Biochemical and electrophysiological approaches for studying the stoichiometry of ORAI channels

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The dynamics of intracellular Ca^{2+} signals govern a wide variety of cellular functions. Especially for long lasting processes cells rely on the so-called store-operated Ca^{2+} entry pathway. STIM1 proteins in the endoplasmic reticulum (ER) sense a decrease of the Ca^{2+} concentration, then react by clustering and trapping of ORAI1 proteins, located in the plasma membrane, to form functional Ca^{2}+ channels in close apposition to the ER. ORAI channel stoichiometry may thus change during different functional states (i.e. at rest, and during channel activation). The assembly and stoichiometry of ORAI channels remains controversial with dimeric, tetrameric as well as hexameric assemblies being reported. To address these possibilities we generated cells lacking endogenous ORAI1-3 channels using CRISPR-Cas9 mediated gene deletion. We used these cells to express monomeric ORAI1 channels or ORAI1 concatenated to a second ORAI1 or an ORAI2 subunit. We expressed *ORAI1* with a genetically encoded HA tag and treated cells with cell membrane (im)-permeable cross linkers. Our results showed that the stoichiometry of the ORAI1 multimers did not change upon activation of cells. Furthermore, blue native gel analysis showed that ORAI1 exists mainly as a multimeric complex of an estimated molecular weight of at least a hexameric channel. Electrophysiological and fura-2 based Ca^{2+} imaging analyses of heterodimeric ORAI1-ORAI2 channels with varied concatenation sequence, indicate that the biophysical properties of the channel are mainly determined by the channel occupying the second subunit position.

[1] Hou, X., Pedi, L., Diver, M. M. and Long, S. B., Science 338, 1308-13. (2012).