CHLOROPLAST DEVELOPMENT IN CHAROPHYCEAE

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INTRODUCTION

Members of the Charophyceae are particularly suited to studies on nuclear-chloroplast relationships. The chloroplasts in the elongated internodal cells are arranged in regular rows. Unlike the nuclei and most of the cytoplasm, they are stationary and do not participate in the active streaming of the protoplasm. It is thus possible to maintain plastids in one section of the cell in the dark and those of another segment under controlled illumination. In such an arrangement, the streaming nuclei and cytoplasm are exposed intermittently to both conditions.

We recently made use of this subcellular arrangement to demonstrate that maintenance of full photosynthetic capability in mature chloroplasts of *Nitella* requires direct light stimulation. (1) Illumination of the nuclei, cytoplasm, and neighboring chloroplasts of the same cell is not sufficient.

This report concerns further observations which indicate that the controls regulating the morphological and functional development of the chloroplasts are complex and that, furthermore, the development of chloroplasts can be reversed in this alga.

METHODS

Cultures of *Nitella* sp. were obtained from the Carolina Biological Supply Co., Burlington, N. C., and maintained at 20°C as described previously (1). A culture of *Chara* sp. was obtained from Dr. W. Proctor, University of Texas, and maintained as described for *Nitella*.

Chara spores were collected periodically from the culture vessels and surface sterilized with sodium hypochlorite and alcohol. After thorough washing they were planted beneath the surface of semisolid medium (0.7% agar in *Nitella* medium) in culture tubes and set aside to germinate on a well-lit laboratory shelf.

RESULTS

Our attention was directed to the factors governing the morphological development of plastids in Charophyceae after an observation concerning one cell in a *Nitella* culture as received from the supply house. The first basal internodal cell of one filament was found to possess a gradient of pigmentation, from almost transparent at the extreme basal end to normal dark-green at the apical end. The degree of pigmentation reflected the morphological development of the plastids, as revealed under the light microscope. Chloroplasts in the pale region of the cell were small and threadlike, while those in the apical end were much larger, ovoid in shape, and closely packed (Fig. 1). This situation was in marked contrast to that seen in other internodal cells, in which the plastids throughout the length were morphologically uniform.

A possible explanation of this phenomenon was that, during development, the basal cell in question was partially submerged in the soil, with only the upper section exposed to light. Differential illumination thus was responsible for observed differences in plastid development.

To examine this possibility, *Nitella* filaments were grown in flasks containing a clay-rich soil overlain with *Nitella* medium. The basal cells of the axes were placed so that their lower half was buried in the soil. The flasks were maintained in 12 hr light, 12 hr dark regime. After several weeks, some of the partially buried cells developed a discontinuity in coloration, similar to that observed previously. Particularly interesting was one group of cells, all originating from a single node, which showed a very distinct variance in pigmentation between the exposed and buried segments of the cells (Fig. 2). Microscopic examination of the buried regions revealed plastids which were small and pale, contrasting sharply with the large, green chloroplasts of the exposed regions of the cells.

It was concluded that such changes in morphology could have resulted from differential degradation of plastids in the buried sections of the cells or, if this cell group represented new growth from a submerged node, from more rapid development of chloroplasts in sections of the cell which became exposed. In either case, clear evidence was provided that phenotypically distinct chloroplasts could be induced within the same cell.

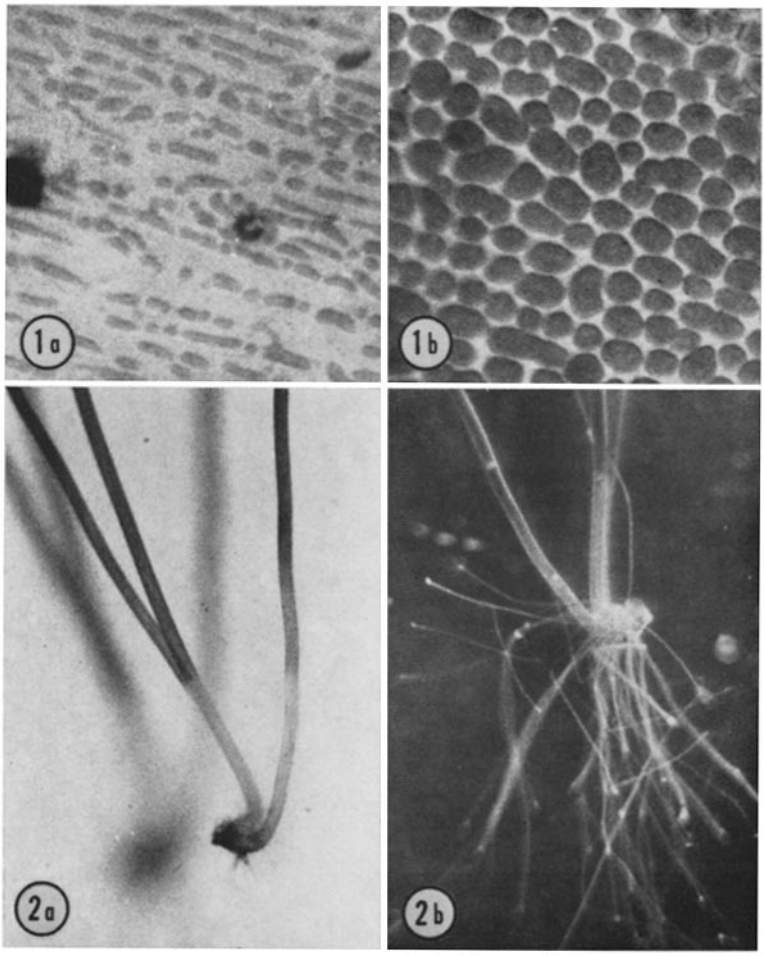

FIGURE 1 Chloroplasts from the basal cell of *Nitella* sp. as described in text, having a gradient in plastid morphology. (*a*) Pale, poorly developed plastids; (*b*) mature pigmented plastids. Photomicrographs were made on living material. Final magnification about 800.

