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Characterization and Function of the Mitochondrial Outer Membrane Peptide-Sensitive Channel

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The PSC (peptide-sensitive Channel), a cationic channel of large conductance, has been characterized in yeast and mammalian mitochondria by three different methods, "tip-dip," patch clamp of giant liposomes, and planar bilayers. The yeast and mammalian PSC share the common property to be blocked by basic peptides such as pCyt OX IV (1–12)Y which contains the first 12 residues of the presequence of cytochrome *c* oxidase subunit IV. The electrophysiological data are consistent with a translocation of the peptide through the pore. Analysis of the frequency of observation of the PSC in different fractions indicates that the channel is located in the outer mitochondrial membrane. Uptake measurements of iodinated peptides by intact mitochondria from a porin-less mutant show that the peptides are translocated through the outer membrane, presumably at the level of PSC. Among the peptides active on PSC, several, such as pCyt OX IV (1–22) and the reduced form of the mast cell degranulating peptide, induce an alteration of the voltage dependence or of the inactivation rate subsisting after washing and which is eliminated only by proteolysis of the interacting peptide. These irreversible effects may account for the variability of the properties of the PSC which would interact with cytosolic or intermembrane cations, peptides, or proteins, thus modulating the channel permeability. Finally, several lines of evidence suggest the participation of the PSC in protein translocation and some interaction with the general insertion pore of the outer membrane translocation machinery.

KEY WORDS: Mitochondria; ionic channels; outer membrane; protein translocation.

INTRODUCTION

The outer membrane of mitochondria is known to be highly permeant. For many years, this permeability was attributed to a unique porin, VDAC (voltage-dependent anion-selective channel), which might represent up to 20% of the outer membrane protein content (Colombini, 1979). However, the presence of other channels in this membrane was suspected (Tedeschi *et al.*, 1987). In 1988 (Thieffry *et al.*, 1988), we described a new cationic channel, later named PSC (peptide-sensitive channel), which was shown to be localized on the outer membrane (Chich *et al.*, 1991). The existence of a pathway different from VDAC was supported by the fact that disruption of the porin gene in yeast was not lethal (Michedja *et al.*, 1989). In this review, we present electrophysiological data on the PSC as well as results of biochemical experiments exploring the function of this channel.

DESCRIPTION OF THE PORE

Electrophysiological Data

Due to the methodology, the channels inserted in tip-dip and planar bilayers may be found in either orientation. The convention for the sign of the trans-

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membrane voltage is thus arbitrary unless the orientation of the channel in the native membrane could be determined, which is the case only for the mammalian channel (Chich *et al.*, 1991). However, since the voltage dependence is not symmetrical, the properties related to the PSC orientation can be described without ambiguity. The conventions used below are identical to those used in all our works on PSC published so far.

Conductance and Voltage Dependence

First studied in bilayers formed at the tip of microelectrodes by the tip-dip method (Ehrlich, 1992), the PSC has been further characterized in patches of giant liposomes or in planar bilayers formed by the Mueller–Rudin method (Mueller *et al.*, 1963). The properties of the channel that have emerged from hundreds of single-channel recordings carried out using the three techniques are now quite clear. In all preparations tested so far, five conductance levels (referred to below as l_1–l_5 in order of decreasing conductance) can be identified.

In mammals (bovine adrenal medulla or adrenal cortex, rat liver), the PSC has a maximum conductance (level l_1) of 750 pS in 150 mM NaCl (Thieffry *et al.*, 1992). It is open at positive potentials ($V_{\text{intermembrane space}} > V_{\text{cytosol}}$) and closes at negative ones. Around −30 mV, the channel exhibits a fast characteristic flicker composed of 220 pS jumps between levels l_1 and l_2 (530 pS) or l_2 and l_3 (310 pS). Below −50 mV, brief transient 100 pS closures from l_3 to l_4 (210 pS) occur and below −100 mV, rare transitions briefer than 1 ms between l_4 and l_5 (110 pS) can be detected. We never observed complete closures. The channel is sensitive to trypsin on one side, the protease abolishing in two steps the voltage dependence (Thieffry *et al.*, 1988; Chich *et al.*, 1991). There is a slight variability in the voltage dependence of the flicker observed at negative potentials, extreme types corresponding to type I and type II channels previously described in tip-dip records (Thieffry *et al.*, 1988).

