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REGULATION OF INTRACELLULAR PROTEIN TRAFFIC*

GÜNTER BLOBEL

*Laboratory of Cell Biology,
The Rockefeller University,
New York, New York*

A cell contains millions of protein molecules. These are steadily being synthesized and degraded. At homeostasis, a given species of protein is represented by a characteristic number of molecules that is kept constant within a narrow range. Very little is known about the cell's accounting procedures, i.e., how it balances and controls biosynthesis and biodegradation.

An important aspect of biosynthesis (Blobel, 1980) as well as biodegradation (Blobel, 1979) is the intracellular topology of proteins. Many protein species spend their entire life in the same compartment in which they are synthesized, others have to be translocated across the hydrophobic barrier of one, or in some cases, two distinct cellular membranes in order to reach the intracellular compartment or extracellular site where they exert their function. Numerous protein species have to be integrated asymmetrically into distinct cellular membranes. For many proteins this requires partial translocation, i.e., selective transfer of one or several *distinct hydrophilic or charged segments* of the polypeptide chain across the hydrophobic barrier of one or two intracellular membranes. Following complete or partial translocation across a translocation-competent membrane(s), subpopulations may undergo further "posttranslocational" traffic (Palade, 1975). Soluble or membrane proteins may be shipped in bulk or by receptor-mediated processes from a translocation-competent donor compartment to a translocation-incompetent receiver compartment. This posttranslocational traffic may be unidirectional (in which case the protein ends up as a permanent resident of a particular cellular membrane) or may follow a

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cyclic pattern between distinct cellular membranes (e.g., recycling of receptors).

The collective term "topogenesis" has been introduced (Blobel, 1980) to encompass protein translocation (partial or complete) across membranes as well as subsequent posttranslocational protein traffic. Not included in these processes that define topogenesis are distinct traffic patterns that may be required for protein degradation. Theoretical considerations on the topology of protein degradation have been presented elsewhere (Blobel, 1979) and will not be dealt with here: in essence, these considerations argue for the existence of three (animal cells) or even four (plant cells) distinct and separate compartments for protein degradation, each containing a distinct set of proteases. Detailed proposals have been made also for protein topogenesis (Blobel, 1980). The essence of these proposals is that the information for intracellular protein topogenesis resides in *discrete* "topogenic" sequences that constitute a permanent or transient part of the polypeptide chain. The repertoire of distinct topogenic sequences was predicted to be relatively small because many different proteins would be topologically equivalent, i.e., targeted to the same intracellular address. Four types of topogenic sequences were distinguished (Blobel, 1980): (i) *Signal* sequences—they initiate translocation of proteins across specific membranes and are decoded by protein translocators that, by virtue of their signal sequence-specific domain and their location in distinct cellular membranes, effect unidirectional translocation of proteins across specific cellular membranes. (ii) *Stop-transfer* sequences—they interrupt the translocation process that was previously initiated by a signal sequence and, by excluding a distinct segment of the polypeptide chain from translocation, yield asymmetric integration of proteins into translocation-competent membranes. (iii) *Sorting* sequences—they act as determinants for posttranslocational traffic of subpopulations of proteins, originating in translocation-competent donor membranes (and compartments) and leading to translocation-incompetent receiver membranes (and compartments). (iv) *Insertion* sequences interact with the lipid bilayer directly and thereby anchor a protein to the hydrophobic core of the lipid bilayer.

An attempt is made here to amplify some of these previous proposals and to discuss some of the recent experimental data that are relevant to these proposals.

I. TRANSLOCATION OF PROTEINS ACROSS MEMBRANES

Translocation is understood here as transport of an entire polypeptide chain across one (or two) membrane(s), proceeding unidirectionally from the protein biosynthetic compartment. Not considered here will be ectopically synthesized proteins (e.g., toxins such as the colicins or diphtheria toxins) although their entry into cells may also require complete or partial translocation of polypeptide chains across a membrane, either the plasma membrane directly or an intracellular membrane, following uptake by endocytosis.

Hypothetical models for intracellular protein translocation must deal with two essential tenets which appear to underly the observed phenomenology of this process. First, the permeability barrier of the membrane appears to be reversibly modified for the passage of each translocated polypeptide chain while being maintained for other solutes. Second, the species of protein to be translocated as well as the type of membrane across which a given protein is translocated are highly specific. Both of these tenets can be readily satisfied by postulating that protein translocation is a receptor-mediated process (Blobel, 1980) in which specificity is achieved by "signal" sequences in the proteins to be translocated and by signal sequence-specific translocation systems that are restricted in their location to distinct cellular membranes.

II. BIOLOGICAL MEMBRANES ENDOWED WITH PROTEIN TRANSLOCATION SYSTEMS

Several signal sequence-specific translocation systems have been postulated to exist (Blobel, 1980). Table I lists the biological membranes or membrane pairs that have been proposed to be endowed each with *one* signal sequence-specific translocation system (in multiple copies) that is able to decode the information of *one* type of signal sequence. Two modes of translocation have been distinguished, a cotranslational and a posttranslational mode. In cotranslational translocation (Redman and Sabatini, 1966; Blobel, 1980) the passage of the polypeptide chain across the membrane appears to be strictly coupled to translation whereas in posttranslational translocation (Dobberstein *et al.*, 1977; Blobel, 1980) the polypeptide can traverse the membrane posttranslationally uncoupled from its synthesis.

