Published in final edited form as:

Semin Cell Dev Biol. 2015 September ; 45: 18–23. doi:10.1016/j.semcdb.2015.09.026.

Cell healing: calcium, repair and regeneration

Alison Moe¹, Adriana E. Golding¹, and William M. Bement^{1,2}

¹Cell and Molecular Biology Graduate Program, Laboratory of Cell and Molecular Biology, University of Wisconsin-Madison 1525 Linden Drive, Madison, WI USA

²Department of Zoology, University of Wisconsin-Madison 1525 Linden Drive, Madison, WI USA

Abstract

Cell repair is attracting increasing attention due to its conservation, its importance to health, and its utility as a model for cell signaling and cell polarization. However, some of the most fundamental questions concerning cell repair have yet to be answered. Here we consider three such questions: 1) How are wound holes stopped? 2) How is cell regeneration achieved after wounding? 3) How is calcium influx linked to wound stoppage and cell regeneration?

Until recently, cell repair was a topic ignored by all but a handful of researchers. However, a series of overlapping findings has moved this subject, if not into the mainstream, at least out of the backwaters of scientific research. In particular, several general types of discoveries have attracted attention to cell healing: cell damage and healing is surprisingly common—many cell types are damaged and heal as a simple consequence of existence (McNeil and Ito, 1989, 1990, McNeil and Khakee, 1992), this apparently includes metastatic cells, which can survive locomotion-induced damage via upregulation of proteins involved in cell repair (Jaiswal et al., 2014); cell repair mechanisms are conserved—damage consistently elicits membrane repair and rapid reorganization of the cytoskeleton in a variety of cell types and organisms (La Claire, 1989; Steinhardt et al., 1994; Miyake and McNeil, 1995; Bement et al., 1999; Mandato and Bement, 2001; Shapire et al., 2008; Abreu-Blanco et al., 2011); cell repair represents a powerful model for studying cell polarization signaling mechanisms (Benink and Bement, 2005; Vaughan et al., 2011; Burkel et al., 2012; Kono et al., 2012; Abreu-Blanco et al., 2014); failed cell repair is linked to disease—pathologies ranging from muscular dystrophies to diabetes may result from, result in, or be exacerbated by deficits in cell repair (Bansal et al., 2003; Han et al., 2007; Muller et al., 2007; Howard et al., 2011). These findings have drawn to cell repair researchers from otherwise disparate fields, and as a consequence, the list of potential molecular participants in cell repair is growing rapidly.

However, in spite of the recent progress concerning the details of cell repair, answers to several “big picture” questions remain elusive. Here we consider three of these questions: 1) How are wound holes stopped? 2) How is cell regeneration achieved after wounding? 3)

Correspondence: wmbement@wisc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

How is calcium inrush linked to wound stoppage and cell regeneration? We do not pretend to have answers to these questions, rather, it is also our hope to clarify what can be a confusing literature and to prompt new ways of considering cell repair.

1) How are wound holes stopped?

It is generally assumed that, unless repaired, plasma membrane breaches will result in rapid cell death as a consequence of inrush of calcium. That is, the calcium concentration outside the cell is several orders of magnitude higher than it is in the cytoplasm and prolonged elevation of intracellular free calcium triggers cell death. Independent of calcium, loss of cytoplasm to the extracellular space would eventually kill the cell. Thus, stopping holes in the plasma membrane is widely viewed as being the most imperative event in the cell repair process.

Contraction

One of the first explicit mechanisms proposed for hole stopping was actomyosin-based contraction (Bement and Capco, 1991; Mandato and Bement, 2001). Here, F-actin and myosin-2 accumulate in the cortex around the wound perimeter, forming a continuous network that closes inward, bringing undamaged plasma membrane and underlying cortical cytoskeleton with it (Fig. 1). This response occurs in frog oocytes and embryos (Mandato and Bement, 2001; Clark et al., 2008) and *Drosophila* embryos (Abreu-Blanco et al., 2011) and may occur in mammalian muscle based on the reported recruitment of myosin-2 (Lin et al., 2012) and the observation that F-actin disruption suppresses lateral recruitment of dysferlin to wounds (McDade et al., 2014). Similarly, EM studies have localized F-actin and/or myosin-2 to sites of cell damage in amoeba and plant cells (Jeon and Jeon, 1975; LeClaire, 1989). Further, transected invertebrate axons pinch inward near the cut site (Gallant, 1988; Krause et al., 1994), a behavior consistent with contraction. Moreover, one of the essential signaling events in the wound-induced contraction pathway—Rho activation—occurs in frog oocytes and embryos (Benink and Bement, 2005), *Drosophila* syncytial embryos (Abreu-Blanco et al., 2014) and in budding yeast (Kono et al., 2012), arguing that it is a fundamental feature of wound repair. The problem with the contraction model is that it seems far too slow to close the hole within the few seconds generally thought to be required for healing (Steinhardt et al., 1994) or to prevent cell death via calcium inrush, with actin and myosin accumulation beginning within a 30 s or more and closure taking 3 or more minutes, depending on the wound size and model system. Further, in some studies, disruption of F-actin has been reported to facilitate, rather than inhibit wound resealing (e.g. Togo et al., 2000).

