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Evidence for acceleration of the rate of elongation of tyrosine aminotransferase nascent chains by dibutyryl cyclic AMP

(ribosomal transit time/H35 hepatoma cells/dexamethasone)

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ABSTRACT **Analogs of cyclic AMP elevate the synthesis of tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase; EC 2.6.1.5) in cultured hepatoma cells and rat liver at a post-transcriptional level but have no discernible effect on total soluble protein synthesis. In order to determine whether cyclic AMP exerts its effect on a step before or after initiation of the synthesis of this enzyme, we have analyzed the ribosomal transit times for both the aminotransferase and total soluble protein in hepatoma cells incubated in the presence or absence of $N^6,O^{2'}$-dibutyryl cyclic AMP. The time required for one ribosome to translate one subunit of the "average" soluble protein (transit time) was about 2 min in cells incubated with or without the cyclic AMP analog. In contrast, the transit time for tyrosine aminotransferase was found to be reduced from 5–8 min under basal conditions to as low as 45 sec after exposure to dibutyryl cyclic AMP. Although the degree of effect varied from experiment to experiment, the relative rate of aminotransferase nascent chain elongation was found to be proportional to the stimulation of its activity. In contrast, dexamethasone did not alter the rate of aminotransferase elongation even though it elevated enzyme activity between 5- and 10-fold. These data are consistent with the hypothesis that induction of tyrosine aminotransferase with cyclic AMP analogs occurs by stimulation of the rate at which ribosomes translate pre-existing mRNA in contrast to adrenal steroids which act by increasing the level of translatable mRNA coding for this enzyme.**

Tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase; EC 2.6.1.5) (aminotransferase) synthesis is stimulated by treatment of cultured hepatoma cells or rat liver with glucocorticoids, insulin, and dibutyryl cyclic AMP (Bt_2cAMP) (1, 2). Considerable work has been directed at determining the mechanisms of action of glucocorticoids in this system, and the results of these investigations have led to the uniform conclusion that steroid hormones induce the aminotransferase by increasing the amount of translatable mRNA. This conclusion has recently been validated by the direct assay of mRNA coding for the enzyme (3, 4). In contrast, aminotransferase induction by both Bt_2cAMP and insulin appears to be the result of stimulation of independent post-transcriptional events, but the mechanisms involved are still not clear (1, 2).

Conceivably, post-transcriptional regulation could be elicited at several points, mRNA processing, ribonucleoprotein (mRNP)–mRNA interconversion, as well as at initiation, elongation, or termination of protein synthesis. Analysis of post-transcriptional events has suggested that initiation of protein synthesis is the predominant control point in the regulation of translation. Fan and Penman (5) have demonstrated that the general depression of protein synthesis that occurs during mitosis in HeLa cells is due to a decrease in the frequency of initiation. On the other hand, the average rate of peptide elongation was unchanged under these conditions (5). Similarly, globin synthesis has been shown to be modulated at initiation either by heme availability or by a specific repressor substance. The rates of alpha and beta chain elongation, although unequal, are not affected by either condition (6, 7).

The possibility of a shift in the mRNA pool between nontranslated mRNP particles and polysomes has also been implicated in post-transcriptional control. The induction of ferritin synthesis by iron has been suggested recently to result from a shift of previously unavailable ferritin mRNA molecules from presumptive cytoplasmic mRNP particles to the polysomes (8). In a like manner, the increase in myosin synthesis that occurs after fusion of myoblasts during muscle cell development has been suggested to result from similar shifts of myosin mRNA (9).

In contrast to the multitude of reports concerning regulation at initiation (5–9), only a handful of published examples of control beyond initiation of protein synthesis can be found. In one such report, analysis of the ribosomal transit time for the synthesis of ovalbumin, conalbumin, and ovomucoid in chick oviduct explants revealed that estrogen seems capable of modulating the rate at which these proteins are assembled at some step beyond initiation (10, 11). In light of measurement of the respective mRNA pool sizes during estrogen treatment (11–14), however, it seems probable that the major contributing factor to the almost exclusive production of these proteins by oviduct cells is the accumulation of mRNA. The 30–40% decrease in transit times (10, 11) does appear to amplify this effect somewhat.

