

Toward understanding GPCR dimers

Charles Parnot & Brian Kobilka

Recent studies on the metabotropic glutamate receptors provide new insights into their signaling mechanisms that may apply to other G-protein coupled receptor (GPCR) families.

G-protein-coupled receptors (GPCRs) mediate cellular responses to the majority of hormones and neurotransmitters, as well as the senses of sight, smell and taste. Although they have been studied for >30 years, it was only recently that GPCR dimers were identified and accepted as physiological entities and not simply biochemical artifacts. The existence of GPCR dimers has been most clearly defined for family C GPCRs. The work by Tateyama *et al.*¹ (Michihiro Kubo's group) and of Kniazeff *et al.*² (Jean-Philippe Pin's group) in *Nature Structural & Molecular Biology* make the case for a strong link between dimerization and activation. They provide new insight into the link between agonist binding to the N-terminal extracellular domains and structural changes in the hydrophobic membrane spanning domains. The structural changes associated with symmetrical dimers could be a key mechanism for ensuring specificity as well as amplifying the conformational changes triggered by binding small molecule ligands.

These results are the latest developments in a series of fascinating studies exploring the structural and functional aspects of dimerization of the γ -aminobutyric acid receptor type B (GABA_B) and the metabotropic glutamate receptors (mGluR)³. Both belong to a relatively small group of GPCRs, the family C, characterized by an unusually large extracellular N-terminal domain linked to a hydrophobic domain (HD) with the seven-transmembrane (TM) topology common to all GPCRs. This N-terminal domain is responsible for ligand binding and has a characteristic structure known as the 'Venus flytrap' (VFT) module (Fig. 1a). A soluble form of the mGluR1 VFT has been crystallized in its open state (empty or antagonist-bound) and in its closed state (agonist-bound)^{4,5}. Interestingly, the VFTs were found as dimers in the crystal, consistent with other experiments showing dimerization of the intact mGluRs. Some crystal forms show that closure of only one VFT in the dimer is

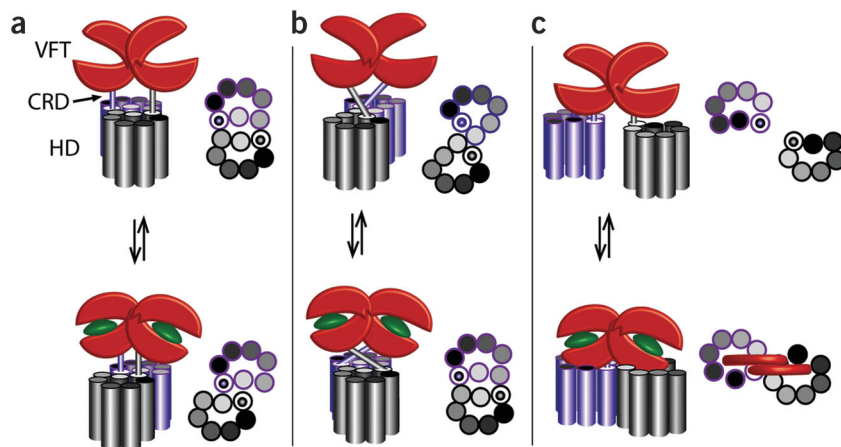


Figure 1 Three structural models that could explain activation of mGluRs. The VFTs (red claw) closes upon agonist binding leading to movement of their C termini closer to each other. The transmembrane segments (black and blue cylinders) are linked to the VFT through a cysteine-rich domain (CRD) (gray). (a) This model assumes no intermolecular rearrangement of the transmembrane segments but only changes in the relative orientation of the HDs. (b,c) In these models, intramolecular rearrangements occur, either indirectly (b) and/or by direct interaction of the VFT with the TM segments (c). For illustration, the VFT in a is linked to TM1 by a direct, rigid connection. In b, the link between the VFT and TM1 is compatible with HD movements observed by Tateyama *et al.*¹.

sufficient to induce a large rearrangement of both protomers, reaching a conformation close to the active state seen when both VFTs are occupied with ligands. In parallel, studies have demonstrated that the GABA_B is an obligate heterodimer GABA_{B1}-GABA_{B2}⁶, where only the GABA_{B1} VFT binds the ligand⁷ while the HD of the GABA_{B2} mediates G-protein activation⁸ (in *trans*).

