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Translocation Gets a Push

Minireview

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Recent years have seen a remarkable concordance of observations concerning the mechanism of polypeptide translocation during secretion in bacteria and in eukaryotic cells. All organisms have some form of signal recognition particle, and diverse membranes from the Escherichia coli inner membrane through the yeast and mammalian endoplasmic reticulum (ER) have a trimeric integral membrane protein complex that comprises the channel or translocase through which nascent secretory polypeptide chains are transmitted. In E. coli, the translocase consists of three genetically and biochemically defined molecules: SecY, SecE, and Band 1 (now called SecG) (Schatz and Beckwith, 1990; Brundage et al., 1992; Douville et al., 1994). In yeast and mammals, the corresponding complex contains Sec61p and two other subunits that in mammalian cells are called Sec61 β and γ (Hartmann et al., 1994). Two of the three subunits of the translocase are homologous in all three sources (Hartmann et al., 1994). A hydrophilic channel activity, with significant connections to the polypeptide translocase, has been detected in E. coli inner membrane and mammalian ER membrane fractions (Simon and Blobel, 1991, 1992; Crowley et al., 1994). Although the proof is not yet in hand, it seems probable that the channel activity is formed by the SecY and Sec61 complexes.

The most extensive enzymologic dissection of the translocase has been achieved for the SecY complex, where the Wickner (Brundage et al., 1992) and Mizushima (Nishiyama et al., 1993) groups have purified the functional entity and reconstituted translocation activity in proteoliposomes using the intact oligomer or from isolated subunits. A direct contact between the nascent secretory chain proOmpA and the SecY subunit has been established by chemical cross-linking experiments (Joly and Wickner, 1993). In eukaryotic ER membranes, cross-linking experiments identify Sec61 as a partner of nascent secretory chains that are engaged to the translocase either by posttranslational or cotranslational insertion (Görlich et al., 1992; Müsch et al., 1992; Sanders et al., 1992).

Divergence of Translocation Mechanisms

Of course, nature has not been so simple, or boring, as to have conserved the translocation mechanism completely. Possibly significant differences in the coupling of translation to translocation, in the choice of energy sources to drive the process, and in the full set of proteins necessary to complete the event have been detected.

The issue of post- versus cotranslational translocation of secretory proteins is not as contentious as in years past because it appears that examples of both are known. The difference is that organisms deploy one or the other pathway in varying degree. On the one extreme, secretion in E. coli is largely posttranslational, while mammalian cells are largely cotranslational, and yeast seems to go both ways.

Unlike in eukaryotes, translocation in E. coli is driven by the combined contributions of ATP and membrane potential. This is not a consequence of the posttranslational lifestyle because at least one example, posttranslational translocation of α-factor precursor in yeast, requires ATP but not membrane potential (Hansen et al., 1986).

Perhaps the key difference between eukaryotes and E. coli is the involvement of three additional (and apparently unique) E. coli proteins. SecA is a peripheral membrane protein that powers translocation by a cycle of ATP hydrolysis (Lill et al., 1990), and SecD and SecF (Pogliano and Beckwith, 1994) are two integral proteins that are required in vivo, but whose contribution to the overall scheme has proven elusive. Recent evidence suggests that SecD and SecF may somehow link membrane potential to the translocase (Arkowitz and Wickner, 1994).

The Role of SecA

A great deal of attention has focused on the mechanism of action of the SecA protein. SecA was defined in E. coli by a genetic screen for pleiotropic, secretion-defective mutants (Oliver and Beckwith, 1981; Schatz and Beckwith, 1990). The gene encodes a hydrophilic, ATP-binding protein. Inverted inner membrane vesicles, washed with urea, are dependent on exogenous SecA protein to engage in the posttranslational translocation, segment by segment, of secretory polypeptides.

