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CHANGES IN PLASTID RIBOSOMAL-RNA AND ENZYMES DURING THE GROWTH OF BARLEY LEAVES IN DARKNESS

HARRY SMITH

Department of Botany, The University, Manchester, 13

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Abstract—During barley leaf growth in darkness, the levels of etioplast ribosomal-RNA, certain etioplast enzymes and Fraction I protein in the apical 1 cm section increase. The levels of certain cytoplasmic enzymes show little significant change during this growth period, whilst cytoplasmic ribosomal-RNA per cell decreases. The increases in the plastid components have been shown to be a true reflection of organelle development, rather than a result of increased numbers of plastids. The increase in plastid ribosomal-RNA just precedes the increases in the plastid enzymes and in Fraction I protein. The results are discussed in relation to the question of the site of synthesis of plastid proteins *in vivo*.

INTRODUCTION

THE POSSIBILITY that plastids may exist in plant cells as semi-autonomous units[1] has in recent years provoked many studies of plastid development.[2] Much of this work has been concerned with the effects of light on plastid development and there is considerable evidence that the appearance of certain biochemical activities in chloroplasts is dependent on light.[2] However, although structural aspects of etioplast development have been intensively studied[2] there has been little investigation of the biochemical aspects of plastid development in the absence of light. Racusen and Foot[3] have shown that ribulose diphosphate carboxylase and Fraction I protein are present in 4-day-old dark-grown bean leaves and that the levels increase during further growth in darkness for 7 days. The cytoplasmic enzymes, catalase and peroxidase did not increase in activity over this period. However, it was not demonstrated that the increase in the plastid enzymes was due to etioplast development rather than to etioplast multiplication. Ingle[4] has recently shown that the level of plastid ribosomal-RNA increased markedly during growth of radish cotyledons in darkness, but again, however, with no indication as to the contribution from plastid multiplication.

The present investigation arose from an observation that plastid ribosomal-RNA was undetectable in extracts of embryonic barley leaf tissue, suggesting that early development of the etioplast may be limited by the level of plastid ribosomes, and thus providing a system suitable for the investigation of the site of plastid protein synthesis. Attempts have therefore been made to determine whether or not a correlation exists between the appearance of plastid ribosomal-RNA and certain plastid enzymes, using dark-grown material in which plastid

[1] A. GIBOR and S. GRANICK, *Science* **145**, 890 (1964).
[2] R. M. SMILLIE and N. S. SCOTT, "Organelle biogenesis: the chloroplast", in *Progress in Molecular and Subcellular Biology* (edited by F. E. HAHN), Vol. I, Springer-Verlag, Berlin, in press (1969).
[3] D. RACUSEN and M. FOOTE, *Can. J. Botany* **43**, 817 (1965).
[4] J. INGLE, *Plant Physiol.* **43**, 1850 (1968).

development occurs relatively slowly. The results are consistent with the hypothesis that plastid proteins are synthesized on plastid ribosomes.

RESULTS

The Experimental System

It is generally characteristic of monocotyledonous, as opposed to dicotyledonous, plants that the growth of the primary leaf is only marginally inhibited in constant darkness. Consequently, in an investigation of plastid development in dark-grown barley leaves it is first essential to define accurately the system under study. Since the leaves of grasses have basal meristems, the various cells of the leaf represent an ontogenetic sequence both temporally and spatially, with the most mature cells situated at the apex and the youngest cells at the base at any point in time. As a result of preliminary experiments it was decided to concentrate investigations on 1 cm apical sections from leaves of increasing length, thus obtaining a temporal developmental sequence. Consequently all the data are expressed in terms of leaf length rather than seedling age. The relationship between leaf length and age under the experimental conditions for dark-grown seedlings is shown in Fig. 1.

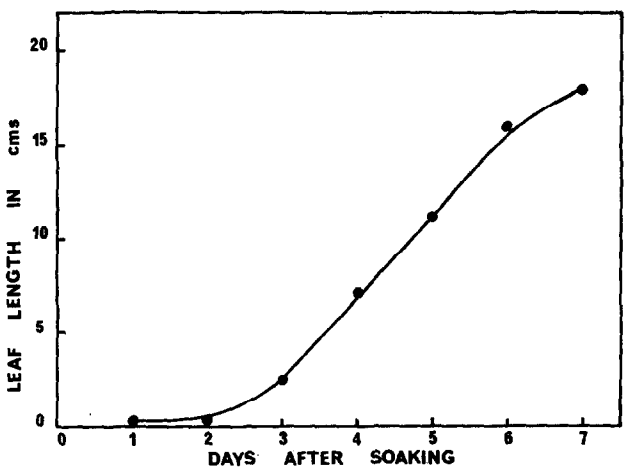

FIG. 1. THE RELATIONSHIP BETWEEN LEAF LENGTH AND SEEDLING AGE IN DARK-GROWN BARLEY.

