JOURNAL OF BACTERIOLOGY, Nov. 1974, p. 741-747

Vol. 120, No. 2
Printed in U.S.A.

Cold-Induced Increase of Glycerol Kinase Activity in *Neurospora crassa*: Rapid Inactivation of the Enzyme In Vivo

M. J. NORTH[1]

Department of Biochemistry, The University, Newcastle upon Tyne, NE1 7RU, United Kingdom

Received for publication 16 August 1974

The glycerol kinase activity induced by incubation of *Neurospora crassa* at low temperatures was rapidly lost when cultures were returned to 26 C. After a short lag, the activity disappeared irreversibly with a half-life of approximately 15 min. The loss of activity was not due to a change in the level of an inhibitor or activator. Glycerol reduced the activity loss but did not completely prevent it, which was an effect that was dependent on protein synthesis. The cold-induced activity was also always lost on addition of cycloheximide at all temperatures tested (0 to 26 C), which indicated continuous inactivation, although cycloheximide did not affect the actual rate of activity loss at 26 C. The basal glycerol kinase activity was not sensitive to cycloheximide. The mechanism responsible for inactivation was destroyed by sonic oscillation. The process is not thought to play a role in the cold-induced increase in activity. Glycerol kinase activity induced at 26 C by glycerol was also lost on addition of cycloheximide and after addition of sucrose.

A study of the control of glycerol kinase (ATP:glycerol 3-phosphotransferase, EC 2.7.1.30) in *Neurospora crassa* has shown a number of interesting features. Increases in activity can be produced not only by incubating cultures in medium containing glycerol (22; J. B. Courtright, Bacteriol. Proc., p. 153, 1971), but also by lowering the growth temperature of cultures growing on sucrose as sole carbon source (22). In this report, evidence is presented for a rapid inactivation in vivo of glycerol kinase. This inactivation was responsible for a loss of the cold-induced activity when cultures were returned to the normal growth temperature (26 C). Some properties of the inactivation are reported and its possible role in the cold-induced changes in activity is discussed.

MATERIALS AND METHODS

Strain. *N. crassa* wild-type strain 74A was used in this study.

Growth conditions and extract preparation. Cultures were grown in Erlenmeyer flasks on Fries minimal medium (1), each with 100 ml of medium (in 250-ml flasks) unless otherwise indicated. All cultures were initially grown at 26 C for 48 h, on medium containing 2% (wt/vol) sucrose as the sole carbon source. Procedures and conditions for inoculation and incubation of cultures and for the preparation of extracts in tris(hydroxymethyl)aminomethane buffer (83 mM, pH 8.0) were exactly as described previously (22). Extracts were normally prepared from 10-ml portions of culture.

Glycerol kinase activity determination. Glycerol kinase activity was assayed by the radiochemical method (adapted from that of Newsholme et al. [21]) of North (22). Protein was assayed by the method of Lowry et al. (17), and glycerol kinase specific activity was determined as nanomoles of glycerol phosphate bound (to DE-81 filter) per hour per milligram of protein.

Determination of protein synthesis. Protein synthesis was determined as the rate of incorporation of [U-^{14}C]leucine into trichloroacetic acid-insoluble material. Cultures were incubated under the required conditions in medium containing labeled leucine (0.92 μM, 22.3 mCi/mmol). Samples (2 ml) were removed at intervals, added to 2 ml of 10% (wt/vol) trichloroacetic acid, homogenized in an M.S.E. overhead homogenizer (Measuring and Scientific Equipment Ltd., London, U.K.) for 30 s, and after it stood on ice for 30 min, the trichloroacetic acid-insoluble material was collected on a glass-fiber filter (Whatman). The filter was washed with 5% (wt/vol) trichloroacetic acid and counted for radioactivity in a Beckman liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.) The rate of protein synthesis was determined as micromoles of leucine incorporated per hour per milligram (dry weight) of mycelium.

Sources. Cycloheximide was supplied by Sigma London Chemical Co. (Kingston upon Thames, U.K.), and radiochemicals were supplied by the Radiochemical Centre (Amersham, U.K.).

[1] Present address: Department of Biology, University of Essex, Colchester, Essex, United Kingdom.

