Protoplasma (2000) 213: 235–245

PROTOPLASMA

Immunocytochemical characterization of early-developing flax fiber cell walls

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Received August 24, 1999
Accepted April 14, 2000

Summary. The deposition and formation of a thick secondary wall is a major event in the differentiation of flax (*Linum usitatissimum*) fibers. This wall is cellulose-rich; but it also contains significant amounts of other matrix polymers which are noncellulosic such as pectins. We have used immunocytochemical techniques with antibodies specific for various epitopes associated with either pectins or arabinogalactan proteins (AGPs) to investigate the distribution of these polymers within the walls of differentiating young fibers of 1- and 2-week-old plants. Our results show that different epitopes exhibit distinct distribution patterns within fiber walls. Unesterified pectins recognized by polygalacturonic acid-rhamnogalacturonan I (PGA/RG-I) antibodies and rhamnogalacturonan II recognized by anti-RG-II-borate complex antibodies are localized all over the secondary wall of fibers. PGA/RG-I epitopes, but not RG-II epitopes, are also present in the middle lamellae and cell junctions. In marked contrast, β-(1→4) galactans recognized by the LM5 monoclonal antibody and AGP epitopes recognized by anti-β-(1→6) galactan and LM2 antibodies are primarily located in the half of the secondary wall nearest the plasma membrane. LM2 epitopes, present in 1-week-old fibers, are undetectable later in development, suggesting a regulation of the expression of certain AGP epitopes. In addition, localization of cellulose with the cellobiohydrolase I-gold probe reveals distinct subdomains within the secondary walls of young fibers. These findings indicate that, in addition to cellulose, early-developing flax fibers synthesize and secrete different pectin and AGP molecules.

Keywords: Arabinogalactan proteins; Fiber; *Linum usitatissimum*; Immunocytochemistry; Polysaccharide; Secondary wall.

Introduction

Flax fibers differentiate from the protophloem cells (Esau 1977), and this differentiation is accompanied by the deposition of a cellulose-rich secondary wall. At maturity, the walls of fiber cells are so thickened that their lumen is nearly filled. Interest in the use of flax fibers for textile and other industrial purposes has increased recently in Europe and North America, and most of the research that is currently undertaken aims to improve not only the quality of fibers but also the processing methods by which they can be isolated (Vandam et al. 1994, Bert et al. 1996, Akin et al. 1996). This requires a complete knowledge of the biochemical nature of the fibers and the surrounding tissues as well as of the synthesis, secretion, and organization of different wall components during flax development.

Unlike cotton fibers, mature flax fibers contain 60 to 70% cellulose, while the remaining material is rich in pectic substances and other noncellulosic polysaccharides that have been analyzed by Raman spectroscopy and other biochemical methods (Morvan et al. 1989, McDougall 1993, Gorshkova et al. 1996, Himmelsbach and Akin 1998). Na_2-EDTA-extracted material from flax fiber cell walls has been shown to contain 4-galactans as the main component (Morvan et al. 1989, Goubet et al. 1995). Similarly, Davis et al. (1990) have reported the presence of rhamnogalacturonan I (RG-I) with short side chains of β-(1→4) galactans in flax fibers. The walls from retted or unprocessed fibers have also been shown to contain various noncellulosic components such as glucomannans, glucuronoxylans, xyloglucan, 4-galactans, and 5-arabinans (McDougall 1993, Akin et al. 1996, Gorshkova et al. 1996, Himmelsbach et al. 1998, Himmelsbach and Akin 1998). Furthermore, polysaccharides consisting of 1,3,6-galactans have also been found in the material extracted from flax fibers (Goubet et al. 1995).

Flax fibers are known for their high tensile strength and rigidity. These physical features reflect the struc-

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tural properties of the wall polymers as well as their spatial organization within the thick secondary wall of fibers. Cellulose, the main component of fibers, is thought to play a predominant role in the buildup of such mechanical properties. However, the other components of the cell wall matrix (e.g., pectins) may provide additional characteristics to the fibers and thus may contribute considerably to their quality (Girault et al. 1997).

