Targeting RNA in the cell

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The typical textbook description of a cell suggests that the genetic information residing in the nuclearly located DNA is transcribed in a regulated fashion and the resulting RNA is processed and transported to cytoplasm, where it is translated and the protein so made is translocated to specific parts of the cell for its structural and/or enzymatic role in the cell's phenotype. However, such simplistic descriptions do not reveal the highly ordered three-dimensional organization of a cell, where every molecule, in fact, has a defined dynamic location. While localized distribution of at least certain specific RNAs in cells had been known for some time, it has become possible only recently to ask how this localized distribution is actually achieved. Application of molecular cell biological approaches has permitted newer insights into the three-dimensional molecular architecture of a cell and how the various molecules are vectorially transported within it to achieve the polarities that a cell has.

It is now clearly established that not only is the primary act of transcription of a given DNA sequence regulated with a certain degree of fidelity, the processing and transport of the transcripts is also a highly regulated process. Studies during the past few years have shown that certain RNAs are targeted to specific subcompartments of the cell where their translational product/s are immediately needed. The importance of such highly specific subcellular localization of RNA and its protein product is most obvious in the zygote cell since the unfolding of the entire body organization during the development of the zygote depends on the molecular asymmetries that are established in the unfertilized or the newly fertilized egg. In what follows, we discuss some recent studies which are attractive for their use of novel combinations of microscopic and molecular techniques to examine (i) the structural components of a cell that transport RNA away from the site of synthesis in nucleus to its final location in a specific subcompartment of the cell, (ii) the signals that tell the cellular transport machinery where to deliver the given RNA molecule and (iii) the components that keep the RNA anchored to the desired subcompartment.

mRNA transport and localization in cells: visuals of the process and mechanism

It has been known that the β-actin protein as well as its mRNA colocalize at the leading edge of lamellipodia in a variety of cell types[1–3]. Apparently, the highly localized distribution of β-actin mRNA in these cell types provides for the compartmentalization of actin synthesis. It has been shown in several cases[4] that the RNA localization signal (zipcode) is present in the 3′ untranslated region (3′ UTR) of mRNA. Kislauskis *et al.*[5] identified the specific 'zipcode' sequence/s by fusing different lengths of the 3′ UTR of β-actin to the reporter β-galactosidase (*lacZ*) gene; this allowed a direct visualization of the intracellular distribution of β-galactosidase activity in transfected chicken embryo fibroblasts by simple X-gal (a chromogenic substrate for β-galactosidase) staining. It was shown that two sequence elements in the 3′ UTR of β-actin mRNA provided the peripheral localization signal to the heterologous β-galactosidase mRNA so that in cells transfected with such chimeric constructs, X-gal staining revealed blue colour at the same place where the β-actin protein was. Of these two peripheral localizing signals, a 54-nucleotide sequence was the main 'peripheral RNA zipcode' while the homologous but less active 43-nucleotide sequence, present further downstream, was composed of 'zipcode elements'. The basic elements of these 'zipcodes' were identified as AATGC and GGACT, which act in concert so that multiple copies of each provide a stronger peripheral localization signal. These authors suggest that the high A/C content in the 54-nucleotide zipcode may also have a role in peripheral localization of the β-actin mRNA. It was also seen that addition of complementary oligonucleotides against the RNA zipcode delocalized the endogenous β-actin mRNA and altered the organization and shape of lamellipodia and stress fibres, without affecting levels of the β-actin mRNA or protein; this showed that the localization of β-actin mRNA is essential for the phenotype of these cells.

