Vol. 99, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 31, 1981 Pages 445-450

DISTINCTIVE CHARACTERISTICS OF PROTEIN SYNTHESIS IN MAIZE EMBRYOS DURING THE EARLY STAGES OF GERMINATION.+

E. SANCHEZ DE JIMENEZ, R. AGUILAR, and S. LOPEZ

Biochemistry Department
Facultad de Química
Universidad Nacional Autónoma de México
México 20,D.F.

Received January 19, 1981

SUMMARY - Quiescent maize embryos were found to contain significant amounts of poly-A-rich pre-formed RNA. ^{14}C-amino acid incorporation into trichloroacetic acid precipitable material was detected at slow rate at the begining of imbibition and fastly increased near 18 to 24 h. Polysomal formation was measured during this period. Addition of - amanitin to the incubation system at two 6h-pulse periods showed significant inhibition of the ^{14}C-amino acid incorporation for the 18-24 h-period, but not for the 0-6 h-period.

INTRODUCTION

The existence of a precise biological clock controlling the synthesis of macromolecules in seed embryos following imbibition has been suggested (1-3). Embryos of wheat,rye, cotton and pea have been reported to contain a stock of pre-formed mRNA molecules (4-6), which seem to support the initial period of protein synthesis following imbibition (7-8). There is, however, no agreement about the role of these mRNA(s) in germination and further embryo development.

With regard to maize, there is only some preliminary data on this subject (9,10), but there is a lack of information about the early events related to resumption of protein synthesis on germinating embryos. Due to the importance of understanding phenomena such as vivipary in maize, the present work was undertaken in an effort to shed some light on the above subject.

+ Grant No. PCAFNAL-790382 from CONACYT (Consejo Nacional de Ciencia y Tecnología, México).

The maize embryos were provided by Dr. Evangelina Villegas from CIMMYT (International Center for the Improvement of Wheat and Maize).

0006-291X/81/060445-06$01.00/0

MATERIALS AND METHODS

Two varieties of Zea mays L. (Crystalline yellow Bajio and Yellow Opaque 2) and Triticum aestivum var. Potam were used as sources of embryos. The seeds were dissected manually.

Batches of 100 to 250 mg of axes or 100 mg of scutella were vacuum infiltrated at 0°C for 20 min and further incubated in a shaking water bath at 25°C in a mixture of 0.3 ml or 1.0 ml containing: 10 to 20 μ Ci of ^{14}C-amino acid mixture (0.1 mCi/mM), 10 mM $MgCl_2$, 50 mM KCl, 10 mM Tris buffer pH 7.6, 10 μg/ml of chloranphenicol and 2% sucrose. For some experiments, α-amanitin (10 μg/ml) was added to the system. The reaction was stopped by cooling at 0°C, and the tissues rinsed with cold water, and frozen until used.

Protein fractionation - Frozen samples of either maize axes or scutella or wheat embryos were ground in a morter, and homogenized in polytron in 6.5 ml of 0.01 M $MgCl_2$, 0.05 M KCl, 0.05 M Tris-HCl, pH 7.6 and 0.005 M B mercaptoethanol. The homogenate was centrifuged at 15 000 x g for 10 min and again at 105 000 x g for 3 h. The supernatant was precipitated with an equal volume of 10% trichloroacetic acid (TCA). The precipitate was dissolved in 0.1 M NaOH and proteins determined by Lowry's Method (11). Aliquots of 10 μl were mixed with 10 ml of Bray's solution and counted in a Packard Scintillation counter. The size of total amino acid pools were measured by the ninhydrin method in an aliquot of the TCA supernatant. 10 μl of the sample were used for radioactive determinations.

Ribosome analysis - The ribosomal pellet was obtained by Beachy et al. method (12), and was purified through 3.0 ml of a 1.0 M sucrose cushion and resuspended in 0.25 ml of 0.02 M KCl, 0.001 M Mg acetate, 0.04 M tris-acetate (pH 8.0). The samples were clarified at 10 000 x g for 15 min and analysed in a 12.5-50% sucrose gradient, in 0.04 M Tris buffer (pH 8.0), 0.02 M KCl, 0.01 M $MgCl_2$ preformed in 17.0 ml tubes of SW 27 Spinco Rotor. The gradients were centrifuged at 26 000 rpm for 2.5 h, fractioned, and U.V. recorded.

