Plastid Transcription Activity and DNA Copy Number Increase Early in Barley Chloroplast Development

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ABSTRACT

Plastid transcription activity and DNA copy number were quantified during chloroplast development in the first foliage leaf in dark-grown and illuminated barley (Hordeum vulgare L.) seedlings. Primary foliage leaves of seedlings given continuous illumination from 2 days post-imbibition reached a final mean length of 15 centimeters at 6.5 days, whereas primary leaves of dark-grown seedlings required 7 days to reach a similar length. Dividing cells were observed in the basal 0.5 to 1 centimeter of primary leaves until 5.5 days post-imbibition. Plastids isolated from cells located in the basal meristem of 4-day-old seedlings were small (~2 micrometers in diameter), exhibited low transcription activity and contained approximately 130 copies of plastid DNA per organelle. Cell size increased from 18 to 60 micrometers in a 1 to 3 centimeter region located adjacent to the leaf basal meristem. In this region, transcriptional activity per plastid increased 10-fold and DNA copy number increased from 130 to 210. Plastid transcriptional activity declined rapidly in illuminated plants with increasing leaf cell age and plastid DNA copy number also declined but with a slower time course. In dark-grown seedlings, plastid transcriptional activity declined more slowly than in illuminated plants while DNA copy number remained constant with increasing cell age. These data show that plastid transcriptional activity and DNA copy number increase early in chloroplast development and that transcriptional activity per DNA template varies up to 5-fold during barley leaf biogenesis.

In monocots such as barley or wheat, leaf cells are produced primarily by a meristem located in the leaf base (5). The meristematic cells of the leaf base contain small (1–2 μm diameter) undifferentiated prochloroplasts (26). In contrast, mesophyll cells located in mature portions of barley leaves contain up to 60 chloroplasts which are 6 to 8 μm in diameter (12, 26). The increase in plastid size during leaf cell development is accompanied by an accumulation of plastid proteins, many of which are encoded by plastid genes (12, 29). The plastid genes are located on a circular DNA which is 120 to 180-kbp in size. This DNA contains up to 137 genes which encode tRNAs, rRNA (16S, 23S, 4.5S, and 5S), and numerous proteins. The proteins encoded by plastid DNA are involved in transcription, translation, and photosynthesis. Therefore, the biogenesis of chloroplasts requires activation of plastid gene expression (reviewed in Ref. 21).

Transcription activity in plastids varies during chloroplast development (10, 22). This could be due to changes in RNA polymerase levels or activity (2, 30). In addition, variation in plastid DNA copy number might influence transcription activity, particularly if plastid transcription is template limited. The amount of DNA in plastids varies considerably (4–7, 15, 17, 20). For example, in wheat, plastid DNA copy number has been reported to increase up to 7.5-fold during leaf development (20). Based in part on this observation, Bendich (3) proposed that increases in plastid DNA could be required for the build-up of chloroplast ribosomes during chloroplast biogenesis. He further suggested that plastid transcription activity and DNA content may be tightly coupled during chloroplast development. As a first step toward addressing these possibilities, we have measured plastid transcription activity and DNA copy number during plastid development in dark-grown and illuminated barley seedlings.

MATERIALS AND METHODS

Plant Growth

Barley (Hordeum vulgare L. var. Morex) seeds were imbibed in distilled water for 1 h with continuous shaking and planted in vermiculite saturated with full strength Hoagland’s solution at a depth of 2 cm. Seedlings were watered with full strength Hoagland’s solution and grown in controlled environmental chambers at 23°C. Seedlings were transferred to illuminated chambers (350 μE·m⁻²·s⁻¹) 2 d after planting or left in darkness throughout the growth period.

Plastid Isolation

Four-d-old seedlings were removed from vermiculite, the seed peeled back and the primary leaf excised at the root-shoot axis. Leaves were subsequently cut sequentially from the leaf base into two 0.5-cm segments, then three 1-cm segments (sections 1 to 5 numbered from the leaf base). Excised leaf segments were immersed in ice-cold water. Leaf sections were also excised from various parts of older primary leaves and treated in a similar manner. Senescence occurs after 8 d growth in darkness, therefore etiolated seedlings were not used beyond this age.

