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What Is the Function of Nitrogen Catabolite Repression in *Saccharomyces cerevisiae*?

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In contrast to the previously held notion that nitrogen catabolite repression is primarily responsible for the ability of yeast cells to use good nitrogen sources in preference to poor ones, we demonstrate that this ability is probably the result of other control mechanisms, such as metabolite compartmentation. We suggest that nitrogen repression is functionally a long-term adaptation to changes in the nutritional environment of yeast cells.

A paradigm for control of nitrogen catabolism in *Saccharomyces cerevisiae* has been developed (4). According to this model, the production of most catabolic enzymes is inducible, with one of the degradative intermediates acting as an inducer. Superimposed on and dominant to the induction process is control by nitrogen catabolite repression. For example, if a poor nitrogen source, such as allantoin or proline, is provided, synthesis of the appropriate degrading enzymes is induced. However, if a "good" and hence repressive nitrogen source, such as glutamine or asparagine, is provided at the same time or subsequently, the production of the allantoin- or proline-degrading enzymes does not occur or ceases; i.e., induction is repressed. It has been presumed for a long time that the combination of induction and catabolite repression enables cells to utilize good nitrogen sources in preference to poor ones. A corollary of this model is that provision of a good nitrogen source to cells growing on a poor one should result in a dramatic shift in metabolism and in the nitrogen source used. However, this view does not address the fate of previously formed enzymes that were degrading the poor nitrogen source or the effects of these enzymes on the mode of metabolism that is used after provision of a good nitrogen source; moreover, it also fails to address the time scale over which repression is implemented, an important consideration if mutants defective in this control mechanism are to be successfully isolated and characterized.

Motivation for the experiments described in this paper came from our previous observation that after an inducer (urea) was removed from the culture medium, enzyme (allophanate hydrolase) activity plateaued but did not decrease (4, 9). In other words, the enzyme appeared to be stable. The number of units of activity per cell decreased as the inverse of cell doubling, leading us to conclude that the intracellular enzyme concentration decreased only due to simple dilution. If repression resulted in the same behavior (i.e., cessation of de novo synthesis and loss of activity only due to dilution), it would be unable to fulfill the function traditionally ascribed to it. In this paper we describe experiments in which we studied both the time course with which repression was implemented and the fate of previously synthesized enzyme. We show that nitrogen catabolite repression is incapable of altering the functional direction of metabolism in the short term. This conclusion necessitates that the previous model for control of nitrogen catabolism be enlarged to include additional regulatory mechanisms that are capable of more rapid responses to a changing external environment and be amended to account for the consequences of the time lag between the onset of repression and its functional implementation.

(A preliminary report of the results has appeared previously [3].)

MATERIALS AND METHODS

Strains and culture conditions. The strains used in this work are described in Table 1. All strains were derived in a strain M-25 genetic background. It should be pointed out that strains in this genetic background are partially resistant to the repressive effects of ammonia. This partial resistance permits reasonable synthesis of enzymes that are sensitive to nitrogen repression. However, the general amino acid permease (*GAP1*), which is highly sensitive to repression, is largely inactivated by the culture conditions which we used.

We used the medium of Wickerham (12) containing 0.6% glucose as the sole carbon source. The nitrogen sources were provided at concentrations of 0.1% unless specified otherwise.

Enzyme assays. Allophanate hydrolase and urea amidolyase were assayed by the methods of Whitney

TABLE 1. Strains of *S. cerevisiae* used in this work

Strain	Genotype
M-25	*MAT***a** *his6 ura1 lys1*
	*MAT*α *ade6 leu1*
M-85	*MAT***a** *his6 ura1 dal1*
	*MAT*α *ade6 leu1 dal1*
M-58	*MAT***a** *his6 ura1 lys1 car1*
	*MAT*α *ade6 leu1 car1*
M-1498	*MAT***a** *his6 ura1 lys1 car1 CAN1*
	*MAT*α *ade6 leu1 car1 CAN1*

et al. (11). Arginase was assayed by the method of Bossinger and Cooper (1), and allantoin uptake was measured by using the procedures of Sumrada and Cooper (8).

