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Cell wall synthesis during growth and maturation of *Nitella* internodal cells

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Abstract. An improved ^{13}C-density-labeling method was used to study cell wall synthesis in rapidly expanding, slowly expanding and recently mature internodes of *Nitella translucens* var *axillaris* (A.Br.) R.D.W. As cells matured, the rate of wall synthesis slowed and the deposition of cellulose microfibrils changed from a predominantly transverse direction in the primary wall of rapidly expanding internodes to a helicoidal array in the secondary wall of mature internodes. The secondary wall was characterized by relatively higher rates of cellulose synthesis and lower rates of pectin synthesis than the primary wall. The synthesis of xyloglucan also decreased markedly at the transition to secondary wall synthesis, while the synthesis of mannose-rich hemicellulose increased. Even though structural differences were striking between the primary and secondary walls of *Nitella*, compositional differences between the two types of wall were quantitative rather than qualitative.

Key words: Cell wall (^{13}C-labeling) – Growth (cell wall) – Microfibril (helicoidal array) – *Nitella* (wall synthesis) – Rhamnogalacturonan – Xyloglucan

Introduction

Internode expansion is highly directional in *Nitella*, and the direction of cell expansion is controlled by the orientation of cellulose microfibrils at the inner cell-wall surface (Richmond 1983). The inner wall of young, rapidly expanding internodes is characterized by a predominantly transverse orientation of cellulose microfibrils (Green 1960; Richmond 1983). Evidence of passive microfibril realignment to a random or predominantly longitudinal orientation during cell elongation led to the interpretation of the *Nitella* wall in terms of multinet growth (Green 1960; Gertel and Green 1977). On the other hand, observations of herringbone patterns in oblique sections of mature *Nitella* cell walls led to the suggestion of a helicoidal wall structure in elongating cells (Neville and Levy 1984) and to the suggestion of inner and outer helicoidal layers separated by a region of transverse microfibrils (Levy 1991).

Although the direction of cell expansion is determined by cellulose orientation, the adjustable yielding properties that control expansion rate are more likely to be determined by the wall matrix (Haughton et al. 1968; Preston 1979; Richmond 1983). The composition of the *Nitella* wall matrix is qualitatively similar to dicotyledonous cell walls (Morikawa 1975), and changes in overall wall composition have been correlated with changes in growth rate (Métraux 1982). However, it has been difficult to study the composition of the newly synthesized inner layer of the cell wall, which controls growth rate. In this paper we describe a convenient, non-radioactive labeling procedure to monitor cell wall synthesis using $^{13}CO_2$ to introduce a density label through the normal photosynthetic pathway. The objective was to compare cell wall synthesis during the transition from rapid internode expansion to internode maturation. Detection by gas chromatography-mass spectrometry (GC–MS) in the selected-ion monitoring mode gave enough sensitivity to analyze cell wall synthesis in small numbers of cells over short periods of time. The sensitivity of this method allowed the first direct comparison between the newly synthesized inner layer of the primary wall of rapidly expanding cells and the helicoidal secondary wall of recently mature cells. This technique provides a particularly useful method to study the transition from primary to secondary wall synthesis in species like *Nitella* in which the primary and secondary walls are similar in composition and cannot be distinguished by other analytical techniques. This paper characterizes two quantitatively distinct pectin fractions of the rhamnogalacturonan type and two hemicellulose fractions, one a xyloglucan and the other rich in mannose. Linkages among sugars of the *Nitella* cell wall are reported for the first time.

Abbreviations: CDTA = trans-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid; GC–MS = gas chromatography-mass spectrometry

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Materials and methods

The plant material used in this study was from a clonal line of *Nitella translucens* var. *axillaris* (A.Br.) R.D.W. (= *Nitella axillaris* Braun) originally collected by Green (1954). Cultures were maintained in an autoclaved soil-extract medium (Richmond 1983) and were subcultured every three to four weeks to maintain consistently rapid growth. Relative growth rates were calculated (Richmond 1983) from measurements of the length of each single-cell internode of three representative plants at 24 h before harvesting and again at the time of harvest. Internodes were combined by growth rate (Table 1) and prepared for cell-wall analysis by cutting the ends of the cells, then gently squeezing out the cytoplasm with fine forceps. To loosen the cuticle, the isolated walls were sonicated for 20 s in 1% sodium dodecyl sulfate (SDS) and 10% octanol, then rinsed several times in 60% ethanol. Any remaining cuticle was removed with forceps. The clean wall tubes were ground to a fine powder in a scintered-glass homogenizer and oven-dried.

