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Biochemistry

A role for asparaginyl-tRNA in the regulation of asparagine synthetase in a mammalian cell line

(Chinese hamster ovary cells/asparaginyl-tRNA synthetase/temperature-sensitive mutant/gene expression)

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ABSTRACT **The expression of asparagine synthetase activity [L-aspartate:ammonia ligase (AMP-forming), EC 6.3.1.1] in cultured Chinese hamster ovary (CHO) cells is regulated by asparagine. After transfer of CHO cells from an asparagine-supplemented medium to a medium lacking asparagine, activity increases 1.5- to 2-fold. If asparagine is added back to the medium, activity returns to control levels. To test the possible involvement of Asn-tRNA^{Asn} in regulating the levels of asparagine synthetase, we have examined the levels of asparagine synthetase in a mutant of CHO cells containing a temperature-sensitive asparaginyl-tRNA synthetase [L-asparagine:tRNA ligase (AMP-forming), EC 6.1.1.22]. Under conditions of limited asparaginyl-tRNA synthetase activity in the mutant, there is a 2- to 3-fold increase in the level of asparagine synthetase activity. Under identical conditions, there is no change in asparagine synthetase activity in the wild type. This correlation between asparaginyl-tRNA synthetase activity and asparagine synthetase levels may be a consequence of a direct role of tRNA^{Asn} in the regulation of the *in vivo* expression of the asparagine synthetase structural gene.**

There is now strong experimental evidence that some transfer ribonucleic acids are specifically involved in the regulation of the synthesis of their cognate amino acid biosynthetic enzymes in bacteria and simple eukaryotes (1, 2). There is also evidence that supports a more general metabolic control of gene expression of many biosynthetic and catabolic operons mediated by guanosine tetraphosphate and uncharged transfer ribonucleic acid (3). Because the roles in protein synthesis of aminoacyl-tRNA synthetases and tRNAs are the same in all cells, it may be reasonable to assume that their regulatory roles will be similar in all cells. In bacteria, studies with amino acid analogs, and with mutants affected in the formation, concentration, or modification of aminoacyl-tRNA, have been particularly useful in demonstrating these regulatory functions (1). Recent advances in mammalian cell mutant methodology have now made it possible to isolate analogous mutants in cultured animal cells (4, 5). Therefore, an evaluation of the significance of tRNA-mediated modulation of gene expression in animal cell systems is now possible. In this communication, we report on the regulatory properties of a temperature-sensitive mutant, PSV1, derived from Chinese hamster ovary (CHO) cells, which is defective in asparaginyl-tRNA synthetase [L-asparagine:tRNA ligase (AMP-forming), EC 6.1.1.22] activity at the nonpermissive temperature (5). This mutant has enabled us to test the relationship between the ability of cells to aminoacylate tRNA^{Asn} effectively and the *in vivo* expression of the biosynthetic enzyme asparagine synthetase [L-aspartate:ammonia ligase (AMP forming), EC 6.3.1.1].

Abbreviation: CHO cells, Chinese hamster ovary cells.

MATERIALS AND METHODS

Growth of Cells. CHO-K1 cells, obtained from the American Type Culture Collection, and PSV1 cells, the generous gift of L. H. Thompson, were grown as monolayers in an atmosphere of 5% CO_2/95% air. The basal media employed were α-MEM (6) (K-C Biologicals) containing 10% dialyzed fetal calf serum (Gibco) and Ham's F-10 (7) containing 10% dialyzed calf serum and 5% dialyzed fetal calf serum.

Preparation of Cell Extracts. Monolayers were rinsed with saline and the cells were detached from the surface by exposure to 0.025% trypsin (Gibco) for 3 min at 37°. Trypsinization was stopped by addition of a 5-fold excess of serum-containing medium. Cells were sedimented by centrifugation for 8 min at 1000 × *g*. After being washed twice in saline, the cells were lysed by suspension in 0.06 M Tris·HCl/5 mM EDTA/1 mM dithiothreitol, pH 7.6 and freeze-thawing twice with dry ice. The extract was centrifuged at 20,000 × *g* for 20 min and the supernatant solution was utilized for enzyme assays. Cells were pooled to yield extracts containing 9–12 mg of protein per ml.

Enzyme Assays. Asparagine synthetase activity was assayed by the aid of high voltage electrophoresis to separate asparagine and aspartic acid (8). Reaction mixtures contained, per ml: *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Tes), pH 7.6, 150 μmol; $MgCl_2$, 7.5 μmol; ATP, 7.5 μmol; L-glutamine, 30 μmol; and L-[^{14}C]aspartic acid (5 μCi/μmol), 2.25 μmol. Enzymes were assayed at 37° under conditions that resulted in a constant rate of reaction for 30 and 60 min. Glutamate dehydrogenase (EC 1.4.1.3) was assayed by the method of Fahien and Cohen (9). Pyruvate kinase (EC 2.7.1.40) was assayed as described by Ibsen and Trippet (10). Protein determinations were performed according to the method of Lowry *et al.* (11).

