The biogenesis and function of eukaryotic porins

M. Dihanich

Friedrich-Miescher-Institute, P.O.Box 2543, CH-Basel (Switzerland)

Summary. Like most other mitochondrial proteins porin is synthesized in the cytosol and imported posttranslationally into the outer mitochondrial membrane. This transport follows the general rules for mitochondrial protein import with a few aberrations: a) porin contains an *uncleaved* NH_2-terminal signal sequence, b) also its carboxyterminus might be involved in the import process, and c) this transport does not seem to require a membrane potential $\Delta\psi$, although it is ATP-dependent. Most likely the actual import step occurs at contact sites between the outer and the inner mitochondrial membrane and involves at least one receptor protein.
Although porin is known to be the major gate through the outer mitochondrial membrane, its absence only causes transient respiratory problems in yeast cells. This could mean a) that there is a bypass for some mitochondrial functions in the cytosol and/or b) that there are alternative channel proteins in the outer membrane. The first idea is supported by the overexpression of cytosolic virus-like particles in yeast cells lacking porin and the second by the occurrence of residual pore activity in mitochondrial outer membrane purified from porinless mutant cells.
Key words. Mitochondria; outer membrane; porin; transport; signal sequence; contact sites; deletion mutant; virus-like particle.

Mitochondria are the organelles for oxidative energy metabolism of eukaryotic cells. Like chloroplasts, they contain their own DNA and are surrounded by two membranes: the inner membrane (IM) carrying enzymes of the respiratory chain, with a protein content of about 70% (wt/wt) and the outer membrane (OM) carrying a few known proteins like monoamine oxidase and many unknown proteins (protein content: 12%[65]). The compartment enclosed by the inner membrane is the matrix space (M), the compartment between the two membranes the intermembrane space (IMS).
The transport of molecules like sugars, amino acids, nucleotides and ions across both mitochondrial membranes is essential for the functioning of mitochondria, since all of the ATP-generating processes take place in their innermost space, the matrix, and the product, ATP, is largely needed outside of the organelle.
Already in 1959 mitochondria were known to be permeable for sugars[33] and in 1965 O'Brian and Brierly demonstrated that also various ions, substrates and even nucleotides can penetrate the outer mitochondrial membrane[38]. The existence of non-specific diffusion pores in mitochondria was convincingly shown by reconstitution of different membrane fractions from *Paramecium aurelia* into planar lipid bilayer membranes[59]. These pores, which also occur in mitochondria of higher eukaryotes and all have an apparent molecular weight of around 30 kDa, were called VDAC (voltage-dependent anion-selective channels) or 'porin' in analogy to similar proteins of bacterial outer membranes[5,6,31,74]. Up to now mitochondrial porins and similar proteins found in the outer membrane of chloroplasts[1] are the only non-toxic unspecific pore proteins known in eukaryotes.

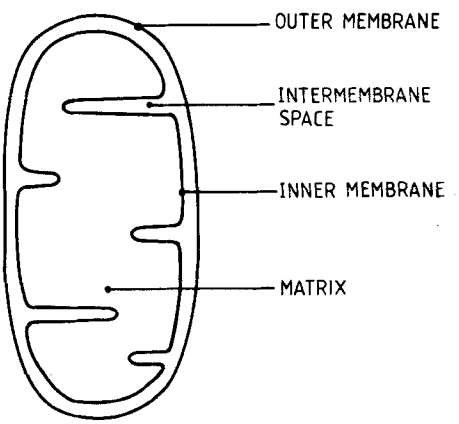

Figure 1. The four major compartments of a mitochondrion (contact sites between the inner and outer membrane have been omitted). Reproduced with permission from Schatz[58].

Biogenesis

The biosynthesis of eukaryotic porin was studied in detail during the past decade and our present models greatly benefit from the progress made in the field of mitochondrial biogenesis as a whole.

Targeting signals

Only a few components of the respiratory chain are encoded by mitochondrial DNA; the majority (> 90%) of all mitochondrial proteins is encoded by nuclear genes,

Table 1. Comparison of eukaryotic pores

Organism	kDa	Exclusion limit (kDa)	Pore size (nm)	Ref.
Paramecium tetraaurela			1.3	2
S. cerevisiae	29.883	5–6	1.7	35, 1
N. crassa	29.979	4–6	1.8–1.9	18, 30
			2	6
Mung bean	30	2–8	2–3	74
Rat liver	31	2–8	1.7	31, 56
E. coli	35–40	0.6–0.8	1.1	1

synthesized on cytoplasmic ribosomes and transported into the mitochondria. This transport in most cases is accompanied by cleavage of an NH_2-terminal signal peptide[32,57] and requires an electrochemical potential across the mitochondrial membranes as well as ATP[16].

The only outer membrane proteins whose biosynthesis has been studied in detail are porin and a 70 kDa OM protein of unknown function. Transport of these two proteins (both from fungi) into the OM essentially follows the same principles as transport of other mitochondrial proteins, but differs in a few points.

These outer membrane proteins are the only mitochondrial proteins whose import does not require transport through a membrane, but only proper integration into a phospholipid bilayer. This needs two kinds of signals: one for targeting to the mitochondria, and one for anchoring in the OM. Since in vitro synthesized porin is protease sensitive, but becomes extremely protease resistent when incorporated into the OM of mitochondria, protease resistance is used as an assay for membrane insertion[17,21,34]. Targeting of in vitro synthesized porin to the mitochondria is specific, since the protein does not interact with microsomal membranes[21].

Characteristically, import of both porin and the 70 kDa protein into the OM occurs without cleavage of a signal peptide[17,21,34,52]. The genes for both proteins have been cloned from yeast libraries[24,35,62] and their deduced amino acid sequences are similar in the NH_2-terminal region (fig. 2)[35].