Aliquots of the cell-wall preparations were extracted sequentially in 50 mM *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid (CDTA) in 50 mM sodium acetate (pH 6.5) at 24° C (twice); 50 mM Na_2CO_3 containing 20 mM $NaBH_4$ at 0° C and 24° C; 1 M KOH containing 10 mM $NaBH_4$ at 0° C and 24° C; and 4 M KOH containing 10 mM $NaBH_4$ at 0° C and 24° C (Selvendren et al. 1985). The extracts were neutralized, desalted by dialysis against distilled water, then lyophilized. The pellet was hydrolyzed in 67% H_2SO_4.

An aliquot of each fraction was methanolized in 1 N methanolic HCl at 85° C overnight (Morrison et al. 1987). The supernatant was divided into two subsamples and dried, then half of each sample was reduced with $NaBD_4$ in 50% ethanol overnight to reduce the uronic acid (esterified during methanolysis) to the corresponding neutral sugar. Reduced and non-reduced sample pairs were hydrolyzed in 2 N trifluoroacetic acid (TFA), reduced with $NaBH_4$ (Albersheim et al. 1967), then acetylated with acetic anhydride and 1-methylimidazole (Blakeney et al. 1983). The resulting alditol acetates were analyzed by gas chromatography on a Perkin-Elmer Sigma 3 gas chromatograph equipped with a DB–23 capillary column (J & W Scientific, Folsom, Calif., USA). Uronic acids were identified and quantitated by comparing reduced and non-reduced sample pairs. Colorimetric assays verified the uronic-acid (Blumenkranz and Asboe-Hansen 1973) and neutral-sugar contents (Updegraff 1969) of the cell-wall preparations. Total protein was determined by the ninhydrin assay, and hydroxyproline was determined by the method of Kivirikko and Liesmaa (1959) on aliquots of wall samples hydrolyzed overnight in 6 N HCl.

Glycosyl linkages were determined by GC–MS of partially methylated alditol acetates prepared by the method of Blakeney and Stone (1985) and analyzed on a Hewlett Packard (St. Louis, Mo., USA) 5890 gas chromatograph with a DB–225 capillary column interfaced to a Hewlett Packard 5970 mass spectrometer. Uronic acid linkages were determined following reduction to the corresponding neutral sugar with $NaBD_4$ catalyzed by carbodiimide (Carpita and Shea 1989). The degree of uronic-acid esterification was determined by reducing aliquots of the pectin-containing fractions without the catalyst, which should reduce only naturally esterified uronosyl residues.

To study cell wall synthesis in cells of different ages, walls were labeled with ^{13}C introduced as $^{13}CO_2$. Highly branched shoots with five visible internodes were selected from rapidly growing plants. After measuring the length of each cell, four or five intact shoots were placed in a 1-l hydrometer cylinder filled with growth medium. The cylinder was sealed with a rubber stopper through which appropriate lengths of glass tubing were inserted. The glass tubing was connected by Teflon tubing to the CO_2-generation chamber via a peristaltic pump (Fig. 1). After the labeling apparatus was sealed, CO_2 was generated by tipping a 4-ml vial of 7 N H_2SO_4 into a flask containing 2.0 g $Ba^{13}CO_3$. The resulting $^{13}CO_2$ was recirculated through the labeling chamber at 20 ml · min^{-1} for periods of 6 to 36 h. Cell walls were isolated, methanolized, and acetylated as described above. The labeled alditol acetates were analyzed by GC–MS in the selected-ion monitoring mode to increase the sensitivity of detection. Labeling was quantitated by comparing the area of the peak at 191 mass units to that of the peak at 187. This pair was chosen because the four-carbon fragment of mass 187 is common to all of the native cell wall sugars, and because none of the sugars gives a fragment of mass 191. Consequently, this peak represents only the fully labeled four-carbon fragment. This method underestimates the true labeling percentage because partially labeled sugars are ignored. However, preliminary experiments determined that the proportion of partially labeled sugars was small after labeling times of 6 h or more. The totally labeled fragment was determined to be a good approximation of total label incorporation for the labeling times used in the experiments reported here.

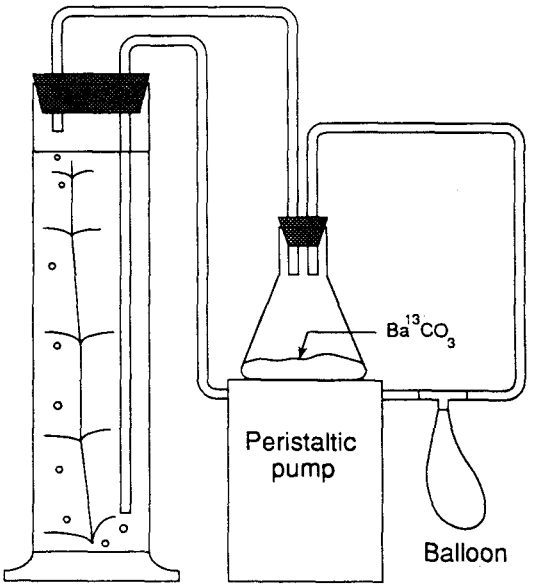

Fig. 1. Apparatus used for labeling cell walls. $^{13}CO_2$ was generated by adding acid to Ba $^{13}CO_3$ in a separate flask and was recirculated through the closed system with a peristaltic pump. A rubber balloon was used to compensate for pressure changes during CO_2 generation

