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Chapter 3

Biosensing Techniques in Yeast: G-Protein Signaling and Protein-Protein Interaction Assays for Monitoring Ligand Stimulation and Oligomer Formation of Heterologous GPCRs

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Additional information is available at the end of the chapter

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Abstract

Guanine nucleotide-binding proteins (G-proteins) act as transducers of external stimuli for intracellular signaling, and control various cellular processes in cooperation with seven transmembrane G-protein-coupled receptors (GPCRs). Because GPCRs constitute the largest family of eukaryotic membrane proteins and enable the selective recognition of a diverse range of molecules (ligands), they are the major molecular targets in pharmaceutical and medicinal fields. In addition, GPCRs have been known to form heteromers as well as homomers, which may result in vast physiological diversity and provide opportunities for drug discovery. G-proteins and their signal transduction machinery are universally conserved in eukaryotes; thereby, the yeast *Saccharomyces cerevisiae* has been used to construct artificial *in vivo* GPCR biosensors. In this chapter, we focus on the yeast-based GPCR biosensors that can detect ligand stimulation and oligomer formation, and summarize their techniques using the G-protein signaling and protein-protein interaction assays.

Keywords: yeast, G-protein, G-protein-coupled receptor, signal transduction, oligomer formation, reporter gene assay, protein-protein interaction

1. Introduction

Guanine nucleotide-binding proteins (G-proteins) are highly conserved among various eukaryotes, and act as signal transduction molecules [1, 2]. In cooperation with seven

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transmembrane G-protein-coupled receptors (GPCRs), G-proteins transduce external stimuli to intracellular signaling and control a wide variety of cellular processes. GPCRs, which represent the largest family of integral membrane proteins and present more than 800 genes in the human genome [3], engage a wide range of ligands. GPCR ligands range from small molecules to large proteins, such as hormones, neurotransmitters, ions, tastants, odor molecules and even light [4]. Thus, GPCRs are involved in various physiological processes, and are the targets of several prescribed drugs [5–8].

Agonist ligand binding to a GPCR causes ligand-specific active conformational changes, and allows the receptor to couple to G-proteins that are composed of Gα, Gβ and Gγ subunits [9]. Subsequently, heterotrimeric G-proteins dissociate from the receptor, and then G-protein signaling generates second messengers such as cyclic adenosine monophosphate (cAMP), inositol phosphates, and intracellular Ca^{2+}. These second messengers trigger different cellular and ultimately physiological responses [10]. During these processes, G-proteins switch from an inactive state to an active state by exchanging a guanosine diphosphate (GDP) molecule from the Gα subunit for guanosine triphosphate (GTP). To resume an inactive state, G-proteins hydrolyze GTP to GDP [11].

Historically, GPCRs transduce signals only as single monomeric entities (homomers) [12]. However, in the past two decades, several studies have shown that GPCRs also transduce signals as heteromers [13–18]. Heteromerization is involved in both the regulation and modulation of GPCR signaling, consequently increasing the potentially large functional and physiological diversity of various GPCR-mediated processes (e.g., ligand binding, receptor biosynthesis, cellular trafficking, maturation, G-protein activation, and internalization) [19–24]. Therefore, heteromerization among GPCRs may provide new opportunities for drug discovery [25, 26]. For example, GPCR heteromers may be new molecular targets for therapeutic treatments, or for developing more potent and selective compounds, such as bispecific or bivalent ligands, with reduced side effects [27–29]. The mechanism of GPCR heteromerization has been under debate, because the identification of individual heteromer pairs is ongoing and the *in vivo* physiological importance of heteromerization has not been well explored. Thus, the search for functional GPCR oligomer pairs is still a challenging task, due to the continued need for elucidation of their physiological roles.

Saccharomyces cerevisiae is an extremely simplistic unicellular eukaryote and an excellent host system for investigating both GPCR signaling and GPCR oligomerization, as the simplicity of this fungus allows for simplified analyses of the more complicated mammalian GPCR signaling [30]. For instance, since haploid yeast cells harbor a monopolistic G-protein (pheromone) signaling pathway, and experience a variety of heterologous GPCR expressions, yeast cells have often been utilized for studies of human and other mammalian GPCRs such as: identification of agonistic ligands, analysis of ligand-mediated signaling properties, and mutational analysis of critical amino acid residues [30–32]. Additionally, yeast two-hybrid (Y2H) techniques can be utilized to investigate exhaustive protein interaction pairs [30], in which the split-ubiquitin membrane Y2H (mY2H) system is suitable for screening membrane protein interaction partners [33] including GPCR heteromer pairs [34]. In this chapter, we focus on yeast-based biosensors that detect ligand stimulation and oligomer formation of GPCRs, and summarize their techniques using the G-protein signaling and protein-protein interaction assays.

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2. G-protein signaling

Heterotrimeric G-proteins, as peripheral membrane proteins, interact with the plasma membrane on the cytoplasmic side. G-proteins consist of three subunits, Gα, Gβ, and Gγ, which are widely conserved in eukaryotic species, and there are various subfamilies within each subunit, especially the Gα subunit. The heterotrimeric G-proteins transduce messages from GPCRs, which regulate important functions such as vision, taste, smell, heart rate, blood pressure, neurotransmission, cell growth, and numerous other processes [10, 35]. When, in response to extracellular stimuli, GPCRs transduce ligand-specific intracellular signaling cascades, they activate a GDP to GTP exchange on the Gα subunit, resulting in Gα dissociation from the Gβγ complex. Free Gα or Gβγ interacts with several downstream effectors including phospholipases, adenylyl cyclases, phosphodiesterases, tyrosine kinases, ion channels, and ion transporters in human and other mammalian cells [36, 37].

2.1. Heterotrimeric G-protein signaling in yeast

S. cerevisiae's pheromone-based mating response provides a valuable model system for characterization of G-protein-mediated GPCR signaling (**Figure 1**) [38], because it allows for simplified analyses of the more complicated signaling pathways employed by higher eukaryotic cells [30]. The yeast pheromone signaling pathway is non-competitive and monopolistic, unlike other higher eukaryotes, and is mediated by a sole heterotrimeric G-protein comprising three subunits, a Gα subunit (Gpa1p) and the Gβγ complex (Ste4p − Ste18p) [39]. Haploid yeast cells of mating type **a** (*MAT***a**) express Ste2p, which binds the peptide pheromone α-factor secreted by cells of the opposite mating type (*MAT*α). Upon pheromone binding, Ste2p undergoes a conformational change and induces a guanine-nucleotide exchange on Gpa1p [40]. Replacement of GDP with GTP on Gpa1p causes a dissociation of the Ste4p − Ste18p complex. Ste4p facilitates binding of the dissociated Ste4p − Ste18p complex to effectors, and results in activation of the mitogen-activated protein kinase (MAPK) cascade [41, 42]. Ste5p scaffold protein binds to the components of a MAPK cascade to bring them to the plasma membrane, and the concentrated kinases on the membrane may facilitate amplification of the signal [43, 44]. As a consequence, the activated yeast pheromone signaling leads to phosphorylation of the cyclin-dependent kinase inhibitor Far1p and the transcription factor Ste12p. These phosphorylated proteins induce G1 cell cycle arrest [45–47] and global changes in transcription [48, 49]. For example, *FUS1* gene expression experiences drastic transcriptional changes in response to yeast pheromone signaling [50, 51]. The *FIG1* gene is also a mating-specific Ste12p target gene [52, 53]. Sst2p is one of the main negative regulators of the yeast pheromone pathway [54] and acts as a GTPase-activating protein (GAP), enhancing the rate of Gα-catalyzed GTP hydrolysis [55–57]. GDP-bound Gα rapidly reassociates with the Gβγ complex, inactivating the pheromone response.

The yeast *S. cerevisiae* is amenable for reporter gene assays investigating agonist-stimulated G-protein signaling. Briefly, yeast cells become available to detect signaling through endogenous or heterologously expressed GPCRs by putting reporter genes, such as *HIS3* (detected by complementation of auxotrophy), *lacZ* (detected by colorimetry), *luc* (detected by luminometry)