RESULTS

Changes in glycerol kinase activity resulting from low-temperature incubation. It has been reported previously (22) that incubation of cultures of *N. crassa* at temperatures below 12 C results in a large increase in glycerol kinase activity. The increase in activity is dependent on protein synthesis and becomes greater as the temperature becomes lower (22). At 4 C (the temperature generally used in these experiments), the specific activity increased from the basal level of 2 to 4 specific activity units to a maximal level of 100 to 120 units after approximately 24 h. The level then decreased slowly to give a specific activity of 70 to 90 units after 48 h of incubation. During incubation at 4 C, there was no net change in the dry weight of a culture, but growth could occur again once the culture was returned to the normal growth temperature (26 C). This change in temperature resulted in a dramatic decrease in the specific activity of glycerol kinase (Fig. 1) at a rate that was too rapid to be accounted for by dilution of preexisting enzyme. The decrease may have represented an actual loss of glycerol kinase activity.

Loss of cold-induced glycerol kinase activity at 26 C. After the transfer from 4 C to 26 C, there was a short lag, and then glycerol kinase activity was lost with a half-life of approximately 15 min (Fig. 2). Addition of glycerol (which induces glycerol kinase at 26 C [22]) reduced the activity loss, but sucrose had no effect. The lag period was eliminated when cold-incubated cultures were resuspended in fresh medium already at 26 C (Fig. 2); the lag may have been due to the time required to warm the cultures. Glycerol also reduced the activity loss after resuspension but again did not prevent an initial fall in activity.

The loss of activity could not be accounted for by differences in the levels of inhibitors (or activators) in extracts prepared from cultures at different times after the temperature change. Dialysis against tris(hydroxymethyl)aminomethane buffer failed to alter the activity of any extract. It was also shown that no extract contained a factor that could alter the activity of another extract with which it was mixed. The change in activity may have represented a change in the level of active glycerol kinase protein.

Irreversibility of activity loss. Once activity had been lost by incubation of cold-incubated cultures at 26 C, it was not possible to regain that activity, either by incubating extracts at 4 C (not shown) or by returning whole cultures to 4 C. Figure 3 shows the lack of effect of reincubation at 4 C on inactivated cultures which were initially incubated at 4 C for 12 h. The same result was obtained with cultures incubated for longer periods at 4 C before their return to 26 C.

Effect of cycloheximide on glycerol kinase activity. The change in temperature from 4 to

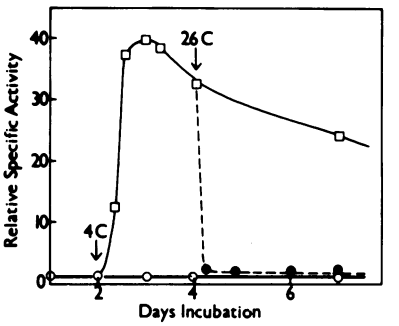

FIG. 1. *Effect of incubation at 4 C and return to 26 C on glycerol kinase activity. Cultures (20 ml) were set up in 50-ml flasks. Some cultures were incubated only at 26 C (○); the rest were transferred to 4 C after 2 days of preincubation at 26 C (□). Some of the latter were returned to 26 C after a further 2 days of incubation (●). At various times whole cultures were harvested and extracted, and the specific activity of the glycerol kinase was determined.*

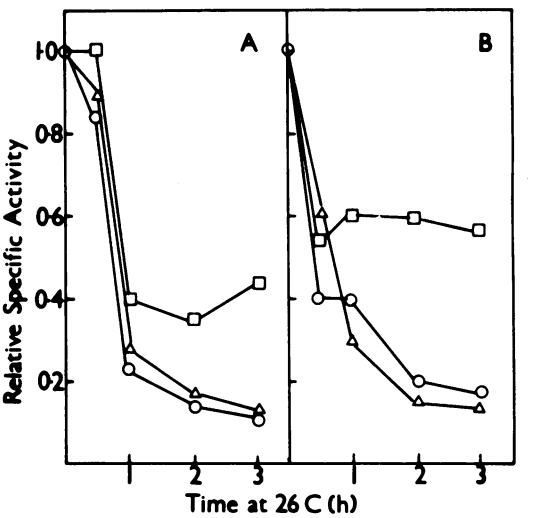

FIG. 2. *Loss of cold-induced glycerol kinase activity at 26 C. After inoculation, cultures were incubated for 48 h at 26 C and then for 48 h at 4 C. They were then either returned to 26 C intact (A) or suspended in fresh minimal medium already at 26 C (B) with additions made as indicated. Samples were removed at intervals and extracted, and the specific activity of glycerol kinase was determined. Symbols: ○, no addition; □, 2% (wt/vol) glycerol; and △, 2% (wt/vol) sucrose.*