In search of a more direct means of analyzing the mechanism by which cyclic nucleotides regulate aminotransferase synthesis, we have turned our attention to measurements of the elongation rates for aminotransferase synthesis in whole cells. The minimal deviation rat hepatoma cell line, Reuber H35, first adapted to continuous culture in 1964 (15), has been used for our studies. These cells synthesize various proteins characteristic of normal rat liver (1, 16–20) and also respond to several hormones that produce responses in rat liver (1–3, 16, 17, 19, 20). Although these cells do not respond to glucagon or isoproterenol (16) because of a lack of receptors,† they do exhibit a strong increase in aminotransferase synthesis in response to cyclic AMP analogs (1, 2, 16, 21, 22), insulin (1, 16), glucocorticoids (1–3), and cholera toxin†. The characteristics of the response of aminotransferase to these agents in H35 cells is virtually identical to

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Abbreviations: aminotransferase, tyrosine aminotransferase; Bt_2cAMP, $N^6,O^{2'}$-dibutyryl cyclic AMP; $NaDodSO_4$, sodium dodecyl sulfate; mRNP, subribosomal mRNA–protein complexes.

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† B. H. Leichtling, Y. F. Su, J. Wimalasena, T. K. Harden, B. B. Wolfe, and W. D. Wicks, unpublished observation.

that in rat liver, thereby providing the necessary physiological basis for the use of these cells which immeasurably simplified the application of the transit time procedure to this problem.

Using the transit time procedure in H35 cells we have discovered the striking fact that Bt_2cAMP appears to act by increasing the rate of elongation of nascent aminotransferase chains. These experiments provide direct evidence that aminotransferase synthesis is controlled by cyclic AMP at the level of translation and suggest that postinitiation control of the synthesis of minor proteins may not be as unlikely as first believed.

MATERIALS AND METHODS

Tissue Culture. The Reuber H35 cell line (H4-II-E-C3) used in these studies was kindly provided by F. T. Kenney, Biology Labs, Oak Ridge National Laboratory, Oak Ridge, TN. Both the original stock cell line and one clone (KRC-7) were used for these studies with similar results. H35 cells were grown as monolayers in Dulbecco's minimum essential medium containing 10% calf and 5% fetal calf serum. Twenty-four hours before harvest, the medium was replaced with serum-free medium. Dexamethasone (1 μM) was added 4 hr before harvest, while Bt_2cAMP (0.5 mM) was added 3 hr before harvest (23). Cells were harvested by washing three times with ice-cold saline while still attached to the dishes, then scraped into an *N*-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid buffer, pH 7.5, containing isotonic KCl and Nonidet P-40 as the lytic agent (21).

Transit Time Measurements. Elongation rates were determined by Palmiter's (10) modification of the method of Fan and Penman (5) with 10 μg of cycloheximide added per ml of lysis buffer to ensure inhibition of further elongation.

Incorporation of ^3H- and ^{14}C-labeled leucine into total soluble protein was analyzed by the filter paper disc method of Mans and Novelli (24). Radioactivity incorporated into released aminotransferase was determined by direct immunochemical precipitation in lysates after centrifugation at 105,000 × *g* for 60 min as described (23, 25). The specificity of the antiserum used has been demonstrated by sodium dodecyl sulfate ($NaDodSO_4$) gel electrophoresis; a single peak of radioactivity at 55,000–60,000 daltons was seen with first precipitates of labeled lysates and only scattered low counts (<5% of the first) were found with the second. In addition, only a single precipitin arc was observed with two-dimensional electroimmunodiffusion. Aminotransferase activity was assayed by a modification (23) of the Diamondstone method (26).

Transit time calculations were made by extrapolating to zero the ratio of tritium to carbon-14 (using a least squares analysis) for total postmitochondrial (released + nascent), total postpolysomal (released), and aminotransferase (released) fractions, then subtracting the postmitochondrial time intercept from either the total soluble protein or aminotransferase postpolysomal intercepts (10). Since the average nascent peptide chain is theoretically half complete at the time of addition of the first radioactive leucine molecule, the observed time differences were doubled to yield actual transit times (5, 10).

