Plastoglobuli and the fine structure of plastids

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Endeavour 27, 144–149 (1968)

Reprinted from Endeavour, Volume XXVII, Number 102
September 1968

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The electron microscope has shown that the chloroplasts of plants contain, in addition to the lamellar thylakoids that perform the light reactions of photosynthesis, numerous distinct spherical bodies, known as plastoglobuli. It is possible to isolate these particles unchanged from the entangling lamellae and their size and number can be shown to vary in different groups of plants.

The ability of plant cells to photosynthesize is located in special organelles, the chloroplasts. As seen in thin sections in the electron microscope, functional chloroplasts show a lamellar fraction (granal and intergranal thylakoids) which is embedded in a chlorophyll-free protein matrix, the stroma. The two phases are separated from the cytoplasm by a double membrane. The thylakoids carry out the photochemical reactions of photosynthesis and the associated electron-transport reactions which lead to the liberation of oxygen, to photophosphorylation (ATP-formation), and the reduction of NADP. The thylakoids consist of 50 per cent lipid and 50 per cent protein, and the composition of the lipid fraction is to a large extent known. Functional thylakoids contain, besides chlorophylls and carotenoids, various phospholipids, the surface-active galactolipids, a sulpholipid, and the group of lipophilic plastid quinones [1].

In the investigation of the ultrastructure of chloroplasts the main interest was at first directed to the origin and fine structure of the photochemically active thylakoids. In the last few years other normal chloroplast structures have also been examined more thoroughly, in particular, the osmiophilic plastoglobuli. Recent work shows that these lipid-containing bodies occur at all stages in the growth and differentiation of plastids: at some stages they may appear in large numbers and at others reach specially large sizes [2].

This article gives an account of their size, frequency, and physiological significance. Of particular interest on the last count is the relation between the globuli content of the chloroplasts and the prominence of their lamellar fraction.

Size and structure of the plastoglobuli in functional chloroplasts

Electron micrograms of chloroplasts after OsO_4 fixation show the thylakoids and also—as round, electron-dense bodies—the osmiophilic plastoglobuli. These lipid inclusions occur in the stroma of the chloroplasts and have no direct contact with the photochemically active thylakoids. They can be recognized clearly only after OsO_4 fixation. The smaller ones (30–100 nm diameter) appear in thin sections as circular, uniformly blackened structures (figure 2), but larger plastoglobuli often show an uneven blackening and are usually elongated in the direction of the long axis of the plastid. The average ratio of length to breadth is 1·5 (figure 3). The strongly staining plastoglobuli are never surrounded by a limiting membrane [2–4].

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The smaller plastoglobuli are not fixed clearly by potassium permanganate, which is often a good means of demonstrating lamellar structures. Larger globuli (diameter >300 nm) are surrounded, after $KMnO_4$ fixation, by a single peripheral membrane, possibly a precipitation membrane. Their contents are mostly transparent and, with after-staining, finely granular (figure 4). The peripheral membrane usually has a jagged, star-shaped configuration, which makes the identification of the globuli rather more difficult.

Individual plant species show definite differences in the size ranges of their plastoglobuli. The smallest diameters lie in the range 30–50 nm, but there are considerable specific differences in the average and maximal sizes (figure 1). An accurate measurement of the size is possible only with isolated particles, since in thin sections the globuli are often cut near the edge. The spherical form is clearly recognizable when isolated particles are shadowed (figure 5). Within a species the size of the plastoglobuli depends on the age of the plant and its physiological condition. In young, functional chloroplasts of *Spinacia* the average diameter is 80 nm, with individual values ranging from 30 to 120 nm. In older spinach chloroplasts the size range lies between 30 and 220 nm, with the greatest frequency at 110 nm.

The increase in size of the globuli has been described by different authors for several species [5, 6]. The youngest stages always show the smallest diameters. In young chloroplasts, still in the stage of thylakoid synthesis and multiplication, the size and number of the plastoglobuli alter very little. Only when the formation of the lamellar system is coming to an end does the size of the globuli increase. Their number may then also rise [2]. In most of the plants so far investigated a continuous increase in the mean diameter of the globuli can be established throughout the period of vegetative growth right up to the final degeneration of the chloroplasts. Certain regularities occur which allow the chloroplasts of different plants to be classified, with respect to the size and number of their plastoglobuli, under two main headings.

