

SPOTLIGHT

Cellular wound healing: A two-step mechanism of plasma membrane repair by annexins and calpains

Sabina Elmi¹ and Jesper Nylandsted^{1,2}

In this issue, Williams et al. (<https://doi.org/10.1083/jcb.202408159>) reveal a two-step plasma membrane repair process upon injury: annexins first stabilize damage sites, then calcium-activated calpains cleave these patches, triggering microvesicle shedding—mirroring the clot-and-scab resolution seen in tissue-level wound healing.

Healing by shedding: A two-phase model of membrane repair

The plasma membrane is essential for maintaining a cell's internal environment. Yet despite its protective role, this barrier is frequently disrupted by mechanical stress, immune attack, or environmental toxins (1). To survive, cells must rapidly repair membrane lesions while restoring their structure and function. Though several repair mechanisms have been described, the sequence of events and molecular players involved in sealing and resolving membrane wounds are still being elucidated (2).

In a new study published in *Journal of Cell Biology*, Williams et al. identify a coordinated, two-step repair mechanism that depends on annexins and calcium-dependent calpain proteases (3). Their findings show that annexins form a transient “scab” over the wounded area, stabilizing the membrane and that calpains subsequently cleave annexins, facilitating the release of this structure as annexin-containing microvesicles (MVs). This work provides a conceptual framework for how cells not only close membrane breaches but also remove damaged material in a regulated manner (Fig. 1).

A closer look at repair-associated vesicles

Extracellular vesicles (EVs) have attracted attention for their roles in intercellular

communication, immune modulation, and as biomarkers of disease (4). Among EVs, MVs—formed by outward budding of the plasma membrane—have been less well characterized than exosomes. However, annexins are consistently found in MVs, and their functions in membrane dynamics suggest they may be more than passive cargo (5, 6, 7).

Williams and colleagues set out to understand how annexin-containing MVs are formed, particularly in the context of membrane injury. Using density gradient centrifugation and proteomics, they identified a distinct population of low-density, annexin-rich MVs, separate from classical CD63⁺ exosomes. These vesicles were enriched in calcium-responsive proteins and plasma membrane components, suggesting their origin from the cell surface following damage.

To model injury, the authors exposed cultured epithelial cells to sublytic concentrations of streptolysin O, a bacterial pore-forming toxin. This treatment triggered a robust, 20–40-fold increase in secretion of annexin A2-positive MVs. Similarly, laser-induced membrane injury led to rapid annexin A2 accumulation at lesion sites, followed by vesicle release over several minutes. These results implicated annexin-mediated patch formation as a key step in membrane repair.

Annexins as dynamic repair scaffolds

The annexin family of proteins binds to negatively charged membrane lipids in the

presence of calcium (8). Previous work has shown that annexins help reseal membrane wounds by inducing membrane curvature, cross-linking lipid bilayers, and forming repair structures at damage sites—properties that are crucial for efficient membrane remodeling and closure (9, 10).

However, the fate of these annexin-based structures after lesion closure remained unclear.

Williams et al. found that annexins within MVs were cleaved, displaying a lower molecular weight than their cytosolic counterparts. This consistent processing pattern suggested that proteolysis was not incidental but functionally important.

They focused on annexin A2, the most abundant annexin in their cell model. Proteomic and biochemical assays revealed that calpains—specifically calpain-1 and calpain-2—were responsible for annexin cleavage. These calcium-activated proteases have previously been implicated in cytoskeleton remodeling and membrane repair, but their specific substrates had remained elusive.

Using selective inhibitors and substrate-trapping mutants, the authors confirmed that calpains directly cleave annexins A1, A2, and A6. They mapped the cleavage site of annexin A2 to its N-terminal region, which is required for membrane binding and interaction with S100A10, a dimerization partner involved in membrane scaffolding.

¹Danish Cancer Institute, Copenhagen, Denmark; ²Department of Molecular Medicine, University of Southern Denmark, Odense, Denmark.

Correspondence to Jesper Nylandsted: jnl@cancer.dk.

