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AMINO ACID INCORPORATION ON PLANT RIBOSOMES: THE TRANSFER SYSTEM

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Key Word Index—*Phaseolus aureus*; *Vicia faba*; Leguminosae; bean; turnip yellow mosaic virus-RNA; poly U; cell-free amino acid incorporation; transfer system.

Abstract—Pre-formed *Vicia faba* phenylalanyl-*t*RNA was active in a TYMV-RNA-directed Transfer System, whereas a similar *t*RNA preparation from yeast was not. Thus, lack of charging of yeast *t*RNA by enzymes from *Phaseolus* was not the only reason why yeast *t*RNA would not function in this Transfer System. In the poly U-directed Transfer System, where both types of *t*RNA were active, the pH and ionic parameters governing the reaction with yeast *t*RNA were more stringent.

INTRODUCTION

LIDDELL and Boulter[1] have defined optimal conditions for TYMV–RNA directed protein synthesis on 80S ribosomes isolated from *Phaseolus aureus*. Whereas *t*RNAs from *P. aureus* and *Vicia faba* were active in the system, those from yeast were not, even though a variety of conditions were tried. In this latter case, therefore, conditions might have inhibited some individual step, e.g. aminoacylation, transfer, polymerization. If a constraint were an inability of the aminoacyl-*t*RNA complex to be formed *in situ*, then the provision of pre-formed aminoacyl–*t*RNA should overcome this translational barrier. The latter system involving charged *t*RNA is called a Transfer System.[2]

This paper reports on an investigation into the conditions required for maximal activity of a Transfer System directed by synthetic and natural messengers and whether the only constraint in the use of yeast *t*RNA in a Complete System is due to lack of charging of yeast *t*RNA.

RESULTS AND DISCUSSION

The Transfer System was investigated using both single- and multi-labelled aminoacyl–*t*RNA. It was first characterized in terms of poly U direction, using both single (phenylalanyl) and multi-labelled aminoacyl–*t*RNAs derived from yeast and *V. faba*.

Poly U-direction

Some aspects of the synthesis of polyphenylalanine in the Transfer System were different from synthesis in the Complete System.[1] The overall activity promoted by poly U was much lower, and the time course of incorporation much shorter in the Transfer System. The reduction in activity compared with the Complete System, could be a consequence of the phenylalanine transferred from the aminoacyl–*t*RNA complex being rate-limiting

[1] LIDDELL, J. W. and BOULTER, D. (1974) *Phytochemistry* **13**, 2065.
[2] BOULTER, D. (1970) *Ann. Rev. Plant Physiol.* **21**, 91.

in the Transfer System whereas in the Complete System ^{14}C-labelled phenylalanine was in excess. Alternatively, since the aminoacyl–*t*RNA preparation might contain non-acylated *t*RNA[3], this might become charged in the incubation with contaminating ^{12}C phenylalanine and ATP resulting in [^{14}C]-polyphenylalanine synthesis becoming diluted by its ^{12}C counterpart, with a consequent reduction of radioactivity/unit peptide chain. In support of this view a marked depression of activity occurred when ATP was added to the yeast aminoacyl–*t*RNA Transfer System. However, addition of ATP might induce Mg^{2+} adsorption rendering sub-optimal the available Mg^{2+}: from other experiments it was known that *t*RNA was not a contaminant of the microsomal preparation.

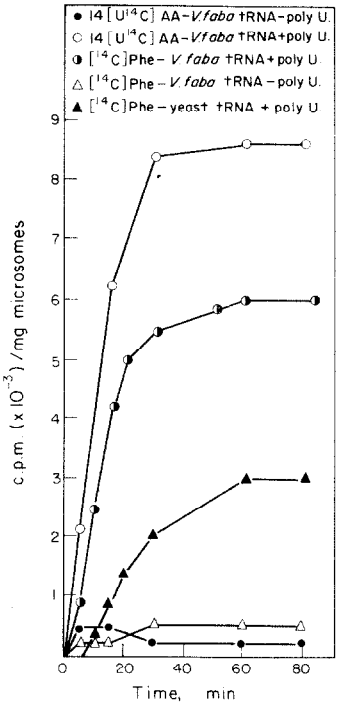

FIG. 1. POLY U-DIRECTION OF Phe INCORPORATION FROM [^{14}C]-Phe-*V. faba tRNA*, 14-[$U^{14}C$]-AMINO ACIDS (AA)-*V. faba* *t*RNA AND [^{14}C]-Phe-YEAST *t*RNA.

