3. M. A. Geeves, K. C. Holmes, *Adv. Protein Chem.* **71**, 161 (2005).
4. S. Hatano, F. Oosawa, *Biochim. Biophys. Acta* **127**, 488 (1966).
5. M. R. Adelman, E. W. Taylor, *Biochemistry* **8**, 4964 (1969).
6. T. D. Pollard, *Annu. Rev. Biophys. Biomol. Struct.* **36**, 451 (2007).
7. H. G. Morrison *et al.*, *Science* **317**, 1921 (2007).
8. D. Lang, A. D. Zimmer, S. A. Rensing, R. Reski, *Trends Plant Sci.* **10**, 542 (2008).
9. A. Mogilner, R. Wollman, W. F. Marshall, *Dev. Cell* **11**, 279 (2006).
10. J. E. Heuser, M. W. Kirschner, *J. Cell Biol.* **86**, 212 (1980).
11. P. J. Hussey, T. Ketelaar, M. J. Deeks, *Annu. Rev. Plant Biol.* **57**, 109 (2006).
12. D. E. Discher, D. J. Mooney, P. W. Zandstra, *Science* **324**, 1673 (2009).
13. B. J. Galletta, J. A. Cooper, *Curr. Opin. Cell Biol.* **21**, 20 (2009).
14. M. Kaksonen, C. P. Toret, D. G. Drubin, *Nat. Rev. Mol. Cell Biol.* **7**, 404 (2006).
15. G. J. Doherty, H. T. McMahon, *Annu. Rev. Biochem.* **78**, 857 (2009).
16. W. M. Brieher, H. Y. Kueh, B. A. Ballif, T. J. Mitchison, *J. Cell Biol.* **175**, 315 (2006).
17. T. P. Loisel, R. Boujemaa, D. Pantaloni, M. F. Carlier, *Nature* **401**, 613 (1999).
18. J. B. Alberts, G. M. Odell, *PLoS Biol.* **2**, e412 (2004).
19. M. Osawa, D. E. Anderson, H. P. Erickson, *Science* **320**, 792 (2008).
20. S. Oliferenko, T. G. Chew, M. K. Balasubramanian, *Genes Dev.* **23**, 660 (2009).
21. A. L. Miller, W. M. Bement, *Nat. Cell Biol.* **11**, 71 (2009).
22. D. Vavylonis, J. Q. Wu, S. Hao, B. O'Shaughnessy, T. D. Pollard, *Science* **319**, 97 (2008).
23. M. Zhou, Y. L. Wang, *Mol. Biol. Cell* **19**, 318 (2008).
24. G. Montagnac, A. Echard, P. Chavrier, *Curr. Opin. Cell Biol.* **20**, 454 (2008).
25. D. Pruyne, A. Legesse-Miller, L. Gao, Y. Dong, A. Bretscher, *Annu. Rev. Cell Dev. Biol.* **20**, 559 (2004).
26. A. Fagarasanu, R. A. Rachubinski, *Curr. Opin. Microbiol.* **10**, 528 (2007).
27. S. G. Martin, F. Chang, *Curr. Biol.* **16**, 1161 (2006).
28. L. Vidali *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13341 (2009).
29. V. Levi, A. S. Serpinskaya, E. Gratton, V. Gelfand, *Biophys. J.* **90**, 318 (2006).
30. A. J. Ridley *et al.*, *Science* **302**, 1704 (2003).
31. R. B. Anderson, D. F. Newgreen, H. M. Young, *Adv. Exp. Med. Biol.* **589**, 181 (2006).
32. T. D. Pollard, G. G. Borisy, *Cell* **112**, 453 (2003).
33. D. R. Kovar, T. D. Pollard, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 14725 (2004).
34. T. M. Svitkina, A. B. Verkhovsky, K. M. McQuade, G. G. Borisy, *J. Cell Biol.* **139**, 397 (1997).
35. T. E. Schaus, E. W. Taylor, G. G. Borisy, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 7086 (2007).
36. C. Yang *et al.*, *PLoS Biol.* **5**, e317 (2007).
37. B. J. Nolen *et al.*, *Nature* **460**, 1031 (2009).
38. T. D. Pollard, W. C. Earnshaw, *Cell Biology* (Saunders, New York, 2007).

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REVIEW

Subcellular mRNA Localization in Animal Cells and Why It Matters

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Subcellular localization of messenger RNAs (mRNAs) can give precise control over where protein products are synthesized and operate. However, just 10 years ago many in the broader cell biology community would have considered this a specialized mechanism restricted to a very small fraction of transcripts. Since then, it has become clear that subcellular targeting of mRNAs is prevalent, and there is mounting evidence for central roles for this process in many cellular events. Here, we review current knowledge of the mechanisms and functions of mRNA localization in animal cells.

