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REVIEW ARTICLE

*t*RNA AND AMINOACYL-*t*RNA SYNTHETASES FROM PLANTS

P. J. LEA and R. D. NORRIS

Department of Botany and Microbiology, University College, Gower Street, London WC1E 6BT

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INTRODUCTION

PREVIOUS reviews of protein synthesis in plants[1,2] have given little space to *t*RNA or aminoacyl-*t*RNA synthetases (amino acid activating enzymes, amino acid ligases), although they have been fully described for other systems.[3–8] Since the elucidation of the machinery of protein synthesis has only recently been attempted in plants, it is of interest to find out to what extent *t*RNA aminoacylation and metabolism resembles that in microorganisms and animals. Reference to microbial and animal systems is made where relevent.

Aminoacyl-*t*RNA synthetases (E.C.6.1.1.–) catalyse the initial step in protein synthesis by attaching specific amino acids to their cognate *t*RNA molecules (Scheme 1).

$$\text{Amino acid} + \text{ATP} + \text{Enzyme} \underset{}{\overset{Mg^{2+}}{\rightleftharpoons}} \text{Aminoacyl-AMP-Enzyme} + \text{PPi} \quad (1)$$

$$\text{Aminoacyl-AMP-Enzyme} + t\text{RNA} \xrightarrow{Mg^{2+}} \text{Aminoacyl-}t\text{RNA} + \text{Enzyme} + \text{AMP} \quad (2)$$

SCHEME 1. ATTACHMENT OF AMINO ACIDS TO TRANSFER RNAs.

To ensure strict fidelity in the translation of the genetic code, the overall reaction must exhibit exclusive specificity. Those plants which synthesize non-protein amino acids structurally akin to those found in proteins,[9] have evolved mechanisms for excluding these

[1] R. J. MANS, *Ann. Rev. Plant Physiol.* **18,** 127 (1967).
[2] D. BOULTER, *Ann. Rev. Plant Physiol.* **21,** 91 (1970).
[3] G. NOVELLI, *Ann. Rev. Biochem.* **36,** 449 (1967).
[4] M. STULBERG and G. NOVELLI, *Enzymes* **6,** 401 (1962).
[5] R. B. LOFTFIELD, in *Protein Synthesis* (edited by E. MCCONKEY), Vol. 1, p. 1, Marcel Dekker, New York (1971).
[6] A. H. MEHLER, *Prog. Nuc. Acid Res. Mol. Biol.* **10,** 1 (1970).
[7] P. J. PETERSON, *Biol. Revs.* **42,** 552 (1967).
[8] A. H. MEHLER and K. CHAKRABURTY, in *Advances in Enzymology* (edited by F. F. NORD and A. MEISTER), Vol. 35, p. 443, Interscience, New York (1971).
[9] L. FOWDEN, in *Progress in Phytochemistry* (edited by L. REINHOLD and Y. LIWSCHITZ), Vol. 2, p. 203, Wiley, New York (1970).

analogues from incorporation. Discrimination may occur at either step (1) or (2) in Scheme 1.[10–12]

Stable aminoacyl-AMP-enzyme complexes have been isolated,[13,14] but the rate of conversion into aminoacylated *t*RNA is slow compared with the turnover numbers for the total reaction.[15,16] A concerted reaction involving *t*RNA, amino acid and ATP has been proposed,[17] where by aminoacyl-*t*RNA's are formed without the production of discrete intermediates. Arguments concerning the reaction mechanism have been discussed.[5,6,18]

AMINOACYL-*t*RNA SYNTHETASES

The pyrophosphate exchange reaction (Scheme 1 reaction 1),[19–21] is very sensitive but exhibits a high endogenous activity with impure preparations due to the liberation of amino acids by proteolytic enzymes. This can be minimized by dilution of the extract,[22,23] which also serves to decrease ATP hydrolysis. Addition of fluoride to inhibit ATP-ases,[24,25] decreases the rate of exchange for some enzymes.[10,21] Pyrophosphate exchange due to enzymes other than aminoacyl-*t*RNA synthetases,[26–28] must be eliminated.

The *t*RNA esterification reaction,[29–32] depends on many variables and these should be carefully controlled.[33] It is subject to the action of nucleases and pyrophosphatases in impure preparations[32,34,35] and is more sensitive to salts,[36] bases[37] and organic solvents,[38] than is the pyrophosphate exchange reaction.

[10] P. J. PETERSON and L. FOWDEN, *Biochem. J.* **97,** 112 (1965).
[11] J. W. ANDERSON and L. FOWDEN, *Biochem. J.* **119,** 677 (1970).
[12] I. K. SMITH and L. FOWDEN, *Phytochem.* **7,** 1065 (1968).
[13] J. E. ALLENDE and C. C. ALLENDE, in *Methods in Enzymology* (edited by K. MOLDAVE and L. GROSSMAN), Vol. XX, p. 210, Academic Press, New York (1971).
[14] K. A. ALIEV and I. I. FILIPPOVICH, *Mol. Biol.* **2,** 364 (1968).
[15] M. YARUS and P. BERG, *J. Mol. Biol.* **42,** 171 (1969).
[16] D. CASSIO, *Europ. J. Biochem.* **4,** 222 (1968).
[17] R. B. LOFTFIELD and E. A. EIGNER, *J. Biol. Chem.* **244,** 1746 (1969).
[18] D. H. GAUSS, F. VON DER HAAR, A. MAELICKE and F. CRAMER, *Ann. Rev. Biochem.* **40,** 1045 (1971).
[19] M. STULBERG and G. NOVELLI, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. V, p. 703, Academic Press, New York (1962).
[20] P. BERG, *J. Biol. Chem.* **222,** 1025 (1956).
[21] J. W. DAVIS and G. D. NOVELLI, *Arch. Biochem. Biophys.* **75,** 299 (1958).
[22] E. MOUSTAFA and M. H. PROCTOR, *Biochim. Biophys. Acta* **63,** 93 (1962).
[23] J. W. ANDERSON and L. FOWDEN, *Plant Physiol.* **44,** 60 (1969).
[24] M. M. ATTWOOD and E. C. COCKING, *Biochem. J.* **96,** 616 (1965).
[25] A. B. LEGOCKI and J. PAWELKIEWICZ, *Acta Biochimica. Polonica* **XIV,** 313 (1967).
[26] L. FOWDEN, D. LEWIS and H. TRISTRAM, in *Advances in Enzymology* (edited by F. F. NORD), Vol. 29, p. 89, Interscience, New York (1967).
[27] W. P. JECKS, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. V, p. 467, Academic Press, New York (1962).
[28] P. BERG, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. V, p. 461, Academic Press, New York (1962).
[29] R. J. MANS and G. D. NOVELLI, *Arch. Biochem. Biophys.* **94,** 48 (1961).
[30] K. MOLDAVE, in *Methods in Enzymology* (edited by S. P. COLOWICK and N. O. KAPLAN), Vol. VI, p. 758, Academic Press, New York (1963).
[31] P. BERG and E. J. OFENGAND, *Proc. Natl. Acad. Sci. U.S.* **44,** 78 (1958).
[32] L. N. VANDERHOEF, R. F. BOHANNON and J. L. KEY, *Phytochem.* **9,** 2291 (1970).
[33] I. B. RUBIN, A. D. KELMERS and G. GOLDSTEIN, *Anal. Biochem.* **20,** 533 (1967).
[34] B. S. VOLD and P. SYPHERD, *Plant Physiol.* **43,** 1221 (1968).
[35] C. MITTERMAYER, R. BRAUN and H. P. RUSCH, *Biochim. Biophys. Acta* **114,** 27 (1966).
[36] E. GRIFFITHS and S. T. BAYLEY, *Biochemistry* **8,** 541 (1969).
[37] R. B. LOFTFIELD and E. A. EIGNER, *Biochemistry* **7,** 1100 (1968).
[38] P. O. RITTER, F. J. KULL and K. B. JACOBSON, *Biochim. Biophys. Acta* **179,** 524 (1969).

Assay of the enzymes involved by the hydroxamate method,[39,40] where the base serves as an aminoacyl acceptor in place of *t*RNA, has recently been discussed.[5] The technique is not suitable for the assay of the synthetases of dicarboxylic acids or their amides, as these amino acids can be converted into β-aspartyl and γ-glutamyl hydroxamates by reactions unrelated to protein synthesis.[41–43] It is possible that the high activities reported for these enzymes in crude tomato root extracts utilizing this method[24] were due to such reactions.

The *t*RNA esterification reaction is the best physiological assay, although the pyrophosphate exchange reaction is important mechanistically.

Isolation and Purification

General methods for the isolation of synthetases[44] and the extraction of enzymes from plant tissue[45,46] have been reviewed. Thioglycollate and metabisulphite increased the specific activity of synthetases in the extract of plumules and radicles from *Phaseolus vulgaris*[23] and tobacco leaf,[47] although the enzymes from the cotyledons of the former were unaffected. Glutathione,[48] mercaptoethanol and dithiothreitol[32] have also been used for a similar purpose. Sucrose did not affect the activity of the enzymes extracted from *Phaseolus vulgaris*.[23] However, individual enzymes may be protected by sucrose or other polyols,[44,49] which help to maintain the integrity of hydrogen bonding within the enzyme molecules. Increase in ATP-ase activity in the presence of sucrose[23] may affect the assay conditions used. Different buffers should be investigated for their effects on individual enzymes.[5,33] PVP,[45,50] EDTA[51] and specific amino acids[52,53] have also been used to supplement the extraction medium. The action of proteases[54,55] can be minimized by either high buffer/tissue ratios or mercaptoethanol.[56] Despite claims that extraction in the presence of liquid nitrogen and glycerol is essential for the extraction of synthetases from pea roots,[49] the enzymes have been successfully extracted from similar tissues in the absence of these materials.[23,24]

Levels of Aminoacyl-tRNA Synthetases in Tissues

Although considerable variation in the levels of individual aminoacyl-*t*RNA synthe-

[39] I. D. Raacke and J. Bové, *Experientia* **16**, 195 (1960).
[40] F. Lipman and L. C. Tuttle, *J. Biol. Chem.* **159**, 21 (1945).
[41] W. H. Elliott, *Biochem. J.* **49**, 106 (1951).
[42] B. N. Grossowicz, E. Wainfan, E. Borek and H. Waelsch, *J. Biol. Chem.* **187**, 111 (1950).
[43] S. Black and N. G. Wright, *J. Biol. Chem.* **213**, 27 (1955).
[44] K. H. Muench and P. Berg, in *Procedures in Nucleic Acid Research* (edited by G. L. Cantoni and D. R. Davis), p. 375, Harper & Row, New York (1966).
[45] W. D. Loomis and J. Battaile, *Phytochem.* **5**, 423 (1966).
[46] J. W. Anderson, *Phytochem.* **7**, 1973 (1968).
[47] J. W. Anderson and K. S. Rowan, *Biochem. J.* **101**, 9 (1966).
[48] P. J. Peterson, Ph.D. Thesis, University of London (1964).
[49] P. C. Scott and R. O. Morris, *Biochem. Biophys. Acta* **185**, 474 (1969).
[50] D. R. Hague and E. C. Kofoid, *Plant Physiol.* **48**, 305 (1971).
[51] L. Fowden and J. B. Frankton, *Phytochem.* **7**, 1077 (1968).
[52] G. Lemaire, M. Dorizzi and B. Labouesse, *Biochim. Biophys. Acta* **132**, 155 (1967).
[53] A. N. Baldwin and P. Berg, *J. Biol. Chem.* **241**, 831 (1966).
[54] L. Beevers, *Phytochem.* **7**, 1837 (1968).
[55] S. M. Hobday and D. A. Thurman, *J. Exptl Bot.* (1972), in press.
[56] D. M. Greenberg, in *Methods in Enzymology* (edited by S. P. Colowick and N. O. Kaplan), Vol. II, p. 54, Academic Press, New York (1955).

tases from different plant tissues has been observed,[11,23,24,47,57–66] a general pattern emerges. Ile-, Leu- and Val-synthetases exhibit high activity, whilst the synthetases for $AspNH_2$,Met,Phe,His,Pro,Ala,Try,Tyr,Cys and Gly are more variable. Arg-, Glu-, $GluNH_2$- and Asp-synthetases exhibit low activity. Comparison of the exchange and esterification reactions leads to the same general conclusions[62] although individual enzyme levels vary considerably. Those enzymes which form strong aminoacyl-adenylate complexes, esterify *t*RNA less efficiently.[14] Thus the total levels observed may be dependent to some extent on the assay method used. Dialysis (which preferentially inactivates some of the enzymes), differences in pH optima[57,62] and high K_m values for individual enzymes,[24,67] may account for some of the variability and low activities obtained.