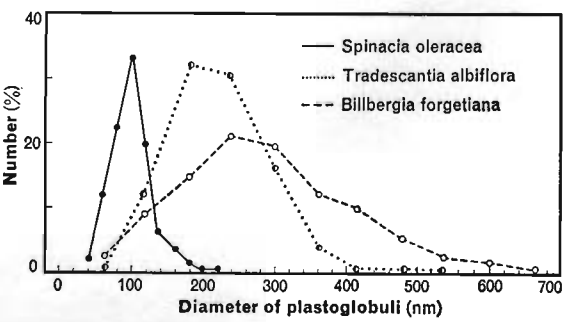
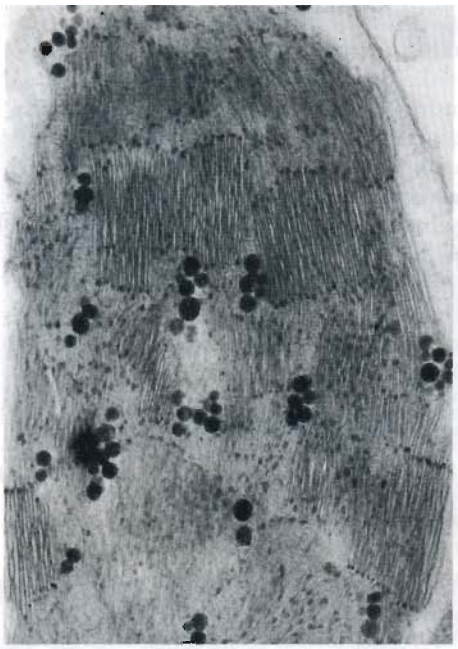


Figure 1 Size distribution of isolated plastoglobuli. (From Lichtenthaler and Sprey, [3])



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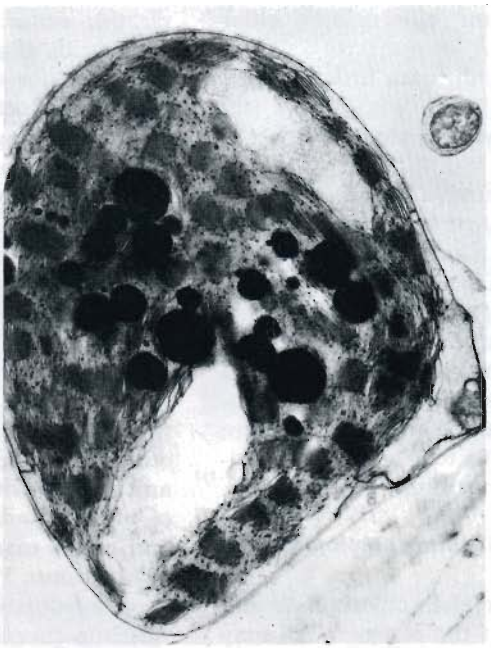

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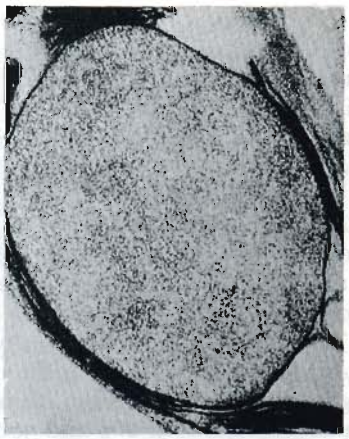

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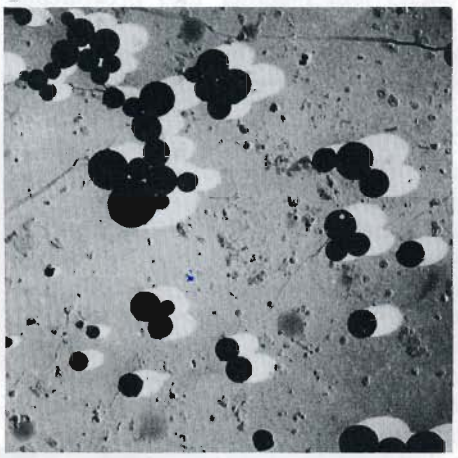

