Functional and Physical Interactions among *Saccharomyces cerevisiae* α-Factor Receptors

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The α-factor receptor Ste2p is a G protein-coupled receptor (GPCR) expressed on the surface of MATa haploid cells of the yeast *Saccharomyces cerevisiae*. Binding of α-factor to Ste2p results in activation of a heterotrimeric G protein and of the pheromone response pathway. Functional interactions between α-factor receptors, such as dominant-negative effects and recessive behavior of constitutive and hypersensitive mutant receptors, have been reported previously. We show here that dominant-negative effects of mutant receptors persist over a wide range of ratios of the abundances of G protein to receptor and that such effects are not blocked by covalent fusion of G protein α subunits to normal receptors. In addition, we detected dominant effects of mutant C-terminally truncated receptors, which had not been previously reported to act in a dominant manner. Furthermore, coexpression of C-terminally truncated receptors with constitutively active mutant receptors results in enhancement of constitutive signaling. Together with previous evidence for oligomerization of Ste2p receptors, these results are consistent with the idea that functional interactions between coexpressed receptors arise from physical interactions between them rather than from competition for limiting downstream components, such as G proteins.

GPCRs comprise a widely distributed superfamly of cell surface receptors responsible for initiating signaling in response to diverse stimuli, including hormones, neurotransmitters, ions, pheromones, and sensory signals. In general, binding of agonists to GPCRs leads to exchange of GTP for GDP bound to the α subunit of a cytoplasmic heterotrimeric G protein. This, in turn, results in dissociation of the trimer into the α and βγ subunits, each of which, in different contexts, is able to activate or inhibit diverse downstream effectors. Non regulatory G protein subunits are present in mammalian cells at high concentration relative to the α subunits, and the functional significance of such sequestration remains uncertain. However, in one of the best-studied cases, that of the γ subunit of a cytoplasmic heterotrimeric G protein. This, in turn, results in dissociation of the trimer into the α and βγ subunits, each of which, in different contexts, is able to activate or inhibit diverse downstream effectors. Non regulatory G protein subunits are present in mammalian cells at high concentration relative to the α subunits, and the functional significance of such sequestration remains uncertain. However, in one of the best-studied cases, that of the heterodimeric GABAr receptor, dimerization appears to be important both for correct trafficking of receptors to the cell surface and for signaling function (6, 32, 42).

Considering the diversity of GPCRs in eukaryotic genomes, there are likely to be important physiological consequences of homo- and hetero-oligomerization of receptors. Signaling pathways may be activated or modulated by the vast number of potential combinations of co-oligomerized receptors. The widespread ability of GPCRs to undergo homo-oligomerization raises the possibility that such associations play important roles in modulating receptor action, as well as competitively inhibiting hetero-oligomerization (21, 36, 41, 58).

The yeast pheromone response is a GPCR-mediated signaling pathway that has served as a model for understanding many aspects of G protein-mediated signaling. Haploid yeast cells of the MATa and MATα mating types secrete the peptide pheromones α-factor and α-factor that bind to receptors Ste2p and Ste3p, respectively, on the opposite cell type, activating a signaling pathway that leads to cell cycle arrest, changes in cell physiology, and altered transcription of a number of genes (12, 26, 47, 51). Pheromone receptors, like all other known GPCRs, are membrane proteins containing seven transmembrane segments. Upon binding ligand, they activate trimeric G proteins exhibiting a high degree of sequence similarity to mammalian G proteins. While the pheromone receptors themselves exhibit no significant sequence similarity to mammalian GPCRs, they are functionally interchangeable with mammalian GPCRs, which, when expressed in yeast, can activate the pheromone response upon binding their cognate ligands (5, 37).

Two types of functional interactions between different coexpressed forms of the α-factor receptor Ste2p have been described. One of these is the dominant-negative behavior of certain mutant receptors that inhibit signaling by coexpressed normal receptors (13, 28). The other is the dominant action of normal receptors in suppressing constitutively active and hypersensitive signaling by coexpressed mutant receptors (14, 23, 43, 50). However, the basis for these interactions between receptors remains unclear.

Two general types of mechanisms for the functional interactions between different forms of the α-factor receptor have been proposed. The first of these is based on sequestration of limiting amounts of a signaling pathway component by the less-active receptor. If receptors compete for access to limiting amounts of a downstream factor, such as the G protein trimer, formation of stable complexes between nonfunctional receptors and G proteins could prevent signaling by coexpressed normal receptors (13, 14, 28). This mechanism would require that receptors form a stable complex with the G protein, even in the absence of receptor activation. The existence of such complexes involving mammalian GPCRs has been reported (16, 38). Evidence for competition
among α-factor receptors for access to G proteins has been provided by reports that functional interactions between receptors can be diminished by overexpressing G protein subunits (13, 14, 28). However, interpretation of these results is complicated by the fact that the loss of dominant effects upon overexpression of G protein was not complete (28) and by the concerns that any imbalance in the expression levels of the three separately encoded G protein subunits can affect activation of the pheromone response pathway (4, 11). The second type of explanation for functional interactions between receptors is based on the extensive evidence for physical interactions between co-oligomerized receptors (10, 17, 39, 55). If signaling requires functional interactions between receptors within an oligomer, co-oligomerization of a normal receptor with a defective mutant receptor could be sufficient to inhibit signaling by the normal receptor and co-oligomerization of a normal receptor with constitutive or hypersensitive mutants could suppress abnormal responses by the mutant receptors. Alternatively, co-oligomerization of mutant and normal receptors could inhibit signaling by causing mislocalization of the normal receptors in cells. Coexpression of different receptors can result in alterations of the patterns of endocytosis compared with the behaviors of the individually expressed forms (55). However, dominant-negative effects of mutant α-factor receptors have been observed even in cases where normal-mutant hetero-oligomers appear to be expressed at the cell surface in quantities sufficient for ligand-dependent activation of signaling (13, 17).

