BRET and FRET given their simplicity and straightforwardness.

Dimersization of GPCRs is a novel mechanism for receptor activation. It remains to be established whether GPCR dimerization occurs in vivo (i.e. in a living organism). Recent studies using energy transfer techniques to demonstrate the existence of dimers in living cells is a major step in this direction.

Selected references

Reply: beyond receptor dimerization

Stephane Angers and Michel Bouvier

We are grateful to Lakshmi Devi for her thoughtful comments on our recent work and her clear review of the latest publications on the oligomerization of G-protein-coupled receptors (GPCRs). As Devi mentioned, the use of resonance energy transfer techniques to study homo- or heterodimerization of different GPCRs has provided the first direct experimental evidence for the existence of receptor oligomers in living cells.

Although the four studies cited in Devi’s article used resonance energy transfer strategies to study receptor oligomerization, this approach could potentially be used to study other aspects of signal transduction events in whole cells. Discrete protein–protein interactions are at the heart of all signal transduction cascades and their spatio-temporal regulation is believed to provide the required level of diversity and selectivity that leads to coordinated cellular responses. Recent studies using yeast two-hybrid screens, co-immuno-precipitation and glutathione S-transferase (GST) pull down assays have revealed an unexpected level of complexity because they led to the identification of a larger than anticipated number of partners that interact with specific signalling molecules. For example, the regulatory protein β-arrestin, which is involved in GPCR desensitization and internalization, has been proposed to interact with Src, NSF (N-ethylmaleimide-sensitive factor), AP-2 (adaptor protein 2), clathrin and numerous receptors. Validation of these interactions in living cells and the study of their dynamic regulation represent major challenges that cannot easily be met using conventional approaches. Resonance energy transfer techniques represent an alternative way to look, in a non-destructive manner, at these interactions in real time. Using bioluminescence resonance energy transfer (BRET), we successfully monitored the agonist-promoted interaction between the β2-adrenergic receptor–luciferase fusion and the cytosolic protein arrestin-3–GFP (green fluorescent protein). This indicates that BRET can be used to study dynamic interactions involving proteins that are located in different cellular compartments under basal conditions but that transiently come into contact on activation of one of the partners. Thus, studying, in real time, each of the interactions proposed for a given protein should allow the construction of dynamic protein–protein interaction models. In addition, resonance energy transfer techniques could be used to screen for novel interactions. This could be particularly useful when considering hydrophobic proteins that cannot easily be translocated to the nucleus of yeast and thus cannot be studied adequately using yeast two-hybrid systems.

Because the elucidation of molecular networks that assemble and disassemble within specific subcellular domains represents one of the next steps in our understanding of cell functions, new techniques that are capable of measuring protein–protein interactions in living cells will offer major advantages over currently existing techniques. Although FRET has been used for some time to study molecular interactions, the recent discovery and development of a large number of fluorescent and luminescent proteins and markers that have spectral properties compatible with their use in either FRET or BRET will undoubtedly lead to a flurry of activity in this field.

Reference


S. Angers, Ph.D. Student, E-mail: angers@magellan.umontreal.ca
and
M. Bouvier, Professor, Department of Biochemistry, Université de Montréal, C.P. 6128, Down-town station, Montréal, QC, Canada, H3C 3J7, E-mail: bouvier@ BCM.umontreal.ca

C O M M E N T

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