The biochemical investigations were concentrated on specific plastid activities which could be studied in crude cell extracts without recourse to cellular fractionation, and thus any changes observed could be due either to plastid development or to changes in plastid number. Figure 2 shows the decrease in number of cells in 1 cm apical sections during leaf growth in the dark and the mean number of etioplasts per cell is shown in Fig. 3. It was found impossible to count etioplasts in preparations from leaves less than 2 cm long since the fixed etioplasts were too small to be distinguished in the microscope using the available methods. It is clear, however, that the number of etioplasts per cell increases during early growth and then reaches a plateau. When the number of cells per section is multiplied by the mean number of plastids per cell, a rough approximation to the total number of plastids per section is obtained (Fig. 3). These data indicate that biochemical changes expressed on a per section basis are a true reflection of etioplast development.

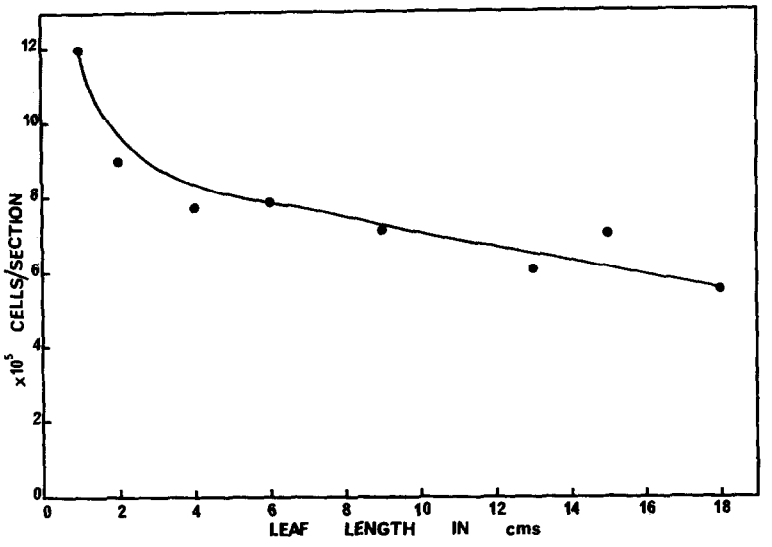

FIG. 2. THE MEAN NUMBER OF CELLS IN 1 cm APICAL SECTIONS OF DARK-GROWN BARLEY LEAVES OF DIFFERENT LENGTHS.

Twenty 1-cm sections were excised and the cells separated in 5% chromic acid for 3 days and counted on a haemocytometer. Each value represents the mean of ten counts for each of three separate preparations.

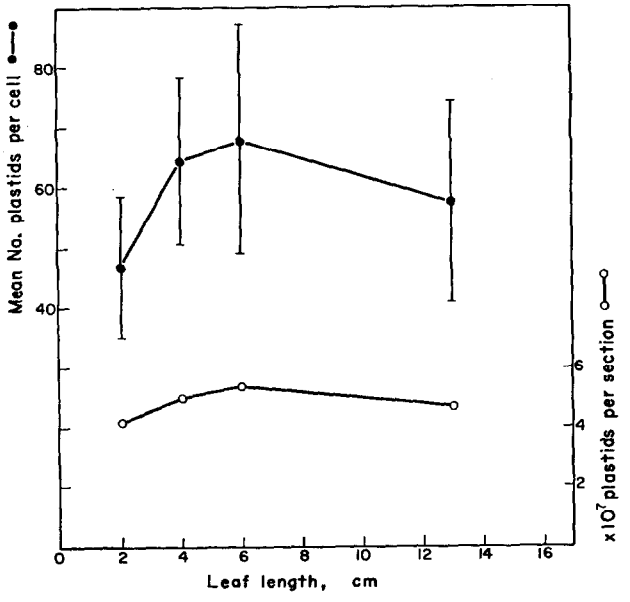

FIG. 3. THE RELATIONSHIP BETWEEN LEAF LENGTH AND THE NUMBER OF PLASTIDS PER CELL (UPPER CURVE) AND PER SECTION (LOWER CURVE).

Solid bars represent standard errors. The lower curve was derived by multiplying the mean number of plastids per cell by the mean number of cells per section (Fig. 2).

The Development of Plastid RNA During Growth in Darkness

The only fraction of plastid RNA which it is at present possible to determine quantitatively is the plastid ribosomal-RNA. The two components of plastid ribosomal-RNA show as discrete bands on polyacrylamide gel electrophoresis[4] (Fig. 5, fractions 1·1 M, and 0·56 M).

