SEVEN-TRANSMEMBRANE RECEPTORS

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Seven-transmembrane receptors, which constitute the largest, most ubiquitous and most versatile family of membrane receptors, are also the most common target of therapeutic drugs. Recent findings indicate that the classical models of G-protein coupling and activation of second-messenger-generating enzymes do not fully explain their remarkably diverse biological actions.

SIGNALLING

An enormous family of over 800 genes encodes receptor proteins that are characterized by a signature seven-transmembrane (7TM) configuration. Members of this family include receptors for many hormones, neurotransmitters, chemokines and calcium ions, as well as sensory receptors for various ODORANTS, bitter and sweet taste, and even photons of light. The odorant-receptor subfamily alone is thought to contain several hundred members. Not only do the 7TM receptors regulate many physiological processes, but drugs that target these receptors — either directly or indirectly — account for most of the medicines sold worldwide. 7TM receptors are commonly referred to as G-protein-coupled receptors (GPCRs), because most of them signal by activating HETEROTRIMERIC G PROTEINS. However, as described below, recent research indicates an increasing number of alternative signalling mechanisms that are available to these receptors.

During the 1980s, there was a growing realization that there are functional analogies between visual signalling in the retina and hormonal signalling in other tissues[1]. It was appreciated that both processes involve a 'receptor' or input receiver, a transducer 'G protein' (transducin in the retina, or G_s in the case of adenylyl cyclase), and an effector enzyme (for example, cyclic GMP phosphodiesterase in the retina, or adenylyl cyclase; FIG. 1). Nonetheless, it was only with the cloning[2] of the gene and complementary DNA for the mammalian β2 adrenergic receptor (β2-AR) for catecholamines in 1986, and the realization that it shares sequence homology and a presumed 7TM structure with the visual receptor, rhodopsin, that the idea of a large gene family of receptors began to emerge. This idea was rapidly confirmed by the cloning of other members of the adrenergic-receptor family, muscarinic cholinergic receptors and several serotonin receptors[3].

Over the past 15 years, much effort has focused on identifying the primary sequences of the receptor proteins that recognize the many known hormones, neuromodulators and PARACRINE factors. This task has been largely completed, and several hundred distinct human 7TM receptors and receptor subtypes have been identified.

Three families of 7TM receptors

These many sequences can be grouped into three distinct families, A, B and C, on the basis of sequence similarity. Sequences within each family generally share over 25% sequence identity in the transmembrane core region, and a distinctive set of highly conserved residues and motifs. Among the three families, little similarity is evident beyond the predicted 7TM architecture.

Family A is by far the largest group, and includes the receptors for light (rhodopsin) and adrenaline (adrenergic receptors) and, indeed, most other 7TM receptor types, including the olfactory subgroup. The olfactory receptors constitute most of these sequences, but nearly 200 non-olfactory 7TM receptors that recognize over 80 distinct ligands have also been functionally characterized[4].

Family B contains only ~25 members, including the receptors for the gastrointestinal peptide hormone family (secretin, glucagon, vasoactive intestinal peptide (VIP) and growth-hormone-releasing hormone),

ODORANT
A chemical substance with a distinct smell. Odorant receptors are a large group of 7TM receptors concentrated in the nasal epithelium that respond to a wide range of odours.

HETEROTRIMERIC G PROTEINS
Guanine-nucleotide regulatory protein complexes composed of α and βγ subunits. They are responsible for transducing signals from 7TM receptors to effectors, including adenylyl cyclases and phospholipases.

PARACRINE
Describing or relating to a regulatory cell that secretes an agonist into intercellular spaces in which it diffuses to a target cell other than the one that produces it.

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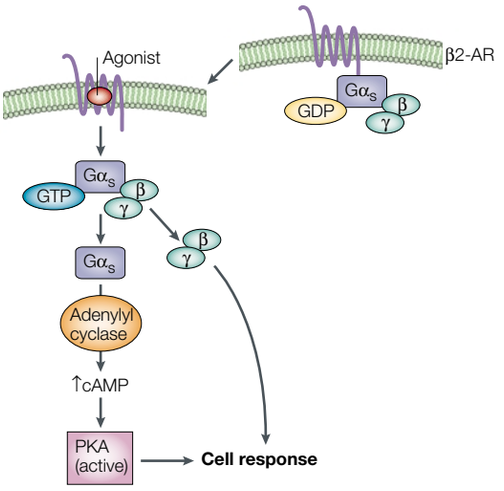


Figure 1 | **Classical examples of seven-transmembrane (7TM)-receptor signalling.** In the absence of agonist, 7TM receptors such as the β2 adrenergic receptor (β2-AR) are in the low-affinity state. After agonist binding, a transient high-affinity complex of agonist, activated receptor and G protein is formed. GDP is released from the G protein and is replaced by GTP. This leads to dissociation of the G-protein complexes into α subunits and βγ dimers, which both activate several effectors. $G\alpha_s$, for instance, activates adenylyl cyclase, which leads to an increase in cyclic AMP (cAMP). This increase in cAMP in turn activates protein kinase A (PKA), which is a serine/threonine kinase that phosphorylates many different substrates, including 7TM receptors, other kinases and transcription factors.

corticotropin-releasing hormone, calcitonin and parathyroid hormone. All family B receptors seem to couple mainly to activation of the effector adenylyl cyclase through the G protein G_s.

Family C is also relatively small, and contains the metabotropic glutamate receptor family, the $GABA_B$ receptor, and the calcium-sensing receptor, as well as some taste receptors. All family C members have a very large extracellular amino terminus that seems to be crucial for ligand binding and activation.

To date, the only member of the receptor superfamily for which a crystal structure has been solved is rhodopsin[5]. This structure, which corresponds to the inactive receptor, confirms the presence of an anticlockwise bundle of 7TM α helices (viewed from the intradiscal or extracellular side) that are connected by loops of varying lengths.

Classical examples of receptor signalling

In 1971, Martin Rodbell conceived the idea[6] that a guanine-nucleotide regulatory protein functionally connects receptors with effectors in the context of hormonal (glucagon) stimulation of the adenylyl cyclase system, generating the second messenger cyclic AMP. The hypothesized protein, G_s (originally termed N_s), was later purified and shown to be heterotrimeric, comprising α, β and γ subunits. The α subunit is responsible for GTP and GDP binding and for GTP hydrolysis, whereas the β and γ subunits are associated in a tightly linked βγ complex[7]. Today, each of these subunits is known to be a member of a gene family; to date 16 α, five β and 12 γ proteins have been cloned.

G proteins are generally referred to by their α subunits. So, the G_s heterotrimeric complex contains $G\alpha_s$; G_q contains $G\alpha_q$; G_i contains $G\alpha_i$; and so on. Four distinct α-subunit subfamilies are recognized: G_s proteins couple to stimulation of adenylyl cyclase; G_i proteins couple to inhibition of adenylyl cyclase as well as to activation of G-protein-coupled inwardly rectifying potassium (GIRK) channels; G_q proteins couple to the activation of phospholipase Cβ; and G_{12} proteins couple to the activation of Rho GUANINE-NUCLEOTIDE EXCHANGE FACTORS (GEFs). However, the combinatorial complexity of αβγ heterotrimers that might form is obviously great and, as yet, relatively little is understood of the specific subunit composition of the G proteins that function in specific pathways.