The studies of Tateyama *et al.*¹ address the effect of ligand binding to the VFTs on the relative position of the HDs. Using fluorescent proteins inserted in the cytoplasmic loops of the HD domains as probes, they monitored the changes in the relative orientation of the two HDs of the mGluR1 α dimers in intact cells. Their results suggest that upon activation by glutamate, the second intracellular loops of the protomers move closer toward each other, while the first intracellular loops move further apart. Their work is the first experimental proof that a relatively large rearrangement of the HDs of a dimer occurs upon ligand binding to the VFTs, largely consistent with the predictions made from the VFT crystal structures.

In the second paper, Kniazeff *et al.*² studied the functional interaction of the two partners of the mGlu5 dimer. They generated mGlu5-GABA_B chimeras—taking advantage of the unique properties of the C-terminal tails of the GABA_{B1} and GABA_{B2} receptors in the protein quality control process—to ensure formation on the cell surface of specific combinations of mGlu5 mutant dimers. For instance, in an mGlu5 dimer examined in this study, only one of the VFT was impaired for glutamate binding, and the response of this dimer to glutamate was biphasic with each phase corresponding to the binding of one ligand to one VFT. Using this and various other engineered dimers with altered binding or coupling defects, Kniazeff *et al.*² show that the closure of one binding domain is sufficient for partial activation, but the closed state of both binding domains is required for full activity. They also show that G-protein activation occurs both in *cis* and in *trans*. Their results generalize the mechanism observed for GABA_B and demonstrate that conformational changes in both the VFTs and the HDs of the two protomers are tightly coupled.

The authors are in the Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, Palo Alto, California 94305, USA.
e-mail: kobilka@cmgm.stanford.edu

What do these studies tell us about the coupling of agonist binding at the VFT to G-protein activation by the HD? Activation of mGluR1 can be explained by several different structural models. Here we discuss a few possibilities. In the simplest model (Fig. 1a), all of the structural changes are restricted to the VFT of each protomer, while the arrangement of the seven TM segments in the HD is unaffected. Binding of the ligand triggers a large rearrangement of the VFTs (as observed in the crystal structure⁴) and accordingly changes the position of the HDs relative to each other. In a more complex model (Fig. 1b), the rearrangement of the VFTs moves the HDs relative to each other, which indirectly forces the seven TM helices within each HD to adopt a new conformation. Finally, in the most complex model (Fig. 1c), in addition to movement of HDs relative to each other, agonist-bound VFTs also directly interact with the HD (or the extracellular loops), changing the relative arrangement of the seven TM segments within each HD.

The simplest model (Fig. 1a) is supported by Tateyama *et al.*¹. They did not observe rearrangement of the intracellular loops within each protomer, suggesting that the movements of the seven TM segments within the HDs does not play a role in G-protein activation. To explain G-protein activation in this model, one has to assume that the G-protein recognizes two distinct regions from each of the two HDs. Only when those two regions come together in the right arrangement is the G-protein activated. In this model, the intermolecular arrangement is the structural determinant for activation, and there is no need for an intramolecular conformational change within individual HDs.

The observed intermolecular movement of HDs reported by Tateyama *et al.*¹ is somewhat unexpected if the link between the C terminus of the VFT and first TM helix (TM1) of the HD is direct and rigid (as in Fig. 1a). From that structure one would predict that agonist-induced juxtaposition of the C termini of the VFTs would bring the two TM1s closer to each other⁴. In contrast, FRET experiments suggest that they move further apart. This could be explained by a less direct link between the VFTs and TM1, where movement of the C termini of the VFT pushes TM1s apart (as in Fig. 1b).

There are arguments in favor of some intramolecular rearrangements of the seven TM segments occurring in the HD of the individual protomers, and this is inconsistent with the simplest model. First, this region is known to undergo important structural changes in family A of GPCRs, including rhodopsin and the β_2 adrenergic receptor, and their sequences

are partly conserved in family C, so some common mechanisms are likely to be found. Second, several allosteric modulators bind the TM regions of GABA_B and mGluR, demonstrating that structural changes in the HDs play a role in activation. For example, Tateyama *et al.*¹ noted that binding of a non-competitive antagonist within the seven-TM bundle altered the agonist-induced movement of the two HDs relative to each other. Thus, some intramolecular rearrangements are likely to occur in the TM and in the intracellular regions of the mGluR in response to ligand binding at the extracellular VFTs. Whether this is through the other two more complicated models discussed here remains unclear. In any case, most of these structural changes may be too subtle to be detected by intramolecular FRET, which is most sensitive to distance changes when fluorophores are 30–70 Å apart.

