in the *bicoid* 3'UTR (Wharton & Struhl 1991). Thus the ectopic localization of *nanos* RNA to the anterior inhibits both *hunchback* and *bicoid* mRNA translation, resulting in the suppression of head development and the formation of a second abdomen. Although both Nanos and Bicoid act as localized translational repressors, these determinants differ in two important respects. First, whereas Bicoid binds directly to *caudal* mRNA to confer both spatial and sequence specificity to this translational control, Nanos provides only the spatial control for *hunchback* regulation (Sonoda & Wharton 1999). Sequence specificity is provided by a second maternal protein, Pumilio, which binds directly to the NREs: Nanos then recognizes this complex to trigger translational repression (Murata & Wharton 1995, Wharton et al. 1998, Sonoda & Wharton 1999). Second, the abdomen can develop normally when both *nanos* and maternal *hunchback* are removed, indicating that the Nanos gradient provides a permissive rather than an instructive signal (Hülkamp et al. 1989, Irish et al. 1989, Struhl 1989).

Nanos and Pumilio are not completely dispensable in the absence of maternal *hunchback* mRNA, however, because they are also required for the proper development of the pole cells once they have formed, and these cells inherit the highest levels of Nanos protein (Kobayashi et al. 1996, Forbes & Lehmann 1998, Asaoka-Taguchi et al. 1999). The direct targets of Nanos translational repression in the pole cells have not been identified, but a number of genes and enhancer trap lines have been shown to be ectopically expressed in these cells in *nanos* mutants, and one of these, *Sex lethal*, is an important target of this pathway because its misexpression disrupts pole cell migration (Asaoka et al. 1998, Deshpande et al. 1999).

In addition to *nanos* mRNA, the development of the pole cells requires at least three other types of localized RNA. *germ cell-less* (*gcl*) mRNA localizes to the posterior of the oocyte after stage 11 of oogenesis, and mutations in the gene or the expression of an antisense RNA severely reduce the number of pole cells that develop (Jongens et al. 1992, Robertson et al. 1999). The exact role of Gcl is unclear, but the protein localizes to the nuclear pores and is able to

induce the formation of ectopic polar buds, but not pole cells, when the mRNA is mislocalized to the anterior of the egg (Jongens et al. 1994). The second RNA is the non-coding transcript *Pgc*, which localizes to the posterior at around stage 11 and is a component of the polar granules (Nakamura et al. 1996). Antisense directed against *Pgc* RNA reduces the amount of *nanos* and *gcl* mRNA at the posterior of the embryo and partially inhibits both pole cell formation and migration, suggesting that the RNA may be required for the structural integrity of the polar granules and the efficient localization and/or function of their components.

The third class of localized RNAs specifically required for pole cell formation are the large and small ribosomal RNAs encoded by the mitochondrial genome (mt rRNA). Unlike *nos*, *Pgc*, and *gcl* RNAs, these transcripts do not localize during oogenesis and instead are exported from the mitochondria in the pole plasm just before the pole cells start to form, where they associate transiently with the polar granules (Kobayashi et al. 1993, Kashikawa et al. 1999). Because embryos injected with ribozymes that cleave the large mt rRNA often fail to form pole cells, these cytoplasmic mt rRNAs seem to have a function in pole cell determination (Iida & Kobayashi 1998). These results raise the question of what possible function these non-coding mitochondrial RNAs could perform in the determination of the pole cells, and one intriguing possibility is that mitochondrial ribosomes are required to translate some of the maternal mRNAs that are localized to the polar granules.

Localized Cytoplasmic Determinants in Other Organisms

Homologues of some of the localized maternal mRNAs that play a role in pole cell development in *Drosophila* have been implicated in the determination of the primordial germ cells (PGCs) in several other organisms, suggesting the existence of a conserved pathway for specifying the germline lineage. Three *nanos* homologues have been identified in the nematode *Caenorhabditis elegans*, and maternal *nos-2* mRNA has been shown to be a component of the P-granules, which segregate into PGCs (Subramaniam & Seydoux 1999, Schisa et al. 2001). Furthermore, the ablation of *nos-2* by RNA interference causes a number of defects in the behavior of the PGCs, all of which are enhanced by mutations in *nos-1*. A similar array of defects are caused by the simultaneous disruption of five of the eight worm *pumilio* homologues. Thus *C. elegans* Nos 1 and 2 seem to fulfil a comparable function in PGC development to *Drosophila* Nanos and probably act in concert with the *Pumilio* homologues to regulate translation in these cells. It is also possible that a *nanos*-like maternal mRNA plays a role in germ cell determination in *Xenopus*. *Xcat-2* mRNA, which encodes a protein with a C-terminal zinc finger domain that is most similar to that found in Nanos, localizes to the vegetal pole of the oocyte in association with the germinal granules (Mosquera et al. 1993, Kloc et al. 1998, 2000). Furthermore, *Xcat-2* RNA is translationally repressed during oogenesis but is expressed in the vegetal blastomeres that give rise to the PGCs (MacArthur et al. 1999). Although the function of *Xcat-2* has not yet been tested directly, the

development of the PGCs does depend on another mRNA, *Xdazl*, which encodes a conserved RNA-binding protein and shows a very similar localization to the germ plasm and subsequent translation in the PGCs (Houston et al. 1998, Houston & King 2000). When maternal *Xdazl* mRNA is depleted using antisense oligonucleotides, the PGCs still form but fail to differentiate normally or migrate into the dorsal mesentery.

Further examples of localized mRNAs that segregate into the germline are provided by Ascidian and zebra fish *vasa* homologues. In *Drosophila*, *Vasa* localizes to the posterior of the oocyte as a maternal protein, where it is required for the assembly of the polar granules and the determination of both the abdomen and the germ cells (Hay et al. 1988a,b; Lasko & Ashburner 1988). In contrast, *vasa* is localized as a maternal mRNA in the 2-cell *Danio rerio* embryo and subsequently segregates, at the 1000-cell stage, into just 4 cells, which are believed to be the PGCs (Olsen et al. 1997, Yoon et al. 1997). Similarly *Ciona intestinalis* maternal *vasa* mRNA localizes to the posterior of the embryo during the second cleavage division and segregates into specific posterior blastomeres (Fujimura & Takamura 2000).

Finally, large mt rRNA is found outside of the mitochondria in the germ plasm of the vertebrate *Xenopus laevis* at the stages when the PGCs are specified (Kobayashi et al. 1998). The germ plasm localizes to the vegetal pole of the *Xenopus* oocyte and condenses after fertilization to form electron-dense particles called germinal granules that bear a strong resemblance to the polar granules of *Drosophila* and the P-granules of *C. elegans*. Although the mt rRNA is not present in the germ plasm initially, the RNA appears in the germ plasm of the 4-cell embryo and associates with the germinal granules themselves as they segregate into the four vegetal blastomeres that give rise to the germline.

As in *Drosophila*, localized maternal mRNAs also have an important function in embryonic axis formation in *Xenopus*. In addition to *Xcat-2*, at least nine other mRNAs have been shown to localize to the vegetal pole of the oocyte, and one of these, *VegT*, functions as a determinant for the endoderm (Zhang et al. 1998, Mowry & Cote 1999). When maternal *VegT* mRNA is ablated by injecting oocytes with antisense oligonucleotides, the resulting embryos fail to form endoderm, and the vegetal blastomeres also lose their capacity to induce mesoderm. *VegT* encodes a T-box transcription factor and determines endodermal fate by directly activating the zygotic expression of the homeobox gene *Bix-1* (Casey et al. 1999). In addition, *VegT* is required for the vegetal expression of the mesoderm-inducing factors, such as *derriere*, and *Xnr-1*, 2, and 4, and probably activates *Xnr-1* expression directly through two T-box-binding sites in its promoter (Clements et al. 1999, Kofron et al. 1999, Hyde & Old 2000). The function of the other vegetally localized mRNAs has not been tested directly, but *Xwnt-11* and *Vg1* mRNAs both encode secreted signaling molecules and can affect the patterning of the embryo when injected elsewhere, suggesting that they may also contribute to the body axial patterning (Ku & Melton 1993, Thomsen & Melton 1993).

Some of the most striking patterns of maternal mRNA localization are seen in the eggs and early embryos of Ascidians, where a number of mRNAs localize first to

the vegetal pole of the egg and then to a small region of cortical cytoplasm within the B4.1 blastomeres at the 8-cell stage (Figure 1F) (Swalla & Jeffery 1996a,b, Yoshida et al. 1996, Satou & Satoh 1997, Sasakura et al. 1998a,b, 2000, Satou 1999). Furthermore, most, if not all, of these mRNAs continue to segregate asymmetrically during subsequent divisions, and end up in 2 cells at the posterior of the embryo at the gastrula stage. The first stages of this segregation are very similar to that of the yellow crescent or myoplasm, which is a specialized region of cytoplasm that specifies the muscle lineage. One of these localized mRNAs, *macho-1*, has recently been shown to fulfil all of the criteria for a muscle determinant (Nishida & Sawada 2001). Antisense ablation maternal *macho-1* mRNA leads to an almost complete loss of the primary muscle cells, whereas injection of the RNA into blastomeres that would otherwise not form any muscle, gives rise to ectopic muscle cells. Thus *macho-1*, which encodes a zinc finger transcription factor, is both necessary and sufficient to specify muscle cell fate. Given the mosaic development of Ascidians, it seems likely that some of the other localized mRNAs will also play a role in patterning the embryo. Among these are a Wnt homologue and a homologue of Mex-3, which is required for anterior-posterior patterning in *C. elegans*; both, therefore, are good candidates for mRNAs involved in axis formation (Sasakura et al. 1998a, Satou 1999).