The asymmetric distribution of specific mRNAs in the cytoplasm was first visualized in the early 1980s, when in situ hybridization techniques were used to detect β-*actin* mRNA in ascidian embryos (*1*). The discovery of differential localization of transcripts encoding cytoskeletal proteins in cultured chicken cells soon gave further prominence to this phenomenon (*2*). Subsequent studies demonstrated that asymmetric mRNA localization contributes to the targeting of diverse types of protein products.

In recent years, the advent of high-throughput approaches has revealed that mRNA localization is much more common than previously assumed. Of expressed mRNA species, 70% were classified as asymmetrically distributed in a large-scale fluorescent in situ hybridization screen in early *Drosophila* embryos (*3*). In addition, large numbers of vertebrate mRNAs are specifically enriched in protrusions of migrating fibroblasts, in neuronal processes, or on spindles (table S1). Thus, mRNA localization has a prominent role in the spatial regulation of gene activity. Here, we provide an overview of the mechanisms and functions of mRNA localization in animal cells. Readers are referred elsewhere for entry points into the seminal work on mRNA localization in fungi and plants (*4*, *5*).

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Mechanisms of mRNA Localization: Illuminating a Multi-Step Process

Four mechanisms are thought to contribute to subcellular localization of specific mRNAs after their transcription: (i) vectorial export from nuclei, (ii) localized protection from degradation, (iii) polarized active transport on the cytoskeleton by using molecular motors, and (iv) localized anchorage. With the exception of vectorial nuclear export, all of these mechanisms are known to contribute to mRNA sorting in animal cells. Combinations of these mechanisms can also be used to localize a single mRNA species.

Protection of mRNAs from degradation (Fig. 1A) plays a crucial role in restricting mRNAs to the germ plasm in *Drosophila* and zebrafish embryos, often in conjunction with local entrapment of transcripts (*6*–*8*). There is also evidence, from the sea slug *Aplysia*, that mRNAs in neuronal processes can be selectively stabilized by interaction with their targets (*9*). However, the molecular mechanisms that locally protect specific messages remain unknown.

Motor-based transport (Fig. 1B) appears to be the predominant mechanism for the localization of mRNAs in animal cells probably because it provides the most rapid method for long-distance translocation of large ribonucleoprotein (RNP) particles through the crowded cellular environment. Live cell-imaging studies in recent years—involving the injection of in vitro synthesized fluorescent mRNAs or labeling transcripts by means of tethering multiple fluorescent proteins—have provided compelling evidence that mRNAs can control their own sorting by recruiting more than one kind of motor and even modulating motor properties.

For instance, in mammalian oligodendrocytes and hippocampal neurons, as well as in *Drosophila* embryos, mRNAs are bound to microtubule-based motor complexes that rapidly switch between bouts of motion in the minus- and plus-end directions (*10*–*12*). Specific mRNAs appear to control net sorting by increasing the relative frequency of movement in one direction through the recruitment of factors that modulate the activities of simultaneously bound opposite polarity motors (*11*).

In the case of delivery of *oskar* mRNA from the nurse cells to the posterior pole of the *Drosophila* oocyte, the frequency of microtubule-based movement in the minus-end and plus-end directions is also altered by specific components of messenger RNPs (mRNPs) (*13*). However, it appears that this comprises sequential, rather than rapidly switching, actions of motors. Localization of *oskar* culminates in a biased walk along a weakly polarized cytoskeleton—driven by the plus end–directed motor kinesin-1—to anchorage sites at the posterior pole (*13*). Vegetal localization of mRNAs in *Xenopus* oocytes may also be based on similar principles, although in this case the concerted action of kinesin-1 and kinesin-2 is crucial (*14*).

Some mRNAs, as is the case for other cellular cargoes, may simultaneously associate with actin-