The total level of aminoacyl synthetase activity in dry seeds is approximately proportional to the protein content.[68] Legumes, in particular soybean, have a high activity of pyrophosphate exchange per gram dry seed.[68] When extracts of any plant material are assayed for pyrophosphate exchange using a mixture of the 20 protein amino acids, the values obtained are lower than the sum of the activities determined when the amino acids are tested individually.[23,47,69,70]

Rates of aminoacyl-*t*RNA synthesis in intact bean leaves are similar to those in leaf discs, but cell-free preparations acylate significantly less *t*RNA.[70] The rate of aminoacylation was more rapid when the plants were grown in the light, although the total amount of charged *t*RNA formed was higher in dark-grown plants.[70]

Alanyl- and Glycyl-tRNA Synthetases

Ala-*t*RNA synthetase from tomato roots,[24] has a K_m for Ala of $2{\cdot}8 \times 10^{-2}$ M, similar to the enzyme from *Lactobacillus arabinosus*.[71] The plant enzyme was unable to activate β-alanine or α-aminobutyric acid, although D-alanine inhibited Ala-dependent pyrophosphate exchange. The fact that no purified Gly-*t*RNA synthetase and low Gly-dependent pyrophosphate exchange has been reported from plant extracts, may reflect an instability of the enzyme similar to that in animals and bacteria.[72,73]

Threonyl- and Seryl-tRNA Synthetases

Thr-*t*RNA synthetase has been partially purified from soybean hypocotyl[74] and *Aesculus hippocastanum* seed.[75] The latter enzyme has a K_m of 10^{-4} M and was insensitive to salts.

[57] G. C. WEBSTER, *Arch. Biochem. Biophys*, **82,** 125 (1959).
[58] G. C. WEBSTER, *J. Biol. Chem.* **229,** 535 (1957).
[59] J. M. CLARK, JR., *J. Biol. Chem.* **233,** 421 (1958).
[60] J. BOVÉ and I. D. RAACKE, *Arch. Biochem. Biophys.* **85,** 521 (1959).
[61] A. MARCUS, *J. Biol. Chem.* **234,** 1238 (1958).
[62] E. MOUSTAFA and J. LYTTLETON, *Biochim. Biophys. Acta* **68,** 45 (1963).
[63] J. D. HENSHALL and T. W. GOODWIN, *Phytochem.* **3,** 677 (1964).
[64] R. J. MANS, C. M. PURCELL and G. D. NOVELLI, *J. Biol. Chem.* **239,** 1762 (1964).
[65] W. HINDER, L. R. FINCH and S. CORY, *Phytochem.* **5,** 609 (1966).
[66] A. LEGOCKI and J. PAWELKIEWICZ, *Bull. L'Acad. Polon. Sci.* **15,** 435 (1967).
[67] P. J. LEA and L. FOWDEN, *Phytochem.* **11,** 2129 (1972).
[68] P. J. LEA and L. FOWDEN, unpublished results.
[69] R. D. NORRIS and L. FOWDEN, unpublished results.
[70] T. C. HALL and K. L. TAO, *Biochem. J.* **117,** 853 (1970).
[71] J. BADDILEY and F. C. NEUHAUS, *Biochem. J.* **75,** 579 (1960).
[72] J. BOKYO and M. J. FRASER, *Can. J. Biochem.* **42,** 1677 (1964).
[73] B. NIYOMPORN, J. DAHL and J. L. STROMINGER, *J. Biol. Chem.* **243,** 773 (1968).
[74] L. N. VANDERHOEF and J. L. KEY, *Plant Physiol.* **46,** 294 (1970).
[75] J. W. ANDERSON and L. FOWDEN, *Biochem. J.* **119,** 691 (1970).

Thr-aminoacylation was inhibited by borrelidine, using either the cytoplasmic or chloroplast enzyme from *Phaseolus vulgaris*.[76] Ser-*t*RNA synthetase has been partially purified from soybean hypocotyl.[32]

Isoleucyl-, Valyl- and Leucyl-tRNA Synthetases

Assay of these enzymes in impure preparations[22,25,47] may give ambiguous results, in accordance with the non-specific nature of the corresponding bacterial enzymes.[77,78] Ile serves as a substrate for the Leu-*t*RNA synthetase from *Aesculus hippocastanum*, although its affinity for the enzyme is 100-fold lower than Leu. Leu-dependent pyrophosphate exchange was inhibited by high concentrations of Ile, and although esterification of *t*RNA was not measured, the analogous situation in bacteria suggests that Ile would not bind to *t*RNA.[77] Although there appeared to be some competition of Leu-stimulated exchange in the presence of Ile, Met and Val, in enzyme preparations from Lupin seeds, these latter amino acids did not effect the aminoacylation of *t*RNA.[25] This may indicate that discrimination against the alternative substrates takes place at the level of the transfer reaction. α-(Methylenecyclopropyl) glycine stimulated pyrophosphate exchange with crude extracts from *Phaseolus aureus*,[48] although it was not activated by purer preparations of the Leu-*t*RNA synthetase from *Aesculus hippocastanum*.[75] This analogue, however, could be acting as a substrate for the Val-*t*RNA synthetase in the former case. Certain analogues of Leu with either a 4 C co-planar ring or with an unbranched carbon skeleton before the γC atom acted as substrates for the *Aesulus* enzyme.[75] Although norleucine acted as a substrate for this enzyme, it was not activated by the lupin enzyme,[25] probably because a crude extract was used with the analogue at non-saturating concentrations. Norvaline and several *N*-acetylderivatives were activated by the lupin enzyme,[25] although the pyrophosphate exchange exhibited by the latter was probably due to a contaminating deacetylase.[79] The MW of 170 000 for the lupin enzyme contrasts with the lower value of 105 000 for the *E. coli* Leu-*t*RNA synthetase.[80] The high sensitivity of the *Aesculus* enzyme to Cs^+ and K^+ and relative inertness of Na^+ on the pyrophosphate exchange,[75] parallels observations with the enzyme from *Pseudomonas aeruginosa*,[81] but contrasts with the situation in several other synthetases from plants, where monovalent cations are increasingly inhibitory to the exchange reaction with increasing charge/radius ratio.[69] The inhibitory action of NH_4^+ is also marked for the Leu-*t*RNA synthetase.[75] Multiple forms of Leu-*t*RNA synthetase have been reported in *Phaseolus vulgaris*[82,83] and soybeans[84,85] as in microbial systems.[86,87] Results must be interpreted with care because of differences in organelle specificity[76] and the formation of artifacts during chromatography.[88] Mixed substrate tests should be applied where multiple forms are suspected.[89,90]

[76] G. Burkard, P. Guillemaut and J. H. Weil, *Biochim. Biophys. Acta* **224,** 184 (1970).
[77] P. Berg, F. H. Bergmann, E. J. Ofengand and M. Dieckman, *J. Biol. Chem.* **236,** 1726 (1961).
[78] F. H. Bergman, P. Berg, and M. Dieckman, *J. Biol. Chem.* **236,** 1735 (1961).
[79] J. I. Harris, *Biochem. J.* **71,** 451 (1959).
[80] H. Hayashi, J. R. Knowles, J. R. Katze, J. Lapointe and D. Söll, *J. Biol. Chem.* **245,** 1401 (1970).
[81] Y. Kaziro, Y. Takahashi and N. Inoue, *J. Biochem. Tokyo* **64,** 181 (1968).
[82] G. R. Williams, *Abstr. 11th. Int. Bot. Cong. Seattle*, p. 239 (1969).
[83] G. R. Williams and A. S. Williams, *Biochem. Biophys. Res. Commun.* **39,** 858 (1970).
[84] J. Kanabus and J. H. Cherry, *Fed. Proc.* **29,** 3670 (1970).
[85] J. Kanabus and J. H. Cherry, *Plant Physiol.* **46A,** 30 (1970).
[86] C. T. Yu and H. P. Rappaport, *Biochim. Biophys. Acta* **123,** 134 (1966).
[87] B. L. Strehler, D. D. Hendley and G. P. Hirsch, *Proc. Natl. Acad. Sci. U.S.* **57,** 1751 (1967).
[88] U. Lagerkvist and J. Waldenström, *J. Biol. Chem.* **242,** 3021 (1967).
[89] T. P. Bennett, *J. Biol. Chem.* **244,** 3182 (1969).
[90] R. B. Loftfield, L. Hecht and E. A. Eigner, *Biochim. Biophys. Acta* **72,** 383 (1963).

Val-*t*RNA synthetase has been partially purified from wheat germ[91] and *Aesculus californica*.[75] As with the *E. coli* enzyme,[92] α-aminobutyric acid inhibits binding of Val to *t*RNA.[91] The Thr-stimulated pyrophosphate exchange by the Val-*t*RNA synthetase from bacteria[78] has not been observed with the plant enzymes. The enzyme from *Aesculus* activated α-(methylene cyclopropyl) glycine and α-(cyclobutyl)glycine. Norvaline stimulated exchange from lupin extracts and this could be due to contaminating Ile- or Val-*t*RNA synthetases, since this compound is activated by both such enzymes from bacteria.[90,93] The bacterial enzyme is sensitive to reagents coupling with SH groups,[25,91] as are the Ile- and Leu- enzymes from plants;[91,94] Mg^{2+} being required for this inhibition.[14] The Val-*t*RNA synthetase from bean chloroplasts is less sensitive to Mg^{2+}, spermidine or mercaptoethanol than is the cytoplasmic enzyme.[76]

The bacterial Ile-*t*RNA synthetase also activates Val, Leu and Met.[78] Since most preparations of this enzyme from plants have been contaminated with other synthetases, the exact position with regard to substrate specificity is not known.[23,47] The enzyme from lupin is protected against thermal denaturation by its cognate *t*RNA,[94] the reaction being Mg^{2+} dependent. No monovalent cation effects were observed on Ile-*t*RNA binding to the enzyme,[94] although they have been recorded elsewhere.[95,96]

Methionyl- and Cysteinyl-tRNA Synthetases

Met-*t*RNA synthetase from wheat germ,[97] catalyses an ethionine-dependent pyrophosphate exchange at a lower concentration than the corresponding bacterial enzyme.[98] It is sensitive to SH-coupling reagents and activated by mercaptoethanol. Partially purified enzymes have been used in the study of protein chain initiation in plants.[99,100]

Cys-*t*RNA synthetase from *Phaseolus aureus* appears to be relatively stable,[68,69] but there are no reports of a purified enzyme from plants.

Some plants are able to accumulate selenomethionine and selenocysteine in large quantities.[101] Non-accumulator plants cannot distinguish between the seleniferous and sulphur amino acid and the former can become incorporated into protein.[102] Accumulator-species, however, do not incorporate the Se analogues into protein[102] which may reflect a discriminatory role of the synthetases.[26]

Aminoacyl-tRNA Synthetases of Dicarboxylic Acids and their Amides

Glu-*t*RNA synthetase, purified 50-fold from *Phaseolus aureus*, *Hemerocallis fulva* and *Caesalpinia bonduc*, exhibits a high K_m (7×10^{-3} M) for the pyrophosphate exchange, which is not lowered by the addition of *t*RNA.[67] This contrasts with the enzyme from *E. coli*[103] and rat liver,[104] where the presence of *t*RNA lowers the K_m for this reaction. The

[91] E. MOUSTAFA, *Biochim. Biophys. Acta* **76,** 280 (1963).
[92] R. B. LOFTFIELD and E. A. EIGNER, *Fed. Proc.* **20,** 1 (1961).
[93] S. L. OWENS and F. E. BELL, *J. Mol. Biol.* **38,** 145 (1968).
[94] W. KEDZIERSKI and J. PAWELKIEWICZ, *Acta. Biochim. Polon.* **XVII,** 41 (1970).
[95] A. PETERKOFSKY, S. J. GEE and C. JESENSKY, *Biochemistry* **5,** 2789 (1966).
[96] D. W. E. SMITH, *J. Biol. Chem.* **244,** 896 (1969).
[97] E. MOUSTAFA, *Biochim. Biophys. Acta* **91,** 421 (1964).
[98] G. A. HAHN and J. W. BROWN, *Biochim. Biophys. Acta* **146,** 264 (1967).
[99] J. P. LEIS and E. B. KELLER, *Biochem. Biophys. Res. Commun.* **40,** 416 (1970).
[100] A. TARRAGO, O. MONASTERIO and J. E. ALLENDE, *Biochem. Biophys. Res. Commun.* **41,** 765 (1970).
[101] I. ROSENFIELD and O. A. BEATH, in *Selenium*, p. 91, Academic Press, New York (1964).
[102] P. J. PETERSON and G. W. BUTLER, *Nature, Lond.* **213,** 599 (1967).
[103] J. M. RAVEL, S. F. WANG, C. HERNEMEYER and W. SHIVE, *J. Biol. Chem.* **240,** 432 (1965).
[104] M. P. DEUTCHER, *J. Biol. Chem.* **242,** 1123 (1967).

plant enzyme appears to be very unstable.[67] Both *threo* and *erythro* forms of γ-methyl and γ-OH-L-glutamic acid as well as γ-OH-γ-methyl-L-glutamic acid are activated by the enzyme from *Phaseolus aureus*, a plant which does not contain any of these substituted glutamic acids in its soluble nitrogen pool. However, the enzyme from *Hemerocallis*, which produces *threo*-γ-OH-L-glutamic acid could only activate the *erythro*-L forms of these compounds. Conversely, the enzyme from *Caesalpinia bonduc*, which synthesizes erythro-γ-methyl-L-glutamic acid, could only activate the *threo*-L isomers. Thus a mechanism has been evolved in each case to exclude the naturally-occurring diastereoisomer from proteins.

