Preface

Confronting competitors is a very tough, but vital task for all living organisms. An important part of the genome is devoted to encoding proteins employed in this competition. Such proteins include many offensive weapons, like toxins, meant to physically eliminate the competitors. Not surprisingly, the cell membrane is often the first target for such weapons. In fact, integrity of the cell membrane is crucial for appropriate cell function and, thereby, for life itself. Similar weapons, collectively called cytolysins or membrane-damaging toxins, form a large family of proteins and polypeptides of bacterial, animal and plant origin which share the capability of altering cell homeostasis. Among them, an increasingly large number are those that have been found to act as pore formers. These molecules inflict a lethal blow to the attacked cells simply by punching an aqueous channel, usually relatively large and poorly selective, through their membranes.

The pore concept was first elaborated to explain the lytic damage imposed by complement to bacteria and other non-self cells, but it was soon realised it could extend to a wider range of lytic agents. Early in 1979, classifications of cytolysins of different origin clearly indicated that many of these could form discrete lesions in the cellular membrane. However, the first direct proof of a pore-forming action had to wait until a few years later when studies were conducted on α-toxin from *Staphylococcus aureus* and aerolysin from *Aeromonas hydrophila*. Since then, pore formation has emerged as a major and widespread mechanism for toxin-mediated cell attack in the plant, animal and microbial world, and a number of books and symposia have been devoted to this topic. So far, hundreds of natural molecules, of peptide or protein nature, have been described to work in this way.

Pore-forming toxins (PFT) are topics of interest not only for people studying their functional role, like physicians or microbiologists, but also for a variety of other scientists, ranging from biochemists to nanoscientists, as it will become clear in the following. PFT are involved in important pathologies or envenomations. For example, large families of leucotoxins have been identified, aimed at contrasting the immune response of the host. They pertain either to Gram positive bacteria, like the cholesterol binding toxins and the α-toxin/bicomponent-toxins family of *Staphylococcus* spp. (reviewed in Chapter 1), or to Gram-negative bacteria, like the RTX superfamily. RTX (for Repeat in ToXins) are characterised by the presence of an octapeptyde repeat forming a variable number of Ca^{++} binding loops (reviewed in Chapter 2). Virtually all pathogenic bacteria, whose genome have been sequenced to date, possess at least one, but usually more, cytolysin of the PFT kind. Often, these toxins, although the parent by the mechanism of action, are completely unrelated by the structure and do not form known families. This is the case, for example, of *Pseudomonas aeruginosa* cytotoxin (Chapter 3) or the vacuolating toxin of *Helicobacter pylori* (Chapter 4), two toxins involved in serious human pathologies: the infection of airways of cystic fibrosis patients and gastric ulcer, respectively.

PFT are not limited to the bacterial world. In fact, plants and animals have adopted similar molecules as tools to contrast microbial infections or as part of their venoms. An example is provided by the family of cytolytic toxins found in the tentacles of sea anemones (*Actiniaria*) described in Chapter 8. Notwithstanding their origin, PFT have some peculiar properties that make them extraordinary tools and study systems. They exist, in fact, in two possible stable structures, a water-soluble and a membrane-inserted form. The polypeptide, once secreted by the producing organism, adopts a temporary water-soluble fold, suitable for diffusion in the organic fluids. When it reaches the target membrane, however, passing through different intermediate states, it ultimately inserts into the lipid and forms a stable pore. While, in most cases, the insertion of proteins in membranes is a complex process that requires the assistance of a number of proteins, besides the one to be inserted, in the case of PFT, insertion is a completely spontaneous, or self-assisted, process. This is apparently achieved through the interaction with specific lipids, but, more importantly, by an aggregation mechanism implying multiple copies of the same or related toxin components (see Chapter 1). For these reasons PFT are unique models with which to study mechanisms, whose importance certainly transcends that of the toxins themselves, for example, the conformational transitions leading to aggregation (protein–protein interaction), those leading to membrane insertion (protein–lipid interaction), and those leading to opening and closing of the pores (gating mechanisms).

