OPINION

# An emergency response team for membrane repair

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Abstract | On demand, rapid $Ca^{2+}$-triggered homotypic and exocytic membrane-fusion events are required to repair a torn plasma membrane, and we propose that this emergency-based fusion differs fundamentally from other rapid, triggered fusion reactions. Emergency fusion might use a specialized protein and organelle emergency response team that can simultaneously promote impromptu homotypic fusion events between organelles and exocytic fusion events along the vertices between these fusion products and the plasma membrane.

Reseal or die. Plasma-membrane disruption is a normal event in the life of many cells — for example, mammalian skeletal[1] and cardiac muscle[2] cells, which reside in mechanically active environments where the disruption frequency is directly dependent on the level of physical activity. Another striking example of membrane disruption under physiological conditions is found in the milk-secreting epithelial cells of the mammary gland[3]. During apocrine secretory events, cytoplasmic constituents (including lipid droplets, organelles and cytosolic proteins) and plasma-membrane fragments are released into the secretory lumen.

Resealing is the membrane-repair process that allows cells to survive disruption, preventing the loss of irreplaceable cell types (such as cardiac myocytes or neurons) or eliminating the cost of replacing large and/or frequently injured cell types (such as skeletal muscle cells or mammary gland epithelial cells). Failure to reseal and consequent $Ca^{2+}$ entry[4] leads to rapid cell death (which can occur within seconds). Therefore, resealing is a necessary rapid emergency response. Resealing requires extracellular $Ca^{2+}$ (REF. 5) (FIG. 1; see Supplementary information S1,S2 (movies)), and has been temporally, spatially and functionally associated with rapid homotypic and heterotypic membrane fusion events, which we focus on here.

As discussed later, $Ca^{2+}$ influx at the site of plasma-membrane disruption — whether this disruption is the result of physical force or the pore-forming protein of a bacterial pathogen — results in the rapid fusion of lysosomes with the plasma membrane. These observations have led to the proposal that lysosomes and their associated protein components represent the key machinery of repair. Below,

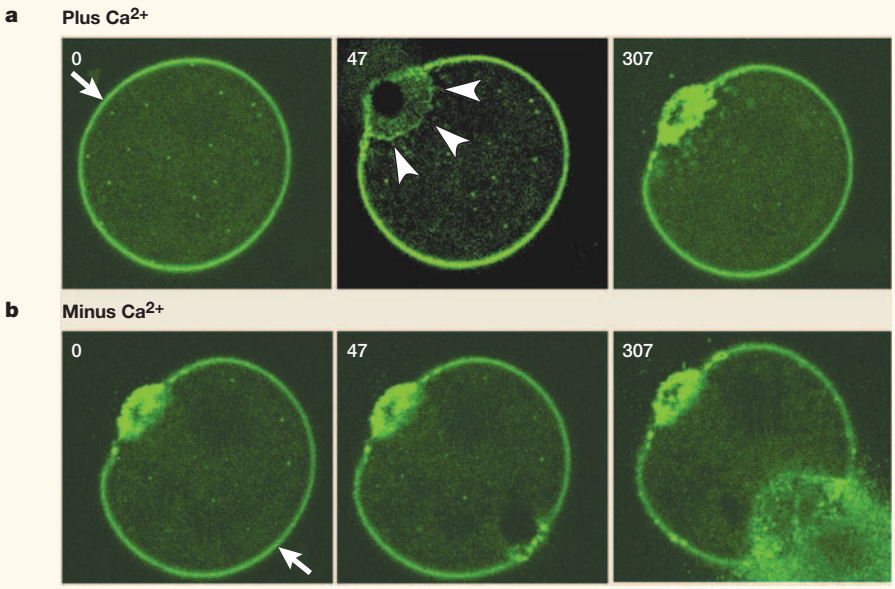



Figure 1 | **The $Ca^{2+}$ dependence and dynamics of barrier formation at sites of membrane disruption in the sea-urchin egg.** A sea-urchin egg was immersed in FM1-43, which is a water-soluble, membrane-impermeant dye that becomes highly fluorescent when it partitions into membranes. The panels show the fluorescent signal that was confocally imaged from the egg before (labelled 0) and 47 and 307 seconds after creating two ~10-μm plasma-membrane disruption sites using a laser beam. **a** | The first disruption site (highlighted by an arrow in the left panel) was made with the egg immersed in artificial sea water that contained ~10 mM $Ca^{2+}$. **b** | The second disruption (highlighted by an arrow in the left panel) was made in the presence of sea water containing no added $Ca^{2+}$. In the presence of $Ca^{2+}$, a membrane barrier stained with FM1-43 forms (see arrowheads). At later time points, membrane that is external to this boundary (internal- and plasma-membrane remnants) continued to take up FM1-43 and became highly fluorescent. By contrast, in the absence of $Ca^{2+}$, no barrier formed and the cytoplasm, which contained fluorescently labelled membrane-bound organelles, spilled out of the egg (see right panel, **b**). For further details, see Supplementary information S1,S2 (movies).

we present arguments that support this idea, but also arguments that support the contrary view, in which a specialized team of protein components and intracellular membranes that are distinct from the lysosomal compartment are required for membrane repair.

