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## Differential Assembly of GPCR Signaling Complexes Determines Signaling Specificity

Pascal Maurice, Abba Benleulmi-Chaachoua, Ralf Jockers

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**Abstract** Recent proteomic and biochemical evidence indicates that cellular signaling is organized in protein modules. G protein-coupled receptors (GPCRs) are privileged entry points for extracellular signals that are transmitted through the plasma membrane into the cell. The adequate cellular response and signaling specificity is regulated by GPCR-associated protein modules. The composition of these modules is dynamic and might depend on receptor stimulation, the proteome of a given cellular context, the subcellular localization of receptor-associated modules, the formation of GPCR oligomers and the variation of expression levels of components of these modules under physiological, for example circadian rhythm, or pathological conditions. The current article will highlight the importance of GPCR-associated protein modules as a biochemical basis for signaling specificity.

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**Keywords (separated by  
' ')** Circadian rhythm - Compartmentalization - Complex assembly - Dimerization - Therapeutic

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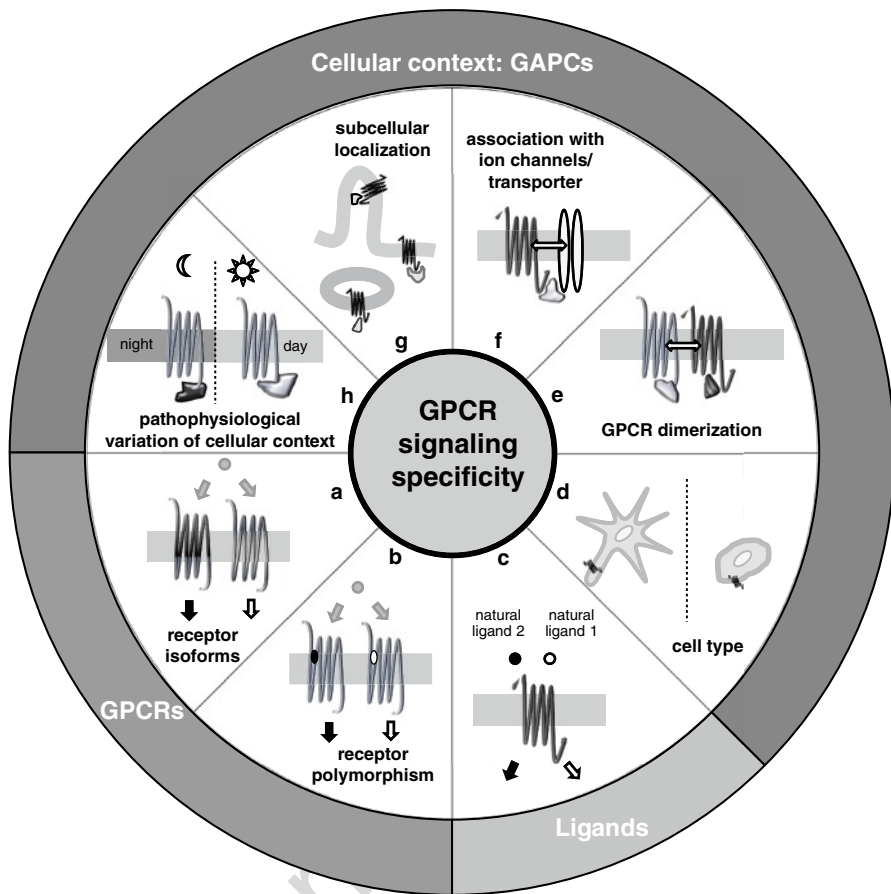
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28 Dimerization • Therapeutic

