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0014-4754/90/020146-08$1.50 + 0.20/0
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Biogenesis of mitochondrial porin: The import pathway

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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

protease resistance. So far the questions of how the chain is folded into the outer membrane and what is the driving force to assume this secondary structure remain unanswered and will be the subject of many future investigations.

Most information about the very early steps of mitochondrial protein import comes from investigations of the import pathways of porin[35,45,46] and of the ADP/ATP carrier of the inner membrane[51,53,71] where the precursors have been trapped at intermediate stages of their import pathways (reviewed by Pfanner et al.[47]). Knowledge about the constituents of the import machinery on a molecular level is now beginning to emerge. The import intermediates which have been characterized in detail will prove to be very useful tools for the identification of these components. The proteins involved which have been identified so far are: cytosolic hsp 70 related heat shock proteins[8,10,41], a matrix-localized hsp 60 heat shock protein, homologous to the bacterial groEL heat shock protein[7], and the two components of the matrix-localized processing peptidase[26,34,54,66,68,69].

The aim of this review is to give an up-to-date account of the import pathway of porin, including energy requirements, precursor recognition and insertion into the outer mitochondrial membrane. Furthermore, the import pathway of porin will be compared to those of other mitochondrial proteins, especially to that of the ADP/ATP carrier, an integral membrane protein of the inner membrane. Finally, a model of the import pathway of porin is presented.

Biogenesis of mitochondrial porin

The porin precursor

Most mitochondrial precursors are synthesized with an N-terminal presequence. Combined genetic and biochemical experiments have demonstrated that presequences of mitochondrial precursors are necessary and sufficient for the specific targeting to the mitochondria[27,29-31]. They display no recognizable sequence homology. From investigations with synthetic peptides it is proposed that mitochondrial presequences can form amphipathic helices where positively charged amino acids are clustered on one side and hydrophobic amino acids on the other side of the helix[55,64].

The presequence presumably has multiple functions. It must contain targeting information which ensures specific recognition of the newly synthesized precursor on the mitochondrial surface. This means that the presequence must be able to interact specifically with surface components of the mitochondrial import machinery. From the proposed amphipathic structure a function in the translocation can be imagined where, after recognition, positive charges of the presequence respond to the membrane potential across the inner mitochondrial membrane (negative inside) while hydrophobic residues facilitate the insertion into the membrane.

Porin precursor contains no cleavable presequence. There are also precursor proteins which are synthesized without a cleavable presequence. While absence of an N-terminal presequence is the rule for outer membrane proteins this is an exception for proteins from other submitochondrial compartments. Best understood examples of precursors lacking presequences are the ADP/ATP carrier (inner membrane), cytochrome *c* (intermembrane space), porin and a 70 kD protein of unknown function from the outer membrane. Targeting information in precursors of this type must be contained in the mature parts of the precursors.

With the ADP/ATP carrier it appears that there is redundant targeting information due to structural characteristics. The primary structure of the ADP/ATP carrier can be divided into three homologous domains[2,3]. By gene fusion studies it has been demonstrated that the N-terminal domain contains targeting information, with the actual critical region residing in the C-terminus of this domain[1,61]. Furthermore, a truncated ADP/ATP carrier consisting of the carboxy-terminal two thirds of the protein contained sufficient information for correct import[48]. For cytochrome *c* it has been found that an altered carboxy-terminus in a mutant cytochrome *c* precursor abolished import[62]. In the case of the 70 kDa outer membrane protein the targeting signal could be confined to the N-terminal 12 amino acids of the protein, followed by a stretch of hydrophobic amino acids which is supposed to serve as a membrane anchor[25,28]. Analysis of the import signals of porin would suggest that targeting information resides in the amino-terminal portion of the protein, which is predicted to form an amphipathic α-helix[35,39]. This helix, in contrast to other mitochondrial signal sequences, does not seem to have a positive net charge. Both amino- and carboxy-terminal portions, however, are required for proper insertion into the outer membrane[22] (import signals of porin are reviewed by Melitta Dihanich in this issue).

