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Ornithine Decarboxylase Activity and Cell Cycle Regulation in *Saccharomyces cerevisiae*

DENIS G. KAY,[1] RICHARD A. SINGER,[1,2]* AND GERALD C. JOHNSTON[3]

Departments of Biochemistry,[1] Medicine,[2] and Microbiology,[3] Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

In the yeast *Saccharomyces cerevisiae*, the specific activity of the enzyme ornithine decarboxylase (ODC) was correlated with overall growth status. The activity of ODC was highest in actively growing cells, whereas the specific activity was lower in slow-growing cultures limited for nitrogen or inhibited by low concentrations of cycloheximide. Specific activities of ODC were also low in cultures arrested in the stationary phase (in the G1 portion of the cell cycle) by starvation for required nutrients. Although correlated with overall growth, ODC activity was not required for growth or cell cycle regulation. Cells continued to grow in the presence of the polyamine spermidine or spermine, which markedly reduced ODC specific activities. Thus, high levels of ODC activity were not necessary for growth, nor were decreased ODC specific activities sufficient to cause cells to arrest in G1. Conversely, one agent (*o*-phenanthroline) which causes growing cells to arrest in G1 did so with no effect on ODC specific activity. Therefore, ODC specific activity changes are not necessary for cell cycle regulation but simply reflect the normal growth status of cells.

Ornithine decarboxylase (ODC; ornithine carboxylase, EC 4.1.1.17) is the initial enzyme of the polyamine biosynthetic pathway and catalyzes the decarboxylation of ornithine to 1,4-diaminobutane (putrescine). This enzyme has served as an indicator of cell growth status in mammalian cells (20). The activity of ODC is characteristically low in resting cells (the G1 phase of the cell cycle) and high in cells during active growth and division. Two observations have suggested that ODC activity may reflect, or be involved in, the cell cycle behavior of animal cells. First, when resting cells are stimulated to divide, there is a marked increase in the specific activity of ODC (20). Second, in synchronously growing cells, increased activity of ODC has been noted in phase G1, before the initiation of DNA synthesis, and again in G2 (13). A mechanism explaining the correlation of ODC levels and growth status has been proposed. In reports describing in vivo correlations (11) and in vitro RNA synthesis assays in the presence of purified ODC (12, 17), it was suggested that in rat liver the ODC enzyme protein itself may be involved in the regulation of RNA polymerase I activity. Since RNA polymerase I, in animal cells (16) and in yeasts (5), is thought to transcribe rRNA genes, growth could be regulated by the effect of ODC on the production of ribosomes.

We have shown that rRNA production is involved in regulation of cell division in the yeast *Saccharomyces cerevisiae*. Treatment of growing cells with the zinc-chelating agent *o*-phenanthroline (OP) or 8-hydroxyquinoline (15), the methionine analog L-ethionine (18), or nalidixic acid (17a) caused cells to be unable to progress through phase G1. The common macromolecular alteration accompanying this cell cycle arrest was a specific decrease in transcription of rRNA genes. These results, coupled with those described above implicating ODC in the regulation of RNA polymerase I, led us to examine the relationship between ODC activity and the cell division cycle in yeasts. Results presented here suggest that ODC activity is usually a good indicator of the growth status of the yeast; however, the enzyme is not required for normal cell cycle regulation.

MATERIALS AND METHODS

Strains, media, and measurement of cellular parameters. The *S. cerevisiae* diploid strain AG1-7 (*ura1 his6*) has been described elsewhere (9). Cells were grown at 30°C in the liquid synthetic medium described previously (6), supplemented with uracil (20 μg/ml) and histidine (4 μg/ml). Cell number was determined as described by Hartwell (2). The proportions of unbudded cells were determined by scoring at least 100 cells with a phase-contrast microscope.

Preparation of cell extract. Cells were harvested by centrifugation and washed once with water. Washed pellets were either frozen at −20°C or processed immediately as follows. Cells were suspended in 1.5 to 3.0 ml of ice-cold TE buffer (50 mM Tris-

hydrochloride, [pH 7.2] and 0.1 mM EDTA) and lysed at 18,000 lb/in^2 in a French pressure cell (American Instrument Co.). Lysates were clarified by centrifugation at 12,100 × *g* and 10°C for 10 min. The supernatant was removed and assayed.

