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Hypothesis

Do cytosolic factors prevent promiscuity at the membrane surface?

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From the point of view of a preprotein, escaping from the cytosol into a specific organelle must seem an arduous, almost impossible task. How is it that preproteins resist the temptation to fold prematurely, to avoid the multiple membrane surfaces in the cell, and manage instead to enter only the translocation apparatus of a single organelle?

Cytosolic factor; Molecular chaperone; Protein import; Organelle biogenesis; Protein sorting; Heat-shock protein

1. INTRODUCTION

Most discussions of protein translocation into mitochondria, peroxisomes, chloroplasts, the nucleus or the endoplasmic reticulum, are written in terms of how the organelle might lure preproteins to it, and assume that the only function of cytosolic factors is to maintain preproteins in a translocation-competent state. This point of view ignores a fundamental problem; what prevents interaction of a preprotein with the multitudinous membrane surfaces across which it must not go? Specific targeting sequences may increase the efficiency of translocation across the proper membrane, but the target organelle may be only a minor component of the cell (Fig. 1). We propose that an important role of cytosolic factors is to prevent the interaction of otherwise promiscuous preproteins with improper membrane surfaces.

2. PREPROTEINS CAN BE TARGETED TO THE INCORRECT ORGANELLE

While the targeting of preproteins to specific organelles occurs with impeccable fidelity in vivo, under appropriate experimental conditions preproteins can exhibit a degree of promiscuity in the membrane surfaces they will recognize. Membrane-bound receptors that recognize preproteins directly can increase the rate of correct targeting, but do not prevent mistargeting. Once placed in the grip of the translocation machinery, mitochondrial preproteins can be translocated into microsome [1.2] and nuclei [3], peroxisomal proteins can be translocated into the mitochondria [4] or microsomes [5], and chloroplast preproteins can be translocated across the membrane of the endoplasmic reticulum [6] or mitochondria [7]. Such promiscuity has been characterized in experiments with the small subunit of ribulose-bisphosphate carboxylase/oxygenase (preSSU). In plants this preprotein is normally translocated into chloroplasts, and in vivo a mechanism must exist to prevent it from being translocated into mitochondria [8]. Nonetheless, preSSU can be imported into the matrix of isolated yeast mitochondria [7,9]; but whereas protease pretreatment of the mitochondria greatly reduces the efficiency of import of most genuine mitochondrial preproteins, it has no effect on the import of preSSU [9]. It appears that preSSU interacts directly with the translocation apparatus in the mitochondrial outer membrane despite its failure to be recognized by preprotein receptors on the mitochondrial surface. It should be noted that, in the experiments described above, preSSU was synthesized in a rabbit reticulocyte lysate, which could not be expected to provide chloroplast-specific cytosolic factors to prevent preSSU from entering the translocation machinery on the mitochondrial surface.

3. MOLECULAR CHAPERONES HAVE TWO DISTINCT FUNCTIONS IN PROTEIN TRANSLOCATION

We propose that cytosolic chaperones help to prevent mistargeting. This idea represents an extension of the 'classical' function of these chaperones, which is to prevent misfolding of preproteins. With the exception of the protein traffic in and out of the nucleus, the translocation of preproteins through membranes seems to require that the preproteins remain loosely folded [10]. Often these loosely folded preproteins contain cleavable

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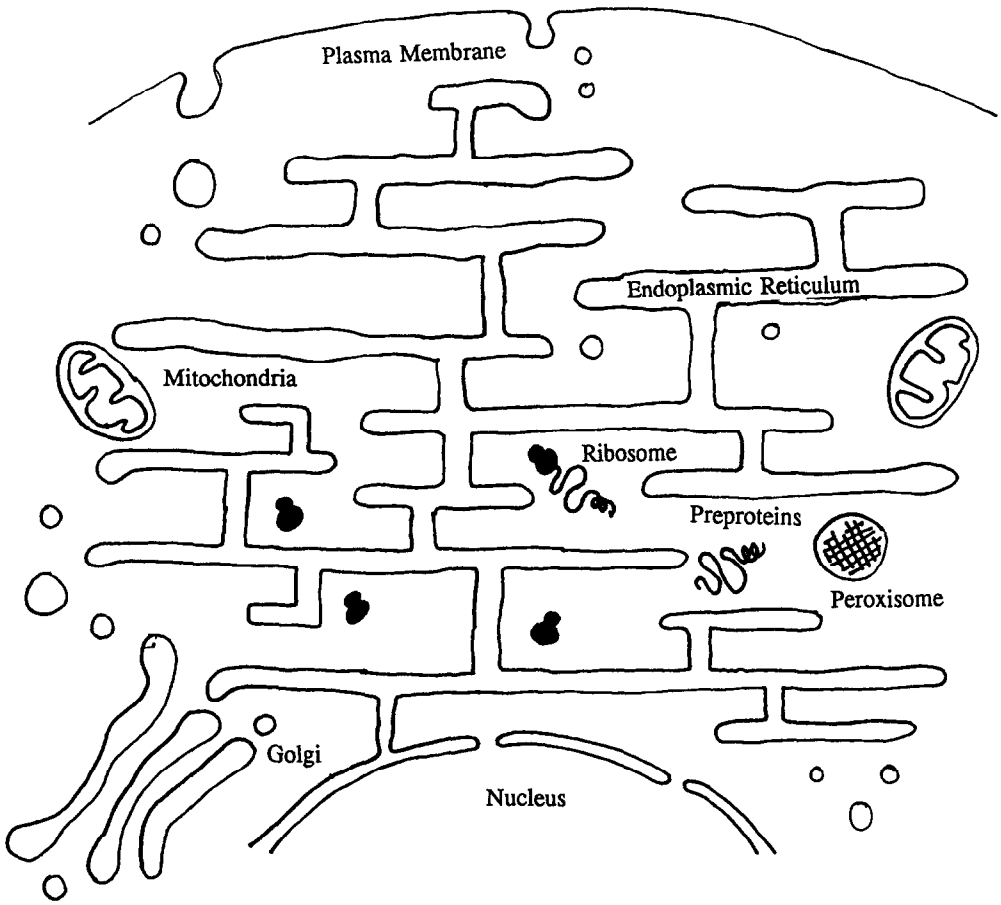