The low activity observed for the $GluNH_2$-*t*RNA synthetase in the absence of *t*RNA in plants,[68,69] may reflect a vital requirement of *t*RNA for the assay of $GluNH_2$-*t*RNA synthetase by the ATP-PPi exchange method as has been shown in *E. coli*,[103] rat liver[104] and pig liver.[105] However, certain bacteria are able to amidate Glu attached to a specific $GluNH_2$ *t*RNA by means of a $GluNH_2$ Glu-*t*RNA synthetase and a $GluNH_2$ or $AspNH_2$ dependent amido transferase.[106] Which of these two systems operates in plants is as yet unknown.

Asp-*t*RNA synthetase from *Phaseolus aureus* activates α-amino malonic acid and *threo*-β-OH-L-aspartic acid by the exchange reaction, but not the *erythro* isomer.[68] $AspNH_2$–*t*RNA synthetase from *Phaseolus*, can utilize isoasparagine and aspartic acid hydroxamate as substrates in the pyrophosphate exchange in place of $AspNH_2$.[68]

Lysyl-, Histidinyl- and Tryptophanyl-tRNA Synthetases

Lys-*t*RNA synthetase from pea seed[107] and wheat germ[97] is unstable and inhibited by SH-coupling reagents, although in the former case, the presence of SH-groups does not lead to reactivation. The enzyme has been partially purified from *Vigna sinensis*[50] and *Delonix regia*.[69] The latter enzyme has a K_m of $1{\cdot}6 \times 10^{-4}$ M for Lys in the exchange reaction. *S*-(2-aminoethyl)-L-cysteine was activated with a K_m of $1{\cdot}2 \times 10^{-3}$ M, but its activity relative to that of Lys was relatively low compared to that observed with the *E. coli* enzyme.[108,109] The enzyme from *Canavalia ensiformis*, with a K_m for Lys of $7{\cdot}3 \times 10^{-5}$ M, apparently activated Arg and homoarginine at high concentrations.[51] This however was found not to be due to the presence of arginase in the extracts, which might produce ornithine, a potential substrate for the synthetase.

His-*t*RNA synthetase from *Delonix regia* has a K_m for His of $1{\cdot}4 \times 10^{-5}$ M[69] and is relatively stable. There is no report of a purified Try-*t*RNA synthetase from plants.

Arginyl-tRNA Synthetase

Arg-*t*RNA synthetase from the chloroplast and cystoplasm of *Phaseolus vulgaris* both activate canavanine, but only the latter exhibited inhibition of Arg-dependent pyrophosphate exchange on addition of the analogue.[76] The chloroplast enzyme was unable to activate Arg in the absence of *t*RNA,[76] as in *E. coli*,[110] but the cytoplasmic enzyme could

[105] L. W. LEE, J. M. RAVEL and W. SHIVE, *Arch. Biochem. Biophys.* **121,** 614 (1967).
[106] M. WILCOX and M. NIRENBERG, *Proc. Natl. Acad. Sci. U.S.* **61,** 229 (1968).
[107] E. MOUSTAFA, *Phytochem.* **5,** 1289 (1966).
[108] E. M. LANSFORD, N. M. LEE and W. SHIVE, *Arch. Biochem. Biophys.* **119,** 272 (1967).
[109] W. H. STERN and A. MEHLER, *Biochem. Z.* **342,** 400 (1965).
[110] A. H. MEHLER and S. K. MITRA, *J. Biol. Chem.* **242,** 5495 (1967).

promote exchange at 10% of the rate when *t*RNA was added. Different Arg-*t*RNA synthetases have also been observed during the development of *Chlamydomonas*[111] and tobacco tissue culture cells.[112] The unstable enzyme from *Canavalia ensiformis* did not activate either homoarginine or canavanine,[51] thus discriminating against the latter compound which is naturally synthesized by the plant. In this respect of substrate discrimination, it would be of interest to study the enzyme from *Indigofera spicata*, a plant producing the potential Arg analogue L-α-amino-ε-amidino caproic acid (indospicine).[113]

Phenylalanyl- and Tyrosyl-tRNA Synthetases

Phe-*t*RNA synthetase from *Phaseolus aureus*, *Leucaena leucocephala*[12] and several *Aesculus* sps.,[11] is less exacting in its amino acid substrate requirements than the Tyr-enzyme, in agreement with results obtained from bacteria.[114–116] Phe-*t*RNA synthetase from *Aesculus hippocastanum* supported pyrophosphate exchange with a variety of Phe analogues, most of which inhibited Phe-dependent exchange at high concentrations.[12] As observed with the bacterial enzyme, Ala molecules substituted on the β-C atom with a coplanar ring[115] or an unclosed coplanar ring[117] of suitable size, served as substrates for the *Aesculus* enzyme. The non-planar homoisoleucine molecule was not utilized.[11] *Aesculus californica*, unlike most *Aesculus* species, contains large amounts of the Phe-analogue, 2-methyl-4-amino-hex-4-enoic acid (AMHA) in its soluble nitrogen pool.[118] This compound is utilized as a substrate for the Phe-*t*RNA synthetase from all plants so far tested, although the enzyme from *A. californica* activates AMHA and similar analogues less efficiently than does the enzyme from non-analogue containing plants.[11] Although the esterification of *t*RNA by the analogue was not investigated, it was assumed that the discriminatory step occurred here, as no AMHA residues were found in *A. californica* protein.[11] Mimosine served as a substrate for the enzyme from mung bean and *Leuceana*, but was not transferred in either case.[12] The low specificity of the Phe-*t*RNA synthetase is reflected in the slight activation of Tyr by the mung bean enzyme[12] and competitive inhibition of the Phe-dependent pyrophosphate exchange by 1-aminophenylethane phosphonic acid.[119] Tyr-, Leu-, Val- and Thr-dependent exchange was unaffected by the corresponding phosphonic acid analogues.[119] Two distinct DEAE-cellulose fractions of Phe-*t*RNA synthetase from wheat seedling chloroplasts differed in MW and K^+ dependence,[120] although heterogeneity due to dissociation[121] could not be ruled out. Although there is a report of instability of Phe-*t*RNA synthetase,[122] the enzyme from most plants appears to be relatively stable.[12,69] Salts had a greater inhibitory effect on the exchange reaction catalyzed by the enzyme from *A. californica*[11] than on the enzyme from other species.[11,12]

[111] R. F. JONES and W. PENG, *Plant Physiol.* **45A,** 9 (1969).
[112] B. M. POLLOCK and P. FILNER, *Plant Physiol.* **46A,** 30 (1970).
[113] M. P. HEGARTY and W. POUND, *Nature, Lond.* **217,** 354 (1968).
[114] R. CALENDAR and P. BERG, *Fed. Proc.* **24,** 217 (1965).
[115] T. W. CONWAY, E. M. LANSFORD, JR. and W. SHIVE, *J. Biol. Chem.* **237,** 2850 (1962).
[116] J. M. RAVEL, M. N. WHITE and W. SHIVE, *Biochem. Biophys. Res. Commun.* **20,** 352 (1965).
[117] J. EDELSON, C. G. SKINNER, J. M. RAVEL and W. SHIVE, *J. Am. Chem. Soc.* **81,** 5150 (1959).
[118] L. FOWDEN and A. SMITH, *Phytochem.* **7,** 809 (1968).
[119] J. W. ANDERSON and L. FOWDEN, *Chem-Biol. Interact.* **2,** 53 (1970).
[120] G. A. LANZANI, L. A. MANZOCCHI, E. GALENTE and F. MENEGUS, *Enzymologia* **37,** 97 (1969).
[121] A. BÖCK, *Europ. J. Biochem.* **4,** 395 (1968).
[122] A. MARCUS, *J. Biol. Chem.* **245,** 955 (1970).

Tyr-tRNA synthetase from mung bean,[12] exhibits a similar substrate specificity to the enzyme from microbial sources,[114,116] although Phe activation was not observed.[123] *p*-Fluorophenylalanine served as a substrate for the enzyme, but neither *m*-tyrosine nor mimosine were activated.

Prolyl-tRNA Synthetase

Pro-tRNA synthetase has been isolated from numerous plant species.[10,48,124–126] Enzyme from plants whose growth is inhibited by azetidine-2-carboxylic acid (A2C), the lower homologue of Pro, activate[10,125] and transfer the analogue to tRNA. However, plants naturally containing A2C, fail to utilize it as a substrate in the absence of tRNA,[10,125] thus preventing the incorporation of the analogue molecule into protein. In the presence of tRNA, a low level of pyrophosphate exchange is observed in the presence of A2C with this type of enzyme,[69] but the analogue fails to bind to tRNA. Enzymes capable of utilizing A2C as a substrate, activate analogues smaller than Pro more efficiently than the enzyme from plants containing A2C. Conversely, larger analogues, such as *cis*-(exo)3,4-methanoproline, are more effectively utilized by the enzyme discriminating against A2C.[124] The maximum level of pyrophosphate exchange relative to Pro exhibited by these compounds never reaches the level observed with the bacterial or animal enzymes although the K_m's are similar.[127,128] Substrate protection constants[129] of Pro with the plant enzymes[130] are 10–15 times lower than that observed for the rat liver enzyme,[131] in agreement with reports of a stable aminoacyl-AMP complex formed with the enzyme from pea seedlings.[14] Instability, high salt sensitivity and high pH optima in the exchange reaction distinguish the enzymes from plants containing A2C from those of non-producer plants.[130]

Intracellular Localization of tRNA and Aminoacyl-tRNA Synthetases

ATP-dependent, amino acid-stimulated protein synthesis has often been observed in subcellular fractions.[132–140] Suitable fractionation and washing procedures for the preparation of intact organelles with a minimum of bacterial contamination, should be em-

[123] R. S. SCHWEET and E. H. ALLEN, *J. Biol. Chem.* **233,** 1104 (1958).
[124] L. FOWDEN, P. J. LEA and R. D. NORRIS, *Proc. Hung. Acad. Sci.* (1972), in press.
[125] P. J. PETERSON and L. FOWDEN, *Nature, Lond.* **200,** 148 (1963).
[126] P. DUNNILL, P. M. DUNNILL, A. BODDY, M. HOULDSWORTH and M. D. LILLY, *Biotechnol. Bioengng* **9,** 343 (1967).
[127] T. S. PAPAS and A. H. MEHLER, *J. Biol. Chem.* **245,** 1588 (1970).
[128] S. J. NORTON, *Arch. Biochem. Biophys.* **106,** 147 (1964).
[129] K. BURTON, *Biochem. J.* **48,** 458 (1951).
[130] R. D. NORRIS and L. FOWDEN, *Phytochem.* **11,** 2921 (1972).
[131] H. Y. K. CHUANG, A. G. ATHERLY and F. E. BELL, *Biochem. Biophys. Res. Commun.* **28,** 1013 (1967).
[132] M. L. STEPHENSON, K. V. THIMANN and P. C. ZAMECKNIK, *Arch. Biochem. Biophys.* **65,** 194 (1956).
[133] N. M. SISSAKIAN, in *Advances in Enzymology* (edited by F. F. NORD), Vol. 20, p. 201, Interscience, New York (1958).
[134] M. J. CHRISPEELS, A. E. VATTER, D. M. MADDEN and J. B. HANSON, *J. Exptl Bot.* **17,** 492 (1966).
[135] N. M. SISSAKIAN, I. I. FILIPPOVICH, E. N. SVETAILO and K. A. ALIYEV, *Biochim. Biophys. Acta* **95,** 474 (1965).
[136] A. A. APP and A. T. JAGENDORF, *Biochim. Biophys. Acta* **76,** 286 (1963).
[137] R. B. FRANCKI, N. K. BOARDMAN and S. G. WILDMAN, *Biochemistry* **4,** 865 (1965).
[138] N. K. BOARDMAN, R. B. FRANCKI and S. G. WILDMAN, *Biochemistry* **4,** 872 (1965).
[139] M. S. BAMJI and A. T. JAGENDORF, *Plant Physiol.* **41,** 764 (1965).
[140] J. M. EISENSTADT and G. BRAWERMAN, *J. Mol. Biol.* **10,** 392 (1964).

ployed.[46,141–151] Most aminoacyl-*t*RNA synthetase activity appears in the non-particulate fraction,[21,59,61,152] although organelle disruption might account for some of this.[153]

Chloroplasts. Tobacco chloroplasts retain enzymes for incorporating amino acids into proteins only when iso-osmotically extracted.[137] This may account for the apparently low levels of activity found in chloroplasts by some workers.[21,59,61,136] However, a high level of specific activity is often associated with the chloroplast synthetases.[60,63,154] In spinach chloroplasts, Tyr-, Met-, and Leu- stimulated pyrophosphate exchange in decreasing order of activity; whilst this order was reversed with the supernatant fraction.[60] The chloroplast synthetases are present in a soluble form,[137,155] and can be precipitated in the 40–70% ammonium sulphate fraction of the chloroplast proteins.[155] Washed grana exhibit no activity.[63] High RNA concentrations are associated with the chloroplast synthetases[135,156] The high acceptor activity of the chloroplast 'pH 5' fraction, apparently double that of the cytoplasmic fraction,[135] was due to a stronger binding of '*t*RNA' to the former.[154] Since the protein synthesizing machinery of the chloroplast differs from that of the cytoplasm,[140] distinctive *t*RNAs and synthetases might be expected. *Vicia faba* chloroplasts contain unique soluble RNAs (possibly *t*RNAs), distinguishable by MAK column profiles.[157] Specific chloroplast *t*RNAs have also been isolated from *Phaseolus vulgaris*.[158,159] Density gradient profiles of chloroplast synthetases indicated fewer components exhibiting synthetase activity than in the cytoplasm.[154] Properties of Val-, Arg- and Leu-*t*RNA synthetases from *Phaseolus vulgaris*[76] and Ile-*t*RNA synthetase from wheat seedlings,[149] differed according to whether they came from chloroplast or cytoplasm. Distinct Phe- and Ile-*t*RNA synthetases have also been recorded from *Euglena* chloroplasts.[160]