## 29 12.1 Introduction

30 Membrane receptors are at the interface between the extra- and intra-cellular  
31 environment and are thus crucial for cellular communication. The superfamily of G  
32 protein-coupled receptors (GPCRs) constitutes the largest family of membrane  
33 receptors with approximately 800 members in humans (Vassilatis et al. 2003;  
34 Fredriksson and Schiöth 2005). They are composed of seven transmembrane  
35 domains connected by short intra- and extra-cellular loops and respond to a large  
36 panel of signals: photons, ions, metabolites, amino acids, lipids, peptides and  
37 proteins. Despite this ligand diversity, the overall architecture and activation mecha-  
38 nism is believed to be highly conserved for these receptors. Many GPCRs are able  
39 to activate heterotrimeric G proteins composed of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits. G proteins are  
40 classified according to the activation of various effectors:  $G_{i/o}$  and  $G_s$  proteins are  
41 typically involved in the inhibition and stimulation of adenylyl cyclase, respectively;  
42  $G_{q/11}$  proteins in the stimulation of phospholipase C and  $G_{12/13}$  proteins in the activa-  
43 tion of small G proteins and cytoskeleton rearrangements. According to the type of  
44 G protein activated, GPCRs have been initially classified as  $G_{i/o}$ -,  $G_s$ -,  $G_{q/11}$ - or  $G_{12/13}$ -  
45 coupled receptors. However, replication of these studies in different cell types and  
46 experimental conditions clearly show that most GPCRs do not couple to one single  
47 G protein but often to several of them. Activation of additional G protein-independ-  
48 ent signaling events, i.e.  $\beta$ -arrestin-dependent pathways, are also increasingly  
49 described. The capacity of GPCRs to signal through multiple pathways together  
50 with the fact that signaling pathways are highly interconnected within networks  
51 raises the fundamental and still largely unsolved question of how signaling specificity  
52 is obtained upon stimulation of cells with a given ligand.

53 A simple way to generate signaling specificity is the expression of different  
54 receptor isoforms responding to the same ligand but triggering different signaling  
55 events (Fig. 12.1a). This strategy is widely used and applies to multiple neurotrans-  
56 mitters (serotonin, dopamine, glutamate, etc.). In other more limited circumstances,  
57 signaling specificity is generated by the expression of different natural ligands tar-  
58 geting the same receptor with different functional outcomes (Fig. 12.1c). Prominent  
59 examples are the  $\beta_2$ -adrenergic receptors ( $\beta_2$ AR) that is activated by epinephrine  
60 and norepinephrine (Reiner et al. 2010) and the parathyroid hormone receptor  
61 (PTHrP) that is activated by PTH and the PTH-related peptide (PTHrP) (Ferrandon  
62 et al. 2009). Epinephrine and norepinephrine induce different conformational  
63 changes within the receptor with different kinetics as monitored with a fluorescently  
64 tagged  $\beta_2$ AR FRET sensor. These differences translate into different capacities to  
65 activate  $G_s$ - and  $\beta$ -arrestin-dependent signaling events. In the case of the PTHrP,  
66 both natural ligands trigger different durations of the cAMP responses in which  
67 PTHrP-promoted actions are short-lived and restricted to the cell surface and



**Fig. 12.1 Schematic illustration of different means to generate GPCR signaling specificity.** (a) Stimulation of different receptor isoforms by the same ligand may result in the activation of different signaling pathways; (b) receptor polymorphisms may modify the functional properties of receptors; (c) different natural ligands may activate the same receptor differently; (d) receptor function may vary depending on the cellular context; (e) GPCR dimerization may modify receptor function and the recruitment of different GPCs; (f) association of GPCRs with ion channels and neurotransmitter transporters may modify receptor function; (g) the subcellular localization of receptors may impact on receptor function; (h) the functional response of receptors may vary depending on physiological or pathological changes of receptor or GPCR expression. Changes in GPCR composition as a function of the circadian rhythm are illustrated

PTH-promoted actions are long-lived and dependent on receptor internalization. 68  
 This idea can be also extended towards inverse agonists as illustrated by melanocortin 69  
 MC3/MC4 receptors and their naturally occurring inverse agonists agouti and 70  
 agouti-related protein (Agrp) that diminish the action of the melanocortin receptor 71  
 agonist  $\alpha$ -melanocyte-stimulating hormone (Adan and Kas 2003). 72

73 The same strategy is increasingly applied by pharmaceutical industry to design  
74 synthetic ligands with biased signaling properties. The idea behind biased ligands is  
75 to develop drugs that activate only a subset of signaling pathways (ideally only those  
76 important for the therapeutic effect) thus significantly decreasing unwanted side  
77 effects of drugs due to the activation of therapeutically irrelevant pathways. The proof-  
78 of-concept for such a strategy has been shown for the nicotinic acid receptor. Nicotinic  
79 acid is one of the most effective agents for both lowering triglycerides and raising  
80 HDL levels. However, the side effect of cutaneous flushing severely limits patient  
81 compliance. Recent evidence suggests that effects on lipid metabolism are mediated  
82 by  $G_{i/o}$  proteins and flushing by  $\beta$ -arrestin (Walters et al. 2009). Drugs with reduced  
83 flushing profiles are currently developed (Shen et al. 2010).