In vitro import has been investigated for porin from mitochondria of the yeast *Saccharomyces cerevisiae*[18,38], rat liver[43] and, in most detail, *Neurospora crassa*[15,35,44-46,52,72]. The precursor protein can be synthesized in vitro in a cell-free translation system programmed with mRNA isolated from the respective organism and detected by immunoprecipitation with specific antibodies[15,18,38,43]. In the case of porin from yeast and *N. crassa*, the cDNA, cloned in an expression vector plasmid, is available[35,39]. The cDNA can be isolated in large quantities from transformed *E. coli* cells and used in an in vitro transcription and translation system to synthesize only the porin precursor. In accordance with the results obtained from the cDNA sequence, no cleavable N-terminal presequence has been detected in any of the in vitro synthesized porin precursors investigated.

Water-soluble porin. Because of the absence of a cleavable presequence, and probably owing to an overall rather

hydrophilic amino acid composition, it was possible to convert purified, mature *Neurospora* porin back to a water-soluble form which displayed a number of characteristics of the precursor form. This water-soluble porin (ws-porin) obtained by acid denaturation and alkaline renaturation has been characterized in detail[44]. The solubility of ws-porin has been investigated by sucrose density-gradient centrifugation. In diluted solutions ws-porin formed a dimer with the tendency to aggregate at higher concentrations.

Conversion of the mature membrane form of porin to the soluble form was reversible. Porin isolated from *Neurospora crassa* mitochondria could be incorporated in planar lipid bilayers where it was functionally characterized with respect to voltage dependence of pore conductivity and anion selectivity[4,14,16]. In experiments with planar lipid bilayers ws-porin could be also reconverted to the membrane form where it displayed pore characteristics indistinguishable from those of the authentic protein. A prerequisite for successful reconstitution was, however, that the membrane contained a sterol. In contrast, for the incorporation of isolated porin into planar lipid bilayers the presence of sterols was not required, presumably because porin purified from *N. crassa* mitochondria contained a large amount of tightly bound ergosterol[16]. Ergosterol is the major sterol in the outer membrane of mitochondria from this organism[21] and may play an important role in the assembly of porin to assume the proper conformation for its biological function. Since ergosterol is present only in the outer membrane but not in the inner membrane of *N. crassa* mitochondria one may speculate that the presence of ergosterol assures the assembly of porin in the outer membrane. Insertion into the inner membrane must be prevented somehow since the formation of pores in it would be fatal for the mitochondrial membrane potential and, therefore, for the whole cell. Ws-porin did not insert spontaneously into sterol-containing lipid bilayers. Only if trace amounts of detergent were present could pore formation be observed.

In import experiments ws-porin displayed properties of the authentic precursor protein[44,45,59]. It could be inserted into mitochondria and could compete for the import of the authentic porin precursor[44,46]. All in all, it appears that ws-porin is the soluble form of an integral membrane protein showing characteristics of the authentic precursor protein. In contrast to precursor proteins synthesized in a cell free system, ws-porin is available as a pure protein in amounts which can be investigated chemically. This provided the opportunity for a thorough investigation of the porin import pathway.

Involvement of cytosolic factors. Recently, the involvement of cytosolic proteins in posttranslational protein import was demonstrated in vivo and in vitro in the yeast system. Members of the hsp 70 heat shock protein family are involved in mitochondrial import as well as in the biogenesis of secretory proteins[8,10,41]. Furthermore, evidence has been presented that in addition to the hsp 70 molecules a second, NEM-sensitive, cytosolic factor is required for import into mitochondria[41]. It has been proposed that a complex with these components renders precursor proteins import-competent. The function of these factors, however, has not yet been defined.

