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Microreview

The MACPF/CDC family of pore-forming toxins

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Summary

Pore-forming toxins (PFTs) are commonly associated with bacterial pathogenesis. In eukaryotes, however, PFTs operate in the immune system or are deployed for attacking prey (e.g. venoms). This review focuses upon two families of globular protein PFTs: the cholesterol-dependent cytolysins (CDCs) and the membrane attack complex/perforin superfamily (MACPF). CDCs are produced by Gram-positive bacteria and lyse or permeabilize host cells or intracellular organelles during infection. In eukaryotes, MACPF proteins have both lytic and non-lytic roles and function in immunity, invasion and development. The structure and molecular mechanism of several CDCs are relatively well characterized. Pore formation involves oligomerization and assembly of soluble monomers into a ring-shaped pre-pore which undergoes conformational change to insert into membranes, forming a large amphipathic transmembrane β-barrel. In contrast, the structure and mechanism of MACPF proteins has remained obscure. Recent crystallographic studies now reveal that although MACPF and CDCs are extremely divergent at the sequence level, they share a common fold. Together with biochemical studies, these structural data suggest that lytic MACPF proteins use a CDC-like mechanism of membrane disruption, and will help understand the roles these proteins play in immunity and development.

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Introduction

Pore-forming toxins (PFTs) are proteins that possess the ability to switch from a water-soluble form to a membrane-inserted pore form. Over 80 different families of PFTs have been characterized (Saier *et al*., 2006), these include short peptides and large globular proteins. PFTs constitute approximately one-third of all the characterized bacterial toxins (Alouf and Popoff, 2006), and are found across both Gram-positive and Gram-negative bacterial species (Parker and Feil, 2005, Aroian and van der Goot, 2007). Although PFTs have been considered traditionally as virulence factors contributing to bacterial invasion and infection, these molecules also play key roles in eukaryotes. Notable examples of eukaryotic PFTs include C9 and perforin, proteins of the immune system (Voskoboinik *et al*., 2006), and venoms used by sea anemones to kill prey (Nagai *et al*., 2002).

A remarkable aspect of PFTs is their ability to exist in both water-soluble and membrane-inserted forms. In the soluble form, the membrane spanning portions of the PFT are commonly stabilized by interaction with the core of the molecule (Tilley and Saibil, 2006). Pore formation thus often involves an extensive conformational change that permits the membrane spanning residues to insert into the lipid bilayer (Tilley and Saibil, 2006).

Pore-forming toxins are broadly classified into two groups depending upon whether membrane spanning is achieved using α-helices (α-PFTs, e.g. colicin) or β-strands (β-PFTs, e.g. perfringolysin O, PFO). While

extensive structural and mechanistic information is available for pore formation by various β-PFTs, the structure of the membrane-inserted form of an α-PFT remains to be determined.

In addition to conformational mobility, another key feature of many PFTs is their ability to self-assemble into doughnut-shaped oligomers (Tilley and Saibil, 2006). Together, oligomerization and conformational change can permit formation of pores that permeabilize membranes and aid processes such as bacterial pathogenesis, for example, through the transport of toxic proteinaceous agents (Tweten, 2005; Aroian and van der Goot, 2007). Depending on the toxin, the pore diameter may vary from 1 to 50 nm (Parker and Feil, 2005; Aroian and van der Goot, 2007).

In this review we focus on the species distribution, structure and mechanism of one of the largest families of β-PFTs, the membrane attack complex/perforin/cholesterol-dependent cytolysin (MACPF/CDC) superfamily.

Cholesterol-dependent cytolysins in bacterial pathogenesis

Pathogenic Gram-positive bacteria such as *Clostridium perfringens*, *Bacillus anthracis* and *Streptococcus pneumoniae* produce CDCs to aid tissue or cell invasion (Table 1). The majority of characterized CDCs are secreted toxins. Exceptions include pneumolysin (PLY), which lacks an N-terminal secretion signal. In many *Streptococcus pneumoniae* strains, it is hypothesized that PLY is released via autolysin mediated bacterial autolysis. However, studies on the WU2 strain reveal release of PLY in the absence of autolysis, suggesting an unconventional secretion mechanism is responsible for toxin release in this strain (Balachandran *et al*., 2001).