Exocytosis

Because healing requires a high concentration of extracellular calcium (see below) and because it is inhibited by toxins that impair the function of SNARES, proteins essential for membrane fusion, exocytosis has also been proposed to heal cell wounds (Steinhardt et al., 1994). Consistent with this mechanism, exocytosis has been detected in response to wounding (Bi et al., 1995; Miyake and McNeil, 1995). However, by definition exocytosis—fusion of an internal compartment with the plasma membrane—cannot occur in a region

that, like a hole, lacks plasma membrane. Thus, exocytosis would likely be effective only for healing very small wounds, and that assumes that membrane inserted into the regions flanking the wound can flow inward (Fig. 1). Since cells can heal wounds that are tens or even hundreds of micrometers in diameter (e.g. Bement and Capco, 1991; Terasaki et al., 1997), it follows that exocytosis cannot be the only mechanism involved in cell repair. Additionally, wounding is thought to be associated with endocytosis and removal of local membrane protrusions by scission (see below) which seems odd if the primary hole stopping mechanism works by inserting new membrane into the plasma membrane.

Patching

A third mechanism proposed for stopping holes is “membrane patching” (Terasaki et al., 1997; McNeil et al., 2000). Here the idea is that internal compartments fuse with each other at the site of the wound, creating a patch where the plasma membrane is missing (Fig. 1). This fusion is accompanied by fusion of the forming patch with the plasma membrane at the edges of the wound, thereby creating a continuous membrane over the wound site. The patching model can explain hole stoppage in both small and large wounds, and is consistent with several experimental findings, most compellingly the demonstration that sea water microinjected into sea urchin eggs is rapidly walled off from the cytoplasm (Terasaki et al., 1997). However, patching has yet to be directly observed in any system and to date there is no consensus on which intracellular compartment or compartments participate in patching. Further, it is not clear how the double-membraned structure predicted by patching would resolve into a single membrane.

Internalization

A fourth mechanism associated with wound repair and proposed for stopping holes is internalization via endocytosis (Idone et al., 2008). Here, plasma membrane-containing holes are simply removed from the cell surface via invagination of the region containing the hole; the invagination would then be pinched off the plasma membrane, similar to the processes of receptor mediated endocytosis or caveolae internalization (Fig. 1). This model is consistent with the observation that mechanical wounding or exposure to plasma membrane pore forming agents triggers endocytosis and that manipulations which inhibit endocytosis inhibit repair (Idone et al., 2008; Corrotte et al., 2013). However, as with exocytosis, such a mechanism would be limited to small wounds as the hole would have to be accommodated by the nascent endocytic compartment.

Externalization

A fifth mechanism proposed for stopping holes is topologically the opposite of internalization, namely, externalization via ESCRT-mediated budding and pinching off of plasma membrane-containing holes (Jimenez et al., 2014; Fig. 1). This model is consistent with localization of ESCRT proteins to wound sites and inhibition of healing following ESCRT inhibition (Jimenez et al., 2014; Jaiswal et al., 2014). Further, the involvement of ESCRTs could explain the results of older EM studies revealing wound-associated compartments comprising vesicles enclosed by other vesicles (e.g. Figure 1 in Miyake and McNeil, 1995), since ESCRTs are well known to promote inward budding of vesicles into endosomes. However, like exocytosis and internalization, ESCRT-mediated budding and

scission is also likely to have an upward limit of utility. Indeed, it was reported that wounds above ~100 nm do not require ESCRTs for hole stoppage (Jimenez et al., 2014) although this point was challenged (Jaiswal et al., 2014).

Plugging

All of the above mechanisms are assumed to stop holes by restoring membrane continuity at the wound site. However, there is a sixth mechanism with the potential to stop holes without establishment of a continuous membrane: plugging (Eddleman et al., 1997). In this model, membranous compartments in the cytoplasm become tightly cross linked to each other and the plasma membrane (possibly by proteins recruited to the wound site), forming a plug that stops or limits movement of ions or other materials into or out of the cell. Plugging has been visualized in transected axons where a variety of membranous compartments accumulate at the cut site over the course of many minutes (Eddleman et al., 1997, 1998) and membranous compartments have been found to form a plug in *Drosophila* syncytial embryo wounds (Abreu-Blanco et al., 2011). Plugging would not have an intrinsic size limit but is necessarily a temporary solution. It is also not clear how efficiently ion inrush could be limited by plugging, although measurements in transected axons indicate that calcium inrush is progressively reduced as plugging occurs (Eddleman et al., 2000). And, as discussed below, it may be that cell holes can remain open longer than is generally thought.

Why has it been so difficult to determine which of these mechanisms are responsible for stopping the hole? It is likely that both experimental and conceptual difficulties are to blame. From the experimental standpoint, the healing response is rapid and complex, making it difficult to draw firm conclusions from fixed samples. Further, it is currently impossible to directly determine wound size in living cells unless the wounds are larger than the limit of resolution of the light microscope. A potential exception is wounds induced by pore-forming toxins, which (presumably) have a minimum diameter of the pore size.

Our assays for hole stopping are also inherently limited. Hole stopping is usually assessed by cessation of dye uptake. Dye uptake-based assays work well for showing differences in rates of hole stoppage, but they are not so good at providing a measure of hole stoppage, as plots typically show a slowing, rather than a cessation of dye incorporation over time.

