

Table 1. Conserved Residues in Ste2

Conserved residues	Mutant substitution	Phenotype	Ref.
Ile53	A, F, L, C	WT	unpub.
Gly60	A, L	Decreased dimerization	(1)
Ala61			
Ile67 (med)	T	CAM	(2)
Phe81 (med)	C	WT	unpub.
Asn84	S,A,Q,C	CAM	(3)
Leu88			
Tyr101 (med)			
Leu138 (med)	R	DN sup	(4)
Glu143 (med)	K, R	LOF	(5)
	A, G	CAM	(6)
	K	DN sup	(4)
	C	WT	(2)
Leu146	R, P	LOF	(5)
	Q	DN sup/sst	(4)
	M	WT	(2)
Gln149	R, P, V, A, T	CAM	(3)
	G, H, N	Sst	(3)
	N, R	DN sup	(4)
	R, K, P	LOF	(5)
	R	CAM	(6)
	H, L	WT	(2)
Ser170	F, C	F=WT; C=LOF	(2)
Ile209 (med)	C	WT	(7)
Ser214	C, L	WT	(2, 7)
Ile215	C, L	WT	(2, 7)
Phe217 (med)	C, L	WT	(2, 7)
Lys225	C, N, E	LOF	(2, 7)
	R	DN sup	(4)
	I	CAM	(2)
Leu226	C, V	WT	(2, 7)
	W	CAM	(6)
many IL3 residues			
Leu247	C	WT	(7)
Ile249	C, M	WT	(2, 7)
	T	Partial LOF	(2)
Gln253	C, L	CAM	(7, 8)
Pro258	L	CAM	(9)
	M,Y,L,I,F, etc	CAM	(10)
Tyr266 (med)	C,D,A	LOF DN	(11, 12)
	F	WT	(2)
Leu287 (med)	F	No preActiv. complex	(13)
	P	Partial LOF	(2)
Leu289	S	CAM, DN sup	(4)
Pro290	C	Partial LOF (trafficking problem)	unpub.

Leu291	C	WT	unpub.
Trp295	C	Partial LOF (trafficking problem)	unpub.
Ala296	T	CAM DN sup	(4)
	P	CAM	(2)

Phenotypes:

WT	wildtype
CAM	constitutively active mutant
LOF	loss of function
Sst	supersensitive
DN sup	suppressor of dominant negative mutation

Table 2. Small Group Conserved Residues in Ste2

Group conserved residue	Mutant substitution	Phenotype	Ref.
Gly56	A, L	Decreased dimerization	(1)
Gly60	A, L	Decreased dimerization	(1)
Ala61			
Ala62			
Ser87	N	CAM	(2)
Thr131	C	WT	(14)
Ser145	P L L, T	LOF Sst/CAM DN sup	(6, 15) (4)
Ser170	F, C	F=WT; C=LOF	(2)
Gly174	C	WT	(14)
Ala185	P C	DN WT	(11) (14)
Ala212	C P, G V	WT CAM CAM DN sup	(7) (2) (4)
Ser214	C, L	WT	(2, 7)
Ser219	C P	WT Antag response	(7) (16)
Cys252 (weak)	L, A G	WT DN sup	(7, 8) (4)
Ser254	F,G,L,W,V,C	CAM	(7, 8)
Ala281	C T	CAM DN sup/CAM	(14) (4)
Ser288	A, P	CAM	(6, 8)
Ser292	A	CAM	(8)
Ser293	A, C	WT	(8), unpub.

Phenotypes: WT wildtype
CAM constitutively active mutant
LOF loss of function
Sst supersensitive
DN sup suppressor of dominant negative mutation

Table 3. Polar Residues in Ste2

Polar	Mutant substitution	Phenotype	Ref.
Gln51	C	WT	(14)
Arg58	G, E	weak LOF	(5)
	D	WT	(5)
Asn84	S,A,Q,C	CAM	(3)
Gln85	A, C, E	WT	(2, 3)
	P	LOF	(17)
His94			
Lys100	C	WT	(14)
Asn132	I, Y	DN	(11)
	T, C	WT	(2, 14)
Gln135	P	DN	(11)
	C	WT	(14)
Glu143	K, R	LOF	(5)
	A, G	CAM	(6)
	K	DN Sup	(4)
	C	WT	(2)
Gln149	R, P, V, A, T	CAM	(3)
	G, H, N	Sst	(3)
	N, R	DN sup	(4)
	R, K, P	LOF	(5)
	R	CAM	(6)
	H, L	WT	(2)
Lys187	C	WT	(14)
Asn216	D	weak CAM	(18)
	D, Y	DN sup	(4)
	C	WT	(7)
Lys225	C, N, E	LOF	(2, 7)
	R	DN sup	(4)
	I	CAM	(2)
Gln253	C, L	CAM	(7, 8)
Asp275	V	DN	(11)
	C	WT	(14)

