Analysis of ion channel stoichiometry within single cells via liquid-phase electron microscopy and modeling

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The stoichiometry of membrane protein complexes forming ion channels varies between different functional states. Most knowledge about the stoichiometry-function relationship has been obtained from pooling materials from many cells, and using methods such as x-ray crystallography, and gel electrophoresis thus obtaining information about population averages only. We have employed liquid-phase scanning transmission electron microscopy [1] to directly image individual channels within intact cells. Two different channels were studied, ORAI1 proteins forming ion channel subunits of the Calcium Release Activated Calcium channel complex [2], and hTMEM16A forming a calcium-activated chloride channel [3]. Quantum dot nanoparticles were specifically attached to the proteins for their detection. Electron microscopy images revealed the individual label locations. This data was analyzed using the pair correlation function, and an analysis of cluster size and frequency was performed. The experimental results were compared to a mathematical model involving cluster probabilities as function of the labeling efficiency from which it was deduced that ORAI1 was present in hexamers in a small fraction, and mostly in monomers and dimers. hTMEM16A, on the other hand, resided in the plasma membrane as dimer only and was not present as monomer.

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