#7: Orai1 Concatemers Reveal a Hexameric Orai1 Channel Assembly

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The multimeric assembly of the CRAC channel has remained a contentious issue despite strong crystallographic evidence of a hexameric structure for both Drosophila and human Orai1. Recent studies suggested concatemeric tetramers of Orai1 mediate authentic CRAC current whereas equivalent hexameric concatemers form only non-selective cation channels. We expressed concatenated human Orai1 constructs with intervening 36-aa linkers. We observed that expression of dimers, trimers, tetramers, pentamers and hexamers of human Orai1 in HEK cells stably expressing STIM1-YFP (HEK STIM1-YFP), all gave rise to similar high levels of Ca2+ entry. All constructs were C-terminally tagged with tdTomato and all were observed to be exclusively PM-expressed. Each construct also gave similar Ca2+ entry when expressed in HEK Orai1 knockout cells stably expressing STIM1. Expressed in HEK STIM1-YFP cells, the Orai1 dimers, trimers, tetramers, pentamer and hexamers all gave rise to authentic CRAC channel activity with similar inward rectification and reversal potential. Substituting wt-Orai1 with the pore-inactive E106A mutant in tetramers, we observed that the initial two N-terminal units of the tetramer are crucial for channel activity. The remaining two C-terminal residues in tetramers are inconsequential for function. In hexamers, the position of inactive mutants in the concatemer are not specific. Substitution with a single E106A mutant monomer in the hexamer had the same effect in reducing channel function at each position. Substitution with two E106A residues resulted in little remaining channel function. We interpret these results to reveal that hexamers are likely the true functional Orai1 channel unit. Tetramers are likely to feed dimers into a hexameric structure, with the two C-terminal residues outside the hexameric ring, explaining why these last two residues in the tetramer can be substituted for E106A mutants without loss of activity. Certainly, the hexameric concatemer gives a fully functional CRAC channel.