RESULTS AND DISCUSSION

To ascertain the fate of a repressible enzyme after the onset of nitrogen repression, we grew two wild-type cultures of *S. cerevisiae* under inducing conditions; in one case urea was provided to induce production of allophanate hydrolase, and in the other arginine was used to induce arginase synthesis. After a short induction time, asparagine was added to each culture. As Fig. 1A and B show, neither enzyme activity decreased over the 5-h duration of the experiment. The number of units of enzyme activity per cell decreased as the inverse of cell doubling (Fig. 1C and D), just as in our previous experiment involving removal of the inducer (9). The observed cellular half-lives of arginase and allophanate hydrolase were estimated to be 120 and 150 min, respectively, or just slightly greater than the doubling time of the cells. As shown previously, this discrepancy arises from the fact that a basal level of enzyme production continues (9). If it is argued that functional switching from one mode of metabolism to another (i.e., a derepressed condition to a repressed condition) requires a 90% loss of active enzyme, then we must conclude that three to four generations of

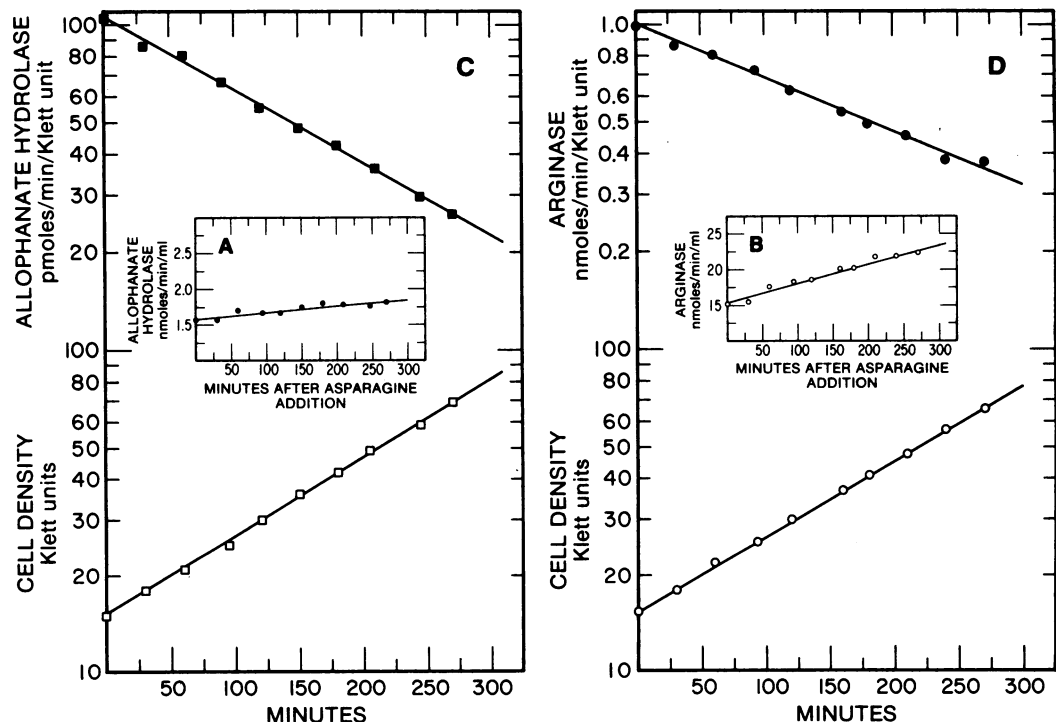

FIG. 1. Effect of the onset of nitrogen catabolite repression on allophanate hydrolase and arginase activities. (A) Strain M-25 was grown overnight to a cell density of 50 Klett units (100 Klett units is equivalent to about 3×10^7 to 4×10^7 cells per ml of culture). At that time the culture was diluted to a density of 6 Klett units with prewarmed, preaerated glucose-ammonia medium. Urea was added to a final concentration of 10 mM, and the culture was allowed to grow to a cell density of 15 Klett units. Asparagine (final concentration, 0.1%) was added at that time, and samples were removed at the times indicated. (B) Experiment performed in the manner described above, except that arginine (final concentration, 10 mM) was provided in place of urea as the inducer. (C and D) The enzyme activities plotted in (A) and (B) were divided by the cell density of each sample. The resulting values were then plotted on semilogarithmic coordinates.

cell growth are required to implement nitrogen catabolite repression. Processes operating over such an extended time are not useful for fine, rapid control of metabolism. These considerations cast doubt on catabolite repression as the most important regulator of short-term nitrogen metabolism and prompted us to look elsewhere for a rapid, sensitive first-line control mechanism.

A second potential means of regulating metabolism is by metabolite compartmentation. To ascertain whether compartmentation is an important mechanism of regulation, we repeated the experiment described above but measured allantoin transport in place of allophanate hydrolase activity. As Fig. 2A and B show, the level of allantoin transport dropped precipitously within minutes of the addition of asparagine to the culture medium. This response time was that expected of a fast-acting regulatory system and leads us to conclude that exclusion of allantoin from cells (a phenomenon termed transinhibition [5]) is perhaps a more immediate response to provision of a good nitrogen source. This interpretation of our results redefines the role of catabolite repression as one associated with steady-state cellular adaptation rather than short-term metabolic regulation. Although the onset of repression (a decrease in mRNA production) may be prompt,the functional consequence of this event is not felt until several generations later.