Cells were prepared for electron microscopy by standard techniques. Shoots were fixed in 1% glutaraldehyde in 10 mM 1,4-piperazinediethanesulfonic acid (Pipes)-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) (3:1 (v/v), pH 6.9) and post-fixed in 1% OsO_4. Cells were cut into open-ended cylinders, then either stained en bloc in 1% uranyl acetate or directly dehydrated in an acetone series before embedding. Silver sections were stained with aqueous uranyl acetate and lead citrate, and viewed at 50 or 75 kV on an electron microscope (11U–B; Hitachi, Tokyo, Japan).

Results

The relationship between cell age and relative growth rate was similar in control and labeled plants (Table 1) even though bubbling $^{13}CO_2$ through the growth medium during labeling lowered the pH of the soil extract from 6.6 to about 5.7. The rapid growth and large size of *Nitella* internodes made it possible to harvest homogeneous samples of cells with similar growth rates. The large cell size also facilitated manual removal of the cytoplasm and cuticle for quantitative determination of wall composition with greater than 94% recovery (Ta-

Table 1. Mean relative growth rate (RGR), cell position, range of cell length and range of relative growth rate for each category of *Nitella* internodal cell isolated for cell-wall analysis. Elongations rates of cells labeled with ^{13}C for 6 h are compared with those of unlabeled control cells

	Mean RGR (% · h^{-1})	Position relative to apex	Range length (mm)	Range RGR (% · h^{-1})
Rapidly growing		Cell 1	1–10	1.5–4.0
Control	2.6[a]			
Labeled	2.5			
Slowly growing		Cells 2–3	10–60	0.05–1.5
Control	0.8			
Labeled	0.9			
Recently mature		Cells 4–5	40–70	0.0–0.05
Control	0.0			
Labeled	0.0			

[a] Least significant difference for relative growth rate was 0.45 at $P<0.05$

Table 2. Composition of *Nitella* cell walls determined colorimetrically at different stages of cell expansion. Uronic acid and non-cellulosic neutral sugars were determined from the supernatant of 2 N trifluoroacetic-acid hydrolyzates and cellulose from 67% H_2SO_4 digests of the trifluoroacetic-acid residues. Protein was determined by the ninhydrin assay on a 6 N HCL digest of cell wall material. Standard errors are given in parentheses ($n=3$)

	Cellulose (% DW)	Non-cellulosic neutral sugars (% DW)	Uronic acids (% DW)	Protein (% DW)
Rapidly growing cells	31.9 (1.8)	26.2 (0.9)	33.7 (1.7)	4.8 (0.4)
Slowly growing cells	34.9 (2.6)	26.0 (1.0)	31.4 (2.2)	5.9 (0.7)
Recently mature cells	36.3 (2.1)	25.4 (1.3)	26.3 (2.5)	6.4 (0.5)

ble 2). Cell walls of rapidly expanding internodes contained 32% cellulose, 26% non-cellulosic neutral sugar, 34% uronic acid and less than 5% protein. As cell expansion slowed, the proportion of cellulose in the wall increased and the proportion of uronic acid decreased (Table 2). The proportion of protein also increased with maturity, although after cuticle removal the protein content of the wall was low at all growth stages. No hydroxyproline was detected in the protein of the *Nitella* wall.

Pectins soluble in CDTA represented 45% of the total carbohydrate in the cell wall of rapidly expanding internodes; the proportion decreased to 37% as growth stopped (Fig. 2). Subsequent extraction with Na_2CO_3 removed additional pectin, which represented 8–9% of the wall. Hemicellulose extracted by 1 M KOH made up 10% of the wall carbohydrate in rapidly expanding internodes and 8% of the mature wall, while the proportion extracted by 4 M KOH increased from 6–10%. The base-insoluble cellulose pellet was hydrolyzed in 67% H_2SO_4, and increased from 31% to 36% of the wall as internodes matured, which is in close agreement with values determined by colorimetric assay (Table 2). Although the

