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Evidence That Specific and "General" Control of Ornithine Carbamoyltransferase Production Occurs at the Level of Transcription in *Saccharomyces cerevisiae*

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Ornithine carbamoyltransferase synthesis is subject to two major regulatory systems in *Saccharomyces cerevisiae*. One system is specific for the arginine biosynthetic enzymes, whereas the other appears to be general, acting on a variety of other amino acid pathways as well. We observed that the synthetic capacity for continued ornithine carbamoyltransferase synthesis had the same short half-life (ca. 5 to 7 min) whether repression of enzyme production was brought about by action of the specific or general control system. We present evidence suggesting that both control systems regulate accumulation of ornithine carbamoyltransferase-specific synthetic capacity, rather than modulating its expression.

The biosynthesis of many amino acids in *Saccharomyces cerevisiae* appears to be modulated by a common, general regulatory system (7, 18, 20). This conclusion was reached after the isolation of two classes of mutant strains. In one class of strains, many enzymes concerned with the production of basic and aromatic amino acids were pleiotropically synthesized in a constitutive manner. The second class of mutant strains was unable to derepress synthesis of these same enzymes even during times of severe amino acid starvation (18, 20). Delforge et al. (7) demonstrated the presence of a general control system by measuring the levels of biosynthetic enzymes in leaky, auxotrophic strains of *S. cerevisiae* grown in the presence and absence of the required amino acid. Starvation for the required amino acid brought about derepression of enzyme production associated with all of the basic and aromatic amino acid biosynthetic pathways they assayed. Derepression of enzyme synthesis was also observed when charging of isoleucyl transfer ribonucleic acid (RNA) was prevented, leading to the suggestion that uncharged transfer RNAs might be acting as the regulatory signal for the general system (15).

All of the arginine biosynthetic enzymes studied thus far (seven out of eight) are subject to the general regulatory system. Five of these enzymes are also under the control of a specific aporepressor, ARGR, which is apparently defective in *argR*$^-$ mutant strains (2, 7). Recently, a second type of mutant strain was isolated which appears to be defective in one element of the arginine-specific regulatory system. Ornithine carbamoyltransferase (OTCase) production remains at a high level in this organism even in the presence of exogenous arginine; i.e., the enzyme is apparently synthesized constitutively. The mutation producing this phenotype was found to be closely linked to the structural gene specifying OTCase (14). These are the phenotypic characteristics expected of a mutation in an operator locus. The existence of presumptive operator and *argR*$^-$ mutations suggests that at least one of the five arginine biosynthetic enzymes may be negatively controlled (14). Also, specific regulation and general regulation of the arginine biosynthetic enzymes seem to operate independently of one another, because inactivation of either control system results in increased levels of enzyme production (7, 14, 15).

It is reasonable to suggest that specific control, involving interaction of the ARGR aporepressor with a presumptive operator locus, might be transcriptional. On the other hand, general control could be either transcriptional or translational. Enhanced levels of many biosynthetic enzymes observed under starvation conditions could result from greater messenger RNA stability or increased translation efficiency rather than increased messenger RNA synthesis. At present, these questions may be approached in yeast only by using indirect methods that measure accumulation of the synthetic capacity to produce a particular enzyme

TABLE 1. *Yeast strains*

Strain no.	Genotype	Phenotype and comments
6716d	*a cargA2*	Arginase-less mutant
BJ210	*α argRII-10*	Arginine-specific regulatory mutants from two different complementation groups but with identical phenotypes; in these strains, the arginine biosynthetic enzymes aren't repressed by arginine, nor are the catabolic enzymes induced
3501a	*a argRI-2*	
MG409	*α argJ*$^{\pm}$	Arginine leaky auxotrophic strain; block unknown
7431d	*α ilv*$^{\pm}$ *cargA-2*	Double mutant carrying the arginase-less mutation and a leaky auxotrophic block in the isoleucine-valine pathway
7561b	*a rna1*	This ascospore was derived by three successive crosses: (i) M304-*105c* (*α his7 ura1 rna1*) × Σ1278b → 7498b (*rna1*), (ii) 7498b (*rna1*) × Σ1278b → 7539c (*rna1*), 7539c × 7368a (*argRI-2 ilv*$^{\pm}$) ascospores derived from this cross: 7561b, 7560b, 7560a, and 7563c
7560b	*α rna1 argRI-2*	
7560a	*a rna1 ilv*$^{\pm}$	Leaky auxotrophic block
7563c	*a rna1 argRI ilv*$^{\pm}$	
7480a	*a argF*$^{+}$*O*$^{-}$	Operator mutant for OTCase
7366b	*α ilv*$^{\pm}$	Isoleucine-valine leaky auxotrophic mutant
7546b	*a argRII-10 argF*$^{+}$*O*$^{-}$	Ascospore carrying two specific regulatory mutations
7552c	*α argF*$^{+}$*O*$^{-}$ *ilv*$^{\pm}$	Contains two mutations, the OTCase operator mutation and a leaky auxotrophic isoleucine-valine block
7368a	*α argRI-2 ilv*$^{\pm}$*-1*	

under various conditions of growth. By using techniques developed by Kepes (11) and modified by Cooper and his colleagues (4, 5, 12, 13), we have obtained data suggesting that both the general and specific control systems of OTCase production probably operate at the level of transcription.

