Creation of molecular biological tools for investigations of G-protein-coupled receptor signalling

Our group is focusing on the intracellular signaling of G-protein-coupled receptors (GPCRs). We are also developing tools for our own investigations, and these tools will be made accessible for other investigators as well. Protein domains can be utilized to create measurement probes and inhibitors with very high selectivity for physiological signals. In this project we want to develop tools of both of these categories.

The most novel family of heterotrimeric G-proteins, the immediate signal transducers of GPCRs, is the $G_{12/13}$ family. Activation of $G\alpha_{12}$ and $G\alpha_{13}$ leads to activation of novel signal transducers such as Rho family small G-proteins. This occurs via interaction of RhoGEFs such as Lsc, which display a highly $G_{12/13}$-specific RGS domain. In this project, Lsc-RGS will be fused to green fluorescent protein (GFP). Translocation of this construct from the cytosol to the cell membrane should occur upon $G\alpha_{12/13}$ activation, and this can be visualized by the help of the GFP fluorescence.

$G\alpha_{12}$ and $G\alpha_{13}$ also interact with Ser/Thr phosphatase type 5 (PP5). The NH_2-terminal regulatory tetratricopeptide repeat (TPR) domain binds $G\alpha_{12}/G\alpha_{13}$ with high specificity. The TPR domain of PP5 should, similar to the RGS-domain of Lsc, translocate from the cytosol to the plasma membrane upon $G_{12/13}$ activation. A PP5-TPR-GFP vector has been produced by another group (Gentile et al) for another purpose, and this can be used as a control for the Lsc-RGS-GFP construct.

Ca^{2+} is a very central intracellular messenger. Ca^{2+} concentration in the cytosol can be elevated via Ca^{2+} release from the intracellular stores or via Ca^{2+} influx. Probably the most general and classical Ca^{2+} signaling pathway is the phospholipase C $\rightarrow$ inositol-1,4,5-trisphosphate (IP_3) -dependent Ca^{2+} release. This exists in all the cell types, and may often mask other Ca^{2+} signaling pathways. Thus, for investigations of the other pathways, it would be very useful to be able to specifically block IP_3-dependent Ca^{2+} signaling, as shown in our recent study (Ekholm et al., Biochem. Biophys. Res. Commun., 2007). *IP_3 sponge*, composed of the modified ligand-binding domain from the mouse IP_3 receptor, which absorbs IP_3 in competition with native IP_3 receptors, has been constructed in Professor Mikoshiba's group (Uchiyama et al, 2002). Although this construct shows a 1000-fold higher affinity for IP_3 than the IP_3 receptor type I, it still has to be expressed at high levels to effectively inhibit IP_3-dependent responses. A plasma membrane-linked IP_3 sponge would be thought to act as a more effective chelator of IP_3. In this project we want to construct two different plasma membrane-linked IP_3 sponges.

Thus, in this project three different expression plasmids will be constructed, a RGS-Lsc-GFP fusion and two different plasma membrane-linked IP_3 sponges. The plasmids will be constructed using basic cloning techniques, i.e. PCR and restriction enzyme digestion. The plasmids, together with the TRP-PP5-GFP fusion, will be expressed transiently in mammalian cell lines, and the expression verified using GFP fluorescence and Western blotting. Functionality will be tested using real-time imaging and the plasmids then used to investigate G-protein-coupled receptor signaling.

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