TABLE I

CELLULAR MEMBRANES PROPOSED TO BE ENDOWED WITH A TRANSPORT SYSTEM (TRANSLOCATOR) FOR THE UNIDIRECTIONAL TRANSLOCATION OF NASCENT OR NEWLY SYNTHESIZED PROTEINS^{a,b}

Mode of translocation	Membrane	Code
Cotranslational	a. Prokaryotic plasma membrane	PPM
	b. Inner mitochondrial membrane	IMM
	c. Thylakoid membrane	TKM
	d. Rough endoplasmic reticulum	RER
Posttranslational (across <i>one</i> membrane)	e. Outer mitochondrial membrane	OMM
	f. Outer chloroplast membrane	OCM
	g. Peroxisomal membrane	PXM
Posttranslational (across <i>two</i> membrane)	h. Mitochondrial envelope	MEN
	i. Chloroplast envelope	CEN

^aFrom Blobel (1980).

^bEach of the translocation-competent membranes listed here (a-i) is proposed to contain only *one* distinct "translocator" (in multiple copies). Each translocator responds to *one* type of signal sequence. Translocation can proceed across a *single* membrane (a-g), or *two* membranes (h-i), cotranslationally (a-d), or posttranslationally (e-i). Suggested abbreviations for these translocation-competent membranes might serve as useful codes. For example, a signal sequence (Si) addressed to the rough endoplasmic reticulum (RER), to the chloroplast envelope (CEN), etc., might be designated Si (RER), Si (RER), Si (CEN), etc. Likewise, a particular signal receptor (SiR), or signal peptidase (SiP), could be classified as SiR (RER), SiR (CEN), or SiP (RER), SiP (CEN), etc.

The conjecture was made (Blobel, 1980), based on possible evolutionary relationships between various cellular membranes (see below, Fig. 4), that the contemporary cotranslational translocation systems (Table I, a-d) were derived from a common ancestral system and that they might be highly conserved. A high degree of conservation has indeed been demonstrated for the rough endoplasmic reticulum (RER) translocation system within the animal and plant kingdoms (Dobbertin and Blobel, 1977). Moreover, it has been demonstrated that a signal sequence of a eukaryotic protein addressed to the RER translocation system can be decoded by its putative analog in the prokaryotic plasma membrane (Talmadge *et al.*, 1980a,b). The existence of two other cotranslational translocation systems, namely, those in the inner mitochondrial membrane and in the thylakoid membrane has been pos-

tulated (Blobel, 1980) because of the presence of membrane-bound polysomes in thylakoid membranes (Chua *et al.*, 1973) and in the inner mitochondrial membrane (Kuriyama and Luck, 1973). These cotranslational translocation systems are most likely involved in partial translocation, i.e., translocation only of a distinct segment of the nascent chain (and not of the entire polypeptide) and therefore function in the integration of membrane proteins (see below).

Posttranslational translocation systems have been postulated (Blobel, 1980) for translocation of cytoplasmically synthesized proteins across a *single* membrane (peroxisomal, outer mitochondrial, outer chloroplast membrane) or across *two* membranes (outer and inner membranes of mitochondria and chloroplasts).

Evidence for the existence of a posttranslational translocation system in the peroxisomal membrane rests on the demonstration that liver catalase and uricase (two enzymes located in the peroxisome) are synthesized by free ribosomes and not by membrane-bound ribosomes (Goldman and Blobel, 1978). Conclusive evidence for the existence of posttranslational translocation systems in the outer mitochondrial membrane (Maccacchini *et al.*, 1979b) and across both outer and inner membranes of chloroplasts (Dobberstein *et al.*, 1977; Highfield and Ellis, 1978; Chua and Schmidt, 1978), and mitochondria (Maccacchini *et al.*, 1979a) was first derived from data of *in vitro* translation and translocation experiments which were subsequently confirmed by numerous laboratories. The existence of a posttranslational translocation system in the outer chloroplast membrane analogs to that in the outer mitochondrial membrane has not yet been demonstrated.

III. SIGNAL SEQUENCES

The existence of a "signal sequence" for translocation across the RER was first postulated on theoretical grounds (Blobel and Sabatini, 1971). Subsequently, cell-free synthesis of secretory proteins showed them to be synthesized as larger precursors (Milstein *et al.*, 1972; Swan *et al.*, 1972; Schechter *et al.*, 1974; Devillers-Thiery *et al.*, 1975) and *in vitro* translocation experiments provided evidence that the sequence extension present in these precursors functions as a "signal sequence" in translocation (Blobel and Dobberstein, 1975a,b; Szczesna and Boime, 1976). Thereafter, signal sequences were discovered, by similar

in vitro approaches, for translocation across the prokaryotic plasma membrane (Inouye *et al.*, 1977; Inouye and Beckwith, 1977), the chloroplast envelope (Dobberstein *et al.*, 1977; Highfield and Ellis, 1978), the two mitochondrial membranes (Maccacchini *et al.*, 1979a), and the outer mitochondrial membrane (Maccacchini *et al.*, 1979b).

Translocation is not always accompanied by cleavage of the signal sequence and there are now numerous examples for uncleaved signal sequences. Further, the signal sequence is not always located at the NH₂-terminus (Lingappa *et al.*, 1979; Garoff *et al.*, 1980) and there may be more than one signal sequence in a polypeptide (Blobel, 1980; Garoff *et al.*, 1980).

The complete primary structure is known for the signal sequence addressed to (i) the RER (numerous examples, see compilation by Steiner *et al.*, 1980), (ii) the prokaryotic plasma membrane (numerous examples, see compilation by Emr *et al.*, 1980), and (iii) the chloroplast envelope (so far only one example, Schmidt *et al.*, 1979).

