NUCLEIC ACIDS IN PLASTIDS AND STARCH FORMATION*

R. SALEMA[1] and N. P. BADENHUIZEN

Department of Botany, University of Toronto, Toronto.

SUMMARY

Chloroplasts lost their starch-synthesizing enzymes (phosphorylase, ADPGα-glucan glucosyltransferase) during a period of darkness. Enzyme activities returned in the light, but this process could be reversibly inhibited with actinomycin D or chloramphenicol. It is concluded that the enzymes are produced in the chloroplast and that starch production is dependent on the functioning of the protein synthesis as directed by the nucleic acids in the chloroplast. The localization and extension of DNA areas in chloroplasts and chloroamyloplasts showed a relationship to the number and size of the starch granules produced. In many cases there was a close contact between DNA areas and starch granules.

DNA and RNA were found in a variety of plastids, in agreement with the expectation expressed by various authors.

It is likely that all plastids contain their own mechanism for nucleic acid-controlled protein synthesis, and that the starch-synthesizing enzymes are always among the proteins thus produced.

1. INTRODUCTION

All types of plastids, so far investigated, contained phosphorylase and had the potentiality to form starch. This also applies to those plastids which, under natural conditions, do not form starch, for instance the large proteinoplasts in *Helleborus corsicus*, described by THALER (1955). Only in very young leaves of 3 mm length these plastids are filled with starch, which is subsequently replaced by proteins. When these proteinoplasts are incubated with glucose-l-phosphate, they produce starch. The presence of phosphorylases appears to be a fundamental characteristic of plastids in general.

Since Leloir's discovery of the so-called starch synthetase, which uses nucleoside diphosphate glucose as substrate, phosphorylase has been discredited as a starch-synthesizing enzyme *in vivo*. A critical analysis showed that the evidence for this concept was insufficient (BADENHUIZEN 1963) and recent work tends to demonstrate a synthetic role for at least one of the phosphorylases found in corn endosperm (TSAI & NELSON 1968).

When studying starch formation in plastids it should be realized that chloroplasts are very specialized since they possess a photosynthetic apparatus. The synthesis of many substances is linked to the process of photosynthesis (RHODES & YEMM 1966). Chloroplasts contain DNA in small scattered areas, and also

* Presented in honour of Prof. Dr. Th. J. Stomps, friend and esteemed teacher of the senior author (N.P.B.), at the occasion of his jubilee with the Royal Netherlands Botanical Society.

[1] Permanent address: Centro de Microscopia Electrónica, Universidade do Porto, Portugal.

RNA and ribosomes (KISLEV *c.s.* 1965; SWIFT 1965). These nucleic acids appear to be responsible for the production of a certain number of proteins, thus giving the chloroplast a measure of autonomy (SMILLIE *c.s.* 1967). The starch, produced by chloroplasts, is metabolically active assimilatory starch in the form of tiny granules scattered in the stroma in between the lamellae of the thylacoids. These granules remain small and their shape is defined by the space available. For this reason assimilatory starch can not be used as a taxonomic character.

Besides phosphorylase, chloroplasts contain ADPG α-glucan glucosyltransferase, as a potential starch-synthesizing enzyme (DOI *c.s.* 1966; FRYDMAN & CARDINI 1964). It was found that the transferase activity was lost from the leaves of plants that had been starved in the dark, and that it reappeared if such plants were re-exposed to light (CHANDORKAR & BADENHUIZEN 1967). Are the two enzymes produced under control of the nucleic acid-directed mechanism of the chloroplast itself? This question could be answered by treating starved chloroplasts with actinomycin D or chloramphenicol in the light. Actinomycin D interferes with RNA production, while chloramphenicol inhibits protein synthesis on the ribosomes and has the advantage that it can do so selectively for chloroplasts. In this publication we report on the results of such experiments.

In contrast, amyloplasts in reserve organs form starch granules which are characteristic for the plant species both in shape and composition and therefore have a certain taxonomic value. When reserve starch is found in two separate organs of one plant, such as tubers and seeds, the shape of the starch granules is different (SEIDEMANN & SCHLIEPE 1963), so that cell differentiation is involved as well.

From studies of corn varieties it has become evident that the characteristics of reserve starch granules (shape and composition) are mainly directed by nuclear genes (ZUBER 1965). Do amyloplasts also contain nucleic acids, and if so, what is the role of their nucleic acids in the production of a starch granule with distinct properties?

Fibrils of DNA have been demonstrated not only in chloroplasts, but also in thylakoid-free chloroplast mutations (RIS 1962; SPREY 1966) and in root leucoplasts (SWIFT 1965; NEWCOMB 1967). We thought it desirable to extend the investigation to other plastid types, especially the amyloplasts, of which there are different forms: colourless, virescent, degenerative and permanently green ones (BADENHUIZEN 1964). The chloroamyloplasts of *Pellionia*, which belong to the last category, are of special interest, because they resemble chloroplasts in structure and development (BADENHUIZEN & SALEMA 1967), are potentially able to photosynthesize, and nevertheless produce reserve starch granules (SALEMA & BADENHUIZEN 1967).