Nuclei. Nuclear amino acid incorporation into protein[132,161,162] and 'pH 5' fractions[59,163,164] have been reported from pea seedlings, spinach leaf and tobacco tissue culture. Although only traces of synthetase activity could be found in pea leaf and

[141] M. HALLAWAY, in *Plant Cell Organelles* (edited by J. B. PRIDHAM), p. 1, Academic Press, New York (1968).
[142] E. C. COCKING, in *Plant Cell Organelles* (edited by J. B. PRIDHAM), p. 198, Academic Press, New York (1968).
[143] J. T. LETT, W. N. TAKAHASHI and M. BIRNSTIEL, *Biochim. Biophys. Acta* **76,** 105 (1963).
[144] C. M. WILSON, *Plant Physiol.* **41,** 325 (1966).
[145] F. PARENTI and M. M. MARGULIES, *Plant Physiol.* **42,** 1179 (1967).
[146] A. A. APP and A. T. JAGENDORF, *Plant Physiol.* **39,** 772 (1964).
[147] P. LADO and M. SCHWENDIMANN, *Ital. J. Biochem.* **15,** 279 (1966).
[148] R. BAXTER and J. B. HANSON, *Planta* **82,** 246 (1968).
[149] C. V. GIVAN and R. M. LEECH, *Biol. Revs.* **46,** 409 (1971).
[150] R. M. LEECH, in *Biochemistry of the Chloroplasts* (edited by T. W. GOODWIN), Vol. I, p. 65, Academic Press, New York (1966).
[151] T. C. HALL and E. C. COCKING, *Plant Cell Physiol. Tokyo* **7,** 343 (1966).
[152] I. BOETTGER, *Wiss. Z. Martin-Luther Univ. Halle-Wittenberg Math-Nat Reche.* **10,** 1119 (1961): *Chem. Abs* **59,** 10473e (1963).
[153] E. GALANTE, G. A. LANZANI and F. MENEGAS, *Ital. J. Biochem.* **16,** 239 (1968).
[154] K. A. ALIEV, I. I. FILIPPOVICH and N. M. SISAKIAN, *Mol. Biol.* **1,** 240 (1967).
[155] S. S. MELIK-SARKISYAN, V. P. GONCHAROV and N. M. SISAKIAN, *Biokhimiya* **30,** 183 (1965).
[156] N. M. SISSAKIAN and S. S. MELIK-SARKISYAN, *Biokhimiya* **21,** 329 (1956).
[157] T. A. DYER and R. M. LEECH, *Biochem. J.* **106,** 689 (1968).
[158] J. H. WEIL, *I.U.B.* Symposium, Leeds (July, 1972).
[159] G. BURKHERD, J. P. VAULTIER and J. H. WEIL, *Phytochem.* **11,** 1351 (1972).
[160] W. E. BARNETT, C. J. PENNINGTON, JR. and S. A. FAIRFIELD, *Proc. Natl. Acad. Sci.* **63,** 1261 (1969).
[161] M. L. BIRNSTEIL, M. I. H. CHIPCHASE and R. J. HAYES, *Biochim. Biophys. Acta* **55,** 728 (1962).
[162] W. G. FLAMM, M. L. BIRNSTEIL and P. FILNER, *Biochim. Biophys. Acta* **76,** 110 (1963).
[163] M. L. BIRNSTEIL and B. B. HYDE, *J. Cell Biol.* **18,** 41 (1963).
[164] M. I. H. CHIPCHASE and M. L. BIRNSTEIL, *Proc. Natl. Acad. Sci. U.S.* **49,** 692 (1963).

pea embryo nuclei,[63] *t*RNA from pea seedling nuclei could be aminoacylated by cytoplasmic and nuclear 'pH 5' fractions.[164] *t*RNA was absent from *Vicia faba* root nucleoli but was present in the extranucleolar chromatin.[165]

Mitochondria. The incorporation of amino acids into protein in soybean[134] and pea seedling[137] mitochondria and observations of pyrophosphate exchange in mung bean[48] and carrot root[21] mitochondria, which parallels observations with rat liver mitochondria, contrasts with reports where little activity has been detected.[60,63,166] However, bacterial contamination could not always be ruled out. Sterile mitochondrial cell-free systems from castor bean endosperm and *Vigna sinensis* were dependent on ATP.[147] Mitochondrial pyrophosphate exchange in carrot root was lost on sonication.[21] Specific mitochondrial *t*RNAs have been isolated from *Phaseolus vulgaris*[158] and a possible mitochondrial Phe-*t*RNA synthetase has been recorded from Euglena.[167] Two of the 6 species of Leu*t*RNA from soybean mitochondria appear to be more predominant than the corresponding cytoplasmic species.[168]

Protein bodies and ribosomes. Proteoplasts from wheat endosperm appear to incorporate protein[169] and contain 'pH5' enzyme and 'soluble RNA'.[170–172] Although there is no evidence for different enzymes or *t*RNAs, the hypothesis was put forward that the enzymes associated with proteoplasts incorporate amino acids into storage protein whilst their corresponding cytoplasmic counterparts incorporated amino acids into cytoplasmic proteins. Evidence from maize[144] and *Vicia faba*[173] protein bodies indicates that most of this incorporation is due to bacterial contamination. Pyrophosphate exchange associated with ribosomal particles, which is enhanced by sonication or salt disruption,[58,59,63] may reflect contamination, as purer preparations exhibit a lower activity.[59]

Aminoacyl-tRNA Synthetases During Development

The specific activity of aminoacyl-*t*RNA synthetases from pea cotyledons was found to increase 8-fold during the first 3 days of germination.[63] The low levels of activity during the first few hours of germination and inhibition of this subsequent rise in specific activity by inhibitors of protein synthesis, was thought to reflect *de novo* synthesis of these enzymes.[63] However, the specific activity of aminoacyl-*t*RNA synthetases from French bean cotyledons was high in the dry seed, and remained constant over the first 6 days of germination.[23] This discrepancy may be partially due to the use of different assay methods, but since most seeds, including pea seeds[48,68] contain high levels of synthetases, the former results with pea cotyledons[63] are difficult to explain. The absolute activity of synthetases from French bean plumules and radicles, increased exponentially over the first 6 days of germination, whilst the specific activity increased during the first 3 days, then gradually decreased.[23] The specific activity of AspNH_2-, His-, Pro-, Cys-, Ile-, Leu-, Phe-, Val- and Tyr-*t*RNA synthetases increased in the plumule during the first 3 days of germination, whilst the activity of the Glu, GluNH_2 and Arg-specific enzymes decreased. The radicle showed a

[165] P. S. Woods and M. Zubay, *Proc. Natl. Acad. Sci. U.S.* **54,** 1705 (1965)
[166] V. M. Craddock and M. V. Simpson, *Biochem. J.* **74,** 10P (1960).
[167] B. J. Reger, S. A. Fairfield, J. L. Epler and W. E. Barnett, *Proc. Natl. Acad. Sci. U.S.* **67,** 1207 (1970).
[168] M. B. Anderson and J. H. Cherry, *Proc. Natl. Acad. Sci. U.S.* **62,** 202 (1969).
[169] R. K. Morton and J. K. Raison, *Biochem. J.* **91,** 528 (1964).
[170] R. K. Morton and J. K. Raison, *Nature, Lond.* **200,** 429 (1963).
[171] R. K. Morton, B. A. Palk and J. K. Raison, *Biochem. J.* **91,** 522 (1964).
[172] R. K. Morton, J. K. Raison and J. R. Smeaton, *Biochem. J.* **91,** 539 (1964).
[173] C. T. Wheeler and D. Boulter, *Biochem. J.* **100,** 53P (1966).

similar pattern of activity, but in addition, increases in Asp-, Ser-, and Gly-*t*RNA synthetases were observed.[23] The absolute activity of aminoacyl-*t*RNA synthetases from tobacco leaf decreased during senescence, but the specific activity did not decrease until the chlorophyll content fell below a certain level.[174]

Kinetin-treated tobacco leaf discs exhibited increased levels of synthetase activity compared with water controls,[174] but this effect was too slow to account for the delay in chlorophyll breakdown and increase in soluble *N* content.[174] The increased levels of aminoacyl-*t*RNA synthetases concomittant with increased amino acid levels observed in pea seedlings,[63] were not observed with senescing tobacco leaves.[174] In rat liver the control of aminoacyl-*t*RNA synthetases may depend on the protein level of the tissue and amino acid pool sizes,[175] but no definite information is available for higher plants. The 'pH 5' fraction from gibberellic acid-treated pea seedlings was 5–8 times more active than that from untreated plants.[63] Cytokinins,[168] red light,[63] or virus infection,[176] have little effect on the level of the enzymes.

Exponentially dividing tobacco cells contain a different Arg-*t*RNA synthetase to that found in non-dividing cells;[112] a situation similar to that in *Chlamydomonas*, where the gametes contain a different Arg-enzyme to that occurring in the vegetative cell.[111] Light induces the chloroplast Ile-*t*RNA synthetase from *Euglena gracilis*, the enzyme being absent from dark-grown organisms and bleached mutants.[167] However, the chloroplast Phe-*t*RNA synthetase from this organism, is non-inducible and coded by nuclear genes.[167]

Extraction and Purification of tRNA from Plants

Apart from a brief discussion by Loening,[177] reviews on extraction and purification of *t*RNA have concentrated on microbial systems.[18,178–180] The method used should remove protein, carbohydrates and other nucleic acids, yet minimize damage to *t*RNA from physical shock and degradative enzymes.

Phenol extraction of nucleic acids from fresh tissue[32] should employ low buffer volumes, but for seed tissue, equal volumes of aqueous extract and phenol are necessary.[68,69,181] Exposure time to the phenol should be minimized to prevent nuclease action,[176,182] but with large-scale preparations from lupin[181] and wheat germ,[183] longer extraction times are more convenient. Since phenol does not completely inhibit the action of ribonuclease,[184–186] bentonite has been used to adsorb these enzymes.[187–191] However, bentonite does not

[174] J. W. ANDERSON and K. S. ROWAN, *Biochem. J.* **101**, 15 (1966).
[175] A. MARIANI, M. A. SPADONI and G. TOMASSI, *Nature, Lond.* **199**, 378 (1963).
[176] Y. HAYASHI, *Virology* **18**, 140 (1962).
[177] U. E. LOENING, *Ann. Rev. Plant Physiol.* **19**, 37 (1968).
[178] K. S. KIRBY, *Prog. Nucleic Acid Res.* **3**, 1 (1964).
[179] A. S. SPIRIN, in *Macromolecular Structure of Ribonucleic Acids*, Rheinhold, New York (1964).
[180] R. W. HOLLEY, in *Methods in Enzymology* (edited by L. GROSSMAN and K. MOLDAVE), Vol. XII, p. 588, Academic Press, New York (1967).
[181] A. B. LEGOCKI, A. SZYMKOWIAK, K. PECH and J. PAWELKIEWICZ, *Acta Biochim. Polon.* **XIV**, 323 (1967).
[182] R. BRAUN, C. MITTERMAYER and H. P. RUSCH, *Biochim. Biophys. Acta* **114**, 527 (1966).
[183] B. S. DUDOCK, G. KATZ, E. K. TAYLOR and R. W. HOLLEY, *Proc. Natl. Acad. Sci. U.S.* **62**, 941 (1969).
[184] R. K. RALPH and A. R. BELLAMY, *Biochim. Biophys. Acta* **87**, 9 (1964).
[185] B. KICHÖFEN and M. BURGER, *Biochim. Biophys. Acta* **65**, 190 (1962).
[186] G. W. RUSHIZKY, A. E. GRECO, R. WHATLEY, JR. and H. A. SOBER, *Biochem. Biophys. Res. Commun.* **10**, 311 (1963).
[187] T. J. BROWNHILL, A. S. JONES and M. STACEY, *Biochem. J.* **73**, 434 (1959).
[188] H. L. SANGER and C. A. KNIGHT, *Biochem. Biophys. Res. Commun.* **13**, 455 (1963).
[189] J. H. CHERRY and H. CHROBOZEK, *Phytochem.* **5**, 411 (1965).
[190] R. E. CLICK and D. P. HACKETT, *Biochim. Biophys. Acta* **129**, 74 (1966).
[191] A. LEGOCKI and K. WOJCIECHOWSKA, *Bull. L'Acad. Polon. Sci.* **XVIII**, 7 (1970).

increase the yields of nucleic acids from woody tissue,[192] and adsorbs only 50% of ribonuclease A from wheat leaf cytoplasm.[193] Increased yields of nucleic acids are obtained by the use of SDS,[189,194,195] but this is due to disruption of organelles,[196] dissociation of nucleic acids from membranes and proteins[197] and inhibition of nucleases.[198] The use of other detergents has been discussed,[176] and extraction of nucleic acids with detergents alone reported.[199,200] Since Mg^{2+} causes RNA to aggregate, it should be omitted from the extraction medium or chelated with EDTA. Zn^{2+},[201] Cu^{2+},[151] or 8-hydroxyquinoline,[32,202] may be used to inhibit ribonucleases. Disadvantages of the phenol extraction include the need for multiple extractions, the formation of emulsions and loss of RNA at the interface.[200] High UV absorbance due to phenol is removed with Sephadex[188] and pigments with chloroform.[203]

Diethyl pyrocarbonate (DEP)[204] inhibits ribonuclease[205] and other enzymes[206,207] by reacting with Try[208] and His[209] residues in the protein without affecting RNA or DNA.[205] RNA from bean and tobacco leaves extracted with DEP or phenol, showed similar sedimentation profiles and levels of protein contamination.[196] Although increased yields of RNA were observed with the DEP method, yields of *t*RNA were lower.[210] However, DEP has been used in the extraction of *t*RNA from Jerusalem artichoke.[211] DEP promotes the ring opening of adenine[212] and loss of amino acid acceptor ability[213–215] although the presence of protein[213] or immediate removal of DEP from the preparation after extraction,[216] will minimize these effects.

