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MINIREVIEW

Nascent Peptide Regulation of Translation

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INTRODUCTION

Translation of mRNA into protein requires the processive decoding of mRNA triplets into amino acids with concurrent formation of peptide bonds. These interdependent events are mediated by the ribosome in association with other cellular components. The complexity of the translation process makes it a likely candidate for regulation at multiple points. Moreover, the existence of small molecules, e.g., antibiotics, that can perturb specific steps in translation argues for the availability of these same steps as targets for regulation. Given this background, it is remarkable that most known examples of translational regulation apparently do not involve altering the catalytic properties of the ribosome. For instance, one major class of regulation involves a mechanism(s) that prevents translation initiation by the binding of specific proteins to critical segments within specific transcripts (7, 10, 24). In another example, the sequence of the mRNA can "confuse" the decoding process, forcing translation to assume an alternative reading frame (2, 4, 18, 30).

Regulation of translation of specific transcripts by the intervention of small, ribosomally targeted *trans*-acting effector molecules is apparently unknown. A reason for this may be that ribosomes constitute a seemingly homogeneous class of cellular macromolecule. A *trans*-acting effector that is active on one ribosome would likely modify many ribosomes in a cell, defeating this strategy as a means of achieving selective alteration of gene expression. On the other hand, *cis*-acting effectors have the potential to induce changes that are unique to the ribosome that is translating a particular transcript. During the past five years, three unrelated examples of *cis* regulation of ribosome function have been described. In each, the effector or co-effector is the nascent peptide (Fig. 1).

TRANSLATION ATTENUATION OF CHLORAMPHENICOL RESISTANCE

Inducible resistance to chloramphenicol in gram-positive bacteria (the *cat* genes) and that due to transposon Tn*1696* (the *cmlA* gene) result from a variation of the attenuation regulatory model termed translation attenuation (5, 20, 21, 28). In both systems, the ribosome binding site for the resistance determinant is sequestered in an RNA secondary structure where it is apparently unavailable for translation initiation (13, 15). Chloramphenicol induces *cat* and *cmlA* translation by stalling a ribosome at a specific site in the translated leader that precedes the domain of secondary structure. For *cat* genes, significant induction occurs only when a ribosome is stalled at leader codon 6 (1). For *cmlA*, translational activation is due to ribosome stalling at leader codon 9 (12).

The inducer of *cat* and *cmlA*, chloramphenicol, inhibits translation elongation at seemingly random sites on mRNA and is an unlikely candidate to provide site specificity to ribosome stalling. Rather, stalling specificity is due to leader codons that precede the stall site (26). These codons specify a 5-mer (for *cat*) or an 8-mer (for *cmlA*) peptide that inhibits peptidyl transferase, the ribosome-associated activity that forms peptide bonds (11, 12, 14). Synthesis of the inhibitory peptides requires translation to the same leader site where ribosome stalling activates *cat* or *cmlA* expression. Thus, the peptides apparently select the site of stalling by *cis*-inhibiting peptidyl transferase. The *cat* 5-mer and the *cmlA* 8-mer bring about significant alterations in the secondary structures of bacterial 23S rRNA and yeast 26S rRNA, suggesting that other activities associated with large-subunit rRNA may also be perturbed by the peptide inhibitors (16). Thus, it is perhaps not surprising that the *cat* 5-mer is also an effective inhibitor of translation termination in vivo and in vitro (23, 27).

RIBOSOMAL BYPASS DURING THE TRANSLATION OF BACTERIOPHAGE T4 TOPOISOMERASE

Gene 60 of phage T4 contains an internal 50 nucleotides of contiguous, untranslated sequence (17). Unlike several other T4 genes involved in DNA metabolism, the insert in gene 60 is not removed by splicing. Rather, the ribosome "hops" over the insert, carrying with it a nascent peptide of 50 residues. The efficiency of the hop approaches 100%, as determined by comparing the level of expression of the wild-type gene with that of a gene with the insert deleted (31). Hopping depends on specific features of the mRNA. Notable are the sequence identity of the "take-off" and "landing" codons and a requirement for a short region of secondary structure at the 5′ end of the untranslated insert. Hopping additionally depends on a sequence of amino acids upstream from the take-off site (31). Amino acids 17 through 45 appear to be most critical, and perhaps not coincidentally this approximates the number of amino acids that can be protected by a translating ribosome.

A plausible analogy has been drawn between a possible role of the nascent peptide in hopping and the antibiotic action of edeine, a basic oligopeptide (31). Edeine blocks P site binding of tRNA. It is conceivable that the nascent peptide might have a similar function, since dissociating the peptidyl tRNA from its attachment with mRNA would seem a likely early and essential event in hopping. Ribosome hopping is genetically "silent," and therefore its detection requires a very careful comparison of gene and protein sequences. Only a single additional example of ribosome hopping has been suggested, but this system seems very different from the T4 example and a role for the nascent peptide has not been proposed (3).

