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Two separate zones of helicoidally orientated microfibrils are present in the walls of *Nitella* internodes during growth

S. Levy*

Laboratoire des Biomembranes et Surfaces Cellulaires Végétales, Ecole Normale Supérieure, Paris

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Summary. The dynamic changes in microfibril architecture in the internode cell walls of the giant unicellular alga *Nitella translucens* were studied during cell expansion. Thin section electron microscopy in conjunction with mild matrix polysaccharide extraction techniques revealed three distinct architectural zones in the walls of fully grown cells. These zones were related to distinct phases of growth by monitoring changes in cell wall architecture of internodes during active cell expansion. The initial microfibril deposition before the onset of active cell growth is helicoidal. A helicoid is a structurally complex but ordered arrangement of microfibrils that has been detected increasingly often in higher plant cell walls. During active cell elongation microfibrils are deposited transversely to the direction of cell elongation as shown in earlier studies by birefringence measurements in the polarizing microscope. The gradual decline in cell elongation corresponds with a final helicoidal deposition which continues after cell expansion ceases entirely.

The continual presence of the initial helicoidal zone in the outer wall region during the whole growth process suggests that these microfibrils do not experience strain reorientation and are continually reorganized, or maintained, in a well ordered helicoidal arrangement.

Keywords: Growth; Cell wall structure; Helicoids; *Nitella*.

Introduction

One of the many roles attributed to the plant cell wall is that of providing both a rigid, yet flexible, barrier allowing controlled enlargement of the plant protoplast. The cell wall is an essential component in regulating the rate and the nature of expansion that the cell will undergo during active growth. The directionality of growth depends in part on the structure of the cell wall, other modulating cell wall factors involved being the localized wall pH (Metraux et al. 1980) and the activity of the so-called wall loosening enzymes (Cleland 1987).

One extreme case of plant cell growth is exhibited by the giant internode cells of the unicellular green alga *Nitella*. As shown by Green (1954, 1958), internode cells are capable of expanding at an exponential rate as they grow from 20 μm to 60 mm in length. Depending on culture conditions expansion occurs either uniformly over the whole cell surface (Green 1954) or in localized acidic regions of the wall (Métraux et al. 1980). To explain the marked predominance of cell elongation over diametric expansion Green (1960) postulated that the cell wall microfibrils were deposited transversely to the axis of elongation and that these transverse microfibrils would be subjected to passive reorientation during growth, such that outer wall regions would possess strain aligned longitudinal microfibrils. Studies of cell wall microfibril orientation have yielded results consistent with these predictions (Green 1958, 1960; Richmond 1983) thereby suggesting that Green's hypothesis provided an adequate explanation for the observed anisotropy in cell growth.

However, the recent finding (Neville and Levy 1984, Hotchkiss and Brown 1987) that helicoidally orientated microfibrils exist in *Nitella* cell walls suggests that growth may be modulated by more cell wall structural parameters than previously envisaged by Green. A helicoid is a composite structure in which parallel microfibril monolayers are packed with a stepwise rotation with respect to their near neighbours (Neville and Levy

* Correspondence and reprints, present address: Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80303-0347, U.S.A.

1985, Levy 1987). Since helicoids are isotropic structures, manifesting no preferred directionality, their role in the anisotropic expansion of a *Nitella* internode warrants further clarification.

This study focuses on the dynamic changes in cell wall architecture in *Nitella* internodes during different stages of growth and more specifically on the distribution of helicoids during the growth process. The observed modifications of microfibril architecture in the walls of fully expanded cells correspond to three distinct phases of growth. The first coincides with development prior to the onset of exponential growth and is characterized by a layer of helicoidally orientated microfibrils. With the transition to active growth, microfibrils appear to be deposited with a unidirectionally transverse orientation, as noted by Green (1958, 1960). Finally, towards the end of the growth phase and continuing even after growth ceases, another layer of distinct helicoidally orientated microfibrils is deposited.

Materials and methods

Plant material and culture methods

Axenic cultures of *Nitella translucens* were grown at 20 °C in 25 cm test tubes filled with an autoclaved culture medium prepared according to Ducreux (1985). Light (42 $\mu E/m^2s$) was provided by neon tubes (White and Daylight) on a 12 h light/12 h dark cycle.

Growth measurements

Apical and internodal cells up to and including the second node were isolated in 9 cm petri dishes filled with culture medium under sterile conditions. New shoots developed from these old shoot apices within 4 days. Cell lengths and diameters were subsequently measured every 4 days using a binocular microscope equipped with an ocular micrometer. From these values relative growth rates (RGR) in cell length and diameter were calculated using the formula

$$RGR = (\ln x_{final} - \ln x_{initial})/(t_{final} - t_{initial}),$$

where x is either cell length or diameter and t is time (Green 1954). The calculated growth rates were finally expressed as percentages.

Electron microscopy

Fixation

Internodal cells were dissected into 1 mm lengths under fixative (2.5% glutaraldehyde, 50 mM sodium cacodylate pH 7.2) immediately after measuring cell dimensions. They were fixed for 2 h at room temperature with agitation then washed in buffer. Specimens were either in-block stained with 2% uranyl acetate for 18 h or matrix polysaccharides were partially extracted with 2% Macerozyme R 10 in 100 mM phosphate-citrate buffer, pH 5.0 at room temperature for 3 h with agitation. Specimens were dehydrated in a graded ethanol series and embedded in Epon/Araldite or LR White resin.

