Journal of Cellular Biochemistry 52:148–152 (1993)

# Spatial Organization of the Synthesis of Cytoskeletal Proteins

**A.B. Fulton**

Department of Biochemistry, University of Iowa, Iowa City, Iowa 52242

**Abstract** The cytoskeleton of most cells is complex and spatially diverse. The mRNAs for some cytoskeletal proteins are localized, suggesting that synthesis of these proteins may occur at sites appropriate for function or assembly. mRNA concentrations were first observed for several oocyte and embryonic mRNAs. Some insight has been gained into the mechanisms that help to position these mRNAs. More surprising to some, many cytoskeletal mRNAs are also localized. Among them are mRNAs for actin, tubulin, intermediate filaments, and a variety of associated proteins. Different mRNAs in the same cell can be located in different places; the same mRNA can be located in different places; the same mRNA can be located differently at different times of development. For example, we observed vimentin mRNA in developing chicken muscle cultures by fluorescent in situ hybridization. We found that vimentin mRNA takes on a variety of positions during myogenesis, ending up located with its cognate protein at costameres. This last pattern is significant because it is too finely structured to have a function in the soluble phase and probably reflects cotranslational assembly of this particular protein. Analogies can be made between oocyte or embryonic positions (animal/vegetal poles, oocyte cortex, and interior) and somatic cell positions (anterior/posterior and cell cortex/cell center). These analogies may point to conserved mechanisms for moving and retaining mRNA. Localization of cytoskeletal synthesis, through the mRNA or by other means, may prove as important for assembling and maintaining differentiated cytoskeletal structures and somatic cells as mRNA location is for organizing the embryo. Mechanisms that permit mRNA localization are likely to be conserved. © 1993 Wiley-Liss, Inc.

**Key words:** cytoskeleton, mRNA localization, vimentin mRNA, cytoskeletal interactions, protein synthesis

A surprise of recent years is that many mRNAs have particular locations in a given cell. Such localized mRNAs include the mRNAs for regulatory proteins, cytoskeletal proteins, and proteins with other spatial functions.

Regulatory proteins that have localized mRNAs include several developmentally critical proteins, such as Vg-1 [Weeks and Melton, 1987], bicoid, maternal cyclin B, several pair-rule genes, and *nanos*. The localization of these mRNAs in oocytes or syncytial embryos has recently been reviewed [Jeffery, 1989; Gottlieb, 1990].

Cytoskeletal proteins with localized mRNAs include actin, tubulin and its associated proteins, and intermediate filament proteins. Some aspects of this have recently been reviewed [Singer, 1992; Russell and Dix, 1992]. Actin mRNA is concentrated at the periphery in fibroblasts and myoblasts [Lawrence and Singer, 1986], the apical surface of some epithelial cells [Cheng and Bjerknes, 1989], and at the cortex of oocytes [Perry and Capco, 1988; Beach and Jeffery, 1990]. mRNA for myosin, the actin-dependent motor, is localized in the myotendinous region of muscles, where new myofibrils are forming [Dix and Eisenberg, 1990; Pomeroy et al., 1991]. In hypertrophic hearts, half of the myosin mRNA is within 10 nm of the myofibril [Eisenberg et al., 1991].

mRNAs for tubulin and its associated proteins have several positions. They concentrate toward the periphery of fibroblasts and myoblasts, in the cell body of neuronal cells [Tucker et al., 1989; Garner et al., 1988; Kleiman et al., 1990], and in the cortex of oocytes [Perry and Capco, 1988]. The mRNA for MAP2 is found specifically in dendrites.

Intermediate filaments derive from a family of genes. Although they differ in tissue expression, vimentin, glial fibrillary acidic protein (GFAP), and neurofilament mRNAs are localized near the nuclei of fibroblasts and myo-

Received January 28, 1993; accepted February 9, 1993.

Address reprint requests to A.B. Fulton, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

© 1993 Wiley-Liss, Inc.

blasts, glial cells, and neuronal cells, respectively [Holmes et al., 1988; Sarthy et al., 1989; Liesi et al., 1986; Trimmer et al., 1991].

