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Utilization of Bioluminescence Resonance Energy Transfer (BRET) to monitor ß-arrestin interactions in living cells

Abstract:
ß-arrestins are multi-functional adaptor proteins that mediate a wide variety of G-protein-coupled receptor (GPCR) functions: from receptor desensitization and internalization serving as scaffolding platforms for the regulation of multiple signaling cascades such as MAP kinases, PI3 Kinases, actin assembly proteins, monomeric GTPases and phosphodiesterases. Proteomics studies and functional assays have revealed nearly 100 scaffolding complexes that assemble on ß-arrestins and yet for a given GPCR, only a small subset appears to form. Thus, the interaction of a ß-arrestin with the GPCR to which it is recruited appears to dictate the available binding sites for downstream effector proteins as well as the location and duration of the ensuing signal. For some time, studies investigating these interactions relied onco-immunoprecipitations and co-localization studies to examine these protein-protein interactions, making it difficult to assess subtle differences in the assembly kinetics and conformation of ß-arrestin/GPCR complexes. Bioluminescence Resonance Energy Transfer (BRET), in which biochemical energy generated by bioluminescent proteins such as Renilla Luciferase excites a nearby fluorophore, has now been widely used to examine interactions between ß-arrestins and different GPCRs. We use BRET to monitor: 1)interactions between two ß-arrestin family members (tagged with luciferase) and 2 different GPCRs (Protease-activated-receptor-2 and neurokinin-1 receptor); 2) conformational changes within ß-arrestin-2 upon recruitment to different GPCRs using an intramolecular BRET biosensor, and 3) receptor-induced direct interactions of ß-arrestins with downstream signaling partners. These studies reveal specific phosphorylation residues within the C-termini of each receptor are important for determining the kinetics of ß-arrestin recruitment, a feature which correlates with their distinct patterns of internalization and ß-arrestin-dependent signaling and that the conformation of ß-arrestin is differentially altered depending upon the interacting protein. These studies suggest that the initial conformational change elicited in ß-arrestins upon their recruitment to a given receptor determines the ultimate signaling patterns and cellular responses observed. These studies point to the potential of BRET technology in the development of pathway-specific drugs targeting GPCRs.