Sectioning and staining

Silver or gold sections were cut with a diamond knife on an LKB ultramicrotome III and were collected on formvar coated copper grids. Sections were either double stained with uranyl acetate and lead citrate (extracted cell walls) or just with lead citrate (in-block stained cell walls). Some sections were stained by the periodic acid-thiosemicarbazide-silver proteinate method (Thiery 1967) which reacts with vic-glycol groups and is therefore a non-specific stain for polysaccharides. This procedure did not improve microfibril resolution over uranyl/lead staining and therefore was not pursued extensively.

Electron microscope observation

All grids were examined on a Philips EM 400 electron microscope, equipped with a goniometric tilting stage, at 80 kV.

Results

Morphological changes during cell growth

Almost all internode cells measuring less than 20 mm in length elongate by between 10–40%/day (Fig. 1). It should be noted that a daily doubling of a cell dimension corresponds to a RGR of 69.3%/day using the compound interest method of calculating rates. Figure 1 also demonstrates that the rate of cell elongation decreases as cell length increases. Therefore cell length provides a general indication of the stage of internodal growth.

The rate of diametric expansion appears to follow a different trend (Fig. 2) and a maximum growth rate is attained when cells are 0.2 mm in diameter or larger. Cell diameters of < 0.1 mm could not be measured with any degree of accuracy using a binocular microscope. An overall comparison of the magnitudes of growth rates in Figs. 1 and 2 reveals a marked predominance in the rate of expansion in cell length over cell diameter indicating a certain degree of anisotropic growth. This anisotropy can be visualized directly in a log-log plot

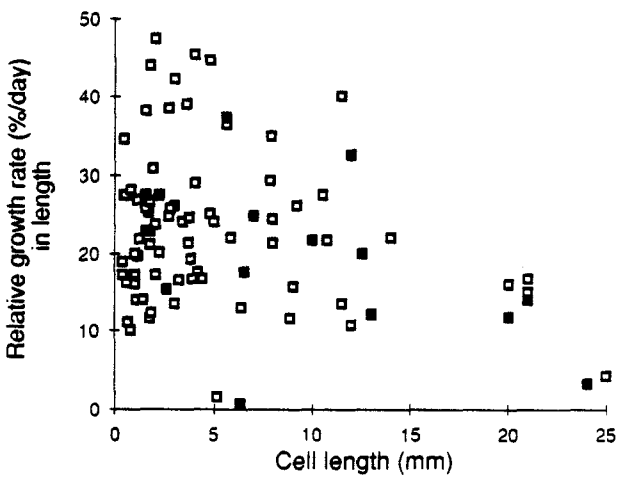


Fig. 1. Relative growth rate in length plotted against cell length. Note that growth rate decreases with increasing cell length. Data from 56 cells. ■ Wall architecture determined

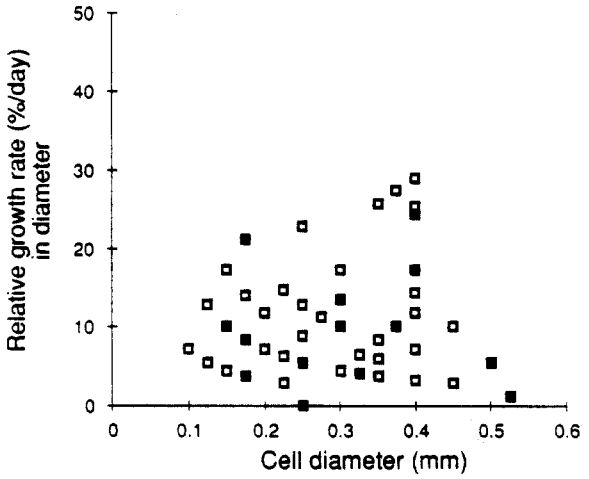


Fig. 2. Relative growth rate in diameter plotted against cell diameter. Note that the rate of growth is at a maximum when cells are 0.2 mm or larger in diameter. ■ Wall architecture determined

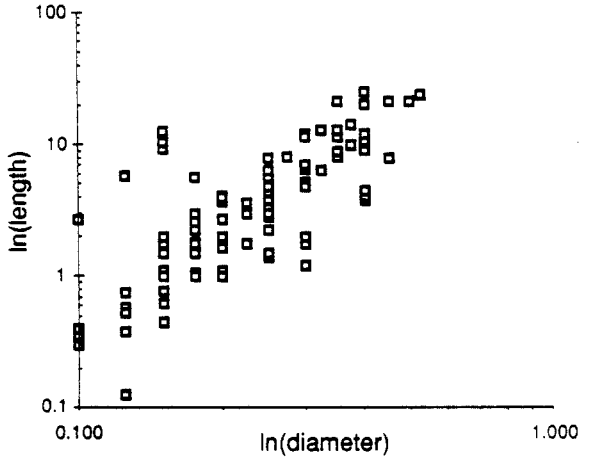


Fig. 3. Natural logarithmic plot of length against diameter. While considerable scatter exists note the overall upsloping linear trend indicating that expansion occurs at an exponential rate and anisotropically over much of the growth process

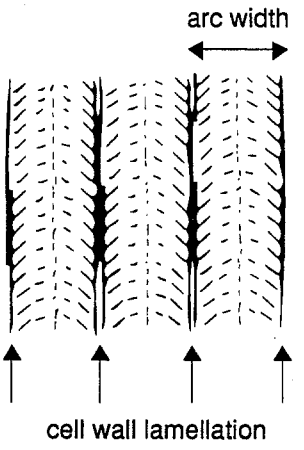


Fig. 4. Diagrammatic representation of the structural features displayed by helicoids when sectioned or fractured obliquely to the surface layers. The arcs are composite structures composed of short microfibrillar lengths. The lamellation occurs when long microfibrillar lengths are exposed in the plane of the section

of cell length against diameter (Fig. 3), on which growth records from 56 different internode cells have been combined. In spite of the scatter in the data points there is an overall tendency toward linearity indicating that both cell dimensions change anisotropically and at an exponential rate over much of the observed growth process.

