Review

Caroline Barisch*, Joost C. M. Holthuis* and Katia Cosentino*

Membrane damage and repair: a thin line between life and death

https://doi.org/10.1515/hsz-2022-0321
Received October 31, 2022; accepted February 3, 2023;
published online February 23, 2023

Abstract: Bilayered membranes separate cells from their surroundings and form boundaries between intracellular organelles and the cytosol. Gated transport of solutes across membranes enables cells to establish vital ion gradients and a sophisticated metabolic network. However, an advanced compartmentalization of biochemical reactions makes cells also particularly vulnerable to membrane damage inflicted by pathogens, chemicals, inflammatory responses or mechanical stress. To avoid potentially lethal consequences of membrane injuries, cells continuously monitor the structural integrity of their membranes and readily activate appropriate pathways to plug, patch, engulf or shed the damaged membrane area. Here, we review recent insights into the cellular mechanisms that underly an effective maintenance of membrane integrity. We discuss how cells respond to membrane lesions caused by bacterial toxins and endogenous pore-forming proteins, with a primary focus on the intimate crosstalk between membrane proteins and lipids during wound formation, detection and elimination. We also discuss how a delicate balance between membrane damage and repair determines cell fate upon bacterial infection or activation of pro-inflammatory cell death pathways.

Keywords: cell death; ESCRT; lysosome; *Mycobacterium tuberculosis*; pore-forming proteins; sphingomyelin.

***Corresponding authors: Caroline Barisch**, Molecular Infection Biology Division, Department of Biology and Center of Cellular Nanoanalytics, Osnabrück University, D-49076 Osnabrück, Germany, E-mail: caroline.barisch@uos.de. https://orcid.org/0000-0002-1493-9006; and **Joost C. M. Holthuis**, Molecular Cell Biology Division, Department of Biology and Center of Cellular Nanoanalytics, Osnabrück University, D-49076 Osnabrück, Germany, E-mail: holthuis@uos.de. https://orcid.org/0000-0001-8912-1586; and **Katia Cosentino**, Molecular Cell Biophysics Division, Department of Biology and Center of Cellular Nanoanalytics, Osnabrück University, D-49076 Osnabrück, Germany, E-mail: katia.cosentino@uos.de. https://orcid.org/0000-0002-3796-3500

Introduction

Cellular membranes are complex assemblies of proteins and lipids that have co-evolved since the origin of life. A highly selective permeability for solutes is key to their fundamental roles as spatial organizers of metabolic pathways and storage devices for energy extracted from the environment. The integrity of cellular membranes is therefore essential for any organism to sustain cellular metabolism and support life. Yet many nucleated cells are not protected by a rigid cell wall. Having a soft membrane bilayer as their only cover, these cells are frequently confronted with acute, potentially lethal membrane damage caused by shear stress, osmotic shock, ischemia, or bacterial pathogens. Consequently, robust membrane repair mechanisms have evolved to restore the integrity of wounded cells and promote their survival. Irrespective of the source of membrane injuries, an aberrant Ca^{2+} influx is a key trigger of pathways that enable cells to plug the wound or remove the lesion (Cooper and McNeil 2015). A detailed understanding of these pathways is desirable as their malfunction underlies various human pathologies while their modulation holds great therapeutic potential (Dias and Nylandsted 2021). This is a challenging task because membrane perforation and repair are complex phenomena that are governed by the collective behavior and dynamic interplay of membrane proteins and lipids.

In this review, we discuss recent insights into how cells exploit an intimate crosstalk between proteins and lipids to detect, constrain and restore lesions in their membranes inflicted by intrinsic or extrinsic stressors. We describe how bacterial pathogens release pore-forming proteins (PFPs) that permeabilize the host membrane system to facilitate their escape from the phagosome and entry into the host cytosol. Many of these so-called cytolysins access the host membrane by binding cholesterol and subsequently oligomerize into ring-shaped pores of about 30–50 nm in diameter. However, cells can also perforate their own membranes through the activation of endogenous PFPs during the execution of various forms of regulated cell death. For instance, gasdermins (GSDMs) cause membrane permeabilization in pyroptosis, a lytic pro-inflammatory

type of cell death. Toxicity depends on the stoichiometry and size of the pores but also on the ability of the cell to mediate their removal via one of several alternative membrane restoration pathways.

Cells are able to sense and respond to various degrees of membrane damage. This allows them to activate a repair mechanism adapted to the magnitude of the injury. Large membrane lesions with a diameter over 100 nm require patching by intracellular vesicles or may undergo engulfment by macrophages. Smaller lesions of up to 100 nm may be sealed by different repair mechanisms that are neither mutually exclusive nor restricted to specific cell types. A recurrent theme is that cells resourcefully exploit ancient molecular machinery used for other tasks to cope with breaches in membrane integrity. For instance, the endosomal sorting complex required for transport (ESCRT) mediates the formation of intraluminal vesicles during multivesicular body biogenesis but also participates in the clearance of minor lesions from the plasma membrane (PM) or lysosomes by promoting an outward or luminal shedding of the affected membrane region (Vietri et al. 2020). Ca^{2+}-activated scrambling and metabolic turnover of sphingomyelin (SM) provide an alternative ESCRT-independent mechanism to restore damaged lysosomes via an inverse involution of the damaged area analogous to ESCRT-mediated membrane repair (Niekamp et al. 2022).

Following removal of the damaged portion of the membrane, the resident lipids lost at the site of injury must be replaced to enable completion of the restoration process and sustain cellular or organellar integrity. We discuss how ER-contact sites are activated on damaged lysosomes in parallel to ESCRT to orchestrate a non-vesicular ER-to-lysosome transfer of lipids required for rapid lysosomal repair. Finally, we focus on how bacterial pathogens exploit a delicate balance between membrane damage and repair to promote their intracellular lifestyle. In analogy, an equilibrium between damage and repair enables cells invaded by pathogens to exploit core mechanisms of programmed cell death pathways for a controlled release of inflammatory molecules to fight infections while staying alive.

How does membrane damage occur?

The PM of cells is continuously exposed to numerous threats that jeopardize its vital barrier function, with potentially lethal consequences. Loss of PM integrity can result from mechanical or chemical trauma, exposure to bacterial cytolysins, or activation of endogenous PFPs in response to danger signals originating from the cell interior (e.g. rise in reactive oxygen species). As lysosomes are the destination of materials taken up from the extracellular environment, they are particularly vulnerable to membrane damage. Leakage of lysosomal contents poses a potential hazard to the cell.

Membrane damage inflicted by mechanical or chemical stressors

The PM of myocytes is particularly susceptible to lesions caused by the forces of muscle contraction. The percentage of wounded myocytes in skeletal muscle of adult rats increases from ~3% at basal condition to ~21% post-exercise (McNeil and Khakee 1992). Excessive exercise generates reactive oxygen species (ROS), which result in oxidation of membrane lipids. Introduction of an oxidized functional group causes the lipid tail to bend toward the water phase and decreases the lipid order in the bilayer (Gaschler and Stockwell 2017; Wong-Ekkabut et al. 2007). Consequently, in myocytes during exercise the PM barrier function is further undermined by lipid peroxidation. Mutations in genes causing muscular dystrophies compromise PM repair. The resulting instability of the PM contributes to a dysregulated Ca^{2+} homeostasis and disease pathology, which is characterized by progressive weakness, atrophy and degeneration of skeletal muscle (Bansal et al. 2003; Cooper and McNeil 2015). Cardiac myocytes experience similar levels of mechanical and chemical stress as skeletal myocytes. Pathological conditions such as a stroke or ischaemia-reperfusion injury generate both osmotic and oxidative stress. The resulting cell swelling and lipid peroxidation exacerbate PM injury in cardiac myocytes which, if not repaired, contributes to irreversible cell loss and heart failure (Dias and Nylandsted 2021).

Lysosomes are the terminal stations for many internalized cargoes and play a crucial role in the degradation of macromolecules, killing of pathogens, and nutrient signalling. Because of their low intraluminal pH and high content of acid hydrolyses and Ca^{2+}, disruption of lysosomal integrity is a potential hazard to cells. At the same time, lysosomes are frequently subjected to damage by materials that they transport or accumulate. Besides intentional damage caused by incoming pathogens that seek access to the host cytoplasm, damage can also arise incidentally by sharp crystals or protein aggregates that can perforate the lysosome-limiting membrane (Papadopoulos and Meyer 2017). For instance, the inability of aged phagocytes to clear the large amounts of cholesterol that are released from myelin after myelin breakdown in demyelinating diseases

leads to phase transition of free cholesterol into crystals, causing lysosomal rupture and inflammasome activation that impair tissue regeneration (Cantuti-Castelvetri et al. 2018). Moreover, the low intraluminal pH of lysosomes favours the accumulation of weak bases that may disrupt lysosomal integrity (Berg et al. 1994). The lysosomotropic drug L-leucyl-L-leucine methyl ester (LLOMe) is converted into a membranolytic polymeric form by dipeptidyl peptidase-I in the lysosomal lumen and is commonly used to trigger lysosomal damage in an acute manner (Thiele and Lipsky 1990a,b). Terminally damaged lysosomes undergo a selective form of autophagy called lysophagy, whereby the organelles are sequestered into an autophagosome before fusion with a healthy lysosome to enable their degradation (Chauhan et al. 2016; Hung et al. 2013; Thurston et al. 2012). However, less severely damaged lysosomes can avoid autophagic degradation and instead be fully repaired. How injured lysosomes are triaged between these opposing fates will be discussed below

Membrane damage inflicted by pathogens

The PM and lysosomal/vacuolar membrane serve as main protective barriers that guard cells against incoming bacterial pathogens. Pathogens like *Salmonella*, *Listeria* and mycobacteria exploit a variety of sophisticated strategies to cross these membrane barriers and gain access to the host cytosol or spread into neighbouring cells and tissues (Figure 1a) (Ammendolia et al. 2021; Flores-Díaz et al. 2016). Bacterial secretion systems and pore-forming toxins (PFTs) are the main causatives of host membrane damage that accompanies phagosomal escape and tissue invasion of these pathogens (Ammendolia et al. 2021). By directly affecting the lipid composition and physical properties of the host membrane, phospholipases (PLases) and sphingomyelinases (SMases) released by bacterial pathogens also facilitate their phagosomal escape and cell-to-cell spreading by making the host cells more susceptible to osmotic lysis (Flores-Díaz et al. 2016).

Bacterial secretion systems

Many pathogenic bacteria use secretion systems to directly inject effector proteins and nucleic acids into host cells to facilitate bacterial uptake and subvert host immune responses (Costa et al. 2015; Gerlach and Hensel 2007). Secretion systems are complex multiprotein structures that have been identified in a wide range of bacteria (Park et al. 2018). Type 3 secretion systems (T3SS) of Gram-negative (Gram-) bacteria comprise injection machineries that are stimulated upon contact with host membranes (Park et al. 2018). Interestingly, the *Shigella* T3SS *per se* causes membrane damage as expression of a functional T3SS without its effectors is sufficient to induce vacuole lysis and bacterial translocation of a laboratory strain of *Escherichia coli* into the host cytosol (Figure 1a) (Du et al. 2016).

Bacterial pore-forming toxins

Pores formed by bacterial PFTs enable the passage of bacterial effectors across host membranes to promote bacterial translocation and cell-to-cell spreading (Peng and Sun 2016). Among the best characterized bacterial PFTs are haemolysins, aerolysins and cholesterol-dependent cytolysins (CDCs) produced by Gram+ bacteria like *Listeria*, and Cytolysin A generated by Gram- bacteria such as *Salmonella* and *Shigella* (Cosentino et al. 2016; Dal Peraro and van der Goot 2016). PFTs reach their target membrane as soluble monomers after recognition of host specific lipids (e.g. cholesterol, phosphatidylcholine [PC], SM) before they oligomerize into pores. CDCs specifically target cholesterol (Lee and Bensinger 2022) and do not affect cholesterol-depleted membranes (Howard et al. 1953; Vadia et al. 2011). One of the best studied CDCs is Listeriolysin O (LLO), which is secreted by *Listeria monocytogenes*, a Gram+ bacterium that causes listeriosis. It is the only cytolysin that can act on both the exoplasmic and cytosolic leaflets of host cell membranes and facilitates vacuolar escape and spreading into neighbouring cells (Figure 1a) (Petrišič et al. 2021). Mutants lacking LLO are avirluent and remain inside the vacuole (Nguyen and Portnoy 2020). The ability of the secreted monomers to bind lipids with high affinity can be exploited to study the subcellular distribution of lipids. Perfringolysin O (PFO), for example is a CDC that is secreted by the Gram+ bacterium *Clostridium perfringens*, the common causes of food poisoning in the United States and Europe. Recently, a number of cholesterol sensors were developed based on either non-pathogenic versions of full length PFO or on its cholesterol-binding D4 domain (Maekawa et al. 2016).

Vacuolar translocation of *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (Tb), requires the pathogenicity locus region of difference (RD1) encoding the type 7 secretion systems (T7SS) ESX-1 and its major effectors EsxA (ESAT-6) and EsxB (CFP-10; (Russell 2016; Simeone et al. 2016; 2021)). EsxA and EsxB are the best studied members of the WXG100 family of putative pore-forming peptides (Poulsen et al. 2014). Interestingly, deletion of the RD1 locus including EsxA and EsxB, severely attenuates *Mtb* (Lewis et al. 2003) and also *Mycobacterium marinum*, a genetically close cousin of *Mtb* that mimics the subcellular conditions of Tb (Barisch et al. 2015; Hagedorn and Soldati 2007).

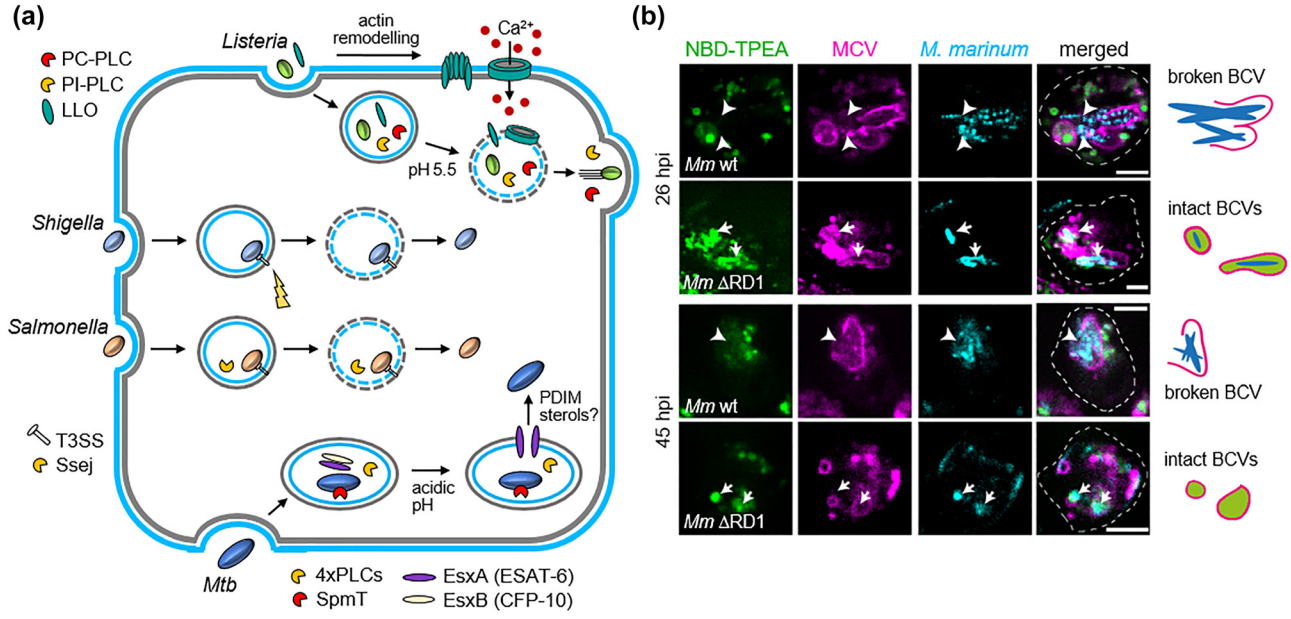

Figure 1: Pathogen-inflicted membrane damage. (a) *Listeria* secretes the cytolysin LLO, the PLases PI-PLC and PC-PLC (with SMase activity) to trigger its own uptake, phagosome escape, and spreading into neighbouring cells. *Salmonella* secretes the PLase SseJ (with cholesterol acyltransferase activity), to destabilize the SCV membrane. The T3SS of *Shigella* is sufficient to mediate the escape of the bacteria into the cytosol. *Mtb* secretes the pore-forming peptide EsxA that acts together with PDIM to promote phagosome escape. (b) The *M. marinum* ΔRD1 mutant that lacks ESX-1 as well as EsxA (ESAT-6) fails to damage the MCV membrane. While zinc, labelled with NBD-TPEA, leaks from damaged MCVs of *M. marinum* wild type, it accumulates in intact MCVs of the ΔRD1 mutant. BCV: bacteria-containing vacuole. Copyright © (Hanna et al. 2021).

In *M. marinum* infected *Dictyostelium discoideum*, ESX-1-mediated damage renders vacuoles leaky for protons and zinc ions (Figure 1a) (Hanna et al. 2021). Since many innate immune host defences rely on the proton gradient of the vacuole their activity is severely impaired upon membrane damage. The mechanism of EsxA-mediated pore formation is not fully understood, however, it is known that EsxA forms a heterodimer with EsxB that interacts with membranes at acidic pH, but not at neutral pH. Low pH leads to the dissociation of EsxB supporting the hypothesis that EsxB serves as a chaperone (De Leon et al. 2012). Recently, it was shown that *Mtb* EsxA acts in concert with the virulence factor phthiocerol dimycocerosate (PDIM), a mycobacterial branched, apolar lipid that is released into host membranes upon infection (Figure 1a) (Augenstreich et al. 2017; Lerner et al. 2018; Quigley et al. 2017). The conical shape of PDIM has been proposed to rigidify host membranes and thus ameliorates EsxA-mediated membrane damage (Augenstreich et al. 2019). This is in line with the fact that mycobacteria lacking PDIM mostly remain inside the vacuole (Lerner et al. 2018) or produce less vacuolar damage (Osman et al. 2020). PDIM shedding was confirmed in studies using the zebrafish/*M. marinum* infection system. Interestingly, here it was shown that PDIM spreading into host membranes is important for infectivity and promoted by its interaction with cholesterol (Cambier et al. 2020).

