Arrangement of Cellulose Microfibrils in Walls of Elongating Parenchyma Cells*

BY G. SETTERFIELD, PH.D., AND S. T. BAYLEY, PH.D.

(From the Division of Applied Biology, National Research Council, Ottawa, Canada)

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ABSTRACT

The arrangement of cellulose microfibrils in walls of elongating parenchyma cells of *Arena* coleoptiles, onion roots, and celery petioles was studied in polarizing and electron microscopes by examining whole cell walls and sections. Walls of these cells consist firstly of regions containing the primary pit fields and composed of microfibrils oriented predominantly transversely. The transverse microfibrils show a progressive disorientation from the inside to the outside of the wall which is consistent with the multinet model of wall growth. Between the pit-field regions and running the length of the cells are ribs composed of longitudinally oriented microfibrils. Two types of rib have been found at all stages of cell elongation. In some regions, the wall appears to consist entirely of longitudinal microfibrils so that the rib forms an integral part of the wall. At the edges of such ribs the microfibrils can be seen to change direction from longitudinal in the rib to transverse in the pit-field region. Often, however, the rib appears to consist of an extra separate layer of longitudinal microfibrils outside a continuous wall of transverse microfibrils. These ribs are quite distinct from secondary wall, which consists of longitudinal microfibrils deposited within the primary wall after elongation has ceased. It is evident that the arrangement of cellulose microfibrils in a primary wall can be complex and is probably an expression of specific cellular differentiation.

INTRODUCTION

Since primary cell walls form the limiting envelope of elongating plant cells, it is essential in gaining an understanding of the mechanism of cell elongation to know how the wall is established and maintained as cell growth proceeds. Recent studies (11, 13) indicate that thin parenchyma walls enlarge over their entire surface, and suggest that the cellulose microfibrils behave as proposed in the multinet model of Roelofsen and Houwink (8). In the multinet model it is assumed that microfibrils are continually deposited in a transverse direction on the inside of the wall, and random disorientation results from stretching of the wall. This mechanism gives rise to a wall structure similar to that described as "tubular texture" by Frey-Wyssling (5). However, several types of primary cell walls showing variations from simple tubular texture have been described. The variations are particularly obvious in thickened primary walls such as epidermal and guard-cell walls of *Arena* coleoptile (2, 10), and collenchyma of several species (1, 4, 6), where the wall contains many layers of longitudinally oriented microfibrils. In addition, however, several authors have described "ribs" of longitudinally oriented microfibrils in the thin primary walls of elongating parenchyma cells (7, 9, 12). These ribs are usually dismissed as "secondary thickening" added to the basic tubular texture, but no direct attempt has been made to demonstrate their relation to the growing wall.

It was decided to reinvestigate the structure of thin primary walls of elongating parenchyma cells in an attempt to provide a more complete description of the behaviour of the cellulose component. The results presented here indicate that the struc-
ture of these walls is considerably more complex
than previously envisaged.

Materials and Methods

The main studies were carried out on parenchyma of *Avena coleoptiles (Avena sativa, Lanark variety)* and onion roots (*Allium cepa*). Additional observations were made on parenchyma of young petioles of celery (*Apium graveolens, Non-Bolting Golden Plume variety*). Coleoptiles of various lengths were obtained from seedlings germinated on damp absorbent paper in the dark. Onion roots about 2 to 3 cm. long were germinated by placing small pickling bulbs over vials of tap water. Celery was grown from seed in a greenhouse.

Unfragmented cell walls free of non-cellulosic material were prepared for examination in the electron and polarizing microscopes by treating parenchyma tissue alternately for an hour each in 0.1 N HCl at about 90°C and 4 per cent NaOH at room temperature. Successive treatments were continued until clean individual cells could be obtained on shaking the tissue in water.

Stained cell walls mounted in water were studied in the polarizing microscope at 5460 A, using a rotating X/12 mica plate in the substage. For the electron microscope, drops of free cell suspension were dried on grids and the grids shadowed at 15° with palladium-gold. Fragments of walls were also prepared for the electron microscope by shattering the extracted tissue on dry ice (9).

Transverse sections of unextracted coleoptiles were studied in the polarizing microscope. Segments of coleoptiles were fixed in 70 per cent ethyl alcohol, dehydrated, and embedded in methacrylate. Sections 1 μm thick, with the methacrylate present, were mounted in water and observed as above.