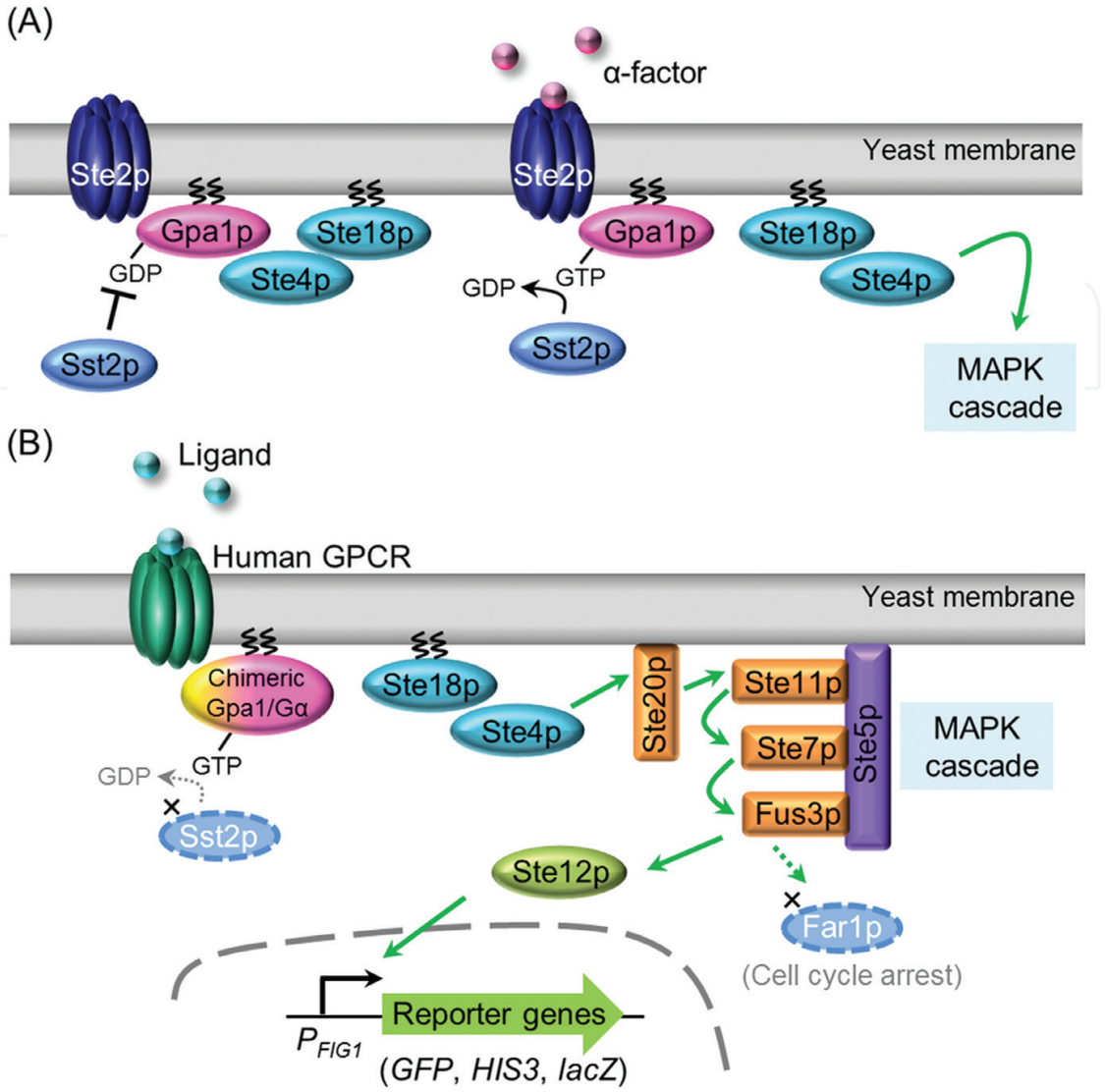

Figure 1. Overview of the yeast pheromone signaling pathway and the human GPCR-expressing yeast signaling biosensor. (A) Schematic illustration of the pheromone signaling pathway. The pheromone signaling pathway is activated, via the heterotrimeric G-protein, when α-factor binds to the Ste2p receptor. The effectors and kinases constitute that MAPK cascades are activated by sequestered Ste4p – Ste18p complex from Gpa1p. Sst2p stimulates hydrolysis of GTP to GDP on Gpa1p and helps to inactivate pheromone signaling. (B) Schematic illustration of typical genetic modifications enabling the pheromone signaling pathway to be used as a biosensor for GPCR activation. Chimeric Gpa1/Gα (transplant) can help to transduce the signal from human GPCRs expressed on the yeast plasma membrane. Transcription machineries, closely regulated by the phosphorylated transcription factor Ste12p, are used to detect activation of pheromone signaling with various reporter genes. *SST2*, *FAR1*, and *STE2* genes are often disrupted to improve ligand sensitivity, prevent growth arrest (cell cycle arrest), and avoid competitive expression of the yeast endogenous receptor.

and gene encoding green fluorescent protein (GFP) (detected via fluorescence), under the expression control of a pheromone-responsive promoter like *FUS1* or *FIG1* [58–60].

2.2. Improvement of the sensitivity of the yeast G-protein signaling

To increase the sensitivity of human GPCR expressing yeast cells, several modifications of yeast-based biosensors have been reported. The yeast's single GPCR (yeast pheromone receptor Ste2p) is often deleted to avoid competitive expression with heterologous GPCRs

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[30]; therefore, expressing human GPCR on the plasma membrane of *ste2*Δ **a**-cells harboring reporter genes facilitates the monitoring of agonist-promoted signaling [30, 61]. The yeast G1-cyclin-dependent kinase inhibitor Far1p, which induces G1 cell cycle arrest in response to signaling, is usually disrupted in positive selection screening to avoid abnormal cell growth [30], because the *far1*Δ strain continues cell growth and improves plasmid retention rates [62]. Removing Sst2p facilitates experiments requiring high ligand binding sensitivity [30, 31, 63], as this removal results in a significant decrease in Gpa1p's GTPase activity by inhibiting the conversion of GTP to GDP.

Yeast Gpa1p is equivalent to mammalian Gα. Gpa1p shares particularly high homology with the human Gα_i classes, and GPCRs from a variety of species, including human, are able to both interact with Gpa1p and activate yeast pheromone signaling [32, 64, 65]. Various genetic modifications allow many other human GPCRs to function as yeast signaling modulators. In one such modification, a chimeric Gpa1p system, referred to 'as "transplants", has' been employed to substitute only five Gpa1p C-terminus amino acids for those of human Gα subunits, of which there are three key families: G$\alpha_{i/o}$, Gα_s, and Gα_q [66]. Indeed, these transplants have allowed functional coupling of various GPCRs (including serotonin, purinergic, muscarinic, and many other receptors) to the yeast pheromone pathway with greater coupling efficiency [32, 66–68].

The use of fluorescent reporter genes can provide the most simple and convenient procedure for comparative quantification of signaling levels, as this removed the need for laborious operations such as sample preparations and enzyme reactions. GFP is commonly chosen as the fluorescent reporter and enhanced green fluorescent protein (EGFP) is often utilized as the GFP. However, the *EGFP* gene was originally codon-optimized for mammalian cells, and it was not suitable for expression in yeast cells [69]. To increase the maximum expression level of GFP and decrease the detection limit of signaling, Nakamura et al. used the tetrameric *Zoanthus* sp. green fluorescent protein (ZsGreen) as a reporter [70]. The use of the *ZsGreen* reporter gene exhibited extremely bright fluorescence and a high signal-to-noise (S/N) ratio in yeast, showing a dramatic improvement in both brightness and sensitivity for GPCR signaling assays compared to a fluorescence reporter system using the *EGFP* reporter gene [70].

2.3. Detection of GPCR agonists by utilizing yeast G-protein signaling

Many heterologous GPCRs (including muscarinic, neurotensin, serotonin, somatostatin, adrenergic, olfactory, and many other receptors) have been functionally expressed in yeast, successfully demonstrating the feasibility of yeast-based GPCR biosensors [31, 32, 63–67, 71–73].

For example, the cyclic neuropeptide somatostatin, known to inhibit growth hormone release, regulates the human endocrine system through somatostatin receptor (SSTR) binding. There are five identified SSTR subtypes (SSTR1 − SSTR5) [74, 75]. SSTR2 and SSTR5 are known to regulate acromegaly patient growth hormone secretion, and are also expressed in most growth hormone secreting tumors [76]. Several researchers demonstrated functional expression of human SSTR2 and SSTR5 in yeasts, and SSTR5 has been often used for constructing yeast-based somatostatin-specific biosensors. To modify the functional expression of human

SSTR5 and somatostatin-specific signaling functions in yeasts, addition of signal sequences derived from secretion or membrane proteins (e.g., prepro- and pre-regions of α-factor, and a N-terminal 20 amino acids of yeast Ste2p; Ste2N) to the N-terminus of the receptor, and implementation of the chimeric Gpa1/Gα_{i3} transplant (see Section 2.2) were tested [77]. Additionally, the *GFP* reporter gene assay (see Section 2.1) was used for evaluating the functional expression of SSTR5 and the signaling response to somatostatin binding. Through these evaluations, yeast cells with improved capabilities as a biosensor capable of detecting somatostatin-promoted signaling (such as potency and efficacy) were successfully constructed. Using this yeast-based biosensor, Togawa et al. performed site-directed mutagenesis of human SSTR5, showing the importance of two asparagine residues (Asn13 and Asn26) on the *N*-linked glycosylation motifs for signaling activation [78]. Furthermore, the artificial signaling circuit formulated a positive feedback loop using Gβ (Ste4p; artificial signal activator, which was set downstream the pheromone-responsive promoter), and was demonstrated to enable highly sensitive agonist detection in SSTR5 expressing yeast [79].

Neurotensin receptor type-1 (NTSR1), a member of the GPCR family, is another example of site-directed mutagenesis of human SSTR5. Neurotensin is the natural ligand of NTSR1, as well as a central nervous system neuromodulator [80]. As neurotensin is also involved in many oncogenic events [81], NTSR1 is a significant therapeutic target. To monitor the activation of human NTSR1 signaling responding to its agonist, a fluorescence-based microbial *S. cerevisiae*-based biosensor was constructed [82]. Successful detection of NTSR1 signaling responding to agonistic ligands was achieved in the Gα-engineered yeast strains IMFD-72 and IMFD-74, which were generated by substituting the Gpa1/Gα_{i3} and Gpa1/Gα_{q} transplants for the intact Gpa1p in modified yeast IMFD-70 strain (*ste2*Δ, *sst2*Δ, *far1*Δ, P_{FIG1}-*EGFP* x2) [82]. *EGFP* genes on the genomes of IMFD-70 and IMFD-72 were replaced with *ZsGreen* genes to generate IMFD-70ZsD and IMFD-72ZsD strains, resulting in the drastic improvement in bright fluorescence and high S/N ratio in the NTSR1 signaling assay [70]. Recently, Hashi et al. modified the expression modes of the human NTSR1 receptor by altering the promoter, consensus Kozak-like sequence, and secretion signal sequences of the receptor-encoding gene [83]. The resulting yeast cells exhibited increased sensitivity to exogenously added neurotensin [83].

