

41. GNRH AND TRH RECEPTORS: MONITORING THE FORMATION OF DYNAMIC PROTEIN COMPLEXES IN LIVING CELLS

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Regulated protein-protein interactions are a key feature of many aspects of receptor activation and deactivation. Genetically encoded luminescent and fluorescent fusion proteins in conjunction with biophysical methods such as bioluminescence resonance energy transfer (BRET) have allowed us to monitor dynamic protein-protein interactions involving G-protein coupled receptors (GPCRs). GPCRs have been reported to undergo oligomerization and the formation of receptor complexes could alter both receptor pharmacology and function to provide an additional level of regulation. Using BRET we have confirmed the existence of oligomeric GPCR complexes in living cells and have been able to quantitatively assess the functional interactions of GPCRs with adaptor proteins such as the β -arrestins as well as with several other partner molecules. We show that subtypes of the TRH receptor (TRHR1 and TRHR2) undergo oligomerization and that each subtype interacts differentially with β -arrestin 1 and 2 isoforms. TRHR2 does not utilize β -arrestin 1, however this interaction does occur when TRHR1 is also present, suggesting that formation of the hetero-oligomeric unit can alter receptor trafficking. Although another GPCR, the GnRH receptor (GnRHR) is also capable of forming oligomers, its unique features make it unable to utilize β -arrestins to promote agonist-dependent internalisation rates. We have investigated interactions between the GnRHR and other proteins and demonstrate a novel interaction between GnRHR and E2F transcription factors involved in cell cycle arrest. By monitoring this interaction with BRET, we observed a rapid loss in binding between GnRHR and E2F after treatment with GnRH. This coincided with a GnRH-mediated change in E2F cellular distribution. GnRH mediates an antiproliferative effect in a range of cells expressing the GnRHR and our finding indicates the involvement of cell cycle arrest. Studies have utilized siRNA to knock-down expression of E2F transcription factors, while chimeric receptors and mutagenesis have been applied to define the sites of interaction between GnRHR and E2F factors. By monitoring dynamic interactions of engineered BRET fusion protein partners involved in GPCR-mediated events, we can more fully understand the mechanisms of ligand-induced processes like receptor trafficking, cell cycle and cellular proliferation.