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Rehabilitation and the single cell

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Abstract

Cellular damage triggers rapid resealing of the plasma membrane and repair of the cortical cytoskeleton. Plasma membrane resealing results from calcium-dependent fusion of membranous organelles and the plasma membrane at the site of the damage. Cortical cytoskeletal repair results from local assembly of actin filaments (F-actin), myosin-2 and microtubules into an array that closes around the original wound site. Control of the cytoskeletal response is exerted by local activation of the small GTPases, Rho and Cdc42. Recent work has given insight into both the membrane fusion and cytoskeletal responses to plasma membrane damage and we propose that Rho GTPase activation results at least in part from the events that drive membrane repair.

Introduction

“The thing about those holes, they won't fill themselves in and neither will the gophers” — Carl Spackler.

Over the past two decades, it has become apparent that holes made in cells do not repair themselves spontaneously, but rather are healed by an active, calcium-dependent process [1]. This process has two functional components: plasma membrane resealing by fusion of intracellular membrane compartments with each other and with the plasma membrane at the damage site, and cytoskeletal reorganization [1,2]. In this review we discuss ideas and issues emerging from the study of cellular wound repair, with an emphasis on findings from the last two years.

The medical relevance of cellular wound healing

Although wound healing is typically studied at the tissue level, tissue damage from physical or chemical insults, therapeutic interventions, or as a natural consequence of tissue function is inevitably accompanied by individual cell damage [3]. If the damage is not repaired, cell death follows from loss of cytoplasm and unabated calcium influx, resulting directly in loss

of tissue integrity. Negative consequences can also ensue indirectly, from release of proteases that attack neighboring cells [4] or provocation of an inflammatory response [5].

Improper plasma membrane repair is a feature of certain genetic diseases, most notably the muscular dystrophies. Extensive muscle plasma membrane damage is a hallmark of muscular dystrophy, and two recent findings indicate that plasma membrane damage is likely to be responsible for some of the symptoms of muscular dystrophies. First, mutations in the gene encoding dysferlin, a 230 kD plasma membrane protein essential for muscle plasma membrane repair [6,7], result in limb-girdle muscular dystrophy [8]. Second, one of the more devastating consequences of muscular dystrophy, cardiomyopathy, can be alleviated in mice by treatment with agents that artificially repair plasma membrane damage by coating wound sites [9**]. Remarkably, a similar result may be obtained naturally in some cells: plasma membrane damage in gastric mucosa and colon results in mucus deposition at the wound site, where it may limit further damage [10**].

The membrane repair response

Thus, impaired cellular wound healing is both a cause of pathologies and a promising target for treatments. Understanding the normal mechanisms of cellular healing is therefore essential. The first event in cellular wound repair is plasma membrane resealing, to stem the inrush of calcium and loss of cytoplasm (Figure 1). Two membrane fusion events fulfill this role: vesicle–vesicle fusion to create a 'patch' in the middle of the wound, and vesicle–plasma membrane fusion to link the patch and plasma membrane [11*]. These membrane fusion events are highly conserved and require extracellular calcium: when external calcium concentration is reduced to less than ~0.3 mM, membrane resealing fails [12]. This fact and the dependence of membrane resealing on exocytosis and core exocytosis machinery such as SNAREs indicate that mechanisms responsible for calcium-dependent exocytosis also control plasma membrane resealing [12–18].

The above scheme explains plasma membrane resealing in a variety of cell types. However, many details of this process are still unclear. The most contentious issue is the identity of the membrane compartments ('sealing organelles') responsible for resealing. To date, the only unequivocal resealing compartment is the sea urchin egg yolk granule [15,18]. Yolk granules undergo heterotypic fusion with the plasma membrane and homotypic fusion with a calcium sensitivity that fits the profile of the healing process itself [15,19]. However, yolk granules have not been described in somatic cells, leading investigators to suggest two alternatives as the most likely candidates: lysosomes and enlargosomes.

Lysosomes were proposed to represent the resealing organelle because they undergo calcium-regulated exocytosis [20] and because manipulations that block lyso-some–plasma membrane fusion inhibit resealing [21]. For example, Synaptotagmin (syt) VII, a broadly expressed calcium-binding protein thought to regulate exocytosis in other contexts, localizes to lysosomes, and a dominant-negative syt VII suppresses lysosome exocytosis and resealing [21]. Further, lysosomal exocytosis and resealing are reduced in syt VII knockout mice [22]. On the other hand, syt VII is not exclusively localized to lysosomes [23] and dominant negative syt VII constructs are unlikely to be specific reagents [24*]. Moreover, lysosome exocytosis is actually enhanced in fibroblasts from syt VII knockout mice [25],

and a small molecule inhibitor of lysosome exocytosis has no apparent effect on plasma membrane resealing ([26**], but see also [27**]). Finally, lysosome exocytosis following calcium elevation is much slower than the resealing response [28] and does not match the expected calcium sensitivity [29], although it should be noted that the assumed calcium sensitivity is based on what extracellular calcium concentration is required for resealing, rather than a direct measurement of the intracellular free calcium concentration in the wound region.

