Intraribosomal Regulation of Expression and Fate of Proteins

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Our studies of SecM (secretion monitor) in *E. coli* have revealed that some amino acid sequences can interact with ribosomal interior components, particularly with gate components of the exit tunnel, thereby interfering with their own translation elongation. Such translation arrest can be regulated by interaction of the N-terminal portion of the nascent polypeptide with other cellular components outside the ribosome. These properties of nascent proteins can in turn provide regulatory mechanisms by which the expression of genetic information at different levels is regulated.

1. Every Amino Acid Residue in a Protein Experiences Time Inside the Ribosome

The protein life cycle comprises birth, maturation, travel, interaction with other proteins, and death or degradation. Although a polypeptide molecule is born by continuous chain elongation at the peptidyl-transferase center (PTC) of the ribosome, the site of this creation, the interface region between the small and large subunits of the ribosome, is well partitioned from the congested cytosol. Thus the new peptide sequence first resides within the large subunit particle. It then has to move through the intraribosomal pathway, (the exit tunnel),[1] as it elongates, until it reaches the surface of the large subunit on the opposite side of the PTC (Figure 1).

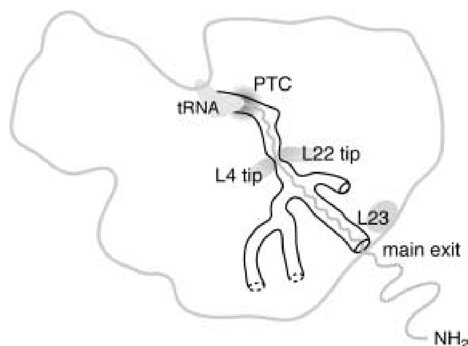

Figure 1. Intraribosomal pathways for nascent polypeptides. The large ribosomal subunit is outlined in gray, the small subunit is omitted. A nascent polypeptide synthesized at the PTC moves through the exit tunnel to emerge from the ribosome. Tips of L4 and L22 are located at the constricted gate of the tunnel. L23 serves as a docking site for a chaperone or a targeting factor.

According to the atomic structure of the *Haloarcula marismortui* ribosome, the exit tunnel is about 100 Å long and its diameter is some 10–20 Å.[2] It has been argued that the dimension of the tunnel precludes tertiary-structure formation of the nascent polypeptide. It is intriguing that the nascent polypeptide segment does not even interact with tunnel wall components. Clearly, if it were otherwise the ribosome could not function catalytically as the general translator of genetic messages.

The PTC-tunnel cavity of the ribosome is essentially composed of RNA. Nissen et al.[2] have argued that the exit tunnel lacks hydrophobic patches so as to minimize interaction with nascent amino acid side chains. However, it was also observed that a portion of the tunnel is significantly constricted. This part of the tunnel is characterized as a place where the tips of two r-proteins, L4 and L22, partially protrude into the cavity (Figure 1), whereas their opposite ends are exposed to the ribosomal surface. What might be the benefit of such a structure for the exit tunnel?

2. The Exit Tunnel May Have Biological Functions

In the past, the biological significance of the ribosomal exit tunnel was not extensively addressed, except for the action of macrolide antibiotics, such as erythromycin, which interact with this part of the ribosome to inhibit protein synthesis.[3, 4] Gavashvili et al.[5] observed three additional conduit-like structures that branched from the main tunnel (Figure 1) and suggested the tempting possibility, that these structures are used differentially by different gene products according to their fate outside the ribosome. Tenson and Ehrenberg[6] summarized observations reported in the literature, suggesting that some nascent polypeptide sequences inside the ribosome are subject to elongation arrest, and that such an intraribosomal process is used for expression regulation of proteins encoded by multicistronic messenger RNA. Some intraribosomal sequences of nascent peptides determine whether the protein is completed with or without a SsrA tag at the C terminus.[7] Our studies on *E. coli* SecM, a secretion monitor protein, provide the best-

