The cytoskeleton and mRNA localization

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The localization of mRNA appears to facilitate protein sorting, so that proteins are synthesized in specific cellular regions. The spatial information on the mRNA may be transduced by proteins that recognize specific localizing sequences on the 3' end and then chaperone the mRNA, presumably along filaments, to its destination. Additional sequences such as poly(A), or the nascent chains of cytoskeleton-associated proteins, may then anchor mRNAs on the cytoskeleton.

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Introduction

Over the past century, microscopists have revealed that the cell is highly organized and compartmentalized. This is most evident in differentiated cells such as muscle, nerve or intestinal epithelium, where the cellular morphology and physiology result from the asymmetric disposition of specialized proteins. Even in the 'undifferentiated' cell, compartments have their particular macromolecular identities, whether they are membrane-limited or not. These are composed primarily of polypeptide complexes unique to their subcellular function. What are the principles by which cells organize and maintain these non-homogeneous distributions of cytosolic proteins?

This question can be redefined as the 'sorting problem'. Tens of thousands of different proteins are synthesized within the cytoplasm and many of them appear to reside in functionally important locations inside or outside the cell. There are three major mechanisms that can account for protein sorting (see Fig. 1): protein targetting, nascent chain targetting and mRNA targetting. A fourth mechanism is highly speculative and involves the targetting of mRNA via its site of nuclear exit. In the first mechanism, the proteins may diffuse but, more likely, are transported through the cytoplasm to the region where they have a high binding affinity (a post-translational sorting mechanism). A paradigm for this mechanism is exemplified by some of the nuclear proteins or their chaperones which contain amino acid sequences for targetting to nuclear pores (Fig. 1a) [1]. This mechanism implies that the cytoplasm must withstand a certain level of chaos if proteins 'search' for their appropriate locations throughout the cell. From this level of disorder the assembly of a complex multipolypeptide structure would appear to be an improbable event. It would be more efficient for the cell to maintain sites of assembly of complex structures compartmentalized within the cytoplasm. This would be greatly facilitated by synthesizing the component proteins at, or near, their site of assembly.

mRNA localization

Sorting of proteins that are concentrated in a particular subcellular region could be effected by localization of their mRNAs. The first evidence for this came from the analysis of oocytes or early embryos, where cytoplasmic polarity is morphologically evident by virtue of inclusions such as yolk or pigment. In these systems, actin or vegetal-specific (vg1) mRNAs can segregate to specific poles [2,3]. In somatic cells, mRNA for actin has been shown to be localized to the leading edge of motile cells, where the corresponding protein is actively polymerizing. Vimentin or tubulin mRNAs were more perinuclear [4]. Actin mRNA has also been shown to be localized apically in intestinal epithelium [5] where actin filaments polymerize to form the microvilli. Actin mRNA appears to become localized where β-actin protein specifically segregates to the lamellipodia of cells undergoing response to injury of a confluent monolayer [6•]. These results indicate that actin mRNA is located where its corresponding protein has functional use, and provide a paradigm for the role of mRNA segregation in cell morphology and physiology.

More recently, a growing list of other systems in which mRNAs are localized has been established: microtubule-associated protein (MAP)-2 mRNA in the dendrites of neurons [7], or mRNA for Gap-43, nuclear factor-68 or tubulin in their cell bodies [8•,9]; mRNAs for myelin-specific proteins in the processes of oligodendrocytes [10]; *bicoid* mRNA in the anterior region of *Drosophila* embryos [11]; and myosin mRNA near sarcomeres in muscle [12,13•]. The mRNAs for α and β subunits of a protein kinase (CaM-KII) are localized to different neuronal regions [14], reflecting functional specializations. These examples continue to add to the evidence that mRNA localization is a means of sorting proteins near to their final destination within cells.

There are at least two major mechanisms that can account for mRNA localization: targetting by the nascent

Abbreviations

MAP—microtubule-associated protein; **vg1**—vegetal-specific.