Most CDC-releasing bacteria identified to date are extracellular pathogens (of either humans or insects) that release their respective CDCs in the extracellular environment. However, at least two pathogens release their CDC (listerolysin O, LLO) inside host phagocytic cells (*Listeria monocytogenes* and *Listeria ivanovii*).

Cholesterol-dependent cytolysins perform a multitude of functions in bacterial infection. For example, these toxins disrupt plasma membranes causing cell death by necrosis (PFO) and facilitating bacterial invasion, or disrupt endosomal or phagosomal membranes to release bacteria into the interior of the cell (LLO). In addition to their pore-forming properties, many CDCs possess pro-inflammatory properties that enhance tissue damage at the site of infection (Cockeran *et al*., 2003). Further, certain CDCs possess the ability to kill cells through alternative mechanisms; for example, in bacterial meningitis caused by *Streptococcus pneumoniae*, PLY has recently been shown to form pores in the mitochrondria of neurons, activating cell death pathways (Braun *et al*., 2007). Table 1 summarizes the role of the known CDCs identified to date.

The structure and membrane insertion mechanism of CDCs

The first structure of a CDC family member, PFO (Rossjohn *et al*., 1997), revealed a flat molecule comprising a box-shaped N-terminal domain [originally annotated as three non-contiguous domains (I–III)] connected to a C-terminal Ig domain (domain 4) (Fig. 1A). An unusual feature of the N-terminal CDC domain is a central four-stranded β-sheet containing a 90° bend at its centre. Two clusters of α-helices [termed transmembrane helices (TMH) 1 and 2] are located at the base of this sheet and are suggested to be responsible for membrane penetration (Shepard *et al*., 1998; Shatursky *et al*., 1999; Fig. 1A). The first cluster of α-helices, TMH-1, is loosely sandwiched between the central β-sheet and the stalk-like β-sheet that links the N-terminal CDC domain to the C-terminal Ig domain, while the second cluster of α-helices (TMH-2) is more solvent exposed. Extensive biophysical and cryo-electron microscopy (cryo-EM) studies suggest that both clusters of α-helices unwind and adopt an amphipathic β-strand conformation in the membrane (Fig. 1B; Shepard *et al*., 1998; Shatursky *et al*., 1999; Tilley *et al*., 2005).

Initial interaction with the membrane, oligomerization and conformational change

The current model for CDC mechanism comprises an initial recognition event at the cell membrane via the C-terminal Ig domain. This is followed by lateral diffusion and association with other CDC molecules to form a pre-pore oligomer (Soltani *et al*., 2007). Cryo-EM and biophysical studies suggest that oligomerization occurs via edge strand hydrogen bonding between the central four-stranded β-sheet of each monomer, i.e. the fourth β-strand of one monomer interacts with the first β-strand of the next (Ramachandran *et al*., 2005; Tilley *et al*., 2005; Tweten, 2005). Following formation of the pre-pore, a triggering event transmitted from the Ig domain to the N-terminal domain permits conformational change and insertion of the transmembrane regions into the membrane. The result of this concerted activity is a giant β-barrel lined pore spanning the membrane. The precise nature of the conformational change remains unclear; however, cryo-EM studies reveal that the N-terminal domain undergoes a significant 'collapse' that brings TMH-1 and TMH-2 in close proximity to the membrane (Tilley *et al*., 2005).

Initially, it was proposed that cholesterol functions as a general receptor for CDCs (hence the family name). For

Table 1. List of current identified members of the CDC subclass.