Bacterial phospholipases and sphingomyelinases

Many bacterial pathogens secrete PLases and SMases to promote infection. It is intriguing that bacteria produce enzymes that can breakdown SM even though they are unable to synthesize this lipid on their own. There are two types of bacterial SMases: SMase C cleaves the ester bond between ceramide and the phosphocholine headgroup whereas SMase D hydrolyses the phosphodiester bond between ceramide-1-phosphate and choline (Flores-Díaz et al. 2016). Similarly, at least five different classes of PLases exist and are present in a wide range of bacteria species (Flores-Díaz et al. 2016). Whereas many of these enzymes hydrolyse either PLs or SM, some have dual activity towards both lipid classes (Monturiol-Gross et al. 2021). *L. monocytogenes* secretes the PLases PC-PLC (with SMase activity) and PI-PLC (Figure 1a). Since PI is mainly enriched in the cytosolic leaflet of the vacuolar membrane, either PI-PLC or PI may exploit LLO-based pores to translocate across the membrane bilayer. DAG released by PI-PLC-mediated PI hydrolysis activates host PKCbeta to facilitate escape of *Listeria* from the phagosome (Poussin et al. 2009). Interestingly, the spreading of the bacteria into neighbouring cells is also mediated by PC-PLC and PI-PLC that operate at the PM (Figure 1a). Recently, it was shown that *L. monocytogenes* generates outer membrane vesicles containing LLO and PI-PLC (Coelho

et al. 2019). These vesicles are released upon infection of epithelial host cells and implicated in mammalian cytotoxicity. Interestingly, depleting PI-PLC in these vesicles reduced the activity of LLO, implying that this phospholipase is essential for LLO-induced damage (Coelho et al. 2019).

Salmonella enterica is a Gram-negative, food-borne enterobacterium that is responsible for a wide range of diseases in humans, depending on the serovar (Kehl et al. 2020). *Salmonella* invades various cell types and replicates inside the so-called *Salmonella*-containing vacuole (SCV). Effector proteins secreted by the T3SS SPI-2 (*Salmonella* pathogenicity island-2) impact on the lipid and protein composition of the SCV and remodel the endosomal system of the host leading to the tubulation of the SCV and the generation of *Salmonella*-induced filaments (SIF; (Kehl et al. 2020)). The SPI-2 effector SseJ modifies the lipid composition of the SCV after activation and interaction with the host Rho GTPase A (Kolodziejek and Miller 2015). Strikingly, SseJ has both PLase and cholesterol acyltransferase activity and generates lyso-PC and cholesterol esters (Christen et al. 2009). The altered lipid composition of the SCV affects its integrity and has a direct impact on bacterial pathogenesis (Figure 1a) (Christen et al. 2009; Ohlson et al. 2005). However, host membrane lipid turnover catalysed by bacterial PLases and SMases might also provide nutrients important for pathogen survival and replication. Pathogenic mycobacteria mainly use host sterols and fatty acids as carbon and energy source during infection. Fatty acids released from triacylglycerols are transferred to the *Mycobacterium*-containing vacuole (MCV) by interactions with lipid droplets (Barisch et al. 2015; Barisch and Soldati 2017a; Caire-Brandli et al. 2014; Peyron et al. 2008). Recently, it was shown that *rv0888* encodes a surface-associated protein that is composed of an *N*-terminal channel domain as well as a *C*-terminal SMase which enables the bacterium to hydrolyse SM, probably to utilize the resulting ceramide and phosphocholine as carbon, nitrogen and phosphate source (Figure 1a) (Speer et al. 2015). Interestingly, the genome of *Mtb* also encodes four PLCs (*plcA-plcD*) (Cole et al. 1998). These enzymes have been first hypothesized to be implicated in phagosome escape. However, the quadrupole *Mtb* mutant was neither impaired in vacuole-to-cytosol transition nor in virulence (Le Chevalier et al. 2015). From studies using *Dictyostelium* as a host and *M. marinum* as pathogenic mycobacterium in which the mechanisms of lipid acquisition and utilization are conserved (Foulon et al. 2022), it became clear that mycobacteria are also able to use fatty acids retrieved from host phospholipids as a carbon source (Barisch and Soldati 2017b). Host fatty acids serve, among others, as important building blocks for mycobacterial membrane lipids and are shuttled into PDIM synthesis to balance out potential toxic side products generated during cholesterol synthesis (Lee et al. 2013; Wilburn et al. 2018). Since PDIM ameliorates EsxA-mediated pore formation (see above), it is feasible that modulating mycobacterial lipid acquisition or turnover might also affect the capability of the bacteria to induce host membrane damage and escape into the cytosol.

Self-inflicted membrane damage in regulated cell death

Strikingly, membrane damage in cells can also be the result of a self-inflicted process orchestrated to induce cell death. This function is triggered as part of an attack/defence mechanism or as a regulatory step in signalling pathways with important implications in inflammatory responses and immunity (Cosentino and García-Sáez 2017; Cosentino et al. 2016; Dunstone and Tweten 2012; Krawczyk et al. 2020; Liu et al. 2021). To this end, organisms evolved genetically-encoded machineries that rely on membrane perforation by PFPs as a final step in the execution of cell death (Cosentino and García-Sáez 2017; Cosentino et al. 2016). Here, we discuss first general mechanisms of pore formation by PFPs and describe the role of PFPs and relevant lipids in the execution of two cell death programs in which membrane pore formation has been best characterized so far, namely apoptosis and pyroptosis. Pore formation in other cell death programs are described elsewhere (Flores-Romero et al. 2020).

PFPs in the execution of cell death

Members of the PFPs super-family, which include bacterial PFTs, share the ability to assemble into supramolecular complexes (or oligomers) that perforate membranes (Figure 2a). This ancient mechanism of membrane damage is exploited in all kingdoms of life (Cosentino et al. 2016; Dal Peraro and van der Goot 2016) and harnessed by mammals as an essential weapon of the immune system against pathogen infection. For example, members of the membrane attack complex (MAC)/perforin family (MACPF), called perforins, are PFPs released by natural killer and cytotoxic T cells to perforate infected target cells and mediate the release of granzymes, i.e. signalling molecules that activate cell death programs in the host cell (Voskoboinik et al. 2015; Zhou et al. 2020). Cells can also use endogenous PFPs to permeabilize their own membranes and kill themselves. This is the case for the apoptotic executers BAX and BAK that permeabilize the mitochondrial outer membrane (MOM) to execute apoptosis, a physiological cell death program essential for maintaining tissue

homeostasis and a correct functioning of the immune system (Cosentino and García-Sáez 2017; Czabotar et al. 2014). Membrane pore formation is also the mechanism used by GSDMs to execute pyroptosis, an inflammatory cell death program activated upon detection of pathogens or dangerous signals inside cells, characterized by PM permeabilization enabling the release of cytokines (Frank and Vince 2019; Lieberman et al. 2019).

PFPs can form a plethora of pores that differ in size, shape and properties (Figure 2a). The secondary structure of the PFP subunit that integrates into the membrane has led to the classification into α- and β-PFPs. α-PFPs, like BAX and BAK, contain α-helical domains and tend to form more flexible pores in comparison to β-PFPs, which contain β-strands that through inter-strand hydrogen bond formation generate a more rigid β-barrel-shaped pore structure (Gilbert 2016). Lipids actively participate in pore formation and architecture. To begin with, pores can be distinguished based on the presence or absence of lipids in the pore structure. Pores solely composed of proteins are classified as protein-lined. In toroidal pores, both proteins and lipids line the pore, whereby the lipid molecules are forced to bend inwards to form a continuum between the two leaflets to shield acyl chains from water exposure (Figure 2a) (Cosentino et al. 2016; Dal Peraro and van der Goot 2016). The high energy cost required to allow this high membrane curvature at the pore rim is called line tension and drives pore closure. However, the presence of proteins at the pore rim reduces this line tension and stabilizes pore opening (Cosentino and García-Sáez 2017; Unsay et al. 2017). In addition, lipids can act as primary receptors to target PFPs to specific membranes and actively contribute to pore assembly and stabilization (Cosentino et al. 2021; Cowan et al. 2020; Gilbert 2016).

Membrane permeabilization by Bcl-2 proteins in mitochondrial apoptosis

Bcl-2 proteins are key regulators of the mitochondrial pathway of apoptosis. A central step in this pathway is MOM permeabilization (MOMP), which allows the release of apoptotic factors like cytochrome *c* and SMAC/DIABLO into the cytosol to activate the apoptotic cascade (Figure 2b). MOMP is primarily executed by the Bcl-2 family members BAX and BAK (Cosentino and García-Sáez 2017; Moldoveanu and Czabotar 2019). In healthy conditions, BAX and BAK continuously shuttle between the cytosol and the mitochondria, with BAX mainly localized into the cytoplasm and BAK at the MOM (Edlich et al. 2011; Todt et al. 2015). Upon apoptosis induction, BAX and BAK accumulate at discrete sites at the MOM to form oligomers that permeabilize the membrane (Figure 2b). Activation of BAX and BAK is regulated by other members of the Bcl-2 family and involves a conformational transition from a globular to an extended membrane-associated conformation that allows homo-dimerization (Figure 2b) (Bleicken et al. 2014; Czabotar et al. 2014; Czabotar et al. 2013; Dewson et al. 2008; Hauseman et al. 2020; Lv et al. 2021; Moldoveanu and Czabotar 2020), a prerequisite for the assembly of higher-order oligomers (Cosentino et al. 2022; Subburaj et al. 2015). BAX/BAK assembly proceeds via inter-dimer association in a disordered and lipid-mediated manner (Dewson et al. 2009; Uren et al. 2017). Intriguingly, BAK dimers have preferential binding sites for lipids, and lipids actively participate into BAK oligomerization by creating a bridge between two dimers (Cowan et al. 2020).

When it comes to the architecture of the α-PFP-based pores formed by BAX and BAK, evidence supports the concept that lipids actively contribute to the pore assembly (Cosentino and García-Sáez 2017; Fuertes et al. 2010; Qian et al. 2008; Unsay et al. 2017). Atomic force microscopy and cryo-EM data of supramolecular structures formed by BAX and BAK revealed not only rings but also arc-shaped arrangements associated to membrane pores, in line with the notion that lipids directly participate in the pore rim, forming a torus in the portion of the pore not covered by proteins (Figure 2B) (Cosentino et al. 2022; Salvador-Gallego et al. 2016; Xu et al. 2013). That BAX and BAK are able to form supramolecular structures with shapes that include lines, arcs and rings, has been also demonstrated directly in apoptotic mitochondria of live cells by super-resolution microscopy. Here, arcs and rings were very heterogeneous in size, ranging from a few to several hundred nanometre and showed a broad stoichiometry distribution (Cosentino et al. 2022; Große et al. 2016; Salvador-Gallego et al. 2016). Altogether, these data support a flexible structural nature of these pores. The contribution of lipids to pore formation may underlie this structural flexibility and explain why these structures are tuneable in size (Bleicken et al. 2013; Salvador-Gallego et al. 2016) and able to evolve over time, allowing the sequential release of cytochrome *c*/SMAC first and mitochondrial DNA afterwards (Figure 2b) (Cosentino et al. 2022; Dewson et al. 2009; McArthur et al. 2018; Nasu et al. 2016; Riley et al. 2018; Uren et al. 2017).

Another relevant aspect is what drives BAX to exclusively target mitochondrial membranes. Recently, we demonstrated that BAX can be recruited to apoptotic mitochondria by activated BAK and the two colocalize in the same supramolecular cluster (Cosentino et al. 2022). However, the fact that BAX and BAK oligomerize at distinctive areas of the mitochondria suggests the presence of recruiting platforms composed by specific proteins and lipids, which include members of the mitochondrial fusion and

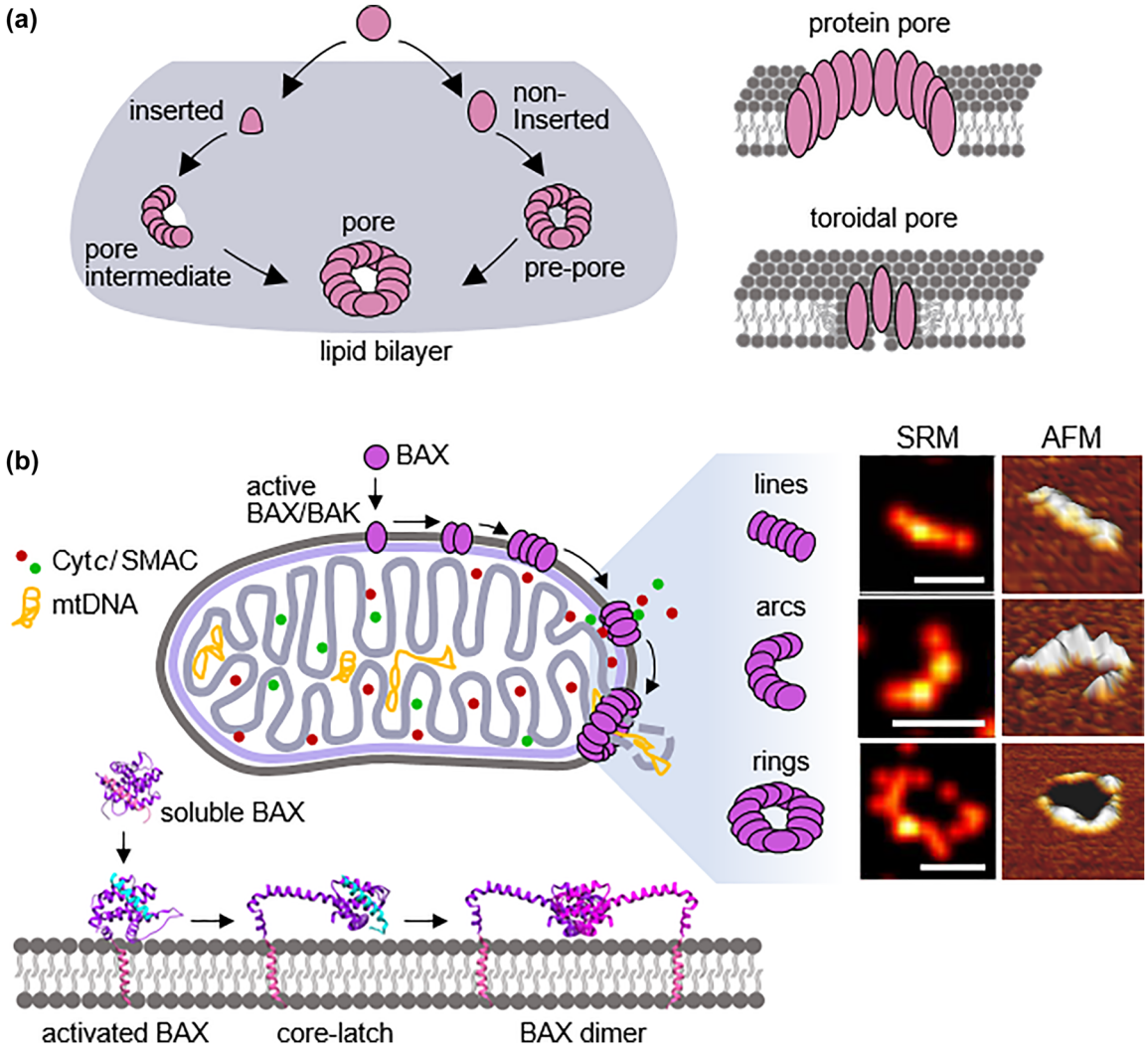

Figure 2: Self-inflicted membrane damage: membrane pore formation in cell death. (a) General mechanism of pore formation by PFPs. After initial protein binding to the membrane, different scenarios can occur. In one model, oligomerization precedes membrane insertion, leading to the formation of "pre-pore" structures on top of the membrane that only after partial or complete assembly into rings, insert into the membrane and induce pore formation. In the second model, monomers insertion occurs before or during oligomerization. In this case, more likely, small intermediate structures can already permeabilize the membrane. PFPs can assemble either in protein pores (no lipid contribution to the pore structure), or in lipid-protein or toroidal pores, where lipids participate to the pore rim. (b) Top: Mechanism of assembly of BAX/BAK at apoptotic mitochondria leading to MOM permeabilization. Pores grow overtime allowing the sequential release of cytc/SMAC and mtDNA. BAX/BAK structures have been observed in the form of lines, arcs and rings. Super-resolution microscopy (SRM) data (scale bar 100 nm) are from (Cosentino et al. 2022) and atomic force microscopy (AFM) data (figure size 250 nm) are from (Cosentino and García-Sáez 2017). Bottom: Structural model of BAX oligomerization. Upon activation by a BH3-only protein (cyan), BAX globular structure (PDB: 1F16; Suzuki et al. 2000) undergoes an initial structural change to allow BAX anchoring to the membrane through insertion of the C-terminal α9-helix (pink), without altering the overall globular fold. A further rearrangement leads to the exposure of the BAX BH3 domain that allows BAX homo-dimerization (PDB: 4BD2; Czabotar et al. 2013).

fission machineries (Jenner et al. 2022; Jiang et al. 2014), the Voltage-Dependent Anion Channel (VDAC) (Chin et al. 2018; Naghdi et al. 2015), cardiolipin (CL) (Kuwana et al. 2002; Lovell et al. 2008) and ceramides (Lee et al. 2011). CL, a mitochondrial specific lipid, has long been implicated in BAX and BAK apoptotic function (Bleicken et al. 2017; Kuwana et al. 2002; Schafer et al. 2009; Unsay et al. 2017). However, data in apoptotic cells lacking CL have challenged its essential contribution in apoptotic pore formation (Raemy et al. 2016). On the contrary, ceramides play a critical role in mitochondrial recruitment of BAX (Ganesan et al. 2010). While mistargeting of newly synthesized ceramides to mitochondria triggers BAX-dependent apoptosis (Jain et al. 2017, 2020; Siskind et al. 2010), mitochondrial ceramides may not directly interact with BAX and BAK to commit cells to death. Our recent work indicates that ceramides exert their

pro-apoptotic activity at least in part by binding directly and specifically to VDAC2 (Dadsena et al. 2019). As VDAC2 serves as molecular platform for mitochondrial translocation of BAX (Lauterwasser et al. 2016), ceramide binding to VDAC2 may trigger apoptosis by stabilizing the mitochondrial pool of BAX.