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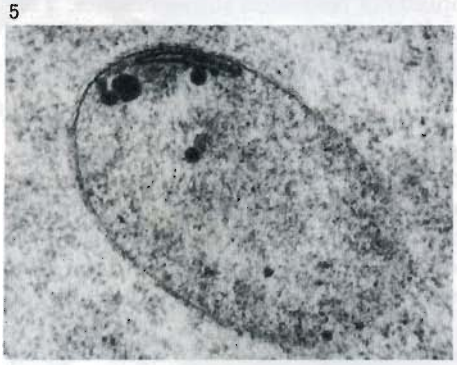

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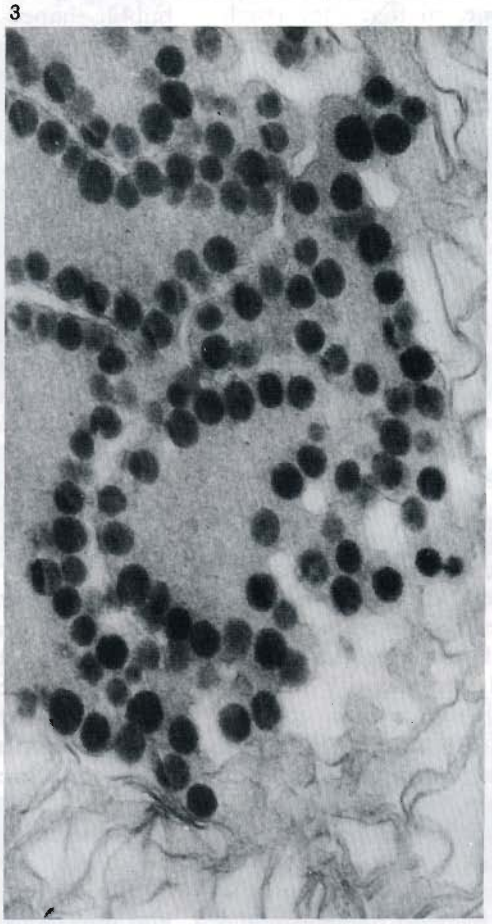

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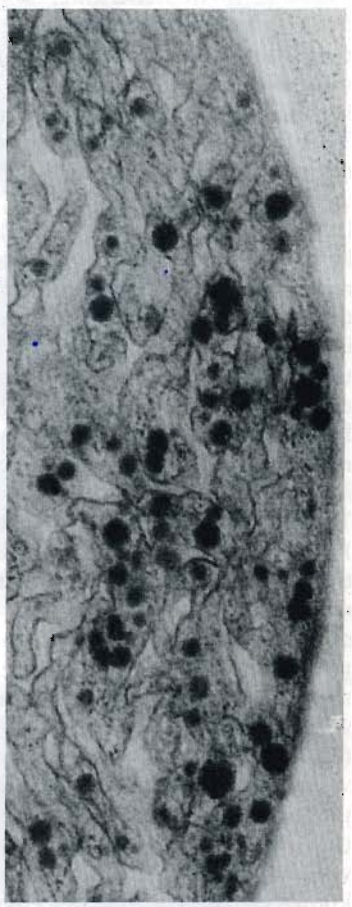

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Figure 2 Chloroplast of *Allium cepa.* (× 26 000)

Figure 3 Chloroplast of *Hoya carnosa.* (× 9000)

Figure 4 Plastoglobuli from *Billbergia forgetiana* ($KMnO_4$-fixation × 43 000). (From Lichtenthaler and Sprey [3] by permission of the publisher.)

Figure 5 Isolated globuli with chromium shadowing. (× 9000)

Figure 6 Proplastid of *Allium cepa.* (× 41 000)

Figure 7 Pyrenoid region of the green alga *Pleurococcus.* (× 43 000)

Figure 8 Blue-green alga *Nostoc.* (× 50 000)

By comparison with the young chloroplasts, older chloroplasts show either:

I. A larger number of plastoglobuli, with little increase in size, or

II. Much-enlarged plastoglobuli with an almost unaltered number.

The chloroplasts of many annuals (*Spinacia*, *Capsella*, *Hordeum*, *Allium*) belong to the first type. The average diameter of plastoglobuli lies, as a rule, between 80 and 120 nm, while individual globuli can attain a maximum of 250 nm. The chloroplasts of perennials (*Billbergia*, *Tradescantia*) or of the leaves of trees and shrubs (*Fagus*, *Aucuba*, *Hoya*) belong, on the other hand, to the second group. Here the average diameters in the older chloroplasts lie between 200 and 300 nm (figure 1); but there are almost continuous graduations to 1 μm, and individual globuli can occur up to a maximum of 2 μm.

