Published in final edited form as:

Trends Biochem Sci. 2014 July ; 39(7): 307–309. doi:10.1016/j.tibs.2014.06.001.

ESCRT: Nipping the wound in the bud?

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Keywords

plasma membrane repair; resealing; wounding

At least four different Ca²⁺-dependent mechanisms were proposed to explain the remarkable process by which eukaryotic cells quickly repair plasma membrane (PM) wounds: 1) fusion of an intracellular membrane patch to the wound ¹; 2) exocytosis-mediated reduction in membrane tension ²; 3) lesion removal by endocytosis, a process triggered by exocytosis of the lysosomal enzyme sphingomyelinase ^{3, 4}; and 4) lesion removal by extracellular shedding of PM blebs ⁵. Due to the importance of maintaining PM integrity, it would not be surprising if multiple pathways were involved in repair. However, there is a surprising consistency in the properties of the resealing process, regardless of the type of injury. Resealing after permeabilization with stable pore-forming proteins is also Ca²⁺-dependent, and occurs as fast as the repair of mechanical wounds. Taken together with the evidence that endocytosis is involved in the repair of cells injured by transmembrane pores or mechanical wounds ^{3, 4}, a recent study by Jimenez *et al.* ⁶ reinforces the view that PM repair occurs primarily through lesion removal.

Jimenez and co-workers investigated a potential role of the endosomal sorting complex required for transport (ESCRT) in PM repair. The ESCRT machinery comprises five distinct protein complexes that interact sequentially with membranes: ESCRTs-0, -I, -II, and -III and the ATPase Vps4. The role of ESCRT-III is to drive formation of membrane invaginations that protrude away from the cytoplasm, a process shown to be associated with viral budding and multivesicular body biogenesis ⁷. Remarkably, Jimenez *et al.* observed very localized recruitment of ESCRT-III subunits to wound sites on the cell surface. Importantly, ESCRT-III recruitment was Ca²⁺-dependent, in agreement with known requisites of PM repair. The endogenous ESCRT-III subunits CHMP3, CHMP2A and CHMP2B and an EGFP-labeled CHMP4B subunit were recruited to lesions created by pore-forming detergents, bacterial toxins, microinjection needles and lasers. However, RNAi

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depletion of CHMP4B or expression of dominant-negative Vps4B caused viability loss only in cells carrying small lesions, suggesting that the importance of ESCRT-III in repair is related to wound size. CHMP4B-EGFP was visualized in association with PM protrusions in laser-wounded cells, leading the authors to propose that small wounds can be eliminated through scission or shedding of ESCRT-III-induced buds.

Recruitment of the ESCRT-III component CHMP4B to the PM did not require fully polymerized microtubules and was, surprisingly, ATP independent. The membrane abscission step, in contrast, appeared to be ATP dependent, consistent with the observed recruitment of the ATPase Vps4 to wound sites. Recognition of ubiquitylated molecules by ESCRT-0 and -I subunits is an important early step in ESCRT-III-mediated membrane remodeling ⁷. Given that ATP is required for ubiquitylation reactions, the authors proposed that injury-induced ESCRT-III assembly bypasses the need for ESCRT-0, -I and II, and might be promoted by ALIX, a protein capable of directly recruiting ESCRT-III to membranes ⁸. Interestingly, Jimenez *et al.* found that ALIX recruitment to wounds requires an intact Ca²⁺-binding domain, revealing a novel potential role of Ca²⁺ influx during PM wounding and repair. However, the ESCRT-I component TSG101 (which binds to ubiquitylated cargo, in addition to ESCRT-0 and -II subunits ⁷) was also recruited to wounds, and surface polyubiquitylation was observed on injured cells. Based on their observations, Jimenez *et al.* suggested that ubiquitylation occurs at a later step to reinforce the initial ESCRT recruitment, rather than initiating it. Given how challenging it is to precisely determine the time course of ubiquitylation in relation to ESCRT recruitment to wound sites, this interesting issue deserves further investigation.

Wounded cells often show extensive PM blebbing, an observation that led several groups to propose that lesions are shed from cells in such blebs. However, studies of cells permeabilized with pore-forming toxins showed that large PM blebs formed in permeabilized cells rapidly retract into the cell body, with no evidence for a role in repair through extracellular shedding ⁹. This conclusion is strengthened by the fact that blocking injury-associated large PM blebs by actin cytoskeleton disassembly does not inhibit PM repair ⁹. Jimenez *et al.* made similar observations, reporting that PM wounding was followed by the rapid formation of blebs of several micrometers in diameter. These large blebs became positive for CHMP4B-GFP and then retracted, following a time course suggested to coincide with ESCRT-III-mediated shedding of smaller membrane buds containing the lesion ⁶ (see also Figure 1). Thus, the wound removal process proposed by Jimenez *et al.*, driven by ESCRT-III-mediated shedding, is not related to the large transient PM blebs that result from Ca²⁺-mediated temporary disruption of the cortical cytoskeleton in injured cells.

Jimenez *et al.* developed an ingenious mathematical model to estimate wound size and rate of closure based on the entry kinetics of the membrane impermeant dye propidium iodide. Using this model, a correlation was found between the time of wound closure and the time of maximum recruitment of CHMP4B. However, a puzzling delay in repair was observed in cells injured with an UV laser, with wounds remaining open for several minutes before resealing. This observation differs from the 10–30 second resealing time previously observed for several types of wounds, ranging from permeabilization with pore-forming

toxins to puncture with microinjection needles^{1, 9, 10}. Extended exposure to elevated intracellular Ca^{2+} and cytosol loss are extremely deleterious for cells, emphasizing the need for fast PM repair, on the scale of seconds. Laser wounding, although useful for coupling localized wounding to live imaging, might lead to denaturation of membrane components due to high local temperatures, perhaps explaining the delayed resealing and large variations in lesion size observed by Jimenez *et al.* Another intriguing possibility is that ESCRT only plays a role in the final stages of the PM resealing process after larger lesions are removed or remodeled by other pathways, such as sphingomyelinase-dependent endocytosis³.

The observed recruitment of ESCRT-III to wounds caused by bacterial pore-forming toxins, which are about 30 nm in diameter and removed from the PM in less than 30 seconds⁹, creates an excellent opportunity for more extensive investigation of the role of ESCRT in repair. The elegant use of correlative scanning electron microscopy by Jimenez *et al.* should now be applied to morphological studies of the abundant, evenly distributed lesions generated by toxin pores. Extensive ESCRT III-mediated budding and shedding should be readily observed in toxin-permeabilized cells, within the 10–30 second resealing time frame. If this does not occur, an alternative role for ESCRT, such as PM remodeling after wound resealing, should be considered. Regardless of the outcome of these future studies, the recently uncovered participation of the ESCRT complex adds an exciting new dimension to the fundamental process of PM repair.

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Figure 1.

Plasma membrane dynamics during wound repair. Large plasma membrane blebs form when cells are wounded, allowing Ca²⁺ entry. However, these large (several microns in diameter) blebs fully retract after repair, not playing a role in lesion removal. Membrane traffic events proposed to mediate plasma membrane repair include: (A) Ca²⁺-dependent lysosomal exocytosis releases acid sphingomyelinase, which triggers lesion endocytosis and subsequent intracellular degradation, and (B) Ca²⁺-dependent recruitment of ESCRT at the injury site generates small membrane buds and extracellular shedding of the lesion.