### Self-sealing?

There is a myth that biological membranes are natural 'self-sealing' entities that can repair a lipid-bilayer discontinuity without assistance. This myth originates from the observation that liposomes reseal rapidly and that red blood cells (RBCs), which lack internal organelles, can also reseal, although only under conditions of very low extracellular $Ca^{2+}$ (REF. 6). In such cases, resealing is probably driven by the unstable increase in free energy that is produced by the disordering of the acyl chains of lipid molecules that are present at the disruption edge (FIG. 2a). Indeed, nucleated cells can reseal small disruptions (< 1 μm; for example, those produced by electroporation) in the absence of $Ca^{2+}$.

However, RBCs do not self-seal under physiological conditions. When their membrane is disrupted by laser irradiation or hypo-osmotic shock in the presence of 1.5 mM extracellular $Ca^{2+}$, unrestricted influx and efflux of a membrane-impermeant dye can be recorded for up to 30 minutes afterwards[7]. Electron microscopy reveals a catastrophic destruction of the cortical cytoskeleton in these RBCs. Other studies indicate that elevated cytosolic $Ca^{2+}$ (ionophore-induced) causes considerable structural changes in the phospholipid bilayer, as well as the cytoskeletal structure, and that, ultimately, if $Ca^{2+}$ levels rise sufficiently high, RBC lysis results[8]. Poorly understood complexities of the plasma membrane and its associated cytoskeleton, as well as the many pathological consequences of $Ca^{2+}$ entry into a cell (even one as relatively simple as an RBC), probably prevent the thermodynamically driven resealing of biological membranes. $Ca^{2+}$ can be seen as a double-edged sword — lethal to a cell if too much enters, but essential as the trigger for resealing and for prohibiting excessive $Ca^{2+}$ entry. So, although self-sealing is certainly applicable to an artificial membrane, for example, that of a liposome or of an RBC under the unusual condition of very low extracellular $Ca^{2+}$, it is not a capacity that is displayed by RBCs under physiological conditions, and is therefore probably not a mechanism of biological significance.

