AN ELECTRON-MICROSCOPIC STUDY OF THE STARCH-CONTAINING PLASTIDS IN THE FERN TODEA BARBARA

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SUMMARY

Gametophytes of Todea barbara grown in vitro have two types of starch-containing plastids. The first type are chloroplasts having well-developed grana regions connected by intervening membranes and little deposition of starch. The other type are amyloplasts usually containing abundant starch deposits and stacks of membranes which resemble the grana of the chloroplast but are not connected. Even when little starch is present, the latter type has very few internal membranes.

Membrane spacing and gross organization of the mature plastid of both types suggests similar morphological development. However, when and where in this development known physiological differences arise is not yet known.

INTRODUCTION

An amyloplast is the organelle within the cell responsible for the elaboration and storage of starch. Polymerization of starch from glucose, however, is not a diagnostic criterion for an amyloplast, for both proplastids and chloroplasts have this capacity (Frey-Wyssling & Mühlethaler, 1965). Under certain environmental conditions, such as high light intensity or continuous exposure to light, chloroplasts can become completely filled with starch (von Wettstein, 1958). In these instances, an amyloplast cannot be distinguished from a 'saturated' chloroplast with any degree of certainty. When such chloroplasts are placed in the dark, the starch is gradually broken down and new internal membranes develop from a quasicrystalline structure called the prolamellar body. Other cells in the same plant contain organelles filled with starch which does not break down, even after 5 days in darkness, and these organelles are regarded by von Wettstein as amyloplasts. However, this criterion permits one to distinguish between the organelles only on the basis of their behaviour with respect to starch storage when placed in the dark.

The literature dealing with the structure of the amyloplast is not extensive and varies with the taxon in question. Electron micrographs of oat endosperm published by Buttrose (1960) show that starch deposition begins in plastids containing numerous very elongated thylakoids. Von Wettstein (1958) has observed that the amyloplasts of Picea store starch in the stroma or in association with a few lamellae. In addition,

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structures are present within the amyloplasts which resemble the prolamellar bodies formed by proplastids which develop in the dark. Setterfield, Stern & Johnston (1959) show micrographs of the amyloplasts of wheat and pea embryos in which no internal membranes are apparent.

This paper describes the amyloplast as found in the gametophyte of a fern, *Todea barbara* (L.) and discusses its structural similarities to the chloroplast.

MATERIALS AND METHODS

Gametophytes of *Todea barbara* were grown *in vitro* on a modified Knudson's solution (DeMaggio, 1961) and maintained at 25 °C on a light regime of 12 h of light and 12 h of darkness. 'Gro-Lux' fluorescent tubes were used as a light source and the illumination on the gametophytes was 130 ft-c.

For electron microscopy, gametophytes were fixed for 3 h in 2.5% glutaraldehyde in 0.05 M cacodylate buffer at a temperature of 4 °C and at a pH of 7.2. They were then washed overnight in several changes of cold cacodylate buffer with 0.25 M sucrose. The material was post-stained in buffered 1% osmium tetroxide for 1 h, dehydrated through a graded ethanol series and embedded in Araldite. Sections were cut on a Huxley microtome, stained with lead citrate (Reynolds, 1963), and examined on a Philips EM 200.

RESULTS

In the vegetative cells of the gametophyte, two types of plastids were observed. The first of these is illustrated in Figs. 1 and 2. Each plastid in this category is traversed by more or less parallel membranous sacs (thylakoids). The sides of adjacent thylakoids become tightly apposed or confluent in regions resembling chloroplast grana, but are separate and less highly ordered in the intervening areas (Fig. 2). It appears that each thylakoid is closed immediately beneath the inner of the pair of membranes limiting the plastid (Fig. 2). Electron-opaque bodies about 0.1 μ in diameter, perhaps representing lipid droplets, together with relatively small deposits of starch frequently occur throughout the finely granular matrix in this plastid type.

The width of the apposed membranes in the grana regions is about 70–80 Å and of the thylakoid lumen about 50–60 Å. The centre-to-centre spacing of constituent membranes of this region was found to be about 140–150 Å.

The major structural features of the second type of plastid are illustrated in Figs. 3–5. This organelle is also limited by a pair of membranes. Similarly, within the plastid are present a granular matrix, electron-opaque droplets, membranes and starch granules, but these components are in strikingly different proportions from those of the first type. Figure 3 exemplifies a plastid in which the content of starch is relatively low, and in which the matrix is extensive and contains numerous stacked membranes with a centre-to-centre spacing of 140–150 Å. In contrast to those of the first type of plastid discussed, these grana-like regions are infrequently linked by intervening membranes.
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A further structural specialization occurring in these organelles is illustrated in Fig. 3. Occasionally, the inner and outer limiting membranes are locally traversed by regularly placed dense structures, repeating at intervals of about 120 Å. One of the two examples of this feature included in Fig. 3 occurs at a constricted region linking the main organelle with a subsidiary portion. The function of those modifications of the plastid envelope is not at present known.

