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# Control of Yeast Mating Signal Transduction by a Mammalian $\beta_2$-Adrenergic Receptor and $G_s$ $\alpha$ Subunit

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**To facilitate functional and mechanistic studies of receptor–G protein interactions by expression of the human $\beta_2$-adrenergic receptor (hβ-AR) has been expressed in *Saccharomyces cerevisiae*. This was achieved by placing a modified hβ-AR gene under control of the galactose-inducible *GAL1* promoter. After induction by galactose, functional hβ-AR was expressed at a concentration several hundred times as great as that found in any human tissue. As determined from competitive ligand binding experiments, hβ-AR expressed in yeast displayed characteristic affinities, specificity, and stereoselectivity. Partial activation of the yeast pheromone response pathway by β-adrenergic receptor agonists was achieved in cells coexpressing hβ-AR and a mammalian G protein ($G_s$) α subunit—demonstrating that these components can couple to each other and to downstream effectors when expressed in yeast. This in vivo reconstitution system provides a new approach for examining ligand binding and G protein coupling to cell surface receptors.**

THE ACTIONS OF MANY EXTRACELLULAR signals (for example, neurotransmitters, hormones, odorants, and light) are mediated by receptors with seven transmembrane domains and by heterotrimeric G proteins (*1, 2*). Such G protein–mediated signaling systems have been identified in organisms as divergent as yeast and man (*1–3*). The mammalian β-adrenergic receptor (β-AR) is a member of the class of ligand-binding receptors with seven transmembrane segments. In response to epinephrine or norepinephrine, the β-AR activates the G protein $G_s$, which in turn stimulates adenylyl cyclase and adenosine 3′5′-monophosphate production (*1, 2*). G protein–coupled pheromone receptors in yeast control a developmental program that culminates in mating (fusion) of **a** and α haploid cell types to form the **a**/α diploid (*3, 4*).

To attain high level expression of the human $\beta_2$-adrenergic receptor (hβ-AR) in yeast, we placed a modified hβ-AR gene under control of the *GAL1* promoter in the multicopy vector YEp24, to give pYβAR2 (Fig. 1). Maximal expression required several manipulations including (i) expression of a transcriptional transactivator protein (the *LAC9* gene product); (ii) replacement of the 5′ untranslated and extreme $NH_2$-terminal coding sequence of the hβ-AR gene with the corresponding region of the yeast *STE2* (α factor receptor) gene; (iii) induction with galactose when cell growth reached late exponential phase; and (iv) inclusion of a β-AR ligand in the growth medium during induction.

A primary function of cell surface receptors is to recognize only appropriate ligands among other extracellular stimuli. Accordingly, we determined ligand binding affinities to establish the functional integrity of hβ-AR expressed in yeast (Fig. 2). An antagonist, $^{125}$I-labeled cyanopindolol ([$^{125}$I]CYP), bound in a saturable manner and with high affinity to membranes prepared from pYβAR2-transformed yeast cells (Fig. 2A). By displacement of [$^{125}$I]CYP with a series of agonists, the order of potency and stereospecificity expected for hβ-AR were observed (Fig. 2B). Binding affinities in yeast were nearly identical to those observed previously for hβ-AR expressed in mammalian cells (Table 1).

A second important function of a receptor is agonist-dependent regulation of downstream components in the signal transduction pathway. Because the pheromone-responsive effector in yeast is not known, indirect biological assays are the most useful indicators of receptor function (*3, 4*). In yeast cells expressing high concentrations of hβ-AR, no agonist-dependent activation of the mating signal transduction pathway could be detected by any of the typical in vivo assays; for example, imposition of $G_1$ arrest, induction of gene expression, alteration of morphology (so-called "shmoo" formation), or stimulation of mating (*5*). A likely explanation for the absence of responsiveness is that the hβ-AR was unable to couple with the endogenous yeast G protein.

