PERIODIC CHANGES IN RATE OF AMINO ACID UPTAKE DURING YEAST CELL CYCLE

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ABSTRACT

Uptake of amino acids is a complex process but in cells growing with ammonia as sole nitrogen source the initial uptake rate of amino acids is a measure of the transport capacity of the uptake system (permease). In synchronous cultures of Saccharomyces cerevisiae amino acids were transported at all stages of the cell cycle. However, for any one amino acid the initial uptake rate was constant for most of the cycle and doubled during a discrete part of the cycle. Thus, for a variety of amino acids the functioning amino acid transport capacity of the membrane doubles once per cycle at a characteristic stage of the cycle. Arginine, valine, and phenylalanine exhibit periodic doubling of uptake rate at different stages of the cell cycle indicating that the transport of these amino acids is mediated by three different systems. Serine, phenylalanine, and leucine exhibit periodic doubling of the uptake rate at the same stage of the cycle. However, it is unlikely that serine and phenylalanine share the same transport system since the uptake of one is not inhibited by the other amino acid. This phenomenon is analogous to the periodic synthesis of soluble enzymes observed in S. cerevisiae.

INTRODUCTION

It is increasingly clear that further insights on the active transport of nutrients and the formation of precursor pools are necessary for an understanding of the kinetics of cell growth and cell division. The present study examines the uptake of different amino acids during the cell cycle of Saccharomyces cerevisiae, which are known to be mediated by specific transport systems, analogous to the individual permeases for amino acids in bacteria. In the fungi Neurospora crassa (14, 21), Penicillium chrysogenum (1, 2), and S. cerevisiae (5, 7, 8, 10, 20) there are specific amino acid transport systems similar to those in bacteria. A general amino acid transport system has been described in S. cerevisiae by Halvorson and Cohen (13) which catalyzes the transport of basic and neutral amino acids. The activity of this system is inhibited when ammonium ions are added to the culture medium (5, 7, 9, 10). In addition to this general amino acid transport system, Grenson and co-workers (5, 7, 10) demonstrated in ammonia grown cells of S. cerevisiae the existence of specific amino acid transport systems for arginine, lysine, and methionine. In the present study the activity of these and other transport systems were examined at different stages of the cell cycle of yeast.

In previous studies we have observed in S. cerevisiae that the activity of a number of enzymes increases discontinuously (stepwise) during the cell cycle. For a given enzyme the enzyme complement per cell remains constant and doubles sharply in each cell division at a stage in the cell cycle characteristic for a particular enzyme (for review see reference 11). The present experiments
are designed to determine whether the functioning transport complement of the membrane increases continuously or stepwise throughout the cell cycle.

MATERIALS AND METHODS

Preparation and Analysis of Synchronous Cultures

Liter cultures of S. cerevisiae Y185, a sucrose-negative diploid were grown on 0.2% synthetic succinate medium (SSM) (18) with 2% glucose as carbon source overnight at 35°C and harvested in the logarithmic phase of growth by filtration through 150-mm BAC-T-FLEX membrane filters (Schleicher & Schuell, Inc., Keene, N.H.). The harvested cells (up to 10^11 cells) were separated according to size and thus age during the cell cycle by zonal centrifugation (12, 18). Small cells (at early stage in the cell cycle) were removed from the zonal rotor, washed free of sucrose, and used to inoculate cultures which grow synchronously. The course of a synchronous culture was monitored by cell number counts using an electronic cell counter (Coulter Electronics, Inc., Fine Particle Group, Hialeah, Fla.).