In contrast to the extensive studies describing the biochemical composition of flax fibers, relatively little is known about the spatial organization of various wall components within the cell walls of mature and immature fibers. In this study, we have used histochemical and immunofluorescence staining as well as immunogold labeling to determine the distribution of specific epitopes associated with different types of pectins and AGPs within the secondary cell wall of fibers during early stages of differentiation. Our results show that early-forming fibers produce and secrete high amounts of pectins and AGPs that exhibit different wall distribution patterns. The abundance of such polymers suggests that they may play an important role in the structural and functional properties of flax fibers.

Material and methods

Plant material and tissue fixation

Seeds of flax (*Linum usitatissimum* L. var. Ariane) were provided by Dr. J. P. Trouvé, La Coopérative Terre de Lin, Normandie, France. Seeds were germinated on moist filter paper in the dark. After three days, seedlings were transferred to an appropriate culture medium as previously described (Morvan et al. 1990) and grown under continuous light for one or two weeks. Hypocotyls were then harvested and small fragments (1–2 mm) were prepared, fixed, dehydrated, and embedded in London Resin White as previously described (His et al. 1997, Jauneau et al. 1997).

Immunogold labeling procedures

The different antibodies used in this study are presented in Table 1.

All incubations were carried out at room temperature, and the experiments were performed as described in Vicré et al. (1998). Thin sections (80–90 nm) mounted on gold grids were incubated in a blocking solution of 3% low-fat dried milk in PBST (10 mM Na-phosphate, 500 mM NaCl, 0.1% Tween-20; pH 7.2) for 30 min. After the grids were blotted dry, they were incubated for 4 h with the following primary antibodies: LM2 (diluted 1 : 5 in PBST), LM5 (diluted 1 : 5 in PBST), LM6 (diluted 1 : 5 in PBST), anti-PGA/RG-I (anti-polygalacturonic acid-rhamnogalacturonan I) (diluted 1 : 5 in PBST), anti-β-(1→6) galactan (diluted 1 : 400 in PBST), and anti-RG-II (diluted 1 : 10 in PBST). The grids were then washed with PBST (containing 0.5% Tween 20) for 1–2 min before their transfer to a droplet of goat anti-rat immunoglobulin G conjugated to 20 nm diameter colloidal gold diluted 1 : 25 in PBST (for LM2-, LM5-, and LM6-labeled sections) or to a goat anti-rabbit immunoglobulin G conjugated to 20 nm diameter colloidal gold solution diluted 1 : 25 in PBST (for anti-β-(1→6) galactan, anti-RG-II, and anti-PGA/RG-I-labeled sections) for 60 min. The sections were washed with PBST (containing 0.5% Tween 20) followed by a distilled-water wash. After immunolabeling, the sections were stained with 2% uranyl acetate for 30 min and Reynolds lead stain (Reynolds 1963) for 30 to 60 s and viewed at 80 kV on a Zeiss electron microscope (EM 109). All micrographs illustrate representative labeling patterns. Control experiments were performed in each case by omission of primary antibodies or their substitution with the preimmune antibodies.

Table 1. Characteristics of the antibodies used in this study

Antibody	Epitope recognized	Reference
Anti-PGA/ RG-I	deesterified polygalacturonic acid	Moore et al. 1986, Lynch and Staehelin 1995
LM5	β-(1→4) galactan	Jones et al. 1997
LM6	α-(1→5) arabinan	Willats et al. 1998
Anti-RG-II	borate-dimeric RG-II complex (epitope not fully characterized)	Matoh et al. 1998
Anti-β-(1→6) galactan	β-(1→6) galactan	Kikuchi et al. 1993
LM2	AGP carbohydrate epitope containing D-glucuronic acid	Smallwood et al. 1996

Light microscopy

Light microscopy was carried out on an Axioskop microscope (Zeiss) equipped with epifluorescence optics.

Immunofluorescence labeling

Immunofluorescence labeling was performed on the same processed tissue used for electron microscopy. Thick sections (500 nm) from London Resin White-embedded plants were cut and mounted on glass microscope slides. Immunostaining was done as described for immunogold labeling except that the secondary antibody was conjugated to fluorescein isothiocyanate (FITC) instead of gold particles and used at a dilution ratio of 1 : 50. Epifluorescence of the immunostained tissue sections was observed with a Zeiss filter set (excitation filter, 485 nm wavelength; barrier filter, 515–565 nm wavelength).