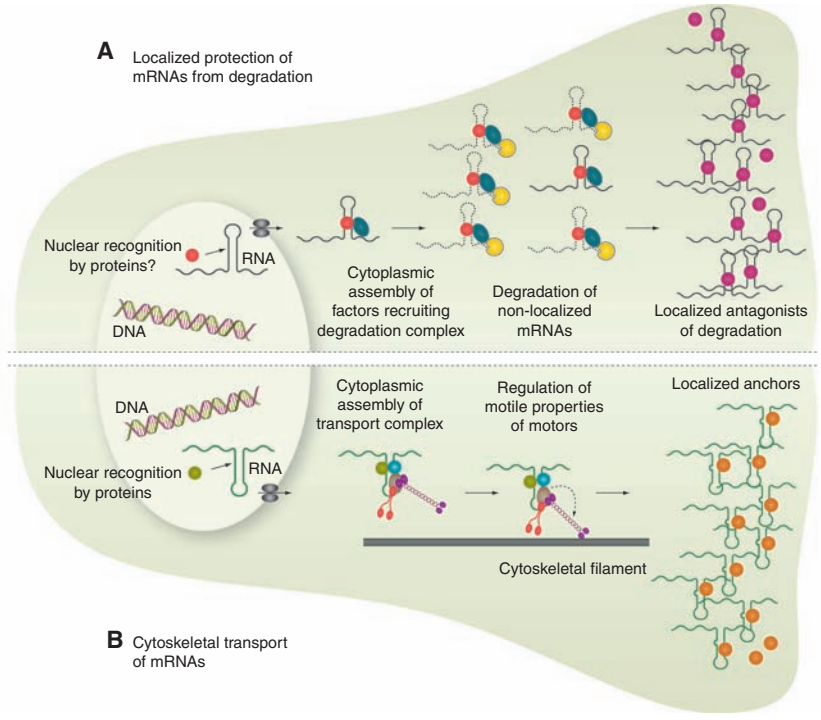

Fig. 1. mRNA localization is a multi-step process. Shown is an illustration of two stylized cells, depicting mechanisms that can contribute to mRNA localization. (**A**) Protection of mRNAs from degradation. Red, nuclear RNA recognition factor; dark blue, cytoplasmic RNA recognition factor; yellow, ribonuclease; purple, agonist of degradation. (**B**) Motor-based transport. Green, nuclear RNA recognition factor; light blue and light gray, cytoplasmic RNA recognition factor; red and purple, molecular motors; orange, anchorage factor. In reality, different combinations of these mechanisms may be used to localize a single mRNA species in the same cell.

and microtubule-based motors, allowing transport to be fine-tuned by switching between different types of cytoskeletal tracks (*15*). Transcripts may also influence the choice of subsets of microtubules by motors. This mechanism has been proposed to contribute to the delivery of *gurken* and *bicoid* mRNAs to the dorso-anterior and anterior regions of the *Drosophila* oocyte, respectively, by the minus end–directed motor dynein and could conceivably be based on differential posttranslational modification of microtubules (*16*, *17*).

Although our understanding of transport mechanisms is increasing, relatively little is known about the processes that contribute to mRNA anchorage. Long-distance transport of mRNPs on microtubules can be followed by transfer to the actin cytoskeleton at the cortex, with entrapment facilitated by the dense network of filaments or associated proteins (*18*, *19*). In other cases, microtubule-based motors may act directly as anchors (*20*) or lead to steady-state mRNA localization through continual active transport (*21*).

Thus, it appears that multiple binding sites within mRNAs recruit combinations of trans-acting factors that regulate the association and activities of different molecular motors as well as mediating interplay with anchorage complexes and translational regulators (see below). Even uniformly distributed mRNAs can be transported to some extent by motors, presumably to facilitate their exploration of space (*11*, *22*, *23*). A key challenge for the future is to understand how the information within asymmetrically localized transcripts is decoded.

Recognition of Localizing mRNAs

Cis-acting RNA localization signals and interplay with translation. The cis-acting elements that mediate asymmetric localization of specific transcripts are referred to as RNA localization signals or zip codes. Depending on the nature of the trans-acting factor they bind, these elements can consist of single-stranded stretches or double-stranded stem loops (*24*). Characterizing these latter types of elements is taxing because recognition may be based on a three-dimensional structure. This is probably the case during transport of several mRNAs toward the minus ends of microtubules in *Drosophila*, where stem loops with relatively little in common at the primary sequence level are recognized by the same RNA-binding protein, Egalitarian (*25*).

Localization signals are typically found within untranslated regions of messages, where they can evolve without the constraints of retaining protein-coding sequences. In cases in which signals are found within coding sequences, their secondary structure may play a role in antagonizing the translational machinery during the mRNA localization process (*26*). Protein production is more commonly silenced during translocation by the recruitment of translational repressors (*27*). In some instances, initiation of protein synthesis at the target site is mediated by the interplay between localized translational derepressors and proteins that bind localization signals. An elegant example of this involves the phosphorylation of the β-*actin* zip code–binding protein ZBP1 by the localized activity of the kinase Src at the cell periphery (*28*). This leads to dissociation of ZBP1 from the transcript at the leading edge of migrating cells, allowing access to the translation initiation machinery.

Trans-acting factors and the assembly of mRNPs. A large number of proteins have been identified with direct roles in mRNA localization complexes. To what extent this reflects discrete pathways at work or functionally related mRNPs containing multiple proteins remains unclear. This latter scenario will be at least part of the story because there are several reports of combinations of well-characterized RNA-binding proteins, such as ZBP1, Staufen, and fragile X mental retardation protein (FMRP), being found in the same complexes. Interactions of the same RNA with multiple trans-acting factors gives scope for redundancy, which may partly explain the difficulties in identifying the molecular links between localizing transcripts and motors in animal cells. However, a complete link has recently been uncovered between mRNA localization signals and dynein in *Drosophila* (*25*), providing an opportunity to probe the molecular details of the assembly and operation of a model RNA:motor complex.