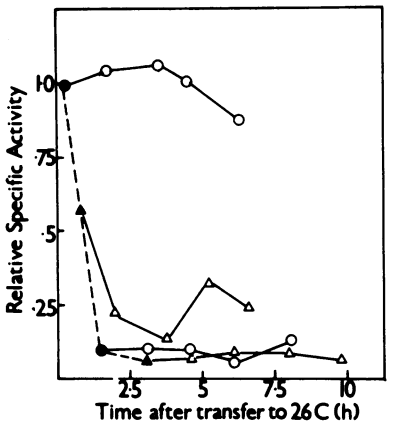

FIG. 3. *Effect of reincubation at 4 C on inactivated glycerol kinase. After inoculation, cultures were incubated for 48 h at 26 C and then for 12 h at 4 C. They were then returned to 26 C for 15, 45, 90, or 180 min before reincubation at 4 C. Samples were removed at intervals for determination of glycerol kinase specific activity. Symbols: ●, ▲, changes in activity occurring at 26 C; ○, △, changes in activity occurring at 4 C.*

26 C might be considered as a switch from inducive to repressive conditions for glycerol kinase. A number of fungal enzymes are known to be inactivated after a change from inducive to repressive or just noninducive conditions (3–5, 11, 16, 27, 29). In certain cases, the activity loss appears to be dependent on the synthesis de novo of some component of the system responsible, since the activity loss is blocked by cycloheximide (16, 27). This was not the case with the inactivation of cold-induced glycerol kinase. The addition of cycloheximide at a concentration known to inhibit protein synthesis in *Neurospora* (8) did not prevent the loss of activity (Fig. 4); instead, it eliminated the lag period and caused an immediate fall in activity at the same rate as that after the lag in the absence of cycloheximide (Fig. 2). In the presence of cycloheximide, glycerol failed to reduce the activity loss (Fig. 4).

Since cycloheximide did not prevent inactivation, the factor(s) responsible must have already been present within the cells at the time of the temperature increase. To determine whether inactivation was switched on by the change to 26 C and to probably repressive conditions, or if it was a continuous process, the stability of the activity at 4 C after addition of cycloheximide was examined. During cold incubation, the specific activity of glycerol kinase increased, reached a maximum and then decreased slowly (Fig. 1). However, whatever the overall change in specific activity, cycloheximide always produced an immediate and, in view of the low temperature, rapid loss of activity (Fig. 5). The activity was always lost with first-order kinetics with a half-life of approximately 3 h at 4 C. Glycerol kinase may have been subject to continuous inactivation; the effect of this was observed as a net loss of activity whenever protein synthesis was inhibited. The immediate effect of cycloheximide indicated that protein synthesis was necessary for the maintenance of the activity, and this strongly supported the belief that increases in glycerol kinase activity are dependent on synthesis de novo of the enzyme.

It is noteworthy that addition of cycloheximide to cultures prior to the cold-induced increase in activity prevented the increase, but did not cause any loss of the basal activity.

Stability of glycerol kinase activity in vitro. Glycerol kinase activity is clearly unstable in whole cells, but in extracts prepared in either tris(hydroxymethyl)aminomethane or phosphate buffer it is relatively stable; for example, the maximal activity loss recorded at 26 C was 25% after 24 h incubation in tris(hydroxymethyl)aminomethane buffer, pH 8.0. Although tris(hydroxymethyl)aminomethane was shown to slow the inactivation rate in whole