MATERIALS AND METHODS

Organisms. The strains used in this work were derived either from the wild-type haploid strain, Σ1278b or from strain M304-105c (*a ural his7 rna1*). Strain M304-105c was a haploid organism constructed by Bossinger and Cooper (4) and contained the Ts-136 mutation located in the *rna1* locus. This mutation was transferred to the Σ1278b genetic background (Table 1) by mating strains Σ1278b and M304-105c and backcrossing an ascospore (7498b) containing the Ts-136 mutation to strain Σ1278b. An ascospore derived from this mating and carrying the Ts-136 mutation (7539c) was then mated with strain 7368a (*argRI ilv*$^{\pm}$). Several strains described in Table 1 were derived from this mating.

Culture medium. Medium 165 (denoted here as M.am) was used in this work and was described previously (14); 0.02 M ammonium sulfate and 3% glucose were supplied as the sole nitrogen and carbon sources.

Cell permeabilization and enzyme assay. OTCase activity was assayed in whole cells permeabilized with nystatin. Cell samples (1-ml each) were removed from exponentially growing cultures at the times indicated and cooled to 4°C in the presence of cycloheximide (final concentration, 8 μg/ml). A 1-ml amount of 0.05 M maleate buffer (pH 6.5) was added to each sample along with 0.6 mg of nystatin, which had been dissolved in 0.1 ml of methanol. The treated cells were then shaken for 40 min at 30°C. After permeabilization, two portions (0.05 and 0.1 ml) of each sample were assayed for OTCase activity. The reaction mixture (1-ml) containing 10 mM L-ornithine, 0.25 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8), and 5 mM lithium carbamoylphosphate was added to the cell sample, yielding a final volume of 1.05 to 1.10 ml. After 30 min of incubation at 30°C, the reaction was stopped by adding 3 ml of 1 N HCl. Reagents required for the Archibald colorimetric assay (1) were added to the assay vessels. With this assay procedure, the maximum difference observed between duplicate samples ranged from 3 to 4%. The reproducibility of cell permeabilization was somewhat less satisfactory and is likely responsible for any scatter observed in our data. OTCase activity is expressed as micromoles of citrulline produced per hour per milliliter of culture.

Chemicals. Nystatin was a Labaz (Belgium) product; ornithine, carbamoylphosphate, and Trizma base were purchased from Sigma Chemical Co., St. Louis, Mo.

Transfer of cells from one medium to another. In a number of the experiments described below, it was necessary to transfer cell samples from one medium to another. This was done by filtering the cultures through membrane filters and suspending the harvested cells in fresh medium. All filtrations were performed with nitrocellulose filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.) and were completed in less than 15 to 20 s.

RESULTS

Repression of OTCase synthesis by arginine. The data presented in Table 2 demon-

TABLE 2. *OTCase activity observed in mutant strains carrying defects in the specific and general amino acid control systems*

Strain and pertinent genotype	OTCase activity[a]		
	M.am	M.am + arginine (1 mg/ml)	M.am + isoleucine + valine (1 mg/ml)
Σ1278b (*wild type*)	29	4	
6716d (*cargA2*)	29	4	
BJ210 (*argRII-10*)	61	48	
3501a (*argRI-2*)	56	51	
7480a (*argF⁺O⁻*)	46	31	
7366b (*ilv±*)	60	15	30
7431d (*ilv± cargA2*)	60	15	30
MG409 (*arg±*)	208	4	
7368a (*argRI-2 ilv±*)	118	85	60
7552c (argF⁺O⁻ ilv±)	100	94	
7546b (*argRII-10 argF⁺O⁻*)	51	41	
7561b (25°C) (*rna1*)	29	4	

[a] Activity is expressed as micromoles of citrulline produced per hour and per milligram of protein.

