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RNA: traffic report

Robert H. Singer

How macromolecules sort to different compartments within cells has been a source of intensive investigation in the field of cell biology. While much information has been generated over the years on how proteins sort to various cellular organelles, one aspect of this sorting, the control of where the proteins are synthesized, has only recently come to be appreciated. In the past few years, the intracellular transport of RNA and the localization of mRNAs in the cytoplasm have received increasing attention. This interest has bubbled up into dedicated meetings*, or sessions within meetings**, as the number of investigators in the field has increased to a critical mass. A common question that pervades all the current discussions is how mRNAs get to specific regions of the cell, or, more teleologically, how does the cell know where they belong, and what is the physiological significance of this? (For a recent comprehensive review, see Ref. 1.) Rapid progress has been made in defining RNA-localization elements ('zipcodes'), which determine the intracellular distribution of a number of RNAs. Clearly, the next step in determining the mechanism of mRNA localization is to identify the proteins that recognize these localization elements and target RNAs to sites in the cytoplasm. Most of the new advances in RNA trafficking revealed at these meetings concern

the complexes of proteins associated with RNA movement, and this report highlights some of the rapid progress being made in this area.

'Zipcodes' and their binding proteins

The *cis*-acting sequences important for RNA localization are now being defined to an increasingly finer resolution. However, with a few exceptions, these sequence elements have not been restricted to small enough RNA segments to allow unequivocal isolation of proteins whose binding is involved in localization. Many, but not all, are in the 3' untranslated region (3'-UTR) of their respective mRNAs.

In chicken fibroblasts, the mRNA for actin is distributed asymmetrically towards the front of the cell, where its translation into actin protein may affect the structure and physiology of the leading edge, which is important in motility². The localization signals of β -actin mRNA in fibroblasts are currently thought to be contained in interspersed repeats of six nucleotides each, suggesting the name 'zipcode', a metaphor relating to the sorting of mail to different postal zones (E. Kislauskis, Worcester, USA). A protein of 68 kDa (A. Ross, Worcester, USA) has been isolated that binds to these zipcode elements. The cDNA encoding this zipcode-binding protein (ZBP) has been

partially cloned and, like some hnRNPs, the protein has at least one RNA-recognition motif (RRM) domain containing RNP1 and RNP2 motifs. It was postulated two years ago that the ZBP, or a complex including the ZBP, is responsive to growth factors since the localization of the actin mRNA is controlled by signal-transduction mechanisms³, and V. Latham (Bronx, USA) now has data suggesting that the small G protein Rho is responsible for this localization. M. Chicurel (Boston, USA) showed that beads coated with RGD peptides (a sequence bound by integrins), which induce focal adhesion formation, cause an increase in polyadenylated RNA around the side of the bead contacting the cell, in a process that may be brought about by a similar mechanism to that involving signal transduction.

J. Carson (Farmington, USA) reported that the localization of myelin basic protein (MBP) in oligodendrocytes appears to require two distinct 3'-UTR elements in its mRNA. The RNA transport signal (RTS) is a 21-nucleotide sequence required for transport from the cell body to the myelin membrane. This sequence binds to a 36-kDa protein that has short stretches of amino acids identical to hnRNP A2 (R. Smith and K. Hoek, Queensland, Australia). The RNA localization signal (RLS) is a less defined sequence that may be necessary to anchor the mRNA in the myelin membrane. The major MBP isoform in myelin binds to its own RNA, and repetitive selection of RNAs that bind to MBP with increasingly higher affinity has identified a consensus sequence, CAGUGU, as the preferred

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ligand. This sequence is contained within the RLS of mRNA for MBP as well as in mRNA encoding connexin 32, a gap junction protein expressed in myelinating cells. This sequence appears in the coding region as well as in the 3'-UTR of a number of other RNAs. The mRNA for Tau has a 91-nucleotide segment in its 4-kb 3'-UTR that appears to be sufficient for its localization to the proximal part of the axon (I. Ginzburg, Rehovot, Israel). A protein that interacts with this segment appears also to interact with microtubules or microtubule-associated proteins.