Fig. 1. Diagram representing the dilemma faced by preproteins which must find their way from cytosolic ribosomes to the surface of a target organelle.

presequences, yet neither in vitro nor in vivo is the mere presence of a targeting sequence sufficient to prevent a preprotein from folding tightly [10–12]. The loosely folded structure of a nascent preprotein can be maintained during translocation by two means. Firstly, translocation can occur co-translationally, so that as the preprotein emerges from the ribosome it immediately encounters the translocation channel across the membrane. This mechanism is employed extensively in the case of translocation into the endoplasmic reticulum [13] and to some degree in translocation of preproteins across the membranes of other organelles [12,14]. Secondly, premature folding of the preprotein can be prevented through the intervention of molecular chaperones. Such a function has been proposed for heat-shock proteins of the HSP70 family [15–17]. HSP70's appear to be involved in maintaining import competence of proteins destined for many intracellular compartments, and therefore probably play no role in targeting specificity.

Other cytosolic chaperones also retard premature folding, but some of these factors have the additional function of delivering preproteins to the appropriate organelles. In the discussion below we focus on three such factors that have been relatively well characterized; signal recognition particle (SRP) [13], which recognizes preproteins destined to enter the secretory pathway, and presequence-binding factor (PBF) [11,18] and mitochondrial import stimulating factor (MSF) which bind specifically to mitochondrial preproteins.

4. A SIGNAL RECOGNITION PARTICLE SURRENDERS PREPROTEINS TO THE ENDOPLASMIC RETICULUM

It is believed that the translocation of most secretory proteins into the endoplasmic reticulum is coupled to translation, although in some cases the two processes can proceed independently both in vitro and in vivo [10,13]. The N-terminal signal sequence of a nascent protein is recognized and bound by a cytosolic factor, SRP. There is evidence that this recognition is GTP-dependent, and it has been suggested that this GTPase function provides a 'proof-reading' mechanism to increase the specificity of recognition of signal sequences by the SRP [20]. N-Terminal signal sequences have no conserved primary structure motifs, but are largely hydrophobic, a characteristic that allows them to fit into

the peptide-binding cleft on SRP. Since preproteins targeted to other membranes contain N-terminal presequences that also show common gross characteristics but no conserved primary structure, it has been speculated that such a proof-reading mechanism, involving other cytosolic factors, might be a more general feature of protein topogenesis [21].

A receptor for SRP exists in the endoplasmic reticulum [13]. Docking of the SRP–preprotein complex to the SRP receptor is required to release SRP from the signal sequence, which is then surrendered to the translocation machinery in the endoplasmic reticulum membrane. Dissociation of SRP from the preprotein also requires hydrolysis of GTP [22]. This step might provide a second opportunity to discriminate against preproteins that had improperly associated with SRP. A number of other proteins that appear to be involved in translocation have been identified in the endoplasmic reticulum [23,24], where distinct receptors for signal sequences and the cytosolic factor (SRP) may exist side-by-side on the membrane surface. This raises the question of whether SRP is actually necessary for the initiation of translocation across the endoplasmic reticulum, or whether interaction with SRP ensures that the preprotein cannot interact with any membrane surface that does not display the SRP receptor.