We included the large proteinoplasts of *Helleborus corsicus* in our study as leucoplasts that do not form starch under natural conditions.

2. MATERIAL AND METHODS

2.1. Treatment of chloroplasts with antibiotics

Plants of *Beta vulgaris* were kept in the dark for about 96 hours. After this period some of the leaf material was immediately fixed for observation with the electron microscope. The rest of the material was cut into pieces of approximately 1 cm^2 and these were placed in small vials, containing the medium to be tested. The vials were illuminated and air was bubbled through the medium. The solution contained actinomycin D in concentrations varying from 10–100 μg/ml dissolved in 0.025 M phosphate buffer, pH 6.1, with 4% sucrose. As a control leaf pieces were similarly treated with buffered sugar solution alone. The duration of the treatment varied from 8–96 hours in both cases. At the end of the period leaf pieces that had been in contact with actinomycin D were either fixed, or they were thoroughly washed with water and then kept in the buffer solution for various periods of time.

Chloramphenicol was used in concentrations of 2 or 3 mg/ml, and the same scheme was followed as described above for actinomycin D.

At the end of each experiment leaf material was fixed in glutaraldehyde, followed by osmium tetroxide, dehydrated in an acetone series and embedded in Epon. In most cases afterstaining with uranyl acetate and lead citrate was applied, as described previously (SALEMA & BADENHUIZEN 1967).

Phosphorylase activity in the plastids was checked by incubation with glucose-l-phosphate (DYAR 1950).

2.2. Detection of nucleic acids in plastids

Three methods have currently been in use to demonstrate the presence of nucleic acids in plastids: 1) Direct localization of DNA material and ribosomes after suitable fixation; 2) Removal of the nucleic acid material by treatment with the appropriate enzymes; 3) Incorporation of labelled specific bases, *viz.* ^{3}H-thymidine for DNA and ^{3}H-uridine for RNA. We have applied these three methods essentially following the prescriptions given by SWIFT (1965) and by KISLEV *c.s.* (1965). In some cases only direct observations with the electron microscope were available, and then we based our interpretation on comparisons with electron pictures of plastid DNA published by various authors, including our own where controls had been applied. We could confirm that the morphology of the alleged DNA material depends on the type of fixation used: clumped after osmium tetroxide fixation, filamentous after the Ryter-Kellenberger fixation. Various tests were applied to chloroamyloplasts from *Pellionia daveauana*, proteinoplasts from *Helleborus corsicus* and amyloplasts from potato tubers. Direct observation was used for chromoplasts from *Solanum pseudocapsicum* and *Physalis pubescens* berries, amyloplasts from corn endosperm, and chloroplasts from *Beta vulgaris.*

In preparation for radioautography the plant material was treated with 0.1% Twin for 20 minutes and then recut in distilled water. For the demonstration of DNA the tissues were then incubated in a sterile aqueous solution containing

100μc ^{3}H-thymidine per ml. Fixation took place in glutaraldehyde, followed by osmium tetroxide, or in formalin to allow for DN-ase action. To test the presence of RNA, similar experiments were done with ^{3}H-uridine. Young leaves required a much shorter period of contact with the tritiated base (4 hours) than old leaves (12–14 hours). Sections were coated with Ilford-L4 or Gevaert NuC 307 photographic emulsion on collodion-covered slides, or the emulsion was applied to the section on a grid by means of a loupe (LETTRÉ & PAWELETZ 1966). Exposure at 4°C took 8 weeks for the 1:5 diluted Ilford emulsion, and 10 weeks for the 3:2 diluted Gevaert emulsion. The preparations were developed with Kodak D-19, Microdol X, or with a mixture that produces point-like silver grains (LETTRÉ & PAWELETZ 1966).

The electron microscope used was a Zeiss EM9.

3. RESULTS

3.1. Chloroplasts

The chloroplasts of our *Beta* plants contained relatively few grana and intergrana connections. The arrangement of these structures was that of a cup, leaving a large part of the stroma unstructured. In between the lamellae of the thylakoids, but never in the unstructured part of the stroma, less dense areas were found, in which the DNA is localized. Throughout the stroma ribosomes were clearly visible.

This picture was unaltered after a dark period of 96 hours, but neither starch nor phosphorylase were present. When such chloroplasts were allowed to photosynthesize in the buffer solution, described above, they produced assimilatory starch granules and contained phosphorylase. When actinomycin D was added in a concentration of 10μg/ml the fine structure remained essentially the same, but no starch was present, except in a few plastids that had formed tiny granules. Ribosomes were abundant, but became less so after prolonged incubation times. Incomplete inhibition of RNA-production at this concentration of the antibiotic has also been found in *Euglena* (POGO & POGO 1964). Phosphorylase production was only partly inhibited.

