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*Topical Review*

# Families of Proteins Forming Transmembrane Channels

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**Abstract.** Channel-forming proteins/peptides fall into over 100 currently recognized families, most of which are restricted to prokaryotes or eukaryotes, but a few of which are ubiquitous. These proteins fall into three major currently recognized classes: (i) α-helix-type channels present in bacterial, archaeal and eukaryotic cytoplasmic and organellar membranes, (ii) β-barrel-type porins present in the outer membranes of Gram-negative bacterial cells, mitochondria and chloroplasts, and (iii) protein/peptide toxins targeted to the cytoplasmic membranes of cells other than those that synthesize the toxins. High-resolution 3-dimensional structural data are available for representative proteins/peptides of all three of these channel-forming types. Each type exhibits distinctive features that distinguish them from the other channel protein types and from carriers. Structural, functional, and evolutionary aspects of transmembrane channel-formers are discussed.

**Key words:** Membranes — Transport — Channels — Porins — Toxins — Holins — Bacteriocins

## Introduction

Classically, solute transporters were defined into two major classes based on mode of transport: channels and simple carriers (Stein, 1967). Channels were thought to allow the relatively free flow of solute through the membrane while carriers were considered to form a complex with the solute and move together with it, at least partially, across the membrane. Primary active transporters and group-translocators couple transport to a primary source of energy. Whether or not the classical concept of a carrier will prove to be correct has yet to be ascertained, but the classical notion of a channel is now well established.

The introduction by Linnaeus of a universal classification system for living organisms in 1758 allowed for the rationalization of the tremendous complexity of biological relationships into an evolutionary framework (Linnaeus, 1758; Olsen et al., 1994). Similarly, the introduction by the international Enzyme Commission (EC) of a universal classification system for enzymes greatly increased our conception of the functional relationships of these proteins (Dixon & Webb, 1979). Although protein/domain classification systems have been suggested, no comparable classification system has yet been proposed for proteins that catalyze vectorial reactions rather than (or in addition to) chemical reactions. To attempt to correct this deficiency, I have developed a universal system of classification for transporters based both on function and on phylogeny.

Early studies revealed that transport proteins could be grouped into families based exclusively on the degrees of similarity observed for their amino acid sequences (Maloney, 1990). The significance of family assignment remained questionable until the study of internal gene duplications that had occurred during the evolution of some of these families established that these families had arisen independently of each other, at different times in evolutionary history, following different routes (Kuan & Saier, 1993; Saier, 1994; Park & Saier, 1996; Pao et al., 1998). This finding led to the use of function and molecular phylogeny for the purpose of facilitating transport protein characterization and classi-

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**Table 1.** Classification of currently recognized families of channel-forming transporters in the TC system

**1.A. α-Type channels**

| | |
|---|---|
| 1.A.1 | The Voltage-gated Ion Channel (VIC) Superfamily |
| 1.A.2 | The Animal Inward Rectifier $K^+$ Channel (IRK-C) Family |
| 1.A.3 | The Ryanodine-Inositol 1,4,5-triphosphate Receptor $Ca^{2+}$ Channel (RIR-CaC) Family |
| 1.A.4 | The Transient Receptor Potential $Ca^{2+}$ Channel (TRP-CC) Family |
| 1.A.5 | The Polycystin Cation Channel (PCC) Family |
| 1.A.6 | The Epithelial $Na^+$ Channel (ENaC) Family |
| 1.A.7 | ATP-gated Cation Channel (ACC) Family |
| 1.A.8 | The Major Intrinsic Protein (MIP) Family |
| 1.A.9 | The Ligand-gated Ion Channel (LIC) Family of Neurotransmitter Receptors |
| 1.A.10 | The Glutamate-gated Ion Channel (GIC) Family of Neurotransmitter Receptors |
| 1.A.11 | The Chloride Channel (ClC) Family |
| 1.A.12 | The Organellar Chloride Channel (O-ClC) Family |
| 1.A.13 | The Epithelial Chloride Channel (E-ClC) Family |
| 1.A.14 | The Nonselective Cation Channel-1 (NSCC1) Family |
| 1.A.15 | The Nonselective Cation Channel-2 (NSCC2) Family |
| 1.A.16 | The Yeast Stretch-Activated, Cation-Selective, $Ca^{2+}$ Channel, Mid1 (Mid1) Family |
| 1.A.17 | The Chloroplast Outer Envelope Solute Channel (CSC) Family |
| 1.A.18 | The Chloroplast Envelope Anion Channel-forming Tic110 (Tic110) Family |
| 1.A.19 | The Influenza Virus Matrix-2 Channel (IVC) Family |
| 1.A.20 | The gp91$^{phox}$ Phagocyte NADPH Oxidase-associated Cytochrome $b_{558}$ (CybB) $H^+$ channel Family |
| 1.A.21 | The Bcl-2 (Bcl-2) Family |
| 1.A.23 | The Small Conductance Mechanosensitive Ion Channel (MscS) Family |
| 1.A.24 | The Gap Junction-forming Connexin (Connexin) Family |
| 1.A.25 | The Gap Junction-forming Innexin (Innexin) Family |
| 1.A.26 | The Symbiotic Ammonium Transporter (SAT) Family |
| 1.A.27 | The Phospholemman (PLM) Family |
| 1.A.28 | The P21 Holin S (P21 Holin) Family |
| 1.A.29 | The λ Holin S (λ Holin) Family |
| 1.A.30 | The P2 Holin TM (P2 Holin) Family |
| 1.A.31 | The LydA Holin (LydA Holin) Family |
| 1.A.32 | The PRD1 Holin M (PRD1 Holin) Family |
| 1.A.33 | The T7 Holin (T7 Holin) Family |
| 1.A.34 | The HP1 Holin (HP1 Holin) Family |
| 1.A.35 | The T4 Holin (T4 Holin) Family |
| 1.A.36 | The T4 Immunity Holin (T4 Immunity Holin) Family |
| 1.A.37 | The ϕ29 Holin (ϕ29 Holin) Family |
| 1.A.38 | The ϕ11 Holin (ϕ11 Holin) Family |
| 1.A.39 | The ϕAdh Holin (ϕAdh Holin) Family |
| 1.A.40 | The ϕU53 Holin (ϕU53 Holin) Family |
| 1.A.41 | The LrgA Holin (LrgA Holin) Family |
| 1.A.42 | The ArpQ Holin (ArpQ Holin) Family |
| 1.A.43 | The Cph1 Holin (Cph1 Holin) Family |
| 1.A.44 | The Urea Transporter (UT) Family |
| 1.A.45 | The Urea/Amide Channel (UAC) Family |
| 1.A.46 | The $H^+$- or $Na^+$-translocating Bacterial Flagellar Motor (Mot) Family |

**1.B. β-Barrel porins**

| | |
|---|---|
| 1.B.1 | The General Bacterial Porin (GBP) Family |
| 1.B.2 | The Chlamydial Porin (CP) Family |
| 1.B.3 | The Sugar Porin (SP) Family |
| 1.B.4 | The *Brucella-Rhizobium* Porin (BRP) Family |
| 1.B.5 | The *Pseudomonas* OprP Porin (POP) Family |
| 1.B.6 | The OmpA-OmpF Porin (OOP) Family |
| 1.B.7 | The *Rhodobacter* PorCa Porin (RPP) Family |
| 1.B.8 | The Mitochondrial and Plastid Porin (MPP) Family |
| 1.B.9 | The FadL Outer Membrane Protein (FadL) Family |
| 1.B.10 | The Nucleoside-specific Channel-forming Outer Membrane Porin (Tsx) Family |
| 1.B.11 | The Outer Membrane Fimbrial Usher Porin (FUP) Family |
| 1.B.12 | The Autotransporter (AT) Family |
| 1.B.13 | The Alginate Export Porin (AEP) Family |
| 1.B.14 | The Outer Membrane Receptor (OMR) Family |
| 1.B.15 | The Raffinose Porin (RafY) Family |