Both sequences exhibit an uninterrupted stretch of uncharged amino acids flanked by basic amino acids in the amino-terminal region corresponding to the cleaved signals of other mitochondrial proteins. Similar features were found later in the cDNA sequence of *N. crassa* porin[30]. When a 70 kDa deletion mutant with slightly impaired respiratory functions was transformed with either the whole wild type 70 kDa-gene or a carboxy-terminally truncated version, only the whole sequence could restore respiratory functions, although both constructs were imported into mitochondria. This shows, that the carboxy-terminal part of the 70 kDa OM protein is required for its function but not for transport[52]. When Hase et al.[23] deleted various portions of the 70 kDa protein, they found that all the information necessary for mitochondrial targeting and anchoring of this protein is localized within its first 41 amino acids. Deletions of only a few amino acids within the first 13 residues disturbed both in vivo targeting and anchoring whereas deletions behind residue 12 resulted in localization of the shortened protein to the mitochondria but failure to integrate in the OM. The authors concluded that separate although overlapping regions of the 70 kDa protein are necessary for targeting to the mitochondria (amino acids 1–12) and anchoring in the OM (amino acids 9–38). Hurt et al.[27] confirmed that the first 12 amino acids of the 70 kDa protein are sufficient to direct a cytosolic protein into the mitochondrial matrix.

Table 2 lists the shortest possible amino acid sequences necessary for targeting and anchoring of various mitochondrial proteins. Similar requirements are found for signals of the endoplasmic reticulum and accordingly, the anchoring signal of the 70 kDa OM protein can func-

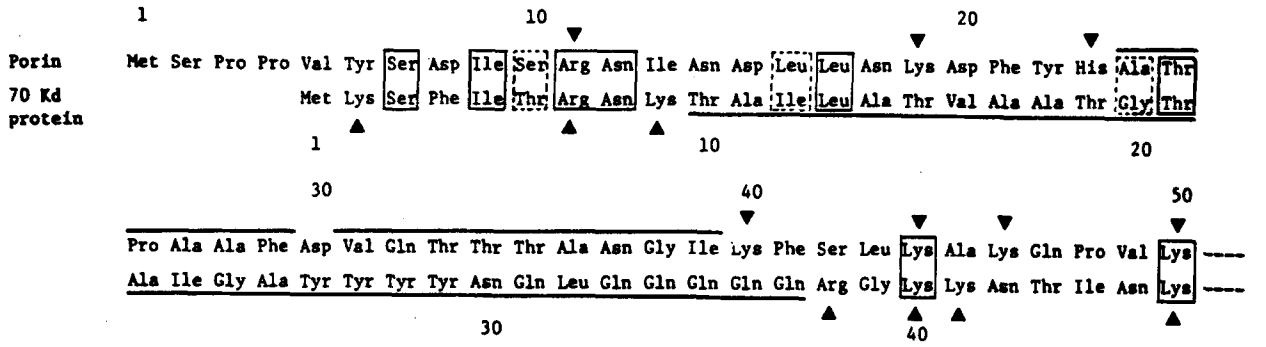

Figure 2. Comparison of the NH_2-terminal sequences of yeast porin and the 70 kDa outer membrane protein. Identical and similar residues are in solid and dashed boxes, respectively. Stretches of uncharged residues are lined above (porin) or underlined (70 kDa protein). Reproduced with permission from Mihara and Sato[35].

Table 2. Minimal length of targeting and anchoring signals (determined by deletion analysis) for mitochondrial and ER-proteins

Protein	Location	Targeting signal[a]	Anchoring (stop transfer) signal[a]	Ref.
δ Ala-synthase	mito (M)	9	–	29
Porin	mito (OM)	≤ 17 N-term.	≤ 37 N-term.	22, Kleene (pers. comm.)
		≤ 62 C-term.	≤ 62 C-term.	
70 kDa OM	mito (OM)	11	29	23, 27
cox. IV	mito (M)	7–12	–	28, 67
cyt c_1	mito (IMS)	16	19	66
various	ER	8–12	8–16	19

[a] Minimal length in amino acids; M, matrix; IM, inner membrane; IMS, intermembrane space; OM, outer membrane.

tion as a stop transfer signal in the ER membrane (Spiess, M., and Baker, K., pers. comm.); vice versa, also the ER-stop transfer sequence of vesicular stomatitis virus G protein can anchor pre-ornithine carbamyl transferase (a matrix protein) in either mitochondrial membrane depending on its position within the precursor molecule[37]. Porin, however, is a special case. According to secondary structure predictions, only its first 18 residues can form an amphiphilic (= charged amino acids on one side, uncharged ones on the other side) α-helix, whereas the remainder most likely folds into amphiphilic β-sheets. This way the porins of *S. cerevisiae* and *N. crassa* could span the outer mitochondrial membrane 12–16 times[30,35]. Since the signal sequences listed in table 2 as well as the signals occurring in most multi-spaning membrane proteins[7,72] belong to the α-helical type, the insertion of porin might follow an unusual route. However, also non-helical peptides can insert into phospholipid membranes, as long as they are amphiphilic[55]. Recently Hamajima et al.[22] and Kleene et al. (pers. comm.) found that disruption of both NH_2- and COOH-terminal sequences of yeast and *N. crassa* porin, respectively, impairs their membrane-insertion. Only the amino terminal sequence is predicted to form an α-helix. Since yeast porin with a deletion from residues 37–98 still inserts into mitochondria in a trypsin-resistant fashion, whereas a deletion between residues 17 and 98 still allows binding but not insertion and a deletion of 61 carboxyterminal amino acids impairs both binding and acquisition of trypsin resistance[22], the NH_2- as well as the COOH-terminal sequence could be involved in targeting as well as anchoring of the protein. Like with the 70 kDa OM protein, the extreme amino terminus contains a targeting signal which is followed by a sequence necessary for anchoring. However, it is not yet clear whether any of these sequences by itself is sufficient for targeting or anchoring of foreign proteins to the mitochondria.

Energy requirement

Another peculiarity of OM proteins is that unlike other mitochondrial proteins[20,47,61] their insertion into mitochondria does not seem to be absolutely dependent on a transmembrane potential $\Delta\psi$[17,21,34], however it requires ATP (see below and next review by R. Pfaller et al.). Therefore $\Delta\psi$ is thought to be necessary only for movement of the NH_2-terminus of the mitochondrial precursor protein beyond the outer membrane[69].

