ROLE OF PHRAGMOPLAST FILAMENTS IN
CELL-PLATE FORMATION

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SUMMARY

With the differential interference microscope (Nomarski system), it is possible to observe
the interaction of filaments of the phragmoplast with micron-size retractile particles and vesicles,
very close to the resolution limit of the microscope, during formation of the cell plate. Filaments
occupying the birefringent zone of the phragmoplast (i.e. remnants of the continuous and
interzonal fibres of the mitotic spindle) shorten and thicken in their mid-region to become
spindle-shaped. The thickenings later fuse laterally to form the cell plate. During the process
of thickening, vesicles can be seen to move toward the plate in close association with the
filaments on both sides of the phragmoplast. These observations, taken together with informa-
tion from electron micrographs of thin sections of Haemanthus endosperm and other dividing
cells, confirm the notion that the fibrillar component of the phragmoplast has a transport
function. The sizes of particles entering the phragmoplast region by saltation suggest that they
correspond to Golgi bodies, which have been suspected of producing vesicles that contribute
to cell-plate formation. Neither light nor electron micrographs have yet provided any insight
into the mechanism of transport of these vesicles.

INTRODUCTION

Cytokinesis in plant cells typically entails the formation of the phragmoplast, cell
plate and cell wall. The phragmoplast is a differentiated region of the plant cell which
forms in late anaphase or early telophase of division between the separating groups of
sister chromosomes. Initially, the phragmoplast forms from remnants of the spindle,
but in later stages differentiation of cytoplasm bordering the previous spindle region
also contributes to the growth of the phragmoplast as a whole. An exception is found
in the endosperm of many plants, for example, Iris, where Jungers (1930) found that
the phragmoplast can also form entirely from the cytoplasm and not from the spindle
remnant. This occurs if the phragmoplast is formed between non-sister nuclei. A
similar situation was reported in the formation of tetrads during meiosis in orchids by
Barber (1942).

In typical plant cells the cell plate originates midway between the former spindle
poles, dividing the phragmoplast into two parts. When first formed, the cell plate
appears as a thin sheet of apparently amorphous substance containing pectins and

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other polysaccharides, as well as lipids (Becker, 1935; Olszewska, 1966). The cell plate is transformed into the middle lamella of the mature cell wall. The peripheral parts of the cell wall are formed by the deposition of cellulose on the two sides of the cell plate.

Some of the earliest details of cell-plate formation in vivo were provided by Strasburger (1875) from studies of cell division in stamen hair cells of Tradescantia. His description of cell-plate formation as the fusion of granules ('Körperchen') led to some fifty years of controversy as to the true nature of these particles (whether they were vesicles, droplets, or granules), and as to the nature of the process by which they fused. Later authors, including Bélaš (1929) and Becker (1938), showed in a series of elegant experiments that the cell plate in its early stages of development could be split longitudinally, indicating either that this structure is double or that its interior is of a soft consistency.

It has also been known since the time of Strasburger (1875) that the phragmoplast has a filamentous texture, but the role of the filaments in the formation of the cell plate has so far remained obscure. Detailed in vitro studies carried out by one of us (Bajer, 1965) suggested that filaments swell rapidly in the region where the cell plate will form, and that these swellings migrate along the long axis of the phragmoplast until they fuse to form the cell plate. Unfortunately, enlargements of single frames of 16-mm film did not demonstrate this point on still photographs as clearly as the details could be seen during projection of the film.

Electron-microscopic studies, especially those of Whaley & Mollenhauer (1963) and of Frey-Wyssling, López-Sáez & Mühlethaler (1964) indicate that small vesicles, apparently formed from the Golgi bodies (dictyosomes), fuse to form the cell plate. Various particles can be seen in the phragmoplast area with the light microscope; their movements have been analysed in detail from cinematographic records (Bajer, 1965). It seems very likely from the similarity in size and position that many of these particles correspond to Golgi bodies that are seen with the electron microscope.

