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Toxicity of 2-Deoxygalactose to *Saccharomyces cerevisiae* Cells Constitutively Synthesizing Galactose-Metabolizing Enzymes

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Analysis of 400 independent spontaneous mutations conferring 2-deoxygalactose resistance upon cells constitutive for the galactose pathway suggests that toxicity is due to 2-deoxygalactose-1-phosphate. Selection for and against growth on galactose in the same strain is now possible; application to systems with transcriptional or translational gene fusions to galactokinase are discussed.

The inducible components of the enzyme system required for galactose utilization in *Saccharomyces cerevisiae* include a specific galactose transport activity (4, 6) and three enzymes in the catabolic pathway, galactokinase, galactose-1-phosphate uridyltransferase (transferase), and uridine diphosphoglucose 4-epimerase (epimerase), respectively (7, 12, 14, 16). The glucose-1-phosphate produced by these inducible enzymes from galactose is then converted to glucose 6-phosphate (by the *GAL5* [*PGM2*] product, one isozyme of phosphoglucomutase; [5]), which in turn enters the glycolytic pathway. A schematic representation of these genes, their relationship to one another, and the interconnected pattern of regulation is shown in Fig. 1.

Yeast cells that are defective in transferase or epimerase will die if grown on galactose, due to accumulation of a toxic intermediate. This property formed the basis of genetic selections demanding growth on galactose of *gal7* or *gal10* strains, which led to the discovery of the regulatory genes for the pathway, *GAL80* and *GAL4* (8, 10, 24). The scheme for regulation postulates that the *GAL4* protein is synthesized constitutively (15, 19, 25) but that its activity is blocked (in the absence of the inducing effect of galactose) by interaction with the *GAL80* protein (Fig. 1). Balanced synthesis of the two proteins appears necessary to maintain normal regulation (11).

Regulation of transcription of the *GAL1-GAL10-GAL7* cluster occurs at the transcriptional level, and the *GAL4* product appears to be a positive effector acting at the respective promoters for each of the genes (30). Certain mutations in the regulatory genes can result in constitutive synthesis of the structural genes. Those designated as *gal80* result in defective repression, whereas those termed here as *GAL4*^{c} (constitutive) (also called *GAL81*) are dominant mutations of *GAL4* that render the activator protein insensitive to the normal repressing action of the *GAL80* product (8, 18).

In a search for a gratuitous (nonmetabolizable) inducer of the galactose-utilizing system, Adams (1) analyzed the effects of 2-deoxygalactose (2DG) on induction of the enzymes. No detectable effects were found, and an additional experiment to test whether 2DG could serve as a substrate for galactokinase was negative. Further studies on *S. cerevisiae* with this analog have not been reported in the literature, but it is toxic to *Salmonella typhimurium* cells induced for the galactose metabolic pathway (2) and also to mammalian cells (31).

To test whether *Saccharomyces* cells were similarly susceptible, two different strains, *gal80* and *GAL4*^{c} (from Y. Oshima), which synthesize the galactose-metabolizing enzymes constitutively, were plated on media of various types, with and without 2DG. Both strains behaved similarly in their responses to 2DG on various media. Most striking was the killing of both strains on minimal medium with glycerol (3%), lactate (2%), or glycerol plus lactate as the carbon source, at concentrations of 2DG as low as 0.02%. When galactose or glucose (at 2%) was used instead of glycerol or lactate or glycerol plus lactate, no significant killing by 2DG was observed. This is evidently related to the relative proportions of the sugars, because toxicity was again evident when galactose or glucose were present in the plates at 0.1%, and 2DG was used at 2%. On YEP plates (1% yeast extract, 2% peptone), 2DG was also toxic to the constitutive strains at 0.02%, as long as no additional carbon sources are added. No differences were found on minimal medium-glycerol-2DG plates as a function of temperature: cells were killed equally efficiently at 23, 30, and 37°C.

In an attempt to decipher the mechanism(s) involved in killing by 2DG, the *gal80* and *GAL4*^{c} strains were plated on YEPD (2% glucose) at 100 to 200 colonies per plate, and these were replicated onto minimal medium-glycerol-2DG plates. Resistant mutants became evident as small colonies that grew from the original colony imprints within 3 to 4 days at 30°C. To guarantee independent events, only one revertant was picked from each original colony. After repurification on the same medium, the revertants were analyzed by complementation against known mutants on galactose indicator plates. The results of examining over 400 independent mutants are presented in Table 1.

Since strains carrying either of the constitutive mutations were resistant to 2DG if they were also *gal2* (Table 1; verified for a reconstructed *gal80 gal2* strain), accumulation of 2DG to toxic levels must require the normal galactose transport activity. The toxic product must then be produced at or after the phosphorylation step, since nearly half of the revertants from a *gal80* parent were *gal1*; a similar number were *gal4*. Surprisingly, survivors from the *GAL4*^{c} parent had a markedly different distribution: only 15% were *gal4*, and 75% were *gal1*. Since the galactokinase levels were similar in both parental strains (data not shown), the simplest interpretations for this ratio difference is that it is slightly more difficult to revert the dominant *GAL4*^{c} gene (to *GAL4*$^+$ or *gal4*) than to eliminate *GAL4*$^+$ function. It is not clear why mutations in *GAL2* (the permease) are so much less frequent than those in *GAL1* and *GAL4*.