*Continued on next page*

**Table 1.** *Continued*

| | |
|---|---|
| 1.B.16 | The Short Chain Amide and Urea Porin (SAP) Family |
| 1.B.17 | The Outer Membrane Factor (OMF) Family |
| 1.B.18 | The Outer Membrane Auxiliary (OMA) Protein Family |
| 1.B.19 | The Glucose-selective OprB Porin (OprB) Family |
| 1.B.20 | The Bacterial Toxin Export Channel (TEC) Family |
| 1.B.21 | The OmpG Porin (OmpG) Family |
| 1.B.22 | The Outer Bacterial Membrane Secretin (Secretin) Family |
| 1.B.23 | The Cyanobacterial Porin (CBP) Family |
| 1.B.24 | The Mycobacterial Porin (MBP) Family |
| 1.B.25 | The Outermembrane Porin (Opr) Family |
| 1.B.26 | The Cyclodextrin Porin (CDP) Family |
| **1.C. Pore-forming toxins** | |
| 1.C.1 | The Channel-forming Colicin (Colicin) Family |
| 1.C.2 | The Channel-forming δ-Endotoxin Insecticidal Crystal Protein (ICP) Family |
| 1.C.3 | The α-Hemolysin Channel-forming Toxin (αHL) Family |
| 1.C.4 | The Aerolysin Channel-forming Toxin (Aerolysin) Family |
| 1.C.5 | The Channel-forming ε-toxin (ε-toxin) Family |
| 1.C.6 | The Yeast Killer Toxin K1 (YKT-K1) Family |
| 1.C.7 | The Diphtheria Toxin (DT) Family |
| 1.C.8 | The Botulinum and Tetanus Toxin (BTT) Family |
| 1.C.9 | The Vacuolating Cytotoxin (VacA) Family |
| 1.C.10 | The Pore-forming Hemolysin E (HlyE) Family |
| 1.C.11 | The Pore-forming RTX Toxin (RTX-toxin) Family |
| 1.C.12 | The Thiol-activated Cytolysin (TAC) Family |
| 1.C.13 | The Channel-forming Leukocidin Cytotoxin (Ctx) Family |
| 1.C.14 | The Cytohemolysin (CHL) Family |
| 1.C.15 | The Whipworm Stichosome Porin (WSP) Family |
| 1.C.16 | The Magainin (Magainin) Family |
| 1.C.17 | The Cecropin (Cecropin) Family |
| 1.C.18 | The Melittin (Melittin) Family |
| 1.C.19 | The Defensin (Defensin) Family |
| 1.C.20 | The Nisin (Nisin) Family |
| 1.C.21 | Lacticin 481 (Lacticin 481) Family |
| 1.C.22 | The Lactococcin A (Lactococcin A) Family |
| 1.C.23 | The Lactocin S (Lactocin S) Family |
| 1.C.24 | The Pediocin (Pediocin) Family |
| 1.C.25 | The Lactococcin G (Lactococcin G) Family |
| 1.C.26 | The Lactacin X (Lactacin X) Family |
| 1.C.27 | The Divergicin A (Divergicin A) Family |
| 1.C.28 | The AS-48 (AS-48) Family |
| 1.C.29 | The Plantaricin EF (Plantaricin EF) Family |
| 1.C.30 | The Plantaricin JK (Plantaricin JK) Family |
| 1.C.31 | The Channel-forming Colicin V (Colicin V) Family |
| 1.C.32 | The Amphipathic Peptide Mastoparan (Mastoparan) Family |
| 1.C.33 | The Cathilicidin (Cathilicidin) Family |
| 1.C.34 | The Tachyplesin (Tachyplesin) Family |
| 1.C.35 | The Amoebapore (Amoebapore) Family |
| 1.C.36 | The Bacterial Type III-Target Cell Pore (IIITCP) Family |
| **1.D. Nonribosomally synthesized channels** | |
| 1.D.1 | The Gramicidin A (Gramicidin A) Channel Family |
| 1.D.2 | The Syringomycin Channel-forming (Syringomycin) Family |
| 1.D.3 | The Syringopeptin Channel-Forming (Syringopeptin) Family |
| 1.D.4 | The Tolaasin Channel-forming (Tolaasin) Family |
| 1.D.5 | The Alamethicin Channel-forming (Alamethicin) Family |
| 1.D.6 | The Complexed Poly 3-Hydroxybutyrate $Ca^{2+}$ Channel (cPHB-CC) Famiy |

fication. The system I have developed is called the transporter classification (TC) system. It has recently been considered for adoption and refined by the transport panel of the International Union of Biochemistry and Molecular Biology (IUBMB) Nomenclature Committee in Geneva, Switzerland (Nov. 29–30, 1999). Its official adoption by the IUBMB Nomenclature Committee is pending.

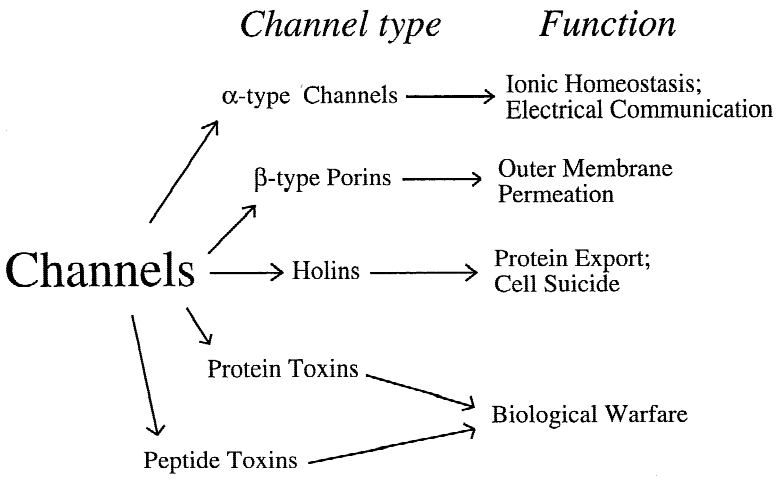


**Fig. 1.** Types of channel-forming proteins and peptides found in nature. These proteins are classified according to their structures, subcellular locations, targets and mechanisms of action (*see* text). Not included are nonribosomally synthesized depsipeptide-type channels and nonproteinaceous channel-forming compounds and complexes (TC subclass 1.D).

In the TC system, all transporters of known mode of action are given primary TC #s according to class as follows: TC class 1: channels; TC class 2: carriers; TC class 3: primary active transporters; TC class 4: group translocators. Each of these classes is then divided into subclasses. For example, class 1 channels are subdivided into α-type channels (subclass 1.A); β-barrel-type porins (subclass 1.B); secreted protein and peptide toxins (subclass 1.C); and nonribosomally synthesized channel formers (subclass 1.D). Each phylogenetic family of transporters then receives a numerical value following the subclass letter. Thus, for example, the voltage-gated ion channel (VIC) family has been assigned the TC #1.A.1; the ligand-gated ion channel (LIC) family has been assigned the TC #1.A.9, and the general bacterial porin (GBP) family has been assigned the TC #1.B.1. Table 1 lists all of the families included in class 1 of the current TC system.

The information on channel proteins presented in this review resulted from an analysis of the families of channel proteins classified in the TC system. This system has been described in a detailed review (Saier, 1998) and in several more recent but less detailed but focused reviews (*see*, for example, Saier, 1999). A description of each of the families of channel proteins discussed here as well as primary references to well-characterized members of each family can be found in our website (http://www-biology.ucsd.edu/~msaier/transport/). The numbering system used in the current TC system is somewhat different from that described in the references cited above as explained in our website.

## Transmembrane Channel Types

Five functional types of integral membrane channel proteins are currently recognized based on structure, function, site of action and size (Fig. 1 and Table 1). The most widely publicized are the α-type channel proteins (TC subclass 1.A) which span the membrane forming a channel lined by amphipathic α-helices. A few families of these proteins are found ubiquitously in the cytoplasmic membranes of prokaryotes and eukaryotes as well as in organellar membranes of eukaryotes. However, many families of these proteins are found exclusively in animals that depend on channel function for bioelectric activity. These channels may either be nonspecific with respect to the solute transported, cation or anion selective, or highly specific for a particular ionic species (Alexander & Peters, 1997; Sakai & Tsukihara, 1998). The high-resolution three-dimensional structure of one such channel protein has been solved (Doyle et al., 1998; Samson, 1998).

A second type of channel protein is the β-barrel-type porin (TC subclass 1.B) found in the outer membranes of Gram-negative bacteria, mitochondria and chloroplasts. Some of these proteins may also prove to be present in other membranes such as the outer membranes of acid fast Gram-positive bacteria (Mycobacteria, Nocardia and Corynebacteria) as well as the nuclear membranes of eukaryotes. The transmembrane pores created by these proteins are formed from amphipathic β-strands arranged in a barrel configuration. High-resolution three dimensional structures of several of these proteins have been determined (Schulz, 1996; Sakai & Tsukihara, 1998).