$NaDodSO_4$ Gel Procedure. $NaDodSO_4$/polyacrylamide gel electrophoresis was performed as described by Weber *et al.* (27). Radioactive protein samples containing either aminotransferase immunoprecipitates or postpolysomal total soluble protein were dissolved in 1% $NaDodSO_4$/3% dithiothreitol and heated at 60° for at least 1 hr and at 37° overnight before electrophoresis. Gel slices were analyzed for radioactivity after treatment with NCS (Amersham-Searle) in a scintillation counter using 0.8% Omnifluor (New England Nuclear) as the scintillant. For determination of the molecular weight average of the newly synthesized soluble proteins, the formula described by Palmiter (10) was used.

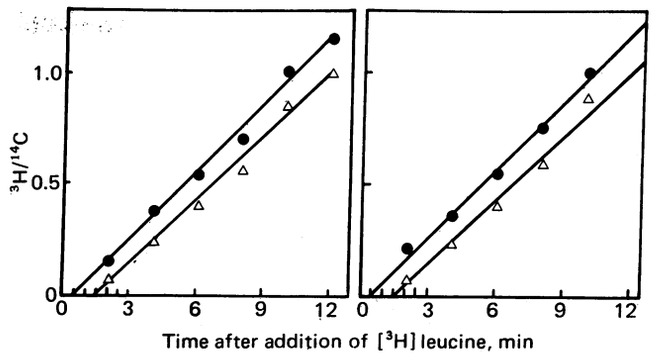

FIG. 1. Effect of Bt_2cAMP on ribosomal transit times for total soluble protein. Confluent H35 cells were incubated (*Left*) without or (*Right*) with 0.5 mM Bt_2cAMP. Three hours later transit time analysis was performed. ●, Postmitochondrial supernatant fractions consisting of completed peptide chains and polysomal bound nascent chains; △, postpolysomal fractions containing only completed chains. Intercept values: ●, (*Left*) 0.45 min, (*Right*) 0.51 min; △, (*Left*) 1.45 min, (*Right*) 1.52 min. Transit times: (*Left*) 2.0 min, (*Right*) 2.02 min. The intercepts in Figs. 1 and 2 were determined by least squares analysis.

RESULTS

Effect of administration of Bt_2cAMP on elongation rates of total soluble protein

Transit time determination for total soluble protein in cells either untreated (*left*) or treated with 0.5 mM Bt_2cAMP (*right*) are illustrated in Fig. 1. The ratio of ^3H to ^{14}C extrapolates to zero within about 30 sec for the polysome-containing postmitochondrial supernatant fractions obtained from either class of cells; thus equilibration of the cells with exogenous radioactive leucine is quite rapid in H35 cells. In either the untreated or Bt_2cAMP-treated cells, the polysome-free supernatant fraction (lower line) extrapolates to zero about 1.0 min after the intercept time for postmitochondrial supernatant, yielding a transit time of 2.0–2.2 min for total soluble protein, a value generally consistent with results in other eukaryotic cells (5, 11, 28–30). It is clear from this experiment that cyclic AMP (as Bt_2cAMP) does not detectably influence the elongation rate of the average soluble protein synthesized by these cells. This result is as expected since cyclic AMP has no reproducible influence on the synthesis rate for total soluble protein of H35 cells (1, 2, 16, 21).

In an effort to ascertain the molecular weight of the "average" protein subunit synthesized by H35 cells to allow calculation of approximate elongation rates, $NaDodSO_4$ gel electrophoresis was performed on a postpolysomal supernatant fraction from cells incubated with ^3H-labeled leucine for 6 hr. The molecular mass of the average subunit using this approach proved to be about 32,000 daltons, consistent with similar measurements made in other eukaryotic cells (5, 30, 31). Assuming an average molecular mass of 130 daltons for each amino acid residue, the average soluble protein subunit consists of about 240 amino acid residues. The approximate elongation rate for total soluble protein calculated from these data is about two amino acids per sec in either the absence or presence of Bt_2cAMP. This value is very similar to the elongation rates re-