From the conceptual standpoint, we have been biased by the notion that hole stopping is or must be extremely fast, occurring within a few seconds or less. This attitude is based on the analogy to regulated exocytosis, on the feeling that calcium inrush must be immediately and completely shut off or the cell will die, and the observation that wound-induced increases in intracellular free calcium cease within 5-10s in wounded sea urchin eggs even when the wounds are extremely large (Steinhardt 1994; McNeil Terasaki 1997). However, more recent studies suggest resealing may often be much slower. For example, fibroblasts require 15 s to 90s to reseal depending on the study (e.g. Togo et al., 1999). Other cell types require longer: 100-300s for syncytial *Drosophila* embryos (Abreu-Blanco et al., 2011), ~60-120s for myoblasts (Azakir et al., 2012), 60-300s for muscle fibers (Bansal et al., 2003; Jaiswal et al., 2014b), 90s for cancer cells (Jaiswal et al., 2014a), 120-240s for HeLa cells (Jimenez et al., 2014), 20-120s for *Xenopus* blastomeres (Clark et al., 2009) and 60s-15m for frog oocytes (Luxardi et al., 2014; our unpublished results).

Clearly, cells can tolerate a hole in the plasma membrane for longer than a few seconds. Indeed, the results from transected axons suggest that complete hole stopping may take hours (e.g. Eddelman et al., 2000). What explains the differences in hole stopping speeds and why don't cells die if the holes remain open for minutes or more? One simple explanation for the differences between resealing rates in sea urchin eggs and other cells is that the former are normally wounded in media containing 10 mM CaCl_2 (which corresponds to the normal concentration of calcium in sea water) but media for other cells typically have 1-2 mM calcium. Since healing is calcium dependent (see also below) higher external calcium concentration may promote more rapid healing. Consistent with this possibility, sea urchin eggs wounded in 2 mM external calcium were observed to take much longer to reseal—90-120s (Steinhardt et al., 1994). With respect to cells surviving relatively lengthy times to hole stoppage, it may be that calcium simply does not diffuse very quickly from the site of damage to the rest of the cell, a possibility suggested by assessment of calcium mobility in cytoplasm (Albritton et al., 1992). Testing this notion will require that studies on healing in different cell types with longer hole stopping kinetics include direct assessment of intracellular free calcium concentration.

Another vexing problem is the identity of the membranous compartments involved in the healing process in general and hole stoppage in particular (Bement et al., 2007). A variety of candidates have been proposed including lysosomes (Reddy et al., 2001), enlargosomes (Borgonovo et al., 2002), and reserve granules (McNeil et al., 2000), but consensus has remained out of reach. And, without knowing the identity of the compartment(s), development of the proper probes for visualization of healing becomes quite difficult. Our suspicion is that we are missing something, perhaps an as-yet unidentified compartment that has evolved specifically for membrane repair. We base this suggestion on the fact that high resolution studies of membranes at wound sites typically reveal a riot of external folds, blebs, and tubes extending into the extracellular space around the wound (e.g. Gingell, 1970; Miyake and McNeil, 1995; Jimenez et al., 2014; Figure 2). Further, such elaborations are apparent even when the wound is comparatively small. For example, SLO toxin, which produces a pore of ~30 nm diameter (Bhakdi et al., 1985), results in protrusions that may extend 6 or more microns into the extracellular space (Atanassoff et al., 2014).

It is also the case that we naturally tend to seek single, simple explanations where possible. But perhaps this is a mistake. As a primordial cell polarization event, the mechanistic similarity of cell repair to cytokinesis has been pointed out previously (Bement and Capco, 1991; Bement et al., 1997; Sonnemann and Bement, 2011) and the comparison appears increasingly apt as more is learned about each process. Animal cell cytokinesis results from several complementary processes: Actomyosin-powered ingression of the plasma membrane brings plasma membrane and cortex together across a large distance, thereby generating a narrow tube of cytoplasm surrounded by plasma membrane (the “midbody”) that separates the nascent daughter cells. After the midbody has formed, it is associated with elaborate membrane extensions (e.g. Crowell et al., 2014), and it undergoes scission in a manner that is thought to involve exocytosis, endocytosis, fusion of the membranous compartments with each other and the plasma membrane, and ESCRT-mediated pinching (Neto et al., 2011; Schiel et al., 2013; Mierzwa and Gerlich, 2014). By analogy, one might imagine that wounds are likewise stopped via collaboration of many processes, with plugging and/or patching

limiting calcium inrush, contraction bringing edges of wounds close to each other and, once the hole is small enough, ESCRTs, endocytosis and exocytosis finishing things off.

2) How is the cell regenerated?

