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Researchers Buzzing Over New Wound-Healing Model

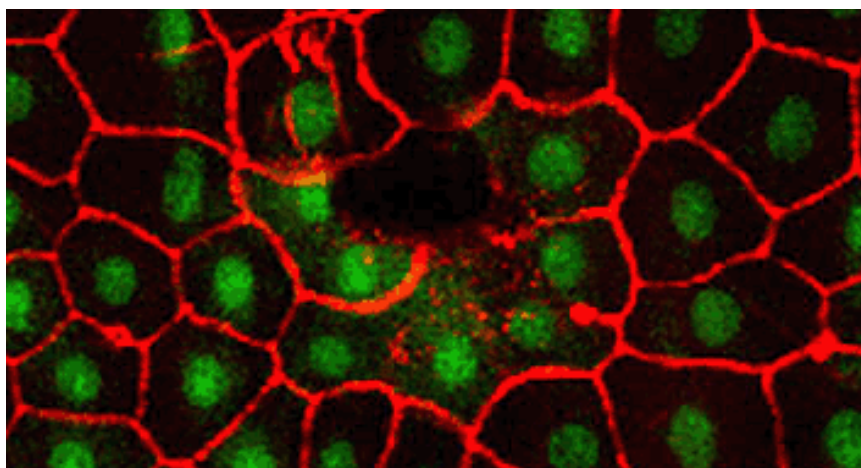


Image Title: The image shows epidermal cell nuclei (green) in fruit fly larvae. The dark hole in the center of the image is a wound that is healing. - Krasnow Lab/HHMI at Stanford University School of Medicine

Howard Hughes Medical Institute researchers have made progress in understanding the genetic program that is deployed to help wounds heal. Their insights come via a somewhat surprising route - hours spent making tiny punctures in fruit fly larvae, then observing and analyzing how the cells recover.

The painstaking analysis reveals that two biochemical pathways are involved in wound healing. And because the work was done in fruit flies, which are amenable to a wide range of genetic tools, there is hope that the new model will guide researchers to points in the wound-healing process that can ultimately be controlled by clinicians. With time and better understanding of how wounds are repaired, doctors may be able to intervene more successfully in cases where wounds fail to heal properly.

"Our hope is that understanding the molecular pathways in the fly, to the extent that they are conserved in humans, might allow the design of mechanistic and pharmacological approaches to promote or alter wound-healing to greatly improve outcomes."

— **Mark A. Krasnow**

The researchers, postdoctoral fellow Michael J. Galko and Howard Hughes Medical Institute investigator Mark A. Krasnow, both at the Stanford University School of Medicine, published their findings in the August 2004 issue of *Public Library of Science Biology*.

Krasnow, whose lab is known for studies of genetic control of lung development, is branching out because he realizes that a simplified model of wound healing is badly needed. "Because of its clinical importance, there has been a huge amount of work in mammalian wound-healing. The challenge has been that the process is so complicated in mammals," said Krasnow. "And it's not possible to conduct the necessary interventional experiments on humans in a clinical setting.

"There have been attempts to study a simplified process in cell culture, but that approach removes the process from its natural context," he said. "What Michael has done is develop a genetically tractable wound-healing model that allows genetic dissection of this complex process."

The fruit fly *Drosophila* has a long history of genetic study, so it made sense to Galko and Krasnow to consider using it as the basis for a simplified model system. Wound-healing is likely quite similar in flies and humans, said Krasnow, since the basic process is probably evolutionarily ancient, with its major components in place since before insects and mammals diverged.

To create a wound, Galko made tiny punctures through the outer cuticle, or exoskeleton, and into the epidermis of fly larvae with a needle about the diameter of a human eyelash. Galko also perfected a technique to create larger wounds that do not form a scab. Without an opaque scab obstructing their view, researchers can see changes in the epidermal cells directly. To produce these scabless wounds, Galko pinched the transparent larval cuticle to damage the epidermal cells beneath the cuticle surface.

Once the researchers created the wounds, they could analyze the genetic changes in the surrounding cells during healing. Any cellular changes could be seen using a microscope. The researchers observed that while puncturing the epidermal cells caused immediate bleeding, a plug that became the basis for scab formation rapidly sealed the wound.

“We saw that the epidermal cells around the wound rapidly began to fuse with each other and orient themselves toward the wound, extending long, fine processes along the plug,” said Galko. “And by eight hours after wounding, the processes from opposite ends of the wound contacted one another and reestablished continuity of the epidermis.”

To study the genetic control of the process, the researchers analyzed the activation of a signaling pathway involving the enzyme Jun N-terminal kinase (JNK), which is activated by cellular stress, and has been associated with the wound-healing process. The researchers found that the pathway was dramatically activated around the fly wounds during healing. Inactivating the pathway inhibited epidermal spreading and epithelial restoration, but did not affect scab formation, indicating that the two processes are separately controlled.

In contrast, when the researchers inactivated another gene, called *lozenge*, they blocked scab formation but the epidermal cells still tried to spread and close the hole in the epidermis. To their surprise, the researchers found that without a scab, the JNK pathway became hyperactive. This showed that scab formation and epithelial reestablishment, although under separate genetic control, can influence each other.

“Wound healing isn't like a bucket brigade putting out a fire,” said Krasnow. “It's more like a modern fire department with different firefighters responding to and containing different parts of the damage, each calling out to let the others know how their task is progressing and if they need help.”

According to Krasnow, the distinct but interdependent genetic responses are evidence of coordination by multiple signals emanating from the wound site. Their findings set the stage for the identification and characterization of those signals and the cellular processes that they control. Further studies, Krasnow said, will involve searching for mutations in fly genes that affect wound healing and tracing the genes involved.

Krasnow said that the new insights into wound healing could have important clinical implications. “Many diabetics suffer such problems as foot ulcers, in which wounds fail to heal. And in some cases of trauma, exaggerated wound-healing responses can create disfiguring scars. Thus, it's extremely important to learn to speed up wound-healing or change it to a scarless process. However, attempting to develop such treatments in the absence of a basic cellular and mechanistic understanding of the process is a huge gamble, and it's difficult to imagine that it could be successful.

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