Membrane repair: Ca^{2+}-elicited lysosomal exocytosis

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Cells in exposed positions are subject to injury and therefore need membrane repair mechanisms. Ca^{2+} entry inevitably follows membrane rupture and recent studies indicate that this elicits repair via Ca^{2+}-activated exocytosis of lysosomes, regulated by lysosomal synaptotagmin VII.

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Current Biology 2001, 11:R971–R974

0960-9822/01/$ – see front matter

The integrity of the plasma membrane is essential for cell survival and most cellular functions. It has been recognized for some time that cells in exposed positions are often injured, resulting in membrane rupture, and therefore need to be able to restore the integrity of their damaged plasma membranes by a repair mechanism. Several years ago, it was proposed that cell membranes could be resealed by a vesicular mechanism similar to neurotransmitter release [1]. Fusion of vesicle membrane with the plasma membrane could plug the gap left by an injury that had caused membrane rupture. As neurotransmitter release is a Ca^{2+}-regulated process, Ca^{2+} would therefore be expected to be critically involved in the process of membrane repair and it was indeed shown that resealing of membrane disruptions is strictly dependent on extracellular Ca^{2+} [1,2].

The difficulty with the idea that Ca^{2+}-regulated exocytosis could be a general mechanism for membrane repair was that this process had been regarded as specific for secretory units, such as endocrine and exocrine cells as well as nerve endings. In recent years, however, several studies have shown that regulated exocytosis occurs in a number of cell types not specialized for secretion, such as fibroblasts and hematopoietic cells [3]. Furthermore, a well established secretory model cell line, PC-12, has been shown to contain not only classical secretory granules, but also an additional population of Ca^{2+}-regulated exocytotic vesicles [4]. Norma Andrews' group [5] has demonstrated that lysosomes behave as Ca^{2+}-regulated exocytotic vesicles in fibroblasts and epithelial cells, and has done much to advance the case for regulated secretion of conventional lysosomes in a number of systems [3]. A very recent study from this group [6] has now shown that Ca^{2+}-regulated lysosomal exocytosis is responsible for the resealing of injured skin fibroblasts.

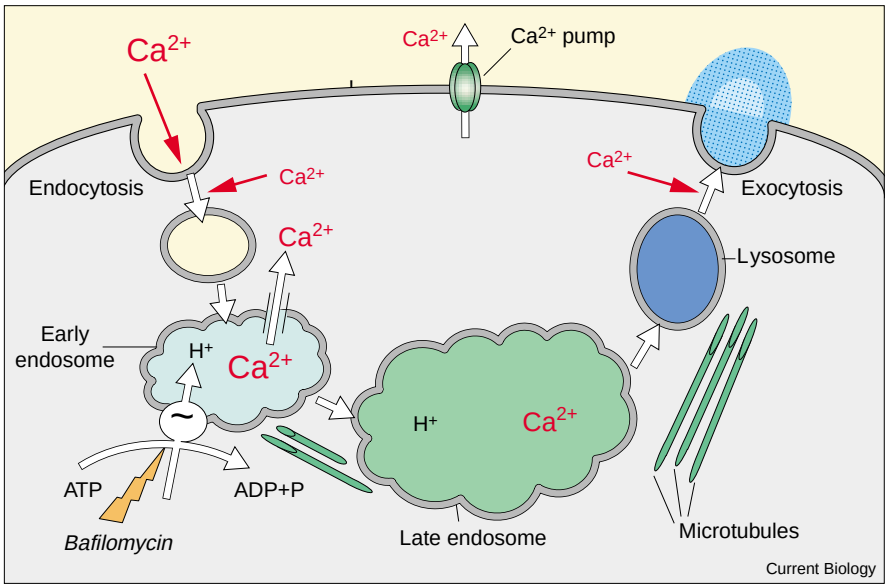
Ca^{2+} in the regulation of exocytosis and endocytosis

The surface area of cells has to be kept reasonably constant, so it is necessary to have a balance between the insertion of internal (organelle) membrane into the plasma membrane (exocytosis) and the retrieval of plasma membrane (endocytosis) (Figure 1). In pancreatic acinar cells stimulated by the secretagogue acetylcholine, the insertion of exocytotic granule membrane into the plasma membrane is followed within seconds by retrieval of the exact amount of fused membrane [7]. It has been generally accepted for more than 20 years that Ca^{2+} is crucial for the control of exocytosis [8,9], but recently it has also become apparent that Ca^{2+} has effects on endocytosis [10–12].