The import pathway

Whereas translocation of proteins across the membranes of the ER can, with few exceptions, only occur cotranslationally, protein import into mitochondria, chloroplasts and peroxisomes as well as protein export from bacteria can occur posttranslationally (i.e. after completion of the new polypeptide, for review see Verner and Schatz[69]). The conformation of the nascent polypeptide chain seems to be crucial for the import competence of a given precursor protein[15,68]. Initial experiments by Eilers and Schatz[15] showed that mitochondrial import can be inhibited by the binding of a specific ligand to a purified precursor resulting in the hypothesis that mitochondrial precursors need to be unfolded in order to be able to enter the translocation machinery. This idea is supported by work done with different mitochondrial as well as secreted proteins[3,36,42,51,60,68]. However, recent experiments by Vestweber and Schatz demonstrate that mitochondria can import artificial precursor proteins which contain a branched polypeptide chain or a large stilbene disulfonate group coupled to their carboxy-terminus[71]. This suggests that proteins need not be completely unfolded for translocation and that the 'translocation-competent' conformation may vary for different precursors[70,71].

An 'import-competent' conformation may be induced, or maintained, by one or more of the following components: a) ribosomes[69], b) heat-shock proteins[4,9], which probably bind to nascent mitochondrial and secretory precursor proteins, helping them to stay in the proper conformation prior to interaction with the target membrane[8], c) ATP[13,14,26,48,49], d) a 'receptor' protein on the mitochondrial surface[39,44,45,54,75–77] whose interaction with the precursor could stabilize its import-competent conformation. The insertion of porin does not seem to require ribosomes, since import of purified 'water soluble' porin is possible[43] (see also the following review by Pfaller et al.). A requirement for ATP has been demonstrated[48]. Evidence for the existence of a receptor protein involved in porin-import mainly stems from two observations: 1) protease-treated mitochondria no longer import porin[30,44,77] and 2) porin competes with other mitochondrial precursors for binding sites on the mitochondrial surface[44].

What happens after newly synthesized porin binds to the outer membrane? Already in 1985 Schleyer and Neupert showed that 'contact sites' between the outer and the inner mitochondrial membrane can trap mitochondrial precursors on their way to the matrix[60], and therefore might be the actual sites on the mitochondrial surface where import is happening. Translocation of a given precursor would then be facilitated through contact with components of the 'import machinery' of the inner membrane[25,40,46,63]. Import of porin can occur through contact sites, as was demonstrated with rat liver mitochondria[41], but also direct import into purified OM-vesicles was observed[21]. This result could mean, that there is more than one import-pathway for porin.

All models to explain this dilemma have to be regarded as highly speculative and up to now the definitive order of the following events is still uncertain:

1) binding of 'import competent' porin to the OM;
2) hydrolysis of ATP;
3) binding to a receptor protein;
4) transfer to contact sites;

5) interaction with the 'import machinery' of the inner mitochondrial membrane;
6) translocation/insertion;
7) (re)folding to the active conformation.

Steps 3 and 4 could be reversed if the receptor were only present or only active in contact sites. Also, the participation of more than one receptor is possible and different import-pathways might be determined by different receptor proteins.

The future awaits the identification of the putative receptor protein(s) and of the components of the 'import machinery'. With these tools in hand it should be possible to obtain a more accurate picture of mitochondrial protein import in general and to understand the peculiarities of the route taken by porin.

In vivo function

Mitochondrial membranes can be isolated from sonicated yeast mitochondria by sucrose-gradient centrifugation yielding the OM as outside-out sealed vesicles[53]. These preparations exhibit a characteristic polypeptide pattern which differs from that of the IM (fig. 3).

By far the most abundant protein of the yeast mitochondrial OM is the 29 kDa porin. Its abundance and its unique role as a general diffusion pore in eukaryotic mitochondria suggests that it could be essential for mitochondrial function. With an exclusion limit of about 6000 Da[1] it might not only be the major gate for different solutes, but might allow even small proteins to enter the mitochondrial intermembrane space.

A porin-less yeast mutant

A valuable test for the in vivo importance of a given protein is to disrupt its gene and thereby construct a null mutant. When this approach was taken[11], it led to the surprising result, that porin is not essential for the survival of yeast cells or even for mitochondrial functions: the porin-deletion mutant was viable, but unable to grow on non-fermentable carbon-sources like glycerol (a test for respiration). However, after about three days, it adapted to growth on glycerol. A similar mutant constructed by Lauquin et al. (pers. comm. and Michejda et al.[33a]) in a different strain of *S. cerevisiae* had even a less pronounced respiratory defect. Analysis of the levels of different mitochondrial proteins in non-adapted mutant

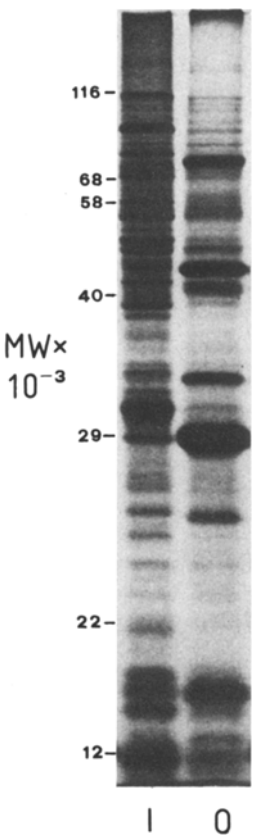

Figure 3. The major proteins of the inner (I) and outer (O) mitochondrial membrane (polyacrylamide gel stained with Coomassie blue). Printed with permission from Riezman et al.[53].