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characterized case in which a nascent sequence interacts with tunnel components as a means of gene expression regulation. [8, 9]

3. SecM Contains an "Arrest Sequence"

Expression of *secA*, which encodes the protein-translocating ATPase in *E. coli*, is controlled at the translation level by the protein export proficiency of the cell. It is up-regulated in response to a secretion defect.[10] An open reading frame *X* (now called *secM*), located upstream of *secA* in the same transcription unit, was known to be involved in the regulation of *secA* expression.[11] We characterized its product, SecM, and showed that it is exported to the periplasm. However, it does not function there as it is rapidly degraded by periplasmic proteases such as Tsp.[8] SecM is an unusual protein, which executes its biological role only in the nascent state with its C-terminal region still embedded in the ribosome.

In fact, our studies established that SecM translation undergoes elongation arrest at Pro166, the fifth codon before the *secM* termination point (Figure 2A), leading to accumulation of a peptidyl-tRNA form of $SecM_{1-166}$.[8, 9] Detailed mutation studies identified the sequence, FXXXXWIXXXXGIRAGP, which is required and sufficient to cause translation arrest during elongation.[9] The arrest sequence works as an independent translation-arresting element even if present in an unrelated sequence.

4. The Ribosome is Not a Universal Translator

Although SecM-elongation arrest is normally transient, and SecM is released when the N-terminal region (already outside the ribosome) of the nascent chain interacts with the Sec protein export machinery, [12] it is prolonged in the absence of active SecM export (see below).[8] Therefore, *E. coli* ribosomes cannot continue translation beyond the sequence FXXXXWIXXXXGIRAGP if it exists in a nonsecreted protein. Thus, the arrest sequence is potentially dangerous. Indeed, overproduction of signal sequence-defective SecM is extremely toxic, presumably due to congestion of a large number of ribosomes. Fortunately, no other *E. coli* proteins have exactly the same sequence. Nevertheless, it has become clear that the *E. coli* ribosome cannot translate *any* genetic message successfully and that genomic sequences should have evolved to avoid sequences that interact nonproductively with ribosomal components.

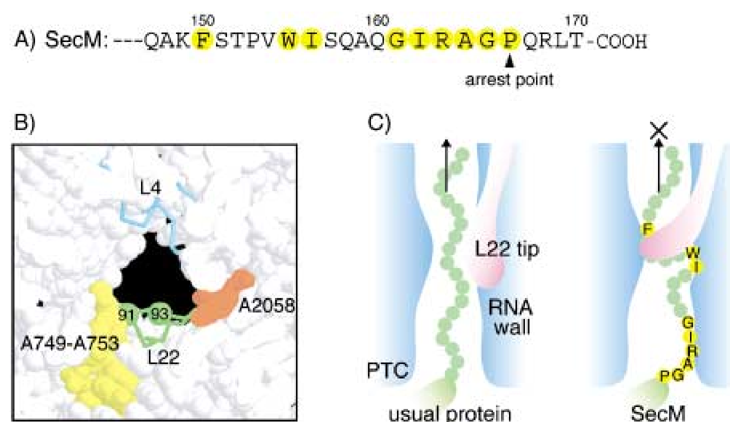

Figure 2. The SecM arrest sequence interacts with the exit tunnel to halt its own elongation. A) Amino acid sequence of SecM carboxy terminal region (Gln147 – Thr170). Translation of secM *is subject to elongation arrest at Pro166 (arrowhead). Residues highlighted by yellow circles are required with exact spacing for elongation arrest. B) Ribosomal alterations that alleviate elongation arrest of SecM. The exit tunnel is viewed from the PTC side with the cytosolic opening shown in black. Mutational alterations that partially circumvent SecM elongation arrest are shown in orange (A2058 of 23S rRNA), yellow (A749 – A753 interval of 23S rRNA) and green (Gly91 and Ala93 of L22). Reproduced from ref. [9] with permission from Elsevier.) C) A working model for the arrest sequence action, as proposed by Berisio et al.[13] When the ribosome is translating a normal protein, the tip of L22 interacts with the RNA wall of the tunnel (left). The SecM arrest segment, however, displaces the L22 tip from the RNA wall, leading to its flipping across the tunnel (right).*