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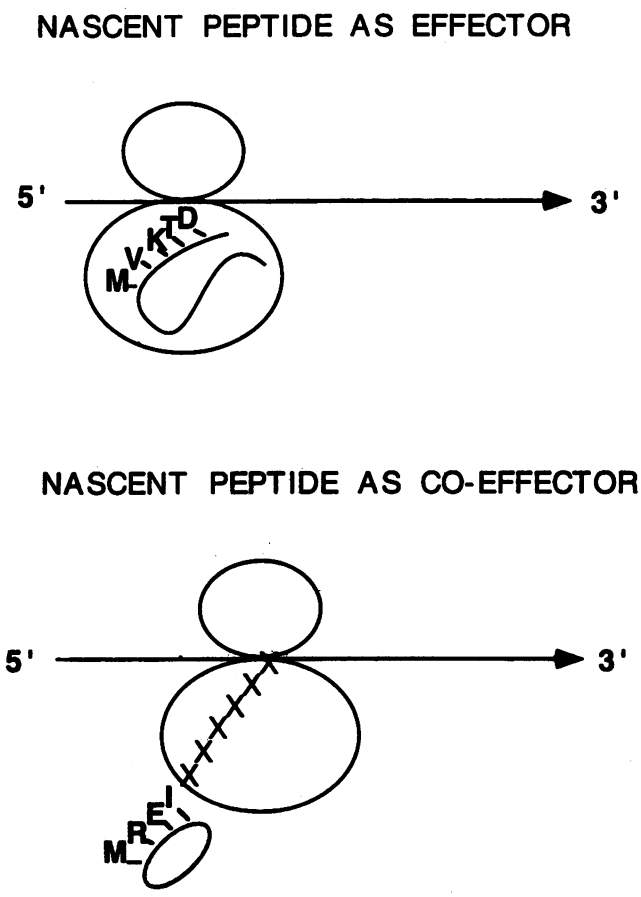

FIG. 1. Two proposed mechanisms of action of nascent peptides in translational regulation. The nascent peptide as effector shows the hypothetical role played by the *cat* leader 5-mer peptide; this model also applies to the *cmlA* leader 8-mer peptide. Both are suspected to interact directly with 23S rRNA since the peptides alter the secondary structure of domains IV and V of phenol-purified RNA (16). The T4 topoisomerase 50-residue nascent peptide probably acts as an effector, although its target in the ribosome is not known. The β-tubulin tetrapeptide acts as a coeffector and brings about mRNA decay through a subsequent event that is triggered by MREI contacting tubulin subunits, shown as a small oval, on the ribosome's surface.

AUTOREGULATED INSTABILITY OF β-TUBULIN mRNA

A very different regulatory role exists for the nascent peptide translated from β-tubulin mRNA. The first four codons of the β-tubulin coding sequence specify a peptide, MREI, that apparently is capable of recognizing free tubulin subunits in the cell (32). High levels of the subunits allow an interaction with the nascent tetrapeptide at the surface of the ribosome that provokes a decrease in the stability of β-tubulin mRNA. Autoregulation due to MREI is also observed when the corresponding codons are fused to the N terminus of a heterologous coding sequence and is lost when the MREI codons are placed internally in the coding sequence (32). Missense mutations in the MREI codons can abolish autoregulation, whereas synonymous codon changes have no effect. Ribosome movement along the transcript is required for autoregulation, suggesting that the events needed for degrading mRNA may depend on concurrent elongation (8).

MREI emerges from the protection of the ribosome after 40 to 45 codons of β-tubulin mRNA have been translated (32). Thus, when MREI appears on the ribosome's surface the codons being translated differ between authentic β-tubulin mRNA and the chimeric MREI-thymidine kinase mRNA. Consequently, it is likely that the interaction between MREI and tubulin subunits determines mRNA instability and not the nature of the codons being translated at the time of the MREI-tubulin interaction. It remains to be determined if the event that triggers mRNA decay is solely the MREI-tubulin interaction or the physical association of tubulin subunits with the ribosome's surface. If the latter were correct, MREI would possibly function primarily as a ligand.

Translation attenuation, ribosome hopping, and autoregulation due to mRNA instability represent three well-documented examples in which the amino acid sequence of the nascent peptide has an active role in translational regulation. In each system, the biologically active peptide exerts its effects only on its translating ribosome, i.e., it exerts a *cis* effect. Genetic studies of other translationally modulated gene systems indicate that nascent peptide effects may also contribute to the observed regulation (6, 9, 19, 22, 29). However, more subtle applications of *cis*-acting peptides could actually be more common, perhaps influencing rates of translation and determining translational pause sites (25). An understanding of the mechanisms that allow the regulatory peptides to provoke a change in ribosome function may aid in correlating ribosome function with structure.

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