84 Furthermore, receptor polymorphisms might modify the signaling profile of the  
85 receptor and thus redirect the functional outcome (Fig. 12.1b). This aspect has to be  
86 considered in the context of personalized drug design.

87 Although the abovementioned cases participate in the generation of signaling  
88 specificity, they only apply to a subset of situations and leave the majority of cases  
89 unexplained. Indeed, the signaling outcome of the stimulation of a particular recep-  
90 tor might largely depend on the cellular context, the precise subcellular localization  
91 of receptors, their clustering and their interaction with signaling modules, whose  
92 composition might vary depending on different physiological or pathological condi-  
93 tions. The present article describes how signaling specificity can be generated under  
94 these different circumstances. We will focus our attention on the importance of  
95 GPCR-associated protein complexes (GAPCs) that might be either involved in the  
96 trafficking and subcellular localization of the receptor or directly in receptor signal-  
97 ing. Importantly, integration of receptors into different preformed signaling  
98 complexes might be a biochemical basis for the generation of signaling specificity.

## 99 12.2 Impact of the Cellular Context

100 Activation of specific signaling pathways and the functional outcome of receptor acti-  
101 vation depend on the cellular context. This may include differences in the functional  
102 response depending on the differentiation state of cells, for example preadipocytes *vs.*  
103 adipocytes, or on the cell types (immune cells, epithelial cells, neurons, etc.).  
104 Differences may occur at the level of receptor expression, receptor localization, the  
105 expression pattern of effector systems and downstream signaling pathways as well as  
106 expression of components of GAPCs (Fig. 12.1d).

107 This is well illustrated by the regulator of G protein signaling (RGS) proteins.  
108 RGS proteins are GTPase-activating proteins that accelerate the GTPase activity of  
109  $G\alpha$  subunits, thereby modulating G protein signaling (Sjogren 2011). In addition to  
110 their selectivity for certain  $G\alpha$  subtypes, one obvious source of specificity is the  
111 tissue- or cell-specific expression pattern of RGS. A good example is the distinct  
112 localization of the two splice variants of RGS9. RGS9, a member of the R7 family  
113 of RGS proteins, is expressed as two splice variants, RGS9-1 in the retina and the

longer form RGS9-2 primarily postsynaptic in dopamine receptor-rich brain regions, such as the striatum (Gold et al. 1997; Mancuso et al. 2010). In the retina, RGS9-1 is exclusively found in the outer segment of photoreceptor cells in which it regulates signaling through transducin  $\alpha$  subunits in rods and cones (Cowan et al. 1998; He et al. 1998). This protein seems to be essential for normal visual perception as demonstrated by the RGS9 knockout mice and humans with mutations in RGS9 (Nishiguchi et al. 2004; Michaelides et al. 2010). In contrast, RGS9-2 is highly expressed in the striatum where it regulates opioidergic and dopaminergic responses (Gold et al. 1997). RGS proteins have been also implicated in regulating cancer progression. In many cases, the link is related to changes in expression. For instance, RGS2 and RGS4 mRNA are both down-regulated in ovarian cancer cell lines, in contrast to RGS6 mRNA which is upregulated, indicating different and specific functional roles for these proteins (for review, see Hurst and Hooks 2009).

### 12.3 GPCR Dimerization

Organization of GPCRs into dimeric clusters has been considered for more than 10 years and is now supported by numerous studies (Milligan 2009) and the crystal structure of two GPCRs (Palczewski et al. 2000; Wu et al. 2010). GPCR heterodimerization is a topic of great interest due to the potential importance of the functional consequences of such interactions on signal transduction pathways. Depending on the two protomers constituting the dimer (the dimer being the minimal functional oligomeric unit), the composition of the GAPCs can vary and generate signaling specificity (Fig. 12.1e). In addition, GPCR heterodimerization offers a plausible solution for the simultaneous binding of many interaction partners to the receptor and would increase the diversity of the activation response of such complexes.

Accumulating evidence in the literature indicates that heterodimerization may modify the G protein coupling profile. For instance, signaling of  $\mu$ - and  $\delta$ -opioid receptor heterodimers is insensitive to pertussis toxin (PTX) treatment in contrast to the individually expressed receptors, suggesting a switch from PTX-sensitive to PTX-insensitive G protein coupling (George et al. 2000). Other studies on dopamine  $D_1$  and  $D_2$  receptor heterodimers demonstrated that the  $G\alpha_s$ -coupled  $D_1$  and the  $G\alpha_i$ -coupled  $D_2$  receptors become  $G\alpha_{q/11}$ -coupled in the corresponding heterodimer (Rashid et al. 2007). These examples illustrate how heterodimerization between two different GPCRs can determine signaling specificity.