Wall architecture at the end of growth

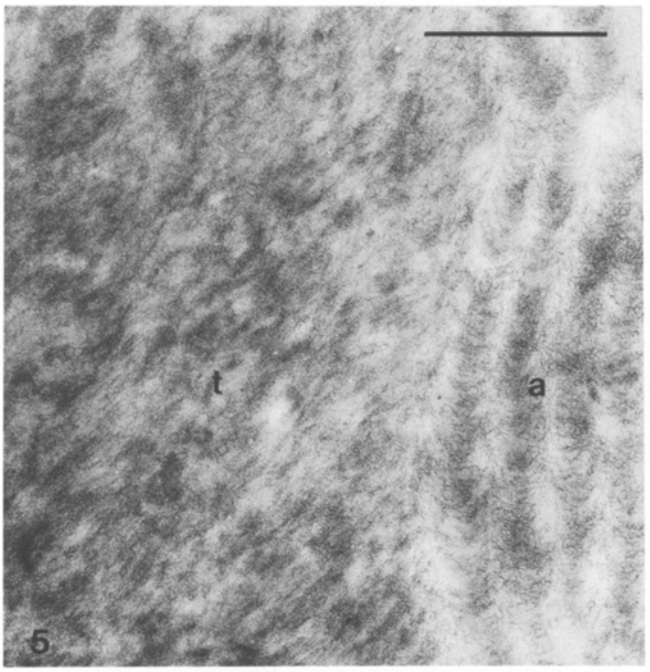
The inner wall regions of cells approaching the end of their expansion exhibit microfibrils organized into rows of arcs when sectioned obliquely for electron microscopy (Fig. 5). The arcing is an illusion produced by microfibrils arranged in a composite manner and when seen in conjunction with lamellation (Fig. 4) it is indicative of the presence of helicoidal superstructures. Microfibrils constituting the arcs are more distinctly visualized after macerozyme extraction (Fig. 6) where arc width is seen to decrease towards the innermost wall region. This ordered helicoidal deposition occurs towards the end of active growth as shown by the 24 mm cell in Fig. 6 which was growing at a rate of 3.3%/day at the time of fixation. The arcing does not always appear in discretely parallel rows as demonstrated by Fig. 7, which is a wall region taken from the same cell visualized in Fig. 6. Variations of arc width suggest either localized instabilities or even an element of overall fluidity in the inner wall helicoid.

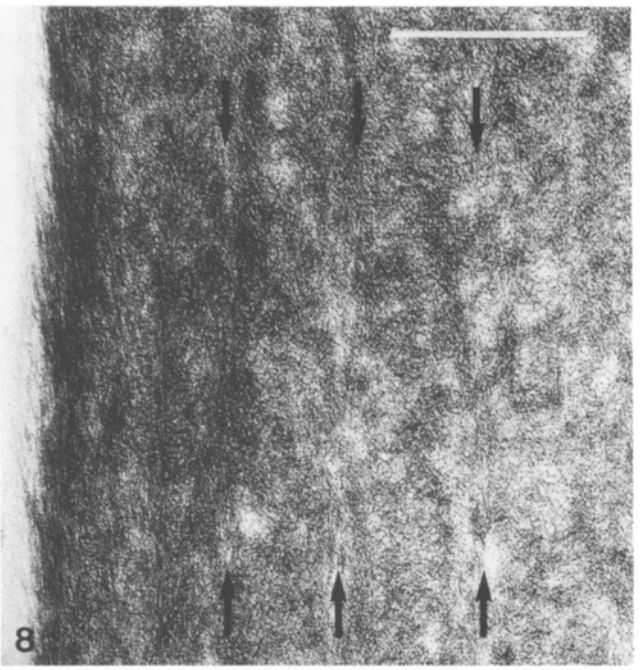
The outer walls of cells approaching the end of growth possess a microfibrillar organization that is not so evidently distinct. An unextracted outer cell wall region does however reveal a degree of lamellation (Fig. 8) which becomes clearer on extraction (Fig. 9). However, it is difficult to discern whether the microfibrillar patterning between the longitudinally orientated microfibrils display arcing.