As expected on evolutionary grounds (Blobel, 1980) and as demonstrated experimentally (Talmadge *et al.*, 1980a,b), the signal sequence addressed to the RER plasma membrane is similar to that addressed to the prokaryotic plasma membrane. At present it is not obvious, at least not from the primary structure of the numerous examples, what features of the signal sequence constitute a consensus structure for the receptor (see below). Elegant experiments with mutants (see review by Emr *et al.*, 1980) and with amino acids analogs (Hortin and Boime, 1980) have shown that replacement in the signal sequence of hydrophobic residues by charged or hydrophilic residues interferes with translocation.

As expected, the primary structure of the signal sequence addressed to the chloroplast envelope (Schmidt *et al.*, 1980) differs dramatically from that addressed to the RER or to the prokaryotic plasma membrane. However, the primary structure of more examples needs to be elucidated before one could recognize features of a consensus structure for the corresponding receptor(s) of the chloroplast envelope translocation system.

It should be emphasized that a signal sequence was postulated only to be involved in the initiation of chain translocation (Blobel and Dobberstein, 1975a). Implicit in this postulate was that the rest of the polypeptide chain must be compatible with the translocation machinery (see "stop-transfer" sequences below); for example, a polyleucine or a non-

secretory protein (Moreno *et al.*, 1980) linked to a signal sequence may be nonpermissive for translocation.

IV. MECHANISMS OF TRANSLOCATION

Until recently, the postulated translocation machinery (Blobel and Sabatini, 1971; Blobel and Dobberstein, 1975a; Blobel, 1980) remained largely undefined, so much so that it was deemed unnecessary (von Heijne and Blomberg, 1979; Wickner, 1979; Garnier *et al.*, 1980; Engelman and Steitz, 1981). Only after the development of an *in vitro* translocation system (Blobel and Dobberstein, 1975b) that was able to reproduce translocation across the ER membrane (isolated in form of closed microsomal vesicles) with apparent fidelity, did it become possible to assay and to characterize the ER's translocation activity *in vitro*. Two approaches were taken to dissect the membrane translocation activity: salt extraction (Warren and Dobberstein, 1978; Walter and Blobel, 1980) and limited proteolysis (Walter *et al.*, 1979; Meyer and Dobberstein, 1980a). Both approaches yielded membrane vesicles that were largely translocation-inactive; translocation activity, however, could be restored by readdition of the salt or tryptic extract. These findings provided an assay for the purification of the active components of the salt extract (Walter and Blobel, 1980) and of the proteolytic extract (Meyer and Dobberstein, 1980b). The purified active component of the proteolytic extract consisted of an apparently single polypeptide chain (Meyer and Dobberstein, 1980b) whereas the purified active component of the salt extract was shown to be an 11 S protein of ~250,000 daltons that consisted of six polypeptide chains which could not be separated from each other by a variety of nondenaturing procedures (Walter and Blobel, 1980). The precise relationship between the purified proteins from the proteolytic and the salt extract remains to be investigated (see below).

Studies on the role of the 11 S protein in the translocation process revealed that it is involved in the recognition of the signal sequence and therefore it was termed "Signal Recognition Protein" (SRP) (Walter *et al.*, 1981). When SRP is present in the cell-free translation system in the absence of salt-extracted microsomal membranes it was found to inhibit selectively only the translation of mRNA for secretory protein (bovine prolactin) but not of mRNA for cytosolic proteins [α and β

chain of rabbit globin (Walter *et al.*, 1981)]. Moreover, SRP was found to bind with a relatively low affinity (apparent K_d 5×10^{-5}) to ribosomes, but was shown to bind with a 6000-fold higher affinity (apparent K_d 8×10^{-9}) when ribosomes are engaged in the translation of mRNA for secretory proteins (Walter *et al.*, 1981). Most interestingly, this high-affinity binding of SRP caused a site-specific and signal sequence-induced arrest of chain elongation (Walter and Blobel, 1981b). The elongation-arrested peptide of nascent preprolactin was shown to be ~ 70 amino residues long (Walter and Blobel, 1981b). Because the signal sequence of nascent bovine preprolactin comprises 30 residues (Jackson and Blobel, 1980) and because about 40 residues of the nascent chain are buried (protected from proteases) in the large ribosomal subunit (Malkin and Rich, 1967; Blobel and Sabatini, 1970), it was concluded (Walter and Blobel, 1981b) that it is the signal sequence of the nascent chain (fully emerged on the outside of the large ribosomal subunit) that causes high-affinity binding of SRP which in turn modulates translation and causes arrest in chain elongation.

Most strikingly, elongation arrest is released upon binding of the elongation-arrested ribosome to salt-extracted microsomal membranes (K-RM) resulting in chain elongation and translocation into the microsomal vesicle (Walter and Blobel, 1981a). Binding of the translating ribosome to K-RM occurs only in the presence of SRP. Further, treatment of K-RM with low concentrations of trypsin abolishes SRP-mediated binding of the translating ribosome to K-RM (Walter and Blobel, 1981a). This latter finding suggests that besides SRP (which could be considered a peripheral membrane protein) integral membrane proteins are required for translocation to proceed. It is likely, but remains to be proven, that it is the hydrophilic cytoplasmic domains of these integral membrane proteins [severed by proteolytic enzymes in such a manner that they retain reconstitutability to their parent molecules (Walter *et al.*, 1979; Meyer and Dobberstein, 1980a)] that have recently been purified (Meyer and Dobberstein, 1980b).

