

Wound Healing Initiated By a Ubiquitous Connexin and a MAGUK Protein

April 16, 2012

ME Arnegard

Gap junction channels play fundamental roles in intercellular communication by electrically and metabolically coupling adjacent cells. In vertebrates, these channels are made up of connexin proteins, which are critical to a large number of physiological processes. For example, connexins and the channels they form help synchronize the contraction of heart muscle cells; they allow astrocytes to feed brain neurons and support the synapses between them; and they provide dynamic communication channels in skin that help direct cell migration and keratinocyte differentiation during wound healing. One of the most striking features of connexins is their rapid 'life cycle'. Most connexins have a half-life of only a few hours, including the most ubiquitous connexin in mammals, Cx43. Regulatory events that underlie channel assembly and rapid turnover directly influence the extent of gap-junctional communication between cells. Moreover, gap junctional channel formation, stability and degradation are all regulated by various protein kinases and other proteins that act as connexin binding partners. One such binding partner is zonula occludens 1 (ZO-1), which is a member of the membrane-associated guanylate kinase (MAGUK) family of proteins. ZO-1 interacts with Cx43 in a manner that depends on its PDZ domain, a common structural motif of 80-90 amino acids found in ZO-1 and other signaling proteins. Another MAGUK, calcium/calmodulin-dependent serine kinase (CASK), is a multi-domain scaffolding protein known to contribute to the assembly of gap junctional components in skin cells and neurons.

Postdoctoral fellow Dr. Lucrecia Márquez-Rosado, principal investigator Dr. Paul Lampe and other members of the Lampe Lab (Human Biology Division) recently set out to better understand how Cx43 and CASK interact in skin and brain cells, as well as the role that these interactions may play in cell migration and wound healing. By conducting co-immunoprecipitation and GST pull-down experiments, Márquez-Rosado *et al.* first showed that Cx43 directly interacts with mammalian CASK *in vitro*. Co-localization of CASK and Cx43 was also detected in living mouse brain astrocytes and human foreskin cells (see figure). The authors further demonstrated that CASK associates with the C-terminal region of Cx43 in a non-PDZ-dependent manner, and that the strongest interaction occurs between CASK and a hypophosphorylated isoform of Cx43. To gain insights into the contributions of Cx43 and CASK to cell migration, Márquez-Rosado and co-authors experimentally expressed one or both proteins in canine kidney epithelial cells, after which they monitored cell

migration in a scratch wound assay. Keratinocyte migration into the wound site was inhibited if either protein was expressed alone. Additional experiments revealed a dynamic pattern of co-localization between Cx43, CASK and CADM1 (a protein that regulates epidermal adhesion and wound repair), which varied in a spatially regulated manner during wound healing. Immediately after wounding, CASK mobilizes to the plasma membrane, where it co-localizes with Cx43 and CADM1 as early as one hour into the healing process. Thus, it is possible that CADM1 may coordinate the later reductions in both CASK and Cx43 that are eventually necessary for proper wound healing to occur. The findings of Márquez-Rosado *et al.* show that the interaction between CASK and Cx43 occurs early in the connexin life cycle. Their results also suggest a plasma-membrane-targeting role for this interaction that clearly affects key physiological processes, such as cell migration and wound healing.

[Márquez-Rosado L, Singh D, Rincón-Arango H, Solan JL, Lampe PD.](#) 2012. CASK (LIN2) interacts with Cx43 in wounded skin and their coexpression affects cell migration. *J. Cell Sci.* 125:695-702.

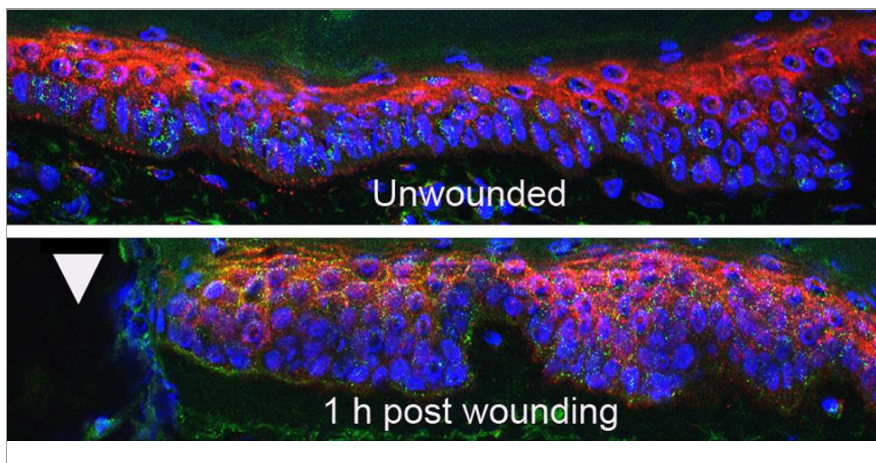


Image courtesy of Paul Lampe

Co-localization of Cx43 and CASK varies spatially and temporally during wound healing. Here, human foreskin explants were analyzed by confocal microscopy following immunostaining with antibodies to Cx43 (red) and CASK (green). Nuclei were counterstained with DAPI (blue). Epidermis (above in each panel) fluoresces strongly, whereas the dermis beneath appears mostly black. The upper panel shows control skin. The lower panel shows skin one hour after being wounded with a punch biopsy (white arrowhead marks wound edge). Note the increasing gradient of green from right to left in the wounded foreskin, indicating greater CASK mobilization, as well as the increased co-localization of Cx43 and CASK (yellow) near the wound margin.