Angiotensin II (Ang II) type 1 receptor (AGTR1) is also a GPCR and its natural ligand, Ang II, is an important effector molecule for the renin-angiotensin system. Thus, AGTR1 controls blood pressure and volume in the cardiovascular system [84, 85]. Interaction of Asn295 with Asn111 may play a role in determining the ligand peptide binding selectivity of AGTR1 receptors [86, 87]. Therefore, a single alanine or serine mutation was introduced at Asn295 of human AGTR1, and the Asn295-mutated (N295A and N295S) AGTR1 was expressed in the IMFD-72ZsD yeast strain [88]. When exposed to Ang II and Ang II peptidic analogs, which differ in affinity toward AGTR1, these cells resulted in successful signal transmissions inside the yeast cells. Additionally, the secretory expression plasmids for angiotensin peptides (Ang II, Ang III, and Ang IV) were transformed into the yeasts expressing AGTR1-N295A or AGTR1-N295S, showing the ZsGreen fluorescence with different intensities according to the respective agonistic activities. In contrast, the monoamine neurotransmitter serotonin (5-HT) regulates a wide spectrum of human physiology through the 5-HT receptor family [89].

Nakamura et al. expressed the human HTR1A in the IMFD-72ZsD strain to enable improved detection of HTR1A signaling in response to the 5-HT [90]. The authors further validated the capability of this improved yeast biosensor for antagonistic ligand characterization and site-directed mutants of human HTR1A.

The rat M_3 muscarinic acetylcholine receptor (M3R) has been used for rapid identification of functionally critical amino acids with random mutagenesis [67]. In this system, the *CAN1* gene coding for arginine-canavanine permease was used as the reporter gene under the control of a pheromone responsive *FUS2* promoter, and in the endogenous *CAN1*-deleted yeast cells. Owing to the cytotoxicity of canavanine, caused by Can1p expression in response to promoted signaling, recombinant strains with inactivation mutations in the M3R receptor could survive on agar media containing canavanine and M3R-specific agonists. In another study, using this yeast platform, "antagonists" atropine and pirenzepine were found to be inverse agonists and low efficacy agonists when coupled to Gpa1/Gα_q and Gpa1/Gα_{12}, respectively [91]. In an extended study, the applicability of this yeast platform to identify allosteric ligand-mediated functional G-protein selectivity was also tested [92].

Human formyl peptide receptor-like 1, which was originally identified as an orphan GPCR, has been used to isolate agonists for functionally unknown GPCRs [93]. Both a library of secreted random tridecapeptides and a mammalian/yeast hybrid Gα subunit were employed for histidine prototrophic selection via the *FUS1 − HIS3* reporter gene. Subsequent peptidic candidate surrogate agonist screens have been successful.

In the case of olfactory receptors (ORs), Minic et al. optimized a yeast system for functional expression of rat I7 OR and subsequent characterization. In engineered yeasts lacking endogenous Gpa1p, the olfactory-specific Gα subunit (Gα_{olf}) was co-expressed. When the receptor was activated by its ligands, MAPK signaling was switched on and luciferase (as a functional reporter) synthesis was induced [71]. Marrakchi et al. successfully expressed human olfactory receptor OR17-40 in yeast based on Minic's biosensor system to detect the conductometric changes [94]. Fukutani et al. improved the firefly luciferase-based biomimetic odor-sensing system [60], and replaced the N-terminal region of mOR226 with the corresponding domain of the rat I7 receptor [95]. They further improved some ORs by the coexpression of either odorant accessory binding proteins or the receptor transporting protein 1 short (RTP1S) [96]. Tehseen et al. demonstrated that the *Caenorhabditis elegans* olfactory GPCR ODR-10 was functionally expressed in yeast by using chimeric Gpa1/*C. elegans* Gα [97]. Mukherjee et al. constructed a medium-chain fatty acid biosensor by using the olfactory receptor OR1G1 that functionally expressed in yeast [98].

2.4. Yeast cell-surface display technology for single-cell signaling assay of GPCR peptides

Yeast cell-surface display technology is a platform to tether functional proteins and peptides expressed in yeast to the cell surface [99–102]. Cell-surface display of peptides can be used as a powerful ligand screening based on the yeast GPCR signaling assay systems [70, 103]. Displaying peptidic ligands by fusing them to an anchor protein in the yeast can enable a series of biological processes within a single cell, from peptide synthesis to agonist detection

against an already expressing cognate GPCR. In such a system, a library of peptides is individually tethered to the plasma membrane on GPCR-producing yeast cells via attachment to a glycosyl-phosphatidylinositol (GPI) anchor. Upon phosphatidylinositol-specific phospholipase C (PI-PLC) cleavage of the GPI, the peptides, which are fused to the anchor protein, are released from the membrane and trapped in the cell wall [103]. In principle, the host cells unconsciously detect the binding of peptidic ligands to relevant receptors on the membrane and report the peptides resulting agonistic activation. Thus, this technique facilitates concomitant library synthesis and identification of peptide ligands at the single-cell level [104, 105].

Ishii et al. have developed a system for cell wall trapping of autocrine peptides (CWTrAP), which activates human SSTR5 signaling using short anchor proteins (e.g., 42 a.a. of Flo1p; Flo42) [103]. The engineered yeast strain concomitantly expressing human SSTR5 and somatostatin peptide successfully induced *GFP* reporter gene expression. Hara et al. demonstrated that the somatostatin displayed on the plasma membrane successfully activated human SSTR2 in yeast [106]. In this system, somatostatin was displayed on the yeast plasma membrane by linking it to the anchoring domain of the GPI-anchored plasma membrane protein Yps1p. Nakamura et al. drastically improved the sensitivity and output of this fluorescence reporter system using the ZsGreen reporter, which is applicable to CWTrAP technology [70].

3. Oligomerization among GPCRs

Many GPCRs have the capacity to form homomers or heteromers that show unique functional and biochemical characteristics including receptor pharmacology, regulation, and signaling [14, 107, 108]. Therefore, GPCR oligomers could be potential molecular targets for the development of new therapeutic agents. Yeast is a potential host for making cell-based biosensors for eukaryotic proteins and biological processes of interest [109], because varied reporting systems are available that can facilitate assays in yeast cells [110–112]. Notably, the "gold standard" for testing protein-protein interactions *in vivo,* Y2H systems, makes use of these reporters [113–115] and has also been used to identify membrane protein interaction partners [116].

3.1. Biophysical RET technologies to study GPCR oligomers in yeast cells

Varieties of resonance energy transfer (RET)-based techniques have promoted the visualization of GPCR oligomers in living cells. Fluorescence resonance energy transfer (FRET) is a strictly distance-dependent energy transfer technique using a cyan fluorescent protein (CFP) as energy donor and a yellow fluorescent protein (YFP) as energy acceptor, but other pairings are also possible [117]. Highly sensitive, bioluminescence resonance energy transfer (BRET) is based on the distance-dependent transfer of energy between a bioluminescent energy donor and a fluorescent acceptor molecule [118, 119].

Overton and Blumer [120] used subcellular fractionation and CFP/YFP FRET to demonstrate that oligomerization of the endogenous mating pheromone Ste2p receptors occurs via a stable association between protomers in yeast. Subsequently, the authors employed FRET in live yeast cells for detection of Ste2p oligomerization with its transmembrane domains [121–124].

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Furthermore, FRET experiments with yeast cells demonstrated the oligomer formation of functional human complement factor 5a (C5a) receptors [125].

BRET was later used to increase the detection sensitivity for Ste2p oligomerization. Increased sensitivity was needed, because the C-terminal regions of full length Ste2p protomers did not reach a proximity sufficient for effective energy transfer [126]. With the BRET system, Gehret et al. [126] demonstrated that mutations previously reported as blocking Ste2p receptor oligomerization decreased but did not completely eliminate oligomerization. Previously, BRET has been employed in yeast to analyze the protein interactions involved with heterogeneous olfactory receptors [127, 128].

3.2. Membrane Y2H technology to study GPCR oligomers in yeast cells

In contrast to FRET and BRET technologies (see Section 3.1), mY2H method is based on transcription-dependent reporter gene assays, permitting colorimetric evaluations with *lacZ* and growth selections with *ADE2* and *HIS3* (detected by complementation of auxotrophies) [129]. Therefore, the split-ubiquitin mY2H approach can be employed both for quantitative assays and for comprehensive screening of protein-protein interactions of membrane proteins [129].

In the split-ubiquitin mY2H system, the N- and C-terminal halves (Nub*G* and Cub, respectively) of ubiquitin are fused to separate membrane proteins (**Figure 2A** and **B**). Nub*G* represent a mutant version of the N-terminal half of ubiquitin that harbors an Ile-13 to Gly substitution. This split-ubiquitin system functions when interaction between the membrane proteins results in ubiquitin reassembly. Notably, Cub is fused to a membrane protein along with an artificial transcription factor (LexA-VP16). Nub*G* has a very low intrinsic affinity for Cub, and therefore can interact with Cub only if the membrane proteins fused to both ubiquitin fragments have affinities for each other [130]. The reconstituted ubiquitin is recognized by ubiquitin-specific proteases, and cleavage liberates LexA-VP16. The released transcription factor then enters the nucleus and induces the transcription of reporter genes, permitting both screening (via *lacZ* expression) and selection (via *ADE2* and *HIS3* expression) based on interactions between membrane proteins.