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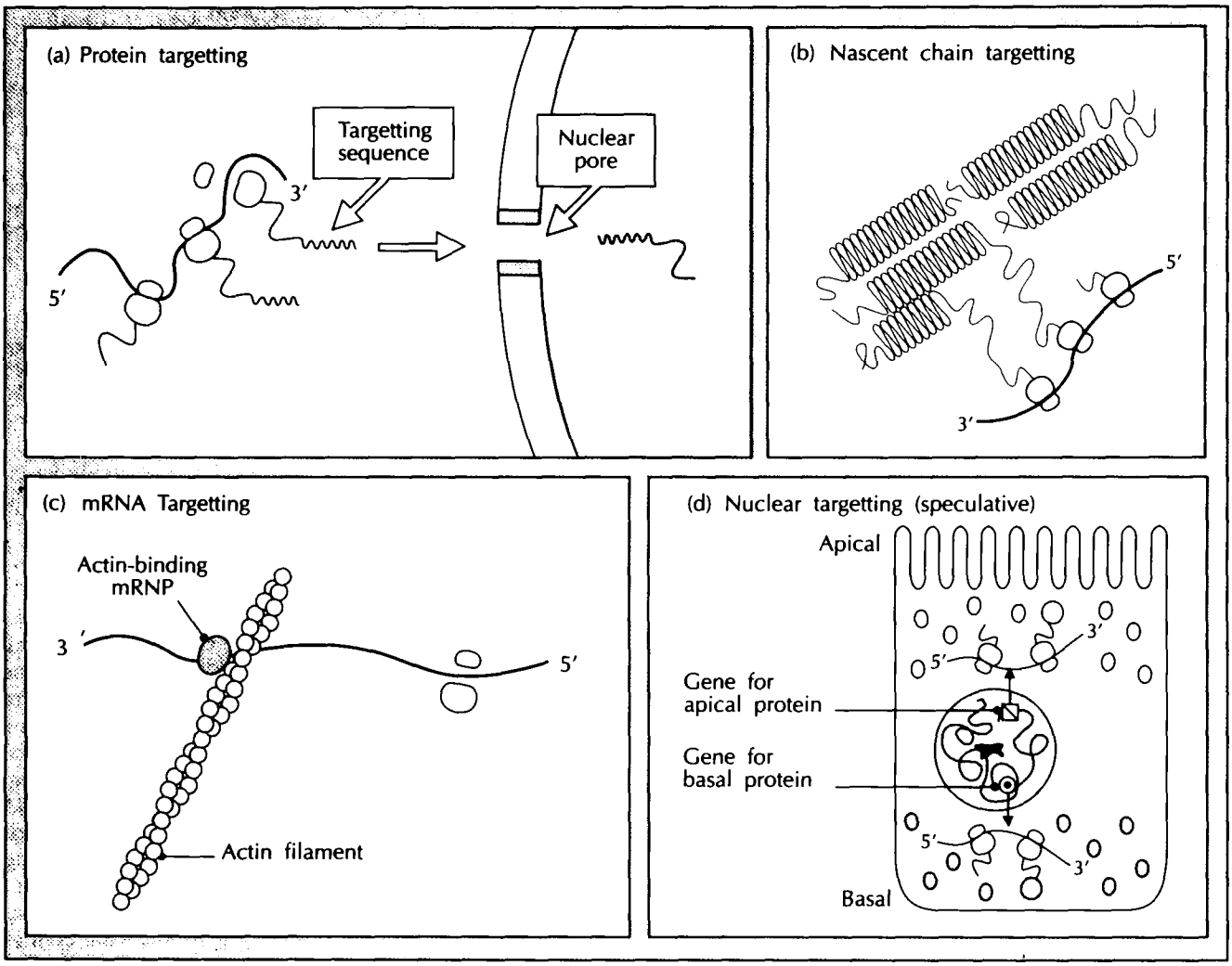

Fig. 1. Some of the possible mechanisms of protein and/or mRNA sorting. In (a), proteins travel through the cytoplasm and are targetted to (e.g.) nuclear pores by virtue of specific amino acid sequences rather than mRNA localization. The nascent chain (b) may also contain specific targetting sequences that direct the mRNA to a particular subcellular region. In this case, nascent cytoskeletal proteins are shown assembling directly into homotypic filaments. Messenger RNA can also be targetted directly rather than via its nascent chain (c). Here, mRNAs become associated with actin filaments: we speculate that this occurs prior to translation. A fourth mechanism for mRNA sorting may occur, as the mRNA exits the nucleus. In this example (d), the genes encoding apical and basal proteins are located at opposite ends of the nucleus. This causes mRNA to exit the nucleus in an asymmetric manner, thus setting up an apical–basal polarity. Combinations of these models are likely to exist; for example, mRNA transport by (c) could be followed by anchoring by (b).

