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Flocculation of *Saccharomyces cerevisiae tup1* Mutants

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Strains of *Saccharomyces cerevisiae* carrying a mutation in the *TUP1* locus exhibited calcium-dependent flocculation. The flocculation had none of the characteristics of sexual agglutination. The flocculation differed from that exhibited by a *FLO1* strain in the effect of pH on cation dependence and sensitivity to chemical inactivation.

Flocculation, a Ca^{2+}-dependent aggregation of yeast cells, is under complex control of a variety of physiological and genetic factors (1, 3, 5, 7, 9). Recently, Miki et al. (4, 5) studied flocculation in genetically defined strains of *Saccharomyces cerevisiae*. They reported that flocculation is a result of activity at *FLO1*, a centromere-linked locus on chromosome I (3). The strain carrying the dominant allele flocculates in defined medium. The recessive allele, *flo1*, does not confer flocculence. Inhibition of flocculation by succinylated concanavalin A or by high concentrations of mannose implies that mannosyl residues are involved in the interaction (4).

Mutations in the *TUP1* locus (also called *FLK1*, *CYC9*, and *UMR7*) on chromosome III affect catabolite repression, mating, and expression of cytochrome *c* isozyme 2, as well as cause cells to clump strongly (2, 6, 8). In the α mating type, *tup1* alleles cause expression of certain **a** cell-specific functions (2). Therefore, we wanted to determine whether clumping of *tup1* strains was due to simultaneous expression of sexual agglutinins of both mating types. This paper reports that the sexual agglutinability of *tup1* cells appears to be similar to that of other haploids. That is, under normal growth conditions, each mating type expressed only the appropriate sexual agglutinin. On the other hand the self-association reaction had the characteristics of a calcium-dependent flocculation reaction similar to that observed in a *FLO1* strain.

Strains XY505-18C (*MATα TUP1 his5 lys1 ura4 gal1 gal2*), XY507-7A (*MAT***a** *tup1-16 his5 lys1 ura4 gal1 gal2*), and XF160-7B (*MATα tup1-16 his3 leu2*) were obtained from Vivian Mackay, Waksman Institute for Microbiology, Rutgers University, Piscataway, N.J. Strains X2180-1A (*MAT***a** *gal2*) and X2180-1B (*MATα gal2*) were obtained from the Yeast Genetic Stock Center, Berkeley, Calif. S646-1B (*MAT***a**/*MATα FLO1*/*FLO1 ade1*/*ade1*) was obtained from Verner Seligy, Molecular Genetics Group, National Research Council, Ottawa, Ontario, Canada. All cells were grown at 25°C in yeast nitrogen base, 2% glucose medium supplemented with amino acids and bases as appropriate. Cells were harvested at densities between 2×10^6 and 1.4×10^7 per ml. Flocculation was determined by an assay similar to an agglutination assay previously described (11), with the following modifications. The buffer was 0.1 M Tris chloride (pH 7.8) containing 2 mM EDTA 5 mM $CaCl_2$, and 10 μg of cycloheximide per ml. The optical density at 660 nm was determined after 5 min of settling. The percentage of cells bound was proportional to the difference in the mean optical density between the experimental tubes and the control tubes without added $CaCl_2$. Values are means of triplicate determinations. The maximum variation between tubes was 10%. Sexual agglutination was assayed by the method of Terrance and Lipke (11). The *tup1* cells were assayed in the presence of 2 mM EDTA unless otherwise noted. Values are means of triplicate determinations. Standard errors averaged 3%.

Table 1 shows a comparison of the self-association reaction of the *tup1* strains with sexual agglutinability. The flocculation of *tup1* cells was inhibited by EDTA, a reagent which did not affect the sexual agglutinability of either the *tup1* strain or the tester cells. Treatment of *tup1* cells with reagents that destroyed sexual agglutinins in tester cells inhibited the sexual agglutination of *tup1* cells but did not affect flocculation. These treatments included conditions that destroyed agglutinability of α cells (trypsinization), **a** cells (dithiothreitol), or both (sodium dodecyl sulfate). Furthermore, masking of the cell-bound agglutinins by soluble complementary agglutinin inhibited sexual agglutination but did not affect flocculation of the *MATα tup1* strain. Flocculation and sexual agglutination displayed differences in the gross characteristics of cell-to-cell binding. Flocculation occurred spontaneously and rapidly in cell suspensions, whereas sexual agglutination required prolonged shaking or sedimentation of cells to promote cell-cell contact. Sexual agglutinates were much more resistant to shear than flocculates and were not visibly affected by resuspension at 2,000 rpm for 6 s, a treatment that disrupted the flocculates. All these results imply that the self-association of *tup1* cells is not due to expression of sexual agglutinins of both mating types.