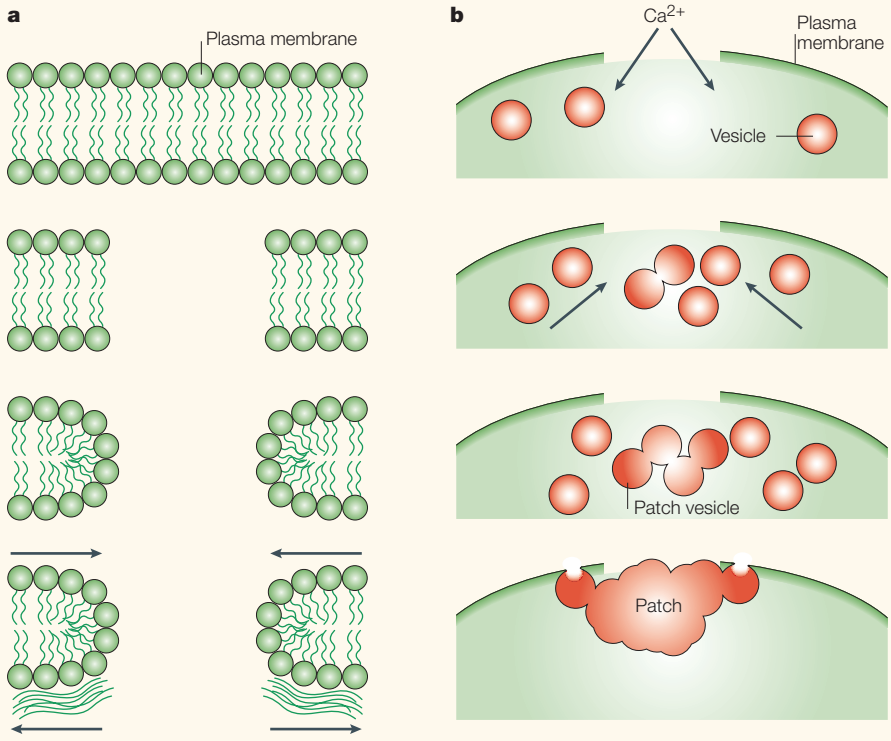



Figure 2 | **Two possible resealing mechanisms. a** | When an intact plasma membrane (top panel) is disrupted, hydrophobic domains of the constituent phospholipids are exposed to water (upper middle panel), which generates a large increase in free energy. This rapidly drives lipid reorientation at the free edge of the disruption, which substantially reduces the net free energy. However, lipid disorder is now present along the curved edge of the disruption site, which provides leftover free energy (line tension) that favours resealing (lower middle panel). In cellular plasma membranes, the adhesion of phospholipid headgroups to the underlying cytoskeleton creates a membrane tension that opposes lipid flow, at least over a disruption site that lacks this cytoskeleton (bottom panel). One model proposes that an exocytic response that is initiated by $Ca^{2+}$ reduces membrane tension sufficiently to allow line tension to dominate — that is, it facilitates the self-sealing capacity of membranes. **b** | An alternative model, which is applicable to large disruptions, is the patch hypothesis. $Ca^{2+}$ enters through a disruption site (top panel), and $Ca^{2+}$-triggered vesicle–vesicle homotypic fusion begins (upper middle panel). Vesicles continue to fuse homotypically to form a large patch vesicle (lower middle panel), and the completion of repair occurs as a result of an exocytic fusion event between the patch and the plasma membrane (bottom panel). Figure modified with permission from REF. 47 © (2003) Annual Reviews.

### Facilitated resealing

Although plasma membranes are not capable of unassisted repair under physiological conditions, it has been proposed[9] that exocytic events can 'facilitate' the self-sealing potential that is inherent in the energetically unfavourable condition of local lipid disorder. As shown in FIG. 2a, an adhesive force is measurable between the phospholipids of the plasma membrane and its underlying cytoskeleton[10], and this 'membrane tension' is predicted to oppose the forces that arise from lipid disorder. Therefore, if a cell could reduce its membrane tension, it could facilitate lipid-disorder-driven resealing. The resealing of a liposome can be slowed or stopped if a tensile force is imposed experimentally on its bilayer, which highlights the potential importance of this parameter[11]. More importantly, studies in fibroblasts have shown that a disruption injury reduces its membrane tension, that this reduction correlates temporally with the exocytic events induced by the disruption, and that the disruption-induced reductions in membrane tension can be blocked by treatments that inhibit exocytosis[9]. Furthermore, agents that measurably reduce membrane tension, such as certain surfactants, increase resealing efficiency[9,12]. How exactly exocytosis leads to a decrease in membrane tension remains unexplained. Moreover, this mechanism cannot explain the repair of large disruptions, for which large segments of missing plasma membrane must be replaced.

### Patching

The cytoplasm of the echinoderm egg can, by itself, form a barrier to the outside world: when sea water (containing physiological $Ca^{2+}$) is microinjected into the egg, it is sequestered behind a membrane barrier as it

leaves the microneedle tip (this sequestration occurs on the sub-second to second timescale)[13]. The homotypic fusion of internal membranes that is triggered by the $Ca^{2+}$ being ejected from the microneedle is the only mechanism that could explain this observation. The yolk granule is the organelle involved in this process, as physically depleting the cytoplasm of this organelle leaves the egg incapable of this sequestration.

Based on these experiments, it was proposed that $Ca^{2+}$ entry into the cytoplasm at a disruption site rapidly triggers homotypic membrane-fusion events, which form a disruption-spanning 'patch' vesicle (FIG. 2b). The annealing of this patch vesicle to the plasma membrane by exocytic events completes repair — that is, it restores plasma-membrane continuity. Several fundamental predictions of this 'patch' hypothesis have been confirmed[14–16]. First, a greatly enlarged vesicle population is present in the region of the cytoplasm that borders a disruption site, both in echinoderm eggs and mammalian cells. Second, the membrane that initially covers a disruption site is derived from an internal source. Third, internal membranes are required for resealing. Finally, the organelle that is required for resealing in the egg — the yolk granule — can undergo rapid $Ca^{2+}$-triggered homotypic fusion *in vitro*.

One challenge has been to understand exactly how, in three dimensions, the exocytic fusion of the disruption-spanning patch vesicle with the plasma membrane proceeds. The exocytic creation of a single, radially expanding fusion pore near the disruption site, which is the standard way of viewing an exocytic event, does not suffice. Such an event, or even several such events, would merely add extra membrane to the disrupted plasma membrane. Membrane continuity would not be restored.

However, we believe that a recently described mechanism, 'vertex fusion'[17,18], might solve this puzzle. According to this model, which was developed to explain vacuole fusion that was imaged using microscopy techniques in *Saccharomyces cerevisiae*, fusion between two membranes can occur at 'vertices'. These vertices are the curvilinear zones of outermost contact between two docked membranes along which the protein machinery of fusion becomes concentrated. Such fusion is not confined to a central, radially expanding pore, but can expand circumferentially along vertices, 'cutting out' a membrane remnant. In FIG. 3, we apply this model to the fusion between a patch vesicle and the plasma membrane that surrounds a disruption site. In the case of considerable disruption of the plasma membrane of a sea-urchin egg, this model can explain the characteristic concave (crater-like) architecture of the repaired surface barrier (FIG. 1). Other predictions of this hypothesis, such as the alignment of fusion-machinery components along the predicted vertices and the presence of organelle membrane extracellular to the boundary of the reformed plasma-membrane, remain to be tested. It will also be interesting to determine whether the process of vertex fusion is used by organelles other than the yeast vacuole and, if so, whether the proteins involved have been conserved throughout evolution.