Historically, the split-ubiquitin mY2H system was employed to screen interacting membrane-associated proteins (not GPCRs) for GPCRs, such as the μ-opioid receptor (MOR) [131, 132] and the M_3 muscarinic acetylcholine receptor (M3R) [133]. Jin et al. identified GPR177, the mammalian ortholog of *Drosophila melanogaster* Wntless, as a novel MOR-interacting protein using the split-ubiquitin mY2H system [131]. Further work showed both enhanced MOR/GPR177 complex formation at the cell periphery and inhibited Wnt secretion in response to morphine treatment, possibly causing decreased neurogenesis. Rosemond et al. investigated the predicted integral membrane protein Tmem147 and discovered that it functions as a novel M3R-associated protein [133]. Additional work also indicated that Tmem147 is as a potent M3R negative regulator, which may interfere with M3R trafficking to the cell surface.

The split-ubiquitin mY2H system has also been applied to identify GPCR heteromers [34]. Nakamura et al. developed a specialized method to screen candidate heteromer partners for target human GPCRs based on the split-ubiquitin mY2H method [34]. The authors noted that mating-associated induction of cell-cycle arrest, which causes robust growth inhibition in yeast, might impair the assessment of reporter gene activity. Therefore, the authors

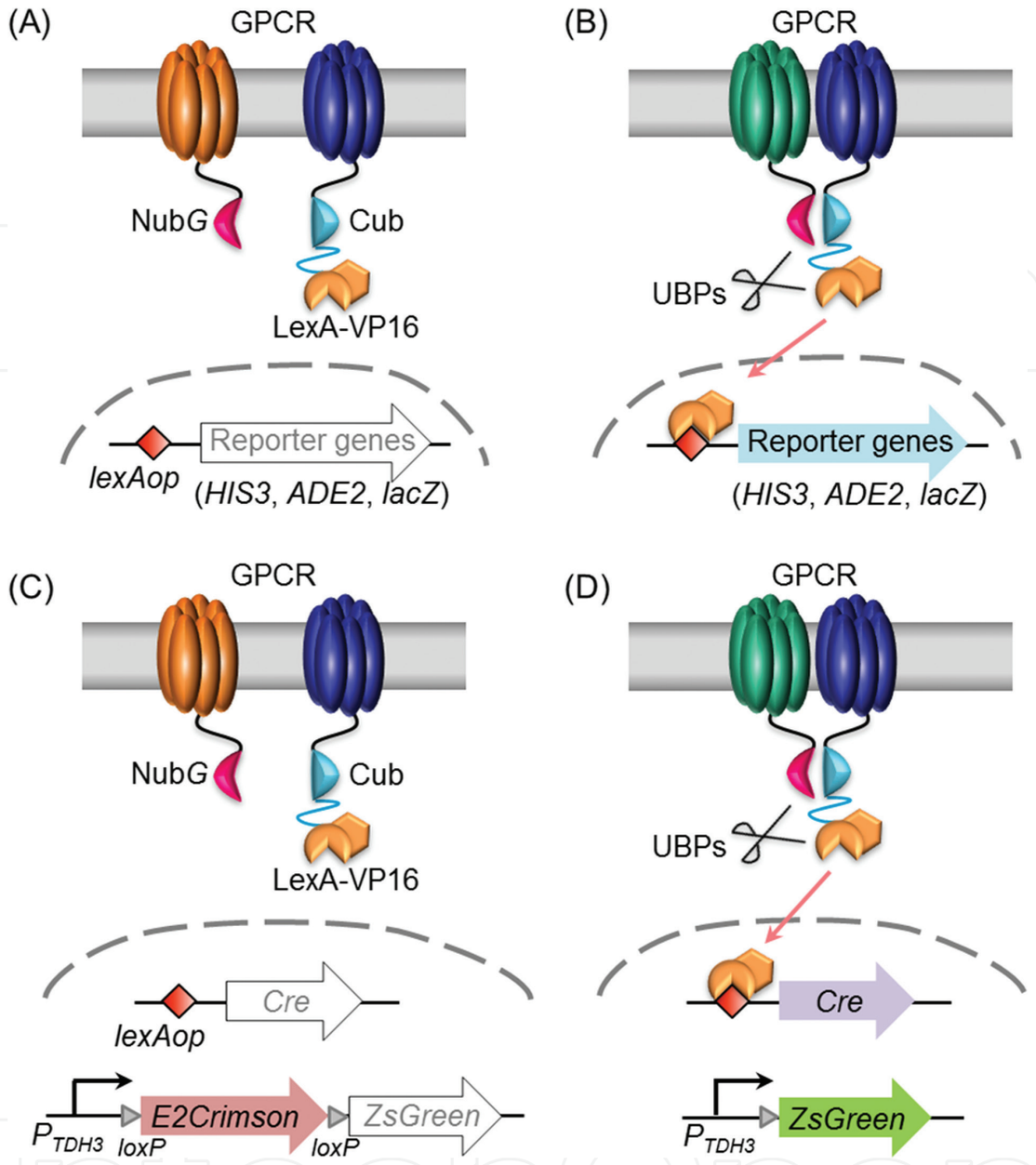

Figure 2. Schematic illustration of the yeast split-ubiquitin mY2H system to study GPCR oligomers. (A and C) No-oligomerization pairs. (B and D) Oligomerization pairs. The candidate GPCR oligomer pairs are fused to respective split-ubiquitin segments (Nub*G* and Cub), and Cub is further fused to an artificial transcription factor (LexA-VP16). Nub*G* and Cub become close in proximity only when the GPCRs form a dimer, leading to the reconstitution of the split-ubiquitin. Ubiquitin-specific proteases (UBPs) can recognize the reconstituted split-ubiquitin, resulting in LexA-VP16 transcription factor cleavage from the Cub-fused GPCRs. LexA-VP16 diffuses into the nucleus where it binds to *lexA*-binding sites on the *lexA* operator (*lexAop*). (A and B) Principal GPCR oligomer pair detection system: the reporter genes such as *HIS3*, *ADE2*, and *lacZ* are placed downstream of *lexAop*, and their expressions are induced when GPCR oligomer pairs interact with each other. (C and D) The reporter switching system for detecting GPCR oligomer pairs: the expressions of two reporter genes (*E2Crimson* and *ZsGreen*) are switched in response to the Y2H readout; one (*E2Crimson*) from ON to OFF and the other (*ZsGreen*) from OFF to ON. Briefly, after the release of the LexA-VP16 transcription factor, the *lexA* operator induces the expression of Cre recombinase, which causes a gene recombination that pops-out the *E2Crimson* gene and alternatively exposes the *ZsGreen* gene. Thus, the formation of GPCR oligomers can be discerned by monitoring the changes from far-red fluorescence to green fluorescence.

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constructed a MAPK signal-defective yeast strain. This modified host permitted the rapid and facile detection, not only of target human GPCR heteromerization, but also of ligand-mediated conformational changes in living yeast cells [34]. Thus, the modified mY2H would be available to identify GPCR heteromer components and potential therapeutic targets for regulating physiological activities.

Furthermore, the authors subsequently designed a reporter switching system that can switch the expressions between two reporter genes (one from ON to OFF and the other from OFF to ON) in response to the Y2H readout (**Figure 2C** and **D**) [134]. Cre/*loxP* site-specific recombination was employed to induce reporter switching. The authors were able to utilize the split-ubiquitin mY2H system to optimize Cre-mediated reporter gene recombination and build a dual-color reporter switching system, which could discern GPCR dimer formation. To demonstrate reporter switching, the authors used a far-red derivative of the tetrameric fluorescent protein DsRed-Express2 (*E2Crimson*) and a tetrameric *ZsGreen* as the two reporter genes. Reporter gene expression was successfully switched in the engineered yeast cells and permitted the detection of the dimerized yeast endogenous pheromone receptor (Ste2p). The authors also validated the applicability of this system for monitoring the formation of human GPCRs homodimers and heterodimers, specifically human serotonin 1A receptor or β2-adrenergic receptor, and confirmed that this system had improved sensitivity when compared with the previous system [134].

Using a modified split-ubiquitin mY2H approach, Sokolina et al. reported the systematic interactome analysis of 48 clinically important human GPCRs in their ligand-unoccupied state [135]. The authors also carried out additional in-depth functional validation on selected GPCR protein-protein interactions using biochemical and cell-based assays as well as knock-out and knock-in animals. The authors found that a G-protein-regulated inducer of neurite outgrowth 2 (GPRIN2) and the GPR37 receptor, both physically and functionally, interact with the serotonin 5-HT4d receptor, a promising target for Alzheimer's disease [135].

3.3. GPCR oligomerization and G-protein signaling

GPCR oligomerization can increase the potential for diversity in the regulation and modulation of GPCR signaling, and thus the specific evaluation of signaling properties among various receptor oligomer pairs. This work has important implications, not only for the development of new drugs, but also for the understanding of signaling networks [22]. This unique system was developed for simultaneous detection of oligomer formation and GPCR signaling activation. This new methodology uses a combination of the split-ubiquitin mY2H assay and a G-protein signaling assay, and is expected to facilitate the identification of physiologically relevant GPCR oligomers [136]. Using this system, Nakamura et al. monitored the physiological relevance of yeast pheromone receptor Ste2p, in both native and mutated forms. In addition, the authors demonstrated the simultaneous detection of homo- and heteromerization, and somatostatin-induced signaling of the human SSTR5 somatostatin receptor [136]. In the future, this system will be useful for identifying agonists that bind to the heteromer, promising to serve as a powerful platform for uncovering the novel functions, modes of action, and potential molecular targets of GPCR heteromerization for the development of new therapeutic agents.