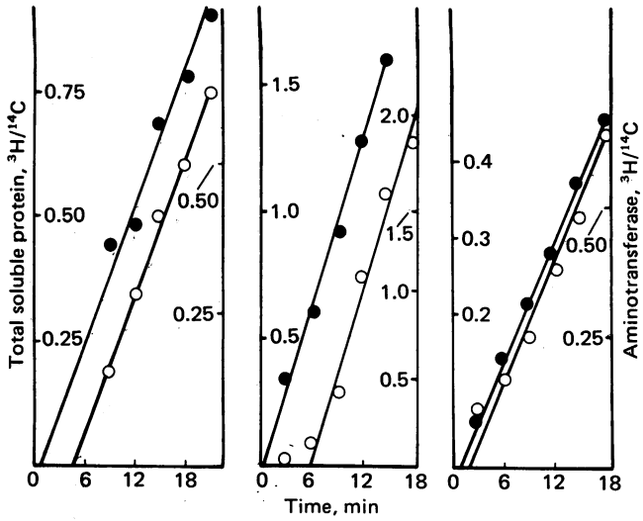

FIG. 2. Effect of Bt_2cAMP on ribosomal transit times for tyrosine aminotransferase. Confluent H35 cells were incubated (*Left*) without or (*Center*) with 1 μM dexamethasone or (*Right*) with 0.5 mM Bt_2cAMP. Three to four hours later, transit time analysis was performed. ●, Postmitochondrial supernatant fraction (as in Fig. 1); ○, immunoprecipitated aminotransferase completed chains. Intercepts: ●, control 0.75 min, dexamethasone 0.23 min, Bt_2cAMP 0.90 min; ○, control 4.89 min, dexamethasone 5.42 min, Bt_2cAMP 1.71 min. Transit times: control 8.3 min, dexamethasone 10.4 min, Bt_2cAMP 1.6 min. The mean specific activity of aminotransferase ± SEM (in units/ml) determined in companion dishes is: control 36 ± 2 (n = 4), dexamethasone 342 ± 8 (n = 6), Bt_2cAMP 109 ± 2 (n = 4).

ported for ovalbumin (10), HeLa total soluble protein (5), globin (6, 29), and pancreatic islet total soluble protein (30).

Effect of Bt_2cAMP on aminotransferase transit times

Monospecific antibodies directed against aminotransferase have been used to demonstrate the specific transit time for this protein. In untreated cells, extrapolation of the ^3H:^{14}C ratio in immunoprecipitates of completed aminotransferase to zero yielded a release time of about 4.9 min (Fig. 2). Since the equilibration time for nascent chain labeling is about 45 sec (see Fig. 1), the half-transit time for aminotransferase under basal conditions in this experiment is 4.14 min. Doubling this value affords a transit time of 8.3 min, more than twice as long as that for total soluble protein. The values for aminotransferase transit time varied between 6 and 9 min from experiment to experiment, with the longer times being more common. The reason for this variation is not clear, but the basal level of aminotransferase fluctuated by about the same extent and the degree of inducibility with Bt_2cAMP also varied from experiment to experiment (see Fig. 3). As previously discussed, the elongation rate of the "average" subunit in the cytosol is about two amino acids per sec. Since the molecular mass of each of its subunits is ~58,000 daltons and the chain is composed of 474 amino acid residues (32–34), the aminotransferase subunit is assembled at the rate of 1.0–1.5 amino acids per sec, roughly half as rapidly as total soluble protein.

In marked contrast to its lack of effect on the transit time of the average H35 cell protein, Bt_2cAMP was so effective at reducing the transit time for aminotransferase that a precise value was sometimes difficult to obtain. The best estimate of the half-transit interval in the experiment illustrated (Fig. 2) was approximately 49 sec. It is important to point out that the degree of increase in aminotransferase activity in this experiment was similar to the degree of reduction in the transit time for aminotransferase.