A third type of channel protein is represented by the toxins that are made in one cell type but targeted to another (TC subclass 1.C). Many of these proteins are made by bacteria. They serve the function of biological warfare, inserting into the membranes of eukaryotes or other prokaryotes, thereby releasing nutrients for the growth of the toxin producers. Three-dimensional structures of some of these toxin proteins are available (Lacy & Stevens, 1998; Sakai & Tsukihara, 1998).

Channel proteins of a fourth type (actually a subdivision of TC subclass 1.A) are produced exclusively by bacteria and their viral parasites and are referred to as

"holins." These small proteins, all of similar size and structure, form oligomeric pores in the membrane that allow export of autolytic cell wall digesting enzymes as well as other enzymes involved in macromolecular degradation. They may also allow the free flow of ionic and neutral solutes across the bacterial membrane prior to disruption of the cell wall. Holins are "apoptotic" proteins, agents of self-destruction. The genomes of many bacteria encode holins suggesting that holin-promoted suicide may have survival value for the bacterial population as a whole (Young & Bläsi, 1995). Although three-dimensional structural data are not available for holins, topological analyses allow specific predictions.

Finally, channel-forming toxic peptides, the fifth and last type of channel formers depicted in Fig. 1 (actually a subdivision of TC subclass 1.C), are produced by all types of organisms, both prokaryotes and eukaryotes, and they functionally resemble toxin proteins in serving as agents of biological warfare. These amphipathic cationic peptide oligomers form channels that usually exhibit a low degree of substrate specificity, allowing nutrient leakage and cell disruption. Some of these peptides have been extensively studied from both structural and functional standpoints but many others are poorly characterized (Klaenhammer, 1993; Bechinger, 1997; Matsuzaki, 1998; Sahl & Bierbaum, 1998). Finally, a distinct class of related molecules, not to be discussed here, includes channel-forming depsipeptides that are biosynthesized in nonribosomal, enzyme-catalyzed processes (TC subclass 1.D; *see*, for example, Burkhart et al., 1998). However, the vast majority of peptide toxins are made by proteolytic processing of ribosomally synthesized polypeptides.

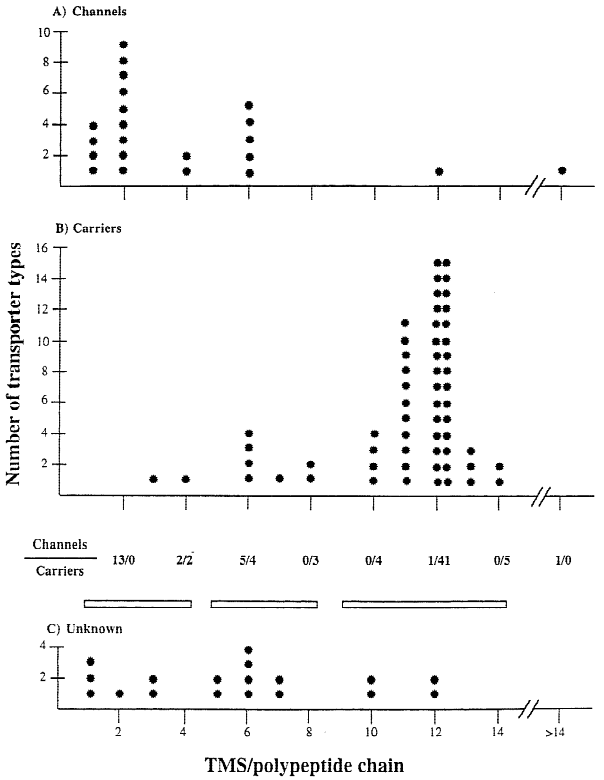


**Fig. 2.** Topology (# TMSs) of (*A*) channel-forming proteins (TC subclass 1.A) *vs.* (*B*) carrier-type proteins (TC subclass 2.A) *vs.* transporters (*C*) of unknown mode of action (TC subclass 9.A). Each entry represents a distinct family or structural type. Thus, each point usually represents a distinct family of transporters. However, when multiple topological types are included within a single family, all such types are presented. The data were generated from the families that were included in the TC system as of April, 1999.

## Channel Protein Topology

There has been considerable discussion concerning the equivalence of channels and carriers. As noted above, the latter proteins are sometimes thought of as channels with intrachannel amino acyl residues that provide stereospecific solute recognition as well as a shuttling mechanism (West, 1997). The observation that some of these carriers can be converted to proteins that appear to function as channels by treatment with chemicals that covalently or noncovalently alter the protein or by imposition of large membrane potentials has provided support for this notion (Dierks et al., 1990*a,b;* Schwarz et al., 1994).

The results summarized in Fig. 2, however, argue against such a premise (*see also* West, 1997). Most families of channel proteins include protein members with from 1–3 α-helical transmembrane spanners (TMSs) although there are exceptions (Fig. 2). In one large superfamily of channel proteins, the voltage-gated ion channel (VIC) superfamily (TC 1.A.1), a single polypeptide chain can have 2, 4, 6, 8, 12, or 24 TMSs. The proposed evolutionary pathway for the appearance of the various VIC family structural types that exist in nature is illustrated in Fig. 3 (*see also* Nelson et al., 1999). The basic channel-forming unit consists of a single TMS with a second TMS providing structural support immediately behind the channel-lining helix (Doyle et al., 1998; Sansom, 1998). In the 2 TMS polypeptide channels of the VIC family, no further structural elements are required for function, but four subunits are required to form the channel (Nelson et al., 1999). The 4 TMS polypeptides arose by intragenic tandem duplication of the element encoding the 2 TMS subunit, and consequently, two rather than four polypeptides comprise the intact channel complex. The 6 TMS $K^+$ channels of the VIC family include a TMS (TMS 1) involved in insertion of the protein into the membrane, three other TMSs (TMSs 2–4) involved in regulation (e.g., voltage sensing), and the two remaining TMSs (TMSs 5 and 6) serving as the channel-forming elements that are homologous to the 2 TMS VIC family members (Fig. 3). The 12 and 24 TMS

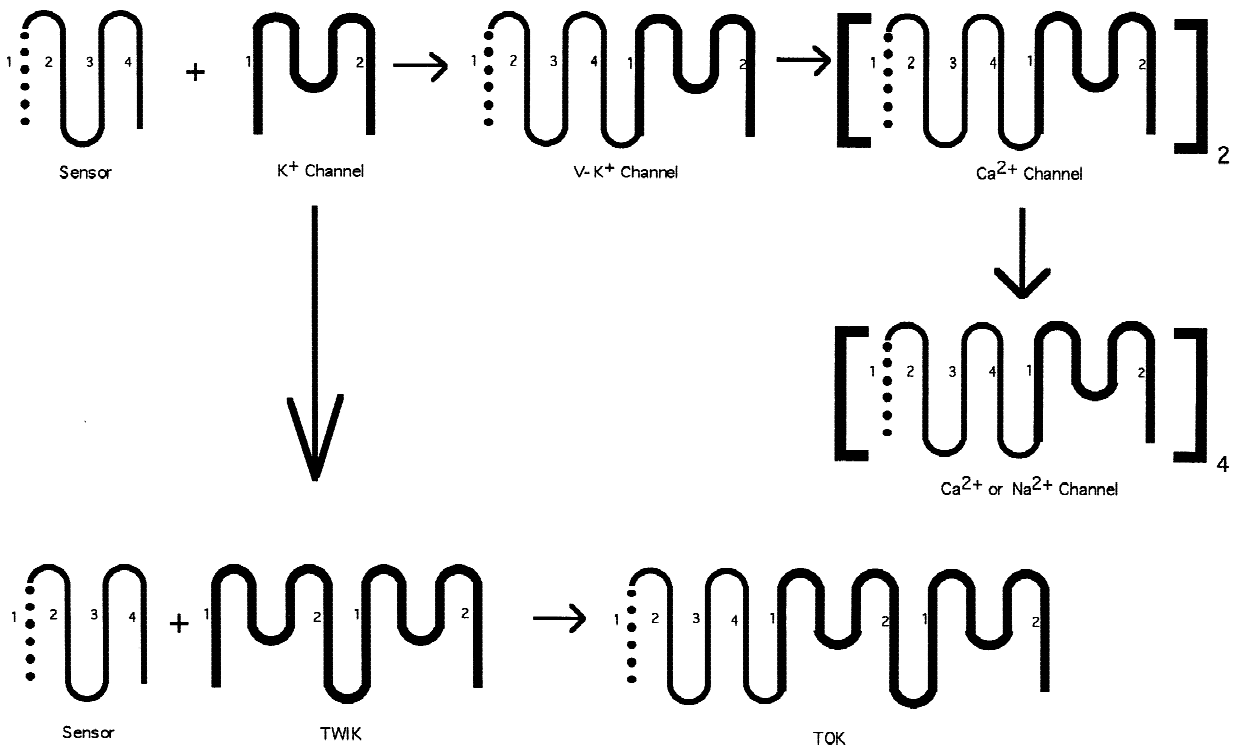


**Fig. 3.** Proposed evolutionary pathway accounting for the currently recognized homologues that belong to the VIC family of channel proteins (TC 1.A.1; Nelson et al., 1999).