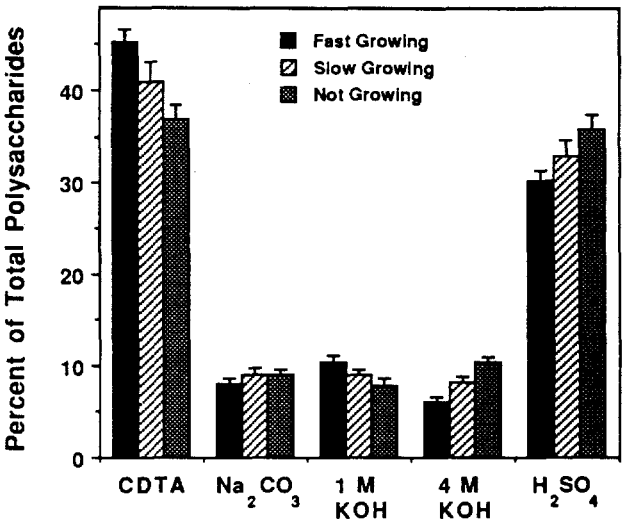

Fig. 2. Distribution of the carbohydrate portion of the cell wall in *Nitella* internodes of different growth rates based on extractability with CDTA, Na_2CO_3, 1 M KOH, 4 M KOH and 67% H_2SO_4

proportions of the polysaccharide fractions changed as cells matured, the composition of each fraction remained similar regardless of growth rate. The composition of the polysaccharide fractions is shown in Table 3, and the predominant linkages of the major sugars are shown in Table 4. The CDTA and Na_2CO_3 extracts both contained pectins of the rhamnogalacturonan type, which differed primarily in the proportions of uronic acid to neutral sugar. The ratio of galacturonic acid to rhamnose in the CDTA fraction was 12:1, compared with 5:1 in the Na_2CO_3 extract. The composition of the 1 M KOH and 4 M KOH fractions indicate two distinct hemicelluloses. The 1 M KOH extract is predominantly a xyloglucan, while the 4 M KOH fraction is rich in glucose and mannose.

Cell wall synthesis was investigated by a photosynthetically introduced ^{13}C label. Over 36 h the mean rate of ^{13}C incorporation into walls of rapidly expanding internodes was 1.3% · h^{-1} (i.e. 46.8% of the wall was labeled after 36 h). Incorporation rates were lower over shorter periods (Fig. 3). We established that labeling times of 6 h or longer were required for accurate quantitation. For labeling times shorter than 6 h, partially labeled sugars represented a significant percentage of the total ^{13}C label and could not be ignored in calculating incorporation rates. The ^{13}C-incorporation rate declined as growth slowed, but wall synthesis continued in recently mature internodes, even after cell expansion had stopped. During short labeling periods, more label was incorporated into the wall matrix than into cellulose, but labeling was similar in the two fractions after 36 h (Fig. 3).

In rapidly expanding internodes, each sugar in the wall matrix was labeled in proportion to its abundance, with a mean labeling rate of 1.3% · h^{-1} (Fig. 4). The mean labeling rate decreased to 0.6% · h^{-1} for slowly expanding cells and 0.1% · h^{-1} for recently mature cells. Labeling rates became less uniform among sugars as cell

Table 3. Composition of cell-wall polysaccharides obtained by sequential extraction of cell walls of *Nitella* internodal cells. Data are expressed as a percentage of the total carbohydrate in each fraction. Standard errors are given in parentheses ($n=9$)

	CDTA extract	Na_2CO_3 extract	1 M KOH extract	4 M KOH extract	Cellulose residue
Rhamnose	5.7 (0.3)	8.1 (0.1)	1.7 (0.3)	1.0 (0.2)	0.0 (0.0)
Fucose	2.1 (0.2)	5.4 (0.1)	10.0 (0.4)	1.9 (0.1)	0.0 (0.0)
Arabinose	4.4 (0.3)	7.8 (0.3)	3.4 (0.3)	1.3 (0.3)	0.0 (0.0)
Xylose	2.3 (0.7)	6.0 (0.1)	21.7 (0.4)	13.2 (0.7)	0.2 (0.0)
Mannose	1.9 (0.2)	2.3 (0.1)	5.5 (0.2)	24.7 (1.1)	3.8 (0.7)
Galactose	12.5 (0.3)	16.5 (0.5)	10.4 (0.3)	6.3 (0.6)	0.0 (0.0)
Glucose	4.0 (0.4)	10.8 (0.4)	45.8 (0.9)	51.6 (1.1)	95.9 (0.7)
Galacturonic acid	67.1 (2.0)	43.0 (1.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Table 4. Linkages of the major sugars of each fraction determined by GC–MS of partially methylated alditol acetates. Linkages are listed in order of relative abundance for each sugar