Expression of a mammalian $G_s$ α subunit can correct the growth defect in yeast cells lacking the corresponding endogenous protein encoded by the *GPA1* gene (*6*). Moreover, because specificity of receptor coupling in mammalian cells is conferred by the α subunit of G proteins (*2*), we reasoned that coexpression of hβ-AR and a mammali-

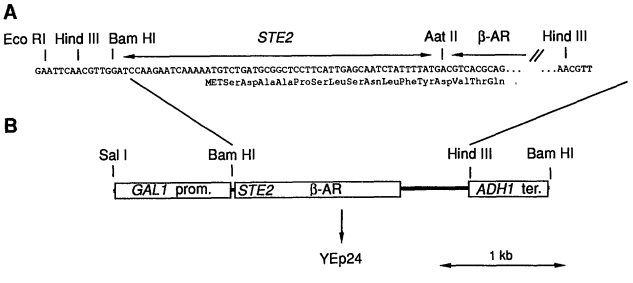


**Fig. 1.** Construction of yeast expression plasmid pYβAR2, in which expression of the hβ-AR gene is under control of the *GAL1* promoter (prom.). (**A**) The 5′ untranslated region and the first 63 bp of coding sequence of the hβ-AR gene in the plasmid pTZNAR (*12*) was removed by Aat II cleavage and replaced with a synthetic oligonucleotide corresponding to 11 bp of noncoding and 42 bp of coding sequence from the *STE2* gene (*13, 14*). The resulting plasmid, pTZYNAR, contains the modified hβ-AR gene flanked by Hind III sites in noncoding sequences. The Hind III–Hind III fragment was isolated from pTZYNAR and inserted into plasmid pAAH5 (*15*) such that the 3′ untranslated sequence of the modified hβ-AR gene was followed by 450 bp containing termination sequences (ter.) from the yeast *ADH1* gene (*15*). (**B**) The plasmid pYβAR2 was constructed by inserting the Bam HI–Bam HI fragment containing hβ-AR and *ADH1* gene sequences into YEp24 (*16*). Where maximum expression was sought, cells were cotransformed with plasmid pMTL9 containing *LAC9*—a homolog of the *S. cerevisiae GAL4* gene, which encodes a transactivator protein required for *GAL1*-regulated transcription (*17*). Cells grown to late exponential phase were induced in medium containing 3% galactose and alprenolol, and grown for an additional 36 hours. Standard methods for the maintenance of cells were used (*18*).

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an $G_s$ α subunit ($G_s$α) in yeast might render the yeast responsive to β-AR ligands. Accordingly, a cDNA encoding rat $G_s$α under control of the copper-inducible *CUP1* promoter was introduced on a second plasmid, pYSK136Gαs (*6*). In yeast (strain NNY19) coexpressing hβ-AR and rat $G_s$α, but containing wild-type *GPA1*, no β-AR agonist–induced shmoo formation—a characteristic morphological change of yeast in response to mating pheromone—was observed (*5*). To prevent interference by the endogenous yeast G protein α subunit, we expressed hβ-AR and rat $G_s$α in *gpa1* mutant cells (strain 8c). Treatment of such cells with the β-AR agonist isoproterenol induced shmoo formation, and this effect was blocked by the specific antagonist alprenolol (Fig. 3).

The isoproterenol-induced morphological response of 8c cells coexpressing hβ-AR and rat $G_s$α suggested that these components can couple to each other and to downstream components of the pheromone response pathway in yeast lacking the endogenous G protein α subunit. To confirm that the pheromone signaling pathway was activated by hβ-AR and rat $G_s$α, we measured agonist induction of the pheromone-responsive *FUS1* gene promoter in a strain of yeast derived from 8c cells (8c1), in which a *FUS1-lacZ* gene fusion had been stably integrated into the genome (*7*) (Fig. 4).

