Comment

Patching the membranes up – new views on cellular healing

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In this issue of the Journal of Cellular and Molecular Medicine (*J. Cell. Mol. Med.*, 7: 197-203, 2003), Meldolesi raises an interesting question, that just shows how many of the fundamental biological processes are taken for granted [1]. Whereas we are every day more and more eager to use cells for playing constructive and fun games of recombination and reconstruction, expressing or repressing proteins and intracellular pathways, we still know very little about some of the fundamental cellular processes involved in these activities. One such Cinderella process is that of cellular healing. A wide range of methods that are used hundreds or thousands of times a day in the scientific community, from cell culturing to intracellular electrophysiological recordings or to electroporation, involve significant transient damage of the continuity of the cellular plasma membrane. In these instances, many of the cells recover and are ready for use, but we do not know how exactly the process of recovery and cellular wound healing takes place. Clearly, the metabolic status of the cell is also of essence since, in last instance, the perturbations of the intracellular milieu resulting from the direct contact with the extracellular medium, with a very different set of ionic concentrations, need to be redressed at some energetic cost. It might also be the case that the cytosolic gel provides a much stronger diffusional barrier than we anticipate. Indeed, assessment of the rate of diffusion of ATP, a molecule that is relatively small, water soluble and thus very diffusible, showed that the intracellular diffusion is

much smaller than that measured in the water solutions [2]. The same is true for another important signaling molecule, the Ca^{2+} ion [3].

Meldolesi brings in another important aspect of cell wound healing. In order for the ionic and general homeostatic systems of the cells to be able to redress the balance disrupted by the cell membrane break, the cell needs, in the first instance, to plug the holes. In essence, the model proposed involves an initial cellular contractile response, triggered by the sudden increase in $[\text{Ca}^{2+}]_i$, followed by another Ca^{2+} -dependent process – regulated exocytosis, that allows the insertion of pre-docked intracellular vesicles or organelles in the gaping holes of the plasma membrane. As pointed out by Meldolesi, this process of regulated exocytosis should not be confused with that of regulated secretion [1]. The latter is the confine of specialized cells, whereas the former process is a more universal process, that can be demonstrated even in cells that do not have secretory activity [4].

As it stands, the proposal for the mechanism of plasma membrane healing in the wake of significant wounds is very interesting, although some of its aspects are open to discussion. For example, Meldolesi proposes that the first step in the process is the initial contraction of the contractile apparatus, but restricted to the subplasmalemmal domain [1]. Because of the concave geometry, the restriction of the contraction to this domain will result in an initial widening of the membrane discontinuity. This is somewhat counter-intuitive, both in the biological common-sense (it would be more beneficial for the initial hole to be reduced as soon as possible rather than enlarged) and also in the common experience of the patch-clamp electrophysiologists that have seen in the microscope many patches being broken

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through, with the resulting flaps of the broken membrane tending to fold one on each other rather than an enlargement of the hole in the plasma membrane. Finally, as proposed, the model depends on a rapid diffusion of the contractile trigger, i.e., the Ca^{2+} ions, through the subplasmalemmal space. However, many studies in the field of Ca^{2+} signaling showed that this subplasmalemmal space is a very powerful Ca^{2+} buffer zone, containing not only various Ca^{2+} buffers but also powerful Ca^{2+} uptake systems (mitochondrial Ca^{2+} uptake, endoplasmic reticulum Ca^{2+} ATPases (SERCAs)) [5]. These systems, most likely, will allow for very limited Ca^{2+} diffusion.

One alternative that could integrate these observations is to consider the 3D architecture of the contractile system in the cells. The Ca^{2+} sensitive contractile proteins do not expand only as a layer in the subplasmalemmal space, but also distribute towards the cytosol, providing powerful anchoring of the subplasmalemmal space into the depth of the cytosol. With this model in mind, it is possible to imagine that an initial injury of the plasma membrane that allows a massive Ca^{2+} entry (the Ca^{2+} gradient across the intact plasma membrane is about 20,000-fold, from the extracellular 2 mM to the resting $[\text{Ca}^{2+}]_i$ of 100 nM) would determine a rapid contraction, more or less restricted to the region of the injured membrane. Such a contraction will induce a rapid reduction of the plasma membrane opening and trigger, also in a localized manner, the process of regulated exocytosis.

The other original aspect of the model is the nature of the organelle that is proposed to be involved in these healing processes. Since cellular wound healing is universal, applicable to all cell types, it cannot involve only the classical secretory vesicles, known to integrate easily, in a Ca^{2+} -dependent manner, in the plasma membrane prior to releasing their content to the extracellular space. Meldolesi proposes a novel type of organelle, the **enlargeosome**, based on their previous observations indicating the small size of these vesicles/organelles, their rapid exocytosis and slow recovery from the membrane surface [4, 6]. If their existence as a separate class of intracellular organelles, with a specific function, remains to be determined, the proposal is nevertheless timely in providing a new angle and perspective on how the cells patch up. Other interesting issues that will be addressed in the future will probably include the determination of the permissible size of the plasma

membrane wound diameter that can be addressed by the proposed mechanism, the relationship between the initial wound and the activation of the cellular homeostatic reserve, the possible existence of specific signalling molecules in addition to and beyond the Ca^{2+} ions, that might couple the injury with the healing process, seen not only as the immediate patch up of the membranes holes but also as an integrated metabolic response. One area that might benefit from such new lines of investigations will be the study of cellular ageing. It is known that ageing is characterised by a significant decrease in the homeostatic reserve of cells, defined as a decreased capacity of the cells to oppose the action of the excessive levels of stimulation, and also with increased vulnerability [7]. The role of cellular microtrauma and the decreased metabolic capacity of such aged cells might indeed be another level of explanation for the increased vulnerability of the aged cells.

One can only hope that such original ideas will spur into action further scientific interest and generate lateral thinking that is, ultimately, the engine that drives good science.

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