RESULTS

Effect of asparagine concentration on asparagine synthetase levels in CHO-K1 cells

Because the mutant cell line, PSV1, was derived from wild-type CHO-K1 cells, it was necessary to examine the normal regulation of this enzyme in the parental cell line prior to investigating the possible effects of this mutation on the regulation of the biosynthetic asparagine synthetase. The levels of asparagine synthetase activity in CHO-K1 cells grown in the presence or absence of asparagine in the culture medium are shown in Fig. 1. A constant basal low level of asparagine synthetase activity was observed in cells maintained in a medium containing asparagine. The level of asparagine synthetase activity increased

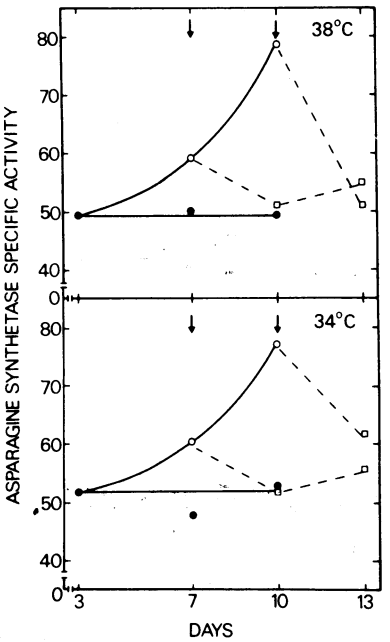

FIG. 1. Asparagine synthetase specific activity in CHO-K1 cells growing with and without asparagine in the growth medium. Cells were grown in F10 plus 0.2 mM asparagine for 3 days. These cultures were divided and passed into the same medium (●—●) or to F10 medium lacking asparagine (○—○). Asparagine was added back to half the cultures growing in medium lacking asparagine at the times indicated by the arrows (○ - - - - □). Specific activities are in terms of pmol asparagine formed per min per mg of protein.

when these cells were transferred to a medium lacking asparagine, and readdition of asparagine resulted in a return of activity to the basal level. In order to compare these results with those obtained with the temperature-sensitive mutant PSV1, the experiments were performed at both 34° and 38°; the results were similar. These results show that asparagine synthetase activity in the parental CHO-K1 cell line responds to the presence or absence of asparagine in the growth medium, as previously shown for other cell lines (12, 13).

Regulation of the levels of asparagine synthetase activity in PSV1

In order to establish whether the response of asparagine synthetase activity levels is affected by free asparagine or by the cells' ability to aminocylate $tRNA^{Asn}$, the levels of asparagine synthetase activity were determined in PSV1 cells at 34° and 38°. It is apparent from the data in Table 1 that growth of the mutant in a medium containing sufficient asparagine (0.3 mM) to maintain repressed levels of enzyme activity in the parental

Table 1. Asparagine synthetase activity in CHO-K1 and PSV1 cells as a function of asparagine concentration and temperature

Strain	Asparagine concentration, mM	Specific activity 34°	Specific activity 38°
PSV1	0.3	49	107
	3.0	41	58
CHO-K1	0.3	41	47
	3.0	41	54

Specific activities (pmol asparagine formed/min per mg protein) were determined after 3 days of growth under the conditions indicated.

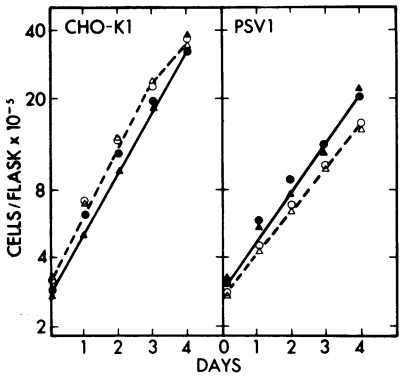

FIG. 2. Growth of CHO-K1 and PSV1 cells at 34° and 38° in α-MEM containing either 0.3 mM asparagine or 3.0 mM asparagine: ○, 0.3 mM asparagine, 38°; △, 3.0 mM asparagine, 38°; ●, 0.3 mM asparagine, 34°; ▲, 3.0 mM asparagine, 34°.

CHO-K1 cell line at both 34° and 38° resulted in similarly repressed levels in the mutant strain at 34°. But when PSV1 cells were grown at 38° in a medium containing this same level (0.3 mM) of asparagine, a marked increase in enzyme activity *in vivo* was observed. At 10-fold higher levels (3.0 mM) of asparagine, a concentration that has been shown to cause a phenotypic reversal of the impaired asparaginyl-tRNA synthetase activity at 38° (5), the regulation of asparagine synthetase activity was restored. These higher levels (3.0 mM) of exogenous asparagine did not lead to any further repression of asparagine synthetase activity in the parental cell line.

The temperatures employed in these experiments were selected in order to minimize differences in growth rates between the mutant PSV1 and parental CHO-K1 cells. This was done because of the evidence for a metabolic regulation, well described in bacteria (14), which couples the rate of synthesis of aminoacyl-tRNA synthetases with growth rates. Minimal differences in growth rates were observed under the conditions employed in these experiments (Fig. 2). It is likely that greater excursions in asparagine synthetase activity levels could be observed at temperatures more restrictive for cell growth.