Since most of the early electron-microscopic studies on the phragmoplast were carried out on permanganate-fixed material, no filamentous elements were detected. However, the more recent studies of Ledbetter & Porter (1963), using glutaraldehyde fixation, have revealed the presence of microtubules, as might perhaps have been expected from the birefringence of the phragmoplast area (Becker, 1938; Inoué, 1953; Inoué & Bajer, 1961).

We have recently recorded some observations on living Haemanthus endosperm cells with the differential interference contrast system of Nomarski (1955) which appear to establish some interesting relationships between the behaviour of the vesicles (or droplets, as one of us has previously called them (Bajer, 1965)), on the one hand, and the filaments of the phragmoplast on the other. These observations have

* The term 'filament' has previously been used by us (Bajer & Allen, 1966) to denote a light-microscopically visible subdivision of what has traditionally been called a 'spindle fibre'. Judging from the fact that the electron microscope reveals many more microtubules in a cross-section of a spindle fibre than there are filaments resolved in the differential interference contrast microscope, it is reasonable to suppose that each of our 'filaments' corresponds to a bundle of
been made possible through the loan to one of us (R. D.A.) of the prototype instrument under development by Dr Horst Piller of Carl Zeiss, Oberkochen, Western Germany. An earlier report (Bajer & Allen, 1966) contains a brief description of the images obtained with this system, and a more complete report is in preparation describing the optimal conditions for contrast and resolution with this new type of instrument (Allen, David & Hirsch, 1966).

MATERIALS AND METHODS

Endosperm of *Haemanthus katherinae* was prepared for microscopic observations according to methods previously described by Molè-Bajer & Bajer (1963). Both 16-mm time-lapse and 35-mm still photographic records were followed by detailed frame-by-frame analysis. Methods used for photographic recording under light-limited conditions have been described elsewhere (Allen, Francis & Nakajima, 1966), as have general methods for improving contrast in polarizing and interference microscopes (Allen, Brault & Zeh, 1966).

RESULTS

A sequence of differential interference contrast photomicrographs (Figs. 2–7) shows the main features of cell-plate formation. Long, thin refractile filaments, initially 0.2–0.3 μ thick and 5–25 μ long, are seen in late anaphase between the chromosome groups (Fig. 2). At least some of these filaments (the thickest and most prominent ones) can be seen extending from or connecting with the arms of sister chromosomes during anaphase, making them 'interzonal fibres' according to the terminology of Schrader (1953), but it is not certain whether the origin of these filaments is intra- or extra-chromosomal (compare Metz, 1934).

As the chromosome groups separate, these filaments shorten and become thicker in their mid-region (Figs. 1 and 2–4). The interzonal region also contains somewhat thinner filaments apparently not derived from the chromosome arms (the 'continuous fibres' of Schrader; see Fig. 3 near the chromosome groups). Whatever the origin of these latter filaments may be, their number increases through anaphase and early telophase. Like the interzonal filaments, the continuous filaments shorten and become spindle-shaped as the larger interzonal filaments had done previously. (In Figs. 4–6 it is possible to see a diminution in the amount of filamentous material in the vicinity of the nuclei.)

In the meantime, at the same stage of mitosis (late anaphase and early telophase), small particles about 1–0 μ in diameter suddenly appear throughout the central region of the phragmoplast, their number increasing linearly with time (Bajer, 1965). Their perhaps a few to a few dozen microtubules. Even though the 'filaments' connecting chromosome ends in the phragmoplast are visibly larger than either the spindle fibre filaments or what appear to be 'continuous filaments' connecting the spindle poles, we have retained the same terminology. This does not necessarily imply that all filaments have either the same structure or a similar function.
manner of appearance suggests migration by saltatory excursions from the neighbouring cytoplasm, although we cannot exclude the possibility of de novo formation from unseen precursors. Subsequently, a small proportion of these particles undergoes saltations toward the equatorial region where the cell plate forms, while the majority migrate toward the region of the former spindle poles.