Cytosolic factors may also be involved in the import of porin, although import of ws-porin did not show such a requirement[45]. The denaturation/renaturation procedure used to prepare ws-porin presumably leads to a conformation different from that of the biosynthetic precursor, which still may enter the authentic porin import pathway. Biosynthetic porin precursor must be somehow different from ws-porin since it displays a different ATP-requirement for import (see next section).

Energy requirements of the porin import

Porin import requires no membrane potential. A general requirement for the import of precursors destined for all submitochondrial compartments except the outer membrane is an electrical membrane potential ($\Delta\Psi$) across the mitochondrial inner membrane. When the membrane potential is destroyed by uncouplers of the respiratory chain like valinomycin or CCCP, or by inhibitors of respiration such as cyanide, import into mitochondria is blocked[17,36,57]. It has been speculated that the membrane potential (negative inside) exerts an electrophoretic effect on the positively charged amino acids contained in the amino-terminal presequences and helps to mediate the translocation across the inner membrane[49]. With porin, a membrane potential is not required for the import[15,18,38,43]. This is in agreement with the absence of a positive net charge in the amino-terminal portion of the precursor polypeptide which is proposed to be the targeting sequence[35,39]. The porin precursor, therefore, would not respond to the membrane potential. Lack of requirement for $\Delta\Psi$ appears to hold for all outer membrane proteins studied so far.

Requirement for nucleoside triphosphates. Recently the involvement of nucleoside triphosphates in mitochondrial import[6,11,50,53] and in the transport of proteins across other subcellular membranes such as the ER[23,40,56,65,67], chloroplasts[13,20] and peroxisomes[32] as well as in the export in *E. coli*[5,19] has been established, as it was possible to effectively deplete the in vitro transport systems of nucleoside triphosphates. Nucleoside triphosphates could not be substituted for by non-hydrolyzable analogues which indicated that the hydrolysis of phosphodiester bonds was required. One may propose three possible roles for the requirement for nucleoside triphosphates. 1) Nucleoside triphosphates may keep precursor proteins in a translocation-competent conformation, i.e. prevent formation of a stable tertiary structure. There is some experimental evidence to support this possibility (reviewed by Eilers and Schatz[12]). This does not, however, exclude the other two possible roles.

2) Precursors may be complexed with cytosolic factors which dissociate by an ATP-dependent mechanism when precursors bind to mitochondria. 3) A component of the import machinery might be phosphorylated during the import of precursors.

In the case of porin, import of the biosynthetic precursor form was found to be blocked when the in vitro import system was depleted of ATP by apyrase (an adenosine-5'-(di)triphosphatase) while mitochondrial ATP synthesis was blocked by oligomycin, an inhibitor of the mitochondrial F_1F_0-ATPase[35, 52]. Inhibition of import could be reversed by addition of ATP or GTP which demonstrated that the lack of nucleoside triphosphates was the reason for inhibition of import.

Interestingly, an ATP requirement has not been observed for the import of ws-porin[52]. Under conditions where import of the biosynthetic precursor form was completely blocked, import of water-soluble porin was not affected. Moreover, if biosynthetic porin precursor was subjected to acid/base treatment, import of this converted precursor form was no longer dependent on ATP. This indicated that the denaturation/renaturation substitutes for the ATP-requiring step in the porin import pathway. The two precursor forms differed in their conformation. Taking susceptibility to proteolysis as a measure for folding[33], the native precursor had a more rigid structure than the precursor after denaturation/renaturation. Both ws-porin and the converted biosynthetic precursor form were three to four-fold more sensitive to proteolysis than the native precursor[52]. This indicated that ws-porin, as a result of the denaturation/renaturation procedure applied in the preparation, assumes a more loosely folded conformation as compared to the biosynthetic precursor.

Components of the import machinery involved in the porin import pathway

Import of precursor proteins into mitochondria is a multi-step process involving, among other things, recognition on the mitochondrial surface, insertion into the outer membrane, and transfer across translocation contact sites (reviewed by Pfanner et al.[47]). In the case of porin the following steps can be distinguished: First, recognition of the precursor on the mitochondrial surface. Second, binding to a common component of the import machinery in the outer membrane. Third, assembly to produce the mature form.