Just as tissue healing is not finished when a clot forms, it is likely that cell repair involves far more than stopping the hole (Sonnemann and Bement, 2011). We use the term “cell regeneration” to refer to the collection of processes hypothesized to return the wound site to its original state, replete with the normal repertoire of plasma membrane lipids and proteins, as well as a fully restored cortical cytoskeleton. Why posit that the repair process continues after the hole is stopped, and perhaps even long after the hole is stopped? First, cell damage triggers exocytosis of lysosomes (Reddy et al., 2001). While the role of this event remains unclear, one consequence is undeniable: fusion of the lysosome with the plasma membrane necessarily changes the latter, as lysosome membranes differ significantly from the plasma membrane in terms of lipid and protein composition (e.g. Schroeder et al., 2010). Likewise, patching would be expected to significantly perturb plasma membrane composition as a consequence of fusion with internal compartment(s). Second, wounding results in loss of the cortical cytoskeleton immediately beneath the wound, which presumably facilitates membrane fusion (Miyake et al., 2001). The cortical cytoskeleton thus has to be repaired. Third, like the clot, the extravagant membrane protrusions and other material that accumulate at wounds likely do not represent functional plasma membrane and these can linger long for 20 minutes or more after wounding (e.g. Gingell, 1970; Eddelman et al., 1997; Marg et al., 2012). Fourth, proteins recruited to wounds can remain there for many minutes to even hours after wounding (e.g. Mellgren, 2010; Marg et al., 2012; Jimenez et al., 2014; our unpublished results). Finally, many of the cells routinely subject to wounds (e.g. muscle, enterocytes, skin; see McNeil and Ito, 1989, 1990, McNeil and Khakee, 1992) are highly differentiated and contain elaborate cortical specializations such as microvilli, cell-cell junctions, and costameres. Such structures, if destroyed during wounding, have to be reassembled, a process that is unlikely to be finished within just a few minutes, given that they may take hours to days to develop in differentiating tissue.

With the exception of patching and plugging, all of the mechanisms described for stopping the hole also have the potential to aid regeneration: exocytosis of vesicles containing appropriate plasma membrane proteins and lipids could replace those lost during wounding and hole stopping. Conversely, endocytosis could remove inappropriate proteins and lipids inserted into the plasma membrane during hole stopping. Scission during externalization could excise protrusions or plasma membrane containing high concentrations of inappropriate proteins and lipids. And contraction could restore the cell to the undamaged state simply by pulling unperturbed plasma membrane and cortical cytoskeleton over the original wound site.

An additional potential contributor to regeneration is wound-induced microtubule assembly and reorganization. Microtubules first disappear from and then accumulate around wounds within ~1-3 minutes as a consequence of both de novo assembly and actomyosin-powered transport (Mandato and Bement, 2003; Togo, 2006). The end result is a radial array of microtubules around the wound that has the potential to transport material to and from the

wound site (Mandato and Bement, 2003). Consistent with microtubules playing a role in regeneration, their loss does not prevent healing of an initial wound, but does slow repair of subsequent wounds (Togo, 2006).

Why should we worry about regeneration of damaged cells? First, failure to recognize regeneration as a part of the cell repair process may lead to the belief that a cell is completely healed when in fact complete healing is hours or even days away, particularly if the cell type in question is highly differentiated. Second, distinguishing between regeneration and hole stopping may help clarify variations in the repair process in wounds of different sizes and in wounds occurring in different cell types. For example, while ESCRTs may not be required for healing of larger wounds (Jimenez et al., 2014), they could nonetheless make important contributions to the regeneration process by excising membrane protrusions. Similarly, even if a given still be useful for rapid removal of inappropriate membrane proteins and lipids inserted as a consequence of exocytosis or patching. Third, it seems probable that pathologies resulting from compromised regeneration rather than hole stopping exist, but have not been discovered because no one is looking for them.

3) What is the cellular and molecular basis of the calcium dependence of cell repair?

If the process of cell repair seems complicated by the potential for multiple hole stopping and regeneration mechanisms, and confusion surrounding the identity of potential repair compartments, at least one feature of cell repair is not in question—the requirement for extracellular calcium. In the absence of extracellular calcium, neither contraction (Bement and Capco, 1991; Bement et al., 1997), exocytosis (Steinhardt et al., 1994; Miyake et al., 1995), patching (Terasaki et al., 1997), internalization (Idone et al., 2008; Corrotte et al., 2013), externalization (Jimenez et al., 2014; Jaiswal et al., 2014), nor plugging (Eddelman et al., 1997, 1998) occur after wounding. Indeed, so important is calcium inrush for repair, wounding in the absence of external calcium is often used as a negative control for the healing process. But the general acceptance of calcium as a key upstream signal for cell repair masks a major mystery: Why does the external calcium concentration have to be so high? For example, repair of sea urchin eggs requires a minimum of 1.2 mM and fibroblast repair a minimum of 0.8 mM external calcium in the presence of physiological concentrations of magnesium (Steinhardt et al., 1994) and similar results have been obtained in other systems (eg Yumura et al., 2014; our unpublished results). Neither the cellular process nor the protein targets of the high calcium are clear. For example, the calcium requirement was originally suggested to support the idea that plasma membrane resealing resulted from exocytosis, based on the well known calcium dependence of regulated exocytosis (Steinhardt et al., 1994). However, uncaging studies show that exocytosis of synaptic vesicles is efficiently triggered at 3-10 μ M intracellular free calcium (Scheggenburger and Neher, 2000), more than an order of magnitude less than the requirement for repair. Similarly, the calcium affinities for the synaptotagmins (syts), which confer calcium sensitivity on SNARE-dependent membrane fusion events (Chapman, 2008), are in the range of 1-20 μ M depending on the syt and the presence of lipid binding partners (e.g. Sugita et al., 2002; Radhakrishnan et al., 2009; van den Bogaart et al., 2012). While it

could be argued that exocytosis of other compartments requires far more calcium, exocytosis of lysosomes occurs at 1 μ M intracellular free calcium (Andrews, 2000). Nor does internalization seem to require high calcium in that it is thought to be triggered by lysosome exocytosis (Corrotte et al., 2013). Similarly, during externalization, calcium sensitivity is thought to be conferred by the EF hand-containing Alg-2 (Jaiswal et al., 2014), but its affinity for calcium is in the 1-3 μ M range (Tarabykina et al., 2000). With respect to contraction, the only known calcium-dependent link to Rho GTPase activation is protein kinase C β (Vaughan et al., 2014), but its affinity for calcium is $\sim$ 5 μ M (Nafelski et al., 2001).

