OBSERVATIONS ON THE MECHANISM OF CHLOROPLAST DIVISION IN HIGHER PLANTS

BY RACHEL M. LEECH

Department of Biology, University of York, England

W. W. THOMSON AND K. A. PLATT-ALOIA

Department of Botany and Plant Sciences, University of California, Riverside, California 92521 U.S.A.

(*Accepted 23 May 1980*)

SUMMARY

The sequence of changes in membranes that occur during the division of young green chloroplasts is described from observations in the electron microscope of thin sections of leaf tissue of *Triticum aestivum* var. Maris Dove, *Atriplex semibaccata* R.Br. and *Sesamum indicum* var. glauca. Comparative observations of the morphological forms of dividing chloroplasts present in suspensions from young wheat leaves were made using phase contrast and Nomarski interference optics and from thin sections. The isthmus region of the dividing chloroplasts has a similar form in all three species and fuzzy plaques of electron opaque material and some invaginations in the isthmus were frequently observed. The significance of these fuzzy plaques is discussed and a possible mechanism for chloroplast division suggested.

INTRODUCTION

The presence of elongated and constricted ('dumb-bell'-shaped) chloroplasts, has been reported by light microscopists and they have also been observed by electron microscopy in thin sections of post-mitotic leaf cells. Careful analysis of the chloroplast complement of cells developmentally younger and older than those in which numerous 'dumb-bell' profiles are observed (Possingham and Saurer, 1969; Boffey *et al.*, 1979) has established beyond reasonable doubt that the constricted chloroplasts are in the process of division. Chloroplasts which have become constricted can also be isolated from leaf tissue and the fission of these plastids followed *in vitro* (Ridley and Leech, 1970; Kameya and Takahasi, 1971). The dividing chloroplasts are about 3 to 4 μm in diameter, and, although smaller in volume and possessing smaller grana, they resemble structurally the mature chloroplasts in the older cells of the same leaf. We term these chloroplasts 'young chloroplasts' to distinguish them from morphologically less complex and smaller proplastids characteristic of meristematic tissue. Since the division of young chloroplasts occurs in leaf cells which will not normally divide again, the resulting increase in chloroplast number is carried forward to the mature leaf cell. As a result of the division of young chloroplasts, a tenfold increase in chloroplast number per cell occurs in spinach leaves and a two to threefold increase in wheat leaves; the process is therefore important in determining the photosynthetic potential of the mature leaf (Leech, 1976)

Although electron micrographs have been published (Boasson, Laetsch and Price, 1972; Cran and Possingham, 1972; Platt-Aloia and Thomson, 1977; Possingham and Rose, 1976; Possingham and Saurer, 1969) showing profiles of

0028-646X/81/010001+14 $02.00 © 1981 The New Phytologist

constricted chloroplasts, the sequence of morphological changes occurring as the chloroplast divides have not been described. Ideas about the mechanisms which initiate and control the division process will remain speculative until the details of ultrastructural changes involved are known. We undertook the present investigation to obtain a detailed ultrastructural description of the changes occurring in the region of the isthmus at fission, and as a necessary step in the assessment of alternative postulates about the mechanism of the division process.

In the absence of any previous observations, evidence was sought for membrane activity of several different kinds. Preparative and observational procedures were chosen which would allow examination of the dividing chloroplasts for evidence, in particular, of invaginations or septum-like development from the inner envelope membrane, for generalized constriction mechanisms including the presence of microfilaments, for changes in distribution of membrane particles at different phases of the division process and for membrane fusion in the isthmus region. The majority of studies were carried out using wheat leaves, from which dividing chloroplasts were also isolated. Dividing chloroplasts from several other angiosperm species were also studied, some of which are presented in the figures and described in the text and legends. Parallel light microscopical observations were also made.

The paper describes the variety of membrane changes which were frequently observed during chloroplast division. Possible models for the mechanism involved in the division process are discussed.

Materials and Methods

Plant material

Seeds of wheat, *Triticum aestivum*, var. Maris Dove, were soaked in running tap water at 20 °C for 17 h with a 1-min surface sterilization in sodium hypochlorite solution (13 % free chlorine) after the first hour. The seeds were sown in Levington Universal Compost (Fisons, U.K.) at a depth of 1 cm and were grown using a photoperiod of 16 h light at 20 °C, 8 h darkness at 15 °C and 70 % RH. The light intensity at the level of the seedlings measured with a solarimeter (Kipp and Zonen), was a 4 mW cm^{-2}. Seedlings were harvested 7 days after sowing, 2 h after the start of the light period; the leaves were cut at their bases and the coleoptiles gently pulled off.

Seedlings of maize, *Zea mays*, var. Kelvedon Glory were grown in the same way as the wheat seedlings but using a photoperiod of 16 h light at 28 °C and 8 h darkness at 23 °C and 80 % RH.

The isolation of dividing chloroplasts

Except where otherwise stated, dividing chloroplasts were isolated from 1-cm wide leaf slices cut from the leaf tissue in the region 1 to 2 cm above the leaf base. Two hundred seedlings were harvested for each isolation. The sections of the leaves were finely chopped with a razor blade in an isolation medium containing 0·4 M sorbitol, 0·75 mM $MgCl_2$, 50 mM Hepes at pH 7·6 (solution A). The slurry was filtered through eight layers of nylon bolting cloth (25 μm) and layered on to 1 ml of isolation medium, but containing 0·4 M sucrose instead of sorbitol. After centrifugation for 5 min at 900$\mathbf{g}_{max}$ the pellet was resuspended in solution A. Observations by phase contrast microscopy and by Nomarski interference optics (Zeiss photomicroscope) showed that in these suspensions between 10 and 20 % of the plastids were in the process of division.