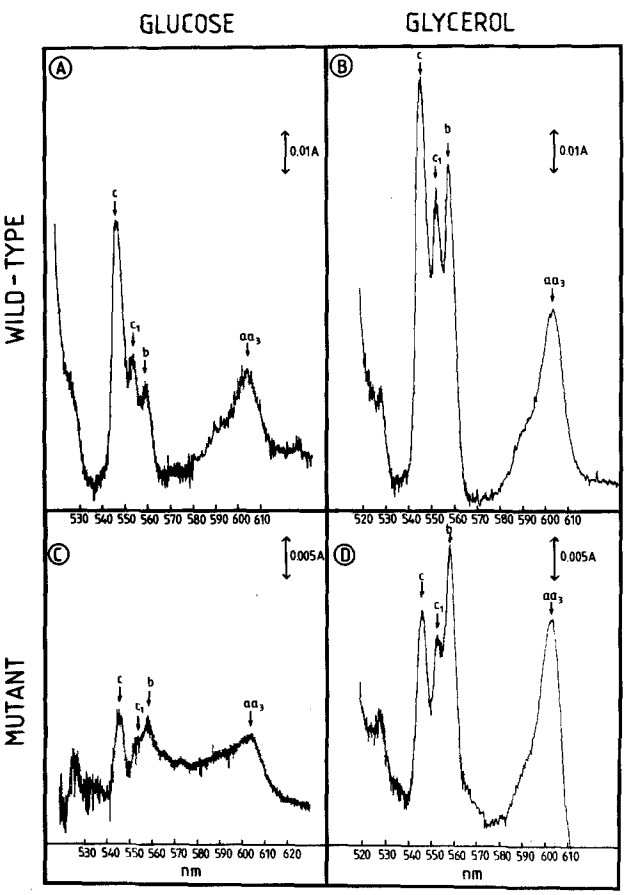

Figure 4. Reduced levels of mitochondrial cytochromes in the por° mutant. The POR$^+$ parent strain and the por° mutant were grown on rich medium containing glucose or, after adaption, on rich medium containing glycerol. Mitochondria were isolated from the cells and reduced minus oxidized difference spectra were recorded at liquid nitrogen temperature at a band-pass of 2 nm, a light-path of 2 mm and the following final concentrations of mitochondrial protein: *A* and *B* at 1.6 mg/ml, *C* and *D* at 2.6 mg/ml. *C* and *D* were recorded at a 2-fold higher sensitivity than *A* and *B*. Reproduced from Dihanich et al.[11].

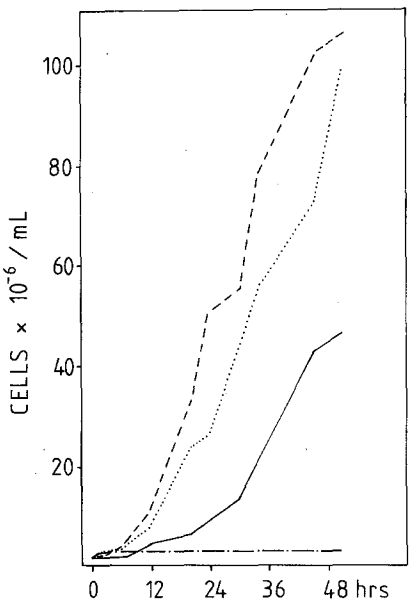

Figure 5. After adaptation, the por° mutant grows well on glycerol. Cells were pregrown to late logarithmic phase in rich medium containing either 2% glucose or 3% glycerol. Aliquots of the precultures were inoculated to 10^6 cells/ml into 100 ml of glycerol-containing rich medium and growth at 30 °C was recorded by cell counting. ..... POR^+ parent pregrown on glucose; ----- POR^+ parent pregrown on glycerol; —·—·—·— por° mutant pregrown on glucose; ——— por° mutant adapted for 5 days on plates containing glycerol-supplemented rich medium and then pregrown on glycerol; reproduced with permission from Dihanich et al.[11].

cells showed that the amounts of all mitochondrial cytochromes were reduced between 5- and 10-fold, whereas other proteins (including OM-proteins) were unchanged. After adaption to glycerol, the cytochrome-levels of the mutant were increased, but not restored to wild type levels, in line with the slow growth of mutant cells on glycerol.

This finding suggests a) that the outer mitochondrial membrane is still permeable in the absence of porin, perhaps because of the presence of other pores and b) that the transport of substrates needed for the biosynthesis or import of mitochondrial cytochromes requires porin whose loss cannot be compensated completely by other pathways.

Accumulation of virus-like particles in porin° mutants during adaptation to glycerol

During adaptation to glycerol, porinless yeast mutants accumulate large amounts of a cytosolic protein of 86 kDa[11] which was identified as the major coat protein of virus-like particles (VLPs[12], fig. 6).

Virus-like particles are found in > 95% of all strains of *S. cerevisiae*. A class of them is known as 'killer-virus' since it produces a membrane-active toxin (see Pattus et al. in this issue). VLPs accumulating in the porinless mutant (L-A type) are known to contain a double-stranded RNA genome which encodes the major coat protein and functions needed for replication of their ds RNA, like an RNA-dependent RNA polymerase[50,64,73]. No pore activity is associated with them[12]. However, recently, accumulation of the same particles was also found in another yeast mutant lacking a mitochondrial

