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Galactose Transport in *Saccharomyces cerevisiae*

I. Nonmetabolized Sugars as Substrates and Inducers of the Galactose Transport System

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The inducible galactose transport system in bakers' yeast carries out the facilitated diffusion of the nonmetabolized galactose analogues D-fucose and L-arabinose. This capacity depends on the activity of the Ga 2 gene. In some strains, D-fucose and L-arabinose are also gratuitous inducers. Mutants in which the inducibility of the galactose pathway enzymes is altered show a parallel alteration of the inducibility of the galactose transport system.

Galactose metabolism in bakers' yeast involves the induction of a specific transport system and the galactose pathway enzymes: for adenosine triphosphate, α-D-galactose-1-phosphotransferase (E.C. 2.7.1.6); for uridine diphosphoglucose, α-D-galactose-1-phosphate uridylytransferase (E.C. 2.7.7.12) and uridine diphosphoglucose 4-epimerase (E.C. 5.1.3.2). The structural gene for the galactose transport system, Ga 2, is on chromosome XII; the structural genes for the enzymes, Ga 1, Ga 7, and Ga 10, respectively, are clustered as a single operon on chromosome II (12, 21). Mutants deficient in the structural genes for the phosphotransferase (or "galactonkinase"), uridylyltransferase, or the epimerase are phenotypically galactose-negative. Mutants deficient in the galactose transport system are galactose-negative in media of low galactose concentrations but are galactose-positive in media of high galactose concentrations (12, 24).

In the experiments reported below, the characteristics of the galactose transport system were studied by use of the nonmetabolized galactose analogues D-fucose and L-arabinose.

MATERIALS AND METHODS

Yeast strains. The haploid strains of *Saccharomyces cerevisiae* used in this study are listed in Table 1. They were kindly supplied by Howard C. Douglas. One diploid strain, L-14, was isolated from a cake of commercial Anheuser-Busch bakers' yeast and is the same yeast used in previous studies (5-7, 27).

Growth conditions. Two types of liquid media were used. The standard medium contained: 1% tryptone (Difco), 0.3% yeast extract (Difco), 0.4% KH_2PO_4 (adjusted to *p*H 7.0), and 2% D-glucose or D-galactose. Induction medium contained: 2% peptone (Difco), 1% yeast extract, 0.2% D-glucose, and 0.2% inducer. The induction medium was used to test the effectiveness of nonmetabolized sugars as gratuitous inducers and to induce the galactose transport system in galactose-negative mutants (12). The sugars and the sugar-free portions of the growth media were autoclaved separately as 2× concentrated solutions and were mixed aseptically before use. The cells were grown overnight in 250 ml of liquid medium in 500-ml flasks on a rotary shaker at 30 C. Each flask was inoculated with the growth from a 24-hr Sabouraud Dextrose Agar (Difco) slant.

Procedure for measurement of sugar transport. The cells were harvested and washed by centrifugation in demineralized water. The volume of the packed cells was determined, and a 50% (v/v) suspension was prepared. In most experiments, 0.75-ml portions of this 50% suspension were added to tubes containing 0.75 ml of a sugar solution containing 2× the desired final sugar concentration. The incubations were carried out at 30 C, and the cells were mantained in uniform suspension by a magnetic stirrer. The cell suspensions and the sugar solutions were temperature-equilibrated before mixing. At the desired intervals (usually six 30-sec intervals), 0.2-ml samples were removed from the incubation mixture and transferred to chilled centrifuge tubes containing 5 ml of ice-cold demineralized water. The cells were centrifuged at 3,000 × *g* for 1 min, and the packed cells were washed with two 5-ml portions of ice-cold demineralized water. After the second wash, the cells were suspended in 2 ml of demineralized water and extracted according to the method of analysis to be used. For radiochemical assay, the cells were extracted by boiling for 10 min. For chemical assay, the cells were incubated in the demineralized water for 1 hr at 30 C. (This extraction was found to be as effective as boiling in releasing ^{14}C-labeled sugars from cells previously incubated in these sugars for short time intervals. It was less efficient than boiling when the cells had reached equilibration with external sugar. The 30 C procedure was used because it produced

TABLE 1. *Yeast strains used*

Strain	Galactose genotype	Galactose phenotype	
		Growth	Inducibility
106-1B	Wild type	+	Inducible
1446-87B	Wild type	+	Inducible
346-3A	ga 1, ga 2	−	Inducible
346-3B	Wild type	+	Inducible
346-3C	ga 1	−	Inducible
346-3D	ga 2	+[a]	Inducible
108-3A	i⁻	+	Constitutive
122-2C-1D	C	+	Constitutive
107-5A	ga 3	+	Slow induction
279-1A	ga 4	−	Noninducible

[a] This strain is galactose-positive only in media of high galactose concentrations (11). The four 346 strains are the tetrad from the same ascus.