Where in the cell are mRNAs earmarked for transport? In many instances, localizing transcripts are first recognized in the nucleus. This is the case for β-*actin* transcripts in chicken fibroblasts, in which the cotranscriptional recruitment of the ZBP2 protein facilitates binding of ZBP1 to the mRNA and its subsequent targeting behind the leading edge (*29*). It has also been revealed, from elegant studies of *Vg1* localization in *Xenopus* oocytes, that important RNA:protein interactions formed in the nucleus can be remodeled in the cytoplasm (*30*), and such events may regulate transitions between critical steps in localization processes. Nuclear history also plays an essential role in cytoplasmic localization of *oskar* mRNA. Deposition of the multicomponent exon junction complex (EJC) during splicing is essential for the translocation of this transcript to the posterior of the *Drosophila* oocyte (*31*), possibly by facilitating switching of the predominant motor activity from dynein to kinesin-1 (*13*). It will be fascinating to discover how the EJC regulates these motors at the molecular level, especially because components of this complex

have been implicated in the localization of functionally important mRNAs within mammalian neurons (*32*).

Functions of mRNA Localization: Cell Polarity and Local Response to Extrinsic Cues

There are several a priori reasons why localizing an mRNA could be advantageous over targeting the protein product directly: (i) increased cost effectiveness because of the production of multiple protein copies from single localized mRNA molecules, (ii) preventing proteins from acting ectopically during translocation, (iii) facilitating the assembly of macromolecular protein complexes by producing a high local concentration of mRNA molecules in microdomains, (iv) distinct properties of newly synthesized proteins, and (v) decentralizing the control of gene expression by permitting local control of translation in response to extrinsic cues. Below, we introduce specific examples that illustrate the importance of asymmetric mRNA localization in key biological processes (see also Fig. 2).

Establishing Embryonic Organization

In *Drosophila*, the differential localization of maternal mRNAs plays a major role in establishing and patterning the dorsal-ventral and anterior-posterior body axes as well as in germ cell specification (table S2). During *Xenopus* development, localization pathways exist in early and late oogenesis that culminate in the vegetal accumulation of transcripts that are important for germline development and patterning of the mesoderm and endoderm (*33*). Differentially localized maternal mRNAs have also been found in ascidians and cnidarians, and many of these transcripts encode proteins with known roles in embryonic patterning (*34*, *35*). Thus, the localization of maternal mRNAs appears to be widely used to establish embryonic organization.

In mammals, an obligatory requirement for localized mRNA determinants in the egg appears to be ruled out by the developmental lability of the early embryo. However, the recent report of apical localization of the message encoding the Cdx2 transcription factor in 8- to 16-cell embryos raises the possibility that mRNA sorting facilitates asymmetric cell fate decisions at later stages (*36*). A function for mRNA localization in influencing embryonic cell lineage choices is also supported by the differential inheritance of messages encoding developmental regulators in snail blastomeres, which is driven by a remarkable process of transcript enrichment at one of the two centrosomes (*37*).

Neurons: mRNA Localization on Demand

The critical importance of posttranscriptional regulation in neurons is illustrated by the high degree of autonomy exhibited by neuronal processes, which often extend great distances from the cell body. This autonomy permits rapid local responses to extrinsic cues and is manifest in the ability of axons and dendrites, respectively, to navigate to guidance cues and undergo certain forms of synaptic plasticity after removal of the soma. It has become increasingly evident that this "decentralization" involves the selective localization and translation of subsets of mRNAs in neuronal processes in response to external stimuli (Fig. 3).

Synapse formation and plasticity. In mammalian hippocampal neurons, strong synaptic activation is accompanied by transcription of the *Arc* gene and rapid trafficking of its mRNA to dendrites, where it localizes selectively to active synaptic sites (*38*). Arc is required for the consolidation of long-term potentiation (LTP), a form of persistent synaptic change, most likely through its ability to regulate actin dynamics and to modulate dendritic spine morphogenesis (*39*). Direct evidence for a requirement for mRNA localization in synaptic plasticity comes from studies of *CamKIIα*. Disruption of dendritic targeting of this mRNA in mice, by replacing the 3′ untranslated region of the endogenous gene with one from a nonlocalizing transcript, impairs LTP and long-term memory (*40*).

mRNA localization is also important for the establishment of synapses. In *Aplysia* sensory neurons, contact with a target motor neuron triggers rapid local concentration of the neuropeptide-encoding *sensorin* mRNA into synapses (*41*). Synaptogenesis is disrupted when *sensorin* mRNA levels are acutely reduced, even before the total concentration of the protein is altered. This indicates not only that mRNA localization is important but that newly synthesized Sensorin could have properties distinct from those of older protein copies. Consistent with an important role for nascent Sensorin, its translation is spatially restricted to active synapses in a stimulus-specific manner (*42*).