Localized mRNAs can also fulfil a similar function in regulating cell fate in the absence of cell division by controlling the behavior of individual nuclei in syncytia. A possible example of this is provided by the apical localization of the *Drosophila* pair-rule transcripts, such as *ftz*, *hairy*, and *eve* mRNAs, in the *Drosophila* syncytial blastoderm embryo (Edgar et al. 1987, Davis & Ish-Horowitz 1991). The localization of these mRNAs ensures that the transcription factors they encode enter the nuclei that transcribed them, which may be important for the precise spatial control of transcription during segmentation in an embryo in which the 5000 nuclei are not separated by cell membranes. RNA localization in a syncytium may also play a role in the determination of the *Drosophila* oocyte, which develops from a cyst of 16 germ cells that are connected by cytoplasmic bridges. A number of transcripts, including *BicD*, *orb*, *egl*, and *osk* mRNAs, are transported from the other 15 cells into this single cell, leading to the suggestion that this localization is required to specify or maintain oocyte fate (Suter et al. 1989, Ephrussi et al. 1991, Kim-Ha et al. 1991, Lantz et al. 1992, Mach & Lehmann 1997).

Control of the Direction of Cell Signaling

Gurken protein signals from the oocyte to the somatic follicle cells twice during *Drosophila* oogenesis, and in each case, the direction of signaling is controlled by the localization of *gurken* mRNA. Early in oogenesis, *gurken* mRNA localizes next to the nucleus along the posterior cortex of the oocyte, where it is translated to produce an EGF-like signaling molecule that induces the adjacent follicle cells to adopt a posterior rather than an anterior fate, and this leads to the polarization

of the anterior-posterior axis of the oocyte (González-Reyes et al. 1995, Roth et al. 1995). The localization of the mRNA seems to be essential for signaling because *maelstrom* mutants, which disrupt this localization, give rise to egg chambers in which the posterior follicle cells adopt anterior fates (Clegg et al. 1997). Later in oogenesis, the oocyte nucleus migrates from the posterior of the oocyte to a point at the anterior margin, and this directs the relocalization of *gurken* mRNA, which forms a cap on one side of the nucleus (Neuman-Silberberg & Schüpbach 1996). The translation of this mRNA produces a second Gurken signal that is secreted in an orthogonal direction to the first, where it induces dorsal follicle cell fates, thereby defining the dorsal-ventral axis of the future embryo (Schüpbach 1987, Neuman-Silberberg & Schüpbach 1996).

mRNA localization also seems to be essential for efficient signaling by Wingless (Wg), a secreted WNT factor that is required for segmentation of the *Drosophila* embryo (Simmons et al. 2001). Endogenous *wg* mRNA is targeted to the apical side of the cells of the embryonic ectoderm, and transgenic rescue constructs that contain the apical localization signals rescue the segmentation defects of a *wg* null mutant. In contrast, constructs that produce basally localized mRNA fail to rescue the mutant even though they produce wild-type amounts of protein that are efficiently secreted. At the moment, it is unclear why the apical localization of *wg* mRNA is necessary for signaling because it signals laterally to other cells in the epithelium. One possibility is that this targets the protein to a specialized apical compartment of the secretory pathway where it can be processed and modified to produce the active factor. On the other hand, this localization may be important because it restricts Wg secretion to the apical side. If, for example, the Wg receptors are also concentrated apically, the polarized secretion of Wg may be necessary to achieve a high enough concentration for efficient signaling.

The transcripts of several other secreted signaling molecules are localized, such as *short gastrulation* (Figure 1A,B), *sevenless*, and *twisted gastrulation* in *Drosophila*, and *Vg1* and *Xwnt11* in *Xenopus*, and it will be interesting to determine whether these localizations are also important for controlling either the efficiency or the direction of signaling (Banerjee et al. 1987, Francois et al. 1994, Mowry & Cote 1999).

Functions of Localized mRNA in Cell Polarity

Almost all examples where the biological importance of mRNA localization has been demonstrated come from experiments on eggs and early embryos, but many mRNAs are asymmetrically distributed in other types of cell. There is often no way to disrupt these localizations, however, and it has therefore been difficult to test their functional significance. One case where this has been possible is the localization of β -actin mRNA to the leading edge of migrating chick embryonic fibroblasts, where actin is actively polymerized to drive cell movement (Kislauskis et al. 1993, Kislauskis et al. 1994). Antisense oligonucleotides directed against the *cis*-acting localization signals in the 3'UTR block the localization of the mRNA

without affecting the rate of β -actin protein synthesis, and this causes the cells to cease migrating and lose their polarized morphology.

Developing neurones also segregate a number of transcripts to either the dendritic or axonal domains of the cell, and this may play an important role in maintaining the highly polarized state of these cells (Bassell et al. 1999). For example, Tau mRNA localizes to the proximal regions of axons, whereas MAP2 mRNA is excluded from axons and accumulates in dendrites (Garner et al. 1988, Kleiman et al. 1990, Litman et al. 1993). In the case of Tau, it is now clear that mRNA localization determines the distribution of the protein since Tau remains in the cell body when the *cis*-acting localization signal is deleted from the transcript and accumulates only in dendrites when fused to the MAP2 3'UTR (Aranov et al. 2001). Because both Tau and MAP2 mRNAs encode proteins that bind to and stabilize microtubules, their polarized distributions may contribute to the different organization of the microtubule cytoskeleton in axons and dendrites.

Recent data also suggest that mRNAs are recruited to sites where cells are under mechanical tension (Figure 1E) (Chicurel et al. 1998). When cultured endothelial cells are exposed to fibronectin-coated beads, both ribosomes and poly-A+ mRNA accumulate in the vicinity of the focal adhesion complexes that form around the beads, and this recruitment is enhanced when tension is applied to the beads. Although no specific mRNAs that localize to these sites have been identified, this recruitment may allow a cell to reinforce junctions that are under stress through the local translation of cytoskeletal components.

Excluding Proteins from Regions of a Cell

Another possible function for mRNA localization is to prevent the synthesis of proteins in regions of a cell where they may be deleterious, and one case where this may be important is the localization of Myelin basic protein (MBP) mRNA to the distal processes of oligodendrocytes (Trapp et al. 1987, Ainger et al. 1997). MBP associates with membranes to cause their compaction and is translated in the cell processes where it is incorporated into the myelin sheath that wraps around axons. If the mRNA was not transported into the cell processes, the protein would have to be synthesized in the cell body where it might interact with and compact the wrong membranes, such as the nuclear envelope or the Golgi, which would both inhibit its movement to the periphery and interfere with the functioning of the cell. The localization of the mRNAs encoding distinct isoforms of proteins that multimerize may serve a similar function by restricting the synthesis of these isoforms to different regions of the cell, thereby preventing the formation of mixed multimers. For example, the mRNA for the M isoform of creatine kinase is localized to the periphery of cultured mouse myoblasts, whereas the B isoform mRNA remains perinuclear, and this may preclude the assembly of MB heterodimers (Wilson et al. 1995). The mRNA for the β isoform of actin localizes to the cell periphery in myoblasts and osteoblasts and to the growth cones of developing neurons. (Figure 1D),

whereas the transcripts for the α and γ isoforms are generally perinuclear, and this may also control the assembly and composition of actin filaments in different parts of the cell (Hill & Gunning 1993, Kislauskis et al. 1993, Bassell et al. 1998, Watanabe et al. 1998).

Role of Localized mRNAs in Synaptic Plasticity

A number of transcripts localize to the dendrites of mature neurons, including mRNAs encoding the α -subunit of calcium-calmodulin kinase II (CaMKII α), Arc protein, MAP2, Fragile X mental retardation protein (FMRP), and the α -subunit of the glycine receptor (Garner et al. 1988, Burgin et al. 1990, Kleiman et al. 1990, Link et al. 1995, Racca et al. 1998). In addition, it is now clear that dendrites have the capacity to translate these mRNAs. Clusters of polyribosomes can be found at post-synaptic sites, and dendritic growth cones that have been isolated from their cell bodies can translate transfected mRNAs (Crino & Eberwine 1996, Steward et al. 1996, Steward 1997). These observations have led to the suggestion that translation of localized mRNAs contributes to the remodeling of individual synapses in response to activity.

One way that mRNA localization could affect synaptic plasticity is if the targeting of the mRNA is controlled by the activity of the synapse. In support of this view, neural stimulation enhances the dendritic localization of a number of mRNAs. For example, depolarization induces an increase in the amounts of BDNF and TrkB mRNA in the dendrites of cultured hippocampal cells and enhances the dendritic localization of L7/Pcp-2 mRNA in Purkinje cells, whereas long-term potentiation (LTP) induces a similar accumulation of CaMKII α and MAP2 mRNAs in hippocampal cells *in vivo* (Thomas et al. 1994, Tongiorgi et al. 1997, Roberts et al. 1998, Wanner et al. 2000). Interestingly, both BDNF and TrkB mRNAs are also recruited to dendrites when cultured hippocampal neurons are treated with BDNF itself, and this factor has been shown to function as a diffusible signal that can induce an increase in the efficiency of synaptic transmission (Righi et al. 2000). However, the most direct evidence in support of this model comes from the observation that the localized stimulation in the dentate gyrus causes the selective and rapid transport of Arc mRNA from the cell bodies to the regions of the dendritic tree that have been stimulated (Steward et al. 1998). This elegant experiment provides the first example of activity-dependent mRNA localization to specific post-synaptic regions and suggests that activity could regulate the strength of individual synapses by increasing the expression of specific proteins through the targeting of their mRNAs. This mechanism may also play a role in neural development since β -actin mRNA has been found to localize to dendritic growth cones in response to the chemoattractant Neurotrophin-3, and this could contribute to the growth of dendrites toward their targets (Zhang et al. 1999).

One consequence of localizing an mRNA is that its translation can now be locally regulated in response to signals in that particular region of the cell, and this provides a second mechanism by which localized mRNAs could influence

synaptic plasticity. One situation where this is likely to occur is the enhancement of synaptic efficiency of hippocampal neurons in culture in response to BDNF and Neurotrophin-3. These factors can increase the efficiency of synaptic transmission in dendrites that have been separated from their cell bodies, but this effect is blocked by inhibitors of translation, suggesting that they act on the dendrites themselves by inducing the translation of localized mRNAs (Kang & Schuman 1996). This has led to the attractive proposal that LTP is controlled in part by the activity-dependent translation of specific dendritic mRNAs, and one of these target transcripts has now been identified as CaMKII α mRNA. The levels of the CaMKII α protein increase in dendrites within 5 min of tetanic stimulation of hippocampal neurons, which is too fast to be explained by transport from the cell body, and this stimulation is blocked by the translation inhibitor anisomycin (Ouyang et al. 1999). Thus activity stimulates the translation of CaMKII α mRNA in dendrites, where this kinase is required for LTP, and two different mechanisms have been proposed to contribute to this translational control (Scheetz et al. 2000, Wells et al. 2000). A second candidate target for activity-dependent translational control is FRMP because its mRNA is recruited to polyribosomes when purified synaptic preparations are stimulated by activation of the glutamate receptor, and recent evidence indicates that this recruitment may be mediated by a signaling pathway involving protein kinase C and P90rsk (Weiler et al. 1997, Angenstein et al. 1998).