Plasma membrane permeabilization by GSDMs in pyroptosis

Pyroptosis is a form of regulated cell death initiated in response to the detection of danger signals, generally referred to as Pathogen- or Damage-Associated Molecular Patterns (PAMPs and DAMPs; Figure 3a) (Broz et al. 2020; Liu et al. 2021). The highly inflammatory nature of this cell death program represents an important defence mechanism against pathogen invasion. Infected cells undergoing pyroptosis secrete inflammatory molecules to recruit immune cells concomitantly to the release of the pathogens into the extracellular milieu. Thereby, pathogens are deprived of their replicative niche and killed by the effector cells of the immune system (Miao et al. 2010). At the molecular level, pyroptosis is initiated by the activation of inflammatory caspases (caspase-1, -4/-5 or caspase-11 in mice), following either a canonical or a non-canonical pathway (Figure 3a). The canonical pathway is mediated by the formation of an activating platform, the inflammasome, where caspase 1 is recruited and activated. Active caspase-1 induces also the maturation of cytokines IL-1β and IL-18. In the non-canonical pathway, intracellular lipopolysaccharide (LPS) binds to caspase-4/-5 or -11, resulting in their oligomerization and activation. Both pathways lead to the downstream cleavage of the GSDM family member GSDMD (He et al. 2015; Kayagaki et al. 2015; Shi et al. 2015). Upon cleavage, the active N-terminal domain of GSDMD translocates to the inner leaflet of the PM where it undergoes a conformational rearrangement and assembles into oligomers that open membrane pores (Ding et al. 2016; Liu et al. 2016). GSDMD pores at the PM allow the release of cytokines IL-1β and IL-18 and eventually induce cell lysis (Figure 3a) (Broz et al. 2020).

GSDMs are an evolutionarily and structurally conserved family, which includes GSDM A, B, C, D, E and DFNB59 in humans and GSDM A1-3, C1-4, D, E and DFNB59 in mice (Broz et al. 2020). Since the seminal discovery on the role of GSDMD in pyroptosis (He et al. 2015; Kayagaki et al. 2015; Shi et al. 2015), GSDM proteins have rapidly emerged as a novel family of PFPs due to their common ability (except for DFNB59) to induce pore formation in artificial membranes. Recent data show that other GSDMs may also directly contribute to PM-mediated pyroptosis (LaRock et al. 2022; Zhou et al. 2020), following different mechanisms of activation, however the cellular settings under which they act are not clear for all members.

Studies in model membrane systems revealed that GSDMD binds to lipids that are exclusively present in the inner leaflet of the PM, such as phosphatidylinositol phosphates (PIPs), phosphatidic acid (PA) and phosphatidylserine (PS) (Ding et al. 2016; Liu et al. 2016; Mulvihill et al. 2018; Sborgi et al. 2016). In contrast, GSDMD does not bind to phosphatidylethanolamine (PE), PC and cholesterol, which are also present in the outer PM leaflet, indicating that GSDMD can only kill the cell from inside. Of note, SM, which is generally present primarily in the outer PM leaflet, promotes GSDMD-N binding, in contrast to cholesterol, which seems to hinder GSDMD insertion (Aglietti et al. 2016; Liu et al. 2016).

The recently solved crystal structure of GSDMA3 and GSDMD (Ding et al. 2016; Liu et al. 2019) and the cryo-EM structure of the pore in artificial systems (Ruan et al. 2018; Xia et al. 2021) have highlighted the importance of the membrane environment in enabling the deep conformational changes that active GSDMs need to undergo to insert and perforate the membrane. In solution, GSDMD is kept in an inactive state through interaction of the *N*-active domain with the inhibitory *C*-terminal domain (Figure 3b). Upon cleavage by inflammatory caspases, however, the two domains separate only in the presence of a negatively charged lipid environment. Membrane insertion requires the formation of elongated β hairpins that assemble a β-barrel pore having a 33-fold symmetry with a pore size of 21 nm (Figure 3b) (Xia et al. 2021)). However, high-resolution (≤2 nm) AFM studies of GSDMD pores in artificial membranes have shown that pore formation is a dynamic process involving heterogeneous GSDMD assemblies in the form of arcs and slits that may evolve to rings, all able to form pores of variable size up to 30 nm (Mulvihill et al. 2018). Although the structural arrangement of the lipids in the vicinity of GSDMD pores remains unknown, the pore opening by intermediate arc structures implies direct involvement of lipids in shaping the pore rim, similar to what observed for BAX/BAK (see above) and the members of the MACP family (Gilbert 2016). If these structures are also validated in cells, the lipid-protein nature of the pores together with the size and shape heterogeneity of GSDMD structures suggest the possibility to modulate GSDMD-pores at the PM for the control of cellular content release. It has been proposed that small GSDMD oligomers may already be sufficient to permeabilize the PM to allow the passage of K^+ and Ca^{2+} ions to activate downstream signalling pathways (de Vasconcelos et al. 2019). Ca^{2+} influx through GSDMD pores activates membrane repair

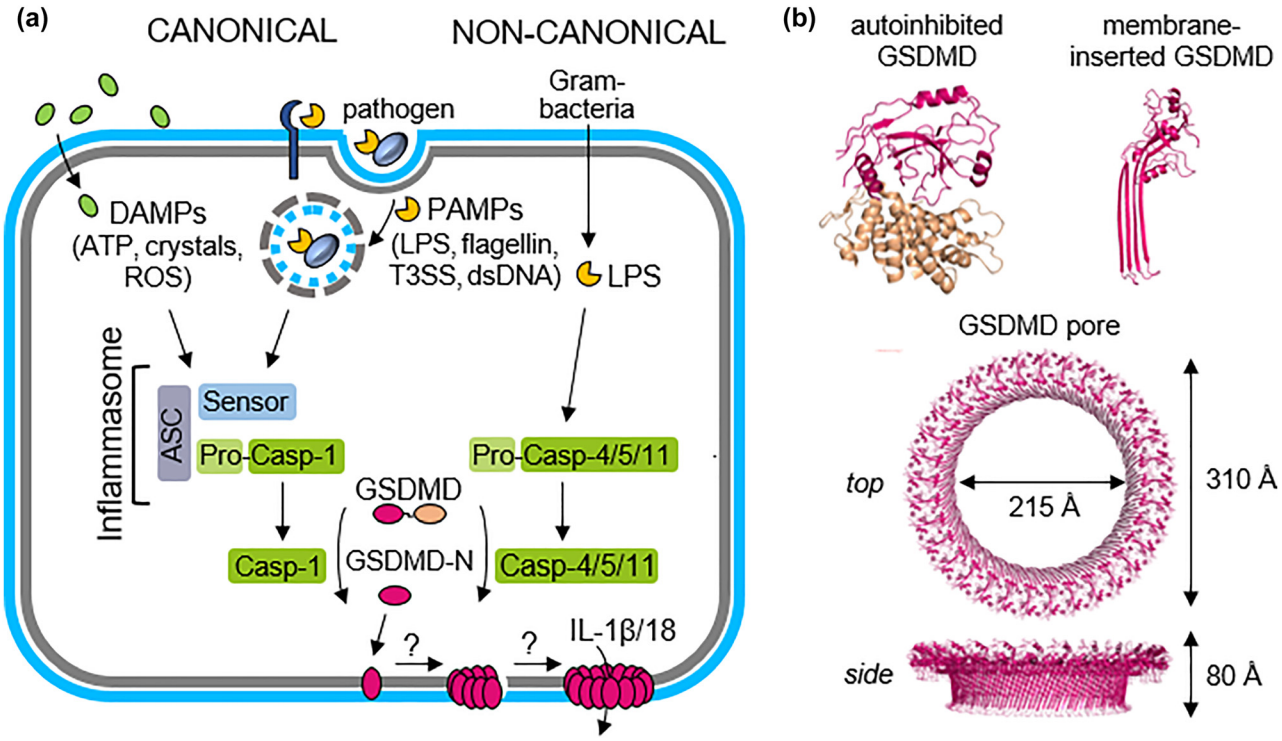

Figure 3: Self-inflicted membrane damage: signalling pathway of pyroptosis. (a) DAMPs and PAMPs activate the canonical and non-canonical inflammasome pathway, involving inflammatory caspases 1 or 4–5 (11 in mice), respectively. These pathways converge on the caspase-mediated cleavage of GSDMD, allowing its active N-terminal domain to translocate to the PM, oligomerize and form pores. The canonical pathway is mediated by the formation of an activating platform, the inflammasome, in which caspase-1 is recruited and activated. Active caspase-1 induces also the maturation of cytokines IL-1β and IL-18. GSDMD pores induce the secretion of IL-1β and IL-18 and eventually lead to cell lysis. (b) Crystal structure of GSDMD in its auto-inhibited form (PDB: 6N9O;(Liu et al. 2019)), and Cryo-EM structure of a single membrane-inserted GSDMD-N unit as well as atomic model of the 33-fold symmetric GSDMD pore (PDB: 6VFE; Xia et al. 2021).

mechanisms (see below), thus rescuing the cell from death (Rühl et al. 2018). Most likely, this repair mechanism has the aim to keep the cell in a sub-lytic status. Modulating the number of GSDMD pores at the PM not only controls cell fate but, importantly, also the release of cytokines and, therefore, the extent of inflammation. Further studies are required to clarify the sub-lytic role of GSDMs pores and how modulation of number, size and shapes of GSDM structures at the PM impact on the strength of the inflammatory responses during pyroptosis.

How is membrane damage sensed?

Injury-mediated Ca^{2+} influx

An appealing theory on the evolution of the biological role of Ca^{2+} as intracellular signalling molecule is based on the ability of Ca^{2+} to effectively precipitate organic anions (Williams 2002). This made it essential for the earliest cells to reject Ca^{2+} from the cytosol. In modern eukaryotic cells, cytosolic Ca^{2+} levels are kept low by Ca^{2+} transporters that actively pump cytosolic Ca^{2+} into the extracellular space or the lumen of Ca^{2+} storage organelles such as the ER and lysosomes. Consequently, cells maintain a 20,000-fold Ca^{2+} gradient across their membranes, with a cytosolic Ca^{2+} concentration at ~100 nM and extracellular or luminal Ca^{2+} levels at several mM (Clapham 2007). The steepness of these gradients along with the evolution of proteins that can bind Ca^{2+} over a million-fold range of affinities (from nM to mM) provide the basis of a highly sensitive and powerful mechanism by which cells can monitor the structural integrity of their membranes. Indeed, leakage of Ca^{2+} into the cytosol is widely recognized as the primary event in the detection of membrane lesions and initiation of membrane restoration pathways. Sea urchin oocytes can repeatedly reseal sequential wounds inflicted by a laser, though they cannot survive a single injury if extracellular Ca^{2+} is removed (McNeil and Kirchhausen 2005). As outlined below, cells contain several Ca^{2+}-binding proteins that function as sensors of membrane injury-mediated Ca^{2+}-influx and initiate wound sealing by membrane

patching, engulfment, shedding or tension reduction. The magnitude of Ca^{2+}-influx depends on the dimension of the membrane wound, which, in the case of toxin-induced pores rely on their size and concentration (Brito et al. 2019).

Injury-mediated cytosolic exposure of glycans

Under homeostatic conditions, the cytosol of mammalian cells is devoid of complex glycans, which are linked to the extracytosolic domains of a large variety of integral membrane proteins that populate the PM and lysosome-limiting membrane. Disruption of PM or lysosome/vacuole integrity, for instance by bacterial pathogens that seek access to the host cytosol, results in cytosolic exposure of these otherwise hidden glycans. This triggers the recruitment of galectins, a family of cytosolic lectins that function as pattern recognition receptors and bind beta-galactoside residues within glycans with high affinity (Johannes et al. 2018; Vasta et al. 2017). In this way, galectins can couple their ability to sense membrane damage to initiate mechanisms that mediate the repair, removal and replacement of damaged organelles. For instance, shortly after lysosomal injury, Galectin-3 recruits ESCRT components to damaged lysosomes to repair them (Jia et al. 2020). At later time points, Galectin-3 interacts with TRIM16, an autophagy-associated E3 ubiquitin ligase that initiates removal of damaged lysosomes by autophagy (Chauhan et al. 2016). Galectin-8 bound to bacterial-containing vacuoles (BCVs) serves as an "eat-me" signal recognized by the autophagy cargo receptor NDP52 to mediate selective autophagy of damaged BCVs (Thurston et al. 2012). Through mTOR inhibition, Galectin-8, Galectin-9 and TRIM16 trigger activation of TFEB, which stimulates expression of numerous lysosomal genes (Jia et al. 2018). Thus, galectins serve a crucial role in coordinating a graded response that enables cells to initially cope and repair endomembrane damage, and then escalate their response towards activating an organelle replacement program when terminally damaged organelles start to accumulate (Jia et al. 2020).

Injury-mediated lipid scrambling

Analogous to glycans, certain lipids show asymmetric distributions across organellar membranes. For instance, SM is highly enriched in the non-cytosolic leaflet of the PM, *trans*-Golgi and endolysosomal organelles while PS is located primarily in the cytosolic leaflet (Holthuis and Menon 2014; Leventis and Grinstein 2010). The translocation of PS across the PM during apoptosis marks dying cells with an "eat-me" signal to enable their timely removal (Segawa et al. 2014). Application of a GFP-tagged version of lysenin, an SM-specific toxin from the earthworm *Eisenia fetida* (Yamaji et al. 1998), indicated that SM becomes readily exposed on the cytosolic surface of BCVs upon endomembrane damage inflicted by Gram negative pathogens like *Shigella flexneri* or *S. enterica* (Ellison et al. 2020). Using an engineered version of equitoxin II (EqtSM) as alternative SM reporter (Deng et al. 2016), we found that lysosome or PM wounding by chemicals, bacterial pathogens or a laser in each case is tightly coupled to a fast, Ca^{2+}-dependent scrambling and cytosolic exposure of SM (Niekamp et al. 2022). The transbilayer movement of SM in response to PM injuries requires TMEM16F, a Ca^{2+}-activated phospholipid scramblase previously shown to catalyse PS externalization in response to elevated intracellular Ca^{2+} (Suzuki et al. 2010). SM scrambling in damaged lysosomes is independent of TMEM16F but impaired when cells are treated with the membrane permeant Ca^{2+} chelator BAPTA-AM, indicating that this process is mediated by a related scramblase (Figure 4a) (Niekamp et al. 2022). The identity of the lysosome-resident, Ca^{2+}-activated SM scramblase remains to be established. Arrival of SM on the cytosolic surface of damaged lysosomes or BCVs invariably precedes glycan exposure and cytosolic entry of bacteria (Figure 4b) (Ellison et al. 2020; Niekamp et al. 2022). This raised the idea that cytosolic SM may serve as an early warning signal to alert cells of an imminent catastrophic breakdown of organelle integrity and bacterial invasion of the host cytosol (Ellison et al. 2020). However, as described below we found that Ca^{2+}-activated scrambling and metabolic turnover of SM by neutral SMases on the cytosolic surface of damaged lysosomes promote their repair (Niekamp et al. 2022). This SM-dependent membrane restoration pathway functions independently of ESCRT and helps prevent lysosome damage-induced cell death.

How is membrane damage repaired?

Breaches in the barrier function of cellular membranes can jeopardize cell function and viability. To counteract the threats of membrane lesions, cells exploit several mechanisms of membrane sealing (Cooper and McNeil 2015; Zhen et al. 2021). Which mechanism is employed depends on wound type (e.g. mechanical rupture, PFP-mediated pore), severity (lesion size, number), location (e.g. PM, lysosome) and cell type (e.g. muscle fiber, macrophage).