Thus, what cellular or molecular targets during cell repair account for its extremely high calcium requirement are unknown and it could be argued that until they are identified, issues concerning the relative importance of different hole stopping mechanisms cannot be settled. One general possibility worth considering is that potential targets with more than one calcium binding site may acquire different functions at extremely high calcium levels (e.g. Lek et al., 2013; Redpath et al., 2014). Alternatively, focussing on proteins that have been implicated in wound repair and which are known to bind calcium with relatively low affinity may be a promising approach. For example, S100A11, an annexin binding protein, has a calcium affinity of $\sim$ 500 μ M (Zimmer and Weber, 2010). Since both S100 proteins (Rezvanpour et al., 2011; Jaiswal et al., 2014a) and annexins (McNeil et al., 2006; Potez et al., 2011; Swaggart et al., 2014) have been implicated in cell repair, they are attractive candidates as targets.

In summary, while the number of molecular participants implicated in cell repair grows at an increasing pace (Jaiswal and Nylandsted, 2015; Boucher and Mandato, 2015), several of the fundamental questions surrounding this important process remain unresolved. We suspect that the answers will require a broadening of outlook, rather than a focus on a particular molecule or process. Regardless, we look forward with excitement to the answers which, one assumes, will be forthcoming as more attention is directed toward them.

Acknowledgements

We are grateful to Nick Davenport and Kevin Sonnemann (University of Wisconsin-Madison) to their ideas about cell repair, and to George Bittner (University of Texas-Austin) for discussing ideas and publications concerning healing of transected axons. The work in our lab is supported by a grant from the National Institutes of Health (GM052932).

References

- McNeil PL, Ito S. Gastrointestinal cell plasma membrane wounding and resealing in vivo. *Gastroenterology*. May; 1989 96(5 Pt 1):1238–48. [PubMed: 2703112]
- McNeil PL1, Ito S. Molecular traffic through plasma membrane disruptions of cells in vivo. *J Cell Sci*. Jul; 1990 96(Pt 3):549–56. [PubMed: 1699955]
- McNeil PL, Khakee R. Disruptions of muscle fiber plasma membranes. Role in exercise-induced damage. *Am J Pathol*. May; 1992 140(5):1097–109. [PubMed: 1374591]
- Jaiswal JK, Lauritzen SP, Scheffer L, Sakaguchi M, Bunkenborg J, Simon SM, Kallunki T, Jäättelä M, Nylandsted J. S100A11 is required for efficient plasma membrane repair and survival of invasive cancer cells. *Nat Commun*. May 8.2014 5:3795. [PubMed: 24806074]
- Steinhardt RA, Bi G, Alderton JM. Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. *Science*. Jan 21; 1994 263(5145):390–3. [PubMed: 7904084]