Translational Control and mRNA Localization

The preceding discussion of synaptic plasticity raises an important issue in mRNA localization, which is that it is frequently coupled to translational control. Indeed, proteins can only be restricted to one region of a cell by localizing their transcripts if their mRNAs are not translated before or during their localization. The prevention of ectopic or premature protein expression is particularly important for maternal determinants, and many of the mRNAs that define the body axes in *Drosophila* are therefore subject to translational control. For example, *oskar* and *nanos* mRNAs can be translated only if they are correctly localized to the posterior pole, because the translation of unlocalized mRNAs is prevented by the binding of repressors to sequences with their 3'UTRs (Gavis & Lehmann 1994; Kim-Ha et al. 1995; Rongo et al. 1995; Dahanukar & Wharton 1996; Smibert et al. 1996, 1999; Dahanukar et al. 1999). In fact, this strict coupling of translation to localization seems to be the primary mechanism for restricting Nanos protein expression to the posterior. Careful quantitation of the amounts of *nanos* mRNA in the anterior and posterior halves of the embryo reveals that most *nanos* mRNA is not localized to the posterior pole, but it is only the ~5% that is localized that can be translationally activated (Bergsten & Gavis 1999). Owing to lack of space, we will not discuss the mechanisms of translational control in detail, but it is worth bearing in mind that the *cis*-acting elements that regulate translation often overlap with the localization signals, and some of the *trans*-acting factors that bind to the 3'UTRs of localized mRNAs regulate both translation and localization.

MECHANISMS FOR THE LOCALIZATION OF mRNAs

Diffusion and Local Anchoring

The localization of *nanos* in the *Drosophila* oocyte is likely to occur by general diffusion coupled to local trapping at the posterior by a localized anchor. The targeting of *nos* mRNA is dependent on components of the pole plasm that localize earlier to the posterior, such as Osk, Vasa, and Tudor (Wang et al. 1994). Although an active transport mechanism cannot be ruled out, it seems unlikely for several reasons. First, the cytoskeleton does not exhibit a defined polarity when *nos* mRNA is localized to the posterior pole, and there is vigorous cytoplasmic streaming in the oocyte at this stage (Gutzeit & Koppa 1982, Theurkauf et al. 1992). Second, *nos* RNA is recruited to the anterior of the oocyte when *oskar* mRNA is mislocalized there by the *bicoid* 3'UTR, but the organization of the cytoskeleton does not seem to change under these circumstances (Ephrussi & Lehmann 1992). Third, the high stability of the unlocalized *nos* RNA argues against local protection from degradation as a mechanism for *nos* RNA localization (Bergsten & Gavis 1999). Several other transcripts such as *gcl*, *Pgc*, and *cyclin B* mRNAs localize to the pole plasm downstream of *oskar*, and it is possible that they localize by a similar mechanism (Raff et al. 1990, Jongens et al. 1992, Nakamura et al. 1996).

The early cleavage divisions of the *Xenopus* embryo require the localization of *cyclin B1* and *Xbub3* mRNAs to the mitotic spindles, and this is probably mediated by their attachment to localized anchors on the spindle microtubules (Groisman et al. 2000). Both transcripts contain cytoplasmic polyadenylation elements (CPEs), and their localization depends on CPE-binding protein, a microtubule-binding protein that decorates the spindle.

Localized Degradation

Maternal *hsp83* mRNA is uniformly distributed in the fertilized egg of *Drosophila* but forms a contracting posterior gradient as the nuclear division cycles proceed and eventually becomes restricted to the pole cells when they form at the posterior of the embryo after nuclear cycle 10 (Ding et al. 1993). Even though the total amount of *hsp83* mRNA decreases dramatically during embryogenesis, the amount of transcript at the posterior remains constant, suggesting that localization is achieved by generalized RNA degradation and local protection at the posterior (Bashirullah et al. 1999). *hsp83* mRNA is probably stabilized at the posterior by components of the pole plasm because this does not occur in mutants that lack this specialized cytoplasm, and it can be induced at the anterior in embryos with ectopic pole plasm at this pole.

Spatial control of degradation in the *Drosophila* embryo also leads to the localization of two other mRNAs, *hunchback* and *caudal*. In these cases, it is translation repression of *hunchback* mRNA by Nanos at the posterior and of *caudal* mRNA by Bicoid at the anterior that reduces the stability of the mRNAs. Thus the resulting anterior-posterior pattern of *hunchback* and *caudal* RNAs is a mirror image

of their translator repressors (Wharton & Struhl 1991, Dubnau & Struhl 1996, Rivera-Pomar et al. 1996).

Localized Synthesis

In multinucleate myofibers, the mRNAs encoding the different subunits of the acetylcholine receptor (AChR) become concentrated beneath the neuromuscular junction (NMJ) (Merlie & Sanes 1985, Fontaine et al. 1988, Goldman & Staple 1989). The mechanisms responsible for the localization of these transcripts are subunit specific. In the case of the α -subunit, it is likely that the focal enrichment of its transcript is from the activity-dependent increase in the number of nuclei near the NMJ. In the case of the δ -subunit, however, an unidentified signal acts locally to activate the expression of this gene in the nuclei close to the synapse and not in nuclei elsewhere in the cell, which is also likely to be the case for AChR ϵ -subunit mRNA (Brenner et al. 1990, Simon et al. 1992).

Localized transcription may also play a role in the localization of *gurken* mRNA in the *Drosophila* oocyte (Neuman-Silberberg & Schüpbach 1993). *gurken* is the only gene shown to be transcribed in the oocyte nucleus, which is transcriptionally quiescent and arrested in meiotic prophase, and this probably contributes to the localization of the mRNA to the apical side of the nucleus (Saunders & Cohen 1999). Cytoplasmic mechanisms must also be involved, however, as some *gurken* RNA seems to be synthesized in the nurse cells (Thio et al. 2000). Finally, localized transcription is the major mechanism for localizing mRNAs in the early *Drosophila* embryo. The first steps in patterning occur at the syncytial blastoderm stage, when the embryo is still a single cell because there are no membranes between the adjacent nuclei. The transcription of the gap and pair-rule genes in specific groups of nuclei can therefore be viewed as a mechanism to localize them within this large cell.

One of the most extraordinary patterns of RNA localization is the accumulation of the mitochondrial large ribosomal RNA (mtlrRNA) outside the mitochondria in *Drosophila* pole plasm, *Xenopus* germ plasm and ascidian myoplasm (Kobayashi et al. 1993, 1998; Oka et al. 1999). In *Drosophila*, the small mitochondrial rRNA has also been shown to be exported from the mitochondria in the pole plasm, where it associates with the polar granules like the mtlrRNA (Kashikawa et al. 1999). Nothing is known about how these rRNAs are exported across the mitochondrial membranes and attached to polar granules, but this process is dependent on the formation of pole plasm, and one factor that may play a role is Tudor protein, which is a component of the polar granules that is also found inside the mitochondria (Bardsley et al. 1993).

Active Transport

The mechanism that is most commonly invoked to explain mRNA localization is active transport along the cytoskeleton, but this is surprisingly difficult to prove. In general, there are two ways to demonstrate that an mRNA is localized by active transport. First, one can observe the directed movement of the RNA in vivo, either

by injecting labeled RNA or tagging the transcript with a labeled protein. However, this type of motion can also be generated by directed cytoplasmic flows that passively move the RNA with the rest of the cytoplasm, and this possibility needs to be ruled out before concluding that active transport is occurring. A second approach is to identify a motor protein that is required for the localization of the RNA. Motor proteins can also contribute to mRNA localization indirectly, for example, by transporting proteins that will anchor the RNA at the correct site or by generating cytoplasmic flows, and it is therefore also essential to prove that the motor is directly linked to the RNA. Because of these caveats, active transport has been conclusively demonstrated in only a few cases. The most convincing example is *Ash 1* mRNA in yeast, which is transported along actin cables by the myosin motor Myo4p (reviewed by Chartrand et al. 2001). Nevertheless, there is strong evidence that a number of mRNAs in higher organisms are localized by active transport, although the majority of these require microtubules rather than actin.

MBP mRNA The first direct visualization of the intracellular movement of an mRNA in living cells was provided by the injection of fluorescently labeled MBP mRNA into cultured oligodendrocytes (Ainger et al. 1993). The injected RNA forms particles in the perikaryon that move as much as $\sim 50 \mu\text{m}$ down narrow processes toward the periphery of the cell at a speed of $12 \mu\text{m}/\text{min}$. Because the mRNA is not extracted by non-ionic detergents, it is probably attached to the cytoskeleton, and localization is disrupted by drugs that affect the microtubule cytoskeleton, but not those affecting actin (Carson et al. 1997). These results strongly suggest that MBP mRNA moves along the microtubules that extend down the processes from the cell body, and the direction and speed of movement are consistent with transport by a plus-end directed microtubule motor. Furthermore, the localization of MBP mRNA is inhibited by antisense oligonucleotides directed against kinesin I, making this a strong candidate for the motor that transports the mRNA.