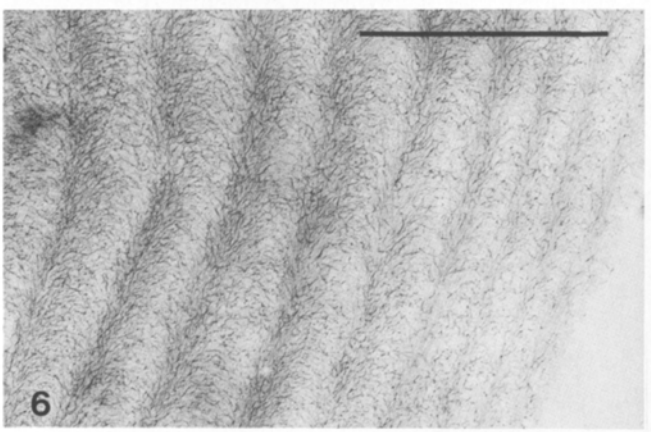
In order to determine whether the outer wall region possess rows of arcs, sections were physical tilted on the goniometric electron microscope stage as helicoidal superstructures alone exhibit a reversal in arc polarity when the two extreme tilt views are examined (Neville and Luke 1969, Bouligand 1972). Vertical, and not oblique, sections were cut and the same regions of the

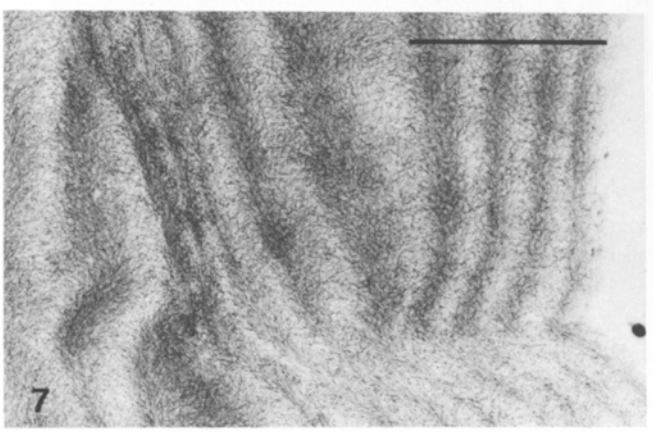
outer wall were visualized at ± 40° on the electron microscope (Figs. 10 and 11). These tilted views display a lamellation effect, whose presence is indicated with arrows, whilst the intervening microfibril orientations combine to produce an arced patterning, whose polarity is seen to reverse on comparing Fig. 10 with Fig. 11.

Another means of producing this arc polarity reversal is achieved by examining the outer wall architecture taken from diametrically opposite points of one inter-









node cell. Figure 17 depicts diagrammatically the expected result from such an observation. Figures 12 and 13 are micrographs from a single oblique section depicting two outer wall regions taken at diametrically opposed points in a 20 mm internode cell elongating by 11.8%/day. The polarity of the arcing effect observable in one row from each region of opposing wall is marked for clarity and it is evident that arc polarity shows reversal when Figs. 12 and 13 are compared. The disparity in arc width dimension seen in Figs. 12 and 13 is probably due to a difference in the obliquity of section plane that traverses each of the diametrically opposed wall regions. The relationship that exists between angle of section and the thickness of observed arcs has been previously demonstrated (Bouligand 1972; Neville and Levy 1984, 1985). These observations at different viewing angles provide additional evidence for the presence of helicoidally oriented microfibrils in the outer wall region, i.e., the wall layers that were deposited during the early phase of cell growth.

The groups of inner and outer helicoids are separated by a region of wall that shows neither arcing nor any form of lamellation. This region of wall, seen in Fig. 5, displays long lengths of microfibrils in parallel alignment which appear to be orientated obliquely to the cell's long axis. Therefore unidirectionally orientated microfibrils are situated between the two helicoidal regions. Furthermore the obliquely-transverse section (Fig. 5) displays microfibrils in the plane of section indicating that these microfibrils are orientated transversely, or near transversely, with respect to cell length.

Wall architecture during growth

The wall architecture of actively growing cells can be clearly visualized only after treatment with macerozyme (data not shown). Other extractants such as EDTA, DMSO, and methylamine were tested during the course of this study but none were able to reveal both wall architecture and preserve simultaneously in vivo microfibrillar arrangements.

Figure 14 shows the cell wall of a rapidly growing cell, elongating by 23%/day, and treated for 3 h with macerozyme at room temperature. Although this relatively harsh extraction regime led to some disruption of the microfibril organization in the inner layers of the walls, it was found to be necessary to reveal the architecture of the outer wall layers.

As seen at higher magnification (Fig. 15) the outer wall region exhibits lamellation coupled with localized arced microfibrillar arrangements. The goniometric tilting test, when performed on these cells, did reveal a difference in the microfibrillar patterning at each extreme of tilt, however, arc polarity reversal was not clearly apparent (data not shown). Nevertheless the microfibrillar appearance observed from oblique sectioning alone (Fig. 15) does provide evidence supporting the occurrence of an outer helicoidal cell wall in actively growing *Nitella* internodes.

Cell wall structural dynamics

Figure 16 demonstrates the relationship between cell diameter, rates of expansion in cell diameter and the types of cell wall architectures observed in ten internodal cells. As seen in this diagram, the formation of helicoids appears to coincide with low growth rates in cell diameter of either small or large diameter cells. In contrast, the deposition of transversely orientated microfibrils coincides with a period of high growth rates, which are associated with cells of intermediate diameter. This kind of relationship was not observed between growth rate in length and wall architecture.