Uptake of Amino Acids

Uptake of amino acids was performed on 2-ml samples removed from synchronous cultures at different stages of the cell cycle. Culture samples were incubated at 25°C in 100 µg/ml cycloheximide and C14-labeled amino acid (0.1 mM) (New England Nuclear, Boston, Mass., and Schwartz Bio Research Inc., Orangeburg, N.Y.). Uptake experiments were terminated by filtering the sample through Millipore filters (Millipore Corp., Bedford, Mass.) and washing cells on the filter five times with 10 ml ice-cold distilled water. Membrane filters with accompanying cells were dried overnight at 60°C and the filter placed in a vial containing toluene, 2,5-diphenyloxazole (POPOP), and the radioactivity of the membrane was determined. Cycloheximide was added to incubation mixtures to inhibit protein synthesis.
synthesis in order to measure uptake without incorporation of amino acids over a 5-min period in the presence or absence of cycloheximide in agreement with the finding in S. cerevisiae by Gresham and Moat (unpublished results). The observation that amino acid uptake in S. cerevisiae can be inhibited by cycloheximide (8) suggests that this experiment must be rechecked for longer time intervals and under different physiological conditions.

In all experiments with synchronous cultures, cells were grown on 0.2% SSM which contains 8 g/liter (NH₄)₂SO₄ as the sole nitrogen source. Thus, in the uptake experiments, the cultures were exposed to amino acids only during the assay procedures.

RESULTS

It is evident upon examination of the uptake of ¹⁴C by exponential cultures of S. cerevisiae previously grown in SSM in the presence or absence of 4 mg/ml of L-arginine that the uptake of amino acids in yeast is influenced by the previous growth conditions of the organism. In ammonium-grown cells (Fig. 1 a) the rate of arginine accumulation is linear for the initial 5 min of incubation and thereafter declines. On the other hand, in cells grown in the presence of arginine the rate of ¹⁴C uptake is considerably lower than in ammonia-grown cells and continues at a linear rate for over 10 min (Fig. 1 b). Similar results were observed for the uptake of other amino acids. In subsequent experiments the uptake of amino acids during the first 3 min of incubation was selected to measure the initial rate of uptake.

Uptake of Amino Acids in Synchronous Cultures

In synchronous cultures of S. cerevisiae grown in 0.2% SSM, exposure to [¹⁴C]histidine for 3 min at different stages of the cell cycle results in uptake of [¹⁴C]histidine at all stages of the cell cycle (Fig. 2). However, the rate of uptake is discontinuous during the cell cycle and doubles during the middle of the cell cycle. Thus, the rate of histidine uptake exhibits a steplike increase(s) during the cell cycle. If the initial uptake of amino acid is a measure of the level of the transport system, this indicates that the functioning histidine transport complement of the membrane doubles once per cell cycle.

To determine whether the discontinuous capacity to transport amino acids during the cell cycle in yeast is a general phenomenon, the initial uptake of other amino acids was examined. As shown in Fig. 3, uptake of [¹⁴C]serine and [¹⁴C]leucine occurs at all stages of the cell cycle of S. cerevisiae grown in SSM. In both instances the uptake rate exhibits a steplike increase once during the cell cycle. The beginning and end of the cell cycle are taken arbitrarily to be the point that cell numbers stop increasing through cell division. By this criterion, the rate of leucine uptake begins to double at approximately 0.4% of a cell cycle.

We have observed in previous studies on periodic enzyme synthesis during the cell cycle that the stage during the cell cycle that periodic enzyme synthesis occurs may vary slightly from experiment to experiment (3). In Fig. 4 is shown the reproducibility of the position in the cell cycle where the rate of leucine transport complement of the membrane begins to double. It can be seen

![Graph](image-url)
FIGURE 3 Uptake of serine and leucine during the cell cycle of *S. cerevisiae*. 2-ml samples were removed from synchronous cultures of *S. cerevisiae* growing in 0.2% SSM. Leucine and serine uptake was determined after the addition of 0.1 ml stock solution of [14C]leucine and [14C]serine to the sample. The stock solution also contained (in 2 ml distilled water) cycloheximide (2 mg), L-serine or L-leucine (2 mM), and 100 µl of [14C]serine (50 µCi/ml; 100 mCi/mmol) or [14C]leucine (50 µCi/ml; 250 mCi/mmol). After a 3-min incubation at 25°C, cells were removed by filtration on Millipore filters, washed, dried at 60°C, and the radioactivity was determined. Zero time controls contained identical materials.