In 8c1 (*gpa1*) cells coexpressing hβ-AR and rat $G_s$α, a dramatic induction of β-galactosidase activity by isoproterenol was observed (Fig. 4). Agonist stimulation was stereoselective and was blocked by addition of alprenolol. Agonist responsiveness was

**Table 1.** Comparison of ligand binding parameters for high-level expression of hβ-AR in yeast [SC261 (pYBAR2, pMTL9)] and monkey kidney [COS-7 (pBC12:BAR)] cells. Yeast data are derived from Fig. 2 and monkey kidney cell data from (*20*). Values represent means ± SEM. ISO, isoproterenol; EPI, epinephrine; NOR, norepinephrine.

| Host cell | $K_d$ (nM) | $B_{max}$ (pmol/mg) | $K_i$ (nM) | | | |
|---|---|---|---|---|---|---|
| | | | (−)ISO | (+)ISO | (−)EPI | (−)NOR |
| Yeast | 0.093 ± 0.013 | 115 | 103 ± 26 | 3670 ± 420 | 664 ± 123 | 6000 ± 1383 |
| COS-7 cells | 0.110 ± 0.009 | 24 | 130 ± 15 | 4000 ± 184 | 360 ± 30 | 5800 ± 373 |

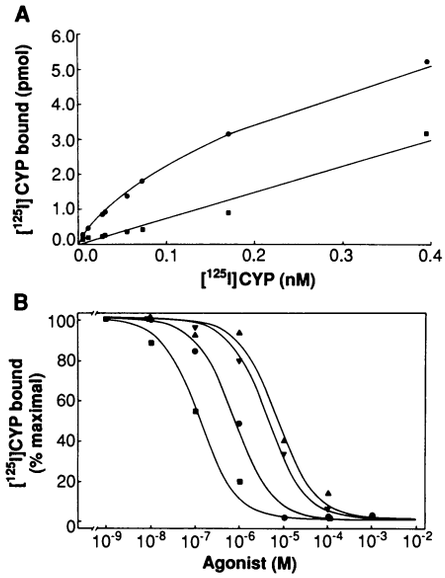


**Fig. 2.** Ligand binding to membranes from pYβAR2-transformed cells. (**A**) $B_{max}$ (maximum ligand bound) and $K_d$ (ligand dissociation constant) values were determined by varying [$^{125}$I]CYP concentrations (5 to 400 pM). Specific binding was defined as the amount of total binding (●) minus nonspecific binding measured in the presence of 10 μM (−)alprenolol (■). A $K_d$ of 93 pM for [$^{125}$I]CYP binding was obtained and used to calculate agonist affinities. (**B**) Displacement of [$^{125}$I]CYP (present at a concentration of 18 pM) with various concentrations of agonists was measured in order to determine apparent low affinity $K_i$ (inhibition constant) values [in the presence of 50 μM guanosine triphosphate (GTP)] for receptor binding: (−)isoproterenol (■), (−)epinephrine (●), (+)isoproterenol (▼), and (−)norepinephrine (▲). Binding data in (A) and (B) were analyzed by nonlinear least squares regression (*19*), and are presented in Table 1. Values given are averages of measurements performed in triplicate, and are representative of two or three experiments. SC261 cells (*MATa ura3-52 trp1 leu2 prb1-1122 pep4-3 prc1-407*) harboring pYβAR2 (*URA3*) and pMTL9 (*LEU2*) were grown in minimal glucose-free selective medium to late log phase [absorbance at 600 nm ($A_{600}$) = 5.0], and then induced with the addition of 3% galactose and 40 μM alprenolol. After 36 hours, cells were harvested and spheroplasts were prepared as described (*16*). Briefly, the spheroplasts were resuspended in 50 mM tris-HCl (*p*H 7.4) and 5 mM EDTA and were lysed by vortex mixing with glass beads for three 1-min periods at 4°C. Crude membranes were prepared from the lysates, and binding assays with [$^{125}$I]CYP were performed as described (*20*).

