

# Repairing a torn cell surface: make way, lysosomes to the rescue

Paul L. McNeil

Department of Cellular Biology and Anatomy, Institute of Molecular Medicine and Genetics, Medical College of Georgia, Augusta, GA 30904, USA  
e-mail: pmcneil@mccg.edu

*Journal of Cell Science* 115, 873-879 (2002) © The Company of Biologists Ltd

## Summary

Biological membranes are often described as 'self-sealing' structures. If indeed membranes do have an inherent capacity for repair, does this explain how a cell can rapidly reseal a very large (1-1000  $\mu\text{m}^2$ ) disruption in its plasma membrane? It is becoming increasingly clear that, in nucleated animal cells, the cytoplasm plays an active and essential role in resealing. A rapid and apparently chaotic membrane fusion response is initiated locally in the cytoplasm by the  $\text{Ca}^{2+}$  that floods in through a disruption: cytoplasmic vesicles are thereby joined with one another (homotypically) and with the surrounding plasma membrane (exocytotically). As a consequence, internal membrane is added to cell surface membrane at the disruption site. In the case of large disruptions, this addition is hypothesized to function as a 'patch'. In sea urchin eggs, the internal compartment used is the yolk granule. Several recent studies have significantly advanced our understanding of how cells survive disruption-inducing injuries. In fibroblasts, the lysosome has been identified as a key organelle in resealing. Protein markers of the

lysosome membrane appear on the surface of fibroblasts at sites of disruption. Antibodies against lysosome-specific proteins, introduced into the living fibroblast, inhibit its resealing response. In gastric epithelial cells, local depolymerization of filamentous actin has been identified as a crucial step in resealing: it may function to remove a barrier to lysosome-plasma membrane contact leading to exocytotic fusion. Plasma membrane disruption in epithelial cells induces depolymerization of cortical filamentous actin and, if this depolymerization response is inhibited, resealing is blocked. In the *Xenopus* egg, the cortical cytoskeleton has been identified as an active participant in post-resealing repair of disruption-related damage to underlying cell cortex. A striking, highly localized actin polymerization response is observable around the margin of cortical defects. A myosin powered contraction occurring within this newly formed zone of F-actin then drives closure of the defect in a purse-string fashion.

Key words: Lysosome, Fusion, Vesicle, Plasma membrane

## Introduction

Since the advent of microsurgical techniques in the early part of the last century, it has been known that animal cells can survive the experimental creation of very large holes ( $>1000 \mu\text{m}^2$ ) in their surface (reviewed in Chambers and Chambers, 1961; Heilbrunn, 1956). Remarkably, these initial observations of a truly remarkable cell capacity failed, for many subsequent decades, to generate further interest. This neglect had two causes. First, with the widespread use of microinjection and other methods for breaching the cell surface, resealing came to be associated primarily with the laboratory setting rather than with any natural, biological one. Resealing permitted cells to survive a microneedle puncture and was therefore a very useful cell property. It was, however, apparently of little biological interest, because a disruption, it was obvious, was merely a laboratory-generated artifact. Considerable work, which is briefly reviewed here, has clearly demonstrated that the mammalian body, in common with other machines that have moving parts, is not immune under normal operating conditions from mechanically induced wear and tear. Plasma membrane disruption is a common and normal event in many mechanically active mammalian tissues, and so resealing, because it permits cells to survive this injury, is a response that has fundamental biological significance.

In addition to suffering from a kind of 'guilt by association',

resealing languished for a second reason. It was thought to have a simple and trivial explanation. Thus, once it was established that the principal cell surface barrier torn by the microneedle was a fluid lipid bilayer, resealing became explicable as simply the thermodynamically determined outcome of the well established principle that 'Membranes hate edges' (Parsegian et al., 1984). According to this view, still current in textbooks, resealing is an ability inherent in all membranes, a response requiring nothing more than that the torn membrane be held at a temperature above its liquid-crystalline transition point. This view is no longer tenable, at least in the case of large ( $>1 \mu\text{m}$ ) disruptions occurring in nucleated animal cells. Rather, as outlined here, resealing is now viewed as the outcome of a dynamic and complex mechanism, one that relies heavily on the participation of numerous cytoplasmic constituents. Recent work, discussed below, strongly implicates lysosomes and the actin-based cytoskeleton as two key cytoplasmic players in the resealing response and actin/myosin-based contraction in the subsequent repair of wound-associated damage to the cell cortex.

## The significance of resealing

Survival is the obvious benefit to a free-living cell of rapidly resealing a plasma membrane disruption. An open disruption

allows potential toxins, such as  $\text{Ca}^{2+}$ , to flood into the cytosol of the 'wounded' cell and diffusible cytosolic components, such as proteins and ATP, to escape. Rapid resealing prevents a rapid death for the wounded cell.

For long-lived, multicellular organisms, too, resealing may often be a beneficial and therefore an evolutionarily favored cell response. For example, physical insults (accidents or attacks) that disrupt tissue integrity are an obvious cause of plasma membrane disruption. However, for a single, isolated tissue injury, it could be argued that resealing is of little consequence: 'once only' cell replacement costs might be of little overall importance, at least in long-lived organisms. It is *only* in cases in which the cells experiencing a disruption during an injury are both irreplaceable and essential to the continued functioning of the organism that resealing has an indisputable value. Importantly, this condition is fulfilled in the case of, for example, a severed nerve. The individual neurons suffering disruptions during the injurious (severing) event cannot be replaced. Therefore they must reseal if they are to survive and subsequently 'grow' back to their targets in a successful re-innervation effort.