chain or targetting by the mRNA itself. In the first case, the affinity of a mRNA for a subcellular region can be augmented or even directed by specific targetting sequences on the nascent chain (a co-translational mechanism). This mechanism is illustrated by proteins that are localized specifically to membranes within the cytoplasm. A receptor–ligand model best describes this system of sorting [15]. Some of the cytoskeletal proteins may sort by this method; it has been suggested that nascent proteins can assemble directly onto either homotypic or heterotypic filaments (Fig. 1b) [16].

The second mechanism involves targetting the mRNA directly to the site of protein localization (Fig. 1c). For example, the process of localizing actin mRNA to the periphery of fibroblasts does not involve the nascent chain, as protein synthesis inhibitors that disrupt the nascent chain by disaggregating ribosomes do not prevent this localization [17•]. Therefore actin mRNA is targetted directly rather than via its nascent chain. Similarly, synthetic vg1 mRNA lacking the initiation codon, when injected into the *Xenopus* oocyte, becomes localized to the vegetal pole, despite the absence of translation [18]. A further example is seen in the case of *bicoid* mRNA, which is localized to the anterior region of *Drosophila* embryos by virtue of specific 3′non-coding sequences [19]. The mechanisms described above may not be the only ones that participate in mRNA localization. For instance, mRNA may exit the nucleus in an asymmetric manner, setting up an apical–basal polarity (Fig. 1d). That mRNA can exit from the nucleus directed to a specific region of the nuclear periphery can be inferred from the visualization of localized Epstein–Barr virus nuclear RNA in a transformed cell [20•]. Localized nuclear export is also

suggested by recent work on the pair-rule genes, which are responsible for apical–basal polarity in the *Drosophila* periplasm [21•].

mRNA targetting involves the cytoskeleton

How does the cell direct mRNAs to the right places? Diffusion of the translational complex would be impeded by physical constraints. Luby-Phelps *et al.* [22] have indicated that mRNA, particularly if loaded with ribosomes, could not diffuse through the cytoplasm, the size limit being a molecular complex with a 260 Å radius of gyration, which is far smaller than a polyribosome. It is possible, therefore, that mechanisms exist to sequester mRNA in a non-translated state during its movement. The movement of mRNA through the cytoplasm may require active transport. This has been suggested by evidence that mRNA translocation to dendrites requires ATP [23]. Once at its destination, mRNA could be anchored to some solid structure in order to prevent its wandering. This two-step model was suggested originally in oocytes where the movement of mRNA occurs over long distances and times [24]. Recently, methods for the non-isotopic detection of mRNA by *in situ* hybridization have been developed, which provide the resolution to distinguish each of these events in somatic cells as well [17•]. The processes of translocation versus anchoring were investigated separately by analyzing the spreading of cells on a substrate where actin mRNA could be seen to move from a perinuclear distribution to the periphery of a cell within 60 min of plating, coincident with the formation of lamellipodia. Some lamellipodia could be seen without actin mRNA, implying that cell polarity may be established before the mRNA can move.

The identity of the cellular mechanism that moves mRNA, anchors it and even controls its expression, is becoming more apparent. It has been an observation for over a decade that mRNA is associated with elements of the cell known as the cytoskeleton [25], and this association is functionally important in that it promotes mRNA translation [26]. The specific filamentous component most likely to be associated with mRNA in somatic cells appears to be actin filaments. Evidence for this comes from electron microscopic *in situ* hybridization analysis [27], as well as from studies involving the actin-disrupter, cytochalasin, which when added to cell cultures causes mRNA to be released from the cytoskeletal framework [28]. Both the localization and anchoring of actin mRNA appear to require microfilaments and not microtubules, as both of these processes are inhibited by cytochalasin, but not by colcemid [29••]. The anchoring of the majority of poly(A) in the cell also appears to rely on actin filaments (K Taneja *et al.*, unpublished data). The data supporting this induce speculation that an actin-binding protein(s) could serve to recognize mRNA for tethering and/or transport within the F-actin compartments. Not only the spatial organization of mRNA within the cell, but also the functional properties of the cell, may involve actin filaments, as the actin-binding protein ABP50 has been shown to be an elongation factor (EF1) required for mRNA translation when associated with F-actin [30••]. Thus, it may be that the translational apparatus is mostly sequestered within the F-actin subcompartment of the cell. The association of mRNA with a particular cytoskeletal compartment may also control mRNA stability. For instance, histone mRNA is associated with cytoskeletal filaments and can be moved to a different subcellular compartment by virtue of gene fusion with sequences encoding a membrane-recognition signal, escaping destabilization when the cell exits S-phase [31•]. The localization of mRNA by cytoskeletal elements is more complex in oocytes and embryos: every major filament system has been implicated. Intermediate filaments have been suggested to play a role in associating with mRNA in the oocyte cytoskeleton [32,33]. However, microtubules have been suggested to act to transport vg1 mRNA, while cortical actin filaments anchor it [34]. Recently, evidence has been presented that a number of steps in the localization of *bicoid* mRNA require microtubules [35••].