Excised leaf sections were sterilized (2% [v/v] sodium hypochlorite, 0.25% [v/v] Tween-20, and 1% [w/v] NaCl) then washed extensively with ice-cold distilled water (12). Leaf sections were homogenized with a Polytron in grinding me-
edium containing 330 mM sorbitol, 40 mM Hepes-KOH (pH 8.0), and 2 mM EDTA. Homogenates of the two basal sections (sections 1 and 2) were filtered through miracloth plus a 10 μm nylon mesh filter, and centrifuged for 10 min at 8900g. Following resuspension in 3 mL of grinding media, the resultant crude organelle fraction was filtered through a 50 μm filter. For leaf sections 3 to 5, initial filtration was carried out with 50 μm filters plus Miracloth. Samples from older leaf sections were prepared as described previously (12). The filtrations aided the removal of cell debris from the plastid populations.

Intact plastids were purified from filtrates by centrifugation through Percoll gradients (12). Plastids were purified on Percoll step gradients (upper layer containing 8 mL of 30% Percoll, the lower layer with 4 mL of 65% Percoll). Step gradients were centrifuged at 3950g for 5 min (sections 4, 5, and older tissue), or 10 min at 8900g (sections 1–3). Intact plastids were recovered from the gradients, washed by diluting at least 1:3 with grinding media, and concentrated by centrifugation at 3950g or 8900g for 5 to 10 min. Plastid pellets were resuspended in grinding media. All manipulations were performed at 4°C.

Plastid Number and Size

Plastid populations were photographed with a Nikon photomicroscope for size measurements. Plastid concentrations were determined by counting on a hemacytometer.

Cell Separation

Leaf tissues were fixed by treating with 5% glutaraldehyde for 30 min at 23°C. Cells were separated by treatment with 50 mM EDTA, 50 mM EGTA as described by Pike and Leech (23). Cell size was measured with a micrometer on a microscope.

Marker Enzyme Analysis and Transcription Assays

Catalase activity was monitored spectroscopically (1), and Cyt c oxidase was monitored as in Brambl (8). Plastid transcription activity was determined using “run on” transcription assays described by Mullet and Klein (22).

DNA Isolation

Fifty μL of extraction buffer (4% [w/v] sodium sarcosyl, 40 mM Tris-HCl [pH 8.0], 20 mM EDTA) containing 200 μg mL⁻¹ proteinase K was added to an equal volume of ca. 5 × 10⁷ plastids. After 20 min at 23°C the solution was adjusted to 1% SDS followed by addition of 150 μL of phenol saturated with 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. The sample was mixed by inversion and then 150 μL chloroform:isoamyl alcohol (23:1; v/v) was added and mixed. Following centrifugation for 5 min, the aqueous phase was removed and saved. The organic phase was re-extracted once with 50 μL RNA extraction buffer (1% SDS, 300 mM NaCl, 20 mM EDTA, 10 mM Tris-HCl, pH 8.0) and the aqueous phases combined. Plastid DNA purified on CsCl gradients (14) was used as a standard for DNA quantitation assays and its concentration was determined spectroscopically (one absorbance unit equal to 50 μg DNA mL⁻¹).

Determination of Plastid DNA Content

Plastid nucleic acid extracted from a known number of plastids was treated with 10 μg mL⁻¹ boiled RNase A for 1 h at 37°C. After digestion, samples were phenol extracted as described above. DNA content was measured by Southern dot blot hybridization. RNase treated plastid DNA from 1 × 10⁶ plastids was blotted onto Gene Screen Plus (NEN) membranes. A standard curve was generated by blotted known amounts of CsCl purified ctdNA onto the same membranes. Blots were probed with a 1.3 kbp PsI-HindIII DNA fragment from the barley rbcl gene labeled by nick-translation (25). The amount of radioactivity hybridized was determined by cutting out radioactive spots and counting by scintillation spectrometry.