Cue-induced mRNA localization in axons. Growing axons navigate in the developing brain using attractive and repulsive cues that stimulate changes in growth and directional steering. β-*actin* mRNA is abundant in *Xenopus* growth cones and is rapidly recruited to the near-stimulus side in response to an attractive gradient (*43*, *44*). Attractive turning is abolished through the specific inhibition of local β-*actin* mRNA translation or disruption of the interaction of Vg1RBP (the *Xenopus* ZBP1 ortholog) with the zip code (*43*, *44*). The picture that emerges of localized translation of mRNAs underlying directionally responsive cell protrusions is strikingly similar to the situation in chicken fibroblasts (see below) and suggests that common mechanisms span the two systems (Fig. 3A).

"On site" versus "distant site" for action. Proteins synthesized from spatially localized mRNAs commonly act "on site." But this is not always the case. The mRNA encoding the transcription factor cyclic adenosine monophosphate (cAMP) response element–binding protein (CREB), which promotes cell survival in dorsal root ganglia neurons, can be translated locally in axons in response to nerve growth factor (*45*). The nascent CREB protein undergoes retrograde transport to the nucleus, where it activates the transcription of target genes. There is evidence that the phosphorylation status of CREB differs depending on its site of translation (*45*), which raises the intriguing possibility that local translation of process-targeted mRNAs controls gene expression in response to distal experience.

Polarized Functions in Other Cell Types

The functional consequences of disrupting mRNA localization have now been tested in many other cell types. These studies have revealed an important role for the localiza-

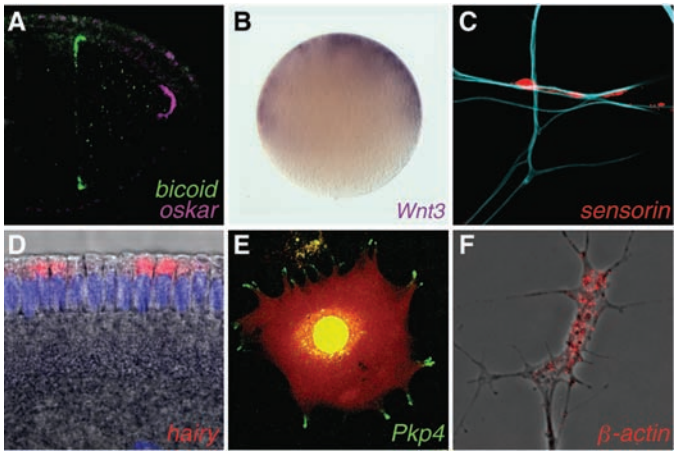

Fig. 2. Examples of asymmetrically localized mRNAs. (**A**) Differential localization of mRNA determinants within the *Drosophila* oocyte. (**B**) Animal localization of a transcript encoding a signaling molecule required for axis development in the egg of a cnidarian, *Clytia*. (**C**) mRNA enrichment in synapses of an *Aplysia* sensory neuron in response to contact with a target motor neuron (blue). (**D**) Apical localization of an mRNA in the *Drosophila* embryo, which facilitates entry of its transcription factor product into the nuclei (purple). (**E**) mRNA localization in pseudopodial protrusions of a cultured mammalian fibroblast (red signal indicates the cell volume). (**F**) mRNA enrichment within a *Xenopus* axonal growth cone. mRNAs were visualized by means of in situ hybridization except in (E), in which the MS2–green fluorescent protein (GFP) system was used. *Drosophila* images are reproduced from (*50*) with permission. [Images were kindly provided by (B) T. Momose and E. Houliston, (C) D.O. Wang and K. Martin, (D) M. Dienstbier, (E) S. Mili and I. Macara, and (F) F. van Horck.]

tion of specific mRNAs in facilitating subcellular protein localization, helping to establish or maintain cell polarity (table S2).

A particularly intriguing example comes from primary chicken fibroblasts. Here, interference with the β-actin zip code through antisense oligonucleotides strongly reduces the persistence of cell movement (*46*). But given that the number of protein molecules encoded by the localizing mRNAs represents only a tiny fraction of the total β-*actin* protein near the leading edge, why is mRNA targeting important? It is conceivable that newly synthesized β-actin monomers polymerize more efficiently than older copies, for instance, because of differential posttranslational modifications or modulation by chaperones. An alternative explanation relates to the potential for transport along a cytoskeletal track to convey multiple β-*actin* mRNA molecules to a small region of the cytoplasm. This could dictate a high local concentration of the protein, aiding rapid polymerization of filaments. The finding that all seven transcripts encoding Arp2 and Arp3 components are localized behind the leading edge lends support to the notion that mRNA targeting controls actin dynamics by facilitating the local assembly of protein complexes (*47*).

But it is not just mRNAs encoding cytoskeletal proteins that are localized in dynamic cells. At least 50 transcript species, coding for proteins with diverse functions, are enriched in pseudopodial protrusions of mouse fibroblasts in response to migratory stimuli (*48*). The localization mechanism is microtubule-associated and appears to be distinct from that used to target mRNAs behind the leading edge of chicken fibroblasts, involving direct roles of the adenomatous polyposis coli (APC) tumor suppressor and FMRP. This study, together with others, opens up new perspectives for elucidating links between mRNA localization and human disease [supporting online material (SOM) text].