Taken together, these data provide the strongest support to date for the most pivotal (and most contested) postulate of the signal hypothesis (Blobel and Dobberstein, 1975b; Blobel, 1980), namely, that protein translocation across the ER is a receptor-mediated process. These data thus definitively rule out alternative hypotheses that have postulated that chain translocation across the ER occurs spontaneously, without the

mediation by proteins, (Bretscher, 1973; Wickner, 1979; Garnier *et al.*, 1980; Engelman and Steitz, 1981). They also rule out translocation models that, although relying on the participation of specific proteins, have postulated a primary interaction of the signal sequence (because of its hydrophobic nature) with the lipid bilayer (DiRienzo *et al.*, 1978; von Heijne and Blomberg, 1979; Steiner, 1980). Thus, the initial events that lead to translocation and provide for its specificity are protein-protein (signal sequence plus ribosome-SRP) and *not* protein-lipid (signal sequence-lipid bilayer) interactions.

The ability of SRP to arrest chain elongation and the finding that microsomal membranes release this arrest is of teleological interest. If this mechanism also operates *in vivo* it would provide the cell with a means to stop the synthesis of secretory proteins (some of which might be harmful if completed in the cytosol) unless sites on the ER are available so that translocation and segregation into the intracisternal space are ensured. These sites in the microsomal membranes could consist of several integral membrane proteins which might form an ensemble undergoing cyclic disassembly and reassembly for each chain translocation event (Blobel and Dobberstein, 1975a). Signal peptidase and core sugar transferase might, as integral membrane proteins, participate in the formation of this ensemble or might be transiently associated with it. Other components of this ensemble might be the so-called ribophorins (Kreibich *et al.*, 1978a,b) although their involvement in protein translocation has not yet been demonstrated.

Because of evolutionary considerations (see below) and because of the documented mechanistic similarity of protein translocation across the prokaryotic plasma membrane (Smith *et al.*, 1977; Randall *et al.*, 1978; Chang *et al.*, 1978, 1979; Emr *et al.*, 1980; Talmadge *et al.*, 1980a) to that across the ER our conjecture is (Blobel, 1980) that there is only *one*, *cotranslational* translocation system in the bacterial plasma membrane and, moreover, that this system will be essentially similar if not identical to that in the ER. However, it should be noted that this view has been challenged and that a posttranslational mode of translocation across the bacterial plasma membrane has been postulated (Wickner, 1979; Koshland and Botstein, 1980).

The discovery of SRP has permitted us to add more detail to and to expand the previously proposed translocation models. The postulated ribosome receptor and signal sequence receptor for the cotranslational

translocation system were envisioned to be integral membrane proteins (Blobel, 1980). Because SRP (presumably a peripheral membrane protein) is, at least in part, endowed with these postulated receptor properties and because additional, integral membrane proteins are required for translocation (translocation activity of trypsinized K-RM cannot be restored by SRP) our present cotranslational translocation model (Walter and Blobel, 1981b) is in detail, not in principle, more complex than previously envisioned (Blobel, 1980).

The discovery of SRP suggests likewise modifications of our models for posttranslational translocation. The latter has been envisioned to be in principle similar to cotranslational translocation except that the existence of only signal sequence receptors (again as integral membrane proteins) but not of ribosome receptors was envisioned (Blobel, 1980). If signal sequence-specific SRP analogs would exist also for the various posttranslational translocation systems and if in turn SRP-specific receptors in various organelle membranes were to control import into organelles, one could envision a cytoplasmic pool of translocation-competent complexes consisting of an SRP analog plus a protein to be imported. The search for these SRP analogs is now under way in our laboratory.

V. INTEGRATION INTO MEMBRANES

Many integral membrane proteins (IMPs) require selective translocation of one or more hydrophilic segment(s) of the polypeptide chain in order to acquire their characteristic asymmetric orientation. How could a selective translocation of discrete segment(s) of the polypeptide chain be accomplished?

In considering theoretical solutions to this problem, an arbitrary definition of possible modes of orientation of the polypeptide chain of IMPs with respect to the hydrophobic core and the hydrophilic environment of the lipid bilayer was proposed (Blobel, 1980). IMPs were classified as monotopic, bitopic, and polytopic (see Fig. 1). The polypeptide chain of monotopic IMPs exhibits unilateral topology—i.e., each molecule possesses hydrophilic domain(s) exposed to the hydrophilic environment on only one side of the membrane. The polypeptide chain of bitopic and polytopic IMPs is bilateral in nature, containing two or multiple hydrophilic domains, respectively, exposed on opposite sides of the membrane.

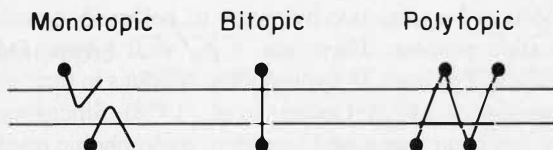


FIG. 1. Classification of integral membrane proteins (IMPs) as monotopic, bitopic, and polytopic. The hydrophobic boundary of the lipid bilayer is indicated by two parallel lines. Solid circles on polypeptide chains indicate major hydrophilic domains. The hydrophilic domain of an individual monotopic IMP is exposed only on one side of the lipid bilayer. A hydrophobic domain is indicated to anchor the polypeptide chain to the hydrophobic core of the lipid bilayer. A monotopic IMP may contain several hydrophilic and hydrophobic segments alternating with each other (not indicated here). However, all hydrophilic domains are unilaterally exposed. The polypeptide chain of bitopic IMPs spans the lipid bilayer once and contains a hydrophilic domain on each side of the membrane. In variants of bitopic IMPs (not indicated), the bilateral hydrophilic domains could be further subsegmented by interspersed hydrophobic domains that are capable of monotopic integration. The polypeptide chain of polytopic IMPs spans the membrane more than once and contains multiple hydrophilic domains on both sides of the membrane. The existence of polytopic IMPs remains to be demonstrated. Two structurally monotopic IMPs located on opposite sides of the membrane could interact via their hydrophobic anchorage domains and form a functionally bilateral ensemble.