TABLE 2. *Time course of L-arabinose and D-fucose uptake at 30 C by wild-type (346-3B) cells grown either on D-glucose or D-galactose medium*[a]

Growth sugar	Transport sugar	Transport time	Amt (mg) of sugar/ml of cell water
		min	
D-Glucose	L-Arabinose	30	0.7
		60	1.1
		90	1.3
		150	2.4
		180	2.5
D-Galactose	L-Arabinose	0.5	3.4
		1	6.7
		1.5	7.4
		2	11.8
		2.5	10.1
		3	10.5
D-Glucose	D-Fucose	0.5	0.07
		2.5	0.21
		4.5	0.34
		6.5	0.45
		8.5	0.51
		10.5	0.65
		12.5	0.72
		14.5	0.83
D-Galactose	D-Fucose	0.5	4.1
		2.5	8.1
		4.5	14.5
		6.5	16.3
		10.5	18.1
		12.5	19.9
		14.5	18.1

[a] The extracellular L-arabinose concentration was 25 mg/ml; the D-fucose concentration was 50 mg/ml.

extracts with low blanks. This is especially important for pentose uptake experiments.)

RESULTS

Facilitated diffusion of D-fucose and L-arabinose. The data in Table 2 compare the time course of uptake of the nonmetabolized analogues of D-galactose, D-fucose and L-arabinose, in glucose-grown and galactose-grown cells. The rate of L-arabinose uptake was well over 100 times greater in galactose-grown cells than in glucose-grown cells; D-fucose uptake was about 50 times greater.

It is significant that the maximum intracellular concentrations did not exceed the extracellular concentrations. To test this point further, the ratio of internal to external concentration of L-arabinose was tested over a wide range of external concentrations. The internal concentration of L-arabinose never exceeded the external concentration (Table 3). Consistent with the nonconcentrative nature of this uptake, uptake was not inhibited by 10 mM azide, cyanide, or arsenate under the identical conditions of exposure as found by Okada and Halvorson (23) to inhibit the inducible α-glucoside active transport in bakers' yeast.

Sugar uptake by transport mutants. Douglas and Condie (11) described galactose transport mutants (ga 2) which could grow on galactose only if the galactose concentration in the growth medium was high. de Rubichon-Szulmajster (24) showed that these mutants have a reduced rate of ^{14}C-D-galactose uptake. L-Arabinose and D-fucose uptake by ga 2 cells grown on galactose also reflect this transport deficiency. Table 4 shows that L-arabinose and D-fucose uptake by the four segregants from an ascus produced from a cross between a galactokinaseless (ga 1) and a transportless (ga 2) strain is dependent on the Ga 2 gene but independent of the Ga 1 gene.

Induction of the transport system by nonmetabolized sugars. Since the galactose transport system is inducible by galactose in galactokinaseless cells in which galactose is not metabolized, it was of interest to determine whether the nonmetabolized galactose anologues could also serve as gratuitous inducers. In some strains, these nonmetabolized sugars do serve as inducers; however, in others they are inactive (Table 5). A cross between an arabinose-inducible strain (1446-87B) and an arabinose-noninducible (106-1B) strain yielded an arabinose-inducible zygote (Table 5). Arabinose inducibility is thus dominant over noninducibility.

Sugar transport in regulatory mutants. Douglas and Hawthorne isolated four mutants in which the inducibility of the galactose pathway enzymes is altered (*see* Table 1). The rate of L-arabinose

TABLE 3. *Concentration ratios of L-arabinose in galactose-grown L-14B cells*[a]

Extracellular molarity	Intracellular-extracellular ratio			
	15 min	30 min	105 min	120 min
1.3×10^{-5}	0.19	0.30	0.45	0.48
1.3×10^{-4}	0.29	0.27	0.62	0.73
1.3×10^{-3}	0.20	0.18	0.72	0.72
1.3×10^{-2}	0.33	0.27	0.71	0.85
1.3×10^{-1}	0.21	0.26	0.76	0.62

[a] Ratios are expressed as intracellular ^{14}C-L-arabinose to extracellular.