Identification of mRNA chaperones

Both mechanisms of mRNA localization require specific sequences either in the protein-coding region (nascent chain targetting) or in the non-coding region (mRNA targetting). Evidence from our laboratory suggests that sequences important for localization also occur in the 3′untranslated region of β-actin mRNA (E Kislauskis, *et al.*, unpublished data) and that the poly(A) tails may be involved in the tethering of many mRNAs to actin (or actin-binding proteins) and, to a lesser extent, vimentin filaments (G Bassell *et al.*, unpublished data). That mRNA contains spatial positioning information [19,21•] is an important modification to our perception that nucleic acids only code for proteins. Now that 3′end localization sequences are being isolated, it will be possible to define the interacting proteins in order to understand further how this spatial-positioning information is transduced. Presumably these proteins also interact with cytoskeletal filaments to act as mRNA motors or anchors. Genetic approaches to these questions appear to represent a fruitful pathway. In *Drosophila*, the anterior determinant *bicoid* requires at least three other maternal genes for localization of its mRNA, and the posterior determinant *nanos* requires at least seven maternal genes, one of which is *oskar*. The *oskar* mRNA is localized to the posterior pole of the oocyte [36•,37•], a considerable distance from its anterior entrance from the nurse cells, and is implicated in the localization of *nanos* mRNA. Protein synthesis affects *oskar* mRNA anchoring at the posterior pole, but not its transport. Of particular interest is the protein encoded by *staufen*, which appears to be required for localizing both anterior- and posterior-pole mRNAs [38•]. Another important protein, Bicaudal-D, may act to chaperone or anchor its own mRNA as well as other oocyte determinants [39•]. Further identification of these proteins should play an important role in the elucidation of the mRNA localization pathway. The localization of maternal and zygotic transcripts in *Drosophila* and *Xenopus* has been reviewed recently in this series by Gottlieb [40]. The dissection of mRNA localization by genetics can be used for somatic cells as well; the chaoptic mutant appears to result from the mislocalization of mRNA in the developing photoreceptors [41].

Conclusion

The region of a cell in which a protein is made has become an important component of gene expression. It is reasonable to expect that there will be a number of mechanisms of mRNA sorting for localizing proteins. These include both direct mRNA targetting and nascent chain recognition. In addition, it is possible that the exit site of mRNA from the nucleus might initiate mRNA asymmetry within the cell. Compartmentalized regulation of mRNA translation or stability could also play a role. It should be appreciated, however, that mRNA localization is a multi-step process and different mechanisms will probably act synergistically. For instance, specific sequences on mRNA may direct translocation to a cellular region and nascent chain stabilization at the site of localization may provide anchoring; subsequent protein targetting may then direct the protein on the short final step to its functional site. This close spatial coupling between translation and functional sites may facilitate feedback regulation on translation, particularly for an autoregulatory mechanism. Furthermore, the sorting of isoforms of a protein family such as actin may rely on mRNA sorting in order to position the particular isoform in its relevant cellular region. The positioning of these translation sites within the spatial context of the cell appears to involve a mRNA–cytoskeleton interaction functioning to translocate and anchor the mRNA. Elucidation of the details of this functional–structural relationship will clarify further one of the principles of cellular organization, and reveal yet another role for these filamentous structures and their accessory proteins.

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