An answer to how the mRNA molecules may possibly remain anchored in cell cytoplasm, essential for the localized distribution, has been provided in a very ingenious study by Bassell *et al.*[6]: single mRNA molecules were seen with an unprecedented resolution and accuracy to be anchored on actin filament intersections in fibroblasts. To achieve this feat, they used different sized gold particles to 'see' simultaneously, at the ultrastructure level, the labelled oligo-dT probe hybridized *in situ* with polyA tail carrying mRNAs and the antibody bound to actin fibres. Furthermore, the oligo-dT probe hybridized with the polyA tails of cytoplasmic mRNAs was used as primer for *in situ* reverse transcription; the *in situ* extended cDNA chains and the oligo-dT–polyA hybrids were covisualized by immunobinding of different sized gold particles. These results unambiguously demonstrated the localization of individual mRNA molecules specifically at intersections of actin filaments. These studies also revealed that the mRNA at these intersections remains in a circular rather than an extended or linear conformation; this may help explain the interactions between the 3′ and 5′ ends of mRNA that are important in regulating translation.

Directed transport and localized anchorage of mRNA during oogenesis in *Drosophila*

The stages of oogenesis provide a remarkable material for study of localized RNAs and the mechanisms that regulate the spatial order in the distribution of different transcripts in different regions of oocyte. It is this asymmetric but highly ordered distribution of different macromolecules in the egg that determines the subsequent cascades of determination events in early embryonic development. The large body of genetic data has made *Drosophila* eggs a material of choice for such studies.

During oogenesis in *Drosophila*, the growing oocyte is nourished and provided with all its RNAs and proteins by the 15 sister nurse cells and the surrounding follicle cells. The nurse cells synthesize the different RNAs and proteins which are transported through intercellular connections to the growing oocyte, where each of these gets localized in a highly ordered and reproducible manner. Among the several different RNAs that are localized in *Drosophila* oocyte and have important roles in embryonic development, those produced by the *bicoid*, *oskar* and *nanos* genes are critical for anterior–posterior axis determination[1]. The *bicoid* (*bcd*) RNA in mature oocyte is localized to the anterior margin and remains confined to the anterior region of embryo, where it directs the development of anterior structures[7,8]. Likewise, transcripts of the *nanos* and *oskar* (*osk*) genes are located at the posterior pole of the egg and are crucial for the posterior development[7].

The localized distribution of these nurse-cell-produced transcripts in oocyte results from a series of events during oogenesis; a number of earlier acting genes are known to affect movement and the characteristic localization of these polarly located early determinants or morphogens[7]. The *staufen* (*stau*) gene plays a critical role in the last step of *bcd* RNA localization, i.e. its release from the egg cortex of mature oocyte into anterior cytoplasm of activated egg and early embryos. *stau* is also important in the final stages of posterior localization of the *osk* mRNA in the egg[7].

Pokrywka and Stephenson[9] found that *bcd*, *osk* and certain other RNAs were dynamically associated with cytoskeletal elements during different stages of oogenesis. These authors developed a simple procedure for fractionation of detergent-insoluble cytoskeleton from the soluble components of developing oocytes and showed that the association of *bcd* RNA with detergent-insoluble pellet was dependent on the stage of oogenesis. In the mature oocyte when *bcd* transcripts remain in the egg cortex at the anterior end, all of these were bound to cytoskeleton elements, but after egg activation, most of the *bcd* RNA was recovered in the soluble fraction. Like the *bcd*, the *osk* transcripts also showed a dynamic association with cytoskeletal components during oogenesis.

Identification of the various proteins associated with the pellet and soluble fractions through Western blotting led Pokrywka and Stephenson[9] to conclude that microtubules, and not microfilaments, are required for the assorting of *bcd* RNA in the pellet fraction and the pellet fraction is enriched in components required for stable anchorage of *bcd* RNA rather than in elements required for initial localization events like transport or docking. It was also clear that the localization of *bcd* transcripts in nurse cells and in deeper cytoplasm of egg involves different mechanism than localization in the anterior cortex of mature oocyte, which alone was pellet-associated.