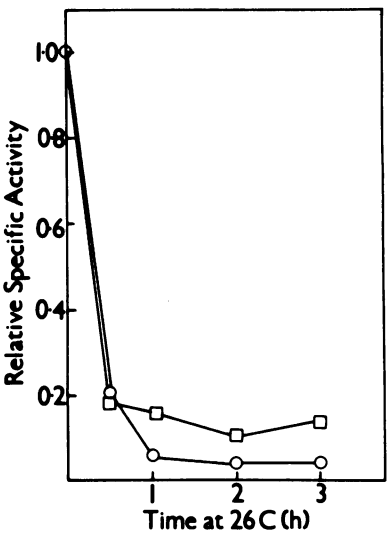

FIG. 4. *Effect of cycloheximide on loss of cold-induced activity at 26 C. After inoculation, cultures were incubated for 48 h at 26 C and then for 48 h at 4 C. The cultures were returned at 26 C and 1.4 μg of cycloheximide per ml was added. To one, no further additions were made (○), but 2% (wt/vol) glycerol was added to the other (□). Samples were removed at intervals for determination of glycerol kinase specific activity.*

cells when cultures were resuspended in the buffer, phosphate buffer had no effect on the inactivation rate in vivo. The differing effects on the activity loss are still observed when both buffers are used at pH 7 (M. J. North, Ph.D. thesis, The University, Newcastle upon Tyne, U.K., 1973).

Glycerol kinase activity may have become stable during the extraction process, presumably through the loss of the inactivating factor(s). This was investigated by using the extraction process described previously (22). Extracts were prepared from a culture after incubation at 4 C, and samples of these were incubated for 1-h periods at 26 C at different stages of extraction, to test whether inactivation could still take place. Table 1 shows that glycerol kinase activity was rendered stable by sonic oscillation.

Homogenization had no effect on inactivation. After homogenization in phosphate buffer, the activity loss was comparable to that in intact cells; thus, preparation of cells in this manner could provide a suitable system for in vitro characterization of the inactivation process.

It is significant that neither extract C nor extract D possessed the total activity originally present in the culture. Both extracts were finally prepared in the buffer used for the incubation at 26 C. If the observed loss of activity had been the result of excretion of glycerol kinase from the cells, the enzyme should have been retained in the final extract and therefore have been detectable. This was not the case. The results suggested that glycerol kinase inactivation was achieved through an intracellular process that was sensitive to sonic oscillation.

TABLE 1. *Effect of extraction process on the stability of glycerol kinase*[a]

Treatment	Nature of incubated sample	Sp act[b]	
		Phosphate	Tris
A. Extracted immediately	—	100	100
B. Further incubation for 1 h at 26 C before extraction	Intact cells	10	15.5
Extraction process interrupted after:			
C. Resuspension in buffer	Intact cells	5.5	50
D. Homogenization	Disrupted cells	14.5	47
E. Sonic oscillation	Disrupted cells	94	122
F. Centrifugation	Cell extract	102	102

[a] After an initial 48 h of incubation at 26 C, a 100-ml culture was incubated at 4 C for 48 h. The culture was divided into two equal portions. From one of these, all extracts were prepared in 50 mM sodium phosphate buffer (pH 6.0), and all extracts from the other prepared in 83 mM tris(hydroxymethyl)aminomethane buffer (pH 8.0). The portions were each further divided into six samples. One of these (A) was extracted immediately, and another (B) was incubated for 1 h at 26 C before extraction. All other samples were extracted immediately, but the process was interrupted by 1 h of incubation at 26 C after the stage indicated. Extraction was then completed as normal, and glycerol kinase specific activity was determined.

[b] Percentage of that in extract A.

Effect of temperature on the rates of overall protein synthesis and glycerol kinase inactivation. The level of glycerol kinase activity appears to depend on the rates of two processes, namely the synthesis of the enzyme and its inactivation. To assess the role of the two processes in the cold-induced changes in activity, the rates of overall protein synthesis and glycerol kinase inactivation were determined at different temperatures. The former was estimated from the rate of leucine incorporation into trichloroacetic acid-insoluble material, and the latter was estimated from the rate of activity loss after the addition of cycloheximide. Since the inhibitor did not affect the actual rate of activity loss after a change to 26 C (Fig. 2 and 4), this was considered to be a valid estimate of the true inactivation rate. When measuring the inactivation rate, it was necessary to preincubate cultures at 4 C to induce a high level of activity. Since it had been found that the rate of inactivation of induced glycerol kinase at a given temperature was constant and not affected by the length or temperature of prior incubation (Fig. 5), the rates shown in Table 2 were considered to reflect the ability of any culture (including those used for the deter-