At a concentration of 30 μg/l and higher actinomycin D completely inhibited starch synthesis. At the same time no phosphorylase could be demonstrated. Although the lamellar structures of the chloroplast seemed to be unaltered, and DNA areas were visible as before, the stroma had assumed a coarse granular aspect and contained very few ribosomes *(fig. 1)*.

When tissues, treated with actinomycin D, were thoroughly washed with buffer solution and then kept in this solution in the light for 48 hours, phosphorylase activity returned and starch granules were formed.

Chloramphenicol completely inhibited starch production in the concentrations used, and again the effect was reversible. However, the stroma retained its ribosomes after treatment with the antibiotic.

We conclude that phosphorylase is one of the enzymes that are produced in

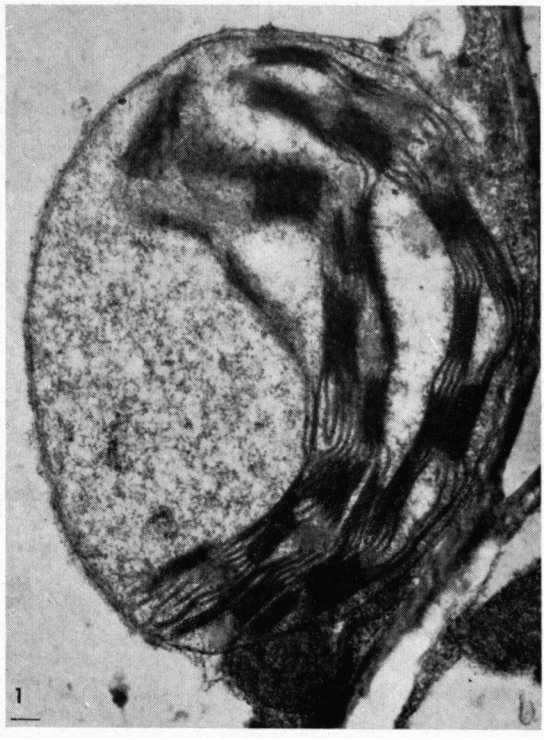

Fig. 1. A chloroplast from *Beta vulgaris*, treated for 96 hours with 100 μg/ml actinomycin D after a 96 hour dark period. Very few ribosomes are left. Fix. GA + Os, afterstaining U + Pb. Final magn. 31,500 ×.

the chloroplasts by their nucleic acids and that there is a correlation between the presence of phosphorylase and the formation of starch.

In *Pellionia* chloroplasts we frequently observed intimate connections between starch granules and DNA areas (SALEMA & BADENHUIZEN 1967). The DNA material in the chloroplasts of *Beta* tended to be clumped after fixation in OsO_4. Often fine threads were seen to connect the central mass with the surrounding stroma and the neighbouring starch granule *(fig. 2)*. Although this is almost certainly an artefact, it also points to a close proximity of DNA and starch.

3.2. Amyloplasts

Although the chloroamyloplasts of *Pellionia* have a structure which is similar to that of chloroplasts, they seem to be less developed, contain fewer grana and ribosomes, and less chlorophyll. They incorporate ^{3}H-thymidine and ^{3}H-uridine and they show fibrillar structures that can be interpreted as DNA material. In the chloroamyloplasts the DNA areas are less frequent, but generally much larger than those found in the chloroplasts. They are characteristically situated near the "pockets" which contain starch precursors (SALEMA & BADENHUIZEN 1967) *(fig. 3)* or the starch granule that has presumably crystallized from this material. The proplastids in the meristem of *Pellionia*, too, contain both nucleic acids.