For all of these cytoskeletal proteins, localized mRNA suggests localized synthesis of their cognate proteins. In several cases, the mRNA is known to be mostly present in polyribosomes and is being actively translated. Locally concentrated synthesis could constrain assembly by creating a locally high concentration of monomer that increases assembly rates or nuclei formation. Locally concentrated mRNA could also be a consequence of assembly, as is discussed below.

Other proteins with localized mRNAs often mediate some aspect of spatial organization at either the cellular or tissue level. Such proteins include the acetylcholine receptor (AchR) [Fontaine et al., 1988], *crumbs* [Tepass et al., 1990], *sevenless* [Banerjee et al., 1987], myelin basic protein (MBP) [Shiota et al., 1989], and histone [Lawrence et al., 1988]. AchR mRNA is near nuclei that underlie neuromuscular junctions. *Crumbs* and *sevenless* mRNAs cluster near the apical surface of epithelial and photoreceptor cells, respectively. MBP mRNA is peripheral in oligodendrocytes.

Localized mRNAs can change location if the cells develop. Most of the developmentally important mRNAs mentioned above relocate either at the transition from oocyte to egg or after fertilization. The cytoskeletal mRNAs found in the oocyte also relocate at these transitions, but their movements do not mirror the movement of the regulatory proteins [Perry and Capco, 1988]. Thus, the cell has mechanisms that permit the independent targeting of mRNAs to distinct locations, whether the mRNAs reach those locations by diffusion or active transport. In neuronal cells, mRNA movement requires metabolic energy and appears to occur on the cytoskeleton [Davis et al., 1987].

Although many oocyte mRNAs rearrange, until recently only one somatic mRNA was known to change location. Actin mRNA becomes increasingly apical during epithelial differentiation [Cheng and Bjerknes, 1989] and more peripheral during cell spreading and migration [Sundell and Singer, 1990; Hoock et al., 1991].

Closer examination of these studies of actin shows why so little is known about changes in somatic cell mRNA distribution. Somatic cells are far smaller than oocytes and the resolution of most in situ hybridization methods to date is only a few fold smaller than the diameter of the cells. For example, autoradiography could only detect a twofold increase of actin mRNA in the apical quarter of the epithelial cells. The actual distribution of mRNA might be a fivefold concentration in the apical 2 μm. Thus, subtle rearrangements will go undetected without a method that offers micron or submicron resolution.

In situ hybridization at the electron microscopic (EM) level promises the most resolution [Singer et al., 1989]. However, like all EM methods, sampling the whole cell is technically demanding because of the small field of view. In addition, the fixations that permit in situ hybridization are not yet those that are optimal for ultrastructure. This promising approach will continue to develop as new methods of fixation and computer-assisted reconstruction become available.

If there is a functional relation between the location of an mRNA and the assembly of its cognate protein, cytoskeletal mRNAs might be expected to rearrange when the cytoskeleton reorganizes. Vimentin filaments rearrange greatly during myogenesis [Pardo et al., 1983; Craig and Pardo, 1983]. To determine whether vimentin mRNA also rearranges at the same time, we refined an existing in situ hybridization method to gain submicron resolution. Using this method, we find that vimentin mRNA takes on a variety of positions during myogenesis, ending up located with its cognate protein at costameres. During muscle development in culture, vimentin mRNA is bipolar in young myoblasts, somewhat perinuclear in elongated myoblasts and spread fibroblasts, and diffuse in young and developing myotubes. In mature myotubes, vimentin mRNA occurs at costameres with vimentin protein [Cripe et al., in press].