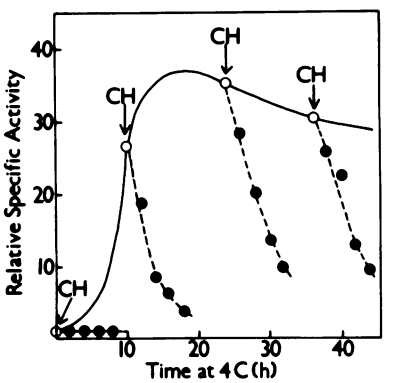

FIG. 5. *Effect of cycloheximide on glycerol kinase activity at 4 C. After inoculation, cultures were incubated for 48 h at 26 C and were then transferred to 4 C. At the times indicated, 1.4 μg of cycloheximide (CH) per ml was added to one of the cultures, and samples were then removed at intervals during continued incubation at 4 C for determination of glycerol kinase specific activity.*

TABLE 2. *Effect of temperature on rates of overall protein synthesis and glycerol kinase inactivation*

Temp (C)	Overall protein synthesis[a]	Glycerol kinase inactivation[b]
26	1.00	1.00
12	0.41	0.50
4	0.16	0.12
0	0.01	0.03

[a] Cultures were incubated at 26 C for 48 h and were then transferred to a reciprocally shaking water bath at the temperature indicated. After 4 h of incubation, labeled leucine was added and its incorporation was followed over a period of up to 4 h. The rate given is relative to that at 26 C.

[b] Cultures were incubated at 26 C for 48 h and at 4 C for 48 h. Cycloheximide (1.4 μg/ml) was then added. The cultures were transferred to a reciprocally shaking water bath at the temperature indicated, and samples were removed at intervals. The rate of inactivation was determined as percent activity lost per hour and is given relative to that at 26 C.

mination of protein synthesis rates) to inactivate glycerol kinase.

Table 2 shows the similar effect of temperature on the rate of both processes.

Inactivation of glycerol kinase activity induced at 26 C. Glycerol kinase activity can be induced at 26 C by incubation in a medium containing glycerol (22) or one of a number of other carbon sources, galactose and deoxyribose in particular (M. J. North, Ph.D. thesis). On addition of cycloheximide, this activity was also lost with a half-life of approximately 15 min. The addition of sucrose to cultures growing in glycerol medium also produced a loss of activity after a short lag. This effect was analogous to that of the temperature increase on cold-induced activity. The effects of cycloheximide and sucrose on glycerol-induced activity are shown in Fig. 6. During the initial incubation period in glycerol medium, the specific activity increased 12-fold to give a level of 35 specific activity units at the time of addition of cycloheximide and sucrose.

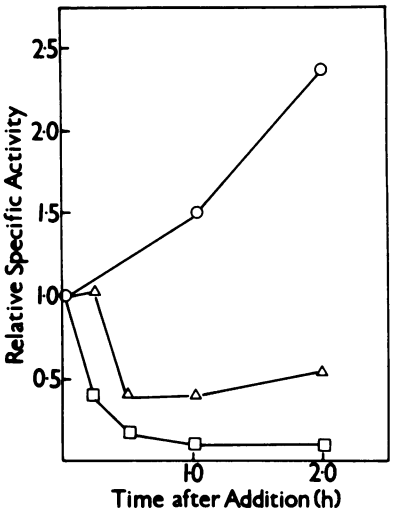

FIG. 6. *Inactivation of glycerol-induced glycerol kinase activity. After an initial 48 h of incubation in sucrose, minimal medium cultures were filtered, washed, and resuspended in fresh medium containing 2% (wt/vol) glycerol as the sole carbon source. Incubation was continued at 26 C for 2 h and additions were then made as indicated. Samples were removed at intervals for determination of glycerol kinase specific activity. Symbols:* ○, *no addition;* △, *2% (wt/vol) sucrose; and* □, *cycloheximide, 1.4 μg/ml.*

DISCUSSION

This report demonstrates that glycerol kinase in *N. crassa* was subject to continuous inactivation, whether the activity is induced by incubation at low temperatures or by incubation in glycerol medium. There was a rapid fall in the level of activity when the synthesis of the enzyme was stopped both nonspecifically by the addition of the protein synthesis inhibitor (cycloheximide), and specifically, as was believed to occur when cold-incubated cultures were returned to the normal growth temperature or when sucrose was added to cultures incubated in glycerol medium.