We report here the results of experiments designed to investigate the basis for functional interactions among yeast α-factor receptors. If the dominant-negative effects arise from competition between receptors for limiting amounts of G protein, the dominant behavior should be diminished either by reduction of the stoichiometric ratio of receptors to G proteins or by covalent tethering of G protein to receptors for limiting amounts of G protein, the dominant-negative effects of mutant receptors in cells. Coexpression of different receptors can result in limiting amounts of G protein, the dominant-negative effects of mutant receptors within an oligomer, co-oligomerization of a normal receptor with constitutive or hypersensitive mutants could suppress abnormal responses by the mutant receptors. Alternatively, co-oligomerization of mutant and normal receptors could inhibit signaling by causing mislocalization of the normal receptors in cells. Coexpression of different receptors can result in alterations of the patterns of endocytosis compared with the behaviors of the individually expressed forms (55). However, dominant-negative effects of mutant α-factor receptors have been observed even in cases where normal-mutant hetero-oligomers appear to be expressed at the cell surface in quantities sufficient for ligand-dependent activation of signaling (13, 17).

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Materials and Methods

Plasmids and yeast strains. Tables 1 and 2 describe the plasmids and strains used in this study. All transformations were performed using the one-step lithium acetate procedure (8). Strains requiring the transformation of two separate plasmids were sequentially transformed to minimize the chances of homologous recombination between plasmids. Oligonucleotide “ON” numbers refer to sequences that are available from us upon request.

The original Saccharomyces cerevisiae host strain from which all the additional strains used in this paper were derived was A230 (MATα arg1− ade2−1 his4−580 lys2−1 trp1−1 tyr1−1 SUP4−3 leu2 ura3 barl−1) carrying a mutation of the BAR1 gene that encodes a protease for α-factor. Strain A232 (28), which has the same genetic background as A230 but also carries a chromosomal deletion of the α-factor receptor gene (STE2), was used for various assays and for determination of Bioluminescence Resonance Energy Transfer (BRET) between tagged receptors. The host strain used to assess constitutive signaling was A529 (STE2+) or A575 (ste2−Δ) containing a deletion of the FAR1 gene (50). For fluorescence microscopy, strains A3087 (STE2+) and A3102 (ste2−Δ) containing a deletion of the PEP4 gene encoding protease A were used as host strains to minimize proteolysis of receptor-containing fusion proteins as described previously (17, 29).

Strain A530 containing a C-terminally truncated allele of STE2 integrated into the genome was created as follows. Plasmid pMD149 (28) was subjected to site-directed mutagenesis (25) using oligonucleotide ON141 to create pMD279 containing a TGA codon in place of the original lysine 304. Following this, pMD279 was digested with SpI and EcoRI and the STE2-containing fragment was subcloned into pMD297 (50) to create pMD299. Next, pMD299 was digested by BspEl and EcoRI and ligated into the similarly digested integrating plasmid pMD364 (50) to create pMD382. pMD382 was integrated into A268 (28) as previously described (50) to create strain A530. To integrate a genomic copy of the C-terminally truncated Y266C-encoding allele, pMD294 (28) was digested with KpnI and Hpal and subcloned into pMD382 to create pMD1481. Following that, pMD1481 was integrated into A268 in the same manner as pMD382 to create A3499. To integrate C-terminally truncated Ste2p containing the E204S mutation, pMD1205 (17) was digested with KpnI and Hpal and then inserted into pMD1481 for integration into the A268 host in the same manner as pMD1481 to create A3507.

Yeast strains containing substitutions of various full-length mutant alleles of STE2 for the genomic copy of this gene were derived from strain A171 (28). Dominant-negative alleles Y266C (pMD294) and N205K (pMD328) (28), C-terminally tagged with 3 copies of the hemagglutinin (HA) epitope, were digested with BspEl and SacI and subcloned into pMD364 (50) to generate pMD277 and pMD278, respectively. Following that, pMD277 and pMD278 were digested with PmlI and transformed into A171, followed by “popping out” of the URA3 marker as previously described (28) to generate A1605 and A1606, respectively, containing the dominant-negative alleles at the chromosomal STE2 locus. Where relevant, untagged STE2 was introduced into the chromosomal locus by digesting pMD310 (50) with NotI to excise the HA epitope and then religating to yield pMD1622. The construct was transformed into A171, following digestion with PmlI, and popped out as described above to create A3808.

To create a construct allowing integration of the STE2-HA fusion construct into the STE2 locus, we first constructed plasmid pMD435, in which a BsrGI site was deleted from the C′ end of the STE2 reading frame and reintroduced after the epitope tag at the C′ terminus. This was accomplished by site-directed mutagenesis of pMD310 (50) with ON207 and ON208. The GPA1 gene was then excised from the pMD288 GPA1-encoding plasmid (28) with BsrGI and SmaI and inserted into BsrGI-digested pMD435 (filling in the BsrGI overhang at the C′ end of the fusion) to generate pMD445. The resulting Ste2p-Gpa1p fusion construct, pMD445, was subjected to site-directed mutagenesis with oligonucleotide ON500 to remove a region from the C-terminal tail of Ste2p from alanine 369 to the start of Gpa1p that has been reported to adversely affect the fusion protein’s ability to signal (34), creating plasmid pMD1052. pMD1052 was digested and transformed into A171 as described previously (28) to generate A2517 containing an integrated copy of the fusion protein. To integrate the constitutive P258L allele of STE2 into the genome, pMD251 (50) was digested with SpI and EcoRI and cloned into pMD297 (50) to create pMD301. Following that, pMD301 was digested with BspEl and EcoRI and subcloned into pMD364 to create pMD384. Last, pMD384 was digested with PmlI, integrated into A268, and popped out to create A532.

To achieve low-level expression of Ste2p, the STE2 gene was inserted downstream of the GAL1 promoter. First, the STE2 gene (tagged at its C-terminal end with 3 tandem repeats of the HA epitope) from plasmid pMD149 (28) was PCR amplified using oligonucleotides ON91 and ON92.
ON321 to introduce SplI and HindIII sites 5' to the start codon. This PCR product was digested with SplI and Hpal and ligated back into pMD149 to generate pMD726. This plasmid was then digested with HindIII and ligated into HindIII-digested pYES2 (Invitrogen) to generate pMD730. To create C-terminally truncated forms of the mutant Y266C STE2 allele tagged with the GFP variant of green fluorescent protein (GFP) used for BRET (3), pMD294 (28) was digested with SplI and Asp718 and ligated into similarly digested pMD1154 (17), generating pMD1173. The