Histochemical staining with methylene blue-basic fuchsin

The staining was performed according to the protocol of Humphrey and Pittman (1974). Briefly, semithin sections (2.5 μm) from London Resin White-embedded samples were prepared and mounted on glass microscope slides. They were then incubated with methylene blue for 20 min at 60 °C, rinsed thoroughly with distilled water, re-incubated in the fuchsin solution for 1 to 5 min, washed again, and observed.

Calcofluor staining of cellulose

Calcofluor staining was done as described by Mori and Bellani (1996). Sections (0.5 μm) from London Resin White-embedded

samples were incubated with 1 mg of the fluorescent probe per liter for 30 min in the dark. After several washes, the sections were observed with a Zeiss filter set (excitation filter, 350–410 nm wavelength; barrier filter, 470 nm wavelength).

Enzyme-gold affinity labeling of cellulose

Cellobiohydrolase-I (CBH-I, EC 3.2.1.91) was generously provided by Dr. B. Vian (University of Paris VI, France). The CBH-I-colloidal gold complex was prepared according to Chanzy et al. (1984) and used on London Resin White-embedded ultrathin sections at a dilution ratio of 1 : 50 for 30 min. Sections were then stained by the periodic acid-thiocarbohydrazide-silver proteinate (PATAg) test (Vian 1986) for a better contrast of the cell walls and observed with a Zeiss electron microscope (EM 109). Control sections were incubated in the presence of 1 mg of carboxylmethycellulose per ml as described by Berg et al. (1988).

Results

Histochemical staining of young flax fibers

We first examined by light microscopy the tissue organization and fiber distribution in 2-week-old flax hypocotyls with methylene blue-basic fuchsin staining (Fig. 1 A–C). The differentiating fibers with thick walls are seen to form an arc-shaped pattern between the cortex and the central cylinder. As shown in Fig. 1 B and C, developing fibers are easily identified as large and irregularly shaped cells with a thick secondary cell wall which is heavily stained red. The thickness of these walls increases rapidly with plantlet development (compare Fig. 1 B with C). Similarly, the number of the fibers increases with development and usually doubles between 1- and 2-week-old plants (data not shown). It is also interesting to note that the thick primary wall of epidermal cells is also intensely stained compared to the wall of cortical cells. These observations show that the onset of fiber differentiation in flax occurs at a very early stage of development (i.e., ca. 1 week after seed germination).

Staining of cellulose in flax fibers

Figure 1 D shows a thin section from 2-week-old plants stained with Calcofluor, a fluorescent dye specific for fibrillar β-glucans of plant cell walls such as cellulose (Maeda and Ishida 1967). A heavy staining of the cellulose-rich secondary wall of differentiating fibers is seen. Similarly, fibers in younger plants (1-week-old plants) are quite heavily labeled with Calcofluor (not shown). This is consistent with the fact that cellulose deposition is an early event of fiber differentiation. We have also probed cellulose in flax fibers with the CBH-I-gold complex followed by PATAg staining at the electron microscopic level. As shown in Fig. 2 A and B, at both stages of flax development, binding of cellulose is mainly associated with the half of the secondary wall closest to the primary wall. This region of the wall is also weakly reactive to PATAg staining. In contrast, the half of the wall closest to the plasma membrane, which is more reactive to PATAg staining, shows almost no binding to the cellulose probe.

Immunolocalization of pectic polysaccharides

Detection of PGA/RG-I epitopes

To examine whether matrix polysaccharides such as pectins are also synthesized and secreted during early stages of fiber differentiation, we have probed for pectins with the anti-PGA/RG-I polyclonal antibodies (Moore et al. 1986; Lynch and Staehelin 1992, 1995). The PGA/RG-I epitope-containing polysaccharides are present and evenly distributed within the secondary wall of fibers (Fig. 3 A). They are also detected at high levels in the primary wall, and particularly in cell junctions. No change in the distribution of the epitope in fiber walls is seen between the two stages of flax development (compare Fig. 3 A with B). This result shows that the cellulosic secondary wall of fibers contains significant amounts of unesterified pectins of the complex PGA/RG-I reflecting an active synthesis and secretion (via the Golgi apparatus) of these polysaccharides by developing fibers.