4. Conclusion

In summary, we focused on yeast-based biosensors employed for the detection of GPCR ligand stimulation and oligomer formation, and described yeast-based techniques using the G-protein signaling and protein-protein interaction assays. Due to their involvement in signal transduction machinery, GPCRs are excellent therapeutic targets for various diseases and clinical indications [137]. The identification of new physiologically relevant GPCR oligomers provides a promising opportunity for drug discovery, based on the effect of allosteric communication between GPCR protomers (each subunit within the oligomer complex) on known pharmacological properties. Thus, approaches for investigating the relationship between oligomerization and GPCR signaling are necessary for creating oligomer-specific bivalent ligands. Additionally, there is great potential for identifying previously undiscovered physiological diversities and therapeutic targets through the generation of comprehensive and interactive GPCR oligomer maps. It is also important to expand our knowledge of the molecular details of GPCR-mediated signal transduction, including the identification of all proteins that interact with clinically relevant GPCRs. Further development of various methods, including yeast-based approaches and the investigation of GPCR oligomers, are expected to facilitate these outcomes in the near future.

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Conflict of interest

The authors declare no commercial or financial conflict of interest.

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References

[1] Wang Y, Dohlman HG. Regulation of G protein and mitogen-activated protein kinase signaling by ubiquitination: Insights from model organisms. Circulation Research. 2006;**99**:1305-1314. DOI: 10.1161/01.RES.0000251641.57410.81

[2] Vögler O, Barceló JM, Ribas C, Escribá PV. Membrane interactions of G proteins and other related proteins. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2008;**1778**:1640-1652. DOI: 10.1016/j.bbamem.2008.03.008

[3] Fredriksson R, Lagerström MC, Lundin LG, Schiöth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Molecular Pharmacology. 2003;**63**:1256-1272. DOI: 10.1124/mol.63.6.1256

[4] Kim TH, Chung KY, Manglik A, Hansen AL, Dror RO, Mildorf TJ, Shaw DE, Kobilka BK, Prosser RS. The role of ligands on the equilibria between functional states of a G protein-coupled receptor. Journal of the American Chemical Society. 2013;**135**:9465-9474. DOI: 10.1021/ja404305k

[5] Nieto Gutierrez A, McDonald PH. GPCRs: Emerging anti-cancer drug targets. Cellular Signalling. 2018;**41**:65-74. DOI: 10.1016/j.cellsig.2017.09.005

[6] Venkatakrishnan AJ, Deupi X, Lebon G, Tate CG, Schertler GF, Babu MM. Molecular signatures of G-protein-coupled receptors. Nature. 2013;**494**:185-194. DOI: 10.1038/nature11896

[7] Katritch V, Cherezov V, Stevens RC. Diversity and modularity of G protein-coupled receptor structures. Trends in Pharmacological Sciences. 2012;**33**:17-27. DOI: 10.1016/j.tips.2011.09.003

[8] Wise A, Gearing K, Rees S. Target validation of G-protein coupled receptors. Drug Discovery Today. 2002;**7**:235-246. DOI: 10.1016/S1359-6446(01)02131-6

[9] Ghosh E, Kumari P, Jaiman D, Shukla AK. Methodological advances: The unsung heroes of the GPCR structural revolution. Nature Reviews Molecular Cell Biology. 2015;**16**:69-81. DOI: 10.1038/nrm3933

[10] Rosenbaum DM, Rasmussen SG, Kobilka BK. The structure and function of G-protein-coupled receptors. Nature. 2009;**459**:356-363. DOI: 10.1038/nature08144

[11] Karnik SS, Gogonea C, Patil S, Saad Y, Takezako T. Activation of G-protein-coupled receptors: A common molecular mechanism. Trends in Endocrinology & Metabolism. 2003;**14**:431-437. DOI: 10.1016/j.tem.2003.09.007

[12] Park PS, Filipek S, Wells JW, Palczewski K. Oligomerization of G protein-coupled receptors: Past, present, and future. Biochemistry. 2004;**43**:15643-15656. DOI: 10.1021/bi047907k

[13] Devi LA. G-protein-coupled receptor dimers in the lime light. Trends in Pharmacological Sciences. 2000;**21**:324-326. DOI: 10.1016/S0165-6147(00)01519-4

[14] Ferré S, Casadó V, Devi LA, Filizola M, Jockers R, Lohse MJ, Milligan G, Pin JP, Guitart X. G protein-coupled receptor oligomerization revisited: Functional and pharmacological perspectives. Pharmacological Reviews. 2014;**66**:413-434. DOI: 10.1124/pr.113.008052

[15] Milligan G. G protein-coupled receptor dimerization: Function and ligand pharmacology. Molecular Pharmacology. 2004;**66**:1-7. DOI: 10.1124/mol.104.000497

[16] White JH, Wise A, Main MJ, Green A, Fraser NJ, Disney GH, Barnes AA, Emson P, Foord SM, Marshall FH. Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature. 1998;**396**:679-682. DOI: 10.1038/25354

[17] Smith NJ, Milligan G. Allostery at G protein-coupled receptor homo- and heteromers: Uncharted pharmacological landscapes. Pharmacological Reviews. 2010;**62**:701-725. DOI: 10.1124/pr.110.002667

[18] Xue L, Rovira X, Scholler P, Zhao H, Liu J, Pin JP, Rondard P. Major ligand-induced rearrangement of the heptahelical domain interface in a GPCR dimer. Nature Chemical Biology. 2015;**11**:134-140. DOI: 10.1038/nchembio.1711

[19] Bouvier M. Oligomerization of G-protein-coupled transmitter receptors. Nature Reviews Neuroscience. 2001;**2**:274-286. DOI: 10.1038/35067575

[20] Bulenger S, Marullo S, Bouvier M. Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. Trends in Pharmacological Sciences. 2005;**26**:131-137. DOI: 10.1016/j.tips.2005.01.004

[21] Devi LA. Heterodimerization of G-protein-coupled receptors: Pharmacology, signaling and trafficking. Trends in Pharmacological Sciences. 2001;**22**:532-537. DOI: 10.1016/S0165-6147(00)01799-5

[22] Jordan BA, Devi LA. G-protein-coupled receptor heterodimerization modulates receptor function. Nature. 1999;**399**:697-700. DOI: 10.1038/21441

[23] Jordan BA, Trapaidze N, Gomes I, Nivarthi R, Devi LA. Oligomerization of opioid receptors with beta 2-adrenergic receptors: A role in trafficking and mitogen-activated protein kinase activation. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:343-348. DOI: 10.1073/pnas.011384898

[24] Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. EMBO Reports. 2004;**5**:30-34. DOI: 10.1038/sj.embor.7400052

[25] Lane JR, Donthamsetti P, Shonberg J, Draper-Joyce CJ, Dentry S, Michino M, Shi L, López L, Scammells PJ, Capuano B, Sexton PM, Javitch JA, Christopoulos A. A new mechanism of allostery in a G protein-coupled receptor dimer. Nature Chemical Biology. 2014;**10**:745-752. DOI: 10.1038/nchembio.1593

[26] Teitler M, Klein MT. A new approach for studying GPCR dimers: Drug-induced inactivation and reactivation to reveal GPCR dimer function in vitro, in primary culture, and in vivo. Pharmacology & Therapeutics. 2012;**133**:205-217. DOI: 10.1016/j.pharmthera.2011.10.007

[27] George SR, O'Dowd BF, Lee SP. G-protein-coupled receptor oligomerization and its potential for drug discovery. Nature Reviews Drug Discovery. 2002;**1**:808-820. DOI: 10.1038/nrd913

[28] Hiller C, Kühhorn J, Gmeiner P. Class A G-protein-coupled receptor (GPCR) dimers and bivalent ligands. Journal of Medicinal Chemistry. 2013;**56**:6542-6559. DOI: 10.1021/jm4004335

[29] Le Naour M, Lunzer MM, Powers MD, Kalyuzhny AE, Benneyworth MA, Thomas MJ, Portoghese PS. Putative kappa opioid heteromers as targets for developing analgesics free of adverse effects. Journal of Medicinal Chemistry. 2014;**57**:6383-6392. DOI: 10.1021/jm500159d

[30] Ishii J, Fukuda N, Tanaka T, Ogino C, Kondo A. Protein-protein interactions and selection: Yeast-based approaches that exploit guanine nucleotide-binding protein signaling. FEBS Journal. 2010;**277**:1982-1995. DOI: 10.1111/j.1742-4658.2010.07625.x

[31] Minic J, Sautel M, Salesse R, Pajot-Augy E. Yeast system as a screening tool for pharmacological assessment of G protein coupled receptors. Current Medicinal Chemistry. 2005;**12**:961-969. DOI: 10.2174/0929867053507261

[32] Brown AJ, Dyos SL, Whiteway MS, White JH, Watson MA, Marzioch M, Clare JJ, Cousens DJ, Paddon C, Plumpton C, Romanos MA, Dowell SJ. Functional coupling of mammalian receptors to the yeast mating pathway using novel yeast/mammalian G protein α-subunit chimeras. Yeast. 2000;**16**:11-22. DOI: 10.1002/(SICI)1097-0061(20000115)16:1<11::AID-YEA502>3.0.CO;2-K

[33] Stagljar I, Korostensky C, Johnsson N, te Heesen S. A genetic system based on split-ubiquitin for the analysis of interactions between membrane proteins in vivo. Proceedings of the National Academy of Sciences of the United States of America. 1998;**95**:5187-5192

[34] Nakamura Y, Ishii J, Kondo A. Rapid, facile detection of heterodimer partners for target human G-protein-coupled receptors using a modified split-ubiquitin membrane yeast two-hybrid system. PLoS One. 2013;**8**:e66793. DOI: 10.1371/journal.pone.0066793

[35] Cotton M, Claing A. G protein-coupled receptors stimulation and the control of cell migration. Cellular Signalling. 2009;**21**:1045-1053. DOI: 10.1016/j.cellsig.2009.02.008

