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mRNA Delivers the Goods

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MOLECULAR BIOLOGY

mRNA Delivers the Goods

In bacteria, RNAs contribute to the proper localization of the proteins they encode.

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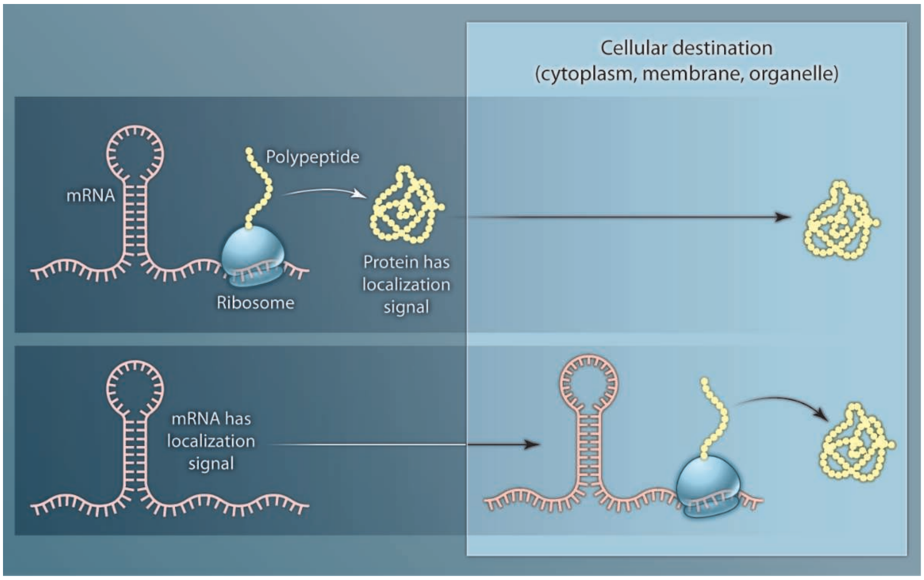
The ability to sort proteins to specific regions within a cell is conserved in all domains of life, even in structurally simple life forms like bacteria (*1*). Regardless of the organism, two parameters dictate how a protein arrives at its proper destination: The location harbors a unique feature that distinguishes it from other subcellular sites; and a specific signal, typically embedded within the protein itself, directs the protein to its target and is recognized at its proper destination (*2*). On page 1081 in this issue, Nevo-Dinur *et al*. (*3*) report that in the bacterium *Escherichia coli*, the localization signal for some proteins may not be contained solely within the protein, but instead can lie within the mRNA that encodes the protein (see the figure).

Nevo-Dinur *et al*. focused on mRNAs encoding proteins that either remain in the cytosol or are inserted into the plasma membrane of *E. coli*. They monitored mRNA localization in live cells by fusing mRNAs of interest with a specific nucleotide sequence that is recognized by an RNA-binding protein that is itself fused to green fluorescent protein. The engineered RNA molecules produced functional proteins that could be tracked in live cells by fluorescence microscopy. The authors observed mRNAs encoding membrane proteins at the periphery of the cells, and those encoding soluble proteins in a helical pattern in the cytosol. Biochemical analysis of fractionated cell lysates confirmed these locations.

According to the well-established signal peptide hypothesis, shortly after a ribosome begins synthesizing an integral membrane protein, translation of the corresponding mRNA temporarily arrests. The ternary complex, consisting of the ribosome, mRNA, and the nascent polypeptide, is then recruited to the cell's secretion machinery; in bacteria, this resides at the plasma membrane. Translation arrest is then relieved and the polypeptide is inserted into the membrane as synthesis resumes. Was the mRNA that Nevo-Dinur *et al*. detected at the cell's periphery simply a passive passenger of the ternary complex during its trip to the plasma membrane? The authors inhibited translation to see what the fate of the mRNAs would be, either by adding translation-arresting antibiotics or by mutating the mRNA to prevent translation initiation. Even in the absence of translation, the mRNAs still localized to the ultimate destination of their encoded proteins.



Where to go? Proteins have been thought to harbor all the information that is required for localizing to their proper destination, whereas mRNAs serve only as templates for protein synthesis by the ribosome. In the bacterium *E. coli*, mRNAs also go to the destination of the proteins they encode, independent of the ribosome or protein synthesis. This suggests that mRNAs harbor information contributing to proper protein targeting in the cell.

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A polycistronic mRNA encodes multiple open reading frames, and each is independently translated into its corresponding protein. Nevo-Dinur *et al*. examined the localization of polycistronic transcripts that encode both membrane proteins and cytosolic proteins. Mutational analysis indicated that a single open reading frame encoding a membrane protein was necessary and sufficient to direct a polycistronic transcript to the membrane, even if it also encoded cytosolic proteins. They also mapped the element responsible for membrane localization within a region of the mRNA that encodes the membrane-spanning domain of the protein. Taken together, the results indicate that mRNAs in *E. coli* contain information that contributes to localizing their corresponding protein products in the cell (cytosol, membrane, or cell poles).