0·5 ml incubations contained: 30 μmol Tris–HCl pH 7·8 at 30°, 40 μmol KCl, 0·1 μmol GTP, 5 μmol GSH, 4 μmol $MgCl_2$, 0·1 mg poly U, 0·5 mg microsomes and 0·2 mg *V. faba* or yeast *t*RNA precharged with either [^{14}C]-Phe or 14-[$U^{14}C$]-AA. Incubation was at 30° and 0·05 ml samples assayed at times indicated. [^{14}C]-Phe–yeast *t*RNA gave no activity in the absence of poly U. Radioactivity/disc determined by the methods of Mans and Novelli.[33,34]

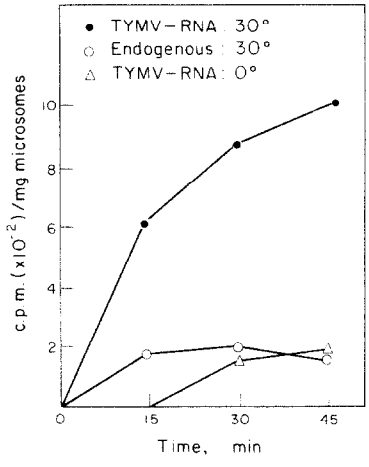

FIG. 2. EFFECT OF TEMPERATURE ON TYMV–RNA-DIRECTED INCORPORATION FROM 14[$U^{14}C$]-AA-*V. faba* *t*RNA.

0·25 ml incubations contained: 15 μmol Tris–HCl pH 7·8 at 0° and 30°, 20 μmol KCl, 0·5 μmol $MgCl_2$, 0·05 μmol GTP, 2·5 μmol GSH, 0·25 mg microsomes, 0·2 mg TYMV–RNA and 0·1 mg *V. faba* *t*RNA precharged with 14 $U^{14}C$ AA. Incubation was at 0° and 30° and 0·05 ml samples assayed at times indicated. Radioactivity/disc determined as in Fig. 1.

The significance of the different rates of synthesis of polyphenylalanine from yeast and *V. faba* aminoacyl-*t*RNAs in the Transfer System (Fig. 1) may also reflect the varying

[3] ALLENDE, J. E. (1969) *Techniques in Protein Biosynthesis* (CAMPBELL, P. N. and SARGENT, J. R., eds.), Vol. 2, pp. 55, Academic Press, London.

amounts of non-acylated *t*RNA present in the respective preparations, since Kaji and Kaji,[4] Kurland,[5] Levin[6] and Seeds and Conway,[7] have all shown that free *t*RNA is bound to the ribosomes in the presence of template. This characteristic may also account for the overall lower incorporation capacity of the Transfer as compared with the Complete System. That this is not the only cause will be seen when the effects of pH are discussed later.

The Mg^{2+} requirement (i.e. provided exogenously), in the poly U-directed Transfer System with both sources of aminoacyl–*t*RNAs was optimal at 8 mM, contrasting with the Complete System at 12 mM (yeast *t*RNA) and 10 mM (*V. faba* *t*RNA).[1] Allende[3] reported a similar reduction in Mg^{2+} requirement between Complete and Transfer Systems derived from wheat embryo. A possible explanation for this reduction is that in the Complete System the extra Mg^{2+} was necessary to meet aminoacylation reaction requirements. Igarashi and Paranchynch[8] record identical Mg^{2+} optima for poly U-directed *E. coli*-derived Complete and Transfer Systems but the range allowing polyphenylalanine synthesis was much less in their Transfer than in their Complete System.

