PLEIOTROPIC PROPERTIES OF A YEAST MUTANT INSENSITIVE TO CATABOLITE REPRESSION

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ABSTRACT

The *flk1* mutation, which was originally isolated in the yeast *Saccharomyces carlsbergenesis*, causes insensitivity to catabolite repression. This mutation has been further characterized and mapped. The gene *flk1* is located on chromosome *III* between *thr4* and *MAL2*, 14 centimorgans from *MAL2*. *flk1* is shown to be allelic to the pleiotropic mutants *tup1*, *cyc9*, and *umr7*; and *flk1* is shown to exhibit an array of pleiotropic properties common to *tup1*, *cyc9* and *umr7*; These results suggest that the *flk1* mutation is not a specific lesion affecting catabolite repression.

A spontaneous mutant of *Saccharomyces cerevisiae*, insensitive to catabolite repression, was isolated and described by SCHAMHART, TEN BERGE and VAN DE POLL (1975). Because this mutant flocculated quickly in liquid media and had a grainy appearance on solid media, it was called "flaky" (*flk1*). It was shown to be insensitive to glucose repression of maltase, α-methyl glucosidase and invertase. We have mapped *flk1* to chromosome *III* between *thr4* and *MAL2* and shown it to be equivalent to the previously described mutation, *tup1*.

Spontaneous mutants of *S. cerevisiae* able to incorporate dTMP into their DNA were isolated by WICKNER (1974). One complementation group of these mutations, *tup1*, was mapped on chromosome *III* between *thr4* and *MAL2*; it was reported to exhibit extreme graininess and abnormal mating behavior in the α but not **a** mating type. In addition, *tup1/ tup1* diploid strains failed to sporulate. Besides the equivalence of *flk1* and *tup1* demonstrated in this paper, recent results have established that *cyc9* (ROTHSTEIN and SHERMAN 1980) and *umr7* (LEMONTT, FUGIT and MACKAY 1980) are also equivalent to *tup1*.

cyc9 mutants were isolated by ROTHSTEIN and SHERMAN (1980) among a group of mutants having increased levels of iso-2-cytochrome *c*. *cyc9*, like *flk1* and *tup1*, maps on chromosome *III* between *thr4* and *MAL2* and is allelic with *tup1*. α *cyc9* strains are clumpy, dry and sterile. In addition, *tup1* strains have elevated levels of iso-2-cytochrome *c* (ROTHSTEIN and SHERMAN 1980).

umr7 was isolated and described by LEMONTT (1976, 1977a,b) among a group of mutants showing defective UV-induced mutation from *CAN1* to *can1* (defective arginine permease). LEMONTT, FUGIT and MACKAY (1980) subsequently found

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that *umr7*, like *cyc9* and *tup1*, maps on chromosome *III* between *thr4* and *MAL2* and showed it to be allelic to *tup1* and *cyc9*, to take up dTMP and to exhibit identical aberrant mating behavior in the α mating type, as well as the characteristic clumpiness (LEMONTT, FUGIT and MACKAY 1980). In addition, LEMONTT, FUGIT and MACKAY (1980) have shown that *cyc9*, *umr7* and *tup1* are all "self-shmooing" in the α mating type, *i.e.*, when stained with fluorescent concanavalin A, they stain like normal **a** cells treated with α factor. It has also been shown that these α mutants do not produce active α factor; that is, when streaked next to a wild-type **a** strain, there is no detectable shmooing response in the **a** strain cells (LEMONTT, FUGIT and MACKAY 1980).

Thus, the *flk1* mutation discussed in this paper and the *tup1 cyc9* and *umr7* mutations discussed in the accompanying papers (ROTHSTEIN and SHERMAN 1980; LEMONTT, FUGITT and MACKAY 1980) are all mutations of the same gene causing a large array of diverse mutant phenotypes.