that while there is some variability in the point during the cell cycle that the doubling occurs, it does, nevertheless, occur at a discrete stage in the cell cycle. Therefore, in determining whether the the uptake of the two amino acids doubles at the same stage of the cell cycle, the uptake of the two amino acids should be followed in the same synchronous culture. For example, serine and phenylalanine transport systems exhibit periodic doubling during the cell cycle, and it can be seen from Fig. 5 which shows the variability of this periodic doubling during the cell cycle that it would be possible in a very few separate experiments with different single-amino acids to conclude that these amino acids exhibit rate doubling at different stages of the cell cycle. However, when the uptake of serine and phenylalanine is followed in the

same synchronous culture, it becomes apparent that the time of doubling the uptake rate for these two amino acids during the cell cycle is indistinguishable (Fig. 6).

Grenson and his colleagues (5, 7, 10) have shown that there are specific amino acid transport systems in yeast for methionine, arginine, lysine, histidine, and dicarboxylic acids (15). It is pos-
sible that this might be reflected in rate doublings of the uptake of these amino acids at different stages of the cell cycle. From the above results, leucine, serine, and phenylalanine uptake rates all double at about the same stage of the cell cycle. However, as shown in Fig. 7, arginine and phenylalanine exhibit periodic doubling of uptake rate once per cell cycle but the doubling occurs at different stages of the cycle for each amino acid, suggesting distinct systems for arginine and phenylalanine uptake. Further, the rate doubling of valine uptake (Fig. 8) occurs at a different stage of the cell cycle than that for phenylalanine and arginine.

It seems plausible that, where two amino acids have uptake rates which double at different stages of the cell cycle, the uptake is mediated by different uptake systems. On the other hand, serine and phenylalanine uptake rates would double at the same stage of the cell cycle if both these amino acids were transported by the same transport system(s). In the latter case, one would expect that excess of one amino acid would inhibit the uptake of the second radioactive amino acid. The results of such experiments are seen in Table I. Although serine and phenylalanine uptake double at the same stage of the cell cycle, unlabeled serine does not inhibit uptake of 14C-labeled phenylalanine uptake. Thus, these two amino acids may have distinct amino acid uptake systems. However, unlabeled arginine inhibits the uptake of 14C-methionine and 14C-lysine, suggesting that this amino acid may compete with methionine and lysine for their respective transport systems.

Figure 6  Uptake of phenylalanine and serine during the yeast cell cycle. 3-ml samples were removed from synchronous cultures of S. cerevisiae growing in 0.5% SSM. Serine (●) and phenylalanine (●) uptake were determined after addition of 0.1 ml stock solution of [14C]phenylalanine or [14C]leucine to the sample. The stock solution contained (in 2 ml distilled water) cycloheximide (2 mg), L-phenylalanine (2 mM) or L-serine (2 mM), and 100 μl of [14C]phenylalanine (50 μCi/μl; 350 mCi/mmol) or [14C]-serine (100 mCi/mmol). After a 3-min incubation at 25°C, cells were removed by Millipore filtration, washed 5 times with 10 ml ice-cold distilled water, dried at 60°C, and the radioactivity was determined. Zero time controls contained identical materials.
FIGURE 7 Relative rate of arginine and phenylalanine uptake during the cell cycle of *S. cerevisiae*. 2-ml samples were removed from synchronous cultures of *S. cerevisiae* growing in 0.2% SSM. Arginine and phenylalanine uptake rates were determined after the addition of 0.1 ml stock solution of [14C]arginine or [14C]phenylalanine to the sample. The stock solution contained (in 2 ml distilled water) cycloheximide (2 mg), L-arginine (2 mM) or L-phenylalanine (2 mM), and 100 µl of [14C]arginine (50 µCi/ml; 200 mCi/mmol) or [14C]phenylalanine (50 µCi/ml; 350 mCi/mmol). After a 3-min incubation at 25°C, cells were filtered on Millipore filters, washed, dried at 60°C, and the radioactivity of cells on the filter was determined. Zero time controls contained identical materials. The beginning and end of a cell cycle are taken to be the times at which cell numbers stop increasing after cell division.