In yeast *Saccharomyces cerevisiae*, identical channels are found both in wild type and in a mutant in which the gene coding for VDAC has been disrupted (kindly provided by G. Lauquin, Université Bordeaux II, France) (Fèvre *et al.*, 1990). Their maximum conductance is slightly higher (850 pS in 150 mM NaCl) and the two main subconductance levels l_2 and l_3 are respectively 330 and 660 pS below the open state l_1 (compared to 220 and 440 pS in mammals) (Thieffry *et al.*, 1992). The voltage dependence is difficult to describe because there exists a large variability among the records, even in the same fraction. For all the channels, the probability of the closed states increases when the potential is decreased below 0 mV. Upon application of a steady-state potential, the channel fluctuates between levels l_1–l_2 or l_2–l_3. The dwell time at levels l_2 and l_3 (tens of milliseconds to seconds at −30 mV) is longer than in mammals. However, the steepness of this voltage dependence is itself variable. Whereas frequent closures already occur at −20 mV in some records, in others similar events are not observed until the voltage is decreased below −50 mV or even lower voltages. The other variable characteristics concern the following points:

—Some channels also close at positive potentials. However, in this case, the voltage dependence is not symmetrical, its steepness depending on the polarity.

—A fast flicker, continuous or occurring in bursts, composed of closures from level l_1 of various amplitudes, is generally present at voltages higher than +40 mV. This pattern is always observed in patches of giant liposomes, but is not constant in tip-dip and planar bilayer experiments.

—In addition to the voltage-dependent fluctuations described above, one observes generally an inactivation at potentials of either polarity (Pelleschi *et al.*, in preparation). After a certain delay, the channel no longer returns from l_2 to l_1 and, after an additional delay, remains at level l_3. At high voltages, brief closures at level l_4 and l_5, respectively 100 and 200 pS below l_3, or a fast flicker among l_3, l_4, and l_5 may occur. For a given channel, the rate of inactivation increases with the voltage magnitude, but this rate is variable from channel to channel. Inactivation is relieved by switching the voltage to 0 mV or to a voltage of reverse polarity. Surprisingly, no inactivation is detected in patches of giant liposomes whereas in tip-dip and planar bilayers records channels devoid of inactivation are rare. Inactivation is suppressed by trypsin. The channel is sensitive to the protease on both faces. The proteolysis prevents the inactivation that would otherwise occur when the compartment where trypsin has been added is made positive with respect to the other one.

—In addition to the 330 and 100 pS current jumps mentioned above, there exist also jumps of various sizes (for instance 260 and 400 pS) corresponding to conductance levels different from l_1–l_5. These levels have, however, a lower probability than the latter.

Selectivity and Ion Permeation

Mammalian and yeast channels exhibit a similar partial cationic selectivity and are permeant to large ions [tetraethylammonium (TEA), glucuronate, arginine]. The sequence of permeability is

$$P_K > P_{Na} > P_{TEA} \approx P_{arginine} > P_{Cl}$$

with $P_{Na}/P_{Cl} \approx 3$ (Thieffry *et al.*, 1988; Fèvre *et al.*, 1990)

Reversible Block by Basic Peptides

Despite their differences, the mammalian and yeast channels have likely identical functions. Both have the same selectivity and five main conductance levels. The presence of couples of current jumps of equal amplitude suggests a dimeric structure, which is in good agreement with the fact that trypsin acts in two steps.

The idea of a common function is further strongly supported by the fact that channels of both origins are reversibly blocked by basic peptides. A typical example is the action of pCyt OX IV (1–12)Y (referred to as peptide M in previous papers), a peptide composed of the first 12 residues of the presequence of cytochrome *c* oxidase subunit IV precursor from yeast *Saccharomyces cerevisiae* and a tyrosine (see Table I). It contains three basic residues and no acidic ones. At micromolar concentrations, the peptide induces a voltage-dependent block irrespective of the side of the channel to which it is applied (Henry *et al.*, 1989). The effect is still observed if the channel has been pretreated by trypsin. If voltages are expressed with respect to that of the compartment to which the peptide is added, the block is characterized by brief closures appearing at potentials below +50 mV the number of which increases with the driving force applied to the peptide. It is relieved if the peptide is washed out. Its voltage dependence, which is related to the compartment to which the peptide is added and not to the orientation of the channel in the bilayer, suggests that the peptide plugs the channel. However, the magnitude and the duration of the block do not increase monotonically with the driving force applied to the peptide as would be expected in a Woodhull model (Woodhull, 1973). Both parameters have their maximum around 0 mV. These data are consistent with a model in which the peptide could go through the pore when the driving force becomes sufficient (Fig. 1) (Thieffry *et al.*, 1992; Fèvre *et al.*, 1994).