Fraction	Sugar residue	Linkage
CDTA extract	Galacturonic acid	1→4
	Rhamnose	1→2; 1→2,4
	Arabinose	Terminal; 1→5
	Galactose	Terminal; 1→4; 1→2
Na_2CO_3 extract	Galacturonic acid	1→4
	Rhamnose	1→2; 1→2,4
	Arabinose	Terminal; 1→5
	Galactose	Terminal; 1→4; 1→2
	Glucose	1→4
	Xylose	1→2
	Fucose	Terminal
1 M KOH extract	Glucose	1→4; 1→4,6
	Xylose	1→2; 1→4
	Galactose	1→2
	Fucose	Terminal
4 M KOH extract	Glucose	1→4; 1→4,6
	Mannose	1→4
	Xylose	Terminal; 1→4
Cellulose residue	Glucose	1→4; 1→4,6
	Mannose	1→4
	Xylose	Terminal

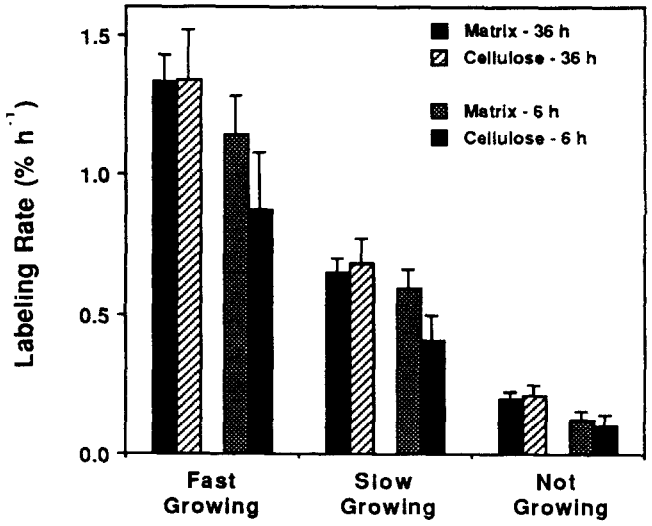

Fig. 3. Rates of incorporation of ^{13}C label into cell-wall polysaccharides in *Nitella* internodes of different growth rates for 6- and 36-h labeling times. Rates are expressed as the percentage of the total polysaccharide (cellulose or matrix) that was labeled per hour

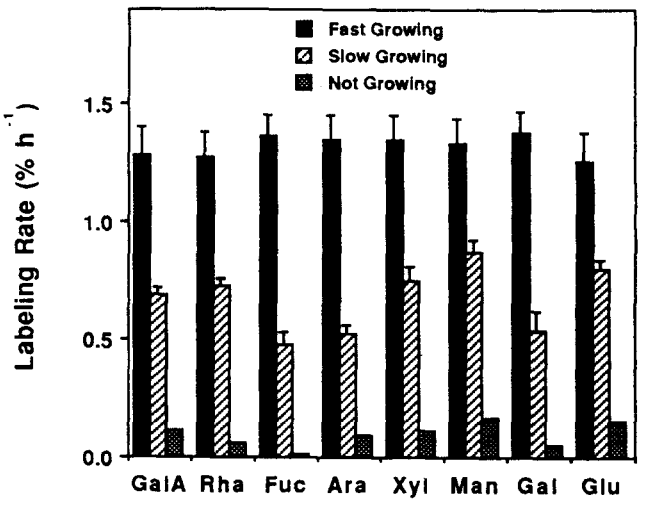

Fig. 4. Rates of incorporation of ^{13}C label into individual sugars of the matrix polysaccharides. Rates are expressed as the mean percentage of the total sugar that was labeled per hour over a 36-h period. For abbreviations see Fig. 7

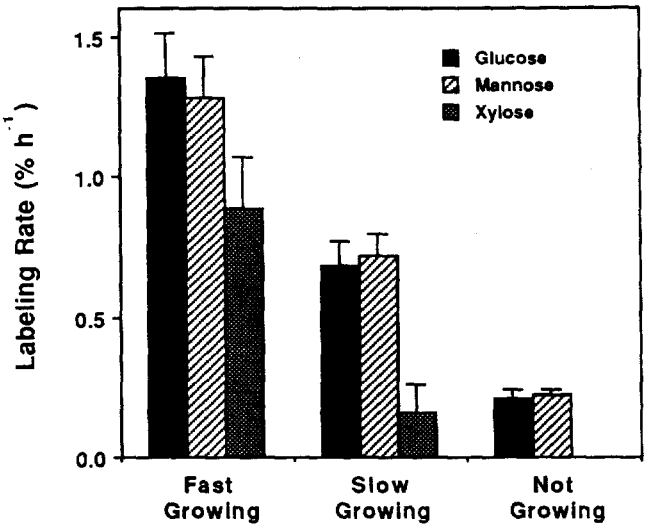

Fig. 5. Rates of incorporation of ^{13}C label into individual sugars of the cellulose fraction. Rates are expressed as the mean percentage of the total sugar that was labeled per hour over a 36-h period

