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Execution Times of Macromolecular Synthetic Processes Involved in the Induction of Allophanate Hydrolase at 15°C

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We have observed that transcription, involved in production of allophanate hydrolase, is completed 2.5 min after the addition of inducer at 15°C. The *rnal* gene product must be functional up until 10 min; protein synthesis is initiated at 20 min and is terminated by 24 min. Two minutes later, active enzyme appears. These results confirm our earlier observations and eliminate any uncertainty that might have clouded identification of the time within the lag period that is occupied by ribonucleic acid synthesis.

Induction of allophanate hydrolase has been shown to involve two major processes. The first is accumulation of a synthetic capacity, which, from the need of ribonucleic acid (RNA) synthesis for its production, is likely to be messenger RNA. The second is expression of this capacity, which requires protein synthesis but not RNA synthesis. In more recent work (1), we determined the times at which various macromolecular synthetic events are completed during induction of allophanate hydrolase at 22°C: transcription appears to be concluded within 1 to 1.5 min after the addition of inducer; the *rnal* gene product functions at 4 min; protein synthesis is initiated at 9 to 10 min; and active enzyme is first detectable at 13 min.

The method used to determine these execution times was a modification of that developed by Kepes (5) and is diagrammed in Fig. 1. At zero time, inducer (urea) was added to a culture growing in permissive conditions. At various times thereafter, portions of the culture were transferred to a nonpermissive condition for the function being tested. This was either an increased temperature, if the function to be tested had been rendered temperature sensitive by mutation, or addition of a specific inhibitor such as lomofungin (6, 7). After transfer, the samples were permitted to express fully whatever functional synthetic capacity they might have accumulated during incubation at the permissive condition. Such an experimental format yields data that are biphasic in nature. Enzyme activity fails to appear in samples of the culture that are shifted to the nonpermissive condition prior to the execution time of the process being tested. Subsequent to that point, it increases linearly with time. The execution time of the function is the extrapolated point of intersection of these two lines. Although interpretation of data yielded by this method is straightforward, some inaccuracy may be observed if the execution time of a function occurs very early in the induction process. In this case, there is time for only a few samples to be taken before the execution point has passed. Hence, the data obtained are not entirely convincing.

This is the situation that exists for measurements of the time needed to complete transcription that is required for production of allophanate hydrolase (see Fig. 7A of reference 1). An execution point of 1 min was observed (1) for the lomofungin-inhibitable step of allophanate hydrolase induction. Although the data are best fitted by a biphasic curve composed of a 1-min lag followed by a linear increase, they can also conceivably be fitted, albeit less well, by a linear curve that extrapolates through zero time. Such ambiguity is not tolerable, because a linear plot beginning at zero time would argue, contrary to all of our other observations, that RNA synthesis is not required for allophanate hydrolase production. Therefore, the present experiments were designed to eliminate the ambiguity by decreasing the temperature (from 22 to 15°C) at which the above-mentioned experiment was performed. This change increases the observed execution times for the various processes and allows collection of sufficient data to permit an unequivocal interpretation. In addition, we measured the time elapsing between the termination of protein synthesis and appearance of active allophanate hydrolase.

The strains used in this work were those described previously (1). All are prototrophic, diploid organisms that carry mutations (*ts*-136 and *ts*-187) of the *rnal* or *prtl* loci in a homozy-

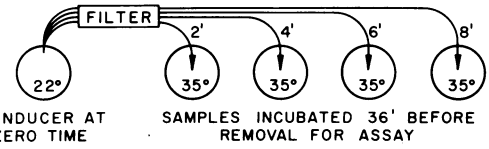

FIG. 1. *Experimental format used to measure the cumulative allophanate hydrolase-specific synthetic capacity.*

gous condition. Strains carrying a mutation in the *rna1* locus are presumed to be defective in the transport of RNA from the nucleus to the cytosol (2, 4). However, additional evidence will likely be required before this conclusion may be considered unequivocal. The remaining strain, which carrys a mutation in the *prt1* locus, is defective in the initiation of protein synthesis at 35°C (3).

The medium used throughout these experiments was that of Wickerham (9). Glucose (0.6%) and ammonium sulfate (0.1%) were provided as sole sources of carbon and nitrogen, respectively. Procedures for manipulating the cultures and shifting them from a medium at one temperature to that at another were described previously (1, 6). The allophanate hydrolase assay was performed as described by Whitney and Cooper (8).