K^+ was an absolute requirement in yeast or *V. faba* aminoacyl-*t*RNA Transfer System, though the optimum for the former (60–80 mM) was much more critical than for the latter (40–100 mM). The high concentrations of K^+ needed for maximal activity are similar to those for the wheat embryo Transfer System[3] and the *E. coli*-derived Transfer System.[9] GTP was essential in the poly U-directed Transfer System using yeast or *V. faba* aminoacyl-*t*RNA complexes.

Striking differences between the yeast and *V. faba* aminoacyl–*t*RNA Transfer Systems were found in their response to variation in pH values. There was no optimum for incorporation in the range pH 7·3–8·5 for the *V. faba* complex, contrasting with an optimum at pH 7·6, for the yeast complex. This indicated that the *P. aureus* transfer enzymes common to both Transfer Systems were much more stringent in their pH requirements for the transfer of the yeast aminoacyl–*t*RNAs. Secondly, the transfer enzymes were much less stringent in their pH requirements than enzymes responsible for aminoacylation to *V. faba* *t*RNA. In the Transfer System with *V. faba* aminoacyl–*t*RNA there was no optimal response in the range of pH 7·3–8·5, contrasting with the discernible optimum of pH 7·8 in the Complete System with that source *t*RNA.[1] Thirdly, *in vitro* systems containing yeast *t*RNA have two different optimal pH responses, one for transfer enzymes (pH 7·6) and the other for aminoacylation (pH 8·1).[1]

Evidently, in the Complete System containing yeast *t*RNA the aminoacylation reaction stage and the transfer stage have dissimilar optima, a condition not apparent in the corresponding *V. faba* tRNA system.

The various time-courses of incorporation in the Transfer System show lag phases with poly U direction.[10] The higher level of activity recorded with the multi-labelled *V. faba* aminoacyl–*t*RNA compared with the single label may mean that more [^{14}C]-phenylalanine is charged to *V. faba* tRNA using the 14-[U^{14}C]-amino acid mixture than using [^{14}C]-phenylalanine; this would be reflected in synthesis if the aminoacyl–*t*RNA transfer-

[4] KAJI, H. and KAJI, A. (1964) *Proc. Nat. Acad. Sci. U.S.* **52**, 1541.
[5] KURLAND, C. G. (1966) *J. Mol. Biol.* **18**, 90.
[6] LEVIN, J. G. (1966) *Federation Proc.* **25**, 778.
[7] SEEDS, N. W. and CONWAY, T. W. (1966) *Biochem. Biophys. Res. Commun.* **23**, 111.
[8] IGARASHI, S. J. and PARANCHYNCH, W. (1967) *Biochemistry* **6**, 257.
[9] NAKAMOTO, T., CONWAY, T. W., ALLENDE, J. E., SPYRIDES, G. J. and LIPMANN, F. (1963) *Cold Spring Harb. Symp. Quant. Biol.* **28**, 227.
[10] SPIRIN, A. S. and GAVRILOVA, L. P. (1969) *The Ribosome*, Springer, New York.

ence of phenylalanine were rate-limiting. On the other hand, results have been published showing that poly U can direct the incorporation of substantial amounts of leucine in an *E. coli*-derived *in vitro* system.[11,12] This anomalous poly U-directed incorporation of leucine has also been demonstrated in 80S ribosomal *in vitro* systems as well as in other 70S systems;[13–16] this effect may be explained in terms of translational errors induced by the non-physiological nature of *in vitro* systems.[17]