Recognition on the mitochondrial surface. Investigations with *N. crassa*, yeast or rat liver have shown that the recognition of precursor proteins by the mitochondria is mediated by a trypsin-sensitive component(s) of the import machinery located on the mitochondrial surface[18, 35, 43-46, 59, 72]. In this respect the import pathway of porin is similar to that of other precursor proteins. Although there has been a report that in the yeast system the import of porin was not affected by trypsin-pretreatment of the mitochondria[18], recently the protease sensitivity of the import of porin into yeast mitochondria could be demonstrated (Kleene and Neupert, unpublished). For porin from *N. crassa*, import was also inhibited when mitochondria were pre-treated with elastase[46], proteinase K or endoproteinase Glu from *Staphylococcus aureus V8* (Pfaller and Neupert, unpublished). The protease treatment degraded sites involved in the specific recognition because not only import was inhibited, but also the association of the porin precursor with the mitochondria. Protease sensitive sites on the mitochondrial surface are presumably the first components of the import machinery where an interaction with precursor proteins occurs and they are, therefore, termed import receptors.

Binding to their surface receptors could directly be demonstrated with a number of precursor proteins when the in vitro import system was depleted of nucleoside triphosphates (see previous section) and the mitochondrial membrane potential was dissipated by uncouplers of the respiratory chain[46, 53]. For example, precursor of the ADP/ATP carrier bound to mitochondria under these conditions was on the correct import pathway, because re-establishment of a membrane potential and re-addition of ATP led to completion of import (chase). Attempts to accumulate porin precursor at its receptor site using a similar approach have failed so far, because of a high degree of unspecific binding under these conditions, i.e. porin precursor bound to mitochondria could not be made to assume the mature form by replacing ATP (Kleene and Neupert, unpublished).

Partial insertion into the outer membrane. Lowering the incubation temperature leads to a markedly reduced porin import rate[15]. Using this approach an insertion intermediate has been characterized. Porin associated with mitochondria at low temperature could be converted into the mature form by increasing the temperature[35, 45]. It was, therefore, a true intermediate on the import pathway.

Employing radioactively-labeled ws-porin as a ligand the low temperature binding sites have been titrated and the binding parameters determined[45] (fig. 1). The number of binding sites of 5–10 pmol/mg mitochondrial protein indicate that this component is of low abundance in mitochondria (for comparison: the content of porin from *Neurospora crassa* is about 100 pmol/mg mitochondrial protein[16]). Ws-porin bound rather tightly with an apparent dissociation constant of about 5 nM, which is comparable to that observed for the binding of hormones to their receptors.

The high-affinity binding sites have been characterized in detail[45]. After pretreatment of mitochondria with trypsin, ws-porin could no longer occupy these sites. A differential sensitivity to added proteases was observed with porin precursor bound to high-affinity binding sites as compared to either the free precursor or to precursor bound to the initial receptor sites which were more sensitive to proteolytic attack. On the other hand, porin precursor bound at low temperature was digested by con-