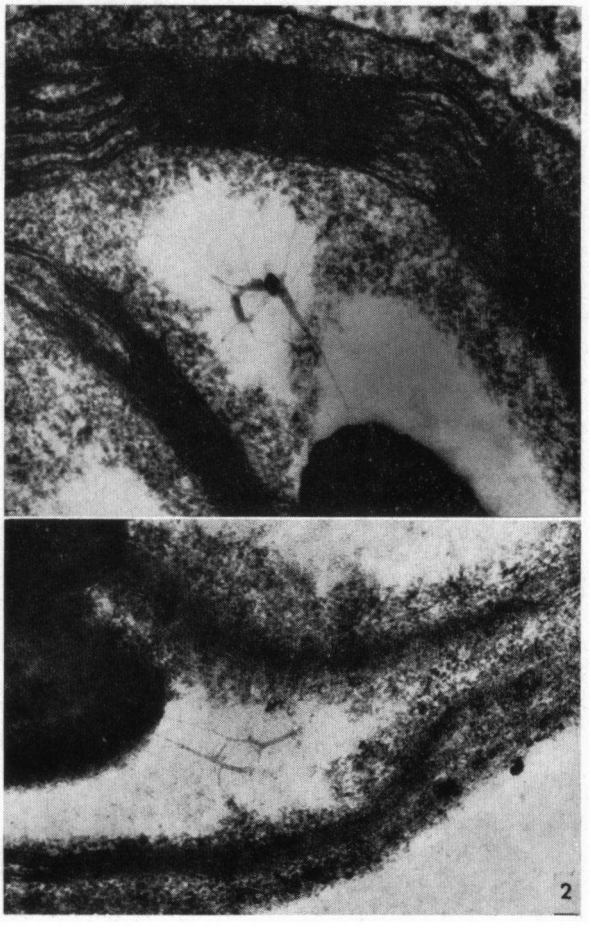

Fig. 2. The two ends of a starch granule in a chloroplast of *Beta vulgaris*. A few grana are visible and the stroma contains many ribosomes. DNA substance partly lumped, partly attached to the stroma and the starch granule with fine threads. The prints have been overexposed to make the DNA material visible. Fix. GA + Os, afterstaining U + Pb. Final magn. 90,000 ×.

The development of a photosynthetic apparatus in the amyloplasts of greening potato tubers has been described elsewhere (BADENHUIZEN & SALEMA 1967). The presence of DNA and RNA was checked with the conventional methods. DNA areas were found in the undifferentiated stroma, surrounding a pocketlike structure, but later they were exclusively situated in the lamellar system. This observation indicates a close connection between DNA and thylakoid formation. Another point of interest is that ribosomes became well-defined stroma structures only after the development of the thylakoid system in the

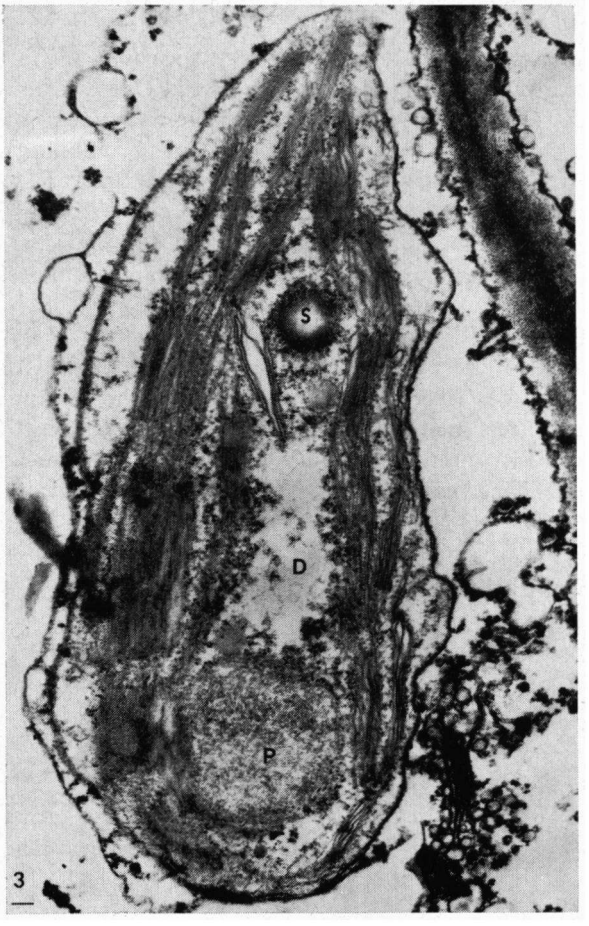

Fig. 3. Chloroamyloplast from a young leaf of *Pellionia*, showing thylakoids, a pocket (P) and a starch granule (S). A large DNA area with fine fibrils (D) is in close contact with the pocket. Kellenberger fix. Afterstaining U + Pb. Final magnif. 28,000 ×.

virescent amyloplasts was well on its way. Since ^{3}H-uridine was incorporated in all stages, whether ribosomes were visible or not, the potato amyloplast should be a good object for the study of ribosome formation.

In fixed material of corn endosperm the amyloplasts contained structures comparable to those in other plastids that could be interpreted as DNA material. After fixation in glutaraldehyde and OsO_4 less electron-dense areas appeared containing a little clumped substance, whereas after Ryter-Kellenberger fixation fine fibrils became visible. In such leukoplasts no ribosomes could be distinguished. If the interpretation is correct, the paucity of DNA would explain why its fibrillar structures were absent in many sections of the amyloplasts.

3.3. Chromoplasts

Of the chromoplasts in the mature berries of the species of *Solanum* and *Physalis* studied, only material fixed in glutaraldehyde and OsO_4 was available. Again less dense areas were found with partly fibrillar material comparable to the DNA areas in chloroplasts. Since these particular chromoplasts were derived from chloroplasts, it is perhaps not surprising that the DNA areas persisted after the lamellar structure had disappeared. The DNA could still have a function in the production of proteins, such as phosphorylase. In some cases chromoplasts become transformed into amyloplasts by forming starch granules.

