ACTIN FILAMENTS AND THE SPATIAL POSITIONING OF mRNAS

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Filamentous actin has been shown to play a major role in the control of mRNA expression. Previous work emphasized RNA-cytoskeletal interactions using biochemical fractionation. More recently, in situ hybridization at the light microscopic and ultrastructural levels has shown that actin, in particular, is directly associated with mRNAs. It is proposed that these interactions play a major regulatory role in how the mRNA is spatially sequestered within the cytoplasm and provide a mechanism for its regulation (Singer, 1992).

Actin has stimulated a major interest in its physical and functional significance for the study of cell biology. Since actin is a major constituent protein of essentially all cells, is strongly conserved evolutionarily and is lethal when its gene is deleted in yeast, it undoubtedly plays a fundamental role in a number of cellular functions. Interest in actin has centered mainly on its regulation within cells and this has led to an extensive study of actin binding proteins which modify the polymerization of the monomer or the structure of the ensuing filaments. Less interest has centered on the synthesis of the protein itself as a means by which to regulate the assembly and ultimately the cell structure and function. This is because the monomers exist in significant concentration within the cytoplasm so that the cell never appears to be limited for availability of the protein. More recent evidence, however, suggests that although actin may exist in significant physical concentration within the cytoplasm, as high as 10 mg/ml, its functional concentration is actually much less, and depends heavily on regulatory proteins such as profilin and β-thymosin which sequester the G-actin monomer, effectively buffering it from polymerization and superimposing an entire regulatory heirarchy on the transition from G to F-actin (Nachmias, 1993). This system of regulation provides an opportunity for the synthesis of actin protein to play a role in the scheme, provided that the new G-actin monomers can be sequestered from the factors that may prevent their utilization. Hence newly synthesized actin may preferentially be incorporated into filaments and therefore exert a disproportionate effect on cell behavior or structure compared to its mass contribution to an existing pool.

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This scheme is more interesting when one considers that actin exists as a number of closely related isoforms (Herman, 1993). Three major isoforms are associated with specific cell types, γ-actin predominates in smooth muscle type cells, and is found also in fibroblasts, β-actin is considered to be the constitutive cytoskeletal form, represented by the fibroblast and α-actin predominates in skeletal and cardiac, and to a less extent in smooth muscle (Paterson and Eldridge, 1984; Sawtell and Lessard, 1989; McHugh et al., 1991; Sawtell et al., 1988). While there is considerable debate on the functional significance of these isoforms (Pardo et al., 1983), sporadic reports suggest that these isoforms are not simply markers for their respective genes expressed during tissue differentiation but rather play a functional role (Rubinstein, 1990). In vitro experiments suggest that the proteins themselves have different affinities for exchange within the sarcomere (Peng and Fishman, 1991). In addition these isoforms have different spatial representations within the cell. The β-actin isoform appears to be involved with cell motility since it predominates at the leading edge of the cell (Hoock et al.,1991; Otey et al., 1988). The α-actin isoform is involved in forming the sarcomere internally, near the nuclei of the syncytial myotube (Lawrence et al.,1989). This sorting of actin isoforms occurs within the cytoplasm even when several isoforms may be present at once (Lawrence et al., 1989; Hill and Gunning, 1993).

It is not entirely clear that the differentiated function of muscle cells, the motility of fibroblasts or the slow contraction of smooth muscle requires different isoforms of actin. Despite evidence that α-actin has the higher affinity for myofilament structures; it has been shown (McKenna et al., 1985) that sarcomeres can readily incorporate microinjected β-actin and sarcomeric actin can associate with non-muscle filaments (Gunning et al., 1984). In contrast, the dramatic changes on cell phenotype affected by an altered ratio of β and γ actin (Schevzov et al., 1992) provide compelling evidence for a functional significance of actin isoforms. The interchangeability of the actin protein isoforms in structures is contrasted with the apparent sensitivity to perturbations of spatial and/or temporal concentrations of actin protein isoforms. Therefore, it is proposed that spatial targeting of actin isoform mRNAs could be the primary means of insuring adequate actin protein synthesis at designated locations and times, thereby affecting specific spatial and temporal control of actin protein concentrations for various cellular processes throughout a cell's life span.

An analysis of the nucleic acid sequence of a variety of actin isoforms reveals two key features: the coding region is highly conserved no matter what the isoform (Vandekerckhove and Weber, 1978) and the non-coding region, particularly the 3' end is not well conserved between different isoforms. However, the 3' UTR is well conserved within the same isoform with little evolutionary divergence (>80% in some regions, Yaffe et al., 1985). Surprisingly, some regions of the actin 3' UTR are more conserved evolutionarily than the coding region, indicating a strong selective pressure on the primary nucleic acid sequence. The specific compartmentalization of the mRNAs for the various actin isoforms is not altogether surprising in view of these considerations. Each of the isoforms can be synthesized in its respective cytoplasmic compartment, presumably having a profound effect on cell structure and cell function. This was first shown for actin mRNA (Lawrence and Singer, 1986) which is localized at the leading edge of the cell where this isoform is actively promoting the extension of the lamellipodia (Wang, 1985; Theriot and Mitchison, 1991). In comparison, the mRNA for α actin is located near the nucleus, presumably consistent with the site of incorporation into sarcomeres (Kislauskis et al., 1993). In fibroblasts, the mRNA for γ actin is located likewise in the perinuclear region whereas β actin mRNA has a more peripheral composition (Hill and Gunning, 1993). Therefore each isoform has its unique site of synthesis. Even when each mRNA can coexist in the same cell, they are each in their respective compartments (Taneja and Singer, 1990; Kislauskis et al., 1993; Hill and Gunning, 1993).

