Volume 285, number 2, 182–188 FEBS 09960 July 1991

ADONIS 001457939100684Q

Minireview

Protein export in prokaryotes and eukaryotes

Theme with variations

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Received 7 May 1991

Protein export in prokaryotes as well as in eukaryotes can be defined as protein transport across the plasma membrane. In both types of organisms there are various apparently ATP-dependent transport mechanisms which can be distinguished from one another and which show similarities when the prokaryotic mechanism is compared with the respective eukaryotic mechanism. First, one can distinguish between transport mechanisms which involve so-called signal or leader peptides and those which do not. The latter mechanisms seem to employ ATP-dependent transport systems which belong to the family of oligopeptide permeases and multiple drug resistance proteins. Second, in signal or leader peptide-dependent transport one can distinguish between transport mechanisms which involve ribonucleoparticles and those which employ molecular chaperones. Both mechanisms appear to converge at the level of ATP-dependent translocases.

ATP-dependent protein transport; Bacterial plasma membrane; Yeast microsome; Dog pancreas microsome

1. INTRODUCTION

Every polypeptide has a unique intra- or extracellular location where it fulfills its function. Two basic facts exist in both prokaryotes and eukaryotes which complicate our attempts to understand this situation: (i) in general, proteins are synthesized in a single compartment, i.e. the cytosol (excluding protein synthesis in mitochondria and chloroplasts of the eukaryotic cells), however, non-cytosolic proteins must subsequently be directed to a variety of different subcellular locations, and (ii) in the case of non-cytosolic proteins the sites of synthesis and of functional location are separated by at least one biological membrane. Consequently, mechanisms must exist which ensure the specific transport of proteins across membranes. In this review we will focus on the export of newly synthesized proteins from prokaryotes and eukaryotes.

By definition, exported proteins in Gram negative bacteria, such as *Escherichia coli*, include proteins which have their functional location in the periplasm, the outer membrane, or the extracellular space (secretory proteins). In eukaryotes, exported proteins include secretory proteins (lower and higher eukaryotes) as well as proteins which have their functional location in or at the cell wall (lower eukaryotes).

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In prokaryotes and eukaryotes there appear to be various ATP-dependent transport mechanisms for protein export. As a first common theme one can distinguish between transport mechanisms which involve so called signal or leader peptides and those which do not (Fig. 1). In *Escherichia coli* most secretory proteins appear to use a leader peptide-independent mechanism for their transport across the plasma membrane [1,2] while the other proteins (periplasmic and outer membrane proteins) appear to use a leader peptide-dependent mechanism. In eukaryotes the signal peptide-independent mechanism seems to take place at the plasma membrane [3,4]. In contrast, the signal peptide-dependent mechanism operates at the level of the membrane of the endoplasmic reticulum. From there the secretory proteins reach the extracellular space by vesicular transport, i.e. fission and fusion of membrane vesicles. A second common theme which has emerged during the last couple of years is that signal or leader peptide-independent transport involves transport components which are related to the bacterial oligopeptide transport systems and the mammalian multiple drug resistance proteins (P-glycoproteins), i.e. a family of ATP-dependent membrane proteins [1–5]. A third common theme is that signal or leader peptide-dependent transport can involve ribonucleoparticles or molecular chaperones. A fourth common theme is that the latter two mechanisms appear to converge at the level of an ATP-dependent translocase. From here on this review will focus on signal or leader peptide-dependent export of proteins.

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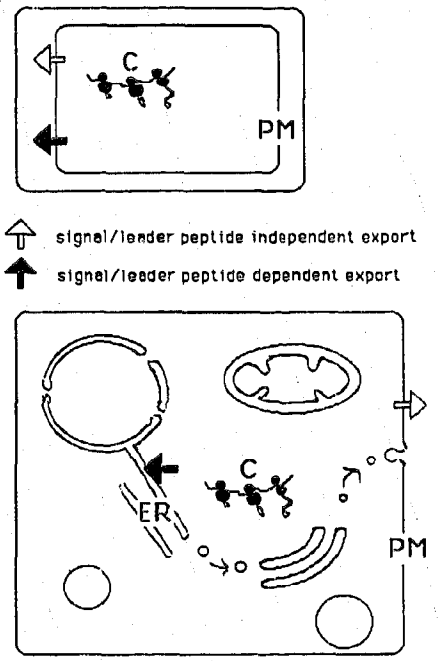

Fig. 1. Protein export in prokaryotes and eukaryotes. C, cytosol; ER, endoplasmic reticulum; PM, plasma membrane.

