How ATP Drives Proteins Across Membranes

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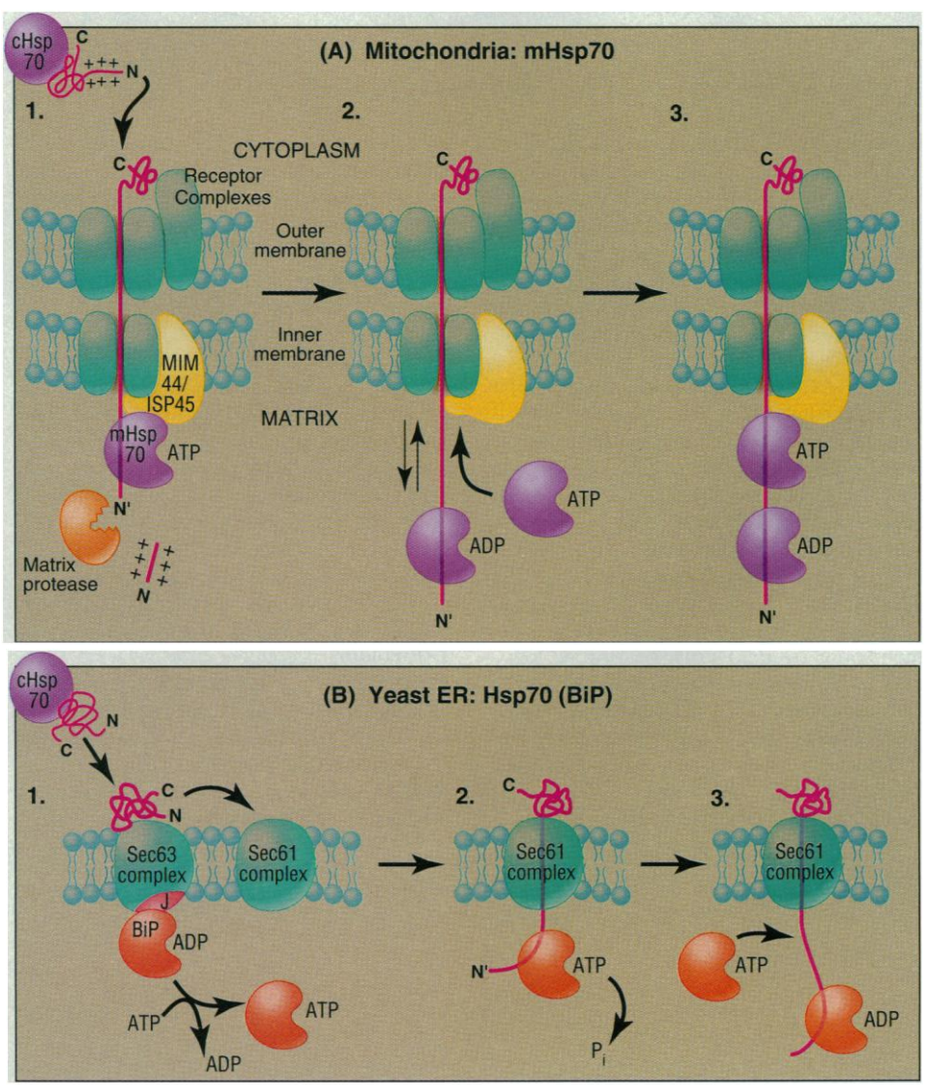
Newly made polypeptides (preproteins) are transported across membranes by translocases, which operate by fundamentally different mechanisms than classical pore proteins or solute transport systems. Pore proteins are open at both ends and conduct up to 10^6 molecules per second, whereas translocases tend to be in proton-tight membranes and take 3 to 60 seconds to transport one preprotein. Classical transport proteins often have a single solute binding site, alternately exposed by subtle conformational changes to the two membrane surfaces, whereas preproteins cross the membrane as extended chains that span the membrane through the translocase. As reported by Ungermann and coworkers on page 1250 of this issue of *Science* (*1*), preproteins in transit can "slide" back and forth through translocase like a thread through the eye of a needle if no energy input is provided. To prevent this slip, preprotein translocases have devised clever ways to couple metabolic energy from adenosine triphosphate (ATP) and electrochemical potentials to preprotein movement.

