IDENTIFICATION OF THE PORE-LINING HELICES OF CONNEXIN 43 GAP-JUNCTIONAL HEMICHANNELS

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The gap-junctional channels that mediate intercellular communication are formed by head-to-head docking of two gap-junctional hemichannels from adjacent cells. The hemichannels are hexamers of connexins, proteins that have four transmembrane helices. The transmembrane helices that line the gap-junctional pore have not been identified, and their identification was the main goal of my dissertation project. To accomplish this goal, I used a combination of molecular biology, biochemical and biophysical techniques that include poly-alanine helix scanning mutagenesis, the substituted cysteine accessibility method and luminescence resonance energy transfer. Using the latter methodology in particular, as well as a new method to produce purified hemichannels of controlled subunit composition, I was able to assign all helices in the available low-resolution cryoelectron microscopy structure published by others, where helices are named A through D, and generate the first model of gap-junctional channels and hemichannels based on experimental structural measurements. In this model, connexin transmembrane helices 1 through 4 correspond to helices A, C, B and D, respectively. Luminescence resonance energy transfer is a powerful method for structural studies of membrane proteins in their native bilayer environment. Taken advantage of this methodology, in combination with the generation of hemichannels of controlled subunit composition, I was also able to determine that PKC-mediated phosphorylation of Ser368 produces a partial closure of the Cx43 hemichannel pore, that this effects requires phosphorylation of all six Cx43 monomers in the hemichannel, and that the decrease in permeability is accompanied by significant conformational changes of the connexin molecules. These changes involve increases of the distances separating the C-terminal ends of the subunits and decreases in the distances separating the pore-lining helices; both changes in inter-subunit distances are of the order of several Angstroms. These results indicate that a simple ball-and-chain mechanism cannot explain the gating of Cx43 hemichannels by PKC-mediated phosphorylation and that a significant re-arrangement of pore helices takes place instead. In summary, my results allowed me to generate an experimentally-based model gap-junctional channels and hemichannels and to gain insight into the molecular mechanism of Cx43 regulation by PKC-mediated phosphorylation.

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