strate the presence of two control systems regulating synthesis of OTCase. A more detailed physiological characterization of these systems has been published elsewhere (7, 14). In wild-type or *cargA2* mutant strains, OTCase production was repressed sevenfold by the addition of L-arginine to the culture. Similar repression, however, was not observed when this experiment was repeated with mutant strains carrying either defective forms of the aporepressor, ARGR (BJ210 and 3501a), or an alteration in the presumptive OTCase gene operator locus (7480a). In an *ilv±* leaky auxotroph (7366b or 7431d), the general control system was turned off due to starvation for isoleucine and valine. This resulted in derepression of OTCase synthesis to the same level observed with the *argR⁻* mutants. However, when isoleucine and valine were added to the medium, thus providing for the deficiency caused by this mutation, enzyme synthesis declined to the levels previously observed for the wild-type strain growing in minimal medium. The specific control system was still functional in these auxotrophic strains as demonstrated by a decline in OTCase production when arginine was added to the medium. Both control systems would be presumably turned off in a leaky arginine auxotroph (MG409), and a highly derepressed enzyme level would be expected. As shown in Table 2, this result was observed. A similar effect was also achieved by combining an *argR* or presumptive operator mutation with a mutation that produced a leaky amino acid auxotroph (strains 7368a and 7552c in Table 2).

Time course of OTCase repression. Figures 1 through 3 depict the time course of enzyme production in cultures of a wild-type strain (Fig. 1), a leaky isoleucine-valine auxotroph (Fig. 2), and an *argRI* mutant strain (Fig. 3) after addition of arginine to the culture medium. Throughout this work an arginase-minus strain (*cargA2*) was used in place of our standard wild type (Σ1278b) because arginine addition to cells growing on M.am medium results in arginase induction (19). We showed previously (3, 16) that arginase normally binds to OTCase in vivo as a means of regulating its activity, an intolerable situation when accurate measurements of the enzyme level are required. Only a transient decrease in enzyme production was observed with the *argR⁻* regulatory mutant (Fig. 3), whereas nearly total cessation of enzyme synthesis occurred in the other two strains (6716d and 7431d; Fig. 1 and 2). Addition of cycloheximide (8 μg/ml), which

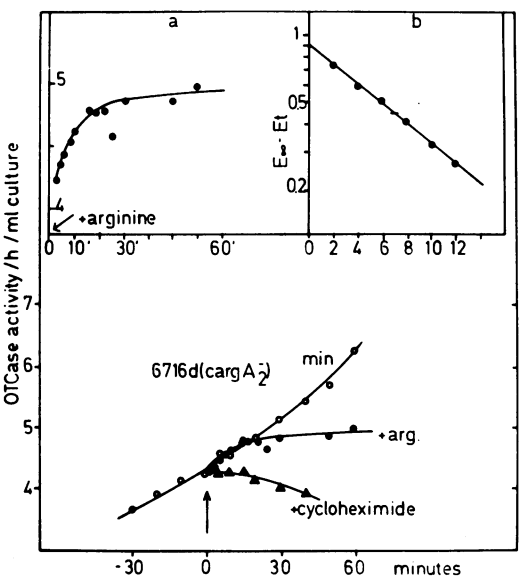

FIG. 1. *Time course for repression of OTCase synthesis after addition of L-arginine (arg). Strain 6716d (arginase minus) was grown at 29°C in M.am medium. At zero time (arrow), the culture was split into three portions: in the control culture* (○), *cells continued to grow in M.am medium; L-arginine was added to the second portion of the culture (final concentration, 0.2 mg/ml)* (●); *and cycloheximide was added to the remaining portion (final concentration, 8 μg/ml)* (▲). *In all three cases, 1-ml samples were removed from the culture at the times indicated. Protein synthesis was stopped by addition of cycloheximide (8 μg/ml), and OTCase activity was assayed as described in the text. Inset (a) depicts an expanded version of OTCase repression by arginine. The plot used for determination of the synthetic capacity half-life is shown in inset (b), where E_∞ is the enzyme level observed at 60 min, and E_t is the level found at the times indicated on the abscissa.*

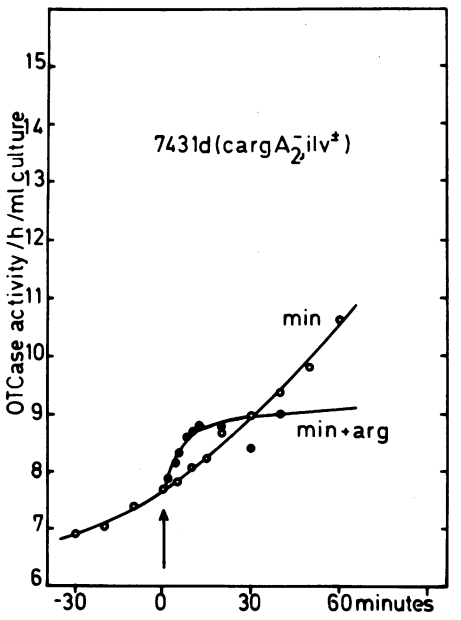

FIG. 2. *Time course for repression of OTCase synthesis by arginine (arg) in a leaky isoleucine-valine auxotroph. Strain 7431d (cargA2 ilv$^\pm$) was grown at 29°C in M.am medium* (○). *At time zero (arrow), L-arginine was added to half of the culture at a final concentration of 0.2 mg/ml* (●). *Samples were removed at the times indicated and processed as described in the legend of Fig. 1.*

is known to act at the level of translational initiation (6), inhibited production of OTCase immediately.