Carbohydrates are removed by treatment with 2-methoxyethanol[178,181] or DEAE-cellulose,[32,76,183,217] although the former does not completely remove nucleoside phosphates and polyphosphates which can be subsequently eliminated by cetyltrimethylammoniumbromide precipitation of *t*RNA.[184,218] Similar quaternary ammonium bases do not affect the acceptor properties of the *t*RNA.[219]

[192] P. H. Li and C. J. Weiser, *Plant Cell Physiol.* **10,** 21 (1969).
[193] D. Hadziyev, S. L. Mehta and S. Zalik, *Can. J. Biochem.* **47,** 273 (1969).
[194] J. Ingle, J. L. Key and R. E. Holm, *J. Mol. Biol.* **11,** 730 (1965).
[195] T. Itoh and T. Hirai, *Ann. Phytopathol. Soc. Japan* **32,** 227 (1966).
[196] F. Solymosy, I. Fedorcsák, A. Gulyás, G. L. Farkas and L. Ehrenberg, *Europ. J. Biochem.* **5,** 520 (1968).
[197] L. V. Gusta and C. J. Weiser, *Phytochem.* **10,** 1733 (1971).
[198] H. Noll and E. Stutz, in *Methods in Enzymology* (edited by L. Grossman and K. Moldave), Vol. XIIB, p. 129, Academic Press, New York (1967).
[199] D. H. Rammler, T. Okabayashi and A. Delk, *Biochemistry* **4,** 1994 (1965).
[200] H. I. Robbins and I. D. Raacke, *Biochem. Biophys. Res. Commun.* **33,** 240 (1968).
[201] G. R. Barker and R. M. Riebe, *Biochem. J.* **105,** 1195 (1967).
[202] K. S. Kirby, *Biochim. Biophys. Acta* **55,** 382 (1962).
[203] A. S. Spirin, *Biokhimiya* **26,** 511 (1961).
[204] T. Boehm and D. Metha, *Ber. Dtsch. Chem. Ges.* **71,** 1797 (1938).
[205] I. Fedorcsák and L. Ehrenberg, *Acta Chem. Scand.* **20,** 107 (1966).
[206] L. Hullan, T. Szontagh, I. Turtóczky and I. Fedorcsák, *Acta Chem. Scand.* **19,** 2440 (1965).
[207] L. A. Pradel and R. Kassoh, *Biochim. Biophys. Acta* **167,** 317 (1968).
[208] C. G. Rosén and I. Fedorcsák, *Biochim. Biophys. Acta* **130,** 401 (1966).
[209] W. B. Melchior and D. Fahrney, *Biochemistry* **9,** 251 (1970).
[210] G. Lázár, I. Fedorcsák and F. Solymosy, *Phytochem.* **8,** 2353 (1969).
[211] G. Jeannin, D. Mellet and A. Kovoor, *J. Exptl. Bot.* (1972), in press.
[212] N. J. Leonard, J. J. McDonald and M. E. Reichmann, *Proc. Natl. Acad. Sci. U.S.* **67,** 93 (1970).
[213] M. Deníc, L. Ehrenberg, I. Fedorcsák and F. Solomosy, *Acta Chem. Scand.* **24,** 3753 (1970).
[214] B. J. Ortwerth, *Biochim. Biophys. Acta* **246,** 344 (1971).
[215] B. Oberg, *Europ. J. Biochem.* **19,** 496 (1971).
[216] P. N. Abadom and D. Elson, *Biochim. Biophys. Acta* **199,** 528 (1970).
[217] R. Monier, M. Stephenson and P. C. Zamecnik, *Biochim. Biophys. Acta* **43,** 1 (1960).
[218] G. N. Zaitseva, Wang T'ing-Chao, A. P. Kalyuzhnaya and A. N. Belozerskii, *Biokhimiya* **29,** 986 (1964).
[219] J. H. Weil and J. P. Ebel, *Biochim. Biophys. Acta* **55,** 836 (1962).

Separation of soluble RNA from high molecular weight (HMW) RNA by high salt concentrations,[220] is best performed with 3M salt to minimize contamination by HMW-RNA.[32,168,221,222] Effective separation on DEAE-cellulose[32,181,221] and MAK columns[189,192,194,223] has been achieved, although loss of acceptor ability by complex formation with methylated albumin, should be avoided.[177,224] Sephadex G200,[191,225,226] and G75,[227] Zn^{2+},[228] ammonium sulphate fractionation,[229] treatment with dimethyl sulphoxide[230] density gradient centrifugation[231] and gel electrophoresis,[232] have been used to separate HMW–RNA from *t*RNA but have not found wide use with plant material.

Countercurrent distribution,[233,234] MAK,[235,236] methylated albumin silicic acid,[237,238] protaminated kieselguhr[191,239] and DEAE-cellulose columns at normal[240] and elevated[241] temperatures, have been used to separate individual *t*RNAs. Hydroxyapatite resolved 4 Ile-, 2 Val- and 3 Leu-*t*RNAs from lupin seeds[234,239] and benzoylated DEAE-cellulose[242] has been used to separate *t*RNAs from wheat germ[99,100,183] and pea root.[243] Reverse-phase chromatography,[244,245] has been successfully employed in the fractionation of isoaccepting *t*RNAs from various plant species.[181,243,246,247] Other separation techniques have, as yet, not been applied to plant *t*RNAs.[248–252]

[220] A. M. CRESTFIELD, K. S. SMITH and F. W. ALLEN, *J. Biol. Chem.* **216,** 185 (1955).
[221] D. G. GLITZ and C. A. DEKKER, *Biochemistry* **2,** 1185 (1963).
[222] K. S. KIRBY, *Biochem. J.* **96,** 266 (1965).
[223] J. D. MANDELL and A. D. HERSHEY, *Anal. Biochem.* **1,** 66 (1960).
[224] H. GOLDIN and I. I. KAISER, *Biochem. Biophys. Res. Commun.* **36,** 1013 (1969).
[225] A. B. LEGOCKI, K. WOJCIECHOWSKA and K. PECH, *Bull. L'Acad. Polon. Sci.* **XVIII,** 63 (1970).
[226] M. REYNIER, M. AUBERT and R. MONIER, *Bull. Soc. Chim. Biol.* **49,** 1205 (1967).
[227] T. A. MCCOY and E. A. CARTER, *J. Chromatogr.* **37,** 458 (1968).
[228] B. K. RAJ and M. S. N. RAO, *Biochemistry* **8,** 1277 (1969).
[229] S. AVITAL and D. ELSON, *Biochim. Biophys. Acta* **179,** 279 (1969).
[230] S. GUTCH, *Biochim. Biophys. Acta* **157,** 76 (1968).
[231] W. GILBERT, *J. Mol. Biol.* **6,** 389 (1963).
[232] U. E. LOENING, *Biochem. J.* **102,** 251 (1967).
[233] B. P. DOCTOR, in *Methods in Enzymology* (edited by L. GROSSMAN and K. MOLDAVE), Vol. XIIA, p. 644, Academic Press, New York (1967).
[234] A. B. LEGOCKI, A. SZYMKOWIAK, M. HIEROWSKI and J. PAWELKIEWICZ, *Acta Biochim. Polon.* **15,** 197 (1968).
[235] A. PETERKOFSKY, *Proc. Natl. Acad. Sci. U.S.* **52,** 1233 (1964).
[236] N. SUEOKA and T. YAMANE, in *Methods in Enzymology* (edited by L. GROSSMAN and K. MOLDAVE), Vol. XIIA, p. 658, Academic Press, New York (1967).
[237] T. OKAMOTO and Y. KAWADEY, *Biochem. Biophys. Res. Commun.* **13,** 324 (1963).
[238] R. STERN and U. Z. LITTAUER, *Biochemistry* **7,** 3469 (1968).
[239] A. B. LEGOCKI and J. PAWELKIEWICZ, *Bull. L'Acad. Polon. Sci.* **XV,** 517 (1967).
[240] R. M. BÖCK and J. D. CHERAYIL, in *Methods in Enzymology* (edited by L. GROSSMAN and K. MOLDAVE), Vol. XIIA, p. 638, Academic Press, New York (1967).
[241] P. L. BERQVIST, B. C. BAGULEY and R. K. RALPH, in *Methods in Enzymology* (edited by L. GROSSMAN and K. MOLDAVE), Vol. XIIA, p. 638, Academic Press, New York (1967).
[242] I. GILLAM, S. MILLWARD, D. BLEW, M. VON TIGERSTROM, E. WIMMER and G. M. TENER, *Biochemistry* **6,** 3034 (1967).
[243] R. O. MORRIS and D. F. BABCOCK, *Plant Physiol.* **46A,** 30 (1970).
[244] A. D. KELMERS, G. D. NOVELLI and M. P. STULBERG, *J. Biol. Chem.* **240,** 3979 (1965).
[245] J. F. WEISS, R. L. PEARSON and A. D. KELMERS, *Biochemistry* **7,** 3479 (1968).
[246] G. BURKARD, P. GUILLEMAUT and J. H. WEIL, *Compt. Rend. Soc. Biol.* **163,** 2731 (1969).
[247] J. H. CHERRY and D. J. OSBORNE, *Biochem. Biophys. Res. Commun.* **40,** 763 (1970).
[248] K. MUENCH and P. BERG, *Biochemistry* **5,** 970 (1966).
[249] A. H. MEHLER and A. BANK, *J. Biol. Chem.* **238,** 2888 (1963).
[250] E. KATCHALSKI, S. YANOFSKY, A. NOVOGRODSKY, Y. GALENTER and U. Z. LITTAUER, *Biochim. Biophys. Acta* **123,** 641 (1966).
[251] G. L. BROWN and G. ZUBAY, *J. Mol. Biol.* **2,** 287 (1960).
[252] G. ZUBAY, *J. Mol. Biol.* **4,** 347 (1962).

Multiplicity of t*RNAs*

The existence of multiple iso-accepting *t*RNAs specific for a single amino acid appears not to show a uniform correlation with the degeneracy of the code.[253] MAK profiles of *t*RNA from wheat seedlings indicated the presence of multiple forms of *t*RNA specific for Arg, Glu, Ser, Val and Lys. The 6 species of Leu*t*RNA from soybean hypocotyl corresponds with the number of Leu-codons,[168] but pea cotyledons contain smaller proportions of Leu$tRNA_{1,4,5,6}$ and little Leu$tRNA_3$.[247] *Phaseolus vulgaris* contains a number of Leu-specific *t*RNAs in both the chloroplasts and mitochondria, and some of these are also found in the cytoplasm.[76,158] Differences in the levels of iso-accepting Val*t*RNA and Leu*t*RNA molecules have also been observed in etioplasts and chloroplasts of *Phaseolus*.[159] Two Met-specific *t*RNAs, as well as a distinct initiator Met*t*RNA, have been isolated from wheat germ.[99,100] Multiple iso-accepting *t*RNAs for Leu, Pro, Thr, Ser, Tyr, Val, Ile, Lys and Arg have also been reported from various plant sources.[50,74,76,112,239,254] Every peak of *t*RNA separated by column chromatography does not necessarily represent a distinct species. Spurious peaks may arise by ribonuclease action,[25,74] slipping of hydrogen bonds, thus altering the tertiary structure,[74,255,256] aggregations of *t*RNA,[50,257–259] changes in base methylation,[260,261] or impurities in the labelled amino acids.[74]

Interaction of t*RNA with Aminoacyl-*t*RNA Synthetases*

Complexes of synthetases with specific *t*RNAs have often been reported in microbial and animal systems.[262–268] Ile-*t*RNA synthetase from lupin is protected against heat denaturation by its cognate *t*RNA.[94] Leu*t*RNA protected this Ile enzyme to a lesser extent, whilst the Leu- and Lys-synthetases were not protected by their respective *t*RNAs. The Mg-dependence of this protection may reflect a conformational change in the *t*RNA or enzyme.[94,269] In contrast to the bacterial synthetases,[270] aminoacylation was unaffected by monovalent cations.[94] The ability of pea seedling synthetases to form aminoacyl-AMP-enzyme complexes, decreased in the order Pro, Arg, Ser, Thr, Val. Whilst the transfer of Arg and Ser to *t*RNA was efficient, Pro aminoacylation was poor.[14] Spermine and spermidine can replace Mg^{2+} in the esterification reaction,[76] as in the enzymes from *E. coli*,[271,272] but the exact role of polyamines is not known.

