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Mini-review

Dealing with damage: Plasma membrane repair mechanisms

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ABSTRACT

Eukaryotic cells have developed repair mechanisms, which allow them to reseal their membrane in order to prevent the efflux of cytoplasmic constituents and the uncontrolled influx of calcium. After injury, the Ca^{2+}-concentration gradient fulfils a dual function: it provides guidance cues for the repair machinery and directly activates the molecules, which have a repair function. Depending on the nature of injury, the morphology of the cell and the severity of injury, the membrane resealing can be effected by lysosomal exocytosis, microvesicle shedding or a combination of both. Likewise, exocytosis is often followed by the endocytic uptake of lesions. Additionally, since plasmalemmal resealing must be attempted, even after extensive injury in order to prevent cell lysis, the restoration of membrane integrity can be achieved by ceramide-driven invagination of the lipid bilayer, during which the cell is prepared for apoptotic disposal. Plasmalemmal injury can be contained by a surfeit of plasma membrane, which serves as a trap for toxic substances: either passively by an abundance of cellular protrusions, or actively by membrane blebbing.

1. Introduction

If one contemplates the seemingly effortless performance of an entire organism, one tends to overlook that its smooth operation critically depends on the integrity of its individual components at the cellular level. Damage control mechanisms are a necessity for the survival of eukaryotic cells since breaches in the plasma membrane lead to uncontrolled calcium and potassium influx and to the efflux of cytoplasmic constituents. Hence, either a potent protective shield or the ability to constantly renew the cellular surface are important factors in cellular survival. Fortunately, Nature has developed a number of methods for the prevention of cellular injuries and more than a few strategies for the repair of plasma membrane lesions.

The majority of eukaryotic cells are exposed to biological or chemical damage and/or mechanical injury. Regardless whether the plasma membrane is perforated by bacterial toxins or complement or whether a membrane lesion consists of a ragged hole, the cell needs to initiate repair quickly in order to prevent catastrophic lysis. Plasmalemmal repair either restores the cell to full functionality or at least enables its regulated disposal through apoptotic pathways.

Cultured cells are the logical choice to investigate the individual membrane repair skills and they have faithfully served as model systems for many years [1]. Mechanical lesions elicited by micropipettes, scraping or lasers [2–7], have been extensively investigated. The cellular reaction to membrane perforation by bacterial toxins or complement has been equally well documented [8–13].

2. Mechanisms of plasmalemmal repair (1) or: what happens to the lacerations?

Membrane lesions of irregular shape and size must be patched by reserve membrane fragments which originate from intracellular reservoirs. Initial experiments addressing this mechanism date back almost one century, when the recovery of a mechanically injured marine invertebrate oocyte was brought about “by the formation of a membrane-like film which prevents extension of the injury” [14]. The Ca^{2+}-dependent, homotypic fusion of intracellular vesicles with the plasma membrane was initially identified by investigating the sea urchin cell membrane after mechanical injury [2]. Studies on the invasion of *Trypanosoma cruzi* confirmed the mechanism [15]. Even extensive lesions can be sealed by membrane patches created by the homotypic fusion of multiple lysosomes into a giant vesicle, which form a disruption-spanning patch and fuse with the exposed edges of the plasma membrane [16]. Another organelle with the ability of providing seals for large wounds is the enlargeosome, a vesicle which has been described in

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PC12 cells, in which lysosomal exocytosis is defective [17]. Enlargeosomes are characterized by their unique membrane marker AHNAK/desmoyokin, a large (~600 kDa) peripheral protein attached to the lumenal side of the organelle's membrane and exposed to the extracellular leaflet upon exocytosis [18]. However, it is as yet unexplained how this giant protein crosses the membrane to transfer to the inside of the enlargeosome vesicle. Nor have other markers for this specific organelle, which is restricted to a few cell types, been described so far.

Inserted membranes originating from lysosomes are clearly identifiable by their expression of lysosomal-associated-membrane protein 1 (LAMP 1) which – after sealing – emerges on the outer leaflet of the plasma membrane [18]. Lysosomal fusion is critically dependent on synaptotagmins [19]. Since during this process, lysosomal β-hexosaminidase is discharged into the extracellular space, measurement of its activity can be used for quantitation of the degree of injury [20,21].

Although in principle, the membrane of any internal organelle should suffice to bridge the plasmalemmal gash [16,22], in practice, lysosomal membranes appear to be the ones most readily employed [16], presumably because they are more amenable to Ca^{2+}-dependent transfer than fractions of the Golgi apparatus or the endoplasmic reticulum.

Since the influx of calcium serves to activate the repair machinery and to guide the repair molecules to the injured site (rev.by Ref. [23]), proteins involved in membrane repair are most often either Ca^{2+}-sensors themselves or act in combination with Ca^{2+} sensitive proteins. In addition, molecules controlling membrane damage often possess fusogenic properties e.g. synaptotagmins, annexins, ferlins (rev. by Ref. [23]).