TABLE 4. *Sugar transport by the four segregant cultures from a single ascus produced by a cross between a galactokinaseless (ga 1) and a transportless (ga 2) strain*[a]

Ascopore	Genotype	Phenotype	Sugar uptake (mg/ ml of cell water)	
			L-Arabinose	D-Fucose
346-3A	ga 1, ga 2	Kinaseless + transportless	0.2	
346-3B	Ga 1, Ga 2	Wild Type	5.4	8.1
346-3C	ga 1, Ga 2	Kinaseless	5.0	
346-3D	Ga 1, ga 2	Transportless	0.3	0.6

[a] The cultures were induced by growth for 24 hr at 30 C in induction medium containing 0.2% glucose plus 0.2% D-galactose. Uptake was measured from an external sugar concentration of 50 mg/ml for 2 min at 30 C.

uptake in the four mutants is shown in Table 6. It is clear from these results that the mutations affect the galactose pathway enzymes and the transport system in a parallel manner. Data on the strictness of the coordination of the control of these separate gene loci will be presented in the second paper of this series.

DISCUSSION

The data presented above show that the non-metabolized sugars, D-fucose and L-arabinose, are substrates of the inducible galactose transport system and that they are transported by a facilitated diffusion mechanism. The nonconcentrative nature of this system distinguishes it from other, previously described inducible transport systems, all of which are active transport systems. This fact serves to emphasize the fundamental similarity between facilitated diffusion and active transport (4, 18, 23). The central, common feature in these two processes is the involvement of a stereospecific carrier or *transporteur.* It is tempting to speculate, therefore, that the product of the transport gene in both cases is the carrier molecule. The energy-coupling mechanism which is unique to the active transport systems would then be an independent step (presumably under independent genetic control). Such a concept is supported by data from the *Escherichia coli* β-galactoside permease system (15, 17, 18).

Irrespective of the basis of the difference between the induced facilitated diffusion and active transport systems, there are a number of interesting parallels between the yeast and the *E. coli* galactose transport systems. One feature of the

TABLE 5. *Induction of the galactose transport system by D-fucose and L-arabinose*[a]

Strain	Amt (mg) of L-arabinose/ml of cell water			
	No-inducer	D-Galactose	D-Fucose	L-Arabinose
346-3B	0.1	5.5		2.2
346-3C	0.0	10.8	5.0	10.1
1446-87B	0.4			3.2
L14-B	0.3	5.0	0.3	0.3
106-1B	0.1	3.7		0.2
Zygote (1446-87B × 106-1B)	0.0			2.4

[a] L-Arabinose uptake was measured at 2 min at 30 C from an external concentration of 50 mg/ml. The inducers were present in the induction medium at 0.2%.

TABLE 6. *L-Arabinose uptake by regulatory mutants grown on galactose induction medium for 24 hr*[a]

Genotype	Inducer	Amt (mg) of L-arabinose/ml of cell water
Wild type	None	0.1
	D-Galactose	5.4
i^-	None	3.1
	D-Galactose	6.5
C	None	2.4
	D-Galactose	7.4
ga 3	None	0
	D-Galactose	0.1
ga 4	None	0
	D-Galactose	0

[a] The extracellular L-arabinose concentration was 50 mg/ml; uptake was measured after 2 min at 30 C.

E. coli galactose system not exhibited by bakers' yeast is the phenomenon of endogenous induction of the remaining galactose genes in galactokinaseless mutants (16, 29). Wu (28) has recently shown that endogenous induction depends upon the capacity of the galactose permease to accumulate an intracellular galactose pool derived from endogenous biosynthesis of galactose. In the absence of the accumulating capacity of the galactose permease (i.e., in galactose permeaseless mutants), endogenous induction is not observed. The absence of the phenomenon of endogenous induction of the remaining galactose genes in galactokinaseless mutants in bakers' yeast may thus be a reflection of the nonconcentrative nature of the yeast galactose transport system.

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