The behaviour of the plastoglobuli during chloroplast breakdown

When the chloroplasts degenerate, their chlorophylls and thylakoids are broken down; but the plastoglobuli may continue to increase their volume, so that they reach their greatest diameters during the chloroplast degeneration stage [6]. Results so far obtained with *Spinacia* seem to show that there are only trifling increases in herbaceous annuals whose chloroplasts belong to Group I. The situation is, however, quite different with deciduous trees belonging to Group II. Thus a marked increase of volume has been measured in *Ginkgo* [6]; and the sun-leaves of beech (*Fagus sylvatica*) also contain particularly large plastoglobuli in their yellow breakdown stage. The mean diameter is 1·5 μ and the maximum 5 μ. Part of the lipids available from the breakdown of the thylakoids is deposited in the plastoglobuli. It depends on the duration of the yellowing stage preceding the death of the chloroplasts whether any synthesis of new lipids occurs. It is possible that during the final stages groups of globuli may run together into larger ones.

The size of plastoglobuli in different taxonomic groups

From micrographs of the fine structure of plastids obtained by the author and others, the occurrence and size of the plastoglobuli has been investigated in the chloroplasts of mosses, ferns, and flowering plants, and in the chomatophores of the algae. In the majority of plants the plastoglobuli show diameters between 30 and 200 nm, with the greatest frequency at 100 ± 30 nm. Globuli of this size are found in the photosynthetic apparatus of the various algal groups, and of mosses, ferns, and flowering plants, though in the last only in young chloroplasts. In the older chloroplasts of group II, large increases of volume may occur as already described, but so far as is known at present this does not seem to happen in the other plants. As regards the size and frequency of their plastoglobuli, the chloroplasts of these plants therefore seem to belong to the group I described above. Symbiotic algae, for example *Pleurococcus* from *Xanthoria parietina* (figure 7), and blue-green algae (figure 8) also have spherical osmiophilic inclusions similar in size to those already described.

Frequency of plastoglobuli at different stages of development and differentiation of plastids

(a) *Proplastids.* In actively dividing cells (meristems) the plastids occur in a much simpler form, namely the proplastids of about 1 μm diameter; these have neither pigments nor thylakoids. They are often distinguished from the mitochondria, which are of similar size, only by containing single plastoglobuli. Even in the following stage, when invaginations of the inner membrane and small starch grains are present, there are only a few plastoglobuli with diameters of 30–130 nm (figure 6).

(b) *Colourless amyloplasts.* In the course of cell differentiation the proplastids may develop into colourless, starch-storing amyloplasts (roots and storage organs). The size and number of their plastoglobuli remain the same as in the preceding, proplastid stage (figure 9).

(c) *Leucoplasts.* Proplastids can also develop into unpigmented leucoplasts (epidermal cells, white petals, etc.). They then contain vesicles resembling thylakoids and globuli with diameters of 30–160 nm. The number of plastoglobuli is very variable; some stages have few and others many (figures 10 and 15).

(d) *Etioplasts.* When seedlings are grown in the dark they become etiolated, and the differentiation of the proplastids into functional chloroplasts does not occur. Small bubble-shaped vesicles join together into prolamellar bodies, often with a lattice structure. The etioplasts contain numerous plastoglobuli (30–90 nm) which usually lie either singly within the prolamellar body or in groups round its edge (figure 11). On illumination, a rapid synthesis of thylakoids follows, while the prolamellar body breaks up and the number of plastoglobuli is usually reduced. Part of the lipid stored during the dark in the plastoglobuli is evidently used in the synthesis of the thylakoids. When formation of the lamellar fraction of the chloroplast is complete, plastoglobuli of the same size as in etioplasts and young chloroplasts appear again.