Figs. 5–9. Oblique sections of the inner and outer wall regions of *Nitella* internode cells approaching the end of growth. Bar: Figs. 5–7 and 9, 1 μm; Fig. 8, 0.5 μm

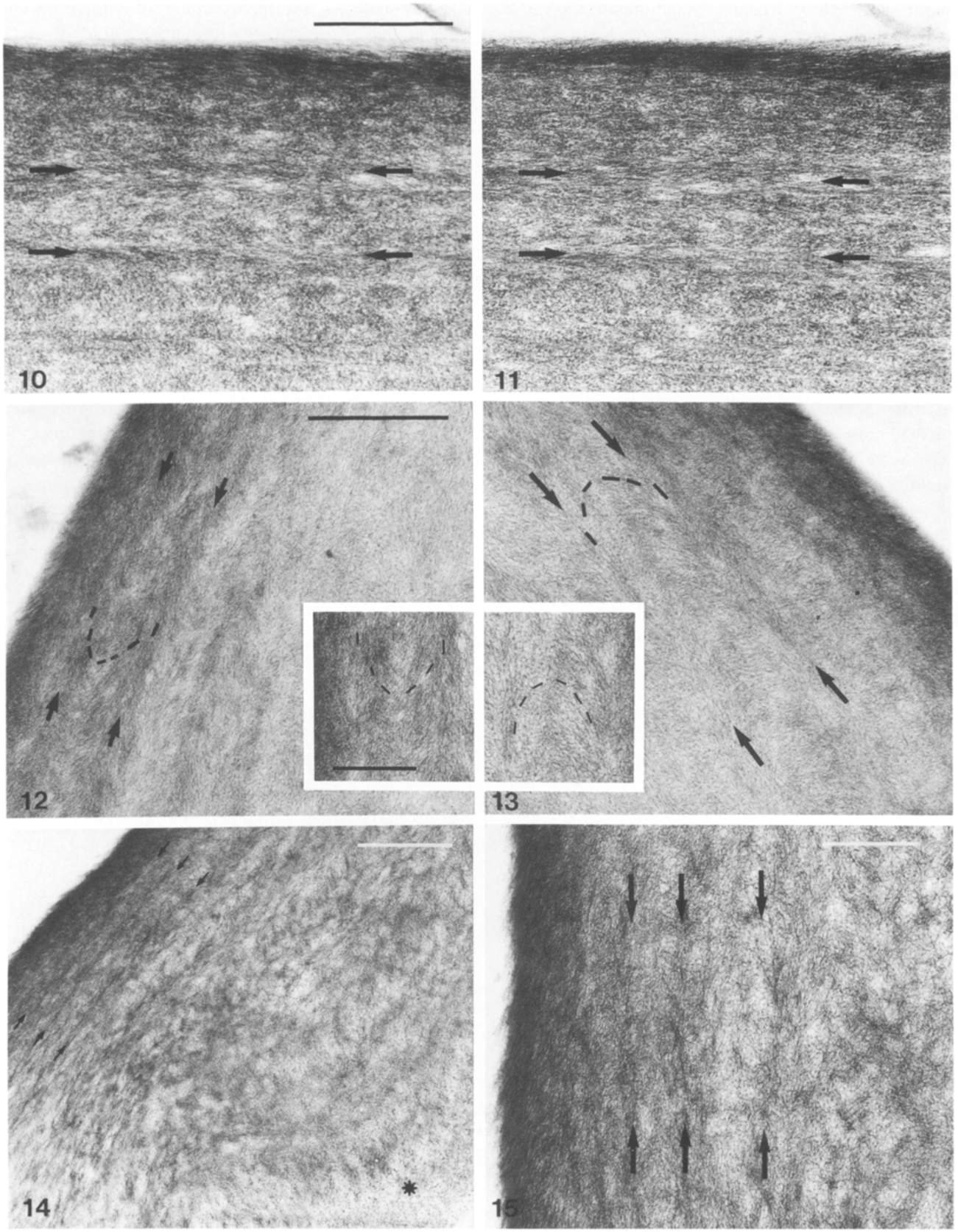
Fig. 5. An oblique-transverse section of the inner wall region. *a* Region nearest the cytoplasm where microfibrils are helicoidally orientated (note the arcing effect). *t* Region with microfibrils in the plane of the section. The long axis of the cell is orientated vertically to the page indicating that these microfibrils are almost circumferentially orientated around the cell. In-block staining

Fig. 6. Inner wall region of a 24 mm cell growing by 3.3%/day at the time of fixation. Note nine rows of arcs progressively decreasing in width towards the cytoplasmic interface. Macerozyme extraction, uranyl/lead

Fig. 7. Inner wall region from the same cell in Fig. 6 displaying arcs of varying width indicating that the helicoidal architecture is prone to structural variations. Macerozyme extraction, uranyl/lead

Fig. 8. Outer wall region. Arrows mark regions where lamellation occurs indicating a helicoidal structure. In-block staining

Fig. 9. Outer wall region of 20 mm cell growing by 11.8%/day. Arrows indicate lamellation between which regions of microfibrillar arcing are situated. Both are indicative of helicoids in the outer wall region. Macerozyme extraction, uranyl/lead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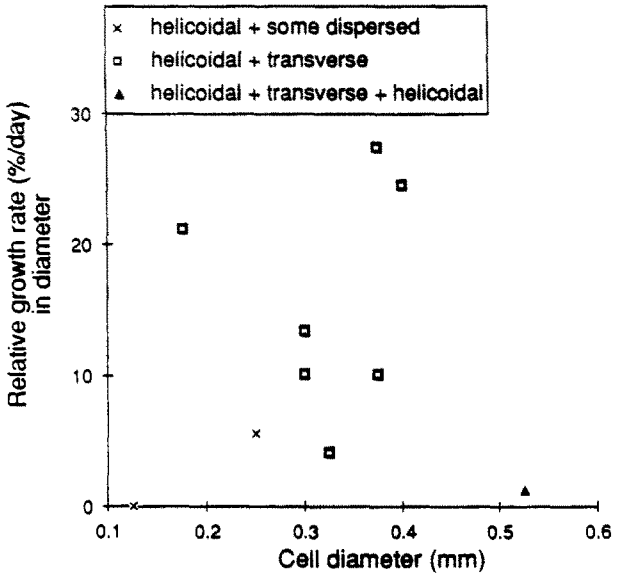