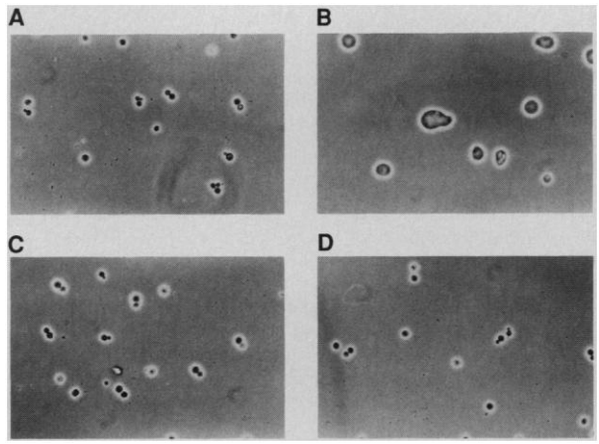


**Fig. 3.** β-Adrenergic receptor agonist–induced morphological changes in yeast. Morphologies of yeast cells cotransformed with pYβAR2, pMTL9, and pYSK136Gαs are shown after incubation with no adrenergic agent (**A**), 100 μM (−)isoproterenol (**B**), 100 μM (−)isoproterenol and 50 μM (−)alprenolol (**C**), and 100 μM (+)isoproterenol (**D**). Results are representative of three or four independent experiments. Yeast strain 8c (*MATa ura3 leu2 his3 trp1 gpa1::HIS3*) (*21*) carrying plasmids pYSK136Gαs (*TRP1*) (*6*), pMTL9 (*LEU2*) (*17*), and pYβAR2 (*URA3*) was maintained on glucose-free minimal selective plates containing 3% glycerol, 2% lactic acid, 50 μM $CuSO_4$, and 3% galactose. Colonies were transferred to similar plates containing 0.5 mM ascorbic acid and the indicated β-AR ligands. After 16 to 20 hours at 30°C, the colonies were transferred to similar liquid media at a density of $10^6$ to $10^7$ cells/ml and examined by phase contrast microscopy.

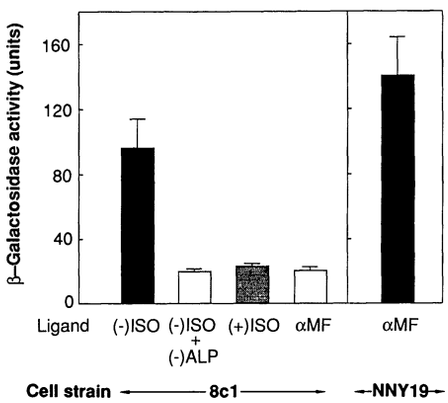


**Fig. 4.** Comparison of β-AR agonist effects on pheromone-inducible gene activity. Strains 8c (see legend to Fig. 3), modified by integrative transformation with YIpFUS102 (*LEU2*) (designated 8c1 in the figure) and strain NNY19 (*MATa ura3 leu2 his3 trp1 lys2 fus1-lacZ::LEU2*) were used in these experiments. These strains were transformed with pYβAR2 and pYSK-136Gαs and maintained on minimal selective plates containing glucose and 50 μM $CuSO_4$. Colonies were inoculated into minimal selective media (3% glycerol, 2% lactic acid, and 50 μM $CuSO_4$), grown to early log phase ($A_{600}$ = 1.0), and induced for 12 hours by addition of 3% galactose. Cells were washed and resuspended in induction media ($A_{600}$ = 5.0) containing 0.5 mM ascorbic acid and the indicated ligands. After a 4-hour incubation at 30°C, cells were harvested, resuspended in 1 ml of Z-buffer (*22*) supplemented with 0.0075% SDS, and β-galactosidase activities were determined in three or four independent experiments as described (*22*). αMF, 10 μM α mating factor; (−)ISO, 50 μM (−)isoproterenol; (−)ALP, 50 μM (−)alprenolol; and (+)ISO, 100 μM (+)isoproterenol.

dependent on expression of both hβ-AR and rat $G_s\alpha$ and required a yeast strain in which the endogenous G protein α subunit was disrupted (*5*). The final β-galactosidase activity achieved in response to isoproterenol in transformed 8c1 cells was comparable to that induced by α factor in nontransformed cells that express *GPA1* (strain NNY19)—although basal β-galactosidase activity in NNY19 cells was considerably lower than in 8c1 cells. Taken together, our results indicate that coexpression of hβ-AR and rat $G_s\alpha$ is sufficient to place key aspects of the mating signal transduction pathway in yeast under catecholamine control. However, the β-AR agonist did not stimulate mating in either 8c cells or NNY19 cells coexpressing hβ-AR and rat $G_s\alpha$ (*5*)—in agreement with recent observations that yeast pheromone receptors, in addition to binding pheromones, participate in other recognition events required for mating (*8*).