Proteins that chaperone maternal mRNAs

The importance of the correct localization of mRNAs that code for morphogens during development is clear from the lethality of mutations that disrupt this process and by the effects of ectopic expression of chimeric RNAs. In *Drosophila* oocytes and embryos, mRNA localization results in a concentration gradient of the encoded morphogen protein that ultimately imposes a spatial pattern to the expression levels of downstream genes and establishes pattern in the embryo. The most widely studied example has been the maternal mRNA for BICOID, the anterior morphogen in *Drosophila*. Localization of maternal mRNAs in *Drosophila* includes an intercellular transport step in which the RNA moves from the nurse cells into the anterior portion of the oocyte in a microtubule-dependent manner. This movement involves the EXUPERANTIA (EXU) protein and another protein, EAP (EXU-associated protein) that interacts with EXU. EAP was isolated by the yeast two-hybrid system and has RRM, but the sequence does not predict that it has motor properties (T. Hazelrigg, New York, USA).

The maternal mRNAs for NANOS and OSKAR are important in the formation of posterior structures and must travel to the distalmost regions of the ooplasm, where they participate in the establishment of the germ plasm as well as morphogenesis. Facilitating this movement is microtubule-dependent cytoplasmic streaming in the ooplasm, which initiates at a particular time during maturation, causing a 'washing machine' effect whereby components, among them the maternal mRNAs, circulate freely⁴. Anne Ephrussi (Heidelberg, Germany) suggested that random collisions of the mRNAs with components in the posterior cortex may result in their anchoring. She reported that, when

fluorescently labelled OSKAR mRNA was microinjected into the posterior oocyte, it could localize correctly, even in the presence of colcemid, but, when injected into the anterior, its localization was sensitive to colcemid, indicating that the microtubule network stimulates translocation of the injected RNA to the posterior pole but is not required for its anchoring *per se*.

In *Xenopus*, maternal mRNAs such as *Vg1* become localized to the vegetal pole of oocytes during oogenesis⁵. The large size of the oocyte makes it possible to distinguish component parts of the localization pathways, such as the transport step as distinct from the anchoring step, or different localization patterns of the different mRNAs at the vegetal pole⁶. There appear to be two pathways for the localization of RNAs at the vegetal cortex. The first, an early pathway during stage 1 of oogenesis, localizes a noncoding RNA, *Xlsirt*, as well as the RNAs *Xcat2* and *Xwnt11*, to a mitochondrial-rich region that eventually becomes the germ plasm⁷. Cytoskeletal components seem not to be required for the localization of these RNAs since the localization is nocodazole- and cytochalasin-insensitive (L. Etkin, Houston, USA), and it is possible that some of them may localize via a diffusion and trapping mechanism (M. L. King, Miami, USA). A second pathway during stages 2 to 4 localizes RNAs such as *Vg1* and uses a microtubule-dependent system.

The *Vg1* mRNA segment required for localization is 340 nucleotides long and binds a number of proteins, some of which do not bind when the localization ability of the construct is lost in a deletion series (K. Mowry, Providence, USA). One of these is a microtubule-associated protein of 69 kDa that appears to become phosphorylated in oogenesis during the time that *Vg1* localizes (J. Yisraeli, Jerusalem, Israel). Other proteins that bind to specific sequences in the 3'-UTR may be membrane-associated proteins and could conceivably control transport of *Vg1* (encoding a secreted protein) and/or its translation (B. Schnapp, Boston, USA).

The best-characterized mRNA-binding protein involved with localization is the *Drosophila* protein STAUFEN, which maintains the localization of both OSKAR and BICOID mRNAs (Fig. 1) and also is required for OSKAR transport and translation. It binds to microtubules, particularly astral microtubules, and has domains for binding to double-stranded RNA that are characteristic of an RNA helicase