If under normal, physiological circumstances plasma membrane disruption affects a large proportion of a tissue's cell population (e.g. is a frequent event) and/or if it affects large cell types, and/or affects cells that are irreplaceable, then resealing might be important under normal as well as pathological conditions. Either it reduces the cost to the organism of complete cell replacement or it prevents an accumulating loss of cells essential for the functioning of the organism. 'Cell wounding', defined as a survivable plasma membrane disruption event marked by the uptake into the cytosol of a normally membrane impermeant tracer, is observable under physiological conditions in the endothelium lining the aorta, the epithelium lining the gastrointestinal tract, the epithelium of skin, and the myocytes of cardiac and skeletal muscle (McNeil, 1993). The frequent occurrence of disruptions in cardiac (irreplaceable) and skeletal (large) myocytes (Clarke et al., 1995; McNeil and Khakee, 1992), under physiological conditions, argues that resealing is a fundamental biological response in mammals. It is possible that resealing is also a cost-effective cell adaptation in the several other tissues mentioned.

The proportion of cells classifiable as wounded typically increases as a function of mechanical load. For example, it rises from ~4% of the myocytes of the triceps muscle of the mouse or rat kept in its cage to ~20% after these rodents are exercised by running downhill (Clarke et al., 1993; McNeil and Khakee, 1992), which results in eccentric (high-force-producing) contractions of this muscle. Resealing is therefore an essential, if widely overlooked, function of the many and diverse cells that reside in mechanically challenging tissue environments of the normally functioning mammalian body.

### **Rapid resealing involves a $\text{Ca}^{2+}$ -dependent, exocytotic response in nucleated animal cells**

The early studies of Chambers and Heilbrunn established the remarkable capacity animal cells have for repairing cell surface tears and showed that this capacity is absolutely dependent on the extracellular presence at near physiological levels of  $\text{Ca}^{2+}$  (Chambers and Chambers, 1961; Heilbrunn, 1956). For example, they observed that, in the presence of extracellular

$\text{Ca}^{2+}$ , the echinoderm egg exhibits a vigorous cytoplasmic reaction beneath a site of surface tearing and that associated with this response is a minimal loss of intracellular material and the retention of cell viability. In the absence of extracellular  $\text{Ca}^{2+}$ , they observed a rapid emptying out of cytoplasm from the egg through the surface injury site. Heilbrunn termed this  $\text{Ca}^{2+}$ -dependent response, which he and others observed in a wide variety of cell types, the 'surface precipitation reaction' (Heilbrunn, 1930). Working before it was known that the main surface diffusion barrier is a phospholipid bilayer, he hypothesized that exposure of cytoplasm to  $\text{Ca}^{2+}$  results in the precipitation of a reparative protein barrier over the surface defect.

The first clue to how exactly  $\text{Ca}^{2+}$  promotes resealing or restoration of disrupted lipid bilayer continuity came from work by Steinhart et al. (Steinhart et al., 1994). They found that resealing is inhibited if fibroblasts or sea urchin eggs are first injected with botulinum or tetanus toxins and then ~60 minutes later wounded by a second microneedle impalement. These toxins are proteases that are thought to specifically target and thereby inactivate members of the SNARE family of proteins required for certain exocytotic events, such as those occurring at the synapse during neurotransmitter release (Schiavo et al., 1992). Therefore the toxin microinjection experiment suggested that exocytosis is required for resealing. Subsequent work, using both endothelial cells (Miyake and McNeil, 1995) and sea urchin eggs (Bi et al., 1995), confirmed that an exocytotic reaction is rapidly evoked in a  $\text{Ca}^{2+}$ -dependent fashion by plasma membrane disruption, that this response is localized to the disruption site and that it is quantitatively related to disruption magnitude.

What is the function in resealing of this exocytotic delivery of internal membrane to the surface of the wounded cell? The plasma membrane adheres to the underlying cortical cytoskeleton (principally filamentous actin). This generates a 'membrane tension' (Raucher and Sheetz, 1999) that opposes the 'line tension' generated by lipid disordering at the free edge of a disruption (reviewed in Chernomordik et al., 1987). It is this line tension that, theoretically, promotes lipid flow over a disruption site, as well as the bilayer fusion event required in completing resealing. If therefore the exocytotic events induced by a disruption could somehow reduce membrane tension, the predicted result would be the promotion of resealing through enhanced, line-tension-driven lipid flow. Consistent with this hypothesis is the observation that a rapid (second timescale),  $\text{Ca}^{2+}$ -dependent reduction in membrane tension is induced by membrane disruption (Togo et al., 1999; Togo et al., 2000). Moreover, treatment of cells with surface active agents, which might reduce membrane or 'surface' tension, enhances resealing and survival (Clarke and McNeil, 1994; Togo et al., 1999).

### **Vesicle-vesicle fusion**

The role for exocytosis that was just described – flow promotion – is hypothesized to be applicable to relatively small (<1  $\mu\text{m}$  diameter) disruptions. The evidence supporting a second hypothesized role for exocytosis – the placement of a reparative 'patch' of internally derived membrane over the disruption site – will now be described.