PAIR-RULE mRNAs Although the position of pair rule transcripts along the anterior-posterior axis of the *Drosophila* blastoderm embryo is determined by localized transcription, these RNAs also need to be localized apical to the nuclei to prevent their diffusion (Edgar et al. 1987, Davis & Ish-Horowicz 1991). Several lines of evidence initially suggested that this localization was achieved by vectorial export from the apical side of the nuclei. First, these RNAs have a half-life of only 6 min, making local stabilization an unlikely mechanism (Edgar et al. 1986). Second, the transcripts are never detected in the basal cytoplasm, even when stabilized, and still localize above the nuclei when they are detached from the cortex, arguing against transport in the cytoplasm (Francis-Lang et al. 1996). However, it was later found that the injection of fluorescently labeled pair-rule mRNAs into the basal cytoplasm results in their rapid and microtubule-dependent localization to the apical side (Lall et al. 1999). Furthermore, several lines of evidence indicate that both pair-rule mRNAs and *wg* mRNA are transported apically by the minus end-directed microtubule motor, cytoplasmic dynein (Wilkie & Davis 2001). First,

the injected mRNAs move at rate of 20–60 $\mu\text{m}/\text{min}$, consistent with an active transport process. Second, two different antibodies against dynein strongly inhibit this localization when they are injected at the same time as the RNA. Third, injected RNAs move at less than half the normal speed in embryos that are mutant for a combination of hypomorphic *dynein* alleles. Although dynein has not been shown to associate with either *wg* or pair-rule mRNAs, these results strongly suggest that dynein transports these transcripts along microtubules toward their minus ends, which lie apical to the nuclei.

BICOID mRNA The localization of *bicoid* mRNA occurs in multiple steps that have different genetic requirements, and several of these steps may involve active transport along the microtubules. *bicoid* is transcribed in the nurse cells and accumulates apically in specialized cytoplasmic structures, probably sponge bodies, before it moves through the ring canals into the oocyte, where it localizes at the anterior margin (St Johnston et al. 1989, Wilsch-Bräuniger et al. 1997). *exuperantia* (*exu*) mutants disrupt both of these localizations, and both endogenous Exu protein and a functional Exu-GFP fusion protein localize to sponge bodies and the anterior of the oocyte, suggesting that they associate with *bicoid* mRNA (Macdonald et al. 1991, Marcey et al. 1991, Wang & Hazelrigg 1994). Time-lapse analysis has revealed that Exu-GFP forms particles that undergo at least three different types of movement during their localization to the anterior of the oocyte (Theurkauf & Hazelrigg 1998). The particles move in a microtubule-dependent manner in the nurse cell cytoplasm at speeds of 5–140 $\mu\text{m}/\text{min}$, but the direction of these movements appears largely random. When the particles are in the vicinity of the ring canals, they show a directed microtubule-dependent movement toward them. They then pass through the ring canals into the oocyte, in a process that is not inhibited by either actin or microtubule-destabilizing drugs. Finally, some of the particles localize to the anterior of the oocyte in a microtubule-dependent manner. These results show that Exu is actively transported along microtubules to the ring canals, and it therefore seems likely this is also the case for *bicoid* mRNA. However, it remains to be proven that *bicoid* mRNA is a component of these Exu particles. Exu has recently been biochemically purified as part of a large RNase-sensitive protein complex, and RT-PCR on this complex demonstrated the presence of *oskar* mRNA but not *bicoid* (Wilhelm et al. 2000). Furthermore, Exu localizes more strongly to the posterior of the oocyte than to the anterior, suggesting that some if not all of the Exu-GFP particles are associated with *oskar* mRNA.

The simplest way to localize *bicoid* mRNA at the anterior of the oocyte would be to trap it as enters from the nurse cells (Frohnhofer & Nüsslein-Volhard 1987). However, the localization of *bicoid* and *oskar* mRNAs within the oocyte requires microtubules, and these seem to be polarized with their minus ends at the anterior and their plus ends at the posterior pole, leading to the suggestion that these RNAs could be localized by minus- and plus-end directed microtubule motors, respectively (Pokrywka & Stephenson 1991, Theurkauf et al. 1992, Clark et al. 1994, Pokrywka & Stephenson 1995, Clark et al. 1997). Furthermore, *bcd* and

osk mRNAs still appear to localize with markers for the minus and plus ends of the microtubules in mutants that alter the organization of the microtubules (Lane & Kalderon 1994, González-Reyes et al. 1995, Roth et al. 1995, Shulman et al. 2000). For example, *osk* mRNA localizes to the center of the oocyte in *gurken* mutants with the plus-end marker kinesin- β gal, whereas *bcd* mRNA is targeted to both the anterior and posterior poles, where the minus ends appear (González-Reyes et al. 1995). This posterior localization of *bicoid* mRNA in *gurken* mutants cannot be explained by the passive trapping mechanism and supports a model in which the RNA is localized to the anterior by active transport toward the minus ends of the microtubules.

Further support for this model comes from the analysis of Swallow, which is required to maintain the anterior localization of *bicoid* mRNA later in oogenesis (St Johnston et al. 1989). Like *bcd* mRNA, Swallow protein localizes to the anterior of the oocyte when it is expressed at stage 10A, and it also localizes to both poles of the oocyte in *gurken* mutants (Schnorrer et al. 2000). Swallow has been shown to bind to the light chain of cytoplasmic dynein, suggesting that it could couple *bcd* RNA to this microtubule motor, which then transports it to the minus ends at the anterior pole. However, *bcd* mRNA has not been detected in Swallow/dynein complexes and is already localized at the anterior when Swallow protein is first expressed. Furthermore, the anterior localization of Swallow does not require Exu or *bcd* mRNA. Thus an alternative possibility is that Swallow is transported by dynein to the site of *bcd* RNA localization where it functions as a localized anchor for a prelocalized mRNA.

The anchoring of *bcd* mRNA during the final stages of oogenesis requires a third protein, Staufen (St Johnston et al. 1991). Staufen protein also co-localizes with *bcd* mRNA at the anterior of the egg, and this localization is dependent on *bcd* mRNA (Ferrandon et al. 1994). Furthermore, Staufen contains five copies of a dsRNA-binding domain, and is therefore likely to bind directly to the RNA (St Johnston et al. 1992). Although Staufen has not been observed to move in association with endogenous *bcd* mRNA, it is recruited by injected RNA into particles that then localize to the poles of the mitotic spindles. The movements of these Staufen/*bcd* RNA particles are microtubule dependent, and they can be seen to oscillate in close proximity to the astral microtubules in time lapse recordings (I.M. Palacios & D. St Johnston, unpublished data). Thus both Swallow and Staufen may anchor *bcd* mRNA by coupling it to microtubules, and it is possible that this process involves motor proteins that either hold the RNA in place or continually transport it back to the anterior.

OSKAR mRNA As discussed above, microtubules are also required for the localization of *oskar* mRNA to the posterior of the oocyte at stage 9. This localization is thought to correspond to the position of the plus ends of the microtubules because a fusion between the constitutively active motor domain of the plus-end-directed microtubule motor, Kinesin I, and β -galactosidase also localizes to the posterior pole at this stage. This suggests a model in which *oskar* mRNA is transported to

the posterior by a plus-end motor, and Kinesin I, itself, has recently emerged as a strong candidate for this activity (Brendza et al. 2000). Although *kinesin heavy chain* is an essential gene in *Drosophila* because it is required for axonal transport, it can be removed from the oocyte by generating homozygous germline clones of a null mutation in the gene. In these egg chambers, *oskar* mRNA is still transported from the nurse cells into the oocyte but fails to move to the posterior pole and remains, instead, at the anterior. Furthermore, this phenotype is not the result of a defect in the microtubule organization because kinesin- β gal localizes normally to the posterior pole. It is possible, however, that Kinesin I plays an indirect role in *oskar* mRNA localization because the endogenous protein has not been shown to localize to the posterior of the oocyte, nor has it been linked to *oskar* mRNA.

One way that Kinesin I could play an indirect role in *oskar* mRNA localization is if it is required to generate cytoplasmic flows in the oocyte that circulate *oskar* mRNA, so that it can bind to a localized anchor at the posterior pole. Experiments in which fluorescently labeled *oskar* mRNA is injected into the oocyte suggest that this mechanism does operate at at least some stages of oogenesis (Glotzer et al. 1997). The polarized microtubule array is only present during stages 7–9 and is replaced at stage 10 by a dense cortical network of microtubules that drive vigorous cytoplasmic streaming (Theurkauf et al. 1992). When the RNA is injected into the oocyte at these later stages, it still accumulates at the posterior pole, and it even localizes in the presence of microtubule-depolymerizing drugs if it is injected close to the posterior pole. However, this accumulation requires Oskar protein, which is known to anchor its own mRNA. Because the translation of Oskar is localization dependent, this means that this protein can only be produced from RNA that has already localized to the posterior pole (Gunkel et al. 1998). Thus streaming toward a localized Oskar protein anchor may account for late *oskar* mRNA localization but cannot explain how the first *oskar* mRNA and protein reach the posterior.

Although the results above strongly suggest that *oskar* mRNA is localized by active transport along microtubules, the actin cytoskeleton may also play a role at some stage in this process, since mutants in the actin-binding protein tropomyosin II prevent this localization and cause the RNA to remain anchored at the anterior (Erdélyi et al. 1995, Tetzlaff et al. 1996). The organization of actin is not noticeably affected in mutant oocytes, however, and it is unclear whether the tropomyosin phenotype results from a subtle defect in the actin cytoskeleton or from some novel function of the protein that is independent of its well-characterized role in stabilizing actin filaments.

Vg1 mRNA *Vg1* mRNA is uniformly distributed throughout the *Xenopus* oocyte in early stages and localizes to the vegetal pole during stages 3 and 4, where it remains anchored for the rest of oogenesis (Melton 1987, Forristall et al. 1995). Several lines of evidence suggest that this localization may involve active transport along microtubules. First, the localization is unlikely to be due to local stabilization because the amount of *Vg1* mRNA remains constant from stage 2 to stage 6, and injected mRNA localizes to the vegetal pole without being degraded (Yisraeli &

Melton 1988). Second, *Vg1* mRNA shows a wedge-shaped distribution in the vegetal cytoplasm during stages 3 and 4, which probably represents transcripts in transit to the vegetal pole, arguing against random diffusion and local anchoring. Third, the movement of the RNA to the vegetal pole requires the microtubule cytoskeleton, whereas anchoring at the cortex in late oogenesis depends on the actin cytoskeleton (Yisraeli et al. 1989). Finally, a proportion of *Vg1* mRNA appears to be attached to microtubules because it co-sediments with polymerized tubulin in microtubule spin-down assays, and this attachment requires Vg1RBP/VERA, a protein that binds to the *Vg1* mRNA localization signal, and co-localizes with the RNA to the vegetal pole (Schwartz et al. 1992, Elisha et al. 1995, Deshler et al. 1997).

