Zygote 8 (Supplement), p. S31–S32. © 2000 Cambridge University Press Printed in the United Kingdom

The mechanism of cell membrane repair

Tatsuru Togo, Janet M. Alderton and Richard A. Steinhardt
Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA

Disruption of plasma membranes is a widespread, common and normal event that occurs in many mechanically challenged tissues (McNeil & Steinhardt, 1997). After injury to the plasma membrane, rapid resealing of the membrane occurs with little loss of intracellular contents.

Analysis of plasma membrane repair in the sea urchin egg and early embryos revealed a new model of the mechanism for plasma membrane repair. Resealing of disrupted plasma membranes required external Ca^{2+} that could be antagonised by Mg^{2+}. Block of Ca^{2+}/calmodulin kinase II, which regulates exocytotic vesicle availability at synapses (Llinás *et al.*, 1991), inhibited membrane resealing. Resealing was also inhibited by botulinum neurotoxins A, B, C1, and tetanus toxin, which disrupt SNARE vesicle docking/fusion proteins. Confocal microscopic observations of exocytotic events in sea urchin eggs and embryos during membrane resealing showed that inhibition of kinesin or myosin motor activity, which are believed to be required for vesicle transport (Goodson *et al.*, 1997), also inhibited membrane resealing and delivery of vesicles to sites of membrane disruption. This pattern of inhibition indicates that membrane repair of micrometre-sized lesions requires vesicle delivery, docking and fusion, similar to the exocytosis of neurotransmitter (Steinhardt *et al.*, 1994; Bi *et al.*, 1995, 1997).

The mechanism of resealing in eggs and embyros was found to be a general property of all cells (Steinhardt *et al.*, 1994; Togo *et al.*, 1999). It is now known that elevated intracellular Ca^{2+} triggers exocytosis in various types of cells (Dan & Poo, 1992; Coorssen *et al.*, 1996), and that endosomal compartments such as lysosomes can behave as Ca^{2+}-regulated exocytotic vesicles (Rodríguez *et al.*, 1997).

In addition, we found that a second disruption of the plasma membrane reseals more rapidly than the initial wound in mammalian cells (Togo *et al.*, 1999). This facilitated response was inhibited by both low external Ca^{2+} concentration and the protein kinase C (PKC) inhibitors bisindolylmaleimide I and Gö-6976. Facilitation was also blocked by brefeldin A, a fungal metabolite that inhibits vesicle formation at the Golgi apparatus (Klausner *et al.*, 1992). PKC activation by phorbol ester facilitated membrane resealing at initial wounding, but this effect was blocked by pretreatment with brefeldin A. These results suggested that at repeated wounding, PKC, activated by Ca^{2+} entry at the first wound, stimulates vesicle formation and delivery from the Golgi apparatus, resulting in more rapid resealing of the repeated membrane disruption (Togo *et al.*, 1999).

The rate of membrane resealing in liposomes has been correlated with lower surface tension (Zhelev & Needham, 1993). In the plasma membrane of living cells, the existence of a closely linked rigid cytoskeleton greatly increases the surface tension, compared with simple lipid bilayers. To confirm whether a decrease in surface tension is necessary for membrane resealing, Swiss 3T3 fibroblasts were treated with a surfactant, Pluronic F68 NF, or cytochalasin D. Artificial decreases in membrane surface tension induced by the addition of Pluronic F68 NF facilitated membrane resealing at initial wounding. Furthermore, Pluronic F68 NF restored membrane resealing even though exocytosis was blocked by tetanus toxin. The same was true for cytochalasin D treatment, which reduced exocytosis without inhibiting membrane resealing. These results suggested that membrane resealing requires a decrease in cell surface tension and under natural conditions this is provided by Ca^{2+}-regulated exocytosis of new membrane near the site of disruption (Togo *et al.*, 1999).

Direct measurements of cell surface tension using a laser tweezer confirmed that membrane wounds result in decreases in surface tension that appear to follow the time course of the exocytotic burst. Normal tension levels were recovered well before repeated wounds, and the decreases in surface tension at repeated wounding were faster than at the initial wound. These results suggested that facilitated resealing depends on a new faster round of exocytosis, rather than a residue of extra membrane from the first. We propose that exocytosis is essential for plasma membrane resealing because it lowers surface tension by the addition of new membrane area.

This research was supported by the National Institutes of Health. We thank Drs T.B. Krasieva, A. Dunn and B. J.

Tromberg, Beckman Laser Institute and Medical Clinic, University of California, Irvine, for supporting our efforts with the laser tweezers.

References

Bi, G.-Q., Alderton, J.M. & Steinhardt, R.A. (1995). *J. Cell Biol.* **131**, 1747–58.

Bi, G.-Q., Morris, R.L., Liao, G., Alderton, J.M., Scholey, J.M. & Steinhardt, R.A. (1997). *J. Cell Biol.* **138**, 999–1008.

Coorssen, J.R., Schmitt, H. & Almers, W. (1996). *EMBO J.* **15**, 3787–91.

Dan, Y. & Poo, M. (1992). *Nature* **359**, 733–6.

Goodson, H.V., Valetti, C. & Kreis, T.E. (1997). *Curr. Opin. Cell Biol.* **9**, 18–28.

Klausner, R.D., Donaldson, J.G. & Lippincott-Schwartz, J. (1992). *J. Cell Biol.* **116**, 1071–80.

Llinás, R., Gruner, J.A., Sugimori, M., McGuinness, T.L. & Greengard, P. (1991). *J. Physiol. (Lond.)* **436**, 257–82.

McNeil, P.L. & Steinhardt, R.A. (1997). *J. Cell Biol.* **137**, 1–4.

Rodríguez, A., Webster, P., Ortego, J. & Andrews, N.W. (1997). *J. Cell Biol.* **137**, 93–104.

Steinhardt, R.A., Bi, G.-Q. & Alderton, J.M. (1994). *Science* **263**, 390–3.

Togo, T., Alderton, J.M., Bi, G.-Q. & Steinhardt, R.A. (1999). *J. Cell Sci.* **112**, 719–31.

Zhelev, D.V. & Needham, D. (1993). *Biochim. Biophys. Acta* **1147**, 89–104.

https://doi.org/10.1017/S096719940013014X Published online by Cambridge University Press