In an initial experiment the embryonic leaves, which were normally 0·5–2 mm in length, were excised from batches of 100 imbibed seeds under a safe-light and the RNA extracted and separated on polyacrylamide gels (Fig. 4). Plastid ribosomal-RNA is not detectable in these extracts either at normal sample loading (Fig. 4*a*) or at extremely high loading (Fig. 4*b*).

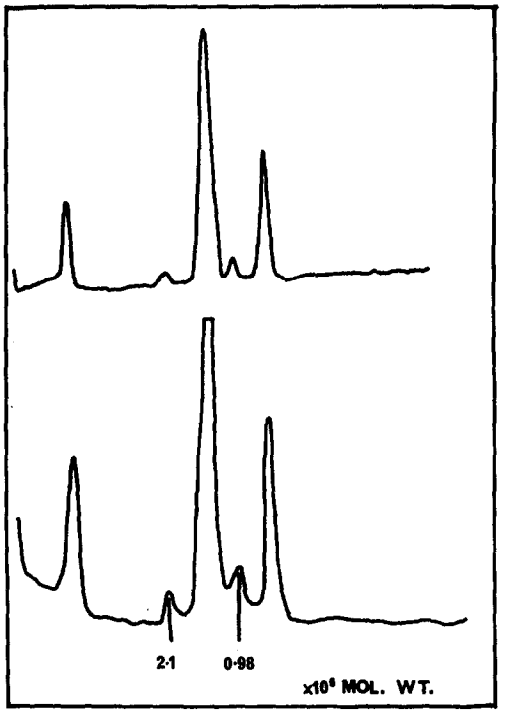

FIG. 4. FRACTIONATION OF NUCLEIC ACIDS FROM EMBRYONIC LEAVES OF 4-hr IMBIBED BARLEY SEEDS.

Total nucleic acid was fractionated on 2·6% gels for 3 hr; (*a*) 20 μg nucleic acid; (*b*) 150 μg nucleic acid.

The RNA fractions running at either side of the main cytoplasmic heavy-ribosomal-RNA fraction are not characteristic plastid fractions. Their molecular weights are calculated to be $2{\cdot}1 \times 10^6$ and $0{\cdot}98 \times 10^6$ respectively. The smaller fraction (0·98 M) has previously been reported by Ingle,[4] whilst the larger fraction has not yet been reported. These fractions are only detectable from very young (i.e. embryonic) leaf tissues and their function and location is unknown.

The development of plastid ribosomal-RNA during leaf growth in darkness was investigated in the following manner. RNA was extracted from 1 cm apical sections of leaves of varying lengths (1–16 cm) and separated on polyacrylamide gels (Fig. 5). In all cases identical total quantities of nucleic acid were loaded onto the gels. It is clear that although

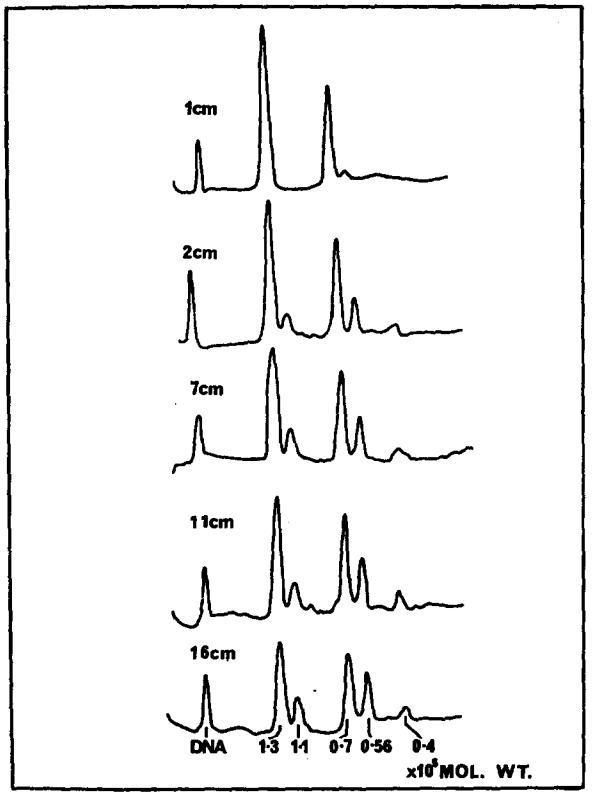

FIG. 5. FRACTIONATION OF NUCLEIC ACIDS FROM APICAL 1 cm SECTIONS OF DARK-GROWN LEAVES OF VARIOUS SIZES.