Electron micrographs of the cortical cytoplasm bordering on

endothelial cell disruption sites suggest that, in addition to vesicle-plasma membrane fusion (exocytotic fusion), vesicle-vesicle fusion is induced locally by a disruption. The cortical cytoplasm surrounding a disruption displays within seconds after its formation a remarkable abundance of abnormally enlarged vesicles (Miyake and McNeil, 1995). What is the role of these enlarged vesicles? An answer to this question was suggested by experiments in which sea water was injected along with fluorescent tracers into the cytoplasm of starfish and sea urchin eggs (Terasaki et al., 1997). Fluorescent seawater containing a normal level of  $\text{Ca}^{2+}$ , but not seawater without  $\text{Ca}^{2+}$ , was immediately sequestered as it left the microneedle orifice behind an impermeant barrier. That this barrier was a membrane was confirmed by electron microscopy, staining with lipidic dyes and its measured impermeability not only to small fluorescent dyes such as fluorescein stachyose (~1000 MW) but also to  $\text{Ca}^{2+}$  and even  $\text{H}^+$  ions.

The sea water injection experiment revealed a key concept: cytoplasm by itself, in the absence of plasma membrane and therefore exocytotic events, can form a membrane barrier to prevent further incursion of the toxic extracellular environment. Moreover, it provided a mechanism consistent with the earlier electron microscope observations: given the scale (vesicles >10  $\mu\text{m}$  in diameter form at pipette tips) and rapidity (second or sub second time scale) of the formation of membrane barrier, the process had to be based on a vesicle-vesicle fusion reaction.

### The patch hypothesis

If the process of de novo barrier formation through vesicle-vesicle fusion, just described, occurred in the cytoplasm beneath a disruption site, and if this barrier was then added by exocytosis to the plasma membrane surrounding the disruption, then resealing would be accomplished by a kind of patching mechanism. This 'patch hypothesis' can be stated in more detail as follows (Fig. 1).  $\text{Ca}^{2+}$  entering through a disruption is hypothesized to evoke a local, chaotic vesicle-vesicle and vesicle-plasma-membrane fusion response. Consequently, a population of abnormally large vesicles is created below the disruption site, and vesicle-plasma membrane fusion events then link the bilayers of some of these vesicles with the plasma membrane. These membrane fusion events continue until a continuous 'patch' of membrane has been erected across the disruption site, blocking further entry of fusion-initiating  $\text{Ca}^{2+}$  ions.

Four key predictions of this 'patch' hypothesis have recently been verified in the sea urchin egg (McNeil et al., 2000). First, native, pre-disruption surface membrane is not present initially over large, resealed disruptions. The membrane covering the disruption site immediately after resealing must therefore be derived from an internal source, as predicted. Second, stratification of organelles induced by egg centrifugation results in a polarization of resealing function. The distribution or availability of internal membrane is therefore a crucial determinant of resealing capacity, as predicted. Third, abnormally large vesicles are readily detected in the cytoplasm underlying a disruption site, both by light and scanning electron microscopy, and the appearance of these is rapid and  $\text{Ca}^{2+}$  dependent (McNeil and Baker, 2001). Vesicle-vesicle fusion is therefore induced locally by  $\text{Ca}^{2+}$  influx through a

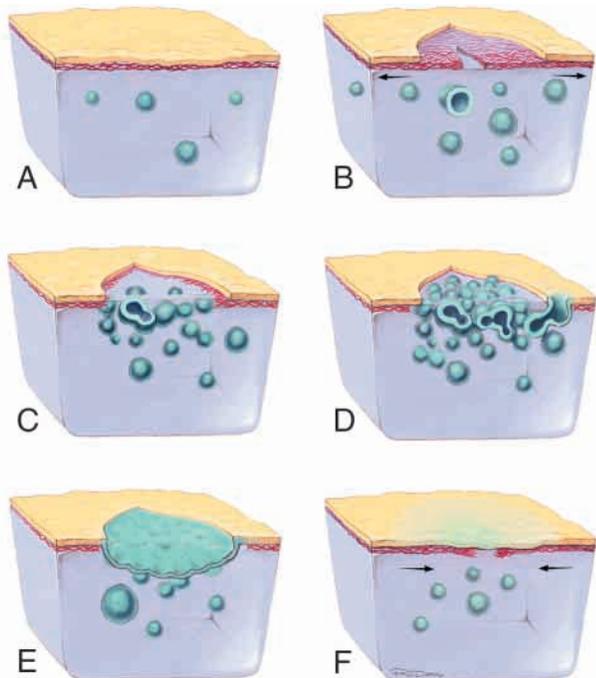
disruption, as predicted. Fourth, an egg organelle (the yolk granule) displays cytosol-independent homotypic fusion in vitro that is initiated  $\text{Ca}^{2+}$  (~10  $\mu\text{M}$  threshold) with a  $T_{1/2}$  of seconds and results in the production of very large (>50  $\mu\text{m}$  diameter) vesicles. The egg therefore possesses a vesicle population capable of homotypic fusion that can occur rapidly in the absence of the time consuming, cytosol-dependent priming steps of other homotypic fusion reactions and that can erect large membrane boundaries, as predicted.