Similar results are obtained with longer peptides derived from the same precursor signal sequence, pCyt OX IV (1–16) and pCyt OX IV (1–23)A19 (Fèvre *et al.*, 1994). Interestingly, dynorphin B, a 13-residue neuropeptide unrelated to mitochondrial biogenesis which, as pCyt OX IV (1–12)Y carries three basic residues and has an amphiphilic structure, has an effect similar to that of the latter peptide (Table I) (Henry *et al.*, 1989). As we shall see below, this peptide is also imported in mitochondria. The effect is clearly related to the charge. Introduction of negative charges in pCyt OX IV (1–12)Y by succinylation suppresses the activity (Henry *et al.*, 1989). Peptides containing acidic residues such as p-gastrin which carries six negative charges, pRIHB, a fragment of the retinoic acid-induced heparin-binding protein carrying three positive and one negative charges, or p-angiotensinogen (1–13) which carries one positive and one negative charge (Table I) are devoid of effect in both compartments whatever the polarity of the transmembrane voltage. It is worth noting that pCyt OX IV (1–12)Y has no effect on VDAC.

The channel described in this section is likely to be similar to that described by Dihanich *et al.* (1989) and by Zoratti *et al.* (1994), working on mitochondria from yeast porin-less mutants.

Biochemical Data

Localization

The tip-dip method allows an assay of the PSC abundance in membrane fractions since the protein content of the monolayer used in such experiments was shown to reflect the composition of the proteoliposomes prepared from the membrane fragments (Pattus *et al.*, 1981). A quantitative evaluation of the PSC abundance can thus be simply obtained by the frequency of observation of the characteristic electrical activity, defined as the total number of channels found divided by the total number of trials. As a matter of fact, increasing the amount of proteins incorporated in the liposomes led to a linear increase of the PSC frequency (Chich *et al.*, 1991).

Localization of the PSC on the outer membrane of mitochondria was shown using mammalian mitochondria (Chich *et al.*, 1991). As described above, the cationic channel of such organelles has a trypsin-

Table I. Inhibitory Effects of Various Peptides

Peptide[a]	Net charge	Inhibitory effect on PSC activity	Import of dynorphin B	Import of pCyt OX IV (1–12)Y[d]	Import of preb2-DHFR
pCyt OX IV (1–12)Y[b] MLSL**R**QSI**R**FF**K**Y	+3	+	+	+	+
pCyt OX IV (1–22)[b] MLSL**R**QSI**R**FF**K**PAT**R**TLASS**R**	+5	+	+	+	+
Dynorphin B[b] YGGFL**RR**Q**F**K**V**VT	+3	+	+	−	−
pAT III[b] **R**NASVL**K**SS**K**NA**KR**YL**R**CNL**K**A	+7	+	+	−	−
rMCD[c] I**K**CNC**KR**H**V**I**K**PHIC**RK**ICGK**N**	+7	+	+	nd	nd
nMCD[c] I**K**CNC**KR**H**V**I**K**PHIC**RK**ICGK**N**	+7	−	−	nd	nd
pRIHB[b] T**R**TGA*E*C**K**QTMLTQ**R**	+2	−	−	−	−
p-Gastrin[b] *E*GPWL*EEEEE*AYG	−6	−	−	−	−
p-Angiotensinogen[b] *D***R**VYIHPFHLVIH	0	−	−	−	−

[a] Bold and italic characters represent, respectively, basic and acidic residues.
[b] Yeast mitochondria (Vallette *et al.* 1994).
[c] Mammalian mitochondria (Juin *et al.* 1995a).
[d] Inhibitory effect on import at 30°C (Vallette *et al.* 1994).
nd = not determined.