Mitochondrial preproteins, initially bound to cytosolic Hsp70 (see figure, part A, step 1), cross two membranes to reach the mitochondrial matrix. After binding to receptors (*2*), the basic amino-terminal "matrix targeting" presequence crosses the outer mitochondrial membrane, is driven across the inner membrane by the membrane electrical potential, and is cleaved by the matrix protease. Three labs have recently found that the ATP-bound form of a matrix heat shock protein of 70 kilodaltons (mHsp70), bound to an inner membrane receptor (called MIM44 or ISP45), awaits the incoming preprotein chain (*1*, *3*). Upon binding the preprotein, Hsp70 hydrolyzes its ATP (step 2), allowing the dissociation of Hsp70 from MIM44 (*3*). It is not yet clear whether Hsp70, bound to both MIM44 and the preprotein, changes conformation to "pull" the transiting polypeptide (*4*). After release from MIM44, Hsp70 prevents the preprotein chain from sliding back to the cytoplasm, but does not obstruct its movement into the matrix. When enough of the preprotein has become exposed to the matrix, a second MIM44-bound Hsp70 can bind (step 3), and the chain is either pulled or "ratchets" (*5*) forward. This cycle uses the energy of ATP binding and hydrolysis to drive preprotein movement into the matrix.

How general is the molecular ratchet (*5*)? Studies by Schekman and his colleagues of protein translocation into the yeast endoplasmic reticulum (ER) suggest that something similar occurs there (*6*). BiP, the form of Hsp70 present within the yeast ER, is bound to the membrane by a "dnaJ" domain of Sec63, a translocase subunit (see figure, part B, step 1). Binding to ATP displaces BiP from Sec63. BiP then binds to the preprotein and hydrolyzes ATP (step 2). Further BiP association (step 3) may drive translocation much as in mitochondrial protein import.

Not all translocation employs the molecular ratchet (*5*) strategy. Protein translocation into the mammalian ER is coupled to translation through the inhibition of translation by the signal-recognition particle (SRP) (*7*) and by the affinity of ribosomes for membrane receptors (*8*). The absence of signal sequence binding (see figure, part C, step 1) by nascent polypeptide–associated complex (NAC) (*9*) allows the formation of an SRP-polysome complex (step 2). This complex associates with docking protein–SRP receptor (step 3), hydrolyzes guanosine triphosphate (GTP) (*10*), and transfers the polysome and nascent chain to the "Sec61 complex" (*11*), a heterotrimer with striking similarity to the translocase first found in the plasma membrane of *Escherichia coli* (see below). As polypeptide elongation resumes, the nascent chain is presumed to be "pushed"



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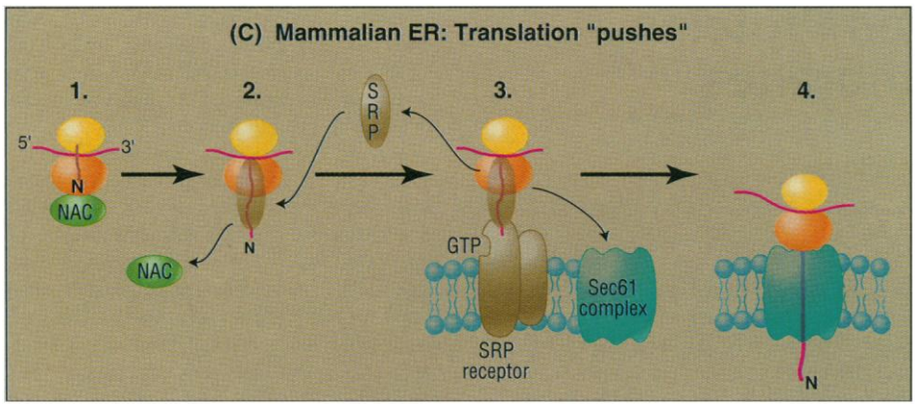

