How Cholesterol-Dependent Cytolysins Bite Holes into Membranes

In a showpiece for electron microscopy (EM), Tilley et al. (2005) used single-particle cryo-EM to visualize the structural rearrangements in the bacterial toxin pneumolysin that occur when it assembles into a membrane-associated prepore and when the prepore subsequently transitions into a fully membrane-inserted pore.

The lipid bilayer defines a cell and allows the cell to adjust the inside conditions to its precise needs. When the integrity of a membrane is compromised, the consequences for the affected cell are usually lethal. Thus, bacterial toxins have evolved that cause cell death by forming transmembrane pores, but, conversely, pore formation has also become a tool of the immune system to attack invading microorganisms. Pores are employed to release cytochrome c from mitochondria in apoptotic cell death, and, more recently, amyloid pores have been implicated in neuronal cell death in neurodegenerative diseases.

The most progress in elucidating the mechanism of pore formation has been made with pore-forming toxins (PFTs). Here, pore formation entails an initial oligomerization of water-soluble monomers into a membrane bound prepore that subsequently inserts into the membrane. PFTs can be classified into \( \alpha \)-PFTs, which use \( \alpha \) helices to form pores, and \( \beta \)-PFTs, which form transmembrane \( \beta \) barrels (reviewed in Parker and Feil, 2005). The cholesterol-dependent cytolysins (CDCs) are among the most widely distributed \( \beta \)-PFTs. CDCs attack cholesterol-rich membranes and form very large oligomeric transmembrane pores. The crystal structure of a CDC, perfringolysin, has been determined in its soluble, monomeric form, revealing four \( \beta \) sheet-rich protein domains (Rossjohn et al., 1997). Domains 1, 2, and 4 are arranged in a linear fashion, whereas domain 3 is nestled against domains 1 and 2 (Figure 1A). Now, how exactly can this soluble protein punch a hole into a membrane?

Obtaining structural information for a fully assembled CDC pore is a much more daunting task than the structural analysis of the soluble, monomeric form. Because the pore is a membrane-embedded structure and contains a large and variable number of subunits, the chances of growing well-ordered three-dimensional crystals are quite poor, discouraging X-ray crystallographic studies. Saibil and colleagues then pursued an alternative approach and succeeded in obtaining first structural information on a CDC pore by cryo-EM studies of pneumolysin (Gilbert et al., 1999). This study was, however, limited to the structure of helical assemblies and the membrane bound prepore. In the latest Cell issue, Saibil and colleagues now present an improved structure for the prepore and, for the first time, a detailed structural model for a fully membrane-inserted CDC pore (Tilley et al., 2005).

By docking and modeling the individual domains of the perfringolysin monomer into the density maps of the oligomeric pneumolysin prepore and pore structures, Saibil and colleagues can now propose a series of conformational changes that occur in the transition from a soluble monomer into a membrane-inserted pore. Each of the four toxin domains plays a specialized role in this highly choreographed process. Briefly, domain 1 serves as a stable scaffold around which the other domains can reorganize. Domain 4 is responsible for binding to the target membrane by inserting a Trp-rich loop into the upper leaflet of a cholesterol-rich lipid bilayer (Figure 1B). As the concentration of the toxin on the membrane surface increases, it begins to assemble into a prepore (Figure 1C) mediated by interactions involving domains 1 and 3. The newly formed intersubunit interactions lead to a change in the relative orientation between domains 2 and 4. This destabilizes the long, thin domain 2 and causes it to both bend and to loosen its contact with domain 3. The transition from prepore to pore occurs when domain 2 completely dissociates from domain 3. Domain 3 contains two sets of three small \( \alpha \) helices, which now refold into two long \( \beta \) hairpins (Shatursky et al., 1999). At the same time, domain 2 buckles fully and moves domain 3 close to the lipid bilayer, punching the freshly formed \( \beta \) hairpins into the lipid bilayer (Figure 1D).

The model Saibil and colleagues put forward for the mechanism of pore formation by CDCs confirms many previously proposed concepts. Still, it is truly remarkable to now have an image of how 38–44 pneumolysin molecules assemble into a ring on the membrane surface and then punch 76–88 \( \beta \) hairpins into the lipid bilayer to form a large pore 320–430 Å in diameter. In addition to showing the strength of cryo-EM to visualize large, complex, and structurally heterogeneous protein assemblies, this study also showcases another advantage of cryo-EM, namely that membrane proteins can be studied in their native environment, the lipid bilayer. It was therefore very easy in this study to identify prepores, which sit on top of an intact membrane, versus the pores, which clearly surround a hole in the membrane. Truly exciting, however, is the observation that the transition from prepore to pore results in a previously unexpected deformation of the membrane surrounding the pneumolysin oligomer, causing the authors to speculate that this deformation may be part of creating the hole in the membrane in the center of the proteinous pore.

Although the cryo-EM study by Saibil and colleagues goes a long way toward defining mechanistic aspects of pore formation by CDCs, a few important questions still await answers. For example, although the authors discuss some possibilities, the exact role cholesterol
Molecular Cell

Figure 1. Pore Formation by Pneumolysin

(A) Crystal structure of the soluble pneumolysin monomer (Rossjohn et al., 1997). The red loop in domain 4 is rich in Trp residues and mediates the initial binding of pneumolysin to the membrane. The red helices in domain 3 have been shown to refold into β hairpins upon membrane insertion (Shatursky et al., 1999).

(B–D) Schematic representation of the structural rearrangements accompanying the pore formation by pneumolysin as proposed by Tilley et al. (2005). (B) Membrane bound monomer, (C) membrane bound prepore, and (D) membrane-inserted pore.

plays in the formation of a CDC pore remains unclear. It is also a puzzling finding that all the imaged prepores are smaller in diameter than the pores. The explanation that comes to mind first, namely that oligomers may have to reach a certain size before they can insert into the membrane, is discounted by the authors on the basis of recent atomic force microscopy studies that showed no significant changes in size when individual prepores converted into pores (Czajkowsky et al., 2004). The authors therefore suggest that larger oligomers may insert faster into the membrane than smaller ones, thus explaining the size difference between pores and prepores by the limited incubation time before the sample was imaged. Of all the unanswered questions, the most intriguing one must be the fate of the hundreds of lipid molecules that disappear from the center of the pore. Imaginative experiments will need to be designed to solve this vanishing act.

Thomas Walz
Department of Cell Biology
Harvard Medical School
Boston, Massachusetts 02115

Selected Reading