PERSPECTIVES: CELL BIOLOGY

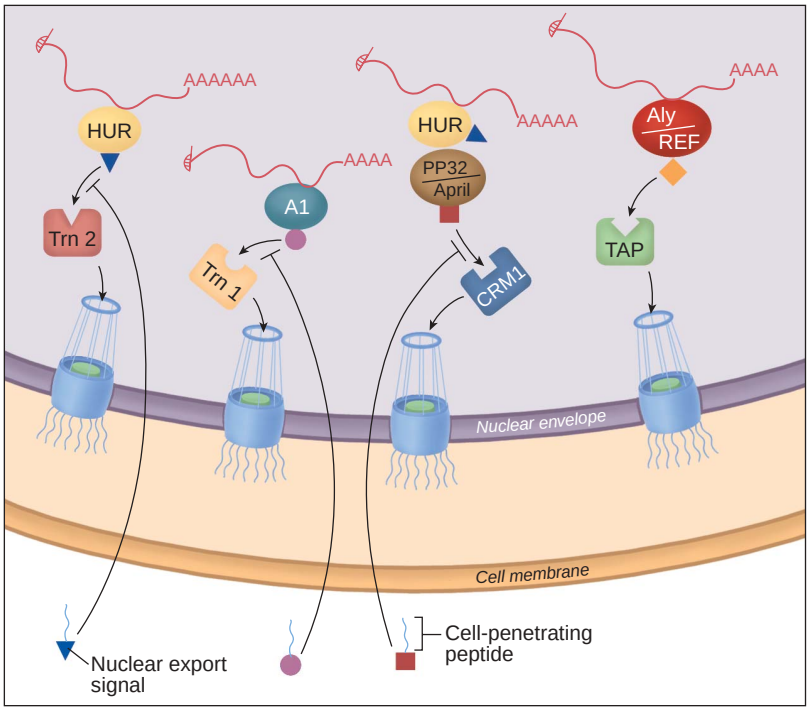
TAPping into mRNA Export

Melissa J. Moore and Michael Rosbash

For researchers trying to elucidate how one protein activity or intermolecular interaction contributes to a complex metabolic pathway, a specific chemical inhibitor can be an invaluable tool. Yet, in living cells, disrupting a single activity is often insufficient to elicit a discernible response. The reason is that many metabolic pathways exhibit functional redundancy—that is, they are made up of multiple interwoven activities that can, at least in part, compensate for one another. Deconvoluting the intricacies of such redundant pathways usually requires disrupting two or more activities at once. For this purpose, a panel of inhibitors, each specific for one component of the pathway, can be priceless. On page 1895 of this issue, Gallouzi and Steitz (*1*) describe the rational design of an inhibitor panel that targets three different routes of messenger RNA (mRNA) export from the nucleus to the cytoplasm of living mammalian cells. They prepared their panel by attaching known nuclear export signals to cell-penetrating peptides (*2*–*6*). This approach to inhibitor design holds the promise of being widely applicable to almost any intracellular activity that relies on transient protein-protein interactions.

Cell-penetrating peptides are short (11 to 34) amino acid sequences with a phenomenal ability to get inside cells. When added to the culture medium, such peptides enter cells incredibly quickly, often achieving maximum intracellular concentrations within 15 minutes. Typical working concentrations are 25 to 200 nM, and cellular transduction efficiencies of 100% are not uncommon. With the exception of yeast, all eukaryotic cells appear to be susceptible, including those difficult to transfect by other means. Although the exact mode of entry remains obscure, it is likely to involve either "inverted micelle" formation or direct membrane penetration, rather than classic uptake pathways such as endocytosis. Peptides possessing cell-penetrating activity include fragments of human NF-κB p50, HIV-1 Tat protein, and the *Drosophila* ANTP (*antennapedia*) transcription factor.