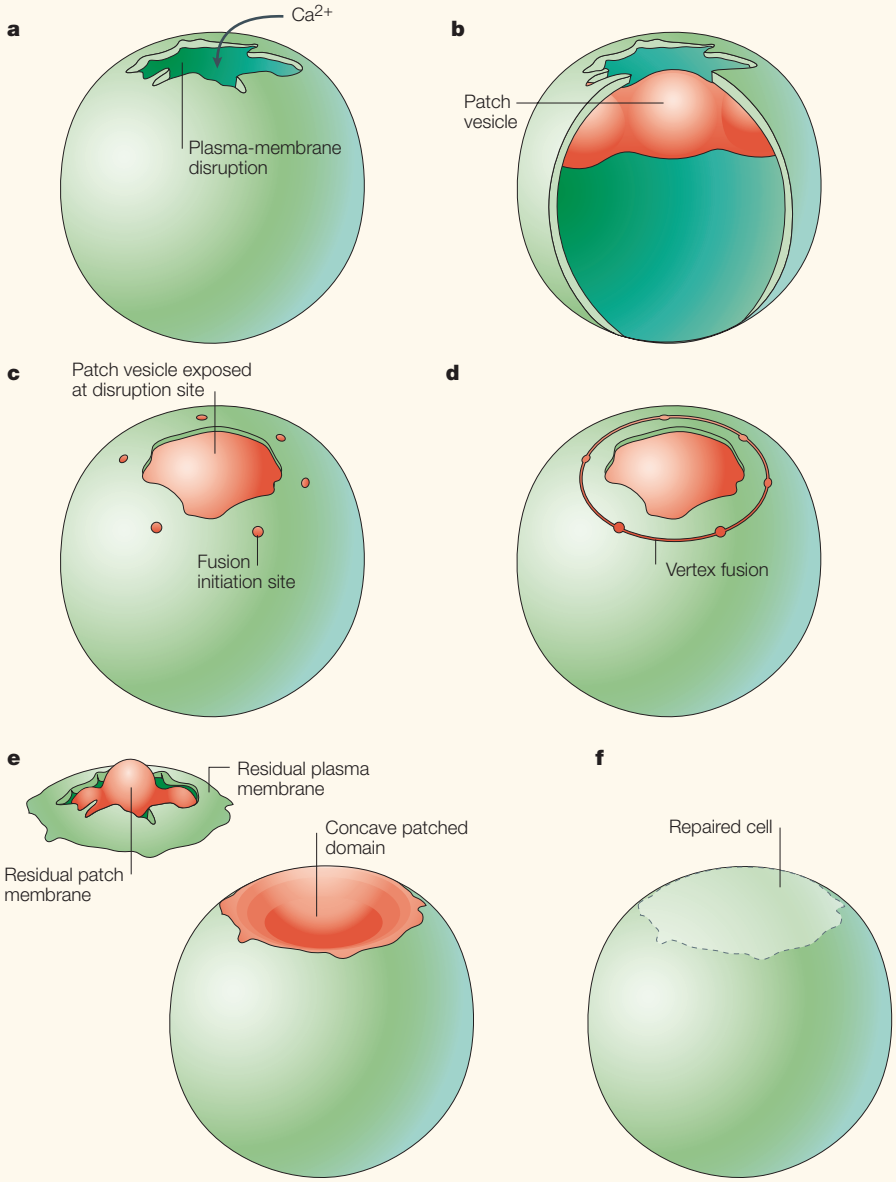


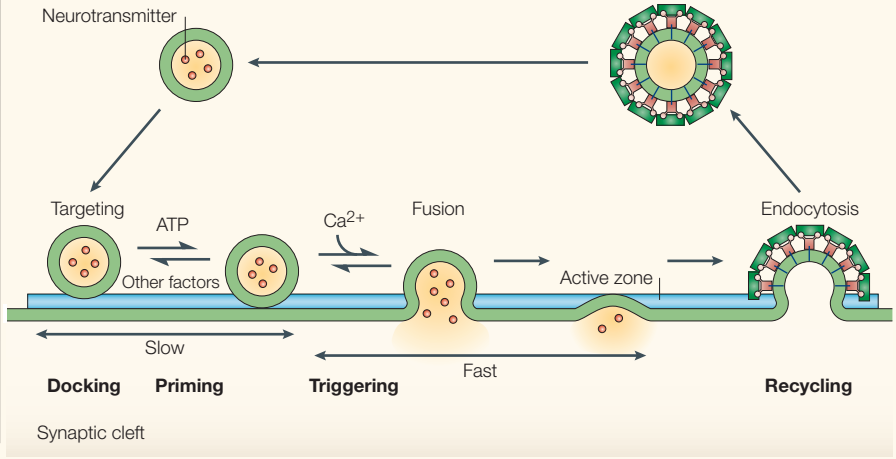

Figure 3 | **'Vertex fusion' and plasma-membrane restoration.** This figure shows 'vertex fusion' as a model to explain how exocytic fusion of a large patch vesicle with the plasma membrane can restore a continuous plasma-membrane barrier. **a** | $Ca^{2+}$ enters through a plasma-membrane disruption site. **b** | Homotypic vesicle fusion creates a patch vesicle. **c** | Several points of fusion are initiated between the patch vesicle and the plasma membrane, which results in several fusion pores. **d** | The lateral expansion of these fusion pores along membrane vertices produces a continuous union of patch and plasma membrane. **e** | The residual plasma membrane and patch membrane are discarded to the extracellular space (the disruption site is resealed using the lower portion of the patch vesicle). Initially, the patched domain of the plasma membrane is concave, which reflects the geometry of the lower portion of the patch vesicle from which it is derived. **f** | Cytoskeletal remodelling restores the normal surface architecture[48].

## Lysosomes — guilt by association?

In mammalian cells, what is the source of the intracellular membranes that are required to close a tear following wounding?

Box 1 | **$Ca^{2+}$-triggered fusion at the synapse: pre-arranging rapidity**

**Before fast, $Ca^{2+}$-triggered fusion can occur at the synapse, synaptic vesicles must first be 'docked' and 'primed', which are relatively slow events that occur at the synaptic cleft (see figure). Docking is mediated by extremely large protein 'tethers', and candidates for such tethers are Bassoon and Piccolo/Aczoronin. The synaptic vesicle is therefore captured and restrained precisely at the site of future exocytic fusion. Priming involves, first, the dissociation of *cis*-SNARE (*N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptor) pairs that were formed in a previous round of fusion, freeing them for the next round. In the synapse, these SNAREs are synaptobrevin/vesicle-associated membrane protein (VAMP) on the vesicle membrane, and SNAP25 (synaptosome-associated protein of 25 kDa) and syntaxin on the plasma membrane. This ATP-dependent dissociation event is catalysed by NSF partnered with a soluble-NSF-attachment protein. *Trans*-SNARE pairing can then take place between vesicle-membrane and plasma-membrane SNAREs. A 'zipping up', which is promoted by conformational changes in the paired SNAREs, then brings the two interacting membranes into closer physical proximity. Finally, $Ca^{2+}$ entry can trigger rapid exocytic fusion (see figure). Synaptotagmin might regulate fusion-pore opening by interacting with SNAREs in a $Ca^{2+}$-dependent manner[42]. In this process, fusion is incomplete — a single fusion pore is open for a time interval that is sufficient only for content release. Rapid budding/endocytosis, which is initiated at the fusion site, ensures that the protein and phospholipid components are efficiently retrieved for re-use. Figure modified with permission from *Nature Reviews Molecular Cell Biology* REF. 43 © (2002) Macmillan Magazines Ltd.