TABLE 1

RNA CONTENT OF MAIZE SCUTELLA AND AXES AND WHEAT EMBRYOS

15 to 20 mg of total RNA from either wheat embryos or maize axes or 1 mg of scutellum RNA were fractionated in 5 ml olygo-dT chromatography colum. The amount of RNA eluated at 0.1 M Tris buffer pH 7.6 was recorded as poly A(+) RNA. Each value represents the average of 3 or 4 repetitions.

BIOLOGICAL MATERIAL		RNA mg/10 g tissue	Poly A (-) RNA (%)	Poly A (+) RNA (%)
Maize	var. Opaque	16.59	98.73	1.23
Axes	var. Bajío	18.33	98.90	1.10
Maize Scutellum		0.53	80.51	19.48
Wheat embryo		13.90	99.28	0.72

Polysomal RNA was extracted by the procedure of Marcus et al. (13) and fractionated by an oligo-dT cellulose column (14).

RESULTS AND DISCUSSION

Total RNA from maize scutella or axes was fractionated through and oligo-dT cellulose column (Table 1). Quiescent maize embryos were found to contain significant amounts of pre-formed mRNAs similarly as has been reported for embryos of other graminae (2,3). It is interesting to notice that maize scutellum, a nondividing embryonic tissue, contains much smaller amounts of total and poly A^+ RNA per gram of tissue (Table 1). This noticeable difference might be related to the specific role for the preformed mRNA in dividing tissues of the embryo during germination (1).

Maize embryos (axes or scutella) or wheat embryos were incubated separately for different times, with a mixture of ^{14}C-amino acids as described in the methods. As shown in figure 1, the incorporation of amino acids into an acid insoluble fraction can be detected very soon after imbibition is initiated. Incorporation proceeds in maize axes at low

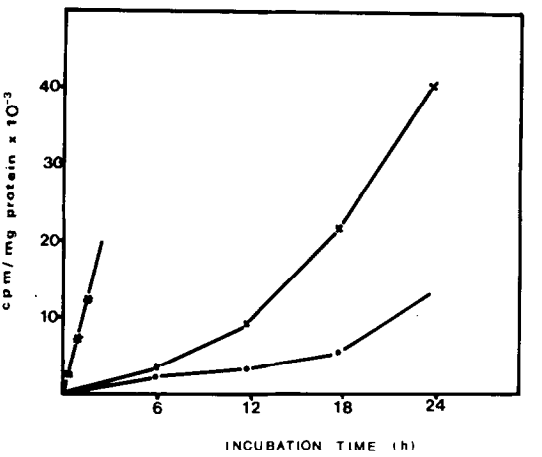

FIGURE 1

Total aminoacid incorporation into TCA precipitable material.- Maize embryos axes (x--x) and scutella (0-0) were incubated separately in a mixture of ^{14}C-amino acids (10 to 20 uCi) at 25°C during 6, 12, 18 and 24 h. Wheat embryos (*-*) during 0.5, 1.0 and 1.5 h. These values have been corrected by the pool size of labeled aminoacids in the cell homogenate.

rate up to approximately 12 h, and it increases while approaching 18-24 h. The initial rate of incorporation in wheat embryos is 8 times higher than in maize axes. (Data corrected by labeled amino acid poools).

Analysis by sucrose gradient centrifugation of the ribosomal fraction was carried out in samples of maize axes at 0, 6, 18 and 24 h of imbibition and at 0 and 6 h, in samples of wheat embryos as a control. Wheat and maize samples showed no polysomes at the dry stage (Figure 2). The characteristic polyribosomal profile was obtained for both after 6 h of imbibition. After 18 to 24 h of imbibition, the polysomal fraction of maize axes did not further increase significantly.

The effect of α-amanitin was analysed at two different 6 h ^{14}C-amino acid pulse periods: between 0-6 h and 18-24 h of imbibition. Total amino acid pools were also measured for both periods. The results indicate that, in the absence of α-amanitin, the amount of labeled amino acid incorporated into trichloroacetic precipitable material is significantly greater for the 18-24 h than for the 6 h period (24,000 vs. 12,600 cpm/mg of protein respectively) as expected from the results showed in