Data shown in Fig. 1 through 3 may be analyzed as described by Kepes (11) and Cooper (4, 12, 13) to determine the stability of the synthetic capacity for continued enzyme production after the onset of repression. The half-life of OTCase-specific synthetic capacity was about 7 min (Table 2). The same values were observed irrespective of the strain in which repression was brought about.

Figure 4 shows the time course of OTCase repression after addition of L-histidine to an *argRII-10* mutant culture treated with 3-amino-1,2,4-triazole. The latter compound inhibits imidazolglycerol phosphate dehydratase, an enzyme of histidine biosynthesis. By lowering the intracellular histidine pool, this inhibitor mimics a leaky auxotrophy and derepresses synthesis of the histidine, lysine, tryptophan, and arginine biosynthetic enzymes. As shown in the figure, OTCase levels were enhanced by the addition of aminotriazole. Addition of histidine to the culture medium restored normal growth and repressed synthesis of OTCase. Since the specific control system was missing in this strain (*argRII-10*), these effects occurred only in response to the general control system. It was not possible to determine the half-life of the OTCase-specific synthetic capacity from these data because repression of enzyme production was not complete. However, it was important to obtain an estimate of synthetic capacity half-life after repression of enzyme synthesis by the general control system. The most desirable way of obtaining such an estimate would have been to add isoleucine and valine to a culture of strain 7431d (*cargA2 ilv$^\pm$*) grown in the presence of arginine. Unfortunately, this experiment was not possible due to the very low levels of arginase produced under these growth conditions. An alternative means of approaching this question was to add arginine to a culture of strain MG409 (*arg$^\pm$*) growing in M.am medium. In this instance, OTCase was synthesized in response to both the general and specific regulatory systems. Enzyme production stopped about 15 min after arginine was added to the medium (Fig. 5). When the data of Fig. 5 were replotted (inset) to estimate the synthetic capacity half-life, a linear semilogarithmic plot was obtained. The half-life estimated from

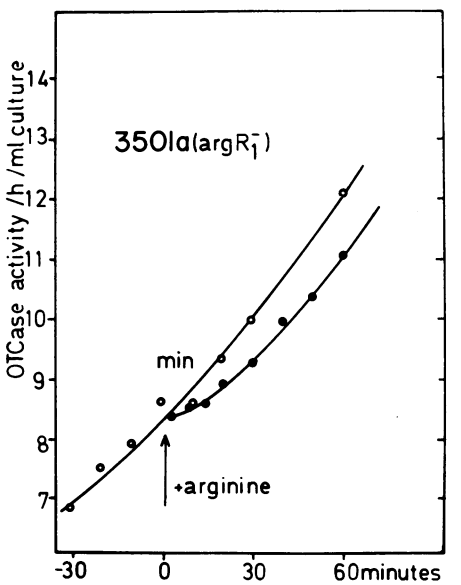

FIG. 3. *Lack of repression of OTCase synthesis in a regulatory mutant (argRI-2). Strain 3501a was grown at 29°C in M.am medium* (○). *At zero time (arrow), the culture was divided in half. L-Arginine was added to one-half of the culture at a final concentration of 0.2 mg/ml* (●), *whereas no further additions were made to the remaining half of the culture. Samples were removed at the times indicated and processed as described in the legend of Fig. 1.*

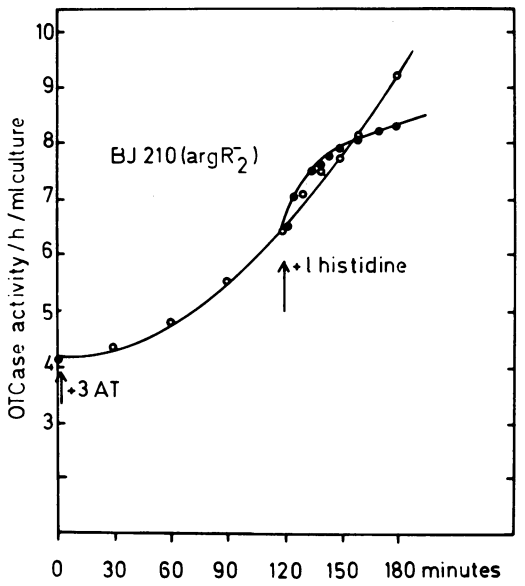

FIG. 4. *Repression of OTCase synthesis by* L-*histidine. Strain BJ210 (argRII-10) was grown in M.am medium at 29°C. At time zero, 3-amino-1,2,4-triazole was added (↑ + 3AT) to the culture at a final concentration of 20 mM. The cells were allowed to derepress OTCase synthesis for 120 min* (○). *At that time,* L-*histidine was added (↑ + l histidine) to half of the culture at a final concentration of 1 mM* (●). *Samples were removed at the times indicated and processed as described in the legend of Fig. 1.*

