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Cell repair: Revisiting the patch hypothesis

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ABSTRACT

Plasma membrane damage elicits a complex and dynamic cellular response. A vital component of this response, membrane resealing, is thought to arise from fusion of intracellular membranous compartments to form a temporary, impermeant patch at the site of damage; however, this hypothesis has been difficult to confirm visually. By utilizing advanced microscopy technologies with high spatiotemporal resolution in wounded *Xenopus laevis* oocytes, we provide the first direct visualization of the membrane fusion events predicted by the patch hypothesis; we show the barrier formed by patching is capable of abating exchange of material across the plasma membrane within seconds. Profound changes also occur to the plasma membrane surrounding wounds; lipid remodeling is accompanied by membrane fusion events, both conventional (e.g., exocytosis) and novel (e.g., "explodosis"). Further, we reveal additional complexity in wound-induced subcellular patterning, supporting existing evidence that extensive interactions between lipid, protein, and ionic signaling pathways shape the cellular wound response.

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The capacity for self-repair is a universal cellular trait[1] and one that is increasingly recognized to have important implications for human health.[1,3,4] And yet, despite this and over a century of investigation,[2] the molecular and cellular mechanisms utilized to sense and respond to damage have been both mysterious and controversial.

The cellular damage response can be conceptualized as bipartite, consisting of an immediate membrane resealing event and a subsequent reorganization of the cortical cytoskeleton required to bring the wound margins together.[5-8] The cytoskeletal response is controlled, at least in part, by local activation of the Rho GTPases[6-8] while the basis of the resealing event has proven elusive. Early studies using electron microscopy (EM) revealed convoluted, multilamellar membrane structures at sites of damage without indicating the likely source of these structures.[9,10] However, pioneering work by McNeil and his colleagues[11-14] led to the patch hypothesis; it posits that cell wounding triggers fusion of intracellular compartments with each other and the plasma membrane (PM) to form an impermeant 'patch' at the site of damage.[12] While the patch hypothesis is supported by several lines of approach,[11-14] the identity of the cellular compartments utilized for patching has been a subject of considerable controversy.[15-16] This controversy, coupled with the fact that the fusion of intracellular compartments predicted by the patching model had never been directly observed, has led to the development of a variety of models for cell repair that do not involve patching (reviewed in ref. 17). However, given the speed of the resealing response, it seemed possible that the hypothetical patching events might occur on a time scale that would frustrate analyses conducted at relatively low temporal resolution. Accordingly, our study[18] was designed to assess the cell damage response using high spatio-temporal resolution imaging.

Live, 4D (3D over time) imaging of a variety of general membrane markers in oocytes of *Xenopus* laevis demonstrated that intracellular compartments do indeed rapidly fuse with each other and the plasma membrane upon wounding to form dynamic, temporary barriers at sites of damage; these fusion events are precisely what was predicted by the patch hypothesis.[12] Based on both size and pre-wounding localization, the "patch" consisted, at least in part, of the membranes of cortical granules.[19] These large secretory compartments are abundant in *X. laevis* oocytes and have also been suggested as a potential source of the "patch" in echinoderm eggs.[20]

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However, because embryos of both *Xenopus*[21] and echinoderms[20] also heal rapidly and yet lack cortical granules, it seems likely that cortical granule membranes are employed simply because they are handy, rather than because they have some special role in healing per se. If this interpretation is correct, another, more general principle emerges: the healing response may be far more plastic than often assumed. That is, cells may simply utilize any available nearby cellular components to perform repairs until the defect has been managed. Differences in wound size, location, or cell type could therefore influence the exact components identified at sites of damage (reviewed in ref. 17).

In addition to providing the first direct demonstration of patching, the results demonstrated an 'uncertainty principle'; each membrane probe revealed a different feature of the healing process while acquisition of imaging data at high temporal resolution often comes at the expense of spatial resolution, and vice versa. These issues masked novel, transient wound behaviors. One such behavior, that we term 'explodosis'—the outward, often violent, exposure of intracellular compartments to the cell surface by rupture—was only clearly resolved by utilizing microscopy technologies with both high spatial and temporal resolution.[22]

We also found that annexin A1 and dysferlin, proteins known to be critical for PM repair in mammalian muscle,[3,23] were recruited to the plasma membrane and membranous compartments at the oocyte wound site, with the annexin concentrated in the interior of the wound (i.e. within an outer ring of active Cdc42, a Rho family GTPase[5-8]). This finding corroborates a recent report[24] wherein wounding of skeletal muscle induces the formation of a "cap" of annexins at the wound center, surrounded by a "shoulder" of proteins including actin and dysferlin. These structures could easily be interpreted as a "patch" or "ring," respectively, if viewed en face, rather than an oblique angle, further exemplifying the importance of obtaining multiple optical sections to obtain a faithful reconstruction of the wound array. Further, our results confirm that long term (>10 min) lipid and cytoskeletal remodeling and sustained calcium signaling is common at wound sites.[25,26] The involvement of Ca^{2+}- and lipid-dependent kinases in GTPase dynamics at wounds[27] suggests crosstalk between gradients of wound-induced signals drive closure of the contractile array and repair of the PM.

While the oocytes and eggs of amphibians and echinoderms have long served as subjects for wound repair studies,[2] it might naturally be wondered whether results obtained with such large cells are applicable to wound repair in somatic cells. Several lines of evidence suggest that results from the *Xenopus* oocyte model are likely to be directly relevant to other cell types: first, as in other model systems, the cell repair response in oocytes is dependent on external calcium.[5,6] Second, the recruitment of actin filaments and myosin-2 to wounds first discovered in frog oocytes[5] has now been observed in a variety of other cell types including mammalian muscle.[24] Third, the wound-induced activation of Rho GTPases first discovered in frog oocytes,[6] while not yet reported in mammalian cells, has recently been observed in *Drosophila* embryos[7] and budding yeast.[28] Fourth, as noted above, 2 proteins implicated in repair of human muscle—annexin[23] and dysferlin,[3] are recruited to frog oocyte wounds in the same pattern observed in wounded human muscle cells.[24]

There is something for every cell biologist to love in cell repair; it represents a nexus of signaling pathways with critical contributions from proteins, lipids, and ions. It is dynamic, inducible, and repeatable. It involves membrane trafficking, membrane fusion and the cytoskeleton. It lies at the boundary between mechanical and chemical. Further, wound repair also represents a "black box;" despite having a defined input (Ca^{2+}) and conspicuous outputs (membrane resealing and cortical contraction), the intermediate steps are thus far poorly-defined. While proteomic analyses[29,30] have identified proteins exposed at the cell surface during damage/repair, few targets have been vetted to demonstrate active participation in the wound response.[5-8,27] Additionally, the rapidity with which repair is initiated suggests the process is driven by complex sequences of post-translational modifications rather than translation of proteins *de novo*.[5] Proteomics has the potential to decipher these signaling events and provide vital information about other cellular contractile events. Further, with high-pressure freezing and EM tomography, it should be possible to assemble informative 3D reconstructions that enable dissection of complex membrane fusion events that underlie the wound repair response. Combined, the above lines of inquiry promise to expand our understanding of this critical, yet enigmatic process.

Abbreviations

EM electron microscopy
PM plasma membrane

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