5. SecM Arrest Sequence Interacts with the Exit Tunnel

We used a reporter LacZα gene with an N-terminally attached SecM arrest sequence to isolate *E. coli* mutants in which elongation arrest of the chimeric protein is partially decreased. [9] Strikingly, all characterized mutations contained an alteration in either 23S rRNA or L22 protein residues located close to the narrowest constriction of the exit tunnel (Figure 2B). The residues affected were A2058 in domain V, an A749 – A753 interval in domain II of 23S rRNA, and Gly91 and Ala93 of L22.

On the other hand, arrest-impairing mutations can occur in any residue in the FXXXXWIXXXXGIRAGP sequence except the Xs. From the spectra of arrest-suppressing amino acid alterations either in L22 or in SecM, we have argued against simple tunnel congestion being responsible for the arrest.[9] The arrest segment may assume a specific configuration within the tunnel such that it prevents further movement along the tunnel or inhibits PTC reaction.

Recently, Berisio et al.[13] determined the structure of a complex formed between the large ribosomal subunit and a macrolide antibiotic, troleandomycin (TAO). It was shown that TAO binds to the RNA wall of the exit tunnel, displacing the β-hairpin region of L22 from the wall and causing it to flip across the tunnel. On the basis of alterations we identified as arrest-suppressing ribosomal mutations, they proposed that the SecM arrest sequence acts like TAO to halt its own passage through the narrow gate of the tunnel, which is occluded by the L22 tip (Figure 2C). This notion provides an interesting working model for SecM action. It is tempting to speculate that regulated flipping of the L22 β-hairpin might have general roles in modulating nascent polypeptide movement and peptide elongation.

6. SecM Monitors Its Own Membrane-Translocation to Control Elongation Arrest

As already mentioned, elongation arrest of SecM is relieved when the N-terminal part of the nascent SecM is engaged in translocation across the membrane (Figure 3B and C). One possibility that accounts for this

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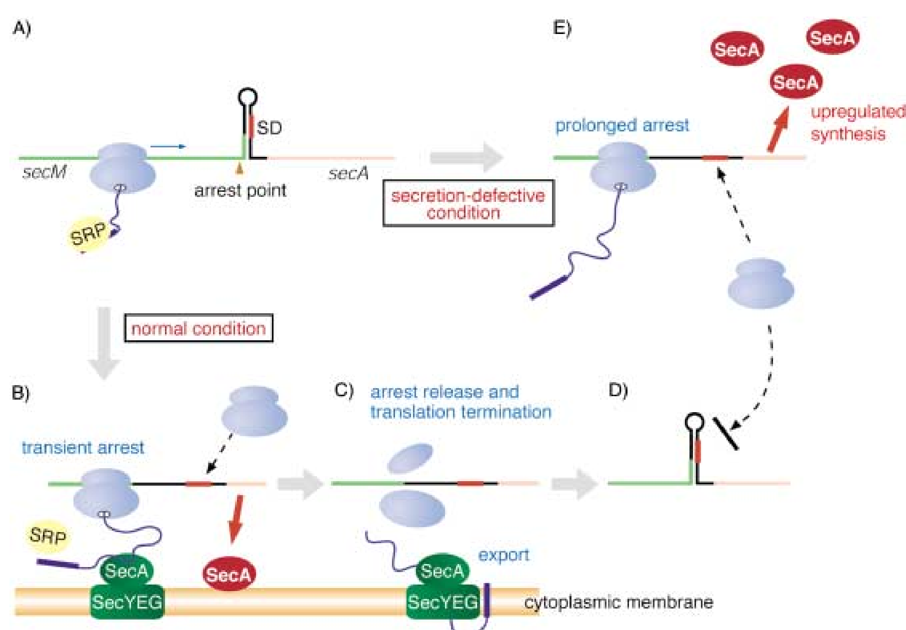