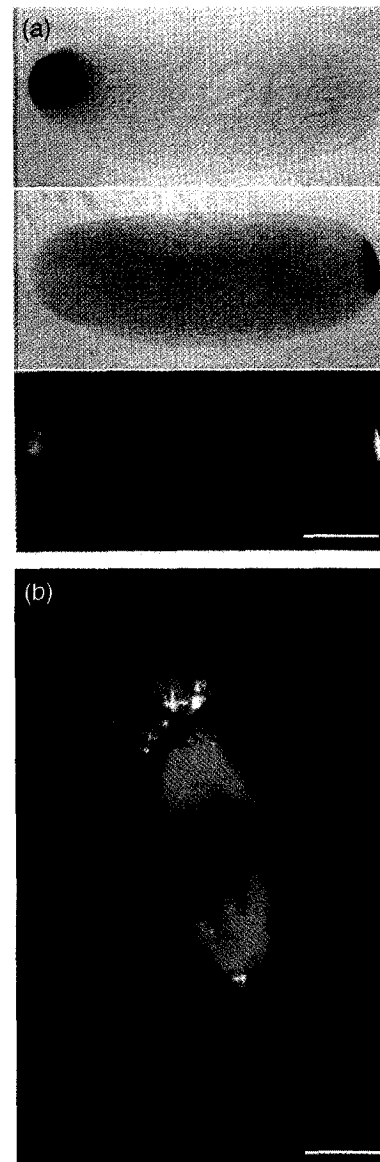


FIGURE 1

STAUFEN in the localization of BICOID and OSKAR mRNAs.

(a) Wild-type *Drosophila* embryos stained by *in situ* hybridization to show the localization of the BICOID (top) and OSKAR (middle) mRNAs, which both colocalize with STAUFEN protein as detected by immunofluorescence (bottom). STAUFEN is required for the localization of both mRNAs, but at different stages of development (see Ref. 8 for more details). Bar, 0.1 mm. (b) STAUFEN particles are microtubule associated. This shows a mitotic spindle in an embryo injected with BICOID 3'-UTR RNA and stained by immunofluorescence for tubulin (green) and STAUFEN (red). The injected RNA recruits endogenous STAUFEN protein to form particles that colocalize with the astral microtubules of the mitotic spindle. Bar, 2.5 μ m. These images were kindly provided by Daniel St Johnston of the Wellcome/CRC Institute, Cambridge, UK.

*Intracellular Trafficking of Protein and mRNA; 16th Hamburg-Blankenese Conference, Germany; 5-19 May 1996. Organized by Gebhard Koch and Dietmar Richter.

**RNA Transport and Localization. Session 9 in The First Meeting of the RNA Society; Madison, WI, USA; 28 May - 2 June 1996. Session organized by E. Lund and R. Singer.

*Sorting and Intracellular Transport of mRNA. The second FASEB Meeting; Snowmass, CO, USA; 22-26 June 1996. Organized by R. Singer and O. Steward; co-organized by H. Lipshitz and L. Etkin.

(S. Grünert, Cambridge, UK)⁸. The localization of maternal mRNAs has been relatively intractable to reduction to small elements mainly because localization in these cases is a complex multistep process and the crucial sequences appear to be distributed widely and have additive effects on localization. *In vivo* localization assays may not be sufficiently sensitive or quantitative to reveal the deletion of individual elements.

Chaperones as granules

In somatic cells, mRNA movement is most easily observed in cells that are asymmetric. The most polarized somatic cells are neurons, and several mRNAs distribute asymmetrically in these cells – mostly between the cell body (soma) and the dendrites (O. Steward, Charlottesville, USA) but occasionally also in axons (D. Richter, Hamburg, Germany). The mRNA for the microtubule-binding protein Tau is concentrated within the proximal axon of cortical neurons, but another microtubule-associated protein, MAP2, is localized to dendrites. Andrew Matus (Basel, Switzerland) described how localization of its corresponding RNA may require the initial translation of the polypeptide. Recently, the growth cone of the extending axon has been identified as a site of localization for the mRNA for β -actin (G. Bassell, Bronx, USA). Interestingly, this mRNA travels on microtubules in neurons and appears to be in granules; but in fibroblasts it is associated exclusively with actin filaments and appears to be less clustered.

In oligodendrocytes, the sorting of mRNA for myelin basic protein (MBP) from the cell body to the extensive processes has also been shown to require microtubules and specific 'granules' that transport the mRNA to sites distant from the nucleus (J. Carson, Farmington, USA). When mRNA for MBP was microinjected into oligodendrocytes, the granules formed as the RNA coalesced with other components necessary for translation, such as ribosomes, tRNA synthetase and the elongation factor EF-1 α . EF-1 α is also an actin-binding protein and its regulation by small fluctuations in pH may control mRNA translation on the actin cytoskeleton by releasing EF-1 α for association with acylated tRNAs (J. Condeelis, Bronx, USA). Granules are also associated with a number of maternal mRNAs. Injection of *BICOID* sequences into oocytes results in the formation of granules containing STAUFEN (see Fig. 1), and three major regions of the *BICOID* 3'-UTR containing

stem-loop structures bind to STAUFEN protein (S. Grünert). Injection of fluorescently labelled *XCat2* and *XIsirt* results in different-sized granules containing one or the other RNA (L. Etkin, Houston, USA). K. Kosik (Boston, USA) showed that, by using the RNA-specific vital dye cyto14, RNA-rich granules can be seen moving bidirectionally in the axon of living neurons.

