Prokaryotic and Eukaryotic Porins: Comparison of Structure and Function

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1 Introduction

1.1 Origin of Bacteria and Mitochondria

The earth formed approximately 4.5 billion years ago. What happened afterward until the oldest known fossils formed about 3.7 billion years ago is still a mystery (Garwood 2012; Nutman et al. 2016). The most accepted hypothesis is that a variety of small molecules were formed from atoms or primitive molecules under the input of energy provided by volcanism, lightnings, fall of meteorites, and high pressure similar as shown in the famous Miller–Urey experiment (Miller 1953; Osinski et al. 2020; Takeuchi et al. 2020). Within such a primordial soup of organic chemicals, cell-like particles may have formed. These cell-like particles represent definitely the origin of life on earth starting more than 4 billion years ago in the Hadean eon (Garwood 2012; Takeuchi et al. 2020). The organisms that originally formed did not leave any evidence of their existence. The oldest indication for life on earth is sedimentary formations of layered structures produced by microbial communities known as stromatolites (Margulis et al. 1986; Allwood et al. 2009; Nutman et al. 2016). The oldest stromatolites are on average about 3.5 billion years old with examples from the Isua supracrustal belt of southwest Greenland that are about 200 million years older (Allwood et al. 2009; Nutman et al. 2016). Stromatolites are sheet-like sedimentary rocks created by the layered growth of photosynthetic microorganisms, such as cyanobacteria, but they also demonstrate the occurrence of diverse and active microbial communities (Gérard et al. 2009). Stromatolites are

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the oldest indication for existence of photosynthesis (Awramik 1992). Communities originally present in the layered structures of stromatolites contained also gram-negative bacteria, because two membranes surround cyanobacteria and phototrophic bacteria (Hansel et al. 1998; Gérard et al. 2009; Bryant and Frigaard 2006). It is very likely that in the outer membrane of these bacteria permeability pathways existed that can be considered as ancestors of porins from modern gram-negative bacteria.

About 4 billion years ago existed another single-cell bacteria like organism, which was designated as the Last Universal Common Ancestor (LUCA) before the bacterial cell lineage divided into different kingdoms (Theobald 2010; Di Giulio 2011; McInerney 2016; Weiss et al. 2016). This organism was identified by comparing the genomes of all its putative descendants of modern time. Around 355 genes were identified, which could have been present in this LUCA (Weiss et al. 2016; Michael 2017). The genes describe a complex life form with many coadapted features, including energy metabolism, the synthesis of amino acids, and the machinery of transcription and translation to allow protein synthesis (Chioccioli et al. 2020; Koonin et al. 2020). It has been proposed that the LUCA may have lived in the deep sea volcanic activity in or near deep sea vents and used presumably chemical components from its environment for the generation of cellular energy (Weiss et al. 2016). However, another view of early life and LUCA suggested that life evolved in ponds of different salinity and pH and in an N_2–CO_2 atmosphere. Direct sunlight and UV light below 200 nm combined with dry–wet cycles might have been very important for the development of life and the LUCA (Sasselov et al. 2020). Subsequently, LUCA divided in the three great superkingdoms or domains of present life on earth: Bacteria, Eukaryota, and Archaea.

The origin of the first eukaryotic cell is still puzzling. Its lineage could be followed from Bacteria and Archaea being probably genomic hybrids of both of them (Hedges 2002; Katz 2012; Koonin 2015). However, as the genomes of many eukaryotic cells, in particular, those of model organisms became known during the last two decades, a similar concept as described above for the LUCA could be introduced in the Last Eukaryotic Common Ancestor (LECA) (Hedges 2002; Grau-Bové et al. 2015; O'Malley et al. 2019). Nevertheless, special eukaryotic features, such as the nucleus and certain aspects of the cytoskeleton, are still not completely understood (Katz 2012). The special property of the LECA is the endosymbiosis of certain gram-negative bacteria between about one and 2 billions of years ago (Margulis 1981). Mitochondria and chloroplasts are descendants from specialist gram-negative bacteria, such as proteobacteria and cyanobacteria that were incorporated into the cytoplasm of the LECA (Gray 2012; Degli 2014; Martijn et al. 2018; Nowack and Weber 2018). This process provided a considerable advantage for the eukaryotic host, because its anaerobic metabolism was complemented by cellular respiration. Similarly, the input of metabolic energy through photosynthesis of sun light allowed the eukaryotic host a considerable evolutionary advantage because it is the prerequisite for plants on terrestrial grounds (Nowack and Weber 2018). As descendants of gram-negative bacteria, mitochondria and chloroplasts have two membranes surrounding the cytoplasm with a special function of the outer membrane similar to that of their endosymbionts.

2 Bacterial Porins

2.1 *Special Features of the Outer Membrane of Gram-Negative Bacteria*

The cell envelope of gram-negative bacteria consists of three different layers, the outer membrane, the peptidoglycan layer, and the inner membrane (Beveridge 1981). The inner membrane is a phospholipid membrane similar to other cytoplasmic membranes. It represents a diffusion barrier for hydrophilic solutes and contains, similar to the mitochondrial inner membrane, the respiration chain, and a large number of specific transport systems that are energy-driven, such as the uptake systems for maltose and maltooligosaccharides or phosphate in the enteric bacterium *Escherichia coli* (Schneider et al. 2012; Rao and Torriani 1990). The outer membrane of gram-negative bacteria plays an important role in life style and physiology. All hydrophilic or hydrophobic solutes including antibiotics that should enter bacteria have to cross this permeability barrier, which means that it acts as a molecular filter. The passive molecular sieving properties of the outer membrane are due to presence of a few major proteins called "porins" (Nakae 1976). The porins are often organized as trimers of three identical subunits and form transmembrane channels that have more general properties, which means that they sort mainly according to the molecular mass of the solutes and not by the structure of the solutes (Benz 1994a; Nikaido 2003). The outer membrane of *Escherichia coli* contains about 10^5 copies of the major outer membrane proteins OmpF and OmpC, which are both general diffusion porins (Steven et al. 1977; Lugtenberg and Van Alphen 1983; Benz et al. 1985b). However, besides the general diffusion porins, the outer membrane of gram-negative bacteria contains also specific porins with binding sites for substrates (Nikaido 2003; Benz 2001). These outer membrane channels are often part of a specific uptake system for solutes (Benz and Orlik 2004).

2.2 *Isolation and Purification of Bacterial Porins*

Porin isolation and porin purification start always with mass production of bacteria. The cells are harvested in the late logarithmic phase. They are washed and resuspended in a small volume before they are homogenized using a French pressure cell, ultrasonication, or a glass pearl machine. The pellet of a subsequent centrifugation step contains the cell envelope. The porins of most enteric gram-negative bacteria are tightly associated with the peptidoglycan layer (Nikaido 1983, 2003; Benz 1988, 1994a; Nikaido and Vaara 1985). This allows the rapid Isolation of the porins via the preparation of the peptidoglycan–protein complex. Most components of the cell envelope are soluble in detergents. The insoluble material is composed of murein, lipopolysaccharides, and a few proteins either covalently bound to or associated with the peptidoglycan. The porins can be released by standard methods

either by digestion of the murein or by the salt extraction method (Nikaido 1983). After the release, pure porin may be obtained by column chromatography using gel filtration or affinity chromatography. When porins are not tightly associated with the peptidoglycan layer, it is possible to apply different steps of solubilization of the cell envelopes with increasing concentration of detergents taking profit from the observation that the bacterial outer membrane is normally less soluble in detergent solutions. The last solubilization step contains the porin, as controlled by SDS-PAGE. This protein fraction could also be applied to affinity chromatography or gel filtration for final porin purification.

