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Temporal Separation of Transcription and Translation in *Neurospora*

J. R. TURNER, K. TERRY,[1] AND W. H. MATCHETT[2]

Biology Department, Battelle Memorial Institute, Pacific Northwest Laboratories, Richland, Washington 99352

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Cells of *Neurospora* accumulated capacity for synthesis of kynureninase in the presence of cycloheximide and a suitable inducer. The capacity was expressed only when the cells were returned to a medium which allowed net synthesis of protein to resume. Subsequent expression did not require synthesis of ribonucleic acid (i.e., was not inhibited by actinomycin D) or the presence of inducer. The relative level of capacity, as judged by the initial rate of appearance of enzyme, increased linearly for 70 min in cells incubated in the presence of inducer plus antibiotic.

In *Neurospora* and other eucaryotes, cycloheximide interrupts protein synthesis but allows some synthesis of ribonucleic acid (RNA) to proceed. Several authors (2, 4) have reported that ribosomal RNA is not accumulated during treatment with cycloheximide. Others (3, 6) have suggested that cycloheximide RNA consists predominantly of high turnover, deoxyribonucleic acid (DNA)-like RNA. In addition, a recent report (5) indicates the appearance of the capacity for synthesis of a proteolytic enzyme in germinating cotton seeds which occurs in the presence of cycloheximide and which is sensitive to actinomycin D.

In the accompanying paper, we described an experiment with *Neurospora*, which suggests that, in the presence of cycloheximide and kynurenine, messenger RNA (mRNA) specific for kynureninase is accumulated in the absence of concomitant translation. We describe here experiments which show the accumulation of capacity to synthesize kynureninase under conditions where protein synthesis is severely inhibited. The accumulation of this capacity, but not its expression, is sensitive to actinomycin D. The capacity, presumably a reflection of the level of mRNA, increases in a linear fashion for at least 70 min, as judged by its initial rate of expression. This rate is about sevenfold higher than the differential rate of synthesis of kynureninase observed during conventional induction experiments.

MATERIALS AND METHODS

Neurospora strains and media. Wild-type *N. crassa* strain 74A was used in these experiments. Cultures for experiments were prepared as described previously (10) and in the accompanying paper (11).

Biochemical methods. The measurement of kynureninase (11) and of kynurenine in crude extracts (10) was performed by a fluorometric method in which anthranilic acid was assayed continuously by monitoring the reaction in the cuvette of a spectrofluorometer.

Reagents and chemicals. Cycloheximide (actidione) was purchased from the Upjohn Co., Kalamazoo, Mich. Actinomycin D and L-kynurenine sulfate, B-grade, were purchased from Calbiochem, Los Angeles, Calif. Ethylenediaminetetraacetic acid (EDTA) was obtained from the Sigma Chemical Co., St. Louis, Mo. Tris buffers were prepared with Sigma-7-9, a preparation of tris(hydroxymethyl)aminomethane obtained from Sigma Chemical Co. All other chemicals were reagent grade.

RESULTS

Induction of kynureninase and its inhibition by cycloheximide. In Fig. 1, the curves of panel A show the time course of induced synthesis of kynureninase by kynurenine and the formation of an intracellular pool of kynurenine. The synthesis of enzyme proceeds rapidly after a lag of about 10 min. Pool formation starts immediately, reaches a maximal value after about 30 min and then declines as the external supply of kynurenine approaches exhaustion. In the presence of cycloheximide (Fig. 1, panel B), induced synthesis of the enzyme fails but pool formation is not disturbed. The effect of cycloheximide is thus exerted on induction and not on transport of the inducer across the cell membrane.

That the failure of induction noted in Fig. 1 is caused by inhibition of protein synthesis is suggested by the results presented in Fig. 2. For this experiment, cycloheximide (4 μg/ml) was added to cells at zero time. After 5 min, kynurenine was added to an initial concentration of 0.05

[1] Present address: Department of Biochemistry, University of Kansas, Lawrence, Kan. 66045.