The patch hypothesis can explain how extremely large disruptions, requiring substantial membrane replacement, are resealed; how, in fact, some cells are able rapidly to replace their entire surface membrane (Rappaport, 1976). Recent work, discussed below, now allows us to name the vesicle population used for patch formation by mammalian cells – lysosomes – and adds, for these cells, an additional early step in the mechanism – actin depolymerization. Moreover, it is becoming clear that a wounded cell's repair work continues after it has patched the surface bilayer discontinuity.

### What vesicles are used for resealing?

Yolk granules clearly are required for resealing of large disruptions made in sea urchin eggs. In the centrifuge-stratified egg, only the end containing yolk granules retains resealing competence; the other end, containing endoplasmic reticulum as well as 'docked' cortical granules, cannot reseat shear-induced disruptions (both ends probably harbor mitochondria) (McNeil et al., 2000). Moreover, as mentioned above, the yolk granule displays  $\text{Ca}^{2+}$ -initiated fusion in vitro, which has properties expected of a fusion reaction capable of supporting resealing (e.g. the speed and capacity to create large boundaries) (McNeil et al., 2000). No other egg fraction similarly displays this  $\text{Ca}^{2+}$ -triggered fusion, except the cortical granule fraction. The cortical granule is, however, unable to support resealing in the stratified egg, and moreover it is absent from resealing-competent fertilized eggs.

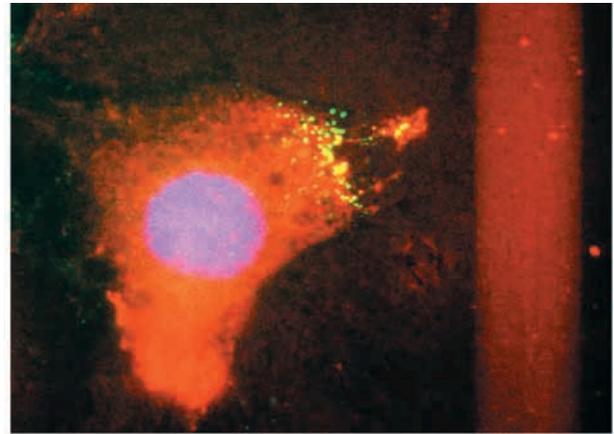
What is the organelle used in cells that lack yolk granules? Considerable indirect evidence had accumulated that pointed to the lysosome. Yolk granules of sea urchin eggs are an acidic compartment (McNeil and Terasaki, 2001) that contains hydrolytic enzymes (Armant et al., 1986), and yolk granules are known to have, in the species studied (Raikhel, 1987; Wallace et al., 1983), an endocytotic origin. Lysosomes of cultured mammalian cells can be induced by elevated  $\text{Ca}^{2+}$  levels, both in vitro (Mayorga et al., 1994) and in situ (Bakker et al., 1997), to fuse with one another (homotypically), as is required for patch formation. Moreover,  $\text{Ca}^{2+}$  induces lysosomes to fuse exocytotically with the plasma membrane (Rodriguez et al., 1997), which is another fusion event induced by disruption. This  $\text{Ca}^{2+}$ -regulated lysosomal exocytosis depends on synaptotagmin (Syt)VII, a lysosomal membrane protein and a member of a  $\text{Ca}^{2+}$ -binding family of proteins long thought to play a role in fusion possibly as  $\text{Ca}^{2+}$  sensors (Geppert and Sudhof, 1998). Antibodies against the C2A domain of this protein and recombinant Syt VII C2A domain peptide both inhibit  $\text{Ca}^{2+}$ -induced (streptolysin-O permeabilized) lysosomal exocytosis (Martinez et al., 2000). Antibodies raised against the C2A domain of a squid synaptogamin inhibited resealing in the giant squid axon and in cultured PC12 cells (Detrait et al., 2000a; Detrait et al.,



**Fig. 1.** The patch hypothesis. (A) An undisturbed cell. The typical subcortical network of F-actin (red) and underlying lysosomes or yolk granules (blue) is emphasized. (B) A large disruption of the plasma membrane occurs.  $\text{Ca}^{2+}$  entering through the disruption initiates deopolymerization of the F-actin network and triggers accumulation of vesicles (lysosomes or yolk granules) powered by myosin and kinesin motor proteins. (C) The accumulating vesicles begin to fuse with one another to create large patch vesicles. (D) Continuing vesicle-vesicle fusion creates more and larger patch vesicles, while vesicle-plasma membrane fusions, now possible owing to dissolution locally of the F-actin barrier, add this membrane to the cell surface. (E) A patch of internal membrane added is thereby added. Resealing is now complete. (F) Post-resealing polymerization of F-actin and its contraction mediated by myosin restores subcortical network continuity.

2000b). However, these axon-resealing studies did not reveal what organelle this antibody targeted. Lastly, earlier studies employing fluorescent dyes taken up endocytotically revealed the involvement of the various compartments, including lysosomes, thus labeled in a disruption-induced exocytotic response (Miyake and McNeil, 1995). Again the organelle involved could not be defined, since this method did not discriminate between the several compartments labeled, for example, endosomes (early, late) and lysosomes.

