DELETIONS OF THE ISO-1-CYTOCHROME *c* AND ADJACENT GENES OF YEAST: DISCOVERY OF THE *OSM1* GENE CONTROLLING OSMOTIC SENSITIVITY

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ABSTRACT

Some of the deletions in the yeast *Saccharomyces cerevisiae* that encompass the *CYC1* gene, which determines iso-1-cytochrome *c*, extend into the *OSM1* gene, causing inhibition of growth on hypertonic media, and into the *RAD7* gene, causing sensitivity to UV light. Two deletions (*cyc1–363* and *cyc1–367*) encompass only the *CYC1* gene, two deletions (*cyc1–366* and *cyc1–368*) encompass the *CYC1* and *OSM1* genes, three deletions (*cyc1–1*, *cyc1–364* and *cyc1–365*) encompass the *CYC1*, *OSM1* and *RAD7* genes, while none of the deletions extend into the closely linked *SUP4* gene.

THE first mutant of yeast deficient in cytochrome *c* was uncovered more than ten years ago during a systematic examination of strains that were defective in mitochondrial function (SHERMAN and SLONIMSKI 1964). This mutant, *cyc1–1*, proved to contain a deletion of the structural gene for iso-1-cytochrome *c*; the *cyc1–1* mutant completely lacks iso-1-cytochrome *c* (SHERMAN, TABER and CAMPBELL 1965), does not revert (PARKER and SHERMAN 1969), nor does it recombine with point mutants including those situated at the extreme ends of the structural gene (SHERMAN *et al.* 1975). Surprisingly, the growth of the *cyc1–1* mutant was found to be inhibited on nutrient medium that contained high concentrations of glucose, although comparable growth rates were observed for normal strains and strains containing the point mutation *cyc1–2* (SHERMAN, TABER and CAMPBELL 1965). Thus, it appeared as if the *cyc1–1* mutant contained an additional defect that was not associated with the point mutant *cyc1–2*. More recently, L. PRAKASH (University of Rochester, unpublished results) discovered that a *cyc1–1* mutant was also more UV sensitive than another *cyc1* mutant and a wild-type strain.

In addition to the availability of the *cyc1–1* mutant, over 100 *cyc1* deletions of various lengths have recently been obtained in specially designed experiments, and their lengths have been estimated from recombination tests with *cyc1* point mutants that have defined lesions corresponding to known positions in the iso-1-

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cytochrome *c* protein (SHERMAN *et al.* 1975). These deletions were generated from crosses having alleles with extensive dissimilarities of sequence in homologous regions of the *CYC1* gene. It was speculated that mispairing due to differences in homology in the region of the *CYC1* gene might lead to deletions during meiosis. Deletions of various lengths, from those covering only two adjacent sites to those encompassing at least the entire *CYC1* gene, were obtained among the *cyc1* mutants derived from the sporulated cultures.

In this paper we describe the phenotypes extrinsic to the lack of iso-1-cytochrome *c* that are associated with the *cyc1–1* deletion and with the *cyc1* deletions recently obtained in the study by SHERMAN *et al.* (1975). The extents of the deletions have been investigated by genetic tests with the *rad7* and *SUP4* genes, which are tightly linked to the *CYC1* locus on chromosome *X* (LAWRENCE *et al.* 1975). The findings established that some of the deletions encompass only the *CYC1* locus, while other deletions also include one or both of the *OSM1* and *RAD7* genes, which result in sensitivity, respectively, to hypertonic media and to UV light.

MATERIALS AND METHODS

Strains: The strains examined in this study include various *cyc1–1* strains, some of the *cyc1* deletions obtained in the study by SHERMAN *et al.* (1975), especially *cyc1–363* through *cyc1–399* and *cyc1–428*, the point mutants *cyc1–13*, *cyc1–179* and others, and the normal strain D311–3A and others. Some of the basic strains are listed in Table 1.

TABLE 1

Basic strains used in this study

Strain	Genotype
D311–3A	**a** *lys2–1 his1 trp2*
B–699	**a** *cyc1–179 lys2–1 his1 trp2*
CL148–21D	**a** *rad7 met3 lys2–1*
CL148–30B	α *rad7 met3 lys2–1 leu1–12*
CL18–157C	**a** *cyc1–13 rad7 ilv3 leu1–12 aro7–1 can1–100 trp5–48 his5–2*
D597–3C	α *cyc1–1 trp1–1 his5–2*
D234–10D	**a** *cyc1–1 his1*
D597–6D	**a** *cyc1–1 trp1–1 aro7–1 leu2–1*
B–3847	α *cyc1–363 arg4–17*
B–3848	α *cyc1–364 arg4–17*
B–3849	α *cyc1–365 arg4–17*
B–4850	α *cyc1–366 arg4–17*
B–4164	α *cyc1–367 arg4–17*
B–4165	α *cyc1–368 arg4–17*
AS428–4B	α *arg4–17 his1 lys2–1*
AS428–2C	**a** *arg4–17 trp2*
AS441–5B	α *cyc1–364 his5–2 lys1–1*
AS442–4C	α *cyc1–365 lys1–1 arg4–17 trp1–1*
AS443–3A	α *cyc1–366 his5–2*
R11	**a** *sup4–o–R his5–2 lys1–1 trp1–1*