RESULTS

Leaf Growth in Dark-Grown and Illuminated Barley Seedlings

The growth of dark-grown and illuminated seedlings was characterized to provide information on the relationship between leaf cell development and chloroplast biogenesis (Fig. 1). Seeds for all treatments were planted and kept in darkness

![Figure 1](image-url)
for 2 d. At this time, germination had taken place, roots protruded from the seed but shoots had not broken the seed surface. Plants to be illuminated were then transferred to an illuminated chamber. The primary foliage leaves of plants grown in darkness increased in length most rapidly between 3.5 and 6 d post-imbibition and reached their final length (14–15 cm) about 7-d post-imbibition (Fig. 1A). Internode elongation accelerated between 5.5 and 6 d post-imbibition about the time meristematic activity in the basal region of the primary leaf ceased. Similar results were obtained with illuminated plants except primary leaves grew somewhat faster and reached their final length sooner than leaves of dark-grown seedlings (Fig. 1B). The basal region of the leaf of 4- to 6-d-old seedlings was tightly rolled and lacked pigment in both dark-grown and illuminated plants. In contrast, apical portions of illuminated leaves of 4- to 8-d-old plants were expanded whereas apical regions of leaves of dark-grown seedlings were narrower and partly folded.

One objective of these studies was to analyze changes in cell and plastid parameters which occur when undifferentiated cells and plastids of the leaf basal meristem develop into mature leaf cells and chloroplast populations. Cells and plastids in early phases of development were obtained from primary foliage leaves of 4-d-old seedlings which were either grown in continuous darkness or grown in darkness for 2 d then illuminated for 2 d. The leaves of 4-d-old seedlings were divided into five sections starting from the root-shoot axis as is shown in Figure 2. Four-d-old seedlings were chosen to examine the early phases of leaf cell and chloroplast development because primary foliage leaves of these plants are growing rapidly (Fig. 1) and dividing cells are found in the basal region of the leaves (Fig. 3). More advanced stages of leaf cell and chloroplast development were examined in 6 to 12-d-old seedlings (Fig. 2). Sections 1 through 5 were marked on 4-d-old seedlings with black ink. The position of the marks were then measured after 2 d of growth. This analysis showed that cells in sections 4 and 5 (2–4 cm from the leaf base) of 4-d-old seedlings are found approximately 5 to 7.5 cm from the leaf base in 5-d-old seedlings, 7.5 to 10 cm from the leaf base in 6-d-old seedlings and 11 to 13 cm from the leaf base in 8 to 12-d-old seedlings. Therefore, cells and plastids from these sections (sections 6–10; Fig. 2) of dark-grown or illuminated plants were analyzed to determine changes which occur during later phases of chloroplast development. Data in Figures 3, 5, 7, and 8 are expressed as a function of leaf section number.

Leaf growth in monocots such as barley occurs primarily by elongation of cells near the leaf base. The size and location of the zone of cell elongation in leaves of 4-d-old barley seedlings was inferred from cell measurements. Cells were separated from leaf sections, mesophyll cell length was measured and this data was plotted as a function of leaf section number (Fig. 3). Mean mesophyll cell length increased from 18 μm to 60 μm between 1 and 2 cm from the leaf base of illuminated seedlings (Fig. 3B). Cells of dark-grown seedlings

**Figure 2.** Diagram showing primary leaves of dark-grown barley 4, 6, and 6 d post-imbibition. Leaf sections used for cell and plastid analysis are indicated by cross-hatch marks and are numbered 1 to 8 adjacent to the designated leaf section. Leaf sections 9 and 10 are 11 to 13 cm from the leaf base in 10- and 12-d-old seedlings, respectively. Illuminated and dark-grown seedlings were sectioned in the same manner.