[253] K. H. Muench and P. A. Safille, *Biochemistry* **7**, 2799 (1968).
[254] M. D. Bick, H. Liebke, J. H. Cherry and B. L. Strehler, *Biochim. Biophys. Acta* **204**, 175 (1970).
[255] B. S. Vold, *Biochim. Biophys. Acta* **182**, 585 (1969).
[256] A. Adams, T. Lindahl and J. R. Fresco, *Proc. Natl. Acad. Sci. U.S.* **57**, 1684 (1967).
[257] J. S. Loehr and E. B. Keller, *Proc. Natl. Acad. Sci.* **61**, 1115 (1968).
[258] H. G. Zachau, *Europ. J. Biochem.* **5**, 559 (1968).
[259] D. Söll, J. D. Cherayil and R. M. Bock, *J. Mol. Biol.* **291**, 97 (1967).
[260] A. Peterkofsky, C. Jesensky and J. D. Capra, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 501 (1966).
[261] J. D. Capra and A. Peterkofsky, *J. Mol. Biol.* **33**, 591 (1968).
[262] U. Lagerkvist, L. Rymo and J. Waldenström, *J. Biol. Chem.* **241**, 5391 (1966).
[263] W. Seifert, G. Nass and W. Zilling, *J. Mol. Biol.* **33**, 507 (1968).
[264] T. Okamoto and Y. Kawade, *Biochim. Biophys. Acta* **145**, 613 (1967).
[265] M. Yarus and P. Berg, *J. Mol. Biol.* **28**, 479 (1967).
[266] O. D. Nelidova, and L. L. Kiselev, *Mol. Biol.* **2**, 60 (1968).
[267] E. C. Preddie, *J. Biol. Chem.* **244**, 3969 (1969).
[268] Y. Takeda, *Biochim. Biophys. Acta* **182**, 258 (1969).
[269] J. R. Fresco, A. Adams, R. Ascione, D. Henley and T. Lindahl, *Cold Spring Harbour Symp. Quant. Biol.* **31**, 527 (1966).
[270] J. Svenson, *Biochim. Biophys. Acta* **146**, 239 (1967).
[271] K. Igarishi and Y. Takeda, *Biochim. Biophys. Acta* **213**, 240 (1970).
[272] K. Igarishi, K. Matsuzaki and Y. Takeda, *Biochim. Biophys. Acta* **254**, 91 (1971).

Homologous and Heterologous Aminoacylation and Species Specificity

The specificity of *t*RNAs and synthetases in a heterologous system is most strict when the organisms belong to widely different taxonomic groups.[273–276] Care must be exercised in drawing conclusions from heterologous systems which superficially appear not to cross-react to a great extent, as very often, the reaction as measured by K_ms is just as efficient.[277]

Pea seed *t*RNA was not esterified to any extent by the Ala-*t*RNA synthetase from pig liver.[278] In contrast to this, a rat liver 'pH5' fraction was able to substitute for spinach chloroplast 'pH5' fraction.[135,136] A higher level of aminoacylation was observed for the lupin homologous system than for heterologous systems in which *E. coli* provided the source of either *t*RNA or synthetase.[191] With maize enzyme and *E. coli* *t*RNAs for Ser, Glu, Val, Asp, Lys, Leu and Phe, the heterologous system was shown to be 0–50% as active as the homologous, although it was more active than the homologous system when Arg-, Ile- and Ala-*t*RNAs from *E. coli* were used with maize enzyme.[279] Phe-*t*RNA synthetases, and *t*RNAs from peas, yeast and algae exhibited little difference in cross-specificity, but the Met-enzyme and Met*t*RNA from peas and algae only slightly reacted with yeast *t*RNA or enzyme.[280] Pea seed and wheat germ homologous system esterified Lys*t*RNA more rapidly than than did the heterologous system.[62] Homologous and heterologous combinations of half molecules of Phe*t*RNA from wheat germ, accepted Phe using the yeast synthetase, and charging of the heterologous combination occurred even when several base pairs in the 3′ and 5′ ends and in the stem of the *t*RNA were changed.[281]

Multiplicity of *t*RNA or synthetase species, or different levels of *t*RNA in different parts of the plant might account for apparently low values for the esterification reaction in unfractionated extracts.[10,167,247] Due to the presence of two Lys-*t*RNA synthetases in peas, incorporation of Lys into pea *t*RNA, using the enzyme from yeast or algae, was only 50% of that observed with the opposite combination.[280] Leu-*t*RNA synthetase from soybean hypocotyl, only charges Leu$tRNA_{1-4}$, whereas enzyme from the cotyledon also charged Leu$tRNA_{5,6}$.[168] Differences in the leucyl binding of soybean or pea *t*RNA with homologous or heterologous enzyme were due to differences in the Leu*t*RNA species present.[247] Multiple Leu-*t*RNA synthetases from soybean and *Phaseolus vulgaris* have a clear specificity amongst the iso-accpeting *t*RNAs.[82,84,85]

*t*RNA and synthetases from the cytoplasm of pea seedlings cross-reacted equally well with those from the nuclei,[164] in contrast to the exclusive specificity reported for pig liver cytoplasmic and nuclear fractions.[278] Aminoacylation of chloroplast or cytoplasmic *t*RNA was most rapid if the synthetase and *t*RNA were from the same subcellular compartment,[14,154,273] the heterologous reaction being 65% of that of the homologous. The reaction between chloroplast enzyme and cytoplasmic *t*RNA was incomplete for Arg, Pro and Ser but not for Val and Thr.[14] With the opposite combination, aminoacylation of *t*RNA by Arg and Val was reduced. Phe*t*RNA and Ile*t*RNA from *Euglena* chloroplasts were only acylated by the chloroplast synthetases.[167] Similarly, 3 Leu*t*RNA's from *Phaseolus*

[273] R. N. GLEBOV, G. N. ZAITSEVA and A. N. BELOZERSKII, *Biokhimiya* **30,** 586 (1965).
[274] S. BENZER and B. WEISBLUM, *Proc. Natl. Acad. Sci. U.S.* **47,** 1149 (1961).
[275] R. RENDI and S. OCHOA, *J. Biol. Chem.* **273,** 3711 (1962).
[276] K. B. JACOBSON, *Proc. Nucleic Acid Res. Mol. Biol.* **11,** 461 (1971).
[277] R. B. LOFTFIELD and E. A. EIGNER, *Acta Chem. Scand.* **17,** S 117 (1963).
[278] G. C. WEBSTER, *Biochem. Biophys. Res. Commun.* **2,** 56 (1960).
[279] K. B. JACOBSON, S. NISHIMURA, W. E. BARNETT, R. J. MANS, P. CAMARANO and G. C. NOVELLI, *Biochim. Biophys. Acta* **91,** 305 (1964).
[280] T. M. ERMOKHINA and M. STAMBOLOVA, *Dokl. Akad. Nauk. SSSR* **164,** 688 (1965).
[281] R. THIEBE and H. G. ZACHAU, *Biochem. Biophys. Res. Commun.* **36,** 1024 (1969).

vulgaris chloroplasts could only be charged by the chloroplast enzyme.[76] However, the three Val-specific *t*RNAs from the cytoplasm could be charged by enzyme from the chloroplast or cytoplasm.[76]

Structure and Base Composition of tRNA

The sequence of 32 *t*RNA species from microorganisms is known,[282] each with a base composition varying between 75 and 85 nucleotides and a minor base content of 10–20%. Tertiary structures have been proposed by several workers.[283–285] The wheat germ Phe*t*RNA sequence is almost identical to that of yeast,[183,286] only 16 of the 76 nucleotides differing, with all but 2 of these being located in double stranded regions. The dihydrouracil loop and stem are exactly the same as in the yeast *t*RNA but the 'Y base' and positions of some of the minor bases differ.[183] Thirteen out of a possible 16 chain-terminal dinucleotide sequences were found in wheat germ soluble RNAs,[287] with an average of one alkali-stable dinucletoide sequence/RNA chain. Alkaline hydrolysis of wheat germ ribonucleates yielded 1 nucleoside/90 nucleotides, adenosine being the most frequently released nucleoside.[288] This is consistent with its position at the 3′ terminus of *t*RNA.[221,289] Variations in the total base composition of 'soluble RNA' from plants have been reported.[190,221,290] No common trends were observed in the terminal nucleotide composition in *Mimosa* epicotyl with increasing age.[291] Modified nucleotides in *t*RNA[292] occur in definite locations in the primary sequence, giving rise to changes in hydrogen-bonding or covalent bonding characteristics which determine the 3-dimensional configuration of the molecule. Methylated bases have been isolated from wheat germ,[293,294] tobacco leaf[295,296] and beet[295] RNA. The faster chromatographic mobility of maize *t*RNAs compared with mouse liver and *Neurospora* *t*RNAs may be due to base methylation.[279]

t*RNA*–m*RNA*–*Ribosome Interaction*

The general pattern of protein synthesis on the ribosome is universal. A complex is formed between an iniator *t*RNA, *m*RNA and the smaller ribosomal subunit, which is dependent on GTP and supernatant factors. The initiator *t*RNA occupies the peptide site whilst non-initiator *t*RNAs bind at the aminoacyl site on the complete ribosome. GTP, factor-dependent transfer of aminoacyl *t*RNA from the aminoacyl site to the peptide site then occurs, the amino acids going into peptide link whilst the deacylated *t*RNA dissociates from the ribosome.

The coding properties of wheat germ *t*RNA coincide with those reported for *E. coli*[297] although small discrepancies could justify the assignment of 3 Val codons in the former

[282] Symposium of the Societé de Chimie Biologique, Strasbourg Dec. 1971.
[283] F. Cramer, H. Doepner, F. von der Haar and E. Schlimme, *J. Cell. Physiol.* **74** (Suppl 1), 163 (1969).
[284] M. Levitt, *Nature, Lond.* **224**, 759 (1969).
[285] P. G. Connors, M. Labanavskas and W. W. Beeman, *Science* **166**, 1528 (1969).
[286] B. S. Dudock, G. Katz, E. K. Taylor and R. W. Holley, *Fed. Proc.* **27**, 342 (1968).
[287] L. Hudson, M. Gray and B. G. Lane, *Biochemistry* **4**, 2009 (1965).
[288] B. G. Lane and F. W. Allen, *Biochim. Biophys. Acta* **47**, 36 (1961).
[289] R. F. Bohannon and J. L. Key, *Plant Physiol.* **45A**, 8 (1968).
[290] R. C. Huang and J. Bonner, *Proc. Natl. Acad. Sci. U.S.* **54**, 960 (1965).
[291] G. N. Brown, *Plant Physiol.* **44**, 272 (1969).
[292] N. Suekoa and T. Kano-Seuoka, *Proc. Nucleic Acid Res. Mol. Biol.* **10**, 23 (1970).
[293] J. W. Littlefield and D. B. Dunn, *Biochem. J.* **70**, 642 (1958).
[294] D. B. Dunn and J. D. Smith, *Trans. Faraday Soc.* **55**, 490 (1959).
[295] J. D. Smith and D. B. Dunn, *Biochem. J.* **72**, 294 (1959).
[296] P. L. Berquist and R. E. F. Matthews, *Biochem. J.* **85**, 305 (1962).
[297] C. Basilio, M. Bravo and J. E. Allende, *J. Biol. Chem.* **241**, 1917 (1966).

case. Some poly-U-directed Leu incorporation was detected,[297] thus conforming with the idea of ambiguity in the code. Poly-I, poly-C and poly-A could not code for their respective amino acids[297] in accordance with the absence of binding of rat liver Pro*t*RNA to wheat germ ribosomes in the presence of poly-C.[298] However, poly-C appeared to enhance incorporation of Pro into peptides on tomato seedling ribosomes.[299] Absence of binding of synthetic polynucleotides may reflect the presence of endogeneous messenger.