Media: The routine nutrient medium contained 1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose, and when needed for solidification, 1% Ionagar (Colab Laboratories). The synthetic minimal medium contained 0.67% Bacto-yeast nitrogen base "without amino acids," 2% glucose, 1.5% Ionagar; various supplements were added to this medium for scoring auxotrophic markers. Chlorolactate medium, used for selection of strains deficient in iso-1-cytochrome *c*, was as described by SHERMAN *et al.* (1974). Hypertonic media were prepared by adding indicated amounts of KCl, ethylene glycol, glycerol, or additional glucose to the nutrient medium described above. Agar concentration in the hypertonic media was increased to 2%. The medium made hypertonic with glucose was prepared by adding a solution of glucose that was autoclaved separately from the other ingredients; all ingredients of other hypertonic media were autoclaved together.

General genetic methods: Procedures standard in yeast genetics were used for analyses of segregations of markers and for construction and characterization of most tester strains. Since a wild-type strain isogenic with any of our *cyc1–1* mutants is not available for comparative study and since this mutation is the focus of the present report, we constructed a pair of comparable strains as follows. A *cyc1–1/CYC1* heterozygote was constructed by crossing a *cyc1–1* mutant, D597–6D, with the normal strain AS428–4B. A *cyc1–1/cyc1–1* homozygote was selected from this diploid on chlorolactate medium and was subsequently verified as having the desired genotype on the basis of its lack of iso-1-cytochrome *c*, ability to sporulate, and possession of other phenotypes characteristic of the *cyc1–1* mutation. As a *CYC1/CYC1* control strain we used a hybrid between AS428–4B and another strain, AS428–2C, from the same pedigree.

Segregation of *cyc1* genes was scored by low-temperature (−190°) spectroscopic examination of intact cells (SHERMAN and SLONIMSKI 1964). The *SUP4-o* gene was scored by the suppression of one or more UAA markers. Growth on various media was determined by spotting cell suspensions, using a rod-type replicator. UV sensitivity was tested by irradiating spots of cell suspensions with a series of UV doses, including 120 Jm^{-2}. In many cases surviving fractions after various doses of UV were determined by irradiating surfaces of plates spread with a known number of cells.

Quantitative growth measurements: Growth was measured in 125 ml side-arm flasks containing 20 ml of either normal or hypertonic media that were inoculated with fresh, stationary-phase cells. Cultures were incubated at 30° with vigorous shaking, and growth was followed by measuring turbidity with a model 800–3 Klett-Summerson photoelectric colorimeter using a no. 62 (560–650 nm) light filter.

Tests for extension of deletions into the SUP4 *locus*: An inactive suppressor mutant *sup4-o-R* was isolated on the basis of its growth on hypertonic media (SINGH 1977). A *SUP4-o* strain containing the UAA alleles *his5–2* and *lys1–1* was plated on hypertonic medium. Genetic analysis of one of the revertants, *sup4-o-R*, that had become auxotrophic for lysine and histidine, revealed that the loss of the suppressor activity was due to a second-site mutation in the *SUP4* locus (see DICAPRIO and HASTINGS 1976; ROTHSTEIN 1977). To test whether certain deletions of the *cyc1* gene extended into the closely-linked *SUP4* locus, strains were constructed that contained the appropriate *cyc1* deletion, as well as the markers *lys1–1* and/or *his5–2*. These strains were then crossed to a strain containing the *sup4-o-R* mutant gene and the UAA markers *lys1–1* and *his5–2*. The diploids were sporulated and the frequencies of *SUP4-o* recombinants were estimated by plating on media lacking either histidine or lysine. Some of the suppressors generated by recombination and containing the *cyc1* deletions were verified to be at the *SUP4* locus by mapping studies.