It was proposed (Blobel, 1980) that all of these orientations could be accomplished by invoking, in addition to the signal sequence, only two additional types of topogenic sequences, termed "stop-transfer sequences" and "insertion sequences." The *stop-transfer sequence* was proposed to contain the information to interrupt the chain translocation process that was initiated by a signal sequence—e.g., by effecting premature disassembly of the translocation system (Blobel, 1980).

Because translocation of the polypeptide chain could be expected to proceed sequentially and asymmetrically in both cotranslational and posttranslational translocation, stop-transfer sequences would be effective means for asymmetric integration of certain IMPs by either modes of translocation (see Table I). There could be as many translocator-specific stop-transfer sequences as there are translocator-specific signal sequences. On the other hand, there could be only one stop-transfer sequence addressed to one component common to all translocators.

The sequence features that constitute a stop-transfer sequence remain to be defined. The stop-transfer sequence may not simply be that stretch of ~25 primarily hydrophobic residues which is found as the transmembrane segment of bitopic IMPs and which might be envisioned to

act as a stop-transfer sequence by virtue of being nonpermissive with the translocation process. There are, e.g., viral bitopic IMPs which possess a stretch of at least 28 hydrophobic residues in their ectoplasmic domain (Scheid *et al.*, 1978; Gething *et al.*, 1978). Since this domain is translocated it is clear that a long stretch of hydrophobic residues per se is not sufficient to stop the translocation process.

The *insertion sequence* functions to anchor a protein monotopically to the hydrophobic core of the lipid bilayer. Insertion would be spontaneous and not mediated by specific proteins. It would not be accompanied by the translocation across the membrane's lipid bilayer of large charged segments of the polypeptide chain. The latter can be achieved only by a signal sequence in a receptor-mediated process.

As is the case for the stop-transfer sequence, the structural features of an insertion sequence remain to be defined. It is conceivable that there are several unique insertion sequences that can distinguish lipid composition and therefore insert only into specific membranes. On the other hand, the specificity of insertion into a distinct membrane may be largely dictated by protein-protein interaction (i.e., by an affinity of a protein to be inserted to another IMP).

Although the precise orientation of the polypeptide backbone with respect to the lipid bilayer is unknown for most species of IMPs, the proposed (Blobel, 1980) hypothetical schemes of multiple topogenic sequences (Fig. 2) can explain any one orientation by what essentially are a limited number of highly redundant mechanisms. It is clear from these examples (Fig. 2) that the integration of most proteins into the membrane requires a signal sequence and a translocator, except for one subgroup of monotopic IMPs (see Fig. 2, upper left example). Thus, most IMPs can be integrated directly only into translocation-competent membranes. Because the translocators themselves are likely to consist of IMPs (see above) that require translocation for their integration into the membrane, it follows that Virchow's paradigm on the ontogeny of cells could be extended to membranes and paraphrased to *omnis membrana e membrana*.

Information about the mechanism of integration can be derived from assays which mimic the *in vivo* situation as closely as possible. Isolation of an IMP with detergents and its subsequent reconstitution into lipid vesicles (Kagawa and Racker, 1971), while important for functional studies, cannot yield such information because it is improbable that

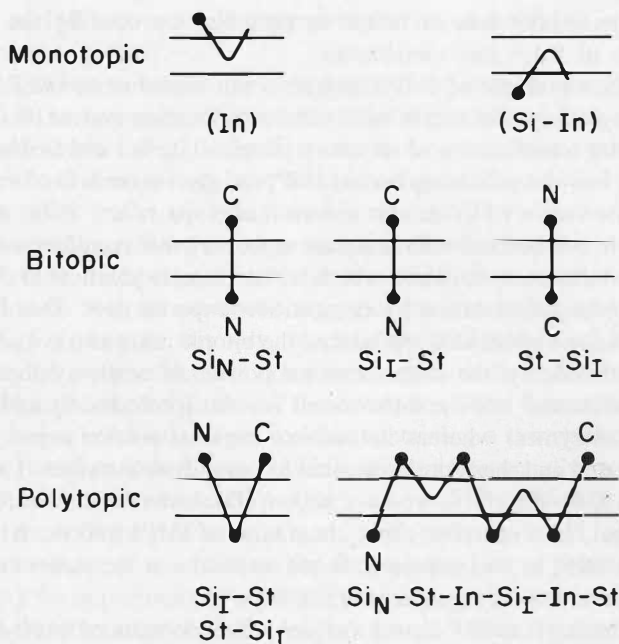


FIG. 2. Program of topogenic sequences for the asymmetric integration into membranes of some representative examples of monotopic, bitopic, and polytopic IMPs (taken from Blobel, 1980). Hydrophobic boundary of the lipid bilayer is indicated by two parallel lines, with the upper line facing the protein biosynthetic compartment. Solid circles represent major hydrophilic domains which, when indicated, contain amino (N) or carboxy (C) terminus of the polypeptide chain. Topogenic sequences are: insertion sequence (In), signal sequence (Si), and stop-transfer sequences (St.). Si_N and Si_I indicate amino-terminal and internal signal sequences, respectively. Examples given here (except for monotopic IMP at upper left) are for cotranslational integration into RER. Similar programs are conceivable also for cotranslational integration into PPM, IMM, and TKM as well as for posttranslational integration into PXM, OMM, OCM, IMM [using Si (MEN)], and ICM/TKM [using Si (CEN)]. An attempt has been made to list topogenic sequences in order of their location along the polypeptide chain starting from the amino terminus. The problems encountered in predicting the order relate to uncertainties as to the order of chain translocation. In particular, in the case of an internal signal sequence (Si_I) there are several possibilities depending on the order of translocation (Lingappa *et al.*, 1979). The orientation of a polytopic IMP such as indicated at the lower right is entirely hypothetical and is illustrated here only to indicate how such a polypeptide chain could be integrated into the membrane by a program of multiple topogenic sequences.

detergents (either free or bound to proteins) are used by the cell to integrate its IMPs into membranes.

