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Commentary

Membrane protein biogenesis: The exception explains the rules

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The transport of most proteins across the bacterial inner membrane or its eukaryotic counterpart, the endoplasmic reticulum, is accomplished in a two step reaction. In the first step, proteins that are destined to leave the cytoplasm are guided or "targeted" to transport sites in the membrane. The prototypical targeting machine is the signal recognition particle (SRP), a ribonucleoprotein complex that binds to hydrophobic leader peptides and transmembrane domains of ribosome-bound nascent polypeptide chains and releases them only after making contact with its membrane-bound receptor (1). Although SRP is required for the transport of essentially all proteins across the endoplasmic reticulum membrane in mammalian cells, it plays a much less central role in yeast and so far has been shown to be required only for the insertion of a subset of polytopic membrane proteins in bacteria (2–4). In microbes, other targeting factors have been identified that function as chaperones to keep partially or fully synthesized passenger proteins in a conformation that is compatible with their transport across the membrane (5–7). In the second step, polypeptides are handed off to a protein conducting channel or "translocon" that facilitates permeation of the membrane barrier. The translocon comprises a conserved heterotrimeric core called the SecY complex (in bacteria) or the Sec61p complex (in eukaryotes) as well as other components that differ in each of the three kingdoms of life (8).

Although the proper localization of many polypeptides strictly depends on this transport pathway, some proteins appear to be able to cross the membrane spontaneously. In particular, the biogenesis of a variety of bacterial inner membrane proteins has been claimed to be "Sec-independent" based on the results of experiments that use conditional *secY* alleles (9–11). In an important paper that appears in this issue of the *Proceedings* (12), de Gier *et al.* reexamine the targeting and transport requirements of several model *Escherichia coli* membrane proteins and find that, in the end, only one protein emerges as a true renegade. A consideration of the factors that distinguish this "exception to the rule" yields significant new insights into the function of both the translocon and the SRP targeting machinery in bacteria.

The protagonists in the present work are wild-type and mutant versions of the familiar bacteriophage M13 procoat and leader peptidase (Lep) proteins. Procoat is a 73-aa polypeptide that contains a typical 23-aa cleaved leader sequence and a single membrane anchor. Unlike most other secreted and membrane proteins that have been examined, procoat synthesized in a cell-free translation reaction integrates into vesicles composed of pure lipid (ref. 13; see Fig. 1). Consistent with this observation, the growth of cells that contain a temperature-sensitive *secY* allele at the nonpermissive temperature has no effect on the membrane insertion of procoat (14). Although the procoat leader sequence initiates transport across the membrane, studies on a naturally leaderless small coat protein of bacteriophage Pf3 suggest that a leader is not required for direct integration into the membrane (15). The observation that small eukaryotic secretory proteins such as prepromelittin (70 amino acids) also appear to traverse the *E. coli* inner membrane in a SecY-independent fashion, however, suggests that the phenomenon of spontaneous transport may not be confined to membrane proteins (16). Although the biogenesis of Lep, which contains two membrane anchors and a large periplasmic domain, requires SecY (14), the insertion of a mutant that is topologically inverted so that only a small loop is transferred across the membrane ("Lep-inv") has been reported to be SecY-independent in both *secY* Ts and Cs strains (9, 17).

A consideration of the energetics of protein transport across a membrane helps to explain how spontaneous transport might occur. The large release of free energy derived from the partitioning of hydrophobic segments into a lipid environment provides a driving force for the direct integration of polypeptides into membranes. Thermodynamics is not the deciding factor, however, because the rate of a chemical reaction is dictated by the size of kinetic barriers and not by equilibrium energies. Thus, the spontaneous insertion of synthetic leader peptides (18) and transmembrane domains (19) into model lipid bilayers that has been demonstrated experimentally is probably caused by the need for very small activation energies. The energy required for the translocation of the small periplasmic segments of proteins like procoat and Lep-inv might still be low enough to permit direct insertion. Moreover, the electrochemical gradient across the *E. coli* inner membrane could help drive the reaction. By contrast, the high energy barrier that must be overcome to translocate the large number of polar and charged amino acids found in most secreted proteins and many integral membrane proteins is probably incompatible with spontaneous transport. Consistent with this argument, the addition of a large tail to the cytoplasmic domain of procoat has no effect on its spontaneous insertion properties (20), but the addition of equally large polypeptide segments to the translocated domain of either procoat or prepromelittin reroutes both proteins into the SecY pathway (16, 21).

