Nascent peptide in the ‘birth canal’ of the ribosome

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All proteins assembled by the ribosome must pass through the nascent-peptide exit tunnel. Some nascent peptides can specifically interact with the tunnel and affect the functions of the ribosome. The molecular mechanisms of such interactions and of the ribosome response are currently unknown. However, a recent study has revealed elements of the tunnel that might be involved in sensing the nascent-peptide sequence.

Nascent-peptide exit tunnel
Assembly of proteins takes place at the ribosome peptidyl-transferase center, at the interface of the large and small ribosomal subunits. On the way out, all of the newly assembled polypeptides, large and small, hydrophobic and hydrophilic, positively charged and negatively charged, must pass through the ribosomal ‘birth canal’, the nascent-peptide exit tunnel. Starting at the peptidyl-transferase center and ending at the solvent side of the large ribosomal subunit, the exit tunnel defines the path for the infant polypeptide out of the ribosome (Figure 1a).

Because the tunnel must enable the passage of different types of polypeptides, it must be promiscuous. The tunnel walls, which are built primarily of RNA, are (in general terms) non-sticky: they are lined with hydrated polar groups and lack any large hydrophobic patches [1]. Promiscuous, however, does not mean indifferent. A growing body of evidence indicates that some polypeptides can specifically interact with the tunnel, and that these interactions might affect translation by stalling the ribosome on its tracks on mRNA [2–4]. The elements of the bacterial ribosomal tunnel that are involved in such interactions are explored in the recent elegant paper from the laboratories of Yanofsky (Stanford University; www.stanford.edu) and Squires (Tufts University School of Medicine; www.tufts.edu/med) [5].

The existence of the exit tunnel in the ribosome was suggested long ago [6], and biochemical experiments have traced its path through the ribosome [7]; but it was the advent of cryo-electron microscopy and ribosome crystallography that clearly showed its existence [1,8]. Although no one has, as yet, been able to see a nascent peptide in the exit tunnel at a high resolution, there is little doubt that the hole seen in the body of the large ribosomal subunit represents the route of the nascent peptide on its way out of the peptidyl-transferase center [9].

At approximately one-third of the tunnel length away from the peptidyl-transferase center (20–35 Å), the nascent peptide reaches a constriction formed by the tunnel walls. The extensions of two ribosomal proteins, L22 and L4, are exposed here in the lumen from opposite walls of the tunnel, restricting the aperture even further. In bacteria, the region of the tunnel between the peptidyl-transferase center and the constriction seems to be the most crucial for the functional interactions of the ribosome with the nascent peptide. In eukaryotes, more remote segments of the tunnel might also be involved in the ribosomal response to the nascent peptide [10,11].

Ribosome stalling on the TnaC nascent peptide
Work in the Yanofsky laboratory has been focused on the investigation of the Escherichia coli tna operon, which encodes the enzymes involved in tryptophan metabolism.

Expression of the tna operon is regulated by translation of a 24-codon tnaC cistron [12]. Stalling of the ribosome at the last sense codon of tnaC, which occurs in the presence of a high concentration of tryptophan, occludes the rho-dependent transcription terminator and activates the expression of functional genes of the operon. The most spectacular observation was that the stalling depends on interaction of the 24-amino acid TnaC nascent peptide with the ribosome [13]. However, the molecular mechanisms of stalling are unclear. The model put forward by Gong and Yanofsky [13] postulates that specific interactions of the nascent peptide with the exit tunnel generate a tryptophan-binding site at, or near, the A-site in the large ribosomal subunit. Binding of tryptophan at this site might hinder functions of the peptidyl-transferase center, thus preventing termination and release of the nascent TnaC peptide from the tRNA. Whatever the actual molecular mechanism of the ribosome stalling, two of the most interesting questions that need to be addressed are which features of the nascent peptide are responsible for specific interactions with the tunnel and which elements of the tunnel serve as a nascent-peptide sensor.

Mutational analysis has shown that two positions in the TnaC peptide, Pro24 (the C-terminal proline) and Trp12 are important for the ribosome stalling, whereas N-terminal amino acids are not essential [13]. In the stalled complex, the nascent peptide remains linked to the tRNAPep and, thus, any possible functional interactions of the nascent peptide with the ribosome must take place in the segment of the tunnel that is proximal to the peptidyl-transferase center. A delightful combination of biochemistry and genetics has enabled researchers to define the tunnel elements that are involved in the interaction with the TnaC-stalling nascent peptide [5]. In the stalled complex, Lys11, a residue preceding the crucial Trp12 of the TnaC nascent peptide, could be crosslinked to A750 in the loop of helix 35 of 23S rRNA (Figure 1b). A mutation

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shows that, like in most other ribosome functions, rRNA might have an important role in interactions with the nascent peptide. However, the proteins that contribute to the tunnel architecture are also important. Most notably, mutations near the tip of the $\beta$-hairpin of protein L22 – specifically those changing the identity of Lys90 – have the most dramatic effect on stalling. Crystallographic studies of large ribosomal subunits have shown that the $\beta$-hairpin of L22 is flexible and can shift its placement in the tunnel, which is compatible with its possible role as a component of the nascent-peptide-sensing mechanism [14,15].