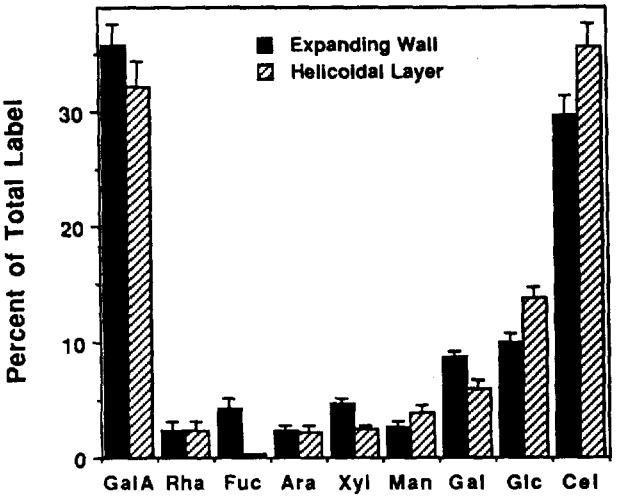

Fig. 7. Distribution of ^{13}C label in the newly synthesized primary cell wall of rapidly expanding internodes and in the helicoidal secondary wall of non-growing internodes of *Nitella*. Data are expressed as a percentage of the total label incorporated into the cell wall. *GalA*, galacturonic acid; *Rha*, rhamnose; *Fuc*, fucose; *Ara*, arabinose; *Xyl*, xylose; *Man*, mannose; *Gal*, galactose; *Glc*, glucose; *Cel*, cellulose

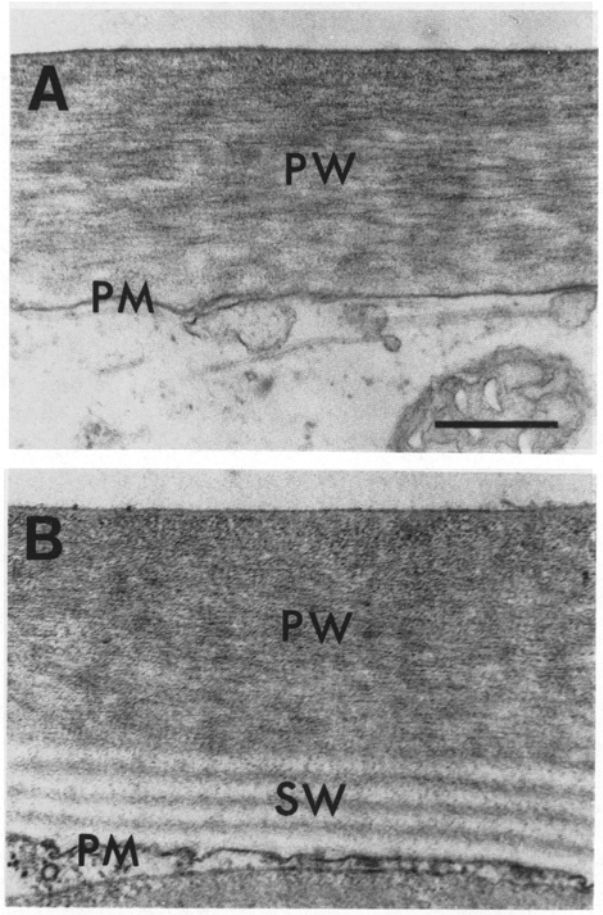

Fig. 6A, B. Transmission electron micrographs of cross-sections of *Nitella* cell walls. **A** The primary wall of a rapidly expanding internode is uniform in texture. **B** A layered secondary wall is deposited subjacent to the primary wall in a recently mature internode. Four helicoidal layers are visible in the secondary wall (a layer is defined as one dark and one light band). *PM*, plasma membrane; *PW*, primary wall; *SW*, secondary wall. ×32000; bar = 0.5 μm

walls matured. Incorporation into mannose and glucose was above the mean for slowly expanding cells, while incorporation into fucose, arabinose and galactose was below the mean. The differences among sugars of the wall matrix, especially the decline in fucose labeling, were even more pronounced in mature internodes.

In the cellulose fraction of the wall, glucose accounted for well over 90% of the dry weight, but small quantities of mannose and xylose were tightly associated with the crystalline cellulose. In the ^{13}C-incorporation studies, glucose and mannose residues were labeled in proportion to their abundance in internodes of all growth categories. However, incorporation into xylose decreased as growth slowed and was not detected in the cellulose fraction of mature internodes (Fig. 5).