Species	Toxin	Toxin abbreviation	Accession code/PDB ID	Functions in disease
Arcanobacterium pyogenes	Pyolysin	PLO	AAC45754	Cytotoxic for murine peritoneal macrophages and J774 cells (dose dependant) (Jost *et al*., 1999)
Bacillus anthracis	Anthrolysin O	ALO	EDT69040	Kills human neutrophils, monocytes and macrophages (Mosser and Rest, 2006)
Bacillus cereus	Cereolysin	CLO	O45105	Uncharacterized
Bacillus sphaericus	Sphaericolysin		BAF96950	Damage to the ganglia of German cockroaches (*Blatetela germanica*) (Nishiwaki *et al*., 2007)
Bacillus thuringiensis	Thuringiolysin	TLO	BT9727_3096*	Uncharacterized
Brevibacillus laterosporus	Laterosporolysin	LSL	–	Uncharacterized
Clostridium bifermentans	Bifermentolysin	BFL	–	Uncharacterized
Clostridium botulinum	Botulinolysin	BLY	–	Evidence that coronary vasconstriction is triggered by BLY causing cardiac dysfunction, leading to systemic hypertension and death in rat model (Sugimoto *et al*., 1997)
Clostridium chauvoei	Chauveolysin	CVL	–	Uncharacterized
Clostridium histolyticum	Histolyticolysin O	HTL	–	Uncharacterized
Clostridium novyi A (oedematiens)	Novyilysin	NVL	–	Uncharacterized
Clostridium perfringens	Perfringolysin O	PFO	P0C2E9/1PFO, 1M3I, 1M3J	Promotes dysfunctional human PMN/endothelial cell adhesion contacts and vascular leukostasis. Inhibits human PMN chemostasis and primes leukocytes for increased respiratory burst (Ellemor *et al*., 1999)
Clostridium septicum	Septicolysin O	SPL	–	Uncharacterized
Clostridium sordellii	Sordellilysin	SDL	–	Suggestion that severity of *C. sordellii*-associated disease may be related to the expression of SDL or lethal toxin (TcsL) (Voth *et al*., 2006)
Clostridium tetani	Tetanolysin	TLY	NP_782466	Observed lysis of rabbit lysosomes in a suspension of the large granule fraction of rabbit liver (Cox *et al*., 1974)
Gardnerella vaginalis	Vaginolysin	VLY	EU522486–EU533488	Species-specific lysis dependant on CD59. Activates p38 mitogen-activated protein kinase pathway, induces IL-8 production by human epithelial cells (Cox *et al*., 1974).
Listeria ivanovii	Ivanolysin	ILO	P31831	Intracellular release of ILO – Does not induce IFN-γ (Kimoto *et al*., 2003)
Listeria monocytogenes	Listeriolysin O	LLO	P13128	Intracellular release of LLO: Suppression of phagocytosis by murine macrophages. Induces the expression of IL-1α, IL-12, IFN-γ, IL-8, macrophage chemotaxis protein 1, adhesion molecules on the surface of human epithelial cells. Activates NF-κB (Kayal *et al*., 1999)
Listeria seeligeri	Seeligeriolysin O	LSO	CAA42996	Strongly induces IL-12 but not IFN-β induces IFN-γ in naïve spleen cells. Requires Toll-like receptor 2 and 4 for signalling (Ito *et al*., 2005)
Paenibacillus alvei	Alveolysin	ALV	P23564	Induced IL-8 expression in human polymorphonuclear leukocyte, lymphocyte, monocyte and basophil cell populations (Konig *et al*., 1994)
Streptococcus canis	Streptolysin O	SLO	Q53957	Uncharacterized (DeWinter *et al*., 1999)
Streptococcus dysgalactiae (ssp. *equisimilis*)	Streptolysin O	SLO	Q54114	Uncharacterized (Gerlach *et al*., 1993)
Streptococcus intermedius	Intermedilysin	ILY	BAA89790/1S3R	Specific for human CD59 (Giddings *et al*., 2004) Essential for *S. intermedius* infection of HepG2 cells (Sukeno *et al*., 2005)
Streptococcus pneumoniae	Pneumolysin	PLY	P0C2J9/2BK1, 2BK2 (28 Å resolution cryo EM)	Directly activates the complement cascade, induces IL-1β and TNFα expression from human monocytes. Inhibition of immunoglobulin production and proliferative response from human lymphocytes, as well as of the bactericidal activity of PMNs and monocytes (Alouf and Popoff, 2006)
Streptococcus pyogenes	Streptolysin O	SLO	P0C0I3	Inhibits chemotaxis and mobility of human PMNs. Induces expression of IL-1β, IL-6 and IL-8 and release of prostaglandin E2 from human keratinocytes. Induces expression of IL-1β and TNFα expression from human monocytes (Ruiz *et al*., 1998)
Streptococcus suis	Suilysin	SLY	CAC94852	Able to lyse epithelial cells. Possible mechanism for entry into bloodstream and brain microvascular endothelial cells leading to increased blood–brain barrier permeability and phagocytosis (Vanier *et al*., 2004)