Thin sections for electron microscopy were prepared as previously described in detail (2, 11). Small segments of tissue were treated with pectinase, fixed in 70 per cent ethyl alcohol, dehydrated, and embedded in methacrylate. Embedding medium was removed from sections, and they were shadowed with palladium-gold.

Observations on coleoptile parenchyma in the polarizing microscope and electron microscope were performed separately on cells near the vascular bundles and midway between bundles. Although there were considerable variations in diameters of cells, no appreciable differences in birefringence or microfibril arrangement were found.

RESULTS

Polarizing Microscope:

Figs. 1 to 3 show walls of parenchyma cells from coleoptiles of varying lengths under the polarizing microscope. The X/12 compensating plate was adjusted to emphasize the structural features of the walls. The structure is similar in all cells; large areas (T) of the walls appear from the sign of the birefringence to consist predominantly of transversely oriented microfibrils, while between these regions are narrow longitudinal ribs (R) with little or no birefringence. The ribs of cells in the same column appear to be aligned (A, Fig. 2), but it will be seen later from sections in the electron microscope that the ribs of lateral neighbouring cells do not necessarily correspond. There is no tendency for the ribs to extend laterally as the cells age. Similar organization is found in parenchyma cells of onion roots and celery petioles.

The optical path difference in the inter-rib region for parenchyma cell walls of young coleoptiles lies between -35 and -70 A with respect to the long axis of the cells, while in the ribs the values range between -20 and +20 A. The measurements were made on whole flattened cells, and therefore refer to two thicknesses of wall. It appears that the birefringence of the longitudinal microfibrils in a rib roughly cancels that of the transverse microfibrils underlying it, although there is considerable variation. Wilson (14) found a similar situation with longitudinal ribs in parenchyma cells of *Elodea canadensis*. This point will be discussed further in connection with the electron microscope results.

In transverse section, the parenchyma walls showed weak birefringence which varied in different regions of individual walls. The optical path differences ranged from roughly 15 to 40 A. The outer epidermal walls, which contain predominantly longitudinally oriented microfibrils (2), had an optical path difference of about 20 A. Thus, the values for parenchyma walls are consistent with the interpretation that some regions of the parenchyma walls contain predominantly longitudinally oriented microfibrils. However, these measurements are subject to considerable variation (2), and the results are of limited value.

Electron Microscope Observations on Intact and Fragmented Walls:

Fig. 4 is a low-magnification electron micrograph which shows the general arrangement of microfibrils in a parenchyma wall. In agreement with the polarizing microscope there are regions (T), containing the primary pit fields (P), composed of microfibrils with a predominantly transverse orientation interspersed with rib regions (R), containing longitudinally oriented microfibrils. This picture is similar to those published by other
authors (7, 12) and similar to those obtained in the present study from parenchyma of onion root and celery petiole. Figs. 5 and 6 are enlarged views showing the detailed microfibril arrangement of unfragmented walls at the junction of rib and interrib regions.

Two main points emerge from these pictures (Figs. 4 to 6). First, these views are of the outside of extracted, but unfragmented cell walls. Since the microfibrils composing the rib are clearly visible, they must be either an integral part of the wall or in extra layers on the outside. They cannot form a secondary wall, since by definition this occurs inside the primary wall. Second, in many areas microfibrils running transversely in the interrib region can be seen to change direction and weave into the rib (points marked C in Figs. 5 and 6). This suggests that these ribs are an integral part of the primary wall and not simply extra outer layers.

The appearance of wall fragments was similar to that of whole walls. However, because of the difficulty of deciding which face of the wall was being viewed it was impossible to say definitely whether longitudinal microfibrils occurred on the inside as well as the outside of the wall.

**Electron Microscope Observations on Transverse Sections:**

In interpreting the electron micrographs of sections shown in Figs. 7 to 18, certain considerations should be kept in mind. The removal of noncellulosic materials by pectinase causes a noticeable loosening of the microfibrillar structure and separation of adjacent walls, thereby giving an exaggerated thickness to the walls. Furthermore, the combination of pectinase treatment, alcohol fixation, and removal of embedding medium completely destroys cytoplasmic structure. Finally, unless the sections are very thin, removal of the embedding medium often results in the microfibril segments, particularly the short segments from microfibrils originally running at right angles to the plane of the section, falling over onto the supporting film. This latter effect is helpful in studying microfibril arrangement, since little detail is visible when the segments remain erect. A more detailed discussion of the interpretation of this type of section is given by Bayley et al. (2).

