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DEVELOPMENT OF AMINOACYL *t*RNA SYNTHETASES IN CULTURED *NICOTIANA TABACUM* CELLS

JOHN L. WRAY, RICHARD E. BRICE and LESLIE FOWDEN*†

Department of Biochemistry, University of St. Andrews, North Street, St. Andrews, Fife, Scotland and *Department of Botany and Microbiology, University College London, Gower Street, London WC1E 6BT

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Abstract—Changes in the activity of aminoacyl *t*RNA synthetases during growth of tobacco XD cells in suspension culture have been determined by the pyrophosphate exchange assay. Alanyl, arginyl, glutamyl, glutaminyl and seryl *t*RNA synthetases showed the lowest activity, whilst lysyl, histidyl, leucyl, isoleucyl, phenylalanyl, threonyl and valyl *t*RNA synthetases were most active. Most synthetases, and total protein, increased to a maximum at around 7 days, just before mid-exponential phase, and then fell.

INTRODUCTION

IN RECENT years plant tissue cultures have proved to be extremely useful tools in investigations into the control of nitrogen metabolism, particularly with reference to the nitrate assimilation pathway,[1–3] and certain aspects of amino acid biosynthesis.[4–7] The advantages of using tissue culture rather than the intact plant are obvious; the cells are more or less homogenous, the culture is aseptic, thus avoiding problems associated with bacterial contamination, and perhaps most importantly, manipulation of the culture medium enables one to grow the cells under any condition at will. Further, the development of methods for preparation of haploid plant cell cultures by anther culture,[8–11] and the preparation of protoplasts from plant cells[12] suggests that it may soon be possible to apply the techniques of biochemical genetics to higher plants.

One area of nitrogen metabolism which has not been examined so intensively as others is the problem of control of aminoacyl *t*RNA synthetases (amino acid-*t*RNA ligases, E.C. 6.1.1.). These enzymes catalyse the two-step transfer of amino acid to *t*RNA in the initial step in protein synthesis:

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† Present address: Rothamstead Experimental Station, Harpenden, Herts. AL5 2JQ.

(1) Amino acid + ATP ⟷ Aminoacyladenylate + pyrophosphate
(2) Aminoacyladenylate + *t*RNA ⟷ Aminoacyl *t*RNA + AMP.

In microorganisms, the aminoacyl *t*RNA products of this reaction also appear to be involved in two other areas of metabolism besides protein synthesis.

Aminoacyl *t*RNA, or some derivative, appears to be active as the corepressor of several,[13–16] but not all,[17,18] amino acid biosynthetic pathways. A requirement for amino acid activation or aminoacyl *t*RNA synthesis is also found in the "stringent response" in *E. coli*. The "stringent response" occurs during amino acid starvation, and involves a reduction in the rate of several biochemical processes, including RNA synthesis, nucleotide synthesis and protein turnover[19–21] Specific inactivation of any of a number of aminoacyl *t*RNA synthetases brings about the "stringent response", even in the presence of the required amino acid.[22,23] The central role of the synthetases in metabolism is thus obvious, although a role for aminoacyl *t*RNA in processes other than protein synthesis in higher plants has not yet been demonstrated.

The presence of these enzymes in higher plants is well documented (see review by Lea and Norris[24]), and reports of changes in their activity during development are legion.[24–29] However, little is known about how the development of these enzymes is controlled in higher plants. Henshall and Goodwin[25] have shown that treatment of pea plants with gibberellic acid leads to an increase in both the free amino acid pool and in the activity of aminoacyl *t*RNA synthetases. However, whether the increased amino acid pool size led to a general stimulation of protein synthesis or whether there was a specific effect on the rate of synthesis of the synthetases is not clear. In the present paper we report results of a survey of changes in the activity of aminoacyl *t*RNA synthetases during growth of tobacco XD cells in suspension culture. Work is currently in progress to examine the factors which might control the changes observed.

RESULTS AND DISCUSSION

Aminoacyl *t*RNA synthetases were assayed by the ATP-pyrophosphate exchange reaction according to the method of Anderson and Fowden.[28] All synthetases were detected, but alanyl, arginyl, glutamyl, glutaminyl, glycyl and seryl *t*RNA synthetases were of very low activity. The low activity of ala, seen both here and in the study of Anderson and

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Fowden,[28] is probably a consequence of the very high K_m for ala in the ATP-pyrophosphate exchange reaction.[30] Both arg[31] and gluNH$_2$[32] probably have a requirement for *t*RNA in the activation step while glu[33] and gly[34] appear to be unstable during extraction. The level of ser activity appears to be lower than that found in other studies (Table 1).