[2] Present address: Battelle Seattle Research Center, Battelle Memorial Institute, Seattle, Wash. 98105.

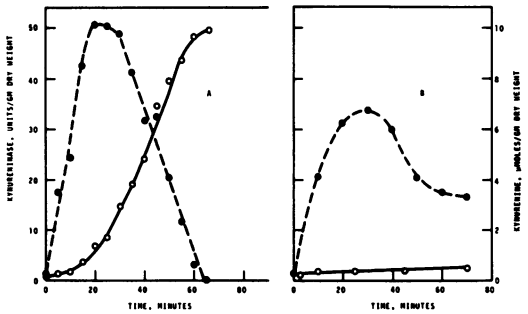

FIG. 1. *Failure of conventional induction in the presence of cycloheximide. (A) No cycloheximide, kynurenine (0.05 μmole/ml) added at zero time. (B) Cycloheximide (4.0 μg/ml) and kynurenine (0.05 μmole/ml) added at zero time. (○) Kynureninase; (●) intracellular kynurenine.*

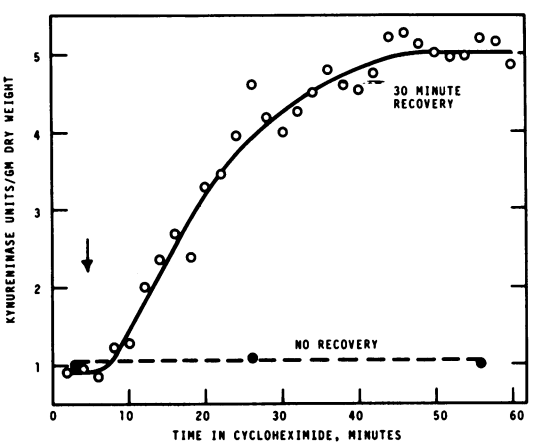

FIG. 2. *Expression of capacity for synthesis of kynureninase. Cycloheximide (4 μg/ml) was added at zero time. Kynurenine (0.05 μmole/ml) was added at 5 min, as indicated by the arrow. At the indicated times samples were washed and either frozen immediately (●) or reincubated for 30 min in minimal medium before freezing (○).*

μmole/ml. At intervals of 2 min thereafter, duplicate samples of cells were removed. One of these was frozen immediately, the other was washed on a filter with fresh minimal medium, resuspended, and reincubated in minimal medium for 30 min. It was then frozen and extracts of each sample were examined for the presence of kynureninase. As expected, the nonrecovered cells showed no induced synthesis of enzyme. That they did have the latent capacity to synthesize enzyme is shown by the cells which were allowed to recover for 30 min. This capacity, as judged by subsequent appearance of enzyme, appeared at 4 min after addition of kynurenine and increases for about 30 min.

Quantitative measurement of capacity. The differential rate of synthesis of an enzyme is generally believed to be directly proportional to the frequency of occurrence of its mRNA in the general pool of mRNA (7). We attempted to evaluate the relative amount of message specific for kynureninase accumulating under the conditions described in Fig. 2, by measuring the initial rate of appearance of enzyme during recovery after various periods of treatment with inducer in the presence of cycloheximide.

The results of these measurements are presented in Fig. 3. These curves show the rates of appearance of kynureninase and the intracellular con-