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**TABLE 1 Yeast strains with plasmids**

| Strain | Plasmid(s) | Host background | Chromosomal allele | Plasmid description |
|--------|------------|----------------|--------------------|---------------------|
| A433   | pMD228     | A232           | STE2Δ              | Empty vector        |
| A454   | pMD228     | A232           | STE2Δ              | Empty vector        |
| A478   | pMD294     | A232           | STE2Δ              | Multicopy STE2 (Y266C) |
| A860   | pMD251     | A232           | STE2Δ              | Multicopy STE2 (F204S) |
| A462   | pMD251     | A232           | STE2Δ              | Multicopy STE2 (F204S) |
| A1690  | pMD730 + pMD284 | A1606    | STE2-Δ            | Multicopy GALI1-STE2 |
| A1694  | pMD730 + pMD284 | A1606    | STE2-Δ            | Multicopy GALI1-STE2 |
| A1717  | pMD730 + pMD284 | A230      | STE2-Δ            | Multicopy GALI1-STE2 |
| A1718  | pMD730 + pMD284 | A232      | STE2-Δ            | Multicopy GALI1-STE2 |
| A2823  | pMD294     | A2517         | STE2-GPA1         | Multicopy STE2 (Y266C) |
| A2824  | pMD328     | A2517         | STE2-GPA1         | Multicopy STE2 (N205K) |
| A2825  | pMD545     | A2517         | STE2-GPA1         | Multicopy STE2 (S184R) |
| A2826  | pMD240     | A2517         | STE2-GPA1         | Multicopy STE2Δ |
| A2827  | pMD559     | A2517         | STE2-GPA1         | Multicopy STE2Δ |
| A2823  | pMD294     | A2517         | STE2-GPA1         | Multicopy STE2 (Y266C) |
| A2958  | pMD1173    | A230          | STE2Δ              | Multicopy STE2 (Δ304–431) (Y266C)-GFP2 |
| A2960  | pMD1244    | A230          | STE2Δ              | Multicopy STE2 (Δ304–431) (Y266C)-GFP2 |
| A3330  | pMD1419    | A230          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3332  | pMD1154    | A230          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3334  | pMD1173    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) (Y266C)-GFP2 |
| A3335  | pMD1244    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) (Y266C)-GFP2 |
| A3336  | pMD1419    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3337  | pMD1141    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3338  | pMD1154    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3339  | pMD1167    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3665  | pMD1422    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3497  | pMD1479    | A259          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3498  | pMD1479    | A575          | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A3501  | pMD730     | A232          | STE2Δ              | Multicopy GALI1-STE2 |
| A3502  | pMD730     | A530          | STE2 (Δ304–431)    | Multicopy GALI1-STE2 |
| A3503  | pMD730     | A3499         | STE2 (Δ304–431)    | Multicopy GALI1-STE2 |
| A3504  | pMD228     | A3499         | STE2 (Δ304–431)    | Multicopy GALI1-STE2 |
| A3517  | pMD730     | A3507         | STE2 (Δ304–431)    | Empty vector         |
| A3518  | pMD228     | A3507         | STE2 (Δ304–431)    | Empty vector         |
| A3568  | pMD1422    | A532          | STE2 (P258L)       | Multicopy STE2 (Δ304–431) |
| A3613  | pMD228     | A532          | STE2 (P258L)       | Multicopy STE2 (Δ304–431) |
| A3614  | pMD240     | A532          | STE2 (P258L)       | Multicopy STE2 (Δ304–431) |
| A3622  | pMD1422    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) |
| A3622  | pMD1422    | A232          | STE2Δ              | Multicopy STE2 (Δ304–431) |
| A3809  | pMD228     | A3808         | UnTagged STE2Δ     | Empty vector         |
| A3810  | pMD739     | A3808         | UnTagged STE2Δ     | Empty vector         |
| A3811  | pMD149     | A3808         | UnTagged STE2Δ     | Empty vector         |
| A3812  | pMD240     | A3808         | UnTagged STE2Δ     | Empty vector         |
| A4186  | pMD1154    | A3087         | UnTagged STE2Δ     | Empty vector         |
| A4187  | pMD1167    | A3087         | UnTagged STE2Δ     | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4188  | pMD1173    | A3087         | UnTagged STE2Δ     | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4189  | pMD1244    | A3087         | UnTagged STE2Δ     | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4190  | pMD1414    | A3087         | UnTagged STE2Δ     | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4191  | pMD1414    | A3087         | UnTagged STE2Δ     | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4192  | pMD1154    | A3102         | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4193  | pMD1167    | A3102         | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4194  | pMD1173    | A3102         | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4195  | pMD1244    | A3102         | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4196  | pMD1414    | A3102         | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |
| A4197  | pMD1419    | A3102         | STE2Δ              | Multicopy STE2 (Δ304–431) (F204S)-GFP2 |

*Unless otherwise indicated, all alleles of STE2 contain a C-terminal HA epitope.*

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GFP$^2$-tagged truncated form of the F204S mutant receptor was con-structed by digesting pMD1205 (17) with SplI and KpnI and ligating the STE2-containing fragment into pMD1173 to create pMD1419. BRET protein fusions to other receptor alleles and to the PMA1 gene used in these experiments were created as previously described (17). The con-struction of other plasmids containing dominant-negative receptor alleles (N205K, S184R) has been described previously (28). To create the multi-copy plasmid pMD1422 expressing C-terminally truncated Ste2p, a KpnI-Sacl fragment from pMD626 (1) containing the truncated receptor was first ligated into KpnI-Sacl-cut vector pMD240 (28), creating plasmid pMD803. The vector pMD228 (28) was then modified by site-directed mutagenesis with oligonucleotides ON622 and ON623 to remove XbaI and Hpal sites, yielding plasmid pMD1383. Finally, a Sacl-SplI fragment containing the truncated STE2 from pMD803 was ligated into Sacl- and SplI-cut pMD1383 to create pMD1422.

**Bioluminescence resonance energy transfer**. A membrane fraction for BRET measurements was prepared using a protocol originally de-scribed by Lin and coworkers (30), modified as previously described (17). Energy transfer measurements were performed using the coelenterazine derivative DeepBlueC (DBC) (Perkin Elmer) as a substrate for the Renilla luciferase, with a Quantamaster spectrofluorometer (Photon Technology International), applying corrections based on signal in the absence of substrate and on strains expressing luciferase alone as described previously (17).

**Liquid assays of FUS1-lacZ induction**. β-Galactosidase assays of the FUS1-lacZ induction were performed essentially as described previously (7, 44) using the fluorescent β-galactosidase substrate fluorescent di-(β-d-galactopyranoside) (FDG). Cells were incubated for 105 min with 0.1% Tween 200 with α-factor. Error bars indicate the standard errors of the means for three independent isolates of the same strain. Because of significant day-to-day variation in the absolute values of the measured β-galactosidase activity, all comparisons between different strains displayed in the figures show assays performed in parallel within the same experiment.