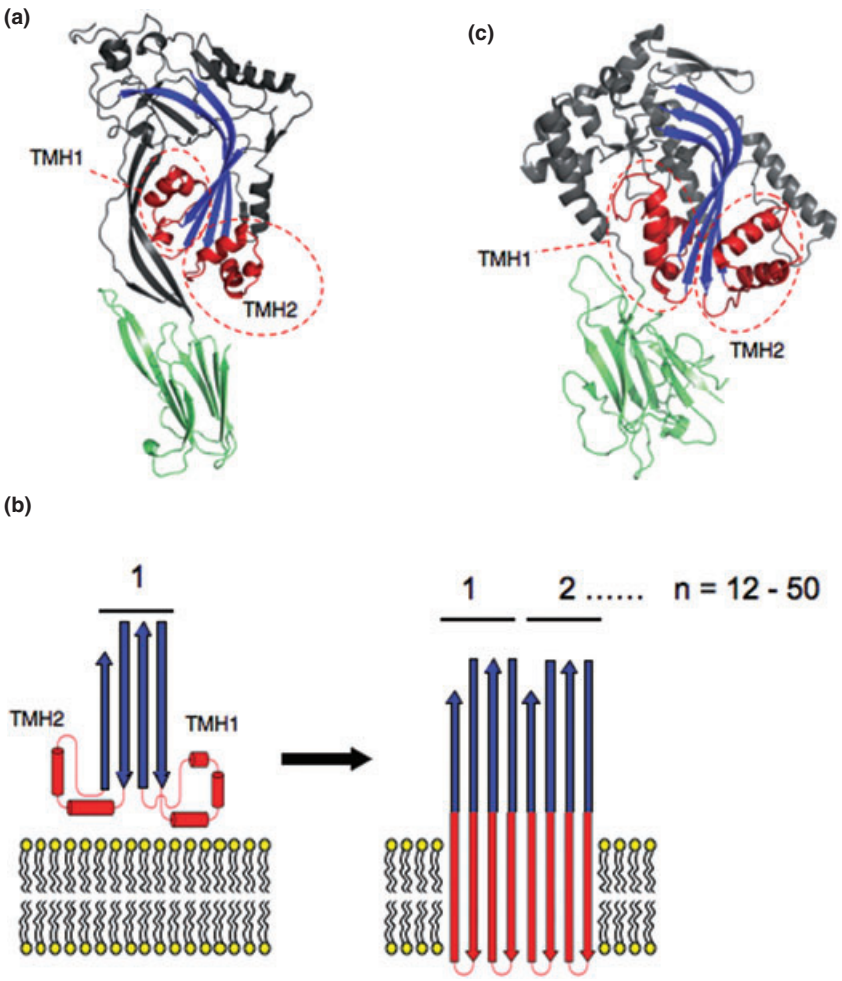

Fig. 1. A. The structure of perfringolysin O [PDB identifier: 1PFO (Rossjohn *et al*., 1997)]. The central β-sheet that contains a 90° bend is in blue. The two transmembrane regions TMH1 and TMH2 are in red and are labelled. The C-terminal Ig domain is in pale green.
B. Schematic showing the molecular mechanism of CDC membrane insertion. The two clusters of α-helices (red cylinders) unwind and insert into the membrane as β-sheets.
C. X-ray crystal structure of Plu-MACPF [PDB identifier 2QP2 (Rosado *et al*., 2007)]. Colouring is as for Fig. 1A, with the central β-sheet in blue and the two clusters of α-helices corresponding to TMH1 and TMH2 labelled. The location of the binding site for CD59 on C8α and C9 is at the TMH2 region.

PFO, biochemical data reveal that cholesterol may function as both a receptor and a trigger for the transition from the pre-pore to the pore conversion. However, recent studies using cholesterol-depleted human red blood cells have revealed that initial membrane interaction and pre-pore assembly of streptolysin O (SLO) and intermedilysin (ILY) does not require the presence of cholesterol (Giddings *et al*., 2003; Soltani *et al*., 2007). For ILY, it has been shown that CD59 functions as a glycoprotein receptor that recruits the CDC to the membrane surface and that cholesterol is instead required to trigger the conformational transition of the pre-pore to the pore form (Giddings *et al*., 2004). Further experiments on ILY demonstrated that a highly conserved region of the Ig domain, the undecapeptide, is not responsible for binding cholesterol-rich membranes. Instead, it was demonstrated that loops adjacent to the undecapeptide loop in ILY, called L1–L3, were responsible for membrane interactions (Soltani *et al*., 2007).