2. RESULTS

The transport of signal or leader peptide-containing exported proteins across the relevant biological membranes can be visualized as a sequence of various steps which include membrane association, membrane insertion and completion of translocation. In the eukaryotic cells the membrane association step may be more complex than in prokaryotes because of the greater variety of membrane types. Recently accumulated evidence on three membrane systems can be summarized in some type of consensus transport scheme (Fig. 2). It appears that there are two cytosolic systems which can contribute to the fidelity of the respective precursor proteins (Table I) (see below for details and references). One system involves ribonucleoparticles (RNP), such as the mammalian signal recognition particle (SRP), plus a receptor for this RNP at the membrane surface and, at least in the eukaryotic cells, the ribosome plus its receptor at the microsomal surface. The mammalian SRP seems to be able to support precursor proteins with respect to membrane specificity (together with its receptor, the docking protein) and, in collaboration with the ribosome, it seems to be able to keep the precursors in a state where the signal peptide is exposed and where the precursor stays water soluble as well as in an 'unfolded' state. In other words, mammalian SRP has a variety of functions which are brought about by the inhibition of elongation of the nascent precursor polypeptide after SRP has bound to the signal peptide as it emerged from the ribosome. Molecular chaperones, on the other hand, only help the precursors to stay soluble as well as loosely folded and to keep their signal peptides exposed. Otherwise the precursors which can make use of this system seem to require structural features which allow them to stay in this form on their own, at least for a certain time.

We have previously shown that ribonucleoparticle-independent transport of proteins into mammalian microsomes is stimulated by the ATP-dependent molecular chaperone hsp70 (see below for details and references). Recently we addressed the question of whether there are additional nucleoside triphosphate requirements involved in this transport mechanism (Klappa, P., Mayinger, P., Pipkorn, R., Zimmermann, M. and Zimmermann, R., EMBO J., in press). We employed a purified presecretory protein which upon solubilization in dimethyl sulfoxide and subsequent dilution into an aqueous buffer was transported into mammalian microsomes in the absence of the cytosolic (termed *cis*-acting) molecular chaperone. We observed that membrane insertion of this precursor protein depends on the hydrolysis of ATP and involves a microsomal protein which is sensitive to photoaffinity labeling with azido-ATP. Furthermore, we found that a microsomal protein with a similar sensitivity towards photoaffinity labeling with azido-ATP is involved in ribonucleoparticle-dependent transport of proteins into mammalian microsomes. The azido-ATP sensitive protein was shown to be distinct from the lumenal (termed *trans*-acting) chaperone BiP. Therefore, we suggested that a hitherto unknown microsomal protein which depends on ATP-hydrolysis is involved in membrane insertion of both, ribonucleoparticle-dependent and -independent precursor proteins.

2.1. *Escherichia coli*

In *E. coli* the pathway which seems to be used by the majority of leader peptide-containing precursors of exported proteins involves *cis*-acting molecular

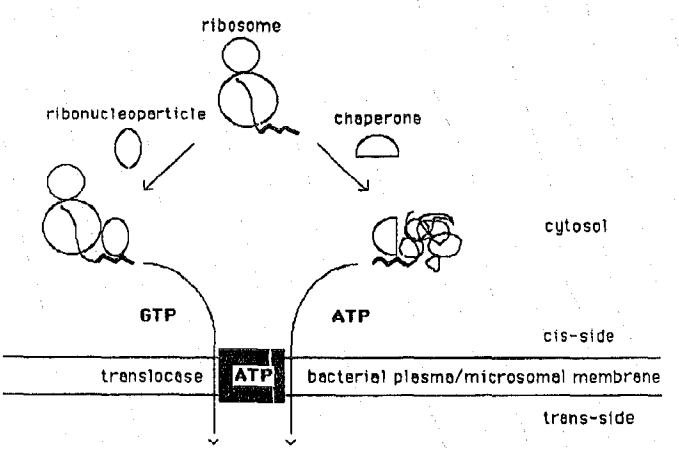

Fig. 2. Consensus model for signal/leader peptide-dependent protein export.