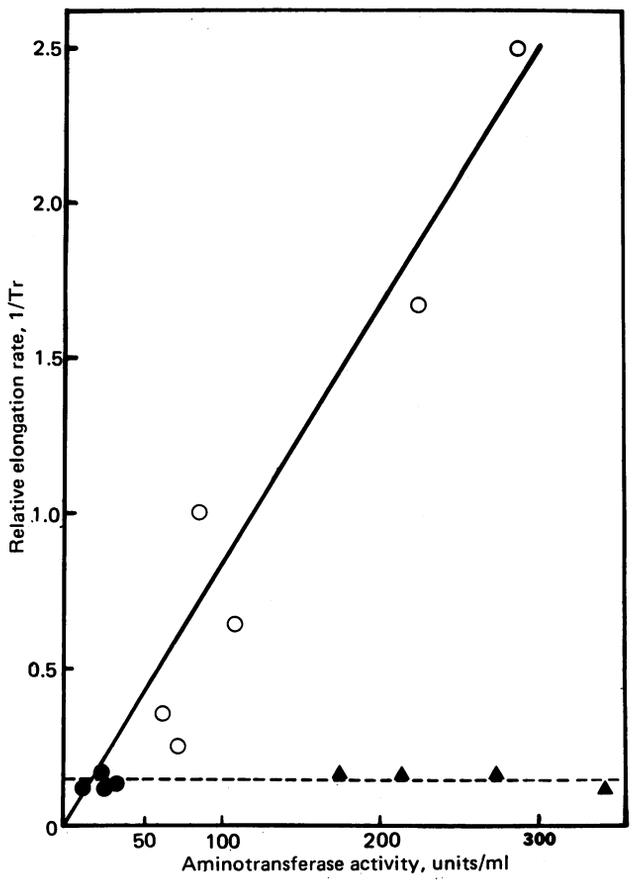

FIG. 3. Effect of Bt_2cAMP and dexamethasone on relative elongation rates for tyrosine aminotransferase synthesis. Aminotransferase transit times were determined in several experiments in H35 cells incubated with (○) 0.5 mM Bt_2cAMP (3 hr) or (▲) 1 μM dexamethasone (4 hr), or (●) with no additions. Relative elongation rate (1/Tr) = transit time^{-1}. The correlation coefficient between the relative elongation rate and aminotransferase activity in Bt_2cAMP and with no additions was 0.92, with $P \simeq 0.001$ (8 degrees of freedom). No significant correlation was found between these parameters in control cells and cells treated with dexamethasone.

Glucocorticoids have been shown directly to elevate the level of mRNA coding for tryptophan oxygenase (35) and tyrosine aminotransferase (3, 4). This type of induction should not involve any alteration in elongation rate, merely an increase in the number of polysomes engaged in the synthesis of aminotransferase (31). In accordance with this expectation, dexamethasone did not shorten the transit time for aminotransferase despite the fact that the enzyme activity was increased 5- to 10-fold in four separate experiments (Fig. 3). Several other transit time experiments were also performed with control and Bt_2cAMP-treated cells, and these results are also illustrated in Fig. 3. It is immediately apparent that, although the degree of induction by the cyclic nucleotide varied from experiment to experiment, the degree of increase in the relative elongation rate (transit time^{-1}) closely parallels the observed increase in aminotransferase activity in each case. As discussed above, the reason for the variation in response to Bt_2cAMP is not clear, but it has been a consistent finding and is also true for dexamethasone and insulin. The correlation coefficient for the relationship between the increase in aminotransferase activity and relative elongation rate was 0.92 ($P \simeq 0.001$), suggesting strongly that elongation of nascent aminotransferase chains is controlled by cyclic AMP.

DISCUSSION

It is clear from several lines of evidence that control of tyrosine aminotransferase synthesis is exhibited at both transcription and translation (1–4). The translational control of its synthesis could be elicited theoretically at initiation, elongation, or termination. It is important to point out that primary stimulation at either elongation or termination would require concomitant secondary increases in the rate of initiation for protein synthesis to be productively affected. Cytoplasmic mRNA processing (such as shifts in the mRNP–mRNA pool) might also be involved (8, 9), but the net result of an increase in available mRNA would be to increase the number of initiations of aminotransferase synthesis as well. However, since Bt_2cAMP causes a consistent reduction in the transit time for aminotransferase, in direct proportion to the degree of increase in enzyme synthesis, Bt_2cAMP apparently exerts its effect on aminotransferase synthesis by influencing the rate of elongation without directly changing the rate of initiation. In contrast, dexamethasone induction is not accompanied by increased translational elongation rates, as would be predicted by the fact that the steroid elevates the aminotransferase mRNA concentration (3, 4).