Figure 3. Role of SecM in upregulation of SecA quantity and quality. A) secM *(green) and* secA *(pink) are cotranscribed into the* secM – secA *mRNA, in which the ribosome-binding site (SD) for* secA *translation (red) is occluded within the stem-loop structure. A ribosome stalled at the arrest point in* secM *will expose the* secA *SD (shown in E). B) Under normal conditions, the N-terminal part of nascent SecM is targeted to the Sec machinery (dark green) by SRP. This cotranslational event also brings the* secM-secA *mRNA into the vicinity of the membrane/Sec machinery. Thus, newly synthesized SecA (red) can readily adopt an active form. C) Elongation arrest is cancelled by ongoing secretion, and translation terminates at the stop codon. D) The stem-loop is reformed at the expense of SecA translation. This completes the normal cycle and the ground state is regenerated. E) Under secretion-impaired conditions, SecM elongation arrest is prolonged, resulting in extended exposure of the SD sequence and a higher* secA *translation initiation frequency. We believe that cotranslational localization of the ribosome/mRNA to the membrane (not shown) is maintained under most variations of physiological conditions.*

arrest cancellation may be a "pulling" mechanism, in which the force generated by the Sec translocation machinery dissociates the arrest sequence of SecM from the wall/gate of the exit tunnel. Alternatively, the ribosome – Sec machinery interaction may allosterically induce a conformational change in the tunnel.

That an impaired signal sequence of SecM prolongs arrest in wild-type host cells indicates that SecM monitors its own export, rather than the cell's general protein-export activity, per se.[8, 11] Of course, defects in Sec factors (other than SecB), or in SRP (signal recognition particle) components, and addition of azide (a SecA inhibitor) also prolong arrest. SecB-independence and SRP-dependence of SecM export are interesting, because these factors are believed to be involved in targeting exported proteins and membrane proteins in *E. coli*, respectively.[14] Together with the fact that the signal peptide of SecM is unusually long,[15] we speculate that SecM uses the targeting system for membrane proteins. SecM may monitor both protein export and integration activities of the cell.

These observations indicate that interacting properties of a nascent chain outside the ribosome can affect intraribosomal polypeptide – tunnel interaction and PTC reaction.

7. Translation Arrest of SecM is Required in vivo for Both Constitutive and Up-Regulated Expression of SecA

The *secM – secA* intergenic mRNA sequence is known to form a stem-loop structure, in which the SD (Shine – Dalgarno) sequence for *secA* translation is sequestered (Figure 3A).[16] When a ribosome stalls at the Pro166 codon of *secM*, it does so while sterically disrupting the secondary mRNA structure, consequently exposing the *secA* SD sequence (Figure 3B and E). This is the presumed mechanism by which SecM elongation arrest acts to elevate the frequency of *secA* translation. Further experiments at our laboratory confirmed that an arrest-abolishing Pro166 to Ala mutation decreases translation of *secA* by about 70 – 75 % in a *cis*-specific manner.[17] This mutation is lethal when introduced into the chromosome unless excess SecA is provided by a plasmid. These results indicate that SecM elongation arrest is required to provide the cell with enough SecA to support viability, even if it is transient in normal secretion-proficient cells (Figure 3B).

By using other viable and weaker *secM* mutations, it was also demonstrated that normal elongation arrest is required for up-regulating SecA levels in response to azide addition and low temperatures (Figure 3E).[17]

8. Does Intraribosomal Peptide – Tunnel Interaction Control the Extraribosomal Fate of a Protein?

In the experimental analyses described above, we observed some inconsistent relationships between cellular SecA abundance and cellular activity of protein export. It became apparent that SecA molecules produced from an engineered configuration of the *secA* gene that lacks the upstream *secM* sequence were less functional than those from the wild-type *secM – secA* gene complex.[17] We interpret this result to mean that cotranslational targeting of SecM to the Sec translocation machinery simultaneously brings the *secM – secA* mRNA into the vicinity of the membrane/Sec machinery (Figure 3B). Transient elongation arrest will ensure this mRNA localization and, hence, the synthesis of SecA in the membrane/translocon-associated state (Figure 3B). Since SecA seems to assume multiple conformations,[18–20] its synthesis in the membrane-proximal location might enable it to directly adopt an active form after biosynthesis.