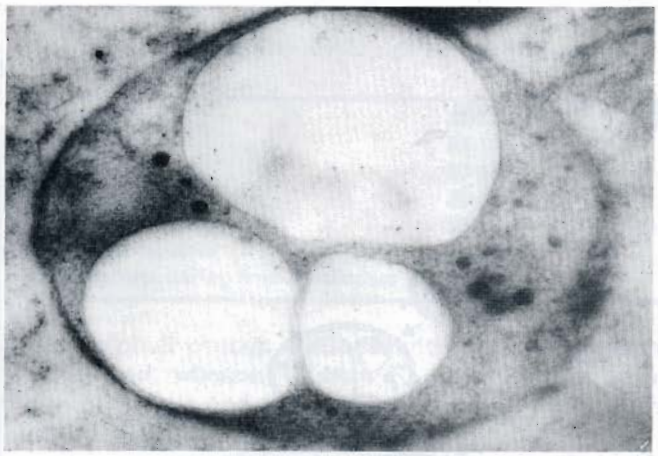
(e) *Chloro-amyloplasts.* Chloroplasts may be transformed, for example in the spongy parenchyma of leaves, to green chloro-amyloplasts which store starch and contain pigments and thylakoids (figure 12). Functionally, they are akin to the amyloplasts of colourless storage tissues. The size of their plastoglobuli corresponds with that of the chloroplasts in the palisade tissue, but they are less numerous.

(f) *Chromoplasts.* During the ripening of the skin of many fruits the chloroplasts (figure 13) are converted into yellow or red chromoplasts (figure 14). Breakdown of the thylakoids is accompanied by synthesis of the secondary carotenoids which cause the change of colour. The number of plastoglobuli is much increased, and the diameter is greater (30–300 nm) than in young chloroplasts.

A scheme of plastid development based on the size and number of the plastoglobuli at the different stages of differentiation is given in figure 15. As regards the origin and multiplication of the thylakoids, this scheme agrees with those developed by other authors [7]. The actual sizes of the plastoglobuli at the different stages of plastid development are summarized in Table 1.

The lipid composition of isolated plastoglobuli

After disintegration of isolated whole chloroplasts the plastoglobuli may be separated from the photochemically active thylakoids by high-speed centrifugation in an ultracentrifuge (1–2 hours at 100 000–150 000 *g*). The lipid-rich plastoglobuli, with a density of less than unity, form a yellowish cream at the top or the inner upper rim



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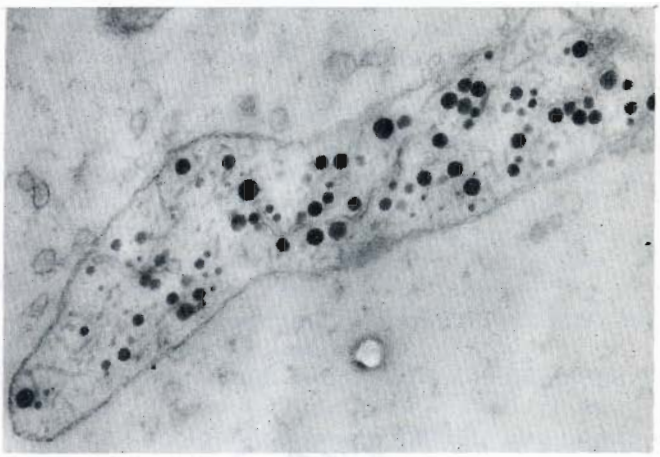

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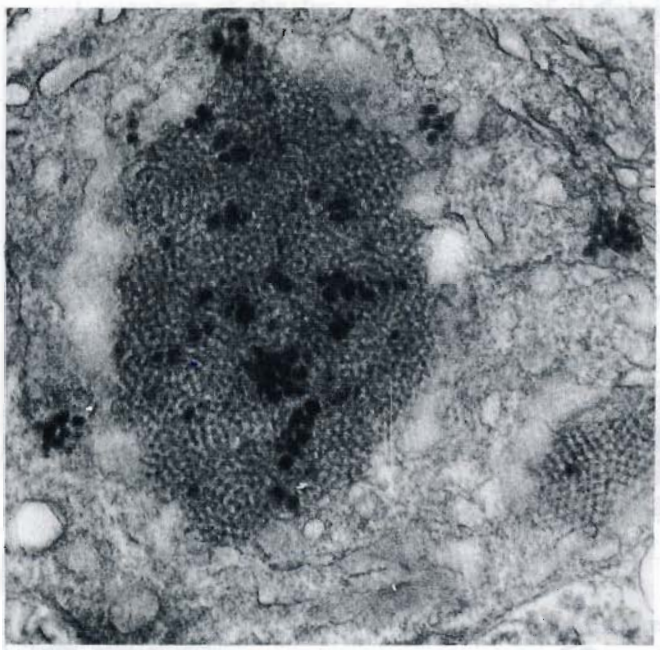