Receptor activation and downstream signalling. Both the α subunit and the βγ dimer signal through the activation, or inhibition, of an ever-expanding list of effectors (TABLE 1). Agonist activation of the receptors induces conformational changes which are, as yet, poorly understood, but which seem to involve, at minimum, rearrangements of membrane helices 6 and 3 (REFS 8,9). This 'activated receptor' can interact with the heterotrimeric G protein, and serves as a GEF to promote GDP dissociation, and GTP binding and activation. Receptors vary in their degree of agonist-independent or constitutive activity to couple to G proteins, and receptor mutants resulting in augmented activity have been found in numerous diseases. In the current model, the activated heterotrimer dissociates into an α subunit and a βγ dimer, both of which have an independent capacity to regulate separate effectors[7] (FIG. 1). Hydrolysis of GTP to GDP — a process that is now known to be regulated by RGS (regulator of G-protein signalling) proteins (see below) — leads to reassociation of the heterotrimer and termination of the activation cycle[10,11]. However, much controversy has surrounded the issue of whether physical dissociation of α from βγ actually occurs during G-protein activation, and the issue remains unsettled[12].

Classical examples of receptor desensitization

Not surprisingly for such an essential cellular function, many mechanisms have evolved for fine-tuning and regulating receptor signalling. Among the most thoroughly studied have been those that rapidly 'dampen' signalling, even in the presence of continuing agonist stimulation — a phenomenon that is referred to as 'desensitization'[13]. Such mechanisms operate at both the level of the receptor as well as downstream at, for example, the level of G proteins.

Rapid dampening of receptor function is usually controlled by receptor phosphorylation, which is mediated by second-messenger kinases (for example, protein kinase A (PKA) and protein kinase C (PKC)), or by a distinct family of G-protein-coupled receptor kinases (GRKs)[14].

GUANINE-NUCLEOTIDE-EXCHANGE FACTORS
Proteins that facilitate the replacement of GDP with GTP in the nucleotide-binding pocket of a GTP-binding protein.

Table 1 | **Examples of heterotrimeric G-protein effectors**

G-protein subunits	Effectors	References
$G\alpha_s$ $G\alpha_{olf}$	↑Adenylyl cyclase RGS-PX1 (GAP, sorting nexin) Calcium channels c-Src tyrosine kinases	6,92,93
$G\alpha_T$ (transducin) $G\alpha_{gust}$ (gustducin)	↑cGMP phosphodiesterase Phosphodiesterase (bitter, sweet taste)	94,95
$G\alpha_{i\ 1,\ 2,\ 3}$ $G\alpha_o$ $G\alpha_z$	↓Adenylyl cyclase, ↑c-Src tyrosine kinases Rap1GAP1	93,96–98
$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14,\ 15,\ 16}$	↑Phospholipase C LARG RhoGEF	99–101
$G\alpha_{12}$, $G\alpha_{13}$	p115 RhoGEF, PDZ-RhoGEF, LARG RhoGEF (Rho activation, stress-fibre formation) E-Cadherin (β-catenin release)	2,99,100,102,103
Gβγ	KIR3.1–3.4 (GIRK K^+ channels) GRKs ↑Adenylyl cyclases (ACII, ACIV) ↑Phospholipases (PLC β1, β2, β3) PI3Kγ	104–109

cGMP, cyclic GMP; GAP, GTPase-activating protein; GEF, guanine-nucleotide exchange factor; GIRK, G-protein-regulated inwardly rectifying potassium channel; PI3K, phosphatidylinositol 3-kinase; RGS, regulator of G-protein signalling.

Second-messenger kinase regulation. PKA- and PKC-mediated receptor phosphorylation directly uncouple receptors from their respective G proteins, and thereby serve as classical negative-feedback regulatory loops. However, they can also mediate 'heterologous' forms of desensitization in which kinase activation by one type of receptor leads to phosphorylation and desensitization of another receptor. PKA-mediated receptor phosphorylation has also been shown, in the case of the β2-AR, to 'switch' coupling of the receptor away from G_s (which elicits desensitization) in favour of enhanced coupling to G_i (REFS 15,16). This facilitates activation of G_i-coupled pathways such as stimulation of the extracellular signal regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway. Several other receptors, including the prostacyclin receptor, also seem to undergo such PKA-mediated 'switching' in their G-protein-coupling specificity[17].

GRK-mediated regulation. A very general mechanism for regulating 7TM-receptor activity is the GRK–β-arrestin system[14,18,19]. This mediates 'homologous' or agonist-specific desensitization, because only the activated or agonist-occupied conformation of the receptors is phosphorylated by GRKs. GRK phosphorylation promotes binding of an arrestin molecule to the receptor, which sterically inhibits further interactions between the receptor and the G protein. The GRKs are encoded by a small family of seven genes[14]. GRK1 is also known as rhodopsin kinase, and GRK2 is known as the β-adrenergic receptor kinase. Other than GRK4 and the two retinal enzymes, GRK1 and GRK7, the other GRKs are fairly widely distributed, and seem to regulate large, overlapping portfolios of receptors, although they do show some receptor specificity (the details of which remain to be determined).

Several factors control the activity of the kinases towards the receptors[14]. Foremost among these are the activated conformations of the receptors themselves, which allosterically activate the enzymes. Also important are the various mechanisms by which the kinases associate with the plasma membrane. These include the interaction of prenylated Gβγ subunits with GRK2 and GRK3; farnesylation of GRK1; and palmitoylation of GRK4 and GRK6. A further layer of regulatory complexity is added by the fact that both PKA and PKC can phosphorylate and activate GRK2, apparently by promoting its Gβγ-mediated membrane association[20,21].

There are four arrestin genes. Two (visual arrestin and cone arrestin) are expressed exclusively in the retina, and two — referred to as β-arrestin-1 (arrestin-2) and β-arrestin-2 (arrestin-3)[18] — are expressed ubiquitously in other tissues. β-arrestin-1/arrestin-2 and β-arrestin-2/arrestin-3 show distinct preferences for different receptors (see below). Receptor phosphorylation is reversed by phosphatases that are localized in intracellular vesicles to which the receptors traffic after stimulation and desensitization[22,23]. As described below, in many cases the β-arrestins have a central role in coordinating processes that are involved in receptor internalization.

Knockout animals have been created in which the various GRK and arrestin genes have been inactivated by HOMOLOGOUS RECOMBINATION. Consistent with the physiological importance of these regulatory systems, all such animals show supersensitivity to various 7TM-receptor-mediated processes (TABLE 2).

Further mechanisms of desensitization. Various other mechanisms contribute to dampening receptor signalling in the face of continuing stimulation. At the level of the receptors, these include receptor degradation, which is often carried out in lysosomes[24], or the dynamic regulation of receptor gene transcription and translation[25]. These processes generally operate over longer periods of time (hours) than the more acute regulatory events that are associated with receptor phosphorylation (seconds to minutes).

