Orchestrating a Symphony of Cellular Repair

February 17, 2014

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Cells are constantly exposed to physical stresses that can cause damage to their plasma membranes (McNeil and Steinhardt, 1997). The efficient repair of membrane damage is critical for cellular survival because membrane rupture can lead to cytoplasmic loss, influx of ions into cells, and ultimately cell death. Cellular wound repair involves two coordinated processes: plasma membrane resealing and cytoskeletal reorganization. Influx of extracellular calcium into cells via membrane ruptures is thought to be the primary driver of membrane resealing, while cytoskeleton remodeling is driven by the accumulation of actin and myosin II in cells around the edge of the wound. Actin and myosin II surround the wound and contract, driving closure of the rupture. The formation of this actin-myosin II (actomyosin) ring is dependent on the action of several GTP-hydrolyzing enzymes (GTPases). In particular, the founding members of the Rho GTPase family, Rho, Rac, and Cdc42 coordinate actomyosin ring formation. However, the individual contributions of these GTPases to wound repair have remained elusive. To elucidate Rho GTPase-mediated control of actomyosin ring formation, postdoctoral fellow Maria Teresa Abreu-Blanco and Jeffrey Verboon in the laboratory of Dr. Susan Parkhurst (Basic Sciences Division) made use of the Drosophila melanogaster embryo as a model of cellular wound repair. They found that Rho GTPases regulate both the cytoskeleton and also each other, giving insight into how local activation of specific regulatory proteins can be used to respond to specific cellular requirements.

The researchers first used genetic and pharmacological inhibition of the Rho1 GTPase to determine its effects on wound repair. Loss of Rho1 activity resulted in assembly of a disorganized actomyosin ring and abnormal wound expansion due to defective actin recruitment. These data indicated that Rho1 was mainly required for actin recruitment and actomyosin ring formation. Notably, myosin II recruitment to the wound was not affected by Rho1 inhibition. Loss of Cdc42 also resulted in decreased actin recruitment to the wound as well as a wider actomyosin ring, and Rac loss was associated with severely compromised actin recruitment with attendant wound expansion. Simultaneous loss of function of Rho1, Rac, and Cdc42 resulted in severely reduced wound healing capacity.

The investigators next examined how Rho GTPases are recruited to wounds by tracking the

movement of fluorescently-tagged Rho1, Rac1/2, or Cdc42 upon membrane insult. Rho1 was the first to appear at the wound at 30-45 seconds after wounding, before accumulation of actin. The appearance of Cdc42 and Rac1 at the wound was after the onset of actin recruitment, 90-135 seconds after wounding. Each GTPase also displayed a characteristic spatial arrangement around the wound: Rho1 was visualized as a sharp ring overlapping within the actin ring, displaying only a small degree of overlap with actin (see figure). Cdc42 was seen as a discrete ring overlapping with actin; and both Rac1 and Rac2 formed diffuse rings as compared to Rho1 and Cdc42.

Interestingly, Abreu-Blanco, et al. also found that crosstalk between Rho GTPases was required for their localization at wound sites. For instance, inhibition of Rho1 GTPase activity resulted in a narrower ring of Cdc42 around the wound site, and loss of Rac1 activity resulted in decreased accumulation of Rho1 and Cdc42 around wound sites, though their distributions around the wound were unchanged. Localization of GTPases to wounds was also dependent on the components of the actomyosin ring. Pharmacological stabilization of actin resulted in delayed GTPase recruitment to the wound, and decreased myosin II levels caused both reduced recruitment and disorganization of GTPase arrays around the wound.

This study shows that the response of the cytoskeleton to plasma membrane injury requires the action of Rho GTPases not only to regulate cytoskeletal rearrangement but also to regulate the spatial arrangement of each other around the wound. Future studies on wound repair in the Drosophila model system include determining how GTPases at wounds are regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) as well as upstream signaling molecules and how they utilize specific downstream effectors to coordinate cytoskeletal rearrangement.

Abreu-Blanco MT, Verboon JM, Parkhurst SM. 2014. Coordination of Rho Family GTPase Activities to Orchestrate Cytoskeleton Responses during Cell Wound Repair. *Curr Biol* 24(2):144-155.

See also: McNeil PL, Steinhardt RA. 1997. Loss, Restoration, and Maintenance of Plasma Membrane Integrity. *J Cell Biol* 137(1):1-4.

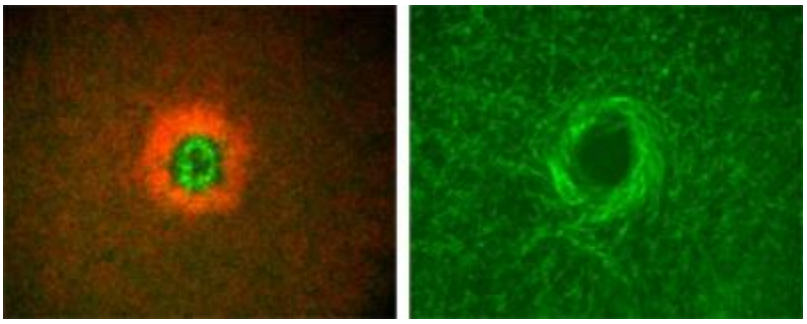

Image provided by Dr. Susan Parkhurst

(Left) Single cell wound repair in an embryo co-expressing a marker for actin (green) and active Rho1 (red), showing that Rho1 accumulates internal to the actin ring. (Right) Single cell wound repair in a Rho family GTPase deficient embryo (simultaneous knockdown of Rho, Rac, and Cdc42) expressing an actin marker (green) and showing actin organization still exists in the absence of the GTPases.