CYCLIC AMP-INDUCED TYROSINASE SYNTHESIS IN NEUROSPORA CRASSA

Jerry F. Feldman* and J. Paige Thayer

Department of Biological Sciences
State University of New York at Albany
Albany, New York 12222

Received October 19, 1974

Cyclic AMP induces the synthesis of tyrosinase in Neurospora crassa. Adenine, adenosine, 3'-AMP, 5'-AMP, and 2',3'-cyclic AMP have no inductive effect while 8-bromocyclic AMP and dibutyryl cyclic AMP are good inducers. Caffeine and theophylline, inhibitors of cyclic AMP phosphodiesterase, also induce tyrosinase. A possible relationship between cyclic AMP induction and previously reported induction by cycloheximide is suggested.

Tyrosinase is normally synthesized in sexually differentiating cultures of Neurospora crassa but not during vegetative growth (1). It is responsible for the conversion of L-tyrosine to melanin, the main pigment of the perithecia and ascospores. Sexual differentiation, as well as derepression of tyrosinase and L-amino acid oxidase, can be induced by starvation conditions (2,3). In addition, de novo synthesis of these enzymes can be stimulated by agents which interfere with protein synthesis, such as cycloheximide, amino acid analogs, and D-aromatic amino acids (4,5). To explain this paradoxical enzyme induction by partial inhibition of protein synthesis, Horowitz et al (5) proposed a model involving a rapidly turning-over protein repressor whose level decreases when protein synthesis is inhibited.

However, protein synthesis inhibitors do not induce tyrosinase well during rapid growth; they are much more effective in cultures which have reached stationary phase. This requirement for some form of starvation or nutrient depletion suggests a similarity to catabolite repression in bacteria (6) and might suggest the involvement of cyclic 3',5'-adenosine monophosphate

*Present address: Biology Board of Studies, Thimann Laboratories
University of California, Santa Cruz, California 95064

(7) (cAMP) in tyrosinase regulation. A possible role for cyclic AMP in catabolite repression in yeast has already been indicated (8) and cyclic AMP (9), adenylate cyclase (10), and cyclic AMP phosphodiesterase (11) have all been identified in Neurospora. We now report that exogenous cyclic AMP as well as inhibitors of cyclic AMP phosphodiesterase derepress tyrosinase in Neurospora crassa.

MATERIALS AND METHODS

Strains. Wild-type strain 69-1113a and the female-sterile mutants ty-1, ty-2 and T22 were obtained from Dr. N.H. Horowitz, California Institute of Technology. Strain 69-1113a had been selected for high tyrosinase production. Properties of 69-1113a, ty-1, and ty-2 have been previously described (2). T22 cannot be induced for tyrosinase synthesis by any of the known conditions for induction but is not a mutation in the structural gene for tyrosinase (Horowitz and Macleod, personal communication).

Growth Conditions. 125 ml Erlenmeyer flasks containing 20 ml of Vogel's (12) salts and 1/2% sucrose were inoculated with 10^5 conidia/ml. Flasks were left standing, shielded from direct light, at 25°C for 4 days unless otherwise noted. At the end of 4 days inducers were added and the flasks transferred to a reciprocating shaker (80 strokes/min) also at 25°C. After 48 hours of shaking the pads were harvested, pressed dry, and wet weights determined. Pads were either assayed immediately or stored at -20°C.

Enzyme Extraction and Assay. Crude extracts were prepared similar to the method of Horowitz et al (2) by grinding pads in a cold mortar and pestle with sand and 10 or 20 parts by weight of 0.1 M Na phosphate buffer, pH 6.0. The homogenate was centrifuged for 15 minutes at 27,000 x g and the supernatant used as the source of enzyme. Tyrosinase activity was determined by the photometric method of Horowitz et al (2) and a conversion factor was used to translate these measurements into Enzyme Commission Units (13). Specific activity was expressed as Enzyme Commission Units/gm wet weight as previously explained (2).

TABLE 1

TYROSINASE INDUCTION BY CYCLIC AMP AND CYCLIC AMP ANALOGS

Addition	Concentration	Tyrosinase Activity
None	---	4.4
3',5'-cyclic AMP	1 mM	9.8
	2 mM	26.8
	5 mM	59.7
Dibutyryl cyclic AMP	5 mM	26.8
8-bromocyclic AMP	5 mM	63.3
adenine	5 mM	11.2
adenosine	5 mM	4.5
3'-AMP	5 mM	8.5
5'-AMP	5 mM	2.2
2',3'-cyclic AMP	5 mM	9.8

Chemicals. 3',5'-cyclic AMP and 5'-AMP were obtained from Schwarz-Mann, Orangeburg, New York. 2',3'-cyclic AMP, 8-bromocyclic AMP, dibutyryl cyclic AMP, 3'-AMP, caffeine, theophylline, and cycloheximide were purchased from Sigma Chemical Company, St. Louis.

RESULTS

Tyrosinase Induction by Cyclic AMP. Addition of 1-5 mM cyclic AMP to 4-day-old stationary phase cultures of wild-type strain 69-1113a results in a 10-12 fold increase in tyrosinase activity (Table 1). This inductive effect is highly specific, since adenine, adenosine, 3'-AMP, 5'-AMP, and 2',3'-cyclic AMP did not significantly raise tyrosinase levels above the controls. Two cyclic AMP analogs which mimic cyclic AMP activity in many other systems (14) - dibutyryl cyclic AMP and 8-bromocyclic AMP - also caused significant induction of tyrosinase.

