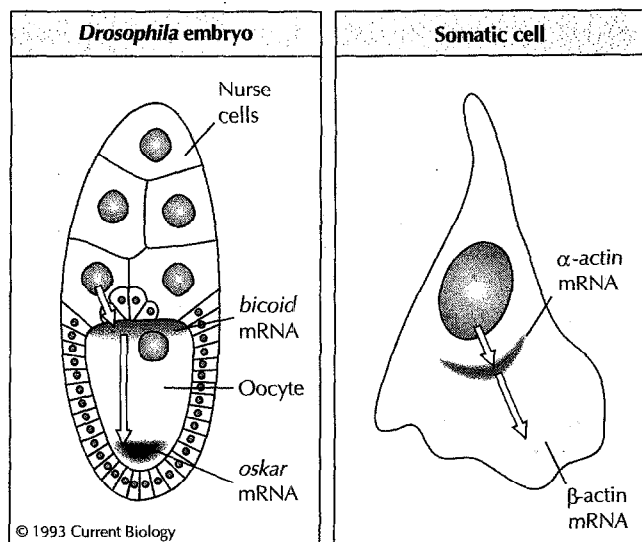


## RNA zipcodes for cytoplasmic addresses

Intracellular localization of mRNAs appears to be determined by sequences in their 3' untranslated regions that are composed of multiple elements.

It is now well established that mRNAs for certain proteins are localized in specific regions of the cytoplasm [1]. Known examples of such localized mRNAs fall into two major classes: cellular mRNAs transcribed in somatic (or zygotic) cells, the localization of which results in synthesis of the encoded proteins at their sites of action [2]; and maternal mRNAs that are asymmetrically positioned in oocytes and involved in establishing the axes of the embryo during early development by setting up concentration gradients of protein 'morphogens' [3] (Fig. 1). Experimental evidence for the specific intracellular localization of both types of mRNA has come from applications of improved *in situ* hybridization techniques. In *Drosophila*, the functional significance of the phenomenon has been shown by the lethal effects of mutations affecting the localization of maternal mRNAs.



**Fig. 1.** Pathways of localization of maternal and somatic mRNAs. Both systems appear to involve compound mechanisms, with multiple possible stopping points for the mRNA. In *Drosophila* oocytes, mRNAs are transported from nurse cells to the anterior ooplasm, where *bicoid* mRNA (red) stops; mRNAs such as *oskar* (green) move on to the posterior ooplasm. In mammalian somatic cells, such as myoblasts, two stopping points have been described so far:  $\alpha$ -actin mRNA stops in a perinuclear region;  $\beta$ -actin mRNA continues on to the leading edge of the cell.

With the phenomenon of mRNA localization now well established, attention has turned in the past few years to the mechanism by which mRNAs are sorted to the correct cytoplasmic compartments. A growing body of evidence supports the view that the localization depends on

specific sequence elements — which we call 'zipcodes' [4] by analogy to a system in which a message can be addressed to a specific postal zone with a minimum of coding information — that occur generally, perhaps even exclusively, within the 3' untranslated region (UTR) of the mRNAs. The localization zipcodes therefore belong to a class of *cis*-acting regulatory elements that occur within mRNA 3' UTRs and that determine properties of mRNAs such as their stability, translation efficiency and responsiveness to autoregulatory mechanisms [5]. The mRNA zipcode determines the site of synthesis of the encoded protein within the cell [4]. Recent results have refined the definition of the zipcodes in *Drosophila* maternal mRNAs and in chicken actin mRNAs.

In two recent papers [6,7], Macdonald and coworkers have dissected the *cis*-acting localization elements of the *Drosophila bicoid* and *oskar* maternal mRNAs (the former is localized at the anterior end of the oocyte and encodes a protein morphogen important in anterior patterning of the embryo [8]; the latter mRNA is localized at the posterior end of the oocyte and is important in organizing the germ plasm [9,10]). Previous work provided evidence that the localization of these mRNAs depends on their 3' UTRs [11,12]. In the recent work [6,7], the elements involved have been defined to short segments within the mRNAs' 3' UTRs. The data suggest that the complex sequence of events by which mRNAs are localized may be mirrored by the complexity of the nucleic acid signals that specify the subcellular compartments to which the mRNAs are directed.