sensitive component responsible for voltage-dependent fast closures. When trypsin is applied to the mammalian channel, the electrical activity is shifted in two steps from its native characteristic pattern (N form of the channel) to a trypsinized form (T form) devoid of rapid fluctuations. Trypsinization of whole mitochondria prior to their incorporation in tip-dip bilayers leads to an increase in the T form frequency and a corresponding decrease in the N form frequency. This shows that, in intact mitochondria, the PSC is accessible to trypsin added to the cytosolic compartment. The PSC is thus likely localized on the outer membrane. Fractionation of membranes from rat liver and adrenal cortex mitochondria was also performed. Fractions enriched in outer membranes had the highest channel frequency. Such experiments do not completely separate mitochondrial membranes. Cross contamination between the outer and the inner membrane fractions might thus explain the presence of PSC activity in both fractions. In a third type of experiments, mitochondria from adrenal cortex were treated with digitonin under conditions where the outer membrane was progressively solubilized. The treatment concomitantly decreased the PSC frequency and the activity of outer membrane markers (hexokinase and monoamine oxidase).

Thus converging evidence indicates the localization of the PSC at the level of mitochondrial outer membrane. Such a localization raises the question of the relationship between VDAC and PSC. The fact that the frequency of observation of the PSC is the same in mitochondria from a yeast mutant with a disrupted porin gene and from the wild type (Fèvre *et al.*, unpublished results) shows that the two activities are different. In a previous review (Thieffry *et al.*, 1994), we suggested that VDAC does not survive the freezing/thawing cycles used in the preparation of proteoliposomes and/or the stay in the monolayer at the air–water interface, which precluded an estimation of the relative abundance of the two channels. From the data of Dihanich *et al.* (1989), the PSC might be 10 times less abundant than the VDAC.

Peptide Import into Mitochondria

The electrophysiological data suggest that the PSC might be permeant to peptides such as pCyt OX

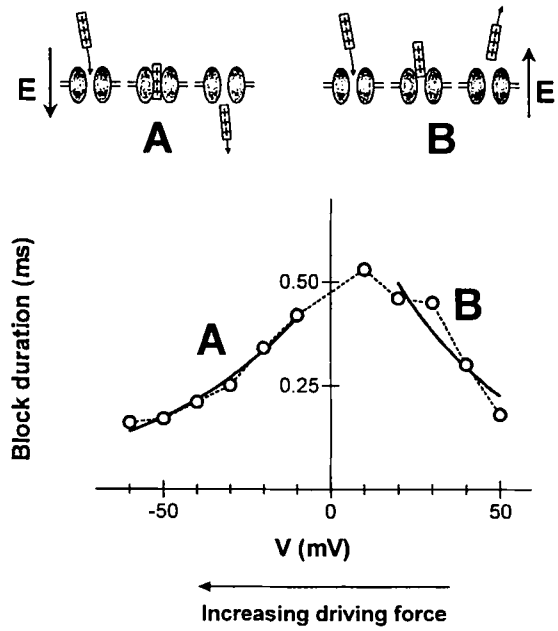

Fig. 1. Tentative model derived from the voltage dependence of the block duration. The experimental values (○) were obtained using a yeast PSC exposed to 8 μM pCyt OX IV (1–16) recorded in a tip-dip bilayer. They concern only peptide-induced closures since they were derived from the additional linear component appearing in semilogarithmic plots of the distribution of the duration of partial closures I_1-I_2-I_1 following peptide addition. The peptide is applied to the *trans* compartment. The voltage in the abscissa is V_{cis}–V_{trans}. Since the peptide carries positive charges, the driving force increases from positive to negative potentials as indicated by the arrow. At positive potentials (B), the electric field *E* tends to repel the peptide from the channel mouth. Decreasing the voltage lowers the field magnitude and the block duration increases with the driving force. The block is relieved only when the peptide leaves the channel mouth, in agreement with the Woodhull model. The experimental data reflect then the block duration *stricto sensu*. At negative potentials, the electric field *E* favors the peptide translocation and its magnitude increases with decreasing potentials. The A part of the curve would then reflect the time necessary for the peptide to be translocated, which is expected to decrease when the driving force increases.