Perspectives

Key principles of mRNA localization mechanisms in animal cells have now been established and many players identified. An important challenge is to piece together a detailed molecular understanding of the interactions that govern the recognition and differential sorting of mRNAs as well as the interplay with translational regulators. In cases in which mRNA localization is regulated by extrinsic cues (Fig. 3), what aspects of the translocation process are being targeted and how? And what is the copy number of mRNAs within the majority of mRNPs (SOM text)? Addressing these questions will benefit from insights from genetically tractable model organisms, including flies and fungi, and from advances in the ability to visualize the composition and behavior of mRNPs in living cells. The use of unbiased genome-wide methods to identify binding sites for specific trans-acting factors (*49*) could also have profound effects on our understanding of the recognition of localizing mRNAs.

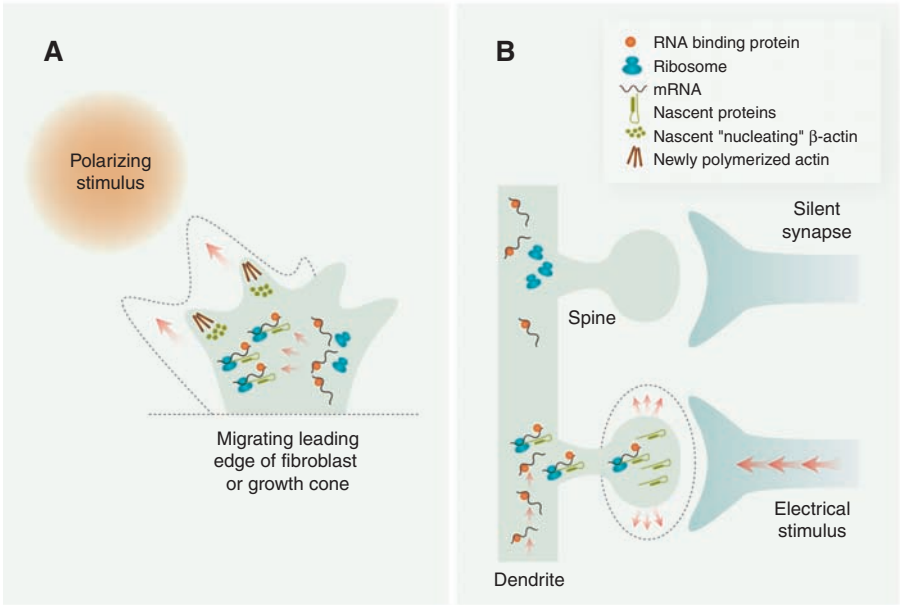

Fig. 3. Extrinsic stimuli elicit changes in subcellular mRNA localization and translation. (**A**) A polarizing stimulus elicits asymmetric localization and translation of mRNAs encoding β-actin and actin regulators on the near-stimulus side of the leading edge of migrating cells, such as fibroblasts and axonal growth cones, thus contributing to polarized cell movement and directional steering. The dashed outline denotes the post-stimulus trajectory. (**B**) Electrical input from presynaptic contacts selectively induces localized trafficking and translation of specific mRNAs in dendrites that mediate changes in spine morphology (dashed outline) and plasticity. Several aspects of these models are speculative.

A large number of studies have now highlighted the importance of subcellular mRNA localization in diverse cellular processes. Nonetheless, several questions remain from a functional perspective. What are the relative contributions of mRNA localization and localized translation to processes such as axon guidance, synaptogenesis, and synaptic plasticity? What is the extent and importance of asymmetric targeting of microRNAs? The requirements for some localizing mRNAs are independent from their translation (table S2); could this reflect a widespread structural role for mRNA in facilitating the assembly of protein complexes?

