Zing Went the Strings of My Hurt

November 19, 2012

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Environmental insults to organisms occur on a frequent basis and require that the organism respond to and correct any resultant homeostatic change. Epithelial tissues are a first line of defense against the external environment and, when injured, can result in microbial invasion and loss of tissue integrity. Therefore, it is critical to determine the molecular pathways that ensure epithelial wound healing.

Several experimental systems exist to study epithelial wound repair, and experimental results have revealed the use of a variety of mechanisms for wound repair including lamellipodial crawling and actomyosin purse string contractility. However, the relative contribution of these mechanisms to wound repair, and their molecular players, remain enigmatic and appear to be context-specific (e.g. embryonic vs. adult tissue repair). The Parkhurst lab (Basic Sciences Division), by utilizing transgenic Drosophila embryos and 4D confocal microscopy, has provided a detailed and thorough investigation of wound repair to reveal key proteins involved in specific repair stages.

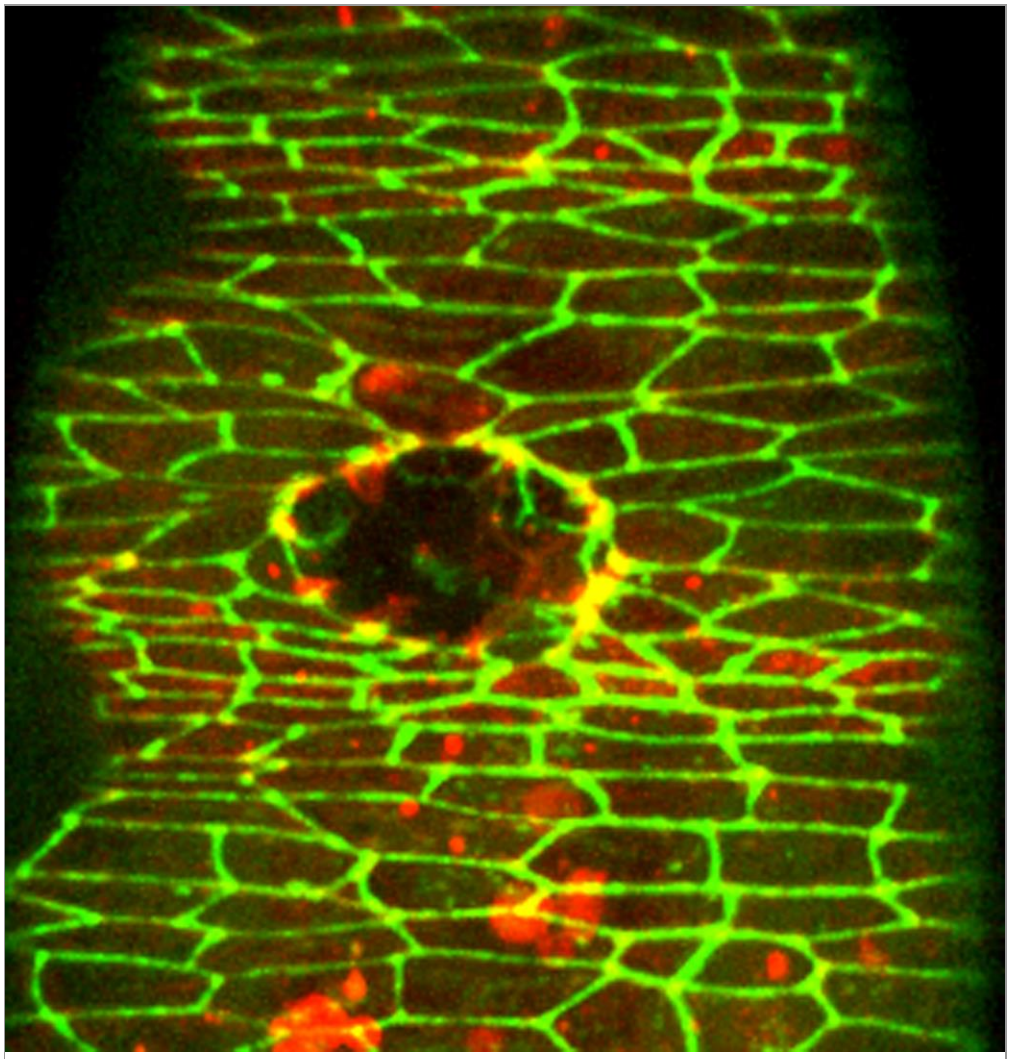
First, live-cell imaging of embryos subjected to laser ablation of a patch of epithelial tissue revealed the overall morphological changes that occurred at and around the site of tissue damage. Four distinct stages were identified: (1) expansion of the wounded area with retraction of the tissue margins and removal of severely damaged cells; (2) coalescence as the wound reaches a maximally retracted area and begins to assemble the cellular machineries required for wound repair; (3) contraction of the wound area as a rapid process to shrink the size of the wound; (4) closure of the final 5% of the wound area and restitution of tissue continuity. Imaging embryos expressing fluorescently tagged actin revealed actin foci along the leading edge of the wound, and dynamic cellular protrusions were evident 5 minutes post-wounding (coalescence). Also, actin patches from leading edge cells coalesced to form a continuous cable that encircled the leading edge of the wound. During contraction, actin became enriched in the actin cable indicating active actomyosin purse-string function. Actin-rich lamellipodia and filopodia (thin plasma membrane protrusions that function in cell mobilization) explored the open space of the wound area during this stage. Lastly, wound closure was achieved through filopodia/lamellipodia contacts from opposing wound edges.

After defining the key stages in wound repair, the localization of candidate proteins that were anticipated to play a role in the process was determined and their function was perturbed to

determine how they affect wound repair. First, myosin II accumulation at the wound edge paralleled that of actin to form the actomyosin purse-string. However, myosin II did not exist in the actin-rich filopodia/lamellipodia. In myosin II mutants, a robust actomyosin cable did not form but instead wound repair was achieved by an increase in the actin-rich protrusions from neighboring or opposing cells interacting with one another to bring the areas closer together.

The actomyosin cable is anchored cell-to-cell by cadherin-based adherens junctions, and E-Cadherin mutant embryos displayed impaired actin cable assembly and function. However, wound closure was once again accomplished by cellular actin protrusions. Given the importance of the actin protrusions in wound repair throughout the repair process, protrusion dynamics were studied by imaging transgenic embryos expressing fluorescently tagged plasma membrane markers. Indeed, cellular actin protrusions emanating from one region of the wound made connections to actin protrusions from other wound regions. The activity of the protrusions was disrupted in Cdc42 mutants (a small ATPase known to regulate filopodial function) to reveal an extended duration of contraction and the absence of closure. Lastly, all stages of wound repair exhibited defects in mutant embryos with defective actin purse string AND protrusion function, suggesting that both mechanisms are necessary for efficient wound repair. The imaging techniques and transgenic animals generated during these studies will fuel subsequent investigations of wound repair and will help to answer a major outstanding question: What are the signaling pathways that respond to and initiate the wound repair process?

Abreu-Blanco MT, Verboon JM, Liu R, Watts JJ, Parkhurst SM. 2012. Drosophila embryos close epithelial wounds using a combination of cellular protrusions and an actomyosin purse string. J Cell Sci. Epub ahead of print, doi: 10.1242/jcs.109066.



Adapted from the manuscript

An epithelial wound in an embryo expressing an e-cadherin-gfp fusion (green) and the actin binding domain of moesin-mcherry (red) (a reporter for actin) roughly 50 minutes after wounding (during the contraction phase).