Structural studies on CDCs have been hampered by their conformational mobility as well as the tendency of these molecules to form oligomers of varying size. Thus much remains to be understood in regards to the mechanism of pore formation by CDCs. Most importantly, the molecular details of the interaction between CDCs and membrane receptors and a precise picture (at atomic resolution) of the conformational change in the family need to be resolved.

Membrane attack complex/perforin-like proteins

In comparison to bacteria, PFTs from eukaryotes are relatively understudied (Smyth and Trapani, 1998; Trapani, 1998). One of the largest mammalian family of PFTs is the MACPF superfamily; so named because of a domain common to proteins of the mammalian membrane attack complex (MAC) and perforin (PF) (Tschopp *et al*., 1986). These molecules perform crucial roles in the defence against bacterial and viral infection as well as in tumour surveillance (Smyth *et al*., 2000; Voskoboinik *et al*., 2006).

Initially identified in the late 19th Century as a lytic factor in blood, the terminal components of complement (C5b, C6, C7, C8α-β-γ and C9) assemble on the surface of Gram-negative bacteria and protozoan pathogens such

as *Leishmania major* to form a large multi-protein complex called the MAC. Complement-associated bacterial cell lysis is optimally achieved in the presence of lysozyme, which hydrolyzes components of the bacterial cell wall (Martinez and Carroll, 1980). However, even in the absence of lysozyme complement pore formation can result in cell death through alternative non-lytic pathways (Martinez and Carroll, 1980). Consistent with these data, deficiency of MAC components results in an increased susceptibility to infection by Gram-negative bacteria such as *Neisseria gonorrhoeae.*

C6, C7, C8α, C8β and C9 all contain a common region called the MACPF domain. Upon binding of C7 to the C5bC6 complex, forming the C5b-7 complex, there is interaction with the surface of the bacteria via C7 through an as yet uncharacterized mechanism. Recruitment of the C8 complex (which comprises the MACPF components C8α and C8β together with the lipocalin C8γ) is followed by membrane insertion of the C8α component (Muller-Eberhard, 1986). Finally, the C5b-8 complex recruits and permits pore assembly by the final component C9 (Bhakdi and Tranum-Jensen, 1978). EM studies also revealed that C9 undergoes conformational change from a ellipsoid to an elongated torus (DiScipio and Berlin, 1999).

Biochemical studies on C8α and C9 have revealed that the MACPF domain is required for membrane insertion and pore formation respectively (Steckel *et al*., 1983). Importantly, all host cells express the MAC inhibitor CD59 that inhibits the membrane inserting activity of C8α and C9, preventing inadvertent lysis of host cells. Deficiency of CD59 can result in an overactivity of complement, uncontrolled host cell lysis and development of paroxysmal nocturnal haemoglobinuria (Walport, 2001).

In 1984, perforin was characterized as a lytic PFT produced by natural killer cells and cytotoxic T lymphocytes (Henkart *et al*., 1984; Podack and Konigsberg, 1984). Perforin is stored in cytoplasmic secretory granules and is released on contact to kill virus-infected or transformed cells (Voskoboinik *et al*., 2006). Perforin itself is able to lyse and kill cells by necrosis; however, it also permits delivery of pro-apoptopic proteases (granzymes) into the target cell (Shiver *et al*., 1992, Bolitho *et al*., 2007). Two competing models for granyzme delivery by perforin have been proposed: diffusion of the granzyme through a plasma membrane perforin channel, versus coendocytosis of perforin and granzyme with subsequent disruption of an endosome membrane by perforin to release the granzyme. However, the precise molecular mechanism remains to be understood.

Congenital perforin deficiency results in the commonly fatal immunoregulatory disease of infants, familial haemophagocytic lymphohistiocytosis (FHL) (Voskoboinik *et al*., 2004; 2005). Affected individuals suffer from massive accumulation of $CD8^+$ T cells in organs and a cytokine-storm mediated immunoproliferative disorder that commonly results in severe tissue damage. Currently, the only effective treatment for severe recurrent FHL is a bone marrow transplant (Jabado *et al*., 1997). Overactivity of perforin also results in disease; for example, perforin is critical for the destruction of insulin-producing pancreatic β-islet cells in the NOD mouse model of Type I diabetes (Kagi *et al*., 1997).