25 μg total nucleic acid was loaded in each case and fractionated on 2·6% gels for 3 hr. See Experimental for description of fraction notation.

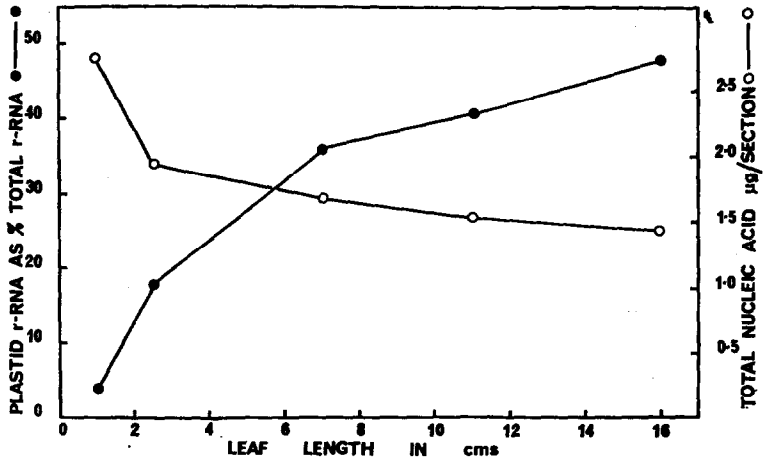

FIG. 6. CHANGES IN TOTAL NUCLEIC ACID PER SECTION, AND IN PLASTID RIBOSOMAL-RNA AS A PROPORTION OF TOTAL RIBOSOMAL-RNA DURING LEAF GROWTH IN DARKNESS.

Total nucleic acid values were obtained by the phenol method; ribosomal-RNA proportions were obtained from area measurements of the scans for Fig. 5.

very little RNA is present in the plastid ribosomal-RNA fractions from the young leaves, these fractions develop steadily as the leaf elongates. In Fig. 6 the amount of plastid ribosomal-RNA is expressed as a percentage of the total ribosomal-RNA. Also in Fig. 6, the total nucleic acid contents per section are presented. These results demonstrate that during growth in the dark the amount of plastid ribosomal-RNA increases steadily until it levels out at about 50% of the total ribosomal-RNA (after approximately 5–6 days' growth). The levels of total nucleic acid per section decrease during this growth period in a manner almost identical with the decrease in cell number per section. The significance of this observation is discussed below.

The Development of Plastid Enzymes During Dark Growth

In order to assess the increasing biochemical capacity of etioplasts during dark growth, the levels of the following enzymes were followed; NAD^+- and $NADP^+$-dependent glyceraldehyde-3-phosphate oxidoreductase, ribulose-1,5-diphosphate carboxylase, alkaline fructose-1,6-diphosphatase and δ-aminolaevulinic acid dehydratase (hydro-lyase). Of these enzymes, $NADP^+$-dependent glyceraldehyde-phosphate oxidoreductase and ribulose-1,5-diphosphate carboxylase are widely recognized as being restricted to the plastids.[5] Alkaline fructose-1,6-diphosphatase is also thought to be a highly specific plastid enzyme, although fructose diphosphatase activity with a neutral pH optimum is known to exist in the cytoplasm and may contribute some activity.[6] Non-specific plant phosphatases in general have an acid pH optimum and are unlikely to contribute to any activity observed at the pH used in these experiments (i.e. pH 8·5). NAD^+-dependent glyceraldehyde–phosphate oxidoreductase, although not thought to be a photosynthetic enzyme *per se*, has been shown in several plants to be converted *in vivo*,[7] *in vitro*,[8] and in isolated chloroplasts[9] into the $NADP^+$-dependent form. This conversion is brought about by the action of NADPH,[8] produced *in vivo*, and in isolated chloroplasts, through photosynthetic electron transport.[7,9] It seems likely, therefore, that it also is present in plastids. δ-Amino laevulinic acid dehydratase is important in porphyrin, and thus in chlorophyll, biosynthesis and although not restricted to the plastids has been shown by Stobart and Thomas[10] to be concentrated there.

To provide an internal control on any observed changes in these plastid enzymes the activities of three cytoplasmic enzymes were also studied: acid phosphatase, glucose-6-phosphate:NADP oxidoreductase and isocitrate:NADP oxidoreductase.

Figure 7 shows the changes in specific activities of the above enzymes in preparations from 1 cm apical sections during growth of the leaves from 1 to 18 cm in continuous darkness. It is quite clear that the specific activities of the "plastid" enzymes (Nos. 1–5) increase steadily during this growth period, whereas those of the "cytoplasmic" enzymes (Nos. 6–8) show no such definite trend. It is also clear that the increases in the "plastid" enzymes are not maintained indefinitely, and that a plateau is reached in all cases, although not simultaneously. Thus δ-aminolaevulinate dehydratase (No. 4) and ribulose-1,5-diphosphate carboxylase (No. 5) reach maximum specific activity at a leaf length of about 8 cm, whilst the other "plastid" enzymes continue increasing in specific activity until a leaf length of 12–14 cm is achieved.