The presence of a *tup1* allele conferred flocculence on both the haploid strains. The parent of XY507-7A (XY505-18C, *MATα TUP1*) was nonflocculent under all tested conditions. Flocculence of both *tup1* strains had the same cation dependence and susceptibility to disruptive reagents. The flocculation of *tup1* strains was remarkable in that it occurred in defined media at all stages of growth under anaerobic as well as aerobic conditions.

The flocculation of *tup1* cells was similar, but not identical, to that of a *FLO1* strain, S646-1B. In both types of cell, Ca^{2+} promoted flocculation at each tested pH. In *tup1* strains, Mg^{2+} but not Mn^{2+} could partially substitute for Ca^{2+} at high pH (Table 2). At pH 4, only Ca^{2+} promoted flocculation significantly above control levels. The flocculation of controls was probably due to poor chelation of endogenous Ca^{2+} at low pH. In contrast, the *FLO1* strain could flocculate in the presence of Mg^{2+} only at pH 4 (data not shown). This result was similar to that previously reported (4).

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TABLE 1. Comparison of flocculation and sexual agglutination of *tup1* cells

Inhibitor[a]	% Cells bound						
	Flocculation		Sexual agglutination				
	α *tup1*[b]	**a** *tup1*	Tester	α	α *tup1*	**a**	**a** *tup1*
None	89	95	**a**	63–82[c]	36[d]	0	ND[e]
			α	0	1[d]	63–82	ND
EDTA	0	0	**a**	80	31	0	1–2
			α	0	0	80	17–31
Trypsin	58[f]	ND	**a**	9	0	ND	ND
Sodium dodecyl sulfate	90	89	**a**	0	0	ND	ND
			α	ND	ND	0	0
a-agglutinin	90	ND	**a**	45	21	ND	ND
Dithiothreitol	ND	87	α	ND	ND	1	0
α-agglutinin	ND	ND	α	ND	ND	27	2

[a] Inhibitors: 20 mM EDTA, added to the assay; trypsin, tested cells were pretreated for 10 min with 10 μg of crystalline trypsin per ml at 30°C in Tris buffer (pH 7.8); 0.1% sodium dodecyl sulfate, added to the assay; 30 mM dithiothreitol, tested cells were preincubated for 30 min at 30°C (pH 7.8); agglutinins, partially purified soluble monovalent glycoproteins isolated from the cells designated by the prefix. α-Agglutinin inhibits sexual agglutinability of **a** cells (11). **a**-Agglutinin inhibits agglutinability of α cells (N. Wagner and P. N. Lipke, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, J26, p. 175).

[b] Cells: **a**, X2180-1A (*MAT***a** *gal2*); α, X2190-1B (*MAT*α *gal2*); **a** *tup1*, XY507-7A (*MAT***a** *tup1-16 his5 lys1 ura4 gal1 gal2*); α *tup1*, XF160-7B (*MAT*α *tup1-16 his3 leu2*).

[c] Values stated as ranges include those for all control experiments.

[d] Flocculation was suppressed by resuspension at high shear.

[e] ND, Not determined.

[f] Control value was 61% for this experiment.

Table 3 shows the ability of several classes of compounds to inhibit *tup1*-mediated flocculation in strain XF160-7B. The cell surface structures mediating flocculation in *tup1* cells were resistant to protease K (Sigma Chemical Co., St. Louis, Mo.) at 10 μg/ml, 10 times the concentration used by Miki et al. (4). At 1 mg of protease per ml, an enzyme-to-cell ratio 1,000 times that which destroys flocculence of *FLO1* cells (4), the flocculation of XF160-7B cells was 5% of the control value, but XY507-7A cells were resistant, retaining 61% of control flocculation after treatment. This difference in susceptibility was the only case observed in which properties of the two *tup1* strains differed. The flocculation of *tup1* cells was inhibited by sulfhydryl reductants only after extensive incubation. Flocculation was reduced by incubation with 1.3 M mercaptoethanol for 90 min, which also destroyed flocculence of *FLO1* cells (4). *FLO1* cells were slightly more susceptible to inhibition by lower concentrations of mercaptoethanol or dithiothreitol than the *tup1*

