

Utilisation du BRET pour suivre le réarrangement structural des complexes pre-formés récepteurs/protéines G au cours de l'activation.

Probing the activation-promoted structural rearrangements within pre-assembled receptor/G protein complexes.

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G protein-coupled receptors (GPCRs) represent the largest family of proteins involved in signal transduction. The generally accepted signalling mode of these receptors involves an initial engagement of G $\square\square$ to the activated receptor followed by a rapid dissociation of G α and G $\beta\gamma$ into free active subunits that regulate the activity of diverse signalling effectors. Classically, this activation process is monitored indirectly through the generation of second messengers by the effectors or the modulation of nucleotide binding to the G α subunit. We have recently developed a bioluminescence resonance energy transfer (BRET) assay that directly monitors in real time the early interactions between GPCRs and the G protein subunits in living mammalian cells (Nature Methods, 2005). In addition to detect basal pre-coupling of the receptors to G \square subunits, BRET measured very rapid ligand-induced increase in the interaction between receptor and G $\alpha\beta\gamma$ complexes ($t_{1/2}$ <300 msec) followed by a slower (several minutes) decrease that reflects receptor desensitization. The agonist-promoted increase in GPCRs/G $\beta\gamma$ interaction was also highly dependent on the identity of the G α subunit present in the complex.

To further get insight on the nature of the agonist-promoted BRET between GPCRs and G protein subunits, we then monitored interactions at the interface between the receptor and the G proteins and between the G protein subunits by BRET between probes inserted at multiple sites within receptor/G protein complexes. Using the data obtained for the $\alpha_{2A}AR/G\alpha_{i1}\beta_1\gamma_2$ complex and based on the available crystals of G $\alpha_{i1}\beta_1\gamma_2$, we propose a model of receptor-mediated G protein activation whereby agonist binding leads to the structural reorganization of a pre-existing receptor/G protein complex that leads to the opening but not the dissociation of the G $\alpha/\beta\gamma$ interface. This conformational change, detected in our BRET assay, may reflect the motion required to allow the exit of the nucleotide exit from the G α subunit, thus reflecting the initial activation event of the G protein by the receptor.