3. Mechanisms of plasmalemmal repair (2) or: what happens to the pores?

Toxins are essential virulence factors for a large number of pathogens. The largest family (~30% of all bacterial toxins) are the pore-forming toxins (PFT). Members include the cholesterol-dependent cytolysins as well as numerous toxins, which are secreted by *Staphylococcus aureus* (α-toxin, leukocidins) [24–26] . Bacterial pore-forming toxins either generate small (0.5–5 nm) or large (20–100 nm) pores [26,27]. Cholesterol-dependent cytolysins are characterized not only by the large size of their pore but also by its localization within cholesterol-enriched membrane microdomains (rev by Ref. [28]). They are produced by numerous pathogens – the most prevalent ones being *Streptococcus pyogenes* (streptolysin O, SLO) and *Streptococcus pneumoniae* (pneumolysin, PLY). Secreted as soluble monomers, these toxins bind to the membrane, oligomerize and are assembled into pre-pores (rev. by Ref. [26]). Conformational changes lead to the insertion of β-hairpins into the membrane and the creation of transmembrane toxin pores of ~300 Å in diameter [29].

PFTs form pathogen-generated trans-membrane pores, which consist of protein-lined channels. However, pore-forming proteins are also produced by the mammalian immune system: the complement system consists of a complex group of proteins (C6–C9) which are able to self-associate into a ring-like structure thus forming the membrane attack complex (MAC) [30]. This ring-like lesion harbours a functional pore which can lead to cell lysis or – at sublytic concentrations – to the release of cytokines from the injured cell [9,31]. The non-lethal MAC pores mediate an increase in intracellular [Ca^{2+}], which triggers intracellular effects ranging from pro-inflammatory cytokine secretion to caspase activation and apoptosis [32].

Perforin is structurally and functionally related to the C9 component of complement pores [33–35]. It is found in cytotoxic T lymphocytes and forms transient pores on the target cell membrane in order to deliver a lethal amount of granzymes [35,36]. It is interesting to note that the pore-forming domains of perforins, of C6–C9 membrane attack complexes and those of CDCs display structural homologies which point to common mechanisms of plasmalemmal insertion [34,37].

An overwhelming attack of pore-forming toxins or MAC complexes, leads to catastrophic cell lysis. However, at sublytic concentrations, membrane lesions can successfully be repaired [9]. Yet, the individual mode of repair remains subject of much scientific discussion and has been suggested to depend on cell type, and/or type of the lesion [23]. Two main routes have been described: microvesicle shedding [9,12,13,21,38–40]; and endocytic removal of membrane pores [8,41]. In addition, a quasi synchronized combination of lysosomal exocytosis and endocytic uptake has been noted [7,42].

Microvesicle shedding has been shown to be instrumental for the removal of complement-induced pores at sublytic concentrations [9] but shedding also eliminates lesions which contain a bacterial toxin pore [12,13,40].

Quarantined by membrane folds and isolated by membrane fusion, the pores are removed from the plasmalemma and expelled into the extracellular space [12,21,40]. Shed in the form of microvesicles, the ejected plasmalemma contains the toxin as well as members of the annexin protein family [12,21,40] (Fig. 1). Annexins are multifunctional membrane proteins, involved in endocytosis, vesicular transport and multivesicular body formation [43–48]. These ubiquitously expressed proteins translocate from the cytoplasm to the plasmalemma upon an increase in intracellular [Ca^{2+}], bind to negatively-charged phospholipids and are capable of fusing membranes [12,40,49]. Their Ca^{2+}-sensing characteristics in combination with their fusogenic properties have previously implicated annexins in membrane repair [5,6,12,21,40]. Since each annexin displays a distinct, but overlapping, Ca^{2+}-sensitivity of membrane binding and most cells are equipped with multiple members, they constitute a broad-range Ca^{2+}-sensor for plasmalemmal perforation. Thus, plasma membrane repair can be effected under conditions of both moderate Ca^{2+}-elevation and massive Ca^{2+} overload [12,40,50]. The expression of several different annexins in any one cell is presumably adapted to the degree of individual hazard.

However, toxin induced microvesicle shedding has also been observed to occur passively in HeLa cells after chemical fixation and subsequent exposure to SLO, indicating that a passive, autonomous, Ca^{2+}-independent process might additionally be involved [13,51].

Recently, the endosomal sorting complex required for transport (ESCRT) has been implicated in the closure of plasmalemmal wounds [7]. The ESCRT subunits are instrumental in the biogenesis of multivesicular bodies and are held to be responsible for membrane deformation observed during vesicle budding. Small plasmalemmal injuries inflicted mechanically or chemically, lead to ESCRT subunit accumulation at the injured site followed by membrane budding and expulsion of the damaged site [7].

Lysosomal exocytosis does not only seal mechanical injuries, but has also been reported to repair toxin-induced membrane perforations [41]. Recent experiments advocate a co-operation between lysosomal patching and endocytic events which are taking place simultaneously during membrane repair [42]. The rapid, Ca^{2+}-dependent endocytosis of patched, SLO induced membrane pores restored membrane integrity in HeLa cells [41]. The endosomes are either ubiquitinated and targeted, via the ESCRT complex, towards lysosomes for their intracellular degradation [52] or they can be sorted into perinuclear multivesicular bodies (MVB) which are removed by exocytosis [7]. Thus, the physical removal of a pore – outwards or inwards – might operate simultaneously within a single cell; its relative importance,