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PERSPECTIVE

A GPCR That Is Not "DRY"

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ABSTRACT

The conserved "DRY" motif (Asp-Arg-Tyr) at the cytosolic surface of rhodopsin-like G protein-coupled receptors has been the subject of much work attempting to understand the mechanisms of receptor activation and interaction with G proteins. Both the acidic (Asp) and basic (Arg) residues of this motif are important for isomerization of receptors between inactive and activated conformations. In this issue of *Molecular Pharmacology*, Rosenkilde et al. (pp. 11–19) show that a novel wild-type receptor, ORF74-EHV2, which lacks the Arg residue, is fully functional, showing both constitutive and ligand-induced activation of G protein signaling. Reintroducing the DRY motif by mutagenesis decreased constitutive activity while retaining ligand-inducible function. This work shows that the conserved Arg side chain is not required for receptor function, but it is important for stabilizing receptors in the inactive conformation.

G protein-coupled receptors (GPCRs) constitute the largest family of membrane proteins in the body. Members of this family mediate communication with the external environment (vision, taste, odor), as well as a broad range of physiological functions, including neurotransmission, cardiac function, hormone responses, and inflammation, and they even contribute to transmission and progression of infectious diseases. GPCRs have proven particularly amenable to modulation by small molecule drugs and are the targets of approximately half of current prescription drugs. The combination of this broad range of biological functions with the clear potential for pharmacological interventions creates enormous interest in the mechanisms by which GPCRs mediate their biological effects.

Despite their diverse biological roles, GPCRs have certain common functions. They all mediate transduction of an extracellular signal across a biological membrane and activate heterotrimeric G proteins within the cell. This occurs via a ligand-induced transition of the receptor from an inactive conformation to an activated conformation that activates G proteins at the cytosolic surface of the membrane. When GPCRs are activated, they also become substrates for phosphorylation by G protein-coupled receptor kinases (GRKs). Phosphorylated activated GPCRs are bound by cytosolic proteins, called β-arrestins, that inhibit interaction with G proteins and cause receptor desensitization, internalization of the receptor, and activation of additional signaling pathways that do not involve G proteins (Ferguson, 2001).

GPCRs consist of seven membrane-spanning helices that are connected by extracellular and cytosolic loops. The rhodopsin-like GPCRs, which are the largest and best-studied family of GPCRs, contain several amino acid motifs that are highly conserved within the family. The conservation of specific amino acid side chains suggests that they participate in the shared function of GPCRs, the ligand-controlled transition between inactive and activated conformations. Side chains of the conserved hydrophilic amino acids form interhelical bonds or interactions that define and stabilize either the inactive or the activated conformation. A particular side chain may stabilize both receptor conformations, by forming distinct interactions with different partners, respectively, in the inactive and activated conformations. The assumption that conserved amino acid motifs contribute to the shared

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ABBREVIATIONS: GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; TM, transmembrane segment.

functions of GPCRs implies that conserved amino acid side chains have similar functions in different GPCRs.

One of the most intensively researched of the conserved motifs is the "DRY" motif, which is located at the cytosolic end of the third membrane-spanning segment (TM3) and named for the single letter designation of its constituent amino acids, Asp-Arg-Tyr. Of these three residues, Tyr is least conserved [67% (Mirzadegan et al., 2003)] and is generally not important for receptor function (Wess, 1998), whereas Asp is conserved as an acidic Asp or Glu residue in 86% of rhodopsin-like GPCRs (Mirzadegan et al., 2003), and numerous mutagenesis studies have shown that the acidic side chain regulates receptor activation and coupling of agonist binding to activation of G protein signaling (Scheer et al., 1996; Wess, 1998; Ballesteros et al., 2001). Arg is one of the most conserved residues in rhodopsin-like GPCRs, and it is now thought to be essential for forming intramolecular interactions that constrain receptors in either the inactive or activated conformation (Rosenkilde et al., 2005) (see below). Analysis of 362 human rhodopsin-like GPCRs showed that only 3% of receptors did not have a basic Arg or Lys at this position and that none of the human GPCRs lacking Arg or Lys has yet been shown to be functional (Rosenkilde et al., 2005).