Assay for ODC activity. The ODC assay used was an adaptation of that of McCann et al. (13). The assay mixture contained 50 mM Tris-hydrochloride buffer (pH 7.2), 6.5 mM L-ornithine, 0.2 mM pyridoxal phosphate, 5 mM dithiothreitol, 0.05 or 0.25 μCi of L-[1-^{14}C]ornithine (New England Nuclear Corp.), and up to 120 μl of extract or TE buffer, in a 200-μl total volume. Reactions were carried out at room temperature (23°C) in capped glass scintillation vials and were initiated by the addition of enzyme. Reactions were stopped by the addition of 100 μl of ice-cold 20% trichloroacetic acid. Evolved $^{14}CO_2$ was trapped in Whatman 3MM paper disks that had been saturated with Protosol (New England Nuclear Corp.) and inserted into the caps of the vials before initiation of the reactions. After the addition of trichloroacetic acid, reaction mixtures were incubated for at least 30 min before the filters were removed and counted.

Protein assay. Protein concentrations in cell extracts were determined by the Lowry method (10). A lysis buffer blank was routinely included.

Chemicals. L-Ornithine, dithiothreitol, pyridoxal phosphate, putrescine, spermidine, spermine, OP, 8-hydroxyquinoline, and L-ethionine were all obtained from Sigma Chemical Co. Bio-Gel P-6 was obtained from Bio-Rad Laboratories.

RESULTS

Characterization of ODC activity. Initially we wished to characterize the ODC activity found in yeasts. Extracts were prepared from exponentially growing *S. cerevisiae* cells and assayed as described above. The K_m for ornithine was 0.5 mM (Fig. 1). The linearity of the Lineweaver-Burke plot in Fig. 1 demonstrates that the enzyme displayed normal Michaelis-Menten kinetics, with no evidence of substrate inhibition. Under our assay conditions, the reaction was linear with time for at least 60 min and with amounts of cell extract up to at least 350 μg of protein per assay (Fig. 1, inset). Inclusion of the serine protease inhibitor phenylmethylsulfonyl fluoride to 1 mM in both the lysis buffer and assay mixture had no effect on the kinetic properties of ODC (data not shown).

Correlation of cell cycle and ODC activity. In mammalian cells, ODC activity is thought to reflect the growth phase of the cells. This correspondence also seems to be true for the yeast *S. cerevisiae*. A marked increase in ODC specific activity, similar to that seen in mammalian cells stimulated to divide, was found when stationary-phase yeast cultures were diluted into fresh medium. The increase in ODC specific activity occurred well after the cells had begun to bud (Fig. 2). For the budding yeast *S.*

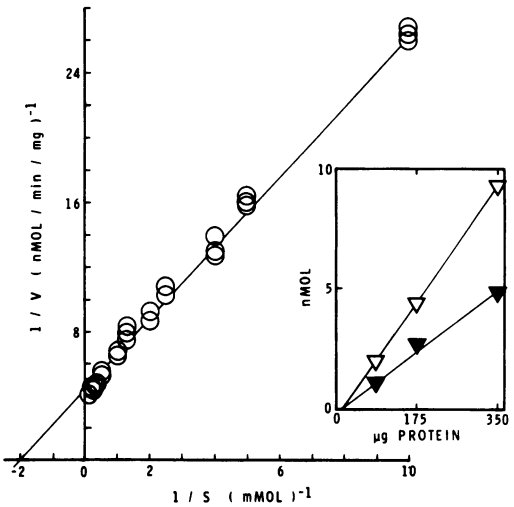

FIG. 1. *Lineweaver-Burke plot of yeast ODC activity versus ornithine concentration. An extract of exponentially growing cells was prepared as described in the text and assayed in triplicate. Regression analysis was used to determine the line. The inset demonstrates the linearity of the assay with the amount of extract protein at both 30 min* (▼) *and 60 min* (▽) *of incubation.*

cerevisiae, cells in the stationary phase are arrested in G1 and have no bud (3). Entry into the S phase corresponds with the appearance of a bud (19), the incipient daughter cell. Similar kinetics were noted for nitrogen-starved cells (9) placed in fresh medium (data not shown). Thus, ODC levels do not increase until after cells have initiated the cell division cycle.