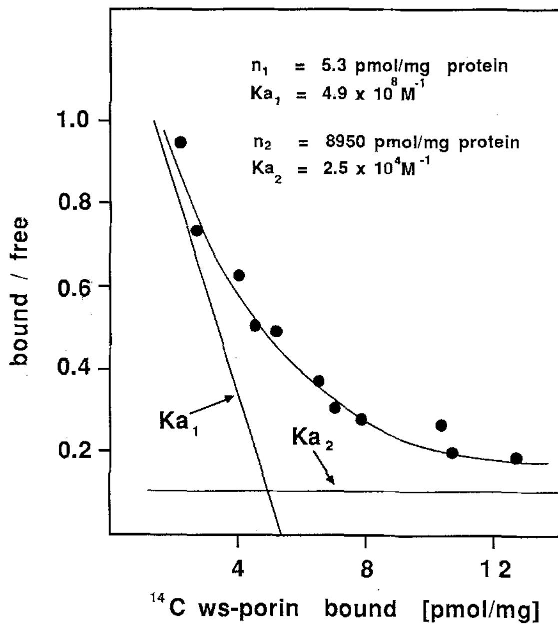

Figure 1. Titration of binding sites for ws-porin on mitochondria. Titration of binding sites was carried out as described[45]. Mitochondria were incubated at 0 °C with increasing concentrations of ^{14}C-ws-porin. Then, bound and free radioactivity were determined and the binding parameters determined by Scatchard analysis. The figure shows a plot of bound/free vs. bound ligand (Scatchard plot). Ka_1: affinity of high-affinity binding sites; Ka_2: affinity of low-affinity binding sites; n_1: number of high-affinity binding sites; n_2: number of low-affinity binding sites.

centrations of protease which did not destroy the assembled porin firmly integrated into the outer membrane. By these criteria it has been concluded that the low temperature intermediate of porin is already beyond the trypsin-sensitive recognition site but not yet assembled. It rather appears that porin bound to high-affinity binding sites is partially inserted into the outer membrane. This partially inserted porin precursor does not represent membrane integrated, mature porin since, in contrast to the mature form, it could still be extracted at alkaline pH. Employing the biosynthetic porin precursor instead of ws-porin the same results were obtained[35]. The high-affinity binding sites have been termed GIP sites (GIP stands for general insertion protein) since they appear to be common to the import pathways of many different precursor proteins[46] (see next section). The properties of free precursor, low temperature intermediate and mature porin are summarized in the table.

Assembly in the outer membrane. Contact sites between the inner and the outer membrane of mitochondria were biochemically and morphologically identified as sites where translocation of precursor proteins into the matrix takes place[58, 60, 63]. One may speculate that contact sites are the place where outer membrane proteins are also assembled. Up to now it has not been shown convincingly that porin or other outer membrane proteins are also imported at contact sites. While import into isolated outer membranes has been observed in yeast[18] and *N. crassa* (Pfaller and Neupert, unpublished) an investigation in

Properties of porin precursor at different stages on its import pathway

Porin precursor	Free	GIP-bound	Assembled
Low prot. K	Sensitive	Resistant	Resistant
High prot. K	Sensitive	Sensitive	Resistant
Alkaline extraction	Soluble	Soluble	Pellet

prot. K: proteinase K; GIP: general insertion protein.

rat liver seems to indicate that porin import into mitochondria of this organism indeed involves contact sites[43]. In his study porin precursor was bound to mitochondria under different conditons, then the mitochondria were fractionated by sonication and separated into outer membrane and a fraction containing inner and outer membrane markers. Porin precursor associated with mitochondria was followed and its distribution compared to that of marker enzymes of outer and inner membrane. After incubation at low temperature for a short period most porin was associated with the outer membrane, while after extending the incubation time the greater part of the precursor cofractionated with vesicles containing outer and inner membrane marker. If the import of mitochondrially associated porin precursor was allowed to go to completion at an elevated temperature porin was predominantly found in the outer membrane again which indicates that after assembly porin was equally distributed over the outer membrane. The intermediates were not further characterized. Since they were obtained at low temperature it is possible that binding to the high-affinity binding sites was observed. One can imagine that after partial insertion into the outer membrane the actual assembly to the mature form (which includes oligomerization to either a dimeric[37] or a trimeric[9] form) occurs at contact sites.

