SPACE LIMITATION FOR PERMEASE INSERTION IN THE CYTOPLASMIC MEMBRANE OF SACCHAROMYCES CEREVISIAE

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SUMMARY

The initial uptake rate of several exogenous substrates was measured in a series of isogenic strains of yeast including haploid, diploid, triploid and tetraploid organisms. For three substrates, whose transport is catalyzed by constitutive permeases, the relative uptake rate (uptake rate per unit of cell mass, or per genome) decreases in the same proportions as the ratio surface to volume. For other substrates, whose transport is inducible, this limitation was not observed. This is interpreted to mean that the space for permease insertion in the cytoplasmic membrane is limited for the constitutive permeases studied. The implications thereof are discussed.

In a study of the synthesis of lac operon proteins as a function of gene dosage in E. coli, Fox (1) observed that whereas a strain of E. coli diploid for the lac operon has twofold β-galactosidase and transacetylase activities and probably twice as much M protein as an haploid, the β-galactosides transport activity is increased by a factor of 1.1 to 1.4 only. As pointed out by the author, these data indicate that some factor other than the M protein is rate limiting for formation of a functional lactose transport system at high levels of operon expression.

We present a possible interpretation of these observations, which is suggested by a study of the uptake of exogenous substances in a polyploid series of yeast. In a series of isogenic yeast strains of increasing ploidy, the biosynthetic potential for a given permease increases with the degree of ploidy. The fourfold genome of a tetraploid in principle allows the cell to produce a

fourfold amount of each of its constituents. However, if the cell membrane is saturated (or nearly saturated) with a given permease in a haploid strain, the cell surface might become more and more limiting for permease insertion with increasing ploidy, since the increase in cell surface is smaller than the increase in cell mass or cell volume. That this appears to be the case for several permeases in yeast is shown by the following observations.

MATERIALS AND METHODS

All the strains used were derived from the previously described strain of Saccharomyces cerevisiae Σ1278b (2). The method for obtaining isogenic polyploid strains and verifying their degree of ploidy by genetic analysis will be described elsewhere.

The uptake rate was measured as previously (2) : after introducing a (^{14}C)-labelled substrate into a culture growing exponentially in M.am medium (3), samples were removed every half minute for 2 minutes, filtered, washed and assayed for radioactivity. Since very precise measurements of initial velocity of uptake were needed, each value was obtained in the following way. At least five measurements of uptake rate of a given substrate were made for each culture, as a function of increase in cell population. An exemple is shown in figure 1.

RESULTS

Cell size and cell shape vary as a function of the degree of ploidy. Cell surface and volume were calculated from measurements on photographs of cultures growing exponentially in M.am medium. The volumes and surfaces were calculated for each cell individually, using the geometrical shape which is the best approximation to actual cell shape (ellipsoid, half sphere + half ellipsoid, or cylinder + 2 half spheres). The mean values, which are given in Table I, were obtained in each case by measuring all the cells of several microscopic fields (50 to 100 cells).