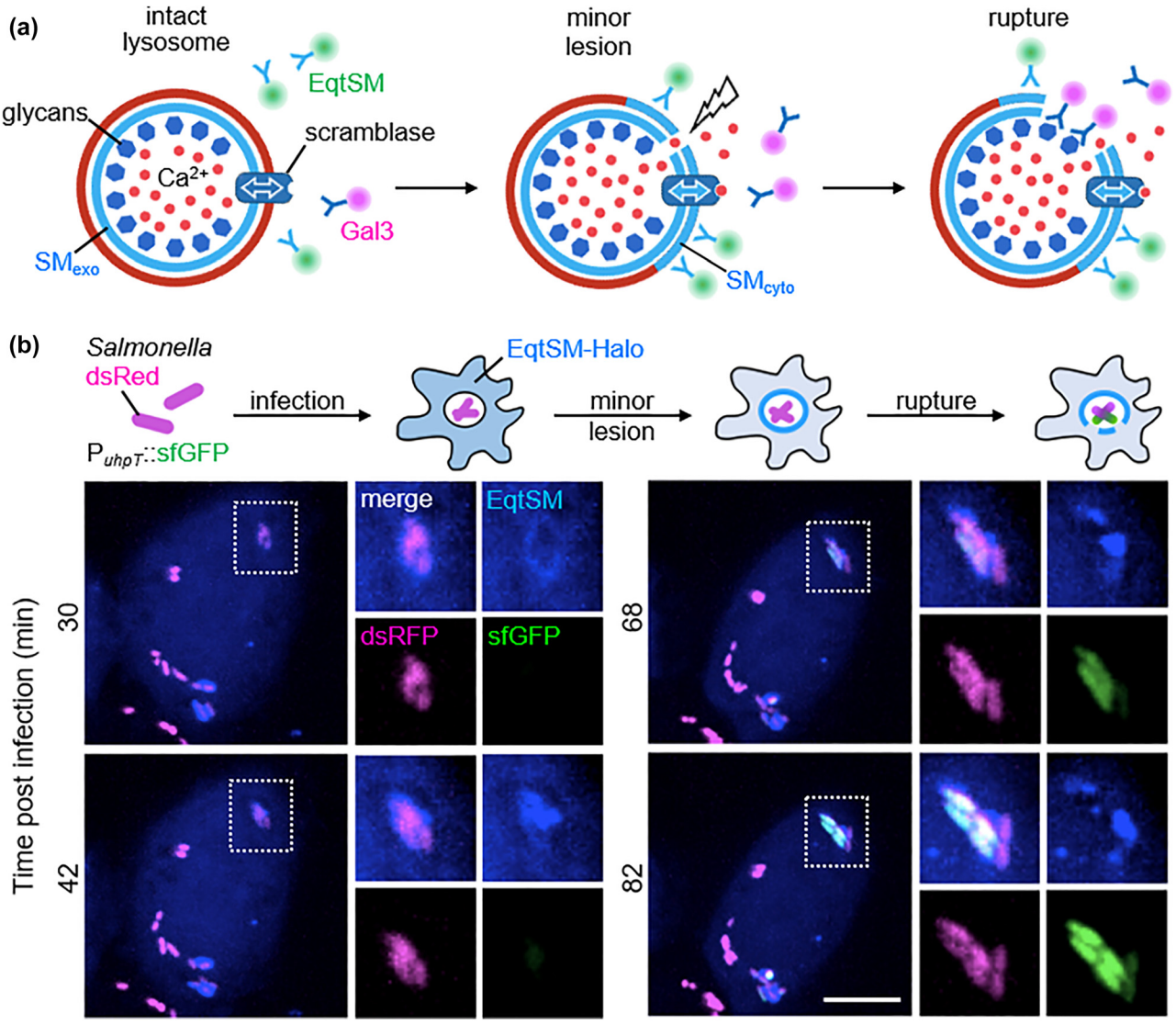

Figure 4: Ca^{2+}-activated SM scrambling precedes catastrophic rupture of SCVs. (a) Minor lesions in the lysosomal/vacuolar membrane cause Ca^{2+} ions to leak from the organelle's lumen into the cytosol, triggering Ca^{2+}-activated scramblases near the injury site. This leads to a rapid exposure of SM on the cytosolic surface of the damaged organelle and mobilization of SM reporter EqtSM. The resulting break in SM asymmetry is an early sign of membrane damage that precedes a catastrophic breakdown of lysosome/vacuole integrity, when glycans that are normally hidden in the organelle's lumen become exposed to the cytosol and bind lectins like galectin-3 (Gal-3). (b) To image *Salmonella*-induced vacuolar damage in real time, HeLa cells expressing Halo-tagged EqtSM were infected with *Salmonella* carrying a plasmid for constitutive expression of dsRed and encoding sfGFP under control of a glucose-6-phosphate (G6P)-inducible promotor. Note that *Salmonella* (magenta) in EqtSM-positive vacuoles (blue) eventually acquire sfGFP fluorescence (green), indicative of a collapse of vacuole integrity and exposure to host cytosol. In contrast, *Salmonella* in vacuoles devoid of EqtSM do not acquire GFP fluorescence. Scale bar, 10 µm. Adapted from Niekamp et al. (2022).

Reduction of membrane tension and self-sealing

Tiny membrane ruptures (<1 nm in diameter), which may occur locally due to mechanical stress, lipid peroxidation or enzymatic damage by host or foreign phospholipases, may seal spontaneously as the lipid disorder or line tension present on the curved edges of the lesion provides a driving force for closing the wound (Gozen and Dommersnes 2014). In the case of wounds inflicted by PFPs, the presence of proteins at the pore rim prevents any spontaneous self-sealing irrespective of the pore diameter, so that cells rely on dedicated mechanisms to either clog the pore or mediate its removal by engulfment or shedding (discussed below). Spontaneous resealing of small ruptures is impaired when the membrane is tethered to an underlying cytoskeleton.

Adhesion to the cytoskeleton augments the membrane tension on both ends of the rupture site, which confers an opposing force for wound closure (Cooper and McNeil 2015; Zhen et al. 2021). Consequently, cellular mechanisms that reduce membrane tension can promote membrane repair by self-sealing. One such mechanism involves a remodelling of the cytoskeleton. Accordingly, compounds that stabilize the actomyosin cytoskeleton hinder PM repair upon mechanical damage, whereas actin-depolymerizing agents foster repair (Abreu-Blanco et al. 2014; Benink and Bement 2005). An alternative mechanism to reduce membrane tension is addition of more membrane through vesicle fusion. Vesicle-mediated repair of membrane lesions is a Ca^{2+}-dependent process and its importance in resealing PM wounds was demonstrated by a lack of repair upon disruption of key components of the vesicle fusion machinery such as SNAREs and synaptotagmins (Ammendolia et al. 2021; McNeil and Kirchhausen 2005; Steinhardt et al. 1994). Synaptotagmin-7 (SYT7) contains two Ca^{2+} and phospholipid-binding C2 domains that sense cytosolic Ca^{2+} and mediate interactions with membranes. SYT7 also controls the assembly of SNARE complexes that catalyze fusion of exocytic lysosomes with the PM in response to cell wounding. Hence, SYT7 acts as a Ca^{2+} sensor that transduces damage-induced Ca^{2+} influx into vesicle fusion to promote PM repair (Reddy et al. 2001).

Clogging and patching

Annexins are abundant Ca^{2+}-binding cytosolic proteins that have the capacity to aggregate and bind to membranes in response to a rise in cytosolic Ca^{2+} levels (Koerdt et al. 2019). Ca^{2+}-dependent binding to negatively charged lipids is a unifying biochemical principle of annexins. Besides serving a potential role in reducing membrane tension (Bouter et al. 2011), annexins form high-order oligomeric structures in a Ca^{2+}-dependent manner that can clog or stabilize wounds in the PM (Bouter et al. 2011; Demonbreun et al. 2016). Their differential sensitivity to Ca^{2+} makes annexins ideally suited to help cells sense the magnitude of a wound and allow a selective mobilization of downstream machinery to complete membrane restoration (Jimenez and Perez 2017; Wolfmeier et al. 2015a). Annexin interaction partners in wound repair include dysferlin. Dysferlin is a transmembrane protein involved in membrane fusion that has been linked to several muscular dystrophies (Bansal et al. 2003; Cooper and McNeil 2015). In stretch-damaged muscle fibers, dysferlin is rapidly recruited to the wound site after Ca^{2+}-induced cleavage by calpains (Redpath et al. 2014; Roostalu and Strähle 2012). The cleaved form of dysferlin resembles synaptotagmins and is thought to promote fusion of numerous intracellular vesicles close to the site of injury to patch up the wound (Bansal et al. 2003). Endomembrane-mediated patching can seal wounds measuring up to several micrometers in diameter (McNeil and Kirchhausen 2005). The origin of the membrane material forming the repair patch is unclear, even though both endosomes and lysosomes have been suggested to contribute.

Removal of lesions by engulfment

PM wounds inflicted by PFPs such as perforin or Streptolysin O (SLO) cannot be eliminated by reducing membrane tension and must be actively removed to restore cell integrity. Ca^{2+}-dependent, SYT7-mediated fusion of peripheral lysosomes with the PM has been widely recognized as a critical step in PM repair after mechanical or PFP-induced injuries (Brito et al. 2019; Reddy et al. 2001). How addition of more membrane to the PM would help eliminate pores formed by PFPs was not well understood. However, subsequent studies revealed that the damage-induced fusion of lysosomes with the PM of cells exposed to perforin or SLO is followed by a burst of endocytosis (Idone et al. 2008; Thiery et al. 2010) after which the internalized pores are captured on the intraluminal vesicles of multivesicular bodies (MVBs) and degraded upon MVB-lysosome fusion (Corrotte et al. 2012) or rerouted to the extracellular medium as exosome-like vesicles (Husmann et al. 2009). Exocytosis of lysosomes allows the release of lysosomal enzymes such as acid SMase (ASM) and cathepsins. Secretion of ASM was found to represent a crucial link between damage-induced lysosomal exocytosis and endocytosis (Andrews et al. 2014; Tam et al. 2010). Upon delivery to the cell surface, ASM cleaves the phosphorylcholine headgroup of SM present in the exoplasmic PM leaflet to generate ceramide, whose cone-shaped structure occupies a smaller membrane area than SM. Ceramides released by SM turnover self-assemble into microdomains that possess a negative spontaneous curvature and can drive membrane invagination in artificial giant liposomes (Alonso and Goñi 2018; Holopainen et al. 2000). Addition of ASM inhibitors or blocking ASM expression disrupt formation of PM-associated ceramide microdomains in SLO-permeabilized cells and inhibit PM repair (Babiychuk et al. 2008; Tam et al. 2010). Moreover, external addition of either recombinant ASM or bacterial SMases promotes PM repair and can bypass the need for Ca^{2+}-triggered lysosomal exocytosis in lesion removal (Tam et al. 2010). This indicated that the essential contribution of lysosomal exocytosis to PM repair is the release of ASM and the ability of this enzyme to trigger endocytosis of the damaged area by a local remodelling of the PM.

Various bacterial pathogens have been shown to subvert ASM-dependent PM repair for cell invasion (Kunz and Kozjak-Pavlovic 2019). For instance, activation of ASM by *Neisseria gonorrhoeae*, the bacterium responsible for the sexually transmitted disease gonorrhoea, is essential for its entry into distinct non-phagocytic cell types (Grassmé et al. 1997). A similar mechanism has been reported to mediate cell invasion by *Staphylococcus aureus,* a human pathogen that causes a wide range of clinical infections (Tong et al. 2015), and *Trypanosoma cruzi*, the parasite that induces Chagas disease (Fernandes et al. 2011). Moreover, recent work showed that SM production is required for phagocytic uptake of *Mtb* (Niekamp et al. 2021). However, whether this is linked to SM turnover by ASM released to the cell surface remains to be established.

Removal of lesions by shedding

While the ASM-dependent repair pathway serves to remove pores and other minor lesions from the PM by driving an inward budding (i.e. towards the cytosol) of the affected area, cells also exploit repair mechanisms whereby such lesions are cleared via outward budding (i.e. away from the cytosol). The latter type of repair mechanism results in a shedding of extracellular vesicles from the surface of wounded cells or intraluminal vesicles inside damaged (e.g. pore-containing) lysosomes or BCVs.

ESCRT-dependent membrane repair

Besides its well-established role in sorting of ubiquitinated membrane proteins into intraluminal vesicles of endosomes (Katzmann et al. 2003), the ESCRT machinery has been recognized as a central catalyst in the trapping and shedding of small wounds (less than 100 nm in diameter). The membrane sealing activity of ESCRT is mediated by ESCRT-III, which comprises α-helical proteins of the CHMP family that assemble into membrane-bound spirals that drive membrane deformation and scission in cooperation with the AAA ATPase VPS4 (Pfitzner et al. 2020; Vietri et al. 2020). ESCRT-III can be recruited to membranes via several alternative mechanisms, which include direct interactions with the ESCRT-I subunit TSG101 and the ESCRT-I-binding protein ALIX. PM damage induced by PFTs, laser radiation, detergents or a glass needle in each case causes a rapid mobilization of ESCRT-I and -III proteins to the site of damage (Jimenez et al. 2014). Time-lapse imaging combined with correlative scanning microscopy revealed that wounding is followed by ESCRT-positive membrane budding and shedding. Depletion of ESCRT-III subunits or VPS4 interfered with PM repair. Energy depletion did not prevent ESCRT recruitment to the wound site but blocked membrane shedding and restoration, supporting the notion that ESCRT-III and VPS4 mediate PM repair by promoting extracellular budding and shedding of PM regions that contain minor lesions (Jimenez et al. 2014). PM damage-induced recruitment of ESCRT is Ca^{2+}-dependent. Ca^{2+}-influx at the wound site results in recruitment of annexin A7 and PDCD6, another Ca^{2+}-binding protein. PDCD6 in turn recruits ALIX, which also binds Ca^{2+}, indicating that these proteins may function as Ca^{2+}-sensors that mediate ESCRT recruitment to the wound site (Scheffer et al. 2014; Sønder et al. 2019).

Analogous to minor PM injuries, small lesions in the lysosome/BCV-limiting membrane caused by bacterial pathogens, lysosomotropic drugs or silica crystals can be repaired via membrane shedding in a process involving ESCRT (Radulovic et al. 2018; Skowyra et al. 2018; Zhen et al. 2021). Thus, ESCRT-III subunits rapidly assemble on the cytosolic surface of injured lysosomes or BCVs. Damage-induced ESCRT recruitment correlates with the onset of small perforations, occurs prior to that of galectin-3 and the lysophagy machinery, and requires Ca^{2+} as well as known ESCRT nucleation factors including TSG101 and ALIX (Figure 5a). Interference with ESCRT recruitment impairs restoration of damaged lysosomes and renders cells more sensitive to lysosome-damaging drugs (Radulovic et al. 2018; Skowyra et al. 2018). Additional studies revealed that BCVs are prone to sporadic ruptures, which are repeatedly repaired by ESCRT. For example, in *Salmonella*-infected cells ESCRT-III subunits are readily recruited to the SCV. Upon removal of ESCRT-III subunit CHIMP3, the morphology of the SCV was drastically altered and the bacteria were more readily exposed to the host cytosol (Göser et al. 2020).

ESCRT is also recruited to the *Mtb*- and *M. marinum*-containing vacuole (MCV) and plays a critical role in restricting mycobacterial growth (López-Jiménez et al. 2018; Mittal et al. 2018; Philips et al. 2008). In *M. marinum*-infected *Dictyostelium*, ESCRT-dependent membrane repair and the autophagy machinery cooperate to reverse pathogen-induced damage of the MCV (López-Jiménez et al. 2018). While ESCRT clears minor membrane lesions in the MCV, the autophagy machinery operates to replace large ruptures by membrane patches. When ESCRT- and autophagy pathways are disrupted, *M. marinum* prematurely escapes to the host cytosol, indicating that both strategies are needed to retain the bacteria inside the phagosome. Interestingly, two *Mtb* effector proteins secreted by the T7SS ESX-3, EsxG and EsxH, counteract membrane repair by inhibiting recruitment of ESCRT to the damaged MCVs, thus favouring the cytosolic translocation of the bacteria (Mittal et al. 2018). Vacuoles

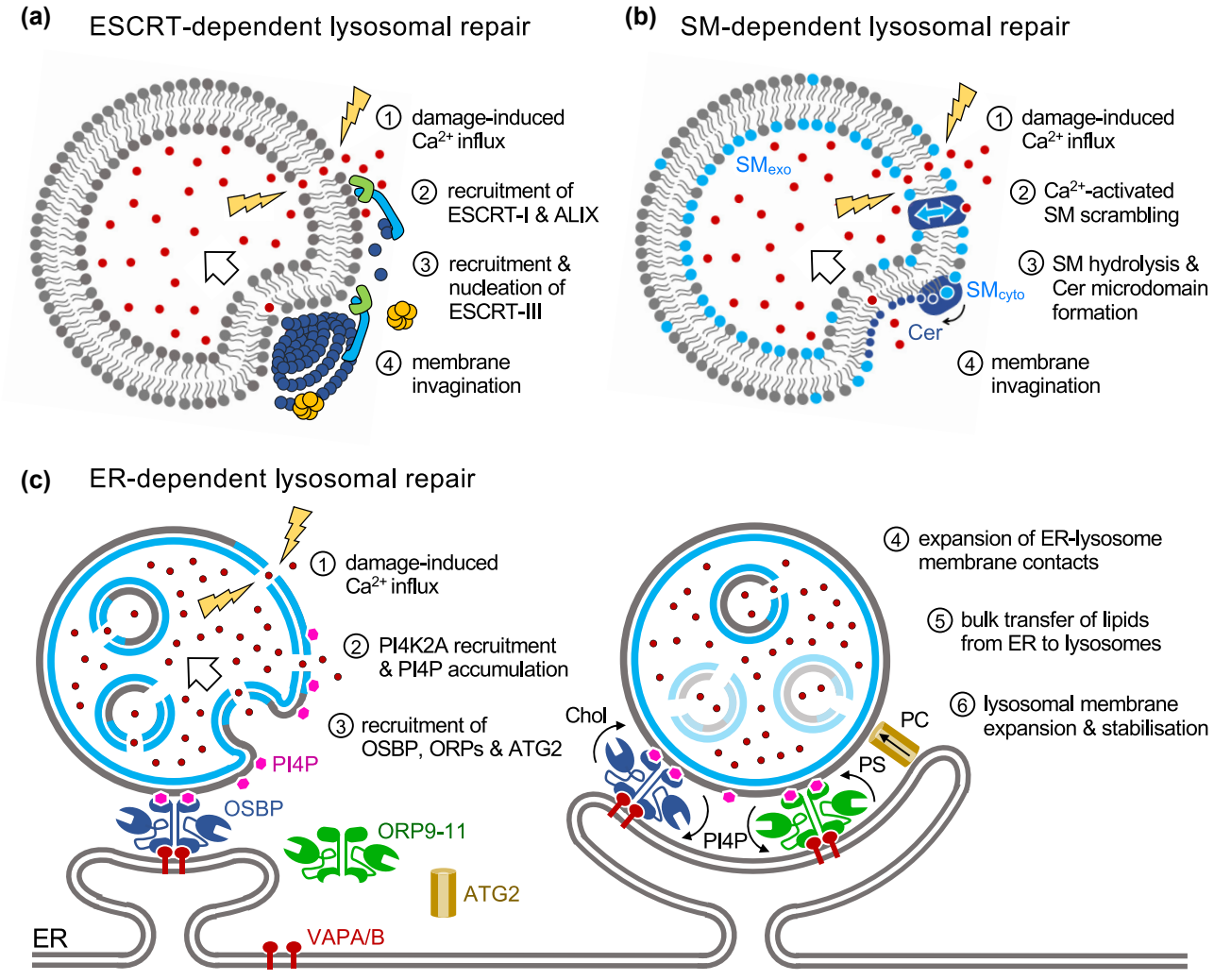

Figure 5: Restoration of damaged lysosomes is mediated by different pathways. (a) Schematic outline of ESCRT-dependent lysosomal repair. (b) Schematic outline of SM-dependent lysosomal repair. (c) Schematic outline of ER-dependent lysosomal repair. See text for further details.

containing the intracellular pathogen *Coxiella burnetii* show reversible ESCRT recruitment, and interference with this recruitment disrupts intravacuolar bacterial replication (Radulovic et al. 2018). Collectively, these findings imply that ESCRT-dependent repair machinery can be subverted by pathogens to promote their intracellular lifestyle.