This conclusion appears unlikely since unlabeled methionine and lysine do not inhibit the uptake of [14C]arginine. Arginine inhibits the uptake of methionine and lysine, but does not compete for the transport of these amino acids.

DISCUSSION

The well-established, discontinuous increase of enzyme activities during the cell cycle in yeast may impose physiological restraints on the capacity of the cell. Thus, it is of interest to examine a complex process such as active amino acid transport in yeast involving both the activity of stero-specific permeation sites in the membrane and coupled energy-yielding reactions of the cell. Not only can any one of a number of steps be rate limiting, but, also shown here, pregrowth of yeast in the presence of ammonium and arginine lowers the initial rate of arginine uptake. We have previously shown (3) that the arginine pool is 2½ times greater in cells grown on 0.2% SSM supplemented with arginine than in cells grown in the absence of this amino acid. Presumably, the expanded arginine pool is capable of feedback regulation of the uptake system. Although the mechanism for this inhibition is not understood, this complication can be minimized by examining the initial kinetics of amino acid uptake in ammonium-grown cultures.

Examination of the ability of yeast cells to transport various amino acids as a function of physiological age in the cell cycle can provide insight into two important questions. First, is the rate of active transport regulated by functions which continuously change during the cell cycle (surface area or cell volume). Second, are the activities of the various specific amino acid transport systems independently regulated. If not, a method is provided for identifying new specific transport systems.

Measurements of cell volume, cell mass, and increases in the major cell components (proteins and RNA) show that these increase continuously over the cell cycle in yeast (6, 16, 17). Although the kinetics of increase in yeast are not as yet clear, it is evident that both volume and cell surface increase continuously throughout the cell cycle in *S. cerevisiae* and therefore might provide the basis for regulating the uptake capacity of amino acids. This possibility is made unlikely by the results shown in this paper. For each of the amino acids examined, the complement present and functioning in the cell membrane does not increase continuously but does, in fact, remain constant for much of the cell cycle after which the uptake activity remains constant until the corresponding stage in the following cycle when it doubles again. These results do not indicate the time in the cell cycle during which the gene for the leucine transport system is transcribed nor necessarily do they show when it is translated into protein; but these results do indicate when it is present within the membrane as a functioning uptake system. The finding that the time of increase in the rate of transport in different amino acids can differ in the cell cycle makes it unlikely that a generalized system (such as energy supply) is regulating the rate of increase observed. However, the periodic doubling of uptake during the yeast cell cycle indicates that a certain caution should be exercised in experiments in which the rate of incorporation into protein or RNA of an
externally added precursor is being investigated. These findings with amino acid transport are very similar to the observations that in *S. cerevisiae*, about 30 enzymes are synthesized periodically once per cell cycle and that for a given enzyme there is a characteristic stage in the cell cycle at which the enzyme complement doubles (for review see reference 11).

It is not unlikely that the initial uptake of arginine (or other amino acids) under the conditions employed here represents the amount of the amino acid transport system present and functioning in the cell membrane. On this assumption the initial rate of amino acid uptake at various stages of the cell cycle is a measure of the amount of transport system incorporated into the membrane at a particular stage in the cell cycle.

Not all amino acids are taken up at the same stage in the cell cycle (Figs. 3-6), an indication that there must be some degree of amino acid uptake specificity. Indeed, it appears that the system responsible for the uptake of arginine is different from that of valine which is different again from that of phenylalanine.

An alternative interpretation of our data is that the periodic increases in amino acid uptake rate during the cell cycle are a result of periodic fluctuations in the level of amino acid pools causing oscillations in feedback regulation. That feedback from an amino acid pool may, under certain conditions, regulate uptake is suggested by our observation that uptake of arginine is diminished
in medium containing arginine (Fig. 1). We do not favor this interpretation because in cells grown in 0.2% SSM (ammonium as the sole nitrogen source) the level of arginine does not fluctuate but remains constant throughout the cell cycle (3). Similar results with a variety of amino acids have been obtained in S. cerevisiae (Tauro, unpublished results) and Schizosaccharomyces pombe (19).

