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Fyn regeneration

A Src family kinase and several other signaling pathways help repair zebrafish fins after wounding.

Though all animals do their best to repair injured tissues—closing wounds and mounting an inflammatory response—some species' repair programs are better than others. Certain amphibians can regrow lost limbs, for example, and zebrafish can completely regenerate injured fins. How these animals sense the initial wound and induce tissue regeneration is unclear, however. Yoo et al. identify several zebrafish signaling pathways that are only transiently activated immediately after wounding but are essential for the subsequent regeneration of damaged fins (1).

Anna Huttenlocher and colleagues at the University of Wisconsin-Madison previously found that wounding zebrafish larvae induced the generation of reactive oxygen species (ROS), especially hydrogen peroxide, which activated the Src family kinase (SFK) Lyn in neutrophils (2). "Lyn functions as a redox sensor to detect hydrogen peroxide and direct neutrophils to the wound site," explains Huttenlocher. "But when we were doing these studies, we noticed that Src family kinases were also activated in the fin epithelium. So we were interested to see what the signaling [in this tissue] did."

Huttenlocher and colleagues, led by Sa Kan Yoo and Christina Freisinger, found that the activation of epithelial SFKs immediately after wounding was also dependent on ROS (1). Inhibiting the ROS-producing enzyme NADPH oxidase, or depleting the hydrogen peroxide–generating enzyme dual oxidase, inhibited SFK activation in the epithelia of wounded fins. The researchers also identified several other early wound signaling events that occurred independently of ROS and SFKs. Wounding zebrafish fins stimulated the activation of the kinase ERK in epithelia and, just like in *C. elegans* (3), induced a burst of calcium release from intracellular stores.

"So wounding induces all of these early signals that are very transient," Huttenlocher says, adding that, because dual oxidase has previously been shown to promote the regrowth of injured tail fins (4), "we wanted to address whether these early signaling events impacted fin regeneration several days later."

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Yoo et al. found that blocking ROS production or SFK activity for one hour before and after wounding impaired the regeneration of zebrafish fins three days later. Inhibiting calcium release or ERK signaling had a similar effect. But regeneration was normal if the signaling pathways weren't inhibited until 3–5 hours after wounding, revealing a brief time window in which early wound signals set up later repair events.

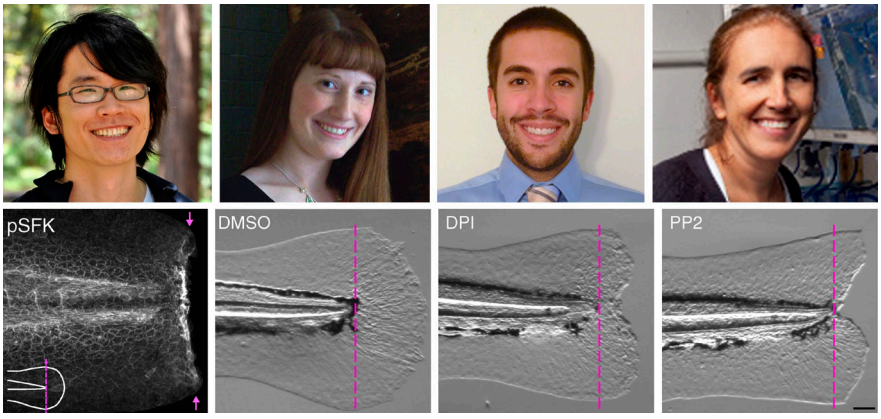
Although inhibiting ROS and SFK signaling caused an abnormal inflammatory response, this wasn't the cause of the fin regeneration defects because fish lacking neutrophils and macrophages still showed impaired fin regrowth after treatment with ROS and SFK inhibitors. Yoo et al. therefore examined whether epithelial SFKs were important for regeneration.

"We found that Yes and Fyn are the two SFKs in the epithelium, and when we depleted Fyn we saw a regeneration defect," Huttenlocher recalls. "So that supported the idea that signaling within the epithelium by the SFK Fyn is critical for the regenerative phenotype."

It remains to be seen how Fyn—or any of the other early signaling pathways induced by wounding—promotes subsequent tissue regeneration. Blocking ROS production or depleting Fyn reduced the proliferation of precursor blastolemal cells in regenerating fins, and, at least in mice, Fyn has also been shown to control differentiation (5). "There are probably changes in gene expression involved in the long-term ability of fins to regenerate," says Huttenlocher. "But we don't yet know the exact targets that mediate this effect."

In the longer term, Huttenlocher is intrigued by the possibility that tweaking these early wound signaling pathways might boost the regenerative capacity of mammalian tissues. "There's evidence that redox signaling is involved in wound healing in mice," Huttenlocher says. "So if you stimulate these pathways, can you improve the healing and regenerative response in mammals?"

Focal Point



PHOTOS COURTESY OF THE AUTHORS

(Top row, left to right) Sa Kan Yoo, Christina Freisinger, Danny LeBert, and Anna Huttenlocher describe several signaling events that occur immediately after wounding and are critical to the subsequent regeneration of damaged zebrafish tail fins. In particular, reactive oxygen species activate the Src family kinase Fyn in the fin epithelium 30 minutes after wounding (bottom row, left). Three days later, a control fish (second from left) has regrown its fin from the initial wound site (dotted line), whereas regeneration is impaired in fish briefly treated with inhibitors of reactive oxygen species (second from right) or Src family kinases (right).

1. Yoo, S.K., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201203154.
2. Yoo, S.K., et al. 2011. *Nature*. 480:109–112.
3. Xu, S., and A.D. Chisholm. 2011. *Curr. Biol.* 21:1960–1967.
4. Rieger, S., and A. Sagasti. 2011. *PLoS Biol.* 9:e1000621.
5. Cabodi, S., et al. 2000. *Mol. Cell*. 6:1121–1129.