**Figure 3.** Mesophyll cell length (○,●) as a function of distance from the leaf base. Results are expressed as mean ± se, n=15. (A) Seedlings grown 4 d in darkness; (B) Seedlings grown 2 d in darkness followed by 2 d illumination. Mitotic index (△,▲) of regions of 4-d-old leaves were measured as described in Figure 1 legend. Values are the mean of five fields from each of five leaf sections ± se. Leaf section numbers are defined in Figure 2.
reached their greatest length 3 to 4 cm from the leaf base (Fig. 3A). The analysis also showed that cell division was occurring in the basal 0.5 to 1.0 cm of the primary leaves of 4-d-old dark-grown and illuminated seedlings (Fig. 3).

Isolation of Plastids from Barley Leaves

Intact chloroplasts and etioplasts can be isolated in a highly purified form from the apical region of 4.5- to 9-d-old barley seedlings using Percoll density gradients (12). However, this procedure has not been used to isolate immature plastids located in basal portions of the leaf. When plastids from the lower portion of 4-d-old barley leaves were separated on Percoll density gradients, it was apparent that these plastids were less dense than plastids from the apical portions of the leaf. Using density marker beads for reference, it was determined that plastids isolated from the basal 1 cm of 4-d-old barley leaves had a density of 1.065 g/mL, whereas plastids from apical regions of 6-d-old illuminated seedlings had a density of 1.092 g/mL. It was found that plastids from the basal 1 to 3 cm of the leaf could be more easily separated from broken plastids and mitochondria by centrifugation on Percoll step gradients which had a 65% Percoll lower step and a 30% Percoll upper step (see "Materials and Methods"). After centrifugation, plastids were collected at the 30%-65% step interface. To test the purity of these plastids, marker enzyme analysis was done (Table I). This analysis indicated that plastids purified on density gradients were largely free of mitochondria and microbodies. The recovery of intact plastids from the crude organelle pellet ranged from 25 to 87% (Table I). The recovery of intact plastids from the filtrate was not determined. However, most plastids and plastid membranes with associated DNA will pellet under the centrifugation conditions used and would therefore be located in the crude organelle pellet. For this reason, we would anticipate recovery of plastids from filtrates to be similar to that from the crude organelle pellet. Plastids isolated from Percoll gradients were greater than 90% intact as judged by phase contrast microscopy (Fig. 4). Plastids isolated from upper sections of the leaf (sections 5 and above) had a mean diameter of 6.7 μm ± 0.8, whereas plastids from the basal 0.5 cm of leaves of 4-d-old seedlings averaged 2.3 μm ± 0.5 in diameter.

Plastid Transcription

Transcription activity was determined in plastids isolated from leaf sections of various developmental stages. A "run on" transcription assay previously developed by Mullet and Klein (22) was used for this analysis. Transcription assays were run for 5 min to minimize the influence of RNA turnover. Pulse-chase experiments showed that RNA turnover would cause at most a 20% underestimation of UTP incorporation during the 5 min assay (see also Ref. 22). Transcription in plastids isolated from basal leaf sections was inhibited by actinomycin D (25 μg/mL) but not by heparin or rifampicin (data not shown). Similar results were previously reported for plastids isolated from apical cells of 4.5-d-old barley leaves (22).

Transcription activity was low in plastids isolated from the basal 0.5 cm leaf section of 4-d-old dark-grown or illuminated seedlings (Fig. 5, section 1). In dark-grown and illuminated plants 32P-UMP incorporation per plastid increased approximately 10-fold to a maximum in plastids isolated from cells 1 to 2 cm from the leaf base (Fig. 5, section 3). Transcription activity rapidly declined in older apical cells of illuminated seedlings. A similar decline in transcription activity was observed in plastids isolated from older apical cells of dark-grown plants although the decrease occurred more slowly.

