Determinants of mRNA localization

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RNA localization provides a mechanism for protein targeting within developing or differentiating cells. Specific *cis*-acting sequences on mRNA mediate this process. Such 'localizer' or 'zipcode' nucleic acid sequences have been restricted to the 3′ untranslated region of several mRNAs. The presence of genetic information denoting a spatial component of translation adds a new dimension to gene expression.

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Introduction

Eukaryotic cells are highly organized to achieve a broad structural and functional diversity. How genetic information mediates different structures and compartmentalizes particular functions remains to be elucidated. Some of the information for cellular organization can be attributed to proteins by virtue of their limited ability to form multipeptide complexes. The role of nucleic acids in establishing subcellular compartments has yet to be fully appreciated. We have postulated that certain mRNAs carry destination codes (determinants) to direct them to specific regions of the cell cytoplasm [1]. The fact that nucleic acids contain spatial information as well as code for amino acid sequences provide support for this hypothesis. RNA localization would thereby establish cytoplasmic diversity through compartmentalization of protein synthesis.

Messenger RNA localization is a mechanism for targeting protein expression in early developing systems and in specialized somatic cells. During oogenesis and early embryogenesis, sequence-specific localization of maternal transcripts precedes embryonic morphogenesis [2–5]. In highly polarized somatic cells, such as neurons and their excessory cells, motile fibroblasts, intestinal epithelia or muscle mRNAs are found spatially coupled to functional domains of the protein that they encode [6,7]. Recent evidence in three different systems (*Xenopus*, *Drosophila* and chicken embryo fibroblasts) has directed our attention towards specific *cis*-acting sequences in the 3′-untranslated region (3′-UTR).

Xenopus: vegetal pole zipcode sequences

The search for maternal factors that act as cytoplasmic determinants in the development of amphibian eggs provided the first evidence of localized concentrations of specific mRNAs. Jeffery *et al.* [8] showed by *in situ* hybridization that actin mRNAs and polyadenylated RNA were preferentially localized near the myoplasm, while histone RNA was homogenously distributed in unfertilized ascidian eggs. Using a molecular genetic approach, Melton and colleagues [9] cloned several maternal mRNAs that were preferentially localized to either the vegetal pole or the animal pole of *Xenopus* oocytes. The best characterized of those clones, *vg-1*, is homogeneously distributed in early stage oocytes and is localized to the vegetal cortex in middle- and late-stage oocytes. The *vg-1* cDNA encodes a transforming growth factor homolog, which possibly functions in mesoderm induction [3,10].

Evidence that *vg-1* localization occurs by an RNA-dependent and not a translational mechanism was provided by Yisraeli and Melton [11]. They observed that microinjected, radiolabeled *vg-1* transcripts that lack a 5′-UTR and an initiation codon co-localize with the endogenous *vg-1*. Definitive proof that the vegetal pole localizer is contained in the RNA has recently been shown by a more detailed analysis of *vg-1* cDNA [12••]. Chimeric transcripts between the β-globin coding region and the *vg-1* 3′-UTR indicate that no less than 340 nucleotides of the *vg-1* 3′-UTR are sufficient to confer vegetal pole localization to an otherwise non-localized reporter transcript. Replacement of 150 nucleotides within this segment delocalized the reporter mRNA. The size of this segment might allow for multiple protein-binding sites. In fact, gel bandshift assays suggest that several as yet uncharacterized proteins bind the *vg-1* zipcode with specificity (K Mowry, personal communication). Whether these proteins function in vegetal localization must be established. Evidence to indicate that microtubules are involved in *vg-1* transport and that actin is required for anchoring at the vegetal cortex has implicated cytoskeletal or cytoskeletal-associated proteins in this process [13]. Ultimately, combinations of biochemical, pharmacological, and molecular biological approaches will elucidate which sequences are involved in the process of the vegetal pole localization.

Drosophila: anterior/posterior zipcode sequences

The anterior–posterior axis in *Drosophila* embryos is established by localized maternal RNAs encoding morphogenetic determinants [14]. At the anterior pole, localiza-

Abbreviations

bsd—*bicoid*; ***eve***—*even-skipped*; ***nos***—*nanos*; ***osk***—*oskar*; ***stau***—*staufen*; **UTR**—untranslated region; ***vg***—*vegetal*.

tion of *bicoid* (*bcd*) mRNA precedes formation of a morphogenetic gradient of Bcd protein, which is essential for the development of the head and thorax. Macdonald and Struhl [15] were the first to provide evidence that *cis*-acting RNA sequences were responsible for localizing *bcd* RNA to the anterior pole. They showed, using RNase protection assays, that a 625 nucleotide portion of the *bcd* 3′-UTR was necessary and sufficient to confer anterior localization to a *lacZ*/α-*tubulin* reporter transcript in transgenic flies. Further deletions in this region prohibited localization of the reporter transcript. These conclusions were confirmed directly using *in situ* hybridization [16••]. The possibility that multiple proteins bind this region was suggested by the conservation of a hypothetical secondary structure among divergent species of *Drosophila* [17]. However, secondary structure considerations have become less important since recent linker-scanning mutagenesis has significantly restricted the size of the anterior-directing zipcode (P Macdonald, personal communication).