As shown in Fig. 2, 26 min elapsed between the addition of inducer and the first detectable

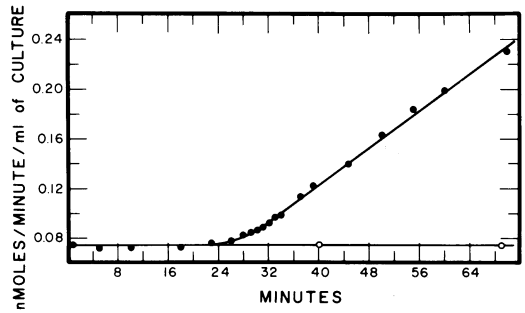

FIG. 2. *Time course of allophanate hydrolase induction at 15°C. A 300-ml culture of wild-type strain M25 was grown to a cell density of about 30 Klett units (green no. 54 filter) at 15°C. One hundred Klett units is approximately equivalent to 3 × 10^7 cells per ml of culture. At this time, the culture was concentrated 10-fold and allowed to equilibrate for 10 min after concentration. After this period, urea (10 mM final concentration) was added to one portion of the culture; the time of urea addition is indicated as zero time. Samples (1 ml each) were removed at the indicated times from both portions of the culture (one to which urea was added, ●; one that received no further additions after concentration, ○) and transferred to test tubes containing cycloheximide (10 μg/ml final concentration) for assay of allophanate hydrolase activity.*

increase in allophanate hydrolase activity. A portion of the culture was incubated in the absence of inducer and served as a measure of basal hydrolase activity. The experimental format depicted in Fig. 1 was used to determine what portion of the 26-min lag time was required for transcription. In this case, portions of the induced culture were transferred, at the times indicated, to flasks containing the RNA polymerase inhibitor lomofungin. Here they were allowed to express fully whatever synthetic capacity they had accumulated prior to the shift. As shown in the upper curve of Fig. 3, 2.5 min passed without a measurable increase in hydrolase activity. Enzymatic activity increased linearly, however, in samples trans-

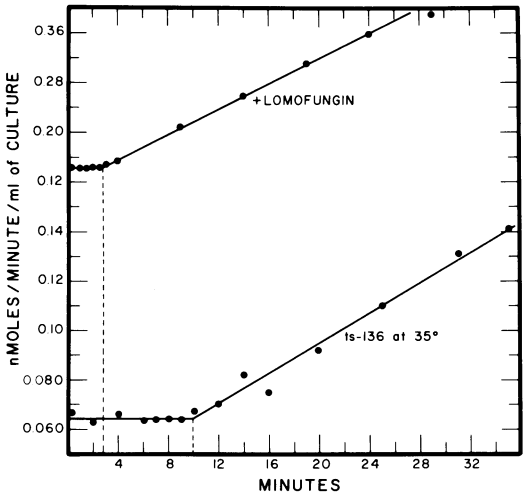

FIG. 3. *Cumulative allophanate hydrolase-specific synthetic capacity measured in the presence and absence of RNA synthesis (upper curve) and rna1 gene product function (lower curve). In the case of the upper curve, a culture of strain M25 was grown to a cell density of about 30 Klett units. The culture was then concentrated 10-fold and allowed to equilibrate for 10 min at 15°C. Following the experimental format outlined in Fig. 1, the culture was induced with urea (10 mM final concentration), and 10-ml samples were removed and added to flasks containing lomofungin (1 μg/ml final concentration, upper curve). After 35 min of incubation, 5-ml samples were taken and assayed for allophanate hydrolase activity. In the case of the lower curve, a strain carrying a temperature-sensitive defect in the rna1 gene product was grown to a cell density of about 30 Klett units. After 10-fold concentration of the culture, urea was added according to the experimental format depicted in Fig. 1. At the indicated times thereafter, 1.0-ml samples were collected by filtration and suspended in 10 ml of fresh medium devoid of urea at 35°C. After 35 min of incubation under these conditions, a 5-ml portion was removed from each sample for assay of allophanate hydrolase.*

ferred subsequent to this time. If this type of experiment was performed with strains carrying temperature-sensitive products of the *rna1* (lower curve of Fig. 3) and *prt1* (upper curve of Fig. 4) genes, execution times of 10 and 20 min, respectively, were observed. The lower curve in Fig. 4 demonstrates the execution time observed when trichodermin, a translation elongation and termination inhibitor, was used to establish the nonpermissive condition. The apparent completion time of protein synthesis was 24 min at this temperature.