3.4. Proteinoplasts of *Helleborus corsicus*

These large plastids can be easiest studied in the leaf epidermis. Their fine structure has been described by HEINRICH (1966), who was unable to detect phosphorylase, but, as mentioned above, our results were positive in this respect. The most conspicuous feature in the plastid is a core which has been termed a vacuole. This vacuole contains material which reacts to fixation fluids

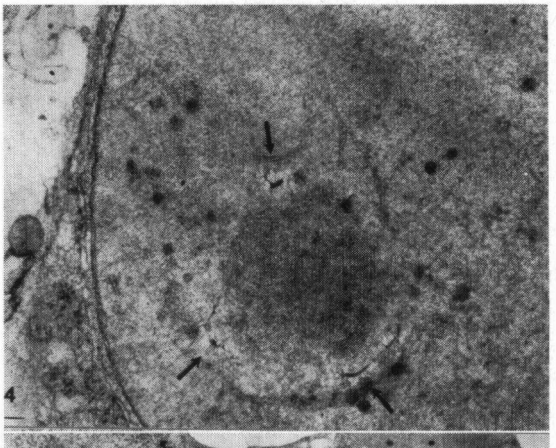

Fig. 4. Part of a proteinoplast from the leaf epidermis of *Helleborus corsicus*. Three DNA areas are visible around a concentration of electron-dense material (arrows). Fix. GA+ Os. Afterstaining U + Pb. Final magnif. 44,000 ×.

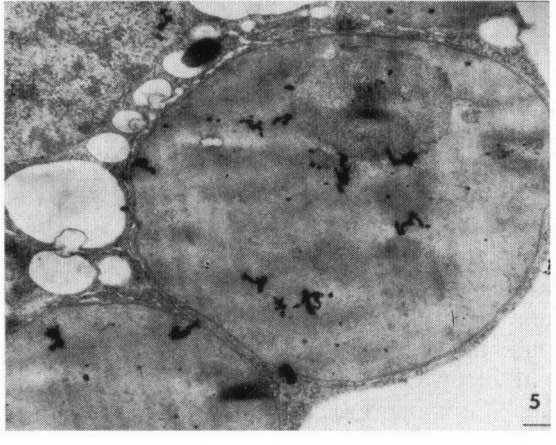

Fig. 5. Proteinoplast of *Helleborus corsicus* after incubation with ^{3}H-thymidine. Emulsion Ilford L4, developer D19. Notable are swellings of the cytoplasm which protrude into the vacuoles. Fix. GA + Os. Afterstaining Pb. Final magnif. 14, 000 ×.

in a way different from that of the stroma. The plastids are very fragile and are easily damaged during the preparation necessary for their observation. Since the vacuole occupies an eccentric position near the periphery, the plastid is easily disrupted at that site and the contents of the vacuole are emptied into the surrounding medium. The little holes left can be seen even with the light microscope. After formol fixation and treatment with various enzymes the electron microscope showed the vacuoles in various stages of discharging the contents, and this made an analysis very difficult.

Around this vacuole lumps of material can be detected after fixation in glutaraldehyde and OsO_4 *(fig. 4)* which we interpret as DNA areas. Their proximity to the vacuole may be an indication that a relationship exists between the DNA and the contents of the vacuole. The presence of DNA was confirmed by the incorporation of ^{3}H-thymidine *(fig. 5)*.

Although ^{3}H-uridine was incorporated as well *(fig. 6)*, no ribosomes of the type characteristic for chloroplasts could be detected *(fig. 4)*.