Receptor signalling can also be regulated at post-receptor levels. One important recent discovery is that of the RGS family of proteins, which serve as GTPASE-ACTIVATING PROTEINS (GAPs) for heterotrimeric G proteins[10,11]. The family consists of at least 25 members, all of which contain a characteristic RGS-homology domain of about 130 amino-acid residues. Different family members have selectivity for distinct G proteins and, in some cases, even for particular receptors, although the mechanisms for such selectivity remain to be determined. RGS proteins accelerate the rate of GTP hydrolysis, so they can speed up the deactivation of a system once stimulation has ceased, or dampen signalling during persistent stimulation. Nonetheless, the physiological role of such proteins in regulating receptor signalling is poorly characterized. Knockout of *RGS9*, which encodes a visual-system protein, clearly slows deactivation of light-activated rhodopsin[26], and knockout of *RGS2* has many effects on T-cell activation, anxiety

HOMOLOGOUS RECOMBINATION
The process by which segments of DNA are exchanged between two DNA duplexes that share high sequence similarity.

GTPASE-ACTIVATING PROTEINS (GAPs) Proteins that inactivate GTP-binding proteins, such as heterotrimeric G proteins and Ras-family members, by increasing their rate of GTP hydrolysis.

Table 2 | **G-protein-coupled-receptor kinase- and β-arrestin-knockout mice**

Genotype	Supersensitive receptor	Physiological consequence	Reference
GRK1$^{-/-}$	Rhodopsin	Photoreceptor degeneration, delayed rhodopsin deactivation	110
GRK2$^{-/-}$ GRK2$^{+/-}$	N/A Cardiac β1-AR	Embryonic lethal Increased contractile response to isoproterenol	111,112
GRK3$^{-/-}$	Odorant receptors Muscarinic cholinergic	Lack fast agonist-induced desensitization Enhanced airway smooth muscle contraction to methacholine	113,114
GRK5$^{-/-}$	Central muscarinic cholinergic receptors	Increased hypothermia, hypoactivity, tremor, salivation and antinociception in response to agonists	115
GRK6$^{-/-}$	Immune-cell chemotactic receptors	Reduced chemokine-induced lymphocyte chemotaxis	116
Arrestin$^{-/-}$	Rhodopsin	Photoreceptor degeneration	110
β-Arrestin 1$^{-/-}$	Cardiac β1-AR	Enhanced contractile response to isoproterenol	117
β-Arrestin 2$^{-/-}$	μ-Opioid receptor	Increased and prolonged antinociceptive effect of morphine, absence of morphine-induced tolerance	118,119
β-Arrestin 1$^{-/-}$/ β-Arrestin 2$^{-/-}$	N/A	Embryonic lethal	56

GRK, G-protein-coupled-receptor kinase; β1-AR, β1 adrenergic receptor.

APELIN PEPTIDES
Vasoactive peptides originally purified from bovine stomach extracts. These peptides interact with the recently de-orphanized 7TM receptor APJ.

TRACE AMINES
Biogenic amines derived from amino-acid metabolism. Once thought of primarily as inactive metabolites, trace amines including tyramine and octopamine are now known to be important neuromodulators.

CLATHRIN
The main component of the coat that is associated with clathrin-coated vesicles, which are involved in membrane transport both in the endocytic and biosynthetic pathways.

CAVEOLAE
Specialized rafts that contain the protein caveolin and form a flask-shaped, cholesterol-rich invagination of the plasma membrane. This might mediate the uptake of some extracellular materials and is probably involved in cell signalling.

ADAPTOR PROTEIN
A protein that augments cellular responses by recruiting other proteins to a complex. They usually contain several protein–protein interaction domains.

AP-2
A member of a family of so-called 'clathrin adaptor proteins', which facilitate the early stages of endocytic vesicle formation through their ability to bind clathrin coats.

DYNAMIN
A GTPase that takes part in endocytosis. It seems to be involved in severing the connection between the nascent vesicle and the donor membrane.

ENDOSOMES
Organelles that carry materials ingested by endocytosis and pass them to lysosomes for degradation or recycle them to the cell surface.

and male aggression[27]. The possible functional redundancy of RGS proteins (for example, there are at least ten forms in the heart) will probably complicate attempts to unravel their physiological roles.

Orphan receptors

The initial cloning of 7TM receptors required either receptor purification to provide a probe for these sequences, or used direct expression-cloning approaches. However, most receptor cDNA clones that have been obtained more recently were identified by similarity to other known receptors. Many receptor-like sequences that have yet to be paired with their endogenous ligands were also found in such searches. This has left the field in a state in which almost 200 such human 'orphan' receptor sequences are known, but in which there are relatively few remaining endogenous ligands whose receptor partners have not yet been identified[4,28]. Indeed, the very existence of so many orphan receptors has led to renewed attempts to identify previously unknown ligands that might activate these receptors. Increasingly, these so-called 'reverse pharmacology' efforts are paying off with the identification of new peptide and small-molecule ligands for orphan receptors, and sometimes leading to new insights into the physiology that is regulated by such receptors[4,28].

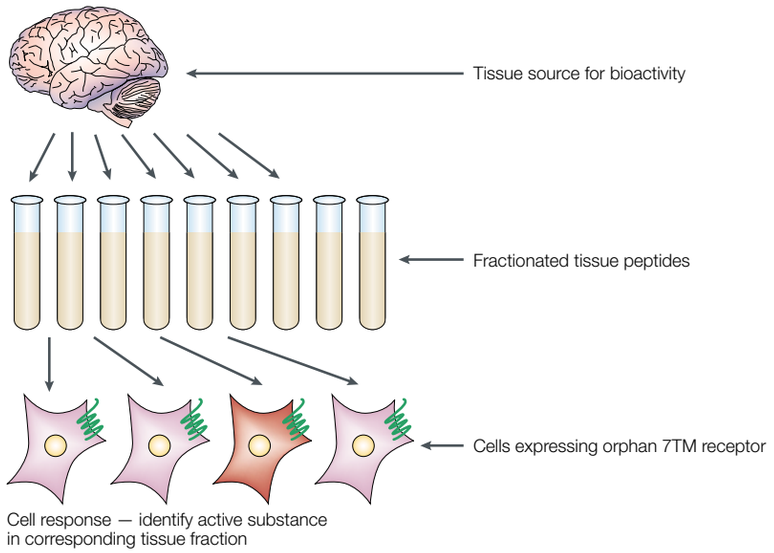
The very first orphan 7TM receptor, called G21, was among the first 7TM-receptor-like sequences cloned, and was identified on the basis of its high similarity to the β2-AR[29]. Further studies established that G21 is not the β1-AR — as it was expected to be — but rather, a 5-hydroxytryptamine (serotonin) receptor, the $5HT_{1A}$ receptor[30]. Over the intervening years, many more receptor sequences have been cloned anonymously and then rapidly matched with their already known ligands by a simple, if laborious, process of elimination. Here we are concerned with advances in understanding the biology of what might be called 'true' orphan receptors — that is, receptors for which there is no known functional ligand, rather than receptors that, for whatever reason, failed to match up quickly with their previously known ligands.

Orphan receptors can be sorted into groups by comparison with other receptors. There are orphan families as well as extreme orphans that are distinct from any other receptors. 'De-orphanizing' orphan receptors (BOX 1) has become an important enterprise due to their therapeutic potential. Notable successes of orphan 'adoptions' within the past few years include the identification of the orexins/hypocretins, ghrelin (growth hormone releasing peptide) and APELIN PEPTIDES as endogenous ligands for orphan 7TM receptors; the identification of the so-called TRACE AMINES (such as β-phenylethylamine, tyramine and octopamine) as agonists of a subfamily of adrenergic/serotonergic-like orphan receptors; the unexpected identification of the insulin-like hormone relaxin as the activator of a gonadotropin-like orphan 7TM receptor; and the recent confirmation of the Bam22 peptide from the *proenkephalin* gene as an active neuropeptide that stimulates an orphan 7TM receptor[4,28,31,32]. In each case, the new appreciation of these endogenous substances as activators of 7TM receptors is rapidly advancing our understanding of previously unappreciated aspects of physiology. The identification of ligands for the remaining orphan receptors will undoubtedly have the same valuable consequences.