Structural studies are certainly paramount and the most informative technique has been, also in this case, X-ray crystallography. Monomeric structures of PFT have recently become available in an increasing number of cases. To derive the structure of peptide PFT and some related smaller molecules however, NMR spectroscopy has proven at least as important, with the additional advantage of operating in a softer, native-like condition. The membrane-bound structure is often the most interesting, but certainly also the most elusive, for the well-known problems posed by membrane proteins: the structure is too disordered for the X-ray technique, but too ordered for the NMR approach. *S. aureus* α-toxin is the one exception, having been crystallised, and structurally solved, in the aggregated pore-forming form, albeit in the presence of a detergent and not in a lipid membrane. For all other membrane-bound protein PFT, the techniques used have lower resolution, and are those classically employed for intrinsic proteins, that is, electron and atomic force microscopy, as well as spectroscopic techniques such as flulorescence, infrared, circular dichroism and mass. With smaller peptides, additional structural data in the lipid environment could be obtained by low-angle X-ray and neutron scattering, by NMR and by theoretical calculations (e.g. molecular dynamics), as also described in Chapter 9.

Comparing the results in the two environments should provide clues about how, and through which intermediate states, the change from water-soluble monomer to membrane-inserted aggregate takes place. Interestingly, at least within protein PFT, and especially in the transmembrane region, the predominant secondary structure observed till now, is the β-sheet, resembling bacterial and mitochondrial porins. The existence of a common ancestor with porins was postulated. Nontheless, PFT forming of predominantly α-helical structures do also exist (called α-PFT as opposed to the previous β-PFT). Examples of α-PFT are colicins, lytic proteins used as lethal arms in the competition between different bacterial strains living on the same host (see Chapter 7). Pore formation by α-PFT implies the insertion in the lipid film of a bundle of helices, which would otherwise remain hidden in the hydrophobic core of the protein. The opening of the pore occurs together with the translocation of a large portion of the protein through the membrane. As explained in Chapter 7, this is in complete analogy with the mechanism of action of diphtheria toxin (DT).

DT is a toxin of the A/B type which are characterised by the presence of two protomers: the B subunit (responsible for cell binding and membrane translocation) and the A subunit (responsible for an enzymatic intracellular activity). In the case of DT, the A and B subunits belong to the same polypeptide chain. Interestingly, even DT is able to open ion channels into cell and model membranes, thus reinforcing its analogy with colicins and PFT. Such translocation mechanism can easily be used to import inside the cell foreign proteins that have been fused to the colicin, suggesting an important biotechnological application, as a transfection tool, for these molecules.

Due to their peculiar self-assembling properties, PFT may also find other interesting applications. They may be used as they are, in the wild form, for applications with either a commercial interest, like the insecticidal toxins of *Bacillus thuringiensis* and *B. sphaericus* widely used in agriculture (see Chapters 5 and 6), or with a basic science interest, for example, the controlled poration of cells in studies of intracellular mechanisms. Pore-forming peptides have also a strong potential as tools in dissecting cellular functions, like the uses of pardaxin described in Chapter 10, or as new-concept antibiotics and antifungals, for example, the metabolites of *P. syringae* described in Chapters 12 and 13. Other applications involve the engineering of PFT to produce new molecules with peculiar properties. Examples range from the production of antitumoral toxins to constituting the basic elements for the construction of sophisticated biosensors. In the case of smaller PFT, chimeric molecules (as those presented in Chapter 11) and even *de novo* designed and constructed peptides are now common strategies.

Finally, a number of peptides deriving, as secondary products, from the spontaneous and, in some cases, misconducted degradation of endogenous proteins, assume the character of pore-forming substances. These include the amyloid peptides characterising more than 20 human amyloid diseases. Amyloidosis (reviewed in Chapter 14) include pathologies that are assuming a greater importance in developed countries, for the risks posed by the ageing of the population (Alzheimer disease) and by the increasingly industrialised food production (Kreutzfeld-Jackob or prion disease).

The Editors