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Translocation and folding of mitochondrial proteins

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Eukaryotic cells are divided into many subcellular compartments (organelles) such as the nucleus, the endoplasmic reticulum, the Golgi-complex, mitochondria, peroxisomes, lysosomes, and (in the case of plants) chloroplasts. Most proteins destined for cell organelles are synthesized as precursor proteins in the cytosol and are translocated into or across the organelle membranes to their functional destination. This intracellular transport of precursor proteins involves several principal steps: (i) the specific targeting of preproteins to the correct organelle via signal sequences on the preproteins and surface receptors on the organelles; (ii) the translocation of the polypeptide chains across the membranes that probably occurs through specific channels; (iii) maturation (proteolytic removal of the signal sequences) and folding of the imported proteins to the functionally active proteins; and (iv) further sorting events such as with proteins destinated for the Golgi-complex, lysosomes or the cell surface that are translocated into the endoplasmic reticulum and are then transferred in membrane vesicles to these compartments. Heat shock proteins (stress proteins) of 70 kd and 60 kd, termed hsp70 and hsp60, play important roles in the biogenesis pathways of organelle proteins.

As model system for the transport processes (i)–(iii) the import of nuclear-encoded proteins into mitochondria will be discussed. Many mitochondrial preproteins carry amino-terminal peptide extensions (presequences) that are positively charged and contain the targeting information required for the interaction of the preproteins with receptor proteins on the mitochondrial surface [1]. Cytosolic hsp70s are involved in keeping the preproteins in a transport-competent conformation by preventing misfolding or aggregation of preproteins. The preproteins appear to be translocated across the mitochondrial outer and inner membranes in an "unfolded" conformation (probably as linear chains) [2]. The membrane potential across the inner membrane drives the presequences into the matrix (the soluble subcompartment inside the inner membrane) [3]. Hsp70s in the matrix directly bind to the polypeptide chains in transit and thereby stabilize their unfolded conformation and support the unidirectional translocation of the preproteins [4]. The imported proteins are then transferred to hsp60 in the matrix where the actual (re)folding of the proteins takes place [5]. The steps involving hsp70s and hsp60 are energy-dependent in that ATP is hydrolyzed.

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Protein folding and assembly in the endoplasmic reticulum

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Being topologically equivalent to the extracellular space, the lumen of the endoplasmic reticulum (ER) provides a unique folding environment for newly synthesized proteins. It is where secretory proteins and membrane glycoproteins fold and assemble into oligomeric complexes before transport to the Golgi apparatus, to vacuolar organelles and the extracellular space. These proteins are, as a rule, synthesized on membrane-bound ribosomes and co-translationally translocated into the ER lumen. Their folding starts already on the nascent chain and continues post-translationally inside the ER. In many instances, the folding of the individual polypeptides is followed by oligomeric assembly inside the ER prior to transport to the Golgi complex.

Numerous studies have shown that the transport to the Golgi is specific only for correctly folded and assembled proteins. This selectivity, combined with ER-associated degradation of the transport-incompetent products, provides an effective quality control system which ensures the structural and functional intactness of proteins secreted and deployed in various organelles. Quality control is essential since the efficiency by which proteins fold in the ER is often quite low, i.e. defective proteins are formed as common side products in the maturation process, and could unless eliminated jeopardize many cellular functions. The quality control system also provides one of the most versatile means for post-translational control of gene expression.

Unlike other compartments in the cell where folding of newly synthesized proteins occurs, the ER is oxidizing and therefore capable of promoting the formation of disulfide bonds which are common in secretory proteins. It is also unique in that it supports co-translational glycosylation and numerous other covalent modifications. As in other compartments of the cell, folding and oligomerization are assisted by folding enzymes and chaperons, but the details of their molecular role remains to be elucidated.

In our work we have analyzed the folding and oligomerization process in the ER of living cells using mainly cell biological techniques. The molecules chosen as our models are well studied viral spike glycoproteins, of which influenza hemagglutinin (HA) is the best characterized. The HA undergoes co- and post-translational folding and disulfide-bond formation, followed by rapid and efficient trimerization.