Receptor internalization

Coincident with the discovery that agonist treatment of 7TM receptors leads to both activation of receptor signalling and receptor desensitization was the discovery that this agonist treatment also leads to the redistribution of the receptor away from the cell surface by a process of endocytosis — also known as internalization or sequestration (FIG. 2)[33,34]. Initially considered solely as part of the process of desensitization, internalization has subsequently been found to promote receptor resensitization and, more recently, to positively regulate receptor signalling[13,35]. Several different pathways of receptor internalization have been described[36]. These include internalization via CLATHRIN-coated pits, CAVEOLAE, or even uncoated vesicles.

Box 1 | **Methods for 'de-orphanizing' seven-transmembrane (7TM) receptors**



Three main approaches have been taken to identify the endogenous ligands for orphan receptors. First, peptides or other putative ligands that are known to have bioactivity in distant species have been found to exist in mammals, and subsequently matched as agonists for orphan receptors. A second related approach has been to identify potential peptides, either biochemically from tissue extracts or by predictive means using the sequences of apparent neuropeptide precursors. These peptides are then synthesized and tested for bioactivity at orphan and known receptors. A distinct third approach has been to use the orphan receptors themselves in functional screens of fractionated tissues, and those that gave rise to an 'active peak' (that is, they include a component that binds to the receptor) are then sequenced or subjected to mass spectroscopy to identify the active principle. This approach is shown above.

UBIQUITIN
A 76-amino-acid protein that can be covalently attached to specific lysine residues in target proteins. This often forms multimeric polyubiquitin chains, which are thought to target the protein for destruction.

PROTEASOME
Protein complex responsible for degrading intracellular proteins that have been tagged for destruction by the addition of ubiquitin.

E1
An enzyme that activates the carboxy-terminal glycine of the small protein ubiquitin, or ubiquitin-like proteins, allowing them to form a high-energy bond to a specific cysteine residue of the E1.

E2
An enzyme that accepts ubiquitin or a ubiquitin-like protein from an E1 and transfers it to the substrate, mostly using an E3 enzyme.

Caveolae-mediated receptor internalization. Caveolae are flask-shaped membrane invaginations that are rich in caveolin proteins as well as cholesterol. The mechanisms by which 7TM receptors are targeted to caveolae are not yet known, but agents that disrupt caveolae prevent internalization of the ET_B endothelin receptors and VIP receptors[37]. An interesting feature of caveolae is that many important components of 7TM-receptor signalling — including several G proteins and adenylyl cyclases — are localized in caveolae, which indicates that caveolae might function as lipid rafts that compartmentalize signalling by bringing together different components of signalling cascades[38].

Internalization, clathrin-coated pits and β-arrestin. The best-characterized mechanism for 7TM-receptor internalization is β-arrestin-dependent receptor internalization, which occurs via clathrin-coated vesicles. β-arrestins mediate this process by directly interacting not only with the agonist-occupied, phosphorylated receptors[39], but also with two components of the clathrin-coated-pit machinery — the heavy chain of clathrin itself[40], and the β2-adaptin subunit of the clathrin ADAPTOR PROTEIN AP-2 (REF. 41). This coordinated interaction of β-arrestin with both clathrin and AP-2, as well as phosphoinositides[42] (components of the inner leaflet of the cell membrane), targets the 7TM receptors to punctate clathrin-coated pits at the cell surface. These pits are then pinched off the cell surface by the actions of the large GTPase DYNAMIN, and the receptors are either rapidly recycled, targeted to larger ENDOSOMES and slowly recycled, or degraded in lysosomes (FIG. 2). Initially it was thought that β-arrestin participated in the *de novo* formation of clathrin-coated pits. However, recent evidence has shown that β-arrestins instead target the receptors to pre-formed pits at the cell surface[43,44].

It has become apparent that β-arrestins also differentially regulate 7TM receptor trafficking. Some receptors recycle rapidly, others recycle more slowly, and some do not recycle at all. The β2-AR is one example of a rapidly recycling receptor. β-arrestin-2 translocates more readily to this receptor than does β-arrestin-1, and the association of either β-arrestin with the receptor is more transient, ending shortly after the receptor is internalized at or near the cell surface. In the case of the slowly recycling receptors, such as the V2 vasopressin receptor, both β-arrestin-1 and β-arrestin-2 translocate to the receptors, and the receptors and β-arrestins remain associated for much longer (up to 4 h) in large endosomes before the receptors are finally recycled[45].

In the past few years, it has become apparent that β-arrestins carry out additional roles in regulating 7TM-receptor internalization through their interactions with additional components of the endocytic machinery. For instance, β-arrestin functions as an adaptor/scaffold that binds proteins such as the non-receptor tyrosine kinase c-Src (REF. 46). The interaction of β-arrestin with c-Src regulates receptor internalization by promoting tyrosine phosphorylation of dynamin. Mutants of c-Src that cannot interact with β-arrestin, as well as mutants of dynamin that cannot be tyrosine phosphorylated by c-Src, function as dominant-negative inhibitors of clathrin-mediated internalization[47,48].

β-arrestin and ubiquitylation. One of the most exciting recent discoveries is that β-arrestin regulates 7TM-receptor internalization and degradation through a novel mechanism that involves ubiquitylation[49]. Ubiquitylation is a post-translational modification in which UBIQUITIN is added — either singly or in multiples — to lysine residues in the target protein. Initially, ubiquitylation was thought to regulate proteins mainly by directing them to the 26S PROTEASOME for degradation. Recently, however, it has also been shown[50] to be important for regulating the internalization of several membrane proteins. Ubiquitylation requires a relay system of three enzymes: a ubiquitin-activating enzyme (E1); a ubiquitin-carrying enzyme (E2); and a ubiquitin ligase (E3). β-arrestin-2 directly interacts with the ubiquitin ligase mouse double minute 2 (MDM2) and other, as-yet-uncharacterized ligases. These interactions lead to the ubiquitylation of both the β2-AR as well as β-arrestin itself. Ubiquitylation of β-arrestin regulates internalization of the β2-AR, as blocking this process prevents internalization of the β2-AR (REF. 49).