There is another way of viewing these results. One could argue that the allantoin permease has a very high turnover rate (a half-life of less than 1 to 2 min would be required to account for the observations described above). If this were the case, then nitrogen catabolite repression of gene expression could bring about a rapid response in permease activity. This explanation could be tested by (i) determining the stability of permease activity under various physiological conditions and (ii) measuring the half-life of the mRNA encoding allantoin permease. At present, we do not favor this explanation for two reasons. First, a permease turnover rate of 1 to 2 min would require that the cognate mRNA turns over even more rapidly; this would be surprising. Second, allantoin transport can be inhibited by D-asparagine (V. Turoscy and T. G. Cooper, manuscript in preparation); this amino acid analog is not readily metabolized and would not be expected to support strong nitrogen catabolite repression. Although these considerations do not eliminate this explanation of our data, they do make it somewhat improbable.

An examination of the literature may provide an even clearer example of metabolite transport playing a primary role in the selective use of nitrogen sources. Brandriss and Magasanik have reported that the proline-degrading enzymes are not sensitive to nitrogen catabolite repression (2). On the other hand, proline transport was observed to be repressed when cells were grown on readily used nitrogen sources (2). Whether proline transport activity is also sensitive to transinhibition, as observed for allantoin uptake, remains to be determined. However, the results of Watson (10) support the hypothesis that proline transport is sensitive to this type of metabolic regulation. This author observed that at low chemostat levels of glutamate plus proline (both equimolar), both nitrogen sources were transported into cells and assimilated. However, a modest increase in the combined nitrogen source concentration above a given threshold resulted in a highly selective use of glutamate and equally efficient exclusion of proline from the cells. In other words, the presence of a good nitrogen source (glutamate) in the medium resulted in exclusion of a poorer one (proline) from the cells (4). Courchesne and Magasanik (6) independently reached this same conclusion from an analysis of data which they accumulated.

It is tempting to generalize this model for short- and long-term catabolic regulation to all of the nitrogen sources used by *S. cerevisiae*. However, the data in Fig. 2C and D prohibit such a generalization; these data were obtained by repeating the allantoin transport experiment described above and measuring arginine accumulation instead. The cells used for the experiment shown in Fig. 2C possessed only the basic amino acid transport system (5); the arginine-specific *CAN1* system was mutationally inactivated, and adding asparagine to the culture medium resulted in a 50% loss of transport activity. In wild-type cells, which possess both the basic amino acid and arginine-specific systems, a decrease of just over 20% was observed (Fig. 2D). Since arginase responded to repression in much the same way as the allantoin-degrading enzymes, it is possible that rapid, fine control of this metabolic system involves additional processes; compartmentation at the cell membrane accounts for only a portion of the needed regulation. This may arise from the fact that arginine serves in a dual capacity (nitrogen source and precursor of protein synthesis), compared with allantoin, whose only role is as a nitrogen source. Our results, which we obtained by directly monitoring arginine uptake after addition of a repressive nitrogen source to the culture medium, contrast somewhat with the conclusion of Courchesne and Magasanik (6) that "nitrogen regulation of arginase synthesis is almost completely at the level of inducer exclusion." The conclusion of these authors was based on measurements of arginase activity un-