Determination of Plastid DNA Levels

We next quantitated the DNA content of plastids isolated from various leaf sections to determine if changes in plastid transcription activity were paralleled by changes in DNA content. DNA was extracted from a portion of the plastids used for transcription assays shown in Figure 5. Recovery of DNA was monitored by adding a known amount of 32P-labeled ctDNA to plastids prior to extraction and determining recovery of label in nucleic acid samples. DNA recovery was greater than 80% in all samples. The DNA isolated from plastids was digested with PvuII and separated on an agarose gel to test its purity and origin (Fig. 6A). Restriction fragments generated by PvuII matched digestion patterns previously reported for barley ctDNA (24) and no other DNA bands were observed.

Plastid DNA content was quantitated by Southern dot blots. Total plastid nucleic acid was treated with RNAse, phenol

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**Table I. Recovery of Marker Enzymes and Plastid DNA in Plastids Isolated from Light and Dark-Grown Barley Leaves**

Values shown are the means of duplicate analyses; standard deviation ranged from 3 to 17%.

<table>
<thead>
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<th>Analysis</th>
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*The location of leaf sections is defined in Figure 2. Leaf sections 1, 2, and 5 were from dark-grown seedlings; section 8 from illuminated seedlings. a, μmoles min\(^{-1}\) Cyt c oxidase; b, μmoles min\(^{-1}\) catalase; c, fmol plastid DNA. ND, not determined. a The 8,900g fraction (sections 1 and 2) or 2,800g fraction (sections 5 and 8) (see "Materials and Methods").
extracted, then blotted onto nylon membranes. To test that RNase treatment removed RNA but did not alter DNA, two experiments were done. First, CsCl purified DNA was treated with RNAse or with RNAse plus DNase and then blotted onto a nylon membrane. The blots were then probed with a \[^{32}P\]-labeled DNA fragment from the \(rbcl\) gene and analyzed by autoradiography (Fig. 6B, lanes 1–3, top). This experiment showed that RNAse treatment did not alter the CsCl purified DNA. When plastid nucleic acid was treated in a similar way, hybridization signals decreased after RNAse treatment due to removal of RNA which hybridizes to the \(rbcl\) probe (Fig. 6B, bottom, lane 1 versus 2). Furthermore, the RNAse insensitive hybridization signal was eliminated by DNase treatment (Fig. 6B, bottom, lane 2 versus 3). This indicates that the RNAse treatment effectively removed RNA which hybridizes to the \(rbcl\) probe from the plastid nucleic acid sample. Increasing amounts of CsCl purified ctDNA were blotted onto nylon membranes to prepare standard curves for each DNA quantitation experiment (Fig. 6C). After hybridization with the \(rbcl\) probe, the radioactive spots were excised and the extent of probe hybridization determined by scintillation counting.

The DNA content of plastids from at least two different experiments was determined and is shown in Figure 7. Duplicate DNA content assays performed on the same set of plastids were within 10% of each other. Somewhat greater variation, indicated by the error bars in Figure 7, was observed from experiment to experiment probably due to differences in plant growth. This analysis showed that plastids isolated from the basal 0.5 cm of primary barley leaves of 4-d-old seedlings contained approximately 130 copies of plastid DNA (Fig. 7). DNA copy number increased to approximately 210 in plastids 1 to 2 cm from the leaf base in both dark-grown and illuminated seedlings. In illuminated seedlings, plastid DNA content declined gradually with increasing cell age, reaching a low value of 50 copies per plastid in the oldest leaf segments examined. Plastid DNA content remained high in apical leaf sections of plants grown 4 to 8 d in darkness (Fig. 7).

**Plastid Number per Cell**

The variation in plastid DNA content observed during chloroplast development could be due to DNA synthesis,
DNA degradation and/or plastid division. To help distinguish between these possibilities the number of plastids per cell was determined in cells from several positions along the leaf blade. Cells were separated from leaf segments and plastid counts were obtained using a microscope. This analysis showed that cells near the base of a 4-d-old barley leaf contain an average of 15 to 18 plastids and that mesophyll cells of more mature leaf cells contained an average of 55 to 60 plastids in both light-grown and dark-grown seedlings (Fig. 8).