Preparation of thin sections for transmission electron microscopy

Each chloroplast pellet was gently released from the sides of the centrifuge tube and resuspended in 3 ml of fixative containing 5 % (w/v) glutaraldehyde in 0·4 M sucrose, 0·03 phosphate buffer (K-Na pH 7·2) (solution B). After 2 h on ice the chloroplasts were repelleted by centrifugation as before and washed in solution B. Post-fixation was in 2 % osmium tetroxide in solution B for 1 h at room temperature, the chloroplasts were again repelleted and then fixed for a further 30 min in 2 % osmium tetroxide in phosphate buffer alone with no added osmoticum. After removal of the osmium the material was dehydrated with graded series of acetone and embedded in Spurr's 'firm' resin (Spurr, 1969).

Preparation of suspensions of dividing chloroplasts

The pellet of isolated dividing chloroplasts was first resuspended and fixed in 3·5 % glutaraldehyde in solution A for 10 min on ice and then spun down at $900\mathbf{g}_{max}$ for 10 min. The resultant pellet was gently taken up into 25 % (v/v) glycerol in solution A. At this stage at least 10 % of the plastids in the suspension showed constrictions typical of dividing chloroplasts. The fixed chloroplasts were stored in 25 % glycerol at minus 26 °C. For freeze-fracturing chloroplasts were repelleted and the pellets gently redispersed to give a viscous paste which was injected into small clefts in brass rivets (using microcapillary tubes) and then frozen at liquid nitrogen temperature. The rivets were loaded on to a stage under liquid nitrogen and the stage was placed in a Leybold–Heraeus Bioetch 2005 automatic freeze-etching machine. The loading temperature was minus 150 °C. The samples were fractured at minus 115 °C and the frozen surfaces were replicated with platinum at +45 °C and with carbon. A vacuum of better than $6{\cdot}0 \times 10^{-7}$ torr was maintained during the operation. The replicas were cleaned in 30 % (w/v) aqueous chromic acid for 12 h and then taken through a dilution gradient to distilled water and dried in a vacuum dessicator. The replicas were observed in a Philips EM 300 electron microscope.

Preparation of leaf material for electron microscopy

Slices 0·2 mm wide were cut from wheat leaves in the regions 17 to 19 mm from the leaf base. The slices were cut in half along their central vein and fixed for 1 h in 2·5 % (v/v) glutaraldehyde in 0·1 M phosphate buffer, pH 7·2. After a short buffer rinse the tissue was post-fixed in 2 % (w/v) osmium tetroxide in buffer for 1 h at room temperature and then dehydrated in a graded series of acetone and embedded in Spurr's 'firm' epoxy resin (Spurr, 1969). Thin sections were cut using a diamond knife on a Sorvall Porter-Blum MT-2 ultramicrotome and picked up on uncoated copper grids; the sections were then stained for 10 min or for 1½ to 2 h with 1 % aqueous uranyl acetate, followed by 1½ to 2 min in Reynolds' lead citrate (Reynolds, 1963). Sections were examined in a Philips EM 300 electron microscope. Samples of leaf material from other species were prepared and examined as described by Platt-Aloia and Thomson (1977).

Results

Light microscope observations of dividing chloroplasts

In young wheat leaves, chloroplasts showing morphological features typical of division are observed only in cells in the tissue between 5 and 25 mm from the

leaf base, i.e. in cells which are only one to one and a half days older than those undergoing their last cell division in the meristem (Boffey, Sellden and Leech, 1980). In order to observe the changes in shape and volume associated with their division, isolated chloroplasts from these cells were observed by light microscopy using phase contrast and also Nomarski optics. Measurements of the average dimensions of the chloroplasts in these suspensions have already been recorded (Boffey *et al.*, 1979). Successive 5-mm sections of leaf tissue cut between 5 and 25 mm from the leaf bases were harvested and chloroplasts isolated from them as described in the Methods section. These suspensions were observed either as suspended drops on inverted cover glasses or conventionally. In the latter case great care was taken to avoid squashing or distortion of the plastids by pressure from the cover glass which was raised on glass chips so that movements of the chloroplasts or progressive changes in their shape could be observed. The percentage of chloroplasts in a state of division varied from 22 % in the chloroplasts from the youngest tissue to 8 % in the chloroplasts from the older tissues (Table 1). The suspending medium was chosen from several used in the preliminary survey, because it caused no osmotic swelling or distortion of the chloroplast. Variations in the osmolarity of the resuspending medium within the range 500 mM to 300 mM did not cause any morphological changes or affect the proportion of dividing chloroplasts in the suspension.

Table 1. *The characteristics of young chloroplasts in the state of division in suspensions isolated from leaf tissue sections of young green wheat leaves*

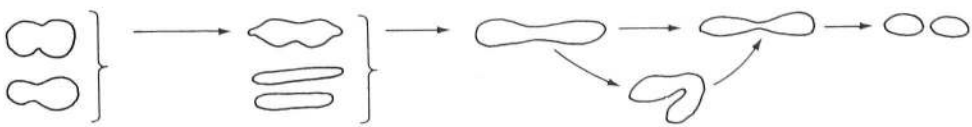
	Number of chloroplasts (percentage of dividing chloroplasts)			
Leaf tissue sections (mm from leaf base)	5	10	15	20
All dividing chloroplasts (percentage of total)	22	13	12	8
Elongated or 'peanut'	84	89	87	98
Figure of 8	13	2	3	0
Beaded	2	2	6	0
L-shaped	1	7	4	2

The criteria used in the classification are explained in the text. Elongated and 'peanut' shaped plastids were present in equal numbers.