It must be remembered that aminoacyl–*t*RNAs labelled with 14-[U^{14}C]-amino acids were essentially for use in TYMV-RNA-directed Transfer Systems, so that the yeast *t*RNA was charged at 12 mM Mg^{2+} with the multi-label, since the yeast aminoacyl-*t*RNA preparation contained representatives of all 20 "protein" amino acids. If the material had been charged at the Mg^{2+} optimum for phenylalanine, i.e. 15 mM Mg^{2+}, valine (and other amino acids) would be esterified to *t*RNA at a low level.[18] The single label yeast aminoacyl–*t*RNA preparation on the other hand, was charged at the phenylalanine Mg^{2+} optimum to facilitate poly U direction in the Transfer System. This would explain the higher levels of polyphenylalanine synthesis in the single-labelled yeast aminoacyl–*t*RNA Transfer System, compared with its multi-labelled counterpart. Since the Mg^{2+} optimum was not so critical for charging with *V. faba* aminoacyl–*t*RNA, this factor was not relevant in that case.

Despite the fact that both sources of aminoacyl–*t*RNA functioned in the Transfer System, the ionic and pH parameters governing the reaction with yeast aminoacyl–*t*RNA were more stringent than those with the *V. faba* complex. Since the provision of pre-formed aminoacyl–*t*RNA still showed differences between yeast and *V. faba* complexes, the restrictive influence(s) occurred after the aminoacylation stage. Allende,[3] Parisi *et al.*,[19] Klink and Richter,[20] Ilan and Lipmann[21] and Cifferi and Parisi,[22] have all reported results which show incompatibilities within heterologous ribosome–transfer enzyme systems, although Boulter *et al.*[17] have concluded that there is a far greater degree of interchangeability of components from different eukaryotic systems than there is between those of eukaryotes and prokaryotes.

TYMV-direction

In the Transfer System, containing 14-[U^{14}C]-*V. faba* aminoacyl–*t*RNAs, TYMV–RNA directed the synthesis of polypeptide by a temperature-dependent reaction (Fig. 2). The Mg^{2+} optimum for this reaction was 2 mM, ostensibly the same as in the Complete System,[1] but completely dependent upon exogenously-added Mg^{2+}; no viral-RNA activity was observed without supplementation. Since the Complete System was exogenously-supplemented with the high-speed supernatant enzyme fraction (containing Mg^{2+}), the

[11] BRETSCHER, M. S. and GRUNBERG-MANAGO, M. (1962) *Nature* **195,** 283.
[12] MATTHAEI, J. H., JONES, O. W., MARTIN, R. G. and NIRENBERG, M. W. (1962) *J. Biol. Chem.* **233,** 657.
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[14] BRETSCHER, M. S. and JONES, O. W. (1967) *Techniques in Protein Biosynthesis* (CAMPBELL, P. N. and SARGENT, J. R., eds.), Vol. 1, p. 217, Academic Press, London.
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[16] FRIEDMAN, S. M., BEREZNEY, R. and WEINSTEIN, I. B. (1968) *J. Biol. Chem.* **243,** 5044.
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[18] LIDDELL, J. W. (1972) Ph.D. Thesis, University of Durham.
[19] PARISI, B., MILANESI, G., VAN ETTEN, J. L., PERANI, A. and CIFERRI, O. (1967) *J. Mol. Biol.* **28,** 295.
[20] KLINK, F. and RICHTER, D. (1966) *Biochim. Biophys. Acta* **114,** 431.
[21] ILAN, J. and LIPMANN, F. (1966) *Acta Biochim. Poland* **13,** 353.
[22] CIFERRI, O. and PARISI, B. (1970) *Progress in Nucleic Acid Research and Molecular Biology* (DAVIDSON, J. N. and COHN, W. E., eds.), Vol. 10, p. 121, Academic Press, New York.

Transfer System optimum possessed a lower Mg^{2+} requirement than the Complete, corresponding to a similar reduction in Mg^{2+} optimum found in the poly U Transfer Systems. This was taken to indicate that a portion of the Mg^{2+} supplied to the Complete System was required for aminoacylation reactions.