Studies of a yeast mutant deficient in the signal sequence-binding subunit of SRP revealed that while the growth rate of this cell line is slowed markedly, protein secretion continues in the absence of functional SRP [24]. The mutant cells exhibited a defect in protein translocation, but the severity of this defect varied for different secreted proteins. Thus some of the proteins destined for secretion were more dependent on SRP-mediated targeting to the insertion complex than others. In vitro assays have been established that allow the translocation of some preproteins across the microsomal membranes independently of SRP and SRP receptor. These preproteins must either be solubilized in 8 M urea and then diluted immediately into the translocation assay, or else maintained in a translocation-competent state by HSP70 [15,25]. It has been proposed that this cytosolic factor-independent secretion involves the unfolded protein bypassing the SRP receptor and interacting directly with the central component of the translocation apparatus [24,25] in a manner analogous to the interaction of preSSU with the translocation apparatus in the mitochondrial outer membrane [9]. In other words, the interaction between SRP and the SRP receptor is not an obligatory first step in the translocation of preproteins across the membrane of the endoplasmic reticulum. What is clear, though, is that once captured by SRP a preprotein must present itself to the translocation machinery at the surface of the endoplasmic reticulum in order to escape. In this scenario, more important than any function of SRP in the actual translocation process is the ability of SRP to prevent the preprotein from accessing a translocation machinery in any membrane other than the endoplasmic reticulum.

5. SPECIFIC MOLECULAR CHAPERONES SURRENDER PREPROTEINS TO THE MITOCHONDRIAL SURFACE

Experiments with purified preproteins have demonstrated that isolated mitochondria, like microsomes and chloroplasts, are able to translocate at least some preproteins in the complete absence of cytosolic factors [26,27]. Such assays will be invaluable in describing components of the actual translocation apparatus, as opposed to receptors that may recognize cytosolic factors. For example, translocation of a purified preprotein depends on an interaction with the mitochondrial outer membrane protein, MOM19, suggesting that this 'receptor' interacts directly with the preprotein [27]. Another mitochondrial outer membrane protein, MAS70, also appears to interact directly with purified preproteins [28].

In order to assay for a function of molecular chaperones in preventing mistargeting, potentially promiscuous preproteins could be incubated together with more than one organelle and partioning between the proper and improper organelles measured. According to our proposal addition of cytosolic factors should retard entry of the preproteins into the improper organelle. For example, we would predict that addition of a cytosolic fraction prepared from leaf extracts would retard the import of preSSU into isolated mitochondria, but allow import into isolated chloroplasts.

While the translocation of some preproteins across the mitochondrial membranes does not require cytosolic factors, in vitro assays have demonstrated that some mitochondrial precursors are poor substrates for translocation until they are bound by cytosolic factors. For example, the presequence of preOTC, the ornithine transcarbamylase preprotein, is necessary and sufficient to direct the protein into the mitochondrial matrix [17], but preOTC has a tendency, in vitro, to assume a conformation that is incompatible with translocation across the mitochondrial membranes. A cytosolic 'presequence-binding factor', PBF, recognizes and binds specifically to the presequence of preOTC and a number of other mitochondrial preproteins, and can maintain them in an import-competent state [18]. A second molecular chaperone has been purified from the cytosol of rat liver that specifically recognizes mitochondrial preproteins [19]. This 'mitochondrial import stimulating factor', MSF, can catalyze the ATP-dependent disaggregation of preproteins, thereby rendering the preproteins competent for translocation. The specific binding of PBF and MSF to the targeting sequences of mitochondrial preproteins suggests that, in addition to their role in maintaining preproteins in a loosely folded state, PBF and MSF could each fulfil an SRP-like targeting

function. A 'PBF receptor' and an 'MSF receptor' might therefore exist on the mitochondrial surface alongside such proteins as MOM19 and MAS70. The development of assays for cytosolic factor-dependent targeting would provide the basis for identifying such 'cytosolic factor receptors'.

6. CYTOSOLIC FACTORS AND PRECISE TARGETING OF PREPROTEINS IN CELLS.

Cytosolic factors involved in targeting preproteins to mitochondria [18,19], chloroplasts [29], the nucleus [30] and the endoplasmic reticulum [13] may act in the following manner. As a nascent preprotein emerges from the ribosome it is potentially vulnerable to capture by any one of these cytosolic factors. Some preproteins are recognized by SRP. Only suitable signal sequences induce the conformational change in SRP that will lock the preprotein in place. Since the reversal of this conformational change requires the intervention of the SRP receptor, the release of the preprotein cannot occur at the surface of any organelle except the endoplasmic reticulum. The translocation machinery might discriminate further to ensure that only preproteins genuinely destined for secretion will get into the endoplasmic reticulum.

Preproteins that are ignored by SRP might instead be captured by factors that recognize targeting sequences for other organelles. These targeting signals will then remain obscured until the cytosolic factor is removed from the preprotein. If this unmasking requires a 'cytosolic factor receptor' on the appropriate membrane surface, misrouting to an improper compartment will be effectively prevented. Although such 'cytosolic factor receptors' have not yet been found, we would predict their presence on the surface of most or all translocation-competent organelles. 'Preprotein receptors' in proximity to the 'cytosolic factor receptors' would provide further discrimination and would ensure efficient translocation only of properly targeted preproteins.

In the same sense that molecular chaperones promote polypeptide folding by preventing misfolding, we propose that targeting factors, such as SRP, MSF and PBF promote specificity in protein targeting by preventing the interaction of preproteins with improper membrane surfaces.

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