3 Study of Bacterial Porin Function

3.1 General Diffusion Pores

Several different methods have successfully been used to reconstitute porin pores into model membrane systems to study their pore properties (Benz et al. 1978; Schindler and Rosenbusch 1978; Nikaido and Rosenberg 1983). The planar lipid bilayer technique has widely been used for the study of channel properties including that of the porins. The lipid bilayer can be formed from lipid solutions in organic solvent across holes (Benz et al. 1978). Alternatively, solvent-depleted lipid bilayers across small holes (diameter < 50 μm) in a Teflon foil may be formed from lipid monolayers with the help of pretreatment of the hole with hexadecane (Schindler and Rosenbusch 1978). The addition of porins to the aqueous phase bathing black lipid bilayer membranes formed by one of these techniques is the simplest reconstitution method. Figure 1 shows a typical reconstitution experiment of this type. The major outer membrane porin OmpF of *Escherichia coli* was added in final concentration of about 10 ng/mL to the aqueous phase bathing a lipid bilayer membrane from diphytanoyl phosphatidylcholine/*n*-decane, which is widely used as a lipid for the formation of bilayers (Janko and Benz 1977). The addition of the porin resulted in a stepwise increase of the membrane conductance. The steps were specific for the

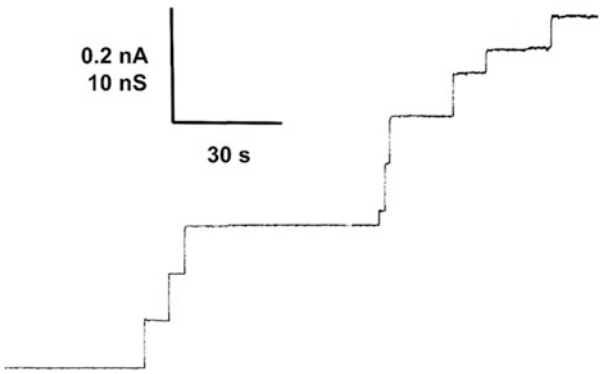

Fig. 1 Stepwise increase of membrane current in the presence of 10 ng/mL OmpF porin of *E. coli* in a 1 M KCl solution (pH 6). The membrane was formed from diphytanoyl phosphatidylcholine/*n*-decane; $T = 20$ °C, $V_m = 20$ mV

Table 1 Single-channel conductance and zero-current membrane potentials V and of different *E. coli* porins in the presence of tenfold KCl gradients

Porin	G (nS)	V_m (mV)	P_{canion}/P_{anion}	Specificity	References
General diffusion porins					
OmpF	4.5	27	3.9	General, cations	Chimerel et al. (2008) and Benz et al. (1985a)
OmpC	2.8	50	26	General, cations	Biró et al. (2010) and Benz et al. (1985a)
PhoE	1.8	−24	0.30	General, anions	Benz et al. (1985a)
K	1.5	46	7.6	General, cations	Benz et al. (1985a)
Lc (HK523)	2.5	44	12	General, cations	Verhoef et al. (1987)
NmpC	1.3	−26	0.27	General, anions	Benz et al. (1985a)
RafY	2.9	20	2.7	General, cations	Andersen et al. (1998)
Specific porins					
LamB	0.16	51	28	Specific, maltose and maltodextrins	Benz et al. (1987)
ScrY	0.70	39	8.6	Specific, sucrose	Schülein et al. (1991), Schülein et al. (1995), and Kim et al. (2002)
BglH	0.56	–	Cation-selective	Specific, beta-glucosides	Andersen et al. (1999)
Tsx	0.010	28	4.2	Specific, nucleosides	Benz et al. (1988a)
TolC	0.080	42	10	Type 1 export (T1SS)	Benz et al. (1993b) and Andersen et al. (2002)

The potential corresponds to the potential at the more dilute side of the membrane. P_{canion}/P_{anion} was calculated using the Goldman–Hodgkin–Katz equation (Benz et al. 1979). The data were taken from references as indicated

presence of the porins and were absent when only detergents were present in the aqueous phase.

Many different ions and other small hydrophilic solutes permeate through the general diffusion pores of the bacterial outer membranes, which is quite understandable because of the large diameter of these general diffusion porins with an exclusion limit for hydrophilic solutes of less than 600 Da (Benz et al. 1979; Nakae 1976; Weiss et al. 1990; Cowan et al. 1992; Baslé et al. 2006). Subsequently, the single-channel conductance of many general diffusion porin pores was approximately a linear function of the specific conductance σ of the bulk aqueous phase as has been shown previously (Benz et al. 1979). Table 1 shows the single-channel conductance of some general diffusion porins and specific porins of *E. coli*.

Ions move through the wide general diffusion pores similar to the way they move in the bulk aqueous phase (Benz et al. 1979). Nevertheless, the porin pores exhibit a certain specificity for charged solutes that can be detected in vivo and in vitro experiments (Nikaido et al. 1983; Nikaido and Rosenberg 1983; Benz et al. 1985b).

Electrophysiology allows also the evaluation of the ionic selectivity by measuring the membrane potential under zero-current conditions. From the measured V_m and the concentration gradient c''/c' across the membrane, the ratio P_{cation}/P_{anion} of the permeability was calculated using the Goldman–Hodgkin–Katz equation (Benz et al. 1979, 1985b). Table 1 shows also the zero-current membrane potentials for different porins of *E. coli.* OmpF and OmpC are present in the strain K-12. Their expression is regulated by the osmolarity of the growth media via the EnvZ/OmpR two-component signaling (Kenney and Anand 2020). PhoE is induced in *E. coli* under the conditions of phosphate starvation (Tommassen and Lugtenberg 1980). NmpC appears in revertants of porin-deficient mutants, whereas porin K was found in *E. coli* strains that form capsules (Benz et al. 1985b). Lc(HK523) is a general diffusion porin found in *E. coli* strains that are highly susceptible to the phage KH523 (Verhoef et al. 1987), and the gene coding for RafY is present on the plasmid pRSD2, which enables *E. coli* to grow on raffinose but is a general diffusion pore and not a specific porin (Andersen et al. 1998). It has to be noted that the selectivity of all these porins is not an absolute one. This means that the permeability ratio P_{cation}/P_{anion} is dependent on the mobility sequence of the ions in the aqueous phase and is generally larger for KCH_3COO than it is for KCl in cation-selective channels. Similarly, the permeability ratio for anion-selective channels is larger for LiCl than it is for KCH_3COO because of the higher mobility of chloride over acetate in the aqueous phase (Benz et al. 1985b).

3.2 *Properties of Specific Porins*

The general diffusion pores are wide water-filled channels. They sort between different substrates mostly according to the molecular mass and to a smaller extent according to the charge of the solutes. The outer membrane of certain gram-negative bacteria contains, besides one or several general diffusion pores, channels that are specific for one class of solutes. These specific porins exhibit completely different properties than the general pores because they contain a binding site for substrates inside the pore, which represents a considerable advantage for the efficient scavenging of substrates (Benz et al. 1987). Similarly, they have a much smaller single-channel conductance than the general diffusion porins because of the closely spaced binding site inside the channel (see Table 1). It has to be noted that specific porins are often induced in gram-negative bacteria when the organisms are grown under special growth conditions. Very often, they are expressed together with an energy-driven inner membrane uptake system such as the mal system for the uptake of carbohydrates or the pst systems for the uptake of phosphate (Bordignon et al. 2010; Mächtel et al. 2019; Nikata et al. 1996; Rico-Jiménez et al. 2016). A completely different approach has to be used for the study of their channel properties, because the substrates for these specific porins are often neutral solutes, which means that simple conductance measurements are not suited to study the channel characteristics. Here we discuss only the carbohydrate-specific porins of *E. coli* (Luckey and Nikaido

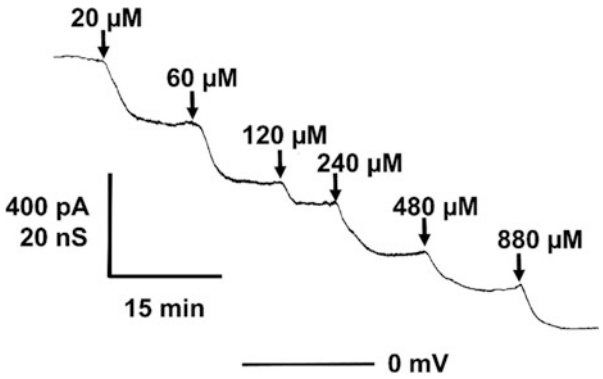

Fig. 2 Titration of LamB-induced membrane conductance with maltotetraose. The aqueous phase contained 50 ng/mL LamB, 1 M KCI, and maltotetraose at the concentrations shown at the top of the figure. The membrane was formed from diphytanoyl phosphatidylcholine/*n*-decane; $T = 25\ ^{\circ}C$, $V_m = 20$ mV. 0 mV indicates the baseline of the experiment at zero voltage

1980; Benz et al. 1986; Schülein et al. 1991; Andersen et al. 1999) and the phosphate starvation inducible porins OprP and OprO of *Pseudomonas aeruginosa* in some detail (Hancock et al. 1982; Siehnel et al. 1992). The properties of specific porins from *E. coli* are listed in Table 1. LamB is specific for maltose and maltooligosaccharides (Benz et al. 1986; Charbit 2003). Scry of *E. coli* is homologous to LamB, but some mutations within the binding site make it specific for sucrose (Kim et al. 2002). The gene coding for BglH in *E. coli* is cryptic but in analogy to bglH of the plant pathogen *Erwinia chrysanthemi*, it is a porin specific for beta-gucosides (El Hassouni et al. 1992; Andersen et al. 1999). Tsx is a monomeric porin that is involved in nucleoside uptake in *E. coli* (Maier et al. 1988; Bremer et al. 1990). Trimers of TolC of *E. coli* and a large number of similar proteins from gram-negative bacteria form a single channel that is involved in Type 1 secretion of proteins and drugs (T1SS) (Benz et al. 1993b; Koronakis et al. 2000).