Localization of RG-II epitopes

Figure 3 C illustrates the immunolocalization of RG-II-like molecules in flax fibers with polyclonal antibodies specific for the borate-RG-II complex isolated from radish (Matoh et al. 1998). Labeling with these antibodies shows a uniform distribution of RG-II epitopes throughout the secondary wall of fibers. Nearly no labeling is found over the middle lamellae and cell junctions between fiber cells. This indicates that the secondary wall of fibers contains RG-II molecules that are probably cross-linked via borate esters.

Detection of β-(1→4) galactan and α-(1→5) arabinan epitopes

To further examine the presence and localization of other types of pectic polysaccharides, we have used two monoclonal antibodies specific for β-(1→4) galactans (LM5) and α-(1→5) arabinans (LM6)

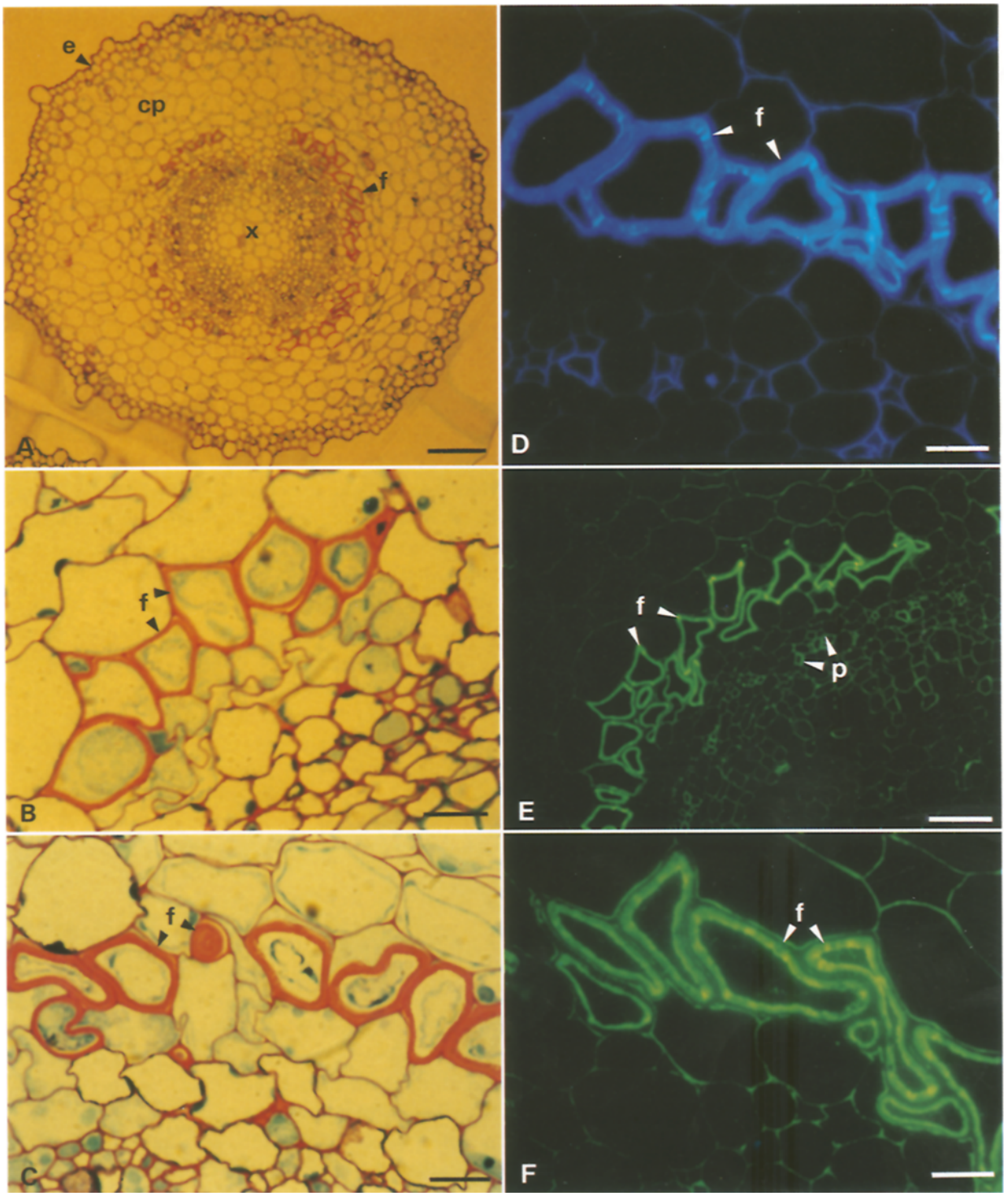

Fig. 1. Histochemical staining (**A–C**) and fluorescence labeling (**D–F**) of wall components in flax fibers. **A** Cross section of 2-week-old flax hypocotyls stained with methylene blue-basic fuchsin. The differentiating fibers (*f*) with thick secondary walls are stained red. **B** and **C** Higher magnifications of fiber cells of 1- and 2-week-old plants stained with methylene blue-basic fuchsin. **D** Section similar to that shown in **B** stained with Calcofluor, a dye specific for fibrillar β-glucans such as cellulose. **E** Immunofluorescence labeling of a cross section of flax hypocotyls with anti-β-(1→6) galactan antibodies specific for plant AGPs. **F** Higher magnification of fiber cells stained with the LM5 antibody, specific for β-(1→4) linked galactans. *cp* Cortical parenchyma; *e* epidermal cells; *f* fibers; *p* protophloem cells; *x* xylem. Bars: A, 0.35 mm; B and C, 0.025 mm; F, 0.02 mm; D, 0.035 mm; E, 0.060 mm