Despite the changes introduced by preparative procedures, considerable order is found in sections, particularly of thickened primary walls (2). This order is reproducible and agrees with that expected from polarizing microscope and x-ray diffraction studies (2), indicating that artifacts which could result in serious misinterpretations are not produced.

Regions of the wall containing microfibrils oriented predominantly transversely can be found in cells of all ages, and are always similar in structure. As described previously (11), microfibrils on the inside of the wall are densely packed and well oriented, while towards the outside they are more loosely organized and less well oriented. This type of organization is shown in young and old cells of both *Avena* coleoptile and onion root in Figs. 7 to 10. In very young cells little disorientation is present (Fig. 9).

An extensive study of cells undergoing elongation failed to reveal any instances in which extra layers of longitudinally oriented microfibrils occurred inside the continuous primary wall where secondary wall would be deposited. It thus seems certain that the ribs are not related to secondary wall.

Two types of longitudinal microfibrillar organization are found, however, in sections of growing cells. The first is illustrated in Figs. 11 to 13, where the entire thickness of wall appears to be composed of longitudinally oriented microfibrils (IR) so that the rib forms an integral part of the wall. Figs. 11 and 12 show regions of this type from young and old coleoptiles, respectively, indicating that the structure persists during growth. Figs. 11 and 12 also indicate that ribs of longitudinal microfibrils do not necessarily occur in the same regions of adjacent walls. In these pictures one wall contains longitudinal microfibrils (IR), while the adjacent wall has the typical transverse structure (T). In Fig. 13, which is from an isolated coleoptile segment grown in auxin and sucrose (3), both adjacent walls contain integral ribs. In the upper wall, the appearance of a rib which has remained erect can be seen (E). The lower wall has fallen over in two directions (the direction changes at P) and clearly shows that no underlying transverse microfibrils have been obscured by the fallen segments of longitudinal microfibrils. A second type of longitudinal microfibrillar organization is shown in Figs. 14 and 15. This consists of an extra, distinct layer of longitudinal microfibrils (OR) outside the continuous primary wall (CPW). These extra layers usually, although not always, occur at the corners formed where several cells meet, and are found in cells of all sizes in both *Avena* and onion.
The integral type of rib seems more frequent in coleoptiles, while outer ribs are definitely more common in the onion root. The two types of rib help to explain the range of optical path differences observed in the polarizing microscope studies on flattened intact cells. Assuming the density of microfibrils in a single layer to be roughly constant throughout, an integral rib would compensate an inter-rib region in the opposite wall, whereas an outer rib of longitudinal microfibrils would compensate only the layer of transverse microfibrils adjacent to it in the same wall, leaving a net negative birefringence due to transverse microfibrils in the opposite wall. Appreciable positive birefringence would be found when two ribs coincided in flattening.

Besides these two types of longitudinal ribs, cells which have ceased elongating show extra layers of longitudinal microfibrils within the continuous primary wall (Figs. 16 to 18). In the coleoptile a single layer of microfibrils, generally around the entire wall, appears during the first day after elongation ceases (SW, Fig. 16). Up to 6 days after elongation no additional layers are found, although by this time the non-cellulosic material becomes very difficult to remove with pectinase. Fig. 17 shows a section of wall in a coleoptile cell, which contains a rib of longitudinal microfibrils outside (OR; not fallen over), the continuous primary wall (CPW) composed of predominantly transverse microfibrils in the center, and a layer of longitudinal microfibrils (SW) on the inside. In the onion root, longitudinal microfibrils are found on the inside of cells 5 to 6 mm. from the root tip. Often several distinct layers are present (SW, Fig. 18).

These inner layers of longitudinal microfibrils meet the classical criteria for secondary walls; they arise after cessation of elongation and form inside the primary wall. Furthermore, the microfibrils seem more compact than in the primary wall, suggesting a higher percentage composition of cellulose as is typical of secondary walls. Since pectinase has removed the encrusting non-cellulosic materials in these secondary walls, it is probable that the non-cellulosic materials are similar to those present in the primary wall. Lignin would appear to be absent, since pectinase is incapable of revealing microfibrils in lignified walls such as those of xylem elements.