The anterior localization pathway is not well understood but must involve a stepwise process. Mutational analyses have identified three genes, *exuperantia*, *swallow* and *staufen* (*stau*), which affect *bcd* mRNA localization at different stages of oogenesis [18–20]. The effects of cytoskeletal inhibitors on eggs that are mutant for these genes suggest that microtubules but not microfilaments are involved in all stages, and that *exuperantia* and *swallow* serve to mediate, stabilize or regulate the association between *bcd* mRNA and microtubules [21]. The Stau protein appears to anchor *bcd* mRNA at the anterior pole [19]. Anterior localization of Stau is dependent on *bcd* RNA (D St Johnston, unpublished data). Whether any of these proteins interact directly or indirectly with the *bcd* localization sequence has yet to be determined.

Two localized maternal RNAs control the development of the abdomen and germline (pole plasm) at the posterior pole. Posterior localization of *nanos* (*nos*) mRNA determines abdominal segmentation [22,23], while *oskar* (*osk*) mRNA directs pole plasm formation [24,25•]. Recent observations indicate that the *nos* 3′-UTR encodes a posterior localization signal [16••]. Gavis and Lehmann [16••] demonstrate that a 780 nucleotide segment of the *nos* 3′-UTR was sufficient to direct the *lacZ* transcript to the posterior pole, independent of its polyadenylation signal. In the case of *osk* RNA, the posterior zipcode appears to be composed of multiple discrete sequences (P Macdonald, personal communication). While primary sequence comparisons between *bcd*, *nos* and *vg 1* 3′-UTRs did not reveal convincing similaritites [16••], Gottlieb [26•] identified a nonamer motif YUGUUYCUG in the 3′-UTRs in four localized RNAs (*bcd*, *nos*, *vg 1* and *an 2*, a *Xenopus* mRNA localized to the animal pole). The significance of this nonomer motif in the *bcd* mRNA was investigated by microinjecting *Drosophila* embryos with synthetic, radiolabeled *bcd* RNAs. The data suggest that a deletion of the nonomer causes the injected RNA signal to diffuse or mislocalize over 30 % of anterior pole, rather than tightly associating with the anterior pole, without actually destabilizing the RNAs. Curiously, this nonomer is not within the 625 nucleotide *bcd* zipcode [15,16••]. It is, however, part of the sequence that confers Nos-dependent destabilization to *hunchback* RNAs in the posterior pole and, when Nos is ectopically expressed, destabilizes *bcd* RNA in the anterior pole [27]. Perhaps this nonomer serves multiple functions in order to maintain morphogenetic gradients. Further analyses of this motif should clarify its role in RNA localization.

The process of localization of *nos* mRNA to the posterior pole requires the function of several genes. This group includes: *cappuccino*, *spire*, *staufen*, *oskar*, *valois*, *vasa*, *tudor* and *mago nashi*. The effects of these mutations on the localization of other members of the posterior group places them in a hierarchical order [24,25•,28,29]. The first molecules to localize at the posterior pole are Stau protein and *osk* RNA [24,28,29]. Since Stau is also required for the anterior localization of *bcd* RNA it may function as an mRNA chaperone that tethers RNA zipcodes to common structures at both poles, such as the cortical cytoskeleton [29]. Cappuccino and Spire are also required to localize *osk* RNA to the posterior pole. Both may form a cytoskeletal framework for posterior localization [30]. The *osk* gene is a key component in the process of pole plasm formation [24,25•]. Evidence indicates that Osk protein acts to induce pole plasm formation. When *osk* mRNA has a *bcd* 3′-UTR, it localizes anteriorly and determines germ cell functions at the anterior pole [25•]. Since mutations that truncate the carboxyl terminus of the Osk protein delocalize *osk* RNA, the maintenance of *osk* RNA localization may require full-length Osk protein [24,28]. Next, Vasa protein becomes posteriorly localized [31,32]. All *osk* mutations also prevent Vasa localization. Lastly, *nos* and *cyclin B* RNAs are localized to the posterior pole [24,33]. Although Osk protein has no obvious RNA-binding motifs, unlike Vasa [31], it may interact specifically with the *nos* RNA because both *nos* and *osk* RNAs are similarly localized in Bicaudal-D embryos, while Vasa protein and *cyclin B* RNA are unaffected [24,31,33]. Expression of reporter genes bearing the posterior zipcodes of *nos* and *osk* RNAs should help to resolve the relationship between protein and nucleic acid determinants of posterior localization.