Tyrosinase Induction by Caffeine and Theophylline. Further evidence for

TABLE 2

TYROSINASE INDUCTION BY THEOPHYLLINE AND CAFFEINE

Addition	Concentration	Tyrosinase Activity
None	---	4.4
Theophylline	5 mM	6.9
	10 mM	31.6
	25 mM	108.8
Caffeine	10 mM	15.9
	25 mM	118.8
	50 mM	182.5

the involvement of cyclic AMP comes from the induction of tyrosinase by caffeine and theophylline, inhibitors of cyclic AMP phosphodiesterase (Table 2). At concentrations which effectively inhibit Neurospora phosphodiesterase activity in vitro (11), both of these compounds cause significant induction of tyrosinase.

Tyrosinase Induction in Female-Sterile Mutants. To attempt to determine whether any relationship exists between cyclic AMP induction and previously reported (5) induction by inhibitors of protein synthesis, cyclic AMP and cycloheximide were added to cultures of three female-sterile mutants whose regulation of tyrosinase was known to be abnormal (2; Horowitz, personal communication). Table 3 shows a strong correlation in the inducibility of tyrosinase in these mutants between these two compounds. Although cyclic AMP always induces less than cycloheximide when added to stationary phase cultures, inducibility in ty-1 is relatively high for both compounds, is significantly reduced in ty-2, and is entirely blocked in T22.

DISCUSSION

The results presented here suggest that cyclic AMP is involved in the regulation of tyrosinase synthesis in Neurospora crassa. When added to stationary phase cultures cyclic AMP causes a concentration-dependent increase

TABLE 3

TYROSINASE INDUCTION IN FEMALE-STERILE MUTANTS

	Tyrosinase Activity		
Additions	ty-1	ty-2	T22
None	2.6	1.9	0.6
Cycloheximide (1.4 uM)	512.0	137.0	0.5
Cyclic AMP (5 mM)	20.4	5.4	0.5

in tyrosinase activity, which most likely represents synthesis of new enzyme, since increase of tyrosinase activity caused by other substances under the same conditions has previously been shown to represent de novo synthesis of the enzyme and to be due to a differential rate of enzyme synthesis rather than a general increase in protein synthesis (4). Further evidence for cyclic AMP involvement comes from induction by the cyclic AMP analogs and by the inhibitors of cyclic AMP phosphodiesterase.

These results involving starvation, cyclic AMP, and cycloheximide in tyrosinase regulation, bear close resemblance to several phenomena in other systems. In E. coli cyclic AMP acts as a positive regulatory factor for operons under the control of catabolite-repression - starvation conditions result in increased cyclic AMP levels which allow the induction of a variety of catabolite enzymes (7). In the slime mold, Dictyostelium discoideum, starvation is necessary for the cyclic AMP-mediated aggregation which ultimately leads to the differentiation of resistant spores able to withstand "hard times" (15).

Even more striking are the similarities with yeast where, under starvation conditions, cycloheximide stimulates RNA synthesis (16) and this stimulation by cycloheximide can be mimicked by cyclic AMP (17). Further analysis of

the possible relationship between cyclic AMP and cycloheximide induction of tyrosinase is now being carried out.

We thank Drs. N. H. Horowitz and W. A. Scott for helpful discussions and comments.

REFERENCES

1. Hirsch, H.M. (1954). Physiol. Plant. 7, 72-97.
2. Horowitz, N.H., Fling, M., Macleod, H., and Sueoka, N. (1960). J. Mol. Biol. 2, 96-104.
3. Horowitz, N.H. (1965). Biochem. Biophys. Res. Comm. 18, 686-692.
4. Horowitz, N.H., Fling, M., Macleod, H., and Watanabe, Y. (1961). Cold Spr. Harb. Symp. Quant. Biol. 26, 233-238.
5. Horowitz, N.H., Feldman, H.M., and Pall, M.L. (1970). J. Biol. Chem. 245, 2784-2788.
6. Magasanik, B. (1961). Cold Spr. Harb. Symp. Quant. Biol. 26, 249-256.
7. Pastan, I. and Perlman, R. (1970). Science 169, 339-344.
8. Van Wijk, R. and Konijn, T.M. (1971). FEBS Letters 13, 184-186.
9. Scott, W.A. (1974). Personal communication.
10. Flawia, M.M. and Torres, H.N. (1972). J. Biol. Chem. 247, 6873-6879.
11. Scott, W.A. and Solomon, B. (1973). Biochem. Biophys. Res. Comm. 53, 1024-1030.
12. Vogel, H.J. (1964). Amer. Naturalist 98, 435-446.
13. Fling, M., Horowitz, N.H., and Heinemann, S.F. (1963). J. Biol. Chem. 238, 2045-2053.
14. Meyer, R.B., Jr. and Miller, J.P. (1974). Life Sciences 14, 1019-1040.
15. Konijn, T.M., Barkley, D.S., Chang, Y.Y., and Bonner, J.T. (1968). Amer. Naturalist 102, 225-233.
16. Gross, K.J. and Pogo, A.O. (1974). J. Biol. Chem. 249, 568-576.
17. Foury, F. and Goffeau, A. (1973). Nature New Biology 245, 44-47.