Phe*t*RNA binding factors have been reported from *Vicia faba*,[300] wheat germ,[301–305] rice embryo,[306] tobacco and pea seedling chloroplasts[140,307] and pea seedlings.[308] The absence of requirements for supernatant factors and GTP for Phe*t*RNA binding to wheat germ ribosomes,[297] may reflect contamination of the preparations with the factors.[306] Although, in the absence of poly-U, GTP had no effect on Phe*t*RNA binding to washed wheat germ ribosomes, DOC-treated ribosomes required GTP, and a supernatant fraction which could be replaced by *E. coli* supernatant.[309] This indicated that the messenger is bound but is not able to be translated. Kinetic data indicate the presence of either 2 subfractions or a co-operative effect of the binding factor on poly-U-stimulated poly-Phe synthesis with wheat germ ribosomes. The factor-ribosome complex increased GTP hydrolysis independently of *t*RNA[301,305] in contrast to the situation in the rabbit reticulocyte system.[310]

The 2 major Met-specific *t*RNAs from wheat embryo,[100,304] exhibit different coding properties.[304] Met*t*RNA$_1$ from wheat resembles Met*t*RNA$_f$ from *E. coli* in that it can interact with the homologous binding enzyme and fails to complex with the heterologous (bacterial) enzyme. Met*t*RNA$_2$ from wheat, however, can form a ternary complex with the enzyme from both organisms.[100,304] The higher non-enzymatic binding affinity of Met-*t*RNA$_1$ to wheat germ ribosomes at low Mg^{2+} concentrations in the presence of ApUpG, may confer on this species of *t*RNA, initiator properties.[100] One of the 3 Met-specific *t*RNAs from wheat germ could be formylated with an *E. coli* transformylase[311] or a transformylase from wheat germ.[99] The presence of Met*t*RNA$_f$ in chloroplasts from *Euglena*,[312] *Phaseolus vulgaris*[313] and wheat leaves,[314] reflects the bacterial-like protein synthesis machinery of these organelles. *N*-Acetyl- and formyl-Phe*t*RNA from yeast could bind to wheat germ ribosomes as well as efficiently as Phe*t*RNA at high Mg^{2+} concentrations,[309] but *N*-blocked, deaminated or denatured *t*RNAs could not bind to the aminoacyl

[298] A. MARCUS and J. FEELEY, *Proc. Natl. Acad. Sci. U.S.* **51**, 1075 (1964).
[299] T. C. HALL and E. C. COCKING, *Biochim. Biophys. Acta* **123**, 163 (1966).
[300] A. YARWOOD, E. S. PAYNE, J. N. YARWOOD and D. BOULTER, *Phytochem.* **10**, 2305 (1971).
[301] A. B. LEGOCKI and A. MARCUS, *J. Biol. Chem.* **245**, 2814 (1970).
[302] C. JEREZ. A, SANDOVAL, J. E. ALLENDE, C. HENES and J. OFFENGAND, *Biochemistry* **8**, 3006 (1969).
[303] M. GATICA, A. TARRAGO, T. M. OJEDA and J. E. ALLENDE, quoted in Ref. 100.
[304] J. E. ALLENDE, in *Techniques in Protein Synthesis* (edited by P. N. CAMPBELL and J. R. SARGENT), Vol. 2, p. 55, Academic Press, New York (1969).
[305] A. B. LEGOCKI and A. MARCUS, *Plant Physiol.* **46A**, 17 (1970).
[306] A. A. APP, *Plant Physiol.* **44**, 1132 (1969).
[307] I. SELA and P. KAESBERG, *J. Virol.* **3**, 89 (1969).
[308] C. Y. LIN and J. L. KEY, *Plant Physiol.* **46A**, 29 (1970).
[309] N. DE GROOT, Y. KAUFMANN and I. SHAFRIR, *Biochem. Biophys. Res. Commun.* **26**, 691 (1967).
[310] W. MCKEEHAN, P. SEPULVEDA, S. Y. LIN and B. HARDESTY, *Biochem. Biophys. Res. Commun.* **34**, 668 (1969).
[311] J. P. LEIS and E. B. KELLER, *Fed. Proc.* **29**, 468 (1970).
[312] J. H. SCHWARTZ, R. MEYER, J. M. EISENSTADT and G. BRAWERMAN, *J. Mol. Biol.* **25**, 571 (1967).
[313] G. BURKARD, B. ECLANCHER and J. H. WEIL. *FEBS Letters* **4**, 285 (1969).
[314] J. P. LEIS and E. B. KELLER, *Proc. Natl. Acad. Sci. U.S.* **67**, 1593 (1970).

site at lower concentrations of Mg^{2+}.[302] Ca^{2+} was unable to replace Mg^{2+} completely in the *t*RNA binding to ribosomes.[315] The minimum structure of *t*RNA for ribosomal binding has been little studied in plants, but in microbial systems, binding is inhibited or altered by acetylation,[316] deletion of the 'Y base',[317] modification of bases,[318] iodination of N^6-(Δ^2-isopentenyl)adenosine (2iPA)[319] or ribonuclease treatment.[320] In every case the aminoacylation function was not impaired, indicating that the correct 3-dimensional configuration around the codon end is required for ribosomal binding.

Cytokinins and t*RNA*

Many cytokinins have been isolated from plant sources[321] and from *t*RNA hydrolyzates by chromatography on LH-20[322–325] or celite.[322,323,326] Their diversity may reflect the presence of multiple *t*RNAs distributed amongst the various subcellular organelles.[322,327] The quantities of cytokinin ribonucleosides in *t*RNA is too small for cytokinin moieties to occur in all *t*RNA molecules.[328,329] Where cytokinins have been localized, they are found adjacent to the 3′ end of the anticodon,[328,330–332] occurring in the sequence A-2iPA-A;[321,328,333] in a position analogous to that of the 'Y base' of yeast and wheat germ Phe*t*RNA.[183,334] 2iPA and *cis*. ribosyl zeatin occur together in wheat germ,[335] pea root[336] and spinach *t*RNA although only the latter compound could be isolated from immature corn kernel *t*RNA.[336] In addition, wheat germ *t*RNA contains the 2-methylthio-derivatives of these two compounds.[324,325] Cytokinins have also been isolated from bacterial and yeast *t*RNA.[322,323,328,337,338] In *E. coli*, cytokinin activity is associated with *t*RNAs having

[315] W. J. McCarthy, A. A. App and W. J. Crotty, *Plant Physiol.* **46A,** 17 (1970).
[316] L. E. Stefanovich, *Mol. Biol.* **3,** 672 (1969).
[317] R. Thiebe and H. G. Zachau, *Europ. J. Biochem.* **5,** 546 (1968).
[318] M. L. Gefter and R. L. Russell, *J. Mol. Biol.* **39,** 145 (1969).
[319] F. Fittler and R. H. Hall, *Biochem. Biophys. Res. Commun.* **25,** 441 (1969).
[320] S. Nishimura and G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* **53,** 178 (1965).
[321] F. Skoog and D. J. Armstrong, *Ann. Rev. Plant Physiol.* **21,** 359 (1970).
[322] W. J. Burrows, D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard and J. Occolowitz, *Science* **161,** 691 (1968).
[323] W. J. Burrows, D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard and J. Occolowitz, *Biochemistry* **8,** 3071 (1969).
[324] W. J. Burrows, D. J. Armstrong, M. Kaminek, F. Skoog, S. M. Hecht, J. T. A. Boyle, L. G. Dammann, N. J. Leonard and J. Occolowitz, *Biochemistry* **9,** 1867 (1970).
[325] S. M. Hecht, N. J. Leonard, W. J. Burrows, D. J. Armstrong, F. Skoog and J. Occolowitz, *Science* **166,** 1272 (1969).
[326] R. H. Hall, *Biochemistry* **4,** 661 (1965).
[327] R. H. Hall, *Prog. Nucleic Acid Res. Mol. Biol.* **10,** 57 (1970).
[328] H. G. Zachau, D. Dütting and H. Feldmann, *Angew. Chem.* **78,** 392 (1966).
[329] M. J. Robins and R. Thedford, *Biochemistry* **6,** 1837 (1967).
[330] B. G. Barrell and F. Sanger, *FEBS Letters* **3,** 275 (1969).
[331] J. T. Madison and H.-K. Kung, *J. Biol. Chem.* **242,** 1324 (1967).
[332] D. J. Armstrong, W. J. Burrows, F. Skoog, K. L. Roy and D. Söll. *Proc. Natl. Acad. Sci. U.S.* **63,** 834 (1969).
[333] H. M. Goodman, J. B. Abelson, A. Landy, S. Brenner and J. D. Smith, *Nature, Lond.* **217,** 1019 (1968).
[334] U. L. Rajbhandary, S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson and H. G. Khorana, *Proc. Natl. Acad. Sci. U.S.* **57,** 751 (1967).
[335] W. J. Burrows, D. J. Armstrong and F. Skoog. *Plant Physiol.* **44A,** 1 (1969).
[336] R. H. Hall, L. Csonka, H. David and B. McLennan, *Science* **156,** 69 (1967).
[337] K. Biemann, S. Tsunakawa, J. Sonnenbichler, H. Feldmann, D. Dutting and H. G. Zachau, *Angew. Chem.* **78,** 600 (1966).
[338] S. M. Hecht, N. J. Leonard, J. Occolowitz, W. H. Burrows, D. J. Armstrong, F. Skoog, R. M. Bock, I. Gillam and G. M. Tener, *Biochem. Biophys. Res. Commun.* **35,** 205 (1969).

U as the first base of the codon. [381,332] However, not all codons beginning with U exhibit cytokinin activity.[339,340]

Labelled benzylaminopurine supplied to cytokinin-requiring soybean and tobacco tissue culture was recovered as the nucleotide in the *t*RNA[341,342] and zeatin could be formed from 2iPA.[343] Randomization of label was greatest when the purine ring was labelled and the 9-substituted derivatives were particularly labile.[342] Although this apparently complete base substitution into *t*RNA led to speculations concerning cytokinin control of *t*RNA synthesis,[342,344] several pieces of evidence refute this. Masking of the 9-position of 6-benzylaminopurine inhibited incorporation into *t*RNA but had no effect on cytokinin activity.[345] Substituted bases are not incorporated *per se*, the substituent groups being added to preformed polynucleotide chains. The absence of cytokinin requiring *E. coli* mutants[345] and presence of the *trans* isomer of zeatin in ethanolic extracts of corn kernel (*t*RNA contains the *cis* isomer)[346] also suggests that cytokinins are not precursors of the modified bases in *t*RNA. Reversible, cytokinin-induced bud formation in moss protonema[347] also suggests that cytokinins do not exert their functional role through incorporation into *t*RNA, but act through some rapidly reversible process.

Mevalonic acid is a precursor of 2iPA in *t*RNA from tobacco callus tissue,[348] and as in microorganisms, acetate is the original precursor.[349] The 2iPA side chain is formed by modification of the preformed *t*RNA, permanganate-treated *t*RNA being able to act as a substrate, whilst iodinated *t*RNA could not.[350] No competitive effect of 2iPA on mevalonic acid incorporation was noted.[348] Cytokinins may be breakdown products of *t*RNA, but since their level in some tissues exceeds that which could be derived from *t*RNA, other routes are necessary. Tobacco pith cells degrade 2iPA into hypoxanthin, inosine and other compounds, some of which exhibit cytokinin activity.[351] Feedback mechanisms for controlling the availability of mevalonate, involving *t*RNA, have been proposed[327] but no evidence from plants is available.

Biosynthesis of t*RNA*

'Soluble' RNA is richer in methylated bases than other types of RNA.[296] The nucleolus is the centre of synthesis and methylation of *t*RNA in pea seedlings[352] in accordance with results from mammalian sources.[353,354] In animals, nuclear turnover of pseudouridine

[339] F. SKOOG, D. J. ARMSTRONG, J. D. CHERAYIL, A. E. HAMPEL, and R. M. BOCK, *Science* **154,** 1354 (1966).
[340] D. J. ARMSTRONG, F. SKOOG, L. H. KIRKEGAARD, A. E. HAMPEL, R. M. BOCK, I. GILLAM and G. M. TENER, *Proc. Natl. Acad. Sci. U.S.* **63,** 504 (1969).
[341] J. E. FOX, *Plant Physiol.* **41,** 75 (1966).
[342] J. E. FOX and C. M. CHEN, *J. Biol. Chem.* **242,** 4490 (1970).
[343] G. A. MIURA and C. O. MILLER, *Plant Physiol.* **44,** 372 (1969).
[344] J. E. FOX and C. M. CHEN, in *Biochemistry and Physiology of Plant Growth Substances* (edited by F. WHITMAN and G. SETTERFIELD), p. 777, Runge, Ottawa (1968).
[345] H. KENDE and J. E. TAVARES, *Plant Physiol.* **43,** 1244 (1968).
[346] D. S. LETHAM, J. S. SHANNON and I. R. MACDONALD, *Proc. Chem. Soc.* p. 230 (1964).
[347] H. BRANDES and H. KENDE, *Plant Physiol.* **43,** 827 (1968).
[348] C. M. CHEN and R. H. HALL, *Phytochem.* **8,** 1687 (1969).
[349] F. FITTLER, L. K. KLINE and R. H. HALL, *Biochemistry* **7,** 940 (1968).
[350] L. K. KLINE, F. FITTLER and R. H. HALL, *Biochemistry* **8,** 4361 (1969).
[351] C. M. CHEN, D. M. LOGAN, B. D. MCCLELLAN and R. H. HALL, *Plant Physiol.* **43A,** 18 (1968).
[352] M. L. BIRNSTEIL, J. L. SIRLIN and J. JACOBS, *Biochem. J.* **94,** 10P (1965).
[353] D. J. COMB and S. KATZ, *J. Mol. Biol.* **8,** 790 (1964).
[354] N. OZBAN, C. J. TANDLER and J. L. SIRLIN, *J. Emb. Ex. Morph.* **12,** 373 (1964).