At the stage when *Vg1* mRNA is localized, the microtubules appear to be organized with their minus ends at the cortex of the oocyte and their plus ends extending toward the center of the cell (Pfeiffer & Gard 1999). Thus active transport of the mRNA would require a minus-end-directed motor such as dynein, although no motor proteins have been found to associate with *Vg1* mRNA. Interestingly, Vg1RBP/VERA protein fractionates with the rough endoplasmic reticulum (RER), and localizes to a vegetal sub-compartment of the RER that has a wedge-shaped distribution similar to that of *Vg1* mRNA in transit (Deshler et al. 1998, Kloc & Etkin 1998). This raises the possibility that *Vg1* mRNA is linked to the motor only indirectly and localizes to the vegetal cortex by hitchhiking with the RER.

A number of other mRNAs localize to the vegetal pole of the *Xenopus* oocyte, and two of these, *fatVg* and *VegT*, probably use the same pathway as *Vg1* mRNA (Lustig et al. 1996, Stennard et al. 1996, Zhang & King 1996, Horb & Thomsen 1997, Chan et al. 1999). A second class, including *Xcat-2*, *Xwnt11*, the *Xlsirts*, *Xpat* and *Xotx1* mRNAs, translocate to the vegetal pole earlier in oogenesis, in association with the fibrillar matrix or the germinal granules of the mitochondrial cloud (Kloc et al. 1993, 1998; Forristall et al. 1995; Kloc & Etkin 1995; Hudson & Woodland 1998; Pannese et al. 2000). It is not known how these mRNAs associate with the mitochondrial cloud, nor how the cloud itself moves to the vegetal pole, but neither process is disrupted by either microtubule or actin-depolymerizing drugs. Nevertheless, this early localization pathway is linked to the late pathway in at least two ways. First, the localization of the *Xlsirt* RNAs is required for the subsequent localization of *Vg1* mRNA (Kloc & Etkin 1994). Second, both *Xcat-2* and *Xpat* mRNAs can localize on the late pathway when they are injected at stage 4 (Zhou & King 1996, Hudson & Woodland 1998).

The microtubule cytoskeleton is required for the localization of a number of other mRNAs in addition to those discussed above. For example, the localization of Tau mRNA to axons is disrupted by microtubule-depolymerizing drugs, whereas β -actin-mRNA is associated microtubules in growth cones (Litman et al. 1994, Bassell et al. 1998). The nature of the requirement for microtubules in these cases is unknown. However, injected *Tau* mRNA has been shown to localize to the vegetal pole of the *Xenopus* oocyte, suggesting a functional link between the localization of *Vg1* mRNA in oocytes and Tau mRNA in axons (Litman et al. 1996).

Actin-Dependent Transport

Unlike *Ash1* mRNA in yeast, it seems that most transcripts in higher eukaryotes that localize via active transport move along microtubules rather than actin. One possible exception is *prospero* mRNA, which moves from the apical to the basal side of *Drosophila* neuroblasts as they enter mitosis in a complex with Staufén, Prospero, and Miranda proteins (Broadus & Doe 1997, Li et al. 1997, Broadus et al. 1998, Ikeshima-Kataoka et al. 1998, Schuldt et al. 1998, Shen et al. 1998). This localization is abolished by the actin-depolymerizing drug Latrunculin A and may be mediated by an unidentified myosin motor, because it is disrupted by high concentrations of BDM, which inhibits myosin activity (Broadus & Doe 1997, Peng et al. 2000, Ohshiro et al. 2001). The actin cytoskeleton does not show any obvious polarity in these cells, however, and it is possible that a myosin anchors the *prospero* mRNA complex to the cortical actin cytoskeleton rather than transporting it. β -actin mRNA localization to the leading edge of motile fibroblasts is also actin dependent, but nothing is known about the mechanism in this case (Hill & Gunning 1993, Kislauskis et al. 1993).

The small number of transcripts whose localization is actin dependent may reflect the different stability and organization of the two types of cytoskeleton in most cells. Since microtubules form stable and long-range structures in interphase, they are ideally suited for transport over long distances in polarized differentiated cell types, whereas actin filaments tend to be shorter and more dynamic. If mRNAs are actively transported along actin, this would probably be over short distances and might, therefore, be difficult to detect with current in situ hybridization techniques.

Multistep Localization

Some mRNAs are localized by more than one of the mechanisms discussed above because their localization occurs either in several steps or by overlapping and partially redundant pathways. For example, the initial accumulation of *nanos* mRNA at the posterior of the *Drosophila* oocyte is mediated by local anchoring in the pole plasm, but this localized RNA is also stabilized after fertilization, whereas the unlocalized transcripts are rapidly degraded (Bashirullah et al. 1999). The localization of *bicoid* mRNA probably involves a number of mechanisms at different stages of oogenesis: the RNA undergoes several different types of movement on its way from the nurse cells into the oocyte and can be targeted there by two redundant pathways (Macdonald & Kerr 1997). Furthermore, the subsequent anchoring of the mRNA requires first *swallow* and then *staufer*, neither of which is involved in its initial transport to the anterior.

Like *bicoid*, other mRNAs that are localized by active transport need to be anchored once they have reached their destination, and these two steps often have distinct requirements. For example, the localization of *Vg1* mRNA to the vegetal pole requires microtubules, whereas anchoring is dependent on the actin cytoskeleton (Yisraeli et al. 1990, Kloc & Etkin 1995). In other cases, the translation of nascent polypeptides plays a role in anchoring, but not in RNA transport. For example, the

tight anchoring of *Ash1* mRNA to the cortex of the daughter cell depends on the translation of Ash1 protein (Gonzalez et al. 1999). Similarly, Osk protein anchors *osk* mRNA at the posterior pole of the *Drosophila* oocyte. In contrast to *Ash1* mRNA, however, Osk protein can act in *trans* to anchor its own mRNA (Rongo et al. 1995).

COUPLING mRNAs TO THE TRANSPORT MACHINERY

Although mRNAs can be localized by a variety of mechanisms, two aspects of this process are universal. First, the transcript must contain *cis*-acting elements that direct its localization. Second, these elements must be recognized by specific *trans*-acting factors that somehow link the RNA to one of the localization mechanisms described above.

Cis-Acting Elements

With the exception of *yemanuclein- α* and *gurken* mRNAs in *Drosophila* and *Ash1* mRNA in *S. cerevisiae*, all the *cis*-acting sequences necessary for RNA localization that have been mapped reside in 3'UTRs (Capri et al. 1997, Chartrand et al. 1999, Gonzalez et al. 1999, Saunders & Cohen 1999, Thio et al. 2000). Most of these localization elements are listed in Table 1 and are not here discussed in detail. Instead, we focus here on some more of the general concepts that have emerged from the analysis of these sequences.

DISTINCT ELEMENTS MEDIATE SPECIFIC STEPS Different steps in the localization of a transcript are often mediated by separate *cis*-acting elements. For example, an 11 nt RNA transport signal (RTS) is sufficient to direct the transport of MBP mRNA from the cell body into the processes of oligodendrocytes, whereas a different region of approximately 340 nt, called the RLR, mediates localization to the myelin compartment (Ainger et al. 1997, Hoek et al. 1998, Munro et al. 1999). Similarly, elements in the 5' non-coding region are required for accumulation of *grk* mRNA in the *Drosophila* oocyte at early stages; sequences in the coding region are necessary for localization to the anterior of the oocyte in mid- to late-stage egg chambers; and the 3'UTR seems to be involved in localization to the dorsal anterior corner of the oocyte (Saunders & Cohen 1999, Thio et al. 2000). A third example is provided by localization of *Xcat-2* mRNA to the vegetal pole of the *Xenopus* oocyte. The early localization with the mitochondrial cloud is mediated by a 227 nt region in the proximal part of the 3'UTR, while the association with the germinal granules requires 164 nt that lie more distal (Kloc et al. 2000). Furthermore, *Xcat-2* RNA can localize to the vegetal pole later in oogenesis when it is injected, and this depends on sequences different from those required for the localization at stage I and II (Zhou & King 1996).

TABLE 1

Cell/RNA	Localization	Mechanism	cis-Acting element	trans-Acting factor	Reference
Mammalian cells					
Neurons					
MAP2	S, D ^a	MT	3' 640 ntds	U	Blichenberg et al. 1999
Tau	A	MT	3' 240 bp	U	Litman et al. 1994, Aronov et al. 1999
β -actin	GC	MT	U	U	Bassel et al. 1998
CaMKII α	D	U	3'	U	Mayford et al. 1996
Arc	S, D	U	U	U	Steward et al. 1998
Tin-5	A	U	U	U	Hannan et al. 1995
Neuropeptide-encoding RNAs	A	U	U	U	Mohr et al. 1991, Jirikowski et al. 1990
GlyR subunits	D	U	U	U	Racca et al. 1998
BDNF	D	U	U	U	Tongiorgi et al. 1997
TrkB	D	U	U	U	Tongiorgi et al. 1997
FMRP	D	U	U	U	Link et al. 1995
Oligodendrocytes					
MBP	Into processes	MT	3' RTS 11 ntds	hmRNPA2, kinesin	Carson et al. 1998
	peripheral membrane	MT	RLR 1131-1473 ntds		
Muscle					
AchR (α - ϵ - δ -)	Postsynapsis	LS	U	U	Goldman & Staple 1989, Brenner et al. 1990
Vimentin	Costameres in myotubes	U	U	U	Fulton & L'Ecuyer 1993
<i>Drosophila</i>					
Neuroblasts					
<i>prospero</i>	Apical to basal	AC	U	Staufen, Miranda	Jan & Jan 2000
<i>inscuteable</i>	Apical	U	U	U	Knoblich et al. 1999
<i>miranda</i>	Apical	U	U	U	Shen et al. 1997
Neuroglia/blast	Daughter cell	U	U	U	Bernardoni et al. 1999
<i>gcm</i>					
Neuromuscular junction:					
<i>DGllur-IIA</i>	Subsynaptic compartment	U	U	U	Sigrist et al. 2000

TABLE 1 (Continued)