What makes cell-penetrating peptides so useful is their capability for carrying cargo. Their cargoes range from small chemical compounds, oligonucleotides, peptides and peptide–nucleic acids to large proteins (>1000 amino acids long), plasmids, and even iron nanoparticles 40 nm in diameter (*2*–*6*). Perhaps even more remarkable is the finding that the Tat peptide transports the enzyme β-galactosidase across the blood-brain barrier in living mice without otherwise compromising this key interface (*5*). Several groups have recently created new intracellular inhibitors by fusing cell-permeable peptides to key protein-protein interaction domains (*2*, *5*, *7*, *8*). When supplied outside of their normal context, such domains compete with their endogenous, full-length counterparts to disrupt functional interactions with other proteins. It is this approach that Gallouzi and Steitz (*1*) have now applied to the study of mRNA export.



A nuclear exodus. Inhibition of specific mRNA export pathways with cell-permeable peptides. The major route of mRNA export from the nucleus may depend on interactions between the mRNA adaptor protein Aly/REF and the export receptor heterodimer TAP:p15, which in turn interacts with the nuclear pore complex. However, new inhibitors that couple cell-penetrating peptides with specific nuclear export signals reveal that some mRNAs use other adaptors and receptors. For example, there are two nuclear export pathways for *c-fos* mRNA. One pathway involves the adaptor protein HuR and the export receptor Trn2, whereas the other involves HuR, its two ligands (pp32 and APRIL) and the export receptor CRM1.

During the past few years, the field of mRNA export has seen striking progress (*9*–*11*). In both yeast (*Saccharomyces cerevisiae*) and metazoan systems, a conserved and generally accepted view has emerged. The major export receptor is TAP (Mex67p in yeast), which connects messenger ribonucleoprotein particles (mRNPs) to nuclear pores. TAP is not itself a strong RNA-binding protein, but interacts predominantly with other components of the mRNP, described as adaptor proteins in the Gallouzi and Steitz paper (*1*). The most conspicuous adaptor protein is Aly/REF (Yra1p in yeast). There is also a new conserved player in the export business, the RNA helicase UAP56 (Sup2p in yeast) (*12*–*15*). Although its biochemical role seems enigmatic, UAP56 manifests a strong interaction with Aly/REF and may contribute to RNP formation or remodeling at an early step of mRNA export.

The prominent part played by the export receptor TAP was unexpected, because the transport field had initially assumed that the key export receptor would be one or more members of the well-studied importin-β family (*9*–*11*). One such family member, CRM1, was the first (and for a while, the only) RNA export receptor to be identified. CRM1 recognizes leucine-rich nuclear export sequences (NES) on the HIV protein Rev, an adaptor protein that

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binds to a structured region of RNA within an HIV intron and thereby facilitates export of HIV pre-mRNA. However, CRM1 appears not to be the major export receptor for cellular mRNAs, because bulk mRNA export is largely unaffected by leptomycin B, a small-molecule inhibitor of CRM1 activity. In contrast, NES-mediated protein export, Rev-mediated RNA export, and small nuclear RNA (snRNA) export are all completely inhibited by leptomycin B. This suggests that redundancy (that is, the presence of another protein with activity identical to that of CRM1) does not explain the lack of an effect of leptomycin B on bulk mRNA export.

Leptomycin B binds tightly, and probably covalently, to CRM1 and is a rapid and potent inhibitor with minimal known side-effects—a dream for cell biologists. However, because no other effective small-molecule inhibitors have been identified, and an excellent in vitro system for export has yet to be developed, RNA export has remained a largely "in vivo" field. It is from this perspective that the paper by Gallouzi and Steitz (*1*) is so valuable. In addition to defining the tasks of new receptors and adaptors for the export of specific mRNAs and raising the profile of CRM1, the authors describe a strategy that should permit the design of many more small-molecule inhibitors. As a demonstration of the power of their approach, they show that there are two pathways for the export of c-*fos* mRNA (see the figure). In one pathway, the HuR adaptor protein interacts with the transportin export receptor, and in the other, HuR interacts with its ligands (pp32 and APRIL) and the CRM1 export receptor.