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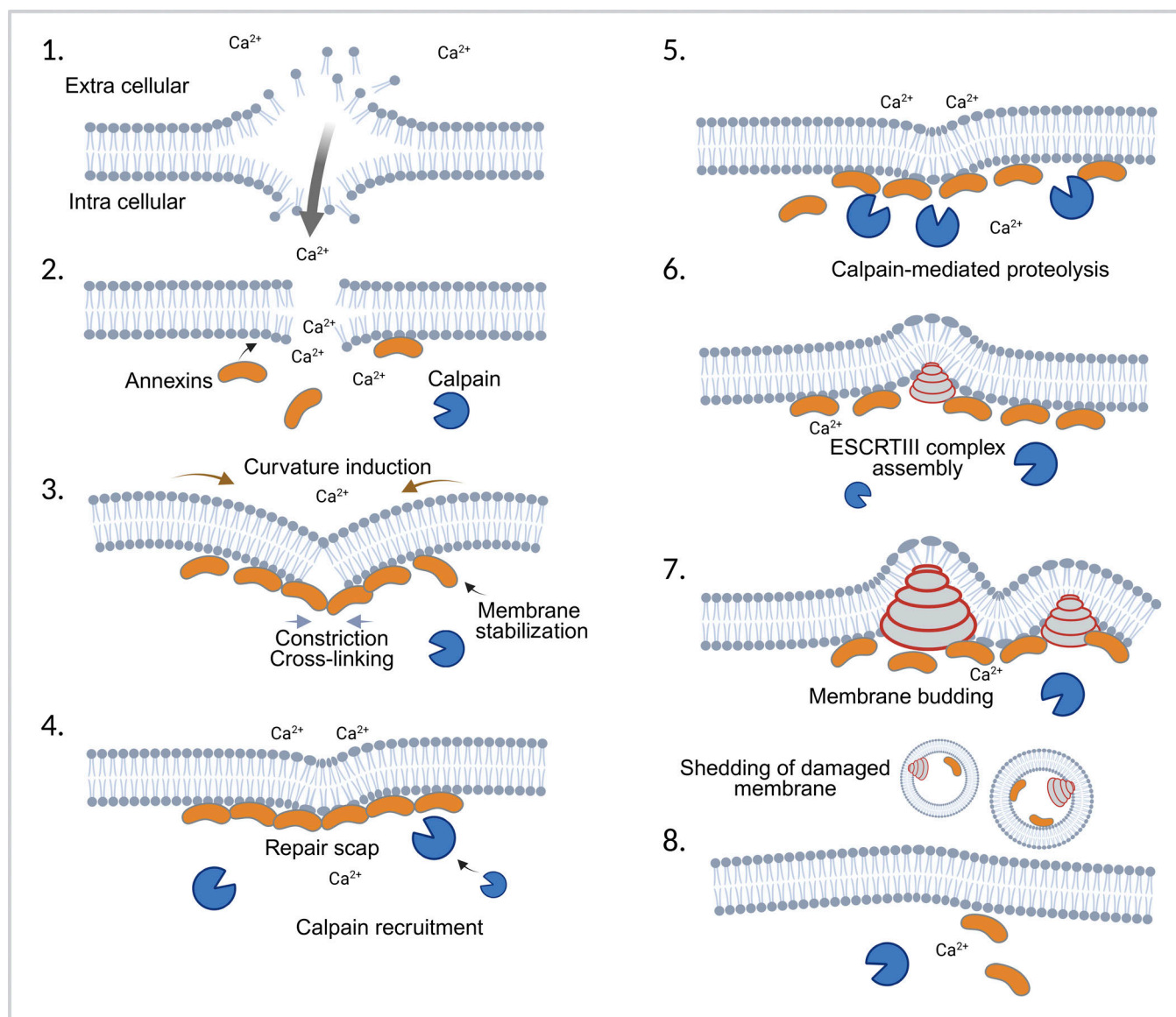


Figure 1. **Williams et al. propose a two-step model of plasma membrane repair.** Upon injury, annexins rapidly assemble into a transient repair cap that stabilizes the lesion. Calcium-activated calpains then cleave the annexins, triggering shedding of the repair cap as annexin-rich MVs. This process involves ESCRT machinery and resembles tissue-level wound healing, where a clot is later shed as a scab. Created in BioRender. Nylandsted (2025) <https://BioRender.com/xzbtu6>.

Cleavage as a functional switch

To determine the consequence of annexin cleavage, Williams et al. generated mutants that either mimicked the cleaved form or resisted calpain processing. Wild-type annexin A2 was efficiently recruited to injury sites and subsequently released in MVs. In contrast, cleavage-resistant mutants formed persistent repair scabs but were poorly secreted. The cleaved form, on the other hand, exhibited reduced membrane binding and failed to support scab formation.

Live-cell imaging using fluorescent cleavage reporters showed that annexin

processing occurred within 1–2 min of injury and was spatially restricted to the lesion site. Vesicle shedding peaked soon after, suggesting that calpain activity temporally and spatially coordinates the transition from membrane sealing to vesicle release.

Moreover, vesicles formed under these conditions were enriched in phosphatidylserine, a lipid normally found on the inner leaflet of the plasma membrane but externalized during damage.

Both perforated and intact vesicles carried cleaved annexin fragments, suggesting

that calpains may contribute not only to the clearance of damaged membranes but also to intercellular signaling or modulation of the immune response.

Linking repair to resolution

Together, the findings support a two-phase model of membrane repair. In the first phase, annexins rapidly accumulate at the damage site, promoting membrane curvature and stabilizing the lesion by cross-linking membrane domains. In the second phase, calpain-mediated cleavage of annexins disrupts these membrane-protein

interactions, allowing the repair scab to bud off as MVs.

This model draws a useful parallel with tissue-level wound healing: an initial clot stabilizes the injury, followed by proteolytic breakdown and removal of the scab. At the cellular level, annexin scabs perform a similar function, and calpains act as internal regulators of scab removal and membrane remodeling.

The study also integrates other repair machinery into this framework. The authors show that ESCRT components—typically involved in membrane budding—contribute to MV formation and that late endosomal fusion is recruited only at higher damage levels (Fig. 1). Thus, cells may employ multiple, damage-tiered repair pathways, with annexin-mediated patching and shedding serving as a primary response to moderate injury.

Broader implications and future directions

This study has several important implications for membrane biology and disease. Williams et al. demonstrate that calpain-mediated annexin cleavage is not merely a byproduct of membrane injury but a crucial step in the repair process. By

promoting the shedding of annexin-containing patches as MVs, calpains enable cells to restore membrane integrity and dispose of repair machinery in an orderly manner. This provides a mechanistic basis for the presence of annexins in MVs and suggests that annexin cleavage products—such as the anti-inflammatory peptides generated from annexin A1—may have extracellular signaling roles, potentially influencing nearby cells or immune receptors.

In physiological contexts where membrane repair is vital, such as in muscle or cancer cells, dysregulation of annexin or calpain function could impair healing or alter intercellular communication. Moreover, annexin-containing vesicles could serve as biomarkers of membrane stress or repair capacity in clinical settings.

Still, key questions remain: What governs the timing and extent of calpain activation? How do annexins and calpains interface with other repair pathways across tissues? And how significantly does this repair-shedding process influence disease progression or resolution?

By bridging the fields of membrane repair, proteolysis, and EV biology, this work offers a coherent and compelling model that is likely to guide future research into

cellular stress responses and therapeutic interventions.

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