$Ca^{2+}$ and $Na^{+}$ channel proteins arose by one and two tandem intragenic duplication events, respectively. These proteins have, in essence, an overall heterotetrameric structure comparable to that observed for the homotetrameric 6 TMS $K^{+}$ transporting channel-forming members of the VIC family (Nelson et al., 1999). While the functionally characterized 2, 4 and 6 TMS proteins of the VIC family are all $K^{+}$ or low selectivity cation channels, and the duplicated 12 TMS proteins are $Ca^{2+}$ channels, the quadruplicated 24 TMS proteins can be either $Ca^{2+}$ or $Na^{2+}$ channels (Hille, 1992; Nelson et al., 1999). The VIC family, which represents one of the few examples of channel proteins with multiple topological types, provides a truly illustrative example of how construction of multi-TMS proteins from the simple 2 TMS precursor may have occurred during evolutionary history (Fig. 3).

In contrast with channels, carriers consist of polypeptides that usually possess 8–14 TMSs (Fig. 2), and in many characterized systems, the monomeric protein has demonstrable transport activity. Oligomerization of this protein when it occurs usually serves a secondary role in regulation, targeting or stability rather than a primary role in transport. Thus, while channels are usually multimers of proteins having 1–3 TMSs, carriers are usually transport-competent monomers of proteins having 10–14 TMSs. Oligomeric carriers may depend on subunit interactions for purposes of stability and/or regulation although dimeric 6 TMS proteins are often required for transport function (i.e., the mitochondrial carrier family). Very few exceptions to this rule have been documented, and these observations therefore have value in assignment of transport mode to proteins revealed, for example, by genome sequencing where functional data are limited or altogether lacking (*see also* West, 1997).

## Evolutionary Origins and Specificities of Ion-Transporting Channels (α-type)

Table 2 presents most of the currently recognized, well-characterized families of α-type ion channel proteins. Unless otherwise indicated, these proteins are found only in animals. TC #s, abbreviations, preferred substrates transported, sizes and numbers of TMSs per polypeptide chain are all tabulated. Oligomeric structure is also indicated when known. Below we briefly discuss each of the families listed in Table 2. More detailed descriptions, additional primary references, names of well-characterized members of these families and database accession numbers that provide easy access to their sequences are all available on our website (http://www-biology.ucsd.edu/~msaier/transport/).

Only two families of channel-forming proteins are currently known to be specific for small neutral molecules. These are the major intrinsic protein (MIP) family (TC #1.A.8) of aquaporins and glycerol facilitators, and the urea transporter (UT) family (TC #1.A.44). While the former family is found ubiquitously in bacte-

**Table 2.** Families of α-type channel proteins (TC subclass 1.A) specific for small molecules (mostly from animals)

| Family name | TC # | Family[1] | Substrates | ~Size (# aas)[3] | # TMSs[4] |
|---|---|---|---|---|---|
| Neutral molecules | 1.A.8 | MIP (ubiquitous) | $H_2O$, glycerol | 300 | $(6)_{2 \text{ or } 4}$ |
| | 1.A.44 | UT | Urea | 400 | $(10)_n$ |
| Cations | 1.A.1 | VIC (ubiquitous) | $K^+$, $Ca^{2+}$, $Na^+$ | 100–2500 | $(2)_4$; $(4)_2$; $(6)_4$; $(12)_2$; $(24)_1$ |
| | 1.A.3 | RIR-CaC | $Ca^{2+}$ | 2500 or 5000 | $(6)_n$ |
| | 1.A.2 | IRK-C | $K^+$ | 400 | $(2)_n$ |
| | 1.A.4 | TRP-C | $Ca^{2+}$ | ~1000 | $(6)_n$ |
| | 1.A.5 | PCC | $Na^+$, $K^+$, $Ca^{2+}$ | ~4000 | $(6)_n$ |
| | 1.A.6 | ENaC | $Na^+$, $C^+$ [2] | 600 | $(2)_4$ |
| | 1.A.7 | ACC | $C^+$ and $Ca^{2+}$ | 600 | $(2)_n$ |
| | 1.A.9 | LIC | $C^+$ and $Cl^-$ | ~500 | $(3–5)_5$ |
| | 1.A.10 | GIC | $C^+$ and $Ca^{2+}$ | ~1000 | $(3–5)_5$ |
| | 1.A.26 | SAT (plants) | $NH_4^+$ | 350 | $(1)_n$ |
| | 1.A.14 | NSCC1 | $C^+$ | 400 | $(4)_n$ |
| | 1.A.15 | NSCC2 (and yeast) | $C^+$ | 400 | $(2)_n$ |
| | 1.A.19 | IVC (viruses) | $H^+$ | 100 | $(1)_4$ |
| | 1.A.20 | CybB (and plants) | $H^+$ | 750 | $(6)_n$ |
| Anions | 1.A.11 | ClC (ubiquitous) | $Cl^-$, other inorganic anions | 400–1000 | $(10–12)_n$ |
| | 1.A.12 | O-ClC | $Cl^-$ | 250–500 | $(2)_n$ |
| | 1.A.13 | E-ClC | $Cl^-$, other anions | | |
| | 1.24 | PLM | $Cl^-$, other anions (inorganic and organic) | 100 | $(1)_n$ |
| Bacterial ion channels | 1.A.22 | MscL | Ions ($C^+ > A^-$) | 130 | $(2)_6$ |
| | 1.A.23 | MscS | Ions ($A^- > C^+$) | 200–1200 | $(2–14)_n$ |

[1] The families listed above are derived exclusively from animals unless otherwise indicated. The exceptions include the MIP, VIC, and ClC families which are found universally in the three domains of life, the NSCC2 and CybB families which are found in animals and one other eukaryotic kingdom, the SAT and IVC families which are found exclusively in plants and in influenza viruses, respectively, and the MscL and MscS families which are restricted to prokaryotes.

[2] $C^+$, monovalent cation.

[3] # aas, number of amino acyl residues per polypeptide chain.

[4] The number of transmembrane spanners (TMSs) is indicated in parentheses. The subscript refers to the homooligometric structure of the protein complex when known. The subscript *"n"* implies that the stoichiometry is not known.

ria, archaea and eukaryotes, the latter family is restricted to animals. The proteins of these two families are exceptional of those that comprise channels as they exhibit 6 and 10 TMSs per polypeptide chain, respectively (Table 2).

The VIC family (TC #1.A.1), mentioned above, is the largest and possibly the oldest of the recognized channel protein families. It is ubiquitous, and VIC family members exhibit varied sizes and topologies (Table 2). Some of these proteins exhibit just 2 TMSs per polypeptide chain while others have 4, 6, 8, 12, or 24 TMSs as discussed from an evolutionary standpoint above. These proteins nevertheless belong in a single family because TMSs 1 and 2 in the 2 TMS proteins are demonstrably homologous to both TMSs 1 and 2 and TMSs 3 and 4 in the 4 TMS proteins, to TMSs 5 and 6 in the 6 TMS proteins, and to TMSs 5 and 6, 11 and 12, 17 and 18, and 23 and 24 in the 24 TMS proteins (Hille, 1992; Nelson et al., 1999).