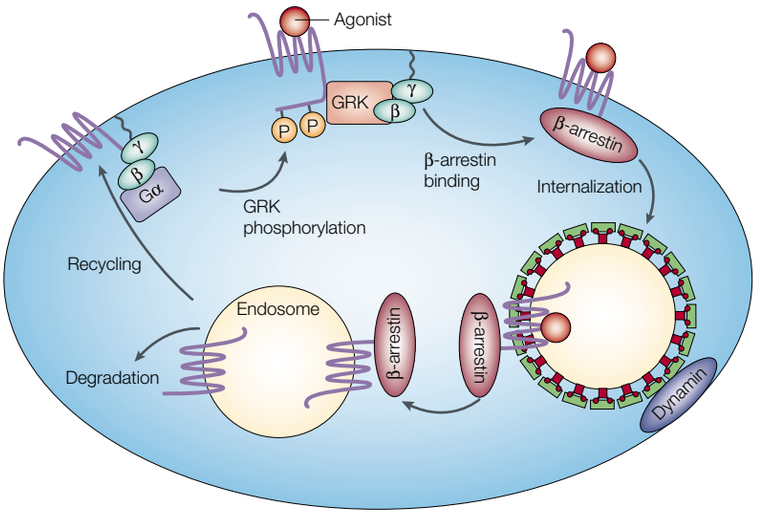


Figure 2 | **Seven-transmembrane (7TM)-receptor trafficking.** Activation of 7TM receptors by an agonist leads to the dissociation of α and βγ subunits. The free βγ dimers recruit G-protein-receptor kinases (such as GRK2 or GRK3) to the receptor, where they specifically phosphorylate agonist-occupied receptors. This, in turn, leads to the recruitment of β-arrestin to the receptor and targets the receptor–β-arrestin complexes to clathrin-coated pits. The receptor is internalized into acidic endosomes and then either dephosphorylated and returned to the cell surface or degraded.

E3
E3 enzymes provide platforms for binding E2 enzymes and specific substrates, thereby coordinating ubiquitylation of the selected substrates.

ADP-RIBOSYLATION FACTOR
(ARF) A small GTPase that regulates the assembly of coats and vesicle budding.

SCAFFOLDING PROTEINS
Proteins that have specific binding sites and are therefore important in the assembly and function of larger molecular complexes.

LYSOPHOSPHATIDIC ACID
(LPA) Any phosphatidic acid that is deacylated at positions 1 or 2.

STRESS FIBRES
Axial bundles of F-actin connecting focal adhesions.

PDZ DOMAINS
Protein-interaction domains that often occur in scaffolding proteins and are named after the founding members of this protein family (Psd-95, discs-large and ZO-1).

β-arrestins and vesicle budding. β-arrestins also modulate receptor internalization through interactions with proteins that are important in vesicle budding. These include *N*-ethylmaleimide-sensitive factor (NSF)[51], and the ADP-RIBOSYLATION FACTOR (ARF) family of small GTP-binding proteins[52] that regulate vesicle budding by recruiting vesicle coat proteins, including the COP coatomers. β-arrestin interacts with ARF6, as well as with its nucleotide-exchange protein, ARF nucleotide-binding-site opener (ARNO), in an agonist-dependent manner[52]. Interestingly, GRK2 interacts with GRK interactor (GIT), which is an ARF GAP[53]. Presumably, then, the localization of GRK2 and β-arrestin at the receptor would bring into close proximity all of the components that are necessary for the nucleotide cycling of ARF6, which is required for proper receptor trafficking.

As well as recruiting β-arrestins for internalization and receptor recycling, the receptors themselves can also interact with proteins that are important for internalization and recycling. Two examples of this are the β2-AR which, through residues in its carboxyl tail, interacts directly with NSF[54], and the interaction of the angiotensin 1A receptor with the small GTP-binding protein Rab5 (REF. 55). Overexpression of NSF in cells expressing the β2-AR leads to an increase in the fraction of receptors that is internalized, and promotes the rapid return of the receptor to the cell surface following the removal of agonist. Interestingly, expression of a dominant-negative form of Rab5 does not block receptor internalization, but instead blocks trafficking of the receptor into the larger endosomes.

Recent evidence indicates that, in many cases, β-arrestin-dependent internalization is a necessary precursor to receptor downregulation — the agonist-induced loss in the total number of expressed receptors. One line of evidence for this is that, in cells that lack both β-arrestin-1 and β-arrestin-2, both internalization and downregulation of the β2-AR are blocked[56]. In addition to requiring β-arrestin, downregulation of the β2-AR requires receptor ubiquitylation. A β2-AR that lacks all potential sites for ubiquitylation, although it is internalized normally, is not downregulated, presumably because the receptor is not targeted to lysosomes[49]. Likewise, for the CXCR4 chemokine receptor, clathrin-mediated endocytosis is a prerequisite for ubiquitin-dependent lysosomal sorting[57]. So, a model has emerged in which β-arrestin, by interacting with endocytic proteins as well as with ubiquitin ligases, orchestrates receptor fate, promoting internalization as well as either recycling or degradation.

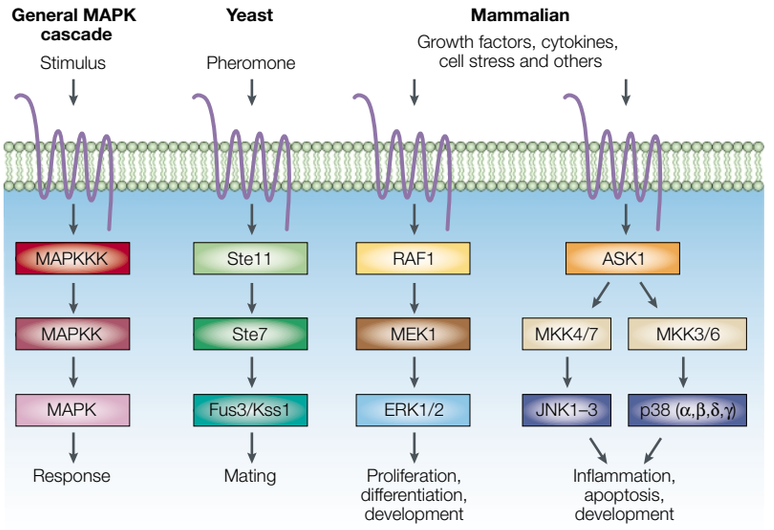
Scaffolding and compartmentalization

Beyond the classical examples of 7TM-receptor signalling that proceeds through G-protein-dependent activation of effectors, 7TM-receptor signalling is now known, in many cases, to involve interactions with adaptor and SCAFFOLDING PROTEINS. These adaptor/scaffolding proteins facilitate the interaction of the receptors with their effectors, ensure specificity in the activation of downstream signalling cascades, and promote the appropriate subcellular localization of these signalling complexes.

Some of these adaptor/scaffolding proteins facilitate the interaction of 7TM receptors with components of classical second-messenger cascades. For example, several of the A-kinase anchoring proteins (AKAPs) — of which there are over 50 — link 7TM receptors with downstream effectors[58], including PKA and the G protein $G\alpha_{12}$. Two of these AKAPs — AKAP79 (REF. 59) and AKAP250 (REF. 60) — have been shown to interact directly with the β2-AR. The interaction of AKAP79 with the β2-AR enhances receptor phosphorylation and promotes ERK/MAPK signalling by this receptor, whereas AKAP250 enhances receptor desensitization. A newly discovered AKAP, AKAP-Lbc, links the $G_{\alpha12}$ family of heterotrimeric G proteins directly to the small GTP-binding protein RhoA (REF. 61). This AKAP selectively interacts with $G_{\alpha12}$ and RhoA, and is itself a RhoA-selective GEF. LYSOPHOSPHATIDIC ACID (LPA) stimulation of cells activates $G_{\alpha12}$, which in turn activates AKAP-Lbc, and this leads to the exchange of GDP for GTP on RhoA and the subsequent formation of STRESS FIBRES. It is not yet known whether this AKAP, like AKAP79 and AKAP250, can interact directly with one of the LPA receptors (Edg-2, Edg-4 or Edg-7).