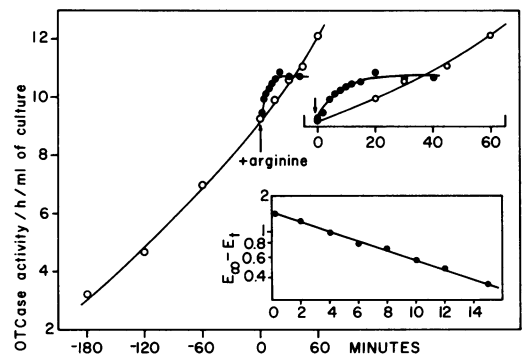

FIG. 5. *Time course for repression of OTCase synthesis after addition of* L-*arginine to a leaky arginine auxotroph. Strain MG409 (arg$^\pm$) was grown at 29°C in M.am medium. Samples were removed for 3 h at the times indicated. After this time (indicated as zero time in the figure), the culture was divided in half. One-half received no further additions, and the second half received* L-*arginine at a final concentration of 0.2 mg/ml (arrow). Both cultures were then sampled for an additional 40 to 60 min. All of the samples were processed as indicated in the text. The synthetic capacity half-life was determined as described in the legend of Fig. 1 (see also inset).*

these data was 7 min. It is significant that the semilogarithmic plot in Fig. 5 exhibited no hint of curvature. Such curvature would indicate the presence of two classes of OTCase-specific synthetic capacity that differ in stability.

OTCase production in strains carrying a defective *rnaI* gene product. Another means of determining the stability of OTCase synthetic capacity is by using mutant strains possessing a defective *rnaI* gene product. This type of strain, which appears to be defective in some step of messenger RNA processing or transport, has been used extensively in the past to determine the synthetic capacity half-life of gross cellular protein (8) and several specific enzymes (J. Bossinger and T. G. Cooper, J. Bacteriol., in press). The half-life values obtained when *rnaI* mutant strains were employed for studies of allophanate hydrolase-specific synthetic capacity were very similar to those observed when enzyme production was terminated by inducer removal or onset of nitrogen repression (4, 12, 13). Hynes and Phillips (9) also used *rnaI* mutant strains for their half-life measurements of polyadenylic acid-containing RNA. As shown in Table 2, the specific activity of OTCase observed with mutant (*rnaI* Ts-136) and wild-type strains growing at 25°C was the same. To ascertain the half-life of the OTCase-specific synthetic capacity, logarithmically growing cultures (25°C) of the wild-type (7561b, *rnaI*) and mutant (7560a, *rnaI argRI*; and 7560b, *rnaI ilv*$^\pm$) strains were sampled periodically for 30 min. After this time, one-half of each culture was collected by filtration and resuspended in an identical medium maintained at 35°C, while the other half remained at 25°C. Both cultures were sampled for another 60 min. About 20 to 30 min elapsed before the cultures incubated at 35°C lost their ability for continued enzyme synthesis (Fig. 6 through 8). All three cultures lost their capacity to produce OTCase, with a half-life of 5 to 6 min (Fig. 9, Table 3). It is significant that the same values were observed whether enzyme production had been due to derepression of the specific or general control systems. The triple mutant 7563c (*rnaI argRI-2 ilv*$^\pm$) exhibited a half-life that was significantly greater than those just cited. This determination was performed several times and always with the same result. The reason behind this increase in synthetic capacity stability is not known. However, at 25°C the triple mutant (7563c [*rnaI argRI-2 ilv*$^\pm$]) grew at the same rate as strain 7560b (*rnaI ilv*$^\pm$).

Specific and general controls act on the accumulation of OTCase synthetic capacity and not on its expression. Transcriptional control, according to the model proposed by Jacob

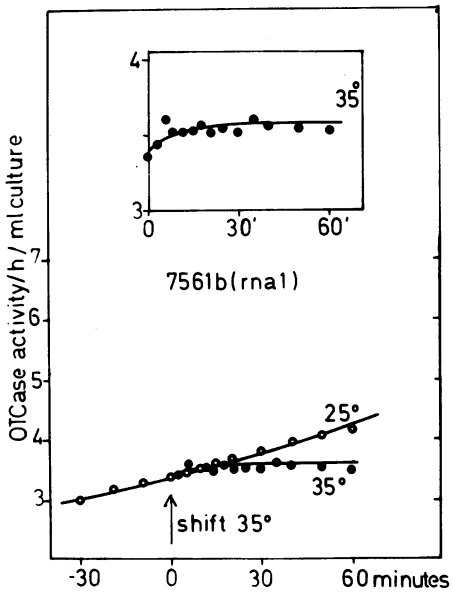

FIG. 6-8. *OTCase synthesis in temperature-sensitive mutant strains grown at the permissive and non-permissive temperatures. All of the cultures were initially grown at 25°C in M.am medium* (○). *At zero time (arrow), one-half of each culture was transferred to medium maintained at 35°C* (●). *Samples were removed from all cultures at the times indicated and processed as described in the legend of Fig. 1.*