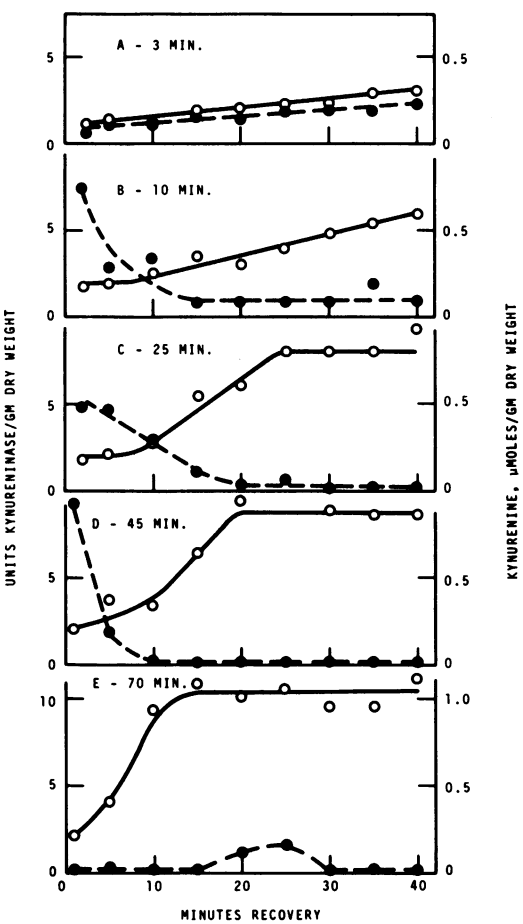

FIG. 3. *Initial rates of synthesis of kynureninase during recovery from treatment with cycloheximide and kynurenine. Cycloheximide (4 μg/ml) was added at zero time. Kynurenine (0.05 μmole/ml) was added at 5 min. Cultures were washed free of cycloheximide and kynurenine and returned to minimal medium at intervals of 3, 10, 25, 45, and 70 min after addition of kynurenine. Samples for determination of enzyme (○) and intracellular kynurenine (●) were removed at 5-min intervals after initiation of recovery. All ordinate scales are linear.*

centrations of kynurenine in recovering cells which had been treated with inducer in cycloheximide for periods from 3 to 70 min. At the time intervals indicated for each panel (Fig. 3), samples of cells were removed from inducer, washed, and returned to minimal medium. Samples were taken from the recovering cells at 5-min intervals during the 40 min of incubation in competent medium. Crude extracts of these samples were examined for kynureninase activity and kynurenine concentration. The rate of appearance of enzyme increased as the length of induction was prolonged. The initial rates of enzyme synthesis (the slopes for the first 20 min of the recovery period shown in Fig. 3) plotted as a function of time in the presence of inducer plus cycloheximide show a linear increase for 70 min (Fig. 4). The dashed curves in the panels of Fig. 3 indicate the intracellular concentrations of kynurenine in the recovering cultures. No apparent relation was found between the intracellular concentration of inducer and the observed rate of synthesis of enzyme. It seems unlikely, therefore, that the increase in activity observed in these cultures was due to initiation of induction at some time after recovery began. We conclude, therefore, that kynureninase-specific messages were produced by the cells during treatment with cycloheximide and inducer such that their relative frequency in the message pool increased linearly for 70 min. They were subsequently translated after the cells were returned to minimal medium.

This conclusion is further substantiated by the experiment presented in Fig. 5 which shows that conventional induction of the enzyme was inhibited by a brief treatment with actinomycin D, but that expression of latent capacity was not.

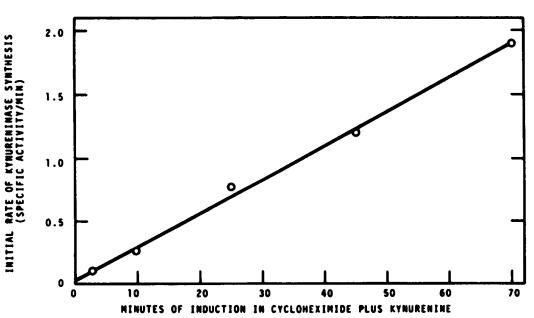

FIG. 4. *Initial rate of synthesis of kynureninase plotted as a function of duration of pretreatment with cycloheximide and kynurenine. Rates were calculated from data of Fig. 3 and are based on synthesis during the first 20 min of recovery. Linear regression analyses were performed using the first five points of each curve. Correlation coefficients for each slope are: A, 0.904; B, 0.907; C, 0.962; D, 0.944; and E, 0.908.*