There are several other examples of adaptor/scaffolding proteins that link 7TM receptors to classical second-messenger pathways. In *Drosophila melanogaster*, for instance, InaD, a large scaffold with several PDZ DOMAINS, interacts with rhodopsin, a G protein, phospholipase C, PKC, and the calcium channel TRP[62]. So all of the proteins in the classical G_q-coupled second-messenger cascade are pulled together, and this undoubtedly facilitates the rapid signalling that is necessary for fly vision.

Box 2 | **7TM receptors activate the ERK/MAPK cascade by several different pathways**



Mitogen-activated protein kinase (MAPK) cascades are phosphorelay systems in which a MAPK kinase kinase (MAPKKK) is phosphorylated and, in turn, phosphorylates a MAPK kinase (MAPKK), which leads to the phosphorylation of a MAPK. This activated MAPK, a serine/threonine kinase, is then shuttled into the nucleus, where it phosphorylates and regulates its target proteins, ultimately leading to gene transcription and cell proliferation. There are three families of mammalian MAPKs: the extracellular signal-regulated kinases 1/2 (ERK1 and ERK2); the c-Jun amino-terminal kinases (JNKs); and the p38 MAPKs. Seven-transmembrane (7TM) receptors are known to activate each of the different families of MAPKs through many different pathways, including both Gα- and βγ-dependent mechanisms, and a particular receptor can activate MAPKs through different pathways, depending on the cell context. 7TM-receptor-activation of MAPKs is conserved from yeast to mammals. In yeast, activation of MAPKs is involved in mating. Activation of the ERK cascades is important for proliferation, development and differentiation. Activation of the JNK and p38 MAPK cascades are important for development, inflammation and apoptosis.

Another mammalian scaffold that brings together components of classical second-messenger cascades is mediated by the Homer family of proteins[63]. The interaction of Homer with the metabotropic glutamate receptor brings with it a number of additional proteins, including several scaffolding proteins and inositol triphosphate ($InsP_3$) receptors. As the metabotropic glutamate receptors mGLUR1 and mGLUR5 are 7TM receptors coupled to phospholipase C activation, this interaction might enhance the efficiency with which signals are transduced from the cell surface to the cell interior.

Bridging receptors with other proteins. Several other receptor–protein interactions have been shown to promote non-classical receptor signalling. These include the direct interaction of the β3-AR with the non-receptor tyrosine kinase c-Src through a proline-rich Src-homology-3 (SH3)-interacting domain in the third intracellular loop of the receptor[64]. This interaction brings c-Src to the receptor in a β-arrestin-independent manner, and leads to ERK/MAPK signalling. Another example of the direct interaction of a receptor with a signalling protein is the association of the angiotensin 1A receptor — through its carboxyl tail — with the Janus kinase, JAK2. This interaction leads to the activation of the transcription factor signal transducer and activator of transcription, STAT (REF. 65).

Many other interactions between additional proteins and 7TM receptors have been described. These include PDZ-mediated interactions, such as the interaction of the β2-AR with the sodium–hydrogen exchanger regulatory proteins (NHERF)1 and NHERF2 (REFS 66,67), and the interaction of the β1-AR with PSD-95 (a post-synaptic-density scaffolding protein)[68]. In addition to the adrenergic receptors, several metabotropic glutamate receptors interact with PDZ-domain-containing proteins. For example, mGLUR7 interacts with PKC through the PDZ-containing protein that interacts with C Kinase (PICK). A recent review[69] details many of the additional interactions of 7TM receptors with these proteins. To date, the biological significance of these interactions is generally not well understood. However, most of these interactions probably enhance the specificity of receptor signalling cascades. Although many of these interactions require agonist activation, the requirement for G-protein coupling is not yet known. Perhaps these interactions represent G-protein-independent signalling that could help to explain how specificity in 7TM-receptor signalling is achieved — that is, how stimulation of two different receptors that are activated by the same ligand and are coupled to the same G protein can lead to distinct physiological outcomes.

β-arrestin as an adaptor/scaffolding protein. Most of the interactions detailed above are between individual receptors and individual proteins. Additional types of adaptor/scaffolding proteins are more general, and can interact with many 7TM receptors to produce biological effects. Among the best-characterized type of these scaffolds is β-arrestin. In addition to its role in receptor

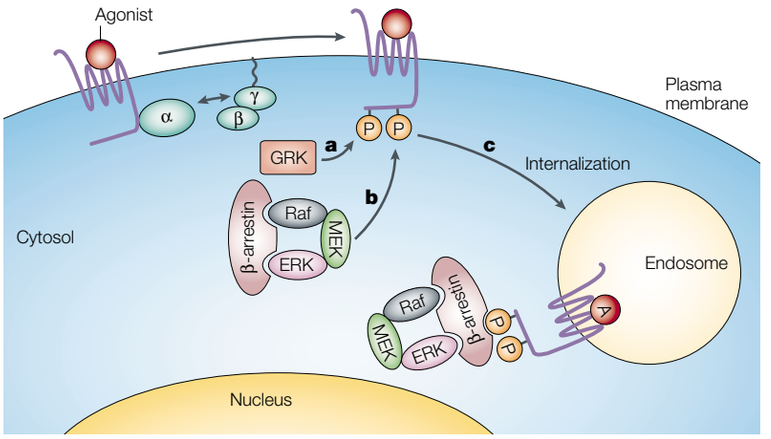


Figure 3 | **β-arrestin scaffolding of mitogen-activated protein kinase (MAPK) cascades.** β-arrestins function as adaptor/scaffolding molecules, which facilitate the activation of two MAPK cascades — the extracellular-signal regulated kinase (ERK)/MAPK cascade and the c-Jun-amino terminal kinase-3 (JNK3) cascade. **a** | After G-protein-regulated kinase (GRK)-dependent phosphorylation of the 7TM receptor, β-arrestin is recruited to the receptor (**b**), and brings with it the components of the MAPK cascade; the MAPK kinase kinase (Raf), the MAPK kinase (MEK1), and the MAPK (ERK). **c** | This association facilitates the activation of ERK/MAPK and targets the active MAPK to the cytosol, thereby decreasing nuclear ERK/MAPK signalling. β-arrestin-dependent scaffolding of the JNK3 is highly analogous — β-arrestin both facilitates JNK3 activation and leads to the agonist-dependent co-localization of the receptor, β-arrestin and the components of the JNK3 cascade in endosomal vesicles.

internalization, β-arrestin has emerged as one of many proteins that serve as adaptor/scaffolding proteins to promote 7TM-receptor signalling.

The first example of the expanding roles of β-arrestins came with the discovery that β-arrestin-1 interacts with c-Src (REF. 70). The β-arrestin–c-Src interaction facilitates the β2-AR-dependent activation of the ERK/MAPK cascade. And the roles of interactions between β-arrestin and c-Src family members are now known to include facilitation of endothelin-receptor-mediated glucose transport by stimulating the translocation of the glucose transporter GLUT4 (REF. 71), and facilitation of interleukin-8-stimulated granule release[72].