RESULTS

UV sensitivity associated with cyc1 *mutations*: In this investigation, we have examined the UV sensitivity of strains containing a number of *cyc1* point mutants, the *cyc1–1* deletion and the following 38 deletions that were generated in the study by SHERMAN *et al.* (1975): six deletions, *cyc1–363* through *cyc1–*

368, which encompass at least the entire *CYC1* locus; 31 deletions, *cyc1–369* through *cyc1–399*, which encompass one end of the gene corresponding to the amino-terminus of iso-1-cytochrome *c*; and the single mutant, *cyc1–428*, which encompasses the other end corresponding to the carboxyl-terminus. Strains bearing the *cyc1–1*, *cyc1–364* and *cyc1–365* alleles were found to be more UV sensitive than the wild-type strains. Thus, only the *cyc1* mutants that contain deletions of the entire *cyc1* locus possess the property of UV sensitivity in addition to the characteristic lack of iso-1-cytochrome *c*. None of the point mutations and other *cyc1* deletions, some of which include the entire locus, are UV sensitive.

In more than 100 tetrads analyzed from crosses heterozygous for *cyc1–1* mutation, the UV sensitivity always segregated with the *cyc1–1* allele. The UV sensitive phenotype of the *cyc1–1* allele does not complement UV sensitivity conferred by the other two *cyc1* alleles, *cyc1–364* and *cyc1–365*, indicating that they are sensitive to UV light due to lesions in the same genetic locus.

These results are compatible with the hypothesis that these deletions extend into an adjacent gene that controls UV sensitivity. This hypothesis could be tested by allelism tests of these deletions with numerous genes in yeast that have been identified as controlling radiation sensitivity (see GAME and Cox 1971). Since LAWRENCE *et al.* (1975) found that the *rad7* gene causing UV sensitivity is closely linked to *cyc1* locus, we have performed complementation tests between *cyc1* deletions causing UV sensitivity and the *rad7* tester strains. Quantitative results of such a test between *cyc1–1* and *rad7* are shown in Figure 1. The two mutations do not complement each other for radiation sensitivity. The *cyc1–1/rad7* diploids exhibit the same level of UV sensitivity as the *rad7/rad7* diploids. Figure 1 also shows that the radiation sensitivity caused by *cyc1–1* is a recessive phenotype. These results establish that the *cyc1–1* deletions include at least part of the *RAD7* gene. Since the other two deletions do not complement *cyc1–1*, they too must extend into the *RAD7* locus.

Growth inhibition of cyc1 *deletions by hypertonic media*: It has long been known that *cyc1–1* strains were inhibited by high concentrations of glucose, whereas increased amount of glucose had no differential effect on several other *cyc1* mutants (SHERMAN, TABLER and CAMPBELL 1965). To determine whether this growth inhibition is specific to glucose or whether it is the increased osmotic pressure of such media that has the inhibitory effect, we have tested growth of *cyc1–1* strains in various types of hypertonic nutrient media. Growth of *cyc1–1* strains is severely retarded if the nutrient medium is made hypertonic by additions of glucose, KCl glycerol, ethylene glycol, or sorbitol. From tests with various concentration of these compounds it was found that normal and sensitive strains can be clearly distinguished with media containing 2 to 2.5 M of either glucose, glycerol, sorbitol or ethylene glycol or with media containing 1 to 1.5 M of KCl. A fermentable carbon source in the growth medium is apparently required for the expression of the osmotic sensitivity of the *cyc1–1* mutation. Normal and *cyc1–1* strains were equally inhibited when the hypertonic glycerol medium did not contain glucose. Similar results were obtained with glycerol or ethanol media that were made hypertonic with KCl.