Chaperones for nucleocytoplasmic transport

mRNA begins its life in the nucleus and must exit to fulfil its biological destiny. Because this aspect of RNA trafficking has been amenable to yeast genetics [the screen is for the retention of poly(A)⁺ RNA in the nucleus]^{9,10}, considerable progress has been made in identifying mutants that are defective in this transport. Yeast genetics will probably also advance our understanding of mRNA localization as the mRNA for the Ash1p protein, a transcription factor involved in mating-type switching, sorts to the bud site (R. Long, Bronx, USA).

The transport of some mRNAs from the nucleus to the cytoplasm requires specific chaperones. One of these is the human immunodeficiency virus (HIV) Rev protein, which is capable of exporting a chimeric RNA containing the HIV RRE (Rev-response element) in yeast (F. Stutz, Waltham, USA). The export of HIV RNA differs from normal RNA-polymerase-II-dependent transport pathways, but appears to overlap with the 5S RNA pathway, as explained by R. Lührmann (Marburg, Germany). The Balbiani ring RNA, a huge, single molecule large enough to be seen by electron microscopy, appears to unwind itself through the nuclear pore, 5'-end first, and become loaded with ribosomes as it is exporting. Some of the proteins associated with the RNA in the nucleus, such as hnRNP A1, travel with the RNA onto the polysomes (B. Daneholt, Stockholm, Sweden). This raises the possibility that hnRNPs that shuttle RNAs from the nucleus to the cytoplasm may also be involved in the cytoplasmic functioning of mRNA and perhaps ultimately their localization.

The polarity of the nucleus may be reflected in a corresponding polarity of mRNA egress from the nucleus. The mRNAs for the pair-rule genes in *Drosophila*¹¹ and the mRNA for *wingless* (H. Krause, Toronto, Canada) sort to apical portions of the blastoderm, and it has been suggested that they get an initial boost towards their site of localization by leaving the nucleus on the apical side. This proposed vectorial

export of mRNAs from the nucleus might combine some of the mechanisms both of localization and of nucleocytoplasmic transport. In addition, the fact that this localized mRNA codes for a secreted protein raises the possibility that the endoplasmic reticulum may play a role in the mRNA sorting pathway.

Proteins involved in expression of localized transcripts

The process of expression of mRNA occurs in the cytoplasm, and includes not only its localization, but also its stability and translation. It is reasonable to propose that these translational control elements and their binding proteins will act synergistically with mRNA localization. The action of BICOID as both a transcription factor and an RNA-binding protein that represses the translation of *CAUDAL* mRNA is well established^{12,13}. The localization of *NANOS* mRNA is coupled with its translation, and both control elements are in the 3'-UTR. The proximal 3'-UTR contains a translational control element, removal of which allows permissive translation. OSKAR relieves the repression of *NANOS* translation, such that *NANOS* is translated only where OSKAR is localized (L. Gavis, Princeton, USA).

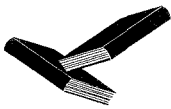
Vg1 mRNA appears to be translated only after it is localized; therefore, a mechanism must exist to suppress this translation. RNA elements that appear to prevent mRNA translation during localization have been found in the 3'-UTR downstream from the localization elements (J. Wilhelm, San Francisco, USA). Clearly, translation of the mRNA before it has localized would compromise the effectiveness of the sorting of the cognate protein. Localization can also result from selective degradation. The maternally synthesized *HSP83* mRNA is localized at the posterior pole of the *Drosophila* zygote because it is stabilized there by sequences in its 3'-UTR. In some way, these sequences are presumed to interact with proteins bound to the 5'-UTR to inhibit degradation of the RNA (H. Lipshitz, Toronto, Canada).

Ultimately, RNA sorting becomes part of the same question as protein sorting: how does the cell regulate molecular spatial distribution? An exciting era is dawning in which these mechanisms of cellular organization are beginning to be revealed.