References and Notes

1. W. R. Jeffery, C. R. Tomlinson, R. D. Brodeur, *Dev. Biol.* **99**, 408 (1983).
2. J. B. Lawrence, R. H. Singer, *Cell* **45**, 407 (1986).
3. E. Lecuyer *et al.*, *Cell* **131**, 174 (2007).
4. K. Zarnack, M. Feldbrugge, *Mol. Genet. Genomics* **278**, 347 (2007).
5. T. W. Okita, S. B. Choi, *Curr. Opin. Plant Biol.* **5**, 553 (2002).
6. K. M. Forrest, E. R. Gavis, *Curr. Biol.* **13**, 1159 (2003).
7. A. Bashirullah, R. L. Cooperstock, H. D. Lipshitz, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7025 (2001).
8. U. Wolke, G. Weidinger, M. Koprunner, E. Raz, *Curr. Biol.* **12**, 289 (2002).
9. J. Y. Hu, X. Meng, S. Schacher, *J. Neurosci.* **22**, 2669 (2002).
10. J. H. Carson, H. Cui, E. Barbarese, *Curr. Opin. Neurobiol.* **11**, 558 (2001).
11. S. L. Bullock, A. Nicol, S. P. Gross, D. Zicha, *Curr. Biol.* **16**, 1447 (2006).
12. J. L. Dynes, O. Steward, *J. Comp. Neurol.* **500**, 433 (2007).
13. V. L. Zimyanin *et al.*, *Cell* **134**, 843 (2008).
14. T. J. Messitt *et al.*, *Dev. Cell* **15**, 426 (2008).
15. J. Krauss, S. Lopez de Quinto, C. Nusslein-Volhard, A. Ephrussi, *Curr. Biol.* **19**, 1058 (2009).
16. V. Van De Bor, E. Hartswood, C. Jones, D. Finnegan, I. Davis, *Dev. Cell* **9**, 51 (2005).
17. N. Vogt, I. Koch, H. Schwarz, F. Schnorrer, C. Nusslein-Volhard, *Development* **133**, 3963 (2006).
18. K. Babu, Y. Cai, S. Bahri, X. Yang, W. Chia, *Genes Dev.* **18**, 138 (2004).
19. J. K. Yisraeli, S. Sokol, D. A. Melton, *Development* **108**, 289 (1990).
20. R. Delanoue, I. Davis, *Cell* **122**, 97 (2005).
21. T. T. Weil, K. M. Forrest, E. R. Gavis, *Dev. Cell* **11**, 251 (2006).
22. D. Fusco *et al.*, *Curr. Biol.* **13**, 161 (2003).
23. L. Chang, Y. Shav-Tal, T. Trcek, R. H. Singer, R. D. Goldman, *J. Cell Biol.* **172**, 747 (2006).
24. A. Jambhekar, J. L. Derisi, *RNA* **13**, 625 (2007).
25. M. Dienstbier, F. Boehl, X. Li, S. L. Bullock, *Genes Dev.* **23**, 1546 (2009).
26. P. Chartrand, X. H. Meng, S. Huttelmaier, D. Donato, R. H. Singer, *Mol. Cell* **10**, 1319 (2002).
27. F. Besse, A. Ephrussi, *Nat. Rev. Mol. Cell Biol.* **9**, 971 (2008).
28. S. Huttelmaier *et al.*, *Nature* **438**, 512 (2005).
29. F. Pan, S. Huttelmaier, R. H. Singer, W. Gu, *Mol. Cell. Biol.* **27**, 8340 (2007).
30. R. A. Lewis, J. A. Gagnon, K. L. Mowry, *Mol. Cell. Biol.* **28**, 678 (2008).
31. C. Giorgi, M. J. Moore, *Semin. Cell Dev. Biol.* **18**, 186 (2007).
32. C. Giorgi *et al.*, *Cell* **130**, 179 (2007).
33. M. L. King, T. J. Messitt, K. L. Mowry, *Biol. Cell* **97**, 19 (2005).
34. C. Sardet, P. Dru, F. Prodon, *Biol. Cell* **97**, 35 (2005).
35. A. Amiel, E. Houliston, *Dev. Biol.* **327**, 191 (2009).
36. A. Jedrusik *et al.*, *Genes Dev.* **22**, 2692 (2008).
37. J. D. Lambert, L. M. Nagy, *Nature* **420**, 682 (2002).
38. O. Steward, C. S. Wallace, G. L. Lyford, P. F. Worley, *Neuron* **21**, 741 (1998).

39. C. R. Bramham, P. F. Worley, M. J. Moore, J. F. Guzowski, *J. Neurosci.* **28**, 11760 (2008).
40. S. Miller *et al.*, *Neuron* **36**, 507 (2002).
41. V. Lyles, Y. Zhao, K. C. Martin, *Neuron* **49**, 349 (2006).
42. D. O. Wang *et al.*, *Science* **324**, 1536 (2009).
43. K. M. Leung *et al.*, *Nat. Neurosci.* **9**, 1247 (2006).
44. J. Yao, Y. Sasaki, Z. Wen, G. J. Bassell, J. Q. Zheng, *Nat. Neurosci.* **9**, 1265 (2006).
45. L. J. Cox, U. Hengst, N. G. Gurskaya, K. A. Lukyanov, S. R. Jaffrey, *Nat. Cell Biol.* **10**, 149 (2008).
46. E. H. Kislauskis, X. Zhu, R. H. Singer, *J. Cell Biol.* **136**, 1263 (1997).
47. L. A. Mingle *et al.*, *J. Cell Sci.* **118**, 2425 (2005).
48. S. Mili, K. Moissoglu, I. G. Macara, *Nature* **453**, 115 (2008).
49. D. D. Licatalosi *et al.*, *Nature* **456**, 464 (2008).
50. S. L. Bullock, *Semin. Cell Dev. Biol.* **18**, 194 (2007).
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REVIEW

The Extracellular Matrix: Not Just Pretty Fibrils

Richard O. Hynes

The extracellular matrix (ECM) and ECM proteins are important in phenomena as diverse as developmental patterning, stem cell niches, cancer, and genetic diseases. The ECM has many effects beyond providing structural support. ECM proteins typically include multiple, independently folded domains whose sequences and arrangement are highly conserved. Some of these domains bind adhesion receptors such as integrins that mediate cell-matrix adhesion and also transduce signals into cells. However, ECM proteins also bind soluble growth factors and regulate their distribution, activation, and presentation to cells. As organized, solid-phase ligands, ECM proteins can integrate complex, multivalent signals to cells in a spatially patterned and regulated fashion. These properties need to be incorporated into considerations of the functions of the ECM.