Transmission electron microscopy was used to compare the overall wall structure of young and mature internodes. In rapidly expanding cells the wall appeared relatively uniform in transverse section (Fig. 6A). In mature cells, the outer wall was similar to that of young cells, but the inner wall had the appearance of discrete layers (Fig. 6B). These layers have been described as helicoidal in structure (Neville and Levy 1984; Levy 1991). In over 40 cells examined, helicoids were not seen in rapidly elongating internodes. On the other hand, in more than 20 mature internodes sectioned, helicoids were always found at the inner surface of the wall. The number of helicoidal layers usually did not increase after the fifth internode (data not shown). Internodes classified as recently mature in the ^{13}C-labeling experiments were at a stage when helicoidal wall material would be deposited. Some cells in the slowly expanding classification also may have had one or more helicoidal layers.

The ^{13}C-labeling patterns in cell walls of rapidly expanding internodes and recently mature internodes provided a comparison between the newly deposited inner layer of the extensible wall and the helicoidal layers of the inextensible mature wall (Fig. 7). The helicoidal layers differed quantitatively, although not qualitatively from the extensible wall. The helicoidal wall incorporated more label into cellulose and less into galacturonic acid than the extensible wall. The helicoidal layers also incorporated more label into glucose and mannose than the extensible wall, but less label into xylose, galactose and particularly fucose than the inner wall of rapidly expanding internodes (Fig. 7).

Discussion

The use of $^{13}CO_2$ provided a convenient and effective method of studying cell wall synthesis without the inherent difficulties of handling radioisotopes. Unlike procedures utilizing ^{2}H- (Sasaki et al. 1989) or ^{13}C-labeled sugars (Greve and Labavitch 1991), in which the choice of substrate may influence the distribution of label, sugars were labeled in proportion to their abundance with the $^{13}CO_2$ method.

Selected-ion-monitoring GC–MS increased the sensitivity of ^{13}C detection sufficiently to allow relatively short (6 h) labeling times. Because the label was introduced photosynthetically, precursor pools were not instantaneously saturated, and for experiments shorter than 6 h, label dilution by a substantial pool of unlabeled substrate became a constraining factor. The abundant starch grains in *Nitella* chloroplasts, observed both by transmission electron microscopy and by staining with iodine-potassium iodide (not shown), are a likely source of the unlabeled substrate. Partial labeling may also account for some of the discrepancy between growth rate and the rate of ^{13}C incorporation over short periods (compare Table 1 and Fig. 3). It is not likely that the difference was due to wall thinning during growth. On the contrary, data from Green (1958) and Métraux (1982) indicate a progressive thickening of the *Nitella* wall during internode elongation. Wall thickening is also evident when the relative rates of ^{13}C incorporation and cell elongation are compared to rapidly expanding, slowly expanding and recently mature internodes, in which wall synthesis continued after growth stopped (Fig. 3). Wall synthesis in mature internodes was visually apparent as a layered pattern in the inner portion of the wall. The pattern was identical to that reported in mature walls of *N. opaca* by Probine and Barber (1966) and to the helicoidal layers described by Levy (1991) and Neville and Levy (1984). The distinctive pattern produced by helicoidal deposition was not seen in walls of rapidly expanding cells (Fig. 6A), in which cellulose is deposited in a predominantly transverse orientation (Green 1958; Richmond 1983). Helicoidal layers became evident only at end of the growth phase (Fig. 6B; Probine and Barber 1966; Levy 1991). The helicoidal portion of the *Nitella* wall can therefore be considered a secondary wall. Secondary wall deposition continued for a relatively short period of time after an internode reached its final length. Deposition in *N. translucens* usually stopped after 5–9 helicoidal layers (data not shown), although Probine and Barber (1966) reported up to 12 layers in *N. opaca*.

Similar shifts in the orientation of cellulose microfibrils were reported at the transition from primary to secondary wall synthesis in cotton fibers (reviewed in Ryser 1985) and in root hairs of several plant species with helicoidal secondary walls (Emons and van Maaren 1987). Secondary-wall composition can be qualitatively different from that of primary walls, as in the heavily cellulosic walls of cotton fibers (Ryser 1985), in seeds that accumulate storage polysaccharides in specialized walls (Bacic et al. 1988), and in lignified sclerenchyma (reviewed in Preston 1974). In fact, secondary-wall composition has been studied primarily in cells that produce thick walls with highly specialized functions. In *Nitella*, which lacks highly specialized cell types, primary- and secondary-wall composition differed quantitatively, but not qualitatively.