Thus it was important to ask more specifically whether exocytosis of lysosomes is triggered by a plasma membrane disruption. The approach Reddy et al. (Reddy et al., 2001) took in answering this question – to look for the disruption-induced appearance on the cell surface of a lysosomal membrane protein – yielded a striking result. Antibodies against the luminal domain of the lysosome-specific protein, Lamp-1 (Granger et al., 1990), do not stain the surface of undisturbed cells, but strongly stain the surface of wounded cells (Fig. 2). This surface exposure of Lamp-1 is  $\text{Ca}^{2+}$ -dependent and localized to disruption sites made with a microneedle. To test the functional importance in resealing of the disruption-induced



**Fig. 2.** Staining of a resealed fibroblast with antibodies against a luminal domain of the lysosome-specific protein, Lamp-1. The right hand side of this cell was severed when the culture dish it was growing in was scratched with a needle (the red streak on the right indicates the needle path). Staining with anti-Lamp-1 was then performed on the living, resealed cell, limiting detection to surface exposed antigen only. Surface exposure of Lamp-1, indicated by the staining, is evident over the resealed disruption site. This provides strong evidence that the local exocytotic response evoked by a disruption utilizes lysosomes. (Photomicrograph courtesy of Norma Andrews, Yale University.)

lysosomal exocytosis, Reddy et al. introduced into living cells antibodies to the C2A (calcium-binding) domain of Syt VII, as well as recombinant peptide fragments of the whole protein. Both of these reagents inhibited the surface appearance of the Lamp-1 luminal domain and cell resealing. These inhibitory effects were observed when the disruption event being monitored was also the route of access of reagent to cytoplasm. Inactivation must therefore have been extremely rapid, since resealing is generally complete in <90 sec in these cells. In an independent test of the role of lysosomes, antibodies against the cytosolic domain of Lamp-1, which have a lysosome-aggregating activity, also inhibited fusion.

These studies, it must be pointed out, do not rule out the participation of other organelles in resealing. When the cortical granules of the sea urchin are ‘undocked’ by treatments with stachyose, resealing is reversibly inhibited (Bi et al., 1995). This is indirect evidence for a cortical granule contribution to resealing, although it remains unclear how specific the stachyose treatment is for cortical granules. Moreover, two separate pools of vesicles, identified on the basis of the timing of their exocytosis and on their susceptibility to myosin/kinesin inhibitors, are required for resealing in urchin eggs (Bi et al., 1997). This is indirect evidence that, in the sea urchin egg, resealing might use both yolk granules and cortical granules, as well as other, as-yet-unidentified organelles. It also remains possible that, in other ‘specialized’ cell types, organelle compartments other than, or in addition to, lysosomes are mobilized for resealing.

#### Rescue by lysosomes: a dangerous expedient?

As mentioned above, plasma membrane disruption is a common and normal cell injury in vivo. Therefore, the lysosomal exocytotic response required for resealing must

occur constitutively and indeed at an enhanced rate during, for example, certain forms of exercise. What prevents consequent release of a battery of potentially destructive hydrolytic enzymes from becoming a net disadvantage to the organism? Two potentially relevant points can be made in answer to this question. First, lysosomal enzymes have a pH optimum of ~3.5. Thus, release into the extracellular environment of a mammalian tissue, which has a pH of ~7.4, severely curtails their activity level. Second, many cells, including liver hepatocytes, which are well placed as a filter of blood, possess cell surface mannose-6-phosphate receptors capable of mediating endocytotic uptake of lysosomal enzymes (von Figura and Hasilik, 1986). Therefore, lysosomal enzymes released as a consequence of cell wounding in vivo will display sub-optimal hydrolytic activity and will be quickly scavenged from the extracellular environment.

### Making way for lysosomes: disruption-induced cortical depolymerization

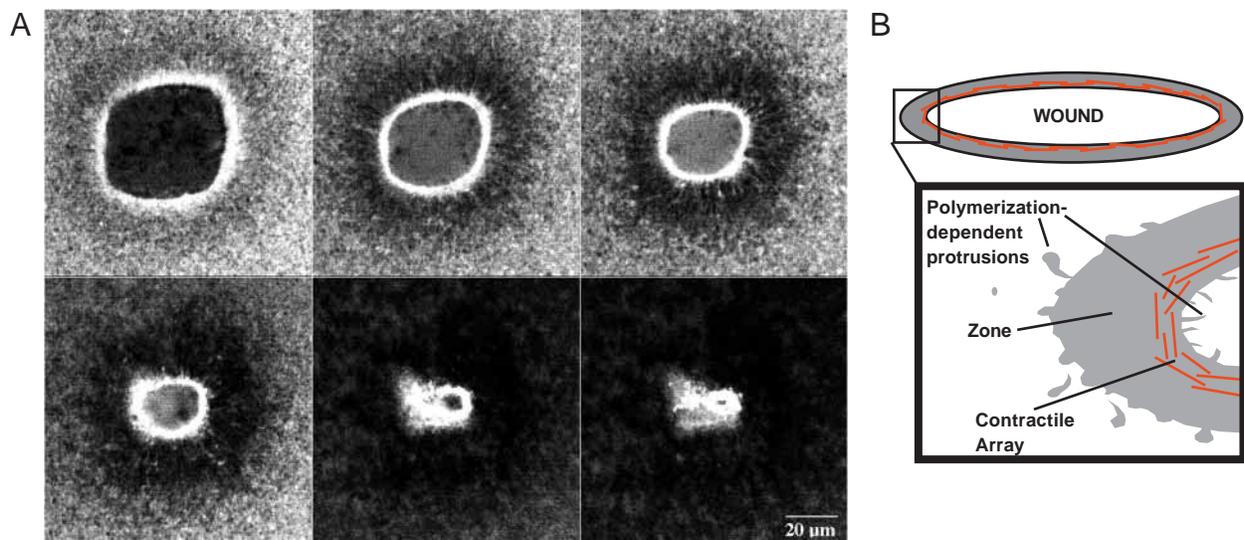
Regulated secretory vesicles are, in most cells, intimately associated with the plasma membrane prior to receipt of an exocytosis-inducing signal. Unlike these 'predocked' vesicles, lysosomes are subcortical organelles and therefore a filamentous actin (F-actin) barrier stands in the way of fusion-productive contact between lysosomes and between lysosomes and the plasma membrane. Recent work suggests that  $Ca^{2+}$ -initiated depolymerization of cortical actin is a pre-requisite for resealing (Miyake et al., 2001). Thus, in rat GM1 epithelial cells, an apparent reduction in F-actin, visualized by staining with phalloidin, was observed at disruption sites. Flow cytometric analysis of phalloidin staining of populations of wounded cells provided a quantitative confirmation that disruption reduces F-actin levels in cells and showed that the decrease is  $Ca^{2+}$ -dependent. Drug-induced stabilization, or