Sphingomyelin-dependent membrane repair

The observation that ESCRT depletion does not completely abolish restoration of damaged lysosomes or vacuoles implied that alternative repair mechanisms may exist. As SM becomes readily exposed on the cytosolic surface of vacuoles damaged by pathogens like *Salmonella* or *M. marinum* (Ellison et al. 2020; Niekamp et al. 2022), we wondered whether SM participates in sustaining vacuole integrity during bacterial infection. To quantify pathogen-induced vacuolar damage, cells were infected with *Salmonella* carrying a dual-color reporter plasmid for constitutive expression of dsRed and encoding superfolder GFP under control of a glucose-6-phosphate-inducible promotor (Figure 4b) (Röder and Hensel 2020). Quantitative flow cytometry revealed that SM removal enhanced vacuole disruption and cytosolic release of *Salmonella* during host cell infection to a similar degree as inactivation of ESCRT-III (Niekamp et al. 2022). Moreover, SM-deficient cells were defective in repair of lysosomes damaged by lysosomotropic drugs, even though the rate and efficiency of ESCRT-III recruitment was unperturbed. Strikingly, ectopic expression of a bacterial SMase targeted to the cytosolic surface of damaged lysosomes enhanced their

repair, also in ESCRT-compromised cells. Conversely, blocking turnover of cytosolic SM through inhibition of endogenous neutral SMases perturbed lysosomal repair and caused otherwise reversible lysosome damage to become lethal (Niekamp et al. 2022). Based on these findings, we postulate that a Ca^{2+}-induced SM scrambling and subsequent SM hydrolysis by neutral SMases mediate an ESCRT-independent mechanism to clear minor lesions from the lysosome-limiting membrane and preserve lysosome function upon injury. Consistent with experiments on SM-containing giant liposomes exposed to external SMases (Holopainen et al. 2000; Nurminen et al. 2002; Trajkovic et al. 2008), we envision that ceramides released by SM turnover in the cytosolic leaflet of damaged lysosomes form microdomains that possess a negative spontaneous membrane curvature and promote an inward budding (i.e. towards the cytosol) of the damaged area. This would result in formation of intraluminal vesicles by which lesions could be cleared from the lysosome-limiting membrane in a process analogous to, but independent of ESCRT-mediated repair (Figure 5b).

A previous study showed that the Ca^{2+}-activated phospholipid scramblase TMEM16F promotes PM repair in cells exposed to the PFT LLO by facilitating the shedding of extracellular vesicles to remove the toxin from the PM (Wu et al. 2020). TMEM16F removal led to a diminished ability of neutrophils, which form the first line of defense against *Listeria*, to resist the toxic effect of LLO. In light of our recent findings, this study raises the possibility that a damage-induced SM scrambling by TMEM16F may drive a sphingolipid-based PM restoration pathway similar to the one operating in lysosomes to preserve the integrity of cells invaded by bacterial pathogens. Even though a critical role of neutral SMases in TMEM16F-dependent PM repair remains to be established, a potential advantage of this mechanism is that it would kick-in instantly upon toxin-induced PM perforation, unlike ASM-mediated PM repair. Indeed, an ultrastructural study provided evidence that toxin-induced active pores are swiftly removed by the shedding of extracellular vesicles whereas endocytosis primarily serves to clear the PM of inactive pores and vesicles that failed to shed (Romero et al. 2017). Another potential advantage is that the TMEM16F-dependent repair pathway may be harder to subvert by bacterial pathogens than the ASM-dependent pathway.

ER-dependent membrane repair

A recent proteome-wide search for proteins that are rapidly recruited to damaged lysosomes yielded, besides multiple ESCRT subunits, several proteins that participate in a phosphoinositide-initiated membrane tethering and lipid transport pathway (Tan and Finkel 2022). These included PI4K2A, an enzyme that generates the lipid messenger phosphatidylinositol-4-phosphate (PI4P), and several members of the oxysterol-binding protein (OSBP)-related protein (ORP) family, which function as lipid transfer proteins that exchange PI4P for sterols or PS at membrane contact sites (Antonny et al. 2018). In cells exposed to lysosomotropic drugs, PI4K2A readily accumulates on damaged lysosomes, generating high levels of PI4P. This results in mobilization of ORP9, ORP10, ORP11 and OSBP as well as in a dramatic expansion of membrane-contact sites between damaged lysosomes and the ER (Figure 5c) (Tan and Finkel 2022). Formation of these contact sites is strictly dependent on the kinase activity of PI4K2A as well as on the ER-resident ORP protein receptors VAPA and VAPB. Removal of PI4K2A, VAPA and VAPB, or the four recruited ORP family members impaired a timely restoration of lysosomes. ORP9, ORP10 and ORP11 were found to transport PS to damaged lysosomes and interfering with their PS transport activity disrupted lysosomal repair. The bulk lipid transfer protein ATG2 is also recruited to damaged lysosomes, and its lipid transfer activity was found to be stimulated by PS and critical for rapid lysosomal repair (Tan and Finkel 2022).

In line with the above findings, recent lipidomic analyses of lysosomes isolated at different time points after membrane damage revealed a gradual increase in phosphoinositide, PS and cholesterol levels (Radulovic et al. 2022). Moreover, the latter study showed that the cholesterol transfer proteins OSBP and ORP1L are primarily responsible for the damage-induced cholesterol accumulation, that lysosomal delivery of cholesterol helps preserve lysosome integrity, and that of OSBP and ORP1L to damaged lysosomes occurs independently of ESCRT. OSBP depletion caused a massive lysosomal accumulation of PI4P and triggered cell death, indicating that OSBP-mediated removal of PI4P after PI4P-mediated membrane repair is essential for cell viability (Radulovic et al. 2022). Collectively, the results indicate that maintenance of lysosomal membrane integrity is critically dependent on a damage-induced, phosphoinositide-mediated membrane tethering and lipid transport pathway. Exactly how transfer of PS, cholesterol and other lipids from the ER mediates lysosome repair remains to be established. However, an ER-mediated lipid supply may be necessary to replace the damaged membrane areas that undergo inward budding and subsequent degradation catalysed by ESCRT- and SM-dependent repair; without such supply, lysosomes undergoing restoration would shrink, with repair processes likely coming to a halt. Another open question is whether an ER-mediated lipid

supply also participates in the restoration of other organellar membranes such as the BCV and PM.

A delicate balance between membrane damage and repair decides cell fate

Over the years, it has become apparent that membrane repair is an active process to sustain membrane integrity in response to microbial- or self-inflicted membrane damage. As such, the coordinate action of membrane repair mechanisms is key to decide the outcome of pathogen infection and to define the balance between cell survival and cell death (Ammendolia et al. 2021; Etxaniz et al. 2020).

Balancing membrane damage and repair in infection

The response of infected cells to membrane damage inflicted by pathogens and in particular PFTs has been extensively studied, providing useful insight about the general mechanisms governing membrane repair (Brito et al. 2019; Romero et al. 2017). The strength of injury-triggered Ca^{2+} signalling may dictate the time required for the cell to restore full membrane integrity and depends on the dimension of the toxin pore, which varies in size and stoichiometry (Dal Peraro and van der Goot 2016; Gilbert 2016). Although counterintuitive, cells damaged by small pores, such the ones generated by the PFTs alpha-toxin from *S. aureus* (around 2 nm in size), require more time to restore homeostasis compared to cells damaged by members of the MACPF/CDCs family, such as LLO or SLO, which have pores in the size range of 30–50 nm (Dal Peraro and van der Goot 2016; Gilbert 2016). Larger pores allow a more rapid and acute variation in Ca^{2+} concentration, in turn, triggering a rapid activation of Ca^{2+}-mediated repair mechanisms (Gekara et al. 2007; Wolfmeier et al. 2015b). On the other hand, excessive and extensive Ca^{2+} influx can compromise signalling and promote cell death (Babiychuk et al. 2009; Gekara et al. 2007).

So far, ESCRT-III-mediated shedding of toxin pores on microvesicles is one of the best characterized mechanism of membrane repair to resist killing by PFTs, such as CDCs (Figure 6) (Keyel et al. 2011; Romero et al. 2017). Oligomerization of the CDCS family members SLO and PFO is sufficient to initiate shedding, and shed vesicles can contain as well pre-pore structures, however, pore formation enhances this process. Interestingly, SLO and PFO mutants defective in oligomerization are removed by endocytosis, possibly as a complementary mechanism to shedding in order to remove remaining inactive toxin at the cell surface (Romero et al. 2017). The CDC family member LLO, whose primary role is to help *Listeria* escaping from the macrophage phagolysosome (see above and Figure 1a), may trigger a different membrane repair response as killing by this toxin is less susceptible to Ca^{2+} impact. This may be linked to a decrease in the extent of vesicle shedding triggered by LLO compared to other CDCs (Maurer et al. 2018).

Besides direct lysis, pathogenic bacteria can also trigger the activation of cell death programs, with consequent activation of counterbalancing mechanisms by the host cell to restore homeostasis. Recently, a dynamic interplay of pyroptosis and ESCRT-mediated damage repair has been proposed in the context of *Mtb* infection (Figure 6) (Beckwith et al. 2020). During *Mtb* infection, PM damage inflicted by the T7SS ESX-1 causes K^+ efflux and, consequently, activation of the sensor NLRP3 leading to inflammasome assembly and pyroptosis. Membrane damage occurs either during the phagocytic uptake or following the escape of the bacteria from the phagosome into the cytosol. The authors suggest that PM damage might be balanced by ESCRT-dependent repair, to further control the timing of inflammasome activation, IL-1 release and pyroptosis (Figure 6).

Balancing membrane damage and repair in cell death programs

How specific membrane repair machineries are activated upon regulated cell death is currently subject of intense research. Variation in Ca^{2+} influx remains central in mediating either cell death or survival and this balance is strictly connected to the extension of PM damage by cell death executors. Importantly, Ca^{2+} influx represents an early event of membrane damage preceding complete PM rupture in cell death programs such as necroptosis and pyroptosis (de Vasconcelos et al. 2019; Ros et al. 2017). This delay provides the time-frame in which cells can still control their fate through the removal of formed pores by membrane repair machineries (Broz et al. 2020; Cao and Kagan 2022; Evavold and Kagan 2019). In the context of pyroptosis, the modulation of GSDM pores at the PM may prevent cells from dying, but not from releasing cytokines (Cao and Kagan 2022). The decoupling of cytokines release from cell death enables a strategic cellular immune response reducing local inflammation in favour of adaptive immunity, which is for example beneficial in disease settings (Zanoni et al. 2016). After formation of functional pores, cells can counteract membrane damage and stay viable through removal of GSDMD pores by the ESCRT-III repair machinery (Figure 6) (Rühl et al.

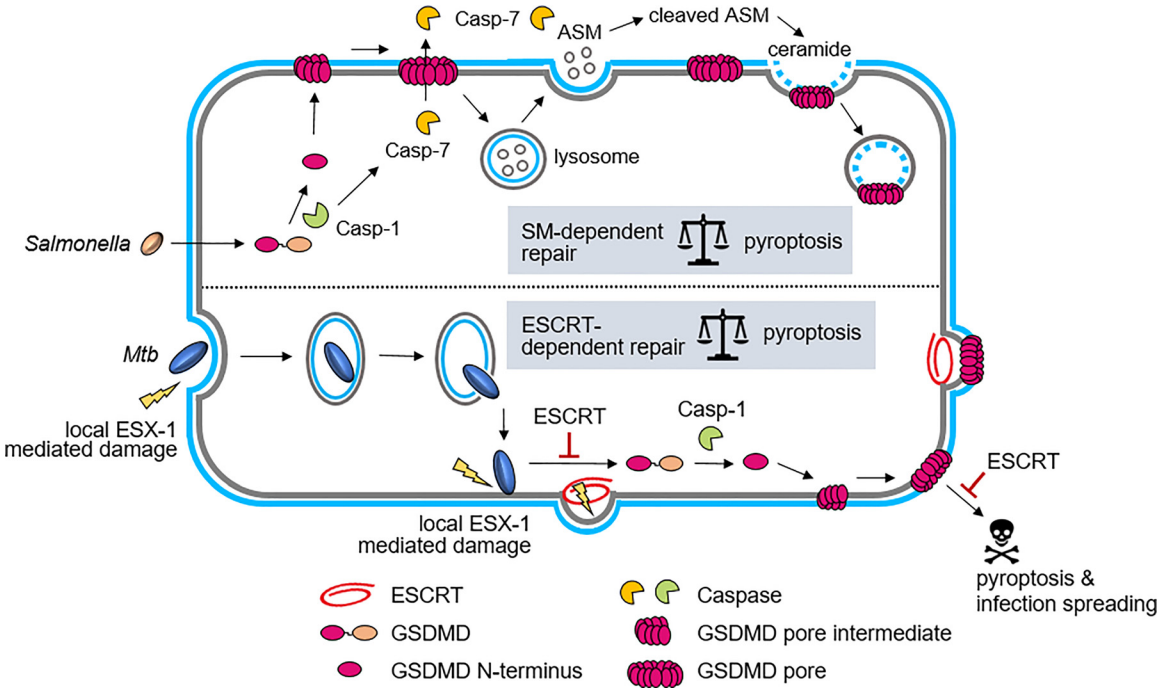

Figure 6: Balancing membrane damage and repair in infection and pyroptosis. The precise timing of cell death is modulated by the elimination of GSDMD-pores by either (i) SM- or (ii) ESCRT-dependent repair. (i) During *Salmonella* infection, Ca^{2+} influx through GSDMD pores leads to the exocytosis of lysosomes and to release of pro-ASM into the extracellular space. Caspase-7 activates ASM which generates ceramide leading to the removal of GSDMD-pores. (ii) ESX-1 mediated PM damage during *Mtb* uptake or cell-to-cell leads to the activation of pyroptosis and the formation of GSDMD-pores. Cell death can be delayed by the removal of the damage site or GSDMD-pores by ESCRT-dependent repair.

2018), a repair mechanism common also to necroptosis and ferroptosis (Gong et al. 2017; Pedrera et al. 2021). Consistently, ESCRT-III deficiency increases GSDMD-mediated permeabilization and pyroptosis (Rühl et al. 2018). In this context, it has been proposed that the membrane curvature induced by GSDMD pores acts as a signal for initiating ESCRT-III-dependent membrane repair (Lee et al. 2015; Wang and Ruan 2022). A recent study has also revealed a ceramide-dependent mechanism of repair of GSDMD pores that is mediated by caspase-7 (Nozaki et al. 2022). Upon infection with *S. Typhimurium*, caspase-7 facilitates the extrusion of intestinal epithelial cells by activating ASM by cleavage. Accordingly, IECs extrusion is incomplete in the presence of inactive ASM or in $Casp7^{-/-}$ mice and organoids. Mechanistically, active ASM generates ceramide that drives PM repair. The repair mechanism is likely initiated by Ca^{2+} influx through GSDMD pores, a process that causes lysosomal exocytosis to release pro-ASM to the cell surface (Figure 6). The authors suggested a model in which the caspase 7-ASM interaction occurs outside of the cell upon passage of caspase-7 through GSDMD pores. Whether this model holds true requires further studies to clarify the site of interaction between caspase-7 and pro-ASM.

Conclusions and outlook

Membrane damage is a persistent threat to cell survival that can be inflicted by various clues, from mechanical stressors and bilayer disrupting bacterial toxins to endogenous PFPs that control distinct cell death programs. To eliminate life-threatening membrane lesions, cells developed a repertoire of membrane restoration pathways. ESCRT-dependent and ESCRT-independent repair mechanisms function in parallel to preserve the structural integrity of the PM, lysosomes and BCVs. While ESCRT- and SM-dependent lysosomal repair pathways appear to operate largely autonomously, both are believed to drive an inward budding of the damaged membrane area and its subsequent degradation in the lysosomal lumen. Lysosomal damage also activates an ER-mediated lipid transfer pathway that supplies injured lysosomes with fresh lipids, presumably to substitute those lost during ESCRT- and SM-dependent repair and maintain the surface area of lysosomes that undergo restoration. Further work is necessary to validate the above concepts and elucidate the crosstalk among the ESCRT-, SM- and ER-dependent lysosomal repair pathways. A plausible reason for the existence

of complementary mechanisms to restore damaged lysosomes is that these organelles are exposed to a wide array of membrane destabilizing agents while leakage of their contents poses an acute threat to cell viability. A thorough characterization of these mechanisms is warranted as a decline in lysosome integrity is not only a driver of physiological aging but also increasingly implicated in numerous age-related diseases.