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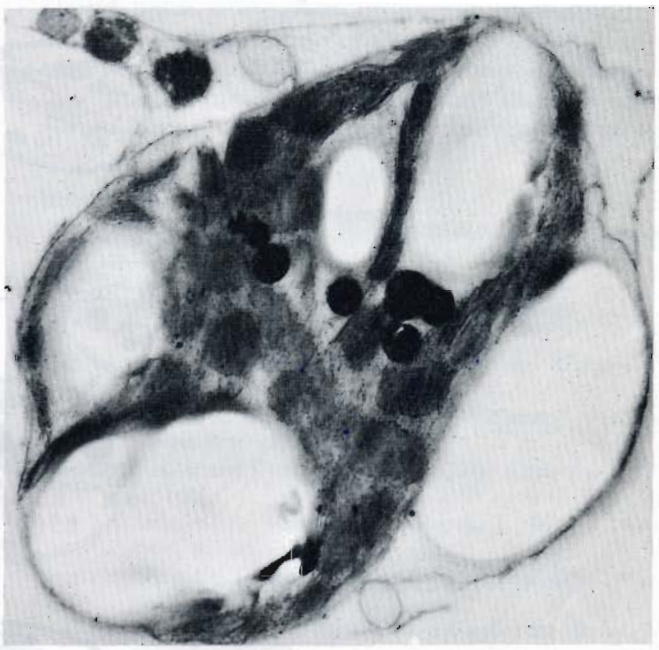

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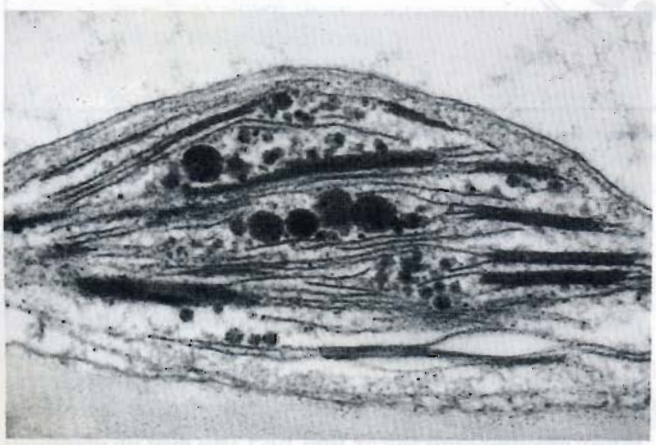

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Figure 9 Amyloplast of *Allium cepa.* (× 26 000)
Figure 10 Leucoplast of *Allium cepa.* (× 24 000)
Figure 11 Etioplast of *Hordeum vulgare.* (× 32 000)

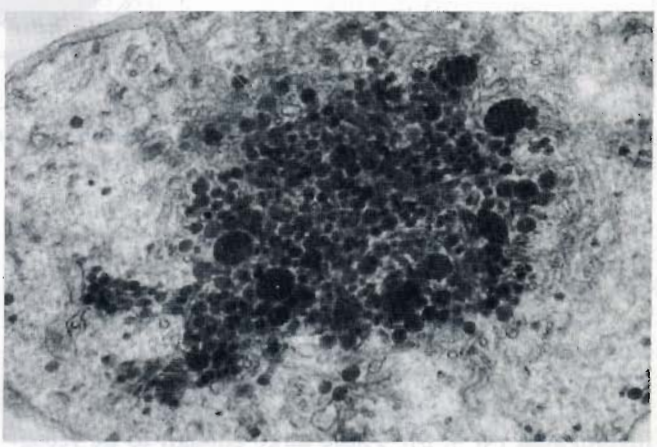

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Figure 12 Chloro-amyloplast of *Hoya carnosa.* (× 10 000)
Figure 13 Chloroplast of *Capsicum* fruit. (× 25 000)
Figure 14 Chromoplast of *Capsicum* fruit. (× 33 000)