TABLE 1. VARIATION OF ACTIVITY OF AMINO ACYL *t*RNA SYNTHETASES WITH CULTURE AGE

Amino acid	Activity (nmol/min/g cells) Day 0	Day 2	Day 4	Day 7	Day 11
Alanine	0·1	0·3	1·0	0·6	0
Arginine	1·0	1·05	1·3	1·6	1·5
Aspartic acid	1·3	0·75	4·1	7·3	0
Asparagine	0·5	1·9	5·1	9·8	1·8
Cysteine	2·7	7·0	16·7	44·0	5·6
Glutamic acid	0·9	0	1·0	3·1	1·5
Glutamine	0·1	0·15	1·5	1·35	0
Glycine	0·6	0·6	2·1	3·7	0
Histidine	2·5	6·7	14·2	37·0	5·0
Hydroxyproline	0	0	0	0	0
Isoleucine	5·3	10·7	26·3	53·7	8·5
Leucine	2·8	6·2	15·7	47·1	6·0
Lysine	1·0	2·3	8·5	11·2	0·7
Methionine	2·8	3·2	7·2	12·8	2·5
Phenylalanine	1·5	2·1	5·8	26·6	6·0
Proline	1·0	2·3	11·0	18·2	1·5
Serine	1·0	0	0·4	2·4	0
Threonine	1·5	3·7	10·0	26·0	5·2
Tryptophan	0·8	1·1	4·5	7·0	0·5
Tyrosine	0·7	1·1	3·1	10·3	6·5
Valine	2·5	5·2	16·2	32·2	1·5

Aspartyl, asparaginyl, lysyl, methionyl, tryptophanyl and tyrosyl *t*RNA synthetases were of intermediate activity, while cysteinyl, histidyl, isoleucyl, leucyl, phenylalanyl, threonyl, valyl and prolyl *t*RNA synthetases exhibited high activity. This activity distribution is very similar to that found in pea,[28] and in such diverse experimental material as myocardial tissue[35] and cells of *Streptococcus thermophilus* KQ.[26] When the activity of individual synthetases was summed they peaked at 7 days, just before mid-exponential phase, but an increase in total activity was seen even while the cells were in lag phase (Fig. 1). The maximum rate of increase in synthetase activity coincided in time with the maximum rate of accumulation of protein (Fig. 2). The variation in activity of the synthetases was very similar to that observed for nitrate reductase in these cells.[1] Individual synthetases increased in activity by different amounts on a fresh weight basis, and most also showed an increase in specific activity, the largest increases being between 3- and 6-fold (cys, his, ile, leu, phe, pro, thr, trp, tyr and val). The fact that all synthetases did not show similar increases may be due in part to their different stabilities.

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Changes in synthetase activity during development have been described by many workers, and in nearly all cases, marked increases in specific activity are seen in tissue undergoing active growth and development. In a study on bean root, Hinde *et al.*[27] found that the synthetases were most active at the root tip, a conclusion supported by Cowles and Key,[29] working with pea. In *Streptococcus thermophilus* KQ,[26] synthetase activity increases 3–5-fold during the growth cycle, reaching a maximum just before mid-exponential phase. Maximum activity of synthetase in XD tobacco cells also reaches a maximum at this time (Fig. 1).

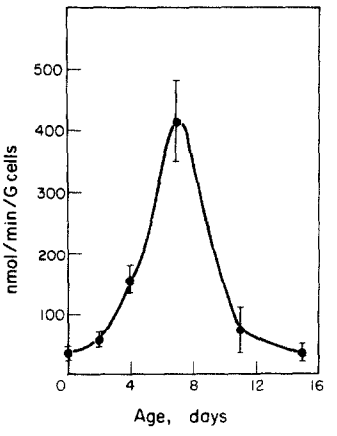

FIG. 1. VARIATION IN TOTAL ACTIVITY OF AMINOACYL *t*RNA SYNTHETASES WITH CULTURE AGE. Calculated by summing the individual activities. Vertical bars denote ± S.E.M.