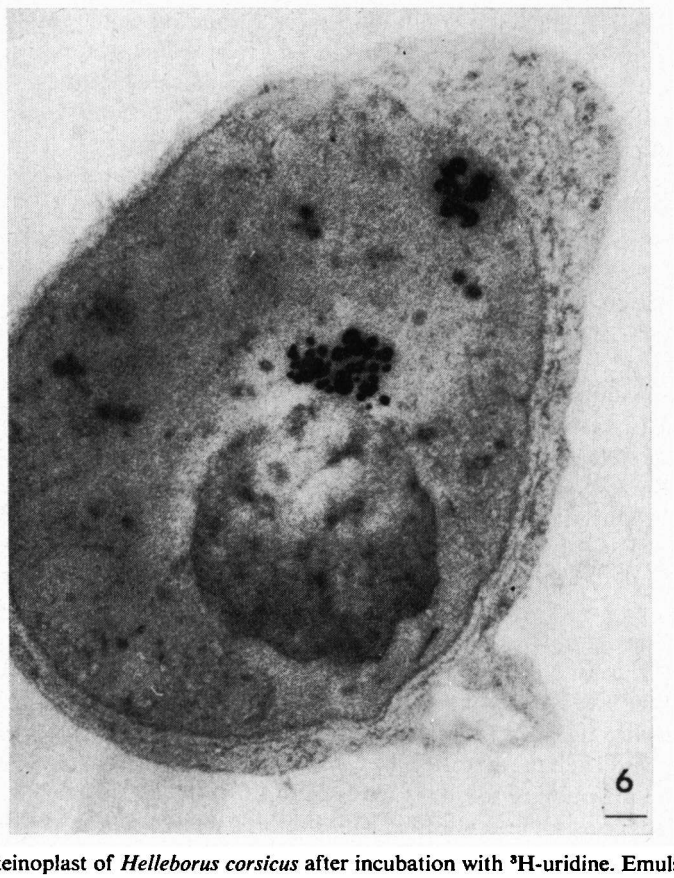

Fig. 6. Proteinoplast of *Helleborus corsicus* after incubation with ^{3}H-uridine. Emulsion Ilford L4, special developer. Fix. GA + Os. Afterstaining Pb. Final magnif. 14,000 ×.

4. Discussion

One of the features that distinguish chloroplasts from all other plastids, including proplastids, is that chloroplasts appear to lose some of their proteins during a period of darkness. This was first demonstrated for ADPG α-glucan glucosyltransferase (Chandorkar & Badenhuizen 1967), but we have now found that it also applies to phosphorylase, and it may be true for other proteins (Smillie *c.s.* 1967). We are here mainly concerned with the enzymes that are thought to be involved in starch synthesis, and of which we consider the phosphorylases to be of prime importance (Badenhuizen 1963). When leaves are re-exposed to light after a dark period the enzymes reappear, and this process can be blocked with antibiotics that interfere with RNA production or directly with protein synthesis. Actinomycin D prevents RNA synthesis (Goldberg & Reich 1964; Pogo & Pogo 1964), but since this will happen both in the nucleus and the chloroplasts, the inhibition of phosphorylase by actinomycin D leaves the question unanswered which of the two organelles is directing the production of phosphorylase. However, the fact that at a concentration of 10μg/l actinomycin D causes only partial inhibition of starch formation would indicate that the site of action is in the chloroplast itself. Moreover, at higher concentrations and long exposure time ribosomes disappear as recognizable entities. Removal of the antibiotic shows that the process is reversible: ribosomes, phosphorylase activity and starch granules all re-appear in the chloroplast. It is therefore necessary that new RNA is formed in the stroma for the reconstitution of normal conditions. Since there is evidence that this RNA is coded by the DNA in the chloroplast (Scott & Smillie 1967) the conclusion that actinomycin D directly interfered with phosphorylase production in the chloroplast seems justified.

The interference with protein synthesis caused by chloramphenicol is less ambiguous (Smillie *c.s.* 1967). Chloramphenicol is thought to prevent the binding of messenger RNA to ribosomes (Penny & Galston 1966; Pogo & Pogo 1965), and it does not affect RNA synthesis. Consequently the ribosomes remain clearly visible in the stroma even after long treatments. Again the process is reversible and needs a sufficiently long exposure time for completion.

From these experiments it would follow that phosphorylase is one of the enzymes produced by the nucleic acid system of the chloroplast itself. Although the presence of phosphorylase appears to be required for the production of starch granules, one has to remember that the enzyme ADPG α-glucan glucosyl transferase follows a similar pattern, so that no conclusion can be drawn from our experiments about the relative importance of the two enzymes in relation to starch synthesis. Both may be acting together and have different functions (Badenhuizen & Chandorkar 1965; Frederick 1968).

We consider the plastids, investigated in this work, as representative for the various types. The fact that they all contain phosphorylase and produce starch, or have the potentiality to do so, lends support to the concept that phosphorylase is produced by these plastids. This concept would gain in probability if it could be demonstrated that all types of plastids contain DNA and RNA. Although the presence of nucleic acids has been firmly established for chloro-

plasts, this is by no means the case for all plastid types, even if one could expect nucleic acids to be present on the basis of relationships between various plastids.

Chloroplasts, chloroamyloplasts *(Pellionia)*, leukoamyloplasts (potato) and proteinoplasts *(Helleborus corsicus)* were found to incorporate ^{3}H-thymidine and ^{3}H-uridine. Ribosomes are clearly defined in the stroma of plastids with a photosynthetic apparatus. We were unable to distinguish them in leukoplasts, and they became only visible during the development of a thylakoid system in light-exposed potato amyloplasts (BADENHUIZEN & SALEMA 1967). Therefore RNA is present even when there are no recognizable ribosomes.

