Phytochemistry, Vol 22, No 1, pp 25–26, 1983
Printed in Great Britain

0031–9422/83/010025–02$03 00/0

CELL-FREE TRANSLATION SYSTEMS FROM NEW SOURCES: PEA COTYLEDONS AND RICE GERM

HEATHER MORTON, I MARTA EVANS, JOHN A GATEHOUSE and DONALD BOULTER

Department of Botany, University of Durham, South Road, Durham, DH1 3LE, U K

(*Revised received* 11 *June* 1982)

Key Word Index—*Pisum sativum*, Leguminosae, pea, polysomes, cell-free translation, reticulocyte lysate, wheat germ, rice germ, pea cotyledons

Abstract—Translation products of polysomes from pea cotyledons in several new *in vitro* cell-free synthesis systems, from rice germ, pea axes, and pea cotyledons were compared with the products obtained in the established wheat germ and rabbit reticulocyte systems

INTRODUCTION

Cell-free translation systems from wheat germ [1] or rabbit reticulocytes [2] have provided much information on the translation of mRNA species and on post-translational modifications of the newly synthesized polypeptides Previous reports from this and other laboratories have described the translation products of polysomes and poly(A)$^+$-RNA isolated from developing pea seed cotyledons by these systems, and shown that storage polypeptide precursors are synthesized and post-translationally modified by removal of leader sequences [3–5] However the post-translational proteolysis that generates the separated legumin acidic and basic subunits and the lower M_r vicilin subunits has not been demonstrated *in vitro* This communication describes the translation of pea polysomes in some additional cell-free synthesizing systems, including the homologous system from pea cotyledons, to assess the possibility of demonstrating processing

RESULTS AND DISCUSSION

Cell-free translation systems were prepared from rice germ, pea axes and pea cotyledons by methods analogous to those used to prepare the wheat germ system [1, 3] The systems were each optimized for concentration of K^+, Mg^{2+}, spermine, creatine phosphokinase and template When programmed with polysomes from developing pea cotyledons, all these systems gave active incorporation of radioactive amino acids into TCA-precipitable material The presence of only limited amounts of endogenous mRNAs is a necessary requirement of a useful *in vitro* cell-free translation system and this criterion was satisfied since all the systems had a low level of endogenous incorporation and gave the same major radioactive polypeptide products when used to translate pea cotyledon polysomes (Fig 1) Clearly special factors favouring the production of certain mRNAs were not present in these systems, including the homologous pea cotyledon translation system Identification of the polypeptide precursors of legumin, vicilin and convicilin was made as previously described [3, 4]

Experiments with aurintricarboxylic acid (ATA) were carried out at a concentration (10^{-4} M) at which it effectively inhibits initiation, but not translation of protein synthesis Both wheat germ and reticulocyte systems were inhibited 90–99% when poly(A)$^+$-RNA was translated under these conditions, but only 30–40% when polysomes were translated, implying that initiation but not elongation is being effectively inhibited in the latter case A similar degree of inhibition of translation of polysomes in the other systems was observed implying that these systems also were initiating Small differences between the translation products of the different systems were observed, e g the reticulocyte system produced more legumin precursor than the plant systems and contained fewer of the low M_r (< 10000) products which could result from premature termination The reticulocyte system gave double bands at 71 000 M_r for convicilin and at 50 000 and 47 000 M_r for vicilin indicating that it was ineffective in leader sequence removal, the plant systems, with the partial exception of that from pea cotyledons, gave only processed products (i e single bands) Since all the systems have been deduced to initiate, the plant systems must have carried out processing, i e leader sequence removal Although the plant translation systems were effective in carrying out limited processing, they did not effect the further proteolysis observed *in vivo*, and whilst an homologous translation system as opposed to a heterologous system may process products, there is no assurance that processing in such systems is physiological

The cell-free translation systems described here are of potential use in further studies, those from pea axes and cotyledons have been studied by other workers [5–7] but with endogenous rather than exogenous templates The system from rice germ is new and is a particularly active system which would seem to be worth further investigation

EXPERIMENTAL

Polysomes and poly(A)$^+$-RNA were prepared from cotyledons of developing pea seeds as previously described [3] The wheat-germ and reticulocyte lysate cell-free synthesizing systems were prepared according to the methods of refs [1] and [2], respect-