- Miyake K, McNeil PL. Vesicle accumulation and exocytosis at sites of plasma membrane disruption. *J Cell Biol.* Dec; 1995 131(6 Pt 2):1737–45. [PubMed: 8557741]
- Bement WM, Mandato CA, Kirsch MN. Wound-induced assembly and closure of an actomyosin purse string in *Xenopus* oocytes. *Curr Biol.* Jun 3; 1999 9(11):579–87. [PubMed: 10359696]
- Mandato CA, Bement WM. Contraction and polymerization cooperate to assemble and close actomyosin rings around *Xenopus* oocyte wounds. *J Cell Biol.* Aug 20; 2001 154(4):785–97. [PubMed: 11502762]
- Schapiro AL, Voigt B, Jasik J, Rosado A, Lopez-Cobollo R, Menzel D, Salinas J, Mancuso S, Valpuesta V, Baluska F, Botella MA. Arabidopsis synaptotagmin 1 is required for the maintenance of plasma membrane integrity and cell viability. *Plant Cell.* Dec; 2008 20(12):3374–88. [PubMed: 19088329]
- Abreu-Blanco MT, Verboon JM, Parkhurst SM. Cell wound repair in *Drosophila* occurs through three distinct phases of membrane and cytoskeletal remodeling. *J Cell Biol.* May 2; 2011 193(3):455–64. [PubMed: 21518790]
- Benink HA, Bement WM. Concentric zones of active RhoA and Cdc42 around single cell wounds. *J Cell Biol.* Jan 31; 2005 168(3):429–39. [PubMed: 15684032]
- Vaughan EM, Miller AL, Yu HY, Bement WM. Control of local Rho GTPase crosstalk by Abr. *Curr Biol.* Feb 22; 2011 21(4):270–7. [PubMed: 21295482]
- Burkel BM, Benink HA, Vaughan EM, von Dassow G, Bement WM. A Rho GTPase signal treadmill backs a contractile array. *Dev Cell.* Aug 14; 2012 23(2):384–96. [PubMed: 22819338]
- Kono K, Saeki Y, Yoshida S, Tanaka K, Pellman D. Proteasomal degradation resolves competition between cell polarization and cellular wound healing. *Cell.* Jul 6; 2012 150(1):151–64. [PubMed: 22727045]
- Abreu-Blanco MT, Verboon JM, Parkhurst SM. Coordination of Rho family GTPase activities to orchestrate cytoskeleton responses during cell wound repair. *Curr Biol.* Jan 20; 2014 24(2):144–55. [PubMed: 24388847]
- Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, McNeil PL, Campbell KP. Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature.* May 8; 2003 423(6936):168–72. [PubMed: 12736685]
- Han R, Bansal D, Miyake K, Muniz VP, Weiss RM, McNeil PL, Campbell KP. Dysferlin-mediated membrane repair protects the heart from stress-induced left ventricular injury. *J Clin Invest.* Jul; 2007 117(7):1805–13. [PubMed: 17607357]
- Müller MW, McNeil PL, Büchler P, Ceyhan GO, Wolf-Hieber E, Adler G, Beger HG, Büchler MW, Friess H. Acinar cell membrane disruption is an early event in experimental acute pancreatitis in rats. *Pancreas.* Nov; 2007 35(4):e30–40. [PubMed: 18090229]
- Howard AC, McNeil AK, Xiong F, Xiong WC, McNeil PL. A novel cellular defect in diabetes: membrane repair failure. *Diabetes.* Nov; 2011 60(11):3034–43. doi: 10.2337/db11-0851. Epub 2011 Sep 22. [PubMed: 21940783]
- Bement WM, Capco DG. Analysis of inducible contractile rings suggests a role for protein kinase C in embryonic cytokinesis and wound healing. *Cell Motil Cytoskeleton.* 1991; 20(2):145–57. [PubMed: 1751967]
- Clark AG, Miller AL, Vaughan E, Yu HY, Penkert R, Bement WM. Integration of single and multicellular wound responses. *Curr Biol.* Aug 25; 2009 19(16):1389–95. [PubMed: 19631537]
- Lin P, Zhu H, Cai C, Wang X, Cao C, Xiao R, Pan Z, Weisleder N, Takeshima H, Ma J. Nonmuscle myosin IIA facilitates vesicle trafficking for MG53-mediated cell membrane repair. *FASEB J.* May; 2012 26(5):1875–83. [PubMed: 22253476]
- McDade JR, Archambeau A, Michele DE. Rapid actin-cytoskeleton-dependent recruitment of plasma membrane-derived dysferlin at wounds is critical for muscle membrane repair. *FASEB J.* Aug; 2014 28(8):3660–70. [PubMed: 24784578]
- Jeon KW, Jeon MS. Cytoplasmic filaments and cellular wound healing in *Amoeba proteus*. *J Cell Biol.* Oct; 1975 67(1):243–9. [PubMed: 1176533]
- La Claire JW 2nd. Actin cytoskeleton in intact and wounded coenocytic green algae. *Planta.* Jan; 1989 177(1):47–57. [PubMed: 24212271]