The mechanism by which mRNAs can localize in their respective compartments is

beginning to be revealed. Cis acting elements within the 3' UTR of each isoform provide the information to localize the mRNA. When the isoform-specific 3' UTR is fused to a reporter, β-galactosidase, and transfected into fibroblasts, the enzyme will be directed to the cytoplasmic compartment, perinuclear (α-cardiac actin 3' UTR) or peripheral (β actin 3' UTR) dependent on the source of the 3' UTR. The 5' UTR plus coding region has no effect on the distribution of the β-galactosidase, and the enzyme becomes homogeneous throughout the cell (Kislauskis et al., 1993). This ability to distribute the mRNA to different compartments is even more remarkable considering that transfectants can be either myotubes, myoblasts or fibroblasts and yet the mRNA sorts correctly irrespective of the cell type. This indicates that the cell structure can recognize these cis-acting sequences despite a diversity of morphologies. Therefore common elements within each cell type, presumably cytoskeletal components, must contain the mechanism for nucleic acid sorting.

These elements are the common cytoskeletal proteins nearly identical in all cells. Primary among these is the actin filament system. Actin is an essential component of the mechanism which sorts actin mRNA. When cells are plated onto a solid substrate, actin mRNA moves to a peripheral location, as the lamellipodia begin to form. This process is exquisitely sensitive to cytochalasin, but not to colcemid, indicating that actin may be playing a major role in the translocation of this particular nucleic acid. In addition, once the actin mRNA is located in its correct position in the cell, it cannot be dislodged by colcemid but only by cytochalasin. This indicates that the anchoring process as well requires intact actin filaments (Sundell and Singer, 1991). Electron microscopic observations provide a direct visualization of actin mRNA as well as other mRNAs. In Triton- extracted cells which have been dried through the critical point of CO_2, the mRNAs can be visualized by in situ hybridization using colloidal gold antibodies to a biotinated DNA probe. These results revealed that the mRNAs for actin, tubulin and vimentin were associated with cytoskeletal filaments. Double labeling with antibodies suggested that these were most likely actin filaments.

In order to ascertain how general the phenomenon of the actin-nucleic acid association was, poly(A) was used as a marker for the majority of mRNA. Hybridization to fibroblasts was performed with a poly dT probe labelled with biotin and detected by streptavidin conjugated to a fluorochrome. In this way the sensitivity to drugs as well as a colocalization study would provide information as to the identity of the cellular "component" responsible for mRNA anchoring. Similar to the work on individual mRNAs, the colocalization of poly(A) was primarily with the actin filament network. This was determined from release of the poly(A) in fibroblasts using cytochalasin, but not colcemid. Colcemid causes collapse of intermediate filaments around the nucleus and the poly(A) did not substantially codistribute with this system. Using quantitative imaging techniques, it was confirmed that about two-thirds of the poly(A) codistributed with the actin network in fibroblasts (Taneja et al., 1992). Figure 1 demonstrates the colocalization of actin and poly (A). This does not mean that a mRNA molecule associates with actin throughout its entire lifetime, but only that, in the steady-state, most poly(A) is associated with actin. Actin mRNA in the transport phase would be expected to be a minor component of all mRNAs. Based on a ratio between the half life of actin mRNA and time to transport to the leading edge, only about 7% of the detected mRNA would be expected to be in transit. Evidence further suggests that the poly(A) associates the mRNA with actin since digestion of a triton-extracted cytoskeleton by low levels of RNAse A, to which poly(A) is resistant, still retains the poly(A) but the rest of the mRNA is released into the supernatant. Possibly, specialized proteins bridge the nucleic acid by associating both with the poly(A) and the actin. Preliminary evidence suggests that some actin binding proteins can also bind poly(A) (Ross et al., unpublished data).

This association of mRNA with actin may function not only to sequester mRNA in cellular compartments, but also to control its translatability by bringing it into association