Cell/RNA	Localization	Mechanism	cis-Acting element	trans-Acting factor	Reference
Epithelia in eye disc					
Chicken <i>seventless</i>	Apical surface	U	U	U	Banerjee et al. 1987
Fibroblast/myoblast					
<i>β-actin</i>	Leading lamellae	AC	3'/54 ntds/43 ntds	ZBP	Kislauskis et al. 1994 Ross et al. 1997
<i>Xenopus</i>					
Vegetal pole					
<i>Vgl</i>	Vegetal cortex	MT/AC	3' 340 ntds Repeats VMI and EI—E4	VgIRBP/Vera, hnRNPI	See text
<i>VegT</i>	Vegetal cortex	U	U	U	Zhang & King 1996
<i>Xcat-2</i>	GG in MC/germ plasm	MC/MT	3' 403—630nto MC 3' 631—795nto GG	U	Kloc et al. 2000
<i>Xcat-3</i>	Vegetal cortex	U	U	U	Elinson et al. 1993
<i>Xdaz1</i>	Germ plasm	U	U	U	Houston et al. 1998
<i>Xwn1-II</i>	MC/germ plasm	U	U	U	Ku & Melton 1993
<i>Xlsiris</i>	MC/germ plasm	U	repeat elements	U	Kloc et al. 1993
<i>favg</i>	Vegetal cortex	U	3' 25 ntds (FVLEI)	U	Chan et al. 1999
<i>mltrRNA</i>	Germ plasm	U	U	U	Kobayashi et al. 1998
<i>Xpat</i>	Vegetal pole	U	3'	U	Hudson & Woodland 1998
<i>βTrCP</i>	Vegetal pole	U	U	U	Hudson et al. 1996
<i>Xorx1</i>	MC/germ plasm	U	U	U	Pannese et al. 2000
Animal pole: undefined pathway					
<i>An1—4</i>		U	U	U	Rebagliati et al. 1985, Hudson et al. 1996

<i>βTrCP</i> (2.5 Kb)						Hudson et al. 1996
<i>Oct-60</i>		U				Hinkley et al. 1992
<i>PABP</i>		U				Schroeder & Yost 1996
<i>xlan4</i>		U				Reddy et al. 1992
<i>xl-21</i>		U				Kloc et al. 1991
Zebrafish embryo						
<i>vasa</i>	Cleavage plane and PGCs	U				Yoon et al. 1997
DAZ-like	Vegetal pole	U				Maegawa et al. 1999
bruno-like	Vegetal pole	U				Suzuki et al. 2000
Worm embryo						
<i>nos-2</i>	PGCs	U				Subramaniam & Seydoux 1999
Ascidian embryo						
<i>actin</i>	Myoplasm and ectoplasm	U				Jeffery et al. 1983
<i>pems</i>	Myoplasm	U				Yoshida et al. 1996,
						Satou & Satoh 1997
<i>Wnt-5</i>	Myoplasm	U				Sasakura et al. 1998a
<i>POPK-1</i>	Myoplasm	U				Sasakura et al. 1998b
<i>ZF-1</i>	Myoplasm	AC/MT				Sasakura et al. 2000
<i>Pet-1, -2, -3</i>	Posiplasm	AC				Sasakura et al. 2000
<i>mtl/rRNA</i>	Myoplasm	U				Oka et al. 1999
<i>macho-1</i>	Myoplasm	U				Nishida & Sawada 2001
<i>rplL5</i>	Myoplasm	U				Swalla & Jeffery 1996a
<i>YC</i>	Myoplasm	U				Swalla & Jeffery 1995
<i>PCNA</i>	Ectoplasm	U				Swalla & Jeffery 1996b

^aU, unknown; LA, diffusion and local anchoring; LD, localized degradation; LS, local synthesis; MT, microtubules-dependent; AC, actin-dependent; S, soma; D, dendrites; A, axon; GC, growth cones; GG, germinal granules; MC, mitochondrial cloud; PGCs, primordial germ cells.

MULTIPLE PARTIALLY REDUNDANT ELEMENTS The mapping of *cis*-acting localization signals has revealed a number of cases where the localization of the RNA can be mediated by multiple partially redundant elements. The most dramatic example of this is *nanos* mRNA, which contains four different regions in its 3'UTR that direct localization to the pole plasm (Gavis et al. 1996). These elements give only weak localization on their own, but function more efficiently when a single element is present in multiple copies, or when two or more elements are included in the same RNA (Bergsten & Gavis 1999, Crucs et al. 2000). Interestingly, these elements seem to direct localization by interacting with different *trans*-acting factors (Bergsten et al. 2001). A 75-kDa protein has been shown to bind to a 41 nt region of the +2' element that is sufficient to mediate posterior localization when multimerized, and mutants that disrupt this binding abolish localization. However, this factor does not bind to the +1 or +4 elements that also target the RNA to the posterior pole. *Ash1* mRNA also contains four localization elements, each of which is sufficient to target the RNA to the daughter cell (Chartrand et al. 1999, Gonzalez et al. 1999). In contrast to *nanos*, however, all these elements are recognized by a single protein, She2p, although they show no obvious sequence similarity (Bohl et al. 2000, Long et al. 2000).

Other transcripts that contain partially redundant localization elements include *fatvg* and β -actin mRNAs. The *fatvg* 3'UTR contains two regions that are able to target the RNA to the vegetal pole, although localization is more efficient when both are present (Chan et al. 1999). Similarly, the first 54 nucleotides (nt) of the β -actin mRNA 3'UTR can direct the localization of a reporter RNA to the leading lamellae in fibroblasts; however, deletion of this element reduces, but does not eliminate, localization. A 43-nt element with partial homology to the 54-nt region must also be deleted to abolish β -actin mRNA localization completely (Kislauskis et al. 1994).

A more complex form of redundancy is shown by *Vg1* mRNA. The *Vg1* localization element (VLE) has been mapped to a 340-nt region of the 3'UTR, but no part of this region is essential because constructs containing a series of 15-nt deletions spanning the entire VLE all localize normally (Mowry & Melton 1992, Gautreau et al. 1997). Furthermore, although larger-scale deletions reveal essential elements at the 5' and 3' ends of the VLE, these also appear redundant because two copies of the 5' element can direct the localization of a heterologous RNA to the vegetal pole. Mutations in a repeated sequence motif called VM1 or E1 block the localization mediated by this duplicated 5' element, but this is also the case for deletions of other three-repeated sequences, E2, E3, and E4, in the context of the complete VLE (Deshler et al. 1997, Gautreau et al. 1997). Thus localization seems to require co-operative interactions between multiple partially redundant elements, which may correspond to the four-repeated sequence motifs.

The 640 nt *bcd* mRNA localization signal shares many of the complexities shown by the VLE (Macdonald & Struhl 1988). Out of a series of deletions spanning the entire 3'UTR, the removal of only one region of 50 nt (BLE1) strongly disrupts anterior localization, and even in this case, some RNA still localizes to

the anterior during oogenesis (Macdonald et al. 1993). Furthermore, smaller deletions that span the BLE1 localize normally, indicating the presence of redundant elements within this small region. Interestingly, the BLE1 behaves like the 5' element in the VLE: It is not sufficient to direct anterior localization of a reporter RNA on its own but does mediate early localization to the anterior of the oocyte when present in two copies. One reason that the BLE1 is not essential for localization is that there appear to be two redundant pathways for targeting *bcd* mRNA to the anterior of the oocyte, which have different sequence requirements. Pathway A functions during stages 4–10 of oogenesis, and requires a region of 274 nt that contains BLE1, and point mutations within BLE1 disrupt this localization (Macdonald & Kerr 1997). Indeed, the $2 \times$ BLE1 construct localizes mRNAs to that anterior by this pathway (Macdonald & Kerr 1998). Pathway B directs anterior localization from stage 7 onward and requires a much larger region of the 3'UTR. This pathway is not disrupted by point mutants in BLE1, which explains why these mutations only abolish localization during stages 4–6 in the context of the whole localization element.

STRUCTURE Unlike DNA, RNA molecules have the capacity to fold into elaborate secondary and tertiary structures, and this is likely to be important for the function of many of the larger and more complex localization signals. Indeed, one reason why localization elements almost always reside in 3'UTRs may be that these regions of the RNA can be structured without disrupting translation. This feature adds an additional level of complexity to mapping the binding sites for *trans*-acting factors. For example, non-contiguous sequences can base-pair to form RNA stems, and mutations in these elements could therefore disrupt mRNA localization indirectly by perturbing the structure of the RNA.

The role of structure in mRNA localization has been most fully analyzed for the *bicoid* 3'UTR. On the basis of phylogenetic sequence comparisons, the *bcd* localization signal is predicted to form five large stem loops (Figure 2*Ai*) (Macdonald 1990, Seeger & Kaufman 1990). The BLE1 region forms the distal portion of stem-loop V, and the structure of this stem is essential for localization by pathway A, since mutations that disrupt base pairs of the stem abolish localization, whereas double mutant combinations that restore base-pairing, but not the sequence, do not (Macdonald & Kerr 1998). The interaction and localization of Stauf protein with injected *bcd* RNA requires even higher order structure of the 3'UTR. Linker scanning experiments show that this interaction depends on three non-contiguous regions of the RNA that correspond to stem-loop III and the distal regions of stem-loops IV and V (Ferrandon et al. 1994). Mutations that disrupt the double-stranded helix of any of the stems in this region block the recruitment of Stauf, whereas compensatory mutations that restore the structure, but not the sequence, rescue complex formation in five out of six cases (Ferrandon et al. 1997).

To make matters more complicated, the loop at the end of stem III can base-pair with an internal bulge in this stem, and mutations in either of these sequences also

disrupt Staufen binding but can be rescued by compensatory mutations that restore complementarity. More importantly, co-injection of two mutant RNAs that cannot form intramolecular loop-bulge duplexes, but can form an intermolecular duplex with each other, leads to the efficient recruitment of Staufen into particles that localize to the poles of the mitotic spindle. Thus the association of Staufen requires the formation of *bcd* 3'UTR dimers and therefore depends on the quaternary structure of the RNA. Staufen contains five double-stranded RNA-binding domains, but a single dsRBD binds to any dsRNA without specificity (St Johnston et al. 1992, Bycroft et al. 1995). It therefore seems likely that the specificity of this interaction involves the three-dimensional structures of both the RNA and the protein.

