OLIGOMERIZATION OF G-PROTEIN-COUPLED TRANSMITTER RECEPTORS

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Examples of G-protein-coupled receptors that can be biochemically detected in homo- or heteromeric complexes are emerging at an accelerated rate. Biophysical approaches have confirmed the existence of several such complexes in living cells and there is strong evidence to support the idea that dimerization is important in different aspects of receptor biogenesis and function. While the existence of G-protein-coupled-receptor homodimers raises fundamental questions about the molecular mechanisms involved in transmitter recognition and signal transduction, the formation of heterodimers raises fascinating combinatorial possibilities that could underlie an unexpected level of pharmacological diversity, and contribute to cross-talk regulation between transmission systems. Because G-protein-coupled receptors are major pharmacological targets, the existence of dimers could have important implications for the development and screening of new drugs. Here, we review the evidence supporting the existence of G-protein-coupled-receptor dimerization and discuss its functional importance.

BIOGENIC AMINES
A series of molecules that can act as neurotransmitters and include noradrenaline and adrenaline.

ALLOSTERIC
A term to describe proteins that have two or more receptor sites, one of which (the active site) binds the principal substrate, whereas the other(s) bind(s) effector molecules that can influence its biological activity.

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As one of the largest gene families, G-protein-coupled receptors (GPCRs) represent the most commonly used signal-transduction system in the animal kingdom. In humans, it is estimated that ~1,000 distinct members direct responses to a wide variety of chemical transmitters, including BIOGENIC AMINES, amino acids, peptides, lipids, nucleosides and large polypeptides. These transmembrane receptors are key controllers of such diverse physiological processes as neurotransmission, cellular metabolism, secretion, cellular differentiation and growth, as well as inflammatory and immune responses. The GPCRs therefore represent important targets for the development of new drug candidates with potential applications in all clinical fields. Many therapeutic agents used at present act by either activating (agonists) or blocking (antagonists) GPCRs; widely used examples are β-adrenergic receptor agonists for asthma and antagonists for hypertension and heart failure, histamine H_1- and H_2-receptor antagonists for allergies and duodenal ulcers, opioid receptor agonists as analgesics, dopamine receptor antagonists as antipsychotics and serotonin receptor agonists for migraine. The results of studies pursued over the past two decades have provided a wealth of information on the biochemical events underlying cellular signalling by GPCRs.

The proposed membrane topology of the receptors consists of a hydrophobic core of seven transmembrane α-helices that interact to form a three-dimensional barrel within the cytoplasmic membrane[1], an extracellular amino-terminal segment bearing amino-linked glycosylation sites and a cytoplasmic carboxy-terminal tail. Their binding to specific ligands involves multiple interactions between functional groups on the ligands and specific amino acids within the extracellular domains and/or the hydrophobic transmembrane core of the receptor[2]. Classically, the basic transduction unit comprises two elements in addition to the receptor: first, a trimeric (αβγ) G protein; and second, an effector component. Binding of a transmitter promotes ALLOSTERIC interactions between the receptor and the trimeric G protein, leading to the release of GDP and the binding of GTP to the α-subunit. This destabilizes the trimeric complex, allowing dissociation of the G_α•GTP and βγ dimer . The 'activated' G protein, through its G_α•GTP chain, the βγ dimer or both, in turn interacts with and modulates the effector component. Termination of the

signal is achieved via hydrolysis of GTP to GDP by a GTPase activity intrinsic to G_α.

Effector systems known to be modulated by GPCRs using the scheme described above include enzymes such as adenylyl cyclase, phospholipases C and D and cyclic GMP phosphodiesterase, as well as ion channels and antiporters such as the calcium and potassium channels and the Na^+/H^+ exchanger. Recently, additional effector systems that were classically believed to be activated by growth-factor receptors via tyrosine kinase activation were also shown to be modulated by GPCRs[3–8]. In particular, the ERK, p38 and JNK MAP (mitogen-activated protein) kinase signalling pathways were shown to be activated by stimulation of G proteins of the G_q, G_i and G_s families. Depending on the system considered, tyrosine kinases[9], phosphatidylinositol-3-OH kinase[10], Akt/protein kinase B (REF. 7), Src[11] and Ras[12] have all been implicated in this pathway. In addition, G-protein-independent signalling has been documented. For instance, direct interaction of the β_2-adrenergic receptor with the Na^+/H^+-exchanger regulatory factor, NHERF, was shown to modulate the activity of the Na^+/H^+ exchanger type-3 (REF. 13). Other examples include the chemokine[14] and angiotensin II[15] receptors, where direct recruitment and activation of the Janus kinases (JAK) was suggested.

Generally, it was believed that distinct sets of intramolecular interactions within the receptors would characterize the active and inactive conformations upon binding of the ligands. However, recent data indicate that, in addition to the specific intramolecular interactions that could define the activation states of the receptor, intermolecular interactions might also be important. Receptor dimerization as well as interactions with accessory proteins have been documented and proposed as important determinants of GPCR activity. The following sections review the biochemical and biophysical evidence supporting the existence of GPCR homo- and heterodimers. The potential roles and implications of the formation of such receptor dimers are discussed in the light of the most recent data, which indicate that dimerization and oligomeric assemblies might represent the rule rather than the exception for this important class of receptors. In several instances, the formation of oligomeric complexes larger than dimers could explain the data as well as, or in some cases even better[16] than, dimers.

History of GPCR dimerization

The concept that dimerization participates in the activation of transmembrane receptors is well accepted for many growth-factor and cytokine receptors, such as the epidermal growth factor (EGF), platelet-derived growth factor (PDGF), interferon-γ and growth-hormone receptors[17]. By contrast, until very recently, the conventional assumption for GPCRs was that monomeric receptors interacted allosterically with a single heterotrimeric G protein. However, as early as the mid-1970s, several indirect pharmacological observations led investigators to propose that GPCRs might also function as dimers. For instance, complex binding curves for both agonists and antagonists to GPCRs were interpreted as evidence for negative or positive cooperativity that could be explained by site–site interactions among receptors within dimeric or oligomeric complexes[16,18–22]. Atypical binding properties of dopamine antagonists such as [^{3}H]-spiperone, which detects only half of the maximal binding sites seen by ligands of the benzamide family, have also been interpreted as evidence for receptor dimers[23]. Biochemical studies, including photo-affinity labelling of the muscarinic receptor[24], radiation inactivation of α-adrenergic and β-adrenergic[25–27], gonadotropin[28], gonadotropin-releasing hormone[29], dopamine[30] and adenosine A1 (REF. 31) receptors, crosslinking of the glucagon receptor[32], and hydrodynamic properties of cardiac muscarinic receptors[33], also supported the idea that GPCRs might form oligomeric structures.

Despite these observations, the idea that GPCRs could function as dimers or oligomers never gained general acceptance and the prevailing model remained that of a single receptor molecule interacting with a single G protein. This dogma remained unchallenged until the mid-1990s, when *trans*-complementation studies and new biochemical data reopened the question of GPCR dimerization. Most of the evidence taken to support the existence of GPCR dimers would also be consistent with the existence of higher-order oligomers. As available techniques do not allow these possibilities to be readily distinguished, the term dimer is often used, being the simplest form of oligomer that can explain the observations. It is in this context that we use the word dimer throughout this review.

*Co*mplementation and immunoprecipitation

One of the first studies that renewed interest in the possibility that GPCRs could function as dimers was the elegant study by Maggio *et al.*[34], using chimeric α_2-adrenergic/M3 muscarinic receptors composed of the first five transmembrane domains of one receptor and the last two transmembrane domains of the other. When either chimera was expressed alone, no binding or signalling could be detected, but coexpression of the two chimeras restored binding and signalling to both muscarinic and adrenergic ligands. Similarly, coexpression of two binding-defective angiotensin II receptor point mutants rescued the binding affinity for the peptide[35], whereas coexpression of calcium receptors harbouring inactivating mutations in distinct domains was shown to partially rescue calcium-mediated signalling[36]. Such functional *trans*-complementations were interpreted as intermolecular interactions between inactive receptors in a way that restored both ligand-binding and signalling domains within a dimeric complex.