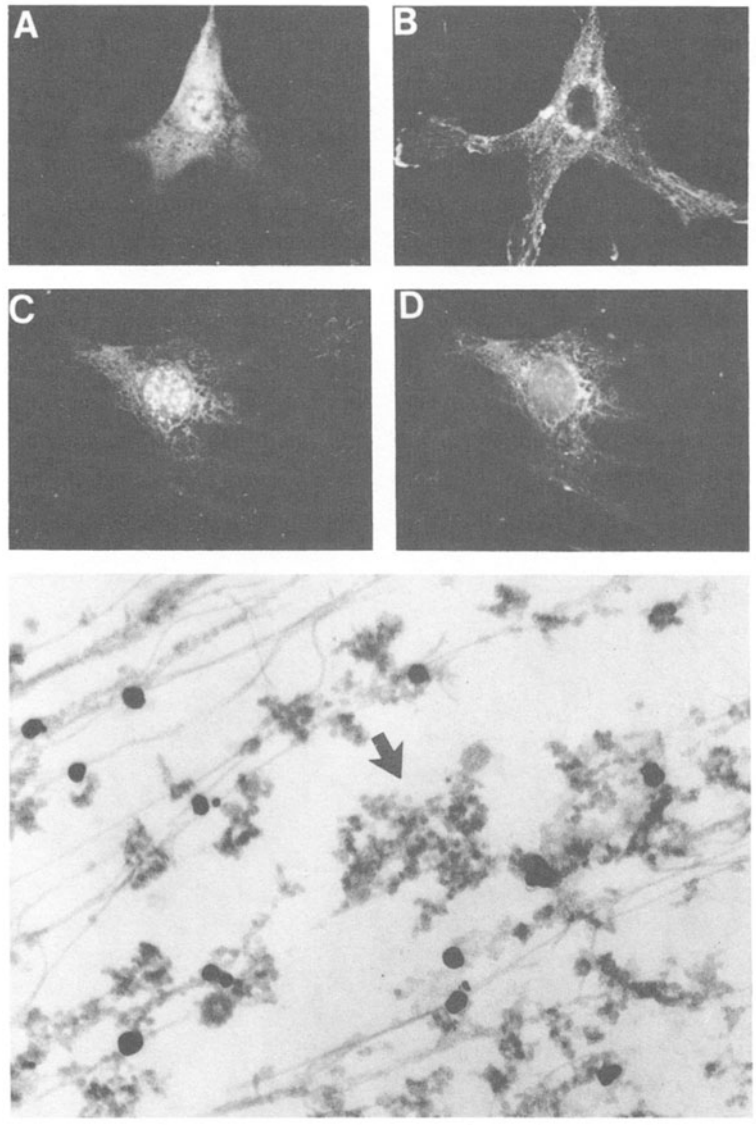

Figure 1. Colocalization of poly(A) and polysomes with actin filaments

Top: Actin antibody immunofluorescence and poly(A).

Human diploid fibroblast cells hybridized to biotinylated poly dT (55 bases) and detected with Texas red-avidin. Cells were immunolabelled with fluorescein-labelled actin antibody (East Acres Biologicals, Southbridge, MA). Exposure time, 45 S on Kodak TMAX 400 ASA film (Eastman Kodak Co.).

Intact cells fixed in formaldehyde: A. poly(A)
B. actin

Triton extracted cells: C. poly(A)
D. actin

Bottom: Polysomes associated with actin filaments.

A triton extracted fibroblast was reacted to an actin antibody (above) and detected with a 1 nm secondary antibody. The cell was then silver-enhanced, embedded in epon, sectioned and stained with uranyl acetate and lead citrate. Polysomes (arrow) can be seen associated with actin filaments; X 70,000.

with the protein-synthesis machinery. Literature over the last two decades has implicated function of mRNA with its cytoskeletal association, particularly in the case of viral mRNAs (see for instance, Farmer et al., 1983; Ben-Ze'ev et al., 1983). More recently, the elongation factor 1 was shown to be identical to an actin binding protein ABP5O in Dictyostelium (Yang et al., 1990). Therefore mRNA may associate with "microcompartments" which contain sequestered components of the translational apparatus. This would also have implications for mRNA localization since the translation of the mRNA would only take place when the mRNA is anchored, and not during translocation. This would effectively segregate transport from translation of mRNA. This suggests that sorting may occur in a translation incompetent state. Additionally, the movement of a very large structure such as a polysome within the relatively viscous cytoplasm would appear prohibitive (Luby-Phelps et al., 1987). Figure 1 (bottom) illustrates the array of polysomes which can be seen associated with actin filaments confirming that protein synthesis is cytoskeletal associated. Since these images are obtained from extracted cells, it does not prove that all mRNA is translated only when associated with actin, but it does confirm that translation occurs when it is associated.

In summary, the mechanism of mRNA movement, anchoring and its functional significance is intimately associated with actin filaments in fibroblasts. We have estimated that at least two-thirds of the newly synthesized proteins in the cell are made within a few tenths of a micron of an actin filament. This not only would facilitate actin-protein interactions but could couple the regulation of the synthesis of proteins with the structure of the cell. One mRNA in particular, that for β-actin may control the spatial distribution of this isoform and may regulate the cellular control of motility or structural polarity. Other isoforms of actin may regulate their respective structure-function relationships, such as sarcomere formation. This spatial regulation is coded for directly by the gene since cis-acting elements on the mRNA control the cytoplasmic compartmentalization of the synthesis of actin isoforms, and this principle may apply to mRNAs for other (e.g., actin-binding) proteins as well. What remains to be done is to identify the bridging proteins, or RNPs which connect the structural part of the cell (i.e., actin filaments) with the function of the mRNAs and define their contribution to the spatial sorting of the mRNAs and their cognate proteins.

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