Lack of effect of growth temperature on other enzymes in CHO-K1 and PSV1 cells

In order to rule out a nonspecific increase in asparagine synthetase activity in PSV1 at 38°, the activities of glutamate dehydrogenase, an enzyme involved in amino acid metabolism, and pyruvate kinase, an enzyme presumably synthesized constitutively, were examined in both CHO-K1 and PSV1 cells grown at both 34° and 38° in the presence of 0.3 mM asparagine. As Table 2 shows, these experimental conditions had no effect on the activities of these enzymes.

Table 2. Lack of effect of growth temperature on activities of glutamate dehydrogenase and pyruvate kinase

Strain	Growth temperature, °C	Specific activity: Glutamate dehydrogenase	Specific activity: Pyruvate kinase
CHO-K1	34	15	4.5
	38	13	4.4
PSV1	34	11	4.5
	38	13	4.4

Specific activities (μmol/min per mg protein) were determined after 3 days of growth at the indicated temperature.

DISCUSSION

The experiments reported here were prompted by our current understanding of the role of tRNA in the regulation of gene expression in bacteria. Experiments analogous to those described here were performed in bacteria by Eidlic and Neidhardt (15) 10 years ago. These workers demonstrated a derepression of the isoleucine and valine biosynthetic enzymes in a temperature-sensitive valyl-tRNA synthetase mutant of *Escherichia coli*. Although their original results did not rule out the possibility that some activity other than the charging of $tRNA^{Val}$ was involved in the derepression of these biosynthetic enzymes, there is now unequivocal evidence that tRNAs can indeed be directly involved in this type of regulation in bacteria (1). Similarly, the correlation in this paper between asparaginyl-tRNA synthetase activity and asparagine synthetase levels in CHO cells may also stem from a direct role of tRNA. The purification of both asparagine synthetase and asparaginyl-tRNA synthetase is necessary in order to assess the roles of enzyme synthesis and degradation in the regulation of the enzyme activity levels reported here.

That asparaginyl-tRNA is involved in the regulation of asparagine synthetase levels in animal cells is not an original suggestion. Gallo *et al.* (16) have demonstrated correlations between the $tRNA^{Asn}$ profiles of asparaginase-sensitive murine leukemia cells (which have low or non-detectable levels of asparagine synthetase) and asparaginase-resistant cells (which have high asparagine synthetase levels). On the basis of these observations, they had proposed that $tRNA^{Asn}$ is involved in the regulation of this biosynthetic enzyme.

As the results reported here show, it would be very worthwhile to utilize the genetic and biochemical approaches previously described in bacteria to evaluate the role in animal cells of other tRNA-related regulatory phenomena. Such regulatory functions include the role of tRNA in the stringent control of stable RNA synthesis (3), the initiation of DNA replication (14), protein degradation (17), autoregulation and attenuation (18), and amino acid transport (19).

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1. Brenchley, J. E. & Williams, L. S. (1975) *Annu. Rev. Microbiol.* **29,** 251–274.
2. Calvo, J. M. & Fink, G. R. (1971) *Annu. Rev. Biochem.* **40,** 943–968.
3. Cashel, M. (1975) *Annu. Rev. Microbiol.* **29,** 301–318.
4. Thompson, L. H., Harkins, J. L. & Stanners, C. P. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3094–3098.
5. Thompson, L. H., Stanners, C. & Siminovitch, L. (1975) *Somatic Cell Genet.* **1,** 187–208.
6. Stanners, C. P., Eliceiri, G. L. & Green, H. (1971) *Nature New Biol.* **230,** 52–54.
7. Ham, R. G. (1963) *Exp. Cell Res.* **29,** 515–526.
8. Horowitz, B. & Meister, A. (1972) *J. Biol. Chem.* **247,** 6708–6719.
9. Fahien, L. A. & Cohen, P. P. (1970) in *Methods in Enzymology,* eds. Tabor, H. & Tabor, C. W. (Academic Press, New York), Vol. 17A, pp. 839–844.
10. Ibsen, K. H. & Trippet, P. (1973) *Arch. Biochem. Biophys.* **156,** 730–744.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
12. Uren, J. R., Summers, W. P. & Handschumacher, R. E. (1974) *Cancer Res.* **34,** 2940–2945.
13. Colofiore, J., Morrow, J. & Patterson, M. K., Jr. (1973) *Genetics* **75,** 503–514.
14. Neidhardt, F. C., Parker, J. & McKeever, W. G. (1975) *Annu. Rev. Microbiol.* **29,** 215–250.
15. Eidlic, L. & Neidhardt, F. C. (1965) *Proc. Natl. Acad. Sci. USA* **53,** 539–543.
16. Gallo, R. C., Longmore, J. L. & Adamson, R. H. (1970) *Nature* **227,** 1134–1136.
17. Goldberg, A. L. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 362–366.
18. Calhoun, D. H. & Hatfield, G. W. (1975) *Annu. Rev. Microbiol.* **29,** 275–299.
19. Quay, S. C., Kline, E. L. & Oxender, D. L. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3921–3924.