**




While studying cell invasion by *Trypanosoma cruzi*, Andrews and her group carried out a series of elegant experiments, which showed the migration of lysosomes to the plasma membrane and their subsequent fusion with the region of the plasma membrane that surrounds the invading parasite[19]. Following these pioneering studies, it was recognized that a transient increase in intracellular $Ca^{2+}$ was required for this process, and that synaptotagmin VII — a membrane protein that is associated with lysosomes and is considered a '$Ca^{2+}$ sensor' — was essential for the targeted fusion of lysosomes[20]. Subsequently, it was shown that functional interference with synaptotagmin VII through the microinjection of antibodies not only prevented the process of lysosome fusion, but also impaired the process of membrane repair following the tearing of the cell surface using glass beads[21].

A simple set of predictions based on these observations is that the functional removal of synaptotagmin VII by gene knockout should result in a considerable defect in *T. cruzi* invasion and in cell-membrane repair, whether at the single-cell or whole-organism level. The former prediction was found to be correct, as embryonic fibroblasts isolated from synaptotagmin-VII-deficient mice were less susceptible to invasion[22]. However, the accuracy of the latter prediction is debatable. Under tissue-culture conditions, the wounding of synaptotagmin-VII-defective cells enhanced the appearance of a cytosolic marker in the media surrounding cells that had been subjected to mechanical stretching[22]. Whether this increased leakage is related to resealing or not remains unknown. Adding to the confusion, $Ca^{2+}$-triggered lysosome exocytosis not only occurs in these cells, but the process of pore formation and fusion between lysosomes and the plasma membrane is significantly accelerated[23]. It is possible that the cells from mice deficient in synaptotagmin VII responded by upregulating alternative protective mechanisms to ensure membrane repair. However, although plausible, this idea does not seem warranted given recent results, which show the normal repair of the plasma membrane of cells expressing synaptotagmin VII that were wounded in the presence of vacuolin — a newly discovered small organic molecule that blocks the wounding- and $Ca^{2+}$-triggered fusion of lysosomes with the plasma membrane[24].