**Immunodetection of receptors**. For determination of cellular receptor expression, relevant strains were cultured to an optical density at 600 nm (OD$_{600}$) of 1 in the appropriate media. A total of 4 × 10$^7$ cells was harvested by centrifugation at 13,000 × g for 5 min, resuspended in 25 mM Tris-HCl (pH 8.0), and recentrifuged. Pelleted cells were resuspended to 2 × 10$^8$ cells/ml in 40 mM Tris-HCl (pH 6.8)–0.1 mM EDTA–5% SDS–9 M urea–5% β-mercaptoethanol–200 μM Pefabloc (Roche). Approximately 0.15 g of 0.5-mm-diameter zirconia/silica beads was added, and the suspensions were subjected to a vortex procedure at 4°C for 5 min. Samples were then centrifuged for 10 min at 13,000 × g, and the supernatant was incubated at 37°C for 5 min. A 5-μl volume (Fig. 1 and lanes 1 to 9 of Fig. 2) or 2.5-μl volume (lanes 10 to 12 of Fig. 2) of supernatant was loaded onto 12% SDS–polyacrylamide gels. Protein was transferred to nitrocellulose filters overnight. The filters were then blocked with phosphate-buffered saline (PBS) supplemented with 0.1% Tween and 5% newborn calf serum for 2 h. Following two washes with PBS and 0.1% Tween, filters were incubated with 1:10,000 mouse-anti-GFP antibodies (Roche), 1:10,000 rat-anti-HA antibodies (Roche), or 1:100,000 rabbit-anti-endo-lase antibodies (a generous gift of Michael Hol-land of the University of California, Davis) in PBS–5% serum for 60 min. The filters were then washed five times with PBS containing 0.1% Tween and 5% serum, incubated for 60 min with 1:10,000 horseradish peroxi-dase-conjugated goat anti-mouse or anti-rat antibodies (Bio-Rad Laborato ries) in PBS-Tween-serum, and then washed four times with PBS containing 0.1% Tween. SuperSignal West Dura Extended Duration sub-strate (Thermo Scientific) was used for chemiluminescent detection of receptor.

**Fluorescence microscopy**. Yeast strains were to be examined were cultured to an OD$_{600}$ of approximately 1 in synthetic defined medium with uracil dropout (SD-ura medium). Cell suspensions were examined using a Nikon Diaphot 200 inverted microscope equipped with a charge-coupled-device (CCD) camera (Princeton Instruments), a 100× objective with a 1.4 numerical aperture (NA), and standard fluorescein isothio-cyanate (FITC) filters. Images were analyzed using the program Metamorph from Universal Imaging.

**RESULTS**

**Effects of altering receptor-G protein stoichiometry on functional interactions between receptors**. Certain forms of α-factor receptors that contain loss-of-function mutations exert domin-ant-negative effects on signaling by coexpressed normal recep-tors (13, 23, 28, 43, 50). The particular receptor alleles exhibiting these effects are diverse; they are readily isolated in genetic screens of randomly mutated receptors (13, 28), and a large fraction of

### Table 2 Yeast strains used for BRET assays

| Strain    | Plasmids$^a$ | Host | β-luc-tagged protein | GFP$^2$-tagged protein | Chromosomal allele |
|-----------|--------------|------|----------------------|------------------------|-------------------|
| A2753     | pMD1155 + pMD1154 | A232 | Ste2p (Δ304–431)     | Ste2p (Δ304–431)       | ste2Δ             |
| A2756     | pMD1168$^a$ + pMD1167 | A232 | Ste2p                | Ste2p                  | ste2Δ             |
| A2789     | pMD1168$^a$ + pMD1154 | A232 | Ste2p                | Ste2p (Δ304–431)       | ste2Δ             |
| A2935     | pMD1199 + pMD1154 | A232 | Ste2p (Δ304–431) (CEN) | Ste2p (Δ304–431)       | ste2Δ             |
| A2940     | pMD1232 + pMD1173 | A232 | Ste2p (CEN)          | Ste2p (Δ304–431) (Y266C) | ste2Δ             |
| A2949     | pMD1232 + pMD1167 $^a$ | A232 | Ste2p (CEN)          | Ste2p                   | ste2Δ             |
| A3347     | pMD1232 + pMD1154 | A232 | Ste2p (CEN)          | Ste2p (Δ304–431)       | ste2Δ             |
| A3510     | pMD1232 + pMD1419 | A232 | Ste2p (CEN)          | Ste2p (Δ304–431) (F204S) | ste2Δ             |

$^a$ Unless otherwise noted, all proteins were expressed from multicopy plasmids.

$^b$ Plasmids pMD1167 and pMD1168 encode STE2–β-luc and STE2–GFP$^2$ fusions that do not contain an HA epitope.

**FIG 1** Cellular level of expression of chromosomally encoded STE2 alleles. Immunoblotting (IB) of whole-cell lysates using antibodies directed against the HA epitope was performed as described in Materials and Methods. Cells were cultured in glucose-containing medium to repress expression from the GALI promoter. Lane 1, GALI–Ste2p expressed in ste2Δ host strain (strain A1718); lane 2, empty vector expressed in STE2$^+$ host strain (A433); lane 3, GALI–Ste2p expressed in STE2$^+$ host strain (strain A1717); lane 4, GALI–Ste2p expressed in ste2Δ (Y266C) host strain (strain A1690); lane 5, GALI–Ste2p expressed in ste2Δ (N205K) host strain (strain A1694). All STE2 alleles present in these strains are tagged with 3 tandem repeats of an influenza hemagglutinin (HA) C-terminal epitope tag.
receptors containing loss-of-function mutations exhibit dominant-negative behavior (28). The frequency at which dominant-negative alleles arise, and the diversity of positions in the receptor sequence at which they are found, indicates that they share an origin in causing a loss-of-receptor function. The affected amino acid residues tend to be located at or near the extracellular surface of the receptor. However, the underlying molecular bases for the losses of function and dominant-negative behaviors of these mutations remain unknown.