Most other examples of enzyme inactivation that are at present known in fungi differ from that of glycerol kinase, since they are not continuous and occur only in the absence of inducive conditions. The triggering of such an inactivation mechanism is a specific response to the withdrawal of inducive conditions. A loss of activity cannot be brought about simply by inhibiting further enzyme synthesis with cycloheximide (3, 5, 27, 29), the inhibitor actually prevents the inactivation of some enzymes after a switch from inducive conditions (4, 16, 29). This specific loss of activity has been termed inactivation repression (4), although it should not strictly be described as repression because it involves a posttranscriptional event.

However, in *Neurospora*, cycloheximide does cause a loss of activity of three inducible transport systems (those for tryptophan [30], sulfate [18], and choline-*O*-sulfate [19]) and the loss of one component of another enzyme, δ-aminolaevulinate dehydratase (20). Hynes (11) has reported that the inhibitor also causes a fall in nitrate reductase activity in *Aspergillus nidulans*, although it has no effect on the activity in *Neurospora* (29) or *Ustilago maydis* (16). A

number of fungal enzymes are thus continuously inactivated.

Many mammalian enzymes are now known to be turned over continuously (25), but at present only two have been reported with half-lives as short as that for *Neurospora* glycerol kinase, ornithine decarboxylase (23), and soluble δ-aminolaevulinate synthetase (6) from rat liver. The rate of inactivation of a number of mammalian enzymes can be regulated (2, 12, 14, 26), and changes in the level of enzyme activity can be achieved by altering the rate not only of synthesis, but also of inactivation. At present, there is no evidence to suggest that such control exists over glycerol kinase inactivation. The effect of glycerol on the loss of cold-induced activity (Fig. 2) can be explained by the glycerol-induced synthesis of new enzyme, since the effect was eliminated when further enzyme synthesis was prevented by cycloheximide (Fig. 4). The effect of sucrose on glycerol-induced activity (Fig. 6) was almost certainly due to the repression of enzyme synthesis, which prevented replacement of inactivated enzyme.

It is not yet possible to say how the loss of activity was achieved. Since it was irreversible, however, it was probably due to the breakdown of enzyme protein. Specific proteases have been reported to be responsible for the inactivation of nicotinamide adenine dinucleotide-dependent (13) and pyridoxal-requiring (15) enzymes in rats and the tryptophan synthase-inactivating enzymes in yeast have also been identified as proteases (7, 24, 28). The sensitivity of glycerol kinase inactivation to sonic oscillation suggested that some structural organization may be required for the process to take place.

The sensitivity of the glycerol kinase assay should have allowed the detection of at least a fourfold fall in the basal level of activity. Since no decrease in activity was observed on addition of cycloheximide, the enzyme responsible for the basal activity was stable in vivo. Therefore, this enzyme may differ from the one responsible for the labile induced activity. This conclusion is supported by the results of a study of glycerol-induced, cold-induced, and basal activities in crude extracts; the properties of the induced activities are the same but differ from those of the basal activity (M. J. North, Ph.D. thesis).

The presence of a system that continuously inactivated glycerol kinase raised the question of whether the cold-induced increase in activity was merely the result of the organism possessing this system. The level of glycerol kinase activity was dependent on the rates of both synthesis and inactivation. One possible explanation for the cold-induced increase was that the enzyme responsible for the induced activity is synthesized continuously, but at 26 C the level of activity was kept low because of the rapid rate of inactivation. When the temperature was lowered so the rates of both synthesis and inactivation would be slowed, and, if the direct effect of the fall in temperature on the inactivation rate were greater than on the synthetic rate, a new, elevated, steady-state level would be achieved. However, this is not thought to be so. There is no evidence that the enzyme responsible for the induced activity was present in sucrose-grown cultures at 26 C, and it can be calculated from the data in Table 2 that, if glycerol kinase synthesis behaved exactly as overall protein synthesis, the fall in temperature from 26 C to 4 C would result in less than a twofold increase in the level of glycerol kinase activity. It must be concluded that as a result of the temperature drop, the rate of glycerol kinase synthesis is increased relative to overall protein synthesis, and that a change in the rate of inactivation is not responsible for the cold-induced increase.