Secretory exocytosis is activated by a rise in the cytosolic Ca^{2+} concentration [8,9]. At synapses between nerve cells, where secretion of neurotransmitters has to be very rapidly and precisely controlled, the high Ca^{2+} concentration required to elicit exocytosis only occurs in spatially restricted active zones. In these zones, the vesicles containing the relevant neurotransmitter are docked near clusters of voltage-sensitive Ca^{2+} channels in the plasma membrane [13]. In chromaffin cells, neuronal R-type Ca^{2+} channels are coupled to the rapid component of secretion [14]. In pancreatic acinar cells, the classical model for the study of regulated exocytosis in electrically non-excitable cells, the high Ca^{2+} concentrations required for stimulated secretion occur specifically at the granule-containing apical pole. The specific Ca^{2+} release from apical endoplasmic reticulum extensions is due to the clustering of Ca^{2+} release channels in this part of the cell [15].

Calmodulin is the prototypical Ca^{2+} sensor [16] and may play some role in regulated exocytosis. In pancreatic acinar cells, calmodulin is translocated in a Ca^{2+}-dependent manner into the apical region, where Ca^{2+}-regulated exocytosis occurs [17]. The essential Ca^{2+} sensor responsible for Ca^{2+}-elicited exocytotic secretion, however, would appear to be synaptotagmin, a low Ca^{2+}-affinity, C_2-domain containing protein [18]. Synaptotagmin I is a synaptic vesicle-associated protein that binds syntaxin, a plasma membrane protein which, together with another plasma membrane protein SNAP-25 and the synaptic vesicle membrane protein synaptobrevin, forms the so-called core complex of membrane fusion. Very recent data obtained from synaptotagmin I null mutant mice [19] has demonstrated directly that synaptotagmin I is essential for synchronous neurotransmission: in the absence of synaptotagmin I, fusion and secretion rates are much reduced and there are prolonged exocytic delays.

Figure 1



A schematic representation of the endocytic–exocytic membrane cycling, involving the generation and use of lysosomes, and the roles of Ca^{2+} in these processes. Endosomes are generated by endocytosis in a process that depends on extracellular Ca^{2+} and appears also to be stimulated by a rise in the cytosolic Ca^{2+} concentration. Release of Ca^{2+} from endosomes occurs in a process that is dependent on the concomitant acidification, mediated by a vacuolar-type H^+ ATPase. The Ca^{2+} release and H^+ uptake appear to be linked, most likely through the need for charge neutralization. The colour change from yellow through green to blue signifies increasing intraorganellar acidification. Lysosomes are generated from late endosomes and can be inserted into the plasma membrane by a Ca^{2+}-regulated exocytotic process, which depends on normal function of microtubules.

The role of Ca^{2+} in the control of endocytosis is less clear. In pancreatic acinar cells, endocytic membrane retrieval following Ca^{2+}-elicted exocytosis depends on the presence of extracellular Ca^{2+} [10], and in fibroblasts the rate of spontaneous endocytosis is reduced to about 50% of the control value after removal of external Ca^{2+} [11]. In a recent study of the Ca^{2+}-dependence of exocytosis and endocytosis in the inner hair cell afferent synapse, it was shown that an increase in the cytosolic Ca^{2+} concentration increased the contribution of a fast mode of endocytosis [12]. In contrast to what has been shown for the regulation of exocytosis, synaptotagmin does not appear to be involved in the control of endocytosis [19].

Cellular Ca^{2+} homeostasis is influenced by both exocytosis and endocytosis. Because of the high Ca^{2+} concentration in secretory vesicles and granules, exocytosis inevitably also means export of Ca^{2+} from cells during the secretory process, and this has been directly demonstrated [20]. As the Ca^{2+} concentration in the extracellular fluid is very much higher than in the cytosol, endocytosis contributes to cellular Ca^{2+} entry and this has been directly visualized in studies on fibroblasts [11]. Ca^{2+} taken into the cell by endocytosis does not stay in the endosomes, but is released into the cytosol by a process that is dependent on the endosomal acidification [11]. Exocytosis and endocytosis are thus regulated by Ca^{2+}, but they also contribute to overall Ca^{2+} homeostasis by exporting and importing Ca^{2+}. The cycling of Ca^{2+} through the endocytic–exocytic pathways, as well as the plasma membrane Ca^{2+} pump, is also essential for the creation of the acidic lysosomes (Figure 1).