This particular pattern of mRNA, which has the same periodicity as the costamere and sarcomere, is significant for physical reasons. Stripes repeating with a spacing of 1.5 μm cannot generate gradients of soluble compounds; diffusion would disperse such gradients. Such a pattern for vimentin mRNA therefore cannot have a function in the soluble phase. In these cells, half of the vimentin assembles during translation [Isaacs et al., 1989]. The close spacing of vimentin mRNA most likely reflects the vimentin nascent chains undergoing assembly. Which pattern is causal is not clear. Whether the vimentin

assembling during translation tethers the mRNA at this position or a zip code on the mRNA delivers the mRNA to the correct address for assembly will be revealed by further experiments.

Few patterns of mRNA are sufficiently localized to exclude functions in the soluble phase. Most patterns reported so far are equally compatible with functions in both the soluble and the solid phases of the cell. In addition, the mass of nascent peptides is much less than the mass of full length peptides. In any given cell, the position of nascent chains might correspond to messenger position even though full length protein has undergone redistribution after assembly.

The sequence in which protein and mRNA redistribute and the patterns of mRNA seen offer insights into possible mechanisms of rearrangement and imply a relation between mRNA position and cytoskeletal synthesis and assembly. To understand that relation, several questions need to be answered. What features of mRNAs allow different ones to be located differently in the same cell? What features of the cytoskeleton allow the same mRNA to be located differently at different times, and different ones at the same time? What is the functional consequence of mRNA location?

Some features of mRNAs that allow different ones to take on different locations are likely to be in the sequence, especially sequences that could form secondary structures. Some mRNAs appear to have zip codes in the untranslated 3′ end [Kislauskis and Singer, 1992]. Further studies such as those of the bicoid and actin 3′ UTRs will make data available to permit comparisons like those made by Gottlieb [1992]. She observed that the nonamer YUGUUYCUG was common to several localized mRNAs and could restore partial localization to deletion mutants that lacked the nonamer. This nonamer is missing from the localized messages An1.a and An1.b, so other signals are also involved. Mix and match experiments (in which constructs encode one cytoskeletal protein but follow it with a different 3′ UTR or other zip code) will further test the sufficiency of such signals. The actin isoforms, which distribute differently in the same cell, may be especially informative [Taneja and Singer, 1990]. mRNAs that become localized when their nascent peptides are "captured" by appropriate sites on the cytoskeleton will also be detected by such experiments, since their localization will reflect the coding sequences, not UTRs.

Some features of the cytoskeleton allow the same mRNA to be located differently at different times, and different mRNAs to localize to the same location. Biochemical evidence that mRNA is bound to the cytoskeleton is available from several systems [Biegel and Pachter, 1992; Hesketh et al., 1991; Pondel and King, 1988; see review in Pachter, 1992]. For most of these cases, biochemical evidence for cytoskeletal attachment is reinforced by morphological evidence of localization in the intact cell. Pharmacological evidence shows that bicoid and actin mRNA require microtubules and microfilaments, respectively, to localize [Pokrywka and Stephenson, 1991; Sundell and Singer, 1991]. The attachment of mRNA is probably specific, because different mRNAs have different elution profiles. The mRNAs that remain after 1 M salt extraction are enriched in localized mRNAs. One of these localized mRNAs encodes a protein with an RNA-binding zinc finger domain that is related to *nanos* [Mosquera et al., 1993]. In the future, zip codes and RNA sequences could be used to isolate cytoskeletal proteins that bind to them specifically. Once such cytoskeletal proteins are identified, the cellular location and cytoskeletal interactions of such "zip code binding proteins" should be determined.

In both oocytes and somatic cells, different mRNAs can occupy different locations. This suggests that cytoskeletal proteins will be found that correspond to positions in oocytes such as "animal half," "vegetal half," "animal cortex," or "vegetal cortex," possibly by separate factors that specify "animal/vegetal" and "cortical/central." Many other messengers appear to be bound without a specific site, while a few such as germ plasm and P granules may be specified uniquely by factors specific for those mRNAs.