Some of the most common chloroplast shapes are illustrated in the photograph shown in Plate 1, No. 1 and by the plasticine models shown in Plate 1, No. 2. The chloroplasts in suspensions are shown in both side and face views. In their most characteristic form the dividing chloroplasts appear to be considerably elongated and narrowed in side view and in face view have a well defined central constriction and often show attenuation at the distal ends of the presumptive daughter plastids so that they resemble 'peanuts' in appearance. Over 80 % of the dividing chloroplasts from all the young tissues examined showed this form in suspension. In view of the considerable changes in shape and volume accompanying division,

growth of chloroplasts during division cannot be ruled out. A small proportion of plastids resembled a conventional figure of eight in side view (Table 1, Plate 1, No. 2) and occasionally extremely constricted forms with very narrow isthmuses connecting the daughter plastids are seen. Another morphological form consistently observed was one in which the two halves of the chloroplast lay approximately at right angles to each other and the two halves were observed to pivot around the central isthmus. Twisting of the two halves centrally about their axis was also observed for 'peanut'-shaped chloroplasts. It is possible we were observing in these movements the results of the operation of forces responsible for the constriction and eventual fission of the daughter plastics.

From numerous light microscopical observations carried out by four different observers in the laboratory, a tentative time sequence can be proposed. Many plastids show 'intermediate' morphology between the forms illustrated here:



The fat 'dumb-bell'-appearance appears to precede the assumption of a 'peanut' shape; subsequently the isthmus becomes progressively narrower, the ends of the chloroplast more rounded and the two daughters separate. On the extremely few occasions when fission was actually observed it was extremely rapid.

Electron microscopical observations of dividing chloroplasts

In thin sections of leaf tissue chloroplast profiles corresponding to all the morphological forms described above were found. Examples of elongated chloroplasts with a variety of relationships between the isthmus and the daughter plastids were observed and profiles in which the two daughter halves were aligned at right angles to each other were also seen. Examples of these profiles are illustrated in Plate 2, No. 1, Plate 3, No. 1 and Plate 5, No. 2.

The electron micrograph in Plate 2, No. 1 shows a presumptive dividing plastid, apparently close to the end of its division cycle: a narrow isthmus connects the two daughter plastids which are still completely joined together. Profiles of dividing chloroplasts in leaf cells (Plate 2, Nos 1, 2; Plate 4, Nos 1, 2, 3, 5, 6) and also in isolation (Plate 1, No. 3; Plate 3, Nos 1, 2, 3; Plate 5, Nos 1, 2), illustrate some of the membrane configurations which may be interpreted as preceeding the formation and subsequent disappearance of the isthmus. The first structural indication that division is occurring is a general constriction of the central region of the chloroplast as illustrated by arrows in Plate 2, Nos 2, 3. Frequently small vacuoles, mitochondria and other organelles are associated with this constriction (not shown). These organelle associations may be significant *in vivo* but cannot in any way be responsible for the maintenance of the constrictions seen in isolated plastids which are removed from their cellular environment (Plate 2, No. 3; Plate 3, No. 1). Plate 2, No. 3 shows a presumptive invagination indicated by, (i), of the inner envelope membrane in an isolated plastid and similar invaginations can also be observed in chloroplasts *in vivo*. The interpretation of such invaginations in isolated plastids is complicated by the slight swelling which occurs on isolation and suggestions that these invaginations may represent the origin of a growing septum should therefore be treated with some caution.

As an alternative method of visualizing the membranes of the constriction zones, replicas of freeze-fractured isolated chloroplasts were also examined. Plate 3, No. 2 shows the surface of the inner envelope membrane of an intact isolated wheat chloroplast. The fracture plane lies between the outer and inner envelope membranes and several small distinct invaginations (i) are visible in the inner envelope membrane in the region of constriction. In fracture faces of the inner envelope membrane (Plate 3, No. 3) no such invaginations are observed. Careful observation of the characteristics of the membrane particles revealed in the freeze-fracture replicas and their distribution patterns in the envelope membranes failed to reveal any distinctive or consistent differences either in the distribution pattern or in the size of the particles in the constriction zone and in the other areas of the plastid envelopes.

As chloroplast division progresses, the constriction zone narrows to an isthmus and several additional changes in the membranes of this region may be observed (Plate 4, Nos 1, 2). Fuzzy plaques of electron opaque material can frequently, but not always, be seen covering or displacing the membranes of the isthmus. Where such plaques are present it is often very difficult to distinguish the envelope membranes in the isthmus region from the plaque material (Plate 4, No. 2). Although they are not always seen in other regions of the plastid surface, these plaques are sufficiently common in the isthmus region to indicate that they represent a real change in the molecular arrangement of the membrane components of the isthmus region at this particular time in the chloroplast division cycle. However, their presence is almost certainly transitory. At this stage in division invaginations of the inner envelope membrane in the region of the isthmus are often observed both in plastids within the leaf cell (Plate 4, No. 3) and after they have been isolated (Plate 4, No. 4).