## Alternative candidate repair organelles

With possibly two exceptions, there are no obvious candidates that correspond to intracellular vesicular compartments that can realistically be considered the source of sufficient membrane to ensure resealing in mammalian cells. There is no evidence to support the abrupt deposition of membranes that originate from secretory granules, the Golgi apparatus or the endoplasmic reticulum, which are all compartments that are involved in constitutive or regulated traffic along the secretory pathway. The fusion of bona fide endosomes, which were labelled by a brief pulsing of fibroblasts with a general marker of endocytosis (FM1-43 dye), has been observed to be induced by plasma-membrane disruption (R. Steinhardt, personal communication). However, to date, enlargeosomes are the only candidate organelle with the potential to provide the missing role. They were identified in a particular clone of PC12 cells that were defective in lysosomal exocytosis, but could reseal their plasma membrane normally after cell wounding[25]. Little is known about this intracellular compartment[26]: it seems to be ubiquitous, it fuses with the plasma membrane in response to an abrupt increase in cytosolic $Ca^{2+}$ and this fusion is not blocked by pre-incubating the cells with vacuolin[24]. So far, it is not known whether enlargeosomes can undergo homotypic fusion following wounding, which is probably necessary for the repair response. Electron-microscopy images taken from any mammalian cell show a huge number of internal membrane structures, including many that cannot be accounted for. So, if enlargeosomes are not responsible for providing the extra membrane that is needed for membrane resealing, then another one of these membrane structures that still awaits identification might be the organelle responsible. It must be emphasized that work beyond the scope of this review, for example, on resealing in sea-urchin eggs, fungi and

plants, indicates that many different compartments might be involved depending on the cell type and the size of the lesion.

### Paradigmatic fusion proteins

A large body of data — biochemical and genetic — identified SNARE (soluble *N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptor)-family proteins as requisite components of the protein machinery that mediates a wide variety of membrane-fusion events, both homotypic and heterotypic in cells from *S. cerevisiae* to humans[27]. Resealing is blocked by the microinjection of bacterial toxins that inhibit SNARE function into fibroblasts and sea-urchin eggs[5]. Toxin microinjection into these cells also blocks disruption-induced exocytic events[28]. These findings were the first to indicate the importance of membrane-fusion events in resealing and, more specifically, of exocytic fusion events. Whether the toxins also function to prevent resealing by blocking homotypic fusion events remains unknown. In any case, the available data strongly indicate that resealing-based fusion uses at least one protein component that is common to other fusion reactions.

The synaptotagmins are another family of proteins that have been established as general components of the exocytic fusion machinery, particularly when the trigger is $Ca^{2+}$ (REF. 29). The inhibition of synaptotagmin VII and synaptotagmin III through the cytoplasmic introduction of function-blocking antibodies or partial peptide sequences partially inhibits membrane resealing in the squid giant axon[30] and in fibroblasts[21]. However, as outlined above, subsequent studies failed to confirm that synaptotagmin VII (and its associated organelle, the lysosome) is an essential component of the resealing-based fusion machinery. Furthermore, a recent study[31] failed to confirm earlier work[21] reporting that the C2A domain of synaptotagmin VII is inhibitory to the resealing of an initial wound, although it found that the C2B domain inhibited resealing. The role of synaptotagmins in resealing therefore remains unresolved.

### Resealing-specific protein components

Why postulate that there are protein components that are specific for membrane-resealing-based fusion events? Our argument is based on several, apparently radical, differences between resealing-based and other, better studied fusion events, and on recent discoveries that are outlined below. First among the differences is the fact that the resealing-based homotypic fusion that has been reconstituted *in vitro* occurs on a sub-second timescale and is complete within seconds, whereas the homotypic fusion that has been characterized in other systems is typically 50% complete on a minute to hour timescale[32]. Second, resealing-based fusion in the reconstituted echinoderm model does not require the participation of cytosolic proteins or ATP, unlike most other reconstituted homotypic fusion systems[15,33]. Third, external $Ca^{2+}$ triggers yolk-granule homotypic fusion, whereas most homotypic fusion systems are part of constitutive fusion pathways — that is, they are not regulated, for example, by being triggered by an exogenous messenger. Fourth, despite being rapid, the homotypic or exocytic fusion events of resealing-based fusion do not use a pre-assembled matrix of site-specific fusion-machinery components. The yolk granule, and probably its equivalent in mammalian cells, is not present in a pre-docked configuration, unlike other known rapid, regulated exocytic vesicles, such as those at synapses[34] (BOX 1). Last, the exocytic fusion events that accomplish resealing must be complete, that is, involve the full incorporation of the lipid and protein components of the patch vesicle into the plasma membrane. Most other regulated exocytic events are, by contrast, 'kiss-and-run' affairs that do not result in complete incorporation, but rather, in line with the role of such events, function primarily to release the vesicular contents rapidly[35] (BOX 1).