Because cell wall synthesis in *Nitella* occurs primarily by apposition (Green 1958), the distribution of ^{13}C label in walls of mature internodes represents the composition of the helicoidal secondary wall. Labeling patterns in the secondary wall indicated polysaccharides fundamentally similar to those in the primary wall of elongating internodes, although there appeared to be a marked shift in the rates of synthesis of specific polysaccharides (Fig. 7). The low rate of ^{13}C incorporation into fucose in secondary walls (Figs. 4, 7) indicated a sharp decrease in the synthesis of the fucose-rich xyloglucan extracted by 1 M KOH. This is consistent with the decrease in the abundance of this polysaccharide fraction in walls of older cells (Fig. 2). The composition and linkages among sugar residues in this fraction are similar to xyloglucans found in primary walls of dicotyledons (Darvill et al. 1980).

In contrast to fucose, incorporation of ^{13}C into mannose and glucose was higher than the mean incorporation rate in secondary walls (Fig. 4), indicating an increase in the rate of synthesis of the mannose-rich hemicellulose extracted by 4 M KOH. This was confirmed by an increase in the proportion of the total label represented by mannose and glucose (Fig. 7), and by an increase in the proportion of the total wall extracted by 4 M KOH as internodes matured (Fig. 2). The glucomannan was more tightly bound to cellulose than was the xyloglucan, and a portion of the mannose-rich hemicellulose could not be removed from the cellulose residue by KOH concentrations up to 8 M (data not shown). To explore the possibility that hemicellulose was physically entrapped in the cellulose, aliquots were dissolved in methylmorpholine-N-oxide, which completely solubilizes the wall (Joseleau et al. 1981). Cellulose reprecipitated from methylmorpholine-N-oxide had the same ratio of mannose to glucose as the original cellulose residue (data not shown), indicating a chemical rather than physical binding of the hemicellulose to crystalline cellulose.

The rate of cell wall biosynthesis declined with cell age (Fig. 3). However, the increase in the percentage of the total ^{13}C label incorporated into cellulose (Fig. 7) indicates that cellulose biosynthesis decreased at a slower

rate than the biosynthesis of matrix polysaccharides. An increase in the ratio of cellulose to pectin in maturing internodes (Table 2, Fig. 2), which was also reported by Métraux (1982), marked the transition from primary to secondary wall synthesis. The increase in the cellulose-to-pectin ratio is even more pronounced in the lignifying secondary walls of dicotyledonous species in which pectin deposition virtually ceases (Bolwell et al. 1985). Pectin synthesis continued at a reduced rate in secondary walls of *Nitella*, as evidenced by the continued incorporation of ^{13}C into galacturonic acid in maturing cell walls (Fig. 4).

The two pectic fractions isolated from the *Nitella* wall were both rhamnogalacturonans with side chains of galactose and arabinose attached at rhamnose residues within the galacturonic acid backbone. The CDTA-soluble pectin had relatively few branch points, and the low ratio of galactose to rhamnose indicates short side chains. The Na_2CO_3-soluble pectin was more highly branched and had a higher neutral-sugar content, with a ratio of rhamnose to galacturonic acid of 1:5, compared with a ratio of 1:12 in the CDTA extract. Galactose and arabinose were also higher in the carbonate extract. The pectin composition was similar to that of dicotyledons, except that in *Nitella* pectins the uronic acid was totally non-esterified, which is in agreement with Anderson and King (1961). Together, the two pectin fractions constituted about half of the carbohydrate of the *Nitella* cell wall. The high content of nonesterified pectin results in a high ion-exchange capacity (Van Cutsem and Gillet 1983) and in high concentrations of exchangeable and non-exchangeable calcium associated with the *Nitella* wall (Wuytack and Gillet 1978).

The cell wall composition reported here was consistent with previous reports for this (Métraux 1982) and other *Nitella* species (Anderson and King 1961; Morikawa 1975). However, by removing the cuticle we found the protein content of the wall to be half that reported by Morikawa (1975) for *N. flexilis*. Many algal cuticles have a high protein content (Hanic and Craigie 1969), and we estimated by Coomassie Blue staining that about half of the extracellular protein of *N. translucens* was localized in the cuticle (data not shown). The low protein content of the wall, and Thompson and Preston's (1968) observation that pronase digestion did not affect the tensile properties of the *Nitella* wall, indicate that wall structural proteins do not play a major role in controlling the rate of cell expansion in *Nitella*. It is more likely that metabolism of xyloglucan, which is the predominant hemicellulose in rapidly expanding internodes, or the high concentration of calcium-linked pectin plays a role in controlling growth rates of younger cells. The timing of the onset of helicoidal wall deposition strongly indicates that the structure and composition of the secondary wall limit growth in mature cells. It has been suggested that the composition of the hemicellulosic component of the wall controls cellulose orientation in helicoidal walls (Neville 1985; Vian and Reis 1991). This suggestion of helicoidal self-assembly regulated by specific matrix polysaccharides deserves close attention in light of the marked shift in hemicellulose synthesis at the onset of secondary wall deposition in *Nitella*.

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