[AU3] Asymmetry between the two protomers of a GPCR dimer may also contribute to signaling specificity (for review, see (Maurice et al. 2011; Kamal et al. 2011)). The recent development of a functional complementation assay allowed to explore the individual contribution of each protomer of class A GPCR dimers (Han et al. 2009). A *trans*-activation model was proposed in which the agonist-occupied protomer can *trans*-activate the second protomer that associates with the  $G\alpha$  subunit to modulate the signal transduction pathway. Another example in which asymmetry downstream of the G protein determines signaling specificity was recently reported for the



155 melatonin  $MT_1/MT_2$  heterodimer and RGS20 (Maurice et al. 2010). In contrast to  
156 the  $MT_2$  receptor, which only couples to  $G_i$  proteins, the  $MT_1$  receptor forms a con-  
157 stitutive ternary complex with both  $G_i$  and RGS20. By using a ligand binding-  
158 deficient  $MT_1^*$  mutant and bioluminescence resonance energy transfer (BRET)  
159 studies with energy transfer probes at different sites of the  $MT_1/Gi/RGS20$  complex,  
160 Maurice et al. showed that activation of the  $MT_2$  protomer, within the  $MT_1^*/MT_2$   
161 heteromer, can *cis*-activate  $G_i$ , which then interacts functionally with RGS20 bound  
162 to the  $MT_1^*$  protomer. This shows that receptors, such as  $MT_2$ , that are insensitive  
163 to a specific GPCR-interacting protein (GIP) can become sensitive to the action of  
164 this GIP in the appropriate heterodimer complex.

165 Agonist binding to GPCRs and G protein activation are followed by the recruit-  
166 ment of G protein-coupled receptor kinases (GRKs) that phosphorylate the receptor,  
167 and of arrestins triggering receptor endocytosis and arrestin-dependent signal trans-  
168 duction (Reiter and Lefkowitz 2006). The receptor/GRK stoichiometry remains  
169 unknown but several studies have reported that binding of only one GRK molecule  
170 to the receptor dimer might be sufficient for full receptor phosphorylation of both  
171 protomers by cross-phosphorylation. This phenomenon has been shown for rhodop-  
172 sin for which recruitment of the rhodopsin kinase to an activated rhodopsin can  
173 cross-phosphorylate multiple nearby non-activated rhodopsin molecules (Shi et al.  
174 2005) and for several other class A GPCR heteromers (Pfeiffer et al. 2002, 2003).  
175 In these studies, GRK cross-phosphorylation of the unligated GPCR within the het-  
176 erodimer was accompanied by co-internalization of this unligated protomer together  
177 with the ligand-bound protomer of the dimer (Pfeiffer et al. 2002, 2003). Cross-  
178 internalization of receptors within GPCR heterodimers may have important func-  
179 tional consequences, as shown in the case of the  $\mu/\delta$ -opioid receptor heterodimer.  
180 Activation of the  $\delta$ -opioid receptor cross-internalizes  $\mu$ -opioid receptors, thereby  
181 severely limiting  $\mu$ -opioid receptor-associated morphine analgesia (He et al. 2011).

182 Taken together, these examples indicate how GPCR heterodimerization, by  
183 acting as scaffolds and by assembling distinct GAPCs, can generate signaling properties  
184 that differ from those observed in the corresponding homodimers thus illustrating  
185 the diversification of the functional properties of receptors by heterodimerization.

## 186 12.4 Complex Formation with Membrane Proteins 187 Other Than GPCRs

188 GPCRs have not only been reported to cluster into homo- and heterodimeric com-  
189 plexes but also with other major transmembrane proteins as diverse as neurotran-  
190 smitter transporters and ion channels, which are privileged pharmacological targets  
191 by themselves (Fig. 12.1f). A physical and functional interaction was reported  
192 between the  $\gamma$ -aminobutyric-acid A ( $GABA_A$ )-ligand gated channel and the dop-  
193 amine  $D_5$  receptor (Liu et al. 2000). Interestingly, this association was shown to  
194 enable mutually inhibitory effects resulting in  $D_5$  receptor-attenuated  $GABA_A$ -  
195 mediated inhibitory postsynaptic currents and  $GABA_A$ -stimulated reductions of  $D_5$

receptor cAMP accumulation. These findings are of primary importance since GABA<sub>A</sub> receptors mediate fast inhibitory synaptic transmission at the vast majority of inhibitory synapses in the brain, thereby playing a fundamental role in brain physiology and pathology.