Somatic cells do not have the same axes as oocytes, but the dimensions "anterior/posterior," "apical/basal," or "cortical/cell center," may be analogous. Somatic cells may therefore use homologous proteins to accomplish localization. The functional consequence of mRNA location for cytoskeletal assembly will only be known when cytoskeletal RNAs are mislocalized. For mRNAs with zip codes, this will require either giving a coding sequence the "wrong" zip code or overexpressing or deleting the mRNA binding protein. For mRNAs that are captured when

their nascent chains bind to the cytoskeleton, mislocalization will be more difficult, but might be accomplished by adding a strong heterologous zip code. It is already clear for the oocyte and embryo that mislocalized mRNAs cause aberrant development [Gottlieb, 1990]; the consequences of mislocalized cytoskeletal synthesis may be equally dramatic, given the many functions of the cytoskeleton.

## REFERENCES


Banerjee U, Renfranz PJ, Pollock JA, Benzer S (1987) Molecular characterization and expression of *sevenless*, a gene involved in neuronal pattern formation in the *Drosophila* eye Cell 49 281–291

Beach RL, Jeffery WR (1990) Temporal and spatial expression of a cytoskeletal actin gene in the ascidian *Styela clava* Dev Genet 11 2–14

Biegel D, Pachter JS (1992) mRNA association with the cytoskeletal framework likely represents a physiological binding event J Cell Biochem 48 98–106

Cheng H, Bjerknes M (1989) Asymmetric distribution of actin mRNA and cytoskeletal pattern generation in polarized epithelial cells J Mol Biol 210 541–549

Craig SW, Pardo JV (1983) Gamma actin, spectrin, and intermediate filament proteins colocalize with vinculin at costameres, myofibril-to-sarcolemma attachment sites Cell Motil 3 449–462

Cripe L, Morris E, Fulton AB (in press) Vimentin mRNA location changes during muscle development PNAS

Davis L, Banker GA, Steward O (1987) Selective dendritic transport of RNA in hippocampal neurons in culture Nature 330 477–479

Dix DJ, Eisenberg BR (1990) Myosin mRNA accumulation and myofibrillogenesis at the myotendinous junction of stretched muscle fibers J Cell Biol 111 1885–1894

Eisenberg BR, Goldspink PH, Wenderoth MP (1991) Distribution of myosin heavy chain mRNA in normal and hyperthyroid heart J Mol Cell Cardiol 23 287–296

Fontaine B, Sassoon D, Buckingham M, Changeux JP (1988) Detection of the nicotinic acetylcholine receptor α-subunit mRNA by in situ hybridization at neuromuscular junctions of 15-day-old chick striated muscles EMBO J 7 603–609

Garner CC, Tucker RP, Matus A (1988) Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites Nature 336 674–677

Gottlieb E (1990) Messenger RNA transport and localization Curr Opin Cell Biol 2 1080–1086

Gottlieb E (1992) The 3′ untranslated region of localized maternal messages contains a conserved motif involved in mRNA localization PNAS 89 7164–7168

Hesketh JE, Campbell GP, Whitelaw PF (1991) c-myc mRNA in cytoskeletal-bound polysomes in fibroblasts Biochem J 274 607–609

Holmes E, Hermanson G, Cole R, de Vellis J (1988) Developmental expression of glial-specific mRNAs in primary cultures of rat brain visualized by in situ hybridization J Neurosci Res 19 389–396

Hoock TC, Newcomb PM, Herman IM (1991) β-Actin and its mRNA are localized at the plasma membrane and the regions of moving cytoplasm during the cellular response to injury J Cell Biol 112 653–665

Isaacs WB, Cook RK, Van Atta JC, Redmond CM, Fulton AB (1989) Assembly of vimentin in cultured cells varies with cell type J Biol Chem 264 17953–17960

Jeffery WR (1989) Localized mRNA and the egg cytoskeleton Int Rev Cytol 119 151–195

Kislauskis K, Singer R (1992) Determinants of mRNA localization Curr Opin Cell Biol 4 975–978

Kleiman R, Banker G, Steward O (1990) Differential subcellular localization of particular mRNAs in hippocampal neurons in culture Neuron 5 821–830

Lawrence JB, Singer RH (1986) Intracellular localization of messenger RNAs for cytoskeletal proteins Cell 45 407–415