Also consistent with the idea of GPCR dimer formation was the observation that several receptor mutants behave as DOMINANT-NEGATIVE mutants when expressed with their cognate wild-type receptor[37–41]. In these cases, dimerization between the wild-type and the inactive receptor was invoked to explain the blunted response observed. This was suggested to be potentially clinically relevant for the calcium-sensing receptor, as some mutants with dominant-negative properties for this receptor are associated with inherited human hypocalcaemic disorders[37].

DOMINANT-NEGATIVE
A mutant protein that can form a heteromeric complex with the normal molecule, knocking out the activity of the entire complex.

Although these *trans*-complementation results indicate that, at least in some conditions, GPCRs can function as dimers, several investigators argued that this was most probably the case only when mutant receptors were considered. At about the same time, however, new biochemical data were starting to support the idea that wild-type GPCRs also existed as dimers. A co-immunoprecipitation approach using differentially epitope-tagged receptors provided direct biochemical evidence to support the existence of β_2-adrenergic receptor homodimers[42].When Myc- and HA-tagged β_2-adrenergic receptors were coexpressed, detection of HA immunoreactivity in fractions immunoprecipitated with the anti-Myc antibody was taken as evidence of intermolecular interactions between the two differentially tagged receptors. The selectivity of the interaction was illustrated by the lack of co-immunoprecipitation of the distantly related Myc-tagged M2 muscarinic receptor with HA–β_2-adrenergic receptor. Similar co-immunoprecipitation approaches have since been used to document the dimerization of several GPCRs, including the $GABA_B$ (REFS 43–45), mGluR5 (REF. 46), δ-opioid[47], calcium[48] and M3 muscarinic[49] receptors.

An interesting feature of many of these dimers is their relative resistance to sodium dodecyl sulphate (SDS) denaturation. Upon SDS–PAGE, they often migrate as molecular species corresponding to twice the expected monomeric receptor molecular mass. This intriguing resistance of the dimers to SDS is not unique to GPCRs and is common to several proteins that form hydrophobic intermolecular interactions[50]. This might explain the recurrent observations, in western-blot analyses, of immunoreactive receptor bands that could correspond to oligomeric complexes. Covalent crosslinking before solubilization was also found to increase the proportion of dimers observed upon western blotting, and this was used to document GPCR dimerization of the β_2-adrenergic receptor (REF. 42), the δ-opioid receptor[51], the metabotropic glutamate mGluR5 receptor[46] and the calcium receptor[48].

In most instances, detection of GPCRs dimers using co-immunoprecipitation, crosslinking and western-blot approaches was achieved in heterologous systems overexpressing the receptor under investigation. However, dimers of the A1 adenosine[52], dopamine D2 (REF. 53) and metabotropic $GABA_B$ (REF. 45) receptors were also observed *in situ* in brain tissue, indicating that the phenomenon is not simply an artefact due to anomalously high levels of expression.

Detecting dimers in living cells

Although fairly convincing, co-immunoprecipitation and western-blot analyses require receptor solubilization, raising the possibility that the observed dimers could be solubilization artefacts. This is a putatively important caveat when considering proteins such as GPCRs that are composed of seven hydrophobic transmembrane domains. Incomplete solubilization could easily lead to aggregation that could be mistakenly interpreted as dimerization. In an effort to assess the existence of GPCR dimers in living cells, several groups took advantage of biophysical assays based on light resonance energy transfer (BOX 1).

Using fusion constructs between a GPCR and bioluminescent (luciferase) and/or fluorescent (green fluorescent) proteins, bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET) were originally used to show homodimerization of the human β_2-adrenergic receptor[54] and the yeast α-mating factor[41] in living cells. More recently, the existence of δ-opioid[55] and thyrotropin-releasing hormone receptor[56] oligomers was also confirmed in intact cells using BRET. FRET between fluorescently conjugated antibodies recognizing differentially epitope-tagged receptors also allowed the detection of homodimers of the SSTR5-somatostatin[57] and δ-opioid receptors[55] in whole cells. Finally, derivatization of luteinizing hormone (LH) with two different fluorophores (fluorescein isothiocyanate and tetramethylrhodamine isothiocyanate) representing a good FRET pair permitted the detection of LH receptor dimers in cells[58].

Detection of BRET and FRET, even in the absence of added agonist, unambiguously shows that many GPCRs can form constitutive homodimers in intact living cells and that GPCR dimerization is not a biochemical artefact. The presence of receptor dimers in the absence of receptor activation by ligands raises the question of the role of dimerization in the activation process. It has been proposed[56] that the constitutive presence of dimers could explain the constitutive activity that has been described for many GPCRs[59]. To our knowledge, however, no study has systematically assessed the effect of inverse agonists, which are known inhibitors of the constitutive 'agonist-independent' activity of the receptors, on constitutive dimerization. Alternatively, constitutive dimerization could be the reflection of a more fundamental role of GPCR dimerization in receptor ontology.

Dimerization in chaperoning and transport

One of the most striking observations to indicate that GPCR dimerization might be important in receptor folding and transport to the cell surface came from studies of the metabotropic $GABA_B$ receptor. Several groups simultaneously reported that coexpression of two isoforms of the $GABA_B$ receptor, $GABA_B$R1 (a or b) (GBR1) and $GABA_B$R2 (GBR2), is a prerequisite for the formation of a functional GABA receptor at the cell surface[43–45,60,61] (FIG. 1). Detailed analysis of this phenomenon revealed that, when expressed alone in mammalian cells, the GBR1 isoforms are retained intracellularly as immature glycoproteins[62]. By contrast, GBR2 is transported to the cell surface even when expressed alone but cannot bind GABA or promote intracellular signalling[43]. When both receptors were coexpressed, the two proteins reached the cell surface as mature proteins and a functional GABA receptor ensued. These data were interpreted as an indication that heterodimerization between GBR1 and GBR2 receptors is necessary for the proper cell-surface expression of a functional $GABA_B$ receptor (FIG. 1). In agreement with this hypothesis, the existence of heterodimers could be demonstrated in the same studies by co-immunoprecipitation of two

SDS–PAGE
(Sodium dodecyl sulphate–polyacrylamide gel electrophoresis). A method for resolving a protein into its subunits and determining their separate molecular weights.

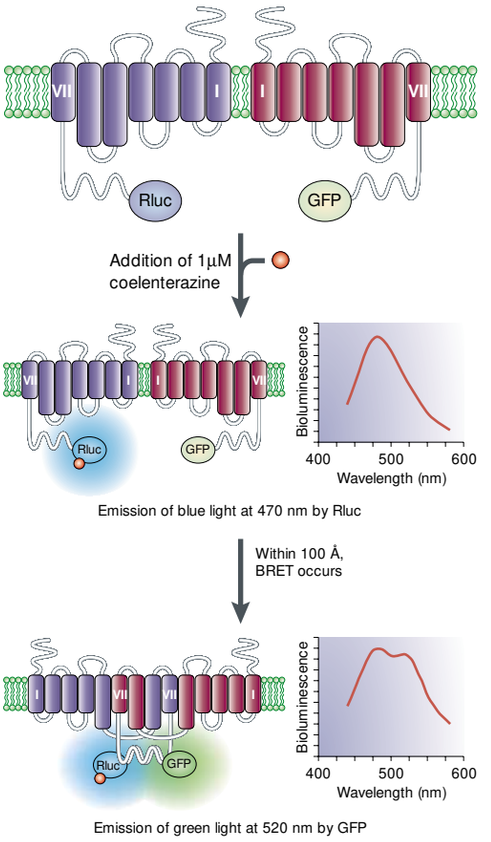
Box 1 | Light resonance energy transfer approaches

Light resonance energy transfer approaches are based on the non-radiative transfer of excitation energy between the electromagnetic dipoles of an energy donor and acceptor. In the case of fluorescence resonance energy transfer (FRET), both the donor and acceptor are fluorescent molecules, whereas for bioluminescence resonance energy transfer (BRET), the donor is bioluminescent and the acceptor is fluorescent. A prerequisite for these processes is that the emission spectrum of the donor and the excitation spectrum of the acceptor must overlap and that the donor and acceptor be in close proximity.