IV (1–12)Y and dynorphin B. In order to see whether such a permeability pathway exists at the level of intact mitochondria, we analyzed the interaction of iodinated peptides with mitochondria isolated from wild-type yeast (Vallette *et al.*, 1994). In the presence of *ortho*-phenanthroline, an inhibitor of metalloproteases, pCyt OX IV (1–12)Y and dynorphin B are imported into the organelle where they are protected from externally added proteinase K. In the absence of *ortho*-phenanthroline, the accumulated peptides are degraded by intramitochondrial metalloproteases. The rate of accumulation observed in the presence of the inhibitor is similar to the rate of degradation in the absence of the inhibitor, thus showing that metalloproteases are not rate-limiting in the import mechanism. In addition, similar rates of import are observed using porin-less mitochondria, indicating that VDAC is not involved in this pathway.

The characteristics of dynorphin B import were determined: (i) this peptide is translocated through the mitochondrial outer membrane directly into the intermembrane space; (ii) accordingly, its import is not dependent on intramitochondrial $\Delta\Psi$; (iii) decreasing the incubation temperature from 30° to 0°C is without any effect on dynorphin B import; (iv) the import involves proteins at the surface of the organelle since pretreatment of mitochondria by trypsin decreases the rate of import.

Roise and co-workers have shown that a mitochondrial addressing peptide can be imported into protein-free phospholipid vesicles in which a valinomycin-induced K^+ diffusion potential was established (Maduke and Roise, 1993). They have concluded that such peptides might be translocated through the lipid phase of mitochondria. Nevertheless, such a mechanism cannot account for dynorphin B import since this phenomenon is $\Delta\Psi$-independent, trypsin-sensitive, and temperature-insensitive. In addition, ^{125}I-labeled dynorphin B import is inhibited by an excess of unlabeled peptide (Table I), showing the involvement of a saturable step at the level of the outer membrane. Dynorphin B import thus appears to occur through an aqueous pore rather than through the phospholipid phase of the outer membrane.

The existence of a saturable step at the level of the outer membrane allowed us to study the effect of an excess of different peptides on dynorphin B import (Table I). Peptides that interact with the PSC such as pCyt OX IV (1–12)Y, pCyt OX (1–22), or pAT III, a 22-residue fragment of antithrombin III (see below for a description of the electrophysiological effect of the latter two peptides) inhibit dynorphin B import into yeast mitochondria whereas peptides inactive on the PSC do not. This result suggests the involvement of the PSC in dynorphin B import (Vallette *et al.*, 1994).

Additional evidence for such an involvement was obtained with the mast cell degranulating peptide (Juin *et al.*, 1995a). This basic peptide in its native form (nMCD) is locked in a tight conformation by two disulfide bridges. Irreversible reduction of these bonds leads to a reduced form (rMCD) that might adopt a linear structure. Both forms interact with the yeast PSC and inhibit dynorphin B import into yeast mitochon-

dria. Interestingly, nMCD is inactive on dynorphin B import into mammalian mitochondria (Table I). Reduction of the peptide is then necessary to obtain an inhibitory effect on peptide import. In a parallel way, nMCD interacts very poorly with the mammalian PSC in conditions similar to those used for import experiments, and reduction of the peptide is required to block efficiently the electrical activity (Table I). These results are interesting in several ways. On one hand, they definitely rule out the hypothesis of the translocation of dynorphin B through the lipid phase of mitochondria: nMCD inhibits such a mechanism in yeast mitochondria and does not in mammalian ones although both types are likely to have similar lipid composition. On the other hand, they strongly argue in favor of the involvement of the PSC in dynorphin B import since changes in the structure of the peptide which induce the block of the mammalian channel also lead to inhibition of dynorphin B import into these mitochondria. The experiments on dynorphin B import thus suggest that the PSC might play the role of a peptide-conducting pore in the mitochondrial outer membrane.

IRREVERSIBLE ACTION OF SOME BASIC PEPTIDES

The effect of basic peptides such as pCyt OX IV (1–12)Y, pCyt OX IV (1–16), pCyt OX IV (1–23)A19, or dynorphin B on the electrical activity is characterized by the fact that it is reversible following washing out of the peptide. Other basic peptides were found which induce changes in the voltage dependence persisting after washing. This kind of effect is interesting since it may explain both the differences between channels of different origin (mammalian and yeast) and the variability in voltage gating and inactivation observed among channels originating from the same mitochondrial fraction.