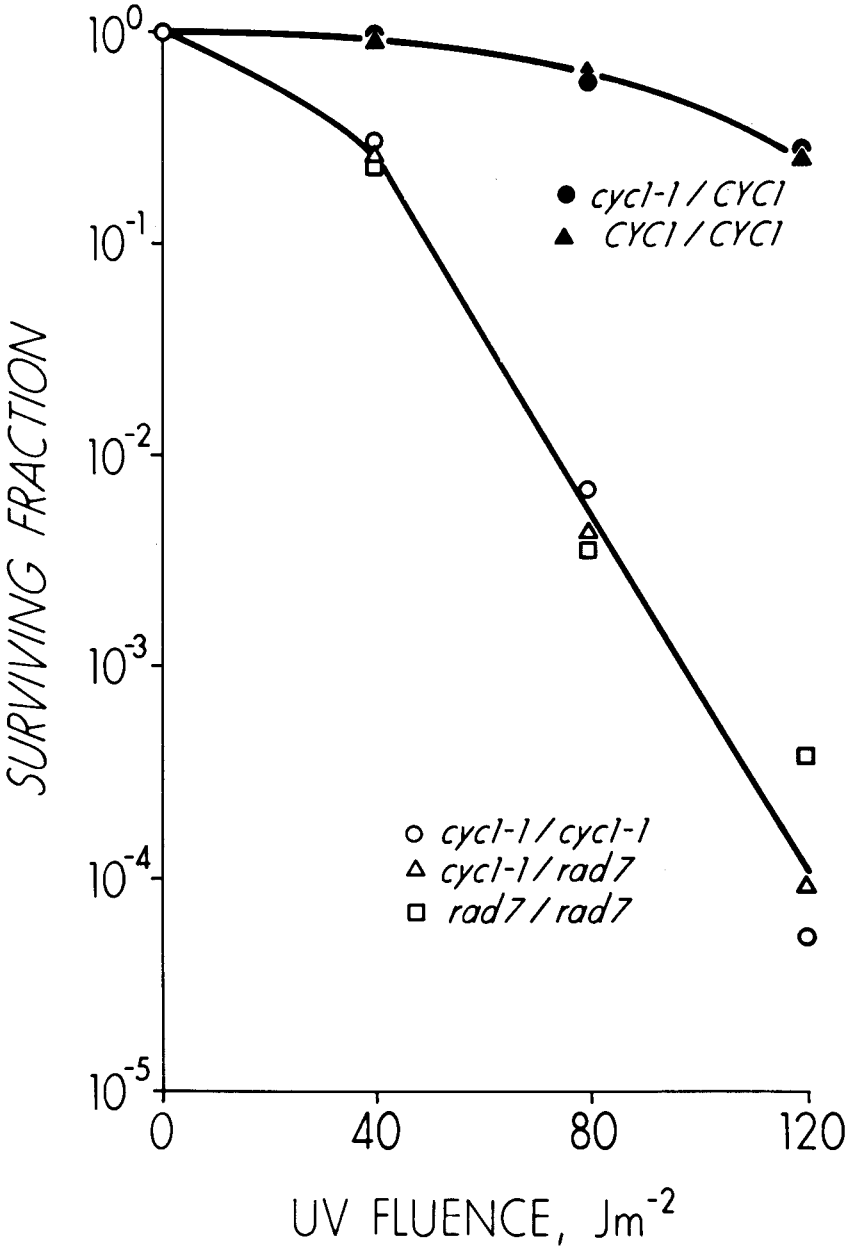

FIGURE 1.—Quantitative complementation test for UV sensitivity of crosses between the *cyc1–1* deletion and the *rad7* point mutant. Appropriate dilutions of stationary phase cells were plated and the surfaces of these plates were irradiated with indicated doses. Surviving fractions were determined from number of colonies produced after five to six days of incubation at 30°.

Figure 2 shows growth characteristics of a *cyc1–1/cyc1–1* and isogenic *cyc1–1/CYC1* strain in regular nutrient medium and in nutrient medium supplemented with 2 M ethylene glycol. Also included in Figure 2 are growth properties of a related *CYC1/CYC1* diploid. All three strains have similar growth rates on the nutrient medium, whereas their growths on the hypertonic medium differ markedly. We emphasize here that the intermediate level of growth of the heterozygote shown in Figure 2 should not be construed to mean that osmotic sensitivity conferred by the *cyc1–1* mutation is a semi-dominant property. From tests with numerous diploids heterozygous for the *cyc1–1* gene, we have established that the osmotic sensitivity is a recessive trait. As mentioned above, the *CYC1/CYC1* diploid is not isogenic with the other two strains; this diploid was a hybrid between two strains, both of which grew well on hypertonic media. As