The presence of maltose and maltooligosaccharides in the growth media of *E. coli* results in the expression of a number of different proteins located in either the inner membrane, the periplasmic space, or the outer membrane (Boos and Shuman 1998). LamB, the protein induced in the outer membrane, is the receptor for phage Lambda and plays an important role for the uptake of maltose. LamB forms small ion-permeable channels in lipid bilayer membranes (see Table 1) (Benz et al. 1986). The binding of carbohydrates to LamB can be studied in experiments with many LamB channels reconstituted in a lipid bilayer membranes. When the reconstitution process saturates, increasing concentrations of carbohydrates were added to the aqueous phase as shown in Fig. 2 for the addition of maltotetraose to a membrane containing about 350 LamB channels. The membrane conductance decreased as a function of the maltotetraose concentration. The data of Fig. 2 (and of similar experiments with other carbohydrates) can be analyzed using a one-site two-barrier model for the carbohydrate transport through LamB (Benz et al. 1987). The conductance $G(c)$ (maximum conductance G_{max} in the absence of substrates) of the

Table 2 Stability constants, K, for the binding of different carbohydrates to the carbohydrate-specific channels of *E. coli*

Carbohydrate	LamB	ScrY	BglH
	K (L/mol)		
Glucose	9.5	8.3	16
Maltose	100	150	50
Maltotriose	2500	550	18
Maltotetraose	10,000	910	n.m.
Maltopentaose	17,000	3300	31
Maltohexaose	15,000	4800	n.m.
Maltoheptaose	15,000	4800	16
Raffinose	46	640	35
Fructose	1.7	1.9	n.m.
Galactose	24	5.3	n.m.
Arbutin	78	n.m.	700
Salicin	97	n.m.	350
Gentiobiose	250	n.m.	245
Cellobiose	6.7	n.m.	21
Cellopentaose	96	58[d]	120

The stability constants for carbohydrate binding to LamB, ScrY, and BglH were taken from Benz et al. (1987), Schülein et al. (1991), and Andersen et al. (1999), respectively

The membranes were formed from diphytanoyl phosphatidylcholine/*n*-decane. The unbuffered aqueous solutions (pH around 6) contained about 199 ng/mL of the porins and 1 M KCl; $T = 25\ ^{\circ}C$; $V_m = 20$ mV. The stability constant, K, is given as the mean of at least three titration experiments similar to that shown in Fig. 2. n.m. means not measured

channel at a given carbohydrate concentration c is given by the probability that the binding site is free:

$$G(c) = \frac{G_{\max}}{(1 + Kc)} \tag{1}$$

where K is the stability constant for the binding of the carbohydrate to the binding site. This means that the titration curve can be analyzed using a Langmuir adsorption isotherm or a Lineweaver–Burk plot (Benz et al. 1986). For the data of Fig. 2, a stability constant K of 9600 L/mol could be calculated corresponding to a half saturation constant of 0.1 mM. In similar experiments, the binding of nucleosides to the nucleoside-specific Tsx protein of E. *coli* (Maier et al. 1988) and the binding of phosphate to the phosphate-specific protein P of *P. aeruginosa* (Hancock and Benz 1986; Benz and Hancock 1987) were determined. The binding sites inside the specific porins lead to a saturation of the substrate flow at high substrate concentration (Benz et al. 1987).

Binding properties of different carbohydrates to the specific porins of *E. coli* are shown in Table 2. The data demonstrate that LamB has an interesting selectivity for long chain maltooligosaccharides although the channel size is much smaller than that

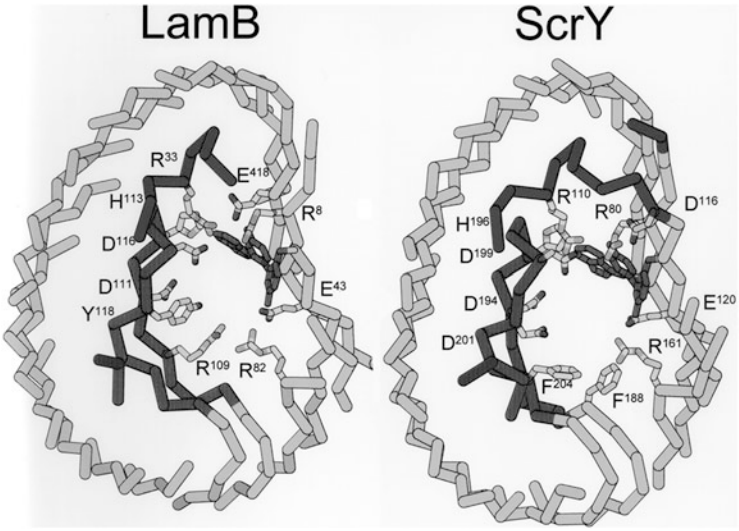

Fig. 3 Cross sections of the *E. coli* LamB monomer (maltoporin) and the ScrY monomer (sucroseporin). The Figure shows loop 3 (dark gray) and the amino acid residues (denoted with their numbers from the mature N-terminal end) that are relevant for passage of carbohydrates and ions through the central constriction of the two porins. The strands of the β-barrel cylinder of the porins are given in light gray. The coordinates of maltoporin were taken from the crystallographic data of Schirmer et al. (1995) (PDB id: 1MAL) and that of sucroseporin from Forst et al. (1998) (PDB id: 1A0S)

of OmpF. On the other hand, LamB is an inefficient channel for the uptake of sucrose (Luckey and Nikaido 1980; Andersen et al. 1995). However, a few amino acid mutations transform LamB in the highly homologous ScrY channel that is able to transport sucrose at much higher rate than maltoporin (Andersen et al. 1995; Kim et al. 2002). Figure 3 shows a cross section of the two carbohydrate-specific porins. The constriction of ScrY for the passage of solutes is indeed much larger than that of LamB, which is also the reason for the lower conductance of the latter (see Table 1).

Another outer membrane channel of the LamB type is BglH, which is specific for beta-glucosides (see Table 2). LamB is again not very well suited for the transport of these carbohydrates, but mutations in the central binding site allow transport at much higher rate through BglH (Andersen et al. 1999). Homologues of LamB are found in many gram-negative bacteria, in particular in *Enterobacteriaceae* (Lun et al. 2014).

