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Induction of Arginase and Ornithine Transaminase in the Fission Yeast *Schizosaccharomyces pombe*

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The induction of arginase and ornithine transaminase in the fission yeast *Schizosaccharomyces pombe* requires the absence of ammonia and the presence of the inducer arginine. It seems that immediate arginase degradation is initiated by starved cells or ones from which arginine has been removed.

Two types of regulation, induction by arginine (6) and inhibition of induction by ammonia (7, 11), control arginase synthesis in most eucaryotic cells. In addition, nitrogen starvation in *Saccharomyces cerevisiae* results in immediate derepression of arginase and ornithine transaminase, enzymes of the arginine breakdown (7). Although the derepression of arginine-degrading enzymes during nitrogen starvation can be seen in a few other yeast species (7), most of them show no effect on the specific activity of either enzyme. The present work establishes the events associated with arginase and ornithine transaminase regulation in the fission yeast *Schizosaccharomyces pombe*. As in *S. cerevisiae*, these enzymes are inducible by arginine and are subjected to inhibition by ammonia, but the macromolecular events associated with their regulation are different from those of *S. cerevisiae* and other yeasts (7).

A wild-type strain 972 h^- of *S. pombe* (originally obtained from U. Leupold, Bern) was used for all experiments. The minimal medium was modified EMM2 (9). In some experiments the ammonia salt in the modified EMM2 medium was replaced by 20 mM glutamate as the nitrogen source. Cultures were grown with magnetic stirring at 35°C. Cell number was determined with a Coulter Counter. For protein determination, samples were taken as described elsewhere (T. Benítez, P. Nurse, and J. M. Mitchison, J. Cell Sci., in press), and proteins were measured by the Folin reaction (5). Arginase and ornithine transaminase were induced by adding arginine to cells growing in medium containing glutamate as the sole nitrogen source to give a final arginine concentration of 20 mM. From those subcultures, 200-μl samples were taken, filtered through filters (Millipore Corp.), washed with 10 mM Tris buffer (pH 8) containing 25 mM $MnCl_2$, and frozen in dry ice. Enzyme assays were done on cells which had been frozen and thawed. Arginase (EC 3.5.3.1) and ornithine transaminase (EC 2.6.1.13) were assayed with slight modifications by the methods described elsewhere (6).

Arginase was induced very soon after the addition of arginine to glutamate-supplemented medium (Fig. 1c; Benítez et al., in press). To investigate whether that addition induced only arginase or some other enzymes of the arginine breakdown, the specific activity of ornithine transaminase was also determined. This activity increased about fivefold in a way similar to that of arginase (Fig. 1a). Arginase and ornithine transaminase show similar patterns in experiments measuring both enzymes. Therefore, the figures give results for only one or the other of them.

Cycloheximide, an inhibitor of protein synthesis, added at a final concentration of 100 μg/ml, prevented further increase in induced arginase and ornithine transaminase activities after about 30 min (Fig. 1a). This result suggested that continued protein synthesis was required for such an increase, although there may be a "precursor delay" between the synthesis of the two enzymes and their activation (8; Benítez et al., in press). When 8-hydroxyquinoline, an inhibitor of RNA synthesis (3), was used at a concentration of 50 μg/ml, the synthesis of both enzymes was found to continue for a short time with a declining rate (Fig. 1a), suggesting that an unstable RNA was responsible for increasing enzyme activity.

No induction of arginase was observed when arginine was added to ammonia-supplemented medium (Fig. 1b). Cells were unable to grow at an ammonia concentration of under 1 mM. The subsequent addition of arginine did not result in arginase induction, although growth was resumed. The results indicate that arginase synthesis is subjected to repression by ammonia. They also suggest that nitrogen catabolite repression has a regulation mechanism separate from that of induction. When cells were shifted from ammonia to nitrogen-free medium, arginase activity declined abruptly, within 1 to 2 min. Such an immediate arginase degradation