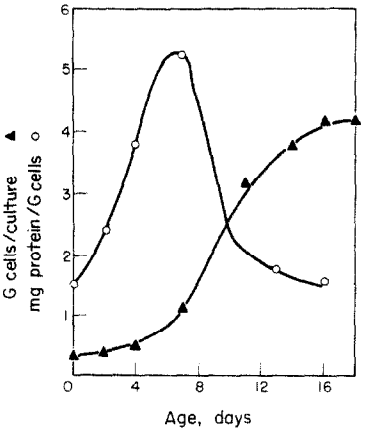

FIG. 2. CHANGES IN FRESH WEIGHT AND PROTEIN LEVELS WITH CULTURE AGE.

However, although such changes are well documented, little is known about the controlling factors. As indicated above, Henshall and Goodwin[25] have suggested that amino acids are involved in the control process since an increase in the free amino acid pool was correlated with an increase in synthetase activity. In the present study an increase in free amino acid levels was also seen, maximum levels occurring just before the maximum synthetase activity. This suggestion however is not supported by the studies of Anderson and Rowan,[36] who showed that treatment of tobacco leaves with kinetin increased the specific activity of the synthetases whilst at the same time causing a decrease in the free amino acid pool.

Data from other systems however implicate amino acids in the control mechanism. In rat liver,[37] protein depletion (starvation) leads to an increase in the activity of these enzymes, suggesting that as the amino acid pool size decreases, the level of aminoacyl *t*RNA synthetases rises to increase the fraction of amino acid involved in protein synthesis. In yeast,[38] removal of valine from the culture medium of cells grown in the presence of this amino acid has been shown to cause a 2-fold increase in the specific activity of valyl *t*RNA synthetase, and use of valine analogues suggests that valyl $t\text{RNA}^{val}$ is a key component in the derepression mechanism. The control of amino acyl *t*RNA synthetases has

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been studied in greatest detail in *E. coli* and *Salmonella typhimurium*, where it has also been shown that restriction of the supply of a particular amino acid, specifically increases the rate of synthesis of its cognate synthetase.[39–45] The involvement of $tRNA^{his}$ has been implicated in the control of *Salmonella* histidyl tRNA synthetase.[42,44] Thus, although there is no clear evidence for an involvement of amino acids (or some derivative) in the control process in higher plants, they appear to be the most likely candidates.

EXPERIMENTAL

Cell line. The XD line of tobacco cells used in this investigation was obtained from Dr. Philip Filner of Michigan State University. The composition of the culture medium and the origin of the cell line have been previously described.[1] The cells were grown at 28–30° on an orbital shaker. Stock cultures were grown in 200 ml of medium in 500 ml conical flasks. Experimental cultures were started by transferring 10 ml aliquots of a 14-day-old culture (0·4 g cells) into 100 ml of medium.

Growth assay. Cells were harvested by filtration on Miracloth (Chikopee Mills Inc., New York, N.Y.) and weighed.

Protein assay. Protein was assayed by the method of Lowry *et al.*[46]

Extraction and assay of aminoacyl tRNA synthetases. Cells were harvested by filtration on Miracloth, weighed and homogenized in a glass–Teflon Potter homogenizer at 4°, in 0·1 M Tris–HCl buffer, pH 7·5 containing 20 mM $MgCl_2$ and 25 mM thioglycollic acid (1 g of cells to 3 ml buffer). The homogenate was centrifuged at 39000 rev/min for 1 hr in an M.S.E. Superspeed 65 preparative ultracentrifuge. A 2-ml aliquot of the supernatant was then passed through a column (20 × 2 cm) of Sephadex G25, previously equilibrated with extraction buffer. The fractions containing protein were pooled and diluted to 25 ml with extraction buffer.

Synthetase activity was measured by pyrophosphate exchange as described by Anderson and Fowden.[28] The reaction mixture contained ATP (2 μmol), ^{32}P-pyrophosphate (2 μmol), amino acid or amide (2 μmol), Tris–HCl, pH 7·5 (50 μmol), $MgCl_2$ (10 μmol), thioglycollate (12·5 μmol) and enzyme in a final vol. of 1 ml. Synthetase activity is expressed as the difference in pyrophosphate exchange in nmol/min between assays with and without added amino acid.

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