BRET[95] is a phenomenon occurring naturally in several marine animals such as the sea pansy *Renilla reniformis* and the jellyfish *Aequorea victoria*. In *R. reniformis*, the luminescence resulting from the catalytic degradation of coelenterazine by luciferase (Rluc) is transferred to green fluorescent protein (GFP), which, in turn, emits fluorescence at its characteristic wavelength on dimerization of the two proteins. The strict dependence on the molecular proximity between donors and acceptors for energy transfer makes it a system of choice to monitor protein–protein interactions in living cells.

As shown in the figure, one can take advantage of this phenomenon to study dimerization of G-protein-coupled receptors (GPCRs). Fusion proteins that link GFP and Rluc to the carboxyl terminus of individual GPCRs are constructed and coexpressed. In the absence of dimerization, the addition of coelenterazine H should lead to a characteristic broad bioluminescence signal with an emission peak at 470 nm, consistent with the spectral properties of Rluc. If homodimerization occurs, the energy transfer between Rluc and GFP (resulting from the proximity between the bioluminescent and the fluorescent fusion proteins) should lead to the appearance of an additional fluorescence signal with an emission peak at 530 nm that is characteristic of the GFP used (namely the red-shifted YFP)[54].

FRET can be used in the same way, using GPCRs fused to GFPs that have overlapping spectral properties (typically the CFP and the YFP). In this case, the initial energy is provided by direct excitation of the fluorescent donor with a light source[41]. Both the fluorescence emission of the acceptor and the quenching of the fluorescence of the donor can be used to quantitate the energy transfer. Antibodies[57] or ligands[58] that bind to the receptors can also be coupled to fluorophores that can be used as FRET pairs. Other variations of the FRET technique that have been used to study GPCR dimerization include photo-bleaching FRET[57] and time-resolved FRET[55]. In photo-bleaching FRET, the efficacy of energy transfer is indirectly determined by measuring the photo-bleaching time of the energy donor (upon sustained excitation) in the presence and absence of the energy acceptor. The energy transfer between the donor and the acceptor results in a slowing down of the photo-bleaching. Time-resolved FRET takes advantage of the long-lived fluorescence of fluorophores such as the lanthanide chelate Europium^{3+}, which allow delayed FRET measurements while reducing the background resulting from the short-lived autofluorescence[96].



receptors bearing different immunological tags. The observation that the transcripts of the two receptor subtypes are coexpressed in many regions of the brain[44,45], and that endogenous GBR1 and GBR2 could be co-immunoprecipitated from a cortex membrane preparation derived from rat brain[45], adds support to the physiological relevance of this phenomenon.

The idea that emerged from these studies is that GBR2 serves as a MOLECULAR CHAPERONE that is essential for the proper folding and cell-surface transport of GBR1 and of a functional metabotropic GABA receptor. Whether, once at the cell surface, the dimer is the functional receptor, or whether the maturation and transport of GBR1 to the cell surface is sufficient, is discussed more extensively in the next section. In any case, the idea that GBR2 serves as a chaperone and escort protein for GBR1 has been further supported by a recent study identifying an endoplasmic reticulum (ER) retention signal within the carboxyl tail of GBR1 (REF. 63). According to the model proposed by the authors, GBR1–GBR2 dimerization involving a COILED-COIL INTERACTION of the carboxyl tail would serve to hide the ER retention signal, thereby allowing ER export and plasma membrane targeting of the dimer.

The role of dimerization as an early event involved in receptor maturation and transport is further supported by the observation that the dominant-negative effect of truncated forms of the V2 VASOPRESSIN receptor resulted from the interaction of the truncated mutants with full-length receptors, leading to the intracellular retention of the complex[38]. The early onset of dimer formation is also confirmed by the fact that mutants of the vasopressin receptors that cause nephrogenic diabetes because they are retained in the ER and never reach the cell surface[64] also form dimers (J. P. Morello, D. Bichet and M. B., unpublished observations). The dominant-negative effects of a truncated form of the CCR5 chemokine receptor (CCR5Δ32) over its wild-type counterpart were also attributed to its propensity to dimerize with the wild-type receptor in the ER, thereby promoting intracellular retention of the heterodimer[39]. As the wild-type receptor is a major co-receptor for HIV entry[65], it has been proposed that this dominant-negative effect of CCR5Δ32 could explain the slow onset of AIDS in patients who are heterozygous for this mutation[66,67]. The intracellular retention of the wild-type dopamine D3 receptor upon coexpression of a splice variant form,

MOLECULAR CHAPERONE
A protein that assists in the non-covalent assembly of a protein complex but does not participate in its function.

COILED-COIL INTERACTION
A type of protein–protein interaction that involves interlacing of two helical domains.

VASOPRESSIN
Antidiuretic hormone.

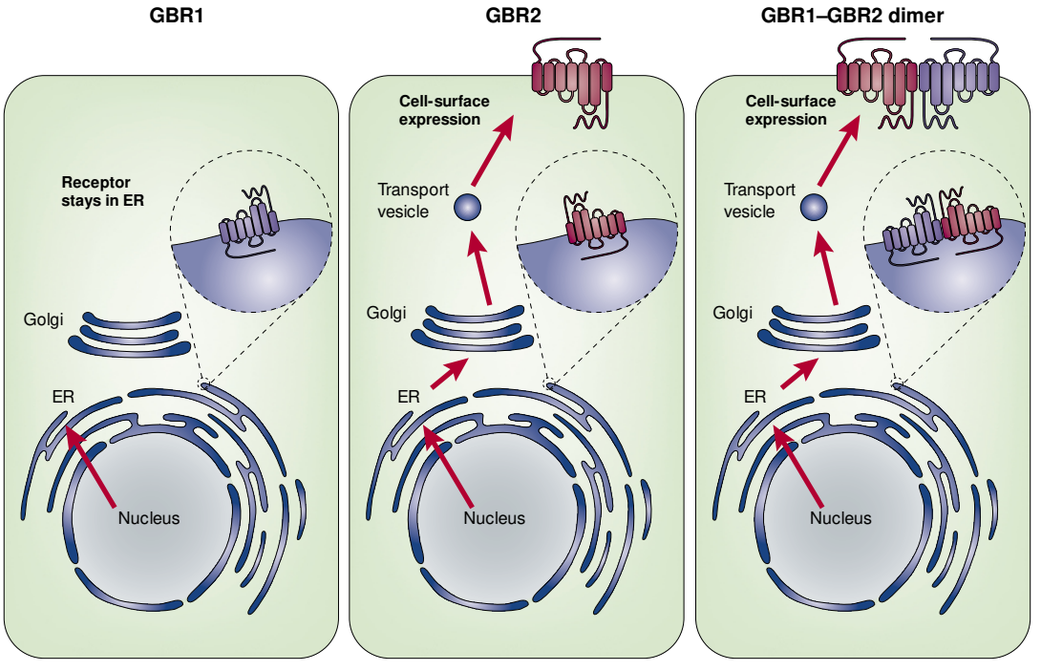


Figure 1 | **Role of homo- and heterodimerization in the transport of G-protein-coupled receptors.** When expressed alone, the $GABA_BR1$ (GBR1) receptor is retained as an immature protein in the endoplasmic reticulum (ER) of cells and never reaches the cell surface. By contrast, the GBR2 isoform is transported normally to the plasma membrane but is unable to bind GABA and thus to signal. When coexpressed, the two receptors are properly processed and transported to the cell surface as a stable dimer, where they act as a functional metabotropic $GABA_B$ receptor.