The ionotropic *N*-methyl-D-aspartate (NMDA) receptor was also reported to physically interact with the D<sub>1</sub> receptor (Lee et al. 2002) and more recently with the mGlu5a receptor (Perroy et al. 2008). NMDA receptors play an important role in the regulation of activity-dependent neuroplasticity and excitotoxicity and the interaction with D<sub>1</sub> was reported to modulate NMDA receptor-mediated functions in hippocampal culture neurons (Lee et al. 2002). Interaction with mGlu5a allows reciprocal modulation of both receptors; the mGlu5a receptor decreases NMDA receptor currents and the NMDA receptor strongly reduces the ability of the mGlu5a receptor to release intracellular calcium (Perroy et al. 2008). These studies illustrate the richness in the functional interactions between a GPCR and other unrelated transmembrane receptors, leading to a much higher degree of effector specificity and subcellular signaling pathways.

The nociceptin (ORL1) receptor has been shown to physically and specifically interact with another type of ion channel, the voltage-gated N-type Ca<sub>v</sub>2.2 calcium channel (Beedle et al. 2004). Calcium influx through N-type calcium channels in dorsal root ganglion (DRG) neurons is an essential step in the transmission of nociceptive signals at the spinal level. Specific interaction between ORL1 and Ca<sub>v</sub>2.2 has been shown to result in an agonist-independent, receptor concentration-dependent inhibition of the channel *in vitro* and in DRG neurons (Beedle et al. 2004). These findings raised the possibility that the activity of N-type calcium channels, and consequently transmission of pain signals, may be regulated by difference in GPCR (ORL1) receptor density. Further characterization of the ORL1/Ca<sub>v</sub>2.2 interaction showed that prolonged exposure of ORL1 to its cognate agonist nociceptin triggers co-internalization of ORL1 and the ion channel thus down-regulating calcium entry in DRG neurons (Altier et al. 2006).

Preferential interactions have also been reported between GPCRs and neurotransmitter transporters. The dopamine D<sub>2</sub> and orphan GPR37 receptor have been shown to interact with the dopamine transporter (DAT), one of the key presynaptic components involved in regulating dopaminergic tone. The physical coupling between DAT and D<sub>2</sub> facilitates the recruitment of intracellular DAT to the plasma membrane, thus enhancing dopamine reuptake in primary cultures of rat midbrain neurons. Disruption of the D<sub>2</sub>/DAT interaction in mice decreases synaptosomal dopamine uptake and increases locomotor activity (Lee et al. 2007). Interestingly, opposite effects have been reported following interaction of DAT with GPR37 (Marazziti et al. 2007). *Gpr37*-null mutant mice show enhanced DAT-mediated dopamine uptake in striatal membrane samples, with a significant increase in the number of membrane DAT molecules. Taken together, these two studies demonstrate how, according to the nature of the GPCR engaged in a complex with a neurotransmitter transporter, opposite functional effects can be observed.

Formation of stable GPCR-effector complexes within the plasma membrane is also exemplified by GPCRs and G protein-gated inwardly-rectifying K<sup>+</sup> (GIRK)

241 channels, particularly of family 3 (Kir3). Kir3 channels represent a family of ion  
242 channels that mediate the postsynaptic inhibitory effect of many neurotransmitters  
243 and drugs of abuse. In 2002, a first study reported the formation of stable complexes  
244 of Kir3 with D<sub>2</sub>-like, D<sub>4</sub> and  $\beta_2$  adrenergic receptors using co-immunoprecipitation  
245 and BRET experiments (Lavine et al. 2002). Further studies reported formation of  
246 such complexes with other GPCRs such as GABA<sub>B</sub> receptors (David et al. 2006;  
247 Ciruela et al. 2010; Fowler et al. 2007), and muscarinic M<sub>2</sub>, serotonin 1<sub>A</sub>, adenosine  
248 A<sub>1</sub>, and LPA<sub>1</sub> receptors (Jaen and Doupnik 2006).

249 Overall, these different studies suggest that assemblies containing these compo-  
250 nents may represent a common organizational premise needed to determine  
251 specificity and efficacy of signal transduction and may be critical to assure the speed  
252 of signaling.