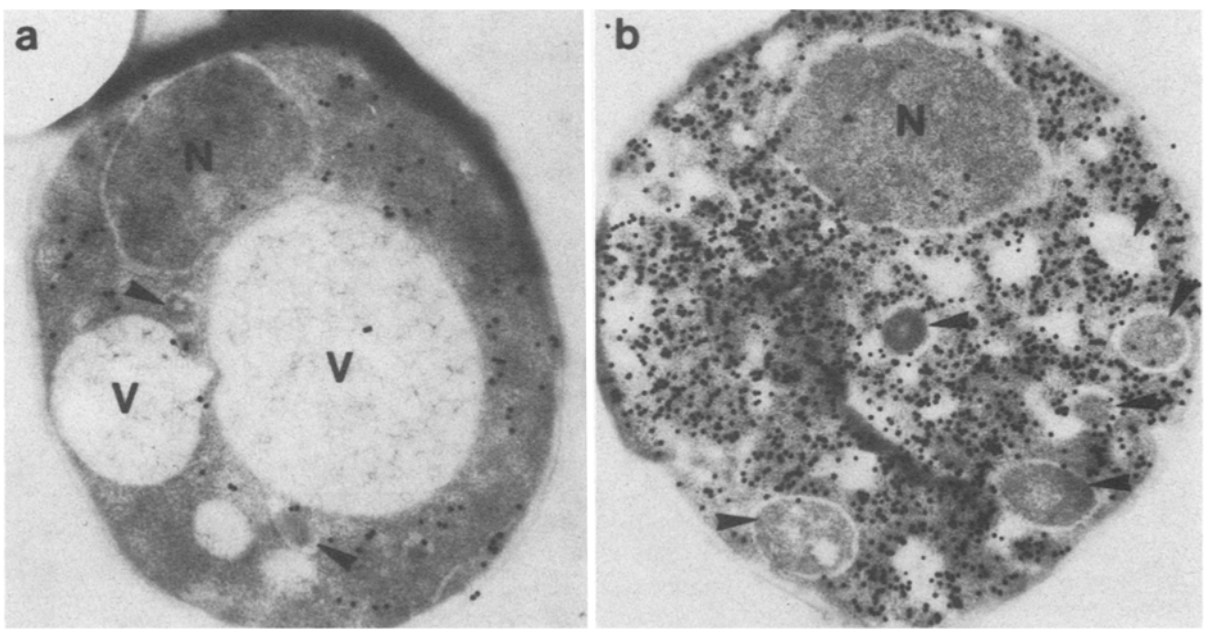

Figure 6. Adapted por° mutants accumulate large amounts of virus-like particles in the cytosol: *a* glucose-grown; *b* glycerol adapted. Virus-like particles were visualized by immunogold-labeling using polyclonal antisera against the 86 kDa major coat protein. N, nucleus; V, vacuole; arrowheads, mitochondria[12].

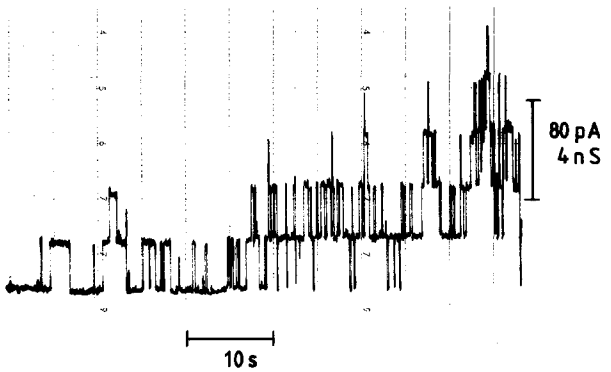

Figure 7. Single-channel recording of a diphytanoyl phosphatidylcholine/n-decane membrane after the addition of mitochondrial outer membranes of the yeast mutant HR125-5A to the aqueous phase. The aqueous phase contained 100 ng/ml outer membranes, and 1 M KCl, pH 6. The applied voltage was 10 mV; the current prior to the addition of the protein was less than 0.5 pA. Taken with permission from Dihanich et al.[10].

nuclease (Dieckman et al., pers. comm.). Furthermore, some yeast strains become respiration deficient after having been cured of VLPs, suggesting a connection between VLP- and mitochondrial functions[12]. Characterization of all the activities associated with VLPs and their products should tell us whether they create a bypass for a mitochondrial product or which of their products enters the mitochondria through OM-pores.

Alternative pores in the outer mitochondrial membrane

The existence of alternative gates through the OM is suggested by the fact that porinless mutants are viable and grow even on non-fermentable carbon-sources. In order to identify these alternative pores, mitochondrial OMs from porin° mutants and wild type cells were purified by sucrose gradient centrifugation as described previously (fig. 3). When detergent-solubilized OMs from the porin° mutant were reconstituted into lipid bilayer membranes, pore activity was observed. Control experiments with detergent alone were negative; control experiments with wild type cells showed two different kinds of pores. The alternative pores found in mutant OMs had a single-channel conductance of 0.21 nS in 0.1 M KCl and behaved like general diffusion pores with a slight preference for cations (fig. 7). Their extrapolated diameter of 1.2 nm is smaller than that of eukaryotic porin (1.7 nm) and corresponds more closely to bacterial pores. Until now the protein corresponding to this activity has not been identified with certainty[10,33a].

Acknowledgments. I would like to thank C. Dieckmann, G. Lauquin, R. Kleene and M. Spiess for communicating results before publication, H. Riezmann, R. Sato and G. Schatz for providing some of the figures shown in this review and most of all G. Schatz for his generous support and for critically reading the manuscript.