A key prediction of the biophysical theory is that the translocon functions primarily to reduce the activation energy of transport across a membrane by providing a hydrophilic environment through which polar polypeptide segments can pass. Several types of experiments have provided convincing evidence that the translocon can indeed form an aqueous channel (22–24). de Gier *et al.* now add another wrinkle to the story by analyzing the insertion of procoat, a new derivative of procoat that contains an extremely hydrophobic leader sequence ("H1-procoat"), and Lep-inv in a new light. Instead of shifting cells that contain a conditional *secY* allele to a temperature that is nonpermissive for growth, they depleted translocons by turning off expression of the gene that encodes the SecE subunit. Given that a previous study already has raised the suspicion that temperature shifts do not inactivate mutant translocons completely (24), the depletion approach is clearly more reliable (even though it has its own drawbacks). Based on the logic described above, it would be expected that H1-procoat insertion requires no more energy than procoat or

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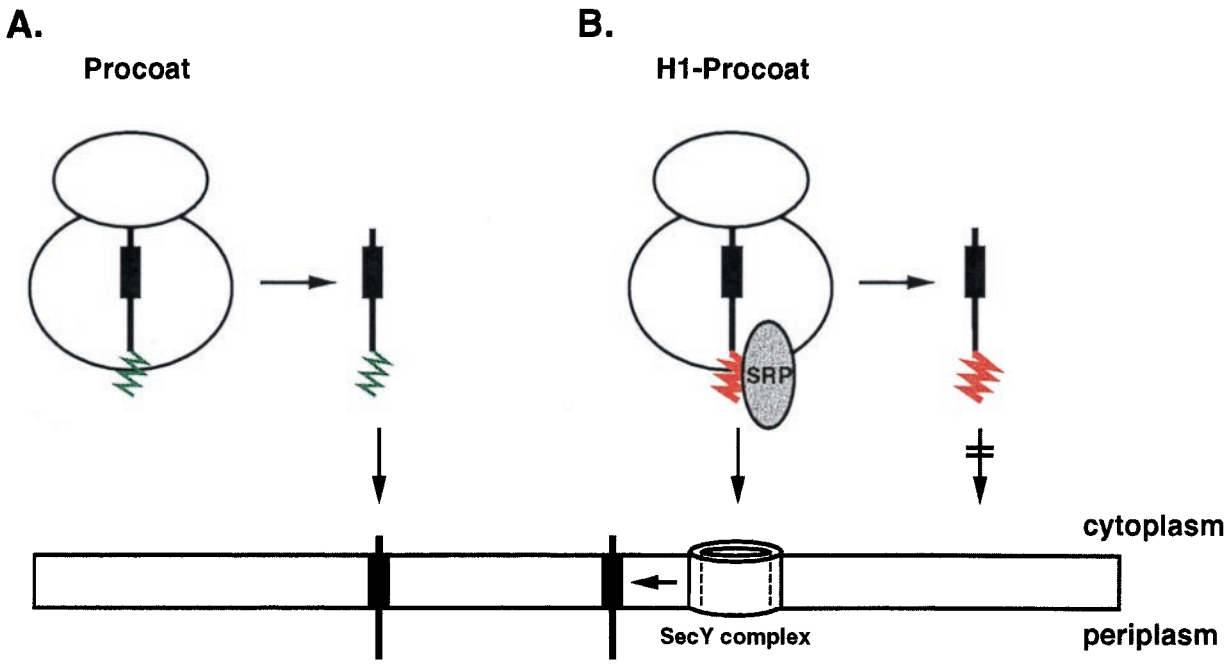

FIG. 1. The biogenesis of the M13 procoat and H1-procoat proteins in *E. coli*. The major coat protein of bacteriophage M13 is synthesized as a 73-aa precursor (procoat) that contains a typical cleaved leader sequence (green). Wild-type procoat (*A*) appears to insert into the inner membrane spontaneously. The insertion of H1-procoat (*B*), a procoat derivative that contains an extremely hydrophobic leader sequence (red), depends on both the cotranslational SRP targeting pathway and the SecY translocation complex. The modification of the leader presumably prevents direct insertion by either changing the physical properties of the molecule or increasing the probability of aggregation in the cytoplasm.

Lep-inv and therefore would be SecY-independent. Surprisingly, the insertion of both H1-procoat and Lep-inv was sharply inhibited by SecE depletion. Under the same conditions, however, wild-type procoat still could bypass the translocon requirement. Besides demonstrating the perils of using conditional alleles to study cell physiology and prompting a wholesale reevaluation of previous work, these observations clearly imply that the translocon has a second function in addition to reducing the kinetic impediment to the transport of polar residues.