## 253 12.5 Physiological Changes, Illustrated by Circadian 254 Rhythmicity

255 The cellular state is constantly changing depending on the cell cycle and the devel-  
256 opmental stage of the organism. An interesting regular modification of biological  
257 systems is imposed by the biological clock that generates circadian rhythmicity at  
258 the cellular level. It is estimated that at least 10 % of all genes are cycling in a cir-  
259 cadian manner, which has obviously important functional consequences on cellular  
260 homeostasis (Lowrey and Takahashi 2004). Two recent examples illustrate the  
261 direct impact of circadian rhythmicity on GPCR function (Fig. 12.1h).

262 In mammals, the hypothalamic suprachiasmatic nucleus (SCN) is the central  
263 pacemaker, generating an endogenous and self-sustained circadian activity and  
264 coordinating rhythms of numerous peripheral tissues (Sujino et al. 2003). Circadian  
265 rhythmicity is generated at the cellular level but how the phase and period of circa-  
266 dian oscillation of different SCN neurons is maintained remains largely unknown.  
267 Oscillation in intracellular cyclic adenosine monophosphate (cAMP) levels is  
268 known to be an important component of circadian rhythmicity. The cAMP system  
269 can be manipulated by several means. cAMP production is controlled by stimulat-  
270 ing G<sub>s</sub> or G<sub>i</sub> protein-coupled receptors that activate or inhibit adenylyl cyclases,  
271 respectively. Degradation of cAMP occurs through phosphodiesterases. In addition,  
272 RGS proteins such as RGS16 may inhibit the function of G<sub>i</sub> proteins by accelerating  
273 their GTP-GDP exchange rate. Interestingly, RGS16 is expressed in a circadian  
274 manner in the dorsomedial part of the SCN with peak levels during dawn modulat-  
275 ing cAMP signaling in a highly regulated spatiotemporal manner (Doi et al. 2011).  
276 Ablation of the *Rgs16* gene eliminates the circadian cAMP production and, as a  
277 result, lengthened the circadian period of the locomotor activity rhythm thus dem-  
278 onstrating the importance of defined circadian activation patterns for behavioral  
279 outputs. This is further underlined by the fact that cAMP levels in *Rgs16* knockout  
280 mice remained constant at an elevated level demonstrating that not elevated cAMP  
281 levels *per se* but rather their rhythmicity are important. Taken together, rhythmic

changes in the expression of an important component of GPCR signaling, RGS16, may define signaling specificity in a temporal manner. 282 283

Another example illustrating the impact of the circadian rhythm on GPCR signaling has been shown in a peripheral organ, the liver (Zhang et al. 2010). In hepatic cells, intracellular cAMP levels appear to be regulated by a core component of the circadian rhythm generating cellular machinery, cryptochrome proteins (Cry). Circadian control of gene expression is achieved by two transcriptional activators, Clock and Bmal1, which stimulate Cry1, Cry2 and Period repressors that feedback on Clock-Bmal1 activity (Reppert and Weaver 2002). Recent evidence indicates that Cry may have additional, extra-nuclear, functions in hepatocytes (Zhang et al. 2010). Cry protein expression is rhythmic with peak levels during the night-day transition. By interacting with G<sub>s</sub> proteins, Cry diminishes intracellular cAMP levels in a circadian manner due to its circadian expression pattern. This may be of physiological relevance as the liver clock regulates the gluconeogenic genetic program through Cry-mediated inhibition of CREB activity during fasting. Furthermore, hepatic overexpression of Cry1 lowered blood glucose concentrations and improved insulin sensitivity in insulin-resistant db/db mice suggesting that enhancing Cry activity might be beneficial in type 2 diabetes treatment. Taken together, circadian expression of proteins involved in the regulation of GPCR signaling provides a powerful means to control GPCR signaling specificity. 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301

## 12.6 Localization of GPCRs at Different Subcellular Compartments 302 303

GPCR signaling specificity can be modulated depending on receptor localization at different subcellular compartments (Fig. 12.1g). Targeting of receptors to these compartments determines receptor function due to the specific physicochemical properties of each compartment (membrane rigidity and lipid composition) and the enrichment of GAPCs and signaling pathway components in these compartments. Microdomains (lipid rafts and caveolae) are specialized compartments of the plasma membrane that are highly structured zones of low fluidity due to their enrichment in cholesterol and sphingolipids. The structural integrity of caveolae is further maintained by a coat composed of caveolin protein at the cytoplasmic side. Microdomains have been suggested to constitute a scaffolding zone of GPCR signaling cascades that also modify GPCR trafficking and favor crosstalk with other signaling pathways. The precise mechanism of GPCR targeting into these microdomains remains unclear, in particular whether some GPCRs show higher intrinsic affinity for microdomains than others or whether microdomain-enriched GAPCs target GPCR to these domains (Insel et al. 2005). Furthermore, the impact of microdomains on GPCR localization and signaling specificity may vary depending on the tissue, as some cells, such as adipocytes, are richer in microdomains than others. 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320

