On the Translocation of Proteins Across Membranes

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A fundamental process in membrane biology is the translocation of large hydrophilic proteins across intact membranes. In bacterial cells, proteins that are components of the outer membrane or the periplasmic space, as well as proteins that are secreted into the medium, are first synthesized in the cytoplasm and then must somehow cross the cytoplasmic, or inner, membrane as individual polypeptide chains. In eukaryotic cells, all secretory and lysosomal proteins are initially translocated across the endoplasmic reticulum membrane; while almost all of the proteins of mitochondria, chloroplasts and peroxisomes are synthesized in the cytoplasm and are specifically translocated across one, two, or sometimes three, membranes to arrive at their specific destinations. In addition to such normal physiological processes, there are problems of practical interest associated with such translocations: e.g., in the production and secretion of foreign proteins in bacteria and other cells by gene cloning techniques; and in the possible selective translocation of protein toxins across the plasma membranes of tumor cells.

Closely related to the problem of the complete translocation of a hydrophilic polypeptide across a membrane is the problem of the intercalation of amphipathic integral proteins into membranes. Almost all known integral proteins span the membrane, with hydrophilic domains exposed to the aqueous phase on both sides of the membrane, and a hydrophobic domain within the bilayer. The intercalation of an integral protein into a membrane can be viewed as one of *partial* translocation of the polypeptide chain across the membrane.

Despite the crucial importance of these phenomena, and the intensive experimental studies of the last decade, we do not yet know how translocation works. In this paper, we assume that there is a unitary mechanism for all translocations of polypeptides across, or into, membranes, which, with relatively minor modifications, can accommodate the diversity of translocations known to exist. The molecular components, thermodynamic constraints, energy requirements, and other factors that are likely to be involved in such a mechanism are first briefly examined. We then suggest a mechanism that conforms to these restrictions. This work is fully described in three papers¹⁻³⁾ to which reference should be made for details and the citation of others' studies.

The primary restrictions on a unitary mechanism of translocation are considered to be as follows:

- 1) The specificity of targeting of a polypeptide chain to its appropriate (organelle) membrane, and its capacity for translocation, are determined by a "signal sequence" of 20-40 amino acid residues generally located near the NH₂-terminus of the chain. The signal sequence is usually, but not always, cleaved from the chain upon translocation.
- 2) Two general classes of signal sequences, hydrophobic and hydrophilic, have to be accommodated, with substantial heterogeneity of sequences within each class.
- 3) The post-signal sequences appear to be largely permissive in translocation; i.e., they seem to have little bearing on the specificity or the process of translocation.
- 4) Translocation can occur either co-translationally or post-translationally *in vivo* or *in vitro*.
- 5) A polypeptide chain appears to

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be translocated neither by a linear threading of the chain residue by residue across the membrane, nor by the entire molecule at once, but rather by successive *segments* of the chain sequence. 6) Energy is required for translocation. 7) Thermodynamic considerations require that the ionic groups of the polypeptide chain must largely remain in contact with water, and the hydrophobic residues shielded from water, during translocation.

These restrictions together strongly indicate that translocation cannot occur directly through the lipid bilayer, but must require the mediation of a set of special integral membrane proteins (translocator proteins, TP's). We propose a unitary mechanism for translocation which assigns a specific structure to TP's, namely, that of a trans-membrane aggregate of n homologous polypeptide subunits, with a trans-membrane water-filled channel running down the central axis of the aggregate. This structure enables the TP: first, to bind the signal sequence; second, to "seed" the formation of successive "sub-domains" of the chain within a subunit interface of the TP; third, to displace (translocate) in an energy-dependent manner these successive sub-domains one after the other until the entire chain is translocated; throughout which entire process, the ionic residues of the chain remain in contact with water *via* the water-filled channel in the TP. This model (2) will be described in detail.

REFERENCES

- 1) P. A. Maher and S. J. Singer : *Proc. Natl. Acad. Sci. USA* (1986)
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