Bacteriorhodopsin chimeras containing the third cytoplasmic loop of bovine rhodopsin activate transducin for GTP/GDP exchange

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The mechanisms by which G-protein-coupled receptors (GPCRs) activate G-proteins are not well understood due to the lack of atomic structures of GPCRs in an active form or in GPCR/G-protein complexes. For study of GPCR/G-protein interactions, we have generated a series of chimeras by replacing the third cytoplasmic loop of a scaffold protein bacteriorhodopsin (bR) with various lengths of cytoplasmic loop 3 of bovine rhodopsin (Rh), and one such chimera containing loop 3 of the human beta(2)-adrenergic receptor. The chimeras expressed in the archaeon Halobacterium salinarum formed purple membrane lattices thus facilitating robust protein purification. Retinal was correctly incorporated into the chimeras, as determined by spectrophotometry. A 2D crystal (lattice) was evidenced by circular dichroism analysis, and proper organization of homotrimers formed by the bR/Rh loop 3 chimera Rh3C was clearly illustrated by atomic force microscopy. Most interestingly, Rh3C (and Rh3G to a lesser extent) was functional in activation of GTPgamma(35)S/GDP exchange of the transducin alpha subunit (Galphat) at a level 3.5-fold higher than the basal exchange. This activation was inhibited by GDP and by a high-affinity peptide analog of the Galphat C terminus, indicating specificity in the exchange reaction. Furthermore, a specific physical interaction between the chimera Rh3C loop 3 and the Galphat C terminus was demonstrated by cocentrifugation of transducin with Rh3C. This Galphat-activating bR/Rh chimera is highly likely to be a useful tool for studying GPCR/G-protein interactions.

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