Pseudomonas aeruginosa is one of the major causes responsible for hospital-acquired infections and chronic lung infections in cystic fibrosis patients (Pachori et al. 2019; Malhotra et al. 2019). The organism shows a high resistance toward many antibiotics (Lambert 2002; Nikaido 2003; Gellatly and Hancock 2013; Chevalier et al. 2017). One of the reasons for this resistance is the lack of a large number of general diffusion porins in the outer membrane. Instead, it contains substrate-specific channels of the OprD family dependent on growth conditions, which provide highly controlled solute transport across the outer membrane

Table 3 Single-channel conductance of OprP and OprO of *Pseudomonas aeruginosa* in 0.1 M KCl and 1 M KCl solution buffered with 10 mM MES, pH 6

Porins	Single-channel conductance *G*(pS)		Phosphate binding	Diphosphate binding
	0.1 M KCl	1 M KCl	K (L/mol)	K (L/mol)
OprP	160	260	770	310
OprO	240	440	220	1450

Monophosphate- and diphosphate-mediated inhibition of chloride conductance of OprP and OprO in 0.1 M KCl, 10 mM MES, pH 6, at an applied voltage of 50 mV at room temperature. The stability constants for chloride conductance inhibition were measured in titration experiments similar to that of Fig. 2. They represent means of three experiments and were taken together with the single-channel data from Ganguly et al. (2017)

(Sugawara and Nikaido 1994; Hancock and Brinkman 2002; Tamber and Hancock 2003; Sugawara et al. 2012). Type 1 efflux pumps (T1SS) support the high resistance of *P. aeruginosa* for antibiotics, such as MexAB of the inner membrane in combination with OprM, which is a TolC-like outer membrane channel (Hancock and Brinkman 2002; Chevalier et al. 2017). Phosphate starvation leads to the expression of two homologous outer membrane porins, OprP and OprO that are specific for phosphate and pyrophosphate, respectively (Hancock et al. 1982; Siehnel et al. 1992). The properties of these specific porins can be measured in single-channel and titration experiments similar to those shown above for OmpF (Fig. 1) and LamB (Fig. 2). The results of these measurements are given in Table 3. The single-channel conductance for OprP and OprO in KCl of different concentration saturates at high concentration indicating the presence of a binding site for phosphate (Benz and Hancock 1987; Benz et al. 1993a; Hancock et al. 1992). Addition of phosphate and diphosphate blocks ion (chloride) transport through both specific porins because they are single-file channels similar to the specific porins of the LamB-family, which means that no solute can pass the OprP and OprO channels when the binding site is occupied by phosphate or diphosphate, respectively. The stability constant for the block shows clearly the preference of the channels for either phosphate or diphosphate (Ganguly et al. 2017). Again, only a few amino acids are exchanged inside the binding sites of the two channels to create the specificity for phosphate or diphosphate.

4 Structure of General Diffusion and Specific Porins

Porins from the bacterial outer membrane are almost exclusively formed by β-barrel cylinders. This has presumably to do with the synthesis and translation of the porins. They are synthesized in the cytosol and posttranslationally transported by the *sec* system to the periplasmic space. Integration and assembly of the β-barrel proteins into the bacterial outer membrane are performed by their interaction with the β-barrel assembly machinery (BAM) of the outer membrane (Doyle and Bernstein 2019). The important part of this machinery is an outer membrane protein BamA (also known as Omp85), which acts together four lipoproteins—BamB, C, D, and E (Hart

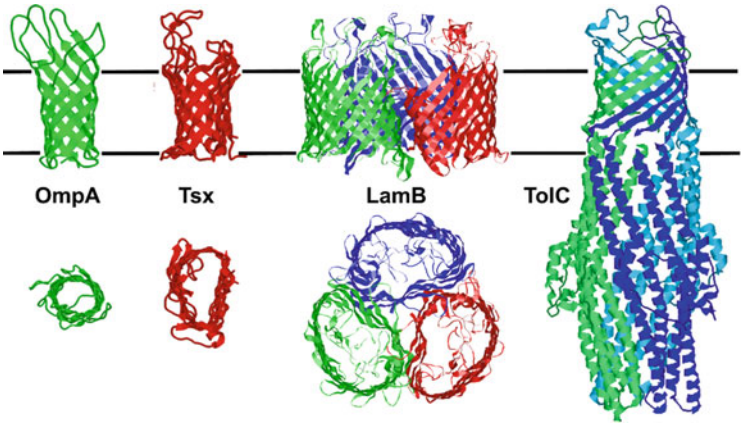

Fig. 4 Bacterial outer membrane proteins. The proteins have usually a β-barrel cylindrical structure with long external loops and short periplasmic turns. The 3D structure of the proteins is shown in direction to the cell surface (up) and the periplasmic space (down). The lower structures show the view on the proteins from the cell surface. OmpA (PDB code: 1BXW) refers to the membrane-spanning part of the protein (Pautsch and Schulz 2000). It is not an outer membrane channel but represents a membrane anchor. Tsx (PDB code: 1TLY) is a substrate-specific porin for nucleosides of *E. coli* (Maier et al. 1988; Ye and van den Berg 2004). LamB (PDB code: 1MAL) is a carbohydrate-specific porin (Schirmer et al. 1995). TolC (PDB code: 1EK9) is an outer membrane channel composed of three subunits forming a single pore that together with an inner membrane transport system and adaptor proteins form efflux pump for drugs and secretion systems for proteins (T1SS) (Koronakis et al. 2000; Masi and Wandersman 2010)

et al. 2020). BamA is also a β-barrel protein. Members of the BamA-Omp85 superfamily of protein have also an essential function in the assembly of outer membrane proteins of mitochondria, plastids, and chloroplast (Ulrich and Rapaport 2015; Ranava et al. 2018; Ganesan and Theg 2019). In the case of bacteria, the interaction of BamA with the outer membrane protein to be newly assembled into the OM is not fully understood, but it seems that the first β-strand of the open configuration of BamA plays an important role in the assembly of the outer membrane proteins (Doyle and Bernstein 2019). Similarly, the interior surface of the BamA cylinder plays also an important role in this process.

The β-barrel cylinders of outer membrane porins are formed by amphipathic β-strands, which means that on average each second amino acid is either hydrophilic or hydrophobic. The hydrophobic amino acids are preferentially localized toward the lipid and LPS side chains. The hydrophilic amino acids are directed to the interior of the β-barrel cylinder. Ten amino acids in a β-strand are sufficient to cross a lipid bilayer, whereas 20 amino acids are needed to cross a membrane in the case of hydrophobic or amphipathic α-helices. Figure 4 shows the 3D structure of a variety of outer membrane proteins from *E. coli*. The structures are shown with their cell surface exposed structures directed upward and their periplasmic side downward. Shown is the membrane-localized part of OmpA that comprises eight β-strands that do not conduct ions but represent a membrane anchor (Pautsch and Schulz 2000).

Another form of OmpA is designated as "slow porin" and should have 16 β-strands, but it comprises presumably only a minor fraction of OmpA (Sugawara and Nikaido 1994). Other outer membrane proteins with eight β-strands, such as OmpW of *Caulobacter crescentus*, may serve in contrast to OmpA as outer membrane channel (Benz et al. 2015). The nucleoside-specific channel of *E. coli* and other enteric bacteria has 12 β-strands (see Fig. 4) (Ye and van den Berg 2004). The general diffusion porins of *E. coli* listed in Table 1 are all trimeric of three polypeptide subunits with 16 β-strands (not shown in Fig. 4). Specific porins are also very often trimeric but have 18 β-strands, such as LamB shown in Fig. 4. TolC is a special outer membrane pore because it is a trimer forming one outer membrane pore (12 β-strands) with 12 long α-helical strands extending over the periplasmic space (Koronakis et al. 2000). The α-helical cage (see Fig. 4) links the inner membrane part of Type 1 secretion systems (T1SS) with TolC, which results in direct energy-driven export of proteins and drugs from the cytoplasm to the cell surface (Koronakis et al. 2000). Typical for all outer membrane proteins of gram-negative bacteria is the tilting of the β-strands in given angle from the normal of the membrane plain (see Fig. 4).

5 VDAC (Mitochondrial Porins)

5.1 Function of the Mitochondrial Outer Membrane

Mitochondria are the energy-producing cell organelles of eukaryotic cells. In the introduction section, we discussed already the origin of mitochondria. According to the molecular analysis of proteins coded and produced within mitochondria, it is very likely that a strictly aerobic and presumably gram-negative bacterium from the α-proteobacterial lineage is the ancestor of mitochondria. This means that the precursor of the mitochondrial outer membrane could be the outer bacterial membrane and its filter properties were transferred during evolution to the mitochondrial outer membrane pores. Nevertheless, there exist differences. In particular, mitochondria have generally small circular genomes like bacteria, but they do not contain enough genes needed to code for its whole proteome. This means that since the event of endosymbiosis, which occurred around 1–2 billion ago, many mitochondrial genes came under the control of the nucleus, including all genes coding for proteins of mitochondrial outer membrane and the intermembrane space. On the other hand, mitochondria retain much of their own independent genetic material coding in particular for proteins of the respiration chain exhibiting homology to bacterial respiration chains (Atteia et al. 2004). The reason for this is still a matter of debate.