In addition, β-arrestin functions as an adaptor/scaffolding protein that leads to the activation and subcellular targeting of two different MAPK cascades. Our understanding of the role of β-arrestin in facilitating MAPK activation was expanded with the discovery that β-arrestin can interact with the last three kinases in two different MAPK cascades — the ERK/MAPK cascade and the c-Jun amino-terminal kinase 3 (JNK3) cascade[73–75] (BOX 2; FIG. 3). β-arrestins bring the last three kinases in both the JNK3 and ERK/MAPK cascades into close proximity with each other and with the receptors, and this facilitates the transfer of information from the receptor to its effector. Surprisingly, however, instead of targeting the MAPK to the nucleus, the β-arrestin-mediated scaffolding of the MAPK cascades leads to cytosolic retention of the active kinase[73–75]. So, after receptor activation, β-arrestin promotes internalization of the receptor, and co-localization of the receptor, β-arrestin and the components of the MAPK cascade in large endocytic vesicles. This localization of active MAPK in the cytosol enhances overall MAPK activation, but inhibits the traditional nuclear signalling of these kinases[73,74,76]. In the case of the ERK/MAPK cascade, this cytosolic retention of active ERK/MAPK prevents agonist-stimulated cell proliferation. However, these β-arrestin-scaffolded pathways are only one of many ways by which 7TM receptors can activate MAPK cascades (FIG. 3), and the cellular context is critical for determining which of the various MAPK pathways is used.

Undoubtedly, more 7TM-receptor scaffolds will be discovered. But the β-arrestin MAPK scaffolds nicely illustrate many of the characteristics of scaffolding proteins. First, they enhance the overall activation of the proteins in the cascade. Second, they ensure specificity by bringing only those proteins that should be activated into close proximity. For example, as well as JNK3, there are two additional JNK isoforms, JNK1 and JNK2. Yet β-arrestin-2 only facilitates the activation of JNK3, even though JNK1 and JNK2 can be activated by the same upstream kinases[75]. Third, as described above, these scaffolds compartmentalize signalling cascades, regulating the biological effects of the proteins by bringing them into close proximity with their ultimate targets. By scaffolding these MAPKs, β-arrestin brings the activity and subcellular localization of the active MAPK under the control of the receptor.

7TM-receptor dimerization

In the traditional view of 7TM-receptor signalling, a single cell-surface receptor protein is activated by the binding of a single agonist ligand, and this ligand-activated receptor can then activate many G proteins, one at a time. This model is supported by protein-reconstitution studies, in which a purified receptor protein (as defined by ligand binding) and purified G protein together adequately recapitulate the agonist-stimulated GDP release and GTP binding that are the hallmarks of G-protein activation by receptors[77,78]. However, the issue of whether a single receptor protein is competent to function on its own remains unsettled and controversial.

In other cellular signalling models, cell-surface receptors function as dimers or even higher multimers. In signalling by receptor tyrosine kinases, for example, dimeric receptors are essential for function, as each monomer phosphorylates the other monomer during the ligand-stimulated 'autophosphorylation' that leads to the fully active state of the kinase[79].

Two types of evidence have been used to show that some 7TM receptors similarly function as dimers. First, there are examples of 7TM receptors that absolutely require a heterodimeric interaction to function, although these are few and seem limited in generality. Second, on the basis of biochemical and biophysical studies, many 7TM receptors have been reported to be present in heterodimers, with variable effects on receptor function. These cases differ in that such dimerization is not essential for receptor function, although it might modulate it. For instance, in the case of the κ and

OPIOID RECEPTORS
These 7TM receptors are produced at high levels in the nervous system. They are important for modulating pain responses. Many analgesic drugs target these receptors, including codeine, morphine and heroin.

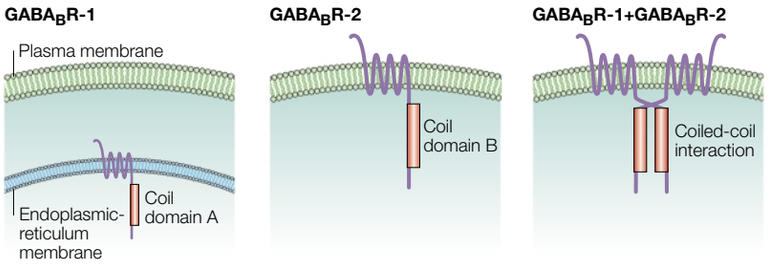


Figure 4 | **Heterodimerization of the GABA$_B$ receptor produces a functional receptor.** The GABA$_B$ receptor is an obligate heterodimer that is only functional when both the GABA$_B$R-1 and GABA$_B$R-2 are co-expressed in the same cell. When the GABA$_B$R-1 is expressed alone, it is trapped in vesicles within the cell, whereas the GABA$_B$R-2 alone is expressed on the cell surface, but cannot bind GABA or activate G proteins. When both receptor subunits are expressed in the same cell, the receptors interact through coiled-coil domains in their carboxyl tails. They are then expressed on the cell surface, bind GABA and activate G proteins. The GABA$_B$ receptor is one of only a few obligate receptor heterodimers currently known. In other cases, receptor heterodimerization can alter either ligand preferences or G-protein coupling.

δ OPIOID RECEPTORS, heterodimerization enhances agonist-induced inhibition of adenylyl cyclase, as well as altering the receptor pharmacology[80].

In the case of the γ-aminobutyric acid (GABA)$_B$ receptor, recent studies[81–83] have shown conclusively that dimerization is essential for function. Unlike most 7TM receptors, the GABA$_B$ receptor consists of an obligate heterodimer of the GABA$_B$R-1 and GABA$_B$R-2 subunits. Each subunit is a member of the family C of 7TM receptors. The GABA$_B$R-1 subunit contains the ligand-binding site, but when this protein is expressed alone, it does not reach the cell surface. The GABA$_B$R-2 subunit makes it to the cell surface, but it does not bind GABA. When co-expressed, though, the B1 and B2 subunits associate through a coiled-coil interaction of their cytoplasmic carboxy-terminal domains, and the B2 subunit helps the B1 subunit to traverse to the cell surface (FIG. 4). Allosteric interactions between the B1 and B2 subunits are required for optimal GABA$_B$ receptor G-protein signalling[83].

Taste receptors that recognize sugars and amino acids also require heterodimerization of family C 7TM receptors. Sweet responses require co-expression of taste receptor 1, member 2 (T1R2) and taste receptor 1, member 3 (T1R3) receptor proteins, whereas umami (amino-acid) responses require co-expression of the T1R1 and T1R3 receptor proteins[84,85]. These receptor dimers can be co-immunoprecipitated, but whether this dimerization affects trafficking, or whether it is primarily structural, remains to be determined.

A similar situation exists for the calcitonin receptor-like receptor (CLRL) receptor protein, a family B receptor. When expressed alone, it seems to be non-functional, but when co-expressed with a member of the receptor-activating-modifying (RAMP) protein family, CLRL becomes a functional receptor; with RAMP1, it functions as the calcitonin-gene-related-peptide (CGRP) receptor, whereas with RAMP2 or RAMP3, it is the adrenomedullin receptor[86]. Unlike the case with the GABA$_B$ receptor, this associated subunit does not resemble a 7TM receptor itself. Instead, RAMP proteins are small, single-transmembrane-domain proteins. Nevertheless, it is now clear that RAMP protein association directs appropriate plasma-membrane transport of the CLRL receptor and modifies its pharmacology[87,88]. However, this example is unlikely to be general, as RAMP proteins have not been reported to be required for the function or cell-surface expression of any other 7TM receptor, and other RAMP-like proteins have not been identified for other receptors.