MATERIALS AND METHODS

Media: Complete medium (YPD) contained the following: yeast extract, 1%; peptone, 2%; dextrose, 2%; agar, 1.5%. Minimal medium contained the following: yeast nitrogen base without amino acids, 0.67%; dextrose, 2%; agar, 1.5%. This was supplemented with adenine sulfate, 20 mg/l; leucine, 30 mg/l; lysine, 30 mg/l; threonine, 200 mg/l; histidine, 20 mg/l; and/or tryptophan, 20 mg/l, as required. Maltose-fermentation test plates contained the following: yeast extract, 1%; peptone, 2%; maltose, 2%; ethidmium bromide, 10 μg/ml (ethidmium bromide suppresses residual growth of nonfermenting strains); agar, 1.5%. Prespore medium contained the following: yeast extract, 0.8%; peptone, 0.3%; dextrose, 10%; and agar, 1.5%. Sporulation medium contained the following: potassium acetate, 1%; yeast extract, 0.1%; dextrose, 0.05%, agar, 1.5%. dTMP medium contained the following: yeast extract, 0.15%; peptone, 1%; dextrose, 2%; sulfanilamide, 6 mg/ml; aminopterin, 50 μg/ml; and dTMP, 100 μg/ml, prepared according to WICKNER (1974).

Genetic methods: The haploid strains used are listed in Table 1. Diploids were isolated on minimal medium lacking the complementary requirements of the parents. Tetrad dissection and analyses were carried out by the method of HAWTHORNE and MORTIMER (1960).

Because of their graininess and tendency to clump even on solid media, the *flk1* strains would not transfer uniformly, if at all, using velveteen. Therefore, replica plating was done using a 32-point metal innoculator.

To assay large numbers of segregants for the *dep* (derepressed or glucose insensitive) phenotype, the following qualitative assay, which is a modification of the method of MOWSHOWITZ (1976), was developed: segregants were grown overnight on a liquid prespore medium that contains high levels of glucose. The tubes were scored for flocculence; then, 10 μl samples were spotted on to filter paper (Whatman #1) in a pattern. After complete drying to permeabilize the cells, the filter papers were sprayed with a solution of p-nitro-phenyl-α-D-glucoside (PNPG), 1 mg/ml in potassium phosphate buffer, 0.05 M, pH 6.9. Hydrolysis of PNPG due to α-glucosidase activity produces an immediate bright yellow color in the spots containing derepressed segregants. As a control for the reliability of the filter paper assay, standard derepressed and normal strains were simultaneously tested with each set of assays. In addition, a series of quantitative enzyme assays to measure α-glucosidase levels in the presence of high glucose were run on segregants from several tetrads to corroborate the results of the qualitative assays, using the method previously described by MOWSHOWITZ (1976).

dTMP uptake was assayed by the ability of the strain to grow on YPD medium containing sulfanilamide, aminopterin and dTMP, as described by WICKNER (1974).

TABLE 1

Saccharomyces strains used

Strain	Genotype	Source	Importance
100	**a** *mal trp5 flk1*	R. NEEDLEMAN	Source of *flk1*
109	*α ade2 his4 thr4 leu2 tup1–66*	R. WICKNER	Source of *tup1*
AT18	**a** *ade1 ade2 ura1 trp1 his7 lys2 gal mal tup1–18*	R. WICKNER	Source of *tup1*
AT68	**a** *ade1 ade2 ura1 trp1 his7 lys2 gal mal tup1–68*	R. WICKNER	Source of *tup1*
143	**a** *MAL2 ade1 his4 FLK*	This laboratory	Source of *MAL2*
149	**a** *MAL2 lys1 his4 FLK*	This laboratory	Source of *MAL2*
26	*α MAL2 ade1 leu2 thr4 lys2*	This laboratory	Source of *MAL2*
M31–7G	**a** *MAL4 his2 FLK*	This laboratory	Source of *MAL4*
132	*α MAL3 lys FLK*	This laboratory	Source of *MAL3*
10	**a** *ade1 ade2 ura1 tyr1 his7 lys2*	L. HARTWELL	parent strain of *tup* mutants
13	*α his1*	N. KHAN and N. EATON	Standard laboratory strain
194	*α mal his6*	This laboratory	Standard laboratory strain
118	**a** *ade1 lys2 flk1*	This laboratory	Source of *flk1*
M60–13F	*α lys1 trp5 flk1*	This laboratory	Source of *flk1*
M67–15H	*α his2 lys1 flk1*	This laboratory	Source of *flk1*
M60–11G	**a** *mal2 leu2 flk1*	This laboratory	Source of *flk1*
M45–17	*α mal lys1 flk1*	This laboratory	Source of *flk1*
M60–1C	**a** *lys1 trp mal flk1*	This laboratory	Source of *flk1*
M59–2D	*α mal lys1 ade1 FLK*	This laboratory	Standard laboratory strain

Cell morphology after vegetative growth was scored qualitatively by microscopic observation as either the normal oval shape with or without a bud, or the abnormal elongated shmoo shape. Cultures of strains classed as shmoo shaped also contained a proportion of unbudded normal cells.

