Targeting RNA in the cell

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The typical textbook description of a cell suggests that the genome information residing in the nuclearly localized DNA is transcribed in a regulated fashion and the resulting RNA is processed and transported to the 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 distributions of at least certain specific RNAs in cells have 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 new insights into the three-dimensional molecular architecture of a cell and how the various molecules are necessarily compartmented within it to achieve the polarities that a cell has.

It is now clearly established that not only is the primary, yet well-conserved transcription of a given RNA sequence regulated at 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 compartments of the cell when their translational products are immediately required. The importance of such highly specific subcellular localization of RNA and its protein product is most obvious in the type of cells since the unfolding of the entire body organization during the development of the zygote depends on the molecular machinery 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 techniques of microscopic and molecular techniques to examine the structural components of a cell that transport RNA away from the site of synthesis in the nucleus to its final location as a specific microscopic region of the cell, (b) the signals that tell the cellular transport machinery where to deliver the given RNA molecule and (c) the components that keep the RNA confined to the desired compartment.

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

It has been known that the β-cathepsin G as well as its mRNA colocalize at the farthest edge of lamellipodia in a majority of cell types.1,2 Apparently, the subcellular localization of β-cathepsin G mRNA in these cell types provides a means for the compartmentalization of this protein. It has been shown in several cases that the mRNA localization signal (LBS) is present in the 3' untranslated region (3'UTR) of mRNA. Kikutani et al.3 classified the specific LBS sequence by analyzing different lengths of the 3'UTR of β-cathepsin G to the vector 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 histochemical methodology for β-galactosidase staining. It was shown that two upstream elements in the 3'UTR of β-cathepsin G mRNA provided the preferential localization signals to the human β-galactosidase mRNA so that in cells transfected with such chimeric constructs, X-gal staining revealed reclining color at the same plane where the β-cathepsin was seen. Of these two upstream signals, a 54-nucleotide sequence was the main "recognition signal" while the hepta-repeats but not active 43-nucleotide sequence, present further downstream, was composed of "repeatable elements.

The basic elements of the "recognition signals" were identified as AAGC and CAAGC, which act in concert to the multiple copies of each provide a stronger preferential localization signal. These authors suggest that the high A/C content in the 54-nucleotide sequence may also have a role in the renal localization of the β-cathepsin G mRNA. It was also seen that addition of complementary oligonucleotides against the RNA species de-localized the endogenous β-cathepsin mRNA and altered the organization and shape of lamellipodia and stress fibers, without affecting levels of the β-cathepsin G or protein, this showed that the localization of β-cathepsin mRNA is essential for the phenotype of these cells. An answer to the mRNA transport may possibly remain sequestered in cell cytoplasmic core, essential for the localised distribution, have been provided in a very ingenious study by Bast et al.4,5 Single mRNA molecules seen were seen with an immunoprecipitation study to be included in actin filament intermediates in fibroblasts. To achieve this feat, they used different sized gold particles to "see" simultaneously, at the same 3D level, the labeled oligo-dT probe hybridized in cells with poly(A) tail carrying mRNAs and the antibody bound to actin fibers. Furthermore, the oligo-dT probe hybridized with the poly(A) tails of cytoplasmic mRNAs was used as prime for in situ reverse transcription in sections in situ extended CDNA chains and the oligo-CT-primed, hybridized were confirmed by immunobinding of different sized gold particles. These results simultaneously demonstrated the localization of individual mRNA molecules specifically at intersections of actin filaments. These studies also revealed that the mRNAs at these intersections remain in a circular rather than an extended or linear configuration, this may help explain the intersection between the 3' and 5' ends of mRNAs that are important in regulating translation.