D3nf, which is truncated before the sixth transmembrane domain, has also been attributed to early dimerization in the ER[68]. The potential regulatory role of this naturally occurring variant in dopamine receptor function remains to be investigated, but the overlap in the distribution of wild-type and D3nf in pyramidal neurons of rat brains[69] certainly makes it worth exploring.

Role of dimerization in signal transduction

The strongest evidence supporting a role for GPCR dimerization in signal transduction once the receptor has reached the cell surface also comes from work carried out on the $GABA_B$ receptor. As mentioned above, mutation of the ER retention signal within the carboxyl tail of GBR1 results in the transport of GBR1 to the cell surface, but for this mutant receptor to respond functionally to GABA, it had to be coexpressed with GBR2 (REF. 63). This strongly suggests that the formation of a GBR1–GBR2 dimer is essential for signalling and that the simple cell-surface targeting of GBR1 is not sufficient. Although entirely consistent with the idea that the dimer represents the signalling unit, one cannot exclude the possibility that GBR2 might be required for the proper folding of GBR1 in the ER, and that the mutant GBR1 expressed alone might not reach the correct conformation.

Additional evidence indicating a role for dimerization in GPCR function comes from the observation that a peptide derived from the proposed dimerization interface of the β_2-adrenergic receptor (see the section on architecture and three-dimensional organization) inhibits both dimerization and receptor-stimulated adenylyl cyclase activity[42]. This result can easily be reconciled with a two-state receptor model, assuming that the monomer and dimer represent the inactive and active conformations respectively. Obviously, these results alone do not prove that the dimer represents the active form of the receptor or that dimerization is even required for activation. Indeed, it could be proposed that the peptide prevents signalling by disrupting intramolecular interactions within the receptor monomer, and that loss of dimers is a consequence rather than the cause of receptor inactivation. Supporting this possibility is the observation that a similar peptide derived from the dopamine D1 receptor inhibits dopamine signalling without affecting dimerization[70]. However, the demonstration that bivalent anti-β_2-adrenergic receptor antibodies, but not their monovalent FAB FRAGMENTS, function as agonists and stimulate receptor activity[71] lends further support to the idea that activation might result from dimerization.

In a slightly different context, antibody-promoted dimerization had previously suggested a role in gonadotropin-releasing hormone receptor activity. Crosslinking of gonadotropin-releasing hormone peptide antagonists with specific antibodies converted the antagonists to agonists, suggesting that induction of receptor dimerization/aggregation is sufficient for activation[72,73]. Similarly, dimerization of the occupied LHRH (LH-releasing hormone) receptor was proposed as the mechanism leading to LH release from pituitary cells[74]. In a more recent study, Carrithers and Lerner[75]

FAB FRAGMENT
The antigen-binding portion of an antibody.

showed that covalent dimerization of a peptidic α-MSH (melanocyte-stimulating hormone) receptor antagonist transformed it into an agonist, also consistent with the view that dimerization of this GPCR might be sufficient to activate G-protein signalling. More recently, additional indirect evidence supporting a role for dimerization in receptor activity came from FRET studies. FRET between fluorescent LH derivatives was observed in cells expressing a wild-type receptor, but not a receptor that can bind LH but is unable to transmit the signal, indicating that these signalling-deficient receptors were unable to form dimers[58].

Interestingly, an anti-CCR5 receptor antibody that promotes dimerization was found to activate receptor-promoted calcium mobilization and cell migration[40], but to inhibit its function as an HIV co-receptor and thereby prevent viral entry into cells[76]. This indicates that oligomerization might have both positive and negative influences on distinct receptor functions. A role in signal termination was also invoked for the dimerization of the δ-opioid receptor, as truncation of the receptor carboxyl tail was found to inhibit both dimerization and agonist-induced endocytosis, a process involved in receptor desensitization[51].

Although increasing evidence supports the idea that GPCR dimerization might be an important aspect of receptor function, how it does so and whether dynamic regulation of the oligomeric state is involved in normal receptor activity remain highly debated issues.

Expanding receptor diversity

The existence of GBR1–GBR2 dimers explicitly showed that non-identical receptors can form stable dimers and raised the intriguing possibility that additional heterodimers between distinct receptor subtypes could exist. This possibility was rapidly confirmed in 1999 when Jordan and Devi showed that, when coexpressed in the same cells, Myc-tagged κ-opioid and flag-tagged δ-opioid receptors could be co-immunoprecipitated and therefore existed as a stable complex[47]. By contrast, no heterodimerization between κ- and μ-opioid receptors was observed. Interestingly, the pharmacological properties of the κ–δ heterodimer were found to be different, both from those of each receptor expressed individually and from what would be expected from a simple mixture. For instance, the heterodimer showed no significant affinity for either κ- or δ-selective agonists or antagonists but showed high affinity for partially selective ligands. However, selective ligands were found to bind the heterodimer synergistically when added simultaneously. At the functional level, the more-than-additive effect of selective κ- and δ-agonists on the stimulation of MAP kinase activity was interpreted as a synergistic action of the drugs on the heterodimer.

As some of the properties of the heterodimer were similar to those reported for the proposed κ2-opioid receptor subtype[77], Devi and colleagues[47] proposed that heterodimerization between opioid receptor isoforms might account for some of the receptor subtypes that have been identified pharmacologically but for which no gene or cDNA has been found, despite large-scale efforts. Alternatively, these heterodimers could serve as receptors for as yet undiscovered endogenous opioid peptides. If heterodimerization is a general feature of the GPCR class of receptors, this would offer a theoretical basis for a pharmacological complexity that was unanticipated. Also, it would provide a molecular mechanism that could explain some aspects of the cross-talk regulation observed between different signalling systems.

Although the generality of the phenomenon remains to be shown, recent studies suggest that heterodimerization is most probably not restricted to the GBR1/GBR2 and κ/δ-opioid receptor cases. Co-immunoprecipitation studies revealed stable association of the angiotensin I and bradykinin B2 (REF. 78), dopamine D1 and adenosine A1 (REF. 79), μ- and δ-opioid[80] and β_2-adrenergic and δ-opioid receptors, whereas a combination of co-immunoprecipitation and FRET approaches convincingly showed heterodimerization between somatostatin SSTR5 and SSTR1 (REF. 57), and SSTR5 and dopamine D2 (REF. 81) receptors.

In some of these cases, the functional consequences of the heterodimerization observed in heterologous expression systems might help to rationalize cross-talk regulatory processes that have been postulated *in vivo*. For instance, the functional antagonism between the vasoconstrictor and vasodilator actions of angiotensin II and bradykinin could involve mutual regulatory influence at the receptor level. This is an intriguing concept, considering that these two signalling systems are already interconnected by the angiotensin-converting enzyme that releases angiotensin II from its precursor and inactivates bradykinin. It was observed that coexpression of the angiotensin I and bradykinin B2 receptors in HEK-293 cells increases the efficacy and potency of angiotensin II, but reduces the ability of bradykinin to stimulate inositol-phosphate production[78]. Whether this truly reflects receptor heterodimerization remains to be investigated. However, the fact that similar regulatory influences between the two receptor systems could also be observed in the smooth muscle cell line A10 that endogenously expresses both angiotensin I and bradykinin B2 receptors, and that these two receptors are coexpressed in several tissues including smooth muscle, glomerular mesangium and renal medulla, lends more support to a possible physiological role for heterodimerization.