In principle, because cell-penetrating peptide inhibitors should inhibit their target proteins rapidly, they are superior to other "functional knockout" approaches such as RNA interference (RNAi). RNAi prevents translation of a protein by destroying its mRNA; however, a long-lived protein will continue to be active long after its synthesis has stopped. The more rapidly a peptide affects RNA export, the more likely it is that the peptide directly (rather than indirectly) inhibits export of the target RNA. This logic has been applied to the interpretation of yeast temperature-sensitive export mutants: In some cases, mRNA retention is detectable within 5 minutes of a shift to the nonpermissive temperature, suggesting that the wild-type proteins contribute directly to mRNA export. From this perspective, it is conceivable that these designer inhibitory peptides will generate a mammalian cell tool kit that will parallel the temperature-sensitive mutant collection available to yeast geneticists.

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PERSPECTIVES: NATURAL PRODUCT SYNTHESIS

The Art of Total Synthesis

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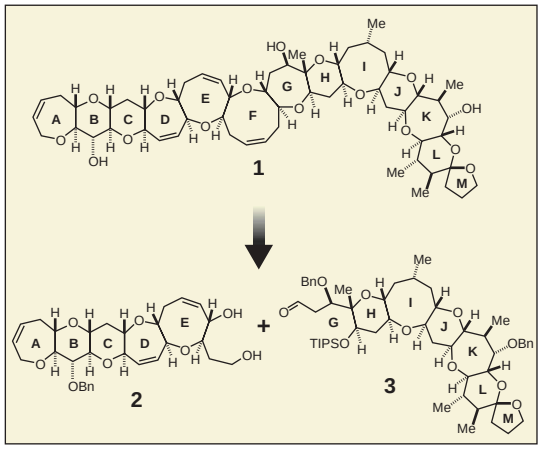
The total synthesis of complex natural products remains the most difficult, daunting, and challenging endeavor in organic chemistry. It is also the most humbling, exhilarating, and formative entreprise in our science. The sizes and complexities of the natural products synthesized today bear no resemblance to the substrates that were targeted in the beginning (*1*). The assembly of complex natural products has stimulated the development of powerful synthetic methodologies that enable organic chemists to build, in a shorter time and more efficient manner, structures of previously undreamed complexity. The desire to imitate nature has led to the discovery and establishment of powerful biomimetic approaches, as exemplified by the Johnson synthesis of steroids (*2*).

The remarkable synthesis of vitamin B12 by Eschenmoser (*3*) and Woodward (*4*) marks the start of the modern natural product synthesis. Before this work, organic synthesis was performed primarily to nail down the structure of particular molecules. But the structure of vitamin B12 was known through the pioneering crystallographic work of Dorothy Crowford Hodgin. The emphasis thus shifted to exploring new synthetic routes to make this complex material from simple starting materials.

The synthesis took 11 years and involved more than 90 separate reactions performed by over 100 co-workers. The stereochemical puzzles involved in the synthesis led to the Woodward-Hoffman rules, which spell out how the electronic structures of molecules reorganize during reactions. The vitamin B12 synthesis revolutionized theoretical chemistry, and the Woodward-Hoffman rules paved the way to the use of orbital theory by the chemical community (*5*).

The next milestone in organic chemistry was the discovery by Barton that organic molecules could be assigned a preferred conformation and that the chemical and physical properties of a molecule could be interpreted in terms of that preferred conformation (*6*). This discovery helped to guide synthetic pathways. Retrosynthetic analysis, which entails going backward from a target molecule to starting materials, was introduced by Corey and Cheng (*7*) and its relevance demonstrated by a number of exquisite total syntheses. The advent of organometallic chemistry and the realization that metal complexes could perform unique transformations resulted in a major leap forward in the complexity and size of the molecules that could be prepared (*8*). Analytical tools have evolved in parallel. For example, powerful nuclear magnetic resonance spectrometers can now routinely detect



The key strategic disconnection.

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