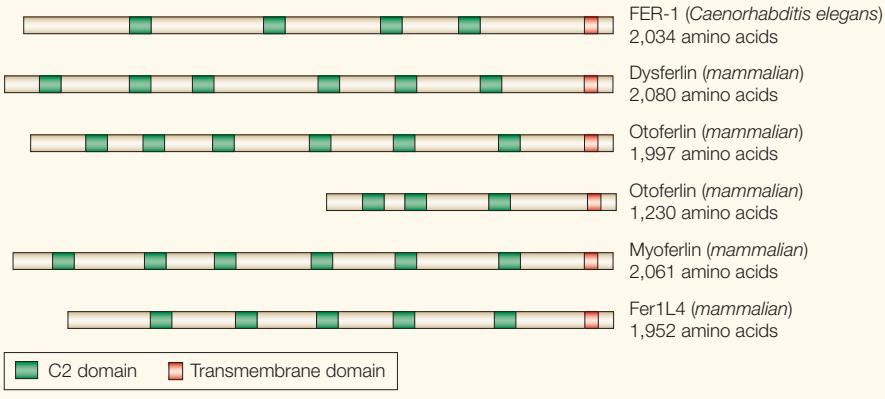
Have any protein candidates been identified as possibly fitting the role of repair-specific fusion components? The ferlins (BOX 2) are a family of transmembrane proteins with considerable homology to the synaptotagmins, which were mentioned earlier as having a possible $Ca^{2+}$-sensing role

Box 2 | **The unfurling of the ferlins**

**The discovery of a role for dysferlin in membrane resealing raises many new questions about its molecular characteristics, as well as about the ferlin-protein family as a whole. All four mammalian family members have several cytosolic C2 domains and a single-pass transmembrane domain at their C terminus[44] (see figure). The C2 domain is a common motif and is thought to be especially important in $Ca^{2+}$-triggered events[42]. $Ca^{2+}$ binding by a C2 domain neutralizes locally negatively charged aspartyl groups. This, in turn, strongly promotes C2-domain interactions with phospholipids and with other proteins. For example, following $Ca^{2+}$ binding, synaptotagmin interacts with members of the SNARE (*N*-ethylmaleimide-sensitive fusion protein (NSF) attachment protein receptor) family of proteins through its C2 domains, and might thereby trigger $Ca^{2+}$-regulated fusion at the synapse[45].**

**As discussed in the main text, mutations in dysferlin result in a form of limb-girdle muscular dystrophy in humans[36,37]. In *Caenorhabditis elegans*, mutations in another ferlin, FER-1, result in immobile spermatids[38] (please refer to the main text for further details). Another member of the mammalian ferlin family — otoferlin — is also associated with disease[46]. It is present in inner ear hair cells, which, perhaps importantly, are deformed by sound input. Mutations in otoferlin result in progressive hearing loss, which would be a predicted consequence of the failure of these crucial cell types to reseal.**

**Myoferlin, the third member of the mammalian family, is widely distributed in mammalian tissues[42]. As yet, its loss or mutation has not been shown to be associated with disease, although its levels are upregulated in dystrophin-deficient muscle from the mouse model for Duchenne muscular dystrophy. Nothing is known about the function of Fer1L4, the most recently discovered member of the mammalian family. Figure modified with permission from REF. 44 © (2004) Elsevier Science.**



in resealing. One member of the ferlin family — dysferlin — is present in cardiac and skeletal muscle, where it associates with the plasma membrane and with a yet-to-be-identified vesicular compartment. In humans, mutations in dysferlin result in a form of limb-girdle muscular dystrophy[36,37]. In *Caenorhabditis elegans*, mutations in another ferlin, FER-1, result in immobile spermatids[38]. These spermatids move in an amoeboid fashion, and evidence indicates that FER-1 functions to promote the vesicle fusion that is necessary to supply the advancing pseudopod of the spermatid with new membrane. This finding in *C. elegans*, together with the known molecular properties of dysferlin (BOX 2), indicates that dysferlin might function in the membrane-fusion events that are required for resealing. Skeletal muscle fibres are, as already mentioned, normally exposed to disruption-producing levels of mechanical stress, and therefore must have a robust resealing capacity. If this were lacking, muscle cell death and muscle wasting would be predicted to ensue. Indeed, *dysferlin*-null mice show a severe resealing deficiency and develop a pronounced limb-girdle muscular dystrophy[39]. Moreover, dysferlin becomes concentrated in normal muscle cells at plasma-membrane sites of repair, as is predicted by the patch hypothesis[39]. Although dysferlin might have other as-yet-unknown roles, none are known in other membrane-fusion events. We propose that its primary role might therefore be as part of the emergency response team that mediates resealing-based fusion events.

The annexins A1 and A2, similar to dysferlin, are $Ca^{2+}$-activated phospholipid-binding proteins that have long been considered potential fusogens and that can promote the fusion of liposomes[40]. However, despite considerable effort, their role in promoting the fusion of biological membranes has remained controversial. Recent work showed that, in addition to dysferlin, annexins A1 and A2 are concentrated at sites of muscle-cell plasma-membrane disruption[41]. Functional evidence for the involvement of these annexins will be needed before their role in resealing, which is indicated by this spatial coincidence, can be accepted.