FIG. 6. *Time course of OTCase production in a strain that contained a mutation of the rna1 locus.*

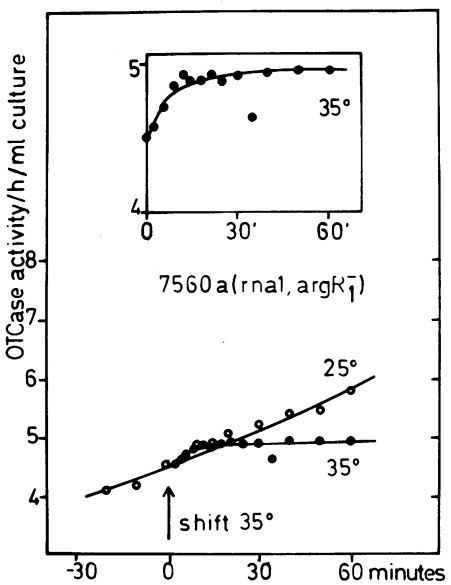

FIG. 7. *Results with a strain that contained mutated forms of the rna1 and argRI loci.*

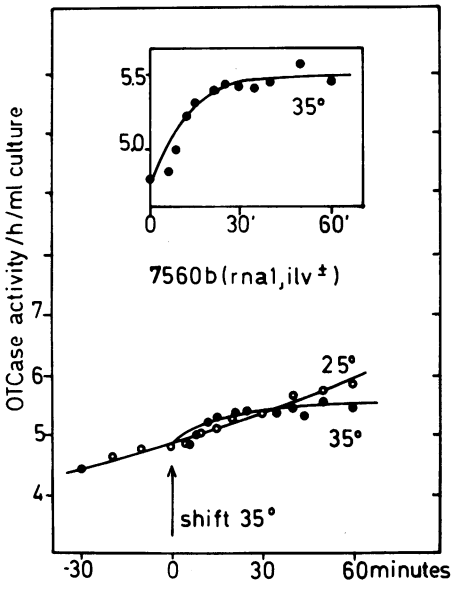

FIG. 8. *Results with a strain that contained mutations in the rna1 and ilv loci.*

TABLE 3. *Half-life of the synthetic capacity for OTCase produced in various mutant strains of S. cerevisiae*

Strain	Means used to arrest production of enzyme sythetic capacity	Synthetic capacity half-life observed (min)
6716d (*cargA*)	Addition of arginine (0.2 mg/ml)	7, 6.8
7431d (*ilv± cargA*)		7
3501a (*argRI-10*)		NR[a]
MG409 (*arg±*)		7
7561B (*rna1*)	Increase in temperature of culture medium: 25°C to 35°C	4.8
7560b, (*ilv± rna1*)		5.5, 5.7
7560b (*ilv± rna1*)		6
7563c (*rna1 argRI-2 ilv±*)		

[a] NR, No repression.

and Monod (10), blocks accumulation of enzyme-forming potential, whereas translational control inhibits the expression of accumulated synthetic capacity. To determine whether control of OTCase production occurred at the level of synthetic capacity accumulation or expression, we monitored the expression of this synthetic capacity in the presence and absence of conditions that cause general and specific repression of enzyme production. Strain 7431d (*cargA2 ilv±*) was grown in minimal medium, and samples were removed periodically for 30

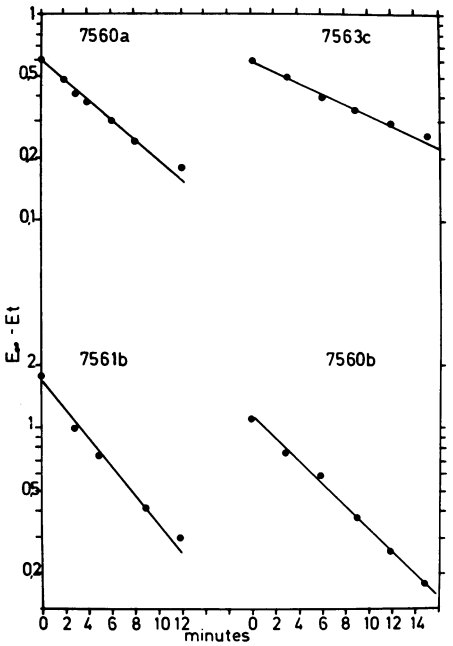

FIG. 9. *Determination of the half-lives of OTCase-specific synthetic capacity in strains 7560a (rnal ilv±), 7560b (rnal argRI-2), 7561b (rnal), and 7563c (rnal argRI ilv±). The method used for these determinations was described in the text and in the legend of Fig. 1 (inset).*