An obvious role of caveolae in determining the functional outcome of GPCR signaling is provided by caveolae-localized PAR1 receptors that phosphorylate 321 322

323 caveolin-1 in a Src-dependent manner. Phosphocaveolin-1 then binds to the SH2  
324 domain of the C-terminal Src kinase (Csk) resulting in a rapid decrease in Src kinase  
325 activity. Thus, PAR1-induced Src activation is down-regulated by recruiting Csk  
326 specifically in caveolin-1 containing microdomains (Lu et al. 2006).

327 GnRH receptors have been shown to constitutively localize within microdomains  
328 as part of a preformed signaling complex composed of caveolin-1, c-Src, protein  
329 kinase C, Ras, MAPK kinase 1/2, ERK1/2, tubulin, FAK, paxillin, vinculin, and  
330 kinase suppressor of Ras-1 (Dobkin-Bekman et al. 2009). Stimulation by GnRH  
331 induces molecular rearrangement of the complex, the rapid dissociation of some  
332 components and the phosphorylation of FAK and paxillin by ERK1/2. Whereas acti-  
333 vation of ERK1/2 typically induces their nuclear translocation and phosphorylation  
334 of transcription factors, scaffolding into microdomain-associated multiprotein sig-  
335 naling complexes, as described here, helps to maintain the activated ERK1/2 pool  
336 within the cytoplasm and to phosphorylate FAK and paxillin at focal adhesions.

337 Primary cilia are appendages present at the cell surface on most mammalian cells  
338 involved in signal sensing and transmission (Goetz and Anderson 2010). Some  
339 GPCRs, including somatostatin receptor 3, serotonin receptor 6, smoothened,  
340 dopamine D<sub>1</sub> receptor and melanin-concentrating hormone receptor 1 localize to cilia.  
341 The importance of the localization and signaling of GPCRs in primary cilia is sug-  
342 gested by altered signaling caused by the absence of cilia formation (Berbari et al.  
343 2009). Some molecular determinants directing localization of GPCRs to cilia have  
344 been identified in the third intracellular loop, which most likely binds to cytoskeleton  
345 proteins involved in the transport into and out of cilia (Berbari et al. 2008). The func-  
346 tions of cilia are defined by the signaling proteins localized to the ciliary membrane  
347 such as adenylyl cyclases, heterotrimeric G proteins and  $\beta$ -arrestins. What makes  
348 primary cilia so special compared to other cell surface-exposed membrane regions,  
349 remains still an open question and warrants further investigation.

350 Many GPCRs contain a PSD-95/Disc large/Zonula occludens 1 (PDZ) ligand  
351 sequence composed of three amino acids at their carboxyl-terminal extremity  
352 (Heydorn et al. 2004). These PDZ ligands are recognized by PDZ domains, which  
353 are present in almost 200 proteins. PDZ domains are scaffolding domains per excel-  
354 lence that are involved in GPCR trafficking and subcellular localization. The post-  
355 synaptic density protein 95 (PSD95) contains three PDZ domains and interacts with  
356 the serotonin 5-HT<sub>2C</sub> receptor and other GPCRs in post-synaptic membranes  
357 (Becamel et al. 2002). PSD95 is also part of large molecular networks such as the  
358 PSD95/GKAP/Shank/Homer3 complex that physically and functionally connects  
359 NMDA receptors (PSD95 binding partner) to metabotropic glutamate 5 receptors  
360 (mGluR5) (Homayoun and Moghaddam 2010). Homer three proteins assemble into  
361 tetramers bridging GKAP and mGluR5. Interestingly, this complex can be disrupted  
362 by the monomeric immediate early gene Homer1a that blocks the interaction  
363 between GKAP and mGluR5 by competing with Homer3. This indicates a dynamic  
364 regulation of the presence of mGluR5 in the complex and possibly its localization  
365 to post-synaptic membranes (Bertaso et al. 2010).