When continuous membrane damage overwhelms the cellular repair machinery, cells may resort to evolutionary-designed cell death pathways for restoring organismal homeostasis. In this context, a particular intriguing topic for future investigations is how cells deal with membrane damage inflicted by extrinsic and endogenous PFPs. For instance, pathogenic bacteria release their PFTs at concentrations sufficient to elicit host cell responses that support their intracellular lifestyle while avoiding killing the host. Consequently, ultra-structural and super-resolution microscopy of bacteria-inflicted wounds along with the localization of mobilized host membrane repair machinery may reveal novel mechanistic insights relevant for antimicrobial strategies to fight bacterial infections. However, as toxin pores vary considerably in size and stoichiometry, each type may elicit a distinct cellular repair mechanism, thus complicating any extrapolation. Additionally, further investigations are necessary to decipher the dynamic interplay between membrane damage caused by endogenous PFPs such as GSDM, which participates in pro-inflammatory cell death pathways, and membrane repair pathways. Recent studies revealed that a delicate balance between GSDM-mediated PM permeabilization and repair enables cells to decouple the release of cytokines from cell death, thus suppressing local inflammation while promoting intercellular signaling to boost adaptive immune responses. Unraveling how cells modulate the action of GSDMs and other endogenous PFPs to meet physiological demands provides another promising area for future research.

Acknowledgements: We gratefully acknowledge the Deutsche Forschungsgemeinschaft for financial support of the work carried out in the laboratories of the authors (SFB944-P14 to J.H., SFB944-P25 to C.B., and SFB944-P26 to K.C.). C.B. is member of the SPP2225.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: This work was financially supported by the Deutsche Forschungsgemeinschaft (SFB944-P14 to J.H, SFB944-P25 to C.B, SFB944-P26 to K.C).

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Abreu-Blanco, M.T., Verboon, J.M., and Parkhurst, S.M. (2014). Coordination of Rho family GTPase activities to orchestrate cytoskeleton responses during cell wound repair. Curr. Biol. 24: 144–155.

Aglietti, R.A., Estevez, A., Gupta, A., Ramirez, M.G., Liu, P.S., Kayagaki, N., Ciferri, C., Dixit, V.M., and Dueber, E.C. (2016). GsdmD p30 elicited by caspase-11 during pyroptosis forms pores in membranes. Proc. Natl. Acad. Sci. U. S. A. 113: 7858–7863.

Alonso, A. and Goñi, F.M. (2018). The physical properties of ceramides in membranes. Annu. Rev. Biophys. 47: 633–654.

Ammendolia, D.A., Bement, W.M., and Brumell, J.H. (2021). Plasma membrane integrity: implications for health and disease. BMC Biol. 19: 71.

Andrews, N.W., Almeida, P.E., and Corrotte, M. (2014). Damage control: cellular mechanisms of plasma membrane repair. Trends Cell Biol. 24: 734–742.

Antonny, B., Bigay, J., and Mesmin, B. (2018). The oxysterol-binding protein cycle: burning off PI(4)P to transport cholesterol. Annu. Rev. Biochem. 87: 809–837.

Augenstreich, J., Arbues, A., Simeone, R., Haanappel, E., Wegener, A., Sayes, F., Le Chevalier, F., Chalut, C., Malaga, W., Guilhot, C., et al. (2017). ESX-1 and phthiocerol dimycocerosates of *Mycobacterium tuberculosis* act in concert to cause phagosomal rupture and host cell apoptosis. Cell Microbiol. 19: 7.

Augenstreich, J., Haanappel, E., Ferré, G., Czaplicki, G., Jolibois, F., Destainville, N., Guilhot, C., Milon, A., Astarie-Dequeker, C., and Chavent, M. (2019). The conical shape of DIM lipids promotes *Mycobacterium tuberculosis* infection of macrophages. Proc. Natl. Acad. Sci. U. S. A. 116: 25649–25658.

Babiychuk, E., Monastyrskaya, K., Potez, S., and Draeger, A. (2009). Intracellular Ca^{2+} operates a switch between repair and lysis of streptolysin O-perforated cells. Cell Death Differ. 16: 1126–1134.

Babiychuk, E.B., Monastyrskaya, K., and Draeger, A. (2008). Fluorescent annexin A1 reveals dynamics of ceramide platforms in living cells. Traffic 9: 1757–1775.

Bansal, D., Miyake, K., Vogel, S.S., Groh, S., Chen, C.C., Williamson, R., McNeil, P.L., and Campbell, K.P. (2003). Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 423: 168–172.

Barisch, C., Paschke, P., Hagedorn, M., Maniak, M., and Soldati, T. (2015). Lipid droplet dynamics at early stages of *Mycobacterium marinum* infection in *Dictyostelium*. Cell Microbiol. 17: 1332–1349.

Barisch, C. and Soldati, T. (2017a). Breaking fat! How mycobacteria and other intracellular pathogens manipulate host lipid droplets. Biochimie 141: 54–61.

Barisch, C. and Soldati, T. (2017b). *Mycobacterium marinum* degrades both triacylglycerols and phospholipids from its *Dictyostelium* host to synthesise its own triacylglycerols and generate lipid inclusions. PLoS Pathog. 13: e1006095.

Beckwith, K.S., Beckwith, M.S., Ullmann, S., Sætra, R.S., Kim, H., Marstad, A., Åsberg, S.E., Strand, T.A., Haug, M., Niederweis, M., et al. (2020). Plasma membrane damage causes NLRP3 activation and pyroptosis during *Mycobacterium tuberculosis* infection. Nat. Commun. 11: 2270.

Benink, H.A. and Bement, W.M. (2005). Concentric zones of active RhoA and Cdc42 around single cell wounds. J. Cell Biol. 168: 429–439.

Berg, T.O., Strømhaug, E., Løvdal, T., Seglen, O., and Berg, T. (1994). Use of glycyl-L-phenylalanine 2-naphthylamide, a lysosome-disrupting cathepsin C substrate, to distinguish between lysosomes and prelysosomal endocytic vacuoles. Biochem. J. 300: 229–236.

Bleicken, S., Hantusch, A., Das, K.K., Frickey, T., and Garcia-Saez, A.J. (2017). Quantitative interactome of a membrane Bcl-2 network identifies a hierarchy of complexes for apoptosis regulation. Nat. Commun. 8: 73.

Bleicken, S., Jeschke, G., Stegmueller, C., Salvador-Gallego, R., García-Sáez, A.J., and Bordignon, E. (2014). Structural model of active bax at the membrane. Mol. Cell 56: 496–505.

Bleicken, S., Landeta, O., Landajuela, A., Basañez, G., and García-Sáez, A.J. (2013). Proapoptotic BAX and BAK proteins form stable protein-permeable pores of tunable size. J. Biol. Chem. 288: 33241–33252.

Bouter, A., Gounou, C., Bérat, R., Tan, S., Gallois, B., Granier, T., d'Estaintot, B.L., Pöschl, E., Brachvogel, B., and Brisson, A.R. (2011). Annexin-A5 assembled into two-dimensional arrays promotes cell membrane repair. Nat. Commun. 2: 270.

Brito, C., Cabanes, D., Sarmento Mesquita, F., and Sousa, S. (2019). Mechanisms protecting host cells against bacterial pore-forming toxins. Cell. Mol. Life Sci. 76: 1319–1339.

Broz, P., Pelegrín, P., and Shao, F. (2020). The gasdermins, a protein family executing cell death and inflammation. Nat. Rev. Immunol. 20: 143–157.

Caire-Brandli, I., Papadopoulos, A., Malaga, W., Marais, D., Canaan, S., Thilo, L., and de Chastellier, C. (2014). Reversible lipid accumulation and associated division arrest of *Mycobacterium avium* in lipoprotein-induced foamy macrophages may resemble key events during latency and reactivation of tuberculosis. Infect. Immun. 82: 476–490.

Cambier, C.J., Banik, S.M., Buonomo, J.A., and Bertozzi, C.R. (2020). Spreading of a mycobacterial cell-surface lipid into host epithelial membranes promotes infectivity. Elife 9: e60648.

Cantuti-Castelvetri, L., Fitzner, D., Bosch-Queralt, M., Weil, M.T., Su, M., Sen, P., Ruhwedel, T., Mitkovski, M., Trendelenburg, G., Lütjohann, D., et al. (2018). Defective cholesterol clearance limits remyelination in the aged central nervous system. Science 359: 684–688.

Cao, A. and Kagan, J.C. (2022). Gasdermin pore forming activities that promote inflammation from living and dead cells. J. Mol. Biol. 434: 167427.

Chauhan, S., Kumar, S., Jain, A., Ponpuak, M., Mudd, M.H., Kimura, T., Choi, S.W., Peters, R., Mandell, M., Bruun, J.A., et al. (2016). TRIMs and galectins globally cooperate and TRIM16 and galectin-3 co-direct autophagy in endomembrane damage homeostasis. Dev. Cell 39: 13–27.

Chin, H.S., Li, M.X., Tan, I.K., Ninnis, R.L., Reljic, B., Scicluna, K., Dagley, L.F., Sandow, J.J., Kelly, G.L., Samson, A.L., et al. (2018). VDAC2 enables BAX to mediate apoptosis and limit tumor development. Nat. Commun. 9: 1–13.

Christen, M., Coye, L.H., Hontz, J.S., LaRock, D.L., Pfuetzner, R.A., Megha, and Miller, S.I. (2009). Activation of a bacterial virulence protein by the GTPase RhoA. Sci. Signal. 2: ra71.

Clapham, D.E. (2007). Calcium signaling. Cell 131: 1047–1058.

Coelho, C., Brown, L., Maryam, M., Vij, R., Smith, D.F.Q., Burnet, M.C., Kyle, J.E., Heyman, H.M., Ramirez, J., Prados-Rosales, R., et al. (2019). Listeria monocytogenes virulence factors, including listeriolysin O, are secreted in biologically active extracellular vesicles. J. Biol. Chem. 294: 1202–1217.

Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K., Gas, S., Barry, C.E., 3rd, et al. (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393: 537–544.

Cooper, S.T. and McNeil, P.L. (2015). Membrane repair: mechanisms and pathophysiology. Physiol. Rev. 95: 1205–1240.

Corrotte, M., Fernandes, M.C., Tam, C., and Andrews, N.W. (2012). Toxin pores endocytosed during plasma membrane repair traffic into the lumen of MVBs for degradation. Traffic 13: 483–494.

Cosentino, K. and García-Sáez, A.J. (2017). Bax and Bak pores: are we closing the circle? Trends Cell Biol. 27: 266–275.

Cosentino, K., Hermann, E., von Kügelgen, N., Unsay, J.D., Ros, U., and García-Sáez, A.J. (2021). Force mapping study of actinoporin effect in membranes presenting phase domains. Toxins 13: 669.

Cosentino, K., Hertlein, V., Jenner, A., Dellmann, T., Gojkovic, M., Peña-Blanco, A., Dadsena, S., Wajngarten, N., Danial, J.S.H., Thevathasan, J.V., et al. (2022). The interplay between BAX and BAK tunes apoptotic pore growth to control mitochondrial-DNA-mediated inflammation. Mol. Cell 82: 933–949.e939.

Cosentino, K., Ros, U., and García-Sáez, A.J. (2016). Assembling the puzzle: oligomerization of α-pore forming proteins in membranes. Biochim. Biophys. Acta Biomembr. 1858: 457–466.

Costa, T.R., Felisberto-Rodrigues, C., Meir, A., Prevost, M.S., Redzej, A., Trokter, M., and Waksman, G. (2015). Secretion systems in Gram-negative bacteria: structural and mechanistic insights. Nat. Rev. Microbiol. 13: 343–359.

Cowan, A.D., Smith, N.A., Sandow, J.J., Kapp, E.A., Rustam, Y.H., Murphy, J.M., Brouwer, J.M., Bernardini, J.P., Roy, M.J., Wardak, A.Z., et al. (2020). BAK core dimers bind lipids and can be bridged by them. Nat. Struct. Mol. Biol. 27: 1024–1031.

Czabotar, P.E., Lessene, G., Strasser, A., and Adams, J.M. (2014). Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat. Rev. Mol. Cell Biol. 15: 49–63.

Czabotar, P.E., Westphal, D., Dewson, G., Ma, S., Hockings, C., Fairlie, W.D., Lee, E.F., Yao, S., Robin, A.Y., Smith, B.J., et al. (2013). Bax crystal structures reveal how BH3 domains activate Bax and nucleate its oligomerization to induce apoptosis. Cell 152: 519–531.

Dadsena, S., Bockelmann, S., Mina, J.G.M., Hassan, D.G., Korneev, S., Razzera, G., Jahn, H., Niekamp, P., Müller, D., Schneider, M., et al. (2019). Ceramides bind VDAC2 to trigger mitochondrial apoptosis. Nat. Commun. 10: 1832.

Dal Peraro, M. and van der Goot, F.G. (2016). Pore-forming toxins: ancient, but never really out of fashion. Nat. Rev. Microbiol. 14: 77–92.

De Leon, J., Jiang, G., Ma, Y., Rubin, E., Fortune, S., and Sun, J. (2012). *Mycobacterium tuberculosis* ESAT-6 exhibits a unique membrane-interacting activity that is not found in its ortholog from non-pathogenic *Mycobacterium smegmatis*. J. Biol. Chem. 287: 44184–44191.

de Vasconcelos, N.M., Van Opdenbosch, N., Van Gorp, H., Parthoens, E., and Lamkanfi, M. (2019). Single-cell analysis of pyroptosis dynamics reveals conserved GSDMD-mediated subcellular events that precede plasma membrane rupture. Cell Death Differ. 26: 146–161.

Demonbreun, A.R., Quattrocelli, M., Barefield, D.Y., Allen, M.V., Swanson, K.E., and McNally, E.M. (2016). An actin-dependent annexin complex mediates plasma membrane repair in muscle. J. Cell Biol. 213: 705–718.

Deng, Y., Rivera-Molina, F.E., Toomre, D.K., and Burd, C.G. (2016). Sphingomyelin is sorted at the *trans* Golgi network into a distinct class of secretory vesicle. Proc. Natl. Acad. Sci. U. S. A. 113: 6677–6682.

Dewson, G., Kratina, T., Czabotar, P., Day, C.L., Adams, J.M., and Kluck, R.M. (2009). Bak activation for apoptosis involves oligomerization of dimers via their α6 helices. Mol. Cell 36: 696–703.

Dewson, G., Kratina, T., Sim, H.W., Puthalakath, H., Adams, J.M., Colman, P.M., and Kluck, R.M. (2008). To trigger apoptosis, Bak exposes its BH3 domain and homodimerizes via BH3:groove interactions. Mol. Cell 30: 369–380.

Dias, C. and Nylandsted, J. (2021). Plasma membrane integrity in health and disease: significance and therapeutic potential. Cell Discov. 7: 4.

Ding, J., Wang, K., Liu, W., She, Y., Sun, Q., Shi, J., Sun, H., Wang, D.-C., and Shao, F. (2016). Pore-forming activity and structural autoinhibition of the gasdermin family. Nature 540: 150.

Du, J., Reeves, A.Z., Klein, J.A., Twedt, D.J., Knodler, L.A., and Lesser, C.F. (2016). The type III secretion system apparatus determines the intracellular niche of bacterial pathogens. Proc. Natl. Acad. Sci. U. S. A. 113: 4794–4799.

Dunstone, M.A. and Tweten, R.K. (2012). Packing a punch: the mechanism of pore formation by cholesterol dependent cytolysins and membrane attack complex/perforin-like proteins. Curr. Opin. Struct. Biol. 22: 342–349.

Edlich, F., Banerjee, S., Suzuki, M., Cleland, Megan M., Arnoult, D., Wang, C., Neutzner, A., Tjandra, N., and Youle, Richard J. (2011). Bcl-xL retrotranslocates Bax from the mitochondria into the cytosol. Cell 145: 104–116.

Ellison, C.J., Kukulski, W., Boyle, K.B., Munro, S., and Randow, F. (2020). Transbilayer movement of sphingomyelin precedes catastrophic breakage of enterobacteria-containing vacuoles. Curr. Biol. 30: 2974–2983.e2976.

Etxaniz, A., González-Bullón, D., Martín, C., Alonso, M.T., and Ostolaza, H. (2020). Irreversible versus repairable membrane poration: differences in permeabilization elicited by *Bordetella* adenylate cyclase toxin and its hemolysin domain in macrophages. FEBS J. 287: 1798–1815.

Evavold, C.L. and Kagan, J.C. (2019). Defying death: the (w)hole truth about the fate of GSDMD pores. Immunity 50: 15–17.

Fernandes, M.C., Cortez, M., Flannery, A.R., Tam, C., Mortara, R.A., and Andrews, N.W. (2011). *Trypanosoma cruzi* subverts the sphingomyelinase-mediated plasma membrane repair pathway for cell invasion. J. Exp. Med. 208: 909–921.

Flores-Díaz, M., Monturiol-Gross, L., Naylor, C., Alape-Girón, A., and Flieger, A. (2016). Bacterial sphingomyelinases and phospholipases as virulence factors. Microbiol. Mol. Biol. Rev. 80: 597–628.

Flores-Romero, H., Ros, U., and Garcia-Saez, A.J. (2020). Pore formation in regulated cell death. EMBO J. 39: e105753.

Foulon, M., Listian, S.A., Soldati, T., and Barisch, C. (2022). Chapter 6 – conserved mechanisms drive host-lipid access, import, and utilization in *Mycobacterium tuberculosis* and *M. marinum*. In: Fatima, Z. and Canaan, S. (Eds.). Biology of mycobacterial lipids. Academic Press, pp. 133–161, Available at: https://www.sciencedirect.com/book/9780323919487/biology-of-mycobacterial-lipids#book-description.

Frank, D. and Vince, J.E. (2019). Pyroptosis versus necroptosis: similarities, differences, and crosstalk. Cell Death Differ. 26: 99–114.