**DISCUSSION**

Chloroplast development in monocots occurs during the conversion of meristematic cells of the leaf base into mature mesophyll cells of the expanded leaf. In barley, primary leaf mesophyll cells contain an average of 60 photosynthetically active chloroplasts which average 6.7 μm in diameter (12, 26) (Figs. 3, 7). In contrast, cells in the leaf basal meristem contain 10 to 15 prochloroplasts which are nonphotosynthetic and average 2.3 μm in diameter. Therefore, the build-up of mesophyll cell photosynthetic capacity involves an increase in plastid number and the activation of nuclear and chloroplast genes which provide components of the photosynthetic apparatus.

**Plastid Transcription Increases Early in Chloroplast Development**

In this paper, we report that prochloroplasts isolated from cells of the leaf basal meristem have low transcription activity. The low level of plastid transcription activity in the prochloroplasts is reasonable because these organelles are small, contain few ribosomes and have little photosynthetic apparatus (reviewed in Ref. 21). However, when cells of the leaf basal meristem stop dividing and enter the region of cell elongation 1 to 3 cm above the leaf basal meristem, transcription activity increases 10-fold per plastid and up to 30-fold per cell. The increase in plastid transcription precedes a build-up in plastid RNA, ribosomes, cytoplasmic RNA encoding plastid proteins and accumulation of the photosynthetic apparatus (JC Rapp, BJ Baumgartner, JE Mullet, unpublished). Furthermore, if the increase in plastid transcription activity is prevented then the photosynthetic apparatus does not accumulate and undifferentiated plastids are found in expanded leaves (18, 19). These data suggest that a key early event in leaf chloroplast biogenesis is the activation of plastid transcription.

The molecular basis of the increase in plastid transcription described above is unknown. Activation of transcription could result from increased DNA copy number in plastids, DNA modification, a change in DNA conformation (30), activation of preexisting RNA polymerases (2) or synthesis and accumulation of higher levels of RNA polymerase. In this paper we show that plastid DNA copy number increases from approximately 130 to 210 during the period when plastid transcription increases 10-fold (Figs. 5 and 7). The increase in plastid DNA content may stimulate transcription activity. However, the less than 2-fold change in DNA copy number seems insufficient to entirely account for the 10-fold increase in transcription activity per plastid and the 5-fold increase in transcription activity per DNA template.

**Plastid DNA Copy Number Build-up**

Barley mesophyll cells 3 to 8 cm above the leaf base contained 8,000 to 12,000 copies of plastid DNA which is distributed in 60 plastids (Table II). Similar high plastid DNA copy numbers have been reported in leaf cells of spinach (up to 12,000) (17), and pea (10,000) (15). In contrast, embryonic cells and undifferentiated cells of meristems contain much...
Table II. Plastid DNA Content and Transcription Activity in Cells of Dark-Grown and Illuminated Barley Seedlings

<table>
<thead>
<tr>
<th>Leaf Sectiona</th>
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<td></td>
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<td>DNAa</td>
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<tr>
<td>7</td>
<td>16</td>
<td>3,300</td>
</tr>
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</table>

a Leaf sections are defined in Figure 2. b Plastid transcription (moles of UMP incorporated x 10^-18·cell^-1·hr^-1) calculated from the data in Figures 5 and 8. c Number of plastid DNA copies per cell calculated from the data in Figures 7 and 8.

less plastid DNA. For example, in wheat, cells of seed leaf primordia contain 30-fold less plastid DNA than mature leaf cells (20). The increase in plastid DNA copy number per cell which occurs during mesophyll cell development is due to an increase in DNA per plastid and an increase in plastid number per cell. In wheat, plastids of leaf mesophyll cells contain 7.5-fold more DNA than plastids of leaf primordia in seeds (20). A similar situation probably exists in barley. It is interesting to note therefore, that the DNA content of plastids in the barley leaf basal meristem is already quite high compared to the highest copy numbers found in plastids 1 to 2 cm from the leaf base and above (130 versus 210). This suggests that a significant increase in DNA copy number per plastid probably takes place during formation of the leaf basal meristem from cells of the seed leaf primordia. Furthermore, this increase in plastid DNA copy number must have occurred prior to the increase in plastid transcription activity observed in elongating cells making it a very early event in chloroplast biogenesis.