As the isthmus narrows still further and chloroplast division approaches completion, membrane delineation becomes difficult and there is considerable localized disorder of the membrane materials particularly at the neck of the isthmus (Plate 4, No. 5). Progressive increased disorder may lead to localized formation of myelin-like material between the two daughter plastids as seen in Plate 4, No. 6 and Plate 5, No. 1. These formations may be interpreted as a transitory stage prior to membrane reorganization. In isolated chloroplasts the myelin-like aggregations are not encountered. In the final stages of division, prior to separation, contiguous double membranes appear to be present around the two daughter plastids (as can be seen in Plate 5, Nos 1, 2). We interpret such arrangements as representing the final stage of division before the separation of the two new daughter chloroplasts.

Discussion

From measurements of the rate of cell expansion in wheat leaves, the number of chloroplasts per cell and the proportion of these chloroplasts in the process of division, the lifetime of a division profile of a wheat chloroplast has been estimated to be about 20 min (Boffey *et al.*, 1979). When the fission of a chloroplast is followed *in vitro* (Ridley and Leech, 1969; Leech, 1976), the phase when the narrow isthmus may be observed is relatively transitory and the disappearance of the isthmus and the separation of the two daughter plastids is so rapid that the two processes appear to be simultaneous when observed *in vitro*. If it is assumed that the relative length of the phases of the division cycle are the same during division

of chloroplasts *in vivo*, then in the leaf the isthmus would be observable for 5 min or less. It is perhaps not surprising therefore that observations of the ultrastructure of the chloroplast in the final stages of division reveal a variety of different morphological features and that it is particularly difficult to establish a definite sequence of events. Suggestions about the mechanisms of constriction and fission must remain speculative but the fuzzy plaques and the invaginations observed in the region of the isthmus were found frequently enough for us to regard their presence as significant. Based on these observations a model can be proposed which suggests a possible mechanism for the separation of the plastids.

It is suggested that the fuzzy plaques represent a component, possibly proteinaceous, which is associated with the formation of the constriction zone. If this component were distributed sparsely in the earlier phases of isthmus formation, for example in a series of narrow bands, or was particularly hydrophobic, it would be difficult if not impossible to identify or visualize it in conventionally prepared thin sections. We observed the most distinct plaques when the constriction of the isthmus was most advanced and it is possible that visualization at this stage may result from increasing accumulation or aggregation of the components or loss of their hydrophobicity or from all three of these processes.

Only very small invaginations of the inner envelope membrane of the isthmus have ever been seen and although we have searched extensively we have never found evidence for proliferation or fusion of these invaginating membranes.

Changes in charge distribution of the membrane components rendering them more hydrophobic (Barber, 1979) would almost certainly provide sufficient force to effect fusion of the membrane components or their realignment in new configurations. If there were much stress due to forces parallel with the long axis of the chloroplast and in the direction of the tips of the chloroplast, physical reorganization of membrane components would probably be very rapid resulting in few and small myelin-like structures. If there were less stress promoting molecular reaggregation then the disorganized membrane material would remain in large myelin-like assemblies (as observed in Plate 5, No. 1). The absence of plaques and myelin-like membrane material in the isolated plastids is explained on this model since on isolation of the plastids from the cell the gel/sol constraints of the cytoplasm are absent and the components of the reassembling membrane would be dispersed.

All our own observations on chloroplast division could be explained along the lines of the theoretical model for cytokinesis proposed by Greenspan (1977) and simulated artificially later (Greenspan, 1978). He suggests that changes in the dynamic instability of surface tension forces within the cell membranes are sufficient to effect formation of a constriction zone. On this model, chloroplast division would be initiated by a change in the relative contribution of polar and equatorial surface tensions leading to contraction of the surface towards the equator. Additional experimental approaches will need to be used to see if this is indeed the mechanism involved in the initiation and completion of chloroplast division.

ACKNOWLEDGEMENTS

We are most grateful to Dr Stephen Boffey, Dr Gun Sellden, Raymond Ellis and Caroline Dean for advice and skilled technical assistance during several phases of this work. This work was supported by the Science Research Council, U.K. and by a grant from the National Science Foundation, PCM 74–19927.

REFERENCES

BARBER, J. (1979). Energy transfer and its dependence on membrane properties. In: *Chlorophyll Organisation and Energy Transfer in Photosynthesis. CIBA Foundation Symposium* **61**, 283–297. Excerpta Medica, Amsterdam.

BOASSON, R., LAETSCH, W. H. & PRICE, I. (1972). The etioplast/chloroplast transformation in tobacco: correlation of ultrastructure, replication and chlorophyll synthesis. *American Journal of Botany*, **59**, 217–233.

BOFFEY, S. A., ELLIS, J. R., SELLDEN, G. & LEECH, R. M. (1979). Chloroplast division and DNA synthesis in light-grown wheat leaves. *Plant Physiology*, **64**, 502–505.

BOFFEY, S. A., SELLDEN, G. & LEECH, R. M. (1980). The influence of cell age on chlorophyll formation in light-grown and etiolated wheat seedlings. *Plant Physiology*, **65**, 680–684.

CRAN, D. G. & POSSINGHAM, J. V. (1972).Two forms of division profile in spinach chloroplasts. *Nature New Biology*, **235**, 142.

GREENSPAN, H. P. (1977). On the dynamics of cell cleavage. *Journal of Theoretical Biology*, **65**, 79–99.

GREENSPAN, H. P. (1978). On fluid mechanical simulations of cell division and movement. *Journal of Theoretical Biology*, **70**, 125–134.

KAMEYA, T. & TAKAHASI, N. (1971). Division of chloroplasts *in vitro*. *Japanese Journal of Genetics*, **46**, 153–157.