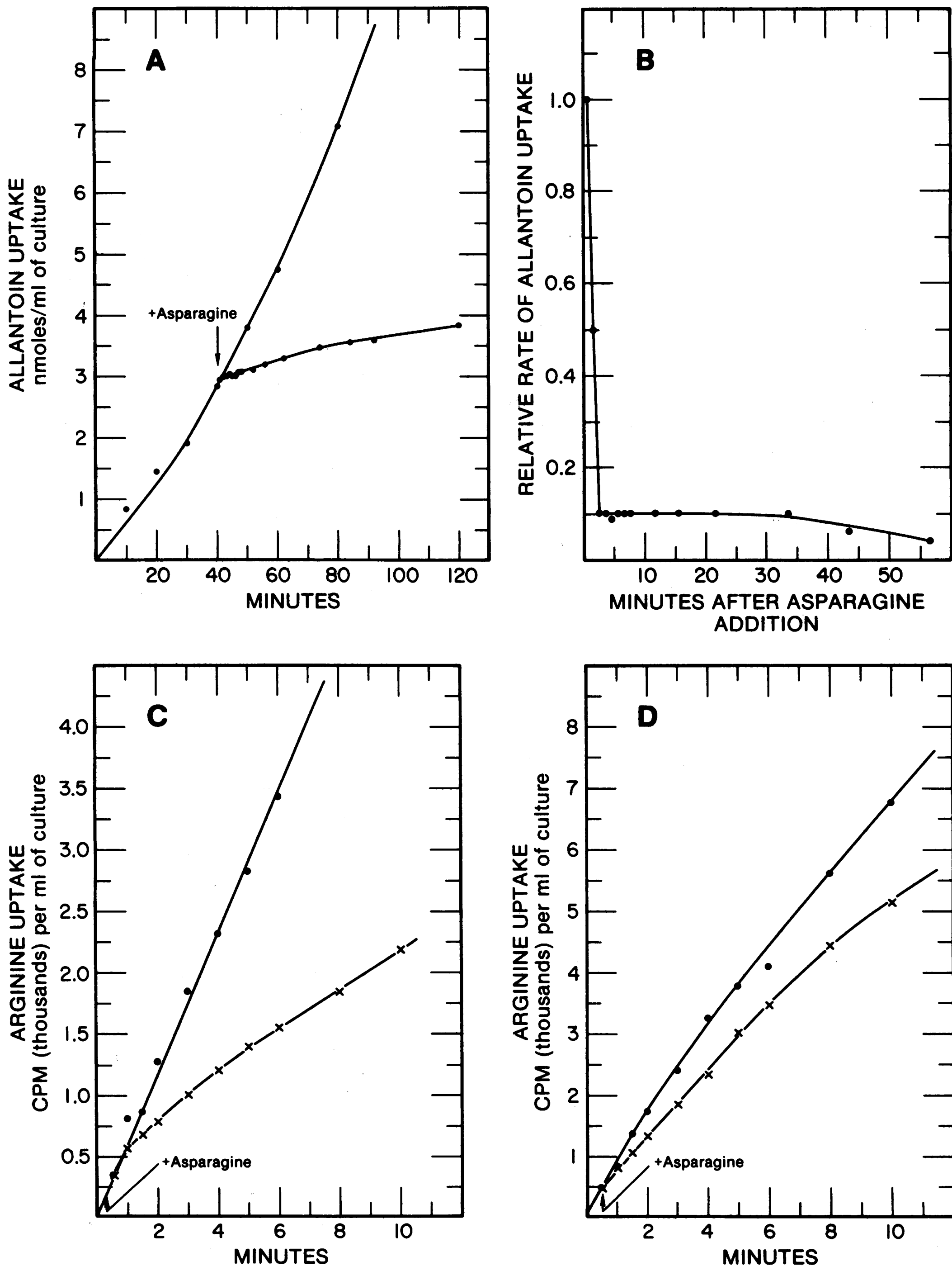

FIG. 2. Effect of the onset of nitrogen catabolite repression on allantoin and arginine transport. (A) Strain M-85 was grown overnight in glucose-ammonia medium in the presence of hydantoin acetate (final concentration, 1 mM) as inducer to a cell density of 50 Klett units. A sample of the culture was collected by centrifugation at room temperature and suspended in an identical amount of prewarmed, preaerated glucose-ammonia medium. After 5 min of equilibration at 30°C, a portion of this culture was transferred to a flask containing radioactive allantoin (final concentration, 0.1 mM; 0.2 μCi/μmol). Samples were removed at the times indicated to assay for allantoin uptake. At 40 min after the start of the experiment, asparagine (final concentration, 0.1%) was added to the medium, and sampling was continued as before. (B) The data shown in (A) expressed as the rate of allantoin uptake relative to the rate of uptake observed at the time that asparagine was added to the medium. (C) Results of an experiment conducted in a manner identical to the experiment described above, with the following exceptions. Strain M-58 was used instead of strain M-85, and radioactive arginine (2.5 μCi/μmol) accumulation was monitored instead of allantoin accumulation. Asparagine was added 30 s after the start of the experiment, and the samples were processed as described above. (D) Results of an experiment performed exactly as described above for (C), except that strain M-1498 was used instead of strain M-58.

der various culture conditions rather than on direct measurements of arginine exclusion from cells. In our view, the level of arginase activity observed experimentally represents the summation of all regulatory mechanisms that are operating and hence can be misleading at times.

In summary, we have reported results suggesting that nitrogen catabolite repression is functionally a long-term response to rather stable changes in the environment. Short-term responses to more subtle environmental variations probably occur by one or more distinct mechanisms. In the case of allantoin metabolism and perhaps in the case of proline metabolism, control of transport activity seems to play an important role. On the other hand, for arginine, transport accounts for only part of the needed control. This perspective of nitrogen catabolite repression in a yeast is similar in some ways to the ideas expressed by Magasanik and colleagues about carbon catabolite repression in bacteria (7). However, it is important to point out that the size of the intracellular metabolite pools and their compartmentation and potential for participation in metabolic regulation are likely to be quite different in yeasts than in bacteria. These results may modify future approaches to the isolation of mutants defective in this type of regulation. They are also likely to stimulate a search for additional levels of control that are implemented over a shorter time period than previously considered.

ACKNOWLEDGMENT

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