Directed transport and localized anchorage of mRNA during morphogenesis in Drosophila

The stages of morphogenesis provide a remarkable model for study of localized RNAs and the mechanisms that regulate the spatial order in the distribution of different mRNAs in different regions of the body. It is this symmetric but highly ordered distribution of different mRNAs in the egg that determines the subsequent movements of segmentation genes in early embryonic development. The large body of genetic data has made Drosophila egg 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 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 oskar and nanos genes are crucial for anterior-posterior axis determination. The loci of these RNAs in the oocyte are localized to the anterior margin and remain confined to the anterior region of embryos, where it directs the development of anterior structures. Like oskar, transcripts of the nanos and ocellus (osk) genes are located at the posterior pole of the egg and are crucial for the posterior development.

The localization of these nurse-cell-produced transcripts in oocyte results from a series of events during oogenesis. A number of existing acting genes are known to affect movement and the characteristic localization of these polarly located early determinants during oogenesis. The mRNA (oskar) gene plays a critical role in the last step of localization in nurse RNAs, i.e., its release from the egg cortex of mature oocyte into anterme cytoplasm of activated egg and early embryos. osk is also important in the final stages of posterior localization of the osk mRNA in the egg. Piekarska and Stephanowicz 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 osk 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 cytoskeletal elements, and after egg activation, most of the osk 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 Piekarska and Stephanowicz to conclude that microtubules, and not microfilaments, are required for the assembling 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 mechanisms than localization in the anterior cortex of mature oocyte, which shows a wavelike association.

The bcd mRNA 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 shown that the bcd mRNA is associated with osk RNA to mediate its binding with cytoskeletal components that transport the complex to the posterior pole. Other studies have shown that the plus ends of microtubules within the oocyte are polarized towards the posterior pole. A visual demonstration of the posterior fanning of the plus ends of microtubules in osk was provided by Clark et al. In fuses transformed with a chimeric gene lacking the kinesin domain on its 5' end and the β-galactosidase coding region on the 3' end: this fusion protein returns the properties of kinesin (moving along microtubules towards its plus end) 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 bcd 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 bcd protein is freed and quickly recruited for bcd localization.

Ferrandon et al. have very convincingly documented a mutual binding of the bcd 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 bcd mRNA. In vitro transcribed bcd RNA (full-length mRNA or the 3' UTR or variously mutated 3' UTR) was microinjected at ectopic sites in 2-3-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 bcd proteins; the normal bcd 3' UTR rapidly recruiting the bcd protein around itself to result in microscopically visible aggregates which moved during cell cycle stages in a microtubule-dependent manner. Using deletion and linker-scanning sub-stitutions, these nonmutant localization signals in stems I and II and distal regions of stems IV and V of the predicted secondary structure of the 3' UTR were identified. The RNA-dependent localization of bcd RNA at the site of egg activation and its anterior-specific localization to anterior cortex of oocyte via different but overlapping sequence signals. Aggregation of the cellular bcd protein at early embryos with the microinjected appropriate 3' UTR RNA and their movement with microtubules showed that the bcd transcripts and the bcd mRNA are dependent on each other for complex formation and localization. It is interesting to note that during oogenesis the bcd transcripts and the bcd protein do not recognize each other at that time the bcd protein is involved in localizing the osk transcripts. Only after delivering the osk transcripts at the posterior pole, the bcd protein quickly move to the anterior of egg and bind with the bcd RNA for its localization and anchorage at the anterior region. Since both the osk and bcd embryos the localization of the bcd protein depends on the appropriate RNA (osk in oocyte and bcd in early embryos) and vice versa. Ferrandon et al. suggest that perhaps the same microtubule motor is involved in both these transcript movements. However, even the events that make the bcd protein change its affinity from osk to bcd transcripts in the mature egg are not known. Also, if the bcd-βc complex has to move on the same microtubule motors as the osk-βc complex, a change in the microtubule polarity may be necessary. The myosin basic protein mRNA when microinjected into nurse eggs...
deformities also aggregates into large particles that are transported along microtubules in analogous directions. 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 episode” 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 axon and rod transcript localizations showing different stages of organogenesis, different subsets of cytoskeletal components may be involved in transport as well as anchorage of the specific RNA in a stage-dependent manner. With the feasibility of doing sophisticated biochemistry and molecular probing in situ in the cell at high 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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