Findings by the Yanofsky and Squires teams hint that the exact position of the nascent peptide in the tunnel might depend on the peptide sequence. RNA probing has shown that, in the stalled complex, wild-type TnaC nascent peptide protects the rRNA residue A788 in the tunnel from chemical modification. However, when Trp12 of TnaC is mutated to arginine, A788 becomes more accessible, suggesting that the mutant peptide might be placed somewhat differently in the tunnel.

**Stalling on SecM nascent peptide: similar but not the same**

The functional interactions of TnaC with the ribosome provocatively resemble those of the *E. coli* secretion-monitor protein SecM [16]. Stalling of the ribosome during translation of secM is used for the regulation of expression of a downstream secA cistron that encodes important components of the secretion apparatus. Arrest of translation depends on the SecM nascent peptide: the SecM sequence FxxxxWIxxxxGIRAGP$_{166}$ within the ribosome exit tunnel is required for stalling. Mutational studies have revealed the importance of several amino acid positions. Intriguingly, in both TnaC and SecM, stalling occurs at the proline codon, underscoring the importance of the C-terminal proline residue that, in the stalled complex, resides in the peptidyl-transferase active site and is still linked to the tRNA moiety. In addition, in both TnaC and SecM nascent peptides, a tryptophan residue located 11 amino acids (in TnaC) or 10 amino acids (in SecM) from the C-terminal proline is crucial for stalling, which implies the possibility of specific interactions of this residue with the response element(s) of the tunnel. The tunnel components mapped by Nakatogawa and Ito [16] as being important for specific interactions with the nascent SecM peptide overlap, but do not coincide exactly with those that are essential for interaction with the TnaC peptide. Mutations in the loop of helix 35 abolish ribosome stalling at both SecM and TnaC peptides. However, the mutation at A2058, which is also located in the tunnel, has a profound effect on the ribosome response to SecM, but it does not greatly affect the tryptophan-induced stalling during TnaC translation. Furthermore, alterations at neighboring, but different, amino acid positions at the tip of the L22 $\beta$-hairpin affects interactions with the SecM or TnaC nascent peptides. Potentially, different placement of the SecM and TnaC nascent peptides in the exit tunnel might explain the involvement of different rRNA and protein positions in ribosome stalling in these two cases. Other notable differences exist between the ribosome responses to the SecM and TnaC nascent peptides.

in this loop (insertion of an extra adenine residue at position 751) in addition to substitutions at another rRNA residue, U2609, located in the tunnel closer to the peptidyl-transferase center, alleviates stalling. This
case of SecM, stalling interferes with translation elongation, whereas, at tnaC, the ribosome stalls at the last sense codon and fails to terminate translation (even though in the stalled complex, the TnaC peptide is also puromycin unreactive, suggesting some major perturbations of the peptidyl-transferase function).

Concluding remarks
Despite the beauty of the experimental systems used and the elegance of the approaches, the mechanism of the ribosome response to the stalling nascent peptides remains largely obscure. It seems that, in the best-characterized cases, nascent-peptide-dependent translation arrest in bacteria primarily affects the functions of the peptidyl-transferase center. But what is the molecular mechanism of the peptidyl-transferase response? What exactly are the interactions between the specific nascent peptide and the sensor element in the tunnel? How is the signal communicated from the tunnel back to the peptidyl transferase: is it wired through the ribosome or through the nascent peptide? Yet another question, and probably one of the most important: is a sequence-specific ribosome interaction with the nascent peptide a cute exception to the ‘non-sticky’ tunnel rule, or is it a general phenomenon that is involved in the expression of many cellular proteins?

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Chutes and Ladders: the search for protein kinases that act on AMPK

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AMP-activated protein kinase (AMPK), a key regulator of energy homeostasis in mammalian cells, is, in turn, regulated by long-sought upstream protein kinases (AMPKKs). Following the recent identification of the tumor-suppressor kinase LKB1 as an AMPKK, a broader role for AMPK in metabolic economy has been unveiled by a new body of work from three groups that implicates the Ca²⁺/calmodulin-dependent protein kinase kinases as AMPKKs. We suggest that PKE (protein kinase

‘energy’ or ‘economy’) is now an apt name for this kinase, which regulates both cellular and whole-organism energy homeostasis.

Introduction
For more than three decades, the journey to find upstream protein kinases that act on the AMP-activated protein kinase (AMPK) has often felt like a game of ‘Chutes and Ladders’. Chutes and Ladders (also known as ‘Snakes and Ladders’) is a game of rewards and consequences

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