Fig. 16. Graph summarizing the changes in wall architecture from 10 internodes of a known growth rate in diameter plotted against cell diameter. Helicoidal architecture appears to coincide with low growth rates in cell diameter

Discussion

Three architecturally distinct zones have been observed in the walls of *Nitella* internode cells that have attained their maximum dimensions. These are, two helicoidal regions, located at the inner and outer zones of the cell wall, and a connecting layer of transversely orientated microfibrils (Fig. 17).

Inner wall helicoid

The cell walls of mature *Nitella* internodes, which were presumed to be no longer elongating, have previously been shown to possess helicoidal arrays of microfibrils in the inner wall regions (Neville and Levy 1984, Hotchkiss and Brown 1987). The helicoid in the cell walls of *Nitella opaca* and *N. translucens* was revealed by employing two different electron microscopic visualization techniques: thin sectioning (Neville and Levy 1984) and freeze fracture (Hotchkiss and Brown 1987).

By studying oblique thin sections of unextracted cell walls from internodes of a known growth status (Figs. 5–7), it has been possible to confirm the presence of a helicoidal zone adjacent to the plasma membrane and to define the conditions under which this layer is laid down. These findings indicate that this helicoidal deposition is present in walls of cells approaching the end of their elongation phase and continues to be deposited after elongation stops. Inner wall helicoids are most prominent in cells exceeding 20 mm in length under current culture conditions.

Outer wall helicoid

The outer wall helicoid is present in both growing and non-growing cells. Although their arcs are never as clearly seen as those associated with inner wall helicoids, the lamellation effects are readily observed.

The occurrence of helicoidally orientated microfibrils in the outer wall has been demonstrated by subjecting this wall region to a goniometric tilting test and by viewing diametrically opposite points of the wall from one cell. These critical tests produced reversals in arc polarity on comparing tilt extremes (Figs. 10 and 11) or by viewing opposing wall surfaces (Figs. 12 and 13). Bouligand (1965) demonstrated that helicoidal superstructures, when viewed obliquely to the surfaces of each component layer, produced arcs. It is possible to obtain two oblique views of helicoidally organized microfibrillar layers and it has been previously demon-

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Figs. 10 and **11.** Goniometric tilting of vertically sectioned cell walls. A 24 mm cell elongating by 3.3%/day. Macerozyme extraction. Bar: 0.5 μm

Fig. 10. Visualization of an outer cell wall sectioned longitudinally and tilted by − 40° on the goniometric electron microscope stage. Arrows mark the lamellation effect. Microfibrils between the arrows constitute the arcing effect

Fig. 11. Same longitudinal section as in Fig. 10 tilted by + 40°. Microfibrils form an arc of the opposite polarity to that visualized in Fig. 10

Figs. 12 and **13.** Oblique-transverse section revealing the outer wall region of diametrically opposed points in a 20 mm internode cell elongating by 11.8%/day. Arrows mark the lamellation effect. Broken lines delineate the polarity of one row of arcs. Note the reversal in arc polarity on comparing Fig. 12 with Fig. 13. Bar: 1 μm. **Insets** Higher magnification of Figs. 12 and 13 showing the microfibrils that delineate one row of arcs. Arc polarity reversal is apparent on comparison of the insets. Bar: 0.5 μm

Figs. 14 and **15.** Wall architecture of actively growing cells. Macerozyme extraction

Fig. 14. The whole cell wall of a 1.5 mm cell growing by 23%/day. Arrows indicate lamellation in the outer wall. The inner wall microfibrillar architecture (*) is disrupted by the extraction process. Bar: 1 μm

Fig. 15. Higher magnification of the outer wall lamellation (arrows) seen in Fig. 14. Bar: 0.5 μm