The increase in glycerol kinase activity resulted from a relative increase in enzyme synthesis under conditions when overall protein synthesis was reduced. It is interesting to note that derepression of tyrosinase in *Neurospora* also occurs under conditions of restricted protein synthesis, in this case caused by starvation (10), or the addition of amino acid analogues (9, 10), or protein synthesis inhibitors (8). Horowitz et al. (8) have proposed that the synthesis of tyrosinase is controlled through a rapidly turned-over repressor whose level is reduced when protein synthesis is restricted. Glycerol kinase activity is little affected at 26 C by amino acid analogues or by low levels of cycloheximide (M. J. North, Ph.D. thesis), and it is therefore unlikely that a similar mechanism is responsible for the cold-induced increase in activity. It could have been achieved, however, through a direct effect on the stability of a macromolecule involved in the control of glycerol kinase synthesis (e.g., a cold-labile regulatory protein), or through changes in the pool size of an inducer or repressor as a result of metabolic changes at the low temperature.

The continuous inactivation of an enzyme allows its activity to be kept under tight control. Any change in the rate of synthesis is immediately reflected in the level of activity. It is tempting to speculate that glycerol kinase has some special and as yet unknown role in *Neurospora*, for which the additional control allowed by the otherwise wasteful process of continuous

inactivation is necessary. The increase in activity induced by incubation at low temperatures may then also be explicable in terms of such a special role.

ACKNOWLEDGMENTS

I wish to thank K. Burton and other members of the Department of Biochemistry at Newcastle upon Tyne for their helpful advice and discussions.

The work was supported by a Science Research Council Research Studentship.