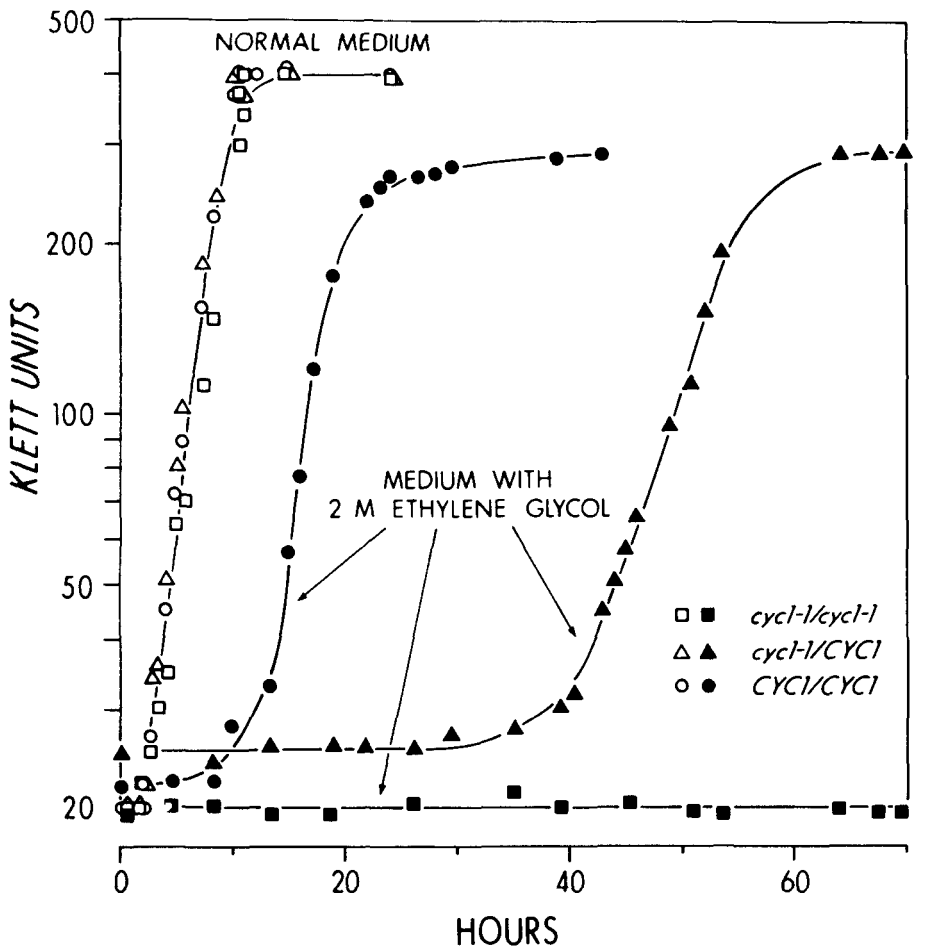

FIGURE 2.—Growth characteristics of the normal and osmotic-sensitive strains in normal and hypertonic media.

we will discuss later, various normal strains differ in their ability to grow on media with increased osmotic pressure.

The growth inhibition by addition of increasing amount of ethylene glycol to the normal nutrient medium were determined after an arbitrary period of 50 hours from the time of inoculation. The growth of sensitive and normal strains begin to differ significantly at about 1 M of ethylene glycol (Figure 3).

Deletions causing osmotic sensitivity extend into OSM1: The experiments described above have established that the *cyc1–1* deletion included at least part of the *RAD7* gene and that it also prevents growth in hypertonic media. To determine if osmotic sensitivity is an inherent property of radiation-sensitive strains or of such strains that completely lack iso-1-cytochrome *c*, we have determined UV and osmotic sensitivities of many appropriate strains described above and listed in Table 2. The results established that the UV sensitive strains are not necessarily osmotic sensitive, nor are the two properties interdependent. The *cyc1–13 rad7* double mutant, like the *cyc1–1* mutant, lacks iso-1-cytochrome *c* and is sensitive to UV light, although its growth is not inhibited by hypertonic