[5] R. M. SMILLIE, W. R. EVANS and H. LYMAN, *Brookhaven Symp.* **16**, 88 (1963).
[6] R. M. SMILLIE, *Nature* **187**, 1024 (1960).
[7] H. ZIEGLER and I. ZIEGLER, *Planta* **65**, 369 (1965).
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[9] B. MÜLLER and H. ZIEGLER, *Planta* **85**, 96 (1969).
[10] A. K. STOBART and D. R. THOMAS, *Phytochem.* 7, 1313 (1968).

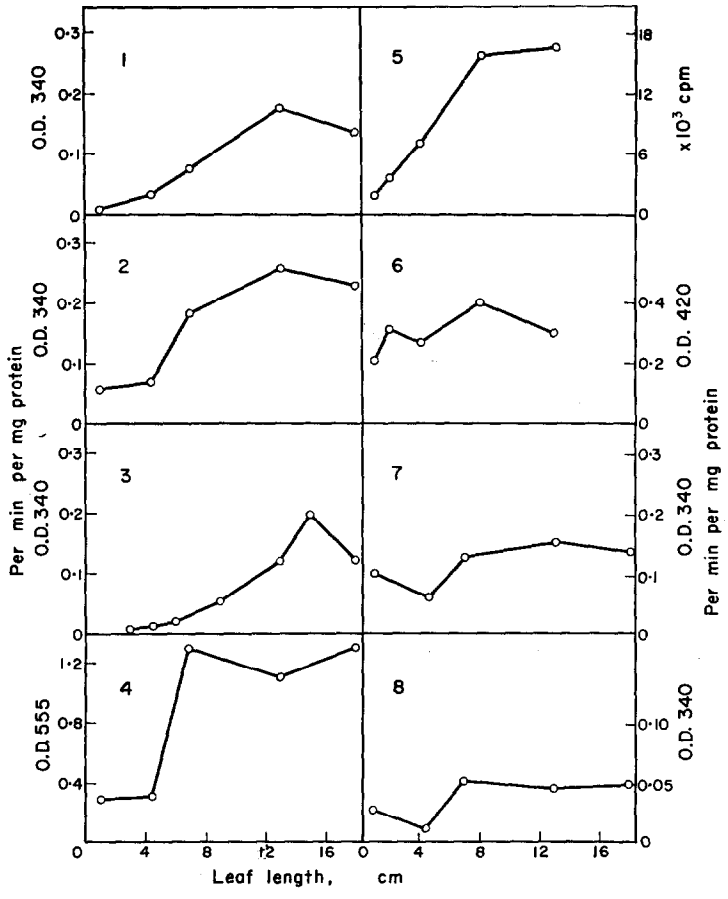

FIG. 7. THE CHANGES IN SPECIFIC ACTIVITY OF PLASTID AND CYTOPLASMIC ENZYMES DURING LEAF GROWTH IN DARKNESS.

1. $NADP^+$-dependent glyceraldehyde-phosphate oxidoreductase; 2. NAD^+-dependent glyceraldehyde-phosphate oxidoreductase; 3. alkaline fructose diphosphatase; 4. δ-aminolaevulinate dehydratase; 5. ribulose diphosphate carboxylase; 6. acid phosphatase; 7. isocitrate:NADP oxidoreductase; 8. glucose-6-phosphate:NADP oxidoreductase.

The Development of Fraction I Protein During Growth in Darkness

A major component of the protein complement of plastids is the so-called Fraction I protein. This soluble (i.e. non-structural) protein is considered to contain ribulose-1,5-diphosphate carboxylase,[11,12] and comprises up to 36 per cent of the total soluble protein of the chloroplast.[13] In order to determine whether the development of this major plastid component proceeded in parallel with that of the other components described above, and to provide a demonstration of net protein synthesis, total soluble protein extract of 1 cm apical sections were separated by polyacrylamide gel electrophoresis. The quantities of Fraction I protein were determined by the dye adsorption/elution method and the results are presented

[11] G. VAN NOORT and S. G. WILDMAN, *Biochim. Biophys. Acta* **90**, 309 (1964).
[12] J. P. THORNBER, S. M. RIDLEY and J. L. BAILEY, *Biochem. J.* **96**, 29c (1965).
[13] U. HEBER and E. TYSZKIEWICZ, *J. Exp. Botany* **13**, 185 (1962).

in Fig. 8. Here again it can be seen that the development of Fraction I protein proceeds steadily until it reaches a plateau at a leaf length of 13–18 cm.