[36] Dupré DJ, Robitaille M, Rebois RV, Hébert TE. The role of Gbetagamma subunits in the organization, assembly, and function of GPCR signaling complexes. Annual Review of Pharmacology and Toxicology. 2009;**49**:31-56. DOI: 10.1146/annurev-pharmtox-061008-103038

[37] Ritter SL, Hall RA. Fine-tuning of GPCR activity by receptor-interacting proteins. Nature Reviews Molecular Cell Biology. 2009;**10**:819-830. DOI: 10.1038/nrm2803

[38] Bardwell L. A walk-through of the yeast mating pheromone response pathway. Peptides. 2005;**26**:339-350. DOI: 10.1016/j.peptides.2003.10.022

[39] Elion EA. Pheromone response, mating and cell biology. Current Opinion in Microbiology. 2000;**3**:573-581. DOI: 10.1016/S1369-5274(00)00143-0

[40] Alvaro CG, Thorner J. Heterotrimeric G protein-coupled receptor signaling in yeast mating pheromone response. Journal of Biological Chemistry. 2016;**291**:7788-7795. DOI: 10.1074/jbc.R116.714980

[41] Leberer E, Thomas DY, Whiteway M. Pheromone signalling and polarized morphogenesis in yeast. Current Opinion in Genetics & Development. 1997;**7**:59-66. DOI: 10.1016/S0959-437X(97)80110-4

[42] Leeuw T, Wu C, Schrag JD, Whiteway M, Thomas DY, Leberer E. Interaction of a G-protein beta-subunit with a conserved sequence in Ste20/PAK family protein kinases. Nature. 1998;**391**:191-195. DOI: 10.1038/34448

[43] Elion EA. The Ste5p scaffold. Journal of Cell Science. 2001;**114**:3967-3978

[44] Pryciak PM, Huntress FA. Membrane recruitment of the kinase cascade scaffold protein Ste5 by the Gβγ complex underlies activation of the yeast pheromone response pathway. Genes & Development. 1998;**12**:2684-2697. DOI: 10.1101/gad.12.17.2684

[45] Chang F, Herskowitz I. Identification of a gene necessary for cell cycle arrest by a negative growth factor of yeast: FAR1 is an inhibitor of a G1 cyclin, CLN2. Cell. 1990;**63**:999-1011. DOI: 10.1016/0092-8674(90)90503-7

[46] Chang F, Herskowitz I. Phosphorylation of FAR1 in response to alpha-factor: A possible requirement for cell-cycle arrest. Molecular Biology of the Cell. 1992;**3**:445-450. DOI: 10.1091/mbc.3.4.445

[47] McKinney JD, Cross FR. FAR1 and the G1 phase specificity of cell cycle arrest by mating factor in Saccharomyces cerevisiae. Molecular and Cellular Biology. 1995;**15**:2509-2516. DOI: 10.1128/MCB.15.5.2509

[48] Dolan JW, Kirkman C, Fields S. The yeast STE12 protein binds to the DNA sequence mediating pheromone induction. Proceedings of the National Academy of Sciences of the United States of America. 1989;**86**:5703-5707

[49] Song D, Dolan JW, Yuan YL, Fields S. Pheromone-dependent phosphorylation of the yeast STE12 protein correlates with transcriptional activation. Genes & Development. 1991;**5**:741-750. DOI: 10.1101/gad.5.5.741

[50] McCaffrey G, Clay FJ, Kelsay K, Sprague GF Jr. Identification and regulation of a gene required for cell fusion during mating of the yeast Saccharomyces cerevisiae. Molecular and Cellular Biology. 1987;**7**:2680-2690. DOI: 10.1128/MCB.7.8.2680

[51] Hagen DC, McCaffrey G, Sprague GF Jr. Pheromone response elements are necessary and sufficient for basal and pheromone-induced transcription of the FUS1 gene of Saccharomyces cerevisiae. Molecular and Cellular Biology. 1991;**11**:2952-2961. DOI: 10.1128/MCB.11.6.2952

[52] Zeitlinger J, Simon I, Harbison CT, Hannett NM, Volkert TL, Fink GR, Young RA. Program-specific distribution of a transcription factor dependent on partner transcription factor and MAPK signaling. Cell. 2003;**113**:395-404. DOI: 10.1016/S0092-8674(03)00301-5

[53] White JM, Rose MD. Yeast mating: Getting close to membrane merger. Current Biology. 2001;**11**:R16-R20. DOI: 10.1016/S0960-9822(00)00036-1

[54] Chasse SA, Flanary P, Parnell SC, Hao N, Cha JY, Siderovski DP, Dohlman HG. Genome-scale analysis reveals Sst2 as the principal regulator of mating pheromone signaling in the yeast Saccharomyces cerevisiae. Eukaryotic Cell. 2006;**5**:330-346. DOI: 10.1128/EC.5.2.330-346.2006

[55] Dohlman HG, Song J, Ma D, Courchesne WE, Thorner J. Sst2, a negative regulator of pheromone signaling in the yeast Saccharomyces cerevisiae: Expression, localization, and genetic interaction and physical association with Gpa1 (the G-protein alpha subunit). Molecular and Cellular Biology. 1996;**16**:5194-5209. DOI: 10.1128/MCB.16.9.5194

[56] Apanovitch DM, Slep KC, Sigler PB, Dohlman HG. Sst2 is a GTPase-activating protein for Gpa1: Purification and characterization of a cognate RGS-Gα protein pair in yeast. Biochemistry. 1998;**37**:4815-4822. DOI: 10.1021/bi9729965

[57] Dohlman HG, Apaniesk D, Chen Y, Song J, Nusskern D. Inhibition of G-protein signaling by dominant gain-of-function mutations in Sst2p, a pheromone desensitization factor in Saccharomyces cerevisiae. Molecular and Cellular Biology. 1995;**15**:3635-3643. DOI: 10.1128/MCB.15.7.3635

[58] Ishii J, Matsumura S, Kimura S, Tatematsu K, Kuroda S, Fukuda H, Kondo A. Quantitative and dynamic analyses of G protein-coupled receptor signaling in yeast using Fus1, enhanced green fluorescence protein (EGFP), and His3 fusion protein. Biotechnology Progress. 2006;**22**:954-960. DOI: 10.1021/bp0601387

[59] Evans BJ, Wang Z, Broach JR, Oishi S, Fujii N, Peiper SC. Expression of CXCR4, a G-protein-coupled receptor for CXCL12 in yeast. Identification of new-generation inverse agonists. Methods in Enzymology. 2009;**460**:399-412. DOI: 10.1016/S0076-6879(09)05220-3

[60] Fukutani Y, Ishii J, Noguchi K, Kondo A, Yohda M. An improved bioluminescence-based signaling assay for odor sensing with a yeast expressing a chimeric olfactory receptor. Biotechnology and Bioengineering. 2012;**109**:3143-3151. DOI: 10.1002/bit.24589

[61] Pausch MH. G-protein-coupled receptors in Saccharomyces cerevisiae: High-throughput screening assays for drug discovery. Trends in Biotechnology. 1997;**15**:487-494. DOI: 10.1016/S0167-7799(97)01119-0

[62] Ishii J, Tanaka T, Matsumura S, Tatematsu K, Kuroda S, Ogino C, Fukuda H, Kondo A. Yeast-based fluorescence reporter assay of G protein-coupled receptor signalling for flow cytometric screening: FAR1-disruption recovers loss of episomal plasmid caused by signalling in yeast. The Journal of Biochemistry. 2008;**143**:667-674. DOI: 10.1093/jb/mvn018

[63] Ladds G, Goddard A, Davey J. Functional analysis of heterologous GPCR signalling pathways in yeast. Trends in Biotechnology. 2005;**23**:367-373. DOI: 10.1016/j.tibtech.2005.05.007

[64] Leplatois P, Josse A, Guillemot M, Febvre M, Vita N, Ferrara P, Loison G. Neurotensin induces mating in *Saccharomyces cerevisiae* cells that express human neurotensin receptor type 1 in place of the endogenous pheromone receptor. European Journal of Biochemistry. 2001;**268**:4860-4867. DOI: 10.1046/j.0014-2956.2001.02407.x

[65] Price LA, Kajkowski EM, Hadcock JR, Ozenberger BA, Pausch MH. Functional coupling of a mammalian somatostatin receptor to the yeast pheromone response pathway. Molecular and Cellular Biology. 1995;**15**:6188-6195. DOI: 10.1128/MCB.15.11.6188

[66] Erlenbach I, Kostenis E, Schmidt C, Hamdan FF, Pausch MH, Wess J. Functional expression of M1, M3 and M5 muscarinic acetylcholine receptors in yeast. Journal of Neurochemistry. 2001;**77**:1327-1337. DOI: 10.1046/j.1471-4159.2001.00344.x

[67] Li B, Scarselli M, Knudsen CD, Kim SK, Jacobson KA, McMillin SM, Wess J. Rapid identification of functionally critical amino acids in a G protein-coupled receptor. Nature Methods. 2007;**4**:169-174. DOI: 10.1038/nmeth990

[68] Pausch MH, Lai M, Tseng E, Paulsen J, Bates B, Kwak S. Functional expression of human and mouse P2Y12 receptors in Saccharomyces cerevisiae. Biochemical and Biophysical Research Communications. 2004;**324**:171-177. DOI: 10.1016/j.bbrc.2004.09.034

[69] Kaishima M, Ishii J, Matsuno T, Fukuda N, Kondo A. Expression of varied GFPs in Saccharomyces cerevisiae: Codon optimization yields stronger than expected expression and fluorescence intensity. Scientific Reports. 2016;**6**:35932. DOI: 10.1038/srep35932