biologically induced enhancement, of the cortical F-actin cytoskeleton severely decreases resealing. By contrast, actin depolymerization, induced by DNase 1, enhances resealing. Dissolution of a cortical filamentous actin barrier standing in the way of lysosome-lysosome and lysosome-plasma membrane contact must therefore be a crucial early step in the resealing mechanism (Fig. 1).

Earlier studies suggested that the cytoskeleton is an aid, as well as an obstacle, to resealing. Antibodies to kinesin and a general inhibitor of myosins, both inhibited resealing (Bi et al., 1997; Steinhardt et al., 1994). One possible role for these motors might be to move vesicles into the disruption site and hence to promote contacts leading to the vesicle-vesicle and exocytotic fusion events required for resealing. The remarkable concentration of vesicles observed by electron microscopy to occupy cortical disruption sites in endothelial cells (Miyake and McNeil, 1995) is consistent with this conjecture, but, so far, the disruption-induced vesicular movements that might be powered by kinesin and/or myosin have not been directly observable.

### Continuing repairs and defensive preparations

Resealing prevents disaster (e.g. cell death), but restoration of full cell function might require additional, follow-up repairs. For example, a membrane-disrupting force penetrating deeply into the cell will necessarily damage the cortical cytoskeleton as well. Even in the absence of such direct damage, the F-actin depolymerization response consequent to disruption (see above) is predicted to disrupt normal cytoskeletal structure locally. Recent work has suggested how repair of such damage is accomplished (Bement et al., 1999; Mandato and Bement, 2001). The cortex underlying a laser wound to a *Xenopus* oocyte is initially ( $t=5-10$  minutes, for a 50  $\mu\text{m}$  diameter wound) depleted of its normal meshwork of F-actin and



**Fig. 3.** (A) Post sealing repair of wound-related damage to the cortical F-actin network. A *Xenopus* oocyte injected with fluorescent actin was wounded (circular profile) with a laser and then imaged at intervals of 3 minutes. An intensely labeled zone of F-actin assembly is seen around the wound site in the first panel and, in subsequent panels, is seen to contract, purse-string-style, restoring continuity to the cortical actin network. (B) Experimental analysis of the zone of actin polymerization indicates that it contains at its interface with the wound site a 'contractile array' consisting of F-actin and myosin 2. Finger-like, polymerization-dependent protrusions of F-actin extend into the wound site and may facilitate final closure. (Micrographs and drawing courtesy of C. Mandato and W. Bement, University of Wisconsin.)

associated myosin 2. Whether this F-actin depletion (henceforth referred to as the cortical wound) is caused by the laser injury or by the disruption-induced depolymerization response just described above is not known but remains an interesting question. In any case, repair of the cortical wound is initiated by assembly of an F-actin and myosin 2 network in a zone bordering the wound site. A narrow ring, consisting of concentrated F-actin and myosin 2, appears within this assembly zone, and this ring is demonstrably contractile (Fig. 3). This contraction, which occurs in a purse-string fashion, is required for closure of the cortical wound. The final stage of closure is associated with the formation of 'fingers' of F-actin along the sides of the now closely apposed wound borders. Similar actin structures are formed by separate cells contacting each other during dorsal closure in *Drosophila*, another of numerous remarkable parallels between tissue (multicellular repair of tissue defects) and individual cell (membrane disruption) wound healing (Woolley and Martin, 2000). Apparent contacts between these opposing fingers are observed, and their contraction might power final closure of the cortical wound.

After resealing a disruption, and repairing associated cortical damage, can a cell then prepare itself to better withstand future injury? Fibroblasts reseal a second plasma membrane disruption more rapidly than a first made ten minutes earlier (Togo et al., 1999). Drug inhibitor and activator experiments suggest that this 'facilitated resealing' is dependent on enhanced protein kinase C activity and enhanced vesicle production by the Golgi apparatus. Such an enhancement of vesicle production might target lysosomes, which are, of course, supplied with membrane (and enzymes) by Golgi-derived shuttle vesicles. An increase in the size and/or number of lysosomes should enhance resealing (see above) and might, therefore, constitute the mechanistic basis of the facilitated resealing response.