Curiously, as early as 1975, mRNA molecules were detected in eukaryotic cells that remained associated with endoplasmic reticulum membranes, even after their associated ribosomes were removed from the ER (*4*). As a result, a model was considered in which association of mRNA with the ER membrane could direct ribosomes to the secretion machinery. However, subsequent overwhelming evidence for the signal peptide hypothesis suggested that information encoded within the proteins themselves is sufficient for proper localization and thus did not absolutely require a localization signal harbored within the mRNA (*5*, *6*). As a result, years of subsequent research focused largely on proteins that localize to specific regions in the cell and the factors that recognize them. More recent studies, though, have revived models that invoke a more active role for mRNAs in protein localization. For example, in yeast, mRNAs encoding proteins that are destined for secretion via the ER may be exported from the nucleus solely by virtue of encoding a signal peptide sequence, even in the absence of a nuclear export complex (*7*). Similarly, the 5′ sequence of mRNAs that encode proteins secreted by the bacterial type III protein secretion machinery have been implicated in harboring information required for secreting the proteins they encode (*8*). Nevo-Dinur *et al*. now suggest that mRNA

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signals may be found in widely divergent protein localization pathways, not just during protein secretion.

Additional work will reveal whether mRNA signals represent a general protein localization strategy or if they remain exceptional examples. For example, a recent report revealed that several transcripts in *E. coli* and *Caulobacter crescentus* also localize specifically, but that they remained very close to the chromosomal site where they were synthesized (*9*). In any case, the observations that mRNA molecules localize specifically within a bacterium predict the existence of pathways that mediate this localization, and these components will need to be identified. It appears that mRNA can contribute not simply to encoding proteins, but to delivering them as well.

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PSYCHOLOGY

Science Starts Early

Infants and children grasp surprisingly sophisticated correlational and causal patterns.

Frank C. Keil

Infants and young children can exhibit striking confusion about how the world works, from failing to grasp that wind causes waves, to being mystified about how babies are created. Indeed, some researchers have characterized a child's knowledge of the world as a bundle of misconceptions awaiting replacement with correct concepts through education (*1*).

Evidence is mounting, however, that young children are often quite adept at uncovering statistical and causal patterns and that many foundations of scientific thought are built impressively early in our lives. This growing understanding of how children acquire many of the thinking skills used in science has implications not only for education but also for understanding how all of us make scientific progress in the face of ignorance and incomplete knowledge.

For cognitive psychologists, scientists have long presented an intriguing puzzle. Whether a biologist or a geologist, scientists routinely, and with seeming ease, call upon a diverse set of cognitive skills to do their jobs. They detect correlations, often between seemingly unrelated phenomena. They infer causation from these correlations. If all goes well, they uncover the mechanisms that explain it all—and then share their knowledge and build upon it by acquiring knowledge from others.

Each of these abilities has early origins. Consider, for example, how children respond to the challenge of noticing correlations as they encounter them in the flow of experience. For instance, an infant learning language, upon hearing streams of syllables, not only has to notice how often certain syllables occur but also needs to infer higher-order patterns arising from those syllables. One study (*2*) showed that 5-month-old infants can handle this challenge by rapidly tracking not only the sounds of the syllables but also visual patterns associated with each syllable. In the experiment, infants looking at a computer screen were repeatedly presented with abstract patterns of syllables and shapes. An "ABB" pattern, for instance, could be represented by certain shapes corresponding to the syllables "di ga ga." When presented with a new pattern (ABA) with new syllables—such as "le ko le"—the infants looked longer at the shapes on the screen than if the new syllables were in the old ABB pattern. This suggests that they recognized it as a new, unfamiliar correlation.

Other research (*3*) has found that 6-month-olds can take the next step and infer causation from certain kinds of correlations. In these experiments, researchers measured how long infants looked at animations showing "collisions" of shapes. In some animations, one object "launched" a second one, causing it to move, as when two billiard balls collide. When shown animations in which the balls reversed roles, infants looked longer at the new pattern than at the original one. They did not react as strongly, however, when the original and reversed animations contained half-second gaps between the moment when the first object stopped moving and the second one started to move. This suggests that the infants recognized these events as noncausal, or unrelated.

Later studies showed that infants make causal interpretations by integrating information in ways that closely mirror adults. For instance, they will form causal interpretations based on information that is collected in brief time windows after the occurrence of the critical event (such as a possible collision) (*4*); these post-event decision-making windows are similar to those measured in adults. Thus, it appears that certain sequences of events automatically elicit thoughts of causation at all ages.

Older infants expand on these inferences of causality to sense more abstract and subtle causal relationships. In one recent study (*5*), 11-month-olds were shown animations of two sets of blocks: one initially ordered into a neat array, the other scattered into disorder. Then, a screen obscured the blocks and either a lifelike "animate" agent appeared, such as an object with a face, or an "inanimate" agent, such as a ball. Finally, the screen was removed, revealing that either an orderly stack of blocks had become disordered or the opposite. By measuring how long the infants looked at various combinations, the researchers concluded that the infants learned that only the animate object could cause disordered blocks to become orderly but that both the animate and inanimate agents could scatter an orderly pile.

Once out of infancy, children become able to examine more complex networks of correlations to infer causal patterns, including hidden ones, and they readily do this using sample sizes too small for traditional statistical tests of significance. They are particularly sensitive to the usefulness of "intervening on a system"—or manipulating conditions—to separate causal links from those that are just correlational. For example, when confronted with a novel box consisting of gears and a switch, preschool children are easily able to figure out cause-and-effect relations, and rule out mere cor-

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