The hβ-AR stimulates adenylyl cyclase in animal cells via the action of $G_s\alpha$. In contrast, mating factor receptors in yeast trigger their effector via the action of the G protein βγ subunits (*9*). Our results indicate that activation of hβ-AR in yeast leads to dissociation of mammalian $G_s\alpha$ from yeast G protein βγ subunits, and it is the βγ subunits that presumably elicit the response.

The ability to control the yeast pheromone response pathway by expression of a heterologous adrenergic receptor and its cognate G protein α subunit may facilitate structural and functional characterization in yeast of mammalian G protein–coupled receptors. By scoring for growth arrest or β-galactosidase induction, the functional properties of mutant receptors can now be rapidly tested. Similarly, as additional genes for putative G protein–coupled receptors are isolated (*10, 11*), numerous ligands can be screened to identify those with activity toward the unidentified receptors. Finally, our results underscore the conservation of function between analogous receptor and receptor-coupling components of signal transduction pathways in yeast and mammals.


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# Rhodopsin Mutants That Bind But Fail to Activate Transducin


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**Rhodopsin is a member of a family of receptors that contain seven transmembrane helices and are coupled to G proteins. The nature of the interactions between rhodopsin mutants and the G protein, transducin ($G_t$), was investigated by flash photolysis in order to monitor directly $G_t$ binding and dissociation. Three mutant opsins with alterations in their cytoplasmic loops bound 11-*cis*-retinal to yield pigments with native rhodopsin absorption spectra, but they failed to stimulate the guanosine triphosphatase activity of $G_t$. The opsin mutations included reversal of a charged pair conserved in all G protein–coupled receptors at the cytoplasmic border of the third transmembrane helix (mutant CD1), replacement of 13 amino acids in the second cytoplasmic loop (mutant CD2), and deletion of 13 amino acids from the third cytoplasmic loop (mutant EF1). Whereas mutant CD1 failed to bind $G_t$, mutants CD2 and EF1 showed normal $G_t$ binding but failed to release $G_t$ in the presence of guanosine triphosphate. Therefore, it appears that at least the second and third cytoplasmic loops of rhodopsin are required for activation of bound $G_t$.**


PHOTOACTIVATED RHODOPSIN, META-rhodopsin II (MII), binds to $G_t$ and catalyzes guanosine diphosphate (GDP)–guanosine triphosphate (GTP) exchange (*1*). The GTP-bound form of the α subunit of $G_t$ then dissociates from MII and interacts with a guanosine 3′,5′-monophosphate (cyclic GMP) phosphodiesterase, a process that ultimately results in the generation of a neural signal (*2*). Evidence suggests that the cytoplasmic surface of rhodopsin (Fig. 1A) participates in $G_t$ binding and activation. The sites of rhodopsin that interact with $G_t$ have been proposed to include loop CD (*3*), loop EF (*4, 5*), or a combination of these loops, as well as a third loop formed by the COOH-terminal tail (Fig. 1A) (*6*). To further study the specificity of interaction between rhodopsin and $G_t$, we prepared rhodopsin mutants in which cytoplasmic loops CD or EF were altered by amino acid replacements or deletions (Fig. 1B). In mutant CD1, a charged pair ($Glu^{134}Arg^{135}$) was mutated to $Arg^{134}Glu^{135}$ (*7*). A GluArg or AspArg charged pair is conserved at the cytoplasmic border of helix C in all known G protein–coupled receptors. In mutant CD2, a segment of 13 amino acids (residues 140 to 152) in loop CD was replaced by an unrelated sequence of equal length. A 14–amino acid segment (residues 137 to 150) was deleted from loop CD to form mutant CD3, and a 13–amino acid segment (residues 237 to 249) was deleted from loop EF to form mutant EF1. The mutant opsin genes were expressed in COS-1 cells, regenerated with 11-*cis*-retinal, and purified in detergent solution by an immunoaffinity procedure (*4, 8*).

Ultraviolet (UV)-visible spectroscopy and in vitro guanosine triphosphatase (GTPase) assays were performed on these opsin mutants (Table 1). Mutant CD3 did not bind


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