- Gallant PE. Effects of the external ions and metabolic poisoning on the constriction of the squid giant axon after axotomy. *J Neurosci.* May; 1988 8(5):1479–84. [PubMed: 3130467]
- Krause TL, Fishman HM, Ballinger ML, Bittner GD. Extent and mechanism of sealing in transected giant axons of squid and earthworms. *J Neurosci.* Nov; 1994 14(11 Pt 1):6638–51. [PubMed: 7965066]
- Togo T, Krasieva TB, Steinhardt RA. A decrease in membrane tension precedes successful cell-membrane repair. *Mol Biol Cell.* Dec; 2000 11(12):4339–46. [PubMed: 11102527]
- Bi GQ, Alderton JM, Steinhardt RA. Calcium-regulated exocytosis is required for cell membrane resealing. *J Cell Biol.* Dec; 1995 131(6 Pt 2):1747–58. [PubMed: 8557742]
- Terasaki M, Miyake K, McNeil PL. Large plasma membrane disruptions are rapidly resealed by Ca²⁺-dependent vesicle-vesicle fusion events. *J Cell Biol.* Oct 6; 1997 139(1):63–74. [PubMed: 9314529]
- McNeil PL, Vogel SS, Miyake K, Terasaki M. Patching plasma membrane disruptions with cytoplasmic membrane. *J Cell Sci.* Jun; 2000 113(Pt 11):1891–902. [PubMed: 10806100]
- Idone V, Tam C, Goss JW, Toomre D, Pypaert M, Andrews NW. Repair of injured plasma membrane by rapid Ca²⁺-dependent endocytosis. *J Cell Biol.* Mar 10; 2008 180(5):905–14. [PubMed: 18316410]
- Eddleman CS, Ballinger ML, Smyers ME, Godell CM, Fishman HM, Bittner GD. Repair of plasmalemmal lesions by vesicles. *Proc Natl Acad Sci U S A.* Apr 29; 1997 94(9):4745–50. [PubMed: 9114062]
- Eddleman CS, Ballinger ML, Smyers ME, Fishman HM, Bittner GD. Endocytotic formation of vesicles and other membranous structures induced by Ca²⁺ and axolemmal injury. *J Neurosci.* Jun 1; 1998 18(11):4029–41. [PubMed: 9592084]
- Eddleman CS, Bittner GD, Fishman HM. Barrier permeability at cut axonal ends progressively decreases until an ionic seal is formed. *Biophys J.* Oct; 2000 79(4):1883–90. [PubMed: 11023894]
- Togo T, Alderton JM, Bi GQ, Steinhardt RA. The mechanism of facilitated cell membrane resealing. *J Cell Sci.* Mar; 1999 112(Pt 5):719–31. [PubMed: 9973606]
- Azakir BA, Di Fulvio S, Kinter J, Sinnreich M. Proteasomal inhibition restores biological function of mis-sense mutated dysferlin in patient-derived muscle cells. *J Biol Chem.* Mar 23; 2012 287(13):10344–54. [PubMed: 22318734]
- Luxardi G, Reid B, Maillard P, Zhao M. Single cell wound generates electric current circuit and cell membrane potential variations that requires calcium influx. *Integr Biol (Camb).* Jul 24; 2014 6(7):662–72. [PubMed: 24801267]
- Allbritton NL, Meyer T, Stryer L. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science.* Dec 11; 1992 258(5089):1812–5. [PubMed: 1465619]
- Bement WM, Yu HY, Burkel BM, Vaughan EM, Clark AG. Rehabilitation and the single cell. *Curr Opin Cell Biol.* Feb; 2007 19(1):95–100. [PubMed: 17174083]
- Reddy A, Caler EV, Andrews NW. Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes. *Cell.* Jul 27; 2001 106(2):157–69. [PubMed: 11511344]
- Borgonovo B, Cocucci E, Racchetti G, Podini P, Bachi A, Meldolesi J. Regulated exocytosis: a novel, widely expressed system. *Nat Cell Biol.* Dec; 2002 4(12):955–62. [PubMed: 12447386]
- Gingell D. Contractile responses at the surface of an amphibian egg. *J Embryol Exp Morphol.* Jun; 1970 23(3):583–609. [PubMed: 4990699]
- Bhakdi S, Trantum-Jensen J, Sziegoleit A. Mechanism of membrane damage by streptolysin-O. *Infect Immun.* Jan; 1985 47(1):52–60. [PubMed: 3880730]
- Atanassoff AP, Wolfmeier H, Schoenauer R, Hostettler A, Ring A, Draeger A, Babiychuk EB. Microvesicle shedding and lysosomal repair fulfill divergent cellular needs during the repair of streptolysin O-induced plasmalemmal damage. *PLoS One.* Feb 21; 2014 9(2):e89743. [PubMed: 24587004]
- Sonnemann KJ, Bement WM. Wound repair: toward understanding and integration of single-cell and multicellular wound responses. *Annu Rev Cell Dev Biol.* 2011; 27:237–63. [PubMed: 21721944]
- Miyake K, McNeil PL, Suzuki K, Tsunoda R, Sugai N. An actin barrier to resealing. *J Cell Sci.* Oct; 2001 114(Pt 19):3487–94. [PubMed: 11682608]