min (denoted as −30 min to zero time in Fig. 10). At time zero and various other times between 0 and 40 min, samples were removed from the culture, transferred to fresh medium, and allowed to express whatever OTCase synthetic capacity they had accumulated. The 40-min expression period was carried out either in medium containing 0.2 mg of L-arginine per ml (closed circles) or in medium devoid of arginine (open squares). After 40 min of incubation, cycloheximide was added to the cells and enzyme activity was measured. The rate of OTCase synthesis was the same in the presence and absence of arginine (Fig. 10A). The experiment shown in Fig. 10B was performed in exactly the same way, but 0.1 mg of isoleucine per ml and 0.1 mg of valine per ml were added in place of arginine. As observed before, enzyme levels found in samples allowed a 40-min expression in M.am medium (open squares) fell on an extension of the curve derived from samples removed during the first 40 min of growth without further expression of synthetic capacity (open circles). Cells incubated with isoleucine and valine during the expression period (closed circles) produced OTCase at the same rate as the control cultures. However, these samples

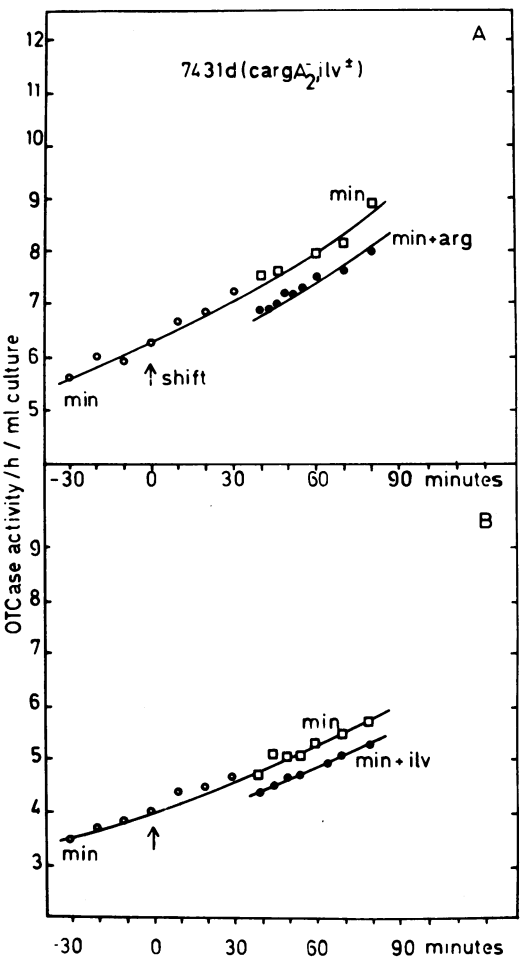

FIG. 10. *Accumulation and expression of OTCase-specific synthetic capacity in the presence of L-arginine (A) or isoleucine plus valine (B). Strain 7431d (cargA2 ilv±) was grown in M.am medium at 29°C. Samples were removed from the culture for 30 min (indicated −30 min to zero time in the figure) and processed as described in the legend of Fig. 1. At zero time, and at the times indicated between 0 and 40 min, samples were transferred from the original culture to fresh medium and allowed to express whatever OTCase synthetic capacity they had accumulated. Expression of synthetic capacity was carried out in M.am medium containing L-arginine (●) or in M.am medium without an added amino acid (□). The control cells were transferred from M.am medium to M.am medium. After 40 min of expression, cycloheximide was added to the cells, and enzyme activity was measured as described in the legend of Fig. 1. ○, Samples removed during the first 40 min of growth without further expression of synthetic capacity. (B) Same as (A), except that expression of the synthetic capacity was carried out in the presence or absence of isoleucine and valine at a final concentration of 0.1 mg/ml each.*

contained less enzyme than samples incubated in the absence of amino acids. The two curves (open squares and closed circles) were separated by about 20-min worth of enzyme production. If, as shown in Fig. 1 through 3, 20 to 30 min is required for total loss of enzyme-forming capacity, then samples incubated in the presence of an amino acid were devoid of enzyme-forming potential for about 10 to 20 min of the 40-min expression period, accounting for the displacement. Inhibition of synthetic capacity expression in this experiment would have been signaled by a large decrease in the rate of enzyme production when an amino acid was present in the expression medium.