Partial dependence on GTP was shown, demonstrating that the microsomal preparation used here was contaminated with GTP. K^+ ion was an absolute requirement for viral-RNA-directed (and poly U) incorporation. The formation of the ternary complex, ribosome–template–aminoacyl–*t*RNA, is specifically stimulated by K^+ or NH_4^+ cations.[10] Stimulation may range from strong[23] to slight.[24] Mg^{2+} is essential for the formation of the ternary complex and from the Mg^{2+} optimum determined in the present work, it may be deduced that binding of the aminoacyl–*t*RNAs with ribosomes would use GTP and Transfer factors. Only for *in vitro* systems which function above 10 mM Mg^{2+}, a somewhat arbitrary figure, are GTP and Transfer factors not required.[4,5,23,25–28]

Spirin and Gavrilova[10] have pointed out that K^+ serves another purpose in the suppression of "non-specific binding" of *t*RNA or aminoacyl–*t*RNA species to the ribosome in the absence of template, or where template is not ribosome-bound. This non-specific complex, readily formed at 0–4° needs neither energy source nor protein factors for formation. In the absence of K^+, in the work reported here, it was noted in binding assay experiments with 14-[$U^{14}C$]-*V. faba* aminoacyl–*t*RNAs that zero-time samples showed high radioactivity, possibly owing, in part, to non-specific aminoacyl-binding.

Yeast 14-[$U^{14}C$]-aminoacyl-*t*RNA was inactive in the TYMV–RNA-directed Transfer System, i.e. provision of yeast aminoacyl–*t*RNA did not overcome the barrier observed previously in the TYMV–RNA-directed Complete System.[1] This shows that there are constraints, besides those involved in aminoacylation reactions. It has been shown that the optimum conditions for yeast *t*RNA are very different from those of *V. faba* *t*RNA in the Complete and Transfer Systems directed by poly U. It is therefore of interest to determine whether a yeast high-speed supernatant enzyme fraction would permit TYMV–RNA direction of a *P. aureus* microsomal system with yeast *t*RNA. If it should not be effective, then other conditions may be necessary for successful viral-RNA translation. Aviv *et al.*,[29] have shown that the incompatibility between *t*RNAs derived from unicellular organisms and the ascites tumour cell, is partly due to inability of the synthetases of the latter to charge certain *E. coli* and yeast *t*RNAs. However, their data suggest that other factors, possibly involving the favoured use of specific degenerate codon classes by the virus and the ascites tumour cell, are involved. They suggest that the barriers of heterology may extend to less closely related higher organisms, to specialised organs of a single species, to organs at various stages of development or even to certain viruses and their hosts.

EXPERIMENTAL

Biological materials, including TYMV–RNA, and the source of chemicals and radiochemicals, were as described previously.[1,30]

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[29] AVIV, H., BOIME, I. and LEDER, P. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 2303.
[30] PAYNE, E. S., BOULTER, D., BROWNRIGG, A., LONSDALE, D., YARWOOD, A. and YARWOOD, J. N. (1971) *Phytochemistry* **10**, 2293.

Transfer RNA was aminoacylated by the method of Ravel *et al.*[31] Single-label ([^{14}C]-phenylalanine) or multi-label was used to charge *V. faba* *t*RNA and yeast *t*RNA. The multi-label was composed of 14 individually purified [^{14}C]-amino acids, including phenylalanine, together with 6 [^{12}C]-amino acids, in *ca* the same proportions as in the typical algal protein hydrolysate CFB 104.

The Transfer System from *Phaseolus aureus* and the methods of radioactive assay, were as described previously.[1,32]

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[32] YARWOOD, A., PAYNE, E. S., YARWOOD, J. N. and BOULTER, D. (1971) *Phytochemistry* **10,** 2305.
[33] MANS, R. J. and NOVELLI, G. D. (1960) *Biochem. Biophys. Res. Commun.* **3,** 540.
[34] MANS, R. J. and NOVELLI, G. D. (1961) *Arch. Biochem. Biophys.* **94,** 48.