[70] Nakamura Y, Ishii J, Kondo A. Bright fluorescence monitoring system utilizing *Zoanthus* sp. green fluorescent protein (ZsGreen) for human G-protein-coupled receptor signaling in microbial yeast cells. PLoS One. 2013;**8**:e82237. DOI: 10.1371/journal.pone.0082237

[71] Minic J, Persuy MA, Godel E, Aioun J, Connerton I, Salesse R, Pajot-Augy E. Functional expression of olfactory receptors in yeast and development of a bioassay for odorant screening. The FEBS Journal. 2005;**272**:524-537. DOI: 10.1111/j.1742-4658.2004.04494.x

[72] King K, Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ. Control of yeast mating signal transduction by a mammalian beta 2-adrenergic receptor and Gs alpha subunit. Science. 1990;**250**:121-123. DOI: 10.1126/science.2171146

[73] Liu R, Wong W, IJzerman AP. Human G protein-coupled receptor studies in *Saccharomyces cerevisiae*. Biochemical Pharmacology. 2016;**114**:103-115. DOI: 10.1016/j.bcp.2016.02.010

[74] Raynor K, Murphy WA, Coy DH, Taylor JE, Moreau JP, Yasuda K, Bell GI, Reisine T. Cloned somatostatin receptors: Identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides. Molecular Pharmacology. 1993; **43**:838-844

[75] Raynor K, O'Carroll AM, Kong H, Yasuda K, Mahan LC, Bell GI, Reisine T. Characterization of cloned somatostatin receptors SSTR4 and SSTR5. Molecular Pharmacology. 1993;**44**: 385-392

[76] Jaquet P, Saveanu A, Gunz G, Fina F, Zamora AJ, Grino M, Culler MD, Moreau JP, Enjalbert A, Ouafik LH. Human somatostatin receptor subtypes in acromegaly: Distinct patterns of messenger ribonucleic acid expression and hormone suppression identify different tumoral phenotypes. The Journal of Clinical Endocrinology & Metabolism. 2000;**85**:781-792. DOI: 10.1210/jcem.85.2.6338

[77] Iguchi Y, Ishii J, Nakayama H, Ishikura A, Izawa K, Tanaka T, Ogino C, Kondo A. Control of signalling properties of human somatostatin receptor subtype-5 by additional signal sequences on its amino-terminus in yeast. The Journal of Biochemistry. 2010;**147**:875-884. DOI: 10.1093/jb/mvq023

[78] Togawa S, Ishii J, Ishikura A, Tanaka T, Ogino C, Kondo A. Importance of asparagine residues at positions 13 and 26 on the amino-terminal domain of human somatostatin receptor subtype-5 in signalling. The Journal of Biochemistry. 2010;**147**:867-873. DOI: 10.1093/jb/mvq022

[79] Fukuda N, Ishii J, Kaishima M, Kondo A. Amplification of agonist stimulation of human G-protein-coupled receptor signaling in yeast. Analytical Biochemistry. 2011;**417**:182-187. DOI: 10.1016/j.ab.2011.06.006

[80] White JF, Noinaj N, Shibata Y, Love J, Kloss B, Xu F, Gvozdenovic-Jeremic J, Shah P, Shiloach J, Tate CG, Grisshammer R. Structure of the agonist-bound neurotensin receptor. Nature. 2012;**490**:508-513. DOI: 10.1038/nature11558

[81] Dupouy S, Mourra N, Doan VK, Gompel A, Alifano M, Forgez P. The potential use of the neurotensin high affinity receptor 1 as a biomarker for cancer progression and as a component of personalized medicine in selective cancers. Biochimie. 2011;**93**:1369-1378. DOI: 10.1016/j.biochi.2011.04.024

[82] Ishii J, Oda A, Togawa S, Fukao A, Fujiwara T, Ogino C, Kondo A. Microbial fluorescence sensing for human neurotensin receptor type 1 using Gα-engineered yeast cells. Analytical Biochemistry. 2014;**446**:37-43. DOI: 10.1016/j.ab.2013.10.016

[83] Hashi H, Nakamura Y, Ishii J, Kondo A. Modifying expression modes of human neurotensin receptor type 1 alters sensing capabilities for agonists in yeast signaling biosensor. Biotechnology Journal. 2018;**13**:e1700522. DOI: 10.1002/biot.201700522

[84] Griendling KK, Lassègue B, Alexander RW. Angiotensin receptors and their therapeutic implications. Annual Review of Pharmacology and Toxicology. 1996;**36**:281-306. DOI: 10.1146/annurev.pa.36.040196.001433

[85] Mehta PK, Griendling KK. Angiotensin II cell signaling: Physiological and pathological effects in the cardiovascular system. American Journal of Physiology Cell Physiology. 2007;**292**:C82-C97. DOI: 10.1152/ajpcell.00287.2006

[86] Groblewski T, Maigret B, Larguier R, Lombard C, Bonnafous JC, Marie J. Mutation of Asn111 in the third transmembrane domain of the AT(1A) angiotensin II receptor induces its constitutive activation. Journal of Biological Chemistry. 1997;**272**:1822-1826. DOI: 10.1074/jbc.272.3.1822

[87] Balmforth AJ, Lee AJ, Warburton P, Donnelly D, Ball SG. The conformational change responsible for AT1receptor activation is dependent upon two juxtaposed asparagine residues on transmembrane helices III and VII. Journal of Biological Chemistry. 1997;**272**:4245-4251. DOI: 10.1074/jbc.272.7.4245

[88] Nakamura Y, Ishii J, Kondo A. Construction of a yeast-based signaling biosensor for human angiotensin II type 1 receptor via functional coupling between Asn295-mutated receptor and Gpa1/Gi3 chimeric Gα. Biotechnology and Bioengineering. 2014;**111**:2220-2228. DOI: 10.1002/bit.25278

[89] Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. International Union of Pharmacology Classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacological Reviews. 1994;**46**:157-203

[90] Nakamura Y, Ishii J, Kondo A. Applications of yeast-based signaling sensor for characterization of antagonist and analysis of site-directed mutants of the human serotonin 1A receptor. Biotechnology and Bioengineering. 2015;**112**:1906-1915. DOI: 10.1002/bit.25597

[91] Stewart GD, Sexton PM, Christopoulos A. Detection of novel functional selectivity at M3 muscarinic acetylcholine receptors using a *Saccharomyces cerevisiae* platform. ACS Chemical Biology. 2010;**5**:365-375. DOI: 10.1021/cb900276p

[92] Stewart GD, Sexton PM, Christopoulos A. Prediction of functionally selective allosteric interactions at an M3 muscarinic acetylcholine receptor mutant using Saccharomyces cerevisiae. Molecular Pharmacology. 2010;**78**:205-214. DOI: 10.1124/mol.110.064253

[93] Klein C, Paul JI, Sauvé K, Schmidt MM, Arcangeli L, Ransom J, Trueheart J, Manfredi JP, Broach JR, Murphy AJ. Identification of surrogate agonists for the human FPRL-1 receptor by autocrine selection in yeast. Nature Biotechnology. 1998;**16**:1334-1337. DOI: 10.1038/4310

[94] Marrakchi M, Vidic J, Jaffrezic-Renault N, Martelet C, Pajot-Augy E. A new concept of olfactory biosensor based on interdigitated microelectrodes and immobilized yeasts expressing the human receptor OR17-40. European Biophysics Journal. 2007;**36**:1015-1018. DOI: 10.1007/s00249-007-0187-6

[95] Fukutani Y, Nakamura T, Yorozu M, Ishii J, Kondo A, Yohda M. The N-terminal replacement of an olfactory receptor for the development of a yeast-based biomimetic odor sensor. Biotechnology and Bioengineering. 2012;**109**:205-212. DOI: 10.1002/bit.23327

[96] Fukutani Y, Hori A, Tsukada S, Sato R, Ishii J, Kondo A, Matsunami H, Yohda M. Improving the odorant sensitivity of olfactory receptor-expressing yeast with accessory proteins. Analytical Biochemistry. 2015;**471**:1-8. DOI: 10.1016/j.ab.2014.10.012

[97] Tehseen M, Dumancic M, Briggs L, Wang J, Berna A, Anderson A, Trowell S. Functional coupling of a nematode chemoreceptor to the yeast pheromone response pathway. PLoS One. 2014;**9**:e111429. DOI: 10.1371/journal.pone.0111429

[98] Mukherjee K, Bhattacharyya S, Peralta-Yahya P. GPCR-based chemical biosensors for medium-chain fatty acids. ACS Synthetic Biology. 2015;**4**:1261-1269. DOI: 10.1021/sb500365m

[99] Ueda M, Tanaka A. Genetic immobilization of proteins on the yeast cell surface. Biotechnology Advances. 2000;**18**:121-140. DOI: 10.1016/S0734-9750(00)00031-8

[100] Kondo A, Ueda M. Yeast cell-surface display—Applications of molecular display. Applied Microbiology and Biotechnology. 2004;**64**:28-40. DOI: 10.1007/s00253-003-1492-3

[101] Gai SA, Wittrup KD. Yeast surface display for protein engineering and characterization. Current Opinion in Structural Biology. 2007;**17**:467-473. DOI: 10.1016/j.sbi.2007.08.012

[102] Pepper LR, Cho YK, Boder ET, Shusta EV. A decade of yeast surface display technology: Where are we now? Combinatorial Chemistry & High Throughput Screening. 2008;**11**:127-134. DOI: 10.2174/138620708783744516

[103] Ishii J, Yoshimoto N, Tatematsu K, Kuroda S, Ogino C, Fukuda H, Kondo A. Cell wall trapping of autocrine peptides for human G-protein-coupled receptors on the yeast cell surface. PLoS One. 2012;**7**:e37136. DOI: 10.1371/journal.pone.0037136