SecA makes contact with four elements of the translocase–substrate complex: the SecY complex, the secretory preprotein, the SecB chaperone, and anionic phospholipids. A complex of proOmp and the chaperone SecB interacts stoichiometrically with the SecA protein, which in turn is positioned on the membrane through contact with the SecY complex (Hartl et al., 1990). By itself, the SecA protein possesses a weak ATPase activity. However, in association with the substrate-bound translocase, SecA ATPase is dramatically activated.

SecA recognizes all portions of the secretory substrate; maximum SecY complex–stimulated SecA ATPase is achieved in the presence of signal peptide and mature portions of a secretory precursor (Lill et al., 1990). Aside from its affinity for the SecY/E membrane-binding site, SecA also can bind and become at least partially embedded in pure phospholipid vesicle membranes (Ulbrandt et al., 1992), which suggests that the environment of a translocating chain could include the SecY protein and lipid. However, cross-linking studies have shown that the nascent secretory chain is in the vicinity of the SecA and SecY proteins and not adjacent to phospholipids (Joly and Wickner, 1993). Furthermore, at least in the eukaryotic ER, the nascent chain is embedded in a distinctly polar environment (Crowley et al., 1994).

SecA, a Transient Membrane-Spanning Protein

Until very recently, one had the impression that SecA oper-

ated at the cytosolic surface of the translocase to feed segments of a secretory chain into the channel without actually penetrating the channel itself. This picture has changed radically with the discovery, reported independently by the Oliver (Kim et al., 1994) and Wickner (Economou and Wickner, 1994) laboratories, that an arm of the SecA protein accompanies the nascent chain into the channel such that the SecA protein, though exclusively polar in its sequence, becomes transiently a membrane-spanning protein.

Kim et al. (1994) began with the observation that the SecA protein, which in normal cells partitions between the cytosol and the membrane, became nearly exclusively membrane bound when SecD and SecF (and possibly other gene products near this locus) were overexpressed in transformed E. coli cells. Such cells were active in protein secretion, implying that the membrane bound SecA must be competent and that the supply of protein from the *secD secF* locus must normally be limiting for membrane recruitment of SecA.

In normal cells most of the membrane-bound SecA can be eluted by extraction with pH 11 carbonate, usually taken as an operational definition of a peripheral membrane protein. However, in SecD- and SecF-overexpressing cells, essentially all of the SecA remains membrane bound even after carbonate extraction.

One can imagine a conformational change giving rise to this behavior, particularly since SecA can partially embed in a pure phospholipid vesicle. This seems not to be the case, however, as Kim et al. (1994) went on to show that a portion of the SecA protein is accessible to proteolysis and biotinylation on the periplasmic surface of the inner membrane. Careful control experiments using proteins exclusively located on the inner surface of the inner membrane revealed that membrane-bound SecA is unusually accessible to the action of external modifying reagents. Kim et al. (1994) concluded that SecA may be positioned within and through the SecY/E channel or at the very least be exposed to external reagents by embedment within the channel. A similar situation was documented last year in the disposition of the hisP subunit of the histidine permease in Salmonella typhimurium (Baichwal et al., 1993). This protein, also an exclusively polar ATP-binding protein, is accessible to proteolytic attack in intact spheroplasts and thus may span the bilayer by being sandwiched between its two integral membrane partners, hisQ and hisM.

Wickner's group used the cell-free proOmpA translocation reaction to probe the cycle of SecA embedment in the membrane. Starting with radiolabeled SecA protein, mixed with nonradioactive membranes and proOmpA, Economou and Wickner (1994) found that the 102 kDa protein could be degraded to an ~30 kDa fragment by concentrations of trypsin or proteinase K that completely degraded the pure protein. The 30 kDa fragment was preserved unless translocation reactions were treated with protease and detergent or protease was allowed access to the interior of the inverted inner membrane vesicle (corresponding to the periplasmic surface of the inner membrane) by sonication or freeze–thaw treatment. Importantly, the 30 kDa fragment was not revealed when any one or combination of the essential elements of the translocation reaction was omitted (proOmpA, membranes, ATP) or if incubation was conducted at 0°C.