The specific activity of ODC was highest in cells from cultures growing exponentially. This activity began to decrease approximately two generations before the stationary phase and was less than 10% of maximal values by the time cells entered the stationary phase (Fig. 3). To determine whether the ODC activities were always correlated with growth status, we employed several treatments to specifically arrest cells in the G1 phase of the cycle. These treatments included the addition of compounds such as OP, 8-hydroxyquinoline, or L-ethionine, which limit progression of cells through G1 (8, 18), as well as starvation for required nutrients such as uracil or histidine. (Supplementation experiments showed that the stationary phase in this medium was a result of histidine depletion.) The results of these G1-arresting treatments are presented in Table 1. Starvation or treatment of cells with 8-hydroxyquinoline or L-ethionine caused the cells to arrest in G1 and were accompanied by decreases in the specific activities of ODC. Treatment of the cells with

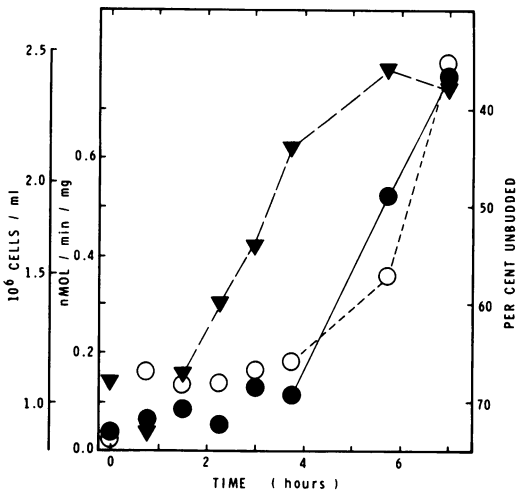

FIG. 2. *Specific activity of ODC during outgrowth from the G1-arrested state. Cells were grown overnight to the stationary phase, collected, and suspended to 10^6 cells per ml at time zero in fresh medium. Samples were removed periodically for determination of cell number* (○) *and proportion of unbudded cells* (▼) *and for extract preparation and ODC assay* (●).

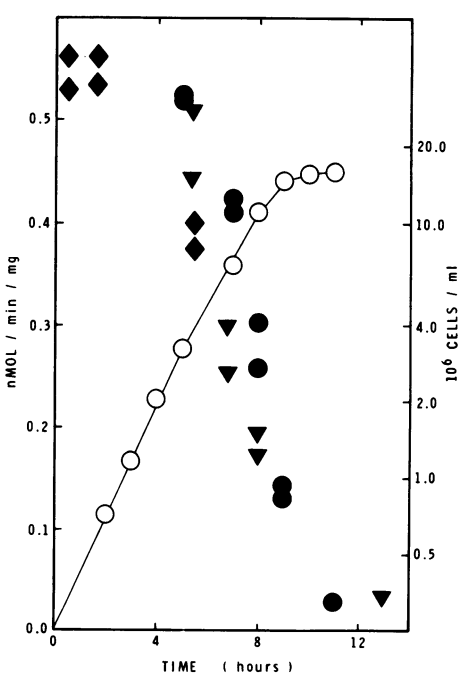

FIG. 3. *Specific activity of ODC during growth arrest. Cells were grown until the stationary phase was reached, and samples were removed periodically for determination of cell number* (○) *and for extract preparation and ODC assay* (◆, ▼, ●). *The three sets of symbols for ODC specific activity represent the results of three independent experiments.*

TABLE 1. *ODC levels in G1-arrested cells*

Arresting treatment[a]	ODC (nmol/min per mg)	% of control
Control	0.55	100
Histidine depletion	0.025	4.5
Uracil depletion	0.19	34
L-Ethionine	0.32	58
8-Hydroxyquinoline	0.31	57
OP	0.53	96
OP + ethionine	0.64	116

[a] Cells were arrested in phase G1 by growth in medium containing reduced concentrations of uracil (0.5 μg/ml) or by the addition of L-ethionine (10 μg/ml), 8-hydroxyquinoline (20 μg/ml), or OP (20 μg/ml). Cell cycle arrest was followed by monitoring the proportion of unbudded cells. Cell extracts were prepared for assay 4 h (for OP and 8-hydroxyquinoline) or 8 h (for ethionine and OP plus ethionine) after the addition of arresting agents or after growth in low-uracil (0.5 μg/ml) medium. All cultures were at less than 3 × 10^6 cells per ml at harvest.

OP, which also caused G1 arrest, did not, however, lead to a decrease in the ODC specific activity. Thus, it appears that decreased ODC specific activity is not required for G1 arrest, although it may be a normal consequence of alterations leading to G1 arrest.

Effect of growth rate on ODC activity. The experiments discussed in the previous section showed that in most cases nongrowing cells, arrested in G1, had decreased levels of ODC activity; however, decreased specific activity was not always correlated with G1 arrest. To determine whether the ODC activities were correlated with growth rate, we examined cells growing at decreased rates. Growth rates were depressed by employing nitrogen sources other than ammonia (6) and by the addition of limiting concentrations of cycloheximide (4). ODC specific activities in slowly growing cultures were decreased (Table 2). Therefore, ODC activity is, in general, proportional to growth rate.

