Phosphorylating Communication

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Gap junctions are intercellular connections between adjacent cells that allow for communication by facilitating the diffusion of ions and other metabolites between cells. These connections are composed of integral membrane proteins from the connexin family. Connexin43 (Cx43) is the most ubiquitous member of this protein family and it is the most widespread in different tissues. Previous studies have shown that proteasomal inhibition increases gap junction size (Dunn, et al., 2012); however, it is unclear how this activity is regulated. In a recent report published in the *Journal of Cell Science*, Drs. Clarence A. Dunn and Paul D. Lampe (Divisions of Human Biology, Public Health Sciences, and Translational Research Program) demonstrate that Akt phosphorylates Cx43 at amino acid S373 (pS373). This phosphorylation acts as a molecular switch to mediate gap junction size and intercellular communication both in vitro and in vivo in response to injury.

Cx43 S373 is predicted to be an Akt substrate. To confirm this prediction, the authors generated a phospho-specific antibody targeting this site. Using this antibody they showed that, like other Akt substrates, proteasome inhibition increased pS373 levels (3.2+/-0.4 fold, $p=0.007$). This increase in pS373 was completely eliminated with the addition of the Akt1 inhibitor MK2206. By immunofluorescence studies, the authors found that pS373 preferentially localized to the center of larger gap junctions. Similarly, cells expressing a phosphomimetic S373D mutant formed larger gap junctions ($p\leq0.003$), while a non-phosphorylatable S373A mutant Cx43 failed to form gap junctions. These differences in gap junction size had a functional impact, as the larger gap junctions in S373D mutants increased intercellular communication, allowing the cells to recover from photobleaching faster than wildtype Cx43 ($p<0.001$). In contrast, S373A mutants recovered more slowly than wildtype ($p=0.03$). Previous studies have shown that the protein ZO-1 associates with Cx43 and that disrupting this interaction also leads to larger gap junction formation (Rhett, 2011). Therefore, Dunn tested the impact of pS373 on this interaction and found that pS373 or S373D effectively eliminated ZO-1:Cx43 interaction, while non-phosphorylated S373A mutants strongly interacted with ZO-1 and the cell membranes lacked punctate structures characteristic of gap junctions. These results confirm that the phosphorylation state of S373 acts as a molecular switch for Cx43:ZO-1 interaction.

To test the importance of pS373 during injury response, the authors subjected cells to scratch wounding and hypoxic conditions. Under both conditions, cells displayed a rapid and significant

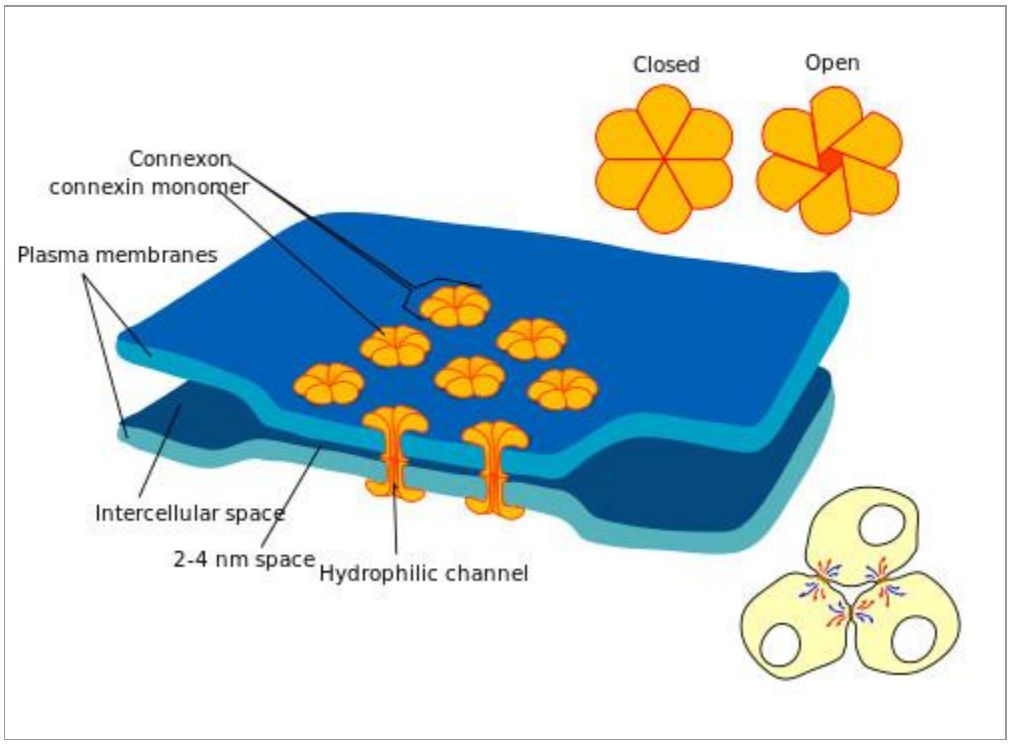
increase in pS373 levels with a concomitant increase in gap junctional Cx43 which then decreased over time. In more biologically relevant models, mouse hearts subjected to 30 minutes of no-flow ischemia had significant increases in pS373 levels (3.6+/-0.4 fold increase, p=0.001) and pronounced relocalization of Cx43 from the ends of myocytes to punctate structures along the myocyte edges. Similarly, an epidermal wound assay found that pS373 levels increased significantly (43+/-11%, p=0.003) at the wound edges relative to control skin.

Taken together, this study demonstrates that Akt-mediated phosphorylation of Cx43 governs its interaction with ZO-1 to control the size of gap junctions and regulate intercellular communication. The rapid accumulation of phosphorylated Cx43 at sites of tissue damage suggests that the phosphorylation of S373 acts as a molecular switch to maintain intercellular communication, perhaps as a mechanism to activate and recruit factors to the site of injury.

Dunn CA and Lampe PD. Injury-triggered Akt phosphorylation of Cx43: a ZO-1-driven molecular switch that regulates gap junction size. *J Cell Sci*. 127:455-464.

See also: Dunn CA, Su V, Lau AF, Lampe PD. 2012. Activation of Akt, not connexin 43 protein ubiquitination, regulates gap junction stability. *J Biol Chem*. 287(4):2600-7.

See also: Rhett JM, Jourdan J, Gourdie RG. 2011. Connexin 43 connexon to gap junction transition is regulated by zonula occludens-1. *Mol Biol Cell*. 22(9):1516-28.



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Gap junctions, composed of connexin proteins (orange), are integral membrane proteins that connect the cytoplasmic spaces of adjacent cells to facilitate intercellular communication.