Such dominant-negative effects were originally detected using mutant alleles expressed from multicopy plasmids in cells that also contain a chromosomal copy of a normal STE2+ gene (13, 28). Overexpression of G protein subunits in these cells was reported to partially reverse the dominant-negative effects of some of these mutations (13, 28). This led to the suggestion that the dominant effects are mediated by stable association of the mutant receptors with limiting amounts of G protein, restricting access of coexpressed normal receptors to G proteins and downstream elements of the pheromone signaling pathway. (The number of α-factor receptors has been reported to approximately equal the number of trimeric G proteins in cells containing normal chromosomal copies of the genes encoding receptors and G protein subunits [20, 56].) However, oligomerization of α-factor receptors has now been demonstrated based on fluorescence (10, 40) and bioluminescence (17) energy transfer, communoprecipitation of differentially tagged receptors (55), and cotrafficking of different receptor alleles (55), raising the possibility that functional interactions might, instead, be mediated by biochemical interactions among co-oligomerized receptors. Interpretation of experiments involving overexpression of G protein subunits is complicated by the fact that subtle differences in the relative amounts of the negative regulatory G protein α subunits with respect to positive regulatory β and γ subunits can lead to either increased or decreased responses to α-factor (4, 11). Furthermore, the reported suppression of the dominant-negative effects by G protein overexpression was partial and was observed only with certain dominant alleles (13, 28). This could indicate either that G protein titration is not the primary mechanism of action of dominant-negative receptors or that the G protein subunits could not be overexpressed at levels that are high enough to saturate all the dominant-negative receptors. In the latter case, further increases in the ratio of G proteins to receptors would be expected to lead to more complete suppression of the dominant-negative phenotype. However, it would be difficult to achieve such high levels without altering the balance of subunit biosynthesis or inducing secondary effects on the overall responses of the pheromone pathway arising from the multiplicity of roles of subunits (35, 48, 57).

To explore a wider range of ratios of G protein to receptor than had been examined previously without altering the balance of G protein subunit levels, we tested for dominant receptor effects in cells that contain severely diminished levels of receptors but express G protein subunits from their normal chromosomal loci. Reduction of cellular levels of normal α-factor receptors was accomplished by placing the STE2+ gene under the control of the GAL1 promoter and repressing expression by growing cells in the presence of galactose and the absence of glucose in the culture medium. Under these conditions, cellular levels of Ste2p were less than 20% of the levels in cells that express the receptor from the normal chromosomal STE2 locus (Fig. 1; compare lane 1 with lane 2). The response of cells to α-factor is relatively insensitive to the overall level of Ste2p expression—reduction of levels of receptor to 5% of normal has previously been reported to have little effect on the pheromone response (46). In accordance with this, we found that, under conditions in which the GAL1 promoter is repressed, cells expressing the GAL1-controlled wild-type STE2 gene as their only receptors exhibit levels of α-factor-dependent induction of the FUS1-lacZ reporter that are nearly identical to those of cells expressing receptors from the normal chromosomal STE2 locus (Fig. 3A). Furthermore, these types of manipulations of receptor expression did not affect the abundance of the Gα subunit Gpa1p, based on immunoblotting of whole-cell extracts (Fig. 2).

Reduction of the levels of dominant-negative receptors (compared to the results of previous studies in which dominant-negative alleles were expressed from multicopy plasmids) was accomplished by integrating single copies of mutant ste2 alleles into the chromosomal STE2 locus in place of the normal STE2 gene. Overall cellular levels of Ste2p receptors expressed from single-copy genes are at least 5-fold lower than those of receptors expressed from multicopy plasmids in these strains (see Fig. 2) (1, 17, 28) but remain in excess of those of GAL1-controlled receptors (Fig. 1). Two previously characterized mutants forms of Ste2p containing...
normal receptors, since cells exhibited ample capacity to increase expression of plasmid-encoded receptors in the presence of a co-expressed chromosomal STE2\(^+\) allele (Fig. 2) and since, as we have previously reported, the presence of high levels of dominant-negative receptors in cells does not prevent cell surface expression of normal receptors that are competent to bind ligand (17). In that previous study, cells coexpressing dominant-negative alleles and normal chromosomal STE2 exhibited levels of \(\alpha\)-factor binding that were similar to the levels observed for normal chromosomally encoded STE2 expressed alone whereas there was no detectable binding of \(\alpha\)-factor to similar strains in which the dominant receptors were expressed alone.

**Effects of dominant-negative receptors on receptor-G protein fusions.** To determine the extent to which functional interactions between receptors might reflect competition for access to G proteins, we tested the effects of providing preferential access of recessive receptors to G proteins by covalently fusing them to G protein subunits. Thus, we examined the effects of dominant-negative mutant receptors on coexpressed fusion proteins in which normal receptors are covalently linked to Gpa1p, the G protein \(\alpha\) subunit. The dominant-negative alleles tested included those containing the substitutions N205K and Y266C (see above) as well as S184R, which was originally identified as a loss-of-function mutation (49) that was then found to have dominant-negative effects (28).

To test the effect of fusing Gpa1p to recessive mutant receptors, it was necessary to develop a yeast strain in which the only copy of GPA1 is the one fused to the receptor. In creating such a strain, we avoided the creation of intermediate strains that completely lacked GPA1, as we have previously found it difficult to maintain stable signaling-competent strains following deletion of the endogenous GPA1 gene (28, 44). Thus, to create the strain containing STE2-GPA1 as the only copy of GPA1, we first recreated an exact copy of a previously reported functional STE2-GPA1 fusion construct in which the last 62 amino acids of the carboxyl-terminal tail of the receptor were deleted (34). This fusion construct was integrated into the chromosome at the STE2 locus before deletion of the endogenous GPA1 gene. The strain created in this way exhibits a hypersensitive \(\text{FUS1-lacZ}\) response to \(\alpha\)-factor typical of that seen for unfused receptors with truncations of the C-terminal tail (Fig. 4) (23, 43).

Expression of dominant-negative receptors containing the substitutions Y266C, N205K, and S184R from multicopy plasmids in a host containing a chromosomal STE2-GPA1 as its only copy of GPA1 resulted in significant dominant-negative effects on signaling (Fig. 4), just as coexpression with the same mutant receptors inhibited signaling by normal unfused Ste2p. Furthermore, coexpression of normal receptors with the hypersensitive STE2-GPA1 fusion construct suppressed the hypersensitivity of the fusion, much as the hypersensitivity of unfused truncated receptors is suppressed by coexpression with normal receptors (23, 43). Thus, providing preferential access of active receptors to G protein by covalently fusing one to the other does not confer the protection from inhibitory effects of less-active receptors that would be expected if these inhibitory effects resulted from sequestration of G proteins by the less-active receptors.