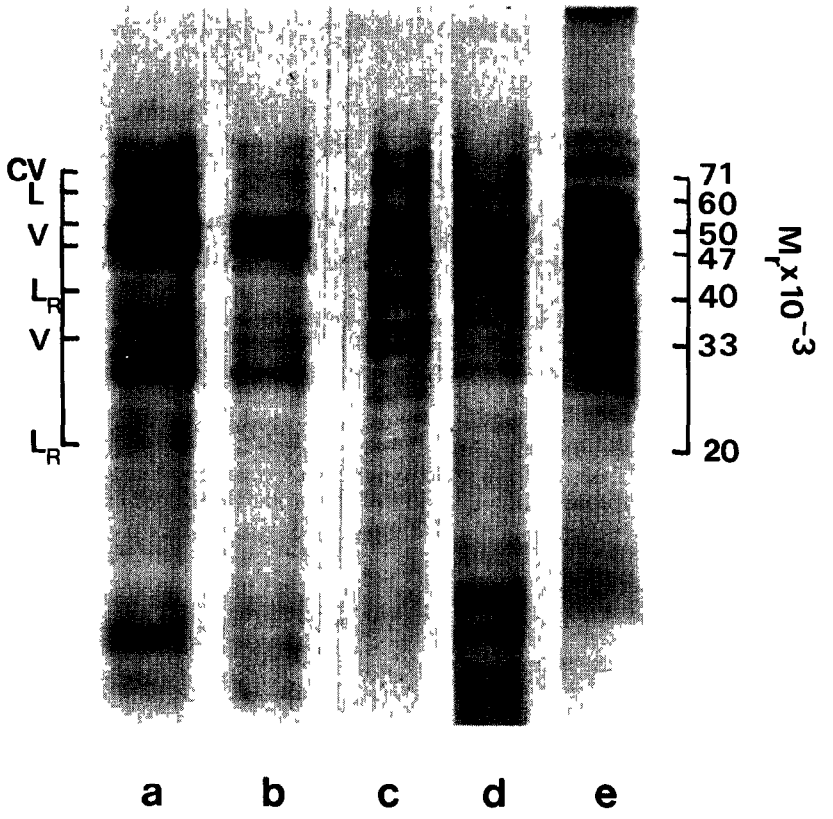

Fig 1 [^{3}H]Leucine labelled translation products of polysomes in various cell-free synthesizing systems Polysomes were isolated from cotyledons at 14 days after flowering (middle-stage of seed development) Track a, wheat germ, track b, rice germ, track c, pea cotyledon, track d, pea axes, track e, rabbit reticulocyte lysate MW markers cv, convicilin, L, unreduced legumin, L_R, reduced legumin, v, vicilin

ively, and were optimized [3, 4] The cell-free synthesizing system from rice-germ ($A_{260} = 11\,2$, $A_{260}/A_{280} = 1\,3$) was prepared exactly as for the wheat germ system [3] Translation of polysomes was carried out under the conditions established for the wheat germ system, with very similar incorporation being obtained The cell-free system from pea axes was produced by aseptically removing axes from partly dried seeds (25 days after flowering) and preparing an extract from them essentially as described in ref [6] The optimized assay system contained 100 μg polysomes, 5–10 μCi [^{3}H]leucine, 19 unlabelled amino acids at 50 μM each, 2 mM DTT, 1 mM ATP, 20 μM GTP, 8 mM creatine phosphate, 5 μl cell extract ($A_{260} = 22\,2$, $A_{260}/A_{280} = 1\,4$), 75 mM KCl, 1 5 mM Mg acetate, 60 μM spermine and 8 μg/ml creatine phosphokinase (CPK) in a 10 μl vol Incorporation increased to a maximum of 60% of added counts after 30 min at 30° Pea cotyledons at mid-development (14 days after flowering) were used to prepare an extract essentially as described in ref [8] The extract was centrifuged at 149 000 g for 90 min and the supernatant ($A_{260} = 9\,9$, $A_{260}/A_{280} = 1\,6$) was taken and used as the *in vitro* synthesizing system Optimized conditions were as in the axes system except for 25 mM KCl, 5 mM $Mg(OAc)_2$, 5 μg/ml CPK and 75 μM spermine Incorporation increased to a maximum of 10% after 45 min

Translation products from all systems were analysed by SDS-PAGE on 17% acrylamide gels followed by fluorography as previously described[3] Inhibition studies were carried out by performing translation in the presence of varying concns (10^{-6}–10^{-2} M) of ATA in some assays polysomes were pre-incubated for up to 30 min in the presence of ATA (10^{-5}–10^{-4} M) to remove 'run-off' product

Acknowledgement—We thank Dr B Juliano (International Rice Research Institute, Los Banos, The Phillipines) for the gift of rice germ

REFERENCES

1 Gordon, M E and Payne, P I (1976) *Planta* **130**, 269
2 Pelham, H B R and Jackson, R J (1976) *Eur J Biochem* **67**, 247
3 Evans, I M, Croy, R R D, Hutchinson, P, Boulter, D, Payne, P I and Gordon, M E (1979) *Planta* **144**, 455
4 Croy, R R D, Gatehouse, J, Evans, I M and Boulter, D (1980) *Planta* **148**, 49 and 57
5 Higgins, T J V and Spencer, D (1981) *Plant Physiol* **67**, 205
6 Peumans, W J, Carlier, A R and Schreurs, J (1980) *Planta* **147**, 302
7 Peumans, W J, Delaey, B M, Manickam, A and Carlier, A R (1980) *Planta* **150**, 286
8 Beevers, L and Poulson, R (1972) *Plant Physiol* **49**, 476