Although the structure of only a few localization elements has been investigated, there are at least two other cases where it is likely to be important. A 44-nt sequence in the 3'UTR of *K10* mRNA is both necessary and sufficient for direct localization to the anterior of the *Drosophila* oocyte and is predicted to form a simple stem loop. All mutations that disrupt this stem block localization, whereas mutations that alter only the primary sequence have little or no effect on *K10* mRNA localization (Serano & Cohen 1995). The 340-nt element required for MBP mRNA to localize to the myelin compartment has also been predicted to contain a stable secondary structure. This localization element shows 70% homology between human, mouse, and rat, and computer analysis predicts a conserved RNA secondary structure for all three (Figure 2*Aii*) (Ainger et al. 1997).

Trans-Acting Factors

Only a few *trans*-acting factors involved in mRNA localization have been identified so far, in most cases, by biochemically purifying RNA-binding proteins that recognize *cis*-acting localization elements. The essential role of localized maternal mRNAs in *Drosophila* axis formation has allowed a complementary approach in which proteins required for mRNA localization are identified in genetic screens for mutants that disrupt embryonic patterning, and some of these are RNA-binding proteins required for the localization of specific transcripts.

STAUFEN The first RNA-binding protein proven to play a role in RNA localization is Staufen, which was originally identified in a *Drosophila* genetic screen as a mutant that disrupts both anterior and posterior patterning (Schüpbach & Wieschaus 1986). Subsequent work has shown that it plays an essential role in the localization of three different RNAs. Staufen co-localizes with *osk* mRNA throughout oogenesis (Figure 2*Biii*) and is required both for its microtubule-dependent localization, and for the anchoring and translation of the mRNA once it has reached the posterior pole (Ephrussi et al. 1991, Kim-Ha et al. 1991, St Johnston et al. 1991). Later in oogenesis and during early embryogenesis, Staufen is also recruited to the anterior pole, where it anchors *bcd* mRNA and enhances its translation (St Johnston et al. 1989, 1991; Micklem et al. 2000). Finally, Staufen is required for the actin-dependent localization of *pros* mRNA in neuroblasts, and the protein

co-localizes with the RNA throughout the cell cycle (Broadus & Doe 1997, Li et al. 1997, Broadus et al. 1998).

Staufen contains five dsRNA-binding domains, of which three, dsRBDs 1, 3, and 4, have been shown to bind dsRNA in vitro (St Johnston et al. 1992, Micklem et al. 2000). Although Staufen has not been shown to bind specifically to these mRNAs, several other lines of evidence suggest that it binds directly to the *osk*, *bcd*, and *pros* localization signals. First, the localization of Staufen to the anterior or posterior of the oocyte depends on its interaction with the appropriate mRNA, since an increase in the quantity of *bcd* or *osk* mRNA induces a corresponding increase in the amount of Staufen that co-localizes to that pole (Ferrandon et al. 1994). Second, the 3'UTRs of *bcd* and *pros* mRNAs recruit Staufen into particles when they are injected into the embryo (Ferrandon et al. 1994, Schuldt et al. 1998). Finally, the dsRNA-binding activity of Staufen dsRBD3 is required for the localization of *bcd* and *osk* mRNAs, although this has not been tested for *pros* mRNA (Ramos et al. 2000).

Although dsRBD2 and 5 do not bind dsRNA, they have both been highly conserved during evolution, and appear to link Staufen/RNA complexes to different localization pathways. dsRBD2 contains an insertion sequence at an identical position in all species, and the domain does bind RNA when this insertion is deleted. The removal of this insertion from full-length Staufen blocks the transport of *osk* mRNA to the posterior of the oocyte but has no effect on the localization of *pros* mRNA. Thus this domain appears to be specifically required to couple Staufen/RNA complexes to the microtubule-dependent localization pathway.

In contrast, deletion of dsRBD5 abolishes the localization of Staufen and *pros* mRNA in neuroblasts without affecting the transport of *osk* mRNA to the posterior of the oocyte (Schuldt et al. 1998, Micklem et al. 2000). This domain binds to Miranda protein, which co-localizes with Staufen and *pros* mRNA both before and after their localization to the basal side of the neuroblast (Fuerstenberg et al. 1998, Matsuzaki et al. 1998, Schuldt et al. 1998, Shen et al. 1998). Furthermore, the localization of Staufen and *pros* mRNA requires Miranda, but not vice versa, indicating that Miranda functions as an adaptor protein linking Staufen to an actin-based localization pathway. Miranda provides the first example of an essential *trans*-acting factor that recognizes an RNA-binding protein associated with a localized mRNA, but it is still unclear how Miranda itself is localized.

The mammalian homologues of Staufen have also been implicated in mRNA transport (Marión et al. 1999, Wickham et al. 1999). In rat hippocampal neurons, Staufen is found in large RNA-containing particles that are associated with microtubules, and these particles have been observed to move along dendrites in time-lapse films of Staufen-GFP (Kiebler et al. 1999, Köhrmann et al. 1999). The RNAs in these particles have not been identified, but one candidate is MAP2 mRNA because Staufen was isolated in a yeast three-hybrid screen for proteins that interact with the MAP2 dendritic-targeting element (DTE) (Monshausen et al. 2001). This could be due to non-specific binding of Staufen dsRBDs to dsRNA, however, and two other proteins have been shown to bind specifically to the DTE (Rehbein et al. 2000).

Vg1RBP/VERA AND ZBP-1 *Xenopus* Vera or Vg1RBP, which contains five RNA-binding motifs, one RRM, and four KH domains, is one of the six proteins that bind to the 340-nt VLE required for the localization of *Vg1* mRNA (Schwartz et al. 1992, Mowry 1996, Deshler et al. 1997, 1998, Havin et al. 1998). The protein Vg1RBP/Vera has been reported to bind both to the VM1 repeat that is necessary for the localization of the duplicated 5' element of the VLE and to the E2 elements, which are required for the localization of the complete VLE. In both contexts, mutations that abolish binding of this protein disrupt the localization of the RNA, and therefore it seems likely that Vg1RBP/Vera is required for the microtubule-dependent transport to the vegetal pole. Furthermore, the protein co-localizes with the RNA and associates with microtubules and the rough ER (Elisha et al. 1995, Deshler et al. 1997).

Surprisingly, Vg1RBP/Vera is 78% identical to ZBP-1, a 68-kDa protein involved in the actin-dependent localization of β -actin mRNA in chicken fibroblasts. ZBP-1 binds to the 54-nt *cis*-acting localization element, which contains a 6-nt tandem repeat, and mutation of this repeat reduces both ZBP-1 binding and mRNA localization (Ross et al. 1997). This motif is not present in either VM1 or E2, but it shows some similarity to the latter, suggesting that Vg1RBP/Vera and ZBP-1 recognize related but distinct sequences (Deshler et al. 1998). The discovery that these two highly related proteins are involved in microtubule and actin-dependent mRNA localization in different species suggests that, like *Staufen*, members of this family of proteins may mediate localization along both types of cytoskeleton.

hnRNP PROTEINS Heterogeneous nuclear riboproteins are abundant nucleic acid-binding proteins that have a variety of functions in the nucleus. However, a subset of the hnRNPs shuttle into the cytoplasm and can play important roles in the regulation of the translation, stability, and localization of specific cytoplasmic mRNAs (Shyu & Wilkinson 2000). One of these hnRNPs is VgRBP60, which shows 87% amino acid identity to the human hnRNPI/PTB (polypyrimidine tract-binding protein) (Cote et al. 1999). VgRBP60 accumulates at the vegetal pole of the oocyte and shows a striking co-localization with *Vg1* mRNA. More importantly, the protein binds to the VM1 element, and a single point mutation that prevents the binding of just this protein abolishes localization of the duplicated VLE 5' element to the vegetal pole. Thus VgRBP60/hnRNPI appears to play an essential role in the cytoplasmic localization of *Vg1* mRNA.

Another hnRNP, hnRNPA2, binds to the MBP mRNA transport signal (RTS), and has been implicated in the localization of this RNA. Immunolocalization of the protein shows that it is located not only in the nucleus of oligodendrocytes but also in granules found in the cell body and processes, as previously described for the MBP mRNA (Hoek et al. 1998). Point mutations of the hnRNPA2-response element (A2RE) that eliminate binding to hnRNPA2 also markedly reduced the ability of this region to support RNA transport. Furthermore, treatment with antisense oligonucleotides directed against hnRNPA2 disrupt transport of injected MBP mRNA (Munro et al. 1999). Interestingly, the HIV genome also contains two A2RE-like sequences, which bind hnRNPA2, and viral RNAs that include these

sequences localize in oligodendrocytes, suggesting that the hnRNPA2 couples the viral RNA to the same localization pathway as MBP mRNA (Mouland et al. 2001).

One of the most abundant hnRNPs in the *Drosophila* embryo is hrp40 or Squid, the homologue of human hnRNPA1. In *squid* mutant oocytes, *gurken* mRNA fails to localize to the anterior dorsal cortex (Kelley 1993). Although Squid does not show any obvious co-localization with *grk* mRNA, it can be UV-cross-linked to two different regions of the *grk* 3'UTR (Norvell et al. 1999). Furthermore, rescue experiments indicate that two of the three isoforms of the protein, Squid A and S, function in different steps in the localization of Grk activity. The expression of Squid S in a *squid* mutant rescues *gurken* mRNA localization. This isoform is concentrated in the nuclei and binds more strongly to K10, another nuclear protein required for *gurken* mRNA localization (Neuman-Silberberg & Schüpbach 1993, Serano et al. 1995). In contrast, Squid A rescues the dorsal localization of Grk protein, but not *grk* mRNA, suggesting that it plays a role in *gurken* translational regulation. Unlike Squid B and S, Squid A is cytoplasmic and interacts more strongly than the other isoforms with Bruno protein, which has been implicated in translational repression of both *grk* and *osk* mRNAs (Webster et al. 1997).