**Interactions between full-length and C-terminally truncated receptors.** Truncation of the C-terminal tail of mutant \(\alpha\)-factor receptors has been reported to eliminate dominant-negative effects of receptor mutations based on long-term halo assays of

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**FIG 3** Dominant-negative effects of mutant alleles of STE2 are maintained at low levels of receptor expression. (A) Assays of \(\text{FUS1-lacZ}\) induction in strains that all express a wild-type Ste2p receptor under the control of a \(\text{GAL1}\) promoter. Cells were maintained under repressing conditions in SD medium containing 2% glucose. Results are shown for strains that coexpress wild-type Ste2p, the dominant-negative allele Ste2p (Y266C) or Ste2p (N205K), or no receptor from the chromosomal locus (strains A1717, A1690, A1694, or A1718, respectively). (B) Assays of \(\text{FUS1-lacZ}\) induction in strains that express STE2 from a normal chromosomal locus or contain a deletion of the normal locus. Pheromone responses are shown for a strain containing a vector with no insert in a host strain containing a chromosomal \(\text{ste2}\)-\(\Delta\) deletion (A434), for a strain containing a vector with no insert in a \(\text{STE2}\) host (A433), and for a strain expressing the dominant-negative allele Y266C from a multicopy plasmid in a \(\text{STE2}\)-\(\Delta\) host (A478).
growth arrest (14). The results presented in Fig. 5 confirm that a similar loss of dominant behavior upon C-terminal truncation of receptors was observed in short-term assays of FUS1-lacZ induction in response to \( \alpha \)-factor. These experiments focused on dominant-negative receptor alleles containing the Y266C and F204S substitutions, since these are the alleles for which the effects of C-terminal truncation have been most extensively studied (14). Overexpression of truncated receptors containing the Y266C or F204S substitutions resulted in little or no inhibition of the signaling of a coexpressed chromosomally encoded full-length normal receptor (Fig. 5), whereas similar expression of full-length receptors containing these same substitutions caused significant inhibition of the coexpressed normal receptor (14, 28) (Fig. 3B).

The lack of dominant effects of truncated receptors was previously interpreted within the paradigm of the G protein titration model as evidence for a role of the C terminus of the dominant receptors in maintaining a stable interaction with the G protein, leading to sequestration of the G protein from coexpressed normal receptors (14). However, if dominant-negative effects of receptors result from physical interactions between oligomerized receptors, rather than from competition for access to G proteins, an alternative explanation of the effects of C-terminal truncation is required.

One possible alternative explanation for the effects of truncation on dominant-negative mutants could have been that removal of the C-terminal tail reduces the tendency of these receptors to co-oligomerize with normal full-length receptors. However, we used Bioluminescence Resonance Energy Transfer (BRET) to demonstrate association between truncated and full-length receptors. BRET allows detection of close proximity between receptors with lower backgrounds and smaller corrections for channel cross-talk than the related approach of fluorescence resonance energy transfer (17). Figure 6 shows BRET transfer between Renilla luciferase-tagged full-length receptors and truncated receptors tagged with the GFP\(^2\) variant of green fluorescent protein (3).
full-length receptors and the high efficiency observed for transfer between truncated receptors (Fig. 6). This is consistent with a previous proposal (17) that the increased efficiency seen for truncated receptors arises from decreased spacing between tags attached directly to transmembrane regions of truncated receptors compared with tags attached via the 130-amino-acid C-terminal tails. Figure 6 also shows that dominant-negative mutations in truncated receptors do not significantly reduce their abilities to co-oligomerize with full-length normal receptors. We had previously reported, based on BRET measurements, that co-oligomerization between full-length normal and full-length dominant-negative receptors occurs just as efficiently as the oligomerization of each species with itself (17). The data in Fig. 6 indicate that there is also efficient co-oligomerization of full-length normal receptors with C-terminally truncated dominant-negative receptors. These measurements were performed using strains expressing the normal full-length receptors from single-copy plasmids and the mutant truncated receptors from multicopy plasmids, which approximates the expression levels of the different receptor forms that result in dominant-negative effects on the pheromone response (13, 28).

Comparison of the pheromone responses of cells expressing full-length and truncated mutant receptors in the absence of any coexpressed normal receptors (Fig. 7) suggests that one reason for the lack of dominant effects of the truncated mutant receptors is that their signaling is simply not sufficiently impaired to provide significant dominant-negative behavior. Dominant-negative effects of a mutation are generally no stronger than the defects observed for the mutant expressed alone. This places a lower limit on the strength of the defect that can be expected to result in dominant-negative behavior.

The signaling competence of the truncated mutant receptors is particularly apparent at high concentrations of α-factor. Thus, in the presence of 10 μM α-factor, Fus1-LacZ induction in response to α-factor in yeast strains expressing A full-length or (B) truncated alleles of STE2-GFP2 fusions from multicopy plasmids. The strains shown in panel A express full-length receptor alleles as follows: A3334 (containing Y266C), A3336 (containing F204S), and A3338 (no substitutions). Strains shown in panel B express truncated receptor alleles as follows: A3335 (containing Y266C), A3337 (containing F204S), and A3339 (no substitutions).
region generally enhances the sensitivity of receptors to pheromone (23, 43).

Since we found that reduction of overall levels of receptor expression while maintaining normal levels of G protein subunits enhances the dominant-negative effects of full-length mutant receptors, we tested whether similar reductions in receptor expression might allow detection of dominant effects of C-terminally truncated mutant receptors. Thus, strains were created in which expression of the full-length STE2+ gene could be repressed under the control of the GAL1 promoter and in which normal and mutant truncated receptors were expressed from single chromosomal copies of the relevant STE2 alleles under the control of the normal STE2 promoter. Assays of FUS1-LacZ induction under conditions in which the GAL1 promoter was repressed showed that C-terminally truncated mutant receptors are, in fact, capable of exerting substantial dominant-negative effects (Fig. 8).

Despite the fact that the hypersensitivity of truncated receptors is generally recessive (23, 43, 50), the assays of FUS1-LacZ induction in response to α-factor (Fig. 8) showed that the hypersensitivity of truncated (but otherwise normal) receptors expressed from the chromosomal STE2 locus is not completely suppressed by coexpression of galactose-repressed normal receptors. Based on fitting of the responses to sigmoidal dose-response curves, the 50% effective concentrations (EC50s) for truncated receptors coexpressed with galactose-repressed full-length receptors remain 2- to 3-fold lower than the EC50s for the full-length receptors expressed alone. This is most likely explained by the very high ratio of truncated receptors to full-length receptors seen when the cells were maintained on glucose. A similar effect of expression levels on the recessive behavior of constitutively active alleles of STE2 has been observed previously (50).