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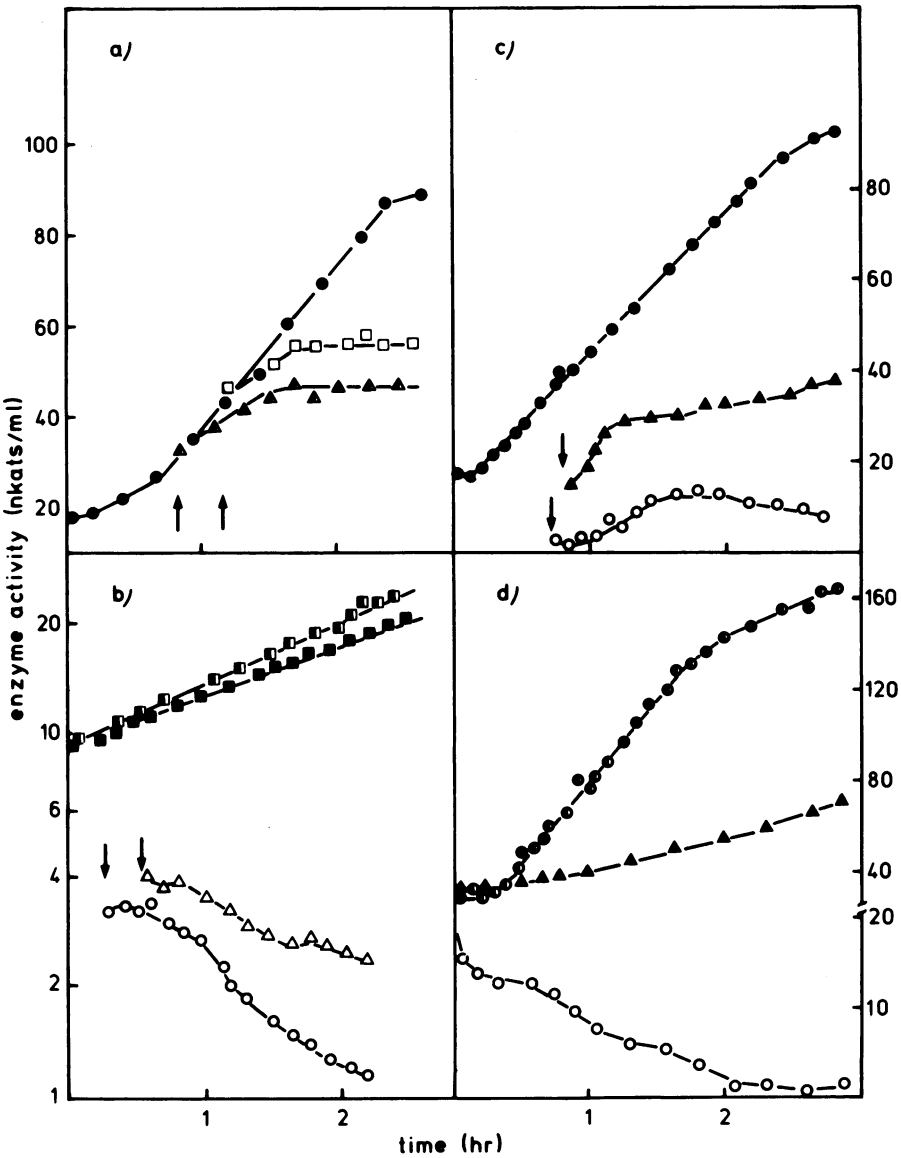

FIG. 1. *Arginase and ornithine transaminase induction in S. pombe. Enzyme activities are expressed in nkat/ml (1 nkat = 1 nmol of substrate transformed per hour). (a) Ornithine transaminase induction after adding arginine to glutamate-supplemented medium at zero time (●). Arrows show the time at which part of the culture was divided into two and cycloheximide (□) or 8-hydroxyquinoline (▲) was added. (b) Arginase in cells growing on ammonia-supplemented medium (◧) or the same medium with arginine added at zero time (■). Arrows show the time at which part of the former culture was divided into two, filtered, and resuspended into nitrogen-free medium (○) or that supplemented with 1 mM glutamate (△). (Notice the log scale). (c) Arginase induction after adding arginine to glutamate-supplemented medium at zero time (●). Arrows show the time at which part of the culture was divided into two, filtered, and resuspended into nitrogen-free (○) or glutamate-supplemented medium (▲). (d) Arginase induction after shifting the cells from glutamate- to arginine-supplemented medium. Cells were grown on glutamate. At zero time, the culture was divided into three, filtered, and resuspended into arginine- (◐) or glutamate-supplemented medium (▲) or nitrogen-free medium (○).*

contrasts with the results reported in nitrogen-deprived cells of some other yeast species (7, 10). The addition of small quantities of glutamate (1 mM) did not significantly modify the degradation process (Fig. 1b).

A similar decline was observed, once the en-

zyme had been induced, when the culture was shifted to nitrogen-free medium (Fig. 1c). The effect was shown to be different when cells, once induced, were shifted to glutamate-supplemented medium. In other yeast species, removal of the inducer does not affect the increase of enzyme activity for some time. After that, enzyme activity falls off until it reaches a constant level (1). However, in *S. pombe*, removal of the inducer had a more drastic effect, since arginase activity declined until it reached basal level; its specific activity was reduced about one-fourth to one-fifth (Fig. 1c).

Cells were also grown on glutamate-supplemented medium and, at zero time, distributed into three different flasks containing glutamate- or arginine-supplemented or nitrogen-free medium (Fig. 1d). When cells were shifted from glutamate- to arginine-supplemented medium the induction effect on arginase was the same as when arginine was added to glutamate-supplemented medium. Arginase activity in the nitrogen-free medium declined in a way similar to that observed in Fig. 1b and 1c. In contrast to some other yeast species, this enzyme cannot be derepressed by starving the cells of nitrogen.