The demonstration of DNA *in situ* can be done best in chloroplasts and chloroamyloplasts, where the removal of fibrillar structures by means of DN-ase leaves "empty" areas between the lamellae. We have noticed that the DNA areas in chloroplasts are limited to the structured parts of the plastid. The inclusion of DNA areas in the developing lamellar system was particularly clear in greening potato amyloplasts (BADENHUIZEN & SALEMA 1967), and indicates a close relationship between DNA and lamellar synthesis. During recent work on the isolation of DNA molecules from chloroplasts a close association was found to exist between DNA and the chloroplast lamellae, and the authors suggested that there may be a highly organized spatial orientation (WOODCOCK & FERNÁNDEZ-MORÁN 1968). Pictures, like the one shown in *fig. 2*, may be the consequence of such an association, although the DNA material had been profoundly changed by the fixation used.

The connection between DNA and starch granules, shown in *fig. 2*, may be entirely fortuitous, but it would be possible only if both were in close proximity. Since newly synthesized RNA was found to first appear in direct contact with the DNA (GIBBS 1967), phosphorylase could be produced *in situ* as well. Given a sufficient supply of substrate, starch molecules would be formed and a starch granule would evolve in close association with the DNA area. The chloroplasts investigated had numerous small DNA areas and produced many small assimilatory starch granules. In contrast the DNA areas in the chloroamyloplasts of *Pellionia* were few and large, closely associated with "pockets" (SALEMA & BADENHUIZEN 1967) or starch granules. The starch is of the reserve type and only one or a few granules are formed per plastid. It is therefore possible that the distribution and quantity of the DNA is of influence in starch granule formation.

It is much more difficult to demonstrate DNA in leukoplasts. In general the formol fixation as a preliminary treatment before DN-ase application proved to be unsatisfactory for amyloplasts. One may rely for the interpretation on a similarity shown to the appearance of DNA in fixed chloroplasts, but no positive test is available. The identification of "empty" areas after the action of DN-ase is also less certain and all one can say is that certain structures have disappeared. With these restrictions in mind we believe that it is nevertheless possible to recognize structures that would be interpreted as DNA material, had they occurred in chloroplasts. There is little doubt, for instance, that the arrows in *fig. 4* point to DNA areas. If this were accepted, than their close

association with the "vacuole" in the proteinoplast is again of considerable interest.

Even if the direct demonstration of DNA in some plastids meets with difficulties and requires the scanning of many photographs, the incorporation of labelled thymidine and uridine shows that both DNA and RNA are present in various plastid types. It is therefore likely that all plastids contain these nucleic acids and that they have the mechanism to produce some of their proteins, if not all. Our results indicate that phosphorylase is one of them.

Phosphorylase is the basic enzyme, or one of a complex, that produces starch molecules in the stroma of the plastid. These molecules can be seen to crowd at the surface of the growing starch granule (SALEMA & BADENHUIZEN 1967; BADENHUIZEN 1962). The size of these molecules and the way they become associated to make a wheat-, potato-, or other starch granule is controlled by nuclear genes and depends upon many factors (BADENHUIZEN 1963). Amyloplasts from different plant species show characteristic morphological features (BADENHUIZEN 1962). The chemical composition of the stroma, which sets the stage for the type of starch produced, will be equally under genetical control by the nucleus. The plastid has the necessary apparatus to carry out the orders, issued by the nucleus, by producing the necessary proteins in a prescribed quantity and conformation. It can, therefore, be expected that gene mutations will alter the activity and properties of the phosphorylases and other proteins, and in this manner influence the type of starch produced.

ACKNOWLEDGEMENTS

The work, reported in this paper, was financed by the National Research Council of Canada, the Corn Industries Research Foundation (Washington, D.C.) and the Ontario Department of University Affairs. One of us (S.) held a Fellowship of the Calouste Gulbenkian Foundation. Gifts of actinomycin D by Merck, Sharp and Dohme of Canada Ltd. (Montreal) and chloramphenicol by Parke, Davis and Co. Ltd., are gratefully acknowledged.

REFERENCES

BADENHUIZEN, N. P. (1962): The development of the starch granule in relation to the structure of the plastid. *Proc. Kon. Nederl. Akad. Wetensch. Amsterdam.* **65** C: 123–135.

— (1963): Formation and distribution of amylose and amylopectin in the starch granule. *Nature* **197**: 464–467.

— (1964): A note on green amyloplasts. *Revista Biol.* (Lisboa) **4**: 113–120.

— & K. R. CHANDORKAR (1965): UDPG alpha-glucan glucosyltransferase and amylose content of some starches during their development and under various external conditions. *Cereal Chem.* **42**: 44–54.

— & R. SALEMA (1967): Observations on the development of chloroamyloplasts. *Revista Biol.* (Lisboa) **6**: 139–155.

CHANDORKAR, K. R. & N. P. BADENHUIZEN (1967): The fate of ADPG alpha-glucan glucosyltransferase during amylolytic corrosion of starch granules, and its relation to starch granule structure. *Cereal Chem.* **44**: 27–38.