MACPF proteins are eukaryote CDCs: implications for function and dysfunction

In the absence of structural information, and based upon bioinformatic studies, it was originally proposed that C9 and perforin insert into membranes using two predicted amphipathic α-helices that map to the most conserved region of the MACPF domain (residues 292–333 of the human C9 sequence; Peitsch *et al*., 1990). Therefore, it was postulated that C9 and perforin belonged to the α-PFT class of toxins. Several recently determined structures challenge this hypothesis. The X-ray crystal structure of Plu-MACPF (a MACPF domain containing protein from *Photorhabdus luminescens*; Rosado *et al*., 2007; Fig. 1C), the MACPF domain of C8α (Hadders *et al*., 2007) and the MACPF domain of C8α in complex with C8γ (Slade *et al*., 2008) reveal that the MACPF domain is homologous to the N-terminal portion (domains I–III) of CDCs (Fig. 1C). In particular, conservation of a complex core fold, including the membrane spanning clusters of helices (TMH1-2 or CH1-2) suggests strongly that MACPF proteins and CDCs form pores using a analogous mechanism (Fig. 2). Interestingly, it appears that highly conserved MACPF signature sequences (Ponting, 1999) map to regions that may be crucial for controlling conformational change and

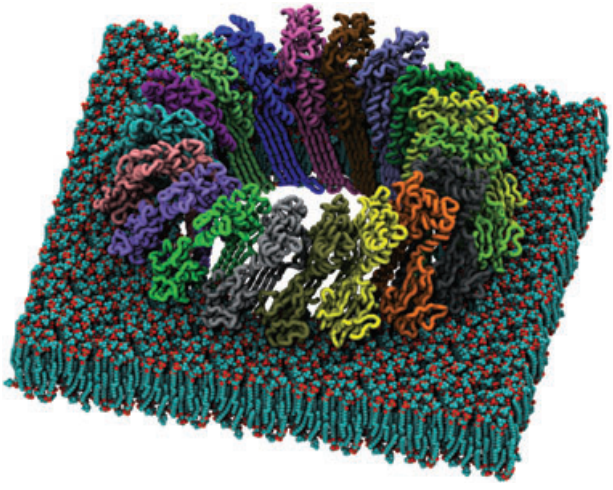

Fig. 2. Model of the pore form of a MACPF proteins in a lipid bilayer (using the Plu-MACPF structure as a template, PDB ID: 2QP2).

unfurling of the membrane spanning regions for the MACPF subclass of the MACPF/CDC family of proteins.

The structural data provide a framework upon which we can start to understand disease-linked variants of perforin (Rosado *et al*., 2007). In a previous study, the rather puzzling observation was made that many disease-linked variants of perforin map to two regions that are most poorly conserved across the superfamily (Voskoboinik *et al*., 2006). The structural data reveal that these two regions comprise the putative perforin transmembrane sequences. These data present an exception to the rule that functional regions of proteins generally contain the highest degree of sequence conservation. A possible reason for this apparent paradox is that membrane spanning regions depend on physiochemical properties (hydrophobicity), rather than absolute sequence. Furthermore, it is important to note that not all MACPF proteins insert into membranes (e.g. C6) and thus do not require amphipathic sequences in TMH1-2/CH1-2. Indeed, many members of the family may play important non-lytic functions that are discussed at the end of this review.

The structural data also provide an explanation for the control of C8α and C9 by the host cell factor CD59. The CD59 binding site on both proteins maps to the second putative transmembrane sequence TMH-2/CH-2 (Fig. 1C). Thus, it is suggested that CD59 controls MAC function by directly interfering with the assembly of the transmembrane pore (see Fig. 1C).