Lawrence JB, Singer RH, Villnave CA, Stein JL, Stein GS (1988) Intracellular distribution of histone mRNAs in human fibroblasts studied by in situ hybridization Proc Natl Acad Sci USA 85 463–467

Liesi P, Julien JP, Vilja P, Grosveld F, Rechardt L (1986) Specific detection of neuronal cell bodies, by in situ hybridization with a biotin-labeled neurofilament cDNA probe J Histochem Cytochem 34 923–926

Mosquera L, Forristall C, Zhou Y, King ML (1993) A mRNA localized to the vegetal cortex of *Xenopus* oocytes encodes a protein with a *nanos*-like zinc finger domain Development 117 377–386

Pachter JS (1992), Association of mRNA with the cytoskeletal framework Its role in the regulation of gene expression Crit Rev Eukaryot Gene Express 2 1–18

Pardo JV, Siliciano JD, Craig SW (1983) A vinculin-containing cortical lattice in skeletal muscle Transverse lattice elements ("costameres") mark sites of attachment between myofibrils and sarcolemma Proc Natl Acad Sci USA 80 1008–1012

Perry BA, Capco DG (1988) Spatial reorganization of actin, tubulin, and histone mRNAs during meiotic maturation and fertilization in *Xenopus* oocytes Cell Differ Dev 25 99–108

Pokrywka NJ, Stephenson EC (1991) Microtubules mediate the localization of bicoid RNA during *Drosophila* oogenesis Development 113 55–66

Pomeroy ME, Lawrence JB, Singer RH, Billings-Gagliardi S (1991) Distribution of myosin heavy chain mRNA in embryonic muscle tissue visualized by ultrastructural in situ hybridization Dev Biol 143 58–67

Pondel MD, King ML (1988) Localized maternal mRNA related to transforming growth factor beta mRNA is concentrated in a cytokeratin-enriched fraction from *Xenopus* oocytes Proc Natl Acad Sci USA 85 7612–7616

Russell B, Dix DJ (1992) Mechanisms for intracellular distribution of mRNA In situ hybridization studies in muscle Am J Physiol 262 C1–8

Sarthy PV, Fu M, Huang J (1989) Subcellular localization of an intermediate filament protein and its mRNA in glial cells Mol Cell Biol 9 4556–4559

Shiota C, Miura M, Mikoshiba K (1989) Developmental profile and differential localization of mRNAs of myelin proteins (MBP and PLP) in oligodendrocytes in the brain and in culture Brain Res Dev 45 83–94

Singer RH (1992) The cytoskeleton and mRNA localization Curr Opin Cell Biol 4 15–19

Singer RH, Langevin GL, Lawrence JB (1989) Ultrastructural visualization of cytoskeletal mRNAs and their associated proteins using double-label in situ hybridization J Cell Biol 108 2343–2353

Sundell CL, Singer RH (1990) Actin mRNA localizes in the absence of protein synthesis J Cell Biol 111 2397–2403

Sundell CL, Singer RH (1991) Requirement of microfilaments in sorting of actin messenger RNA Science 253 1275–1277

Taneja KL, Singer RH (1990) Detection and localization of actin mRNA isoforms in chicken muscle cells by in situ hybridization using biotinated oligonucleotide probes J Cell Biochem 44 241–252

Tepass U, Theres C, Knust E (1990) *crumbs* encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia Cell 61 787–799

Trimmer PA, Phillips LL, Steward O (1991) Combination of in situ hybridization and immunocytochemistry to detect messenger RNAs in identified CNS neurons and glia in tissue culture J Histochem Cytochem 39 891–898

Tucker RP, Garner CC, Matus A (1989) In situ localization of microtubule-associated protein mRNA in the developing and adult rat brain Neuron 2 1245–1256

Weeks DL, Melton DA (1987) A maternal mRNA localized to the vegetal hemisphere in *Xenopus* eggs codes for a growth factor related to TGF-beta Cell 51 861–867