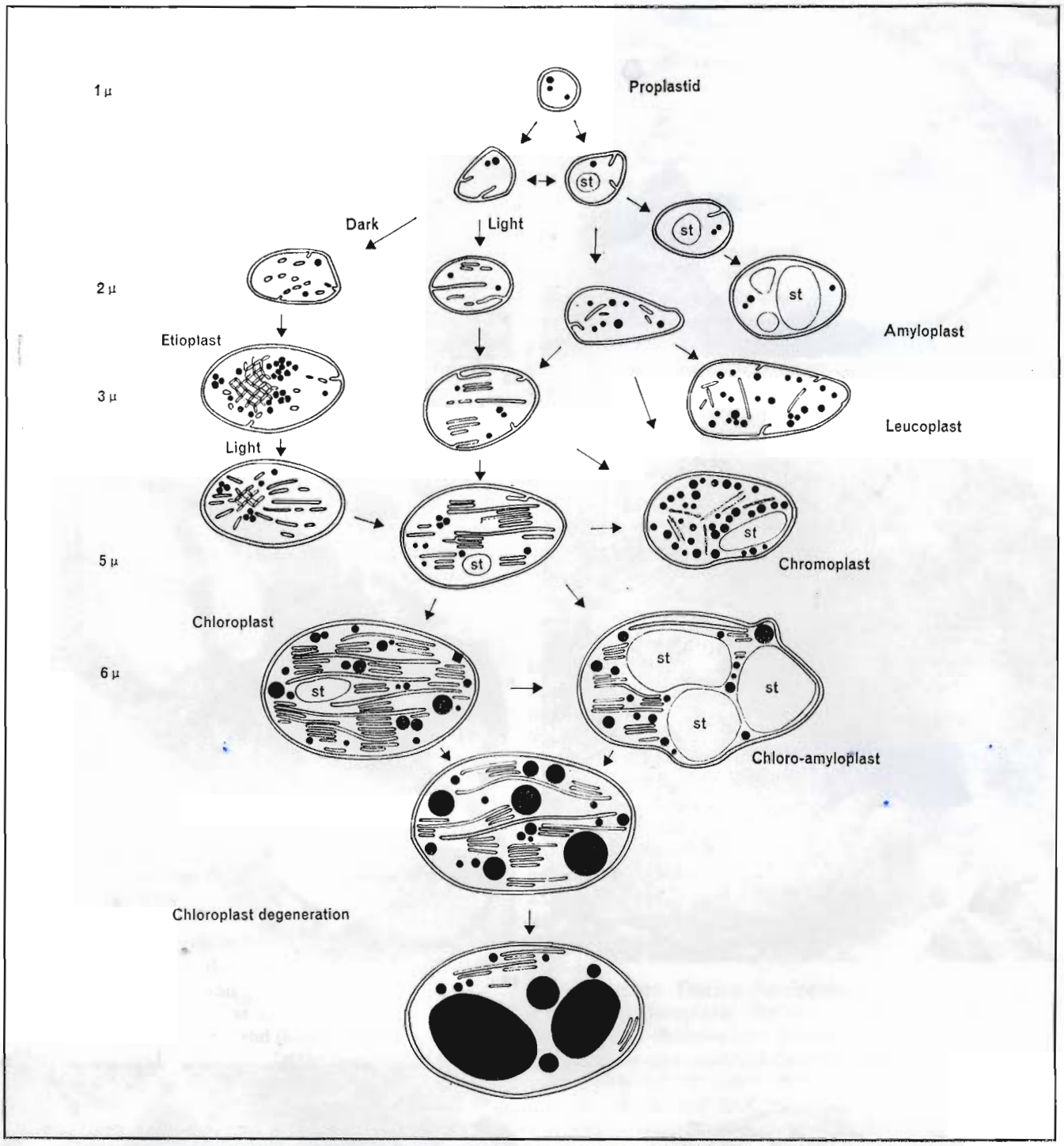

Figure 15 Scheme of plastid development depending on the size and number of their osmiophilic plastoglobuli. St = starch. (After Lichtenthaler [2].)

of the centrifuge tube. Several methods have been used for disintegrating chloroplasts prior to centrifugation, namely short periods of ultrasonic treatment, osmotic treatment with a hypotonic buffer solution, grinding with alumina, use of a needle valve disintegrator, and shaking with glass beads in a cell homogenizer [3, 5, 8, 9].