Altered ODC specific activities. Decreased enzyme activity in inhibitor-treated or starved cultures may be due to the presence of an inhibitor or to the absence of an activator of the enzyme. Attempts to detect activators or inhibitors of ODC were made by mixing, in various proportions, extracts of untreated cultures and of cultures arrested in G1. These mixing experiments failed to demonstrate the presence of dissociable activators or inhibitors of ODC activity (Table 3). The results obtained were those expected from a simple dilution of a high-activity extract with a low-activity extract. Passage of high-activity and low-activity extracts through gel permeation columns (Bio-Gel P-6), to sepa-

TABLE 2. *Effects of slow growth on ODC levels*

Nitrogen source[a]	Generation time (h)	ODC, (nmol/min/mg)	% of control
$(NH_4)_2SO_4$	2	0.47	100
$(NH_4)_2SO_4$ + cycloheximide	7	0.055	12
Threonine	9	0.035	7
Tryptophan	5.6	0.025	5

[a] Cells were grown in medium containing the indicated nitrogen sources (added to 0.1%) or cycloheximide (0.06 μg/ml), to limit growth rates, and were harvested for extract preparation at approximately 10^6 cells per ml.

TABLE 3. *Effect of mixing high- and low-activity extracts*[a]

Ratio of volumes added	nmol/min per assay mix		
	Expected	Observed	Observed/expected
Control: Ethionine-treated			
90:10	0.137	0.150	1.09
75:25	0.138	0.145	1.05
50:50	0.143	0.131	0.92
25:75	0.146	0.160	1.10
10:90	0.149	0.161	1.08
Control: Uracil-depleted			
90:10	0.123	0.126	1.02
75:25	0.105	0.108	1.03
50:50	0.073	0.075	1.03
25:75	0.042	0.044	1.05
10:90	0.023	0.024	1.04

[a] Extracts of cultures treated with ethionine, depleted for uracil, or growing exponentially were mixed in various proportions, and the resultant enzyme activities in the mixes were determined. Specific activities of the extracts were: untreated, 0.52; ethionine treated, 0.36; and uracil depleted, 0.22 nmol/min per mg of protein.

rate high-molecular-weight components from low-molecular-weight material, also had no effect on ODC specific activities. Thus, crude extracts did not contain low-molecular-weight modulators of ODC activity.

We also determined the distribution of ODC activity during extract preparation. For both high-activity and low-activity extracts, enzyme activity was measured in pellets and supernatants from the 12,100 × *g* centrifugation step. In extracts from both cultures, 75% of the total activity resided in the supernatant fraction, and the remaining 25% was found in the pellet. The sum of supernatant and pellet activities equalled the total activities in the crude unfractionated lysates; thus, this centrifugation step was not differentially removing some modulator of ODC activity.

When we stored extracts from both starved and actively growing cultures, we found that neither extract exhibited significant changes in ODC specific activity, even after 12 h of storage at 4°C. This stability makes it likely that differing ODC specific activities result not from in vitro artifacts but instead from in vivo events occurring during cell cycle arrest.

Effect of OP on ODC levels. As noted above, the cell cycle-active compound OP caused G1 arrest of yeast cells but without decreasing ODC specific activity. This anomalous behavior of ODC activity during cell cycle arrest suggested that OP had secondary effects on the mechanism causing loss of ODC activity. To test this hypothesis, cells were treated with both OP and the arresting agent L-ethionine, which when added alone led to reduced ODC specific activities. The presence of OP abolished the decrease in ODC specific activity normally found during L-ethionine treatment (Table 1). Thus, in addition to causing G1 arrest, OP must also block the loss of ODC activity. This result is consistent with the observation that OP, in similar concentrations, inhibits the carboxypeptidase activity of peptidase α from a *Saccharomyces* strain (1).

Effects of polyamines on ODC levels. Cells grown in the presence of the polyamine spermidine or spermine, products of the polyamine biosynthetic pathway, had extremely low levels of ODC activity (Table 4). In contrast, cells grown in the presence of putrescine, the immediate product of ODC activity, contained near-normal levels of ODC. Although low ODC levels in the presence of spermine may simply be accounted for by slow growth (Table 2), this cannot be the case for growth in the presence of spermidine. Thus, spermidine may, in fact, repress the synthesis of ODC while supplying polyamine functions normally dependent on ODC activity. The active ODC enzyme protein itself may, therefore, be dispensable for normal cell growth.