One of the most important questions remaining about GPCR dimerization is to understand why dimerization is beneficial, why such a mechanism has evolved. In almost all cases, ligands have at best moderate effects on the dimerization of family A GPCRs. In the case of family C GPCRs, it is well documented that dimer formation is an irreversible process that happens during biosynthesis, that receptor-receptor interaction is not dependent on ligand binding at all, and that functional GPCRs do not exist as monomers at the plasma membrane³. But if agonist binding does not regulate dimer formation, then what is the purpose of having dimers? One possibility is that dimerization plays a role in the quality control process during biosynthesis, as shown for the GABA_B⁶ and for the β_2 adrenergic receptors¹⁰. In the case of heterodimers, one could also argue that combinations of two distinct GPCRs might be a way to provide pharmacological and functional diversity¹¹.

As explained above, rearrangement of the dimer shown by Tateyama *et al.*¹ might be required for G-protein activation, which would give dimerization a major role in the activation process. Alternatively, individual HDs could be capable of G-protein activation, but dimerization may guarantee specificity by enabling tight structural and functional coupling of the protomers, as in the study of Kniazeff *et al.*². In the context of allostery, this kind of coupling is also known as 'concerted activation', a model that could explain many of the intriguing properties of the mGluR and GABA_B dimers. For mGluR, this concept is particularly well supported by the VFT crystal structure where, even with only one ligand, the dimer can already achieve an active state similar to a dimer with two bound ligands. Applying the concerted activation concept to

the whole mGluR would require that both of the seven-TM helical bundles in the two protomers be both in either the 'inactive' or the 'active' conformation. Consistent with the results of Kniazeff *et al.*², having one ligand bound to the dimer would then be sufficient to push the equilibrium toward the 'active-active' state, with binding of both ligands pushing it even further. It could potentially explain the observation that G-protein activation occurs both in *cis* and in *trans*. In addition, it could also explain the observed positive cooperativity for a binding-impaired mutant: the mutant only responds to agonist when paired with a wild-type partner but not when forming a homodimeric receptor.

In summary, the two studies discussed here provide insight into the mechanism by which binding of a small molecule ligand to a large hydrophilic extracellular domain leads to activation of intracellular G-proteins. Do these lessons apply to other GPCRs known to form dimers? Clearly, the simplest model (Fig. 1a) is not sufficient by itself to explain the activation of family A GPCRs, where ligand binding has been experimentally shown to change the relative orientation of the seven TM segments in each protomer⁹. It is possible however, that agonist-induced conformational changes in the seven-TM domain lead to changes in the arrangement of the two protomers within a dimer. This rearrangement could then present G-protein interaction sites in the right orientation. FRET techniques similar to that used by Kubo's group could help to address these questions.

While compelling evidence exist for a functional link between dimerization and G-protein coupling in the case of family C receptors, such evidence remains scarce in the case of other GPCRs. Hopefully, the recent work by the Kubo and Pin labs^{1,2} will foster new ideas and new experimental approaches to investigate the role of dimerization in receptor activation and coupling to G-proteins.

1. Tateyama, M., Abe, H., Nakata, H., Saito, O. & Kubo, Y. *Nat. Struct. Mol. Biol.* **11**, 637–642 (2004).
2. Kniazeff, J. *et al. Nat. Struct. Mol. Biol.* **11**, 706–713 (2004).
3. Pin, J.P., Galvez, T. & Prezeau, L. *Pharmacol. Ther.* **98**, 325–354 (2003).
4. Kunishima, N. *et al. Nature* **407**, 971–977 (2000).
5. Tsuchiya, D., Kunishima, N., Kamiya, N., Jingami, H. & Morikawa, K. *Proc. Natl. Acad. Sci. USA* **99**, 2660–2665 (2002).
6. Margeta-Mitrovic, M., Jan, Y.N. & Jan, L.Y. *Neuron* **27**, 97–106 (2000).
7. Galvez, T. *et al. J. Biol. Chem.* **275**, 41166–41174 (2000).
8. Margeta-Mitrovic, M., Jan, Y.N. & Jan, L.Y. *Proc. Natl. Acad. Sci. USA* **98**, 14649–14654 (2001).
9. Gether, U. *Endocr. Rev.* **21**, 90–113 (2000).
10. Salahpour, A. *et al. J. Biol. Chem.* JBC online, 20 May 2004; 10.1074/jbc.M403363200.
11. Terrillon, S. & Bouvier, M. *EMBO Rep.* **5**, 30–34 (2004).