5.2 Isolation of VDAC

The mitochondrial outer membrane pores observed in *Paramecium* mitochondria were named VDAC (voltage-dependent anion selective channel, also known as mitochondrial porin or eukaryotic porin) because of their voltage dependence and anion selectivity (Schein et al. 1976). Early attempts to isolate and purify the pore-forming protein started always from isolated mitochondria (Roos et al. 1982). Rat liver mitochondria were subfractionated by means of density gradient centrifugation, and a 32 kDa protein was identified as VDAC with similar properties as described for the pore from fractionated *Paramecium* (Schein et al. 1976; Colombini 1979; Roos et al. 1982). Similar pores were found in mitochondria of different organisms using similar or other methods (Zalman et al. 1980; Freitag et al. 1982). The most efficient method to isolate the mitochondrial pore VDAC started from whole mitochondria and used a hydroxyapatite column as an essential step (De Pinto et al. 1987a). The reason for this method is presumably that the mitochondrial pore is deeply buried in the mitochondrial outer membrane. After digestion of the mitochondrial outer membrane with detergent, the protein is also deeply buried in the detergent micelle. As a consequence, the mitochondrial pore is found in the pass-through of the hydroxyapatite column because it does not interact with the column material (De Pinto et al. 1987a). Final purification can be achieved by standard biochemical protocols. Many different species of VDAC have been isolated and purified using this method (De Pinto et al. 1987b; Schmid et al. 1992; Wiesner et al. 1996; More recent studies of VDAC start from its gene since the primary sequence of the mitochondrial pore was known from amino acid sequencing (Kayser et al. 1989). Subsequently, pore-forming proteins from mitochondria of different origin and also from plastids were heterologously expressed in *E. coli* where the protein was found in inclusion bodies (Peng et al. 1992; Fischer et al. 1994; Heins et al. 1994; Popp et al. 1996, 1997; Engelhardt et al. 2007). The mitochondrial pore could be reconstituted from the protein using detergents, lipids, and sterols (Popp et al. 1995, 1996; Engelhardt et al. 2007; Tateda et al. 2011; Mlayeh et al. 2017; Lopes-Rodrigues et al. 2020).

5.3 Reconstitution of the Mitochondrial Porin (VDAC)

The reconstitution of eukaryotic porin in membranes was essentially similar as described above for the bacterial porins. The addition of small amounts of protein results in a conductance increase of many orders of magnitude. After an initial rapid increase for 15–20 min, the membrane conductance increased at a much slower rate. This slow conductance increase continued usually until membrane breakage. When the rate of conductance increase was relatively slow (as compared with the initial one), it was shown for different mitochondrial porins that the membrane conductance was a linear function of the protein concentration (Colombini 1979; Freitag

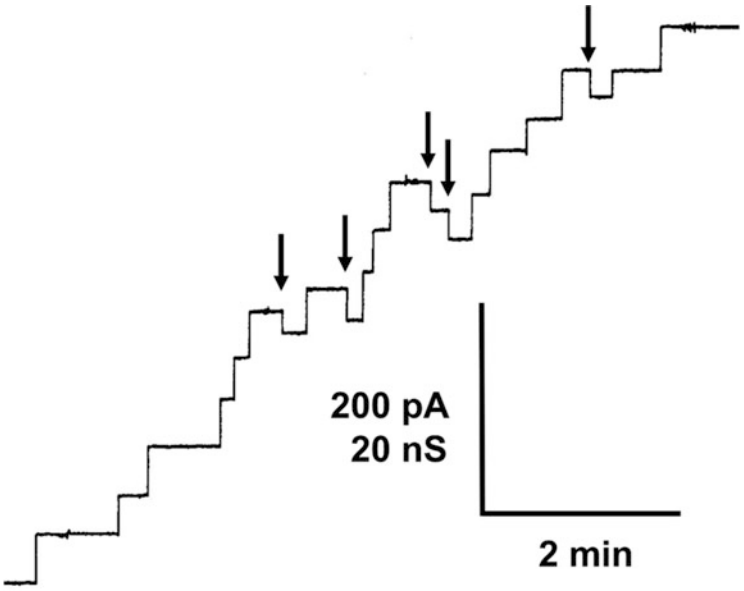

Fig. 5 Current fluctuations observed after the addition of 10 ng/mL Porin 31HL to a 1 M KCl solution bathing a black membrane of diphytanoyl phosphatidylcholine/*n*-decane; $T = 20\ ^{\circ}C$, $V_m = 10$ mV. The arrows indicate closing of pores in subconductance levels

et al. 1982; Roos et al. 1982; Ludwig et al. 1988). The addition of smaller amounts of mitochondrial porin on the order of ng/mL from different eukaryotic cells allowed the resolution of step increases in conductance as shown in Fig. 5 for human VDAC1 (Porin 31HL; Benz et al. 1992).

Most of the conductance steps were directed upward, and closing steps were only rarely observed at small transmembrane potentials of about 10 mV (see Fig. 5). The closing events were very often smaller in size than the onset of the pores (arrows in Fig. 5). This indicated that the eukaryotic pore switched in sublevels of the open pore and did not close completely. The most frequent value for the single-channel conductance of Porin 31HL (hVDAC1) in 1 M KCl was about 4 nS. Most eukaryotic porins studied to date had a similar single-channel conductance under identical conditions (1 M KCl) with the exception of eukaryotic porins from plants (see Table 4). In these cases, very often two conductance levels around 1.5 nS and 3.5 nS in 1 M KCl were observed. Experiments using different salts indicated some anion selectivity of the pore (Roos et al. 1982; Benz 1985; Benz et al. 1992). The single-channel conductance of the eukaryotic porins was approximately a linear function of the bulk aqueous salt concentration (Colombini 1979; Roos et al. 1982). This result indicated that the pore does not contain a binding site for anions inside the channel or a cluster of positive charges, which means that eukaryotic porins form a general diffusion pore in their open state similar to bacterial porins (Benz 1994a).

Table 4 Single-channel conductance of mitochondrial porins (VDACs) from different eukaryotic cells in 1 M KCI, pH 6

Eukaryotic porin (VDAC)	*G* (nS)	References
Neurospora crassa	4.5	Freitag et al. (1982)
Rat liver	4.3	Roos et al. (1982)
Beef heart	4.0	Benz et al. (1985a)
Rabbit liver	4.0	Benz et al. (1985a)
Rat brain	4.0	De Pinto et al. (1987b)
Rat kidney	4.0	De Pinto et al. (1987b)
Pig heart	3.5	De Pinto et al. (1987b)
Yeast	4.2	Ludwig et al. (1988)
Paramecium	4.5 2.4	Colombini (1979) and Ludwig et al. (1989)
Human VDAC1 (Porin 31HL)	4.3	Benz et al. (1992)
Pea mitochondria	1.5 and 3.7	Schmid et al. (1992)
Pea root plastids	1.5 and 3.7	Fischer et al. (1994)
Maize root plastids	1.5 and 3.7	Fischer et al. (1994)
Solanum tuberosum POM 34	2.0 and 3.5	Heins et al. (1994)
Maize mitochondria	1.5 and 3.7	Carbonara et al. (1996)

All pores formed by these proteins were anion selective in their open state (i.e., at small transmembrane voltage)

5.4 Voltage Dependence of VDAC (Eukaryotic Porin)

All mitochondrial porins studied to date were found to be voltage-dependent (Schein et al. 1976; Freitag et al. 1982; Roos et al. 1982; De Pinto et al. 1987b; Benz et al. 1992). The pore conductance is reduced at membrane potentials larger than 20 mV. Figure 4 shows that human porin (hVDAC1) switched already occasionally at 10 mV in subconductance states that were smaller than the open state. The voltage dependence of the mitochondrial porins can be demonstrated on the single-channel level and in multichannel experiments. Figure 5 shows an experiment of the latter type. About 50 hVDAC1 pores were reconstituted in a diphytanoyl phosphatidylcholine/*n*-decane membrane, when the experiment started by application of −10 mV to the *trans*-side of the membrane followed by application of 10 mV. In a next step, −20 mV followed by +20 mV were applied, which resulted already in a more or less symmetrical decrease of the membrane current. Higher voltages with negative and positive sign resulted in an even higher decrease of the current following a voltage step. The decrease saturated at very high voltages near 100 mV. The decay of the membrane current following a voltage step could be described by single exponential functions with decreasing time constant for increasing voltages (Ludwig et al. 1989; Benz 1994b, 2004). It has to be mentioned that the reopening of the mitochondrial pore after the release of the membrane potential was much faster than the closing of the pore and needed presumably only a few ms (Benz 2004).