LITERATURE CITED

1. Beadle, G. W., and E. L. Tatum. 1945. Neurospora. II. Method of producing and detecting mutations concerned with nutritional requirements. Amer. J. Bot. **32**:678–686.
2. Cihak, A., C. Lamar, and H. C. Pitot. 1973. L-Tryptophan inhibition of tyrosine aminotransferase degradation in rat liver *in vivo*. Arch. Biochem. Biophys. **156**:188–194.
3. Ferguson, A. R., and A. P. Sims. 1974. The regulation of glutamine metabolism in *Candida utilis*: the inactivation of glutamine synthetase. J. Gen. Microbiol. **80**:173–185.
4. Ferguson, J. J., M. Bohl, and H. Holzer. 1967. Yeast malate dehydrogenase: enzyme inactivation in catabolite repression. Eur. J. Biochem. **1**:21–25.
5. Gancedo, C. 1971. Inactivation of fructose-1,6-diphosphatase by glucose in yeast. J. Bacteriol. **107**:401–405.
6. Hayashi, N., B. Yoda, and G. Kikuchi. 1969. Mechanism of allylisopropylacetamide-induced increase of δ-aminolaevulinate synthetase in liver mitochondria. IV. Accumulation of the enzyme in the soluble fraction of rat liver. Arch. Biochem. Biophys. **131**:83–91.
7. Holzer, H., T. Katsunuma, E. G. Schött, A. R. Ferguson, A. Hasilik, and H. Betz. 1973. Studies on a tryptophan synthase inactivating system from yeast, p. 53–60. *In* G. Weber (ed.), Advances in enzyme regulation, vol. 11. Pergamon Press, Oxford.
8. Horowitz, N. H., H. M. Feldman, and M. L. Pall. 1970. Derepression of tyrosinase synthesis in *Neurospora* by cycloheximide, actinomycin D and puromycin. J. Biol. Chem. **245**:2784–2788.
9. Horowitz, N. H., M. Fling, H. M. Feldman, M. L. Pall, and S. C. Froehner. 1970. Derepression of tyrosinase synthesis in *Neurospora* by amino acid analogues. Develop. Biol. **21**:147–156.
10. Horowitz, N. H., M. Fling, H. Macleod, and Y. Watanabi. 1961. Structural and regulative genes controlling tyrosinase synthesis in *Neurospora*. Cold Spring Harbor Symp. Quant. Biol. **26**:233–238.
11. Hynes, M. J. 1973. The effect of lack of a carbon source on nitrate reductase in *Aspergillus nidulans*. J. Gen. Microbiol. **79**:155–157.
12. Jost, J.-P., E. A. Khairallah, and H. C. Pitot. 1968. Studies on the induction and expression of enzymes in rat liver. V. Regulation of the rate of synthesis and degradation of serine dehydratase by dietary amino acids and glucose. J. Biol. Chem. **243**:3057–3066.
13. Katunuma, N., E. Kominami, S. Kominami, and K. Kato. 1972. Mode of action of specific inactivating enzymes for pyridoxal enzymes and NAD-dependent enzymes and their biological significance, p. 289–306. *In* G. Weber (ed.), Advances in enzyme regulation, vol. 10. Pergamon Press, Oxford.
14. Kay, J. E., V. J. Lindsay, and A. Cooke. 1972. Ornithine decarboxylase in phytohaemagglutinin-stimulated lymphocytes: control of degradation rate by amino acids. FEBS Lett. **21**:123–126.
15. Kominami, E., K. Kobayashi, S. Kominami, and N. Katunuma. 1972. Properties of a specific protease for pyridoxal enzymes and its biological role. J. Biol. Chem. **247**:6848–6855.
16. Lewis, C. M., and J. R. S. Fincham. 1970. Regulation of nitrate reductase in the basidiomycete *Ustilago maydis*. J. Bacteriol. **103**:55–61.
17. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
18. Marzluf, G. A. 1972. Control of the synthesis, activity and turnover of enzymes of sulfur metabolism in *Neurospora crassa*. Arch. Biochem. Biophys. **150**:714–724.
19. Marzluf, G. A. 1972. Genetic and metabolic control of sulfate metabolism in *Neurospora crassa*: a specific permease for choline-*O*-sulfate. Biochem. Genet. **7**:219–233.
20. Muthukrishnan, S., K. Malatha, and G. Padmanaban. 1972. δ-Aminolaevulinate dehydratase, the regulatory enzyme of the haembiosynthetic pathway in *Neurospora crassa*. Biochem. J. **129**:31–37.
21. Newsholme, E. A., J. Robinson, and K. Taylor. 1967. A radiochemical enzymatic activity assay for glycerol kinase and hexokinase. Biochim. Biophys. Acta **132**:338–346.
22. North, M. J. 1973. Cold-induced increase of glycerol kinase in *Neurospora crassa*. FEBS Lett. **35**:67–70.
23. Russel, D. H., and S. H. Snyder. 1969. Amine synthesis in regenerating rat liver: extremely rapid turnover of ornithine decarboxylase. Mol. Pharmacol. **5**:253–262.
24. Saheki, T., and H. Holzer. 1974. Comparisons of the tryptophan synthase inactivating enzymes with proteinases from yeast. Eur. J. Biochem. **42**:621–626.
25. Schimke, R. T. 1973. Control of enzyme levels in mammalian tissues, p. 135–187. *In* A. Meister (ed.), Advances in enzymology, vol. 37. John Wiley and Sons, New York.
26. Schimke, R. T., E. W. Sweeney, and C. M. Berlin. 1965. The roles of synthesis and degradation in the control of rat liver tryptophan pyrrolase. J. Biol. Chem. **240**:322–331.
27. Schneider, R. P., and W. R. Wiley. 1971. Regulation of sugar transport in *Neurospora crassa*. J. Bacteriol. **106**:487–492.
28. Schött, E. H., and H. Holzer. 1974. Purification and some properties of tryptophan synthase inactivase II from yeast. Eur. J. Biochem. **42**:61–66.
29. Subramanian, K. N., and G. J. Sorger. 1972. Regulation of nitrate reductase in *Neurospora crassa*: stability in vivo. J. Bacteriol. **110**:538–546.
30. Wiley, W. R., and W. H. Matchett. 1968. Tryptophan transport in *Neurospora crassa*. II. Metabolic control. J. Bacteriol. **95**:959–966.