- Crowell EF, Gaffuri AL, Gayraud-Morel B, Tajbakhsh S, Echard A. Engulfment of the midbody remnant after cytokinesis in mammalian cells. *J Cell Sci.* Sep 1; 2014 127(Pt 17):3840–51. [PubMed: 25002399]
- Neto H, Collins LL, Gould GW. Vesicle trafficking and membrane remodelling in cytokinesis. *Biochem J.* Jul 1; 2011 437(1):13–24. [PubMed: 21668412]
- Schiell JA, Childs C, Prekeris R. Endocytic transport and cytokinesis: from regulation of the cytoskeleton to midbody inheritance. *Trends Cell Biol.* Jul; 2013 23(7):319–27. [PubMed: 23522622]
- Mierzwa B, Gerlich DW. Cytokinetic abscission: molecular mechanisms and temporal control. *Dev Cell.* Dec 8; 2014 31(5):525–38. [PubMed: 25490264]
- Schroder BA, Wrocklage C, Hasilik A, Saftig P. The proteome of lysosomes. *Proteomics.* 2010; 10:4053–4076. [PubMed: 20957757]
- Marg A, Schoewel V, Timmel T, Schulze A, Shah C, Daumke O, Spuler S. Sarcolemmal repair is a slow process and includes EHD2. *Traffic.* Sep; 2012 13(9):1286–94. [PubMed: 22679923]
- Mellgren RL. A plasma membrane wound proteome: reversible externalization of intracellular proteins following repairable mechanical damage. *J Biol Chem.* Nov 19; 2010 285(47):36597–607. [PubMed: 20810652]
- Mandato CA, Bement WM. Actomyosin transports microtubules and microtubules control actomyosin recruitment during *Xenopus* oocyte wound healing. *Curr Biol.* Jul 1; 2003 13(13):1096–105. [PubMed: 12842008]
- Togo T. Disruption of the plasma membrane stimulates rearrangement of microtubules and lipid traffic toward the wound site. *J Cell Sci.* Jul 1; 2006 119(Pt 13):2780–6. [PubMed: 16772335]
- Yumura S, Hashima S, Muranaka S. Myosin II does not contribute to wound repair in *Dictyostelium* cells. *Biol Open.* Sep 19; 2014 3(10):966–73. [PubMed: 25238760]
- Schneggenburger R1, Neher E. Intracellular calcium dependence of transmitter release rates at a fast central synapse. *Nature.* Aug 24; 2000 406(6798):889–93. [PubMed: 10972290]
- Sugita S, Shin OH, Han W, Lao Y, Südhof TC. Synaptotagmins form a hierarchy of exocytotic Ca(2+) sensors with distinct Ca(2+) affinities. *EMBO J.* Feb 1; 2002 21(3):270–80. [PubMed: 11823420]
- Radhakrishnan A, Stein A, Jahn R, Fasshauer D. The Ca2+ affinity of synaptotagmin 1 is markedly increased by a specific interaction of its C2B domain with phosphatidylinositol 4,5-bisphosphate. *J Biol Chem.* Sep 18; 2009 284(38):25749–60. [PubMed: 19632983]
- van den Bogaart G, Meyenberg K, Diederichsen U, Jahn R. Phosphatidylinositol 4,5-bisphosphate increases Ca2+ affinity of synaptotagmin-1 by 40-fold. *J Biol Chem.* May 11; 2012 287(20):16447–53. [PubMed: 22447935]
- Andrews NW. Regulated secretion of conventional lysosomes. *Trends Cell Biol.* Aug; 2000 10(8):316–21. [PubMed: 10884683]
- Tarabykina S, Møller AL, Durussel I, Cox J, Berchtold MW. Two forms of the apoptosis-linked protein ALG-2 with different Ca(2+) affinities and target recognition. *J Biol Chem.* Apr 7; 2000 275(14):10514–8. [PubMed: 10744743]
- Nalefski EA, Wisner MA, Chen JZ, Sprang SR, Fukuda M, Mikoshiba K, Falke JJ. C2 domains from different Ca2+ signaling pathways display functional and mechanistic diversity. *Biochemistry.* Mar 13; 2001 40(10):3089–100. [PubMed: 11258923]
- Rezvanpour A, Santamaria-Kisiel L, Shaw GS. The S100A10-annexin A2 complex provides a novel asymmetric platform for membrane repair. *J Biol Chem.* Nov 18; 2011 286(46):40174–83. [PubMed: 21949189]
- Lek A, Evesson FJ, Lemckert FA, Redpath GM, Lueders AK, Turnbull L, Whitchurch CB, North KN, Cooper ST. Calpains, cleaved mini-dysferlinC72, and L-type channels underpin calcium-dependent muscle membrane repair. *J Neurosci.* Mar 20; 2013 33(12):5085–94. [PubMed: 23516275]
- Redpath GM, Woolger N, Piper AK, Lemckert FA, Lek A, Greer PA, North KN, Cooper ST. Calpain cleavage within dysferlin exon 40a releases a synaptotagmin-like module for membrane repair. *Mol Biol Cell.* Oct 1; 2014 25(19):3037–48. [PubMed: 25143396]
- Zimmer DB, Weber DJ. The Calcium-Dependent Interaction of S100B with Its Protein Targets. *Cardiovasc Psychiatry Neurol.* 2010;2010, 728052.

- McNeil AK, Rescher U, Gerke V, McNeil PL. Requirement for annexin A1 in plasma membrane repair. *J Biol Chem*. Nov 17; 2006 281(46):35202–7. Epub 2006 Sep 19. PMID: 16984915. [PubMed: 16984915]
- Potez S, Luginbühl M, Monastyrskaya K, Hostettler A, Draeger A, Babiychuk EB. Tailored protection against plasmalemmal injury by annexins with different Ca²⁺ sensitivities. *J Biol Chem*. May 20; 2011 286(20):17982–91. [PubMed: 21454475]
- Swaggart KA, Demonbreun AR, Vo AH, Swanson KE, Kim EY, Fahrenbach JP, Holley-Cuthrell J, Eskin A, Chen Z, Squire K, Heydemann A, Palmer AA, Nelson SF, McNally EM. Annexin A6 modifies muscular dystrophy by mediating sarcolemmal repair. *Proc Natl Acad Sci U S A*. Apr 22; 2014 111(16):6004–9. [PubMed: 24717843]
- Boucher E, Mandato CA. Plasma membrane and cytoskeleton dynamics during single-cell wound healing. *Biochim Biophys Acta*. Jul 22; 2015 1853(10 Pt A):2649–2661. doi: 10.1016/j.bbamcr.2015.07.012. [Epub ahead of print] Review. [PubMed: 26209916]
- Jaiswal JK, Nylandsted J. S100 and annexin proteins identify cell membrane damage as the Achilles heel of metastatic cancer cells. *Cell Cycle*. 2015; 14(4):502–9. [PubMed: 25565331]

Figure 1.

Schematic diagram depicting the various mechanisms proposed to stop holes made in cells. In each case, the black line with gap represents the plasma membrane with a wound-induced hole, black circles represent membranous compartments in the cell and healing progresses from top to bottom. Green lines in “Contraction” represent cortical cytoskeleton; yellow dots in “Internalization” represent machinery powering endocytotic invagination and pinching; blue dots in “Externalization” represent ESCRT machinery powering scission; red dots in

“Plugging” represent proteins crosslinking membranous compartments. See text for description of mechanisms.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Confocal micrograph showing membrane extensions extending into the extracellular space around a wound made in a *Xenopus* egg. The wound (which is obscured by the extensions) is ~5 μm in diameter; the region occupied the extensions is ~60 μm in diameter.