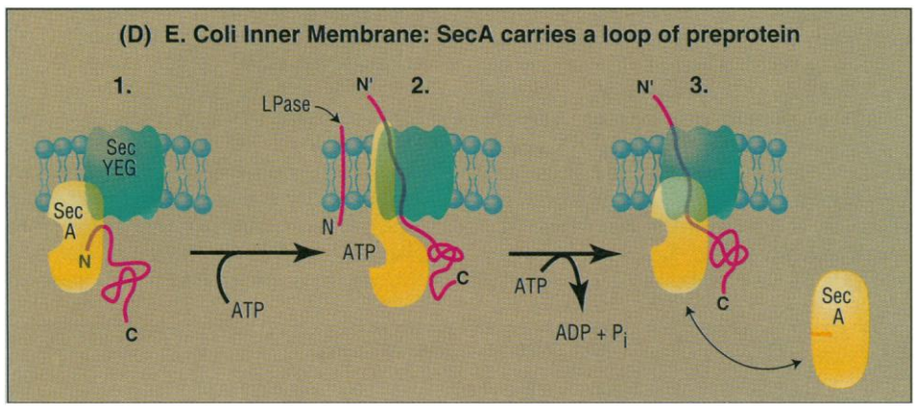

through the Sec61 complex (step 4) to which the ribosome is bound. However, some in vitro translocation into mammalian ER can occur late in translation (*12*), a reaction which might yet utilize BiP.

Translocation across the *E. coli* plasma membrane employs a different strategy for coupling ATP to preprotein movement. *Escherichia coli* preprotein translocase is comprised of SecA, an inherently polar protein, bound to acidic membrane lipids and to SecYEG, a heterotrimeric membrane-embedded protein (*13*). Both the leader and mature domains of preproteins are recognized by SecA (*14*) (see figure, part D, step 1). Preproteins activate SecA for the binding and hydrolysis of ATP. ATP drives a large (30 kilodalton) region of SecA to insert into, and across, the membrane (step 2) (*15*), bearing a "loop" of approximately 20 amino acyl residues of the preprotein across the membrane (*16*). Cross-linking studies (*17*) indicate that the translocation pathway is comprised of both SecA and SecY. A second ATP is consumed to drive the deinsertion of SecA, completing a cycle that translocates 20 amino acyl residues of preprotein. Deinserted SecA (step 3) rapidly exchanges with cytosolic SecAs. When not complexed with SecA·ATP, the preprotein translocates in response to the membrane protonmotive force (*16*).

Why do bacteria, yeast ER and mitochondria, and mammalian ER employ such different modes of coupling high-energy phosphate to protein translocation? The rapid rates of protein synthesis in yeast and bacteria favor posttranslational translocation. Furthermore, *E. coli* preproteins do not begin translocation until they reach a "critical molecular weight" of about 25 kilodaltons (*18*), reflecting the fact that critical information specifying translocation is encoded in the mature domain (*14*, *19*). Folding is coupled to translation (*20*), but translation is not coupled to translocation (*18*). Thus, ribosome "pushing" does not work for bacteria. Furthermore, the bacterial periplasm is home to potent hydrolases that preclude the presence of nucleoside triphosphates and an Hsp70 "ratchet." Yeasts also translate faster than mammals, and protein import into yeast mitochondria and ER is not coupled to ongoing translation (*21*). The MIM44 of mitochondria and the dnaJ-like domain of the yeast ER translocase, which bind the Hsp70 of each organelle at the membrane, have no known counterpart in the mammalian Sec61 complex. Nature appears to have evolved several distinct mechanisms for coupling ATP energy to preprotein movement through the membrane-embedded translocase domains.

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