The IRK-C family (TC #1.A.2) includes members resembling the 2 TMS VIC family members, and in this case there is enough sequence similarity to strongly suggest homology (Nelson et al., 1999). The RIR-CaC, TRP-CC, and PCC families (TC #1.A.3, 1.A.4, and 1.A.5, respectively) exhibit transmembrane domains topologically resembling those of the 6 TMS members of the VIC family. On this basis as well as their ion specificities and complex regulatory patterns, we suggest that these families may represent distant constituents of the VIC superfamily. However, this suggestion cannot be established on the basis of statistical analyses of their primary sequences.

The next two families listed in Table 1, the ENaC and ACC families (TC #s 1.A.6 and 1.A.7, respectively), do not exhibit enough sequence similarity between them to prove common descent. However, they may nevertheless share a common ancestry for the following reasons: (i) they are of the same size (about 600 residues), (ii) they exhibit very similar topologies with two TMSs and large cysteine-containing extracytoplasmic loops, and (iii) they are specific for cations. Similar arguments apply to the ligand-gated ion channel (LIC) and glutamate-gated ion channel (GIC) families (Tables 1 and 2; TC #s 1.A.9 and 1.A.10). Members of these latter two

families (i) exhibit the same unusual pentameric structure, (ii) serve as neurotransmitter receptors and (iii) exhibit varied and sometimes broad and overlapping ionic specificities.

All of the families discussed above are either ubiquitous (MIP and VIC) or restricted to animals. The symbiotic $NH_4^+$ channel transporter (SAT) family is one of the few recognized channel families restricted to a eukaryotic kingdom other than animals. Members of the SAT family occur only in plants. While the nonspecific cation channel family 1 (NSCC1) is animal specific, NSCC2 family members are found in both animals and yeast.

Of particular interest are the proton-transporting channel protein families, the influenza virus matrix-2 (VY2) channel (IVC) family and the oxidase-associated cytochrome $b_{558}$ (CybB) family. Proteins of the former family facilitate viral uncoating in endosomes of the host animal cell (Pinto et al., 1997). The M2 channel of influenza virus allows protons to enter the virus's interior where they weaken protein-ribonucleic acid interactions, thus promoting transcriptional activation of the viral genetic material (Pinto et al., 1997). The CybB proteins are NADPH oxidases in their C-terminal domains (the last 2/3rds of these proteins) and a 6 TMS proton channel in their N-terminal domain (the first 1/3rd of these proteins). The oxidase transfers electrons from NADPH in the cytoplasm to $O_2$ on the external side of the membrane, and the released protons cause membrane depolarization and cytoplasmic acidification (Henderson et al., 1997; Henderson, 1998). Efflux of $H^+$ through the channel provides charge compensation, preventing the fall in internal pH. Arachidonic acid, which allosterically activates the oxidase, simultaneously opens the channel. This system provides a remarkable example of how hybrid protein construction, fusing a channel domain with an enzyme domain, allows coordination of scalar and vectorial processes so as to circumvent the toxic consequences of the enzyme's action (Henderson et al., 1997; Henderson, 1998). Multiple homologues of these so-called "respiratory burst oxidases" found in animals are encoded within the genomes of plants such as *Arabidopsis thaliana* (Torres et al., 1998).

Only four channel protein families in addition to the LIC family discussed above, include members that selectively transport inorganic anions. The ubiquitous $Cl^-$ channel (ClC) family (TC #1.A.11) includes functionally characterized members that transport a variety of anions in addition to chloride (Foskett, 1998). The organellar ClC (O-ClC) family (TC #1.A.12) is distinct from the ClC family with members that exhibit different sizes, topologies and specificities (Table 2; Landry et al., 1993). Similarly, the epithelial ClC (E-ClC) family (TC #1.A.13) exhibits features that differ from those of both the ClC and O-ClC families (Ran & Benes, 1992; Agnel et al., 1999). Finally, the PLM family (TC #1.A.27) exhibits an unusually large range of transport substrates, being able to equilibrate a number of structurally dissimilar organic anions including lactate, glutamate, isothionate and gluconate as well as inorganic anions (Kirk & Strange, 1998).

**Table 3.** Substrate selectivites of bacterial outer membrane porins[1]

| Channels | Number of types | Example | TC # |
|---|---|---|---|
| Nonselective | 9 | OmpF of *E. coli* | 1.B.1.1.1 |
| Anions | 3 | Porin32 of *C. acidovorans* | 1.B.1.6.1 |
| Phosphate | 2 | PhoE of *E. coli* | 1.B.1.1.2 |
| Pyrophosphate | 1 | OprO of *P. aeruginosa* | 1.B.5.1.2 |
| Cationic compounds | 4 | OprD2 of *P. aeruginosa* | 1.B.25.1.1 |
| Heavy metals | 1 | CnrC of *A. eutrophus* | 1.B.17.2.1 |
| Nucleotides | 1 | NmpC of *E. coli* | 1.B.1.1.4 |
| Nucleosides | 1 | Tsx of *E. coli* | 1.B.10.1.1 |
| Monosaccharides | 1 | OprB of *P. aeruginosa* | 1.B.19.1.1 |
| Oligosaccharides | 2 | LamB of *E. coli* | 1.B.3.1.1 |
| Fatty acids | 1 | FadL of *E. coli* | 1.B.9.1.1 |
| Short chain amides and urea | 1 | FmdC of *M. methylotrophus* | 1.B.16.1.1 |
| Toluene | 1 | XylN of *P. putida* | 1.B.9.2.1 |
| Drugs | 1 | OpcM of *B. cepacia* | 1.B.17.3.3 |
| Iron-complexes | 2 | FepA of *E. coli* | 1.B.14.1.1 |
| Vitamins and cofactors | 2 | BtuB of *E. coli* | 1.B.14.3.1 |
| Complex carbohydrates | 3 | AlgE of *P. aeruginosa* | 1.B.13.1.1 |
| Proteins | 6 | PapC of *E. coli* | 1.B.11.2.1 |

[1] All of the porins listed are believed to function passively with the exception of the proteins of the OMR family (TC #1.B.14) which take up iron complexes and vitamin $B_{12}$ by a concentrative mechanism, accumulating the substrates in the periplasm of the Gram-negative bacterial cells.

Two families of bacterial-specific channel proteins, MscL and MscS, long recognized on the basis of electrophysiological studies (Martinac et al., 1987, 1990) include the well-characterized MscL protein of *E. coli* (Sukharev et al., 1996) and the relatively poorly characterized channels of the MscS family (Levina et al., 1999). The physiological functions of both family members concern rapid cellular adaptation to osmotic downshift (Levina et al., 1999). While MscL family proteins exhibit fairly uniform size and topological features, MscS family proteins are highly variable in this regard.

## Specificities of Bacterial Porins (β-type)

Table 3 presents the range of substrate specificities exhibited by β-barrel-type outer membrane porins of Gram-negative bacteria and provides an example of a porin exhibiting the stated specificity. A TC # is provided to allow easy access to the protein and its sequence

accession number (*see* our website). Many of these porins are either nonspecific or only moderately selective, or their physiological functions may be to export macromolecules, specific proteins or carbohydrates (*see* Table 1 and our website). However, various porins are known that serve the primary function of transporting a specific compound or group of compounds (Table 3). In some cases these porins exhibit fairly strict specificity, but in other cases the function specified may be dictated by the induction properties of the porin rather than by its actual transport capability. In many cases the range of substrates transported by a porin is not known.

Several porin types are anion-selective, and a few of these function to transport a specific anion such as phosphate or pyrophosphate. Others are specific for cationic compounds. However, porins are also known that specifically transport a range of organic compounds. Nutrient transport substrates include nucleosides and nucleotides, sugars, fatty acids, urea, and short chain amides (Table 3). Toxic hydrophobic substances (organic solvents and drugs) may be exported via other porins. Finally, specific outer membrane receptors recognize and transport iron-siderophore complexes and vitamin $B_{12}$ in a process that depends on the ExbBD-TonB protein complex. ExbBD probably serves as an $H^+$ channel in the cytoplasmic membrane that allows the pmf across this membrane to drive the active uptake of these iron scavengers and vitamin $B_{12}$ across the outer membrane (Locher et al., 1998; Larsen, Thomas & Postle, 1999). Accumulation of these molecules in the periplasm occurs against a greater than 1,000-fold concentration gradient. Presumably, the ExbBD-TonB protein complex provides a transmembrane proton transport pathway, and translocation of protons down the proton electrochemical gradient (the proton motive force or pmf) induces conformational changes in the outer membrane porin. This may possibly occur by opening and closing the channel in response to energy input in a fashion that allows accumulation of the substrate in the periplasm. The details of the mechanism by which outer membrane transport is energized remains one of the intriguing unanswered questions in membrane biology.