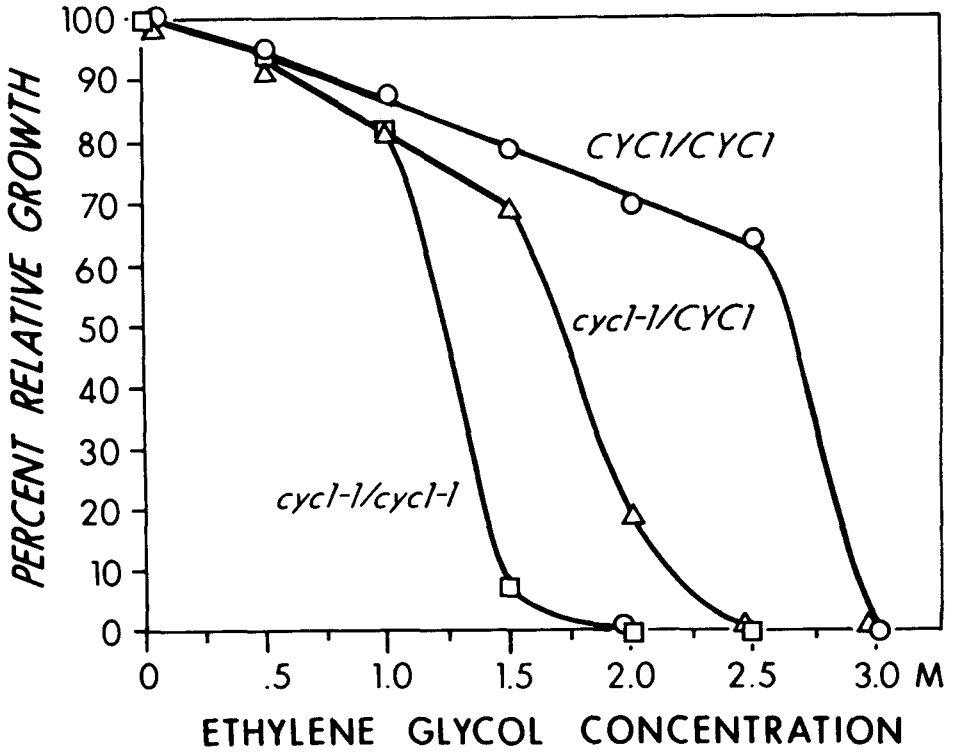

FIGURE 3.—Relative growth of normal and osmotic sensitive strains in media with various levels of hypertonicity. Stationary phase cells were used to inoculate the normal nutrient medium and nutrient medium containing various amounts of ethylene glycol. The cell density were determined by measuring turbidity after 50 hr of growth at 30°. Cell concentrations in normal nutrient medium were used as 100% growth.

TABLE 2

Phenotypes and genetic constitutions of various normal and mutant strains

Strain	Relevant genotype	Phenotypes			Genetic constitution		
		Iso-1-cyto. *c*	Osmotic sensitivity	UV sensitivity	*CYC1*	*OSM1*	*RAD7*
D311-3A	*CYC1+ RAD7+*	Normal	Normal	Normal	Normal	Normal	Normal
B-699	*cyc1-179 RAD7+*	Absent	Normal	Normal	Point mutant	Normal	Normal
CL148-21D	*CYC1+ rad7*	Normal	Normal	Sensitive	Normal	Normal	Point mutant
CL180-157C	*cyc1-13 rad7*	Absent	Normal	Sensitive	Point mutant	Normal	Point mutant
D597-6D	*cyc1-1*	Absent	Sensitive	Sensitive	Deletion	Deletion	Deletion
B-3847	*cyc1-363*	Absent	Normal	Normal	Deletion	Normal	Normal
B-3848	*cyc1-364*	Absent	Sensitive	Sensitive	Deletion	Deletion	Deletion
B-3849	*cyc1-365*	Absent	Sensitive	Sensitive	Deletion	Deletion	Deletion
B-3850	*cyc1-366*	Absent	Sensitive	Normal	Deletion	Deletion	Normal
B-4164	*cyc1-367*	Absent	Sensitive*	Normal	Deletion	Normal*	Normal
B-4165	*cyc1-368*	Absent	Sensitive	Normal	Deletion	Deletion	Normal

* The osmotic sensitivity in the B-4164 strain complemented the osmotic sensitivity of *cyc1-1* strains and was not due to a *osm1* defect (see text).

media. Conversely, *cyc1–366* and *cyc1–368* are osmotic sensitive, but not UV sensitive. It is important to note that these two mutants, like the *cyc1–1* mutation, contain complete deletions of the *CYC1* locus.

Analysis of meiotic progeny from diploids heterozygous for the *cyc1–366* mutation has shown that the osmotic sensitivity and the lack of iso-1-cytochrome *c* co-segregate, indicating that the growth inhibition on hypertonic media is caused by either the *cyc1–366* mutation itself, or by a linked gene. Complementation tests among various deletions listed in Table 2 have established that five deletions have defects in the same gene causing osmotic sensitivity. These results indicate that the *cyc1* deletions include at least part of an adjacent gene that determines osmotic sensitivity of yeast. We have designated this new gene *OSM1*.

Other genes preventing growth on hypertonic media: In these studies, we have tested the growth of several hundred different strains on hypertonic media. There is high incidence of apparently independent mutations that inhibit growth on hypertonic media. Twelve of the 210 *cyc1* mutations described by SHERMAN *et al.* (1974) had reduced growth on hypertonic media. Tests with 38 from the set of the 104 *cyc1* deletions recently isolated (SHERMAN *et al.* 1975) showed that the *cyc1–367* deletion was osmotic sensitive, similar to the four deletions, *cyc1–364*, *cyc1–365*, *cyc1–366* and *cyc1–368* described above and listed in Table 2. However, analysis of diploid strains indicated that the osmotic sensitivity of the *cyc1–367* deletion strain and of the *cyc1* point-mutant strains is complemented by the *cyc1–1* mutation. Furthermore, pedigree analysis with several of the point mutants revealed that osmotic sensitivity in these cases was not linked to *CYC1* locus. Thus, these strains contain mutant genes other than *osm1* that cause osmotic sensitivity. We have located one gene, *osm2*, on the right arm of chromosome *XVI*; no recombination between *osm2* and *aro7* was observed in eight tetrads analyzed.

