THE CO-ORDINATED SYNTHESIS OF THE LARGE AND SMALL SUBUNITS OF RIBULOSE BISPHOSPHATE CARBOXYLASE DURING EARLY CELLULAR DEVELOPMENT WITHIN A SEVEN DAY WHEAT LEAF

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1. Introduction

The synthesis of ribulose bisphosphate carboxylase (RUBPC) requires the co-operation of protein synthesis in two cellular compartments: the large subunit is synthesized in the chloroplasts and the small subunit is produced as a higher relative molecular mass precursor in the cytoplasm. Investigations into the degree of co-ordination of the synthesis of the two subunits have so far only been possible using leaves (1) or leaf cells (2) whose metabolism has been perturbed. In seedlings of temperature-sensitive varieties of rye deficient in 70 S plastid ribosomes Feierabend and Wildner [1] were able to detect the synthesis of the small subunit in the absence of synthesis of the large subunit. Barraclough and Ellis [2] showed that in isolated soybean leaf cells in which the synthesis of the small subunit had been inhibited with cycloheximide, the synthesis of the large subunit continued for 4 h after the inhibitor treatment. A pool of free small subunits can also be detected in isolated tobacco leaf protoplasts [3]. It is clear from these observations that the synthesis of one subunit of RUBPC can continue in the absence of the synthesis of the other subunit under experimental conditions. The tightness of coupling of the synthesis of the two subunits under physiological conditions remains to be explored.

In order to investigate the in vivo situation we have studied the in vivo synthesis of the two subunits during normal chloroplast development in a naturally grown leaf. The young leaves of *Graminaceous monocotyledons* grown under a diurnal light regime are particularly suitable for such a study since all the cell division occurs in a basal meristem resulting in a gradient of cellular and plastid development from the base to the tip of the leaf [4]. We have described the developmental sequence in young wheat leaves, determined the age of the cell in sequential leaf slices [5] and used these leaves to study development [4–7]. Wheat leaves are used in the present study to investigate the synthesis of the large and small subunits of RUBPC during cell and plastid development. The results show that the synthesis of the two subunits is tightly co-ordinated during cellular development in a normal light grown leaf since changes in their synthesis occur simultaneously. By analysis of the products of in vitro translation of RNA isolated from these leaves we are able to account for the changing synthesis of the two subunits as development proceeds by changes in their translatable mRNAs.

Abbreviations: RUBPC, ribulose bisphosphate carboxylase (EC 4.1.1.39); LSU, large subunit of RUBPC; SSU, small subunit of RUBPC; pSSU, precursor to the SSU.

2. Experimental

2.1. *Labelling of protein in excised leaves*

Seven day old wheat leaves (*Triticum aestivum* var. Maris Dove) were grown and harvested as in [7] except that the leaves were cut under water. Ten leaves were placed in sterile water containing 200 μCi [^{35}S]methionine (>600 Ci/mmol, Radiochemical Centre, Amersham) for 3 h at 20°C. These ten leaves together with eighty other leaves which had not been labeled were aligned at their bases and 5 mm slices cut progressively up the leaf, commencing either from the leaf base or 2.5 mm above it. The leaf slices were frozen in liquid nitrogen.

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2.2. *Estimation of incorporation into the subunits of RUBPC*

The frozen leaf slices were ground in a pestle and mortar in a total volume of 600 μl of 25 mM Tris–HCl (pH 8.0), 2 mM EDTA, 2 mM phenylmethylsulphonylfluoride, 100 mM 2-mercaptoethanol. The homogenate was centrifuged at 10 000 × *g* for 3 min. Aliquots of the supernatant were prepared for electrophoresis and polyacrylamide gel electrophoresis carried out as in [8]. After staining, the large and small subunit bands (identified by comigration of the purified enzyme and partial proteolytic mapping [9]) were excised from the gel with a razor blade. Each gel slice was dissolved overnight at 50°C in 0.2 ml H_2O_2 (100 vol.) in a scintillation vial. The vials were cooled and 4 ml of a 1:2 mixture of Triton X-100–toluene containing 0.5% (w/v) 2,5-diphenyloxazole (PPO) added and the radioactivity estimated by scintillation counting in a 1216 Rackbeta counter (LKB-Wallac).

Polyacrylamide slab gels used for autoradiography were dried and exposed to Kodak Blue Brand X-ray film at –20°C for 20 days.

2.3. *In vitro synthesis of the subunits of RUBPC*

RNA extracted from the leaf slices according to [10] was washed three times with 3 M potassium acetate (pH 5.5), dissolved in 500 μl 0.3 M potassium acetate (pH 5.5) and reprecipitated with 2.5 vol. ethanol. 1 μg was used to stimulate [^{35}S]methionine incorporation in a reticulocyte lysate (Radiochemical Centre, Amersham) in vitro cell free translation system. Incubations were carried out for 1 h at 30°C. The samples were treated and electrophoresed as in [8]. The bands of the large subunit and the precursor to the small subunit were excised from the gels after they had been located by autoradiography. Their identity was checked by partial proteolytic mapping [9]. The gel slices were treated as above to estimate incorporation into the subunits.