The porin import pathway in relation to the import pathways of other precursors

In competition experiments ws-porin has been employed to block import of other precursor proteins. Ws-porin not only competed for the import of the biosynthetic precursor form of porin but also for the import of precursors destined for the other submitochondrial compartments[45, 46]. Concentrations of ws-porin required for half-maximal competition were comparable to concentrations of unlabeled ws-porin required to compete for half of the high-affinity binding of radioactive-labeled ws-porin. The only exception was cytochrome *c*, which is known to follow a unique import pathway in that it is imported without a protease-sensitive surface receptor into the intermembrane space where the mature protein is formed by the covalent attachment of heme[42, 70]. Competition could only be observed when ws-porin was able to occupy the high-affinity binding sites[46]. At elevated temperatures, under import conditions where high-affinity binding sites were occupied only transiently, ws-porin competed very inefficiently for import with other precursor proteins. When ws-porin was rendered incom-

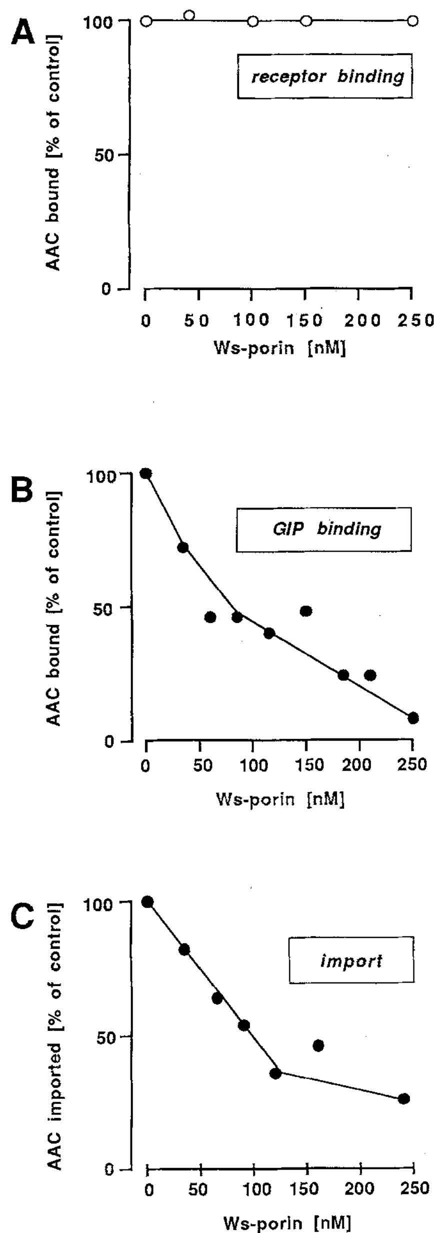

Figure 2. Competition with the ADP/ATP carrier precursor on different stages in its import pathway by ws-porin. Competition experiments with ws-porin were carried out as described[45,46]. Binding and import of ^{35}S-labeled precursor of the ADP/ATP carrier was performed in the presence of increasing concentrations of unlabeled ws-porin. Bound or imported precursor was analyzed by polyacrylamide gel electrophoresis and fluorography. Fluorographs were quantified by densitometry. *A* Competition of binding of ADP/ATP carrier precursor to surface receptors. *B* Competition of binding of ADP/ATP carrier precursor to GIP sites in the outer membrane. *C* Competition of import of ADP/ATP carrier precursor.

petent for specific binding and import by repeated freezing and thawing, no competition could be observed. ADP/ATP carrier import was similar to porin import in that two steps of recognition could also be resolved. In the absence of ATP and without a membrane potential the carrier precursor bound to proteinaceous receptors on the mitochondrial surface[46,53] (see previous section). Binding to sites in the outer membrane comparable to the high-affinity binding sites for porin, however, was observed if the membrane potential was dissipated[51]. The parameters for binding of the ADP/ATP carrier to these sites in the outer membrane were determined by Scatchard analysis and were comparable to those for the high-affinity binding sites for porin[46].