### Future challenges

A convincing hypothesis for explaining how cells reseal large tears in their surface can now be outlined, but many questions remain, especially at the molecular level.  $\text{Ca}^{2+}$  entering through a disruption is the likely trigger of the vesicle-vesicle and vesicle-plasma membrane fusion events that result in the construction of a vesicular patch and its annealing to the disruption margins. Recent work clearly identifies synaptotagmin as a key player in the resealing response and suggests that this protein, which is known to have two  $\text{Ca}^{2+}$ -binding domains, is an important  $\text{Ca}^{2+}$ -sensing component of the fusion response. This hypothesized  $\text{Ca}^{2+}$ -sensing role, which could greatly strengthen the general notion that synaptotagmin has a  $\text{Ca}^{2+}$ -sensing function in membrane fusion, obviously warrants further experimentation. And what is the identity of the remaining components of the fusion machinery? Proteins of the SNARE family are implicated in the exocytotic events of resealing, but their involvement in the vesicle-vesicle fusion step of resealing has not been confirmed. Moreover, the in vitro fusion reaction has some unorthodox properties that suggest that its molecular elucidation could yield some surprises: unlike other homotypic fusion systems, reconstituted in vitro, yolk granule fusion does not require cytosol, ATP or GTP, and it is, moreover, extremely rapid (on

a timescale of seconds rather than many minutes). The molecular basis of the actin depolymerization response also remains unknown. Finally, it is now clear that the response to plasma membrane disruption is a complex and dynamic one. Additional interesting adaptations to disruption surely remain to be discovered. Does, for example, the wounded cell switch on, in addition to the cortical-repair response, other 'clean-up' activities? Are there longer-term changes in wounded cell behavior, dependent perhaps on changed patterns of gene expression, that constitute an adaptive response to mechanical stress? Our recently rekindled interest in how cells cope with a life threatening but normal and perhaps, in the life of many cells, inevitable injury promises answers to these and many more unforeseen questions arising in this rapidly emerging field of cell biology.

### References

- Armant, D. R., Carson, D. D., Decker, G. L., Welply, J. K. and Lennarz, W. J. (1986). Characterization of yolk platelets isolated from developing embryos of *Arbacia punctulata*. *Dev. Biol.* **113**, 342-355.
- Bakker, A. C., Webster, P., Jacob, W. A. and Andrews, N. W. (1997). Homotypic fusion between aggregated lysosomes triggered by elevated  $[\text{Ca}^{2+}]_i$  in fibroblasts. *J. Cell Sci.* **110**, 2227-2238.
- Bement, W. M., Mandato, C. A. and Kirsch, M. N. (1999). Wound-induced assembly and closure of an actomyosin purse string in *Xenopus* oocytes. *Curr. Biol.* **9**, 579-587.
- Bi, G.-Q., Alderton, J. M. and Steinhardt, R. A. (1995). Calcium-regulated exocytosis is required for cell membrane resealing. *J. Cell Biol.* **131**, 1747-1758.
- Bi, G. Q., Morris, R. L., Liao, G., Alderton, J. M., Scholey, J. M. and Steinhardt, R. A. (1997). Kinesin- and myosin-driven steps of vesicle recruitment for  $\text{Ca}^{2+}$ -regulated exocytosis. *J. Cell Biol.* **138**, 999-1008.
- Chambers, R. and Chambers, E. L. (1961). Explorations into the nature of the living cell. Cambridge, Massachusetts: Harvard University Press.
- Chernomordik, L. V., Melikyan, G. B. and Chizmadzhev, Y. A. (1987). Biomembrane fusion: a new concept derived from model studies using two interacting planar lipid bilayers. *Biochim. Biophys. Acta* **906**, 309-352.
- Clarke, M. F. C., Khakee, R. and McNeil, P. L. (1993). Loss of cytoplasmic basic fibroblast growth factor from physiologically wounded myofibers of normal and dystrophic muscle. *J. Cell Sci.* **106**, 121-133.
- Clarke, M. S., Caldwell, R. W., Chiao, H., Miyake, K. and McNeil, P. L. (1995). Contraction-induced cell wounding and release of fibroblast growth factor in heart. *Circ. Res.* **76**, 927-934.
- Clarke, M. S. F. and McNeil, P. L. (1994). Syringe loading: a method for inserting macromolecules into cells in suspension. In *Cell Biology: A Laboratory Handbook.*, vol. 3 (ed. J. E. Celis), pp. 30-36. San Diego, CA: Academic Press.
- Detrait, E., Eddleman, C. S., Yoo, S., Fukuda, M., Nguyen, M. P., Bittner, G. D. and Fishman, H. M. (2000a). Axolemmal repair requires proteins that mediate synaptic vesicle fusion. *J. Neurobiol.* **44**, 382-391.
- Detrait, E. R., Yoo, S., Eddleman, C. S., Fukuda, M., Bittner, G. D. and Fishman, H. M. (2000b). Plasmalemmal repair of severed neurites of PC12 cells requires  $\text{Ca}^{2+}$  and synaptotagmin. *J. Neurosci. Res.* **62**, 566-573.
- Geppert, M. and Sudhof, T. C. (1998). RAB3 and synaptotagmin: the Yin and Yang of synaptic membrane fusion. *Annu. Rev. Neurosci.* **21**, 75-95.
- Granger, B. L., Green, S. A., Gabel, C. A., Howe, C. L., Mellman, I. and Helenius, A. (1990). Characterization and cloning of lgp110, a lysosomal membrane glycoprotein from mouse and rat cells. *J. Biol. Chem.* **265**, 12036-12043.
- Heilbrunn, L. V. (1930). The surface precipitation reaction of living cells. *Proc. Am. Philos. Soc.* **LXIX**, 295-301.
- Heilbrunn, L. V. (1956). Dynamics of Living Protoplasm. New York: Academic Press.
- Mandato, C. A. and Bement, W. M. (2001). Contraction and polymerization cooperate to assemble and close actomyosin rings around *Xenopus* oocyte wounds. *J. Cell Biol.* **154**, 785-797.
- Martinez, I., Chakrabarti, S., Hellevik, T., Morehead, J., Fowler, K. and Andrews, N. W. (2000). Synaptotagmin VII regulates  $\text{Ca}^{2+}$ -dependent exocytosis of lysosomes in fibroblasts. *J. Cell Biol.* **148**, 1141-1149.