So far, the plastoglobuli of only a few plants have been investigated [3, 5, 8]. Those large enough to be recognized under the light microscope can be stained with fat reagents. Small globuli persist as separate particles after isolation at 4°C; but at temperatures above 10°C they run together into larger ones that can be stained with fat-soluble dyes. Their behaviour in the centrifuge also points to a high lipid content.

The main lipids of isolated plastoglobuli are the lipophilic plastidquinones. These are fat-soluble quinones of benzo- or naphthoquinone type which occur as biological oxidation-reduction catalysts in the photochemically active thylakoids. Their occurrence in the osmiophilic plastoglobuli shows that they can also be deposited outside the thylakoids. Individual substances identified include plastoquinone-45, α-tocoquinone and its chromanol, α-tocopherol (Vitamin E), and the naphthoquinone, Vitamin K_1. Compared with whole chloroplasts the

Table I
Size of the plastoglobuli (in nm) at different stages of plastid differentiation

Proplastids	30–130
Etioplasts	30–100
Leucoplasts	30–160
Chromoplasts	30–300
Young chloroplasts	30–150
Older chloroplasts	30–2000
Chloroplasts during final breakdown	30–5000

plastoglobuli contain predominantly the benzoquinone derivatives, whereas Vitamin K occurs only in lower concentrations and is therefore localized chiefly in the thylakoids.

It is not yet certain what other chloroplast lipids are contained in the plastoglobuli. Galactolipids have indeed been demonstrated in the globuli-fraction in two cases [5, 8], but the possibility that they originated in a contamination of the fraction by pieces of thylakoid cannot be excluded. Chlorophylls and carotenoids do not occur in the plastoglobuli of chloroplasts. A trace of pigment found at first was no longer present when the fraction had been purified by the removal of thylakoid fragments. Phospholipids, sulpholipid, and free fatty acids are other possible components.

The lipid composition of the plastoglobuli seems in fact to depend upon the stage of development. Thus, during the breakdown of chloroplasts, the plastoglobuli contain carotenoids as well as lipoquinones. Likewise, the more numerous globuli of etioplasts and chromoplasts may store, alongside their lipoquinones, carotenoids and secondary carotenoids.

The function of plastoglobuli as stores of excess lamellar lipids outside the thylakoids

Investigation of the occurrence and abundance of globuli in plant plastids has shown:

1) that the plastoglobuli are normal lipid components of the photosynthetic apparatus of plants of varied taxonomic status and occur at all stages of plastid differentiation, and

2) that there is a close connexion between plastoglobuli-content and the development of the lamellar fraction in chloroplasts. If thylakoid synthesis is prevented or the thylakoids break down there is an increase in the number or volume of the plastoglobuli.

The etioplasts can be cited as a typical example. Lamellar lipids synthesized in the dark are deposited in the plastoglobuli and not in thylakoids, because there are none. Globuli are also numerous in *Xantha*-3 mutants of barley, which are unable to form functional thylakoids; and the same happens when thylakoid development is prevented by lack of nutrients or by treatment with chemicals [10].

After the completion of the lamellar fraction, an increase in the number of plastoglobuli may occur with the ageing of the chloroplasts. In both stages excess lamellar lipids are stored in the plastoglobuli. The predominant lipids involved are the lipophilic plastidquinones whose syntheses continue steadily after those of the thylakoids and chlorophylls are finished. The plastoglobuli thus represent in chloroplasts an extra-thylakoidal reservoir for excess lipids.

In the breakdown of the thylakoids during the final collapse of the chloroplasts, or in the conversion of chloroplasts to chromoplasts, there is also usually an increase in either the number or volume of the plastoglobuli. Here they are acting as post-thylakoidal stores of lipid.

Whether the plastoglobuli act solely as storage sites of plastid lipids outside the thylakoids, or possibly have other physiological functions over and above this, can only be settled by further work. A complete analysis of their lipid composition at the various stages of plastid differentiation will bring us nearer to solving the problem.

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