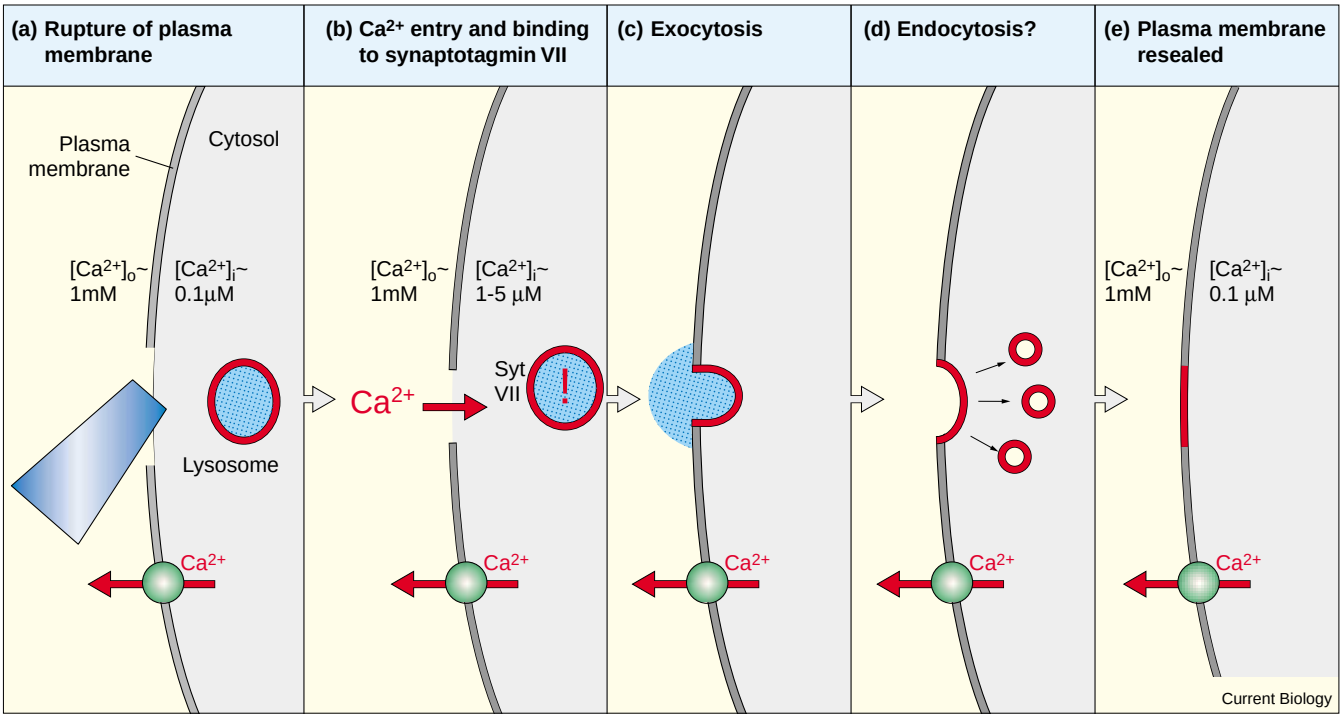
Regulated exocytosis of lysosomes

Lysosomes are acidic organelles, containing acid hydrolases, which play an important role in the digestion of phagocytosed material [3]. Lysosomes are derived from, and in equilibrium with, late endosomes [3] (Figure 1). The acid pH in the lysosomes is therefore influenced by the endosomal acidification process, which is due to the action of a bafilomycin-sensitive vacuolar proton ATPase (Figure 1). The endosomal acidification depends on Ca^{2+} loss from the endosomes to the cytosol (Figure 1), perhaps because of the need for charge compensation. The Ca^{2+} loss from the endosomes depends on the acidification, as blockage of the proton ATPase prevents the loss of imported Ca^{2+} from the endosomes to the cytosol [11].

Lysosomes have traditionally been viewed as terminal degradative compartments, but evidence has emerged from studies of fibroblasts and epithelial cells demonstrating that they can behave as Ca^{2+}-regulated exocytotic vesicles [5]. Elevation of the cytosolic Ca^{2+} concentration in normal rat kidney fibroblasts, using either a Ca^{2+} ionophore or the peptide agonist bombesin, was found to induce release of the lysosomal enzyme β-hexosaminidase and the appearance on the plasma membrane of the lysosomal glycoprotein lpg120. This and other evidence indicates that Ca^{2+} can stimulate lysosomal exocytosis [5].