1 Benz, R., Porin from bacterial and mitochondrial outer membranes. CRC Cr. Rev. Biochem. *19* (1985) 145–190.
2 Benz, R., Ludwig, O., de Pinto, V., and Palmieri, F., Permeability properties of mitochondrial porins of different eukaryotic cells, in: Achievements and Perspectives of Mitochondrial Research, vol. 1: Bioenergetics, pp. 317–327. Eds E. Quagliariello et al. Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam 1985.
3 Chen, W. J., and Douglas, M., The role of protein structure in the mitochondrial import pathway: unfolding of mitochondrially bound precursors is required for membrane translocation. J. biol. Chem. *262* (1987) 15605–15609.
4 Chirico, W. J., Waters, M. G., and Blobel, G., 70 k heat shock related proteins stimulate protein translocation into microsomes. Nature *332* (1988) 805–810.
5 Colombini, M., A candidate for the permeability pathway of the outer mitochondrial membrane. Nature *279* (1979) 643–645.
6 Colombini, M., Pore size and properties of channels from mitochondria isolated from *Neurospora crassa*. J. Membr. Biol. *53* (1980) 79–84.
7 Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H., Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3A resolution. Nature *318* (1985) 618–624.
8 Deshaies, R. J., Koch, B. D., and Schekman, R., The role of stress proteins in membrane biogenesis. TIBS *13* (1988) 384–388.
9 Deshaies, R. J., Koch, B. D., Werner-Washburne, M., Craig, E. A., and Schekman, R., A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. Nature *332* (1988) 800–805.
10 Dihanich, M., Schmid, A., Oppliger, W., and Benz, R., Identification of a new pore in the mitochondrial outer membrane of a porin deficient yeast mutant. Eur. J. Biochem. *181* (1989) 703–708.
11 Dihanich, M., Suda, K., and Schatz, G., A yeast mutant lacking mitochondrial porin is respiratory-deficient but can recover respiration with simultaneous accumulation of an 86-kd extramitochondrial protein. EMBO J. *6* (1987) 723–728.
12 Dihanich, M., VanTuinen, E., Lambris, J. D., and Marshallsay, B., Accumulation of viruslike particles in a yeast mutant lacking a mitochondrial pore protein. Molec. Cell. Biol. *9* (1989) 1100–1108.
13 Eilers, M., Hwang, S., and Schatz, G., Unfolding and refolding of a purified precursor protein during import into isolated mitochondria. EMBO J. *7* (1988) 1139–1145.
14 Eilers, M., Oppliger, W., and Schatz, G., Both ATP and an energized inner membrane are required to import a purified precursor protein into mitochondria. EMBO J. *6* (1987) 1073–1077.
15 Eilers, M., and Schatz, G., Binding of a specific ligand inhibits import of a purified precursor protein into mitochondria. Nature *322* (1986) 228–232.
16 Eilers, M., and Schatz, G., Protein unfolding and the energetics of protein translocation across biological membranes. Cell *52* (1988) 481–483.
17 Freitag, H., Janes, M., and Neupert, W., Biosynthesis of mitochondrial porin and insertion into the mitochondrial membrane of *Neurospora crassa*. Eur. J. Biochem. *126* (1982) 197–202.
18 Freitag, H., Neupert, W., and Benz, R., Purification and characterization of a pore protein of the outer mitochondrial membrane from *Neurospora crassa*. Eur. J. Biochem. *123* (1982) 629–636.
19 Garoff, H., Using recombinant DNA techniques to study protein targeting in the eukaryotic cell. A. Rev. Cell Biol. *1* (1985) 403–445.
20 Gasser, S., Daum, G., and Schatz, G., Import of proteins into mitochondria: energy-dependent uptake of precursors by isolated mitochondria. J. biol. Chem. *257* (1982), 13034–13041.
21 Gasser, S., and Schatz, G., Import of proteins into mitochondria: *in vitro* studies on the biogenesis of the outer membrane. J. biol. Chem. *258* (1983) 3427–3430.
22 Hamajima, S., Sakaguchi, M., Mihara, K., Ono, S., and Sato, R., Both amino- and carboxy-terminal portions are required for insertion of porin into the outer mitochondrial membrane. J. Biochem. *104* (1988) 362–367.
23 Hase, T., Müller, U., Riezman, H., and Schatz, G., A 70-kd protein of the yeast mitochondrial outer membrane is targeted and anchored via its extreme amino terminus. EMBO J. *3* (1984) 3157–3164.
24 Hase, T., Riezman, H., Suda, K., and Schatz, G., Import of proteins into mitochondria: nucleotide sequence of the gene for a 70-kd protein of the yeast mitochondrial outer membrane. EMBO J. *2* (1983) 2169–2172.
25 Hay, R., Boehni, P., and Gasser, S., How mitochondria import proteins. Biochim. biophys. Acta *779* (1984) 65–87.
26 Hurt, E. C., Unravelling the role of ATP in posttranslational protein translocation. TIBS *12* (1987) 369–370.
27 Hurt, E. C., Müller, U., and Schatz, G., The first twelve amino acids of a yeast mitochondrial outer membrane protein can direct a nuclear-