The formation of the SecA fragment was found kinetically to precede the completion of translocation of proOmpA; saturation binding experiments showed that it formed at the high affinity binding site previously characterized as the SecY/E complex. Multiple roles for ATP were indicated in experiments that test the formation and deinsertion of membrane-embedded forms of SecA. Economou and Wickner (1994) found that hydrolyzable ATP, perhaps bound to a second ATP-binding site on SecA, was necessary to unlock the inserted form and to allow exchange with a fresh source of SecA protein.

This cycle of ATP binding and hydrolysis correlated with previous studies from Wickner's group that show proOmpA segments of ~20 amino acids were transferred across the membrane as units (Schiebel et al., 1991). Furthermore, the existence of a deeply embedded species of SecA explains why translocation-arrested forms of proOmpA can be chemically cross-linked to SecY and SecA, even when the cysteine available for cross-linking is positioned at or near the periplasmic surface of the membrane (Joly and Wickner, 1993).

Transducing Energy into Work

The Kim et al. (1994) and Economou and Wickner (1994) results offer a significant explanation for the transduction of energy from ATP hydrolysis into work in the form of unidirectional penetration of a secretory polypeptide. The prediction is that a fairly dramatic conformational change in ATP-bound SecA produces a piston-like action that drives an ~20 amino acid length segment of translocating substrate into and through the SecY translocase and that ATP hydrolysis releases SecA from its cargo, allowing retraction of the piston for capture of the next segment to be translocated.

Are Bacteria Unique?

Something this significant cannot have been the sole invention of one organism, even a clever one like E. coli. Yet there is no obvious correlate of SecA in eukaryotes. Hsc70 molecules in the cytosol or lumen of the ER (BiP) could play a role similar to SecA. Although cytosolic hsc70 is required for certain posttranslational translocation events, its role seems more as a chaperone to keep secretory cargo from aggregating (Chirico et al., 1988; Deshaies et al., 1988). Thus, the requirement for hsc70 in the translocation of yeast α-factor precursor is obviated by denaturation of the substrate in urea, whereas SecA is required even for fully unfolded secretory cargo.

BiP, an hsc70 on the lumenal side of the membrane, seems a more promising candidate. Genetic and biochemical evidence in yeast suggests that BiP (known in yeast as KAR2) is required for the translocation of the full range of secretory and membrane proteins, including representatives of the post- and cotranslational lifestyles (Sanders et al., 1992; Vogel et al., 1990). Denaturation of α-factor presursor does not diminish the requirement for BiP in an in vitro translocation reaction, and nascent secretory chains can be chemically cross-linked to BiP and Sec61

(Müsch et al., 1992; Sanders et al., 1992). The obvious difference between BiP and SecA is that the two are topologically on opposite sides of the membrane. Thus, whereas SecA would be pushing, BiP would be pulling. Consequently, the nature of the conformational change that accompanies ATP and protein substrate binding would differ for SecA and BiP.

Why these different topological strategies would have evolved is not clear, although ATP-dependent events need not be restricted to the cytosol in eukaryotes as they are in bacteria. The use of BiP and ATP sequestered in the ER lumen may present the eukaryotic cell with some additional insurance that secretory proteins will be inserted only into the ER. Thus, BiP would serve a proofreading role as well as transducing the force required to drive protein import. Bacterially secreted proteins must all pass through the inner membrane, and for this purpose the fidelity achieved by SecA and SecY/E may be sufficient.

The role of BiP as an analog of SecA in mammalian cells is unclear. Although ER lumenal proteins stimulate translocation in opened and then resealed microsomes (Nicchitta and Blobel, 1993), a fully purified and reconstituted translocation reaction shows no dependence on BiP (Görlich and Rapoport, 1993). It may be that proteoliposomes containing BiP will be more active or will promote more rounds of translocation than is achieved with the less complex proteoliposome. If not, then one must look elsewhere, perhaps to the large ribosomal subunit for the piston that drives translocation in the mammalian ER.

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