In a recent abstract, Pomato *et al.* have reported (36) evidence that directly supports our results. Using a postmitochondrial liver system in which only completion of nascent aminotransferase chains was occurring, this group observed that prior Bt_2cAMP treatment did not increase the number of chains initiated. In contrast, glucocorticoid treatment significantly increased the number of chains initiated *in vivo*, as expected (3, 4).

A direct indication of postinitiation control of aminotransferase synthesis by cyclic AMP has been reported (37, 38). In these studies, very high levels of cyclic AMP (0.1–1 mM) were effective *in vitro* in releasing aminotransferase activity from washed polysomes obtained from selected neonatal rats. For unknown reasons this effect could not be duplicated with washed polysomes prepared from older animals. Since cyclic AMP could evidently "release" aminotransferase activity, the authors suggested that the nucleotide acted to increase aminotransferase synthesis by stimulating termination of completed nascent chains. Although the protocol used in our studies cannot discriminate fully between effects during elongation or termination, it seems unlikely that an alteration in the rate of aminotransferase termination is involved for the following reasons:

(*i*) Synthesis of total soluble protein does not seem to be limited by termination, based on the similar rates observed for the elongation of H35 total soluble protein and globin nascent chains (6) for which the termination rate is known, and is not rate limiting.

(*ii*) Under basal conditions, aminotransferase is assembled only slightly more slowly than total soluble protein (1.0–1.5 amino acids/sec for the enzyme compared to about 2 amino acids/sec for total soluble protein). If termination were rate limiting for aminotransferase synthesis, the enzyme would be expected to exhibit a much slower rate of elongation than total soluble protein.

(*iii*) The elongation rate for aminotransferase synthesis after stimulation with cyclic AMP is far greater than that for total soluble protein (up to about 10 amino acids/sec for the enzyme compared to 2 for total soluble protein). If the termination of aminotransferase were made non-rate-limiting by treatment with cyclic AMP, then the rate of elongation would not be expected to exceed that of total soluble protein.

The arguments presented above, although reasonable, do not completely eliminate a potential action of cyclic AMP on termination. In order to distinguish between these possibilities, it will be necessary to measure the rate of aminotransferase termination either by direct or indirect means. Unfortunately, the carboxyl terminus of aminotransferase is not yet known, so that direct measurements of termination cannot be performed by existing methodology (6). However, we are currently attempting to measure the rate indirectly through determination of the size of polysomes engaged in aminotransferase synthesis.

Very few reports of any sort of elongation control have appeared in the past. Palmiter (10) observed changes in the transit times for three specific proteins in chick magnum after estrogen treatment, auxin treatment of pea epicotyls has been reported to alter transit times for total soluble protein (39), and thyroxine appears to change the elongation rate for total soluble protein in liver (40). With the possible exception of thyroxine treatment of thyroidectomized rats, none of these changes amounts to much more than 25% alterations in the elongation rate and, at least in the first two instances (10, 39), are apparently minor secondary effects with the primary one being an increase in mRNA content resulting from hormone treatment. The thyroxine effect also appears to result from altered levels of elongation factor (41) and is a nonspecific effect of the hormone. We believe that this is the first report in which a selective large increase in the rate of nascent chain elongation appears to account fully for the increase in the synthesis of a specific protein in eukaryotic cells caused by an inducer.

A large number of reports of regulation of specific protein synthesis apparently at the translational level by cyclic AMP have appeared over the past few years (2). It is conceivable that some of these will prove to be due to a mechanism similar to that operative for aminotransferase. The dearth of reports of regulation of protein synthesis at the primary level of elongation may well be due to the understandable emphasis in the past on major cellular proteins or on total protein. It seems clear that the synthesis of most major proteins is limited by initiation, but tyrosine aminotransferase is a decidedly minor protein comprising only ~0.25% of newly synthesized soluble protein under basal conditions.‡

Iynedjian and Hanson have recently reported (43) that Bt_2cAMP treatment increases the level of translatable mRNA coding for phospho*enol*pyruvate carboxykinase in rat liver. We have presented indirect evidence for a translational effect of Bt_2cAMP on the synthesis of this enzyme (44). The basis for this apparent discrepancy is not clear unless it is a reflection of an action of cyclic AMP at both the levels of transcription and translation, as has been suggested to be the case with pineal *N*-acetyltransferase (45).

