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Electron microscopy and functional analysis of recombinant innexin gap junctions

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Innexin is a molecular component of invertebrate gap junctions, which have an important role in neural and muscular electrical activity in invertebrates. Although the structure of vertebrate connexin26 was revealed by X-ray crystallography [1], the structure of innexin channels remains poorly understood. To study the structure of innexin gap junction channels, we expressed and purified Caenorhabditis elegans innexin-6 (INX-6) gap junction channels, and characterized their molecular dimensions and channel permeability using electron microscopy (EM) and a fluorescent dye transfer assay, respectively [2]. Negative-staining and thin-section EM of isolated INX-6 gap junction plaques revealed a loosely packed hexagonal lattice. We performed single particle analysis of purified INX-6 channels with negative-staining and cryo EM. Based on the negative-stain EM images, the class average of the junction form had a longitudinal height of 220 Å, a channel diameter of 110 Å in the absence of detergent micelles, and an extracellular gap space of 60 Å, whereas the class average of the hemichannels had diameters of up to 140 Å in the presence of detergent micelles. Cryo EM images revealed rotational peaks that could be related to the INX-6 subunits. Structural analysis of the reconstituted INX-6 channels with single particle analysis and electron tomography suggested that the oligomeric number of the INX-6 channel was distinct from that of the dodecameric connexin channel. Dye transfer experiments indicated that the INX-6-GFP-His channels were permeable to 3-kDa and 10-kDa dextran-conjugated tracers. These findings indicate that INX-6 channels have a characteristic oligomer component that differs from that in connexin gap junction channels.

[1] *S. Maeda, S. Nakagawa et al., Nature, 2009, 458, 597–602,* ***[2]*** *A. Oshima, T. Matsuzawa et al., J. Biol. Chem., 2013, 288, 10513-10521*

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