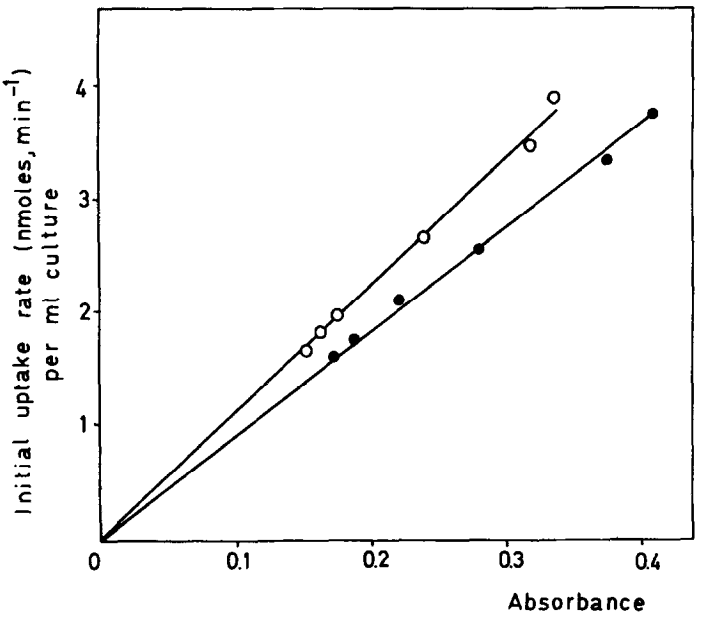

Fig. 1. Each point corresponds to a determination of initial uptake rate made on a sample of culture growing exponentially in M.am medium.
Initial uptake rate of 0.04 mM ^{14}C-L-arginine in Σ1278b, α haploid strain : (○), and in D_2Σ1278b, αα diploid strain : (●).

TABLE I

Surface and volume of Saccharomyces cerevisiae

as a function of degree of ploidy

	haploid	diploid	triploid	tetraploid
Strain	Σ1278b	D_1Σ1278b	T_3-3002	Q103
Mating type	α	αα	aαα	aaαα
Mean surface (μ^2)	45.3	70.7	95.7	114.4
Mean volume (μ^3)	29.3	54.7	83.0	104.5
Ratio surface/volume	1.54	1.29	1.15	1.09
Relative s/v ratio	1	0.83	0.74	0.70

The ratio dry weight to absorbance does not vary as a function of the degree of ploidy.

The initial velocity of uptake of several substrates in a polyploid series is shown in Table II. For arginine, lysine and uridine, the uptake rate per cell mass decreases with increasing degree of ploidy in the same proportions as the ratio cell surface to cell volume. However, the uptake rate of methionine and that of leucine do not seem to be subject to the same limitation.

TABLE II

Initial uptake rate of several exogenous substrates as a function of degree of ploidy

	haploid	diploid	triploid	tetraploid
Relative surface to volume ratio	1	0.83	0.74	0.70
0.02 mM substrate	Initial uptake rate (nmoles/mg protein/minute)			
L-arginine	20	16	15	14
	1	0.80	0.75	0.70
L-lysine	7	5.6	-	4.8
	1	0.80	-	0.70
Uridine	1.10	0.92	0.85	0.82
	1	0.83	0.77	0.73
L-methionine	5.0	5.2	-	-
	1	1	-	-
L-leucine	0.40	0.38	0.35	0.40
	1	0.95	0.92	1

Underlined data are relative values. - : not tested for. Substrates in the first column are taken up by specific permeases (2,3,4,5,6). Methionine and leucine uptake are increased after induction (6). The uptake test here is made on uninduced cells (minimal medium M.am).

DISCUSSION

Absence of full expression of the genetic potential is clear in the case of uptake of arginine, lysine and uridine by the corresponding specific (2,4,3) permeases in a polyploid series of yeast, in contrast to full expression of a non-regulated intracellular enzyme like tryptophan synthetase (7). The fact that limitation of uptake rate is proportional to decrease in cell surface to cell volume ratio strongly suggests that limitation is due to lack of space for insertion of the transport system in the membrane. If this is the right interpretation, it means that the membrane is already saturated with the arginine, the lysine and the uridine permeases respectively in haploid cells. That this is not the case for methionine and leucine uptake is in agreement with the observation that these amino acids are transported by inducible systems (6), since if the membrane were saturated with these uptake systems in the uninduced haploid cells no induction could be observed.

The fact that space for insertion appears to be limited for some (constitutive) permeases but not for other (inducible) uptake systems suggests that the sites for permease fixation in the membrane might be specific, as proposed by Anraku (8).

In *E. coli* diploid for the lac operon, two structural genes for β-galactoside permease are present in a cell whose surface is not increased. If the membrane of a haploid cell is nearly saturated with β-galactoside permease, no doubling of uptake activity could be observed.

The reported observations raise the question of what happens to the permease molecules which do not find a place in the membrane. They might accumulate in the cytoplasm, as seems to be the case in *E. coli* for the β-galactoside permease (1), but alternatively there might be a special type of regulation of permease formation involving the permease itself as a regulatory factor of its own synthesis.

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