Table I

Proteins and complexes involved in protein translocation across *E. coli* plasma membrane, yeast microsomal membrane and canine microsomal membrane

Complex/protein	*E. coli*	Yeasts	Mammals
RNP	4.5 S RNA	7 S RNA	7 S RNA
			72 kDa-p SRP72
			68 kDa-p SRP68
	48 kDa-p ffh-p,GTP-bp	54 kDa-p SRP54,GTP-bp	54 kDa-p SRP54,GTP-bp
		30 kDa-p sec65-p	19 kDa-p SRP19
			14 kDa-p SRP14
			9 kDa-p SRP9
RNP-receptor	55 kDa-p ftsY-p,GTP-bp		69 kDa-p DPα-su,GTP-bp
			30 kDa-p DPβ-su,GTP-bp
ribosome-receptor			180 kDa-p
cis-acting chaperone	16 kDa-p secB-p	70 kDa-p hsp70,ATPase	70 kDa-p hsp70,ATPase
translocase	102 kDa-p secA-p,ATPase		
	49 kDa-p sec Y-p	41 kDa-p sec61-p	45 kDa-p
	15 kDa-p Band 1-p	30 kDa-p sec62-p	
	14 kDa-p secE-p	31.5 kDa-gp	34 kDa-gp SSRα-su
		23 kDa-p	23 kDa-gp SSRβ-su
		73 kDa-p sec63-p	
trans-acting chaperone		78 kDa-p BiP,ATPase	78 kDa-p BiP,ATPase
signal/leader peptidase	37 kDa-p lep	19 kDa-p sec11-p	18/21 kDa-p SPC18/21-su
			23 kDa-gp SPC23-su
others	65 kDa-p secD-p		65 kDa-gp ribophorin I
	35 kDa-p secF-p		63 kDa-gp ribophorin II

BiP, immunoglobulin heavy chain binding protein; bp, binding protein; DP, docking protein; gp, glycoprotein; hsp, heat shock protein; kDa, kilodalton; p, protein; RNP, ribonucleoparticle; SPC, signal peptidase complex; SRP, signal recognition particle; SSR, signal sequence receptor; su, subunit

chaperones, such as the ATP-independent secB-protein, and does not involve ribonucleoparticles (Table I) [6–8]. It has been shown that secB-protein can be substituted for by dnaK, i.e. the bacterial hsp70, and by trigger factor as well as groEL [9,10]. The RNP-dependent pathway seems to represent an alternative mechanism. The ffh- (also termed P48-) protein was first identified as a homologue of SRP54-protein and has since been shown to interact with 4.5 S RNA and to be involved in protein export of pre-β-lactamase [11–14]. The definition of ftsY-protein as RNP-receptor is based on its sequence similarity to the α-subunit of docking protein (SRP-receptor) [13,14]. It may be pure coincidence that the coding region for ftsY-protein is part of an operon which also codes for ftsE-protein, another member of the nucleotide binding protein-family [5]. However, there is no direct evidence suggesting that a RNP-receptor exists in the bacterial plasma membrane. Furthermore, there is no reason to assume that a ribosome receptor exists in the bacterial plasma membrane. As a matter of fact, biochemical evidence suggests there is none (i.e. in contrast to the ribosomes which are bound to mammalian microsomes, salt or puromycin is sufficient for the removal of ribosomes from bacterial plasma membranes). There is also no reason to assume that there is a signal peptide receptor in the bacterial membrane since the problem of membrane specificity does not exist in the bacterial cytosol.

Genetic and biochemical evidence suggests that both pathways involve an ATP-dependent translocase, i.e. the peripheral membrane ATPase secA-protein and the two integral membrane proteins secE- and secY-protein (Table I) [15–25]. Biochemical evidence points to a third integral membrane protein as a component of the translocase, i.e. Band1-protein [20]. Furthermore, there is a general requirement for a membrane electrochemical potential. According to genetic evidence proteins secD and secF also are general transport components and act late in translocation [26]. However, no specific function has been assigned to these proteins as yet. Leader peptidase is a single subunit enzyme and is not directly involved in protein export [27]. Although this enzyme and its eukaryotic counterparts show overlapping substrate specificities, it does not show any sequence similarities to any of the known subunits of eukaryotic signal peptidase.