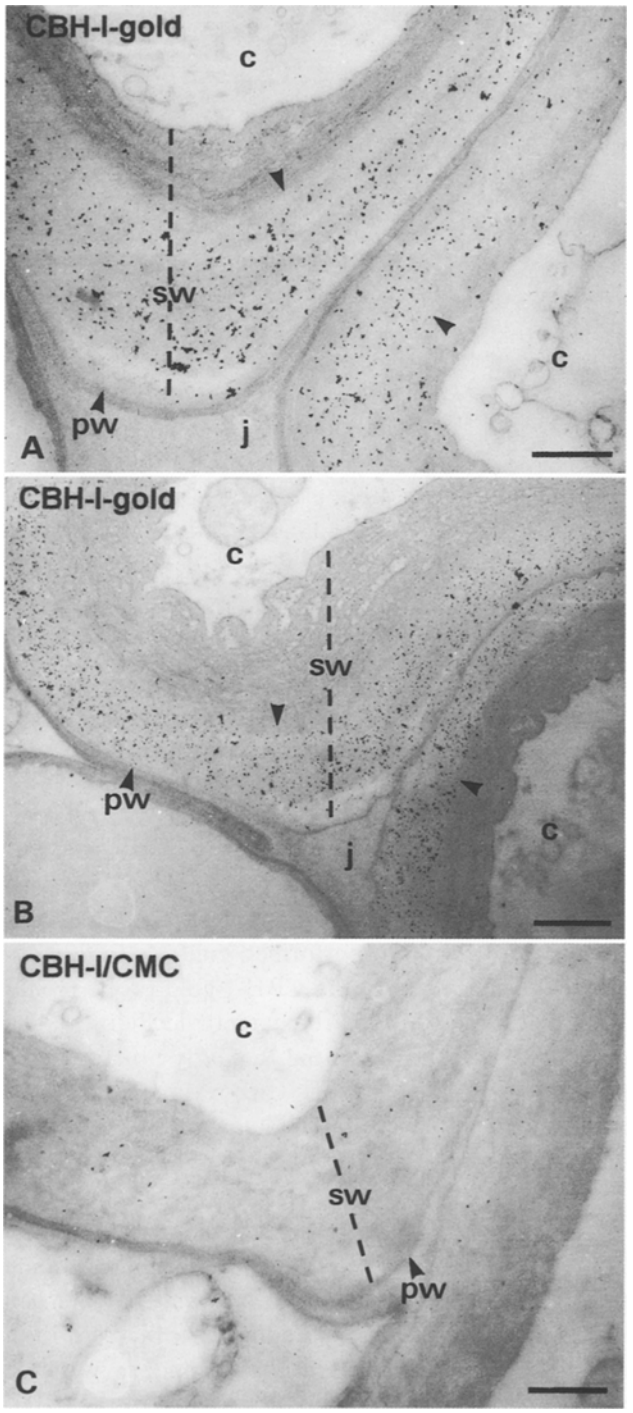

Fig. 2. **A** and **B** Affinity labeling of cellulose with the CBH-I-gold probe in flax fibers followed by PATAg staining. Note that most of the labeling is associated with that half of the secondary wall nearest the primary wall in either 1- (**A**) or 2-week-old (**B**) plants. **C** A control experiment showing the specificity of the CBH-I-gold probe: few gold particles are seen associated with the wall when the CBH-I-gold complex was preincubated with carboxylmethylcellulose before use. *c* Cytoplasm; *j* cell junction; *pw* primary wall; *sw* secondary wall. Arrowheads indicate the limit between the CBH-I-gold-labeled and nonlabeled areas of the secondary wall. Bars: A and C, 0.7 μm; B, 1.25 μm

(Jones et al. 1997, Willats et al. 1998). These are usually associated with the side chains of the pectic polysaccharide RG-I, although they can be found as free molecules in flax plants (Davis et al. 1990, Goubet et al. 1995, Gorshkova et al. 1996, Girault et al. 1997). The LM5 and LM6 antibodies have been found to recognize an RG-I-rich polysaccharide fraction of flax (data not shown). In addition, LM5 binds strongly to isolated β-(1→4) galactans of flax fibers (P. Knox pers. commun., Girault et al. unpubl.). Attempts are being made to establish whether these antibodies bind to flax AGPs.