We have, most recently, analyzed the effects of a reducing agent on the folding of spike glycoproteins in the ER of infected

CHO15B cells. We found that, when added to living cells infected with influenza virus, dithiothreitol (DTT) prevented the formation of disulfide bonds in proteins synthesized. It also induced reduction of already folded and oxidized monomers, but did not reduce the trimeric form. The reduced molecules in the ER remained only partially folded and incapable of trimerization and intracellular transport. When the reducing agent was washed out, the proteins immediately started to fold and became fully and correctly oxidized and transport-competent. The results indicated that the oxidizing conditions within the ER could be rapidly modified using external reducing agents, and that the ER was able to rapidly re-establish reducing conditions once these were removed. Importantly, they showed that co-translational folding and disulfide bond formation were not required for normal folding.

Taking advantage of the DTT effect, we demonstrated, moreover, that folding of influenza hemagglutinin is energy dependent. We established that metabolic energy is required to 1) support the correct folding and disulfide bond pairing in this well-characterized viral glycoprotein, 2) rescue misfolded proteins from disulfide cross-linked aggregates, and 3) maintain the oxidized protein in its folded and oligomerization-competent state.

Taken together our results indicate that the ER is a dynamic folding environment where folding of newly synthesized polypeptide chains occurs alongside unfolding and refolding processes as well as reactions involved with oligomeric assembly. The successful maturation of functional, transport-competent proteins is clearly a complex phenomenon involving numerous cellular components including ATP-driven factors.

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The pathway of chaperone-assisted protein folding

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Proteins fold spontaneously in vitro thus demonstrating that the amino acid sequence contains all the information required to specify the conformation of the native state [1]. Consequently, it was assumed that also in vivo folding (acquisition of tertiary structure) and assembly (acquisition of quaternary structure) occur without the help of additional components. This view has changed only recently when it was discovered that newly-synthesized proteins acquire their functionally active conformations in a complex process which is mediated by a group of proteins collectively termed "molecular chaperones" [2]. These components, mostly constitutively-expressed stress proteins, occur in the cytosol as well as within subcellular compartments. They apparently have the general ability to recognize unfolded or partially denatured proteins without specificity for defined sequence motifs [3].

We are interested in understanding the function of members of the Hsp70 and Hsp60 families of heatshock proteins in the process of in vivo protein folding. Both classes of components, although structurally unrelated, utilize the energy of ATP-hydrolysis to release bound substrate proteins. While Hsp70s act as monomers or dimers, Hsp60s function as high molecular weight oligomeric structures composed of two stacked seven-subunit rings. Recent evidence suggests that Hsp70 and Hsp60 family members act sequentially in protein folding [4–6] determined by their differential specificity for structural elements exposed at different stages of the folding pathway. Hsp70s recognize completely unfolded polypeptide chains as they emerge from ribosomes or at the trans-side of membranes following translocation into organelles such as mitochondria [7]. The newly-made proteins, adopting the conformation of partially folded intermediates, are then transferred to Hsp60 which is required for their folding and assembly to the native state [6]. Proteins appear to acquire stable tertiary structure by a process of step-wise, ATP-dependent release from the surface of the Hsp60 complex [8].

Using the purified heatshock proteins of E. coli we have reconstituted the pathway of chaperone-mediated protein folding. Our results show that Hsp70 (the DnaK protein of E. coli) functionally depends on two additional heatshock proteins, DnaJ and GrpE. DnaJ and DnaK cooperate in stabilizing the folding polypeptide chain in an intermediate conformational state. GrpE serves as a coupling factor that stimulates the ATPase activity of DnaK thus allowing the transfer of the associated folding intermediate to Hsp60 (the GroEL protein of E. coli). We propose that this hierarchical action of chaperone components represents a general paradigm for the folding of newly-synthesized proteins within cells. This would suggest that different compartments in which protein folding occurs have to contain structurally and/or functionally equivalent chaperone proteins [9].

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