In addition, there is wide variation in growth of "normal" strains on hypertonic media. Thus, designation of a strain as sensitive or normal is relative. A striking example of this variability may be seen in Figure 2. From tests with numerous strains, we have established that osmotic sensitivity caused by *cyc1–1* mutation is a recessive property. The growth characteristics of the *cyc1–1/CYC1* strain on the hypertonic medium are definitely that of a normal strain. However, the specific *CYC1/CYC1* strain exhibits better growth on the hypertonic medium than does the specific *cyc1–1/CYC1* strain. As pointed out above, the growth difference between the heterozygote and the homozygote does not mean that the osmotic sensitivity of *cyc1–1* mutants is semi-dominant.

Our studies have also revealed that certain strains carry mutations that confer sensitivities to high concentrations of some but not all compounds. These mutations can be distinguished from genes causing general osmotic sensitivity. For example, the iso-1-cytochrome *c* deficiency and osmotic sensitivity, as scored on media made hypertonic with glucose or glycerol, clearly segregated together in the meiotic progeny from the *cyc1–366* heterozygote described above. However, on hypertonic KCl or ethylene glycol media, the two phenotypes segregated independently. From tetrad analysis of crosses among various segregants from the

above cross, we found that the diploid was heterozygous for a mutation that caused extreme sensitivity to high concentration of KCl. Segregation of this mutation interfered with the scoring of osmotic sensitivity of *cyc1–366* on KCl and ethylene glycol media. Thus, when the cross was homozygous for the putative gene causing KCl sensitivity, the osmotic sensitive phenotype of *cyc1–366* mutant could be followed on all but KCl media; as expected, no segregant grew on media containing high concentration of KCl. When the hybrid did not contain the gene causing KCl sensitivity, growth inhibition caused by the *cyc1–366* mutation could be demonstrated on all types of hypertonic media.

We have described an example in detail to emphasize that there are varieties of mutations in yeast that cause general and specific growth inhibition on different types of hypertonic media. Another class of mutations that cause osmotic-sensitive growth is comprised by a number of amber and ochre suppressors (SINGH 1977).

Deletions encompassing CYC1, RAD7 *and* OSM1 *do not extend into* SUP4: *CYC1*, *RAD7*, *SUP4* and *CDC8* form a cluster of closely linked genes on the right arm of chromosome *X* (LAWRENCE *et al.* 1975). The *RAD7–SUP4–CDC8* genes may be contiguous since only one reciprocal recombination between *rad7* and *SUP4* was observed among 272 tetrads, and no reciprocal recombination between *SUP4* and *cdc8* was observed among 226 tetrads (LAWRENCE *et al.* 1975). We have investigated the possibility that some of the deletions described in this report extend into the *SUP4* locus, which codes for a tyrosine tRNA and which can mutate to form UAA and UAG suppressors. If a deletion extends into the *SUP4* locus, an active suppressor would not be expected to be recovered together with the deletion. Since the order of the relevant genes appears to be *CYC1–RAD7–SUP4–CDC8*, only the deletions that include *CYC1* and *RAD7* are expected to cover *SUP4* and possibly *CDC8*. There is, *a priori*, reason to believe that none of the deletions extend into *CDC8* gene because this is apparently an essential gene (HARTWELL 1971), and none of the deletion mutations cause conditional or recessive lethality. In any event, the results show that none of the deletions examined extend even into the *SUP4* locus.

Tetrad analysis of the cross D-672 (*cyc1–1* × *SUP4-o*) previously established that the *cyc1–1* deletion does not extend into the *SUP4* locus since one tetratype tetrad and several conversion tetrads were observed among the 233 tetrads analyzed (LAWRENCE *et al.* 1975). The recovery of *cyc1–1 SUP4–o* segregants that still contain an active UAA suppressor indicates that the *cyc1–1* deletion did not destroy the functioning of the *SUP4* locus.

We have extended the analysis to the *cyc1–364* and *cyc1–365* deletions, which encompass the *CYC1*, *OSM1* and *RAD7* genes. In addition we have analyzed the *cyc1–366* deletion, which does not include *RAD7* and is therefore not expected to extend into *SUP4*. Strains individually carrying each of the deletions were crossed to a strain carrying an inactive form of the suppressor, *sup4-o-R*, having a second-site mutation within the *SUP4* locus. By use of homozygous UAA markers, the frequencies of *SUP4–o* recombinants were determined before and after meiosis. The results, summarized in Table 3, showed that all three hybrids

TABLE 3

The frequencies of SUP4-o *recombinants per 10^6 asci or per 10^6 cells obtained from crosses between* cyc1 *deletions and the* sup4-o-R *mutant*