For adenosine A1/dopamine D1 heterodimerization, the coexpression of these two receptors in cortical neurons and their widely reported antagonistic interactions in the central nervous system might offer a physiological rationale. Interestingly, Ginés *et al.*[79] found that in fibroblasts, in which they could co-immunoprecipitate the A1 and D1 receptors, pretreatment with both adenosine and dopamine agonists, but not with either individually, reduced the signalling efficacy of the D1 receptor upon subsequent stimulation. Whether this mechanism contributes to the known A1-induced inhibition of D1 receptor function in the brain remains to be formally tested.

Similarly, colocalization of the dopamine D2 receptor and somatostatin SSTR5 in cortical and striatal neurons, and the rich clinical and behavioural literature

indicating interactions between the somatostatinergic and dopaminergic systems, supports the idea that the heterodimerization observed by FRET in heterologous expression system might have physiological relevance[81]. In this case, the functionality of the heterodimer was demonstrated by showing that coexpression of the D2 receptor with a SSTR5 mutant (Δ318 SSTR5), which binds somatostatin but does not signal when expressed alone, imparts a somatostatin response to the cells. This somatostatin-mediated inhibition of adenylyl cyclase activity was blocked by a dopamine antagonist, indicating that SSTR5 and D2 heterodimerize to constitute a functional receptor. Also, synergistic binding of dopaminergic and somatostatinergic agonists was observed upon coexpression of wild-type SSTR5 and D2 receptors. As for the other examples reported above, further work is now required to determine if such functional heterodimers can provide an explanation for the reported reciprocal enhancement of each of these signalling systems upon administration of both somatostatin and dopamine *in vivo*.

Despite increasing evidence that GPCR heterodimerization might be a general phenomenon, a word of caution is provided by a recent study by McVey and colleagues[55]. In their study, these authors clearly demonstrate that homodimerization of the β_2-adrenergic and δ-opioid receptors can be detected by co-immunoprecipitation, BRET and time-resolved FRET approaches. However, heterodimerization between these two receptor types was detected only after co-immunoprecipitation, and no significant BRET or FRET signals were observed upon their coexpression. Given that co-immunoprecipitation requires solubilization of the receptor, and that BRET and FRET were carried out in living cells, the authors concluded that this heterodimerization might represent a biochemical artefact resulting from nonspecific aggregation of these hydrophobic proteins in the co-immunoprecipitation conditions. This conclusion contrasts sharply with that of Jordan *et al.*[82], who also observed co-immunoprecipitation of the β_2-adrenergic and δ-opioid receptors but additionally found that a selective β_2-adrenergic agonist promoted internalization of the δ-opioid receptor in living cells, indicating that a functional heterodimer was expressed at the surface of these cells. The apparent contradiction between these two studies could be due to the inability of FRET and BRET to detect the heterodimer as a result of distance constraints (BOX 1), or might indicate that the β_2-adrenergic receptor could promote the internalization of the δ-opioid receptor by a process that is independent of heterodimerization. In any case, it emphasizes that great care must be taken in the interpretation of data generated in this emerging field.

Stable association between distinct receptor subtypes is not the only type of heterodimerization in which GPCRs engage. Recent studies have indicated that interaction with newly discovered accessory proteins as well as with receptors of completely distinct classes can have important consequences for GPCR expression and function (BOX 2 and BOX 3).

As already indicated, a pharmacological diversity that exceeds what can be accounted for by molecularly defined and cloned receptors has been proposed for several receptors. These include: the atypical β-adrenergic pharmacology often referred to as the β_4-receptor, CALCITONIN-gene-related peptide 1 (CGRP1) and CGRP2 subtypes, C5a receptor subtypes, ET_B receptor subtypes, galanin receptor subtypes, additional metabotropic glutamate receptor subtypes, a neuropeptide Y3 receptor, $\mu_{1,2}$-, $\delta_{1,2}$- and $\kappa_{1,2,3}$-opioid receptor subtypes, platelet-activating-factor (PAF) receptor subtypes, additional prostanoid receptor subtypes, additional neurokinin receptor subtypes and subtypes of the vasoactive intestinal peptide PAC_1 receptor. Whether some of these unaccounted-for pharmacologically identified subtypes could be explained by heterodimerization between already cloned receptors or between such receptors and accessory proteins remains an open question. Obviously, other explanations such as alternative splicing, post-translational modifications or the existence of additional genes coding for these receptors could also be responsible for this diversity.

A definitive demonstration that heterodimerization does indeed contribute to the pharmacological diversity and regulation of GPCRs will require colocalization and simultaneous expression of potentially heterodimerizing receptors to be documented in native tissues in each case. Correlation between coexpression and the functional consequences imparted by heterodimerization will remain the ultimate criteria to judge the general physiological importance of this phenomenon.

Dynamic regulation of GPCR dimerization

Given the potential role that dimerization could play in receptor transport, function and pharmacological specificity, the question of the dynamic regulation of dimer formation becomes central in understanding receptor activation and regulation processes. However, when the effects of agonist stimulation were considered for various receptor homo- and heterodimers, different groups obtained somewhat different results. When co-immunoprecipitation and/or western blots were used to assess the extent of dimerization, agonists were found to increase[42,76,81] (for β_2-adrenergic, SST5 and chemokine CCR5 homodimerization), decrease[51,79] (for δ-opioid homodimerization and dopamine D1/adenosine A1 heterodimerization) or to have no effect on (for κ-opioid and M3 muscarinic homodimerization as well as angiotensin I/bradykinin B2 heterodimerization)[78,83,84] the extent of dimerization.

Such variability could be attributed to the poor quantitative power of the co-immunoprecipitation and western-blot approaches. However, the more quantitative energy-transfer techniques also led to considerably variable results. In the cases of the δ-opioid[55] and yeast α-mating factor[41] receptors, the BRET or FRET basal signals (indicative of constitutive dimerization) remained unaffected by the addition of agonists, whereas for the β_2-adrenergic[54] and thyrotropin-releasing hormone[56] receptors, agonists promoted an increase in BRET signals above the basal levels. When considering

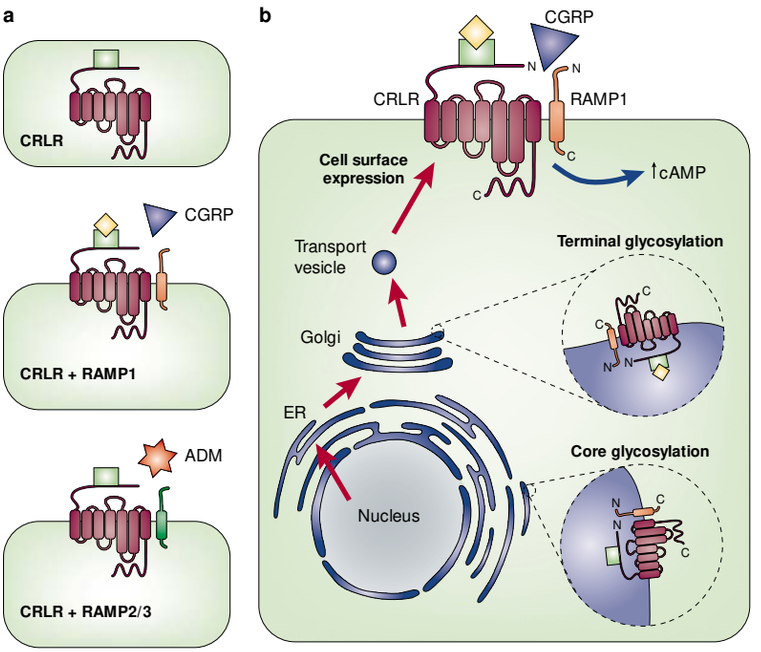
CALCITONIN
A polypeptide hormone, consisting of 32 amino-acid residues, that regulates calcium and phosphate levels in the blood.

HOMOTROPIC
Interaction between proteins of the same class.

ADRENOMEDULLIN
A hypotensive peptide hormone secreted by the medulla of the adrenal gland.