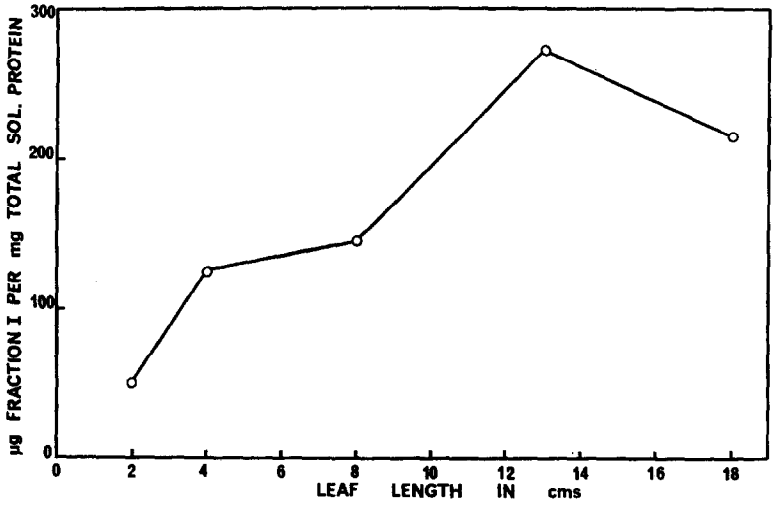

FIG. 8. CHANGES IN FRACTION I PROTEIN LEVELS DURING LEAF GROWTH IN DARKNESS.

Total soluble protein was extracted from 1 cm apical sections, separated by electrophoresis on 7·5% polyacrylamide gels for 1·5 hr and stained with amido schwarz. The de-stained gels were sliced, the dye eluted and recorded spectrophotometrically. The values for the Fraction I protein zone were converted to µg by comparison with a standard curve.

DISCUSSION

It is clear from these results that considerable biochemical development occurs in the barley etioplast during leaf growth in darkness. Thus, although plastid ribosomal-RNA is electrophoretically undetectable in extracts from the embryonic leaves of 4-hr imbibed seeds (Fig. 4), these fractions develop steadily during leaf growth (Fig. 5). The greater part of the plastid-RNA is synthesized as the leaf grows to 8 cm in length, the rate of increase during subsequent growth being very much lower (Fig. 6). It is interesting to observe that the development of large quantities of etioplast ribosomal-RNA during leaf growth in the dark occurs simultaneously with a decrease in total nucleic acid in those sections (Fig. 6), although the decrease in cell number per section over this time (Fig. 2) suggests that total nucleic acid per cell remains relatively constant. It seems likely, although there is at present no evidence, that plastid transfer-RNA molecules are also produced during this time period. Thus it must be concluded that the increase in the RNA of the etioplasts is balanced by a decrease in the RNA of the rest of the cell. It could be speculated, therefore, that degradation of cytoplasmic nucleic acids provides precursors for the synthesis of the etioplast nucleic acids.

The accumulation of plastid ribosomal-RNA during the growth in darkness of radish cotyledons has recently been reported.[4] The time-course of the appearance of etioplast-RNA in radish cotyledons is similar to that in barley leaves, but the level of cytoplasmic-RNA simultaneously increases in the radish, whereas it decreases in barley leaves. This may be a manifestation of the different patterns of development of etiolated leaves of monocotyledonous and dicotyledonous plants. Furthermore, it is not possible to determine whether the

increases in etioplast-RNA in the radish cotyledons are the result of an increase in plastid number, or whether they are true developmental changes within the organelles. In the barley leaf section system, it is clear that leaf growth from 2 to 18 cm does not lead to an overall change in the number of etioplasts in the sections examined (Fig. 3). It is thus reasonable to conclude that the changes in the barley leaf sections during this growth period are due to increased levels of ribosomal-RNA within each plastid.

The development of the barley etioplast is clearly reflected in the levels of the plastid enzymes investigated. Thus, during leaf growth in darkness, ribulose diphosphate carboxylase, NAD^+- and $NADP^+$-dependent glyceraldehyde phosphate oxidoreductases, alkaline fructose-diphosphatase and δ-aminolaevulinate dehydratase increase in specific activity, each reaching a peak at a leaf length of 8–13 cm followed by a plateau (Fig. 7). A similar pattern is observed in the amount of Fraction I protein detected (Fig. 8). This major plastid component increased from *ca.* 4 per cent of the total soluble leaf protein at a leaf length of 2 cm, to a maximum of *ca.* 27 per cent at a leaf length of 13 cm. Thus, it is clear that a considerable increase in the net protein content of the etioplast takes place during leaf growth in darkness. It is not yet known whether this increase is due to protein synthesis within the etioplast, or to synthesis in the cytoplasm followed by transport across the plastid membrane. Comparisons with the properties of mature chloroplasts suggests that Fraction I protein is likely to be synthesized within the etioplast. However, unpublished observations (D. R. Berry and H. Smith) have shown that although considerable incorporation of ^{14}C-leucine into etioplast protein occurs in excised etiolated leaf sections, very little of the label is located in the Fraction I component separated by electrophoresis.