2.2. *Yeasts*

Genetic and biochemical evidence suggests a role of the *cis*-acting chaperone, hsp70, and a second (NEM-sensitive) protein which has not been identified as yet (Table I) [28,29]. However, there also is RNP-dependent protein transport in yeast [30–32]. The SRP54-protein was first identified as homologue of mammalian SRP54-protein and has since been shown to be essential for cell growth. The sec65-protein was identified genetically as a transport component and according to the sequencing data contains a domain which has a striking similarity to mammalian SRP19-protein (Stirling, C. and Schekman, R., personal communication). At this point it is unclear which pathway is the one which is predominantly acting.

We assume that the two pathways converge at the level of a putative signal peptide receptor which only has been functionally characterized so far [33]. Genetic evidence suggests that the membrane proteins sec61-, sec62- and sec63- (also termed ptl1- or npl1-) protein are generally involved in protein transport (Table I) [34–37]. However, no specific function has been assigned to any of these proteins as yet. The sec63-protein contains a domain which has a striking similarity to bacterial dnaJ-protein, a protein which is known to functionally interact with dnaK, the bacterial hsp70 homologue [36]. The sec61-protein has been found to have extensive sequence similarity to the bacterial secY-protein (Stirling, C. and Schekman, R., personal communication). Biochemical evidence suggests that the sec61-, sec62- and sec63-proteins transiently form complexes with a 31.5 kDa glycoprotein and a 23 kDa protein, i.e. two proteins which are reminiscent of two mammalian ER-proteins which have been termed α- and β-subunits of the signal sequence receptor (SSR) [38]. From the behaviour of sec61-protein with respect to complex formation with the other proteins one could speculate that it is functionally related to either the 45 kDa signal peptide receptor which has been described for mammalian microsomes or to the azido-ATP sensitive component which we have functionally defined for canine microsomes. Furthermore, the *trans*-acting chaperone BiP (*KAR2*-gene product) has been shown to have a role in transport [39]. Yeast signal peptidase contains more than one subunit and is not directly involved in protein transport [40].

2.3. *Mammals*

The RNP-dependent pathway seems to be used by the majority of presecretory proteins and has been analyzed in great detail (Table I) [13,14,41–50]. It involves SRP and its receptor in the microsomal membrane, docking protein (SRP-receptor) and the ribosome and its receptor. In addition, ribophorins I and II seem to be involved in this mechanism [51,52]. However, no specific function has been assigned to the latter two proteins as yet. As an alternative to this elaborate system, a molecular chaperone can function at least in the case of certain precursor proteins [53–59]. The *cis*-acting chaperone has been characterized as hsp70 and seems to collaborate with a second soluble (NEM-sensitive) protein which has not been identified as yet [59].

We assume that the two pathways converge at the level of a putative signal peptide receptor which may be identical to the 45 kDa protein which was characterized as a signal sequence-binding protein in microsomal

membranes (Table I) [60]. The so called SSR-subunits appear to be part of the translocase and can be expected to be generally involved [61–65]. Besides these proteins biochemical evidence points to additional membrane proteins being involved, one of which is sensitive to photoaffinity labeling with azido-ATP. We recently addressed the question of what stage of ribonucleoparticle-dependent transport is affected after photoinactivation of microsomes by azido-ATP (Zimmermann, R., Zimmermann, M., Mayinger, P. and Klappa, P., FEBS Lett., in press). Thus, a nascent presecretory protein was employed. We observed that the nascent precursor protein did not become associated with the signal sequence receptor complex after photoaffinity labeling of microsomes with azido-ATP. We concluded that the microsomal protein which is sensitive to photoaffinity labeling with azido-ATP acts prior to the signal sequence receptor complex. This protein could well be functionally related to secA-protein of the bacterial translocase. The signal peptidase of higher eukaryotic organisms contains at least two different subunits and is not directly involved in protein transport [66–69]. One of the two subunits has been shown to occur as a pair of homologues and to be highly similar to the yeast sec11-protein.