Mating-hormone response was assayed by exposing the mutants to hormones known to be produced by normal tester strains in a confrontation test on agar (DUNTZE, MACKAY and MANNEY 1970; MACKAY and MANNEY 1974). Mating-hormone response was considered positive if a strain exhibited both G1 arrest and shmoo formation in response to the hormone. Mating-hormone production was considered positive if hormone produced by a strain elicited both G1 arrest and shmoo formation in a tester strain known to exhibit a normal response to active hormone in the confrontation test.

Cells were stained for observation using fluorescent microscopy by using concanavalin A labelled by reaction with fluorescein isothiocyanate (FITC-ConA) as previously described (TKACZ, CYBULSKA and LAMPEN 1971) to treat yeast cultures by the method of TKACZ and MACKAY (1979). Acetone-fixed specimens were mounted in buffered glycerol and observed under epi-ilumination with a Zeiss fluorescence microscope.

RESULTS

When we began our study of the *flk1* mutant, our interest was in its derepressed phenotype. Because flocculent strains are extremely difficult to work with, we had hoped to separate derepression from flocculence, although it had been

previously reported that the two characters were closely linked (SCHAMHART, TEN BERGE and VAN DE POLL 1975). In dissecting the appropriate crosses, we found that flocculence could not be separated from depression; in a total of 96 complete tetrads dissected, plus another 100 random spore segregants, we saw no separation of flocculence and derepression. These results confirm those of SCHAMHART, TEN BERGE and VAN DE POLL (1975) that the *flk1* and *dep* phenotypes are probably pleiotropic effects of a single mutation. From the crosses done in an attempt to separate *dep* and *flk1*, it became clear that the locus responsible was linked to *MAL2*. (Crosses involving *flk1* and *MAL3* or *flk1* and *MAL4* showed no linkage.) Using appropriate three-point crosses, we were subsequently able to map *flk1* on chromosome *III* between *thr4* and *MAL2*, 14 centimorgans from *MAL2* (Table 2).

As we continued to study the *flk1* mutant, it became evident that the mutation affected mating behavior in the α mating type. All **a** *flk1* strains mated normally with α strains, but α *flk1* strains fell into three categories of mating behavior: (1) α strains that mated normally with **a** strains, (2) nonmaters, and (3) α strains that mated with either **a** or α strains in an equal but low frequency (bisexual). Upon noting the apparent similarity in phenotypic properties and map position between *flk1* and the three other pleiotropic mutants, *tup1*, *umr7* and

TABLE 2

Mapping of flk1 *locus*

Interval tested		Cross*	PD	NPD	T	cM‡
flk1–MAL2		1	8	0	3	
		2	8	0	3	
		3	10	0	4	
		4	14	0	6	
	Total		40	0	16	14.2
flk1–MAT†		1	1	0	10	
		2	1	0	9	
		3	2	0	12	
		4	6	0	17	
	Total		10	0	48	42
flk1–thr4		4	9	0	11	28
thr4–MAT		4	11	0	7	18
MAL2–thr4		4	5	0	15	38

* Cross 1: 100 × 149
2: 143 × M 45–17
3: M 60–11 G × 59–2 D
4: M 60–1C × 26

† Due to the frequent ambiguity of the mating-type phenotype of the α *flk1* genotype (see explanation in text), α *flk1* segregants could act as α, nonmater, or bisexual. Since all tetrads used for this analysis were clearly real tetrads as measured by 2:2 segregation of all markers except mating type, the **a** segregants were scored directly and the remaining two segregants were classified as α.

‡ Centimorgans were calculated on the basis of the equation in MORTIMER and HAWTHORNE (1969).

cyc9, we proceeded to determine whether *flk1* was, in fact, allelic to the others and whether the array of phenotypic properties was shared by all four mutants.

