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VIEWPOINT

Spatial Organization of mRNA Within Cells

The series of Prospect articles on this subject concern the mechanisms by which cells develop and maintain intracellular organization. A pervasive theme involves the hypothesis that intracellular structural and functional diversity can come about by targeting mRNAs to regions of the cell where their cognate proteins are used. A good paradigm for this model is represented by the polarized cell, where asymmetric morphology may be represented by a concomitant distribution of specific proteins. These are the cell types which first showed a localized distribution of mRNA: e.g., the leading edge of the chicken embryonic fibroblast, the vegetal pole of the *Xenopus* oocyte, or the dendrites of neurons.

We are now past the point where mRNA localization surprises us, but more questions remain. What are the mechanisms that direct mRNAs to specific regions in the cell and then anchor them there? Recent evidence indicates that the cytoskeleton is a component of this mechanism. One approach has been to visualize mRNAs directly and to describe their cytoskeletal interactions. Ultrastructural work reviewed by Bassell in this series shows that most mRNA attaches directly to the actin cytoskeleton and that subcompartments of filamentous actin exist which may delineate diversity in binding sites within this structural compartment of the cell. In addition, other filament systems in the cell appear to play a role in the compartmentalization of mRNA. Intermediate filaments can also bind mRNA, and so can microtubules but to a much lesser extent. These various filament systems may have either anchoring or transport properties or both. The variety of addresses in the cytoplasm may depend on the number of different interactions that mRNA can make within, and in combination with, cytoskeletal "microenvironments." The term "microenvironment" can be defined in the most reductionist view, as the cytoplasmic volume controlled by a single mRNA (about 0.25 μm^3 in our estimation). Within this region of cytoplasm, a high concentration of protein corresponding to the controlling mRNA would result. At one extreme, the cytoplasm could be seen as a collection of blocks with different protein compositions. However, since these proteins are being synthesized with the mRNA attached to filaments, a system most likely exists to "channel" newly synthesized proteins to nearby sites.

During the development of a differentiated cell, the process by which specialized intracellular structures become assembled could also involve mRNA localization. For instance, in the case of muscle development, the formation of the sarcomere and its accessory components would be facilitated by the creation of a localized "assembly plant." Work reviewed by Fulton in this series has shown that in some cases nascent chains associate directly with cytoskeletal filaments, a process Fulton calls "cotranslational" assembly. This may speed the assembly process. The assembly of the sarcomere, for instance, must result from some cooperation between protein-protein interactions and localized protein synthesis where the effective concentration of the assembling polypeptides is increased by virtue of this physical coupling. In the case of vimentin mRNA, the synthesis and assembly occur in the developing costameres.

Since steady-state (and not newly synthesized) mRNA is visualized by in situ hybridization, the analyses so far have been confined to mRNA which has anchored to the cytoskeleton presumably at its final destination. However, how it moves to its site is as yet unexplained. Are there motors, or could mRNA diffuse along the cytoskeleton or within preferred channels and then bind to "receptors?" Work by Luby-Phelps, in this issue, deals directly with the question of passive vs. active mobility within the cytoplasm. The passive diffusion of macromolecules the size of ribosomes is severely limited by the gel-like viscous cytomatrix. Therefore, an mRNA fully loaded with ribosomes would have essentially no mobility and would be difficult to

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Address reprint requests to Robert H. Singer, Department of Cell Biology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA 01655.

move to a cellular destination. This would suggest that mRNA must translocate in untranslated form, possibly in specialized channels comprising most of the solvent compartment. The channels suggested by Luby-Phelps represent yet another view of intracellular motility where facilitated diffusion may be directing mRNAs along preferential pathways followed by subsequent binding in the excluded (cytomatrix) compartment. Alternatively, a motor system using actin, microtubules, or both actively moves mRNA. Because of diffusion limitations, assembly of macromolecular complexes would be facilitated by mRNAs for components being sequestered in the same microenvironment. The biophysical approaches applied by Luby-Phelps will provide important models for the mechanisms involved in mRNA transport.

Finally, there is the question of function. For the case of obvious cellular asymmetry, the structure-function relationship centers on the synthesis of components unique to a particular cellular region. In this case, interpretation of the significance of mRNA localization is facilitated by the ease by which one can determine the coincidence between cell morphology and mRNA localization. However, we assume that the mRNA in this region is actively translated. Association with the cytoskeleton appears to have a functional significance for mRNA translation. The literature over the years has consistently suggested, using viral systems, for instance, that mRNA is only translated when associated with filament systems. Therefore, the cytoskeletal filaments not only may move or anchor mRNA, but also provide a regulatory role. The work reviewed by Edmonds ties together these two very important fields: the structural components of the cytoskeleton and their function. An actin binding protein in *Dictyostelium*, ABP50, is also the elongation factor EF1α, providing the linkage between the spatial regulation of mRNA and its translation. This latter work relates directly to the concept of "microcompartmentalization" of mRNAs as originally raised by Bassell in this series. In this scheme, localization of mRNA seen by high resolution provides a view where the cell may be a mosaic of individual mRNA binding sites. These receptors may bind a generic sequence such as poly(A) and the specificity of localization may be in the transport. Alternatively, the receptors may bind particular sequences. Regulation of mRNA translation could be facilitated by cycling off and on the receptor. Possibly conformational changes could occur to the mRNP after binding to the receptor and this may regulate its expression. For instance, translation could occur when the 5′ end is brought into contact with appropriate factors sequestered at the 3′ end. The role of poly(A) binding proteins in interacting with the 60s ribosomal subunit and initiating translation supports this model (Sachs AB, Davis RW: Cell 58:857, 1989). At the intercellular level, this regulation could be effected by extracellular signals operating through signaling pathways such as phosphorylation.

Our job now is to synthesize a coherent scheme for the interrelationship of spatial and functional mRNA regulation, and the factors which provide the transduction of nucleic acid sequence into spatial information. Over the coming years, further information will be revealed by new approaches which will allow more precise study of cellular microenvironments, possibly in living cells, and their role in mRNA regulation.

Robert H. Singer
Department of Cell Biology
University of Massachusetts Medical Center
Worcester, Massachusetts 01655