Voltage Gating

An example of such an irreversible modification of the voltage dependence is the effect of pCyt OX IV (1–22), again a part of the presequence of cytochrome *c* oxidase subunit IV precursor (Table I) (Fèvre *et al.*, 1994). A striking difference between mammalian and yeast PSC is the existence in the former of a characteristic flicker between levels l_1, l_2, and l_3 at voltages around −30 mV. As mentioned above, this flicker no longer occurs if the channel is pretreated by trypsin on its cytosolic side. If a proteolyzed channel is exposed to pCyt OX IV (1–22) after washing out the protease, it exhibits again a voltage dependence like that observed before proteolysis. In particular, the channel shows again fast fluctuations around −30 mV. These fluctuations are in turn suppressed by trypsin and can be restored by a new exposure to the peptide.

If the peptide is applied to the other side of the channel, it induces changes in the voltage dependence at potentials of reverse polarity. If a native channel is treated in this way, it now closes at both positive and negative potentials and exhibits, after washing of the peptide, comparable, though not strictly identical, fluctuations around +30 and −30 mV.

The peptide is also active on both sides of yeast PSC. It induces a voltage-dependent gating which resembles the natural gating of mammalian channels except for its kinetics which are slower. As in mammalian preparations, these changes are abolished by trypsin added to the side exposed to the peptide and are restored by a second exposure to the peptide following elimination of the protease.

Similar effects are obtained using pOAT(1–34), a peptide containing the first 34 residues of the presequence of rat liver ornithine aminotransferase precursor (Ono and Tuboi, 1988) or pAT III, a molecule unrelated to mitochondrial biogenesis (Table I).

Inactivation

As mentioned above, yeast PSC may inactivate during application of steady potentials. In some planar bilayer records, one may also observe an inactivation in two steps of mammalian PSC during long application of voltages of either polarity, but this is rare and the study of inactivation was entirely carried out on yeast channels using protocols similar to those which were used for the study of VDAC voltage dependence (5 mHz voltage sawtooth) (Wunder and Colombini, 1991). In fact, similarities in the characteristics of inactivation between VDAC and PSC make the distinction between the two channels difficult if the selectivity is not determined (Thieffry *et al.*, 1994). The reduced form of mast cell degranulating peptide (rMCD), increases the rate of inactivation irreversibly at concentrations as low as 0.2 μM (Pelleschi *et al.*, in preparation). The peptide is active on both sides. However, its application to one compartment accelerates only the inactivation that occurs when the compartment to

which rMCD is added is positive with respect to the other one. Channels pretreated by trypsin on both faces, which therefore no longer inactivate, remain sensitive to the peptide which then restores a fast inactivation when a positive voltage is applied to the compartment where the peptide is added.

The effect of rMCD on intact or proteolyzed PSC persists after washing the peptide out. Like the natural inactivation, the rMCD-induced inactivation is suppressed by trypsin applied to the side of the channel that has been exposed to the peptide.

PSC or PSCs?

The results obtained with peptides inducing the irreversible modifications described above indicate that PSC or associated molecules carry binding sides on both faces of the channel. Channels treated by protease before or after exposure to the peptide remained equally sensitive to peptide application. Trypsin thus interrupts the interaction by acting on the peptide and not on the channel itself.

The irreversible effects of basic peptides on PSC are similar to the effect of the modulator on VDAC (Holden and Colombini, 1988; Liu and Colombini, 1992). Together with the similarities mentioned above, they suggest that PSC, though different from VDAC, might be a cationic porin. In bacteria, several types of porins are present in the same membrane (Nikaido, 1994). At the present time, we do not know if the variability in PSC characteristics reflects the existence of different molecular channel structures. However, in view of the irreversible effects observed, it appears likely that this variability rather results from strong interactions between cytosolic or intermembrane molecules with only one type of cationic porin, interactions which could *in vivo* modulate the flow of molecules through the channel.

IS THE PSC INVOLVED IN PROTEIN TRANSLOCATION?

Two possible roles might be assigned to the PSC in mitochondria. In the first hypothesis, this channel could be the cationic counterpart of the VDAC. The PSC would allow the transport of positively charged molecules into the mitochondria as VDAC does for anionic components. Import of basic peptides would not necessarily be physiologically relevant to protein import and would be only an *in vitro* property reflecting the ability of the pore to translocate positively charged components of low molecular weight. In the second hypothesis, the PSC would be related to the protein translocation machinery of the mitochondrial outer membrane where it would act as a protein-conducting channel. In the latter case, known elements of the translocation machinery should be associated with PSC. We shall discuss this hypothesis in the following section.