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Cell cycle in motion

Progress in Cell Cycle Research (Volume 1)

edited by Laurent Meijer,
Silvana Guidet and H. Y. Lim Tung,
Plenum Press, 1995. \$75.00
(373 pages) ISBN 0 306 45280 4

Progress indeed! Just thumb through this compilation of reviews to see for yourself that cell-cycle research is an 'exploding area', as the editors proclaim. *Progress in Cell Cycle Research* is a collection of 29 reviews, each of which can stand alone. Topics range from DNA replication, RNA transcription and protein translation to signal transduction, cell biology and immunology and include many organisms: yeasts, frogs, flies, mice, men and more. It is a dizzying array.

The eukaryotic cell cycle is traditionally divided into G1 (the first growth phase after quiescence or the previous division), S (synthesis of DNA), G2 (growth again) and M (mitosis). Entry into the cell cycle, that is progression through G1 and initiation of DNA replication, is controlled in response to growth factors, hormones, cytokines and other signals as well as by stress and nutrient availability. How these conditions are sensed, which signal-transduction cascades are activated and how these ultimately regulate cell-cycle progression are addressed in several of the reviews. For example, two chapters are dedicated to mitogen-activated protein (MAP) kinase 'modules', a conserved kinase cascade

that has been plugged into various signal-transduction pathways by the caprices of evolution. Other chapters deal with cAMP-dependent kinase, ligand-activated G proteins, and tyrosine kinases. An especially fascinating chapter by Richard Pearson and George Thomas describes how control of protein translation by S6 kinase may be a crucial part of the mechanism for controlling entry into G1.

Several of the reviews deal with the machinery that directly governs cell-cycle events. In all eukaryotes, the cell cycle is globally regulated by cyclin-dependent kinases (CDKs), which, as the name implies, are activated by periodically expressed cyclins. In the simplest model, rising kinase activity first triggers the G1–S transition, and then, at higher levels, triggers the G2–M transition. Cyclins are degraded as cells exit M phase, thus resetting the kinase to G1 levels. Of course, nature is not so simple. For one thing, different cyclins are responsible for specific cell-cycle transitions. Three chapters are dedicated to the cyclins functioning in G1 and S phase. In one, Krisi Levine, Authur Tinkelenberg and Frederick Cross describe the circuitry of G1 cyclins that controls START (commitment to a new cell cycle) in the well-characterized yeast *Saccharomyces cerevisiae*. This gives a sense of the subtlety and fine tuning of CDK activity that results in orderly cell-cycle progression.

In addition to regulation by cyclins, CDK activity is controlled by phosphorylation. Negative control of CDK activity by phosphorylation is the primary mechanism controlling the G2–M transition in most eukaryotes. Mitosis is initiated when the Cdc25 phosphatase removes the CDK inhibitory phosphate (reviewed by Catherine Jessus and Rene Ozone). Although a regulatory function is not certain, phosphorylation of CDKs in the 'T-loop'

is required for their activity (reviewed by John Shuttleworth).

Recently, it has become apparent that significant CDK regulation is mediated by CDK inhibitory proteins. Two chapters describe such inhibitors, p27^{KIP1} in mammalian cells (Andrew Koff and Kornelia Polyak) and p40^{SIC1} in yeast (Micheal Mendenhall *et al.*); we await the next volume in this series for a review of the tumour-suppressor class of p15/p16-related inhibitors of CDK activity.

These examples provide a taste of the smorgasbord of reviews, but fail even to mention a number of the interesting topics reviewed such as chemical CDK inhibitors and mechanisms of chromosome segregation. While providing the reader with a variety of cell-cycle-related subjects, this collection of reviews is just that: a collection. Some articles are outstanding, including Chapter 5, 'Mechanism of action of rapamycin: new insights into the regulation of G1-phase progression in eukaryotic cells' (Gregory Wiederrecht *et al.*) and Chapter 10, 'The role of cyclin E in the regulation of entry into S phase' (Karsten Sauer and Christian F. Lehner). Others are difficult to read and may appeal only to the specialist as a compilation of details and references. It is clear that the reviews were written independently because the 'facts' are sometimes quite different from one chapter to the next. Perhaps this illustrates the value of a compiled edition of reviews – it is easy enough to flip back and forth to see where individuals have different perspectives. The editors' goal to offer '... a series of reviews covering the firmly established facts rather than covering conflicting and unconfirmed results' seems unrealistic. The book as written provides a reasonable compromise between 'firm facts' and up-to-date research.

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