It should be stressed that the two types of plastid described above typically occur together within each of the vegetative cells of the gametophyte. While the micrographs have prominently been selected to illustrate aspects of plastid structure, they also include other details of nuclear and cytoplasmic organization, indicated in the figure captions.

DISCUSSION

The plastids described in this paper can be divided into two well-defined categories on the basis of quantitative differences in the proportions of their internal structures. In this material the organelles containing abundant internal membranes and limited deposits of starch are apparently chloroplasts. Their highly organized differentiation into grana and intergrana regions is characteristic of typical chloroplasts and they also occur in far greater numbers, within each gametophyte cell, than do the other plastids described.

On the other hand, we believe the organelles depicted in Figs. 3 and 5 to be amyloplasts. They commonly have less highly developed internal systems of membranes and relatively large deposits of starch. However, even when very small amounts of starch are present, their complement of internal membranes is limited and the stroma is extensive. The starch is always stored in the stroma, either without or accompanied by only a few short thylakoids.

Although no information on the morphogenesis of amyloplasts is available from this study, the organelles illustrated in Figs. 4 and 5 appear to represent more advanced stages of starch accumulation. In each instance, internal membranes are discernible but, together with the stroma, appear to become progressively reduced as starch deposits increase in size. It seems most unlikely that these are examples of chloroplasts saturated with starch, rather than amyloplasts. The low light intensity and 12 h photoperiod under which the gametophytes were grown would presumably not support photosynthesis to the extent that starch would be stored in such quantities in the chloroplast.

None of the amyloplasts observed in this study exhibited pro-lamellar bodies since they were described from actively photosynthesizing tissue and not from areas of the plant not exposed to light. In all other respects, the structure of the amyloplast of Todea corresponds closely to that of Picea. The amyloplasts of barley (Buttrose, 1960) have much elongated thylakoids which are not disposed in parallel stacks, whereas the amyloplasts of Todea are relatively shorter and aligned into grana-like structures. It should be pointed out that the term 'grana-like' was suggested by the similarity in appearance and membrane spacing between these structures and the...
grana of the chloroplasts. The function of the internal membranes of the amyloplast is not known, although it has been suggested, by analogy with the cristae of the mitochondrion, that they might be associated with starch-synthesizing enzymes (Buttrose, 1960).

The ontogenetic relationships which may exist between the various types of plastid within a cell have long been a subject of conjecture. Membrane spacing and gross organization of the mature plastids of both types studied here suggest that they may be derived by similar morphological development. However, when and where known physiological differences arise in this developmental sequence has not yet been established. In this preliminary study, morphological evidence for the presence of DNA and RNA in Todea plastids has not yet been obtained, though elsewhere these organelles have been shown (Gibor & Granick, 1964; Jacobson, Swift & Bogorad, 1963) to contain an autonomous genetic system which in part controls their biochemical properties.

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REFERENCES


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Fig. 1. Low-power electron micrograph of a chloroplast in a cell of Todea gametophyte. Note the well-defined grana (g) and intergrana membranes (ig), and the dual membranes limiting the organelle (*). Within the stroma (sm) are situated small electron-opaque granules, presumably of lipid material (l). Numerous small starch granules are included (s) together with a larger ovoid starch granule (g). × 48000.

Fig. 2. Portion of a chloroplast at higher magnification. Note the grana (g), intergrana membranes (ig) and the restricted granular stroma (sm). The two membranes of the chloroplast envelope are resolved (*). It appears that the internal membranes of this organelle are deposed as parallel series of sacs (thylakoids). These are closed peripherally (thin arrows) and the surfaces of adjacent thylakoids become confluent or tightly apposed at intervals to form the grana regions (thick arrows). × 120000.
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Fig. 3. An amyloplast within a gametophyte cell of Todea. The organelle is limited by an envelope of two membranes (•) and exhibits an extensive granular matrix (sm). Within the latter occur lipid droplets (l), large starch granules (s) and well-defined membranous structures (g') somewhat resembling chloroplast grana (cf. Fig. 2). Within the cytoplasm surrounding the amyloplast occur mitochondria (m), cisternae of the rough-surfaced endoplasmic reticulum (er) and a portion of a membrane-limited vacuole. \( \times \) 62 000. The numerals 1 and 2 indicate the specialized peripheral areas described in the text. Area 1 is shown enlarged in the inset, \( \times \) 120 000.
Starch-containing plastids
Fig. 4. A field similar to that shown in Fig. 3 illustrating an amyloplast with more extensive deposits of starch (s) and lipid droplets (l). Small numbers of grana-like membranes (g') are present within the restricted stroma (sm) and the organelle is largely filled with starch granules up to 1.5 μm in diameter and is limited by an envelope of two membranes (•). Other cytoplasmic structures are present as in Fig. 3. × 60,000.
Fig. 5. Several amyloplasts from the gametophyte of *Todea* replete with starch deposits (s) and containing lipid droplets (l) and very sparse grana-like membranes (g'). Note the lobate nucleus (n) and inner and outer membranes of the nuclear envelope (ne), mitochondria (m) and vacuoles (v). \( \times 48,000 \).
Starch-containing plastids