The co-ordinated synthesis of the subunits of ribulose bisphosphate carboxylase in a wheat line with alien cytoplasm

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1. INTRODUCTION

The large subunit of ribulose bisphosphate carboxylase (RUBPC) is encoded and synthesized in the chloroplast [1,2] whereas the small subunit is encoded in the nucleus and synthesized as a higher M_r precursor in the cytoplasm [3,4]. The synthesis of these subunits is closely co-ordinated during early cellular development in normally-grown first leaves of wheat, with changes in the synthesis of the 2 subunits and their translatable mRNAs occurring simultaneously [5]. Whether this co-ordination in the synthesis of the subunits of RUBPC is the result of inherent developmental patterns in the expression of the 2 subunit genes, or whether there is a signal co-ordinating their synthesis as in the hypothesis that the SSU acts as a positive control signal on the synthesis of the LSU [6,7], needs to be answered.

To elucidate the pattern of control we have examined the cellular co-ordination of the synthesis of LSU and SSU of RUBPC in a wheat line in which a nucleus has been introduced into an alien plastid and cytoplasmic background. Several wheat lines with alien cytoplasms were available but all except the one chosen for these experiments appeared to undergo normal leaf development. The chosen wheat line has the nuclear genetic component from the hexaploid wheat *Triticum aestivum* cv. Chinese Spring and the cytoplasm from a diploid wheat *Aegilops umbellulata*. There is no transfer of plastids or cytoplasm from the male gamete during fertilization in wheat and the wheat line we used had been backcrossed with *Triticum aestivum* cv. Chinese Spring for 9 generations to ensure a pure *Triticum aestivum* cv. Chinese Spring nuclear background. The first leaves of the chosen line *T. aestivum* cv. Chinese Spring (*umbellulata*) were a much paler green than had been observed for any other wheat species. Indeed, the chlorophyll/plastid value in the cross was only 25% of the value for plastids of a similar developmental stage in *T. aestivum* cv. Maris Dove. Chlorophyll accumulation, therefore, appears to be disturbed in this cross. Chloroplast division is also disturbed and occurs over a longer period in the cross than in its hexaploid parent although the final no. chloroplasts/cell (135) is similar. Cell and plastid areas were ~10% higher in the cross than in *T. aestivum* cv. Chinese Spring, its nuclear parent. The transfer of the plastids into a foreign nuclear background clearly produces some temporary but not permanent disturbance in cell development and so this wheat cross provides us with a valuable natural system in which to explore the effects of a foreign nuclear background and a disturbance in development on the co-ordination of the synthesis of the 2 subunits of RUBPC.

The in vivo synthesis of the two subunits of RUBPC during cellular development in the wheat line *T. aestivum* cv. Chinese Spring (*umbellulata*) was, therefore, studied using the developmental sequence of cells and plastids which is found extending from the base to the tip of young wheat leaves [8–10]. The results show that the control mechanisms involved in the synthesis of RUBPC are not affected by the disturbed cellular development of

Abbreviations: RUBPC, ribulose bisphosphate carboxylase (EC 4.1.1.39); LSU, large subunit of RUBPC; SSU, small subunit of RUBPC; M_r, relative molecular mass

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the cross and that although the plastids are in a foreign nuclear background the syntheses of LSU and SSU are still tightly co-ordinated.

2. EXPERIMENTAL

2.1. *Plant material*

Seeds of *Triticum aestivum* cv. Chinese Spring (*umbellulata*) were a kind gift from Dr Colin Law of the Plant Breeding Institute (Maris Lane, Trumpington); 8-day leaves were grown as in [10].

2.2. *Incorporation of [^{35}S]methionine into the subunits of RUBPC*

Leaves (20) were harvested under water and were labelled with [^{35}S]methionine (<600 Ci/mmol, Radiochemical Centre, Amersham) as in [5]. The incorporation into the 2 subunits of RUBPC (identified in [5]) was done as in [5].

3. RESULTS

The [^{35}S]methionine enters the leaves 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.

Fig.1A shows the incorporation of [^{35}S]methionine into the 2 subunits of RUBPC in the leaf slices of the wheat cross. This 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. It is clear that both subunits undergo the same changes in incorporation along the leaf, with a small burst of synthesis at 4 cm from the leaf base and a major peak at 8 cm, before the incorporation declines (fig.1A). Fig.1B shows a quite separate labelling experiment and illustrates the reproducibility of the labelling pattern of the subunits of RUBPC in this wheat cross. The incorporation again shows a small peak at 4 cm in both subunits (in this experiment there was an additional increase in the incorporation into the subunits at 3 cm) and the major peak at 8 cm from the leaf base. The actual cpm values for [^{35}S]methionine incorporation into the 2 subunits differed by 5-fold between the labelling experiments due to differences in transpiration rate causing different total [^{35}S]-methionine incorporation in the different parts of the leaf.