To investigate whether the effect of starvation on arginase reflected a situation of total macromolecular breakdown or selective protein degradation, growth (as determined by protein accumulation) and cell division (as determined by cell number count) were measured. Under conditions of nitrogen deprivation (Fig. 2b), little protein accumulated, whereas the number of cells increased at least threefold (Fig. 2a). Since cell number increase (and, therefore, DNA) during this process needs protein synthesis, of which there has been little, it is suggested that nitrogen deprivation is accompanied by extensive protein degradation. There may also be preferential loss of protein subpopulations (i.e., arginine breakdown enzymes). This suggestion is supported by the fact that some unrelated enzymes (basal sucrase and maltase) remained active during nitrogen starvation (T. Benítez, unpublished data).

The results of growth and cell division on arginine- or glutamate-supplemented medium are also shown in Fig. 2a and 2b. Cells in the arginine-supplemented medium grow slightly faster than those in the glutamate-supplemented one. This result is corroborated by the cell number data. Our nutritional shift-down results supported the evidence that cell size at division diminishes at slow growth rates (2) (as occurs in nitrogen depletion). Nuclear division and cell division are stimulated after the shift, even though there is very little increase in protein accumulation (Fig. 2a and 2b).

The data suggest that induction of arginase

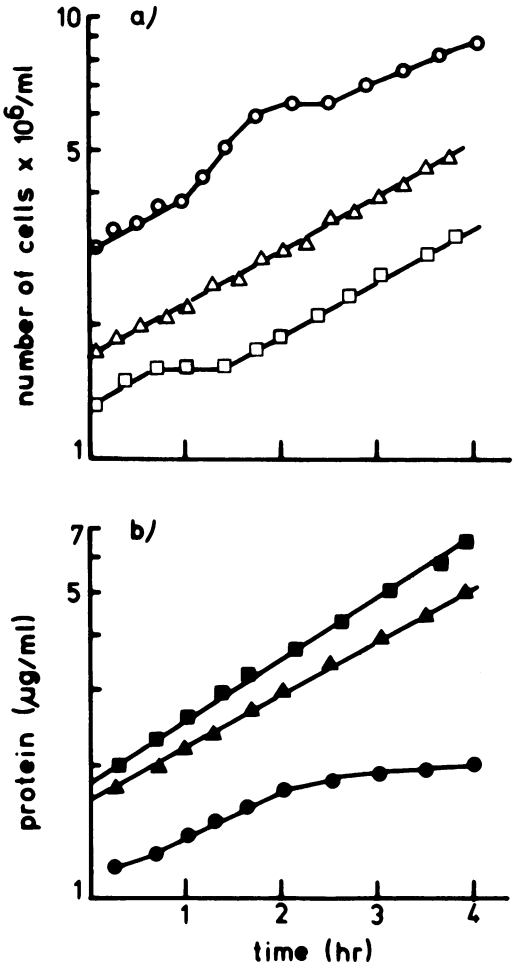

FIG. 2. *Effect on cell number and protein of the shift from glutamate-supplemented medium (▲, △) to either the same medium with arginine added at zero time (■, □) or nitrogen-free medium (●, ○). The protein values are plotted on an arbitrary relative scale so that the actual micrograms of protein per milliliter and the scale values are related by a factor of 13.5 (▲), 10.0 (■), and 35 (●).*

and ornithine transaminase in *S. pombe* produces specific synthetic capacities requiring the presence of an inducer, functioning RNA, and protein synthesis. This evidence argues for transcriptional control, although it does not unequivocally demonstrate RNA synthesis as a requirement for enzyme production. A second conclusion concerns the stability of the specific synthetic capacities for arginase and ornithine transaminase. The enzymes seem to be synthesized de novo, since the addition of cycloheximide or 8-hydroxyquinoline inhibits their increase within minutes.

After inducer removal or shift to nitrogen-free medium, our results differ greatly from those

reported in other yeast species (1, 10). The immediate arginase degradation is difficult to explain, since the enzyme seems to be stable under cycloheximide or 8-hydroxyquinoline inhibition.

The situation might be different in starved cells. If the induction of arginase under nitrogen starvation were simply contingent upon the presence of arginine in the amino acid pools of the cells, little or no effect on arginase specific activity could be expected. No immediate derepression of arginase occurs in *S. cerevisiae* if the arginine of the amino acid pools is eliminated from ammonia-starved cells (10).

Proteins are extensively degraded during starvation to supply growth requirements. Degradation begins immediately upon shift to nitrogen-free medium, and patterns of protein synthesis in growing and starving cells differ (4). Our results might reflect the fact that under conditions of nitrogen deprivation, enzymes such as those for arginine catabolism are selectively (preferentially) degraded, whereas in yeasts like *S. cerevisiae* (7) those enzymes are newly synthesized.

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