## Channel-Forming Proteins that Function in Biological Warfare

Table 4 lists a number of pore-forming protein and peptide families, the members of which prove toxic to a target cell other than the producer cell. Of the 14 protein toxin families listed, all but one, the yeast killer toxin K1 of *Saccharomyces cerevisiae*, are produced by bacteria. While most of these toxin types are designed to attack animal cells, colicins target bacteria other than the producer strain. This bacterial-specific toxin family is a large and well-defined family that includes a number of homologous pore-forming toxins produced by *E. coli* and its close relatives (Stroud et al., 1998). These proteins function to kill other closely related bacteria. The mechanism of killing is complex, involving recognition of an outer membrane receptor, translocation across the outer membrane in a pmf-dependent process, and formation of a channel in the cytoplasmic membrane (Braun, Pilsl & Gross, 1994; Cramer et al., 1995; Gonaux, 1997; Lazdunski et al., 1998; Stroud et al., 1998).

**Table 4.** Families of protein and peptide toxins (mostly from bacteria)[1]

| TC # | Family or superfamily | Approximate size (# aas) | # TMSs |
|---|---|---|---|
| Proteins | | | |
| 1.C.1 | Colicin | 600 | $(4?)_n$ |
| 1.C.2 | ICP | 500–1500 | $(6?)_n$ |
| 1.C.3 | αHL | 400 | $(2\beta)_7$ |
| 1.C.4 | Aerolysin | 400 | $(2\beta)_7$ |
| 1.C.5 | ε-toxin | 300 | ? |
| 1.C.6 | YKT-K1 (yeast) | 300 | ? |
| 1.C.7 | DT | 340 | $(2?)_n$ |
| 1.C.8 | BTT | 600 | $(2?)_n$ |
| 1.C.9 | VacA | 1290 | $(?)_{12}$ |
| 1.C.10 | HlyE | 305 | ? |
| 1.C.11 | RTX-toxin | 900–1700 | $(3)_n$ |
| 1.C.12 | TAC | 450–600 | ? |
| 1.C.13 | Ctx | 250 | ? |
| 1.C.14 | CHL | 600 | ? |
| Peptides | | | |
| 1.C.16–1.C.35 | Cationic amphipathic peptides (20 families) | 20–100 | (1 or 2)$_n$ |
| Depsipeptide | | | |
| 1.D.1 | Gramicidin | 15 | — |

[1] Families listed can be further identified by reference to Table 1 and our website. The abbreviations are: # aas, number of amino acyl residues; # TMSs, number of putative transmembrane α-helical spanners.

Insecticidal crystal proteins belonging to the ICP family are called δ-toxins. They resemble colicins in one important respect in that they are produced by a very limited range of bacteria (*Bacillus thuringiensis* strains), and they target very specific cells in a narrow range of organisms, most of which prove to be insects (Schnepf et al., 1998). Each δ-toxin is specific for a particular invertebrate or a narrow range of such organisms (Aronson, 1993; Knowles & Dow, 1993). Their phylogenetic characterization (Bravo, 1997) has resulted in a new proposal for naming the members of the ICP family (Crickmore et al., 1998).

α-Hemolysins (αHL; sometimes called β- or γ-toxins; TC #1.14; Song et al., 1996) and aerolysins (TC #1.15; Parker et al., 1996) are structurally (and probably evolutionarily) related toxins. They need to be activated by the animal hosts of the producing bacteria in sequential proteolytic processes that must precede assembly of the heptomeric complex and insertion into the mem-

brane. While αHLs are produced exclusively by Gram-positive bacteria, aerolysins are produced by both Gram-positive and Gram-negative bacteria as well as plants. The toxins produced by species of *Aeromonas* are among the best-characterized (Parker van der Goot & Buckley, 1996).

The ε-toxin family includes proteins produced exclusively by Gram-positive bacteria. Some are specific for mammals (Petit et al., 1997), but others target insects such as mosquitoes (Chan et al., 1996; Liu et al., 1996). Proteolytic processing by the host is a characteristic shared by these toxins as well as the α-hemolysins and aerotoxins discussed in the previous paragraph.

Diphtheria toxin (DT) and the botulinum and tetanus toxins (members of the BTT family) are exoneurotoxins that exert their effects cytoplasmically. They resemble each other in that they are secreted as single polypeptide chains but are cleaved to yield disulfide linked heterodimers (Binz et al., 1994; Kachel et al., 1998). The N-terminal domain of the heavy chain forms a channel in the animal cell membrane that serves as the translocation pathway for the toxic light chain (London, 1992; Binz et al., 1994; Lesieur et al., 1997; Montecucco, 1998 [1.25 & 1.35]).

Members of the cytohemolysin (CHL) family, all from Gram-negative bacteria, resemble other toxins discussed above in requiring extensive host-mediated proteolysis before activation becomes possible. The monomeric end product of proteolysis pentamerizes in the presence of cholesterol-sphingolipid-containing membranes to generate a water filled pore of about 1.5 nm diameter that lyses the cell (Alm Stroeher & Manning, 1988; Zitzer et al., 1999).

Several other families of bacterial toxins have been classified, but structural information is available only for some of the constituent proteins of these families (Lacy & Stevens, 1998). These families include the vacuolating cytotoxin (VacA) family (Czajkowsky et al., 1999), the hemolysin E (HlyE) family (Ludwig et al., 1999), the RTX toxin family (Westrop et al., 1997) and the thiol-activated cytolysin (TAC) family (Rossjohn et al., 1997). It is clear that many different types of toxins evolved independently of each other to form very different structural elements that serve the unified function of creating pores in target cells. The extent of this structural diversity will be an important topic of study for years to come.

## Holins

The Holin functional superfamily consists of at least sixteen distinct families of proteins that exhibit common structural and functional characteristics but which do not exhibit statistically significant sequence similarity between members of distinct families (*see* Tables 1 and 2 and our website). They are encoded within the genomes of Gram-positive and Gram-negative bacteria as well as those of the bacteriophage of these organisms (Young & Bläsi, 1995). The primary function of holins appears to be the transport of murein hydrolases across the cytoplasmic membrane to the cell wall where these enzymes hydrolyze bonds in the peptidoglycan cell wall polymer as a prelude to cell lysis. When chromosomally encoded, these enzymes are therefore autolysins. Holins may also facilitate leakage of electrolytes and nutrients from the cell cytoplasm, thereby promoting cell death (Martin López & García, 1998).

Although the amino acid sequences of holins are divergent, they generally exhibit (i) small sizes (60–145 amino acyl residues), (ii) two, three or four putative transmembrane spanners (TMSs), depending on the protein, each separated by a putative β-turn linker region, (iii) a hydrophilic N-terminus that is localized to the cytoplasm, and (iv) a highly polar, charge-rich C-terminal domain. The N- and C-terminal hydrophilic regions generally function to regulate holin-mediated channel formation (Barenboim et al., 1999; Bläsi et al., 1999).

Murein hydrolases lack N-terminal signal sequences, and therefore are not believed to be transported via the general secretory pathway (TC #3.A.5). Holins undoubtedly form homo-oligomeric complexes that generate pores in the cytoplasmic membrane. These pores are thought to provide a passive transport pathway for their substrate proteins. Whether or not they function in conjunction with other proteins is not known. If not, transport of substrate proteins such as the murein hydrolase is not likely to be driven by ATP hydrolysis as is true for many other protein export systems (TC #3.A.1; 3.A.5-3.A.7).

## Channel-Forming Amphipathic Peptides (CAPs)

Organisms from all three domains of life synthesize small proteins (or large peptides) which are degraded to even smaller hydrophobic or amphipathic, bioactive peptides (*see* Tables 1 and 2, Klaenhammer, 1993; Bechinger, 1997). Many of these peptides exhibit antibiotic, fungicidal, virucidal, hemolytic and/or tumoricidal activities by interacting with membranes and forming transmembrane channels that allow the free flow of electrolytes, metabolites and water across the phospholipid bilayer. They generally appear to function in biological warfare although other possibilities have been considered (Nes et al., 1996). There are many designations given to these bioactive peptides. They include the magainins, cecropins, melittins, defensins, bacteriocidins, etc., each from a different biological source. The proteins in each family within this functional superfamily are homologous, but they exhibit little or no significant sequence similarity with members of the other families. Thus, several of these families may have evolved independently. However, common structural features ob-

served between members of distinct families suggest that at least some of them share a common ancestry (Riley, 1998).