Basic peptides active on the PSC, either reversibly or irreversibly, are derived from mitochondrial presequences or from peptides and proteins unrelated to mitochondrial physiology such as dynorphin B or pAT III. Peptides of the latter class have, however, some of the features characterizing mitochondrial presequences such as a high content of basic and hydrophobic amino acids and the absence of acidic residues. Several studies have shown that all presequences interact with common elements of the translocation machinery and that, as a result, the addition of a synthetic mitochondrial presequence of a given preprotein inhibits the *in vitro* import of other mitochondrial proteins with different presequences (Glaser and Cumsky, 1990). We have tested the effect of basic peptides active on PSC on the import into yeast mitochondria of a preprotein, the cyt b_2-DHFR precursor (yeast cytochrome b_2 fused to the N-terminus of rat dihydrofolate reductase). This chimeric protein is imported through the conservative pathway to the matrix prior to being exported to the intermembrane space (Rassow *et al.*, 1989). Peptides were used at concentrations allowing maximal block of the PSC. Saturating concentrations of peptides corresponding to presequences inhibit precursor import whereas peptides of the second class, such as dynorphin B, have no effect (Vallette *et al.*, 1994). In addition, the import of ^{125}I-labeled pCyt OX IV (1–12)Y is not inhibited by an excess of peptides of the second class. These results suggest the existence of two pathways for peptide import. The first one, which is probably similar to that followed by inner membrane and matrix preproteins, would be used by presequences. The second one, leading to the intermembrane space, would be used by nonpresequence peptides and some proteins such as cytochrome *c* heme lyase (Lill *et al.*, 1992). As discussed above, the second pathway is likely to involve the PSC.

The current view on protein import indicates that translocation across outer and inner membranes can be dissociated (Segui-Real *et al.*, 1993) and that upon translocation a dynamic contact site is probably consti-

tuted (Pfanner *et al.*, 1992). In addition, it has been shown that the outer membrane translocation apparatus exists as a multi-subunit complex in *Neurospora crassa* and *Saccharomyces cerevisiae* mitochondria (for a review see Kiebler *et al.*, 1993). The complex contains at least three components partially exposed to the cytosol, MOM 19/ Mas 20, MOM 22/ Mas 22, and MOM 72/ Mas 70 (mitochondrial outer membrane proteins of 19, 22, and 72 kDa in *Neurospora crassa* and mitochondrial assembly proteins of 20, 22, and 70 kDa in *Saccharomyces cerevisiae*) which function as receptors for different subsets of mitochondrial proteins. Several other proteins such as MOM 38/ ISP 42 (import site protein of 42 kDa) are parts of a general insertion pore (GIP) and, therefore, are likely to be components of the putative protein-conducting channel. According to this view, discrimination between peptides of the second class and presequences might occur at the level of the different receptors located on the outer membrane cytosolic side. This hypothesis is consistent with the observation that the imports of pCyt OX IV (1–12)Y and dynorphin B are differently affected by a mild trypsin treatment that cleaves the receptors for protein precursors (Vallette *et al.*, 1994). It is also supported by the recent finding that, after such a treatment, import of ^{125}I-labeled pCyt OX IV (1–12)Y is inhibited by an excess of dynorphin B, thus showing that proteolysis abolishes the discrimination between presequences and peptides of the second class (Juin *et al.*, 1995b). The translocation of both classes of peptides (and of proteins) could therefore occur at the level of a common channel.

It is thus tempting to speculate that GIP, the aqueous protein-conducting pore of the outer membrane translocation machinery, is the PSC or a part of it. That PSC belongs to the import complex is supported by recent immunodepletion experiments showing that the frequency of observation of PSC is decreased in fractions solubilized, incubated in the presence of an anti-ISP 42 antibody, and reconstituted (unpublished results). Other experiments in progress also suggest an association of the PSC with the outer membrane protein import machinery (Juin *et al.*, 1995b). However, the direct demonstration of the PSC involvement in protein translocation will require further experiments such as the reconstitution of the PSC activity from purified components of the protein import machinery.