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These results suggest that SecM's role is not only to ensure enough SecA translation, but also to synthesize SecA in a biologically active form. Can the concept of nascent chain–tunnel interactions affecting the fate of a protein be generalized or extended? Mutations of different SecM arrest sequence residues give different degrees of arrest release kinetics;[9] this points to the possibility that elongation speed can be modulated by sequence features that pass through the tunnel. Localized variation of the elongation speed might affect outside events such as cotranslational folding, subunit association, or subcellular targeting. Consistent with such a view, the exit region of the ribosome surface appears to be surrounded by docking sites for some folding catalysts (such as trigger factor) and targeting factors (such as SRP) (Figure 1).[21–25] It would be even more interesting if the existence of differentially used multiple exit pathways in the ribosome could be corroborated.[5]

It was postulated that a nascent segment within the ribosome that is destined to become a transmembrane segment transmits a signal, presumably via the ribosome, to alter the gating property of the associated translocon channel.[26, 27]

Ribosomes should not be regarded as merely old-fashioned robust protein-synthesis factories, but as dynamic devices fully equipped with information technology (Figure 4). Indeed, ribosomes undergo dynamic structural changes during translation.[28] To define structural changes induced by specific polypeptide sequences within the PTC-tunnel cavity of the ribosome and correlate them with biological outputs promises to be a challenging area of research.

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Keywords: biosynthesis · elongation arrest · exit tunnel · proteins · protein secretion

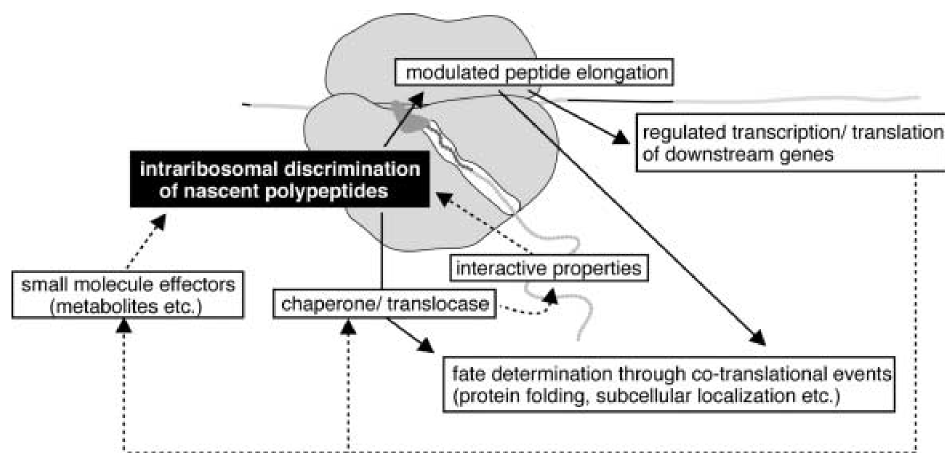

Figure 4. Biological regulation involving intraribosomal protein states. Every portion of a nascent elongating polypeptide may be sampled by the exit tunnel for unrestricted PTC reaction-coupled movement or modulation of elongation speed. Such modulation could affect cotranslational processes outside the ribosome or, alternatively, expression of downstream genes (solid arrows). Signals may also be transmitted via ribosomal conformational change to affect outside factors, such as translocon components and ribosome associated chaperones. Conversely, the ribosome–nascent chain complex can receive information from the outside that provides feedback control for the nascent chain–tunnel interaction and elongation speed (dotted arrows). Small effector molecule influencing nascent chain behavior within the ribosome have also been described.[29]

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