It is particularly clear from interference microscope images (Figs. 3-7) that one important process involved in the formation of the cell plate is the transport of a considerable amount of organic matter toward the highly phase-retarding cell plate, as it develops. It is also clear from frame-by-frame analysis of our 16- and 35-mm films that some kind of co-operation between particles and filaments in the phragmoplast is responsible for this mass transport. During the medial thickening of the filaments, there is an association between these filaments on the one hand and both the larger particles described above and very small vesicles, of dimensions less than a micron, on the other (Figs. 4-7). The size of these vesicles is very likely at or below the limit of resolution of the light microscope. It is sometimes possible to see, during projection of the film, particles or vesicles 0.2-0.5 μ in diameter within or attached to thickenings in the filaments. Minute thickenings initially bear the appearance of a cluster of small subunits very close to the resolving power of the film emulsion (in this case 16-mm plus-X film); hence they are difficult to demonstrate on prints, but show clearly on projection. At first the thickenings are not lined up exactly in register, but within about 10 min their ranks straighten and fuse laterally (compare Figs. 5 and 6). Some of these events were inferred from previous film records (Bajer, 1965), but the clarity and contrast in differential interference contrast images now captured on film...
Phragmoplast filaments

has provided considerably more detail on the form changes of the filaments and on their association with particulate matter destined to participate in cell-plate formation.

In Haemanthus endosperm, the cell plate typically forms first at the periphery and 'grows' toward the centre, but the reverse may occur, as may also simultaneous formation of the cell plate along its entire length. There seems to be a positive correlation between the sites of early cell-plate formation and high population densities of 'interzonal fibres' appearing between chromosomal arms (e.g. compare Figs. 2 and 4). The position of the chromosomal arms in turn depends on the degree of flattening of the cells under surface tension. As the formation of the cell plate spreads laterally from its point of initiation, micron-size particles merge with the swollen, now spindle-shaped filaments, and migrate laterally in the cell-plate region, giving the impression of the oozing of a semi-liquid material in a channel between parallel membranes of the newly formed cell plate. The swellings on the spindle-shaped filaments also show a tendency to migrate laterally, even though they maintain their filamentous connections, and as a result the entire thickened filament frequently bends.

The association between particles and fibrils may persist for a considerable time (up to 20 min) and accounts for the wavy appearance of the cell plate for some time after its formation. The newly formed cell plate is very soft and becomes progressively thinner until it finally bends into innumerable wrinkles, indicating that until that time, little or no cellulose deposition has occurred. The wrinkling occurs at the same time as the birefringence of the phragmoplast disappears (Barber, 1942).

DISCUSSION

We shall now consider whether conclusions of physiological significance may be obtained by considering the present results in the light of recent findings with the electron microscope. In view of the severe temporal and spatial sampling problems with the electron microscope, it is essential that we attempt to reconstruct probable events in the cell by comparing motion picture records with electron micrographs of cells at known stages of mitosis. Such efforts at synthetic reconstruction of events near the limit of resolution with the light microscope are now possible because of the very high quality images obtained with the Zeiss differential interference optics. Our present results indicate that more can be accomplished with this technique than was previously possible with phase contrast or polarized light.

First, it seems probable that the refractile filaments that we have observed in the late-anaphase/early-telophase phragmoplast are composed of the bundles of microtubules seen in electron micrographs of cross-sections of the phragmoplast by Esau & Gill (1963), E. H. Newcomb & H. T. Bonnett (1965, unpublished observations), Harris & Bajer (1965) and Pickett-Heaps & Northcote (1966). These same elements are probably also responsible for the diffuse birefringence observed in the Haemanthus endosperm phragmoplast by Inoué & Bajer (1961).