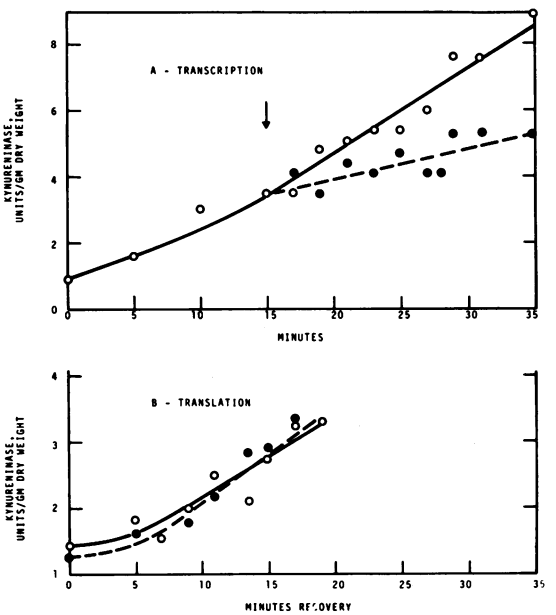

FIG. 5. *Sensitivity of transcription but not translation to actinomycin-D. (A) Kynurenine (0.05 μmole/ml) was added to a logarithmically growing culture at zero time. At 15 min (indicated by the arrow), the culture was divided into two subcultures. One subculture (●) was harvested, washed, and resuspended in Tris buffer (0.1 M, pH 8.0) containing EDTA (0.1 M) and actinomycin D (2 μg/ml); the second subculture (○) was similarly treated except that actinomycin D was omitted. Both subcultures were incubated at 30 C for 4 min, after which they were harvested, washed, and resuspended in minimal medium containing kynurenine (0.05 μmole/ml). (B) Cycloheximide (4 μg/ml) and kynurenine (0.05 μmole/ml) were added to a logarithmically growing culture at zero time. After 70 min of incubation at 30 C, the culture was divided into two subcultures. One subculture (○) was harvested, washed, and resuspended in Tris buffer (0.1 M pH 8.0) containing EDTA (0.1 M) and actinomycin-D (2 μg/ml); the second subculture was similarly treated except that actinomycin D was omitted. After 4 min, both cultures were harvested, washed, and resuspended in minimal medium. Zero time at recovery represents the time of resuspension in minimal medium.*

Panel A (Fig. 5) depicts a conventional induction experiment in which the induced culture was divided after 15 min. One-half (closed circles) was treated with actinomycin D (2 μg/ml) and EDTA (0.1 M) in Tris buffer (0.1 M, *p*H 8.0) for 4 min. The other half (open circles) was treated the same with a solution lacking actinomycin D. After treatment, both were returned to minimal medium containing L-kynurenine (0.05 μmole/ml). Induction immediately following treatment with actinomycin D was inhibited to about 50% of the con-

trol value (Fig. 5). Panel B (Fig. 5) shows the results obtained when cells which had been preinduced for 70 min in the presence of kynurenine plus cycloheximide were similarly treated with actinomycin D immediately before recovery. No obvious effect of actinomycin D was observed on the expression of latent capacity (i.e., translation of performed messages).

DISCUSSION

The experiments described here show that the capacity to synthesize kynureninase can be formed by cells under conditions where inducer is present in the cells but net synthesis of protein is severely inhibited. The subsequent expression of this capacity requires neither the presence of inducer nor the synthesis of RNA. These experiments also show that in conventional induction where transcription and translation are proceeding simultaneously, interruption of either protein synthesis or RNA synthesis prevents appearance of enzyme.

Several studies in bacteria have demonstrated clearly that derepression and induction occur as the result of increased representation of a particular species of message RNA in the general population of these molecules (1, 7–9). Thus, the conclusion that the differential rate of synthesis of a particular enzyme is proportional to the relative abundance of its message RNA (7) is now generally accepted.