LEECH, R. M. (1976). The replication of plastids in higher plants. In: *Cell Division in Higher Plants*. (Ed. by M. M. Yeoman), pp. 135–159. Academic Press, London.

PLATT-ALOIA, K. & THOMSON, W. W. (1977). Chloroplast development in young sesame plants. *New Phytologist*, **78**, 599–605.

POSSINGHAM, J. V. & SAURER, W. (1969). Changes in chloroplast number per cell during leaf development in spinach. *Planta*, **86**, 186–194.

POSSINGHAM, J. V. & ROSE, R. J. (1976). Studies of the growth and replication of spinach chloroplasts and the location and segregation of their DNA. In: *Genetics and Biogenesis of Chloroplasts and Mitochondria* (Ed. by Th. Bücher *et al.*), pp. 187–290. Elsevier/North Holland, Amsterdam.

RIDLEY, S. M. & LEECH, R. M. (1970). Division of chloroplasts in an artificial environment. *Nature*, **227**, 463–465.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, **17**, 208–212.

SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research*, **26**, 31–43.

EXPLANATION OF PLATES

PLATE 1

No. 1. Dividing chloroplasts isolated from young wheat leaves observed using phase contrast (left hand side of plate) and Nomarksi interference optics (right hand side of plate). The chloroplasts have been chosen to illustrate the morphological forms in Table 1 and further described in the text.

No. 2. Plasticine models illustrating the range of morphological forms observed in suspensions of isolated dividing chloroplasts from young wheat leaves.

PLATE 2

No. 1. A dividing chloroplast from a young leaf of sesame (*Sesamum indicum*, var. glauca). Note the narrow isthmus, arrow, between the two daughter chloroplasts. × 30000.

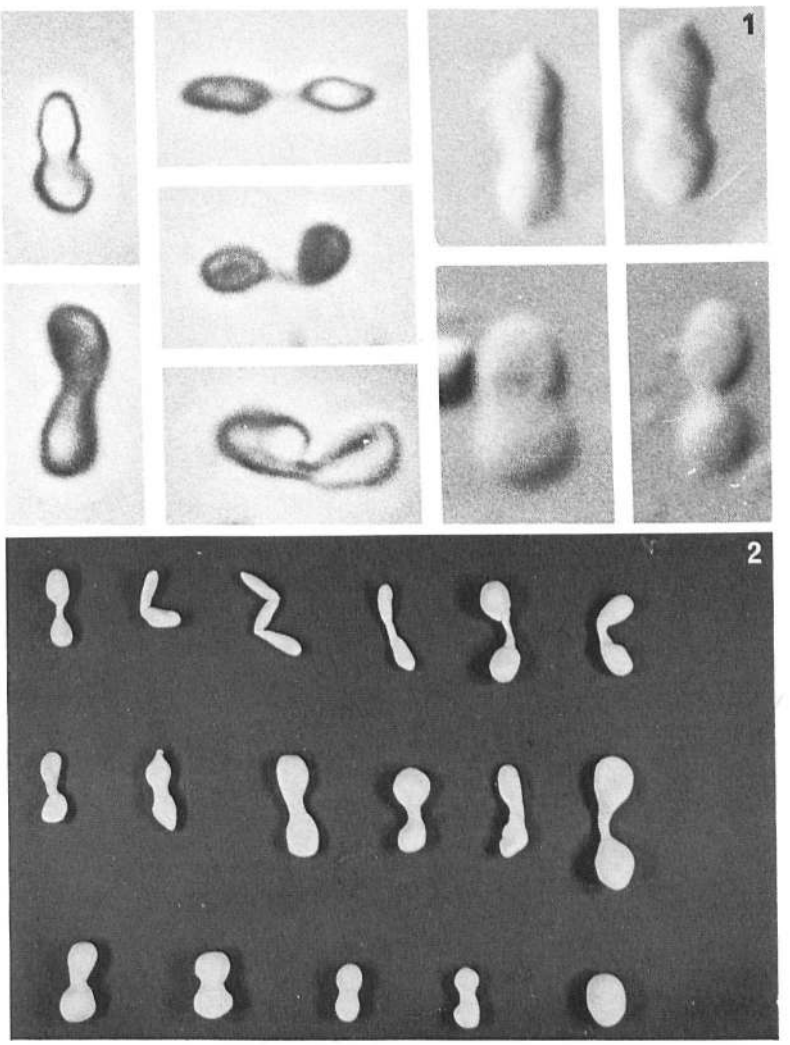
No. 2. A dividing chloroplast from a young leaf of *Atriplex semibaccata* R.Br. This chloroplast is early in division as indicated by the shallow nature of the constriction band, arrows. × 25000.

