RNA localization: different zipcodes, same postman?

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RNA-localization mechanisms involve specific sequences in the localized RNA and proteins that bind to these sequences and mediate the interaction with cytoskeletal elements. Until recently, it seemed as though two separate types of mechanism were operating for mRNA localization – involving interaction with either microtubules or actin microfilaments. However, it is now clear that some of the protein components involved in mRNA localization can participate in both microtubule- and actin-dependent localization pathways. This, combined with new evidence for evolutionary conservation of some of these proteins, suggests a previously unanticipated uniformity in mRNA-localization mechanisms.

One of the most important events in cell differentiation and development is the determination of asymmetry. In almost all cells, this asymmetry is required for subsequent function, as in the polarity of neurons that transmit directional signals or in the polarity of specific morphogens in oocytes that drive different cell fates in the embryo. Morphological asymmetry requires the sorting of proteins unequally in the cell, and one mechanism for doing this is to synthesize the proteins in their relevant locations. The discovery that RNAs can be localized specifically within oocytes or somatic cells provided a direct link between nucleic acid information and cellular compartmentalization[1,2]. The mechanisms involved in this process have been investigated persistently over the past decade. These studies have shown that many localized mRNAs contain 'zipcodes', specific targeting regions usually found in the 3′-untranslated region (3′-UTR)[3]. These sequences, most of which are necessary and sufficient for localization, appear to be unique in each mRNA, and often multiple and variable in length[4]. Efforts have also been directed towards identifying proteins involved in the entire localization process. It seemed logical that there would be proteins that recognize these localization sequences, transport the RNA, anchor it, and then control its expression and stability. Recent evidence from *Drosophila*, *Xenopus*, chicken fibroblasts and mouse neurons has begun to identify these proteins and suggests that RNA localization mechanisms might be conserved. Recently, two lines of investigation have moved the field towards convergence regarding the mechanism that localizes RNA in oocytes, embryos and somatic cells and the proteins involved with the cytoskeletal elements on which these RNAs travel. One comes from discoveries on localization in the neuroblast lineage in *Drosophila*, the other from work on localization in *Xenopus* and fibroblasts.

RNA localization in development

Genetic analysis of determinant localization in *Drosophila* oocytes has revealed a cascade of genes important in localization of *BICOID*, an anteriorly localized RNA, and *OSKAR* and *NANOS*, posteriorly localized RNAs. One such gene, *staufen*, is required for localization of both posterior (*OSKAR*) and anterior (*BICOID*) RNAs[5,6]. *staufen* encodes a protein that contains five copies of a conserved double-stranded (ds) RNA-binding domain. The C-terminus of Staufen interacts with other proteins involved in localization (see below). When microinjected into *Drosophila* embryos, the 3′-UTR of *BICOID* mRNA interacts separately with Staufen, forming particles associating with microtubules and translocating in a microtubule-dependent manner[7]. This suggests that Staufen is involved in microtubule-dependent transport of localizing RNAs. Another RNA-localization system involving microtubules has been described in *Xenopus* oocytes. This system is active in late stage III to early stage IV of oogenesis and localizes *Vg1* mRNA, which encodes a transforming growth factor (TGF)-β-like protein, to the vegetal cortex[2]. As is the case for *BICOID*, there are cis-acting elements in the 3′-UTR of the mRNA and *trans*-acting proteins involved in the localization[2]. *Cis*-acting elements have been mapped to the 3′-UTR of the *Vg1* message and at least one *trans*-acting protein has been cloned (see below).

More recently, Staufen has been shown to play a role in asymmetric distribution of a mRNA specifying lineage in somatic cells. This mRNA, *PROSPERO*, encodes a homeodomain transcription factor required for differentiation of the early neuroblast lineage[8,9] (Fig. 1). Prospero is sorted differentially into the nuclei of ganglion mother cells (GMCs)[10], where it acts to induce gene expression. Staufen interacts with the 3′-UTR of *PROSPERO* mRNA and is necessary for its localization during mitosis[11]. The C-terminus of Staufen interacts with Miranda[12], whose function is required for the asymmetric localization of Staufen itself. Miranda is a membrane-associated protein with a predicted coiled-coil domain and might serve to tether the complex to the cortex of the dividing neuroblast. During mitosis, a complex of *PROSPERO* mRNA plus Prospero, Staufen and Miranda proteins forms on the apical side of the sensory organ precursor cell and is translocated into GMCs[12]. Miranda is then degraded and Prospero enters the nucleus. Two other proteins, Inscuteable and Numb, also are localized asymmetrically during this mitosis[13]. Inscuteable is necessary to orient the spindle and also binds to the C-terminus of Staufen[14]. Numb is a membrane protein, but its