Finally, prior to $Ca^{2+}$-triggered fusion, yolk granules in the echinoderm egg become reversibly tethered to one another to form large aggregates of more than 100 granules *in vitro* (A. McNeil and P.L.M., unpublished observations). Washing the yolk granules with mildly chaotropic salt abolished this tethering reaction and prevented the $Ca^{2+}$-triggered formation of the large fusion products that are characteristic of tethered granules. Protein factors that were present in the eluted fraction could be substantially enriched by anion-exchange chromatography. Under native conditions, the enriched fraction behaved as a high molecular weight (> 650 kDa), multisubunit complex of at least seven proteins. Monoclonal antibodies that were directed against this protein complex could immunodeplete the tethering activity, which confirms the role of the complex in granule tethering. These antibodies selectively stained the surface of yolk granules in the intact egg. So, these results indicate a new role for tethering — it can promote the formation of large vesicular fusion products, such as those required for successful resealing. These resealing tethers, as with tethers in other systems, might be specific for this single function.

## Implications and future directions

If we are correct in suggesting that novel protein components and even organelles are required for resealing, then the identification of these components will require approaches that go beyond testing candidates that have been derived, by analogy, from the study of other fusion events. As the history of the fusion field has shown, the analysis of human diseases, *in vitro* reconstitution that permits the biochemical purification of the components and genetic screening are all possible routes that will aid future discoveries in this area.

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Competing interests statement
The authors declare no competing financial interests.

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OPINION

# Autophagy: dual roles in life and death?

*Eric H. Baehrecke*

Abstract | Autophagy is an evolutionarily conserved mechanism for the degradation of cellular components in the cytoplasm, and serves as a cell survival mechanism in starving cells. Recent studies indicate that autophagy also functions in cell death, but the precise role of this catabolic process in dying cells is not clear. Here I discuss the possible roles for autophagy in dying cells and how understanding the relationship between autophagy, cell survival and cell death is important for health and development.

The development of multicellular organisms involves an important balance between cell growth, cell division and cell death. The coordination of these cellular programmes ensures appropriate organ and body size[1], and the disruption of cell division and death can result in abnormal growth typical of cancer[2]. Other factors, including the cellular response to growth signals, the interactions between cells and the extracellular matrix, metabolic rate, nutritional status and nutrient uptake, might also deviate during abnormal growth[3]. Therefore, it is important to consider all these factors when investigating the mechanisms that regulate normal and abnormal growth and development.

Programmed cell death has an important role in all animals, by forming and deleting structures, controlling cell numbers and eliminating abnormal damaged cells[4]. Three forms of cell death have been defined in dying cells of developing animals on the basis of morphology, location and dependency on lysosomes[5,6]. Type 1 programmed cell death (or apoptosis) is defined by the condensation of the nucleus and cytoplasm, the association of chromatin with the nuclear periphery, DNA fragmentation, membrane blebbing, and the engulfment and lysosomal degradation of the dying cell by a phagocyte[7]. Type 2, or autophagic, cell death differs from apoptosis by the presence of autophagic vacuoles (autophagosomes) and autophagolysosomes in the dying cell that are used for self-degradation. This process is independent of phagocytes. Type 3 (or non-lysosomal) cell death involves the swelling of organelles and the lysosome-independent formation of 'empty spaces' in the cytoplasm, and has similarities to necrosis. Apoptosis and autophagic cell death have been observed in many types of developing animals, whereas non-lysosomal cell death does not seem to be as common in non-pathological conditions. Several other forms of cell death have been described on the basis of studies in derived cell lines (reviewed in REF. 8), but it is not yet clear if these cell morphologies are relevant in physiological situations.

The degradation and recycling of the building blocks of organelles, proteins and other components of the cytoplasm is important for the maintenance of cellular homeostasis. Two general mechanisms are used for large-scale degradation of components of the cytoplasm; short-lived regulatory proteins are destroyed by the ubiquitin–proteasome system, and long-lived structures and proteins are targeted to the lysosome by autophagy[9]. Several forms of autophagy have been described[10,11], but here I focus on macroautophagy (hereafter referred to as autophagy) because of its association with type 2 autophagic cell death.

Many of the genes that regulate autophagy have been conserved in organisms that are as different as yeast and humans, and these organisms all seem to use autophagy as a cell survival mechanism during nutrient deprivation[12]. By contrast, little is known about the possible role of autophagy in type 2 autophagic cell death. The presence of autophagic structures in dying cells of diverse organisms led to the hypothesis that autophagy can have a causative role in autophagic cell death[4,6]. Recent work supports this hypothesis[13,14], but alternative models exist, and the precise function of this catabolic process in autophagic cell death is uncertain[12]. Here I will briefly introduce the mechanisms that regulate autophagy during cell survival in higher animals, and discuss the roles that autophagy might have in cell death. Recent studies indicate that apoptosis and autophagic cell death are associated either through complementary pathways of activation, or by the presence of both active caspases and autophagy in a common dying cell. The relationship between caspase activation and autophagy might

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