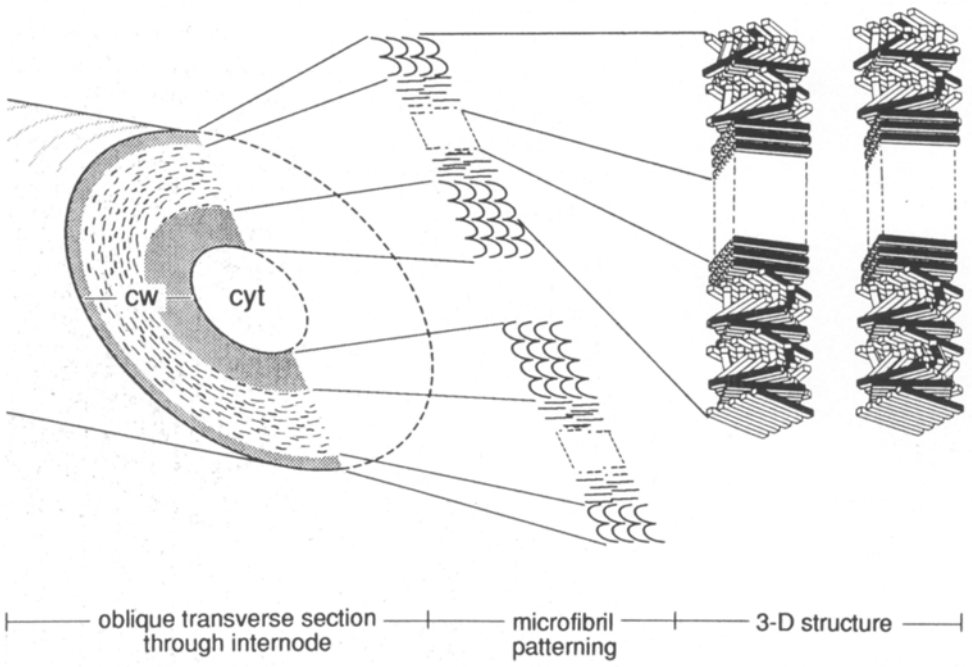

Fig. 17. An obliquely transverse section of a *Nitella* internode cell depicting the relative distribution of the three distinct microfibrillar architectures in the wall. The microfibril organization constituting the two-dimensional patterns seen in the electron microscope from different wall regions is elaborated into three-dimensions and shown as a stereo-pair (for method, see Levy 1987)

strated that arc polarity reverses when two such views are compared (Neville and Luke 1969, Livolant et al. 1978, Neville 1980). A 3-D computer modelling technique has been developed to display graphically the expected results of the tilting test (Levy 1987).

The outer wall constitutes the initial extracellular secretion by the cell at the onset of growth. The microfibrillar architecture in the outer walls of growing (Fig. 15) and non-growing (Figs. 8–13) cells provide evidence supporting the existence of a helicoidal architecture in the outer walls of cells at the beginning of growth. However, it was not possible to find a short (0.125–1 mm in length), growing cell with a completely helicoidal wall. This could be because matrix extraction disrupts the microfibrillar architecture in thin walls. In fact, a certain degree of disruption is even evident in the inner wall of the young cell shown in Fig. 14. Alternatively, a completely helicoidal cell wall may exist in recently formed internode cells (approximately 20 µm in length; Green 1958), which were not considered in this study due to technical limitations.

Previous studies of cell wall architecture

The inner wall helicoid found in cells approaching the end of growth would appear to correspond to the isotropic wall described by Green (1958, 1960) using polarizing microscopy. Green (1960) and Gertel and Green (1977) also describe a random microfibrillar layer in the outer wall of cells at the end of elongation. An ordered helicoidal architecture, possessing an integral number of turns, when viewed perpendicularly to its component layers would be detected as an isotropic or random microfibril arrangement in the polarizing microscope. The failure of polarizing microscopy, X-ray diffraction and surface replicas of microfibrils to provide sufficient structural information for the unequivocal detection of helicoidal cell wall architectures has already been discussed (see Roland and Vian 1979, Neville and Levy 1984, Levy 1987).

The measured birefringence of young, growing internodes indicates that microfibrils are arranged in a transverse fashion in the cell wall (Green 1958; Richmond 1977, 1983). Replicas of dried inner wall surfaces and freeze fracture of walls in growing cells show microfibril orientation to be mainly transverse (Green 1958, Probine and Preston 1961) and occasionally randomly dispersed (Hotchkiss and Brown 1987). These techniques have not been able to reveal the outer wall helicoid that constitutes approximately 25% of the whole wall (Fig. 14). This helicoidal layer may be too thin to contribute significantly to the birefringence to indicate the

presence of an isotropic microfibril arrangement. Furthermore, there may be a low probability of a fracture plane cleaving the outer wall region, to display this particular helicoidal zone.

Formation of different cell wall architectures during different stages of growth

Figure 3 indicates that growth is anisotropic over much of the internodal cell's expansion phase in agreement with Green's (1954) findings. A value for anisotropy can be derived by calculating the gradient of the line that passes through the data points. The scatter in the data suggests that anisotropy may not have the same value for each cell and log-log plots of data records from individual cells confirm this (data not shown). Figure 2 suggests that the rate of diametric expansion follows a different pattern than the rate for elongation, especially at cell diameters between 0.1–0.2 mm. If diametric expansion occurred at a different rate then this would be seen as a change in the gradient through the data points in Fig. 3. In fact the most obvious departures from linearity do occur at small values of cell diameter. It will be necessary to test further this preliminary observation by measuring rates of diametric expansion for cell diameters < 0.1 mm, using more precise measuring techniques than those currently employed.

If the rates of diametric expansion did prove to be low for values of < 0.1 mm then this may permit a possible explanation for the observed changes in wall architecture during growth. As seen in Fig. 16 there appears to be a causal relationship between the growth rate in cell diameter and the accumulation of architectural types in the wall. Before active growth of an internode cell of approximately equal dimensions starts, it possesses a wall that is in part, if not completely, helicoidal (Fig. 16; cells between 0.10–0.25 mm in diameter). This architecture reinforces the cell wall in a multidirectional fashion such that the cell can expand in any desired direction. Such cells expand in diameter and length, exhibiting the maximum rate of diametric expansion between 0.3–0.4 mm in diameter. With time these cells have walls possessing an increasing amount of transversely orientated microfibrils which ultimately restrict diametric expansion and promote cell elongation. At the cessation of growth (> 0.5 mm in diameter), cells are usually in excess of 20 mm in length and a helicoidal reinforcement at this stage would prevent external forces, such as those incurred by bending, from compromising the wall mechanically.