The first example of IMP integration into membranes (RER) under physiological conditions, in an *in vitro* translocation system [developed for *in vitro* translocation of secretory proteins (Blobel and Dobberstein, 1975b)] was that of a bitopic viral IMP, the glycoprotein G of vesicular stomatitis virus (VSV). It was shown (Lingappa *et al.*, 1978) that this protein is synthesized with a signal sequence, that is addressed to the ER translocation system and which is functionally identical to that of a secretory protein (shown by competition experiments). This *in vitro* translocation system also reproduced the bitopic asymmetric orientation of G with fidelity; the amino-terminal portion of newly synthesized G was translocated into the microsomal vesicles (protected by added proteolytic enzymes) whereas its carboxy-terminal portion remained untranslocated and therefore accessible to proteolytic enzymes (Lingappa *et al.*, 1978). Recently, we have shown (D. Anderson, P. Walter, and G. Blobel, in preparation) that integration of IMPs into the RER also requires SRP, as was expected, based on results of the earlier competition experiments (Lingappa *et al.*, 1978).

The finding that SRP causes a signal sequence-induced arrest in chain elongation (Walter and Blobel, 1981b) should be useful for mapping the location [NH₂-terminal or internal (Lingappa *et al.*, 1979)] of a signal sequence in those IMPs that contain an uncleaved signal sequence (Bonatti and Blobel, 1979; Schechter *et al.*, 1979). The same approach should be useful also for mapping the location of multiple signal sequences (Garoff *et al.*, 1980).

Together with the rapidly accumulating information on the primary structure of a variety of IMPs and on their precise topology in the membrane, SRP and the *in vitro* translocation system can also be expected to yield detailed information on the mechanism of integration of those IMPs with other than a simple bitopic orientation.

VI. PHYLOGENY OF MEMBRANES, PROTEIN TRANSLOCATION, AND COMPARTMENTS

How then could biological membranes with their characteristic asymmetry of proteins have evolved if their assembly depended on the development of a protein translocation system which, because it was made up

in part of IMPs, was itself dependent for its assembly on a protein translocation system?

In an attempt to retrace the "phylogeny" of membranes one could distinguish between precellular and cellular stages of evolution. Starting with lipid vesicles (Fig. 3) the first step in the precellular evolution of biological membranes may have been monotopic integration of proteins into the outer leaflet of lipid vesicles via insertion sequences. Such vesicles could have functioned as capturing devices to collect, on their outer surface, components involved in replication, transcription, and translation as well as metabolic enzymes present in the surrounding medium (Fig. 3A). In this way, much of the precellular evolution and assembly of macromolecular complexes (such as the ribosome) may have proceeded on the surface of these vesicles rather than within vesicles. By vesicle fusion, larger vesicles containing a synergistic assortment of functions could have evolved, resulting essentially in the formation of "inside-out cells" (Fig. 3A and B) (Blobel, 1980). Concurrent with the evolution of such inside-out cells could have been the development of mechanisms for the translocation of proteins, thus providing the opportunity to segregate proteins, to colonize (with monotopic IMPs) the interior leaflet of the vesicle's lipid bilayer, and to integrate bitopic IMPs. Toward this end, the ribosome-membrane junction could have been remodeled and the insertion sequence could have evolved into a signal sequence so as to achieve first a cotranslational mode of translocation. The development of the stop-transfer sequence (perhaps as a variant of the signal sequence) to integrate bitopic IMPs may have concluded the precellular evolution of the cotranslational mechanism for the assembly of membranes. The posttranslational mode of translocation may have evolved from the cotranslational mode by transposing the information that might be contained in a ribosomal protein and adding it to the signal sequence for cotranslational translocation. The integration of bitopic IMPs into the lipid bilayer permitted the development of transport systems and signaling systems. This set the stage for evolution to continue within a closed system (the primordial cell) effectively sealed from some of the hazards of the surrounding medium by the lipid bilayer but able to communicate with the outside via the lipid bilayer-integrated transport and signaling systems. The primordial cell (Fig. 3D) may have possessed two membranes, a plasma membrane delimiting the newly generated endoplasmic compartment,