(ψ) and incorporation of 5-methyl cytosine and ψ into *t*RNA has been observed,[353,355–357] the ψ-labelled *t*RNA being chased into the cytoplasm.[358] In *Vicia faba* meristems, pulse-labelled *t*RNA appeared rapidly in the cytoplasm.[165] Much of the label was probably due to the addition of the CCA end to the *t*RNA molecules.[359] This reaction, catalysed by CTP, ATP-terminal nucleotidyl transferase, adds C,C then A, sequentially to the incomplete *t*RNA molecule at the 3′ terminus.[360,361] In pea root and soybean, 85–90% of the molecules of *t*RNA have an intact 3′ terminus.[32] Little is known about *t*RNA transcription in plants. Hybridization of chloroplast DNA with chloroplast *t*RNA indicated that there was sufficient genetic material present to code for 20–30 *t*RNA molecules.[362] However, since iso-accepting *t*RNA species exist in the chloroplast,[159] this might represent the lower limit.[362] Hybridization of bean leaf Leu*t*RNA with DNA from the chloroplast, was better than that observed with nuclear DNA.[83,363] New *t*RNA synthesis has been demonstrated in enucleated *Acetabularia*[364] and tobacco leaf chloroplasts.[365]

In bacteria, *S*-adenosyl methionine serves as a methyl group donor at the polynucleotide level for the methylation of bases in *t*RNA,[366,367] and Cys serves as a precursor for the 2-methylthio derivatives.[368,369] A high reciprocal specificity between methylating enzymes and *t*RNA was found for spinach and various mammalian sources[370] and wheat germ and *E. coli*.[371] RNA from a given source, whilst fully methylated with respect to its homologous enzyme, could offer new methylation sites for the enzyme from a different species.[372] Methylation of *E. coli* *t*RNA with enzyme from wheat embryo produced N^2-dimethylguanosine ($m_2{}^2$G) in the sequence G–C–$m_2{}^2$G–C.[371] With homologous (wheat) and heterologous (wheat and *E. coli*) systems, methylation of bases exceeded sugar methylation.[371] With wheat enzyme and *t*RNA, pyrimidine nucleosides were the primary targets for methylation, whilst the mixed system favoured methylation of purine nucleosides. The heterologous system was more extensively methylated than the homologous.[371] Met could not completely reverse the inhibition of methylation of sugar beet *t*RNA by ethionine.[373] Methyl-deficient *t*RNA species have a higher ambiguity in recognizing the correct *m*RNA codon.[374] The ability to charge Leu*t*RNA from sugar beet is correlated with the degree of *in vivo* methylation of the molecule,[373] in agreement with results from bacteria.[375]

[355] J. L. SIRLIN, K. KATO and K. W. JONES, *Biochim. Biophys. Acta* **48,** 421 (1961).
[356] S. OSAWA, *Biochim. Biophys. Acta* **42,** 244 (1960).
[357] M. SLUYSER and L. BOSCH, *Biochim. Biophys. Acta* **55,** 479 (1962).
[358] J. JACOB and J. L. SIRLIN, *Biochem. J.* **84,** 88P (1962).
[359] J. PREIS, M. DIECKMANN and P. BERG, *J. Biol. Chem.* **236,** 1748 (1961).
[360] L. I. HECHT, P. C. ZAMECKNIC, M. L. STEPHENSON and J. F. SCOTT, *J. Biol. Chem.* **233,** 954 (1968).
[361] H. L. SÄNGER and C. A. KNIGHT, *Biochem. Biophys. Res. Commun.* **13,** 455 (1963).
[362] K. K. TEWARI and S. G. WILDMAN, *Symp. Soc. Exptl. Biol.* **24,** 147 (1970).
[363] G. R. WILLIAMS, *Plant Physiol.* **46A,** 30 (1970).
[364] H. G. SCHWEIGER, W. L. DILLARD, A. GIBOR and S. BERGER, *Protoplasma* **64,** 1 (1967).
[365] K. K. TEWARI and S. G. WILDMAN, *Biochim. Biophys. Acta* **186,** 358 (1969).
[366] L. R. MANDEL and E. FLEISSNER, *Fed. Proc.* **21,** 379 (1962).
[367] L. R. MANDEL and E. BOREK, *Biochem. Biophys. Res. Commun.* **4,** 14 (1961).
[368] M. L. GEFTER, *Biochem. Biophys. Res. Commun.* **36,** 435 (1969).
[369] R. S. HAYWARD and S. B. WEISS, *Proc. Natl. Acad. Sci. U.S.* **55,** 1161 (1966).
[370] E. WAINFAN and B. LANDSBERG, *FEBS Letters* **19,** 144 (1971).
[371] J. L. NICHOLS and B. G. LANE, *Can. J. Biochem.* **47,** 863 (1969).
[372] P. R. SRINIVASAN and L. BOREK, *Proc. Natl. Acad. Sci. U.S.* **49,** 529 (1963).
[373] B. P. STONE, C. D. WHITTY and J. H. CHERRY, *Plant Physiol.* **45,** 636 (1970).
[374] U. Z. LITTAUER, M. REVEL and R. STERN, *Cold Spring Harbor Symp. Quant. Biol.* **31,** 501 (1966).
[375] L. S. SHUGART, B. H. CHASTAIN, G. D. NOVELLI and M. P. STULBERG, *Biochem. Biophys. Res. Commun.* **31,** 404 (1968).

Role of t*RNA in Development*

Several theories have been forwarded for *t*RNA-mediated control of protein synthesis and differentiation.[292] Differences in levels or species of *t*RNA during development or in certain physiological states of the organism have often been demonstrated.[376–379] The level of *t*RNA in *Pinus resinosa*, which is low in the dormant embryo, increases slightly on germination.[380] Similar results were obtained with *Mimosa* epicotyl.[291] *t*RNA was lost from wheat embryos within 10–15 hr of germination, but the level was restored in 20 hr by rapid resynthesis.[34] Cold hardening of apple twigs increased levels of soluble RNA, whilst dehardening led to a reduction in the total level.[381] Leaves from pea seedlings contain relatively more *s*RNA than the roots or stem.[190] Auxins generally bring about an increase in RNAs including *t*RNA.[382] Cytokinin activity is most abundant near the tip of the radicle of pea seedlings, where the *t*RNA concentration is highest.[383]

Modification of several species of *t*RNA was observed in differentiating wheat seedlings,[34,384] differences in Pro-, Lys- and Ser-*t*RNAs being especially noticeable. This could be due to a change in one degenerate species or the formation of a new species. Similar results were recorded for benzyladenine-treated lupin embryos,[191] but aminoacylation of *t*RNA in different parts of the seed was maintained at the same level except for an increase in the acceptor activity of Ala*t*RNA and one species of Ile*t*RNA in the epicotyl. Kinetin, IAA and 2*i*PA had no effect on the levels of *t*RNA, although kinetin-treated embryos exhibited a higher ψ/U ratio for extracted *t*RNA.[225] 6-benzyladenine-treated soybean seedlings exhibited increased aminoacylation of Leu*t*RNA$_{5,6}$ and decreased acylation of Leu*t*RNA$_1$ in the hypocotyl, compared with control plants, whilst the cotyledons contained more Leu*t*RNA$_{5,6}$.[247] However, cytokinins had no effect on Tyr*t*RNA.[254] Leu*t*RNA$_{5,6}$ may arise by modification of pre-existing species, experiments with ethionine-treated excised cotyledons indicating that differences in methylation might be involved.[385] However, cycloheximide, 6-methylpurine or ethionine did not decrease the relative amount of Leu*t*RNA$_{5,6}$ extracted, suggesting that Leu*t*RNA$_{1,4}$ are preferentially destroyed,[386] in agreement with the results of Bick and Strehler.[387]

*t*RNA from mature cells of soybean, acylated more Ser and Leu per unit *t*RNA than did *t*RNA from dividing cells.[289] Slight increases in Lys-, Phe-, Tyr- and Met-acylation were also observed. No quantitative differences in levels of iso-accepting *t*RNAs were observed in dividing or non-dividing pea root cells, except in the case Tyr*t*RNA. Non-dividing cells exhibited decreased levels of Tyr*t*RNA$_{2,3}$ relative to Tyr*t*RNA$_1$; this change occurring during the transition from the elongation to the maturation stage. Decrease in Tyr*t*RNA$_3$ in the transition from the division to elongation phase was also noted.[74] A similar decrease of Tyr*t*RNA$_2$ was found in cotyledons of soybean on germination but the level of Tyr*t*RNA$_3$ and 2 of the 6 Leu-specific *t*RNAs increased.[254] Exponentially-growing tobacco pith

[376] T. KANO-SUEOKA and N. SUEOKA, *J. Mol. Biol.* **20,** 183 (1966).
[377] L. C. WATERS and G. D. NOVELLI, *Proc. Natl. Acad. Sci. U.S.* **57,** 979 (1967).
[378] J. C. LEE and V. M. INGRAM, *Science* **158,** 1330 (1967).
[379] I. KANEKO and R. H. DOI, *Proc. Natl. Acad. Sci. U.S.* **55,** 564 (1966).
[380] S. SASAKI and G. N. BROWN, *Plant Physiol.* **45A,** 7 (1969).
[381] P. H. LI and C. J. WEISER, *Plant Cell Physiol. Tokyo* **10,** 21 (1969).
[382] J. L. KEY, *Ann. Rev. Plant Physiol.* **20,** 449 (1969).
[383] K. C. SHORT and J. G. TORREY, *SEB Symposium*, University Coll. London (Jan. 1972).
[384] B. S. VOLD and P. S. SYPHERD, *Proc. Natl. Acad. Sci. U.S.* **59,** 453 (1968).
[385] R. VENKATARAMAN, P. DELEO, M. B. ANDERSON and J. H. CHERRY, *Plant Physiol.* **46A,** 9 (1970).
[386] R. VENKATARAMAN and P. DELEO, *Phytochem.* **11,** 923 (1972).
[387] M. D. BICK and B. L. STREHLER, *Proc. Natl. Acad. Sci. U.S.* **68,** 224 (1971).

cultures acylate 30% more Arg than cells in the stationary phase, rapidly-growing cells containing a different synthetase as well as a different Arg*t*RNA.[111] This agrees with the situation in *Chlamydomonas*, where the mature organism contains 3 Arg-specific *t*RNAs but the gametes contain only one.[111] Specific changes in bean leaf Leu*t*RNA species occurs during the greening process.[83] Differences in rates of amino acid incorporation into protein in etioplasts and chloroplasts,[388] may reflect differences in specific *t*RNAs in these organelles.[159,389] Exposure of dark-grown *Euglena* to light results in the formation of 3 new chloroplast *t*RNAs.[160]

No changes in the cytokinin content of *t*RNA from tomato plants was detected when infected with *Agrobacterium tumefaciens*.[390] However little work has been reported on changes in *t*RNA levels in infection of plant material by pathogens. Changes in species of *t*RNAs during development could reflect their possible function in inactivating old messages beginning with a common codon sequence,[292] the presence or absence of a unique *t*RNA determining the reading of the message.[292] Uniqueness in a *t*RNA species could be due to hypermodified nucleosides specific for certain protein molecules, thereby making possible a mechanism for activating or deactivating *t*RNA by rapid structural alterations in the 3-dimensional configuration.[327] The role of *t*RNA in the control of RNA synthesis[391,392] and in the end-product repression of the enzymes of amino acid biosynthesis,[393,394] has not as yet been studied in plant systems.

CONCLUSIONS

Compared with the abundant literature available concerning the animal, bacterial and yeast aminoacyl-*t*RNA synthetases and *t*RNAs, the study of these molecules in plants is still in its infancy. Aminoacylation in plants, poses interesting problems with regard to the substrate specificity of individual synthetases from plants producing certain amino acid analogues. As yet, no crystalline synthetase has been prepared from a plant, nor have any mechanistic investigations been carried out with a purified enzyme. There is no reason to suppose that the plant synthetases on the whole are any more unstable than the corresponding bacterial or animal enzymes. Further investigations into these enzymes is urgently required, especially as regards their relationship with the iso-accepting *t*RNAs in the plant cell. Also, little is known about the role of *t*RNA in differentiation in plants and how its function is regulated by methylating enzymes and other enzymes involved in its biosynthesis.

The difference in *t*RNA species and synthetases in chloroplasts from those in the cytoplasm also warrants a more thorough investigation. Results to date, indicate that the chloroplast synthetases and *t*RNAs are more akin to those of bacteria in their properties, whilst those from the cytoplasm differ considerably. Whether or not the cytoplasmic enzymes are similar to those of the mammalian cytoplasm, is open to conjecture. The comparative properties of synthetases from tropical plants and plants from temperate climates might also be of interest, especially since many synthetases are temperature-labile.

[388] H. E. Drumm and M. M. Margules, *Plant Physiol.* **45,** 435 (1970).
[389] W. Merrick and L. Dure, *Proc. Natl. Acad. Sci. U.S.* **68,** 461 (1970).
[390] T. B. Johnson, C. Cross and R. Baker, *Biochim. Biophys. Acta* **199,** 521 (1970).
[391] L. Eidlich and F. Neidhart, *J. Bacteriol.* **89,** 706 (1965).
[392] L. Williams and M. Freundlich, *Biochim. Biophys. Acta* **179,** 515 (1969).
[393] M. Freundlich, *Science* **157,** 823 (1967).
[394] L. Williams and M. Freundlich, *Biochim. Biophys. Acta* **186,** 305 (1969).

The site of transcription of individual *t*RNA molecules, especially of those in the chloroplast and the sites of synthesis of individual chloroplast synthetases, remains to be fully formulated, as does the control of the levels of individual *t*RNAs. However, the complete role of *t*RNA in the plant cell is not yet fully clear, especially since in bacteria, it may control RNA synthesis or act as a repressor molecule.

With the increasing interest shown in *t*RNA and aminoacyl-*t*RNA synthetases in plants over recent years, it is expected that many of these problems will be tackled in the near future in order to clarify the pivotal role of *t*RNA in cell metabolism.

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