3. DISCUSSION

3.1. *Consensus Model*

It seems reasonable to assume that the mature part within a precursor protein determines folding of the precursor protein and that the signal or leader peptide interferes with folding of the precursor to the native conformation of the mature part to a lower or higher degree, depending on the particular precursor protein. At some point precursor proteins interact with ribonucleoparticles or molecular chaperones (Fig. 2). This interaction has to be reversible, however, in order to eventually allow translocation. Membrane association of the precursor proteins occurs via some type of receptor protein. With the help of the translocase the signal or leader peptides are then inserted into the membrane, most likely in the form of a loop structure which is made up of the signal or leader peptide plus the amino terminus of the mature part (Fig. 2). Therefore, in order to become inserted into the translocase, the precursor has to unfold, at least partially, starting at its amino terminus. This may well represent the point where ATP-hydrolyzing subunits of the respective translocase come into action (see below). In order for translocation to progress, the protein on the *cis*-side has to unfold further. However, the energy for complete unfolding of a precursor protein may be as low as 10 kcal/mol, i.e. the initial hydrolysis of one ATP would be sufficient to drive such an unfolding reaction. Completion of translocation may then be driven by the membrane electrochemical potential or by binding to the *trans*-acting molecular chaperone, BiP, or by spontaneous refolding on the *trans*-side of the target membrane (see below). It is tempting to speculate that the translocase contains a component which, by analogy to what has been proposed for the molecular chaperones, interacts with the polypeptide chain backbone of the precursor protein in transit.

3.2. *Open Questions*

Even twenty years after the signal hypothesis was first put forward one of the major open questions is whether the components of the translocase form a pore, i.e. an aqueous channel through which the precursor protein passes in transit, or whether the translocase is a set of enzymes which facilitates translocation at a lipid/protein interface. Precursors in transit were found to be extractable by aqueous perturbants. This was taken as an indication that the environment of the polypeptide in transit is proteinaceous. However, the validity of these methods is clearly limited.

Another open question is related to the facts that ATP-requirements were observed to translocate a protein from one side of the membrane to the other and that the precursor proteins in transit were found to have a rather extended structure [70]. The question is whether the ATP-requirement is related to an unfolding machinery. For the bacterial membrane the answer seems to be a clear yes [20–24]. However, this point has not yet been directly proven. It is not at all clear in the case of the microsomal membrane of both lower and higher eukaryotes. It is clear that precursor proteins have to be unfolded to be translocated and that unfolding has to occur on the *cis*-side of the respective membrane [70]. Cytosolic chaperones and ribonucleoparticles may help to keep precursor proteins and nascent precursor polypeptides, respectively, in a loosely folded conformation [6–10,28,29,58,59]. However, further unfolding must probably occur on the membrane surface. The question is, where does the energy for unfolding come from? Practically all precursor proteins carry signal or leader peptides which are cleaved off during or after translocation by signal/leader peptidase. Thus, in principle, the differences between the free energies of precursor versus mature forms of a protein could be sufficient to drive unfolding at the surface. As an alternative to unfolding, the ATP-requirement could be directly related to the actual transport process, e.g. in providing the energy for membrane insertion.

Yet another open question which has been a burning one for the last couple of years is how the microsomal transport apparatus can operate without the membrane electrochemical potential while the latter seems to be strictly required in the case of leader peptide-dependent bacterial protein export [21–24]. A possible solution to this apparent dilemma may reside in the recent observation that protein transport into yeast microsomes in-

volves the *trans*-acting molecular chaperone BiP [39]. However, a similar requirement for BiP in mammalian microsomes has yet to be shown. In any case it is tempting to speculate that binding of the precursor protein in transit to the *trans*-acting molecular chaperone provides the energy which in the bacterial system is provided by the membrane electrochemical potential.

Acknowledgements: We thank Dr Randy Schekman for communicating results prior to publication. The authors' work on this subject was supported by the Sonderforschungsbereich 236 (grant B22) and by the 'Fonds der Chemischen Industrie'.

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