TABLE 2. Metal ion dependence of flocculation in *tup1* cells[a]

Added cation	Concn (mM)	% Agglutinable cells at:		
		pH 4.0	pH 6.9	pH 7.8
None		43	0	0
Ca^{2+}	1.2	90	ND[b]	84
Ca^{2+}	10	91	82	ND
Mn^{2+}	10	54	8	21
Mn^{2+}	100	12	12	9
Mg^{2+}	10	53	29	49
Mg^{2+}	100	46	44	59

[a] The assay buffers were 0.1 M sodium acetate (pH 4.0 and 4.7) or 0.1 M Tris chloride (pH 6.9 and 7.8) containing 1 mM EDTA. Results at pH 4.7 were similar to results at pH 4.0. The data are from strain XF160-7B. Results were similar when strain XY507-7A was assayed.

[b] ND, Not determined.

TABLE 3. Effect of potential inhibitors of flocculation

Inhibitor[a]	% of control flocculation[b]	
	tup1	*FLO1*
None	100	100
1 M Tris chloride (pH 8.0)	100	92
1% Triton X-100	100	100
5 M NaCl	33	80
8 M urea	46	65
1.3 M mercaptoethanol[c]	30	42
30 mM dithiothreitol	94	79
Protease K (10 μg/ml)[c]	94	15
Concanavalin A (1 mg/ml)[d]	63	ND[e]
1 M glucose	70	107
1 M galactose	96	100
1 M mannose	45	88
33 mM mannose-6-phosphate	93	ND
0.25 M α-methyl mannoside	83	96

[a] Inhibitors were added to the flocculation buffer unless otherwise stated.

[b] Control values ranged from 84 to 93% aggregated cells. The data for the *tup1* cells are from strain XF160-7B. Results were similar with strain XY507-7A. Because Tris has been reported to inhibit flocculation of *FLO1* strain, it was assayed in 0.1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) containing EDTA and cycloheximide. Experiments in Tris buffers gave qualitatively similar results in this assay.

[c] Incubation was for 30 min at 30°C at pH 7.8.

[d] Incubation was for 40 min at 30°C in the presence of 5 mM Mn^{2+} and Ca^{2+}. Inhibition was prevented by inclusion of 50 mM α-methyl mannoside.

[e] ND, Not determined.

strains. The *tup1* strains were inhibited to a greater extent by mannose, however. To ensure that our results were qualitatively equivalent to those obtained from other assays, we assayed the flocculence of XF160-7B cells by the method of Woof (12) as modified by Miki et al. (5). The cells were suspended at 7×10^7/ml in Tris buffer containing 2 mM EDTA and transferred to a 1-cm cuvette. Addition of 5 mM $CaCl_2$ initiated flocculation. The rapid decrease in optical density was monitored at 660 nm. Inhibition of flocculation led to a decrease in the rate of change of optical density. The flocculation was inhibited in both assays by pretreatment of cells with concanavalin A or 1 mg of protease K per ml. In neither assay was flocculation reduced significantly by treatment with 30 mM dithiothreitol, protease K at 10 μg/ml, or concanavalin A in the presence of 50 mM α-methyl mannoside.

In summary, the self-association of *tup1* cells appeared to be due to flocculation and not to sexual agglutination. Flocculation was maximal in the presence of Ca^{2+}, but Mg^{2+} could substitute at pH 7.8. The flocculation of the two *tup1* strains was similar, differing only in the lability of the cell surface components to protease K. Despite differences between flocculation mediated by *tup1* (recessive, chromosome III) and *FLO1* (dominant, chromosome I), the phenomena were remarkably similar. Both reactions are Ca^{2+}-dependent and resistant to sodium dodecyl sulfate, Triton X-100, and pH changes. On the other hand, there were quantitatively different responses to several perturbants listed in Table 3 and to ionic substitution. Therefore, mutations in at least two loci can induce flocculation with different molecular characteristics. The difference could be due to independent mechanisms or, if the *TUP1* product prevents expression of the *FLO1* product, to the influence of different genetic backgrounds on a single mechanism.

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