We investigated whether ws-porin competed for either of the two binding sites on the import pathway of the ADP/ATP carrier. As shown in figure 3, ws-porin did not compete in binding to the receptor sites on the mitochondrial surface[46]. In contrast, ws-porin competed as efficiently with the carrier precursor in binding to sites in the outer membrane as in import into the inner membrane[45]. While the initial recognition of the porin and the ADP/ATP carrier precursor appear to be mediated by different surface receptors, the high-affinity binding sites of the porin import pathway are shared by other precursors. Apparently, there is a common component of the import machinery at the level where precursors are inserted into the outer membrane. This component, corresponding to the high-affinity binding sites in the import pathway of porin is, therefore, called general insertion protein (GIP)[46].

Pretreatment of *N. crassa* mitochondria with elastase abolished import of porin and other precursor proteins but not of the subunit β of the F_1-ATPase (F_1 β)[46,72]. This indicated that the initial recognition, e.g. binding to import receptors, of F_1 β and porin display different characteristics. On the other hand, competition experiments suggested that the import pathways of both precursors share at least one common component. Competition by ws-porin with F_1 β import into elastase-treated mitochondria has been employed to determine at which level the import pathways of F_1 β and porin share a common component. After elastase treatment, import of F_1 β was no longer competed with by ws-porin, indicating that this component seems to be involved after the initial recognition of precursor proteins by import receptors but before the membrane potential-dependent translocation across contact sites[46].

A working hypothesis on the import pathway of mitochondrial porin is summarized in figure 3. After synthesis on cytoplasmically localized ribosomes the soluble porin precursor is transported to mitochondria. This may involve hsp 70 molecules or so far unknown cytosolic factors. On the mitochondrial surface it is recognized by a trypsin-sensitive component which has characteristics of an import receptor. Cytosolic factors may dissociate and the porin precursor is inserted into the outer membrane; this process is mediated by GIP. This represents the step where ATP is required, either by GIP or a so far unknown component. After binding to GIP, assembly to the active pore protein occurs; this involves the final formation of porin dimers or trimers. Assembly of porin is then completed in the outer membrane or in contact site regions between outer and inner membranes which

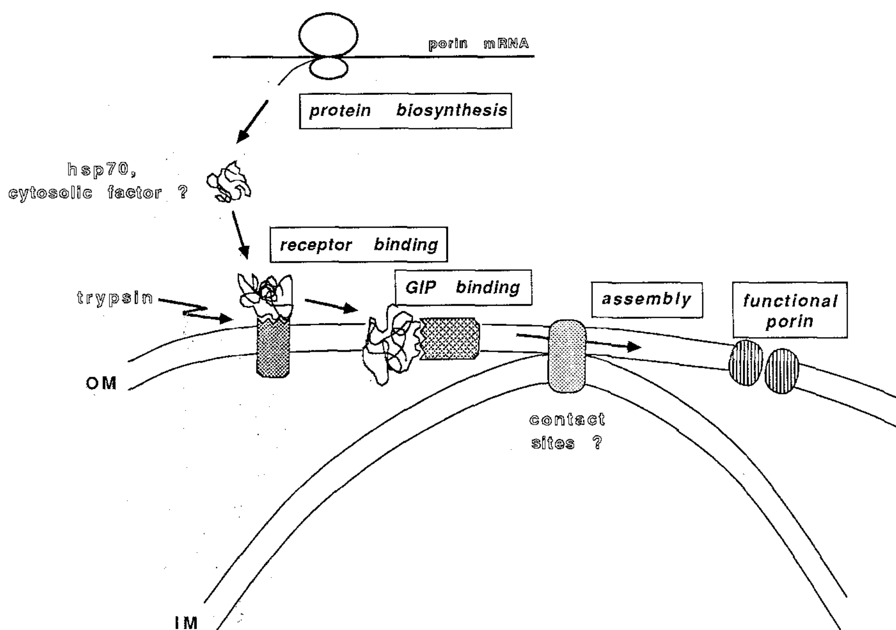

Figure 3. Working hypothesis on the porin import pathway. Porin precursor is synthesized on free cytoplasmic ribosomes and released into the cytosol. Hsp 70 related heat shock proteins and/or other cytosolic factors may keep the precursor in a competent form for import. This process may involve the hydrolysis of nucleoside triphosphates. Then the porin precursor binds specifically to a protease-sensitive import receptor on the mitochondrial surface. In a subsequent reaction the precursor becomes partially inserted into the outer membrane. This step is mediated by GIP and requires nucleoside triphosphates. The final step of the import pathway is the assembly to produce the functional, pore-forming protein. This may occur either directly in the outer membrane or at translocation contact sites, and involves formation of dimers or trimers.