Grenson and his colleagues (7, 10) have suggested that there are distinct transport systems for lysine and arginine, but that while lysine may enter the cell by means of the arginine system, arginine cannot use the lysine transport system. If lysine is transported by the arginine system, then lysine should inhibit competitively the uptake of 14C-labeled arginine in synchronous and exponential cultures. Our results (Table I) indicate that whereas arginine inhibits the uptake of 14C-labeled lysine, the converse does not occur. Thus, at least in this strain of S. cerevisiae Y185, lysine does not enter through the arginine uptake system to any appreciable extent. This conclusion is consistent with the observation that there is only one increase in the rate of lysine during the cell cycle (Carter, unpublished observations). However, it is possible that lysine can enter the arginine transport system but that the affinity (Km) for lysine and the maximum velocity of the reaction are very low.

Crabeel and Grenson (4) demonstrated in S. cerevisiae that histidine can enter the yeast cell by two distinct histidine permeases which have differing affinities for histidine. The observation (Fig. 2) that there is only one stage in the cell cycle in which the rate of histidine uptake doubles suggested that the two transport systems for histidine are inserted into the membrane in a functioning form at an identical stage of the cell cycle. However, in a more rigorous examination of the two histidine uptake systems during the cell cycle it would be appropriate to employ several concentrations of histidine in the assay medium: a low concentration at which Crabeel and Grenson (4) observed the second system to be predominant. As observed in Fig. 4 the rate of doubling for serine and phenylalanine uptake occurs at the same stage of the cell cycle. However, inhibition experiments (Table I) make it likely that there are distinct transport systems involved in the uptake of serine and phenylalanine; serine uptake is not inhibited by serine.

Our results suggest that there may be more specific amino acid transport systems in yeast than have hitherto been reported. Specific amino acid uptake systems can be differentiated on the basis of differences in the stage at which the rate doubling of amino acid uptake occurs in the cell cycle (Fig. 6). While our results indicate the activity of the transport system within the membrane, they may not reflect the uptake of amino acids during the cell cycle of cells grown in medium supplemented with amino acids. We have shown that if arginine is added to cells grown in minimal medium the initial uptake of arginine remains constant for only a few minutes, after which it declines, presumably caused by feedback by the enlarged amino acid pool. Thus, it is apparent that the accumulation of amino acids by yeast is influenced by other factors in addition to the transport system levels.

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### Table I

**Initial Uptake of [14C]Amino Acids in the Presence of Other Unlabeled Amino Acids**

| [14C]Amino acid | Competing amino acid | Percent of control |
|-----------------|----------------------|-------------------|
| Arginine        | Lysine               | 101               |
| Arginine        | Methionine           | 103               |
| Phenylalanine   | Serine               | 100               |
| Phenylalanine   | Valine               | 108               |
| Lysine          | Arginine             | 76                |
| Methionine      | Arginine             | 69                |

Exponential cultures were grown in 0.2% SSM containing (NH4)2SO4 as the sole nitrogen source. 2-ml samples were taken at intervals and the uptake of [14C]amino acids was determined after the addition of 0.1 ml stock solution containing (in 2 ml distilled water) cycloheximide (2 mg), 14C amino acid (2 mM), and 100 µl of either [14C]arginine (50 µCi/ml; 200 µCi/mmol), [14C]methionine (100 µCi/ml; 250 µCi/mmol), [14C]lysine (50 µCi/ml; 300 µCi/mmol), or [14C]serine (50 µCi/ml; 150 µCi/mmol). Where indicated unlabeled amino acids (2 mM final concentration) were added to test the specificity of the uptake system. Uptake of [14C]amino acid in the presence of unlabeled amino acid is expressed as the percentage of the initial rate of uptake of the [14C] amino acid incubated alone.
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