DISCUSSION

It is clear that the structure of the thin primary wall of parenchyma cells is considerably more complex than previously considered. The simple concept of "tubular texture" (5) with secondary thickenings arising and extending laterally during elongation (7) is incorrect. As shown here, true secondary wall arises inside the primary wall after elongation ceases, while the ribs are already an intimate part of the primary wall during the early stages of elongation. Furthermore, the multinet model for wall growth as originally presented by Roelofsen and Houwink for plant hairs (8) is too simple to account for the observed structure in the parenchyma cells studied here.

It appears that throughout elongation the primary wall contains microfibrils running both transversely and longitudinally. Moreover, the areas with different orientation are not independent, but are intimately linked by microfibrils changing direction and running from one area to the other. Change of microfibrillar direction within individual walls has also been noted in collenchyma cells of celery (4), and probably occurs at the transition from the outer to radial walls in coleoptile epidermal cells (2). During growth, microfibrils are presumably deposited with both longitudinal and transverse orientation. Elongation of the wall would not affect orientation of the microfibrils, or portions of microfibrils, with longitudinal orientation, but it would cause a disorientation of microfibrils running transversely. Thus the wall would have, as found, a multinet appearance in the inter-rib regions (Figs. 7 to 10) and a merging of rib and inter-rib regions (Figs. 5 and 6).

The external layers of longitudinal microfibrils are a further complication of primary wall structure. These layers are similar to those found in the early thickenings of celery collenchyma cells (4). In collenchyma extra layers of longitudinal microfibrils appear to arise outside the inner, intact layer of the primary wall. It has been suggested that formation and growth of microfibrils may take place remote from the organized cytoplasmic surface through polymerization of low-molecular weight precursors by enzymes bound in the wall. A similar mechanism may operate in the formation of the outer ribs described here, although it is also possible that the cytoplasm penetrates the thin continuous wall to deposit the outer microfibrils.

At any rate, it appears that thickened primary walls containing many layers of longitudinal microfibrils (2, 4, 6) are not entirely distinct structures, but merely extreme modifications of thin primary wall. In fact, a graded series of primary wall types may be found in higher plants. The plant hairs
studied by Roelofsen and Houwink (8) possess no longitudinal microfibrils and grow by the simple multitnet mechanism. The parenchyma walls described here show roughly an equal occurrence of transverse and longitudinal microfibrils in the main wall and the beginnings of extra outer layers of longitudinal microfibrils. Collenchyma walls (1, 4, 6) show an extensive development of the extra layers of longitudinal microfibrils around the entire wall, while epidermal cells (2) and guard cells (10) in the Arena coleoptile have extra layers of longitudinal microfibrils precisely localized in definite walls. This leads to the conclusion that the form of the primary wall is not primarily a passive result of external physical forces, but is precisely determined as part of specific cellular differentiation. In this respect, primary walls would then be similar to secondary walls, which possess very distinctive forms in different cell types and species.

Since almost nothing is known of the biochemistry of cellulose formation or of the mechanisms of microfibril formation or growth, it is impossible at present to see how the cell builds up the complex structure of these elongating walls. Particularly puzzling are the means by which precise microfibril orientation is controlled. Furthermore, maintenance of intricate wall form during rapid cell growth requires a delicate balance between the rates of elongation and new wall formation. This balance may be partially explained by the finding that primary wall deposition is dependent upon wall extension taking place (3).

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References
EXPLANATION OF PLATES

PLATE 204

FIGS. 1 and 2. Polarizing micrograph of extracted, but unfragmented parenchyma cell walls from an *Avena* coleoptile 10 to 15 mm. long. The compensator has been adjusted to emphasize the structural detail. Areas showing (negative) birefringence (T) contain primary pit fields (P) and are separated by longitudinal ribs (R) with little or no birefringence. 8 mm. objective. × 340.

FIG. 3. Same as Figs. 1 and 2, except cell is from a coleoptile 50 mm. long. × 410.

FIG. 4. Electron micrograph of an extracted, but unfragmented parenchyma cell wall from a coleoptile 15 mm. long, showing the same structural features as Figs. 1 to 3. × 8900.
(Setterfield and Bayley: Parenchymal cellulose microfibrils)
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Figs. 5 and 6. Enlarged views of extracted, but unfragmented coleoptile parenchyma walls at the junction of rib and inter-rib regions. The unlabelled arrow indicates the major axis of the cell. Longitudinally oriented cellulose microfibrils are present in the rib region (R), and transverse microfibrils surround the pit fields (P) in the inter-rib region (T). Microfibrils can be seen to change direction (C) and weave from rib to inter-rib regions. Fig. 5, X 17,000. Fig. 6, X 17,000. (The 1μ line in Fig. 5 should be the same length as that in Fig. 6.)
(Setterfield and Bayley: Parenchymal cellulose microfibrils)
**Fig. 7.** Transverse section of portions of inter-rib regions of two adjacent parenchyma cell walls in a coleoptile 20 mm. long. The unlabelled arrows point toward the centers of the cells. Near the lumina (L) the walls are composed of microfibrils compactly arranged and transversely oriented, while toward the intercellular region (IC) considerable disorientation is present. Remains of cytoplasm (Cy) are present. × 35,000.