TABLE 4. *Effects of growth in polyamine-supplemented medium on ODC levels*

Polyamine[a]	Generation time (h)	ODC (nmol/min per mg)	% of Control
None	2	0.506	100
Putrescine	2	0.416	82
Spermidine	2.5	<0.022	<4.3
Spermine	5	<0.026	<5

[a] Cells were grown for over five generations in medium containing 10 mM putrescine or spermidine or for three generations in medium containing 10 mM spermine. Cells were then harvested for extract preparation and ODC assay.

DISCUSSION

Experiments presented here show that in the yeast *S. cerevisiae* the specific activity of the enzyme ODC is generally a good indicator of cell growth status. The activity of ODC was high in the early- to mid-log phase of rapidly growing cultures. The specific activities were lower in cultures growing at lower rates under nitrogen limitation or in limiting concentrations of cycloheximide. Specific activities of cultures arrested in the stationary phase by starvation were also decreased.

The correlation of ODC activity and growth status is an imperfect one, however. The continued growth of cells in the presence of the polyamine spermidine or spermine, which markedly reduced ODC specific activities, shows that high levels of ODC activity are not necessary for growth, nor are reduced ODC specific activities sufficient to generate cell cycle arrest. Conversely, the fact that OP causes G1 arrest without affecting ODC specific activity shows that decreases in ODC specific activity are not necessary for cell cycle regulation, nor are high levels of ODC activity sufficient for active growth.

In the experiments presented here, there were no indications of dissociable modulators of ODC activity either in the high-activity or low-activity extracts. Also, it is unlikely that the changes in ODC specific activity that we observed were due to the type of reversible enzyme modification found for ODC from another lower eucaryote, *Physarum* (14, 15). In that system, the ODC enzyme was found to convert from high-activity to low-activity forms, differing mainly in K_m values for the cofactor pyridoxal phosphate. Our assays of yeast ODC were all performed at a relatively high (0.2 mM) pyridoxal phosphate concentration, manyfold greater than the reported K_m for the cofactor (15). Furthermore, since the substrate concentrations used here were saturating, our measurements of ODC specific activity most likely reflect levels of ODC enzyme protein.

It has been suggested (11, 12, 17) that in mammalian cells ODC functions as an initiation factor for RNA polymerase I. This relationship probably does not exist in yeasts for the following reasons. The activity of RNA polymerase I, as reflected in the production of precursor rRNA, is important in yeast cell cycle regulation (8, 17a, 18), but decreased ODC levels were not always correlated with decreased rRNA production and cell cycle arrest. For example, growth of yeast cells in the presence of spermidine decreased ODC specific activities to very low levels (Table 4). Under these conditions, growth rates were similar and G1 arrest mediated by L-ethionine was unaffected (unpublished data). Moreover, these low levels of ODC may reflect the amounts of active enzyme protein present and not simply enzyme activity, since the addition of spermidine to the in vitro assay system had only small effects on the activity of ODC (data not shown). Thus, we feel it is unlikely that in yeasts the ODC enzyme protein significantly modulates RNA polymerase I activity.

Two further aspects of ODC regulation require comment. First, ODC specific activity began to decrease approximately two generations before the cultures entered the stationary phase (Fig. 3). This observation suggests that cells metabolically respond to (in this case) histidine depletion long before any cell division response is manifested. Second, the levels to which ODC specific activity declined upon G1 arrest mediated by 8-hydroxyquinoline and L-ethionine, which both arrest cells within one cell cycle (8, 18), were not as low as those found for G1 arrest induced by starvation (Table 1). The reason for this difference may lie in the less-immediate cell cycle response brought about by starvation. In these experiments, uracil starvation was obtained by suspending cells in medium containing uracil at low concentrations (0.5 μg/ml). Cells were able to undergo approximately 2.5 doublings under these conditions, increasing in concentration from 0.2×10^6 to 1.3×10^6 cells per ml before arresting in phase G1. Therefore, in fashion similar to that demonstrated for histidine starvation (Fig. 3), during uracil starvation there may be functional responses, such as decreases in ODC specific activity, occurring before the cell division response is seen. A response that decreases ODC specific activity manifested over two generations, as found for G1 arrest induced by starvation, may well be expected to result in ODC levels lower than found for a similar response operating during the more-rapid, first-cycle G1 arrest caused by 8-hydroxyquinoline or L-ethionine.

In summary, ODC levels are normally correlated with cell growth status in the yeast. However, our results indicate that a change in ODC specific activity is simply a response to, but not a cause of, normal cell cycle regulation.

ACKNOWLEDGMENTS

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