- Mayorga, L. S., Beron, W., Sarrouf, M. N., Colombo, M. I., Creutz, C. and Stahl, P. D.** (1994). Calcium-dependent fusion among endosomes. *J. Biol. Chem.* **269**, 30927-30934.
- McNeil, P. L.** (1993). Cellular and molecular adaptations to injurious mechanical force. *Trends Cell Biol.* **3**, 302-307.
- McNeil, P. L. and Baker, M. M.** (2001). Cell surface events during resealing visualized by scanning electron microscopy. *Cell Tissue Res.* **304**, 141-146.
- McNeil, P. L. and Khakee, R.** (1992). Disruptions of muscle fiber plasma membranes. Role in exercise-induced damage. *Am. J. Pathol.* **140**, 1097-1109.
- McNeil, P. L. and Terasaki, M.** (2001). Coping with the inevitable: how cells repair a torn surface membrane. *Nat. Cell Biol.* **3**, E124-E129.
- McNeil, P. L., Vogel, S. S., Miyake, K. and Terasaki, M.** (2000). Patching plasma membrane disruptions with cytoplasmic membrane. *J. Cell Sci.* **113**, 1891-1902.
- Miyake, K. and McNeil, P. L.** (1995). Vesicle accumulation and exocytosis at sites of plasma membrane disruption. *J. Cell Biol.* **131**, 1737-1745.
- Miyake, K., McNeil, P. L., Suzuki, K., Tsunoda, R. and Sugai, N.** (2001). An actin barrier to resealing. *J. Cell Sci.* **114**, 3487-3494.
- Parsegian, V. A., Rand, R. P. and Gingell, D.** (1984). Lessons for the study of membrane fusion from membrane interactions in phospholipid systems. *Ciba Found. Symp.* **103**, 9-27.
- Raikhel, A. S.** (1987). The cell biology of mosquito vitellogenesis. *Mem. Inst. Oswaldo Cruz.* **82** Suppl. 3, 93-101.
- Rappaport, R.** (1976). Furrowing in altered cell surfaces. *J. Exp. Zool.* **195**, 271-278.
- Raucher, D. and Sheetz, M. P.** (1999). Characteristics of a membrane reservoir buffering membrane tension. *Biophys. J.* **77**, 1992-2002.
- Reddy, A., Caler, E. V. and Andrews, N. W.** (2001). Plasma membrane repair is mediated by Ca<sup>2+</sup>-regulated exocytosis of lysosomes. *Cell* **106**, 157-169.
- Rodriguez, A., Webster, P., Ortego, J. and Andrews, N. W.** (1997). Lysosomes behave as Ca<sup>2+</sup>-regulated exocytotic vesicles in fibroblasts and epithelial cells. *J. Cell Biol.* **137**, 93-104.
- Schiavo, G., Benfenati, F., Poulain, B., Rossetto, O., Polverino de Laureto, P., DasGupta, B. R. and Montecucco, C.** (1992). Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. *Nature* **359**, 832-835.
- Steinhardt, R. A., Bi, G. and Alderton, J. M.** (1994). Cell membrane resealing by a vesicular mechanism similar to neurotransmitter release. *Science* **263**, 390-393.
- Terasaki, M., Miyake, K. and McNeil, P. L.** (1997). Large plasma membrane disruptions are rapidly resealed by Ca<sup>2+</sup>-dependent vesicle-vesicle fusion events. *J. Cell Biol.* **139**, 63-74.
- Togo, T., Alderton, J. M., Bi, G. Q. and Steinhardt, R. A.** (1999). The mechanism of facilitated cell membrane resealing. *J. Cell Sci.* **112**, 719-731.
- Togo, T., Krasieva, T. B. and Steinhardt, R. A.** (2000). A decrease in membrane tension precedes successful cell-membrane repair. *Mol. Biol. Cell* **11**, 4339-4346.
- von Figura, K. and Hasilik, A.** (1986). Lysosomal enzymes and their receptors. *Annu. Rev. Biochem.* **55**, 167-193.
- Wallace, R. A., Opresko, L., Wiley, H. S. and Selman, K.** (1983). The oocyte as an endocytic cell. *Ciba Found. Symp.* **98**, 228-248.
- Woolley, K. and Martin, P.** (2000). Conserved mechanisms of repair: from damaged single cells to wounds in multicellular tissues. *BioEssays* **22**, 911-919.