The evidence presented here (Fig. 5) indicates that transcription, involved in production of allophanate hydrolase, is completed 2.5 min after the onset of induction at 15°C. The unknown function performed by the *rna1* gene product is concluded by 10 min; protein synthesis is initiated at 20 min and is terminated by 24 min. Two minutes later, active enzyme appears. These results confirm our earlier observations of induction at 22°C and eliminate any uncertainty that might have compromised identification of the time occupied by RNA synthesis during induction of allophanate hydrolase at 22°C. Here we have shown clearly that RNA synthesis accounts for about 10% of the induction lag period. This is in good agreement with the value of 1 min or about 8% of the

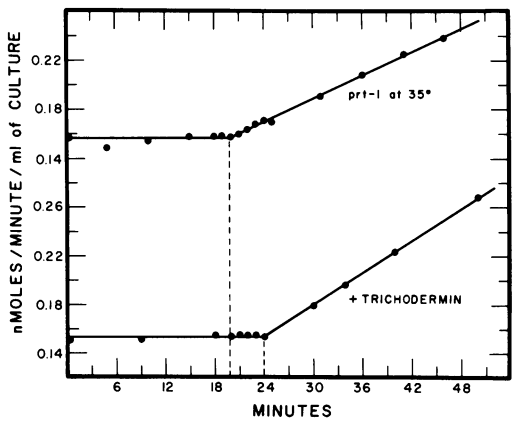

FIG. 4. *Cumulative allophanate hydrolase-specific synthetic capacity measured in the presence and absence of protein synthesis initiation (upper curve) and elongation and termination (lower curve). The procedures used in the case of the upper curve were identical to those used to determine the execution time of the rna1 gene product. However, in this instance, a strain carrying a temperature-sensitive defect in the prt1 gene product was used. The procedures used to obtain the data depicted in the lower curve were identical to those used to determine the execution time of transcription (upper curve of Fig. 3). However, in this case trichodermin (30 μg/ml final concentration) was used in place of lomofungin.*

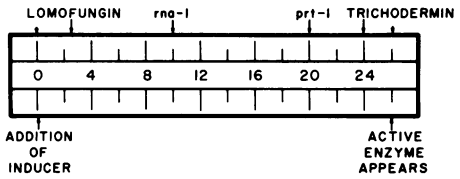

FIG. 5. *Sequence and timing of the macromolecular synthetic events involved in the induction of allophanate hydrolase at 15°C.*

lag period that we reported previously (1). It is interesting that the times required to complete all of the processes up to the initiation of protein synthesis are increased by 2- to 2.5-fold, whereas protein synthesis requires only about 1 min more at 15°C than it does at 22°C. This is in agreement with our earlier observation (6) that the rate of expression of hydrolase-specific synthetic capacity is less temperature dependent than its production.

There exists a period of 2 min between completion of protein synthesis and appearance of active enzyme. This may reflect the time needed for the newly synthesized polypeptide chain to assume its active conformation. If this is true, there is conceivably sufficient time for some processing of the polypeptide chain to occur. However, such speculation must be tempered, for the present, by the fact that this 2-min period may simply reflect a difference in the resolution capabilities of procedures used to obtain the 26-min value (Fig. 2) and the point of protein synthesis termination (lower curve of Fig. 4).

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LITERATURE CITED

1. **Bossinger, J., and T. G. Cooper.** 1976. Sequence of molecular events involved in induction of allophanate hydrolase. J. Bacteriol. 126:198-204.
2. **Hartwell, L. H., H. T. Hutchinson, T. M. Holland, and C. S. McLaughlin.** 1970. The effect of cycloheximide upon polyribosome stability in two yeast mutants defective respectively in the initiation of polypeptide chains and in messenger RNA synthesis. Mol. Gen. Genet. 106:347-361.
3. **Hartwell, L. H., and C. S. McLaughlin.** 1969. A mutant of yeast apparently defective in the initiation of protein synthesis. Proc. Natl. Acad. Sci. U.S.A. 62:468-474.
4. **Hutchinson, H. T., L. H. Hartwell, and C. S. McLaughlin.** 1969. Temperature-sensitive yeast mu-

tant defective in ribonucleic acid production. J. Bacteriol. 99:807-814.
5. **Kepes, A.** 1963. Kinetics of induced enzyme synthesis: determination of the mean life of galactosidase-specific messenger RNA. Biochim. Biophys. Acta 76:293-309.
6. **Lawther, R. P., and T. G. Cooper.** 1975. Kinetics of induced and repressed enzyme synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. 121:1064-1073.
7. **Lawther, R. P., S. L. Phillips, and T. G. Cooper.** 1975. Lomofungin inhibition of allophanate hydrolase synthesis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. 137:89-99.
8. **Whitney, P. A., and T. G. Cooper.** 1972. Urea carboxylase and allophanate hydrolase. Two components of adenosine triphosphate:urea amido-lyase in *Saccharomyces cerevisiae*. J. Biol. Chem. 247:1349-1353.
9. **Wickerham, L. J.** 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J. Bacteriol. 52:293-301.