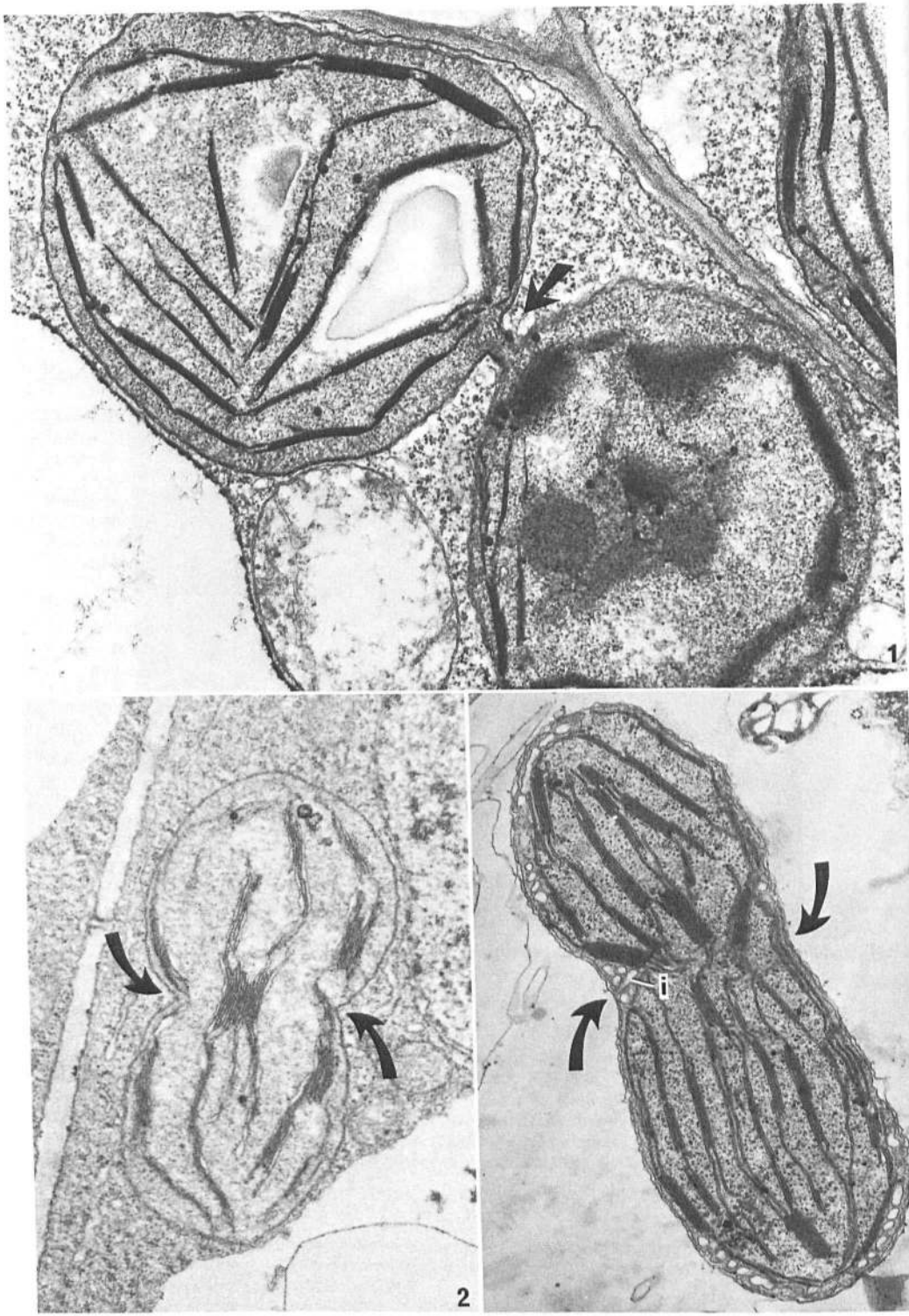
No. 3. An isolated chloroplast from maize. Note the presumptive constriction band, arrows, and the invagination, i, of the inner envelope membrane where the constriction is most advanced. × 30000.

PLATE 3

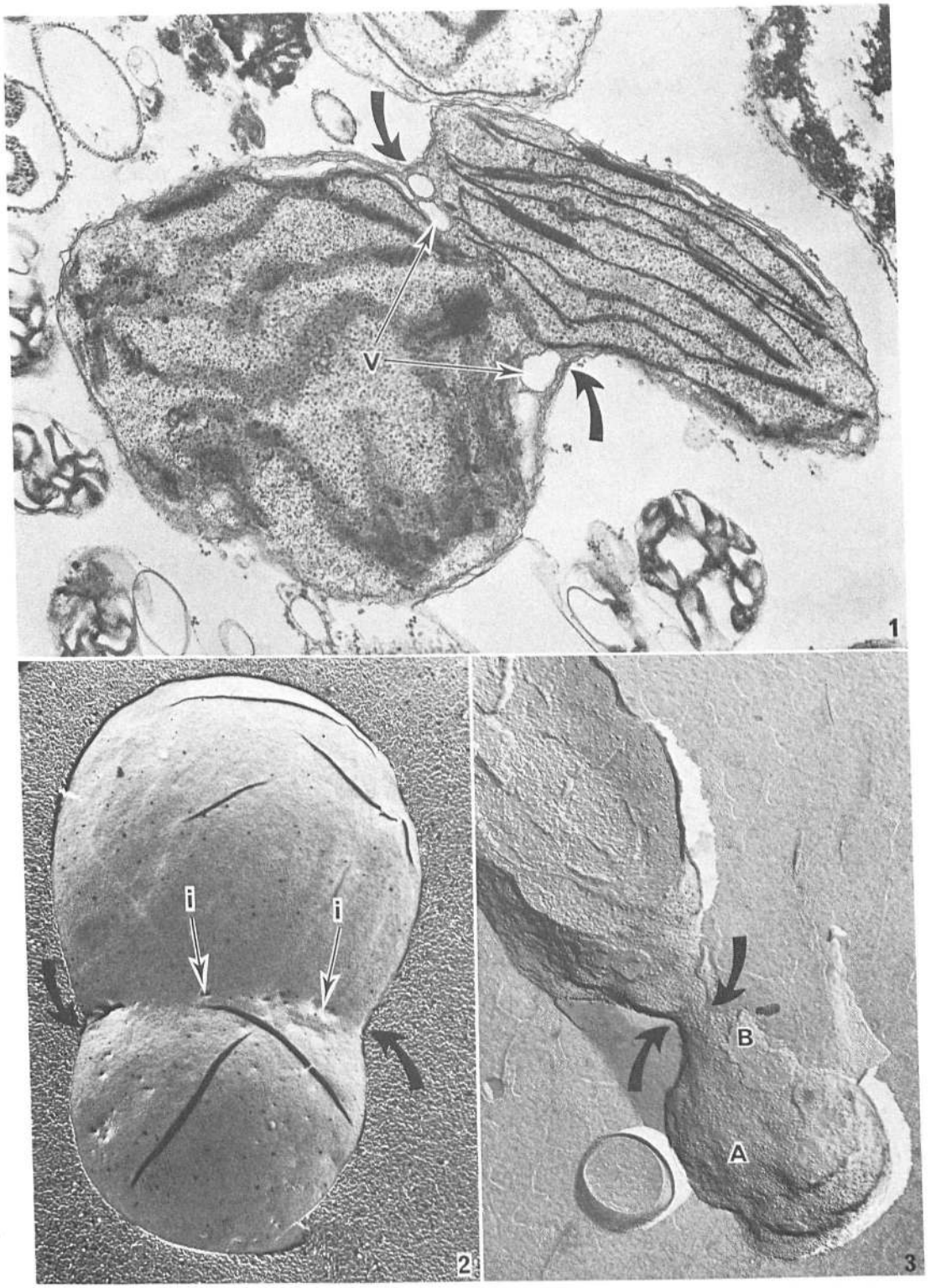
No. 1. An isolated, dividing chloroplast of maize. The constriction band by the arrows. Note the orientation of the axes of the two daughter chloroplasts are in different planes and that the 'vesicles', located within the constriction band are swollen. × 33000.