**Fig. 8.** Same as Fig. 7, except that the cells are from a coleoptile 50 mm. long about the time elongation has ceased. × 35,000.

**Fig. 9.** Transverse section of portions of inter-rib regions of two adjacent parenchyma cell walls in the terminal millimeter of an onion root. The unlabelled arrows point toward the centers of the cells. The two walls are closely appressed and are composed almost entirely of transversely oriented microfibrils. × 35,000.

**Fig. 10.** Same as Fig. 9, except that the cells are from the region 3 to 4 mm. from the tip of the root. As in Figs. 7 and 8, the amount of disorientation of microfibrils increases from the lumen (L) to the intercellular (IC) side of the walls. × 42,000.
(Setterfield and Bayley: Parenchymal cellulose microfibrils)
Fig. 11. Transverse section of portions of two adjacent parenchyma walls near a junction between several cells in a coleoptile 10 mm. long. One wall shows the typical appearance of the inter-rib region (T) (cf. Figs. 7 and 8), while the other wall is composed entirely of longitudinally oriented microfibrils (IR). The region IR presumably represents a section of a longitudinal rib which is an integral part of the main primary wall. The unlabelled arrows point toward the centers of the cells. IC, intercellular region; L, lumen. X 38,000.

Fig. 12. As Fig. 11, except that the cells are from a coleoptile 50 mm. long about the time elongation has ceased. X 34,000.

Fig. 13. Similar to Figs. 11 and 12, except that the cells are from an isolated coleoptile segment grown for 20 hours in 2 per cent sucrose plus 8 p.p.m. indoleacetic acid. Both walls contain only longitudinal microfibrils (IR). The lower wall has fallen over in two directions, the direction changing at P. The rib in the upper wall has remained erect (E). X 37,000.
(Setterfield and Bayley: Parenchymal cellulose microfibrils)
FIG. 14. Transverse section of the junction of three parenchyma walls in a coleoptile 50 mm. long about the time elongation ceased. Outside the continuous primary wall (CPW), which at these points contains predominantly transverse microfibrils, "outer ribs" of longitudinal microfibrils (OR) occur in the intercellular region (IC). Unlabelled arrows point toward the centers of the cells. × 24,000.

FIG. 15. Transverse section of two adjacent parenchyma walls 3 to 4 mm. from the tip of an onion root. The two continuous primary walls (CPW) are composed largely of transverse microfibrils. Two outer ribs (OR) composed of longitudinal microfibrils are present in the intercellular region. × 29,000.
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**Fig. 16.** Transverse section of two adjacent parenchyma walls from a coleoptile 50 mm. long, 1 day after cessation of elongation. Inside each primary wall (CPW) is a layer of secondary wall (SW) composed of densely packed longitudinal microfibrils. Unlabelled arrows point toward the centers of cells. IC, intercellular region. × 35,000.

**Fig. 17.** Transverse section of a single parenchyma wall in a coleoptile 50 mm. long, 2 days after cessation of elongation. The unlabelled arrow points toward the center of the cell. On the outside, adjacent to the intercellular region (IC), is an outer rib (OR) of longitudinal microfibrils standing erect. In the middle of the wall is the continuous primary wall (CPW), containing predominantly transverse microfibrils. On the inside is a layer of secondary wall (SW) composed of longitudinal microfibrils. × 42,000.

**Fig. 18.** Transverse section of a single parenchyma wall, 5 to 6 mm. from the tip of an onion root. On the outside is the primary wall (CPW). Inside is the secondary wall (SW) composed of several layers of longitudinal microfibrils. A small section of an outer rib (OR) is visible in the lower left. The unlabelled arrow points toward the center of the cell. IC, intercellular region. × 42,000.
(Setterfield and Bayley: Parenchymal cellulose microfibrils)