To determine if *tup1* and *flk1* strains possessed the same pleiotropic properties, we tested WICKNER's *tup1* strains for flocculence and derepression, and tested our *flk1* strains for dTMP uptake and failure to sporulate. We found that the *tup1* strains were both flocculent and derepressed, like *flk1*, while the *flk1* mutants were able to take up dTMP, like *tup1* (Table 3). To test the *flk1* strains for failure to sporulate, it was necessary to construct *flk1/flk1* diploid strains (and *tup1/tup1* controls); this was difficult to do because of the inherent aberrant mating behavior of the mutants, as discussed above. We were able to construct two strains of each homozygous mutant genotype, *flk1/flk1* and *tup1/tup1* (and two *flk1/tup1* strains for complementation tests, see below) by carrying out the mating in liquid YPD (30°) and then plating a heavy lawn of washed cells on the appropriate minimal medium lacking complementary amino acids. In our hands, the *tup1/tup1* diploid strains each exhibited less than 1% sporulation, while the two *flk1/flk1* diploid strains showed 1% and 20%, respectively (Table 4). Diploid strains made from the parental wild-type strains were also tested for sporulation. Normal diploid strains constructed from the *tup1* parental strains had 50–60% sporulation (Table 4); normal diploid strains made from the *flk1* parental strains gave 90–95% sporulation (Table 4).

Although the sporulation frequencies for the *flk1/flk1* and *tup1/tup1* diploid strains were not the same, both frequencies were significantly lower than that of the diploid strains constructed from the appropriate parental strains. Therefore, both *tup1* and *flk1* mutations seemed to cause poor sporulation in addition to the three other phenotypic properties: flocculence, derepression and ability to take up dTMP.

Since *flk1* and *tup1* mutants shared all four properties in common and mapped in the same region of chromosome *III*, *flk1/tup1* diploid strains were examined

TABLE 3

Phenotypes of tup1 *and* flk1 *haploids*

Strain	Relevant genotype	Phenotypes* FLK	DEP	TUP
10	a *TUP FLK*	+	+	+
194	α *TUP FLK*	+	+	+
AT18	a *tup1*	flk	dep	tup
109	α *tup1*	flk	dep	tup
100	a *flk1*	flk	dep	tup
100R	a *FLK*†	+	+	+
118	a *flk1*	flk	dep	tup
67–15H	α *flk1*	flk	dep	tup
60–13F	α *flk1*	flk	dep	tup

* Normal phenotypes were scored as +; flocculent strains were scored as flk; derepressed strains were scored as dep; strains growing on YPD medium containing dTMP(sulfanilamide and aminopterin were scored as tup.

† Revertant of strain 100.

TABLE 4

Phenotypes of tup1 *and* flk1 *diploids*

Cross	Relevant genotype	Diploid phenotypes* FLK	DEP	TUP	% Sporulation
Control crosses using normal strains:					
(1) 10† × 194‡	**a** *TUP* × *α TUP* [control for crosses 6&7]	+	+	+	60%
(2) 100R§ × 13‡	**a** *FLK* × *α TUP* [control for crosses 8&9]	+	+	+	90%
Heterozygous crosses involving mutant × normal alleles:					
(3) 10 × 109	**a** *TUP* × *α tup1*	+	+	+	50%
(4) AT18 × 194	**a** *tup1* × *α TUP*	+	+	+	60%
(5) 100 × 149	**a** *flk1* × *α TUP*	+	+	+	>95%
Homoallelic crosses:					
(6) AT18 × 109	**a** *tup1* × *α tup1*	flk	dep	tup	4%
(7) AT68 × 109	**a** *tup1* × *α tup1*	flk	dep	tup	4%
(8) 118 × 60–13F	**a** *flk1* × *α flk1*	flk	dep	tup	1%
(9) 100 × 67–15H	**a** *flk1* × *α flk1*	flk	dep	tup	20%
Heteroallelic (*tup/flk*) crosses:					
(10) AT18 × 60–13F	**a** *tup1* × *α flk1*	flk	dep	tup	3%
(11) AT68 × 60–13F	**a** *tup1* × *α flk1*	flk	dep	tup	12%

* tup, dep and flk phenotypes were scored as in Table 3; % sporulation was calculated on the basis of number of sporulated cells per 1000 cells viewed under the light microscope.
† Strain 10 is the wild-type parent of the *tup1* strains.
‡ Strains 194, 13 and 149 are normal laboratory strains which are + for the flk, dep and tup phenotypes.
§ Strain 100R is a revertant of strain 100, chosen for reversion of the dep phenotype.

for complementation to determine if the two mutations were allelic. The two *tup1/flk1* diploid strains constructed (Table 4) were found to exhibit all four mutant properties; that is, they did not complement. Therefore, *flk1* and *tup1* appear to be allelic; and flocculence, derepression, dTMP uptake and low sporulation in the diploid strains are probably the pleiotropic effects of a single mutation.

