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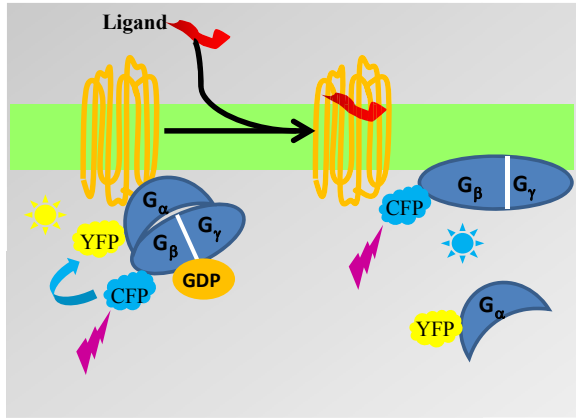

FIGURE 4.18 Functional activity of test compounds can be determined with double-labeled GPCR assemblies. Attaching two FRET compatible fluorescent proteins to different subunits of the G-protein complex enables a FRET interaction in the absence of an activating ligand. Activation of the GPCR by a functional agonist will separate the donor/acceptor pair, suppressing FRET emission. Functional antagonists will block separation of the FRET pair, preserving FRET emission.

in close enough proximity (Figure 4.18). As discussed in Chapter 3, in the absence of the natural ligand or agonist, the G-protein complex associates with the GPCR and GDP. Irradiation of CFP in the inactive complex produces fluorescence in the YFP through a FRET-mediated pathway. An activating ligand, however, will cause the G-protein complex to disassociate, disrupting the FRET interaction, and irradiation of CFP will produce CFP fluorescence rather than YFP fluorescence. This assay system can be used to identify both agonists and antagonists. Agonists will activate the system in the absence of the natural ligand, leading to an increase in CFP fluorescence at the expense of YFP fluorescence. On the other hand, the presence of an antagonist in the presence of the natural ligand will result in decreased activation of the GPCR signaling pathway, maintaining YFP fluorescence at the expense of CFP fluorescence. Variations on this general strategy have been employed to successfully study multiple GPCRs and their related signaling pathways.[43]

FRET technology has been successfully employed to study many other biochemical processes such as conformational changes in proteins,[44] assembly and dissociation of protein complexes,[45] and the distribution and transport of lipids.[46] Biosensors have also been designed to take advantage of FRET interactions. Proteins designed with molecular recognition sites that either bring together or separate a donor/acceptor FRET pair can be used to detect and quantitate analytes in solution (i.e., small molecules, proteins) by monitoring fluorescence at the proper wavelength.[47] This technology has been widely adopted since its initial development, and a