AMYLIN
A peptide consisting of 37 amino-acid residues that is secreted with insulin and might act to modulate its stimulatory effects on glucose metabolism in muscle. Also known as islet amyloid peptide.

Box 2 | Heterodimerization of CRLR and RAMP



HOMOTROPIC interactions between G-protein-coupled receptors (GPCRs) are not the only type of protein–protein interaction shown to influence their functional expression. Recently, a new class of membrane proteins that can interact with GPCRs and affect their activity profile has been identified. These new proteins were discovered while studying the expression of a complementary DNA that encoded a putative GPCR, which did not lead to the expression of a functional receptor. Specifically, a cDNA named calcitonin-receptor-like receptor (CRLR), which showed 55% overall identity to the calcitonin-receptor gene, was proposed to encode the receptor for the calcitonin-gene-related peptide (CGRP)[97]. However, various attempts to show that it was indeed the CGRP receptor failed because it was impossible to demonstrate any type of functional expression[98].

Several years later, using an expression-cloning approach in oocytes, McLatchie *et al.*[99] isolated a new gene product that conferred CGRP signalling when expressed in oocytes. Surprisingly, the new gene encoded a relatively small protein containing only one putative transmembrane (TM) domain and a short cytosolic tail. Obviously, the structure of this protein did not conform to the general seven-TM-domain topology of GPCRs. Further studies showed that this single TM-domain protein had to be coexpressed with the CRLR gene product to confer CGRP binding and signalling[99]. The newly discovered protein was therefore considered as a co-receptor and was named receptor-activity-modifying protein (RAMP). Sequence data analysis and additional cloning experiments led to the identification of at least three isoforms of RAMP (1, 2 and 3). As the figure shows, although coexpression of CRLR with RAMP1 generated a CGRP receptor, coexpression with RAMP2 or RAMP3 produced a receptor with the pharmacological properties of the ADRENOMEDULLIN receptor, suggesting that the nature of the RAMP could determine the pharmacological properties of a receptor produced from a unique GPCR-encoding gene. Taken with the observation that CRLR expressed in the absence of RAMP is retained intracellularly and does not reach the cell surface, these results led to the suggestion that RAMPs act as chaperone/escort proteins that facilitate the maturation and targeting of the receptors, and also as direct activity modifiers through intermolecular interactions once the receptors have reached the cell surface. Coexpression of RAMP1 and RAMP3 with a cDNA encoding the calcitonin receptor (CTR) allows the expressed receptor to bind AMYLIN in addition to calcitonin, showing that the actions of RAMP are not limited to CRLR[100]. However, in this case, RAMP was not required for the transport of the CTR receptor to the cell surface and seemed to act solely as an activity modifier. Therefore, as is the case for dimerization among GPCRs, heterodimerization with RAMP is involved both in endoplasmic-reticulum (ER) export and transit to the plasma membrane, and in the modulation of receptor function at the cell surface. The relative importance of these roles seems to vary from case to case.

Despite intense efforts to identify additional members of the RAMP family through databank analysis or sequence homology cloning, no additional genes or cDNAs have been found so far, indicating that this family of proteins has only a few members, or if additional members do exist, that they have important sequence differences. Supporting the latter possibility is the identification, in *Caenorhabditis elegans*, of a single transmembrane domain protein named ODR4 that shares no sequence homology with the RAMPs but is required for the cell-surface targeting and functional expression of the seven-TM olfactory receptor, ODR10 (REF. 101)**.**

VINBLASTIN
An alkaloid that arrests mitosis in metaphase by binding to spindle microtubules.

CYTOCHALASIN
Any of a group of fungal metabolites that interfere with the assembly and diassembly of actin filaments. One of the consequences of treating cells with these agents is that cleavage of the cytoplasm after nuclear division is prevented.

DYNAMIN
A protein involved in the formation of microtubule bundles and in membrane transport.

the heterodimerization of the D2 and SSTR5 receptors, Rocheville *et al.*[81] found that FRET between D2 and SSTR5 fluorescently labelled antibodies could be observed only in the presence of either somatostatin or dopamine. Similarly, using fusion constructs between the gonadotropin-releasing hormone receptor and green and red fluorescent proteins, Cornea *et al.*[85] found a FRET signal that was entirely agonist-dependent.

Agonist-promoted energy transfer was distinguishable from macro-aggregation processes such as patching, capping and internalization, as in the case of the gonadotropin-releasing hormone receptor it was not inhibited by VINBLASTIN, CYTOCHALASIN or EGTA[85]. A contribution of the receptor internalization process to the agonist-mediated increase in BRET was also excluded for the β_2-adrenergic and thyrotropin-releasing hormone receptors as the BRET increase was not prevented by a dominant-negative mutant of DYNAMIN (dynamin K44A) that blocks receptor endocytosis via clathrin-coated vesicles[54,56]. Also, coexpression of two receptors that are known to be internalized via the clathrin-coated pit pathway (the β_2-adrenergic receptor–Rluc and the chemokine CCR5–YFP) did not result in any BRET, even in the presence of agonists for the two receptors[54], indicating that clustering of receptors into clathrin-coated pits is not sufficient to promote BRET.

As the contribution of receptor clustering and internalization was excluded as a likely explanation for agonist-induced increase in energy transfer, several authors[56,81,85] concluded that agonists promote dimer formation. However, great care must be taken before jumping to that conclusion. Although an increase in the number of dimers would lead to a larger BRET or FRET signal, a different interpretation could account for the changes in energy transfer. Both BRET and FRET efficacy vary with the sixth power of the distance between the

Box 3 | Heterodimerization of dopamine D5 and $GABA_A$ receptors

G-protein-coupled receptors (GPCRs) and ion-channel receptors belong to two entirely different classes of protein and until recently, no data indicated that they could physically interact. However, as the two main classes of proteins involved in neurotransmission, it would not be surprising if they did. In fact, the reported dopamine-receptor-mediated modulation of $GABA_A$-stimulated synaptic activity would support such an interaction. Addressing this possibility, Liu *et al.*[102] provided convincing biochemical and functional data indicating that the dopamine D5 receptor physically interacts with the GABA-operated chloride channel $GABA_A$ receptor. Using hippocampal neurons and transfected fibroblasts, the authors demonstrated the direct binding of the carboxy-terminal portion of the D5 receptor to the second intracellular loop of the $GABA_A$ receptor γ2 subunit. This interaction was found to be dependent on the presence of agonists for both receptors, indicating that only the activated forms interacted.

In cells coexpressing the two receptors, the D5-mediated stimulation of adenylyl cyclase was inhibited by GABA, whereas the GABA-induced chloride current was decreased by the activation of the dopamine receptor, indicative of reciprocal receptor cross-talk. This functional cross-talk was shown to be dependent on the physical interaction between the two receptors, as it could be blocked by the expression of mini-genes expressing either the carboxyl tail of D5 or the γ2 subunit of $GABA_A$ that both inhibited the formation of the complex. The physiological relevance of the interaction is further supported by the observation that a D5 agonist decreased the amplitude of the $GABA_A$-mediated miniature inhibitory postsynaptic current in cultured hippocampal neurons under conditions where the second-messenger-mediated pathways (PKA and PKC) were blocked. This regulatory effect was prevented by the addition of a peptide derived from the carboxyl tail of the D5 receptor, supporting the idea that it resulted from a direct interaction between the channel and the GPCR.

As no interaction could be observed between $GABA_A$ and the D1 dopamine receptor, the ability of D5 to form a heterodimer with the ionotropic receptor might represent the first clearly defined functional role differentiating these two dopamine receptor subtypes. The fact that D5, but not D1, is preferentially targeted to the dendritic shafts and the cell soma/axon area of cortical and hippocampal neurons, where inhibitory GABAergic neurons form major postsynaptic contacts[103], supports the physiological relevance of such a selective interaction.