encoded cytochrome oxidase subunit to the mitochondrial inner membrane. EMBO J. *4* (1985a) 3509–3518.
28 Hurt, E. C., Pesold-Hurt, B., Suda, S., Oppliger, W., and Schatz, G., The first twelve amino acids (less than half of the pre-sequence) of an imported mitochondrial protein can direct mouse cytosolic dihydrofolate reductase into the yeast mitochondrial matrix. EMBO J. *4* (1985b) 2061–2068.
29 Keng, T., Alani, E., and Guarente, L., The nine amino-terminal residues of δ-aminolevulinate synthase direct β-galactosidase into the mitochondrial matrix. Molec. cell. Biol. *6* (1986) 355–364.
30 Kleene, R., Pfanner, N., Pfaller, R., Link, T. A., Sebald, W., Neupert, W., and Tropschug, M., Mitochondrial porin of *Neurospora crassa*: cDNA cloning, in vitro expression and import into mitochondria. EMBO J. *6* (1987) 2627–2633.
31 Linden, M., Gellerfors, P., and Nelson, B. D., Purification of a protein having pore forming activity from the rat liver mitochondrial outer membrane. Biochem. J. *208* (1982) 77–82.
32 Maccechini, M.-L., Rudin, Y., Blobel, G., and Schatz, G., Import of proteins into mitochondria: precursor forms of the extramitochondrially made F1-ATPase subunits in yeast. Proc. natl Acad. Sci. USA *76* (1979) 343–347.
33 Malamed, S., and Recknagel, R. O., The osmotic behavior of the sucrose-inaccessible space of mitochondrial pellets from rat liver. J. biol. Chem. *234* (1959) 3027–3030.
33a Michejda, J., Guo, X. J., and Lauquin, G. J. M., Bioenergetic consequences of the lack of mitochondrial porin: identification of a putative new pore, in: Anion Carriers of Mitochondrial Membranes, pp. 225–235. Eds A. Azzi et al. Springer, Heidelberg/New York 1989.
34 Mihara, K., Blobel, G., and Sato, R., In vitro synthesis and integration into mitochondria of porin, a major protein of the outer mitochondrial membrane of *Saccharomyces cerevisiae*. Proc. natl Acad. Sci. USA *79* (1982) 7102–7106.
35 Mihara, K., and Sato, R., Molecular cloning and sequencing of cDNA for yeast porin, an outer mitochondrial membrane protein: a search for targeting signal in the primary structure. EMBO J. *4* (1985) 769–774.
36 Müller, G., and Zimmermann, R., Import of honeybee prepromelittin into the endoplasmic reticulum: energy requirements for membrane insertion. EMBO J. *7* (1988) 639–648.
37 Nguyen, M., Bell, A. W., and Shore, G. W., Protein sorting between mitochondrial membranes specified by position of the stop-transfer domain. J. Cell Biol. *106* (1988) 1499–1505.
38 O'Brien, R. L., and Brierly, G., Compartmentation of Heart Mitochondria. I. Permeability characteristics of isolated beef heart mitochondria. J. biol. Chem. *240* (1965) 4527–4531.
39 Ohba, M, and Schatz, G., Protein import into yeast mitochondria is inhibited by antibodies raised against 45-kd proteins of the outer membrane. EMBO J. *6* (1987a) 2109–2115.
40 Ohba, M., and Schatz, G., Disruption of the outer membrane restores protein import to trypsin-treated yeast mitochondria. EMBO J. *6* (1987b) 2117–2122.
41 Ono, H., and Tuboi, S., Integration of porin synthesized in vitro into outer mitochondrial membranes. Eur. J. Biochem. *168* (1987) 509–514.
42 Park, S., Liu, G., Topping, T. B., Cover, W. H., and Randall, L. L., Modulation of the folding pathways of exported proteins by the leader sequence. Science *239* (1988) 1033–1035.
43 Pfaller, R., Freitag, H., Harmey, M. A., Benz, R., and Neupert, W., A water-soluble form of porin from the mitochondrial outer membrane of *Neurospora crassa*. J. biol. Chem. *260* (1985) 8188–8193.
44 Pfaller, R., and Neupert, W., High-affinity binding sites involved in the import of porin into mitochondria. EMBO J. *6* (1987) 2635–2642.
45 Pfaller, R., Steger, H. F., Rassow, J., Pfanner, N., and Neupert, W., Import pathways of precursor proteins into mitochondria: multiple receptor sites are followed by a common membrane insertion site. J. Cell Biol. *107* (1983) 2483–2490.
46 Pfanner, N., Hartl, F.-U., Guiard, B., and Neupert, W., Mitochondrial precursor proteins are imported through a hydrophilic membrane environment. Eur. J. Biochem. *169* (1987) 289–293.
47 Pfanner, N., and Neupert, W., Transport of proteins into mitochondria: a potassium diffusion potential is able to drive the import of ADP/ATP carrier. EMBO J. *4* (1985) 2819–2825.
48 Pfanner, N., Pfaller, R., Kleene, R., Ito, M., Tropschug, M., and Neupert, W., Role of ATP in mitochondrial protein import: conformational alteration of a precursor protein can substitute for ATP requirement. J. biol. Chem. *263* (1988) 4049–4051.
49 Pfanner, N., Tropschug, M., and Neupert, W., Mitochondrial protein import: nucleoside triphosphates are involved in conferring import-competence to precursors. Cell *49* (1987) 815–823.
50 Pietras, D. F., Diamond, M. E., and Bruenn, J. A., Identification of a putative RNA dependent RNA polymerase encoded by a yeast double stranded RNA virus. Nucl. Acid Res. *16* (1988) 6225.
51 Randall, L. L., and Hardy, S. J. S., Correlation of competence for export within lack of tertiary structure of the mature species: a study *in vivo* of maltose-binding protein in *E. coli*. Cell *46* (1986) 921–928.
52 Riezman, H., Hase, T., van Loon, A. P. G. M., Grivell, L. A., Suda, K., and Schatz, G., Import of proteins into mitochondria: a 70 kilodalton outer membrane protein with a large carboxy-terminal deletion is still transported to the outer membrane. EMBO J. *2* (1983c) 2161–2168.
53 Riezmann, H., Hay, R., Gasser, S., Daum, G., Schneider G., Witte, C., and Schatz, G., The outer membrane of yeast mitochondria: isolation of outside-out sealed vesicles. EMBO J. *2* (1983a) 1105–1111.
54 Riezman, H., Hay, R., Witte, C., Nelson, N., and Schatz, G., Yeast mitochondrial outer membrane specifically binds cytoplasmically synthesized precursors of mitochondrial proteins. EMBO J. *2* (1983b) 1113–1118.
55 Roise, D., Theiler, F., Horvath, S. J., Tomich, J. M., Richards, J. H., Allison, D. S., and Schatz, G., Amphiphilicity is essential for mitochondrial presequence function. EMBO J. *7* (1988) 649–653.
56 Roos, N., Benz, R., and Brdiczka, D., Identification and characterization of the pore-forming protein in the outer membrane of rat liver mitochondria. Biochim. biophys. Acta *686* (1982) 204–214.
57 Schatz, G., How mitochondria import proteins from the cytoplasm. FEBS Lett. *103* (1979) 203–211.
58 Schatz, G., 17th Sir Hans Krebs Lecture: Signals guiding proteins to their correct locations in mitochondria. Eur. J. Biochem. *165* (1987) 1–6.
59 Schein, S. J., Colombini, M., and Finkelstein, A., Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from Paramecium mitochondria. J. Membr. Biol. *30* (1976) 99–120.
60 Schleyer, M., and Neupert, W., Transport of proteins into mitochondria: translocational intermediates spanning contact sites between outer and inner membranes. Cell *43* (1985) 339–350.
61 Schleyer, M., Schmidt, B., and Neupert, W., Requirements of a membrane potential for the posttranslational transfer of proteins in mitochondria. Eur. J. Biochem. *125* (1982) 109–116.
62 Suissa, M., Suda, K., and Schatz, G., Isolation of the nuclear yeast genes for citrate synthase and fifteen other mitochondrial proteins by a new screening method. EMBO J. *3* (1984) 1773–1781.
63 Teintze, M., and Neupert, W., Biosynthesis and assembly of mitochondrial proteins, in: Cell Membranes: Methods and Reviews, vol. 1, pp. 89–114. Eds E. Elson, W. Frazier and L. Glaser. Plenum Press, New York 1984.
64 Tipper, D. J., and Bostian, K. A., Double-stranded ribonucleic acid killer systems in yeasts. Microbiol. Rev. *48* (1984) 125–156.
65 Tzagoloff, A., Mitochondria. Ed. P. Siekevitz. Plenum Press, New York 1982.
66 van Loon, A. P. G. M., Brändli, A. W., Pesold-Hurt, B., Blank, D., and Schatz, G., Transport of proteins to the mitochondrial intermembrane space: the 'matrix-targeting' and the 'sorting' domains in the cytochrome c_1 presequence. EMBO J. *6* (1987) 2433–2439.
67 Verner, K., and Lemire, B., Tight folding of a passenger protein can interfere with the targeting function of a mitochondrial presequence. EMBO J. *8* (1989) 1491–1495.
68 Verner, K., and Schatz, G., Import of an incompletely folded precursor protein into isolated mitochondria requires an energized inner membrane, but no added ATP. EMBO J. *6* (1987) 2449–2456.
69 Verner, K., and Schatz, G., Protein translocation across membranes. Science *241* (1988) 1307–1313.
70 Vestweber, D., and Schatz, G., A chimeric mitochondrial precursor protein with internal disulfide bridges blocks import of authentic precursors into mitochondria and allows quantitation of import sites. J. Cell Biol. *107* (1988a) 2037–2043.
71 Vestweber, D., and Schatz, G., Mitochondria can import artificial precursor proteins containing a branched polypeptide chain or a carboxy-terminal stilbene disulfonate. J. Cell Biol. *107* (1988b) 2045–2049.
72 Wessels, H. P., and Spiess, M., Insertion of a multi-spanning membrane protein occurs sequentially and requires only one signal sequence. Cell *55* (1988) 61–70.