One of the major reasons for carrying out these experiments was to determine whether or not a correlation existed between the levels of plastid ribosomal-RNA and plastid enzymes in the early stages of leaf growth, and to thus obtain evidence on the question of the location of plastid protein synthesis *in vivo*. It is clear from Figs. 6, 7 and 8 that the patterns of increase in plastid RNA and proteins are similar. Furthermore, the changes in cytoplasmic enzymes are not correlatable with the changes in plastid RNA.

Detailed examination of the time-courses of these processes indicates that the accumulation of etioplast ribosomal-RNA precedes that of the plastid enzymes and of Fraction I protein. It can be derived from Fig. 6 that 50 per cent of the increase in etioplast ribosomal-RNA has occurred before the leaf attains a length of 4·7 cm. At this stage of development the increases in the plastid proteins (with the exceptions of ribulose-diphosphate carboxylase and Fraction I protein) were all less than 10 per cent of the final total increases. In the cases of ribulose-diphosphate carboxylase and Fraction I protein, 35 per cent and 27 per cent respectively of the total increases had occurred at this stage. Thus, this evidence is consistent with the view that the plastid proteins are synthesized on the plastid ribosomes, and that in the early stages of etioplast development plastid protein synthesis is limited by the availability of plastid ribosomes. As the plastid ribosomes are accumulated during leaf growth, the synthesis of the plastid enzymes can take place.

The existence of a plateau, or in some cases a decline, in the specific activity of the plastid enzymes as the leaf grows from *ca.* 13 to 18 cm, indicates the existence of mechanisms that regulate the levels of these enzymes in the etiolated leaf. It is possible that this may be an unspecific regulation due to the limitation of the energy supplies. However, the data of Graham *et al.*,[14] working on other monocotyledonous plants grown for several days in

[14] D. Graham, M. D. Hatch, C. R. Slack and R. M. Smillie, *Phyhcto*(1970.)

darkness, demonstrate that brief irradiation with red light is sufficient to markedly increase the specific activity of the plastid enzymes, probably through the agency of phytochrome,[15] indicating that a more subtle mechanism is likely to be operating.

EXPERIMENTAL

Plant Materials and Culture Conditions

Seeds of barley (*Hordeum vulgare*, cv. Proctor) were obtained from Carters Seeds Ltd., London, soaked in running tap-water for 4 hr and sown in 5 cm of vermiculite. Seedlings were grown in a dark controlled-temperature room at 25°. All manipulations were performed under a dim green safelight consisting of a 15 W daylight fluorescent tube filtered through three layers of Cinemoid No. 29 Primary Green (Strand Electrics, Ltd., London). In operations involving dark-grown leaves of less than 7–10 cm it was necessary to excise the leaves from the coleoptile.

Cell and Plastid Counts

Cell counts were carried out by the method of Brown and Rickless[16] with the modification that the leaf sections were left in the 5% chromic acid solution for 3 days. Plastid numbers per cell were made by the method of Possingham and Saurer.[17] Apical sections of leaves were cut into *ca.* 1 mm² pieces, fixed in 2% aqueous glutaraldehyde for 2·5 hr, and the cells subsequently separated by incubation in N HCl at 60° for 1 hr. The suspensions were placed on a microscope slide, and the cover-slip pressed firmly onto the cells, thus liberating the plastids which nevertheless remained in close contact. At least 50 counts were made for each suspension.

Nucleic Acid Extractions and Separations

Total nucleic acid was extracted by the tri-isopropyl-naphthalene sulphonate/4-aminosalicylate/phenol method of Kirby[18] as modified by Loening and Ingle.[19] Total nucleic acid was measured at 260 nm using an extinction coefficient obtained with yeast highly polymerized RNA (Sigma Chemical Co. Ltd.): $E^{1\%}_{1cm} = 220$.