Overall, heterodimer formation was interpreted as a newly discovered mechanism by which GPCRs and ligand-gated channels mutually regulate each other in the control of synaptic signalling efficacy.

energy donor and acceptor, and they are also sensitive to their dipole orientations (BOX 1). Therefore, conformational changes promoted by the binding of an agonist to a pre-existing dimer could lead to significant changes in energy-transfer efficacy. The extent to which the conformational change imposed by a specific receptor agonist is communicated to the receptor domain where the fluorophores have been attached could determine whether a change in signal is observed. In some cases, the signal could go from undetected to very significant, whereas in others the high constitutive signal (reflecting an already optimal transfer) would not be affected by the binding of the agonist.

Such an explanation is entirely compatible with the chaperoning role evoked above for the dimerization of several GPCRs, as their dimerization occurs in the ER, long before the agonists can influence dimer formation. The recent crystallographic resolution of the three-dimensional structure of the extracellular amino-terminal domain of the metabotropic glutamate receptor is also consistent with such a model of constitutive dimerization. Indeed, this part of the receptor, constituting its ligand-recognition and binding domain, was found to exist as a dimer whether glutamate was present or not, with the addition of glutamate leading to a significant conformational change of the pre-existing dimer[86].

Clearly, additional studies are required to determine unambiguously if dimer assembly can be regulated by the activation state of the receptor. The role of post-translational modification, as well as interaction with other proteins involved in signal transduction (for example, G proteins, β-arrestins, receptor-activity-modifying protein (RAMP)), also remains to be determined. These are crucial questions for understanding the mechanisms underlying receptor activation, and they take on a special importance when considering the heterodimers. Receptor selectivity would have to be considered in an entirely new light if receptor monomers were found to exchange between homodimers and different heterodimers once they have reached the cell surface and become activated.

Architecture of GPCR dimers

The only available data that directly address the three-dimensional structure of GPCR dimers is the recently solved structure of the extracellular ligand-binding (amino-terminal) region of the metabotropic glutamate receptor mGluR1 (REF. 86). The crystal structure shows that a single disulphide bridge between Cys140 of each monomer connects the two PROTOMERS (FIG. 2). However, the authors of this study argue that this disulphide bond cannot act as a scaffold because of its location within a disordered segment of the protein. The dimer interface was proposed to consist mainly of the helical packing between α-helices (helices B and C) in each monomer.

On the basis of co-immunoprecipitation and western-blot studies, the formation of intermolecular disulphide bonds within the extracellular amino-terminal portion of the receptors has also been proposed to contribute to the formation of GPCR dimers. For mGluR1, mutation of Cys140 interfered with the detection of dimers[87,88]. Similarly, disulphide bonding within the large extracellular amino-terminal domains of mGluR5 (REF. 46) and the calcium-sensing[48] receptor was found to be important for covalent dimerization. Finally, cysteines located in extracellular loops two and three of the M3 muscarinic receptor were also invoked in the dimerization of this receptor[83]. For mGluR5 (REFS 87,89) and the calcium-sensing receptor[90], however, the disulphide bond was found not to be the only point of contact, and non-covalent interactions involving both the extracellular and transmembrane domains were proposed. The idea that hydrophobic interactions involving transmembrane domains could be involved in GPCR dimerization was first proposed for the β_2-adrenergic receptor[42]. The idea came from an elegant series of experiments by Engleman and colleagues[91,92] that combined the use of synthetic peptides, site-directed mutagenesis and biophysical techniques. They showed that specific residues located in the transmembrane domain of GLYCOPHORIN A are essential for the formation of the hydrophobic interactions that stabilize the protein dimers. On the basis of an analogy with this proposed motif, glycine and leucine residues located within the sixth transmembrane segment of the β_2-adrenergic

PROTOMERS
Identical subunits in an oligomeric protein complex.

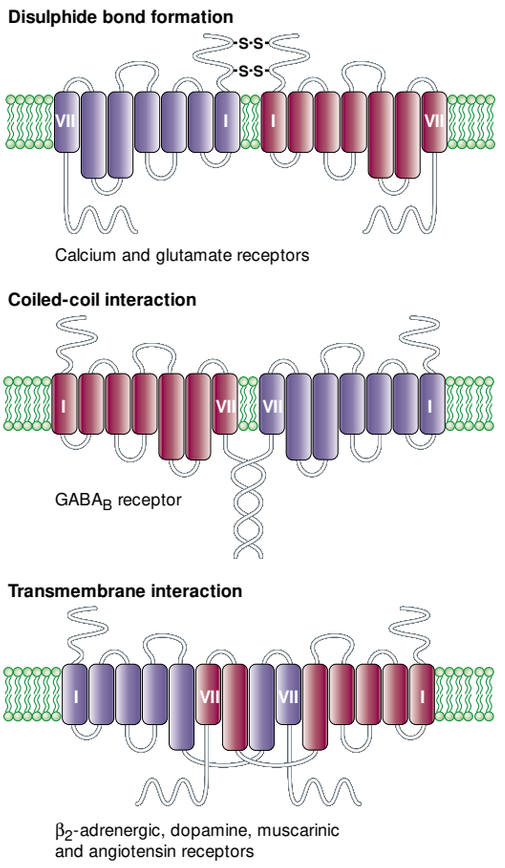


Figure 2 | **Molecular determinants of G-protein-coupled-receptor dimerization.** Distinct intermolecular interactions were found to be involved for various G-protein-coupled receptors. Covalent disulphide bonds were found to be important for the dimerization of the calcium-sensing and metabotropic glutamate receptors. A coiled-coil interaction involving the carboxyl tail of the GBR1 and GBR2 receptors is involved in the formation of their heterodimer. Finally, for monoamine receptors such as the β_2-adrenergic and dopamine receptors, interactions between transmembrane helices were proposed to be involved.

receptor were suggested as part of the dimerization interface for this receptor[42]. Similar results were obtained for the dopamine D2 receptor[53]. However, the fact that peptides derived from several transmembrane domains of D2 could also block dimerization suggests that transmembrane α-helices might have a more general role in receptor folding and oligomerization and might not be limited to strict consensus sequences.

As mentioned previously, yeast two-hybrid screens[43] pointed to coiled-coil domains within the carboxyl tails of GBR1 and GBR2 as important molecular determinants of GABA$_B$ receptor heterodimerization (FIG. 2). However, mutagenesis studies revealed that, although it was important for receptor function and to mask the ER retention signal, the coiled-coil motif was not necessary for dimer formation, as deletion of the carboxyl tail did not abrogate it[63]. It is therefore likely that the carboxyl tail participates in but is not essential for dimerization. Although it lacks a consensus sequence for a coiled-coil domain, the carboxyl tail of the δ-opioid receptor was also invoked as a determinant of dimerization[51].

Clearly, results available so far are too fragmentary to establish a general molecular mechanism for GPCR dimerization. The different dimerization modes proposed in the different studies (FIG. 2), rather than reflecting different strategies used by receptors of different classes, most probably indicate that multiple sites of interaction are involved in the assembly and stabilization of receptor dimers.

Computational studies led Gouldson *et al.*[93] to propose two alternative three-dimensional models that could describe GPCR dimers (FIG. 3). Molecular-dynamics simulations, correlated-mutation analysis and evolutionary-trace analysis all support the involvement of transmembrane helices five and six in the dimerization interface, but cannot distinguish between the domain-swapped and contact-dimer models. Interestingly, the two models predict that the third intracellular loop originating from each monomer would be parallel within the dimer. This could have important functional implications given the role proposed for this receptor domain in G-protein engagement and stimulation. Although some of the biochemical and functional data might be more easily rationalized by one model than another, the validation of any of these models awaits additional studies. The most direct test will certainly come from the resolution of the structure of a GPCR dimer crystal. Unfortunately, the recent crystallization of rhodopsin did not allow the issue of dimerization to be directly addressed. The crystal revealed an antiparallel dimer with respect to the plane of the membrane that certainly resulted from the crystallization process. Most probably, solubilization led to the disassembly of physiologically relevant dimers and the antiparallel dimer formed to minimize packing energy during crystallization. Nevertheless, the resolution of this structure still provided indirect information that could be analysed in the context of the dimer hypothesis. Indeed, the size of the intracellular surface exposed to the cytosol is too small to account for the simultaneous interaction of the receptor with both α- and βγ-subunits of the G protein. As it is generally accepted that such concomitant interaction is required for function, it could suggest that a receptor dimer is needed for a complete and productive interaction with a single heterotrimeric G protein. Of course, this intriguing speculation will require a more formal demonstration.