To test whether *α flk1* strains produce normal *α* factor, we assayed the *flk1* mutants for the "self-shmooing" and con A staining properties. Indeed, the *flk1* strains exhibited properties identical to the other mutants in the allelic set (LEMONTT, FUGIT and MACKAY 1980; ROTHSTEIN and SHERMAN 1980). The **a** *flk1* strains produce normal **a** factor and respond normally to *α* factor as assayed by a confrontation test with wild-type *α* strains (see METHODS). *α flk1* strains do not produce normal *α* factor; that is, when streaked next to a wild-type **a** strain, there is no detectable shmooing response in the wild-type **a** strain. Furthermore, *α flk1* strains appear to be "self-shmooing"; they shmoo spontaneously and do not require extraneous **a** factor. When the *α flk1* "self-shmoos" are stained with fluorescent concanavalin A, they stain like normal shmoos made in response to hormone. The shmoo tip fluoresces more brightly than the body of the shmoo. These results indicate that all of the mutants exhibit aberrant mating behavior in the *α* mating type, in addition to the four properties described above.

A spontaneous revertant of *flk1*, isolated because it was no longer derepressed, was found to have simultaneously lost its flocculence and its ability to grow on dTMP. Similarly, revertants of *umr7* were also shown to have simultaneously lost the entire array of pleiotropic phenotypes: graininess, mating-type abnormality, ability to "self-shmoo" in the α mating type and ability to take up dTMP (LEMONTT, FUGIT and MACKAY 1980). The simultaneous reversion of all five properties confirms that all five are the pleiotropic effects of a single mutation.

DISCUSSION

It is clear from the data presented here and in the accompanying papers by LEMONTT, FUGIT and MACKAY (1980) and ROTHSTEIN and SHERMAN (1980) that *flk1*, *tup1*, *umr7* and *cyc9* are allelic. The alleles express similar pleiotropic phenotypes, do not complement in **a**/α diploids, and can be mapped at the same locus on chromosome *III*.

When the *flk1* mutant was originally isolated, it was thought that further analyses of this mutant might elucidate the factors mediating the catabolite repression response in yeast. It has been known for some time that cAMP is the probable mediator of catabolite repression in *E. coli* and, indeed, in prokaryotes in general. In wild-type yeast, it was found that cAMP level varies in similar fashion; that is, it is lower in repressed cells and higher in derepressed cells. In addition, a positive correlation has been demonstrated between degree of catabolite repression and the levels of cAMP-binding protein, adenyl cyclase and protein kinase (SCHAMHART, TEN BERGE and VAN DE POLL 1975). There is more recent evidence that addition of cAMP to a repressed culture triggers derepression with the same kinetics as release from repression by growth on ethanol (MAHLER and LIN 1978).

In the *flk1* strain, no correlation was found between the alterations in catabolite repression and the levels of cAMP. The catabolite repressed enzymes were repressed to varying degrees from 0% for maltase to 59% for succinate dehydrogenase, but the levels of cAMP were essentially the same as in the wild type; therefore, the phenotype of the *flk1* mutant was originally taken as evidence against the role of cAMP in catabolite repression in yeast. Given the pleiotropic nature of the *flk1/tup1/umr7/cyc9* mutation described here (and elsewhere), it seems likely that this mutant is not really a derepression mutant, and its possession of wild-type cAMP levels does not argue against the role of cAMP in yeast catabolite repression.

What is the primary cause for the *flk1* or pleiotropic phenotype? A cell surface defect might explain abnormal thymidylate uptake or flocculence, as well as certain mating type abnormalities; a mating-type defect could explain the sporulation and mating defects, but it is difficult to correlate the range of possibilities presented by the vast array of pleiotropic effects of this single mutation (see LEMONTT, FUGIT and MACKAY 1980; ROTHSTEIN and SHERMAN 1980 for further discussions). We hope that further studies of the locus and its phenotypic properties will elucidate the system.

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