Phenotypes: WT wildtype
CAM constitutively active mutant
LOF loss of function
Sst supersensitive
DN sup suppressor of dominant negative mutation

REFERENCES

1. Overton, M. C., Chinault, S. L., and Blumer, K. J. (2003) Oligomerization, biogenesis, and signaling is promoted by a glycophorin A-like dimerization motif in transmembrane domain 1 of a yeast G protein-coupled receptor, *J. Biol. Chem.* 278, 49369-49377.
2. Martin, N. P., Celic, A., and Dumont, M. E. (2002) Mutagenic mapping of helical structures in the transmembrane segments of the yeast α -factor receptor, *J. Mol. Biol.* 317, 765-788.
3. Parrish, W., Eilers, M., Ying, W. W., and Konopka, J. B. (2002) The cytoplasmic end of transmembrane domain 3 regulates the activity of the *Saccharomyces cerevisiae* G-protein-coupled α -factor receptor, *Genetics* 160, 429-443.
4. Lin, J. C., Duell, K., Saracino, M., and Konopka, J. B. (2005) Identification of residues that contribute to receptor activation through the analysis of compensatory mutations in the G protein-coupled α -factor receptor, *Biochemistry* 44, 1278-1287.
5. Sommers, C. M., and Dumont, M. E. (1997) Genetic interactions among the transmembrane segments of the G protein coupled receptor encoded by the yeast STE2 gene, *J. Mol. Biol.* 266, 559-575.
6. Sommers, C. M., Martin, N. P., Akal-Strader, A., Becker, J. M., Naider, F., and Dumont, M. E. (2000) A limited spectrum of mutations causes constitutive activation of the yeast α -factor receptor, *Biochemistry* 39, 6898-6909.
7. Dube, P., DeCostanzo, A., and Konopka, J. B. (2000) Interaction between transmembrane domains five and six of the α -factor receptor, *J. Biol. Chem.* 275, 26492-26499.
8. Dube, P., and Konopka, J. B. (1998) Identification of a polar region in transmembrane domain 6 that regulates the function of the G protein-coupled α -factor receptor, *Mol. Cell. Biol.* 18, 7205-7215.

9. Konopka, J. B., Margarit, S. M., and Dube, P. (1996) Mutation of pro-258 in transmembrane domain 6 constitutively activates the G protein-coupled α -factor receptor, *Proc. Natl. Acad. Sci. USA* 93, 6764-6769.
10. Stefan, C. J., Overton, M. C., and Blumer, K. J. (1998) Mechanisms governing the activation and trafficking of yeast G protein-coupled receptors, *Mol. Biol. Cell* 9, 885-899.
11. Dosil, M., Giot, L., Davis, C., and Konopka, J. B. (1998) Dominant-negative mutations in the G-protein-coupled α -factor receptor map to the extracellular ends of the transmembrane segments, *Mol. Cell. Biol.* 18, 5981-5991.
12. Lee, B. K., Lee, Y. H., Hauser, M., Son, C. D., Khare, S., Naider, F., and Becker, J. M. (2002) Tyr266 in the sixth transmembrane domain of the yeast α -factor receptor plays key roles in receptor activation and ligand specificity, *Biochemistry* 41, 13681-13689.
13. Dosil, M., Schandel, K. A., Gupta, E., Jenness, D. D., and Konopka, J. B. (2000) The C terminus of the *Saccharomyces cerevisiae* α -factor receptor contributes to the formation of preactivation complexes with its cognate G protein, *Mol. Cell. Biol.* 20, 5321-5329.
14. Lin, J. C., Duell, K., and Konopka, J. B. (2004) A microdomain formed by the extracellular ends of the transmembrane domains promotes activation of the G protein-coupled α -factor receptor, *Mol. Cell. Biol.* 24, 2041-2051.
15. Marsh, L. (1992) Substitutions in the hydrophobic core of the α -factor receptor of *Saccharomyces cerevisiae* permit response to *Saccharomyces kluyveri* α -factor and to antagonist, *Mol. Cell. Biol.* 12, 3959-3966.
16. Abel, M. G., Lee, B. K., Naider, F., and Becker, J. M. (1998) Mutations affecting ligand specificity of the G-protein-coupled receptor for the *Saccharomyces cerevisiae* tridecapeptide pheromone, *Biochim. Biophys. Acta-Mol. Cell Res.* 1448, 12-26.
17. Leavitt, L. M., Macaluso, C. R., Kim, K. S., Martin, N. P., and Dumont, M. E. (1999) Dominant negative mutations in the α -factor receptor, a G protein-coupled receptor encoded by the STE2 gene of the yeast *Saccharomyces cerevisiae*, *Mol. Gen. Genet.* 261, 917-932.

18. Parrish, W. R. (2001) The cytoplasmic end of transmembrane domain three regulates the activity of the G protein-coupled α -factor receptor, Ph.D. Thesis, Department of Molecular Microbiology, State University of New York at Stony Brook, Stony Brook, NY.