Concluding remarks

After 20 years as an 'underground' concept, in the past five years GPCR dimers have gone from being a controversial idea to a widely accepted hypothesis. Although its universality still needs to be established, the increasing number of reports that have used various approaches to describe GPCR dimers has led to the general acceptance that at least some of them exist as oligomeric assemblies involving more than one receptor. The use of biophysical approaches that do not require cell disruption, such

GLYCOPHORIN A
A carbohydrate-rich sialoglycoprotein that is abundant in erythrocyte membranes.

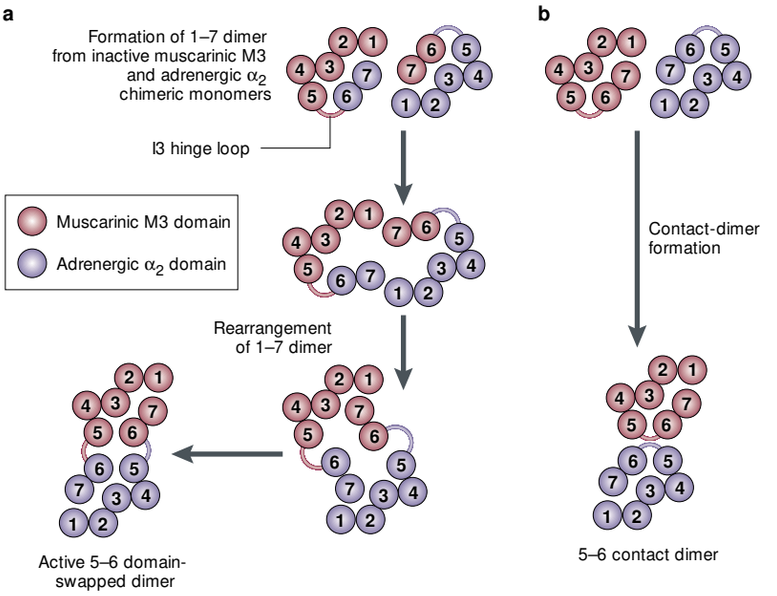


Figure 3 | **Alternative three-dimensional models showing dimers of G-protein-coupled receptors.** Two models have been proposed for the general three-dimensional organization of G-protein-coupled-receptor dimers. **a** | First is the domain-swapping model in which each functional unit within the dimer is composed of the first five transmembrane domains of one polypeptide chain and the last two of the other. Such a model is useful to rationalize the functional complementation observed when mutant or chimeric receptors are coexpressed. **b** | Second is the contact model in which each polypeptide forms a receptor unit that touches the other through interactions involving transmembrane domains five and six.

as light energy transfer, has certainly helped to reassure us that they are not biochemical artefacts. Also, the absolute requirement of heterodimerization for the transport of a functional metabotropic $GABA_B$ receptor to the cell surface has strongly reinforced the idea that dimerization is functionally important. Whether the main role of dimerization will be in the chaperoning and cell transport of receptors, or in the control of signalling specificity and efficacy, remains an open question for most receptors. However, a growing body of evidence arguing in favour of important roles for dimerization in all GPCR classes largely justifies further investigation. This is particularly true when considering the veritable revolution that it might trigger in the development and screening of drugs that target GPCRs for their therapeutic actions. For instance, defining the oligomerization interface and its molecular dynamics could offer new pharmacological targets. Compounds that modulate receptor dimerization without interfering with the hormone-binding pocket could represent a new class of non-competitive drugs with distinct selectivity and activity profiles.

One of the most intriguing promises is offered by the possibility that heterodimerization might be a common phenomenon among GPCRs. In addition to exponentially increasing the number of pharmacologically distinguishable targets, the definitive demonstration of their physiological relevance will markedly change the way that high-throughput screens are conceived for this class of receptor. For one, combinatorial expression systems rather than systems expressing unique receptor isoforms would have to be considered. Also, assays aimed at monitoring the dimerization state of receptors could be imagined.

The pharmacological reality of such considerations is supported by the recent report that the anticonvulsant, antihyperalgesic and ANXIOLYTIC AGENT gabapentin is an agonist for the GBR1a/GBR2 heterodimer but is esssentially inactive on the GBR1b/GBR2 complex[94]. In CA1 pyramidal neurons of rat hippocampal slices, gabapentin was found to activate postsynaptic inward rectifier K^+ currents, probably via the GBR1a/GBR2 heterodimer, but it did not depress $GABA_A$ signalling presynaptically, indicating a selectivity of action that relies in part on the expression of a specific $GABA_B$ receptor heterodimer. Such selective agonism might be part of its therapeutic advantage as an anticonvulsant. Obviously, the possibilities become staggering when the proposed interactions between GPCRs and other classes of receptor (for example, ionotropic), or accessory protein (for example, RAMP), are taken into account.

Oligomeric assembly of proteins, allowing expanded diversity with a limited number of modular elements, is the rule rather than the exception in biology. When considering the nervous system, the existence of homo- and heterodimers of neurotransmitter GPCRs offers an attractive hypothesis that could underlie the high degree of diversity and plasticity that is characteristic of such a highly organized and complex system.

Despite the excitement raised by the discovery of GPCR homo- and heterodimers, many questions still need to be answered before we can fully appreciate their real contribution to normal physiology. Among them, the general importance of heterodimerization in explaining pharmacological diversity and cross-talk regulation processes is likely to attract considerable attention. Although technically more demanding, it will also be necessary to assess the occurrence and roles of heterodimers in native tissues to validate each of the observations made in heterologous expression systems. If heterodimerization is found to be a general process in the nervous system, understanding the mechanisms that direct the selectivity of interactions between receptors will become a central question in

ANXIOLYTIC AGENT
A drug used to reduce anxiety, such as benzodiazepines.

Links

DATABASE LINKS **EGF | PDGF | interferon-γ | muscarinic receptors | α-adrenegic receptors | β-adrenegic receptors | gonadotropin-releasing hormone receptor | dopamine receptors | adenosine A1 receptor | glucagon receptor | α_2-adrenergic receptor | M3 muscarinic receptor | angiotensin II receptor | β_2-adrenergic receptor | M2 muscarinic receptor | $GABA_B$ receptor | mGluR5 | δ-opioid receptor | SSTR5 | CCR5 | κ-opioid receptor | μ-opioid receptor | SSTR1 | angiotensin I receptor | bradykinin B2 receptor | RAMP | mGluR1**

FURTHER INFORMATION **Domain swapping in G-protein-coupled-receptor dimers**

ENCYCLOPEDIA OF LIFE SCIENCES **G-protein-coupled receptors**

neuropharmacology. One extensively studied aspect will certainly be the potential dynamic regulation of dimer formation during the activation/inactivation cycle of the receptors. The question of whether dimers are constitutive and stable throughout the life of the receptor, or can undergo rounds of monomerization/dimerization with potential exchanges between receptor subtypes, is already a highly debated issue. The prospect of such dynamic regulation has many potential implications for our understanding of the neurotransmission process, and this will undoubtedly lead many researchers to investigate the mechanisms underlying oligomeric assembly of the most important class of neurotransmitter receptors.

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