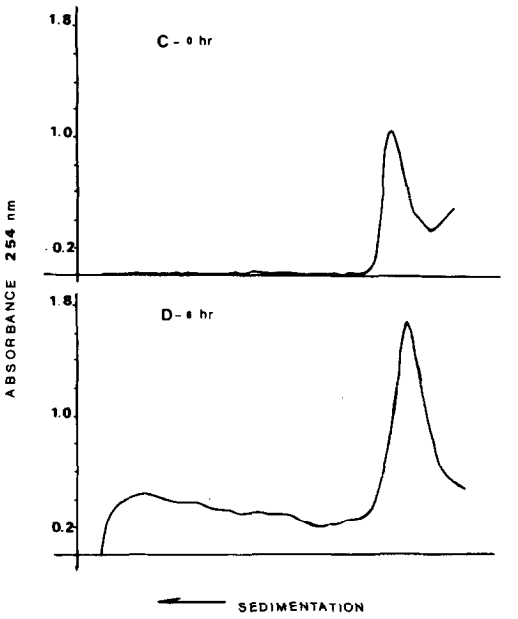

FIGURE 2
Sucrose gradient sedimentation of polyribosomes.- Approximately 6 A_{260} units of polyribosomes extracted from either maize axes or wheat embryos (200 mg) were sedimented on 12.5 to 50% linear sucrose gradient, and monitored at 254 nm in a LKB 8300 Uvicord II with a Beckman recorder 10". Different times of incubation were applied to the samples as follows: A-Wheat 0 h, B-Wheat 6 h, C-maize 0 h, D-maize 6 h, E-maize 18 h, F-maize 24 h.

Figure 1. The α-amanitin inhibited significantly the amino acid incorporation during the 18-24 h period (47.5%) while it has a negligeable effect during th 0-6 h period (5%). These experiments have been repeated 3 times with ^{14}C-amino acid mixture and 3 times with specific ^{14}C-amino acids (leucine or lysine) with very similar results. Therefore, it can be stated that the process of protein synthesis in maize, is mainly supported by the pre-formed mRNA during the early stages of germination. The translation of the newly synthesised mRNA seems, however, to be delayed. The effect of α-amanitin in maize axes is noticeable up to 18-24 h of imbibition, while in wheat embryos it is already significant after 5 h of imbibition (15).

The rate of protein synthesis, as well as the amount of polysomal fraction observed during the early stages of germination (figs 1 and 2)

are significantly lower in maize axes than the observed for wheat embryos, (monosome to polysome ratio: 0.62 and 0.88 respectively), regardless of the amounts of pre-formed mRNA present (Table 1), and much smaller than the capacity displayed by full active plant tissues (16). These findings suggest that the onset of protein synthesis in germinating maize embryos, is not limited by the amount of mRNA. This process should rather be controlled by other factor(s) which might determine the rate of translation (17) and even the type of messages to be translated, as seems to be the case for fertilized sea urchin eggs (18). The apparent increase in efficiency of translation observed in maize axes between the 0-6 and the 18-24 h-period, concomittant to the change from preferential translation of pre-formed to newly synthesised mRNA, seems to be in accordance with this hypothesis.

REFERENCES

1. Dure, S. L. (1977) In: The Physiology and Biochemistry Seed Dormancy and Germination. Elsevier North Holland Biomedical Press. pp. 335.
2. Gordon, E. M. and Payne, P. I. (1976) Planta 130: 269.
3. Bewley, J. D. (1979) Ann. Rev. Plant Physiol. 30: 195.
4. Weeks, P. D. and Marcus, A. (1971) Biochim. Biophys. Acta 232: 671.
5. Ajtkhozhin, M. A., Doschanov, K. H. J. and Akhanov, A. U. (1976) Febs Lett. 66: 124.
6. Caers, L. I., Reumans, W. J. and Carlier, A. R. (1979) Planta 144: 491.
7. Spiegel, S., Obendorf, L. and Marcus, A. (1975) Plant Physiol. 56: 502.
8. Tobías, A., López, S. and Sánchez de Jiménez, E. (1976) J. Cell Biol. 20: 302.
9. Van de Walle C. and Bernier, G. (1976) Plant Physiol. 157: 632.
10. Deltour, R. (1977) R. C. Acad. Sc. Paris. 284: 1643.
11. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193: 265.
12. Beachy, R. N., Thompson, J. F. and Madison, J. T. (1978) Plant Physiol. 61: 139.
13. Brooker, J. D., Tomaszewski, M. and Marcus, A. (1978) Plant Physiol. 61: 145.
14. Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69: 1412.
15. Thompson, E. and Lane, B. (1980) J. Biol. Chem. 255: 5965.
16. Ruzicska, P., Mettrie, R., Dorokhov, L., Premecz, G., Olah, T. and Farkas, G. L. (1979) Planta 145: 199.
17. Dure, S. L., Capdevila, M. A. and Greenway, C. S. (1980) In: Genome Organization and Expression in Plants (C. J. Leaver, ed.) Plenum Publishing Corp. New York. pp. 127.
18. Woods, D. E. and Fitsche, W. (1978) Cell Differentiation. 7: 103.