DOI, A., K. DOI & Z. NIKUNI (1966): ADP-D-glucose -α- 1, 4-glucan α-4-glucosyltransferase

in spinach chloroplasts. Partial purification and some properties of the enzyme. *Biochim. Biophys. Acta* **113**: 312–320.

DYAR, M. T. (1950): Some observations on starch synthesis in pea root tips. *Amer. J. Bot.* **37**: 786–792.

FREDRICK, J. F. (1968): Biochemical evolution of glucosyltransferase isozymes in algae. *Ann. N. Y. Acad. Sci.* **151**: 413–423.

FRYDMAN, R. B. & C. E. CARDINI (1964): Soluble enzymes related to starch synthesis. *Biochem. Biophys. Res. Commun.* **17**: 407–411.

GIBBS, S. (1967): Synthesis of chloroplast RNA at the site of chloroplast DNA. *Biochem. Biophys. Res. Commun.* **28**: 653–657.

GOLDBERG, J. H. & E. REICH (1964): Actinomycin inhibition of RNA synthesis directed by DNA. *Feder. Proc.* **23**: 958–964.

HEINRICH, G. (1966): Die Feinstruktur der "Proteinoplasten" von Helleborus corsicus. *Protoplasma* **61**: 157–163.

KISLEV, N., H. SWIFT & L. BOGORAD (1965): Nucleic acids of chloroplasts and mitochondria in Swiss chard. *J. Cell Biol.* **25**: 327–344.

LETTRÉ, H. & N. PAWELETZ (1966): Probleme der elektronenmikroskopischen Autoradiographie. *Naturwiss.* **53**: 268–271.

NEWCOMB, E. H. (1967): Fine structure of protein-storing plastids in bean root tips. *J. Cell Biol.* **33**: 143–163.

PENNY, P. & W. GALSTON (1966): The kinetics of inhibition of auxin-induced growth in green pea stem segments by actinomycin D and other substances. *Amer. J. Bot.* **53**: 1–7.

POGO, B. G. T. & A. O. POGO (1964): DNA dependence of plastid differentiation inhibition by actinomycin D. *J. Cell Biol.* **22**: 296–301.

— (1965): Inhibition by chloramphenicol of chlorophyll and protein synthesis and growth in Euglena gracilis. *J. Protozool.* **12**: 96–100.

RHODES, M. J. C. & E. W. YEMM (1966): The development of chloroplasts and photosynthetic activities in young barley leaves. *New Phytol.* **65**: 331–342.

RIS, H. (1962): Ultrastructure of certain self-dependent cytoplasmic organelles. *Electron Microscopy. 5th Internat. Congress, Philadelphia,* vol. 2, p. XX-I.

SALEMA, R. & N. P. BADENHUIZEN (1967): The production of reserve starch granules in the amyloplasts of Pellionia daveauana N. E. Br. *J. Ultrastruct. Res.* **20**: 383–399.

SCOTT, N. S. & R. M. SMILLIE (1967): Evidence for the direction of chloroplast ribosomal RNA synthesis by chloroplast DNA. *Biochem. Biophys. Res. Commun.* **28**: 598–603.

SEIDEMANN, J. & W. SCHLIEPE (1963): Zur Kenntnis der Stärke der knollig verdickten Wurzeln von Phaseolus coccineus L. *Biol. Zentralbl.* **82**: 727–731.

SMILLIE, R. M., D. GRAHAM, M. R. DWYER, A. GRIEVE & N. F. TOBIN (1967): Evidence for the synthesis in vivo of proteins of the Calvin cycle and of the photosynthetic electron-transfer pathway in chloroplast ribosomes. *Biochem. Biophys. Res. Commun.* **28**: 604–610.

SPREY, B. (1966): Beiträge zur makromolekularen Organisation der Plastiden. II. Zur Verbreitung von DNS in Plastiden. *Z. Pflanzenphysiol.* **54**: 386–394.

SWIFT, H. (1965): Nucleic acids of mitochondria and chloroplasts. *Amer. Naturalist* **99**: 201–227.

THALER, I. (1955): Die Leukoplasten von Helleborus. *Protoplasma* **44**: 437–443.

TSAI, C. Y. & O. E. NELSON (1968): Phosphorylases I and II of maize endosperm. *Plant Physiol.* **43**: 103–112.

WOODCOCK, C. L. F. & H. FERNÁNDEZ-MORÁN (1968): Electron microscopy of DNA conformations in spinach chloroplasts. *J. Mol. Biol.* **31**: 627–631.

ZUBER, M. S. (1965): Genic control of starch development. In: R. L. Whistler and E. F. Paschall (editors), *Starch: Chemistry and Technology.* Vol. I, pp. 43-63. Academic Press, New York and London.