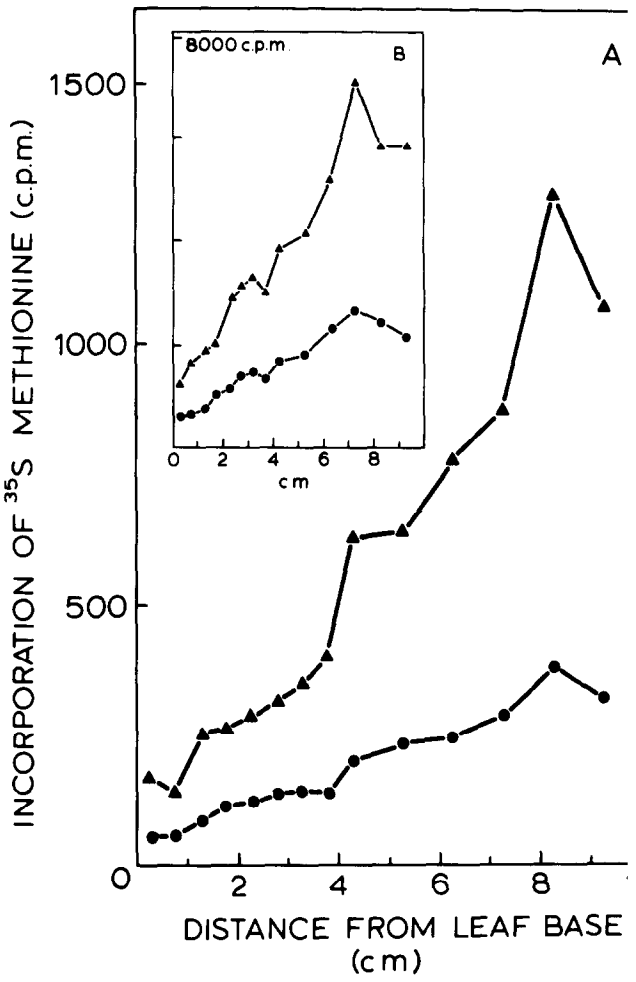

Fig.1. The pattern of incorporation of [^{35}S]methionine into LSU and SSU of RUBPC during cellular development in *T. aestivum* cv. Chinese Spring (*umbellulata*). (A) and (B) show the pattern of [^{35}S]methionine incorporation into the 2 subunits of RUBPC in two separate labelling experiments. The 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. (▲—▲) cpm incorporated into LSU; (●—●) cpm incorporated into SSU.

Fig.2A shows the percentage changes in incorporation of [^{35}S]methionine into LSU and SSU over this developmental period and it is very clear from

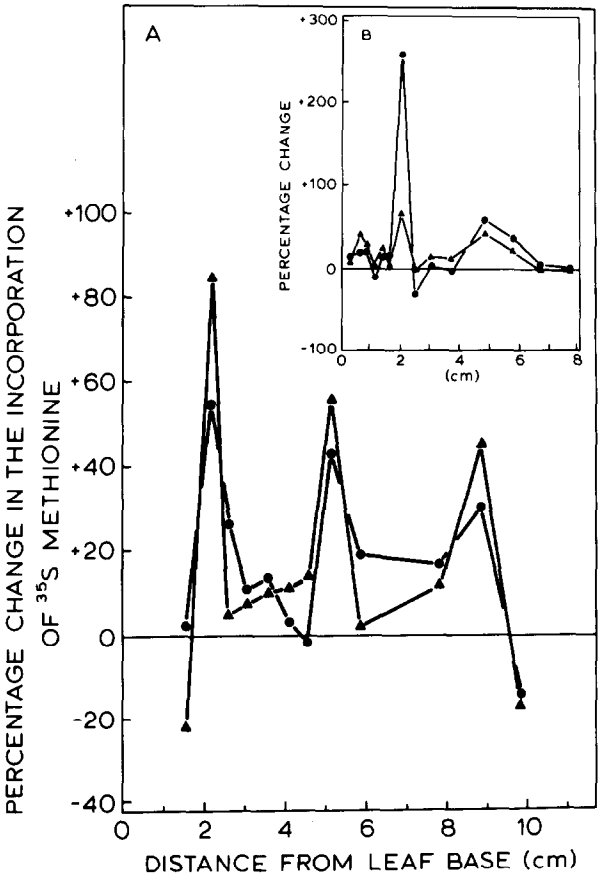

Fig.2. (A) The percentage changes in the incorporation of [^{35}S]methionine into LSU and SSU of RUBPC during early cellular development in the cross *T. aestivum* cv. Chinese Spring (*umbellulata*). The percentage changes were calculated from the values in fig.1(A): (▲—▲) % changes in incorporation in LSU; (●—●) % changes in incorporation in SSU. (B) The insert shows the percentage changes in the incorporation of [^{35}S]methionine into LSU and SSU of RUBPC during early cellular development in *T. aestivum* cv. Maris Dove, a modern hexaploid wheat.

the similarities in the % change of incorporation into the 2 subunits that although the subunits are synthesized in cellular compartments which are genetically alien to each other they still undergo simultaneous changes in [^{35}S]methionine incorporation. Comparison with the percentage changes during development in a genetically 'normal' modern hexaploid wheat *Triticum aestivum* cv. Maris Dove (fig.2B) indicates that in both species the 2 subunits undergo 3 major peaks of incorporation during this developmental period. The major quantitative difference is in the magnitude of synthesis of SSU during the second co-ordinated burst of synthesis which is much larger in the cv. Maris Dove.

4. DISCUSSION

The results show that the cellular co-ordination of the synthesis of the 2 subunits of RUBPC shown in *T. aestivum* cv. Maris Dove [5] also occurs in a wheat line which has the nucleus of a hexaploid wheat with an alien cytoplasm from a diploid wheat. This line has a disturbed cellular development but the co-ordination of the synthesis of the 2 subunits of RUBPC is as tightly controlled in the cross as in the normal situation in *T. aestivum* cv. Maris Dove [5]. This suggests that there is not an inherent developmental pattern for the expression of the 2 subunit genes but there is a continuing active signal co-ordinating the synthesis of LSU and SSU during cellular development and that this signal is common to both the diploid and the hexaploid wheats.

The results from this work are in line with and therefore support the hypothesis [6] that the small subunit of RUBPC acts as a positive control signal on the synthesis of the large subunit of RUBPC in the chloroplast.

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