To understand the role that the SecY complex plays in the biogenesis of proteins like H1-procoat and Lep-inv, it is first necessary to consider another observation made by de Gier *et al*. They found that beefing up the hydrophobicity of the marginally hydrophobic procoat leader (to the point at which it actually could qualify as a transmembrane domain) affects the targeting requirements of the protein as well (see Fig. 1). Although SRP depletion had no effect on the transport of wild-type procoat, it severely impaired the insertion of H1-procoat. This is a remarkable result given that bacterial SRP, like its eukaryotic counterpart, probably works in a cotranslational mode (26). Because amino-terminal targeting signals are not exposed outside a translating ribosome until at least 60 amino acids are synthesized (27), the time window during which SRP can bind to H1-procoat must be very short. Taken together with the observation that the *E. coli* particle has a higher affinity for H1-procoat than for wild-type procoat, this result suggests that bacterial SRP is calibrated to capture quickly those proteins whose biogenesis is likely to fail if their targeting sequences are exposed or if they are released from ribosomes into the cytoplasmic environment.

Based on an analogy to eukaryotic SRP, it is likely that the *E. coli* homolog "protects" nascent membrane proteins by coupling their synthesis directly to their insertion. In mammalian cells, proteins that are targeted by SRP are fed into the translocon in a temporally and spacially optimized fashion by virtue of a large number of interactions between SRP, the SRP receptor, the ribosome, and the translocon that all contribute to the formation of a large macromolecular machine (1, 28). The observation that H1-procoat nascent chains (but not procoat nascent chains) generated in a cell-free extract could be cross-linked to both SRP and the translocon suggests that that at least some aspects of this coordinated assembly process are conserved in bacteria. Perhaps other targeting factors cannot substitute for SRP because they lack the ability to mediate an effective interaction between passenger proteins and the membrane-bound translocation complex.

In light of the SRP requirement for H1-procoat and Lep-inv biogenesis, one possible explanation for the SecY requirement is that theoretical considerations do not take into account subtle features of these proteins or the composition of the bacterial membrane itself that simply forbid direct integration. In this view, proteins like wild-type procoat beat the system only because they possess unique physical or chemical properties that are yet to be appreciated. Ordinarily, SRP may be obliged to steer nascent polypeptides to the membrane, where, based on evidence that the translocon recognizes hydrophobic leader sequences (29–32), the SecY complex binds to the amino-terminal targeting peptide and facilitates insertion of the growing chain.

An alternative explanation for the SecY-dependence of the integration of highly hydrophobic proteins like H1-procoat holds that, as predicted by theory, they have an inherent ability to insert directly into the bacterial inner membrane. The problem, though, is that, once the polypeptide chain reaches a critical size or is released from ribosomes, the insertion process competes with the formation of aggregates in the cytoplasm. According to this model, the much less hydrophobic procoat protein bypasses the SRP and translocon requirements by inserting into the membrane before it has a chance to follow a nonproductive pathway. The binding of SRP presumably shields the hydrophobic leader of H1-procoat, but the SecY requirement suggests that this is only a temporary fix. In the absence of the translocon, SRP-ribosome-nascent chain complexes might be incapable of docking on the membrane. SRP then eventually might release its undelivered cargo, and once again the prospect of aggregation in the cytoplasm would loom large.

Regardless of which explanation is correct, the important message to take away from these experiments is that the SecY complex is needed to ensure both the transfer of hydrophilic segments across the bacterial inner membrane and the insertion of hydrophobic segments into the lipid bilayer. Thus, the SecY-independent insertion of procoat probably represents a rather special case. The observation that the membrane integration of this simplest of bitopic proteins lies precariously perched on the edge of SecY-dependence casts doubt on the possibility that the insertion of more elaborate polytopic proteins bypasses the translocon. Indeed, as the size and sophistication of a membrane protein increases, the complexity of its biogenesis is likely to be multiplied. For example, the intricacy of establishing the correct topology of a protein may correlate with the total number of domains. Evidence that the SecY complex participates in the topogenesis of membrane proteins as well as in their insertion already has been reported (33). The major challenge that lies ahead, given that the SecY complex probably plays a near universal role in membrane protein biogenesis, is to elucidate the mechanism by which it mediates this remarkable process.

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