Two types of approach were used to characterize the localization elements. In the first, *bicoid* transgenes were made with deletions in the region encoding the mRNA 3' UTR, and the deleted transgenes assayed for their ability to rescue partial anterior development of *bicoid*<sup>-</sup> mutant embryos (known to depend on correct localization of the *bicoid* transcripts). In the second, transgenes encoding chimeric mRNAs with sequences taken from the *bicoid* or *oskar* 3' UTR were made, and again the localization of the chimeric mRNAs was assayed in the transgenic flies. Given the presence of endogenous *bicoid* and *oskar* mRNA in both sets of transgenic flies, heterologous sequences served as 'tags' for *in situ* hybridization to the transgene-encoded mRNA. For the *bicoid* mRNA, one of the deletions resulted in a defect that could not be corrected by increasing the transgene dosage. This 50 nucleotide element of the 3' mRNA was

termed bicoid localization element 1 (BLE1). The situation for *oskar* mRNA seems even more complex, as multiple overlapping or redundant elements were identified.

Although BLE1 falls within the 680 nucleotide 3' UTR sequence shown in previous work to be important for localization [11], the new study failed to verify the importance of other sequences previously implicated in localization. Part of the answer to this conundrum may be that the different assays — rescue of a defective mutant phenotype, localization of mRNAs fused to heterologous sequences and *in situ* hybridization to endogenous mRNAs — measure different aspects of the localization pathway. The first assay measures the presence of a functional protein; small amounts of the protein may be sufficient for rescue so that higher dosage may compensate for defects in mRNA localization. Only *in situ* hybridization can verify that the phenotypic rescue does indeed reflect correctly localized mRNA. Furthermore, two copies of BLE1 are required and can direct only the early steps of localization: mRNA with BLE1 as its only *bicoid*-derived control element becomes delocalized past embryonic stages 9 or 10. Other deletions in the *bicoid* 3' UTR create subtly different localization phenotypes — for example, presence of the mRNA in nurse cells, or prolonged and diffuse anterior localization — which suggest that multiple elements interact additively, sequentially or redundantly to determine the wild-type localization pattern.

The sequences regulating localization of *oskar* mRNA were similarly studied by constructing a series of deletions in the *oskar* 3' mRNA UTR, this time fused to reporter sequences in chimeric transcripts [7]. The results suggest that the localization process can be dissected into three major steps. Like *bicoid* RNA, *oskar* RNA is transported from the nurse cells and accumulates first at the anterior margin of the oocyte. Unlike *bicoid* mRNA, however, *oskar* mRNA is then transported to the posterior pole of the oocyte. The effects of the different deletions suggest that different, but overlapping or redundant, regions of the *oskar* mRNA 3'UTR are responsible for each of these steps. It is possible that the multiple *cis*-acting elements act independently to mediate the different transport steps, though it is difficult to analyse them independently because the transport events are temporally as well as spatially sequential. Although it was not a focus of these studies, stabilization of the mRNA at its final destination could be an additional factor determining the mRNA's subcellular distribution. This view is supported by the observation some of the mislocalized mRNAs are present at significantly lower levels in the embryo than the correctly localized, wild-type mRNA.

Specific localization of mRNAs also occurs in somatic cells, and sequences that confer the localization pattern have been defined in the 3' UTR of actin mRNA.  $\beta$ -actin mRNA localizes to the leading edge of motile cells. The *cis*-acting localization determinants of  $\beta$ -actin

mRNA have been analysed by fusing sequences encoding segments of the mRNA to a *lacZ* reporter gene and transiently expressing the resulting constructs in transfected cells [13]. In this system, the distribution of  $\beta$ -galactosidase activity within the cytoplasm is used as an indirect assay of fusion mRNA localization. The cell's leading edge stained blue when correct *cis*-acting elements were fused to the enzyme coding region. These results showed that the 3'UTR of actin mRNA, like the 3'UTRs of *Drosophila* and *Xenopus* maternal mRNAs [14] and also *Drosophila* zygotic mRNAs [15], contain the localization determinants.

$\beta$ -actin is one of a number of actin isoforms and is expressed in most cells constitutively. Another actin isoform,  $\alpha$ -actin, is expressed in differentiating muscle, where it contributes to sarcomere development. The mRNA for this actin isoform is localized around the nucleus, in contrast to the  $\beta$ -actin mRNA which moves to the peripheral region of the cell. At early stages of differentiation,  $\alpha$ - and  $\beta$ -actin mRNAs are present in the same cells, in perinuclear and peripheral compartments, respectively. Fusion of the respective 3' UTR to *lacZ* mRNA confers localization in the appropriate compartment, whereas fusion of the coding regions has no effect on localization. This indicates that each actin isoform mRNA has a distinct zipcode that ensures it is compartmentalized correctly, irrespective of the presence of mRNA for the other actin isoform or the particular morphology of the cell. This suggests another role for mRNA localization, in the morphogenesis of differentiated structures. The linkage of 3' UTRs bearing different zipcodes to similar coding regions allows nearly identical proteins to be expressed in distinct subcellular compartments.