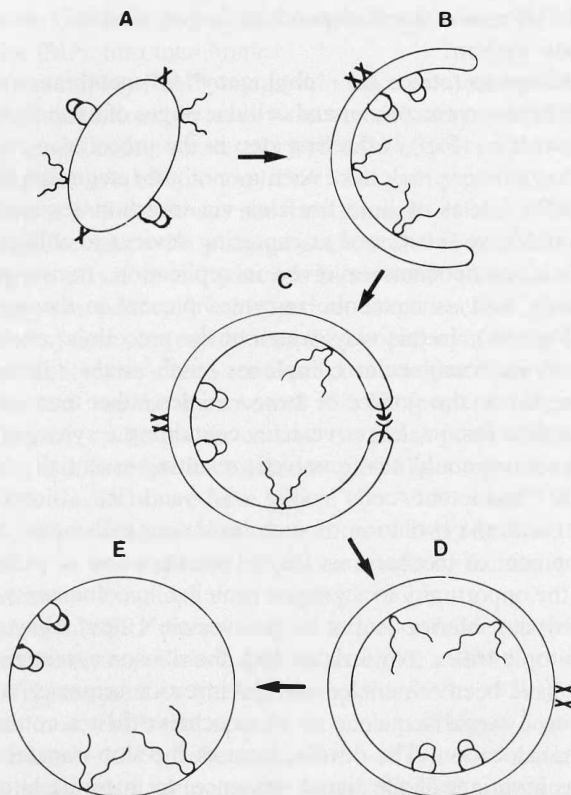


FIG. 3. Schematic illustration of various theoretical stages of precellular evolution on the surface of vesicles culminating in the formation of a primordial cell (taken from Blobel, 1980). (A) Vesicles containing monotopic IMPs (not indicated) are able to bind various macromolecules (X) and macromolecular complexes, among them chromatin and ribosomes. (B) Nonrandom distribution of bound components on the vesicle surface and beginning invagination. (C) Formation of a "gastruloid" vesicle, perhaps able to open and to close via protein-protein interaction of bitopic IMPs at the orifice. (D) Fusion at the orifice, resulting in a primordial cell delimited by two membranes. (E) Loss of the outer membrane. D could have evolved into Gram-negative bacteria and E into Gram-positive bacteria and eukaryotic cells (see Fig. 4).

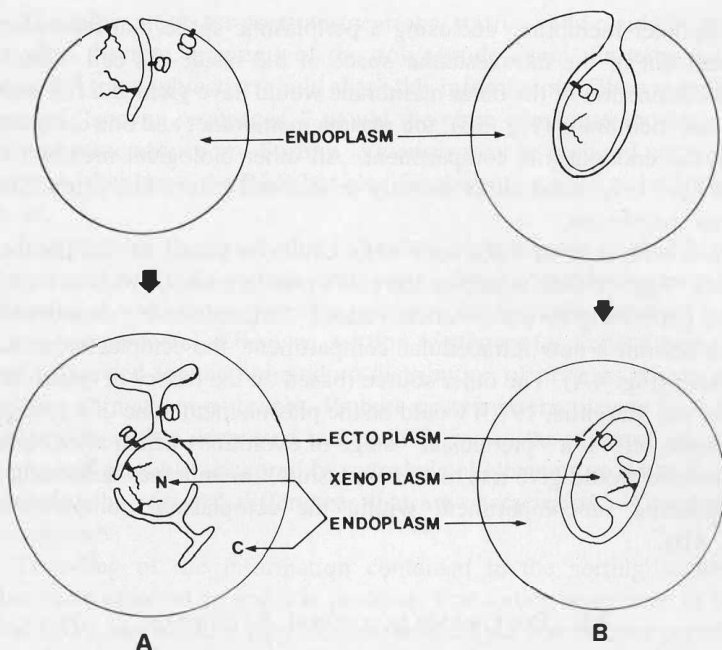


FIG. 4. Schematic illustration of the evolution of intracellular membranes and compartments (taken from Blobel, 1980). (A) Aggregation of certain membrane functions in the plane of the pluripotent plasma membrane. Nonrandom removal of these functions from the plasma membrane by invagination and fission results in the formation of a nuclear envelope (pore complexes omitted) continuous with the endoplasmic reticulum (rough and smooth) and generates an ectoplasmic compartment. The endoplasmic compartment is thereby subdivided into nucleoplasm (N) and cytoplasm (C). Note, however, that N and C remain connected via nuclear pores that do not have a membranous barrier. Other intracellular membranes that are distinct from the endoplasmic reticulum, such as lysosomal, peroxisomal, and Golgi complex membranes, also could have developed by invagination from the plasma membrane or could be outgrowths of the endoplasmic reticulum. (B) Symbiotic capture of another cell, generating an additional xenoplasmic compartment. Green plant cells have two such xenoplasmic compartments (mitochondrial matrix and chloroplast stroma). Only the inner mitochondrial membrane and the inner chloroplast membrane (including derived thylakoid membrane) would be of xenoplasmic origin, whereas the outer mitochondrial and chloroplast membranes would be of orthoplasmic origin, like all other cellular membranes. The proposed terminology may be useful for describing the precise topology of IMPs (see Fig. 1). For example, monotopic IMPs of the thylakoid membrane may be exposed ectoplasmically (i.e., toward the intradisc space) or xenoplasmically (i.e., toward the stroma); bitopic IMPs of the outer mitochondrial membrane have an ectoplasmic and an endoplasmic domain, etc.

and an outer membrane enclosing a periplasmic space that represents the remnant of the intravesicular space of the inside-out cell. Subsequent elimination of the outer membrane would have yielded a cell with only one membrane (Fig. 3E), the plasma membrane, and one compartment, the endoplasmic compartment. All other biological membranes could have originated either directly or indirectly from this primordial plasma membrane.

The membranes of eukaryotic cells could be traced to two distinct sources (Fig. 4). One would be the cell's own primordial plasma membrane, generating by invagination various "orthoplasmic" membranes which delimit a new intracellular compartment, the ectoplasmic compartment (Fig. 4A). The other source (based on the theory of endosymbiosis; see Margulis, 1970) would be the plasma membrane of a foreign symbiotic cell (at a "prenuclear" stage of evolution) which after being interiorized would give rise to "xenoplasmic" membranes delimiting a xenoplasmic subcompartment within the ectoplasmic compartment (Fig. 4B).

VII. POSTTRANSLOCATIONAL PATHWAYS

The nonrandom removal of distinct membrane functions from a pluripotent primordial plasma membrane during evolution would generate a number of highly differentiated intracellular membranes that lack a translocator and that are physically not continuous (at least not permanently) with translocation-competent membranes. These translocation-incompetent membranes (or the subcompartments they enclose) therefore must receive their translocation-dependent, constitutive IMPs (or segregated proteins) from translocation-competent membranes (or subcompartments).