### Eukaryotic CAPs

Several families of eukaryotic CAPs, each from a different group of organisms, are recognized. These families will be listed below and briefly described.

#### *Family 1.C.16*

Magainins are a group of amphipathic peptides (21–26 amino acyl residues in length) from the skin and intestines of frogs. They form right-handed α-helical structures in membranes and serve bactericidal, fungicidal and virucidal functions (Matsuzaki, 1998). They thereby provide defense against infectious agents. As revealed by their synergistic behavior, they can form homo- and/or hetero-oligomeric transmembrane channels.

#### *Family 1.C.17*

Cecropins are produced by insects, particularly under conditions of infection. Cecropins A, B and D are close homologues consisting of 35–39 amino acyl residues (Gudmundsson et al., 1991). They are found in the pupae of the cecropin moth, but related homologues named lepodopteran, bactericidin, moricin and sarcotoxin are produced by other insects.

#### *Family 1.C.18*

Melittin (26 residues) is the best studied of the insect peptides toxins. It is found in the venom of the European honey bee, *Apis mellifera.* Three-dimensional structures of melittin have been elucidated (Terwilliger & Eisenberg, 1982; Bechinger, 1997).

#### *Family 1.C.19*

Defensins are produced by mammals. Their precursors vary in size (35–95 amino acyl residues). The active peptides have antibacterial, antifungal and antiviral activities. Three-dimensional structures of defensin-1 have been solved both by x-ray crystallography (1.9 Å resolution) and by NMR (Hill et al., 1991; Pardi et al., 1992).

### Nonribosomally Synthesized Depsipeptides

Alamethicin (TC #1.D.5; 20 residues), from the fungus, *Trichoderma viride*, is an α-methyl alanine-rich peptide that, like melittin, probably forms a flexible, oligomeric, helix-bend-helix structure. Bundles of such amphipathic helices are probably oriented with their hydrophilic faces pointing inwards to form the surfaces of the pores and the hydrophobic side chains interacting with the phospholipid membrane matrix. While this structural model is most widely accepted, other possibilities can be entertained (Marsh, 1996; Bechinger, 1997). A number of depsipeptides have been shown to form transmembrane channels (*see* Table 1).

### Prokaryotic CAPs

Bacteriocins are bacterially produced peptide antibiotics with the ability to kill a limited range of bacteria, usually but not always those that are closely related to the producer bacterium (Klaenhammer, 1993; Riley, 1998). Many of them exhibit structural features typical of members of the eukaryotic channel-forming amphipathic peptides. That is, they are usually synthesized as small precursor proteins or peptides which are processed with proteolytic elimination of their N-terminal leader sequences, and the resultant mature peptides form one, two or more putative amphipathic transmembrane α-helical spanners (TMSs). For those with two TMSs, a characteristic hinge region that separates the two putative transmembrane segments is usually present. A similar structural arrangement occurs for example in the two-TMS Cecropin A proteins (TC #1.C.17).

Many bacteriocins are encoded in operons that also encode an immunity protein and an ABC transport system (TC #3.A.1) with a protease domain at the N-terminus (Venema et al., 1995*a,b;* Martínez-Bueno et al., 1998). The ABC system exports the bacteriocin while the protease domain cleaves the N-terminal leader sequence. A few bacteriocins are exported via the type II general secretory pathway rather than by ABC-type export systems. In some cases, expression of the bacteriocin-encoding operon is induced by a bacteriocin-like peptide which acts in conjunction with a two component sensor kinase-response regulator to effect induction.

#### *Families 1.C.20–1.C.24*

Class I lantibiotic bacteriocins are small membrane-active channel-forming peptides of less than 5 kDa (Sahl & Bierbaum, 1998). They contain the unusual amino acids lanthionine and β-methyl lanthionine, as well as dehydrated residues. One member of family 1.C.22 is the thiol-activated peptide, Lactococcin B, included in Class IIc by Klaenhammer (1993).

#### *Families 1.C.24–1.C.26*

Class II non lanthionine-containing heat-stable bacteriocins are small membrane active peptides of less than 10 kDa characterized by a Gly-Gly$^{-1}$ Xaa$^{+1}$ processing site

in the bacteriocin precursor. Each one is processed by a protease domain that is covalently linked to the ABC-type bacteriocin export permease (e.g., Nes et al., 1996; TC #3.A.1.42.2). Mature bacteriocins are predicted to form amphipathic helices with varying amounts of hydrophobicity. Subgroups in Class II bacteriocins include:

*IIa, Family 1.C.24*

*Listeria*-active peptides exhibit a consensus sequence in the N-terminus of Y-G-N-G-V-X-C. Many other homologous peptides, not tabulated under TC #1.C.24, are also members of this family.

*IIb, Families 1.C.25 and 1.C.26*

Members of these families form poration complexes requiring two distinct peptides for activity (Allison Fremaux & Klaenhammer, 1994). They therefore form hetero-oligomeric complexes.

*IIc, Family 1.C.27*

Members of this family include Sec-dependent peptides requiring the Type II General Secretory Pathway (IISP; TC #3.A.5) for export.

Many CAP bacteriocins have been identified in addition to those tabulated here, but those listed are among the best characterized with respect to evidence for channel formation in target bacterial membranes. Class III and IV bacteriocins (Klaenhammer, 1993) are large heat-labile proteins that function by mechanisms unrelated to those of CAPs.

In addition to CAPs, several other channel-forming peptide toxins are produced by bacteria. Examples include colicin V, which is not a true colicin and probably functions as a bacteriocin (TC #1.C.31; Gilson Mahanty & Kolter, 1990), and well-characterized ion transporting substances that are synthesized enzymatically in bacteria such as the $K^+$ carrier Valinomycin and other ion-translocating channel-forming peptides such as syringomycin and syringopeptin (Bender Alarcón-Chaidez & Gross, 1999). Finally, archaea produce peptide toxins, but the modes of action of most of these agents are not well understood (Riley, 1998).

## Organismal Distribution of the Different Classes of Channel Proteins

Table 5 summarizes the distribution of recognized channel-forming proteins/peptides in the three domains of life. Of the currently recognized α-type channel families, most are specific to eukaryotes, a few are synthesized exclusively in bacteria, and a few are ubiquitous.

**Table 5.** Distribution of channel families in the three domains of life[1]

| Channels | # Families per group | | | | | | |
|---|---|---|---|---|---|---|---|
| | B | A | E | BA | BE | AE | BAE |
| Protein channels | 3 | 0 | **17** | 0 | 0 | 0 | **3** |
| Protein toxins | **12** | 0 | 1 | 0 | 1 | 0 | 0 |
| Peptide toxins | **10** | 0 | **4** | 0 | 0 | 0 | 0 |
| Holins | **11** | 0 | 0 | 0 | 0 | 0 | 0 |
| Porins | **22** | 0 | 1 | 0 | 0 | 0 | 0 |
| Total | **58** | 0 | **23** | 0 | 1 | 0 | **3** |

[1] This table includes all of the channel families that were included in the TC system as of March, 1999. Table 1 presents a more up-to-date tabulation of these systems (current as of December, 1999). Entries that are doubly underlined represent the major types of families that are bacterial- (B), archael- (A) or eukaryotic- (E) specific, or that are found in all three domains of life (BAE).

A more precise breakdown of these channel families is presented in Table 6. In addition to the three ubiquitous channel families (VIC, MIP, and ClC), another possible ubiquitous channel family is the MIT (metal ion transporter) family (TC #9.A.17) where the mode of transport is not well established (Kehres Lawyer & Maguire, 1998; Smith & Maguire, 1998; Smith et al., 1998*a*,*b*). These proteins exhibit only 2 or 3 TMSs suggesting a channel-type mode of action. Of the eukaryotic-specific α-type channel protein families, all but a few are restricted to animals and their viruses. The exceptions include the nonselective cation channel-2 (NSCC2) family which has representation in both animals and yeast, the NADPH oxidase-associated cytochrome $b_{558}$ (CybB) $H^+$-channel family with representation in plants and animals, and the plant-specific symbiotic ammonium transporter (SAT) family (Table 5).