As these fusion genes were tested in transfected cells in culture, thousands of cells could be examined for mRNA localization and the data subjected to statistical analysis to compare the various constructs. This allows subtle quantitative differences in localization patterns to be correlated with specific sequences. Using this approach, the fine structural elements of  $\beta$ -actin mRNAs have been rigorously characterized, providing evidence for redundant, short (~50 nucleotide) elements that are necessary and sufficient for localization. Comparison of these elements from chicken  $\beta$ -actin mRNA with the equivalent region of human  $\beta$ -actin mRNA revealed conserved motifs that may represent the basic peripheral zipcode.

Clearly what remains is to elucidate the mechanism by which the zipcodes are read. Identification of the zipcode sequences allows a search for specific RNA-binding proteins that recognize them. The expectation is that such proteins will turn out to be transporters, anchors or regulators (of, for example, translation or RNA stability). In some cases, part of the sorting process may occur within the nucleus [16]. At this time, we have no idea how many different zipcodes there will turn out to be. More refined microscopic, genetic

and molecular techniques are likely to reveal different mRNA 'postal zones' in the cytoplasm.

*Acknowledgments:* The author thanks Ruth Lehmann, David Ish-Horowicz and Ed Kislaukis for their useful comments on this article

## References

1. SINGER RH: The cytoskeleton and mRNA localization. *Curr Opin Cell Biol* 1992, 4:15-19.
2. LAWRENCE JB, SINGER RH: Intracellular localization of messenger RNAs for cytoskeletal proteins. *Cell* 1986, 45:407-415.
3. ST JOHNSTON D, NÜSSEIN-VOLHARD C: The origin of pattern and polarity in the *Drosophila* embryo. *Cell* 1992, 68:201-219.
4. KISLAUSKIS EH, SINGER RH: Determinants of mRNA localization. *Curr Opin Cell Biol* 1992, 4:975-978.
5. JACKSON RJ: Cytoplasmic regulation of mRNA function: the importance of the 3' untranslated region. *Cell* 1993, 74:9-14.
6. MACDONALD PM, KERR R, SMITH JL, LEASK A: RNA regulatory element BLE1 directs the early steps of *bicoid* mRNA localization. *Development* 1993, 118:1233-1243.
7. KIM-HA J, WEBSTER PJ, SMITH JL, MACDONALD PM: Multiple RNA regulatory elements mediate distinct steps in localization of *oskar* mRNA. *Development* 1993, 119:169-178.
8. BERLETH T, BURRI M, THOMA G, BOPP D, RICHSTEIN S, FRIGERIO G, NOLL M, NÜSSEIN-VOLHARD C: The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J* 1988, 7:1749-1756.
9. EPHRUSSI A, DICKINSON IK, LEHMANN R: *Oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* 1991, 66:37-50.
10. KIM-HA, SMITH JL, MACDONALD PM: *Oskar* messenger-RNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell* 1991, 66:23-35.
11. MACDONALD PM, STRUHL G: *Cis*-acting sequences responsible for anterior localization of *bicoid* mRNA in *Drosophila* embryos. *Nature* 1988, 336:595-598.
12. EPHRUSSI AL, LEHMANN R: Induction of germ cell formation by *oskar*. *Nature* 1992, 358:387-392.
13. KISLAUSKIS EH, LI Z, SINGER RH, TANEJA KL: Isoform-specific 3' untranslated sequences sort  $\alpha$ -cardiac and  $\beta$ -cytoplasmic actin mRNAs to different cytoplasmic compartments. *J Cell Biol* 1993, 123:165-172.
14. MOWRY KL, MELTON DA: Vegetal messenger RNA localization directed by a 340 nt RNA sequence element in *Xenopus* oocytes. *Science* 1992, 255:991-994.
15. DAVIS I, ISH-HOROWICZ D: Apical localization of pair-rule transcripts requires 3' sequences and limits protein diffusion in the *Drosophila* blastoderm embryo. *Cell* 1991, 67:927-940.
16. DAVIS I, FRANCIS-LANG H, ISH-HOROWICZ D: Mechanisms of intracellular transport localization and export in early *Drosophila* embryos. *Cold Spring Harbor Symp Quant Biol*, in press.

---

Robert H. Singer, Department of Cell Biology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, Massachusetts 01655, USA.