73 Wickner, R. B., Double-stranded RNA replication in yeast: the killer system. A. Rev. Biochem. *55* (1986) 373–395.
74 Zalman, L. S., Nikaido, H., and Kagawa, Y., Mitochondrial outer membrane contains a protein producing nonspecific diffusion channels. J. biol. Chem. *255* (1980) 1771–1774.
75 Zimmermann, R., Hennig, B., and Neupert, W., Different transport pathways of individual precursor proteins in mitochondria. Eur. J. Biochem. *116* (1981) 445–460.
76 Zwizinski, C., Schleyer, M., and Neupert, W., Transfer of proteins into mitochondria: precursor to the ADP/ATP carrier binds to receptor sites on isolated mitochondria. J. biol. Chem. *258* (1983) 4071–4074.
77 Zwizinski, C., Schleyer, M., and Neupert, W., Proteinaceous receptors for the import of mitochondrial precursor proteins. J. biol. Chem. *259* (1984) 7850–7856.

0014-4754/90/020146-08$1.50 + 0.20/0

Biogenesis of mitochondrial porin: The import pathway

R. Pfaller, R. Kleene and W. Neupert

Institut für Physiologische Chemie der Universität München, Goethestr. 33, D-8000 München 2 (Federal Republic of Germany)

Summary. We review here the present knowledge about the pathway of import and assembly of porin into mitochondria and compare it to those of other mitochondrial proteins. Porin, like all outer mitochondrial membrane proteins studied so far is made as a precursor without a cleavable 'signal' sequence; thus targeting information must reside in the mature sequence. At least part of this information appears to be located at the amino-terminal end of the molecule. Transport into mitochondria can occur post-translationally. In a first step, the porin precursor is specifically recognized on the mitochondrial surface by a protease sensitive receptor. In a second step, porin precursor inserts partially into the outer membrane. This step is mediated by a component of the import machinery common to the import pathways of precursor proteins destined for other mitochondrial subcompartments. Finally, porin is assembled to produce the functional oligomeric form of an integral membrane protein wich is characterized by its extreme protease resistance.
Key words. Porin; biogenesis; mitochondrial outer membrane; import receptors; GIP; ATP.

Introduction

The biogenesis of mitochondrial proteins is a rather complex process. Only a small percentage of the proteins are synthesized within the mitochondrion. Most proteins are encoded in the nucleus and are translated on free ribosomes in the cytosol. They are made as soluble precursor proteins which are then subsequently transferred to the mitochondria. In order to maintain the identity of the organelle, targeting of precursors to the mitochondria must occur in a highly specific manner. The crucial event is the initial interaction with the mitochondrial surface. After this step of recognition, the precursor proteins penetrate into and through the mitochondrial membranes. Most precursors enter the matrix space and, during maturation, reach their final destination in the intermembrane space, inner membrane or matrix. In contrast, proteins of the outer membrane do not undergo this complicated process but are directly assembled into the membrane without making a detour. During recent years considerable information has been obtained on the mechanistic principles of how the proteins of mitochondria are translocated across and inserted into membranes after being synthesized on ribosomes in the cytosol (see chapter by Melitta Dihanich in this issue and a recent thorough review on mitochondrial protein import in general by Hartl et al.[24]).

The import pathway of the major outer membrane protein, porin, has been subject to many detailed investigations and several essential aspects of the porin import pathway have been elucidated. Post-translational import of the porin precursor requires specific recognition on the mitochondrial surface which includes binding to receptor sites and the subsequent interaction with a component in the mitochondrial outer membrane which mediates partial insertion of the precursor into this membrane. Furthermore, import requires the hydrolysis of nucleoside triphosphates but, as is the case with all outer membrane proteins studied to date, no energized inner membrane is required. In contrast to the majority of imported proteins, the porin precursor contains no cleavable amino-terminal presequence.

The least understood step in the import pathway of porin is the final assembly in the outer membrane. On the basis of the amino acid sequence, an amphiphilic membrane-spanning β-sheet was proposed to be the predominating structural motif[35] (see chapter by C. Manella in this issue) which is in accordance with the observed extreme