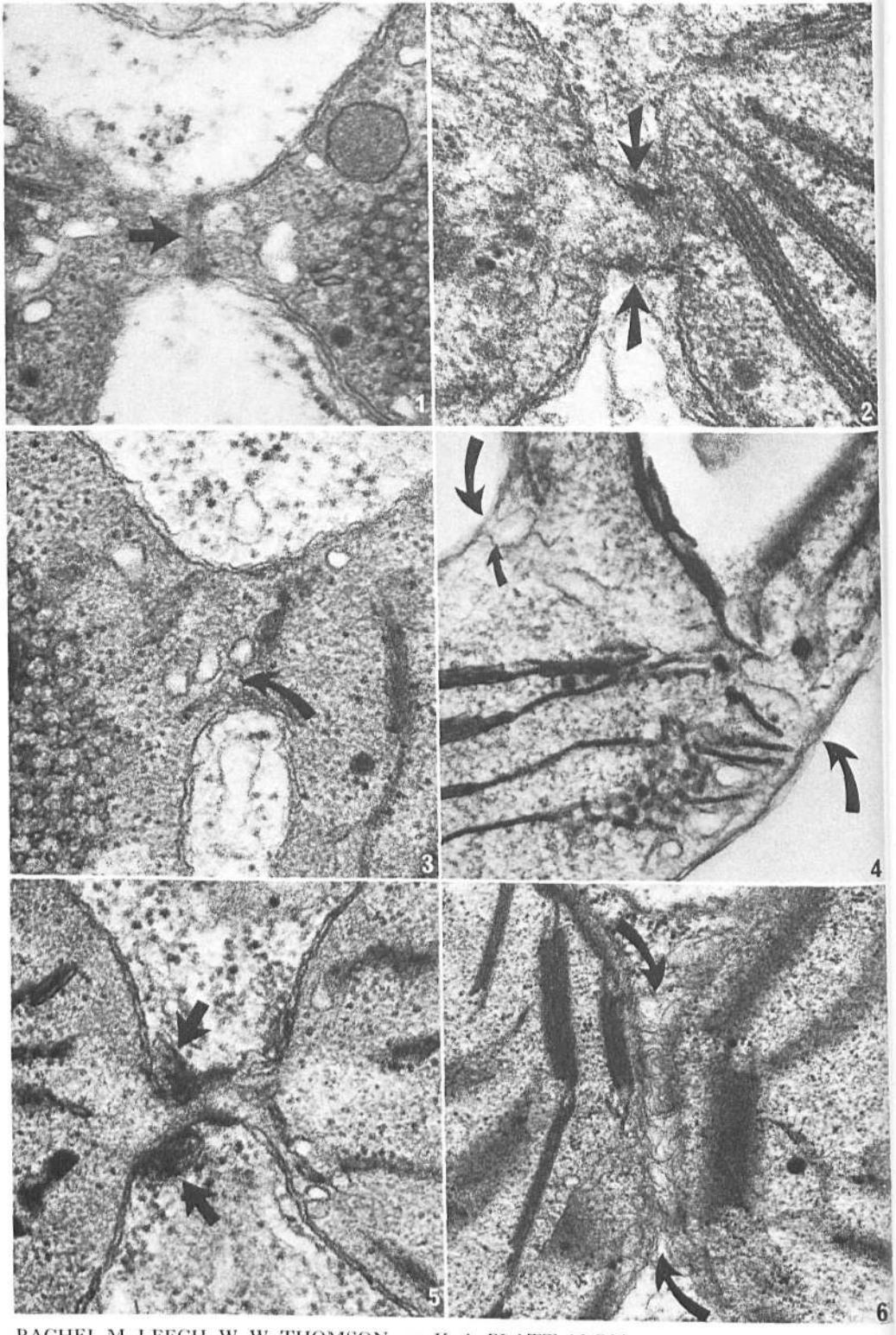
No. 2. A replica of an isolated, dividing chloroplast of wheat which was prepared by freeze-fracture. The absence of particles in the fracture face is indicative that the fracture plane passed between the inner and