DISCUSSION

OTCase synthesis is subject to two major regulatory systems. One system, which is specific for the arginine biosynthetic enzymes, involves interaction of an aporepressor, ARGR, with a presumptive operator region near the OTCase structural gene; the second system appears to be general, because it acts simultaneously on a variety of other amino acid pathways as well. We have presented evidence suggesting that both control systems probably regulate accumulation of OTCase-specific synthetic capacity rather than its expression. This conclusion was reached as a result of two experimental approaches. The first directly assayed the ability of arginine and isoleucine-valine to repress expression of previously accumulated OTCase synthetic capacity. These agents normally repress enzyme production via the specific and general control systems, respectively. In neither case was expression of accumulated synthetic capacity depressed by amino acid addition. Although this experiment does not prove that control of OTCase production is exerted at transcription, it does point to this process as the most likely point at which synthetic capacity accumulation could be modulated.

If both the general and specific control systems operate by regulating the amount of OTCase synthetic capacity, then its stability should be the same regardless of how repression is exerted. In other words, the capacity for continued enzyme production should decay at the same rate whether repression was brought about in response to the specific system or to the general system. Consistent with this expectation, we observed only one decay rate even though repression was elicited in a variety of mutant strains. One of these strains was producing OTCase because it was starved for isoleucine and valine (general derepression, shown in Fig. 2), whereas, in another, production was in response to an absence of arginine from the medium (specific derepression, shown in Fig. 1). In Fig. 5 both general repression and specific repression of enzyme production were exerted simultaneously. Here, as before, only one stability class of OTCase synthetic capacity was found.

In each case where an amino acid was added to a logarithmically growing culture, the rate of enzyme production increased over that seen in the control culture. We have no explanation for this behavior, but we have noticed that the experimental culture exhibited a corresponding increase in absorbance. Whether the increase in absorbance reflected an increase in cell number or cell size has not been determined.

When cycloheximide was added to a culture synthesizing OTCase, enzyme activity not only stopped increasing, but also decreased. This phenomenon has also been observed for allophanate hydrolase (13) and arginase (Bossinger and Cooper, in press). Studies currently in progress suggest that the loss of enzyme activity may be due to proteolytic digestion of cellular protein that is triggered by an inhibition of protein synthesis initiation (T. G. Cooper and R. Sumrada, manuscript in preparation).

A second method of measuring OTCase synthetic capacity half-life involved using strains carrying a temperature-sensitive *rnaI* gene product. Here, the synthetic capacity for enzyme synthesis decayed with a half-life of 5.5 min regardless of the means used to derepress OTCase production (isoleucine-valine starvation or *argR*$^-$ mutation). If one considers the difference in temperature at which the determinations were performed, there is good agreement between the values obtained with *rnaI* mutant strains and those found upon adding a repressing agent to a derepressed culture. As just discussed, the second experimental approach to the problem of general and specific regulation of OTCase synthesis allows one to distinguish between two hypotheses. One hypothesis postulates that, if both controls operate at transcription, then the synthetic capacity synthesized in response to either control system should be indistinguishable from that produced in response to the other; i.e., only one synthetic capacity half-life should be observed. If, on the other hand, the general control system functions by increasing the synthetic capacity stability, two species with different half-lives would be expected: one for the specific system and another for the general system. Our data support the former postulate. Unfortunately, experiments measuring the synthetic capacity half-life of an enzyme do not permit evaluation

of a model in which the general control system functions by increasing or decreasing the translation efficiency of the presumptive enzyme messenger RNA without altering its stability. Such a model postulates that regulation occurs at the expression of a synthetic capacity, a requirement that is at odds with the observations reported in Fig. 10.

OTCase production increased in *rna1* mutant cultures shifted to 35°C over the levels observed in the control cultures (25°C). This behavior was also seen with allophanate hydrolase (4) and was ascribed to an increased rate of protein synthesis at the higher temperature.

The half-life of OTCase-specific synthetic capacity (ca. 5 min) is three to four times more labile than that observed for gross protein synthesis per se (20 min) (8) or polyadenylic acid-containing RNA (17 min) (9, 17). Bossinger and Cooper (in press) suggested that there are at least two distinct classes of messenger RNA molecules in a yeast cell: those that are relatively stable (15- to 20-min half-life) and those that are four- to sevenfold more labile. The labile species have been suggested to be responsible for production of inducible or repressible proteins that must respond rapidly to a changing environment, whereas the more-stable species might be responsible for proteins needed continuously and in large amounts. OTcase is an enzyme that is subjected to a rather complex regulatory scheme both at the level of gene expression (7, 14) and enzyme activity (3, 16). In this regard, the results presented here seem to support the hypothesis constructed by Cooper and his collaborators. It is reasonable to assume that, if regulatory elements are present in a cell to bring about control of gene expression for a certain class of enzymes, this regulation must allow rapid adaptation to the cell environment. Even with the short synthetic capacity half-life observed for OTCase, the enzyme is still produced for 15 to 20 min after addition of arginine to the culture medium. To prove that the different levels of OTCase observed under repressive and derepressive conditions indeed reflect different amounts of messenger RNA, direct in vitro measurements will be needed.

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