Plastid DNA content per cell, however, continues to increase once cells leave the leaf basal meristem. This increase is due primarily to a 4-fold increase in plastid number per cell with DNA copy number per plastid being maintained between 150 and 210. Plastid replication and DNA synthesis must occur in dividing cells of the leaf base otherwise plastid number per cell and plastid DNA copy number would decrease. The continuation of these processes after cell division stops leads to a 4- to 5-fold increase in plastid number and plastid DNA per cell. On the other hand, plastid transcription activity per cell increases 30-fold when cells stop dividing and are displaced 1 to 2 cm from the leaf base. We would argue that this increase does not simply reflect the continuation of events initiated in the leaf basal meristem but is the result of an activation process initiated soon after cell division ceases.

The amount of plastid DNA in cells and plastids was similar in the basal 3 cm of dark-grown and illuminated seedlings. Small differences in the time courses of accumulation are probably due to slightly slower leaf cell development in dark-grown seedlings (Fig. 1). In contrast, plastid DNA content per pea leaf cell was reported to increase 2-fold when 5-d-old dark-grown plants were illuminated with a 5-min pulse of red light and then incubated for 24 h (28). In addition, illumination of 7-d-old dark-grown pea plants for 3 to 4 d caused a 2- to 4-fold increase in plastid DNA per cell (27). The lack of a large difference in plastid DNA content of dark-grown and illuminated barley plants is probably due to the similarity of leaf growth and plastid development in the two conditions (27) (Fig. 1). This contrasts the situation in peas where leaf development is inhibited in the absence of light (9).

Plastid Transcription and DNA Copy Number in Older Leaf Cells

Plastid transcription activity declined with increasing leaf cell age eventually reaching the low levels found in prochloroplasts of the basal meristem. The decrease in plastid transcription occurred faster in illuminated plants than dark-grown plants but both plastid populations eventually reached similar low activities. In dark-grown plants, plastid DNA copy number did not change significantly in apical cells of 4-d-old to 8-d-old seedlings. Therefore, the decline in plastid transcription in apical cells of these plants can not be due to a decline in DNA template level. In illuminated seedlings, plastid DNA copy number gradually decreased from 210 to 50 with increasing cell age (Fig. 7). However, the decrease in plastid transcription was greater than and preceded the decrease in plastid DNA content. When plastid transcription activity is expressed on an equal DNA template basis, it is clear that transcription per DNA copy decreases about 5-fold over this phase of plastid development. Other researchers have also noted a decrease in plastid DNA copy number during the latter phases of chloroplast development (6, 15, 16, 17). In the previous studies (16, 17) the decrease in DNA copy number was attributed to a more rapid decline in plastid DNA synthesis than plastid division (dilution of DNA copies). The decrease in plastid DNA copy number observed in older illuminated plants observed in this study cannot be accounted for by dilution of DNA copies and must be due to DNA degradation.

In a previous study (13), we noted that plastid ribosomal RNA content declined in older illuminated leaves. The decline in plastid DNA observed here and ribosomes previously reported probably reflects decreased demand for protein synthesis in mature chloroplasts. In contrast to illuminated plants, plastid DNA content and ribosomal RNA (12) do not decline in older dark-grown seedlings. Furthermore, plastid transcription activity declined more slowly in dark-grown compared to illuminated seedlings. These results may indicate that light-dependent events in chloroplast maturation lead to the decline in plastid transcription, rRNA and DNA levels. In the absence of light-mediated chloroplast maturation, it appears that a capacity for transcription and translation is maintained in older dark-grown barley leaves. Recent studies (11) indicate that this latent capacity is reactivated when older dark-grown seedlings are illuminated.

LITERATURE CITED