Cross	*cyc1-364* × *sup4-o-R*	*cyc1-365* × *sup4-o-R*	*cyc1-366* × *sup4-o-R*
Before sporulation	0.5	0.3	0.1
After sporulation	74.4	74.1	71.2

Three petri dishes, each containing from 3 to 6 × 10^7 colony-forming units, were examined for each cross, both before and after sporulation.

produced *SUP4-o* recombinants with about the same frequency. A number of the *SUP4-o* recombinants were analyzed for the presence of the *cyc1* alleles; both the *CYC1* normal allele and the *cyc1* deletion alleles were recovered from all three crosses. These results thus show that none of the deletions tested included any part of the *SUP4* locus.

DISCUSSION

We have shown in this investigation that some of the deletions that encompass the *CYC1* gene also extend into the *OSM1* gene, preventing growth on hypertonic medium, and into the *RAD7* gene, causing UV-sensitivity. The deletions extending into all three genes, *CYC1*, *OSM1* and *RAD7*, include *cyc1-1*, *cyc1-364* and *cyc1-365*. The deletions encompassing the two genes *CYC1* and *OSM1* include *cyc1-366* and *cyc1-368*. All of these *cyc1* deletions that extend into adjacent genes encompass the entire *CYC1* locus. Two deletions, *cyc1-363* and *cyc1-367*, encompass the entire *CYC1* locus, but do not extend into adjacent genes. In addition, none of the deletions that encompass only one or the other terminus of the *CYC1* locus were found to extend into the *OSM1* or *RAD7* genes. No *cyc1* deletion was observed to extend into the *SUP4* locus, which is tightly linked to the *rad7* gene. A deletion map, summarizing these findings, is presented in Figure 4. Since *osm1* point mutants are not available, the order of the *CYC1* and *OSM1* genes can not be determined. However, the lack of deletions covering

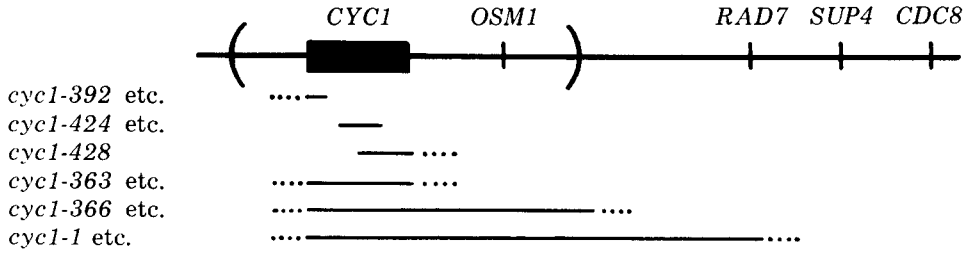

FIGURE 4.—A summary deletion map of the *CYC1* region. The order of the *CYC1* and *OSM1* genes is unknown.

only *CYC1* and *RAD7* is consistent with the order *CYC1–OSM1–RAD7*. Also the orientation of the *CYC1* locus can not be established from these results since the deletions that partially cover the *CYC1* locus do not extend into the *OSM1* or *RAD7* genes.

The results suggest that the *CYC1*, *OSM1* and *RAD7* genes are contiguous and establish that no essential genes are located within the region of this gene cluster. A genetic distance of 1 to 1.5 cM for the separation of the *cyc1* and *rad7* mutants was determined from the meiotic analysis of point mutants (LAWRENCE *et al.* 1975). Since the total length of the chromosomes exceeds 3000 cM (MORTIMER and HAWTHORNE 1975), the longest deletions constitutes less than 0.03% of the total yeast genome. Meiotic analysis indicated that the genetic distances between *rad7* and *SUP4* and between *SUP4* and *cdc8* were even shorter, less than 0.5 cM. Thus it appears as if this short chromosomal region may be comprised of only the five genetic loci, *CYC1–OSM1–RAD7–SUP4–CDC8*.

The lesion in the *osm1* mutant remains elusive since there may be a variety of mechanisms by which mutations prevent growth on hypertonic media. A special class consists of some but not all nonsense suppressors that cause growth inhibition on hypertonic media (SINGH 1977). Mutants inhibited in hypertonic media are reminiscent of mutants inhibited by elevated temperature. Both osmotic sensitivity and temperature sensitivity arise by mutation of any one of a large number of different loci and both types of mutants are easily detected among unselected survivors of mutagenic treatments. Perhaps osmotic-sensitive mutants, analogous to temperature-sensitive mutants, have alterations of essential proteins, making them unusually sensitive to hypertonic conditions.

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