Drosophila: apical/basal zipcode sequences

In the early blastoderm stage of *Drosophila* embryos, segmentation gene transcripts exhibit different patterns of localization in the periplasm surrounding the cortical layer nuclei [34••]. At the end of the blastoderm stage, cell membranes invaginate to subdivide the periplasm of an individual cell into apical (above nuclei) and basal (below nuclei) periplasm. The *gap* genes are the first zygotic genes to be transcribed and they appear in the apical and basal periplasm. These genes activate the transcription of pair-rule genes in reiterated stripes of approximately four nuclei wide. Pair-rule genes are localized in the apical region. To investigate the mechanism of apical localization, Davis and Ish-Horowicz [34••] expressed hybrid transcripts between a β-galactosidase reporter gene and the various segmentation gene sequences in transgenic flies. They observed that RNA sequences that direct three pair-rule genes, *fushi tarazu*, *hairy* and *even-skipped*, to the apical periplasm, and sequences directing the human α-*globin* mRNA to

the basal periplasm both occur in the 3′-UTR. Of the apical localization sequences, *eve* RNA was the most restricted. The last 125 base section of the *eve* 3′-UTR, 98 nucleotides of which is upstream of the polyadenylation site, was shown to confer apical localization to the reporter transcript. Deletion of 59 nucleotides on the 5′-side of the 124 base segment caused the hybrid mRNAs to delocalize, but destruction of the polyadenylation site did not affect apical localization. No obvious secondary structure was uncovered in this region. A somewhat surprising observation was that the *bcd* 3′-UTR and the tubulin 3′-UTR sequences also contain an apical signal. An 863 nucleotide fragment of *bcd* 3′-UTR localized reporter transcripts apically, but close to the nucleus and as foci, as *bcd* is not normally expressed in these cells.Its localizer sequences may be recognized by some universal cellular mechanism. It is also possible that several compartments exist in the apical periplasm, or that the process of localization occurs in multiple stages, or that *bcd* mRNAs are at a default position in these cells.

The process of apical RNA localization is unlikely to be similar to localization of mRNAs for morphogens. Pair-rule transcripts are very unstable ($t_{1/2}=6$ min) [35] and do not diffuse significantly in the cytoplasm, unlike mRNAs for the morphogens that localize over the course of days and hours (*bcd*, *osk* and *nos*). Therefore, the mRNA is unlikely to be transported within the periplasm. Rather, transcripts must leave the nucleus in a polarized fashion. A nuclear, rather than a cytoplasmic sorting mechanism is proposed because independent transformants, which are expected to integrate randomly within the chromatin, yield apical localization.

Chicken embryonic fibroblasts: peripheral zipcode

In cultured chicken embryonic fibroblasts, cytoplasmic mRNAs that encode actin, vimentin and tubulin are localized near their respective functional domains [1]. Actin mRNA, in particular, is localized to the distal regions of the leading lamellae and filopodia, while vimentin and tubulin mRNAs are concentrated around the nucleus. More recently, other groups have shown actin mRNA localized in different polarized cell systems, including intestinal epithelium and cultured fibroblasts [36,37].

Experiments designed to address the mechanism of actin RNA localization, using protein synthesis inhibitors, have shown that peripheral localization occurs by a translation-independent mechanism [38]. To characterize the localization determinant directly, fusion genes between chicken β-actin cDNA sequences and the β-galactosidase reporter gene were transiently expressed in chicken embryonic fibroblasts. The spatial distribution of the β-galactosidase activity and *in situ* hybridization products in transfectants revealed that a maximum of 53 nucleotides in the proximal portion of the 3′-UTR sequences was sufficient to confer peripheral localization to a non-localized reporter (EH Kislauskis, RH Singer, unpublished data). Experiments are currently underway to characterize the proteins that interact with this region. Candidates include various localized actin-binding proteins as both phases of actin localization, transport and anchoring involve the actin cytoskeleton [39].

Conclusions

At present, the ubiquity of RNA localization determinants is unknown. RNA localization is not the only way to target proteins. Proteins themselves have targeting signals for the endoplasmic reticulum and nucleus, or may self-assemble with polymers and macromolecular complexes. Therefore, mRNA localization may serve to increase local intracellular concentrations to promote these interactions. Conversely, the effects of non-localized protein synthesis may cause non-productive, promiscuous interactions between polypeptides, or lethal consequences in the case of morphogens.

A high degree of complexity of spatial information may exist in the genome, encoded in the 3′-UTR of genes. Some of this information may be used within the nucleus where transcripts may be directed to the cytoplasm in a polarized fashion. Within the cytoplasm, these determinants may specify a few, or many, compartments. Families of nearly identical proteins more often differ in their 3′-UTRs than in their coding sequences (e.g. actin). The mRNAs for the different isoforms may denote different compartments where each isoform is synthesized. During muscle differentiation, for instance, co-localization of sarcomeric actin mRNA with other mRNAs encoding contractile proteins could establish a sarcomere assembly complex.

The extent to which non-coding elements function in gene expression is becoming more evident. Sequences for stability (see Peltz and Jacobson, this issue, pp 979–983), translational regulation [40] or nuclear export [41], as well as the spatial determinants we have described all provide for a highly regulated control of the expression of the mRNA. It remains for work over the next year to characterize the proteins that bind to these localizer sequences and to determine the level of complexity needed for transduction of spatial signals.

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