3. Results and discussion

The methionine pools in the wheat leaves (estimated as 10 nM [11,12]) are saturated with [^{35}S]-methionine within 20 min using our labelling technique; this is in a labelling period of 3 h. The methionine enters at the leaf bases and is taken up the leaf by transpiration thus forming a gradient of [^{35}S]methionine, the highest concentration being at the leaf base. A similar gradient of [^{35}S]methionine incorporation into polypeptides was also found in the leaf, resulting from a constant 50–60% of the [^{35}S]methionine present in each leaf slice being incorporated into polypeptides.

The [^{35}S]methionine incorporation into the LSU and SSU of RUBPC isolated from leaf slice 0–0.5 cm are 15 540 cpm and 4480 cpm respectively representing 62 000 000 and 17 900 000 cpm/mg chlorophyl respectively. Thus a similar degree of incorporation of label into the two subunits as found in [1] is occurring. The partial proteolytic digests of both isolated subunits produced by the Cleveland technique [9] using *Staphylococcus aureus* V8 protease gave patterns identical to the purified subunits indicating no major contaminants were being included in the gel slices. The label in the subunits was insufficient to allow an analysis by 2-D electrophoresis.

Fig.1 shows the incorporation of [^{35}S]methionine

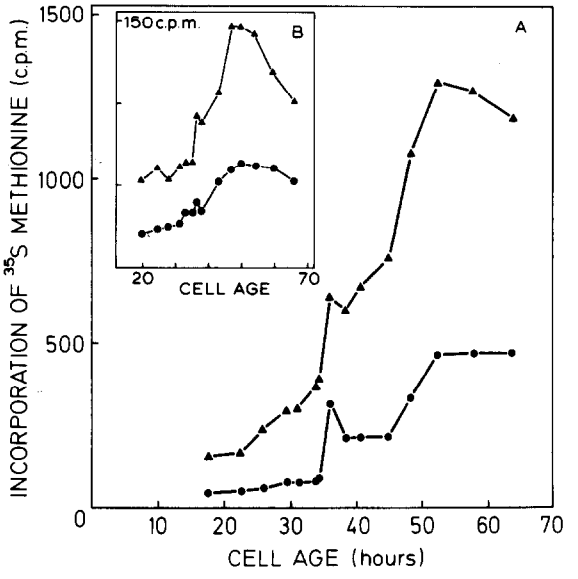

Fig.1. The pattern of incorporation of [^{35}S]methionine into the LSU and SSU of RUBPC during early cellular development in young wheat leaves. A and B show the pattern of [^{35}S]methionine incorporation into the two subunits of RUBPC in two separate labelling experiments and illustrate the reproducibility of the pattern of labelling during cellular development. The incorporation into LSU and SSU isolated from the different developmental stages was calculated on the basis of equal [^{35}S]methionine incorporation in each leaf slice to negate the effect of the gradient of [^{35}S]methionine incorporation. The method for the calculation of the cell age of the leaf slices has been published in [7]. ▲——▲ cpm incorporated into LSU; ●——● cpm incorporated into SSU.

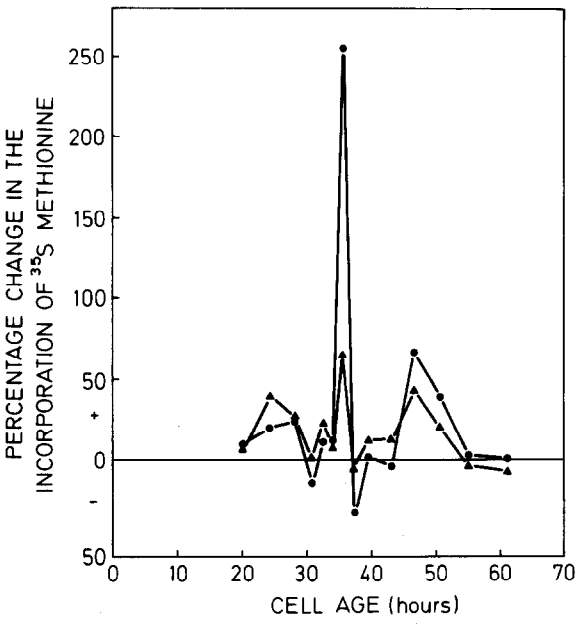

Fig.2. The percentage changes in the incorporation of [^{35}S]-methionine into LSU and SSU of RUBPC during early cellular development in young wheat leaves. The percentage changes were calculated from the values in fig.1A. ▲——▲ percentage changes in incorporation into LSU; ●——● percentage changes in incorporation into SSU.