Fuertes, G., García-Sáez, A.J., Esteban-Martín, S., Giménez, D., Sánchez-Muñoz, O.L., Schwille, P., and Salgado, J. (2010). Pores formed by Baxα5 relax to a smaller size and keep at equilibrium. Biophys. J. 99: 2917–2925.

Ganesan, V., Perera, M.N., Colombini, D., Datskovskiy, D., Chadha, K., and Colombini, M. (2010). Ceramide and activated Bax act synergistically to permeabilize the mitochondrial outer membrane. Apoptosis 15: 553–562.

Gaschler, M.M. and Stockwell, B.R. (2017). Lipid peroxidation in cell death. Biochem. Biophys. Res. Commun. 482: 419–425.

Gekara, N.O., Westphal, K., Ma, B., Rohde, M., Groebe, L., and Weiss, S. (2007). The multiple mechanisms of Ca^{2+} signalling by listeriolysin O, the cholesterol-dependent cytolysin of *Listeria monocytogenes*. Cell Microbiol. 9: 2008–2021.

Gerlach, R.G. and Hensel, M. (2007). Protein secretion systems and adhesins: the molecular armory of Gram-negative pathogens. Int J Med Microbiol 297: 401–415.

Gilbert, R.J. (2016). Protein–lipid interactions and non-lamellar lipidic structures in membrane pore formation and membrane fusion. Biochim. Biophys. Acta Biomembr. 1858: 487–499.

Gong, Y.N., Guy, C., Olauson, H., Becker, J.U., Yang, M., Fitzgerald, P., Linkermann, A., and Green, D.R. (2017). ESCRT-III acts downstream of MLKL to regulate necroptotic cell death and its consequences. Cell 169: 286–300.e216.

Göser, V., Kehl, A., Röder, J., and Hensel, M. (2020). Role of the ESCRT-III complex in controlling integrity of the *Salmonella*-containing vacuole. Cell Microbiol. 22: e13176.

Gozen, I. and Dommersnes, P. (2014). Pore dynamics in lipid membranes. Eur. Phys. J. Spec. Top. 223: 1813–1829.

Grassmé, H., Gulbins, E., Brenner, B., Ferlinz, K., Sandhoff, K., Harzer, K., Lang, F., and Meyer, T.F. (1997). Acidic sphingomyelinase mediates entry of *N. gonorrhoeae* into nonphagocytic cells. Cell 91: 605–615.

Große, L., Wurm, C.A., Brüser, C., Neumann, D., Jans, D.C., and Jakobs, S. (2016). Bax assembles into large ring-like structures remodeling the mitochondrial outer membrane in apoptosis. EMBO J. 35: 402–413.

Hagedorn, M. and Soldati, T. (2007). Flotillin and RacH modulate the intracellular immunity of *Dictyostelium* to *Mycobacterium marinum* infection. Cell Microbiol. 9: 2716–2733.

Hanna, N., Koliwer-Brandl, H., Lefrançois, L.H., Kalinina, V., Cardenal-Muñoz, E., Appiah, J., Leuba, F., Gueho, A., Hilbi, H., Soldati, T., et al (2021). Zn^{2+} intoxication of *Mycobacterium marinum* during *Dictyostelium discoideum* infection is counteracted by induction of the pathogen Zn^{2+} exporter CtpC. mBio 12: e01313–e01320.

Hauseman, Z.J., Harvey, E.P., Newman, C.E., Wales, T.E., Bucci, J.C., Mintseris, J., Schweppe, D.K., David, L., Fan, L., Cohen, D.T., et al. (2020). Homogeneous oligomers of pro-apoptotic BAX reveal structural determinants of mitochondrial membrane permeabilization. Mol. Cell 79: 68–83.e67.

He, W.-t., Wan, H., Hu, L., Chen, P., Wang, X., Huang, Z., Yang, Z.-H., Zhong, C.-Q., and Han, J. (2015). Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. Cell Res. 25: 1285–1298.

Holopainen, J.M., Angelova, M.I., and Kinnunen, P.K. (2000). Vectorial budding of vesicles by asymmetrical enzymatic formation of ceramide in giant liposomes. Biophys. J. 78: 830–838.

Holthuis, J.C. and Menon, A.K. (2014). Lipid landscapes and pipelines in membrane homeostasis. Nature 510: 48–57.

Howard, J.G., Wallace, K.R., and Wright, G.P. (1953). The inhibitory effects of cholesterol and related sterols on haemolysis by streptolysin O. Br. J. Exp. Pathol. 34: 174–180.

Hung, Y.-H., Chen, L.M.-W., Yang, J.-Y., and Yang, W.Y. (2013). Spatiotemporally controlled induction of autophagy-mediated lysosome turnover. Nat. Commun. 4: 2111.

Husmann, M., Beckmann, E., Boller, K., Kloft, N., Tenzer, S., Bobkiewicz, W., Neukirch, C., Bayley, H., and Bhakdi, S. (2009). Elimination of a bacterial pore-forming toxin by sequential endocytosis and exocytosis. FEBS Lett. 583: 337–344.

Idone, V., Tam, C., Goss, J.W., Toomre, D., Pypaert, M., and Andrews, N.W. (2008). Repair of injured plasma membrane by rapid Ca^{2+}-dependent endocytosis. J. Cell Biol. 180: 905–914.

Jain, A., Beutel, O., Ebell, K., Korneev, S., and Holthuis, J.C. (2017). Diverting CERT-mediated ceramide transport to mitochondria triggers Bax-dependent apoptosis. J. Cell Sci. 130: 360–371.

Jain, A., Dadsena, S., and Holthuis, J.C.M. (2020). A switchable ceramide transfer protein for dissecting the mechanism of ceramide-induced mitochondrial apoptosis. FEBS Lett. 594: 3739–3750.

Jenner, A., Peña-Blanco, A., Salvador-Gallego, R., Ugarte-Uribe, B., Zollo, C., Ganief, T., Bierlmeier, J., Mund, M., Lee, J.E., Ries, J., et al. (2022). DRP1 interacts directly with BAX to induce its activation and apoptosis. EMBO J. 41: e108587.

Jia, J., Abudu, Y.P., Claude-Taupin, A., Gu, Y., Kumar, S., Choi, S.W., Peters, R., Mudd, M.H., Allers, L., Salemi, M., et al. (2018). Galectins control mTOR in response to endomembrane damage. Mol. Cell 70: 120–135.e128.

Jia, J., Claude-Taupin, A., Gu, Y., Choi, S.W., Peters, R., Bissa, B., Mudd, M.H., Allers, L., Pallikkuth, S., Lidke, K.A., et al. (2020). Galectin-3 coordinates a cellular system for lysosomal repair and removal. Dev. Cell 52: 69–87.e68.

Jiang, X., Jiang, H., Shen, Z., and Wang, X. (2014). Activation of mitochondrial protease OMA1 by Bax and Bak promotes cytochrome c release during apoptosis. Proc. Natl. Acad. Sci. U. S. A. 111: 14782–14787.

Jimenez, A.J., Maiuri, P., Lafaurie-Janvore, J., Divoux, S., Piel, M., and Perez, F. (2014). ESCRT machinery is required for plasma membrane repair. Science 343: 1247136.

Jimenez, A.J. and Perez, F. (2017). Plasma membrane repair: the adaptable cell life-insurance. Curr. Opin. Cell Biol. 47: 99–107.

Johannes, L., Jacob, R., and Leffler, H. (2018). Galectins at a glance. J. Cell Sci. 131: jcs208884.

Katzmann, D.J., Stefan, C.J., Babst, M., and Emr, S.D. (2003). Vps27 recruits ESCRT machinery to endosomes during MVB sorting. J. Cell Biol. 162: 413–423.

Kayagaki, N., Stowe, I.B., Lee, B.L., O'Rourke, K., Anderson, K., Warming, S., Cuellar, T., Haley, B., Roose-Girma, M., Phung, Q.T., et al. (2015). Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature 526: 666–671.

Kehl, A., Noster, J., and Hensel, M. (2020). Eat in or take out? metabolism of intracellular *Salmonella enterica*. Trends Microbiol. 28: 644–654.

Keyel, P.A., Loultcheva, L., Roth, R., Salter, R.D., Watkins, S.C., Yokoyama, W.M., and Heuser, J.E. (2011). Streptolysin O clearance through sequestration into blebs that bud passively from the plasma membrane. J. Cell Sci. 124: 2414–2423.

Koerdt, S.N., Ashraf, A.P.K., and Gerke, V. (2019). Annexins and plasma membrane repair. Curr. Top. Membr. 84: 43–65.

Kolodziejek, A.M. and Miller, S.I. (2015). *Salmonella* modulation of the phagosome membrane, role of SseJ. Cell Microbiol. 17: 333–341.

Krawczyk, P.A., Laub, M., and Kozik, P. (2020). To kill but not be killed: controlling the activity of mammalian pore-forming proteins. Front. Immunol. 11: 601405.

Kunz, T.C. and Kozjak-Pavlovic, V. (2019). Diverse facets of sphingolipid involvement in bacterial infections. Front. Cell Dev. Biol. 7: 203.

Kuwana, T., Mackey, M.R., Perkins, G., Ellisman, M.H., Latterich, M., Schneiter, R., Green, D.R., and Newmeyer, D.D. (2002). Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 111: 331–342.

LaRock, D.L., Johnson, A.F., Wilde, S., Sands, J.S., Monteiro, M.P., and LaRock, C.N. (2022). Group A Streptococcus induces GSDMA-dependent pyroptosis in keratinocytes. Nature 605: 527–531.

Lauterwasser, J., Todt, F., Zerbes, R.M., Nguyen, T.N., Craigen, W., Lazarou, M., van der Laan, M., and Edlich, F. (2016). The porin VDAC2 is the mitochondrial platform for Bax retrotranslocation. Sci. Rep. 6: 32994.

Le Chevalier, F., Cascioferro, A., Frigui, W., Pawlik, A., Boritsch, E.C., Bottai, D., Majlessi, L., Herrmann, J.L., and Brosch, R. (2015). Revisiting the role of phospholipases C in virulence and the lifecycle of *Mycobacterium tuberculosis*. Sci. Rep. 5: 16918.

Lee, H., Rotolo, J.A., Mesicek, J., Penate-Medina, T., Rimner, A., Liao, W.C., Yin, X., Ragupathi, G., Ehleiter, D., Gulbins, E., et al. (2011). Mitochondrial ceramide-rich macrodomains functionalize Bax upon irradiation. PLoS One 6: e19783.

Lee, I.H., Kai, H., Carlson, L.A., Groves, J.T., and Hurley, J.H. (2015). Negative membrane curvature catalyzes nucleation of endosomal sorting complex required for transport (ESCRT)-III assembly. Proc. Natl. Acad. Sci. U. S. A. 112: 15892–15897.

Lee, M.S. and Bensinger, S.J. (2022). Reprogramming cholesterol metabolism in macrophages and its role in host defense against cholesterol-dependent cytolysins. Cell. Mol. Immunol. 19: 327–336.

Lee, W., VanderVen, B.C., Fahey, R.J., and Russell, D.G. (2013). Intracellular *Mycobacterium tuberculosis* exploits host-derived fatty acids to limit metabolic stress. J. Biol. Chem. 288: 6788–6800.

Lerner, T.R., Queval, C.J., Fearns, A., Repnik, U., Griffiths, G., and Gutierrez, M.G. (2018). Phthiocerol dimycocerosates promote access to the cytosol and intracellular burden of *Mycobacterium tuberculosis* in lymphatic endothelial cells. BMC Biol. 16: 1.

Leventis, P.A. and Grinstein, S. (2010). The distribution and function of phosphatidylserine in cellular membranes. Annu. Rev. Biophys. 39: 407–427.

Lewis, K.N., Liao, R., Guinn, K.M., Hickey, M.J., Smith, S., Behr, M.A., and Sherman, D.R. (2003). Deletion of RD1 from *Mycobacterium tuberculosis* mimics bacille Calmette-Guérin attenuation. J. Infect. Dis. 187: 117–123.

Lieberman, J., Wu, H., and Kagan, J.C. (2019). Gasdermin D activity in inflammation and host defense. Sci. Immunol. 4: eaav1447.

Liu, X., Xia, S., Zhang, Z., Wu, H., and Lieberman, J. (2021). Channelling inflammation: gasdermins in physiology and disease. Nat. Rev. Drug Discov. 20: 384–405.

Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V.G., Wu, H., and Lieberman, J. (2016). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature 535: 153–158.

Liu, Z., Wang, C., Yang, J., Zhou, B., Yang, R., Ramachandran, R., Abbott, D.W., and Xiao, T.S. (2019). Crystal structures of the full-length murine and human gasdermin D reveal mechanisms of autoinhibition, lipid binding, and oligomerization. Immunity 51: 43–49.e44.

López-Jiménez, A.T., Cardenal-Muñoz, E., Leuba, F., Gerstenmaier, L., Barisch, C., Hagedorn, M., King, J.S., and Soldati, T. (2018). The ESCRT and autophagy machineries cooperate to repair ESX-1-dependent damage at the Mycobacterium-containing vacuole but have opposite impact on containing the infection. PLoS Pathog. 14: e1007501.

Lovell, J.F., Billen, L.P., Bindner, S., Shamas-Din, A., Fradin, C., Leber, B., and Andrews, D.W. (2008). Membrane binding by tBid initiates an ordered series of events culminating in membrane permeabilization by Bax. Cell 135: 1074–1084.

Lv, F., Qi, F., Zhang, Z., Wen, M., Kale, J., Piai, A., Du, L., Wang, S., Zhou, L., Yang, Y., et al. (2021). An amphipathic Bax core dimer forms part of the apoptotic pore wall in the mitochondrial membrane. EMBO J. 40: e106438.

Maekawa, M., Yang, Y., and Fairn, G.D. (2016). Perfringolysin O theta toxin as a tool to monitor the distribution and inhomogeneity of cholesterol in cellular membranes. Toxins 8: 67.

Maurer, J., Hupp, S., Pillich, H., Mitchell, T.J., Chakraborty, T., and Iliev, A.I. (2018). Missing elimination via membrane vesicle shedding contributes to the diminished calcium sensitivity of listeriolysin O. Sci. Rep. 8: 1–9.

McArthur, K., Whitehead, L.W., Heddleston, J.M., Li, L., Padman, B.S., Oorschot, V., Geoghegan, N.D., Chappaz, S., Davidson, S., San Chin, H., et al. (2018). BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. Science 359: eaao6047.

McNeil, P.L. and Khakee, R. (1992). Disruptions of muscle fiber plasma membranes. Role in exercise-induced damage. Am. J. Pathol. 140: 1097–1109.

McNeil, P.L. and Kirchhausen, T. (2005). An emergency response team for membrane repair. Nat. Rev. Mol. Cell Biol. 6: 499–505.

Miao, E.A., Leaf, I.A., Treuting, P.M., Mao, D.P., Dors, M., Sarkar, A., Warren, S.E., Wewers, M.D., and Aderem, A. (2010). Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. Nat. Immunol. 11: 1136–1142.

Mittal, E., Skowyra, M.L., Uwase, G., Tinaztepe, E., Mehra, A., Köster, S., Hanson, P.I., and Philips, J.A. (2018). *Mycobacterium tuberculosis* Type VII secretion system effectors differentially impact the ESCRT endomembrane damage response. mBio 9: e01765–e0176518.

Moldoveanu, T. and Czabotar, P.E. (2020). BAX, BAK, and BOK: a coming of age for the BCL-2 family effector proteins. Cold Spring Harbor Perspect. Biol. 12: a036319.

Monturiol-Gross, L., Villalta-Romero, F., Flores-Díaz, M., and Alape-Girón, A. (2021). Bacterial phospholipases C with dual activity: phosphatidylcholinesterase and sphingomyelinase. FEBS Open Bio 11: 3262–3275.

Mulvihill, E., Sborgi, L., Mari, S.A., Pfreundschuh, M., Hiller, S., and Müller, D.J. (2018). Mechanism of membrane pore formation by human gasdermin-D. EMBO J. 37: e98321.

Naghdi, S., Várnai, P., and Hajnóczky, G. (2015). Motifs of VDAC2 required for mitochondrial Bak import and tBid-induced apoptosis. Proc. Natl. Acad. Sci. U. S. A. 112: E5590–E5599.

Nasu, Y., Benke, A., Arakawa, S., Yoshida, G.J., Kawamura, G., Manley, S., Shimizu, S., and Ozawa, T. (2016). *In situ* characterization of Bak clusters responsible for cell death using single molecule localization microscopy. Sci. Rep. 6: 27505.

Nguyen, B.N. and Portnoy, D.A. (2020). An inducible Cre-lox system to analyze the role of LLO in *Listeria monocytogenes* pathogenesis. Toxins 12: 38.

Niekamp, P., Guzman, G., Leier, H.C., Rashidfarrokhi, A., Richina, V., Pott, F., Barisch, C., Holthuis, J.C.M., and Tafesse, F.G. (2021). Sphingomyelin biosynthesis is essential for phagocytic signaling during *Mycobacterium tuberculosis* host cell entry. mBio 12: e031411–e3220.

Niekamp, P., Scharte, F., Sokoya, T., Vittadello, L., Kim, Y., Deng, Y., Südhoff, E., Hilderink, A., Imlau, M., Clarke, C.J., et al. (2022). Ca^{2+}-activated sphingomyelin scrambling and turnover mediate ESCRT-independent lysosomal repair. Nat. Commun. 13: 1875.

Nozaki, K., Maltez, V.I., Rayamajhi, M., Tubbs, A.L., Mitchell, J.E., Lacey, C.A., Harvest, C.K., Li, L., Nash, W.T., Larson, H.N., et al. (2022). Caspase-7 activates ASM to repair gasdermin and perforin pores. Nature 606: 960–967.

Nurminen, T.A., Holopainen, J.M., Zhao, H., and Kinnunen, P.K.J. (2002). Observation of topical catalysis by sphingomyelinase coupled to microspheres. J. Am. Chem. Soc. 124: 12129–12134.