Lysosomal exocytosis requires elevation of the cytosolic Ca^{2+} concentration to about 1–5 μM and is ATP and temperature dependent in a way similar to what is known for the classical secretory process [5]. Furthermore, it has been shown that exocytosis of lysosomes in fibroblasts

Figure 2



Schematic representation of the most important stages in membrane repair, following the generation of a hole in the plasma membrane (**a**). As the Ca^{2+} concentration inside the cell is four orders of magnitude lower than outside the cell, because of a low resting Ca^{2+} permeability and the operation of the plasma membrane Ca^{2+} pump, the rupture of the plasma membrane will inevitably result in a major influx of Ca^{2+} from the external solution into the cytosol, which will activate the Ca^{2+} sensor synaptotagmin VII in the lysosomal membrane (**b**). This activates exocytosis of lysosomes (**c**). Endocytosis, also stimulated by the rise in the cytosolic Ca^{2+} concentration, most likely reduces the surface membrane area back to the control level (**d**). Finally the plasma membrane has been resealed and the normal transmembrane Ca^{2+} gradient can be restored (**e**).

is regulated by synaptotagmin VII, a protein that binds the plasma membrane protein syntaxin at free Ca^{2+} concentrations below 10 µM, and is ubiquitously expressed. Synaptotagmin VII is localized on mature, dense lysosomes in normal rat kidney fibroblasts. To test the involvement of synaptotagmin VII in Ca^{2+}-regulated exocytosis of the lysosomes, antibodies were produced by immunization with the purified recombinant C_2A domain of synaptotagmin VII. Such antibodies specifically and markedly reduced the Ca^{2+}-stimulated secretion of β-hexosaminidase from permeabilized fibroblasts [21]. This indicates that Ca^{2+} regulates lysosomal exocytosis via the Ca^{2+} sensor synaptotagmin VII.

Lysosome exocytosis repairs damaged plasma membrane

When the plasma membrane is punctured, Ca^{2+} must move into the cytosol through the hole created by the puncture, as there is normally an enormous Ca^{2+} gradient across the plasma membrane favouring entry (Figure 2). As the resealing process is Ca^{2+}-dependent and antagonized by Mg^{2+}, which is known to inhibit exocytosis, it is reasonable to suggest that a ubiquitous Ca^{2+}-stimulated exocytosis process could be responsible for the membrane repair (Figure 2). This hypothesis has recently been tested critically by Andrews' group [6]. When normal rat kidney fibroblasts were wounded by scratching with a surgical blade, the amino-terminal domain of the abundant lysosomal membrane glycoprotein Lamp-1 became exposed on the cell surface. This required the presence of Ca^{2+} in the extracellular solution.

Although this type of experiment does not discriminate between intracellular and extracellular effects of Ca^{2+}, further studies clearly implicated the lysosomal Ca^{2+} sensor synaptotagmin VII. Thus, lysosomal exocytosis and membrane resealing were inhibited by the recombinant synaptotagmin C_2A domain or by anti-synaptotagmin C_2A antibodies. Experiments of a more physiological nature were also carried out, in which resealing of primary skin fibroblasts that had been wounded by contraction of the collagen matrix was shown to depend on lysosomal exocytosis [6]. Microtubules probably play a role in the exocytosis

of lysosomes, as the membrane-resealing process was inhibited by nocadozole [6], which inhibits polymerization of tubulin.

Conclusion

Recent work has clarified a major problem in cell biology, namely how cells manage to repair damage to their plasma membrane when they are injured. It turns out that Ca^{2+} entry through holes in the plasma membrane triggers the exocytotic insertion of lysosome membrane plugging the holes in the surface cell membrane and thereby restores the integrity of the injured cell. The exocytosis of lysosomes is regulated in much the same way as exocytosis of secretory granules or vesicles, via the Ca^{2+} sensor synaptotagmin. As the lysosomes are derived from endosomes, there must be careful adjustment of the rates of endocytosis and exocytosis. These processes are both Ca^{2+} dependent and both contribute to import and export of Ca^{2+}.

Acknowledgements

We thank Nina Burdakova for technical assistance. The authors' work is supported by an MRC Programme Grant. O.H.P. is an MRC Research Professor.

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