The most significant donor membrane (subcompartment) is the RER which probably supplies translocation-dependent proteins to essentially all orthoplasmic membranes and ectoplasmic subcompartments (Palade, 1975). Each of the receiving membranes presumably contains a set of IMPs that are permanent residents (either constitutive to a particular receiving membrane or shared by several other orthoplasmic membranes) and a set of proteins in transit [either on their way to their permanent residence or cycling between orthoplasmic membranes (e.g., carrier proteins, see below)].

The information for posttranslocational traffic could reside in one (or several) discrete segments of the polypeptide chain. Proteins with an identical travel objective could share this information. These sequences, termed "sorting sequences," would therefore constitute another group of topogenic sequences. Sorting sequences may be required not only for proteins that leave the RER but also for those that need to be anchored there.

It is possible, however, that individual proteins may be able to reach their target without a sorting sequence(s). They could do this merely by association with another protein (piggybacking) that is endowed with a sorting sequence(s). Likewise, sorting sequences (as defined here) may not be needed for the nonrandom distribution of proteins within physically continuous membranes. Protein-protein interactions to form large ensembles with a decreased rate of diffusion in the plane of the membrane and possibly anchored by cytoskeletal elements could be responsible for the regional differences that are characteristic of continuous membranes.

Decoding of the information contained in the sorting sequences should be effected by specific proteins. For sorting sequences of bilateral IMPs, the effector may be represented by a few distinct peripheral membrane proteins. For sorting sequences of soluble proteins, such as lysosomal enzymes, the effector may be represented by a bilateral IMP that functions as a carrier protein shuttling back and forth between the donor and a receiver compartment. Its ectoplasmic domain may be able to bind reversibly to the sorting sequence(s) of lysosomal enzymes and its endoplasmic domain may contain a sorting sequence for a cyclic traffic pattern between the donor (RER) and receiver compartments [the latter could be represented by a distinct portion of the Golgi apparatus from which primary lysosomes develop (Novikoff, 1976)]. A defect in the carrier could result in secretion of all lysosomal enzymes.

The need for sorting arose from the use of only one translocator for topologically different proteins. The reverse—namely, the potential to use more than one translocator for topologically equivalent proteins—may have arisen when certain membranes (see Table I) acquired a posttranslational translocator. For example, there could be two programs of topogenic sequences for peroxisomal proteins (Table II), both for the "content" proteins of the peroxisome and for those constitutive of the peroxisomal membrane (exemplified by bitopic IMPs). In reality,

TABLE II

ALTERNATE-CHOICE PROGRAMS OF TOPOGENIC SEQUENCES FOR TOPOLOGICALLY EQUIVALENT PROTEINS^{a,b}

Membrane	Bitopic IMPs	Content proteins
Peroxisomal	Si (PXM)-St	Si (PXM)
	Si (RER)-St-So	Si (RER)-So
Inner mitochondrial	Si (IMM)-St	Si (MEN)
	Si (MEN)-St	
Thylakoid	Si (TKM)-St	Si (TKM)
	Si (CEN)-St-So	Si (OCM)-So

^aFrom Blobel (1980).

^bAbbreviations as in Table I; St, stop-transfer sequence; So, sorting sequence. Listed are programs only for bitopic IMPs and content proteins that are not integral membrane proteins. Alternate programs analogous to those shown for the peroxisomal membrane are theoretically possible also for the outer membrane of mitochondria and chloroplasts, whereby the "content" proteins would correspond to proteins that are located in the ectoplasmic compartment (intermembrane space) of mitochondria and chloroplasts (see Fig. 4). Likewise, a program analogous to that shown for the inner mitochondrial membrane is conceivable also for the inner membrane of chloroplasts. For the corresponding "content" proteins in the xenoplasmic compartment there most likely is no alternate program of topogenic sequences: proteins are synthesized either within the xenoplasmic compartment or imported via Si (MEN) or Si (CEN). The alternate programs for bitopic IMPs in the thylakoid membrane are similar to those in the inner chloroplast membrane, except that sorting sequences may be required for the program Si (CEN)-St to distinguish between those bitopic IMPs that remain in the inner membrane and those that continue (by invagination) to become residents of TKM. By the same token, one of the programs [Si (OCM)-So] for the corresponding "content" proteins in the intradisc space is based on the possibility that this space communicates transiently with the ectoplasmic space of chloroplasts.

however, only one program for each group may exist, such as Si (PXM) for peroxisomal content proteins and Si (RER)-St-So for peroxisomal bitopic IMPs, with the alternate program either never developed or eliminated in evolution.

On the other hand, both programs indicated in Table II for the integration of bitopic IMPs into the inner mitochondrial membrane (or the inner membrane of chloroplasts) and into the thylakoid membrane are likely to exist.

Finally, if topogenic sequences behaved in evolution like "transposable" elements one could conceive of "pleiotopic" proteins that are similar in structure and function but different in topology. Pleiotopic proteins could have arisen by the loss or acquisition of a topogenic sequence(s). Such processes may be important (i) for achieving dichotomy in the posttranslocational pathway of proteins (e.g., secretory and lysosomal proteins) or (ii) for achieving either export or retention via binding to membranes [e.g., secreted or membrane-bound form of IgM heavy chains (Rogers *et al.*, 1980)] or (iii) for diversifying the organellar distribution of proteins (e.g., some proteins that may occur both within peroxisomes and the mitochondrial matrix) or (iv) for anchoring polymeric structures in the membrane (e.g., free and membrane-bound forms of cytoskeletal proteins).

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