The involvement of cyclic AMP-dependent protein kinase has been suggested as a mediator of the effects of cyclic AMP analogs on aminotransferase synthesis (22). The precise role played by the kinase in this process, if it is involved indeed, is uncertain. Potential sites of phosphorylation might include nascent aminotransferase chains, elongation factors, or polysomal mRNA-associated protein (46). The first possibility seems

‡ The calculation of % aminotransferase synthesis is based on the steady-state equation: $k_s = [E]\,k_d$; $k_d = 1.6 \times 10^{-4}$/min (16) and [E] = 100 μg of total soluble protein/10^6 cells; hence, $k_s = 16 \times 10^3$ pg/min. For aminotransferase, $k_d = 7.7 \times 10^{-3}$/min (42) and [E] = 5 ng/10^6 cells (assuming a specific activity for pure aminotransferase of 9×10^5 units/mg (34) and 4.5 units/10^6 cells). Thus, aminotransferase is synthesized at 38.5 pg/min per 10^6 cells. Dividing 38.5 pg/min per 10^6 cells by 16×10^3 pg/min per 10^6 cells leads to the conclusion that the enzyme represents ~0.25% of the synthesis of new soluble protein under basal conditions.

extremely attractive in light of the observation that aminotransferase exhibits ^{32}P incorporation into serine residues when either H35 cells or rats are exposed to $^{32}PO_4^{3-}$ (32), a result that we have confirmed. Previous work has shown that the native enzyme is not a substrate for crude or highly purified cyclic AMP-dependent protein kinases from rabbit skeletal muscle or rat liver (W. D. Wicks and D. A. Walsh, unpublished observations). These observations suggest that it is more likely that nascent aminotransferase chains are the true substrates *in vivo*. Should it be demonstrated that aminotransferase elongation is limited by some unfavorable conformation of the growing peptide chains which could be alleviated by phosphorylation of a specific serine residue, then the link between kinase activation and aminotransferase induction would be established.

