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Repair of Single Cell Wounds: Membrane and Rho GTPase Dynamics

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Cells are frequently damaged in the normal course of life and failed repair is a cause and consequence of a variety of pathological conditions. Accordingly, efficient cell repair is a feature of all eukaryotic cells studied to date. Here we will consider the cell repair response in oocytes of the frog, *Xenopus leavis*. Xenopus oocytes were chosen as a model based on their remarkably efficient healing, the enthusiasm with which they express exogenous mRNAs encoding fluorescent probes, and their astonishing tolerance for laser light. Nonetheless, it should be understood that many of the features of the healing response observed in *Xenopus* oocytes are conserved in other cell types and organisms.

Following cell damage, two complementary processes are initiated: a calcium-dependent membrane repair response which is initiated immediately, and calcium- dependent generation of a ring of actin filaments and myosin-2 that closes over the hole, which begins within 20-30 seconds. Using live-cell confocal microscopy with a series of different fluorescent probes, we show that at least one part of the membrane repair response is "patching", a process in which membrane compartments fuse with each other at the site of damage, thereby providing a temporary patch. Patching was originally hypothesized more than two decades ago, but never previously visualized.

After patching, two small Rho GTPases, Rho and Cdc42, are activated around wounds, and then sort into complementary zones of activity, with Rho activity immediately abutting the wound edge, and Cdc42 activity encircling the Rho. These proteins direct local assembly of actin filaments, and local assembly and activation of myosin-2. The Rho and Cdc42 zones are kept separated from each other by a "corral" that encircles the Rho zone. The corral is a ring-like structure comprising F-actin, cortactin, RG1, RG8 and p190 RhoGAP. The latter three proteins are known inactivators of Rho and their presence in the corral could explain why the Rho activity remains confined to the edge of the wound. Consistent with this notion, manipulations that cause breaks in the corral result in leakage of the Rho away from the wound edge. However, complete loss of the corral prevents the Rho zone from forming in the first place. This finding is surprising, in that the loss of Rho inactivators would normally be expected to result in more, not less Rho activity. We speculate that the corral may both limit the spread of Rho activity and recycle Rho back to the Rho zone.

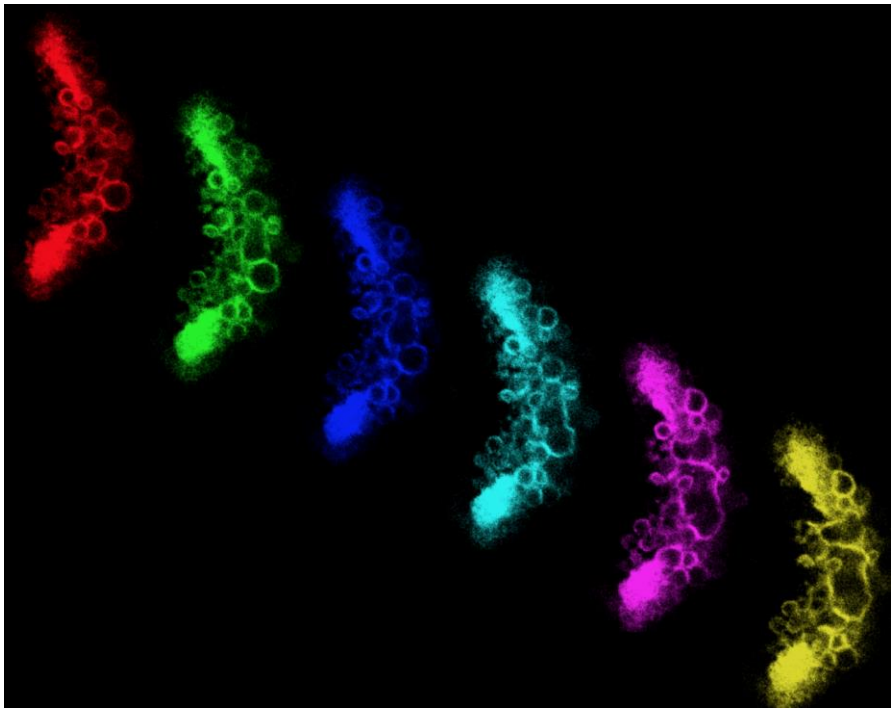

Figure 1. Membrane patching at the site of a single cell wound.

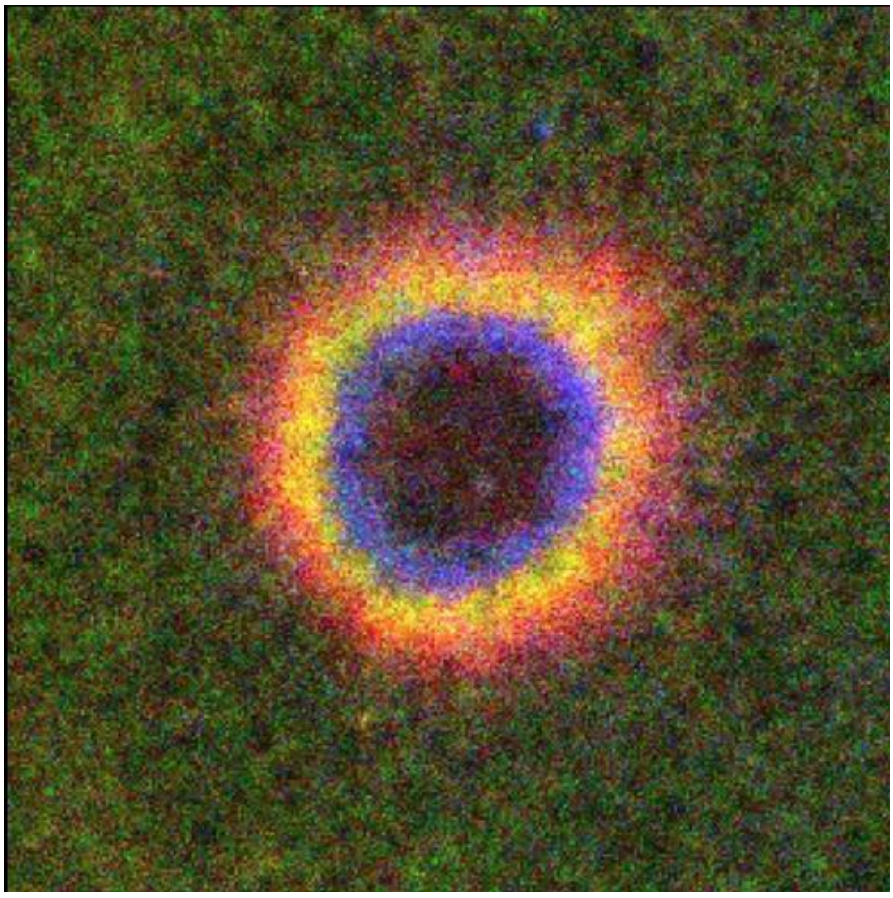

Figure 2. Rho GTPases and cortactin around single cell wounds.

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