

Examination of the Association of KCNQ2 and KCNQ3 K⁺ Channel Proteins As Homomultimers and Heteromultimers Through TIRF Microscopy

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Total Internal Reflection Fluorescence (TIRF) is used in combination with proteins specifically chosen for their ability for Fluorescence Resonance Energy Transfer (FRET) to determine the prevalence and association of M-type KNCQ2 and KCNQ3 proteins in cell membranes. These proteins, in the form of tetramers, form potassium channels. For FRET, Cyan Fluorescing Protein (CFP) is used as the donor fluorophore, while Yellow Fluorescing Protein (YFP) is used as the acceptor. These proteins are connected with the KCNQ channel proteins as an extension of the proteins. Both homomultimers and the heteromultimers of KCNQ2 and KCNQ3 were examined. By photobleaching the energy receiving protein (YFP), the percent of FRET is determined and is used to prove the extent of association of the channel proteins (only those proteins that are immediately adjacent to one another may undergo FRET). KCNQ2 and KCNQ3 expressed together was found to preferentially form multimers, whereas KCNQ2 expressed alone in the membrane seems less inclined to form multimers (and perhaps are expressed mainly as monomers). No conclusive data could be obtained for KCNQ3 homomultimers.