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REFERENCES

Chich, J. F., Goldschmidt, D., Henry, J. P., and Thieffry, M. (1991). *Eur. J. Biochem.* **196**, 29–35.
Colombini, M. (1979). *Nature* **279**, 643–645.
Dihanich, M., Schmid, A., Oppliger, W., and Benz, R. (1989). *Eur. J. Biochem.* **181**, 703–708.
Ehrlich, B. E. (1992). *Methods Enzymol.* **207**, 463–470.
Fèvre, F., Chich, J. F., Lauquin, G. J. M., Henry, J. P., and Thieffry, M. (1990). *FEBS Lett.* **262**, 201–204.
Fèvre, F., Henry, J. P., and Thieffry, M. (1994). *Biophys. J.* **66**, 1887–1894.
Glaser, S. M., and Cumsky, M. G. (1990). *J. Biol. Chem.* **265**, 8808–8816.
Henry, J. P., Chich, J. F., Goldschmidt, D., and Thieffry, M. (1989). *J. Membr. Biol.* **112**, 139–147.
Holden, M. J., and Colombini, M. (1988). *FEBS Lett.* **241**, 105–109.
Juin, P., Pelleschi, M., Sagné, C., Henry, J. P., Thieffry, M., and Vallette, F. M. (1995a). *Biochem. Biophys. Res. Commun.* **211**, 92–99.
Juin, P., Pelleschi, M., Thieffry, M., Henry, J. P., and Vallette, F. M. (1995b). *Eur. J. Cell Biol.*, ECBO Meeting Supplement Volume, A238.
Kiebler, M., Becker, K., Pfanner, N., and Neupert, W. (1993). *J. Membr. Biol.* **135**, 191–207.
Lill, R., Stuart, R. A., Drygas, M. E., Nargang, F. E., and Neupert, W. (1992). *EMBO J.* **11**, 449–456.
Liu, M. Y., and Colombini, M. (1992). *J. Bioenerg. Biomembr.* **24**, 41–46.
Maduke, M., and Roise, D. (1993). *Science* **260**, 364–367.
Michedja, J., Guo, X. J., and Lauquin, G. J. M. (1989). In *Anion Carriers of Mitochondrial Membranes* (Azzi, A., ed.), Springer, Berlin, pp. 225–235.
Mueller, P., Rudin, D. O., Tien, H. T., and Wescott, W. C. (1963). *J. Phys. Chem.* **67**, 534–535.
Nikaido, H. (1994). *J. Biol. Chem.* **269**, 3905–3908.
Ono, H., and Tuboi, S. (1988). *J. Biol. Chem.* **263**, 3188–3193.
Pattus, F., Rothen, C., Streit, M., and Zalher, P. (1981). *Biochim. Biophys. Acta* **647**, 29–39.
Pfanner, N., Rassow, J., Vanderklei, I. J., and Neupert, W. (1992). *Cell* **68**, 999–1002.
Rassow, J., Guiard, B., Wienhues, U., Herzog, V., Hartl, F. U., and Neupert, W. (1989). *J. Cell Biol.* **109**, 1421–1428.
Segui-Real, B., Kispal, G., Lill, R., and Neupert, W. (1993). *EMBO J.* **12**, 2211–2218.
Tedeschi, H., Mannella, C. A., and Bowman, C. L. (1987). *J. Membr. Biol.* **97**, 21–29.
Thieffry, M., Chich, J. F., Goldschmidt, D., and Henry, J. P. (1988). *EMBO J.* **7**, 1449–1454.
Thieffry, M., Neyton, J., Pelleschi, M., Fèvre, F., and Henry, J. P. (1992). *Biophys. J.* **63**, 333–339.
Thieffry, M., Fèvre, F., Pelleschi, M., and Henry, J. P. (1994). In *Molecular Biology of Mitochondrial Transport Systems* (Forte, M., and Colombini, M., eds.), Springer-Verlag, Berlin, Heidelberg, pp. 209–219.
Vallette, F. M., Juin, P., Pelleschi, M., and Henry, J. P. (1994). *J. Biol. Chem.* **269**, 13367–13374.
Woodhull, A. M. (1973). *J. Gen. Physiol.* **61**, 687–708.
Wunder, U. R., and Colombini, M. (1991). *J. Membr. Biol.* **123**, 83–91.
Zoratti, M., Szabo, I., Bathori, G., Starc, T., Wolff, D., and Schatz, G. (1994). *Biophys. J.* **66**, A22.