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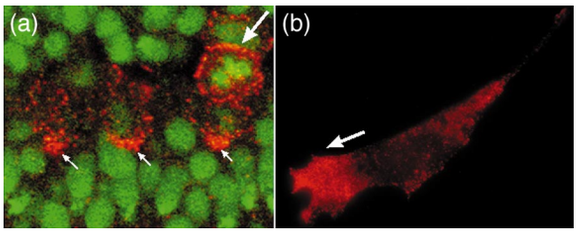

FIGURE 1

RNA localization in developing and differentiated cells. (a) *PROSPERO* RNA (red) localization in neuroblasts in interphase (small arrows) and mitosis (large arrow). Green represents DNA staining. (Images courtesy of C. Doe.) (b) β-actin mRNA localization at the leading edge of a motile fibroblast (large arrow).

precise role in localization is still unclear. Further characterization of how and when these proteins interact should clarify the sequence of binding and mechanism involved. Because Miranda has domains that interact successively with Inscuteable, Prospero and Numb[15] as well as with Staufen, it must play a central role in this process. Its localization also requires an intact actin cytoskeleton.

A similar system of asymmetric cell division and cell-fate determination exists in budding yeast, where the determinant for mating-type switching, the transcriptional repressor Ash1p, is localized asymmetrically to the daughter cell[16,17]. Localization of Ash1p requires localization of its mRNA[18,19], unlike Prospero protein, which can localize independently of *PROSPERO* mRNA localization[11]. However, like neural determination in *Drosophila*, Ash1p localization requires an intact actin cytoskeleton. Localization of *ASH1* mRNA also requires at least five additional genes (*SHE1*, *-2*, *-3*, *-4* and *-5*), one of which is a myosin V (She1p/Myo4p)[16]. It is not known whether myosin is involved in *PROSPERO* mRNA localization.

RNA localization in differentiated cells

Besides serving to segregate determinants during cell division, mRNA localization also provides a means of generating asymmetry in terminally differentiated cells. An example of this can be found in oligodendrocytes, which are involved in myelination of neurons. mRNA encoding myelin basic protein (MBP) is localized to the peripheral processes in oligodendrocytes[20], where myelination occurs. Two *cis*-acting elements have been found in the mRNA, and these interact with specific RNA-binding proteins. One of these *cis*-elements – so-called RTS (RNA-transport sequence) – seems to be important for transport of MBP mRNA into the peripheral sheets on microtubules independently of the presence of the MBP coding region. The RTS binds to hnRNPA2, a protein with a predominantly nuclear distribution[21]. Another *cis*-element – RLR (RNA localization region) – is required for retention of coding-region-containing mRNA at these most distal regions.

In fibroblasts, mRNA encoding actin is localized at the leading edge, where actin polymerization effects motility. The 'zipcode' in the actin mRNA 3′-UTR, which directs the RNA to the leading edge, binds to a protein called zipcode-binding protein 1 (ZBP-1)[22]. Mutations of a double hexanucleotide repeat in this zipcode disrupt localization as well as ZBP-1 binding. The sequence of ZBP-1 indicates that it is a protein with four KH (hnRNP K homology) and one RRM (RNA-recognition motif) RNA-binding domains (Fig. 2). The presence of putative nuclear localization and export signals suggests that ZBP shuttles into and out of the nucleus, but immunofluorescence indicates that it is predominantly cytoplasmic in distribution.

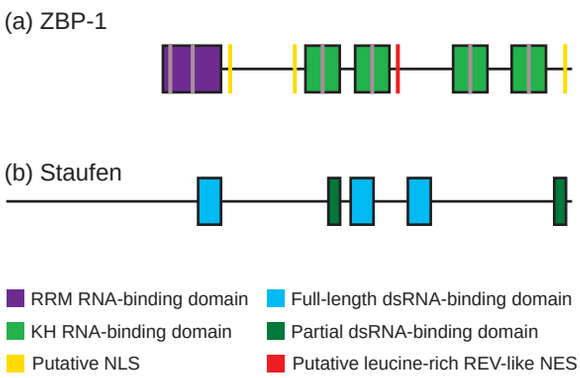

FIGURE 2

Structures of members of two protein families involved in RNA localization. (a) Zipcode-binding protein (ZBP-1); (b) Staufen. Abbreviations: ds, double-stranded; RRM, RNA-recognition motif; NLS, nuclear-localization sequence.

A convergence of mechanisms?

Historically, there has been an assumed mechanistic dichotomy between mRNAs that require microtubules and those that require actin filaments for their localization. As described above, *BICOID* and *OSKAR* localization in *Drosophila* and *Vg1* localization in *Xenopus* require predominantly microtubules, as does mRNA encoding MBP in oligodendrocytes. However, localization of *ASH1* mRNA in yeast, *PROSPERO* mRNA in *Drosophila* and mRNA encoding actin in fibroblasts requires actin filaments[23].