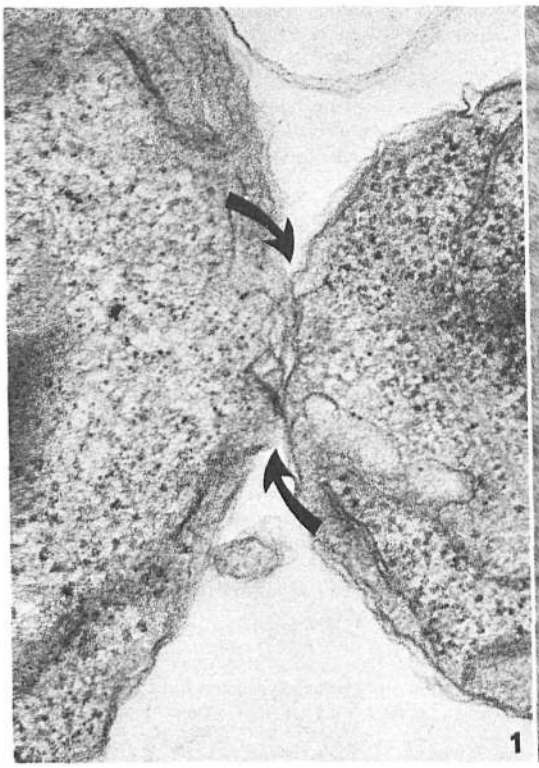

1
2

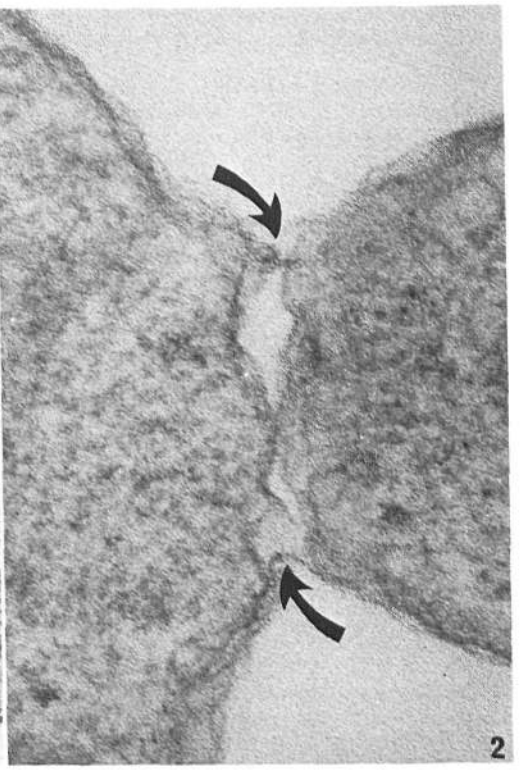


RACHEL M. LEECH, W. W. THOMSON AND K. A. PLATT-ALOIA


V
1
i
i
2
B
A
3


1
2
3
4
5
6


1


2

outer envelope membranes revealing the outer surface of the inner envelope membrane. The constriction band zone is identified by the arrows. The orifices, i, are interpretated as the openings of invaginations in this membrane. × 50000.

No. 3. A replica of an isolated, dividing chloroplast of wheat which was prepared by freeze-fracture. The fracture plane passed through the inner envelope membrane at A, revealing the PF face. The fracture face B is the EF face of the outer envelope membrane. Note the evenly dispersed nature of the particles throughout the PF face including in the constriction zone, arrows. × 40500.

PLATE 4

No. 1. The isthmus between the two daughter chloroplasts of a dividing etioplast of sesame. Note the fuzzy strands (arrow) extending across the isthmus as well as the fuzzy plaques of electron opaque material in the region of the envelope membrane. × 77000.

No. 2. The isthmus between the two daughter chloroplasts of a dividing chloroplast of sesame. Note the plaques of electron opaque material associated with the envelope membranes near the midpoint of the isthmus. × 97000.

No. 3. The isthmus between the two daughter plastids of a dividing etioplast of sesame. Note the apparent invagination (arrow) of the inner envelope membrane in the isthmus region. × 85000.

No. 4. *The constriction zone (large arrows) of an isolated, dividing wheat chloroplast. Note the invagination* (small arrow) of the inner envelope membrane in the constriction zone. × 73000.

No. 5. The isthmus between two daughter chloroplasts of a young, dividing sesame chloroplast. Note the myelin-like membraneous structure (arrows) at the neck of the isthmus and the electron opaque plaque associated with envelope membranes at the midpoint of the isthmus. × 80000.

No. 6. A dividing chloroplast of sesame. Note the myelin-like membraneous configurations (arrows) between the two daughter chloroplasts. × 48000.

PLATE 5

No. 1. A near final stage in the separation of two daughter chloroplasts of a diving, isolated wheat chloroplast. Note the membranes of the two daughter chloroplasts are contiguous in the separation zone (arrows). × 80000.

No. 2. A near final stage in the separation of two daughter chloroplasts of a dividing, isolated wheat chloroplast. As on No. 1, note the various membranes of the two daughter chloroplasts are contiguous in the separation zone (arrows). × 175000.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.