The location of the DRY motif at the cytosolic surface of GPCRs led to the suggestion that it might interact directly with the G protein (for review, see Wess, 1998). Consistent with this idea, initial experiments with mutant receptors showed that receptors lacking the Arg side chain failed to activate G protein signaling (Acharya and Karnik, 1996; Scheer et al., 1996; Ballesteros et al., 1998; Wess, 1998). These results would suggest that the Arg side chain is either directly involved in G protein recognition or involved in stabilizing the receptor in the activated conformation (Capra et al., 2004).

However, some Arg mutant receptors showed enhanced binding affinity for agonist ligands (Scheer et al., 1996, 2000; Alewijnse et al., 2000). According to the extended ternary complex model of GPCR activation (Samama et al., 1993), mutant receptors with enhanced agonist affinity are stabilized in an activated conformation and able to bind to G proteins, even though G protein signaling was disrupted. Consistent with this, a rhodopsin mutant with Gly substituted for Arg was shown to form the activated metarhodopsin II intermediate in the presence of a peptide that mimics the G protein. This observation that a receptor lacking the Arg could be activated and bind to G protein without activating G protein signaling led to a proposal that the Arg side chain directly catalyzes release of GDP from the G protein (Acharya and Karnik, 1996). The question of direct interaction of the Arg side chain with the G protein was addressed using peptides corresponding to the cytosolic end of the TM3 and intracellular loop 2 of the α_{2A} adrenergic receptor (Chung et al., 2002). The peptide with wild-type sequence activated the G protein, stimulating GTPase activity and showing that amino acid residues in this sequence activate the G protein. A peptide with Gln substituted for Arg in the DRY sequence had a similar effect. This wild-type-like activity of the peptide lacking Arg shows that the Arg side chain does not play a direct role in activating the G protein (Chung et al., 2002). It was subsequently discovered that some (Scheer et al., 2000; Barak et al., 2001; Wilbanks et al., 2002) but not all (Scheer et al., 2000; Chung et al., 2002; Capra et al., 2004) receptors with compromised G protein signaling in the absence of the Arg side chain were constitutively phosphorylated and desensitized or internalized. Thus, the mutations seem to stabilize an activated receptor conformation that is phosphorylated and desensitized in the absence of agonist and consequently cannot respond to agonist stimulation, yielding a nonfunctional phenotype (Scheer et al., 2000; Barak et al., 2001; Wilbanks et al., 2002). This suggested that, in these receptors, the Arg side chain stabilizes the inactive receptor conformation, and it was not long before constitutively active Arg mutant receptors were identified (Fanelli et al., 1999; Scheer et al., 2000; Chen et al., 2001), further supporting a role for Arg in stabilizing the inactive receptor conformation. A role for Arg in stabilizing the inactive receptor conformation is also supported by structural data from the rhodopsin crystal, where the Arg side chain forms salt bridges with the preceding Asp side chain and with a Glu side chain in TM6 (Palczewski et al., 2000). As TM3 and TM6 move apart during rhodopsin activation, these interactions are proposed to constrain the receptor in the inactive state, and disrupting them would lead to constitutive activation (Ballesteros et al., 2001). Taken together, the data from many different receptors and mutations suggest that the Arg residue of the DRY motif does not interact directly with G proteins, but it does stabilize both the inactive and the activated conformations of GPCRs.

Now, in this issue, Rosenkilde et al. (2005) add to the debate by showing that a wild-type GPCR with a nonconservative (Thr) substitution for the Arg residue is fully functional. The chemokine-like ORF74 receptor, encoded by the equine herpes virus (EHV2), has a DTW (Asp-Thr-Trp) motif instead of DRY. It constitutively activates signaling through the Gi pathway, and the chemokine ligand CXCL6 acts as a high-affinity agonist. This shows that G protein signaling can occur in a wild-type receptor that lacks the Arg. Reconstitution of the DRY motif, by mutating the Thr and Trp to Arg and Tyr, decreased the constitutive activity while maintaining the ability to respond to CXCL6. These data are interesting because they show that introduction of the Arg residue decreases G protein signaling and stabilizes the inactive receptor conformation. This suggests that ORF74 has evolved a nonconservative substitution for Arg to maximize its constitutive activity. Considering the role of constitutive phosphorylation and desensitization in the development of understanding of the DRY motif function, it would be interesting to know whether ORF74 is subject to phosphorylation by GRKs and whether it interacts with β-arrestin proteins.

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