Equally controversial is the case for enlargosomes, organelles with a composition and morphology distinct from lysosomes, the Golgi, endosomes or the endoplasmic reticulum [30]. Like lysosomes, enlargosomes are broadly distributed, undergo plasma membrane fusion following calcium elevation, and were proposed to mediate plasma membrane resealing [30]. However, more recent work indicates that enlargosomes fuse with the plasma membrane at relatively low free calcium concentrations (1–3 μ M) and do so in a manner that is insensitive to tetanus toxin [31], which was previously shown to block plasma membrane resealing in somatic cells [13].

Thus, the identity of animal cell sealing organelles remains unknown. Interestingly, a peroxisome-related organelle, the Woronin body, may play this role in fungi [32]. However, as these organelles have not been described in animal cells, it is not clear whether these findings will be broadly applicable.

Perhaps, however, it is a mistake to assume that plasma membrane resealing is the exclusive purview of a particular compartment. Instead, it might reflect rapid mobilization and fusion of a variety of organelles. Certainly, it is not difficult to imagine that raising the intracellular free calcium to 1 mM might provoke a local orgy of fusion.

The proteins involved in the resealing response have also been elusive. As noted above, the identification of syt VII as a key player has been challenged. Another candidate is dysferlin. Not only does disruption of dysferlin result in impaired plasma membrane repair in muscle (see above), it binds calcium [33], localizes to sites of membrane damage, and has been shown in other systems to participate in membrane fusion [34*]. Moreover, members of the ferlin family are broadly expressed [35], as expected given the conservation of resealing. Finally, in a very recent study, annexin A1, a calcium-binding plasma membrane protein, has been identified as another potential participant in the resealing response [36**]. Consistent with a role as a general mediator of the resealing response, annexin A1 localizes to damage sites. Moreover, inhibition of annexin A1 function via several independent approaches resulted in inhibition of plasma membrane resealing.

Finally, in the first systematic effort to find proteins required for plasma membrane resealing, a complex required for tethering of yolk granules prior to homotypic fusion was purified from sea urchin eggs [37**]. This complex is comprised of seven major proteins, localizes to yolk granules in fixed samples and is required for formation of large patching compartments from yolk granules *in vitro*. Identification of the individual components of this complex will be of great interest.

The cytoskeletal response to plasma membrane damage

It is not enough for cells to simply reseal the plasma membrane after wounding. They must also restore the wound site to its original state, which entails repopulating the cortical cytoskeleton. It also entails replacement of the patching membrane, which is derived from membranes of organelles (like yolk granules) that presumably differ from the plasma membrane in terms of their lipid and protein composition. Indeed, measurement of ion transport in wounded cells indicates that it remains abnormal well after resealing has finished [38*]. Diffusion into and out of the patch area from the surrounding cytoplasm is too slow to explain the eventual recovery of fully functional plasma membrane, showing that other, more active processes are required.

The cytoskeleton response to wounding underlies these active processes. 30–60s post-wounding, F-actin and myosin-2 accumulate around the wound and then coalesce into a circular array that contracts inward until the original damaged area is covered (Figure 2a). Two features of this array are of particular interest. First, F-actin and myosin-2 accumulation reflects a sharp local increase in assembly, implying that it is templated by localized signals [39]. Second, the array undergoes progressive segregation, concentrating myosin-2 and stable F-actin on the interior and more dynamic actin around the outside [39,40] (Figure 2a). The formation and segregation of the actomyosin array is accompanied by formation of a radial array of microtubules around the wound and microtubules perpendicular to the plasma membrane on the interior of the wound. The microtubule array arises from both new microtubule assembly and physical transport of microtubules by the actomyosin array [40,41]. Conversely, microtubules modulate the actomyosin array. The relative importance of this modulation varies according to wound size, with actomyosin accumulation and closure around the largest wounds being severely compromised by microtubule disruption while smaller wounds are visibly affected, but still capable of closing. Similar scaling effects have been reported for the membrane fusion response [38*].

The cytoskeletal response to plasma membrane damage has been studied most intensively in amphibian oocytes, but there is good reason to think that it is widely conserved. First, F-actin accumulates around fibroblast wounds within 60s [42]. Second, disruption of myosin-2 expression impairs the wound healing response in mammalian cells [43,44]. Third, transected axons constrict at the transection site in a manner consistent with actomyosin-based contraction [45]. Fourth, kinesins have been implicated in the healing response of fibroblasts, indicating the involvement of microtubules [13], while direct visualization of microtubule and EB1 (a microtubule plus-end binding protein) dynamics has shown that microtubules form a radial array around fibroblast wounds by growing toward it [46*].

The above cytoskeletal reorganizations explain how the cortical cytoskeletal network is repaired: F-actin and myosin-2 first assemble around the wound and subsequently move into the wound. They also suggest mechanisms by which plasma membrane function may be fully restored. First, the closing contractile array could physically expel the patched membrane into the extracellular space while pulling undamaged plasma membrane over the wound. Second, microtubules focused around the wound site could serve as transport tracks for vesicles moving to and from the patched area. Consistent with this notion, membranous compartments track toward the wound site on microtubules [46*].