have been shown to be sites of translocation for other precursor proteins. It may be speculated that the components involved in the assembly are organized in a multisubunit complex and that this complex is localized in translocation contact sites.

Investigation of the import pathway for porin has contributed significantly to our current understanding of the early steps of precursor recognition. Now the major interest in mitochondrial protein import research is focussed on the identification and isolation of components of the import machinery, especially those responsible for recognition and translocation. Biochemical, immunological and genetic approaches are being employed to analyze the import apparatus on a molecular level.

Acknowledgments. We thank Rosemary A. Stuart for comments on the manuscript.

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0014-4754/90/020153-09$1.50 + 0.20/0
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Interaction of mitochondrial porin with cytosolic proteins

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Summary. Intracellular phosphorylation is an important step in active uptake and utilization of carbohydrates. For example glucose and glycerol enter the liver cell along the extra intracellular gradient by facilitated diffusion through specific carriers and are concentrated inside the cell by phosphorylation via hexokinase or glycerol kinase. Depending on the function of the respective tissue the uptake of carbohydrates serves different metabolic purposes. In brain and kidney medulla cells which depend on carbohydrates, glucose and glycerol are taken up according to the energy demand. However, in tissues such as muscle which synthesize glycogen or like liver which additionally produce fat from glucose, the uptake of carbohydrates has to be regulated according to the availability of glucose and glycerol. How the reversible coupling of the kinases to the outer membrane pore and the mitochondrial ATP serves to fulfil these specific requirements will be explained as well as how this regulates the carbohydrate uptake in brain according to the activity of the oxidative phosphorylation and how this allows glucose uptake in liver and muscle to persist in the presence of high glucose 6-phosphate without activating the rate of glycolysis.
Key words. Mitochondria; outer membrane pore; hexokinase; glycerol kinase; metabolite exchange; energy metabolism.

The outer mitochondrial membrane contains a specific binding protein for hexokinase

In 1977, Felgner and Wilson[11] characterized a specific binding protein for hexokinase in the outer membrane of rat liver mitochondria. When we incorporated this isolated binding protein into bilayer membranes we observed the same properties concerning conductivity and voltage dependence[14] as those described for the outer membrane pore[9,42]. Vice versa, reconstitution of the pore protein in asolectin vesicles resulted in specific binding of hexokinase[14]. These observations suggested that the outer membrane pore and the hexokinase binding protein were the same proteins which was confirmed by Lindén et al.[29] who proved the identity of the two proteins by two-dimensional electrophoresis and peptide map.
Additional proof of the interaction between hexokinase and the outer membrane pore came from the observation that specific antibodies against porin suppressed the binding of hexokinase to liver mitochondria[7]. Furthermore, yeast hexokinase which was bound in vitro to the isolated outer membrane of yeast mitochondria became cross-linked to the pore protein by dithiobis-(succinimidylpropionate)[26]. One important reason for the specific binding of kinases may be that the exchange of charged metabolites such as ADP and ATP[4,42] across the outer membrane is exclusively possible by passing the pore.

Reversible binding of kinases to the outer membrane of liver mitochondria

Beside hexokinase, reversible binding of other ATP consuming enzymes to the mitochondrial surface in different tissues has been reported namely glycerol kinase[21,31,38,46,47] and ATP citrate lyase[20]. It could be demonstrated that glycerol kinase binds to the pore in experiments with the reconstituted, isolated protein[14]. Furthermore, specific antibodies against porin suppressed the binding of glycerol kinase to isolated mitochondria[38].