To test whether the lack of lesions in *GAL5*, *GAL7*, and *GAL10* was due to statistical fluctuation within the sample size or the inability of mutations in these loci to confer resistance to 2DG, several doubly mutant strains were

constructed. For the alleles used, the following strains were all sensitive to 2DG: *gal80 gal5*, *gal80 gal7*, *GAL4*^{c} *gal7*, and *GAL4*^{c} *gal10*. Doubles with *gal3* have not yet been constructed, and the *gal11* locus (22) was unavailable for testing. Though not all permutations with these loci and *gal80* or *GAL4*^{c} have been examined, there is no reason to suspect that the results from other specific combinations would differ. Since *gal7* lesions cannot confer resistance, the toxicity of 2DG must occur before the transferase reaction. These results strongly suggest that 2-deoxygalactose-1-phosphate (or a metabolic byproduct thereof) is the toxic product, as suggested by Alper and Ames (2) for *S. typhimurium*. Because toxicity was observed on both glycerol and lactate, a metabolic block specific to neither pathway could account for the 2DG effect. Analysis of the alleles conferring 2DG resistance should ultimately elucidate this question.

The unidentified *gal* mutants (Table 1), termed *rdg* (for resistance to 2-deoxygalactose), were all unable to grow on galactose-minimal medium; hence, some defect in metabolism of this sugar is suggested. Thus far, three *rdg* alleles that have been tested are recessive in both *gal80* and *GAL4*^{c} diploid strains. These 2DG-resistant mutations also segregate 2:2 after sporulation; specific linkage to particular genes or to each other has not yet been tested. Some of the *rdg* alleles could be similar to previously identified mutations, such as the dominant, uninducible *GAL80*^{s} (9, 23). The *gal11* mutation, which results in a general depression of the galactose enzyme levels (22), might also confer resistance to 2DG. Similarly, catabolite repression could account for the survival of the constitutive strains when grown on glucose plus 2DG. In the case of the galactose bypass of toxicity, the most plausible explanation is that the affinity-binding constant of galactokinase for 2DG is considerably weaker than for galactose; this may account for the observation of Adams (1) that 2DG was not a substrate for galactokinase, since those experiments had, at most, a 10-fold excess of 2DG over galactose (1 mM versus 0.1 mM).

Regardless of the mechanism of killing by 2DG, it offers a powerful selection against cells that express galactokinase at high levels. Previously, this could only be done in *gal7* or *gal10* strains, making it impossible to do a positive selection for growth on galactose in the same strains. Fortunately, the galactokinase gene (*galK*) from *Escherichia coli* can function in eucaryotes (26). Functional *E. coli-S. cerevisiae* shuttle vectors containing both transcriptional and translational gene fusions to *galK* have been made, and selection for *GAL*$^+$ transformants in a *gal1* yeast host is straightforward (27). The only requirement for using similar vectors in a negative selection with 2-deoxygalactose is a host that is *GAL2* and *gal1* (preferably a nonreverting deletion); in some cases, a *gal80* or *GAL4*^{c} mutation may be advantageous, so that the other enzymes of galactose metabolism are fully induced. Experiments are in progress to utilize this scheme to select for mutations in the import of the galactose regulatory proteins to the nucleus. All indications suggest that negative selections with 2-deoxygalactose will be of general utility in *S. cerevisiae*, and possibly in higher eucaryotes (31), in any system where galactokinase expression can be coupled to structural or regulatory functions.

TABLE 1. Complementation analysis of 2DG-resistant mutants

Complementation group	No. of revertants (%) from parent strain:	
	gal80	*GAL4*^{c}
gal1	73 (46.8)	190 (74.2)
gal2	1 (0.6)	3 (1.2)
gal3	0	0
gal4	68 (43.6)	39 (15.2)
gal5	0	0
gal7	0	0
gal10	0	0
GAL$^+$	6 (3.8)	1 (0.4)
gal (rdg)	9 (5.8)	21 (8.2)

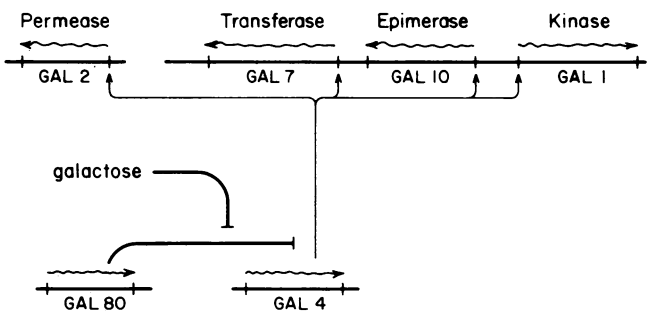

FIG. 1. Galactokinase, transferase, and epimerase are encoded by the three genes *GAL1*, *GAL7*, and *GAL10*, respectively, grouped in a closely linked cluster on chromosome II (3, 7), whose fine structure has been mapped at the DNA level (28, 29). The transport protein is encoded by *GAL2* on chromosome XII (4, 21). Expression of three structural genes is controlled by at least two regulatory genes, *GAL4* and *GAL80* (see the text). It is further subject to catabolite repression (1, 17, 20) and the effects of some partially characterized genes, *GAL3*, *GAL5 (PGM2)*, and *GAL11* (not shown in the figure). Mutations in *GAL3* exhibit pleiotropic defects in melibiose and maltose utilization (13); lesions in *GAL11* appear to affect specifically the galactose enzymes (22). The mechanisms of *GAL3* and *GAL11* participation are as yet unknown.

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