β-Porin families are all restricted to Gram-negative bacteria except for the mitochondrial and plastid porin (MPP) family. All but a couple of the toxin families listed include members that are derived exclusively from bacteria. It should be noted, however, that probable channel-forming toxins have also been isolated from a variety of fungi and other eukaryotic pathogens.

## Channel-Forming Proteins and Peptides for Which High Resolution Structural Data are Available

Table 7 lists the channel-forming proteins for which high resolution structural data are available. In spite of the fact that membrane proteins are hard to crystallize, four of the five categories of channel-forming molecules presented in Fig. 1 are represented, the holins being the one exception. It should be noted that in some of these examples (Table 7), the crystallized species was a soluble form of the protein rather than the channel-forming, membrane-embedded conformer. These examples are

**Table 6.** Kingdom distribution of channel families (TC #1)[1]

| Channel type | Distribution | # Families | TC # |
|---|---|---|---|
| α-Proteins | Ubiquitous | 3 (or 4) | VIC, MIP, ClC (MIT) |
| | Bacteria | 2 | MscL, MscS |
| | Animals | 15 | IRK-C, RiR-CaC, TRP-CC, PCC, ENaC, ACC, LIC, GIC, O-ClC, E-ClC, NSCCl, Bcl-2, Connexin, Innexin, PLM |
| | Animal virus | 1 | IVC |
| | Animals and yeast | 1 | NSCC2 |
| | Animals and plants | 1 | CybB |
| | Plants | 3 | SAT, CSC, Tic110 |
| | Yeast | 1 | Mid1 |
| β-Porins | Bacteria | 25 | All except MPP |
| | Eukaryotes | 1 | MPP |
| Protein toxins | Bacteria | 13 | Colicin, CID, αHL, Aerolysin, ε-Toxin, DT, BTT, VacA, HlyE, RTX-Toxin, TAC, Ctx, CHL |
| | Yeast | 1 | YKT-K1 |
| | Protozoans | 1 | Amoebapore |
| | Animals | 1 | WSP |
| Holins (autolysins) | Bacteria | 15 | 1.A.28–1.A.43; see Table 1 |
| Peptide toxins | Bacteria | 12 | Nisin, Lecticin 481, Lactococcin A, Lactocin S, Pediosin, Lactococcin G, Lactacin X, Divergicin A, AS48, Plantaricin EF, Plantaricin JK, Colicin V |
| | Animals | 7 | Magainin, Ceropin, Melittin, Defensin, Mastoparin, Cathincidin, Tachyplesin |

[1] Full family names can be found in Table 1, and included proteins and their sequence accession numbers are available through our website.

**Table 7.** Channel proteins for which three-dimensional structural data are available

| TC # | Protein | Family | Source | PDB code |
|---|---|---|---|---|
| I α-type Protein Channels | | | | |
| 1.A.22.1.2 | Mechanosensitive channel | MscL | *Mycobacterium tuberculosis* | 1MSL |
| 1.A.1.1.1 | $K^+$ channel, KcsA | VIC | *Streptomyces lividans* | 1BL8 |
| 1.A.9.1.1 | Acetylcholine receptor | LIC | *Torpedo* electric organ | 3MRA |
| II Porins | | | | |
| 1.B.1.1.1 | Porin (OmpC) | GBP | *E. coli* | 1IIV (Theo.) |
| 1.B.1.1.2 | Porin (PhoE) | GBP | *E. coli* | 1PHO |
| 1.B.1.1.3 | Porin (OmpF) | GBP | *E. coli* | 1OPF |
| 1.B.3.1.1 | Maltoporin (LamB) | SP | *Salmonella typhimurium* | 1MAL |
| 1.B.6.1.1 | Porin (OmpA) | OOP | *E. coli* | 1BXW |
| 1.B.7.1.1 | Porin (PorCa) | RPP | *Rhodobacter capsulatus* | 2POR |
| 1.B.14.1.4 | FhuA ferrichrome receptor | OMR | *E. coli* | 1BY5 |
| III Protein Toxins | | | | |
| 1.C.1.1.1 | Colicin Ia | Colicin | *E. coli* | 1CII |
| 1.C.1.2.2 | Colicin E1 | Colicin | *E. coli* | 1COL |
| 1.C.2.1.1 | Cry 1Aa | ICP | *Bacillus thuringiensis* | 1CIY |
| 1.C.2.2.1 | Cry 3Aa | ICP | *Bacillus thuringiensis* | 1DLC |
| 1.C.3.1.1 | α-Hemolysin | αHL | *Staphylococcus aureus* | 7AHL |
| 1.C.4.1.1 | Aerolysin | Aerolysin | *Aeromonas hydrophila* | 1PRE |
| IV Peptide Toxins | | | | |
| 1.C.18.1.1 | Melittin | CAP | Bee venom | 2MLT |
| 1.C.19.1.1 | Defensin 1 | CAP | *Homo sapiens* | 1DFN |
| V Other | | | | |
| 1.A.21.1.1 | Apoptosis regular, Bcl-X(L) | Bcl-2 | *Homo sapiens* | 1MAZ |
| 1.D.1.1.1 | Gramicidin A | Gramicidin A | *Bacillus brevis* | 1GMK |

nevertheless included in our list because the information obtained from such protein structures allows much more concrete postulation of the probable pore-forming structure. Many of these structures have been discussed and analyzed by Lacy and Stevens (1998) and Sakai and Tsukihara (1998). Primary references and detailed discussions of the structures are provided in our website under each family listing (*see* the TC #s provided), as well as the two review references cited above. PDB code numbers provide easy access to the 3-dimensional protein structures tabulated in Table 7. In contrast to carriers and ATP-driven primary active transporters, channel proteins are reasonably well characterized from structural standpoints. It is important to note, however, that new structural types of channel proteins and peptides will undoubtedly emerge as additional structural data become available.

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## Note Added in Proof

While this manuscript was in press, Marini et al. (2000) provided convincing evidence that the soybean SAT1 protein, which had been characterized as an $NH_4^+$ channel on the basis of its ability to complement an $NH_4^+$ transport defect in a mutant strain of *Saccharomyces cerevisiae*, is not in fact an $NH_4^+$ channel protein, but instead is probably a transcription factor. SAT1 apparently restores $NH_4^+$ uptake in the yeast mutant strain by interfering with inhibition of one of the three $NH_4^+$ transporters of *S. cerevisiae*, Mep 3 (Marini et al., 2000). Mep3 is a member of the ammonium transporter (Amt) family (TC #2.A.49). TC #1.A.26 is therefore no longer assigned to the SAT family and has been reassigned to the plant plasmodesmata (PPD) family (*see* our website).

Considerable evidence is accumulating for the presence of multiple porins in the outer mycolate-containing membranes of certain high G+C Gram-positive bacteria. These bacteria include *Mycobacterium tuberculosis* (Senaratne et al., 1998, Kartmann et al., 1999), *Mycobacterium smegmatis* (Niederweis et al., 1999; Raynaud et al., 1999), *Mycobacterium bovis* (Lichtinger et al., 1999), *Nocardia farcinica* (Riess et al., 1998), *Nocardia asteriodes* (Riess et al., 1999) and *Rhodococcus erythropolis* (Lichtinger et al., 2000). One of these proteins is the OmpATb protein of *M. tuberculosis* which has been reported to be a member of the OmpA-OmpF porin (OOP) family (TX #1.B.6.1.3; *see* our website); MspA of *M. smegmatis*, another such protein, is a member of a novel family which we have called the mycobacterial porin (MBP) family (TC #9.B.24) (Niederweis et al., 1999). A third such protein is a partially sequenced protein from *Rhodococcus erythropolis* which we have provisionally specified as the *R. erythropolis* porin (REP; TC #9.C.3) (Lichtinger et al., 2000). The partial sequence available for this last-mentioned protein does not exhibit significant similarity to any sequence present in the current databases.

The available sequence data suggest that the outer membrane porins of Gram-positive bacteria will prove to belong to several distinct families. Although the few fully sequenced proteins currently available from mycolate-containing membranes have been placed under category 1.B (β-barrel porins), it should be noted that structural data are not yet available for any of these proteins. Consequently, these proteins may prove to be more appropriately assigned to a different category in the future.