FIGURE 2 (*a*) Basal cells of *Nitella* sp. grown as described in the text, illustrating visible discontinuity in plastid pigmentation. (*b*) As *a* photographed against a dark background to demonstrate rhizoidal development and complete lack of pigmentation in basal section of the cells. Magnification about 4.

Subsequent experiments have confirmed that chloroplast development can be reversed when portions of the cell are buried in the soil. Fully developed internodal cells were isolated from the middle sections of filaments, and such cells were buried at varying depths in the soil; the cells were maintained under normal culture conditions and examined at intervals. Evidence of plastid discontinuity became apparent after 4 wk; at 6 wk, clear differences in the morphology of the chloroplasts were observed. Plastids in the submerged section were smaller (about 4 μ length compared to 8–9 μ length of mature chloroplasts) and not tightly packed in rows. No detectable cell elongation occurred during the experiment, and we concluded that the plastids in the submerged portions of the cell had undergone a reversal of development.

The degradation of the chloroplast structure was found to be reversible. Cell filaments whose basal internodal cells had gradients of coloration, induced as described above, were removed from the soil and subsequently maintained in liquid culture under even illumination. After 2 wk, the pale sections regained normal pigmentation. Mi-

croscopic examination revealed that the plastids in the basal sections now resembled, in size and shape, the chloroplasts of the exposed end of the cell.

In addition to the production of uneven illumination, a second factor is encountered when cells are partially buried. In our experiments, submerged basal nodes invariably developed several rhizoids. These rhizoids do not possess mature chloroplasts and possibly contain an inhibitor of plastid development which could diffuse into adjacent cells and influence their plastids.

The possible role of the rhizoids was investigated in the following manner. Isolated internodal cells were arranged with only their midsections buried in a ridge of soil. Both ends of the cell, with their attached nodes, were exposed above the soil. After several weeks, cells were found with a differential plastid degeneration in the covered midsection but not in the terminal sections next to the nodes.

The relative independence of the plastid development from the influence of the rhizoids could also be inferred from the recovery experiment described above. In that experiment basal cells with attached rhizoids and gradient of plastid morphology were found capable of developing rows of mature chloroplasts upon exposure to even illumination.

Observations on germinating *Chara* spores suggest, however, that factors other than light participate in the regulation of plastid development. The first elongated internodal cell of the emergent protonema frequently appeared completely bleached. Microscopic examination of such pale cells revealed the presence of rows of very small, pale plastids. In some cases, gradients of plastid development could be observed; those nearest the spore remained least developed. In this case, since the spores were germinated in a clear medium, some factor other than light must have been responsible for the inhibition of plastid development.

DISCUSSION

It has been shown previously (1) that illumination of the nuclei and cytoplasm is insufficient to maintain the full photosynthetic activity of chloroplasts in the dark-maintained portions of a *Nitella* cell. The observations reported here indicate that extended periods in darkness may also lead to loss of structure and pigments, even though the nuclei and other plastids in the same cell are illuminated. This situation differs from the degradation of the chloroplasts of unicellular algae, such as *Euglena*, in the dark (2). In *Euglena*, the decline of the chloroplasts is associated with continuous growth of the culture; it is thus a dilution rather than a degradation.

Hagemann (3) has described cells containing white and green plastids in the border between green and white areas of variegated leaves. In this case it is probable that the heterogeneity reflects differences in the genetic potential of these organelles. With *Nitella*, the phenotypically distinct plastids possess the same geneology (4).

It is likely that, in *Nitella*, light is required to stimulate synthesis of structural components and enzymes of the chloroplasts, as well as pigments. It is also possible that in the absence of light the permeability of the organelle is restricted, resulting in deprivation of metabolites and the cessation of anabolic processes.

The failure of plastid development in emerging protonema of *Chara* is perhaps due to the presence of an abundance of organic metabolites. In *Euglena* (5) and *Chlorella* (6), it is known that presence of organic carbon sources in the medium results in the inhibition or reversal of plastid development, even in the light. It is possible that metabolites which are produced in the germinating spore diffuse into the protonema and inhibit plastid development there. However, the restriction of the inhibition to portions of the cell only is difficult to explain on the basis of a freely diffusible inhibitor.

It has been reported by Ross (7) that colorless rhizoidal cells of *Chara gymnopitys* are capable of limited chloroplast development when exposed to light. This potential, although not expressed in our observations concerning the development of rhizoidal cells of *Chara* sp. cultured under even illumination, does further emphasize the complexity of the controls regulating plastid development in this alga.

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