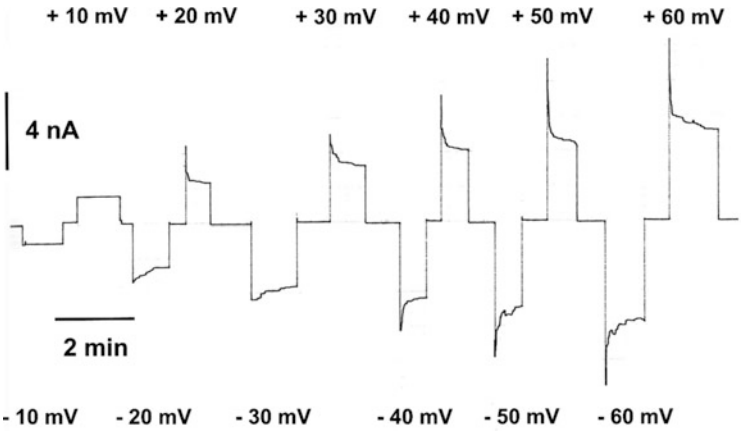

Fig. 6 Relaxation of the membrane current in the presence of hVDAC1 (Porin 31HL). The membrane potential was first switched to −10 mV and then to +10 mV applied to the *trans*-side of the membrane containing about 50 hVDAC1 pores, followed by the application of higher negative and positive voltages. The membrane was formed of diphytanoyl phosphatidylcholine/*n*-decane. The aqueous phase contained 0.5 M KCl (pH 7); $T = 20$ °C

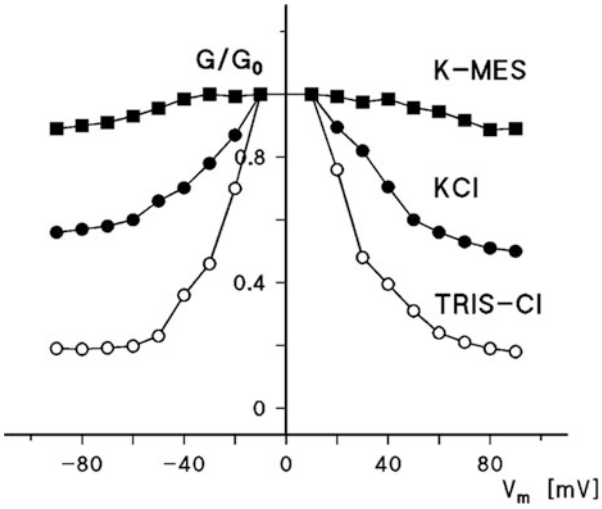

Fig. 7 Ratio of the conductance, G, at a given voltage, V_m, divided by the conductance, G_0, at 10 mV as a function of the voltage. The aqueous phase contained either 0.5 M KCI, 0.5 M K-MES, or 0.5 M TRIS-HCI (pH in all cases 7.2). The *cis*-side contained about 10 ng/mL hVDAC1 [Porin 31 HL (Benz et al. 1992)]. The sign of the voltage is given with respect to the *trans*-side, the side opposite to the addition of Porin 31HL

The steady-state conductance of the experiments shown in Fig. 6 and in similar experiments with other eukaryotic porins showed a bell-shaped curve as a function of the applied voltage when the current at long times after a voltage step was divided by the initial current. Examples are shown in Fig. 7 for hVDAC1 and three different

combinations of anions and cations. The bell-shaped curve could be analyzed as proposed by Schein et al. (1976) by a Boltzmann distribution:

$$N_o/N_c = \exp\left(\frac{nF(V_m - V_0)}{RT}\right) \tag{2}$$

where F, R, and T are Faraday's constant, gas constant, and absolute temperature, respectively, n is the number of gating charges moving through the entire transmembrane potential gradient for channel gating and V_0 is the potential where 50% of the total number of pores are in the closed configuration. The open to closed ratio of the channels, N_o/N_c, may be calculated from the data given in Fig. 6 according to:

$$N_o/N_c = (G - G_{\min})/(G_0 - G) \tag{3}$$

G in this equation is the conductance at a given membrane potential V_m, and G_0 and $G_{\min}$ are the conductance at zero voltage (all pores open) and very high potentials (all pores closed), respectively. Semilogarithmic plots of the data of Fig. 7 could be fitted to straight lines with slopes around 12–15 mV for e-fold changes of the voltage V_m (data not shown). The midpoint potential $V_m = V_0$ for the equal distribution of open and closed pores is about 20 mV. This result indicated that the number of gating charges involved in the voltage-dependent gating of hVDAC1 is approximately two. Similar numbers of gating charges have been found for a variety of eukaryotic porins (Schein et al. 1976; Ludwig et al. 1989).

The observation that the distribution of open and closed pores of Fig. 7 can be explained by a Boltzmann distribution allowed the estimation of the voltage-dependent activation energy of a single channel, $W(V_m)$, which is the energy difference of one mole channels between the open and the closed state. $W(V_m)$ is required for the switching of the eukaryotic porin pores from the open to the closed configuration (Schein et al. 1976). This means that Eq. (2) has the following form:

$$N_o/N_c = \exp\left(-\frac{W(V_m)}{RT}\right) \tag{4}$$

A comparison of Eqs. (2) and (4) shows that $W(V_m) = nF(V_m - V_0)$. The energy needed for channel closure, nFV_0, calculated from the data of Fig. 6 is approximately 6 kJ/mol, an energy that is considerably below the energy of one mol hydrogen bonds.

6 Ionic Selectivity of the Open and Closed States of Eukaryotic Porins

The open state of all mitochondrial porins characterized so far is slightly anion selective for salts with equally mobile cations and anions such as KCl. This means that the ionic selectivity is dependent on the mobility of the ions in the aqueous phase

and may change to cation selectivity for less mobile anions (Benz 2004). Such a behavior is expected for general diffusion pores because ions move through the pores similar to the way they move through the aqueous phase; i.e., the limiting factor for this movement is the aqueous mobility. Zero-current membrane potentials of eukaryotic porins were around −10 mV at the more dilute side of tenfold KCl gradients (Benz 1994b, 2004). This corresponds to an about twofold higher permeability for Cl^- over K^+ according to the Goldman–Hodgkin–Katz equation (Benz et al. 1979, 1985b). The anion selectivity of the eukaryotic porins was higher in LiCl solution because P_{Cl}/P_{Li} was around three [V_m was about −20 mV at the more dilute side (Benz 2004)]. This is the result of the reduced mobility of Li^+ ions as compared to K^+ ions in the aqueous phase. Similarly, the asymmetry potential for a tenfold Kacetate gradient was +14 mV at the more dilute side, which indicated that the anion selectivity changed to a cation one ($P_K/P_{acetate} = 2$), because of the lower mobility of the acetate as compared to Cl^- (Benz 2004).

Figure 7 shows the reduced data for G/G_0 derived from experiments with Porin 31HL as a function of the transmembrane voltage for 0.5 M KCl (pH 7.2), 0.5 M Tris-Cl (pH 7.2), and 0.5 M K-MES (pH 7.2). It is obvious that the voltage dependence was in all cases similar. However, G/G_0 is dependent on the type of the cation and anion present in the aqueous solution. G/G_0 is considerably larger (at high voltages) in K-MES than in KCl or in Tris-Cl. This result could only be explained by the assumption that the subconductance states of Porin 31HL (and those of other mitochondrial pores) have a much lower permeability toward anions than to cations. Otherwise, the results of Fig. 7 cannot be explained. The exact value for the permeability ratio of potassium ions and chloride was difficult to obtain because the mobility of $Tris^+$ and MES^- inside the pore is unknown. However, because of the comparably small mobility of $Tris^+$ and MES^- in the aqueous phase, we are sure that P_K/P_{Cl} of the closed state is at least 10. The value may be even higher if the closed state is impermeable to anions. On the other band, it is also evident from Fig. 7 that potassium ions are almost equally mobile through the open and the closed states, which also suggested that the latter state has completely different properties as the open state.