To further examine the ability of truncated receptors to act in a dominant manner, we tested whether truncated (but otherwise normal) receptors would be capable of suppressing constitutive signaling by a coexpressed constitutively active STE2 receptor containing the P258L substitution (Fig. 9). Unexpectedly, coexpression of the truncated receptors with constitutively active receptors, rather than suppressing constitutive signaling, actually enhanced constitutive FUS1-LacZ induction. As observed in previous studies (14, 50), constitutive FUS1-LacZ induction by the constitutive receptor containing the P258L substitution was reduced by coexpression of full-length receptors and cells expressing truncated, but otherwise normal, Ste2p, as the only α-factor receptor exhibited hypersensitivity to α-factor but no constitutive activity.

Subcellular localization of dominant-negative mutant forms of Ste2p. We used epifluorescence microscopy to compare the subcellular distributions of normal and mutant forms of Ste2p fused at their C termini to green fluorescent protein. Microscopy was performed using yeast strains containing a deletion of the PEP4 gene, a genotype that has previously been shown to minimize cleavage of GFP2 from Ste2p-GFP2 fusions (17, 29). Fusion of GFP2 to full-length normal Ste2p receptors in this background results in a predominantly punctate, intracellular distribution of fluorescence that is not useful for assessing the fraction of receptors correctly targeted to the cell surface (Fig. 10g and h) (29). (The normal signaling responses displayed by full-length GFP2-tagged receptors are attributable to a minority population of such receptors at the cell surface, consistent with the fact that a small number of receptors at the cell surface can activate full signaling responses [46].) Fusion of GFP to the C-terminal of tailless receptors leads to a pattern of fluorescence that is localized predominantly to the cell surface, with some staining of internal compartments (10) (see Fig. 10). The distribution of fluorescence of GFP2 fused to mutant tailless receptors containing the F204S substitution was indistinguishable from that for normal receptors, suggesting that these mutant receptors are effectively trafficked to the cell surface and do not act by trapping co-oligomerized normal receptors in intracellular compartments.

**DISCUSSION**

It has previously been proposed that dominant effects of mutant yeast α-factor receptors on signaling by coexpressed normal re-
receptors (13, 14, 28, 50) result from competition between the different types of receptors for interactions with limiting numbers of heterotrimeric G proteins such that the G proteins are sequestered from actively signaling receptors (14, 28). We report here the results of a series of experiments in which functional interactions between receptors were detected that cannot readily be explained by G protein sequestration.

(i) Reduction of the stoichiometric ratio of receptors to G proteins does not result in any diminution of dominant-negative effects of mutant receptors. If competition between receptors for access to G proteins is responsible for functional interactions between receptors, it should be possible to establish conditions where the ratio of G proteins to receptors is high enough that such competition should disappear. At high levels of receptor expression, using dominant-negative alleles expressed from multicopy plasmids, inhibition of normal receptor function by coexpressed dominant-negative alleles is only partial; cells coexpressing dominant-negative and normal receptors exhibit greater responses to α-factor than cells expressing the dominant receptors alone (14, 28). If receptors are competing for access to G proteins, such retention of residual signaling capability means that the endogenous levels of G proteins must be high enough so that not all G proteins are sequestered by the dominant receptors. In this case, increases in the abundance of G proteins that bring the G protein/receptor ratio closer to 1:1 should relieve the dominant effects of mutant receptors, increasing responses to α-factor. Because of the difficulty of increasing G protein expression levels while maintaining the delicate balance between the inhibitory G~i~ subunit and stimulatory G~s~ and G~y~ subunits, we chose the alternative approach of increasing G protein/receptor stoichiometry by drastically reducing the expression of the mutant and normal receptors without changing expression of G protein subunits. We found that cells in which the ratio of G protein to receptor had been increased in this way exhibited stronger dominant effects than cells expressing higher levels of receptor. This is the opposite of what would be expected if receptors were competing for access to G protein or some other cellular factor present at limiting abundance.

(ii) Dominant effects of certain receptor alleles are not prevented by covalent fusion of coexpressed recessive alleles to G protein α subunits. Dominant behavior of mutant receptors due to their abilities to sequester G proteins should be diminished by providing preferential access of recessive receptors to G proteins by covalently tethering them to G~α~ subunits. However, when tested in two different situations, such fusion of the recessive receptors to G~α~ did not prevent dominant inhibition of signaling: (a) dominant-negative effects of unfused mutant receptors were preserved when they were coexpressed with normal receptors fused to the G~α~ subunit Gpa1p in cells with no other source of G~protein~; (b) hypersensitive responses to α-factor by truncated Ste2p fused to Gpa1p were suppressed upon coexpression with normal full-length unfused receptors. Evidence that covalent fusion of receptors to G~α~ provides preferential access for G protein activation is provided by the previous demonstration that G proteins containing a G~α~ subunit fused to signaling-defective mutant receptors are not effectively activated by coexpressed unfused normal α-factor receptors (27).

(iii) C-terminally truncated mutant receptors can exert dominant-negative effects on coexpressed full-length normal receptors. Dominant effects of truncated receptors are not detected at high levels of receptor expression (14) despite confirmation, using BRET, that the relevant truncated and full-length receptors coligomerize (Fig. 6). However, we found that C-terminally truncated receptors can exert strong dominant-negative effects when...
both the dominant and normal receptors are expressed at low levels in cells. The lack of dominant-negative effects of highly expressed truncated receptors may be due to the inherently enhanced sensitivity of C-terminally truncated receptors to \( \alpha \)-factor. Truncated but otherwise normal receptors are hypersensitive to \( \alpha \)-factor. Truncated receptors containing dominant-negative mutations, when expressed alone, retain high efficacy in the presence of sufficiently high agonist concentrations. Co-oligomerization of normal receptors with these relatively insensitive, but activatable, mutant receptors may shift the conformational equilibrium of the mutant receptors toward an active state that is capable of activating G proteins when the co-oligomerized receptors are present at high enough abundance at the cell surface.