The immunofluorescent reaction of the LM5 antibody (specific for β-(1→4) galactans) with the hypocotyl tissues, and particularly with fiber cells, is shown on Fig. 1F. A close examination of the labeling of fibers shows that the antibody is associated primarily with the secondary wall closest to the plasma membrane (Fig. 1F). This pattern of LM5 distribution within the secondary wall of fibers is confirmed by immunogold electron microscopy in both 1- and 2-week-old plants and shows a gradient increasing from the primary wall to the plasmalemma (Fig. 4A, B). Labeling is also found over the primary wall but not over the cell junctions. In contrast to LM5-recognized molecules, α-(1→5) arabinan epitopes (recognized by the LM6 antibody) are relatively more uniformly distributed within the secondary wall of fibers, particularly in 1-week-old plants (Fig. 4C, D). These epitopes are not detected in the tricellular junctions (see Fig. 4D).

Immunolocalization of epitopes carried by AGPs

To localize AGP-like molecules in flax fiber walls, we have used anti-β-(1→6) galactan and LM2 antibodies, which have been previously characterized and shown to be specific for carbohydrate epitopes of plant AGPs (Kikuchi et al. 1993, Smallwood et al. 1996). Since anti-β-(1→6) galactan antibodies may eventually react with free arabinogalactans (AG), we refer to the epitope-containing molecules as AGP/AG (see below).

Immunofluorescence labeling of a transverse section of flax hypocotyls with anti-β-(1→6) galactan antibodies shows a heavy staining of the secondary cell walls of fibers (Fig. 1E). It is also interesting to note that some other cells are heavily stained with these antibodies (Fig. 1E). A close examination of the epitope distribution by immunogold labeling (Fig. 5A,