The single-channel conductance of the closed state of the eukaryotic porins can be estimated from single-channel conductance experiments at higher membrane potentials of 30 or 40 mV. At these potentials, the open state of the pore has only a limited lifetime because of its voltage dependence. The conductance of the closed state can be subtracted from that of the open state. Table 5 shows the results of this type of measurements obtained for three different salts and two types of eukaryotic porin, yeast (Ludwig et al. 1988) and human VDAC1 [Porin 31HL (Benz et al. 1992)]. The data show that conductance of the closed state of the eukaryotic porins was considerably smaller for Tris-HCl than for K-MES, despite a similar aqueous mobility of K^+ and Cl^-. This result suggested that the closed state(s) of mitochondrial porins is cation-selective.

Table 5 Average single-channel conductance of the open and closed states of yeast (Ludwig et al. 1988) and human (Benz et al. 1992) porins in different 0.5 M salt solutions

Salt	Open state (nS)	Closed state (nS)
Yeast porin		
KCl	2.3	1.3
K-MES	0.95	0.65
Tris-HCl	1.5	0.30
Human porin (Porin 31 HL)		
KCl	2.4	1.4
K-MES	0.70	0.65
Tris-Cl	1.5	0.30

The pH of the aqueous salt solutions was adjusted to 7.2. The protein concentration was between 5 and 10 ng/mL, $V_m = 30$ mV, and $T = 25$ °C. The single-channel conductance of the closed state was calculated by subtracting the conductance of the closing events from the conductance of the initial opening of the pores (see also arrows in Fig. 5)

7 Inhibition of Eukaryotic Pores In Vitro and In Vivo by a Synthetic Polyanion

An amphiphilic synthetic polyanion (a copolymer of molecular mass of about 10 kDa of methacrylate, maleate, and styrene in a 1:2:3 proportion) interferes with metabolite transports in the mitochondrial inner membrane and the ATPase (König et al. 1977, 1982). Experiments with eukaryotic porins in the presence of the polyanion suggested that these effects have nothing to do with a direct interaction between polyanion and inner membrane carriers (Colombini et al. 1987; Benz et al. 1988b). It seems, moreover, that polyanion binds to eukaryotic porin and shifts its voltage dependence. The pore closes already when small negative membrane potentials around 5 mV were applied to the *cis*-side, the side of the addition of the polyanion (Benz et al. 1988a, b; De Pinto et al. 1989). For positive potentials at the *cis*-side, the pore is always in its open configuration, even for very high potentials (Benz et al. 1988b). The polyanion-induced closed state has similar properties as the voltage-mediated closed state as it is clearly shown for its conductance shown in Table 6 (compare also Table 5).

Table 6 Average single-channel conductance of the open and the polyanion-induced closed state of rat liver porin in different 0.5 M salt solutions (Benz et al. 1990)

Salt	Open state (nS)	Closed state (nS)
KCl	2.2	1.2
LiCl	1.8	0.40
K-acetate	1.1	0.85
K-MES	0.88	0.74
Tris-Cl	1.5	0.25

The pH of the aqueous salt solutions was adjusted to 7.2. The membrane voltage was 10 mV at the *cis*-side; $T = 25$ °C. The aqueous phase contained in the measurements of the closed state 0.1 μg/mL polyanion added to the *cis*-side

Experiments with intact mitochondria indicated that the polyanion-induced closed state of rat liver porin resulted in a complete inhibition of mitochondrial kinases that are localized in the intermembrane space between mitochondrial inner and outer membranes. Creatine kinase and adenylate kinase were completely blocked when they were excluded from the external ATP pool by treatment of rat liver mitochondria with 30 μg polyanion per mg mitochondria. Similarly, peripheral kinases, such as hexokinase, were also completely inhibited in these experiments when the enzyme used mitochondrial ATP, but not for utilizing external ATP (Benz et al. 1990; Benz and Brdiczka 1992). Treatment of mitochondria with digitonin, which disrupts the mitochondrial outer membrane, completely restored the activity of the mitochondrial intermembranous kinases (Benz and Brdiczka 1992). This means that the mitochondrial outer membrane pore could regulate mitochondrial metabolism. Compartimentation may also be possible by the folding of the mitochondrial inner membrane (Mannella et al. 2013; Mannella 2020). Voltage-dependent regulation of mitochondrial outer membrane permeability seems to be possible (Benz and Brdiczka 1992). The close apposition of mitochondrial inner and outer membrane in contact sites could lead to a voltage drop across the outer membrane, which could result in pore closure (Benz 1985).

8 Structure of the Pore Formed by Eukaryotic Porins

The primary structure of eukaryotic porins from many organisms, including humans, mouse, fruit fly, plants, yeast, fungi, and *Dictyostelium*, is known to date. With the exception of Porin 31HL (HVDAC1 from humans, obtained by amino acid sequencing) were all derived from their cDNA-sequences. Eukaryotic porins are synthesized on cytosolic ribosomes similar as approximately 1000 other proteins to be imported posttranslationally into mitochondria (Grevel et al. 2019). These proteins do not contain a precursor, which means that they contain an internal sorting signal that is responsible for their import into mitochondria via the mitochondrial outer membrane protein import system TOM (Bausewein et al. 2020; Drwesh and Rapaport 2020). The assembly of the mitochondrial outer membrane β-barrel proteins occurs through the sorting and assembly machinery SAM, similar to the BAM system in gram-negative bacteria (Diederichs et al. 2020). The nucleus of many organisms codes also for more than one sequence. The genomes of mammals code very often for three different protein sequences (De Pinto and Messina 2004; Anflous and Craigen 2004). A comparison of the primary sequences shows that all eukaryotic porins have a similar length (around 280 amino acids), but only a few amino acids are strictly conserved (Benz 2004; Young et al. 2007). This could be the result of the secondary structure of eukaryotic porins because β-depleted sheet structure tolerates many exchanges of amino acids without substantial alterations of secondary structure and of the function as a voltage-dependent pore in a membrane. An example of a highly conserved triplet of amino acids is the GLK motif near amino acid 90, which is present in most of the sequences of eukaryotic porins. It may play an essential but not known role in

eukaryotic porin function. However, it is not the place of ATP binding (Runke et al. 2000). The homology of eukaryotic porin sequences suggests that a common ancestor sequence may have existed. However, a careful phylogenetic analysis performed by Young et al. (2007) demonstrates that paralogs have appeared several times during the evolution of VDACs from the plants, metazoans, and even the fungi, suggesting that there are no "ancient" paralogs within this gene family.

The arrangement of the primary sequence of eukaryotic porins in the pore-forming unit was for a long time the matter of debate. Several models of the pore-forming unit were derived from secondary structure predictions and mutagenesis of eukaryotic porins (Forte et al. 1987; Peng et al. 1992; Benz 1994b; Blachly-Dyson et al. 1990; Blachly-Dyson and Forte 2001; Casadio et al. 2002). One model assumed that the channel is composed of either 12 or 13 ß-strands and the amphipathic N-terminal α-helix as part of the channel (Blachly-Dyson et al. 1990; Song and Colombini 1996). This model was complicated by the question whether N- and C-termini were on the same or different sides of the outer mitochondrial membrane. Another model also based on secondary structure predictions and site-directed mutagenesis suggested that the pore is exclusively formed by 16 β-strands with the amphipathic terminal α-helix in the vicinity of the lipid layer or inside the pore (Popp et al. 1996; Casadio et al. 2002; Young et al. 2007). This model was supported by deletion of 15 amino acids of *N. crassa* porin from the C-terminal end (Popp et al. 1996), because this mutant showed the same pore-forming activity than wild-type porin but had a smaller conductance. The assumption of a ß-barrel cylinder as 3D structure of eukaryotic porins was supported by electron microscopic studies of mitochondrial outer membranes, which suggested that pore has a diameter of about 2.5 nm (Guo et al. 1995).