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1. Wicks, W. D., Van Wijk, R. & McKibbin, J. B. (1973) *Adv. Enzyme Regul.* **11**, 117–135.
2. Wicks, W. D. (1974) *Adv. Cyclic Nucleotide Res.* **4**, 335–438.
3. Nickol, J. M., Lee, K. C., Hollinger, T. G. & Kenney, F. T. (1976) *Biochem. Biophys. Res. Commun.* **72**, 687–693.
4. Roewekamp, W. G., Hofer, E. & Sekeris, C. E. (1976) *Eur. J. Biochem.* **70**, 259–268.
5. Fan, H. & Penman, S. (1970) *J. Mol. Biol.* **50**, 655–670.
6. Lodish, H. F. & Jacobsen, M. (1972) *J. Biol. Chem.* **247**, 3622–3629.
7. Lodish, H. F. (1976) *Annu. Rev. Biochem.* **45**, 39–72.
8. Zahringer, J., Baliga, B. S. & Munro, H. N. (1976) *Proc. Natl, Acad. Sci. USA* **73**, 857–861.
9. Heywood, S. M., Kennedy, D. S. & Baxter, A. J. (1975) *FEBS Lett.* **53**, 69–72.
10. Palmiter, R. D. (1972) *J. Biol. Chem.* **247**, 2770–2780.
11. Palmiter, R. D. (1975) *Cell* **4**, 189–197.
12. Shapiro, D. J., Taylor, J. M., McKnight, G. S., Palacios, R., Gonzales, C., Kiely, M. L. & Schimke, R. T. (1974) *J. Biol. Chem.* **249**, 3665–3671.
13. Palmiter, R. D. (1973) *J. Biol. Chem.* **248**, 8260–8270.
14. Harris, S. E., Rosen, M. J., Means, A. R. & O'Malley, B. W. (1975) *Biochemistry*, **14**, 2072–2081.
15. Pitot, H. C., Peraino, C., Morse, P. A. & Potter, V. R. (1964) *Natl. Cancer Inst. Monogr.* **13**, 229–242.
16. Barnett, C. A. & Wicks, W. D. (1971) *J. Biol. Chem.* **241**, 7201–7206.
17. Munns, T. W., Johnston, M. F. M., Liszewski, M. K. & Olson, R. E. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2803–2807.
18. Peterson, J. A. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 2062–2066.
19. Gerschenson, L. E. & Thompson, E. B. (1975) *Gene Expression and Carcinogenesis* (Academic Press, New York).
20. Haggerty, D. F., Young, P. L., Popjak, G. & Carnes, W. R. (1973) *J. Biol. Chem.* **248**, 223–232.
21. Wagner, K., Roper, M. D., Leichtling, B. H., Wimalasena, J. & Wicks, W. D. (1975) *J. Biol. Chem.* **250**, 231–239.
22. Wicks, W. D., Koontz, J. & Wagner, K. (1975) *J. Cyclic Nucleotide Res.* **1**, 49–58.
23. Wicks, W. D. (1969) *J. Biol. Chem.* **244**, 3941–3950.
24. Mans, R. J. & Novelli, G. D. (1961) *Arch. Biochem. Biophys.* **94**, 48–53.
25. Wicks, W. D., Kenney, F. T. & Lee, K. L. (1969) *J. Biol. Chem.* **244**, 6008–6013.
26. Diamondstone, T. I. (1966) *Anal. Biochem.* **16**, 395–401.
27. Weber, K., Pringel, J. R. & Osborn, M. (1972) in *Methods in Enzymology*, eds. Hirs, C. W. H. & Timasheff, S. N. (Academic Press, New York), Vol. 26, pp. 3–27.
28. Craig, N. (1972) *J. Cell. Physiol.* **82**, 133–150.
29. Craig, N. (1976) *J. Cell. Physiol.* **87**, 157–166.
30. Permut, M. A. (1974) *J. Biol. Chem.* **248**, 2738–2742.
31. Scott, W. A., Shields, R. & Tomkins, G. M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2937–2941.
32. Lee, K. L. & Nickol, J. M. (1974) *J. Biol. Chem.* **249**, 6024–6026.
33. Beck, J. P., Beck, G., Wong, K. Y. & Tomkins, G. M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 3615–3619.
34. Valeriote, F. A., Auricchio, F., Tomkins, G. M. & Riley, D. (1969) *J. Biol. Chem.* **244**, 3618–3624.
35. Schutz, G., Beato, M. & Feigelson, P. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1218–1221.
36. Pomato, N., Nickol, J. M. & Lee, K.-L. (1977) *Fed. Proc.* **36**, 820.
37. Chuah, C. C. & Oliver, I. T. (1971) *Biochemistry* **10**, 2990–3001.
38. Chuah, C. C. & Oliver, I. T. (1972) *Biochemistry* **11**, 2547–2553.
39. Verma, D. P. S., Maclachlan, G. A., Byrne, H. & Ewings, D. (1975) *J. Biol. Chem.* **250**, 1019–1026.
40. Mathews, R. W., Oronsky, A. & Haschemeyer, A. E. V. (1973) *J. Biol. Chem.* **248**, 1329–1333.
41. Nielsen, J. B. K., Plant, P. A. & Haschemeyer, A. E. V. (1976) *Nature* **264**, 804–806.
42. Schimke, R. T. & Doyle, D. (1970) *Annu. Rev. Biochem.* **39**, 929–976.
43. Iynedjian, P. B. & Hanson, R. W. (1977) *J. Biol. Chem.* **252**, 655–662.
44. Wicks, W. D. & McKibbin, J. B. (1972) *Biochem. Biophys. Res. Commun.* **48**, 205–211.
45. Romero, J. A., Zatz, M. & Axelrod, J. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2107–2111.
46. Auerbach, S. & Pedersen, T. (1975) *Biochem. Biophys. Res. Commun.* **63**, 149–156.