In *Neurospora*, no obvious means is available for direct measurement of a specific message RNA. It seems reasonable, however, to assume that the proportionality between frequency of occurrence of a message and the rate of synthesis of the protein for which it codes obtains in this organism. By proceeding on this assumption, we can make certain inferences regarding the relative amount of kynureninase messages which accumulated in our experiments. Although the absolute rates of synthesis of kynureninase in the recovery experiments described here were considerably lower than those observed in conventional cases of induction (Fig. 1, 2, and 3), it should be noted that overall protein synthesis is severely inhibited during the first 30 min of recovery from cycloheximide inhibition (R. C. Kelley, *unpublished data*). A consideration of differential rates of kynureninase synthesis during recovery under the conditions described here and a comparison of them with those observed in conventional induction experiments reveal that after 3 min of transcription, translation proceeds at 60% of the conventional rate. After 10 min of transcription, the rate was equal to the conventional rate, and by 25 min the rate exceeds the conventional by a factor of 3. At 45 and 70 min of transcription, the rates of translation exceed the conventional by factors of 4 and 7, respectively. We conclude, therefore, that during the 70-min period of transcription the frequency of occurrence of kynureninase messages increased linearly to a value which was sevenfold higher than that present under conditions of conventional induction.

The experiments described here indicate that messages transcribed in the absence of translation are somehow stabilized and subsequently accumulated. Could it be that untranslated messages remain in the nucleus and that translation is in some way involved in their removal to the cytoplasm? If this were the case, their intranuclear disposition might protect them from nucleolytic attack to which they would be subjected in the cytoplasm immediately following their normal translation. The curves of Fig. 3 offer some support for this suggestion, since the lifetime of the message appears to be inversely related to its initial rate of translation.

This system offers some experimental approaches to the problem of retrieval of information from the eucaryotic nucleus. It affords a means to study the events leading to initiation of transcription in the absence of net protein synthesis and for studying the relation between translation and removal of messages from the nucleus.

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LITERATURE CITED

1. Edlin, G., G. S. Stent, R. F. Baker, and C. Yanofsky. 1968. Synthesis of a specific messenger RNA during amino acid starvation of *Escherichia coli*. J. Mol. Biol. 37:257–268.
2. Fiala, E. S., and F. F. Davis. 1965. Preferential inhibition of synthesis and methylation of ribosomal RNA in *Neurospora crassa* by actidione. Biochem. Biophys. Res. Commun. 18:115–118.
3. Fukuhara, H. 1965. RNA synthesis of yeast in the presence of cycloheximide. Biochem. Biophys. Res. Commun. 18:297–301.
4. Haidle, C. W., and R. Storck. 1966. Inhibition by cycloheximide of protein and RNA synthesis in *Mucor rouxii*. Biochem. Biophys. Res. Commun. 22:175–180.
5. Ihle, J. N., and L. Dure III. 1969. Synthesis of a protease in germinating cotton cotyledons catalyzed by mRNA synthesized during embryogenesis. Biochem. Biophys. Res. Commun. 36:705–710.
6. de Kloet, S. R. 1965. Accumulation of RNA with a DNA like base composition in Saccharomyces carlsbergensis in the

presence of cycloheximide. Biochem. Biophys. Res. Commun. 19:582–586.
7. Nakada, D., and B. Magasanik. 1964. The roles of inducer and catabolite repressor in the synthesis of β-galactosidase by *Escherichia coli*. J. Mol. Biol. 8:161–181.
8. Spiegelman, S., and M. Hayashi. 1963. The present status of the transfer of genetic information and its control. Cold Spring Harbor Symp. Quant. Biol. 28:161–181.
9. Stubbs, J. D., and B. D. Hall. 1968. Level of tryptophan messenger RNA in *Escherichia coli*. J. Mol. Biol. 37:289–302.
10. Turner, J. R., and W. H. Matchett. 1968. Alteration of tryptophan-mediated regulation in *Neurospora crassa* by indoleglycerol phosphate. J. Bacteriol. 95:1608–1614.
11. Turner, J. R., W. A. Sorsoli, and W. H. Matchett. 1970. Induction of kynureninase in *Neurospora*. J. Bacteriol. 103: 364–369.