Many attempts were performed over the years since the eukaryotic porins were known to crystallize the pores, but they were not successful for a long time. The reason for the failure was probably that the eukaryotic pore is deeply buried in the mitochondrial outer membrane, which means that the proteins do not interact with one another in protein crystals. Finally, three different groups were successful simultaneously in 2008 to derive the 3D structure of mitochondrial porins from human (hVDAC1, two groups) and mouse (mVDAC1, one group) (Bayrhuber et al. 2008; Hiller et al. 2008; Ujwal et al. 2008). They were successful using different techniques. One study used solution NMR to study the structure of recombinant human VDAC1 reconstituted in detergent micelles, but in this case, the position of the N-terminal end of the protein was not properly resolved (Hiller et al. 2008). Another investigation used a combination of x-ray crystallography and NMR spectroscopy (Bayrhuber et al. 2008). The murine VDAC1 (mVDAC1) was crystallized in a lipidic environment with a resolution around at 2.3 Å (Ujwal et al. 2008). All investigations agreed within the basic structure of eukaryotic porins and argued that the derived structure forming a β-barrel with 19 β-strands is generally applicable to the pores formed by all eukaryotic porin pores (Zeth and Zachariae 2018). In two of the investigations, the α-helix at the N-terminal end was found to be located horizontally midway within the pore causing partial narrowing (Bayrhuber et al. 2008; Ujwal et al. 2008). This means that the α-helix has a strategic position to

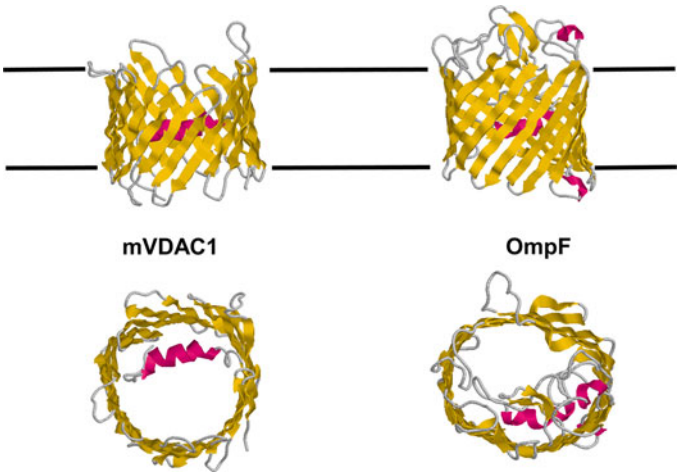

Fig. 8 Structure of the mitochondrial outer membrane pore (mVDAC1) and OmpF of *E. coli*. The proteins have a β-barrel cylindrical structure with the β-strands tilted by 30°–40° toward the surface of the membranes. β-strands within the protein structures are shown in yellow and α-helical stretches in red. The 3D structure of the proteins is shown from the side in direction to the surface of the mitochondrion and the cell (upper structures) and from the surface of the mitochondrion and cell (structures down). mVDAC1 (PDB code: 2JK4) is the 3D structure of mouse mitochondrial porin (Ujwal et al. 2008). OprF (PDB code: 2OMF) represents the structure of the major outer membrane protein of *E. coli* (Cowan et al. 1992)

regulate the passage of metabolites and ions through the eukaryotic porin pore. It is also clear that the α-helix is involved in voltage-dependent channel gating (Popp et al. 1996; Zachariae et al. 2012).

Figure 8 shows cartoons of the 3D structures of hVDAC1 and of OmpF of *E. coli* (Ujwal et al. 2008; Cowan et al. 1992). The mitochondrial pore is formed by 19 β-strands. The dimensions of the eukaryotic β-barrel are: The maximal height of the β-strands is 35 Å, and the width of the β-barrel is 40 Å. Tom40, the central protein of the mitochondrial outer membrane protein import machinery TOM, represents another member of the VDAC superfamily (Zeth and Zachariae 2018). It also shares an odd number of nineteen β-strands. Eighteen strands of these proteins are antiparallel and β-strands one and 19 are parallel. The N-terminal α-helix (amino acids 1–21) is localized inside the pore and represents presumably a stabilizing element, similar as the external loop 3 of OmpF, which folds inside the pore and restricts its permeability (Ujwal et al. 2008; Cowan et al. 1992). It is oriented against the interior wall of the pore and causes something like a gate partially restricting the size of the pore (see Fig. 8). The α-helix has a strategic position within the channel to control the passage of ions and metabolites through the mitochondrial pore. Although there does not exist any homology between the primary sequences of the two pores in Fig. 8, it is clear that they represent highly homologous structures. This has presumably to do with the history of these membrane pores. They first evolved in the bacterial outer membranes of gram-negative bacteria, presumably by multiplication of an ancestral β-hairpin of the structure β-strand-turn-β-strand (Remmert

et al. 2010). The basis for this hypothesis was the identification of clear repeating sequences in many outer membrane β-barrel proteins. The basic repeating sequence was presumably amplified from short peptide modules that may have evolved as cofactors of RNAs (Remmert et al. 2010). This means presumably that all bacterial β-barrel proteins are related to one another, which also could be the reason that they all have an even number of antiparallel β-strands that are composed of multiples of the ancestral ββ-hairpin. Bacterial porins vary in the number of β-strands between 8 and 18. Outer membrane protein with an odd number of β-strands is not known.

The common architecture of bacterial and eukaryotic porins is the β-barrel cylinder spanning the outer membranes of bacteria and mitochondria. This common structure suggests also that both pores have common ancestors (see Fig. 8). As pointed out above based on the bioinformatics approach of Remmert et al. (2010), the bacterial porins evolved by amplification of an ancestral ββ-hairpin. So far, it is not clear if this process was already complete at the time of the LECA, i.e., whether complete β-barrel outer membrane pores existed that time. In general, we have to assume that because the bacterial outer membrane represented already that time a permeability barrier that needed permeability pathways. Pereira and Lupas (2018) argued that the family of SAM50 proteins in mitochondrial outer membranes is clearly of bacterial origin. They suggested that the outer membrane pore of mitochondria evolved in a similar way as bacterial porins from an ancestral ββ-hairpin of bacterial origin at the time of the LECA (Pereira and Lupas 2018). This seems to be possible, but it does not explain the odd number of β-strands in the β-barrel cylinder of the eukaryotic pores. However, if we assumed that the precursor of the mitochondrial pore was already a β-barrel cylinder of 20 β-strands, then it seems to be possible that the original β-strand one was mutated during evolution into the N-terminal α-helical wheel, which plays an important role in voltage dependence of eukaryotic pores. In this respect, it is noteworthy that the eukaryotic pore does not play an only passive role in mitochondrial outer membrane permeability as the bacterial porins do.

9 Role of Eukaryotic Porins in Mitochondrial Metabolism

The possible role of the voltage dependence of eukaryotic porins was already discussed. It seems to be involved in the restriction of metabolite flux across the mitochondrial membrane and in compartimentation of peripheral kinases. However, the mitochondrial pore shows also other features. In particular, when the binding of kinases (Fiek et al. 1982; Adams et al. 1991; De Pinto et al. 2003) and the interaction between porin and other proteins are considered, it seems to be clear that eukaryotic porins play a more active role in regulating of mitochondrial metabolism. In particular, it is suggested that eukaryotic porins are also involved in protein translocation, mitochondria-mediated apoptosis, signal transduction, and the response to different drugs (Shoshan-Barmatz et al. 2006, 2010; Gatliff and Campanella 2012; Kanwar et al. 2020; Grevel and Becker 2020). In this respect, the mitochondrial 18 kDa

Translocator Protein (TSPO), also known under its previous name peripheral benzodiazepine receptor (PBR), plays an important role, because it binds benzodiazepines in different tissues and is involved in cholesterol transfer from the mitochondrial outer to the inner membrane, Ca^{2+} signaling, cytochrome C release, and apoptosis (Gatliff and Campanella 2012; Bader et al. 2019).

10 Conclusions

The outer membranes of gram-negative bacteria and mitochondria have special sieving properties as compared with normal cell membranes. Based on the presence of one or only a few proteins, these membranes have an extremely high permeability for a variety of different solutes. However, the bacterial outer membranes exhibit only passive properties which means their permeability is not voltage-regulated, but still under full genetic control, the permeability of the mitochondrial membrane appears to be regulated by a transmembrane potential. In particular, ADP and ATP and possibly also phosphate cannot pass through the closed states of the eukaryotic porin. In addition, this pore is involved in a variety of important metabolic functions. This means that the pore in the mitochondrial outer membrane has much more important role in mitochondrial metabolism than bacterial pore-forming proteins despite their common origin.

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