Second, it seems quite likely that many of the particles which appear in the phragmoplast before cell-plate formation are Golgi bodies. Electron micrographs of thin sections show many small vesicles associated with the Golgi bodies, and it is generally
assumed that these vesicles have dissociated from the Golgi bodies. Recent electron micrographs of Esau & Gill (1965) and of Pickett-Heaps & Northcote (1966), as well as unpublished work of E. H. Newcomb & H. T. Bonnett and P. Harris & A. Bajer show vesicles adjacent to, or between, microtubules. In later stages electron micrographs show fusion of such vesicles to form the cell plate (Whaley & Mollenhauer, 1963; Frey-Wyssling et al., 1964; Harris & Bajer, 1965). The possibility cannot be excluded that the living phragmoplast may contain other types of particles and filaments that may have failed to be preserved or have escaped notice in electron micrographs due to sampling problems.

At the risk of oversimplification, let us assume that cell-plate formation involves first the transport of vesicles of Golgi origin (but probably also other particles as well) toward the region of the cell plate, and second the fusion of these vesicles to form two apposed membranes.

The problem of transport of particles within cells is a general one, and many cells have solved the problem by one or more processes classified as 'saltatory motion' (Rebhun, 1963, 1964; Parpart, 1964). The motions themselves are generally linear and uniform in velocity within measurement error, suggesting that attachment to a moving filament might be a possible cause. Certainly the mere presence of filaments or fibrils of some kind is associated with many kinds of particle movements (especially in heliozoans, in foraminiferans, and in the mitotic spindles and asters of dividing cells; see Rebhun (1964) for review). Ledbetter & Porter (1963) in particular have emphasized the presence of microtubular structures in cells which exhibit particle motions, and have pointed to their possible implication as force-generating elements in cells. One way in which microtubules might participate in the transport mechanism in cell-plate formation is suggested by the tentacular feeding apparatus in the suctorian, Tokophyra, studied by Rudzinska (1965), in which peristaltic waves are known to occur along what is essentially a ring of microtubules.

A general scheme for the transport of particles and vesicles to the region of the forming cell plate that is consistent with our recorded observations and with recent electron-microscopic findings is as follows: Particle movements predominantly of a saltatory character are brought about by some kind of association with filaments of the phragmoplast. The filaments may play either or both of two roles: (a) they may polarize random saltatory motions without exerting any force on the particles, or (b) they may represent or be associated with contractile elements which are fundamentally force-producing. The appearance of thickenings along the filaments suggests that, if our surmise that a single, microscopically visible filament is actually a bundle of microtubules is correct, it may be possible that vesicles are trapped inside the bundle or arranged around it, and in some way (perhaps by co-operative action of the bundle) are forced toward the site of the forming cell plate. Repeated observations of our films allow us to state that this is what happens. One would have more confidence in such an interpretation if it were definitely established whether the microtubules are elements that are capable of generating forces or are simply skeletal structures.

A remarkable feature of the early stage of cell-plate formation is the way the swell-
ings in the filaments line up prior to fusion. Östergren, Mølë-Bajer & Bajer (1960) once suggested, before the relationship of particles to filaments was observed, that the vesicles (some of which are the swellings to which we refer) might be attached to 'fibres' and pulled by them into an equilibrium position matching tensions applied from the poles. The observations indeed seem to support the hypothesis of Östergren et al.

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Figs. 2–7. Photomicrographs of a dividing *Haemanthus* endosperm cell (× 1970) taken with a planachromatic 100 × oil-immersion lens (N.A. 1.3). The extinction factor was 250 and the condenser N.A. was 1.0.

Fig. 2 shows the chromosome groups at the end of anaphase with interzonal filaments connecting chromosome arms.

Fig. 3 shows shortening spindle-shaped interzonal filaments, a few with medial thickenings. Thinner continuous filaments are visible near the chromosome groups.

Fig. 4 shows medial swellings and the association of particles and vesicles with filaments; the chromosome arms have entirely collapsed.
Fig. 5 shows an increase in the number of filaments and associated material near the forming cell plate. Note that not all of the swellings are in register.

Fig. 6 shows many more particles and small vesicles joining the cell plate. In contrast, the peripheral part of the phragmoplast now seems nearly devoid of vesicles.

Fig. 7 shows the fusion of particles and vesicles to form the continuous cell plate.