The role of C-terminal domains in MACPF function

All CDCs characterized to date contain a C-terminal Ig domain that is critical for interacting with lipid or protein cofactors. Further, this domain is of key importance for triggering conformational change in the N-terminal lytic CDC domain [domains I–III (Polekhina *et al*., 2005)]. Bioinformatic studies reveal that MACPF proteins are also found in concert with one or more C-terminal domains. However, rather than the common Ig domain found in CDCs, a wider variety of C-terminal domain folds are represented in the MACPF branch of the family. For example, perforin contains an EGF-like domain followed by a C2 domain; C8α contains an EGF-like followed by a thrombospondin type 1 domain; mammalian-derived C9 contains an EGF-like domain and Plu-MACPF contains a β-prism domain (Rosado *et al*., 2007). The structure of Plu-MACPF (which appears non-lytic, but binds to membranes) reveals that the β-prism domain is similarly located to the Ig domain of the CDCs. Studies on perforin have revealed that the C2 domain is responsible for initial interaction with the membrane (Voskoboinik *et al*., 2006). Thus for perforin, we propose that C-terminal domain may perform a similar role to the Ig domains of CDCs by interacting with lipids or protein receptors and triggering conformational change and membrane insertion (Fig. 2). However, it is clear that the C-terminal domains of other MACPF proteins perform roles distinct from membrane interaction. For example, the C-terminal domains of C8α are not essential for formation of a functional MAC (Scibek *et al*., 2002; Slade *et al*., 2006).

The broader MACPF proteins family in defence and attack

The development of powerful informatic tools such as PSI-BLAST now permits the identification of a large number (> 500) of MACPF proteins. Predictably, many of these proteins appear to be involved in immune defence or attack (Table 2). Notably, in plants the MACPF protein constitutively activated cell death-1 (CAD-1) is important for defence against bacterial infection. Interestingly, CAD-1 knockouts result in an overactivity of the plant immune response (Morita-Yamamuro *et al*., 2005).

Several organisms use MACPF proteins as weapons of attack. For example, sea anemone venom contains haemolytic MACPF proteins and the malaria parasite uses two MACPF proteins to invade the mosquito midgut and to breach the liver sinusoidal membrane (Ishino *et al*., 2004; Kadota *et al*., 2004).

A variety of pathogenic bacteria produce MACPF proteins; indeed Plu-MACPF from the insect pathogenic enterobacteria *P. luminescens* proved useful for structural studies. It remains to be understood whether these bacterial MACPF domain-containing proteins have pore-forming functions in pathogenesis.

MACPF proteins in development

Interestingly, several MACPF proteins have been identified that may play roles in development rather than immune defence or attack. In most of these cases it is not yet clear whether the MACPF protein has a lytic or non-lytic function.

In insects, Torso-like protein (Tsl) is maternally secreted at the anterior and posterior poles of the oocyte. Through an as yet uncharacterized mechanism, Tsl secretion results in Trunk-mediated activation of the Torso receptor tyrosine kinase. Torso-signalling results in development of anterior and posterior structures. Accordingly, Tsl knockouts are embryonically lethal (Stevens *et al*., 1990).

The sea urchin protein, apextrin, was initially identified in secretory vesicles within eggs (Haag *et al*., 1999). Closely related molecules have been subsequently been identified in several other sea urchin species, Cnidaria [hydrazoans (*Hydra magnipapillata*) and corals (*Acropora millepora*)] (Miller *et al*., 2007). Haag and colleagues initially postulated that the role of apextrin was in cell adhesion in developing embryos (Haag *et al*., 1999). However,

Table 2. List of current identified members of the MACPF subclass.