These results implicate hnRNPs in cytoplasmic mRNA localization, but it is unclear whether they play a direct role in coupling the mRNAs to the transport machinery or if they act as RNA chaperones that fold the RNA so that it can be recognized by other factors. hnRNPs have been described as general nucleic acid-binding proteins, and it is surprising, therefore, that they play such specific roles in the localization of particular mRNAs. However, some hnRNPs do bind with higher affinity to specific RNAs, and this seems to be the case for hnRNPA2. HnRNPA2, which is closely related to hnRNPA1, belongs to a family of proteins that have a modular structure, with two N-terminal RRM s followed by a glycine-rich region. Although the similarities between their RRM s suggest that they should recognize the same RNA, hnRNPA2 has a much higher affinity for A2RE than does hnRNPA1, and this requires cooperative binding of the two RRM s (Shan et al. 2000). Thus the localization of MBP mRNA seems to involve the sequence-specific RNA-binding of hnRNPA2 that is distinct from its weaker non-specific binding to all RNA.

The role of hnRNPs in mRNA localization suggests that the assembly of specific RNA-protein complexes in the nucleus may determine the fate of the mRNA in the cytoplasm. Although no hnRNP proteins have been implicated in its localization, this may also be the case for *oskar* mRNA. Mutants in *mago nashi* and *barentsz* block the movement of *osk* mRNA from the anterior to the posterior of the oocyte, and trace amounts of both proteins transiently co-localize with the mRNA at the posterior pole (Newmark & Boswell 1994, Micklem et al. 1997, Newmark et al. 1997, van Eeden et al. 2001). However, the vast majority of Mago nashi protein is nuclear, whereas Barentsz is predominantly localized around the nurse cell nuclei, probably in association with the nuclear pores (Figure 2*Bi,ii*). Thus these proteins may well be loaded onto *osk* mRNA as it exits the nucleus so that they can direct the localization of *osk* mRNA in the cytoplasm.

PROSPECTS

Although there has been considerable progress in our understanding of the mechanisms of mRNA localization, several important questions remain to be resolved. For example, we still have very little idea of the composition of mRNA localization complexes. While a number of RNA-binding proteins in these complexes have been identified, many more have yet to be discovered, and this will probably require a combination of genetic and biochemical approaches because neither can easily find all types of factors. For example, Staufen cannot be cross-linked to RNA with UV light, which is one of the most common biochemical techniques for detecting RNA-binding proteins. On the other hand, hnRNPs will often be difficult to identify in genetic screens because they are involved in many other aspects of RNA metabolism, and mutations in them are therefore likely to be highly pleiotropic. The binding of many *trans*-acting factors will probably depend on the structure of the localization element, and it will be important to bear this in mind when searching for additional factors.

It seems likely that mRNAs localized by active transport will not be transported as single molecules but will be packaged into larger complexes. A number of localized RNAs are found in granules, whereas Staufen and Exu assemble into large particles with *bcd* mRNA (Ferrandon et al. 1994, Wang & Hazelrigg 1994, Barbarese et al. 1995, Knowles et al. 1996). Indeed, injected HIV mRNA localizes in granules that have been estimated to contain 29 mRNA molecules (Mouland et al. 2001). At present, we know nothing about how these higher-order complexes are formed or if the transport of the mRNAs depends on their formation, but this may represent an additional essential step in mRNA localization.

Another major question in the field is how localized mRNAs are coupled to the motors that transport them. Although this has been solved for *Ash1* mRNA in yeast, the adaptor proteins that link it to the myosin are not conserved, and most examples of mRNA transport in higher eukaryotes involve microtubules rather than actin. The two motor proteins implicated in microtubule-dependent mRNA transport, Kinesin I and dynein, are both required for a variety of other processes such as vesicle trafficking. Thus it is possible that some RNAs are not coupled to motors directly but instead hitchhike on other organelles. The search for the link between the RNA and the motor may therefore be a difficult one, but this will be essential for an understanding of how mRNAs are localized.

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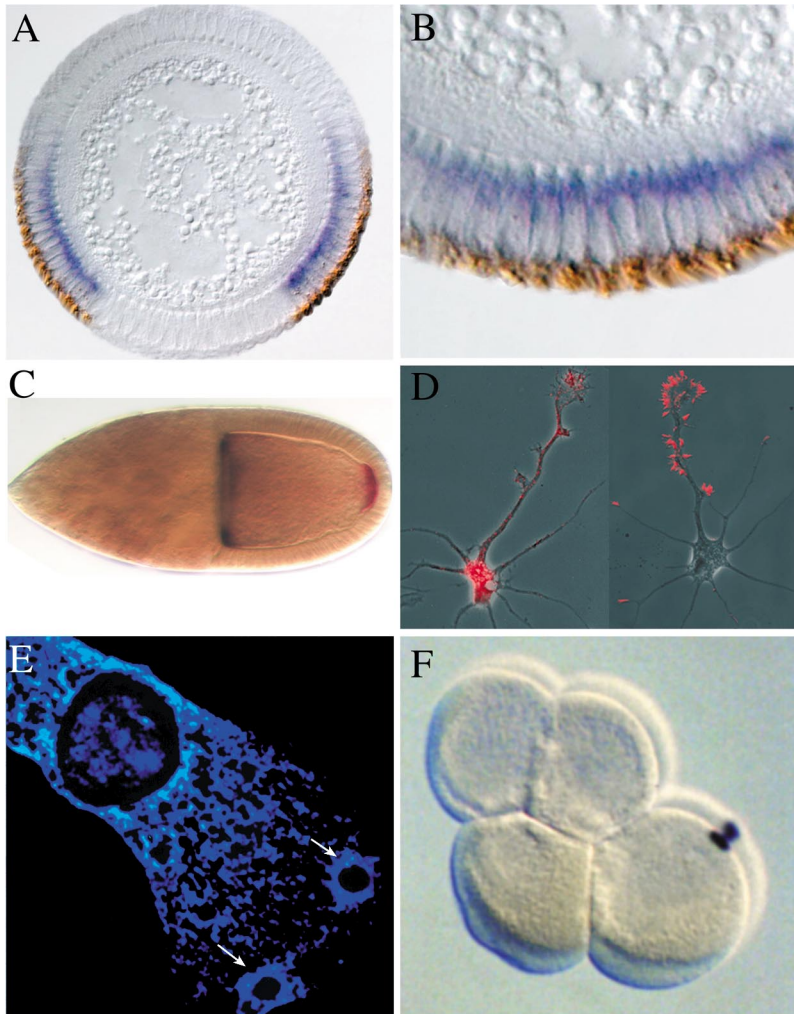


Figure 1 Examples of localized mRNAs. (A,B) *brinker* mRNA (blue) localizes basally in the *Drosophila* blastoderm embryo, whereas *short gastrulation* mRNA (brown) is concentrated apically (courtesy of A. Jazwinska & S. Roth; Jazwinska et al. 1999). (C) *bicoid* (black) and *oskar* (red) mRNAs localize to the anterior and posterior poles of the *Drosophila* oocyte (courtesy of K. Litière). (D) β -actin mRNA (left panel) and protein (right panel) localization in the axonal growth cone of cultured neurons (courtesy of G.J. Bassell; Zhang et al. 1999). (E) Recruitment of total RNA (blue) to focal adhesion complexes after cell binding to extracellular matrix-coated beads (arrows) (courtesy of M. Chicurel & D. Ingber; Chicurel et al. 1998). (F) Localization of HrZF-1 mRNA to the posterior-vegetal cytoplasm in a 8-cell embryo of the ascidian *Halocynthia roretzi* (courtesy of Y. Sasakura & K. W. Makabe; Sasakura et al. 2000).

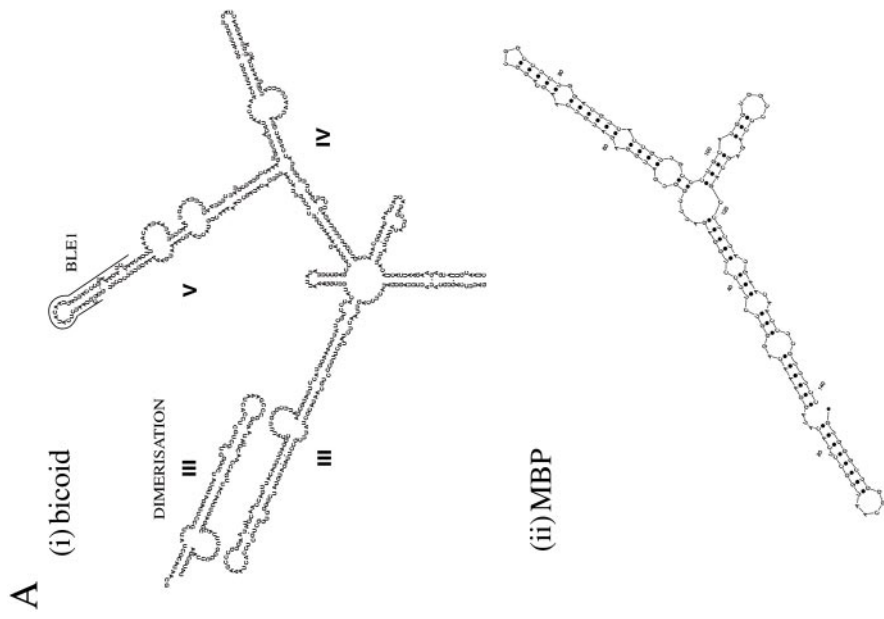


Figure 2 See legend on next page

Figure 2 (See Figure 2 on previous page) (A*i*) Secondary structure of the *bicoid* mRNA localization signal. The intermolecular dimerization domain is indicated by interaction of the external region in stem-loop III of one molecule with the same region in another. BLE1, the element required for the early localization of *bicoid* mRNA, is marked. (A*ii*) Predicted secondary structure for human MBP mRNA from base 1250 to 1390. This region is required for the localization of the transcript to the myelin compartment. Similar structures are obtained with mouse and rat MBP. (B) Mago nashi, Barentsz, and Staufen proteins co-localize with *oskar* mRNA to the posterior of the *Drosophila* oocyte, and they are all required for this localization. However, (i) Mago Nashi is predominantly nuclear, (ii) Barentsz associates with the nuclear envelope, and (iii) Staufen is cytoplasmic, suggesting that the proteins associate with the RNA in different compartments.