Separation of total nucleic acid was performed by polyacrylamide gel electrophoresis on 2·6% gels.[20] In all cases 25 μg of purified nucleic acid was applied to each gel. Gel cylinders were scanned at 265 nm with a Chromoscan recording densitometer (Joyce Loebl Ltd., Gateshead, England). The amounts of individual nucleic acid fractions were estimated by determination of the areas under the peaks. The fractions other than DNA are referred to by their molecular weights as follows: cytoplasmic heavy ribosomal-RNA, 1·3 M (i.e. $1{\cdot}3 \times 10^6$ M.W.); plastid heavy ribosomal-RNA 1·1 M; cytoplasmic light ribosomal-RNA, 0·7 M; plastid light ribosomal-RNA, 0·56 M[21] (see Fig. 5). The RNA fraction with the highest mobility has a molecular weight of $0{\cdot}4 \times 10^6$ (i.e. 0·4 M) and is a breakdown product of the plastid 1·1 M fraction. The breakdown of the 1·1 M fraction also leads to a further 0·7 M fraction which moves with the 0·7 M cytoplasmic fraction, thus rendering estimates of the amounts of the 1·1 M and the 0·7 M fractions unreliable.[21] To overcome this difficulty in estimating the proportions of plastid and cytoplasmic ribosomal-RNA the following procedure has been used: the amount of the 1·3 M fraction was estimated by area measurement and multiplied by 1·5 to give total cytoplasmic ribosomal-RNA; the amount of the 0·56 M fraction was estimated and multiplied by 3·0 to give total plastid ribosomal-RNA. This method is based on the theoretical assumption that the ratio of heavy- to light-cytoplasmic ribosomal-RNA is 2:0, an assumption which has recently been shown to be also true for plastid RNA by Ingle.[21]

Enzyme Extraction and Assays

Samples of 50–100 1-cm apical leaf sections were homogenized in 0·05 M borate buffer, pH 8·0, in a Virtis "23" at 23,000 rpm for 1 min. The homogenate was filtered through four layers of muslin and centrifuged at 30,000 *g* for 20 min. The supernatant was used as the enzyme preparation. All operations and storage of the preparations were carried out at 2–4°, and all assays were completed within 2 hr of preparation.

Ribulose-1,5-diphosphate carboxylase [E.C. 4.1.1.39] was assayed by the method of Smillie[22] except that

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[18] K. S. KIRBY, *Biochem. J.* **96**, 266 (1965).
[19] U. LOENING and J. INGLE, *Nature* **215**, 363 (1967).
[20] U. LOENING, *Biochem. J.* **102**, 251 (1967).
[21] J. INGLE, *Plant Physiol.* **43**, 1448 (1968).
[22] R. M. SMILLIE, *Plant Physiol.* **37**, 716 (1962).

the radioactivity determinations were carried out by scintillation counting using Bray's solution as scintillation fluid.

Glyceraldehyde-3-phosphate:NAD^+oxidoreductase (E.C.1.2.1.12],glyceraldehyde-3-phosphate:$NADP^+$ oxidoreductase [E.C. 1.2.1.13] and fructose-1,6-diphosphatase [E.C. 3.1.3.11] were measured at 340 nm in a Unicam SP800 spectrophotometer using the methods of Latzko and Gibbs.[23]

δ-Aminolaevulinic acid dehydratase (hydrolyase) [E.C. 4.2.1.24] was assayed by the method of Shemin[24] and acid phosphatase [E.C. 3.1.3.2] by the method of Torriani.[25] Glucose-6-phosphate:$NADP^+$ oxidoreductase [E.C. 1.1.1.49] was assayed by the method of Ochoa,[26] and isocitrate:$NADP^+$ oxidoreductase [E.C. 1.1.1.42] by that of Gibbs.[27]

Protein determinations were made by the Lowry method.[28]

Fraction I Protein

Fraction I protein was extracted with total soluble protein by homogenization in phosphate buffer pH 8·0. Known quantities of soluble protein (usually 100 μg) were fractionated by electrophoresis on 7·5% polyacrylamide gels. The gels were stained for 1 hr in amidoschwarz and washed in 7% acetic acid for 2 days. The gels were then frozen in an aluminium tray on powdered dry-ice such that longitudinal expansion was prevented. The frozen gels were sliced into 1 mm sections using a modified McIlwain tissue chopper (Mickle Engineering Ltd., Gomshall, Surrey, England), and the dye in each section eluted in buffer. The relative amounts of dye in each section were estimated spectrophotometrically and the total for the Fraction I peak converted into μg protein using a calibration curve constructed for bovine serum albumin treated in a similar manner.

Acknowledgements—The technical and graphic skill of Mr. Alan Collins is gratefully acknowledged. The author is grateful to Mr. D. R. Berry and Dr. G. R. Stewart for assistance with the Fraction I protein and certain enzyme assays respectively. This work has been supported by an S.R.C. Research Grant.

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