MACPF subclasses	Common names	Description of expression pattern and function
The following subclasses contain proteins with demonstrated lytic activity:		
C9-like	C6, C7, C8α, C8β, C9	Vertebrate membrane attack complex (MAC) Roles within the MAC: C9 – membrane insertion, pore formation and lytic activity C8α, C7 – role in anchoring the MAC to the target membrane C6 and C8β – no detected ability to insert into membranes (Muller-Eberhard, 1986)
Perforin-like	perforin	Released from granules within natural killer and cytotoxic T lymphocytes to lyse targeted cells in the immune response (Voskoboinik *et al*., 2006).
Sea anemone toxins	PsTX-60A PsTX-60B AvTX-60A	Haemolytic toxin released from the sea anenome nemocysts to kill prey. Species include *Phyllodiscus semoni* and *Actineria villosa* (Oshiro *et al*., 2004).
The following proteins have not been demonstrated to have lytic activity:		
Apextrin	apextrin	Located in secretory vesicles in sea-urchin eggs, Apextrin becomes localized to the apical extracellular matrix upon fertilization of the cells in the blastula (Haag *et al*., 1999) Upregulation upon bacterial infection in amphioxus (Huang *et al*., 2007)
Astrotactin	Astrotactin-1 Astrotactin-2	Astrotactin-1 required for neuronal cells migration along glial fibres, possibly neuronal adhesion molecules (Zheng *et al*., 1996)
Chlamydia proteins	–	Hypothetical proteins of *Chlamydia trachomatis, Chlamydophila pneumoniae* and *C. muridium* (Ponting, 1999)
Cyano-bacteria	–	Hypothetical protein of *Trichodesmium erythraeum* (cyanobacteria)
DBCCR/BRINP	DBCCR1 (BRINP1), DBCCR1-like protein 1 (BRINP3), DBCCR1-like protein 2 (BRINP2)	DBCCR-1, deleted in bladder cancer candidate region-1 gene, tumour suppressor gene commonly deleted in bladder cancer. Overexpression of DBCCR-1 suppresses tumour cell growth. Involved in neuronal development (Motomiya *et al*., 2007) Also referred to as FAM5 family of proteins.
EPCS50	EPCS50	EPCS50 expressed in the trophoblast upon implantation of the murine embryo (Hemberger *et al*., 2000)
Fungal proteins	SpoC1-C1C	Expressed during maturation of the conidia (specialized organ for asexual reproduction) of *Emericella nidulans*, mRNA levels drop upon germination (Stephens *et al*., 1999)
Malarial proteins	SPECT2 and MAOP	SPECT2 and MAOP are essential for parasite invasion into the human liver (Ishino *et al*., 2004) and the mosquito host (Kadota *et al*., 2004) respectively
MPS	MPS, MPG	*Macrophage Proliferation-specific Gene-1* detected in differentiated macrophages (Spilsbury *et al*., 1995)
Plant proteins	CAD1	*Arabidopsis thaliana* CAD1 involved in plant immune response (Morita-Yamamuro *et al*., 2005)
Plu-MACPF	Plu-MACPF	Hypothetical protein from the bacteria, *Photorhabdus luminescens* (Rosado *et al*., 2007)
Tsl	Tsl	Torso-like protein (Tsl) from *Drosophila melanogaster,* is hypothesized to activate the receptor, Torso, via the protein Trunk (Stevens *et al*., 1990)

more recent experiments in amphioxus (lancelet) suggest that apextrin may also play a role in immune defence against bacterial infection (Huang *et al*., 2007).

In mammals the large (over 1000 amino acids) proteins astrotactin-1 and -2 play important roles in neural development. Astrotactin 1 is hypothesized to be a neuronal adhesion molecule (Zheng *et al*., 1996) and precursor neuronal cells in the cerebella cortex of humans and mice produce astrotactin-1 in order to migrate along glial fibres. Targeted disruption of astrotactin-1 gene in mice resulted in mice with cerebella that were 10% smaller and reduction in the ability of granule cells to migrate (Adams *et al*., 2002). Similarly human deleted in bladder cancer candidate region-1 (DBCCR-1)/BMP/RA inducible neural-specific protein-1 (BRINP-1) is also thought to play a role in neural development (Kawano *et al*., 2004). Other data also suggest this latter molecule is a tumour suppressor that may modulate the cell cycle. Finally, studies on the mammalian MACPF protein EPCS50, reveal that this protein is produced in the developing trophoblast. It is suggested that this molecule may be involved in trophoblast invasion of the uterine lining (Hemberger *et al*., 2000).

Concluding statements

Recent structural studies have permitted the unification of CDC and MACPF proteins as a single superfamily and thus suggest that MACPF proteins and CDCs share a common mechanism of oligomerization and pore formation. However, important distinctions between the families remain to be understood. Most notably, while all CDCs appear to function as lytic toxins, the same cannot be said for MACPF proteins. Molecules such as C6 and C8β appear non-lytic and perhaps function as scaffolds and/or regulators of lytic molecules such as C9.

Interestingly, several members of the MACPF family appear to perform novel roles in development. While intriguing, a role for proteins of the same family in immunity or development is not unprecedented. For example,

members of the Toll-like receptor family play well-defined roles in fly development and mammalian immunity.

Many unanswered questions remain about the MACPF/CDC family. In particular, the absence of a high-resolution structure of either a MACPF or a CDC in the pore form precludes an understanding of the fine details of the conformational re-arrangements that these remarkable proteins undergo. Future structural and biochemical studies will no doubt start to shed light on these processes as well as addressing the role of lytic or non-lytic MACPF proteins in development.

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