All cells make close contact with the extracellular matrix (ECM), either continuously or at important phases of their lives (for instance, as stem or progenitor cells or during cell migration and invasion). The ECM is well known for its ability to provide structural support for organs and tissues, for cell layers in the form of basement membranes, and for individual cells as substrates for migration. The role of the ECM in cell adhesion and signaling to cells through adhesion receptors such as integrins has received much attention (*1*–*3*), and, more recently, mechanical characteristics of the matrix (stiffness, deformability) have also been recognized to provide inputs into cell behavior (*4*, *5*). Thus, ECM proteins and structures play vital roles in the determination, differentiation, proliferation, survival, polarity, and migration of cells. ECM signals are arguably at least as important as soluble signals in governing these processes, and probably more so. Here, I will emphasize different contributions of the ECM and ECM proteins to cell and tissue behavior, namely their roles in binding, integrating, and presenting growth factor signals to cells.

The Complex Domain Structures of ECM Proteins

There are hundreds of ECM proteins encoded in vertebrate genomes. Many of the genes are ancient, such as those composing the basement membrane toolkit (type IV collagens, laminins, nidogen, perlecan, and type XV/XVIII collagen), which is found in most metazoa, and one can argue that basement membranes were crucial to the evolution of metazoa (*6*). However, many vertebrate ECM proteins and genes evolved much more recently, during evolution of the deuterostome lineage, and that expansion includes not only elaboration of preexisting families (for example, laminins and collagens) but also novel proteins [e.g., fibronectins (FNs) and tenascins]. What purposes are served by this proliferation of ECM proteins? ECM proteins are large and complex, with multiple distinct domains, and are highly conserved among different taxa (Fig. 1). It is not necessary for proteins to be large or complex to generate strong, stable fibrils—intermediate filament proteins and type I collagen provide notable examples to the contrary. So why are most ECM proteins so large, complex, and conserved? Many ECM proteins have dozens of individually folded domains, but in most cases, we understand the functions of only a few of them. What is the purpose of the other domains? The conserved domains are arranged in specific juxtapositions, sometimes controlled by highly regulated alternative splicing. The clear implication is that the specific domains and architectures of ECM proteins contain information of biological importance and evolutionary value. This article will explore that hypothesis in light of recent discoveries concerning representative ECM proteins.

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ECM Proteins and Growth Factor Signaling

One long-standing idea is that the ECM binds growth factors, which is certainly true. Many growth factors [e.g., fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs)] bind avidly to heparin and to heparan sulfate, a component of many ECM proteoglycans (PGs). Hence, a generally held view is that heparan sulfate PGs act as a sink or reservoir of growth factors and may assist in establishing stable gradients of growth factors bound to the ECM; such gradients of morphogens play vital roles in patterning developmental processes. It is also often proposed that growth factors can be released from the ECM by degradation of ECM proteins or of the glycosaminoglycan components of PGs. Those models place the ECM in a distal role, acting as localized reservoirs for soluble growth factors that will be released from the solid phase to function as traditional, soluble ligands. However, some growth factors actually bind to their signaling receptors with heparan sulfate as a cofactor. The binding of FGF to its receptor (FGFR) depends on a heparan sulfate chain binding simultaneously (*7*), and transforming growth factor–β (TGF-β) ligands bind first to integral-membrane PGs that "present" these ligands during signaling (*8*); effectively they act as solid-phase ligands. Such phenomena may well be more widespread than the few, well-studied examples that are currently known. There are also increasing numbers of examples of growth factors binding to ECM proteins themselves, without the involvement of glycosaminoglycans, supporting the notion that the presentation of growth factor signals by ECM proteins is an important part of ECM function.

There are several related concepts that need to be kept separate in thinking about and analyzing functions of the ECM in signaling to cells. First, standard ECM receptors, such as integrins and discoidin domain tyrosine kinase receptors, are themselves signal transduction receptors. Their ligands are specific domains and motifs embedded in the ECM proteins, and ECM-integrin interactions lead to signal transduction responses that are at least as complex and important as those triggered by soluble ligands such as EGF, platelet-derived growth factor, and VEGF (*1*–*3*). Second, and less clearly, there are numerous reports of "cross talk"

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