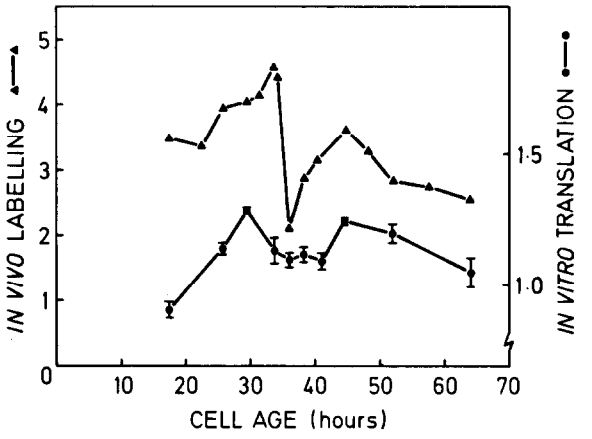

Fig.3. The ratio of incorporation into LSU and SSU of RUBPC after in vivo and in vitro labelling with [^{35}S]methionine during early cellular development in young wheat leaves. ▲——▲ ratio of cpm in LSU:SSU during in vivo labelling data from fig.1A; ●——● ratio of cpm in LSU:pSSU after in vitro translation of the RNA isolated from the sequential leaf sections in a reticulocyte lysate extract. Values are means ± SE for three translations.

into the two subunits of RUBPC during the first 70 h of cellular development in young wheat leaves. Both subunits undergo changes in synthesis simultaneously with a burst of synthesis at 35 h and another more gradual increase peaking at 50 h of cellular development. This pattern of incorporation was very reproducible with the short burst of synthesis at 35 h and the more gradual increase peaking at 50 h, occurring in every experiment (four separate experiments were completed). This is illustrated by comparing A and B of fig.1 which are two independent labelling experiments. The actual cpm values for [^{35}S]methionine incorporation into the two subunits differed by up to 10 fold between labelling experiments due to differences in transpiration rate causing different total [^{35}S]methionine incorporation in the different parts of the leaf.

Fig.2 shows the percentage changes in incorporation of [^{35}S]methionine into LSU and SSU over this developmental period and emphasizes the simultaneous changes in [^{35}S]methionine incorporation which occur in the two subunits. The SSU undergoes a larger increase in synthesis at 35 h of cellular development than the LSU and this is also reflected in the large drop in the ratio of LSU:SSU incorporation in fig.3.

In order to investigate whether the co-ordinated synthesis of the subunits in vivo reflects co-ordinated appearance of their translatable mRNAs, RNA isolated from the sequential leaf slices was translated in a reticulocyte lysate cell-free extract. The ratio of incorporation of [^{35}S]methionine into the LSU compared with pSSU is shown in fig.3. Although the changes in the ratios are much smaller than during in vivo labelling the pattern of the two curves are the same with the peaks and troughs occurring at the same stages of leaf and chloroplast development. This indicates that the changes in the synthesis of the subunits can be accounted for by changes in their translatable mRNAs.

The lower ratio of incorporation of [^{35}S]methionine into the LSU compared with pSSU during translation of the RNA in the reticulocyte lysate extract may be accounted for by (a) the higher methionine content of the pSSU as compared to the mature SSU [13] and (b) a slight preferential translation of the eucaryotic type SSU message compared with the procaryotic type LSU message in the 80 S ribosome-based reticulocyte lysate extract.

Thus during early leaf cellular development in higher plants the synthesis of the two subunits of RUBPC is tightly co-ordinated and this co-ordination appears to extend back to the appearance of their mRNAs in vivo. Observations in experimentally perturbed systems [1,2] have led to the suggestion that SSU acts as a positive control signal affecting chloroplast transcription. If this is the case in normal development then it is clear from the present work that a change in SSU synthesis must be able to cause a change in LSU synthesis in less than 2 h.

It will be interesting to see if there is a close co-ordination of the synthesis of subunits of other multisubunit proteins e.g. coupling factor of the ATP synthase complex whose synthesis is shared by the nuclear/cytoplasmic and chloroplast compartments during normal cellular development.

References

[1] Barraclough, R. and Ellis, R. J. (1979) Eur. J. Biochem. 94, 165–177.
[2] Feierabend, J. and Wildner, G. (1978) Arch. Biochem. Biophys. 186, 283–291.
[3] Hirai, A. and Wildman, S. G. (1977) Biochim. Biophys. Acta 479, 39–52.
[4] Boffey, S. A., Ellis, J. R., Sellden, G. and Leech, R. M. (1979) Plant Physiol. 64, 502–505.
[5] Boffey, S. A., Sellden, G. and Leech, R. M. (1980) Plant Physiol. 65, 680–684.
[6] Sellden, G. and Leech, R. M. (1981) Plant Physiol. 68, 731–734.
[7] Dean, C. and Leech, R. M. (1982) Plant Physiol. in press.
[8] Becker, W. M., Leaver, C. J., Weir, E. M. and Reizman, H. (1978) Plant Physiol. 62, 542–549.
[9] Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102–1106.
[10] Leaver, C. J. and Ingle, J. (1971) Biochem. J. 123, 235–243.
[11] Chapman, D. J. and Leech, R. M. (1979) Plant Physiol. 63, 567–572.
[12] Mills, W. R. and Joy, K. W. (1980) Planta 148, 75–83.
[13] Schmidt, G. W., Devillers-Thiery, A., Desruisseaux, H., Blobel, G. and Chua, N. H. (1979) J. Cell Biol. 83, 615–622.