Taken together, these results document the existence of functional interactions between coexpressed Ste2p receptors that do not appear to arise through competition for limiting amounts of G protein. This does not rule out the possibility that competition for G proteins may occur under certain conditions, such as in cells expressing very high levels of receptors. The simplest explanation for the functional interactions that do not involve competition for G protein is that they arise from the well-documented physical interactions between \( \alpha \)-factor receptors, including the interactions between normal and dominant-negative variants presented in Fig. 6. Such physical interactions could affect signaling either by a mechanism in which one receptor directly modifies the signaling responses of a co-oligomerized receptor or by a mechanism in which the dominant receptors alter the subcellular targeting of coexpressed active receptors, preventing correct localization to the cell surface. However, the following results indicate that the dominant-negative effects are not readily explained by mistargeting of the recessive receptors.

(i) GFP\(^2\)-tagged C-terminally truncated dominant-negative mutant receptors exhibit subcellular localizations that are indistinguishable from the distributions of GFP\(^2\)-tagged truncated normal receptors, with predominant localizations to the cell surface (Fig. 10). Such truncated forms of mutant receptors are capable of exhibiting strong dominant-negative effects at low expression levels when present in excess over normal receptors. It was not possible to use microscopy to test effects of dominant mutations on localization of full-length receptors because of the low yield of surface-expressed tagged receptors in the relevant host yeast strains.

(ii) Previous analysis of ligand binding by cells coexpressing full-length dominant-negative and normal receptors (and therefore defective in \( \alpha \)-factor responses) revealed the presence of a new class of binding sites at the cell surface that exhibited a binding affinity lower than that of normal receptors expressed alone (17). The abundance of such sites was similar to the abundance of sites observed for normal receptors expressed alone in the same host.

(iii) Mutant forms of receptors that dominantly inhibit signaling have previously been reported to be efficiently and stably expressed at the cell surface (13, 33).

Dominant-negative effects that are not due to competition for a limiting component or to mistargeting of the active allele to an incorrect cellular compartment are likely to be the result of physical interactions between co-oligomerized receptors that affect their abilities to be activated by ligand. For example, co-oligomerized receptors may need to undergo a cooperative conformational transition in order to activate G proteins. Such cooperative behavior would not necessarily require the receptors to exhibit any significant cooperativity in ligand binding—no cooperative interactions affecting the affinities of \( \alpha \)-factor receptors for ligand have so far been reported. Evidence for allosteric interactions between co-oligomerized GPCRs affecting G protein activation has been presented in a variety of contexts (15, 19, 52). However, interactions between co-oligomerized Ste2p receptors differ from those involving the hetero-oligomeric GABA\(_B\) receptor in which one subunit appears to bind ligand and the other interacts with G protein (15), since no complementation was observed upon coexpression of a ligand-binding-defective allele of STE2 with an allele that cannot couple to G protein (10).

The unexpected observation that dominant-negative effects of mutant receptors are not only maintained, but actually enhanced, at low expression levels can be explained in several different ways. (i) The ratio of dominant-negative receptors (expressed from the chromosome) to normal receptors (expressed under conditions of glucose repression) at the cell surface may be greater than is the case when the dominant receptors are expressed from multicopy plasmids and the normal receptors are encoded in the chromosome. (ii) In cells expressing high levels of normal and mutant receptors, the minority population of oligomers containing only normal receptors may be present at high enough levels to explain the observed residual level of signaling. However, in cells expressing lower numbers of total receptors, the population of oligomers composed exclusively of normal receptors may be below the very low threshold for normal signaling (46). (iii) Functional interactions between receptors could be mediated by a stoichiometric interaction between receptors and limiting cellular component that is outcompeted when receptors are expressed at high levels (see reference 45).

In addition to characterizing negative interactions between receptors, we uncovered one case in which coexpression of different forms of Ste2p results in signaling responses that are stronger than those seen with either form expressed alone. Coexpression of constitutively active mutant receptors with C-terminally truncated receptors (that do not, when expressed alone, exhibit significant constitutive activity) leads to enhanced constitutive signaling. This enhancement differs from results reported by Dosil et al. (14), who found that truncated normal receptors failed to suppress constitutive signaling by P258L but did not enhance constitutive signaling by the coexpressed receptor. Positive interactions corresponding to allelic complementation between certain coexpressed defective alleles of the \( \alpha \)-factor receptor have been detected previously (10). The synergistic effects on signaling that we have detected may be a direct result of allosteric interactions between co-oligomerized receptors in which removal of C-terminal negative regulatory sequences from one component of a receptor oligomer promotes conversion of an associated full-length constitutive receptor to an activated state. Alternatively, the synergistic effect could be the result of an oligomerization-dependent increase in the efficiency of targeting of constitutive receptor to the cell surface. Cell surface abundances of constitutively active receptors expressed alone are generally very low (24, 50), which may limit the strength of ligand-independent signaling. On the other hand, C-terminally truncated receptors accumulate at increased abundances at the cell surface, compared to full-length receptors (23, 43). Co-oligomerization of C-terminally truncated and full-length receptors has previously been reported to result in enhanced internalization of the truncated receptors (55). The curr-
rent observations suggest that such co-oligomerization may also act in the opposite direction, increasing the abundance of constitutively active receptors at the cell surface by virtue of co-oligomerization with truncated receptors.

Despite the fact that this report has focused on interactions between normal and mutant receptors, the dominant behaviors we observed appear to stem from normal aspects of receptor function rather than from any mutation-induced gain of a novel function. Dominant mutations comprise a relatively abundant class of alleles (13, 28), suggesting that they arise from loss of a normal receptor function rather than from gain of a new receptor function, which would be expected to be rare. Furthermore, even normal receptors are capable of exhibiting dominant effects toward coexpressed hypersensitive and constitutive mutants. This suggests that functional interactions mediated by homo- and hetero-oligomerization of normal receptors may play important roles in regulation of the diverse G protein-mediated signaling pathways in cells. Such regulation could involve interactions that promote signaling, such as those observed in hetero-oligomerization of GABA<sub>G</sub> receptors (6, 32, 42), or negative interactions in which activation of one receptor may inhibit signaling by a co-oligomerized receptor, as reported instances of homo-oligomerization of muscarinic receptors (9) and dopamine receptors (19). In view of evidence that some purified monomeric GPCRs can activate G proteins (53, 54), interactions between co-oligomerized receptors may be functionally relevant only for receptors that are actually constituents of oligomers in cell membranes, acting to modulate the canonical pathways of G protein activation by GPCRs.

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