Signal transduction during cellular wound healing

One of the most astonishing features of cellular wound repair is that what seems like the crudest of all possible stimuli — a plasma membrane hole — elicits a highly precise signal transduction response. Within ~15–20s of oocyte wounding, Rho and Cdc42 are activated. These small GTPases, which are well known to control various aspects of the actomyosin cytoskeleton, subsequently sort into concentric activity zones with active Cdc42 circumscribing active Rho [47**] (Figure 2b). Rho and Cdc42 are required for accumulation of F-actin and myosin-2 around wounds and, perhaps more interestingly, may be responsible for the observed organization of the contractile array. Specifically, experimental disruption of Rho activity inhibits the concentration of active myosin-2 on the interior of the array as well as contractility of the array, while failing to prevent local actin assembly [47**].

Neither Rho nor Cdc42 is activated in the absence of extracellular calcium; thus, either calcium itself or the events triggered by calcium initiate Rho and Cdc42 activation. We favor the latter possibility as we are unaware of any evidence that calcium binds Rho GTPases directly. Further, there is a 10–15s latency between calcium increase, which is thought to be over within a few seconds (e.g. [15,38*]), and Rho GTPase activation, implying the existence of several steps downstream of calcium elevation.

Strikingly, in *Xenopus* eggs, exocytosis of cortical secretory granules (CGs), an event which provides the slow block to polyspermy, triggers activation of Cdc42 on CGs with a latent period of 10–15s after fusion with the plasma membrane [48,49*]. Since membrane damage triggers exocytosis, we propose that at least some of the observed Rho and Cdc42 activation results from wound induced exocytosis via 'compartment mixing'. During compartment mixing, local signals are generated in membranes as a result of fusion of two distinct membrane compartments [49*,50*]. In *Xenopus* eggs, following calcium elevation, levels of plasma membrane DAG transiently increase and DAG incorporates into CG membranes as a result of diffusion when the CGs fuse with the plasma membrane [50*]. Once in the CG membrane, DAG directs sustained recruitment of protein kinase C β , which, in turn, activates Cdc42, which is localized to the CGs prior to exocytosis. Thus, in a similar manner, fusion of other compartments with the plasma membrane following wounding could also lead to local Rho GTPase activation.

While this hypothesis remains to be tested, it has several attractive features. First, it explains why PKC agonists such as phorbol esters promote cellular wound repair [51,52]. Second, it explains the delay between wounding and the onset of Rho and Cdc42 activation — compartment mixing has a built in fuse [49*]. Third, it can also explain why the Rho GTPase activation, and thus actin and myosin-2 assembly, are restricted to the region around the edge of the original wound, rather than over the entire wound area: the middle of the wound is sealed over via membrane generated by vesicle–vesicle fusion while the edges are sealed via membrane fusion of these vesicles with the plasma membrane [11*]. Compartment mixing-dependent signal generation is implicitly dependent on such heterotypic fusion, in that it is the union of membrane components previously kept separate that acts as the trigger [50*].

Conclusions

In conclusion, several key questions are likely to occupy those who study cellular repair in the immediate future: identification of the sealing organelle(s); identification of the proteins directly involved in plasma membrane resealing; characterization of the roles played by the cytoskeleton during healing; and characterization of the signal transduction pathways that link calcium influx to cytoskeletal reorganization.

Acknowledgements

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Figure 1.

A schematic diagram showing the early events of the cellular wound response. Immediately after plasma membrane damage, calcium rushes into the cell. This triggers vesicle-vesicle fusion in the center of the wound, creating a 'patch'. The calcium inrush also triggers fusion of the vesicle with both the plasma membrane (PM) and the patch, thereby sealing the patch to the plasma membrane. Note that vesicle-vesicle fusion and vesicle-plasma membrane fusion could occur simultaneously. See [11*].

Figure 2.

Signal transduction and the cytoskeletal response to plasma membrane (PM) damage. (a) F-actin (red) and myosin-2 (green) segregate into distinct regions around a plasma membrane wound with myosin-2 concentrated on the interior of the array and dynamic actin being more abundant in the outer portion of the array. See [38^{*},39,40]. (b) Active Cdc42 (red) and active Rho (green) sort into distinct activity zones around wounds. The distribution of active Cdc42 parallels that of dynamic actin while the distribution of active Rho parallels that of myosin-2; compare with (a). See [46^{*}]. (c) A schematic showing the expected position of the different membrane fusion events when viewed from the same orientation as (a) and (b). The grey ring, corresponding to where plasma membrane-vesicle fusion occurs [11^{*}], is in the same region where the zones of active Cdc42 and Rho are found (b). (d) Schematic showing the expected position of different membrane fusion events in the same orientation as Figure 1. Cdc42 and Rho activation are hypothesized to be activated as a result of compartment mixing caused by plasma membrane-vesicle fusion at the seam between the patching membrane and the plasma membrane.