Mechanical properties of Nitella helicoidal walls

It is thought that the observed anisotropy in *Nitella* cell growth can be accounted for by elucidating the mechanical properties of the wall (Probine and Preston 1962, Richmond et al. 1980). These properties are defined in part by the organization of the microfibril architecture of the wall that has classically been described as being transversely reinforced.

When walls are stretched uniaxially or multiaxially they display mechanical anisotropy which accounts for nearly all observed growth characteristics (Richmond et al. 1980). The underlying architecture of the inner quarter of the wall has been vindicated as an important factor in controlling the directionality of cell growth (Richmond 1977). These workers were unaware of the helicoid initially secreted before the onset of growth. In fact this region would only be significant mechanically at the time when it constituted the majority of the wall. This would occur early in the cell's development when its dimensions would be prohibitively small for use in a uniaxial/multiaxial extension experiment. In such an experiment a helicoidal cell wall, with its inherent structurally isotropy (Fig. 17), would appear to be mechanically isotropic.

Nitella and higher plant helicoidal cell walls

The *Nitella* cell wall is structurally as complex as many higher plant cell walls. A large number of higher plant cell walls appear to be constructed in a helicoidal fashion (Neville et al. 1976, Neville and Levy 1984, Roland et al. 1987). A study of cell wall dynamics during the growth of epidermal cells in the mung bean hypocotyl shows that the helicoid is a transient structure (Roland et al. 1982). The ordered inner wall helicoid is progressively strained to form so-called herringbone patterns and ultimately a dispersed appearance in the outer wall. These architectures result from a sequential disordering of a helicoid and their occurrence has been predicted by computer graphics (Neville and Levy 1984, Levy 1986). *Nitella* cell walls are reinforced with helicoidal and transversely orientated microfibrils during elongation from 1 to 20 mm in length. Cell elongation leads to the progressive thinning of layers in the wall and an increasing degree of microfibril reorientation (Erickson 1980), these effects being particularly pronounced in the outer wall. Consequently there should be a reduction of the outer helicoidal zone to a thin layer of apparently dispersed microfibrils. However, the outer helicoidal zone is ever present in cell walls even after active growth has stopped (Figs. 8–13)

suggesting that the *Nitella* wall avoids strain reorientation effects. A possible explanation of this phenomenon could be that transverse microfibrils are continually reorganized into helicoids during the growth process. A continual reorganization of hemicellulose-coated microfibrils into helicoids is not unreasonable if helicoidal superstructures in general result from a self-assembly process as proposed by Bouligand (1972). The inner wall helicoid is secreted at the end of growth at which time no strain reorientation of microfibrils would occur and therefore architectural changes would not be expected.

Control of wall architecture by the cell

The reversal of arc polarity seen at diametrically opposed points on the cell surface (Figs. 12 and 13) suggests that the sense of rotation of the microfibrillar layers constituting the helicoid is maintained over the whole cell surface. It therefore appears as though helicoidal structural order is controlled very precisely at the extracellular surface.

How is the construction of a structurally complex wall controlled by the cell? Heath (1974) proposed that cortical microtubules may guide the motion of cellulose synthesizing complexes in the plane of the plasma membrane, resulting in microfibril arrangements that parallel pre-existing microtubule orientations. Giddings and Staehelin (1988) showed that microfibril synthesis originates from synthesizing complexes located between microtubule defined paths on the plasma membrane. Roberts et al. (1985) observed that ethylene induces pitch changes in helical microtubule arrays from epidermal cells of the mung bean hypocotyl, producing a spectrum of microtubule orientations. Furthermore they propose that these dynamic fields of microtubules may be capable of producing the wide variety of microfibril orientations that constitute a helicoid.

Wasteneys and Williamson (1987) have demonstrated that helical microtubule arrays in growing *Nitella* internodes are orientated transversely to the direction of cell elongation. This transverse dominance is lost as the rate of cell growth declines and microtubules are apparently orientated in a random fashion. The end of growth is marked by a slight increase in the number of longitudinally orientated microtubules.

The transverse microtubules, present during much of the elongation phase, may control the deposition of the transversely orientated wall microfibrils. However, it appears unlikely that these albeit dynamic microtubule fields possess enough structural information to control directly the ordering of the helicoidal architectures present just before active growth and when growth ceases. It has been proposed that helicoidal order in the plant cell wall may arise via a self-assembly process (Roland et al. 1977, Neville 1985, Neville and Levy 1985). Self-assembly of this nature would require a defined pH and ionic environment and would be modulated by the stereochemical interactions between certain key molecules (Neville 1985, 1988).

Affinity labelling of the glucuronoxylans (Vian et al. 1986), a hemicellulosic polysaccharide, has localized them to a narrow helicoidally ordered zone forming the transition between the classically defined, unidirectionally orientated, S1 and S2 layers in cells of Linden wood. For self-assembly to be implicated in constructing the helicoidal wall regions of *Nitella* it will be necessary, in the first instance, to demonstrate the presence of certain polysaccharides in the helicoidal regions that are absent from the zone containing transversely orientated microfibrils. Alternatively, certain polysaccharides may reside in the transverse microfibril zone, preventing these microfibrils from forming helicoids. Therefore, these polysaccharides would be absent from the helicoidal zones.

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