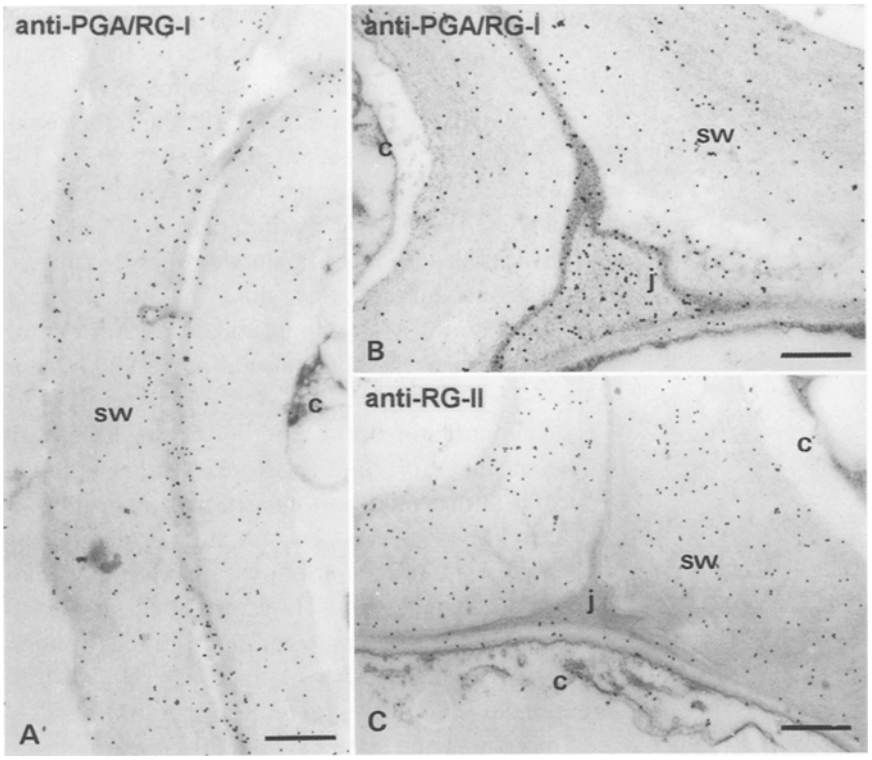

Fig. 3. Immunogold labeling of fiber cell walls with anti-PGA/RG-I (**A** and **B**) and anti-RG-II polyclonal antibodies (**C**). Labeling with both antibodies is uniformly distributed over the secondary wall of fibers. Note the heavy labeling of the cell junctions with anti-PGA/RG-I antibodies (**B**). *c* Cytoplasm; *j* cell junction; *sw* secondary wall. Bars: A, 0.40 μm; B, and C, 0.70 μm

B) demonstrates that the labeling is mostly found over the secondary wall closest to the plasma membrane and also over the plasma membrane (Fig. 5A). However, some fiber cells, especially from 2-week-old plants, show a more uniform labeling all over the secondary wall (Fig. 5B), suggesting an evolution of the distribution pattern of the recognized epitopes during development. Labeling with these antibodies is also found over the plasma membrane and Golgi-derived vesicles in the cytoplasm. In contrast, LM2 epitopes are present at low levels and very close to the plasma membrane (Fig. 5C). LM2 epitopes become less detectable with fiber maturation and almost disappear from the fiber walls of 2-week-old plants (Fig. 5D).

Controls

We have performed different control experiments to assess the specificity of the labeling of flax hypocotyl sections with the different probes used. At the electron microscopic level, no labeling is observed over the walls of fibers after omission of the primary antibodies or their substitution with preimmune antibodies (Fig. 6A–D). Similarly no labeling is observed over the walls of fibers with immunofluorescence staining (not shown). We have also performed controls to demonstrate the specificity of the CBH-I-gold probe (cellulose detection) with carboxylmethylcellulose as a competitor. As shown on Fig. 2, nearly no labeling is found over fiber walls when carboxylmethylcellulose is added to the incubation medium.

Discussion

Different probes were used in this study to examine the spatial organization of various wall polymers in young flax fibers. The main findings are: the pattern of cellulose deposition within the secondary walls of young fibers is heterogeneous as revealed by the CBH-I-gold labeling; different noncellulosic polymers such as pectins (particularly galactans and RG-II) and AGP/AG are abundantly synthesized and secreted during early stages of fiber differentiation; different polysaccharide epitopes exhibit discrete differences in their localization within fiber walls. Such noncellulosic polymers (i.e., pectins) may, in collaboration with cellulose, play an important role in the cohesion, stability, and strength of young fibers.