366 Signaling of GPCRs has been reported not only at the plasma membrane or  
367 specialized plasma membrane sub-compartments such as microdomains, primary

cilia and synapses, but also in intracellular membranes such as endosomes and nuclear membranes (Boivin et al. 2008; Calebiro et al. 2010). GAPCs appear to play an important role under these circumstances as current observations are compatible with the joint translocation of both receptors and their GAPCs from the cell surface to intracellular compartments to prolong their signaling capacity. It remains however unclear why these complexes remain stable during receptor endocytosis and which component is responsible for the targeting of the complex to specific intracellular sites. Although it is tempting to speculate that specific GAPCs participate in this new paradigm of signaling specificity, experimental proof for this hypothesis is still lacking.

## 12.7 Targeting GPCR-GIP Interaction In Vivo: A Promising Approach for Specific Therapeutic Intervention?

An important issue concerns the pathophysiological relevance of such differential assembly of GAPCs. Indeed, the potential of targeting GPCR-GIP interactions as a novel approach for therapeutic intervention is of particular interest and specific disruption of a GPCR/GIP interaction in a given tissue without affecting the interaction of this same GPCR with other GIPs in another tissue represents an attractive strategy for drug discovery. Emerging evidence that illustrate this concept came from studies based on the use of cell-penetrating HIV TAT-conjugated peptides that clearly showed the potential of peptides in disrupting a specific GPCR/GIP interaction.

A first example was proposed by the study of Ji et al. (2006) reporting that intravenous injection in rats of a TAT-conjugated peptide encompassing the PTEN-binding motif located within the i3 loop of 5-HT<sub>2C</sub> receptor suppresses the firing rate of ventral tegmental area (VTA) dopaminergic neurons induced by d9-tetrahydrocannabinol (THC), thereby reproducing the effect of 5-HT<sub>2C</sub> receptor agonists. As 5-HT<sub>2C</sub> receptor agonists, this TAT-peptide also inhibits the rewarding effects of THC (and nicotine) mediated by VTA dopaminergic neurons. However, it does not reproduce the side effects (anxiogenic effects, penile erection, hypophagia, and motor functional suppression) observed following administration of 5-HT<sub>2C</sub> agonists (Ji et al. 2006).

Disruption of the mGluR7a/PICK1 interaction by intravenous injection of a TAT-conjugated peptide was able to trigger absence epilepsy-like seizures in mice and rats, providing evidence that a single PDZ protein/GPCR interaction can result in a specific neurological disorder (Bertaso et al. 2008). More recently, disruption of the interaction between 5-HT<sub>2A</sub> and PDZ proteins in diabetic neuropathic rats by a TAT-conjugated peptide encompassing the nine C-terminal residues of the 5-HT<sub>2A</sub> receptor was shown to induce an antihyperalgesic effect and to strongly enhance antihyperalgesia induced by the selective serotonin reuptake inhibitor (SSRI) fluoxetine. This study thus suggests that 5-HT<sub>2A</sub> receptor/PDZ protein interactions might contribute to the resistance to SSRI-induced analgesia in painful diabetic neuropathy and that disruption of these interactions might be a valuable strategy to

409 design novel treatments for neuropathic pain and to increase the effectiveness of  
410 SSRIs (Pichon et al. 2010).

411 The importance of  $D_1/D_2$  heterodimers in human disease was suggested in a  
412 recent study showing that physical interaction between dopamine  $D_1$  and  $D_2$  receptors  
413 is markedly increased in postmortem brains of patients suffering from major depres-  
414 sion. Administration of an interfering peptide consisting of a sequence located in  
415 the third intracellular loop of  $D_2$  that disrupts the  $D_1$ - $D_2$  receptor complex reduced  
416 immobility in the forced swim test without affecting locomotor activity, and  
417 decreased escape failures in learned helplessness tests in rats (Pei et al. 2010). This  
418 study thus provided evidence for potential implication of GPCR heterodimers and  
419 associated signaling specificity in human diseases.

## 420 12.8 Conclusions

421 Evolution has selected several ways to achieve signaling specificity, by generating  
422 receptor isoforms, receptor polymorphisms, differential localization and targeting  
423 of receptors and pre-assembly of receptors and GAPCs into defined and distinct  
424 signaling modules. The latter holds the potential to explain many of the observed  
425 signaling specificity. However, we are only beginning to define the precise composi-  
426 tion and impact of these modules on receptor signaling. Given the dynamic nature  
427 of these modules, a precise analysis has to be performed for each receptor in a  
428 specific cellular context and under defined physiological and pathological  
429 conditions.

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# Author Queries

Chapter No.: 12      0001645432

<b>Queries</b>	<b>Details Required</b>	<b>Author's Response</b>
AU1	Please provide organisation division for all the authors.	
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Uncorrected Proof