[104] Müller S, Nebe-von-Caron G. Functional single-cell analyses: Flow cytometry and cell sorting of microbial populations and communities. FEMS Microbiology Reviews. 2010;**34**:554-587. DOI: 10.1111/j.1574-6976.2010.00214.x

[105] Yoshimoto N, Tatematsu K, Iijima M, Niimi T, Maturana AD, Fujii I, Kondo A, Tanizawa K, Kuroda S. High-throughput de novo screening of receptor agonists with an automated single-cell analysis and isolation system. Scientific Reports. 2014;**4**:4242. DOI: 10.1038/srep04242

[106] Hara K, Shigemori T, Kuroda K, Ueda M. Membrane-displayed somatostatin activates somatostatin receptor subtype-2 heterologously produced in Saccharomyces cerevisiae. AMB Express. 2012;**2**:63. DOI: 10.1186/2191-0855-2-63

[107] Ferré S. The GPCR heterotetramer: Challenging classical pharmacology. Trends in Pharmacological Sciences. 2015;**36**:145-152. DOI: 10.1016/j.tips.2015.01.002

[108] Milligan G. The prevalence, maintenance, and relevance of G protein-coupled receptor oligomerization. Molecular Pharmacology. 2013;**84**:158-169. DOI: 10.1124/mol.113.084780

[109] Adeniran A, Sherer M, Tyo KE. Yeast-based biosensors: Design and applications. FEMS Yeast Research. 2015;**15**:1-15. DOI: 10.1111/1567-1364.12203

[110] Leskinen P, Virta M, Karp M. One-step measurement of firefly luciferase activity in yeast. Yeast. 2003;**20**:1109-1113. DOI: 10.1002/yea.1024

[111] Naylor LH. Reporter gene technology: The future looks bright. Biochemical Pharmacology. 1999;**58**:749-757. DOI: 10.1016/S0006-2952(99)00096-9

[112] Radhika V, Proikas-Cezanne T, Jayaraman M, Onesime D, Ha JH, Dhanasekaran DN. Chemical sensing of DNT by engineered olfactory yeast strain. Nature Chemical Biology. 2007;**3**:325-330. DOI: 10.1038/nchembio882

[113] Chen J, Zhou J, Bae W, Sanders CK, Nolan JP, Cai H. A yEGFP-based reporter system for high-throughput yeast two-hybrid assay by flow cytometry. Cytometry Part A. 2008;**73**:312-320. DOI: 10.1002/cyto.a.20525

[114] Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, Sakaki Y. A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:4569-4574. DOI: 10.1073/pnas.061034498

[115] Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, Qureshi-Emili A, Li Y, Godwin B, Conover D, Kalbfleisch T, Vijayadamodar G, Yang M, Johnston M, Fields S, Rothberg JM. A comprehensive

analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature. 2000;**403**:623-627. DOI: 10.1038/35001009

[116] Babu M, Vlasblom J, Pu S, Guo X, Graham C, Bean BD, Burston HE, Vizeacoumar FJ, Snider J, Phanse S, Fong V, Tam YY, Davey M, Hnatshak O, Bajaj N, Chandran S, Punna T, Christopolous C, Wong V, Yu A, Zhong G, Li J, Stagljar I, Conibear E, Wodak SJ, Emili A, Greenblatt JF. Interaction landscape of membrane-protein complexes in Saccharomyces cerevisiae. Nature. 2012;**489**:585-589. DOI: 10.1038/nature11354

[117] Milligan G, Bouvier M. Methods to monitor the quaternary structure of G protein-coupled receptors. The FEBS Journal. 2005;**272**:2914-2925. DOI: 10.1111/j.1742-4658.2005.04731.x

[118] Angers S, Salahpour A, Joly E, Hilairet S, Chelsky D, Dennis M, Bouvier M. Detection of beta 2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:3684-3689. DOI: 10.1073/pnas.060590697

[119] Issafras H, Angers S, Bulenger S, Blanpain C, Parmentier M, Labbé-Jullié C, Bouvier M, Marullo S. Constitutive agonist-independent CCR5 oligomerization and antibody-mediated clustering occurring at physiological levels of receptors. Journal of Biological Chemistry. 2002;**277**:34666-34673. DOI: 10.1074/jbc.M202386200

[120] Overton MC, Blumer KJ. G-protein-coupled receptors function as oligomers in vivo. Current Biology. 2000;**10**:341-344. DOI: 10.1016/S0960-9822(00)00386-9

[121] Overton MC, Blumer KJ. Use of fluorescence resonance energy transfer to analyze oligomerization of G-protein-coupled receptors expressed in yeast. Methods. 2002;**27**:324-332. DOI: 10.1016/S1046-2023(02)00090-7

[122] Overton MC, Blumer KJ. The extracellular N-terminal domain and transmembrane domains 1 and 2 mediate oligomerization of a yeast G protein-coupled receptor. Journal of Biological Chemistry. 2002;**277**:41463-41472. DOI: 10.1074/jbc.M205368200

[123] Overton MC, Chinault SL, Blumer KJ. Oligomerization, biogenesis, and signaling is promoted by a glycophorin A-like dimerization motif in transmembrane domain 1 of a yeast G protein-coupled receptor. Journal of Biological Chemistry. 2003;**278**:49369-49377. DOI: 10.1074/jbc.M308654200

[124] Overton MC, Chinault SL, Blumer KJ. Oligomerization of G-protein-coupled receptors: Lessons from the yeast Saccharomyces cerevisiae. Eukaryotic Cell. 2005;**4**:1963-1970. DOI: 10.1128/EC.4.12.1963-1970.2005

[125] Floyd DH, Geva A, Bruinsma SP, Overton MC, Blumer KJ, Baranski TJ. C5a receptor oligomerization. II. Fluorescence resonance energy transfer studies of a human G protein-coupled receptor expressed in yeast. Journal of Biological Chemistry. 2003;**278**:35354-35361. DOI: 10.1074/jbc.M305607200

[126] Gehret AU, Bajaj A, Naider F, Dumont ME. Oligomerization of the yeast α-factor receptor: Implications for dominant negative effects of mutant receptors. Journal of Biological Chemistry. 2006;**281**:20698-20714. DOI: 10.1074/jbc.M513642200

[127] Tehseen M, Liao C, Dacres H, Dumancic M, Trowell S, Anderson A. Oligomerisation of C. Elegans olfactory receptors, ODR-10 and STR-112, in yeast. PLoS One. 2014;**9**:e108680. DOI: 10.1371/journal.pone.0108680

[128] Wade F, Espagne A, Persuy MA, Vidic J, Monnerie R, Merola F, Pajot-Augy E, Sanz G. Relationship between homo-oligomerization of a mammalian olfactory receptor and its activation state demonstrated by bioluminescence resonance energy transfer. Journal of Biological Chemistry. 2011;**286**:15252-15259. DOI: 10.1074/jbc.M110.184580

[129] Nakamura Y, Ishii J, Kondo A. Current techniques for studying oligomer formations of G-protein-coupled receptors using mammalian and yeast cells. Current Medicinal Chemistry. 2016;**23**:1638-1656. DOI: 10.2174/0929867323666160407113353

[130] Johnsson N, Varshavsky A. Split ubiquitin as a sensor of protein interactions in vivo. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**:10340-10344

[131] Jin J, Kittanakom S, Wong V, Reyes BA, Van Bockstaele EJ, Stagljar I, Berrettini W, Levenson R. Interaction of the mu-opioid receptor with GPR177 (Wntless) inhibits Wnt secretion: Potential implications for opioid dependence. BMC Neuroscience. 2010;**11**:33. DOI: 10.1186/1471-2202-11-33

[132] Petko J, Justice-Bitner S, Jin J, Wong V, Kittanakom S, Ferraro TN, Stagljar I, Levenson R. MOR is not enough: Identification of novel mu-opioid receptor interacting proteins using traditional and modified membrane yeast two-hybrid screens. PLoS One. 2013;**8**:e67608. DOI: 10.1371/journal.pone.0067608

[133] Rosemond E, Rossi M, McMillin SM, Scarselli M, Donaldson JG, Wess J. Regulation of M_3 muscarinic receptor expression and function by transmembrane protein 147. Molecular Pharmacology. 2011;**79**:251-261. DOI: 10.1124/mol.110.067363

[134] Nakamura Y, Hashimoto T, Ishii J, Kondo A. Dual-color reporter switching system to discern dimer formations of G-protein-coupled receptors using Cre/loxP site-specific recombination in yeast. Biotechnology and Bioengineering. 2016;**113**:2178-2190. DOI: 10.1002/bit.25974

[135] Sokolina K, Kittanakom S, Snider J, Kotlyar M, Maurice P, Gandía J, Benleulmi-Chaachoua A, Tadagaki K, Oishi A, Wong V, Malty RH, Deineko V, Aoki H, Amin S, Yao Z, Morató X, Otasek D, Kobayashi H, Menendez J, Auerbach D, Angers S, Przulj N, Bouvier M, Babu M, Ciruela F, Jockers R, Jurisica I, Stagljar I. Systematic protein-protein interaction mapping for clinically relevant human GPCRs. Molecular Systems Biology. 2017;**13**:918. DOI: 10.15252/msb.20167430

[136] Nakamura Y, Takemoto N, Ishii J, Kondo A. Simultaneous method for analyzing dimerization and signaling of G-protein-coupled receptor in yeast by dual-color reporter system. Biotechnology and Bioengineering. 2014;**111**:586-596. DOI: 10.1002/bit.25125

[137] Lagerström MC, Schiöth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nature Reviews Drug Discovery. 2008;**7**:339-357. DOI: 10.1038/nrd2518

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