Ohlson, M.B., Fluhr, K., Birmingham, C.L., Brumell, J.H., and Miller, S.I. (2005). SseJ deacylase activity by *Salmonella enterica* serovar Typhimurium promotes virulence in mice. Infect. Immun. 73: 6249–6259.

Osman, M.M., Pagán, A.J., Shanahan, J.K., and Ramakrishnan, L. (2020). *Mycobacterium marinum* phthiocerol dimycocerosates enhance macrophage phagosomal permeabilization and membrane damage. PLoS One 15: e0233252.

Papadopoulos, C. and Meyer, H. (2017). Detection and clearance of damaged lysosomes by the endo-lysosomal damage response and lysophagy. Curr. Biol. 27: R1330–R1341.

Park, D., Lara-Tejero, M., Waxham, M.N., Li, W., Hu, B., Galán, J.E., and Liu, J. (2018). Visualization of the type III secretion mediated Salmonella-host cell interface using cryo-electron tomography. Elife 7: e39514.

Pedrera, L., Espiritu, R.A., Ros, U., Weber, J., Schmitt, A., Stroh, J., Hailfinger, S., von Karstedt, S., and García-Sáez, A.J. (2021). Ferroptotic pores induce Ca^{2+} fluxes and ESCRT-III activation to modulate cell death kinetics. Cell Death Differ. 28: 1644–1657.

Peng, X. and Sun, J. (2016). Mechanism of ESAT-6 membrane interaction and its roles in pathogenesis of *Mycobacterium tuberculosis*. Toxicon 116: 29–34.

Petrišič, N., Kozorog, M., Aden, S., Podobnik, M., and Anderluh, G. (2021). The molecular mechanisms of listeriolysin O-induced lipid membrane damage. Biochim. Biophys. Acta Biomembr. 1863: 183604.

Peyron, P., Vaubourgeix, J., Poquet, Y., Levillain, F., Botanch, C., Bardou, F., Daffe, M., Emile, J.F., Marchou, B., Cardona, P.J., et al. (2008). Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for *M. tuberculosis* persistence. PLoS Pathog. 4: e1000204.

Pfitzner, A.-K., Mercier, V., Jiang, X., Moser von Filseck, J., Baum, B., Šarić, A., and Roux, A. (2020). An ESCRT-III polymerization sequence drives membrane deformation and fission. Cell 182: 1140–1155.e1118.

Philips, J.A., Porto, M.C., Wang, H., Rubin, E.J., and Perrimon, N. (2008). ESCRT factors restrict mycobacterial growth. Proc. Natl. Acad. Sci. U. S. A. 105: 3070–3075.

Poulsen, C., Panjikar, S., Holton, S.J., Wilmanns, M., and Song, Y.H. (2014). WXG100 protein superfamily consists of three subfamilies and exhibits an α-helical C-terminal conserved residue pattern. PLoS One 9: e89313.

Poussin, M.A., Leitges, M., and Goldfine, H. (2009). The ability of Listeria monocytogenes PI-PLC to facilitate escape from the macrophage phagosome is dependent on host PKCβ. Microb. Pathog. 46: 1–5.

Qian, S., Wang, W., Yang, L., and Huang, H.W. (2008). Structure of transmembrane pore induced by Bax-derived peptide: evidence for lipidic pores. Proc. Natl. Acad. Sci. U. S. A. 105: 17379–17383.

Quigley, J., Hughitt, V.K., Velikovsky, C.A., Mariuzza, R.A., El-Sayed, N.M., and Briken, V. (2017). The cell wall lipid PDIM contributes to phagosomal escape and host cell exit of *Mycobacterium tuberculosis*. mBio 8: e00148–e0014817.

Radulovic, M., Schink, K.O., Wenzel, E.M., Nähse-Kumpf, V., Bongiovanni, A., Lafont, F., and Stenmark, H. (2018). ESCRT-mediated lysosome repair precedes lysophagy and promotes cell survival. EMBO J. 37: e99753.

Radulovic, M., Wenzel, E.M., Gilani, S., Holland, L.K., Lystad, A.H., Phuyal, S., Olkkonen, V.M., Brech, A., Jäättelä, M., Maeda, K., et al. (2022). Cholesterol transfer via endoplasmic reticulum contacts mediates lysosome damage repair. EMBO J. 41: e112677.

Raemy, E., Montessuit, S., Pierredon, S., Van Kampen, A., Vaz, F., and Martinou, J. (2016). Cardiolipin or MTCH2 can serve as tBID receptors during apoptosis. Cell Death Differ. 23: 1165–1174.

Reddy, A., Caler, E.V., and Andrews, N.W. (2001). Plasma membrane repair is mediated by Ca^{2+}-regulated exocytosis of lysosomes. Cell 106: 157–169.

Redpath, G.M., Woolger, N., Piper, A.K., Lemckert, F.A., Lek, A., Greer, P.A., North, K.N., and Cooper, S.T. (2014). Calpain cleavage within dysferlin exon 40a releases a synaptotagmin-like module for membrane repair. Mol. Biol. Cell 25: 3037–3048.

Riley, J.S., Quarato, G., Cloix, C., Lopez, J., O'Prey, J., Pearson, M., Chapman, J., Sesaki, H., Carlin, L.M., Passos, J.F., et al. (2018). Mitochondrial inner membrane permeabilisation enables mtDNA release during apoptosis. EMBO J. 37: e99238.

Röder, J. and Hensel, M. (2020). Presence of SopE and mode of infection result in increased Salmonella-containing vacuole damage and cytosolic release during host cell infection by *Salmonella enterica*. Cell Microbiol. 22: e13155.

Romero, M., Keyel, M., Shi, G., Bhattacharjee, P., Roth, R., Heuser, J.E., and Keyel, P.A. (2017). Intrinsic repair protects cells from pore-forming toxins by microvesicle shedding. Cell Death Differ. 24: 798–808.

Roostalu, U. and Strähle, U. (2012). In vivo imaging of molecular interactions at damaged sarcolemma. Dev. Cell 22: 515–529.

Ros, U., Peña-Blanco, A., Hänggi, K., Kunzendorf, U., Krautwald, S., Wong, W.W.-L., and García-Sáez, A.J. (2017). Necroptosis execution is mediated by plasma membrane nanopores independent of calcium. Cell Rep. 19: 175–187.

Ruan, J., Xia, S., Liu, X., Lieberman, J., and Wu, H. (2018). Cryo-EM structure of the gasdermin A3 membrane pore. Nature 557: 62–67.

Rühl, S., Shkarina, K., Demarco, B., Heilig, R., Santos, J.C., and Broz, P. (2018). ESCRT-dependent membrane repair negatively regulates pyroptosis downstream of GSDMD activation. Science 362: 956–960.

Russell, D.G. (2016). The ins and outs of the *Mycobacterium tuberculosis*-containing vacuole. Cell Microbiol. 18: 1065–1069.

Salvador-Gallego, R., Mund, M., Cosentino, K., Schneider, J., Unsay, J., Schraermeyer, U., Engelhardt, J., Ries, J., and García-Sáez, A.J. (2016). Bax assembly into rings and arcs in apoptotic mitochondria is linked to membrane pores. EMBO J. 35: 389–401.

Sborgi, L., Rühl, S., Mulvihill, E., Pipercevic, J., Heilig, R., Stahlberg, H., Farady, C.J., Müller, D.J., Broz, P., and Hiller, S. (2016). GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. EMBO J. 35: 1766–1778.

Schafer, B., Quispe, J., Choudhary, V., Chipuk, J.E., Ajero, T.G., Du, H., Schneiter, R., and Kuwana, T. (2009). Mitochondrial outer membrane proteins assist Bid in Bax-mediated lipidic pore formation. Mol. Biol. Cell 20: 2276–2285.

Scheffer, L.L., Sreetama, S.C., Sharma, N., Medikayala, S., Brown, K.J., Defour, A., and Jaiswal, J.K. (2014). Mechanism of Ca^{2+}-triggered ESCRT assembly and regulation of cell membrane repair. Nat. Commun. 5: 5646.

Segawa, K., Kurata, S., Yanagihashi, Y., Brummelkamp, T.R., Matsuda, F., and Nagata, S. (2014). Caspase-mediated cleavage of phospholipid flippase for apoptotic phosphatidylserine exposure. Science 344: 1164–1168.

Shi, J., Zhao, Y., Wang, K., Shi, X., Wang, Y., Huang, H., Zhuang, Y., Cai, T., Wang, F., and Shao, F. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature 526: 660–665.

Simeone, R., Majlessi, L., Enninga, J., and Brosch, R. (2016). Perspectives on mycobacterial vacuole-to-cytosol translocation: the importance of cytosolic access. Cell Microbiol. 18: 1070–1077.

Simeone, R., Sayes, F., Lawarée, E., and Brosch, R. (2021). Breaching the phagosome, the case of the tuberculosis agent. Cell Microbiol. 23: e13344.

Siskind, L.J., Mullen, T.D., Romero Rosales, K., Clarke, C.J., Hernandez-Corbacho, M.J., Edinger, A.L., and Obeid, L.M. (2010). The BCL-2 protein BAK is required for long-chain ceramide generation during apoptosis. J. Biol. Chem. 285: 11818–11826.

Skowyra, M.L., Schlesinger, P.H., Naismith, T.V., and Hanson, P.I. (2018). Triggered recruitment of ESCRT machinery promotes endolysosomal repair. Science 360: eaar5078.

Sønder, S.L., Boye, T.L., Tölle, R., Dengjel, J., Maeda, K., Jäättelä, M., Simonsen, A.C., Jaiswal, J.K., and Nylandsted, J. (2019). Annexin A7 is required for ESCRT III-mediated plasma membrane repair. Sci. Rep. 9: 6726.

Speer, A., Sun, J., Danilchanka, O., Meikle, V., Rowland, J.L., Walter, K., Buck, B.R., Pavlenok, M., Holscher, C., Ehrt, S., et al. (2015). Surface hydrolysis of sphingomyelin by the outer membrane protein Rv0888 supports replication of *Mycobacterium tuberculosis* in macrophages. Mol. Microbiol. 97: 881–897.

Steinhardt, R.A., Bi, G., and Alderton, J.M. (1994). Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. Science 263: 390–393.

Subburaj, Y., Cosentino, K., Axmann, M., Pedrueza-Villalmanzo, E., Hermann, E., Bleicken, S., Spatz, J., and García-Sáez, A.J. (2015). Bax monomers form dimer units in the membrane that further self-assemble into multiple oligomeric species. Nat. Commun. 6: 8042.

Suzuki, J., Umeda, M., Sims, P.J., and Nagata, S. (2010). Calcium-dependent phospholipid scrambling by TMEM16F. Nature 468: 834–838.

Suzuki, M., Youle, R.J., and Tjandra, N. (2000). Structure of Bax: coregulation of dimer formation and intracellular localization. Cell 103: 645–654.

Tam, C., Idone, V., Devlin, C., Fernandes, M.C., Flannery, A., He, X., Schuchman, E., Tabas, I., and Andrews, N.W. (2010). Exocytosis of acid sphingomyelinase by wounded cells promotes endocytosis and plasma membrane repair. J. Cell Biol. 189: 1027–1038.

Tan, J.X. and Finkel, T. (2022). A phosphoinositide signalling pathway mediates rapid lysosomal repair. Nature 609: 815–821.

Thiele, D.L. and Lipsky, P.E. (1990a). The action of leucyl-leucine methyl ester on cytotoxic lymphocytes requires uptake by a novel dipeptide-specific facilitated transport system and dipeptidyl peptidase I-mediated conversion to membranolytic products. J. Exp. Med. 172: 183–194.

Thiele, D.L. and Lipsky, P.E. (1990b). Mechanism of L-leucyl-L-leucine methyl ester-mediated killing of cytotoxic lymphocytes: dependence on a lysosomal thiol protease, dipeptidyl peptidase I, that is enriched in these cells. Proc. Natl. Acad. Sci. U. S. A. 87: 83–87.

Thiery, J., Keefe, D., Saffarian, S., Martinvalet, D., Walch, M., Boucrot, E., Kirchhausen, T., and Lieberman, J. (2010). Perforin activates clathrin- and dynamin-dependent endocytosis, which is required for plasma membrane repair and delivery of granzyme B for granzyme-mediated apoptosis. Blood 115: 1582–1593.

Thurston, T.L.M., Wandel, M.P., von Muhlinen, N., Foeglein, Á., and Randow, F. (2012). Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 482: 414–418.

Todt, F., Cakir, Z., Reichenbach, F., Emschermann, F., Lauterwasser, J., Kaiser, A., Ichim, G., Tait, S.W.G., Frank, S., Langer, H.F., et al. (2015). Differential retrotranslocation of mitochondrial BAX and BAK. EMBO J. 34: 67–80.

Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T.L., and Fowler, V.G., Jr. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin. Microbiol. Rev. 28: 603–661.

Trajkovic, K., Hsu, C., Chiantia, S., Rajendran, L., Wenzel, D., Wieland, F., Schwille, P., Brügger, B., and Simons, M. (2008). Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science 319: 1244–1247.

Unsay, J.D., Cosentino, K., Sporbeck, K., and García-Sáez, A.J. (2017). Pro-apoptotic cBid and Bax exhibit distinct membrane remodeling activities: an AFM study. Biochim. Biophys. Acta Biomembr. 1859: 17–27.

Uren, R.T., O'Hely, M., Iyer, S., Bartolo, R., Shi, M.X., Brouwer, J.M., Alsop, A.E., Dewson, G., and Kluck, R.M. (2017). Disordered clusters of Bak dimers rupture mitochondria during apoptosis. Elife 6: e19944.

Vadia, S., Arnett, E., Haghighat, A.-C., Wilson-Kubalek, E.M., Tweten, R.K., and Seveau, S. (2011). The pore-forming toxin listeriolysin O mediates

a novel entry pathway of *L. monocytogenes* into human hepatocytes. PLoS Pathog. 7: e1002356.

Vasta, G.R., Feng, C., González-Montalbán, N., Mancini, J., Yang, L., Abernathy, K., Frost, G., and Palm, C. (2017). Functions of galectins as 'self/non-self'-recognition and effector factors. Pathog. Dis. 75: ftx046.

Vietri, M., Radulovic, M., and Stenmark, H. (2020). The many functions of ESCRTs. Nat. Rev. Mol. Cell Biol. 21: 25–42.

Voskoboinik, I., Whisstock, J.C., and Trapani, J.A. (2015). Perforin and granzymes: function, dysfunction and human pathology. Nat. Rev. Immunol. 15: 388–400.

Wang, C. and Ruan, J. (2022). Mechanistic insights into Gasdermin pore formation and regulation in pyroptosis. J. Mol. Biol. 434: 167297.

Wilburn, K.M., Fieweger, R.A., and VanderVen, B.C. (2018). Cholesterol and fatty acids grease the wheels of *Mycobacterium tuberculosis* pathogenesis. Pathog Dis 76: fty021.

Williams, R.J. (2002). Calcium. Methods Mol. Biol. 172: 21–49.

Wolfmeier, H., Schoenauer, R., Atanassoff, A.P., Neill, D.R., Kadioglu, A., Draeger, A., and Babiychuk, E.B. (2015a). Ca^{2+}-dependent repair of pneumolysin pores: a new paradigm for host cellular defense against bacterial pore-forming toxins. Biochim. Biophys. Acta 1853: 2045–2054.

Wolfmeier, H., Schoenauer, R., Atanassoff, A.P., Neill, D.R., Kadioglu, A., Draeger, A., and Babiychuk, E.B. (2015b). Ca^{2+}-dependent repair of pneumolysin pores: a new paradigm for host cellular defense against bacterial pore-forming toxins. Biochim. Biophys. Acta 1853: 2045–2054.

Wong-Ekkabut, J., Xu, Z., Triampo, W., Tang, I.M., Tieleman, D.P., and Monticelli, L. (2007). Effect of lipid peroxidation on the properties of lipid bilayers: a molecular dynamics study. Biophys. J. 93: 4225–4236.

Wu, N., Cernysiov, V., Davidson, D., Song, H., Tang, J., Luo, S., Lu, Y., Qian, J., Gyurova, I.E., Waggoner, S.N., et al. (2020). Critical role of lipid scramblase TMEM16F in Phosphatidylserine exposure and repair of plasma membrane after pore formation. Cell Rep. 30: 1129–1140.e1125.

Xia, S., Zhang, Z., Magupalli, V.G., Pablo, J.L., Dong, Y., Vora, S.M., Wang, L., Fu, T.-M., Jacobson, M.P., Greka, A., et al. (2021). Gasdermin D pore structure reveals preferential release of mature interleukin-1. Nature 593: 607–611.

Xu, X.P., Zhai, D., Kim, E., Swift, M., Reed, J.C., Volkmann, N., and Hanein, D. (2013). Three-dimensional structure of Bax-mediated pores in membrane bilayers. Cell Death Dis. 4: e683.

Yamaji, A., Sekizawa, Y., Emoto, K., Sakuraba, H., Inoue, K., Kobayashi, H., and Umeda, M. (1998). Lysenin, a novel sphingomyelin-specific binding protein. J. Biol. Chem. 273: 5300–5306.

Zanoni, I., Tan, Y., Di Gioia, M., Broggi, A., Ruan, J., Shi, J., Donado, C.A., Shao, F., Wu, H., Springstead, J.R., et al. (2016). An endogenous caspase-11 ligand elicits interleukin-1 release from living dendritic cells. Science 352: 1232–1236.

Zhen, Y., Radulovic, M., Vietri, M., and Stenmark, H. (2021). Sealing holes in cellular membranes. EMBO J. 40: e106922.

Zhou, Z., He, H., Wang, K., Shi, X., Wang, Y., Su, Y., Wang, Y., Li, D., Liu, W., Zhang, Y., et al. (2020). Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. Science 368: eaaz7548.