New evidence suggests that the RNA-localization mechanism has no problem with this dichotomy. First, localization of mRNA encoding actin in neurons occurs on microtubules[24], but the same mRNA is localized on actin filaments in fibroblasts. The next surprising finding was that Staufen, which plays a role in microtubule-dependent localization of mRNAs in oocytes, is also required for the actin-dependent localization of *PROSPERO* RNA[11]. Finally, and most remarkably, determination of the amino acid sequence of proteins binding to *Vg1* mRNA and utilizing microtubules for its transport[25] showed that they are closely related to ZBP-1 (Vera/Vg1 RNA-binding proteins were discovered by different groups but are apparently the same protein)[26–28]. Thus Vera/Vg1-binding proteins and ZBP-1 are almost identical proteins operating on different cytoskeletal elements in different cells of different

species. Additionally, the sequences of zipcodes in these two mRNAs are different.

These four pieces of information have had a significant impact on our thinking about the localization mechanism. How could one protein overcome all these differences? One possibility is that small differences in the amino acid sequence of the binding proteins might account for the differences in nucleic acid binding specificity. Another is that the actual zipcode sequences are not the sole determinants; the proteins might recognize a conserved secondary structure of the zipcodes. Irrespective of these differences, these data indicate a high degree of conservation of the mechanism of localization in these diverse systems. As is the case with Staufen in both somatic and germ cells, zipcode-binding proteins must utilize different means to localize the RNAs to which they bind. In addition, the ZBP and its relatives appear to be a part of a family of KH domain RNA-binding proteins (Fig. 2) whose members have diverse functions. For instance, a protein (KOC)[29] highly homologous to ZBP-1/Vg1/Vera is overexpressed in human cancers, and Vg1/Vera is identical to the *Xenopus* transcription factor B3 (W. Taylor, pers. commun.; GeneBank accession number AF42353). The relationship between all these proteins and their functions clearly merits further research.

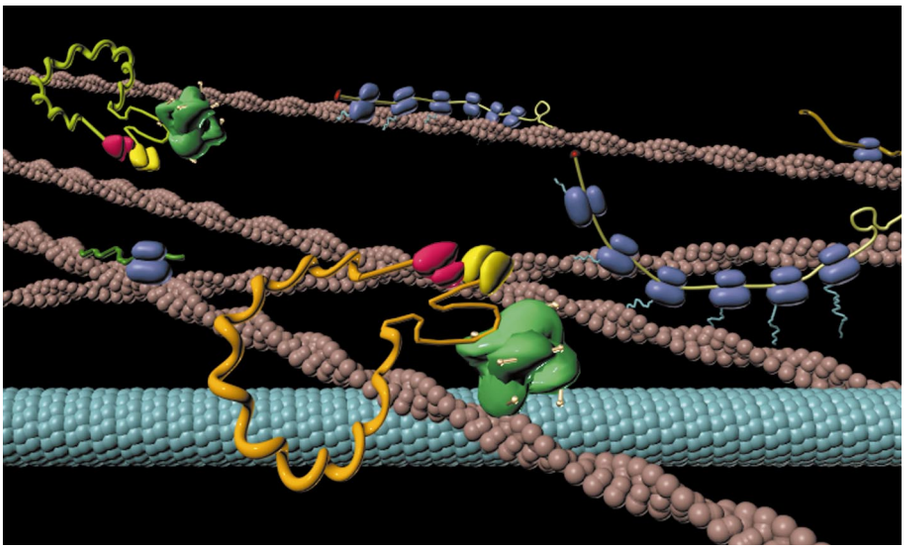

FIGURE 3

A glimpse into the 'RNA-localization world'. Microtubules (light blue) and actin filaments (brown) are thought to be involved in the transport of different mRNAs (orange and light green) to their destination, where they are translated by ribosomes (dark blue). The transport is mediated by a 'locusome' or 'ziposome' (green) – a particle presumably containing RNA-binding and motor proteins (gold) as well as regulatory proteins. During localization, RNA is probably in a compact nontranslatable form bound by several regulatory proteins (red and yellow) at the 5′ and 3′ ends.

It seems likely, therefore, that closely related RNA-binding proteins recognize different zipcodes and transport corresponding RNAs, perhaps switching from one cytoskeletal element system to the other in the process. There are several possible mechanisms for this. One is that the proteins involved are capable of interacting with both cytoskeletal systems through, for instance, dual binding motifs that recognize either actin- or microtubule-based motors (Fig. 3). However, it is more likely that they assemble a complex structure (a 'particle' or 'granule'[30]), with motors and/or binding proteins for either eventuality, that would have regulatable and interchangeable subunits. What these components are and how they are regulated remains to be uncovered. The asymmetric distribution of mRNAs, whether sorted to the axonal growth cone of neurons and required for guidance and motility, or sorted to the bud tip in yeast and required for switching mating type, will need to use a sophisticated and selective transport system to accomplish the final goal of localized proteins and cell asymmetry.

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