The *stau* protein has a very interesting role in localization of both *osk* and *bcd* RNAs at opposite ends of the egg. In the case of *osk* RNA, it has been known that the *stau* protein is associated with *osk* RNA to mediate its binding with cytoskeletal components that transport the complex to the posterior pole[10]. Other studies have shown that the plus ends of microtubules within the oocyte are polarized towards the posterior pole[11]. A visual demonstration of the posterior facing of the plus ends of microtubules in oocyte was provided by Clark *et al.*[12] in flies transformed with a chimeric gene having the kinesin domain on its 5′ end and the β-galactosidase coding region on the 3′ end: this fusion protein retains the properties of kinesin (moving along microtubules towards their plus ends) and also shows β-galactosidase activity, which can be cytochemically visualized as blue staining with the X-gal substrate. Since all the blue staining β-galactosidase activity was seen to move posteriorly (due to the kinesin domain) in oocytes of these flies, it was obvious that the microtubules in oocytes are highly polarized with their plus ends pointing posteriorly. Viewed in this context, it becomes clear that binding of the *stau* protein with *osk* transcripts facilitates attachment to microtubules for transport of the *osk* transcripts to the posterior pole. Once the *osk* transcripts are delivered at the posterior pole, the *stau* protein is freed and quickly recruited for *bcd* localization.

Ferrandon *et al.*[13] have very convincingly documented a mutual binding of the *stau* protein with the *bcd* RNA and the interaction of this complex with microtubules. These authors used an RNA injection assay to map the 3′ UTR of *bcd* RNA necessary for anterior localization and to visualize its specific interaction with the *stau* protein. *In vitro* transcribed *bcd* RNA (full-length mRNA or the 3′ UTR or variously mutated 3′ UTR) was microinjected at ectopic sites in 0 to 1 h-old embryos (the endogenous *bcd* transcripts at this stage are anteriorly localized), and following a brief chase, the embryos were fixed to immunologically monitor the distribution of *stau* protein: the normal *bcd 3′* UTR rapidly recruited the *stau* protein around itself to result in microscopically visible aggregates which moved during cell cycle stages in microtubule dependent manner. Using deletions and linker-scanning substitutions, three noncontiguous localization signals in stem III and distal regions of stems IV and V of the predicted secondary structure of the *bcd* 3′ UTR were identified. The *stau*-dependent localization of *bcd* RNA at the time of egg activation and its earlier *stau*-independent localization to anterior cortex of oocyte use different but overlapping sequence signals. Aggregation of the cellular *stau* protein in early embryos with the microinjected appropriate *bcd* 3′ UTR RNA and their movement with microtubules showed that the *bcd* transcripts and the *stau* protein are dependent on each other for complex formation and localization. It is interesting to note that during oogenesis the *bcd* transcripts and the *stau* protein do not recognize each other; at that time the *stau* protein is involved in localizing the *osk* transcripts. Only after delivering the *osk* transcripts at the posterior pole, does the *stau* protein quickly move to the anterior of egg and bind with the *bcd* RNA for its localization and anchorage at the anterior region. Since in both the oocyte and embryos the localization of the *stau* protein depends on the appropriate RNA (*osk* in oocyte and *bcd* in early embryos) and *vice versa*, Ferrandon *et al.*[13] suggest that perhaps the same microtubule motor is involved in both these transcript movements. However, the events that make the *stau* protein change its affinity from *osk* to *bcd* transcripts in the mature egg are not known. Also, if the *stau–bcd* complex has to move on the same microtubule motors as the *stau–osk* complex, a change in the microtubule polarity may be necessary.

The myelin basic protein mRNA when microinjected into mouse oligo-

dendrocytes also aggregates into large particles that are transported along microtubules in anterograde direction[14]. It is very likely that many more such examples will be known soon and a class of proteins will be identified that recognize the 'RNA zipcodes' and accordingly transport the RNA with the help of cytoskeletal components and deliver them at the specified destination. While microtubules may be principally involved in the transport, the actin filaments may have a greater role in anchoring the localized transcripts. Also, as in the case of *osk* and *bcd* transcript localizations during different stages of oogenesis, different subsets of cytoskeletal components may be involved in transport as well as anchorage of the same RNA in a stage-dependent manner. With the feasibility of doing sophisticated biochemistry and molecular probing *in situ* in the cell at light as well as electron microscopic levels, it should be possible in the near future to have full graphic details of the movement of specific transcripts in cells to their destination and their anchorage after delivery.

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