Higher-order-oligomer formation? In contrast to these clear, if limited, examples of a functional requirement for dimerization, many studies have reported the association of many 7TM-receptor types into higher-order structures[81,82]. Such studies have assessed the functional complementation of two inactive mutant receptors upon co-expression, the co-trafficking of two receptors after the stimulation of one, the co-immunoprecipitation of differentially tagged expressed receptors, and the proximity of receptor proteins in cell membranes using biophysical means (such as fluorescence resonance energy transfer)[81,82]. Structural models have been proposed of dimers of two adjacent 7TM receptors, which fold into distinct receptor bundles that abut each other, or as linked bundles in which the binding pocket is formed from transmembrane helices from distinct receptor proteins[89].

The results of these studies indicate that, on the whole, dimerization is not essential for receptor function, but that it might contribute to the diversity of signalling by altering the specificity of agonist and antagonist interactions. This assumes that ubiquitously required factors (analogous to RAMPs) are not present in all expression systems, and does not adequately address the potential role of receptor homodimerization. At least in the case of rhodopsin, from which an X-ray crystal structure has been determined[5], the receptor appears monomeric in the sense that membrane-spanning domains from two polypeptide chains do not intermingle to create linked dimeric receptors. Clearly much further work will be required to settle this issue.

Future perspectives and conclusions

This discussion of 7TM receptors has highlighted some of the more important questions and emerging directions in this ever-expanding field of research. Despite the many obstacles in obtaining crystal structures of membrane proteins, the structure of inactive rhodopsin[5] gives hope that structures of other 7TM receptors will follow. An important goal is to determine what the structural differences are between the inactive and activated conformations of such receptors, as well as the structure of the receptors in complex with protein partners, such as heterotrimeric G proteins or arrestins. In addition, the development of transgenic mice in which the 7TM receptors have been knocked out has already begun, and will continue to highlight the diversity of the physiological actions of these receptors in

Box 3 | **Yeast seven-transmembrane (7TM)-receptor signalling**

G-protein signalling pathways are remarkably conserved among diverse species. The budding yeast *Saccharomyces cerevisiae* has been a particularly informative model system for delineating signalling pathways by genetic means (for a recent review, see REF. 120). In each of the two haploid yeast mating strains, α and a, 7TM receptors recognize the mating pheromone that is secreted by the other strain, and initiate signalling cascades that lead to cell fusion to form diploid cells, followed by sporulation. The pheromone receptors couple to a heterotrimeric G protein, GPA1, which leads to activation of mitogen-activated protein kinases (MAPKs) through a conserved kinase cascade (see BOX 2).

Remarkably, MAPK activation in yeast was shown to proceed through the G-protein βγ-dimer rather than the GTP-bound α subunit, and this demonstration was a crucial hint that mammalian G-protein pathways might use βγ as well as α as signal transducers. Curiously, activation of adenylyl cyclase in yeast proceeds through activation of the small GTP-binding protein Ras, instead of being mediated by the G-protein α subunit as in higher organisms, whereas MAPK activation in yeast is directly activated through βγ-subunit activation of the complex between Ste5 (a scaffold protein) and Ste20 (the yeast homologue of p21-activated kinase, PAK), instead of through activation of Ras–Raf. So, these two pathway connections are reversed in yeast compared to the equivalent mammalian pathways.

Yeast pheromone signalling does show a desensitization phenomenon, but yeast do not possess G-protein-coupled receptor kinases or arrestin proteins to mediate phosphorylation-dependent homologous desensitization through receptor uncoupling from the G protein. Instead, yeast dampen signalling by directly regulating the G protein. The yeast *Sst2* gene was identified from a mutant strain that was supersensitive to pheromones, and the Sst2 protein is the founding member of the regulator of G-protein signalling (RGS) family. RGS proteins dampen signalling by acting as GTPase-activating proteins for heterotrimeric G-protein α subunits in both yeast and mammalian systems. Yeast pheromone receptors internalize after activation, as do mammalian 7TM receptors. Agonist-activated yeast mating-factor receptors require both phosphorylation and ubiquitylation for internalization. Recent reports[49,57] indicate that mammalian 7TM receptors also undergo agonist-dependent ubiquitylation, although this is not required for internalization but rather for downregulation through degradation in lysosomes. Yeast will certainly continue to be a useful system for defining signalling models.

everything from learning, to metabolism, to regulation of the cardiovascular system.

Investigations of signalling by 7TM receptors are likely to be characterized by a shift away from studying linear signalling pathways towards a consideration of increasingly complex signalling networks in which many pathways are interlinked by protein components that are common to several of them. The combinatorial complexity of such networks is extraordinary and will necessitate different and collaborative research approaches, as has recently been discussed[90].

Studies of adaptor and scaffolding proteins have already indicated important roles for such molecules in orchestrating particular signalling activities of several 7TM receptors. Unclear, as yet, is the extent to which such mechanisms might act as alternatives to classical G-protein-mediated signalling, or whether they simply modulate or fine-tune signals that are transduced primarily through heterotrimeric G proteins. Mounting evidence favours the former hypothesis — for example, in the slime mould *Dictyostelium discoideum*, several signalling pathways persist, even when G-protein signalling has been disrupted[91]. These pathways include calcium influx, ERK/MAPK activation and transcription-factor activation.

And, of course, there are hundreds of orphan receptors that await pairing with their natural ligands, not to mention 'non-traditional' 7TM receptors such as the Frizzled family, for which the ligands (Wnts) have been identified, but for which coupling to G proteins remains controversial.

A deepening understanding of the biology of the 7TM family of receptors — from yeast (BOX 3) to mammals — is likely to pay rich dividends at several levels. First it will provide fundamental insights into the structure and function of what is arguably the most pervasive, ubiquitous and versatile type of signalling molecule known. Additionally, such information will probably lead directly to the development of many new, more selective therapeutic agents, further enhancing the role of 7TM receptors in drug development.

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DATABASES

The following terms in this article are linked online to:

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5-hydroxytryptamine receptor 1A | β2 adrenergic receptor | β-arrestin 1 | β-arrestin 2 | AKAP250 | AKAP79 | AKAP-Lbc | ARF-nucleotide-binding-site opener | AT_{1A} | calcitonin-receptor-like | CXCR4 | Edg-2 | Edg-4 | Edg-7 | ERK1 | ERK2 | Frizzled | G21 | glucagon | GLUT4 | GRK1 | GRK2 | GRK3 | GRK4 | GRK6 | GRK7 | growth-hormone-releasing peptide | $G\alpha_{12}$ | $G\alpha_q$ | $G\alpha_s$ | Homer | interleukin-8 | JAK2 | JNK1 | JNK2 | JNK3 | MEK1 | mGLUR1 | mGLUR5 | mGLUR7 | mouse double minute 2 | NHERF2 | NSF | *proenkephalin* | prostacyclin receptor | Rab5 | RAMP1 | RAMP2 | RAMP3 | *RGS2* | *RGS9* | rhodopsin | secretin | T1R1 | T1R2 | T1R3 | V2 vasopressin receptor | vasoactive intestinal peptide

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