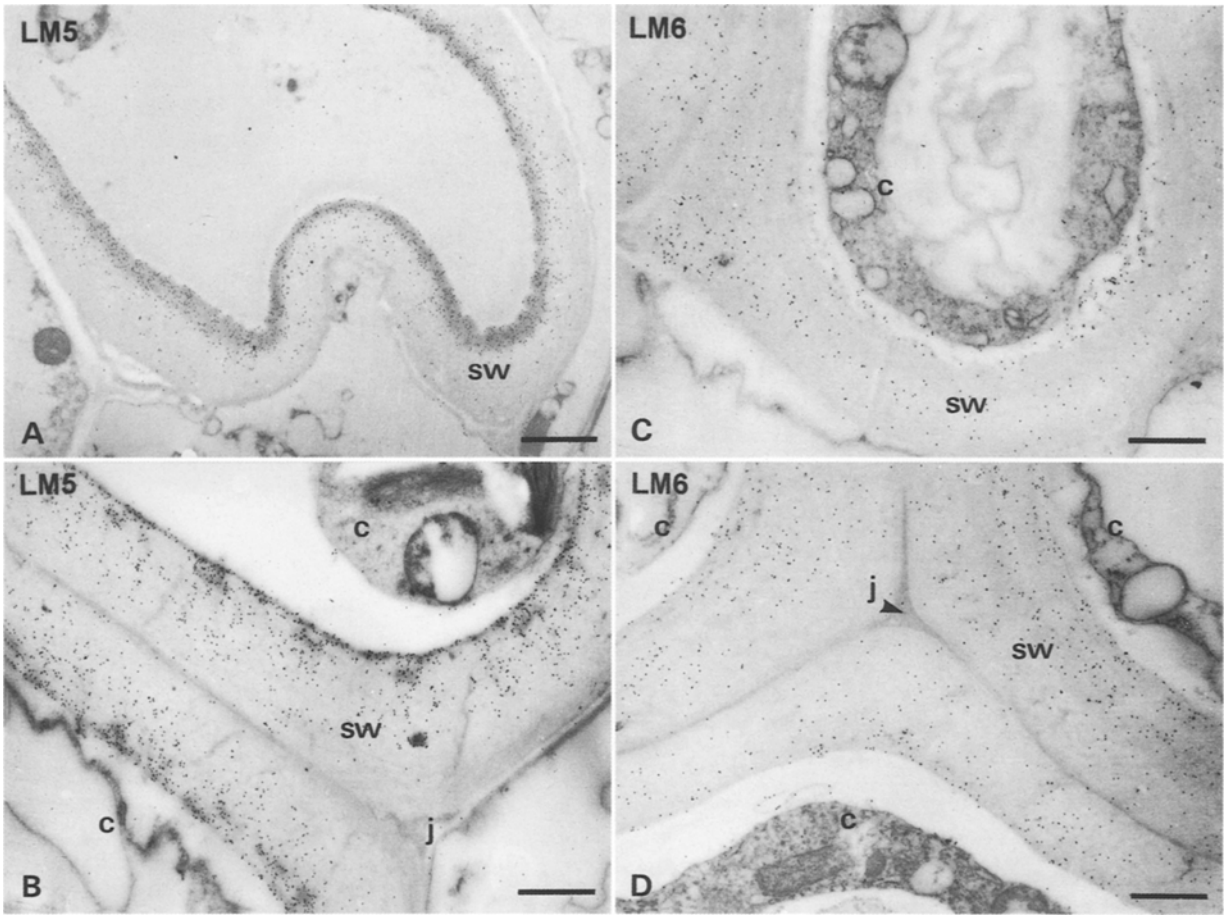

Fig. 4. Immunogold labeling of fiber cell walls with LM5 (**A** and **B**) and LM6 (**C** and **D**) monoclonal antibodies. Note that labeling with LM5 is more localized close to the plasma membrane than LM6 labeling. No labeling is seen over the cell junctions (*j*) with either antibody. *c* Cytoplasm; *sw* secondary wall. Bars: A, 0.50 μm; B and D, 1.15 μm; C, 0.70 μm

Significance of pectin distribution

Different types of pectins are present in the cell walls of flax fibers, suggestive of an active synthesis and secretion of these polysaccharides during early stages of differentiation. The location of these molecules in different wall areas indicates that they may have distinct roles within fiber walls. Unlike galactans and arabinans, unesterified pectins (recognized by PGA/RG-I antibodies) are mainly detected in the middle lamella and three-way junctions between fiber cells. As shown by numerous immunolabeling studies, unesterified pectins are predominantly found in the middle lamellae and cell junctions in different plant species, including flax (Moore and Staehelin 1988, Knox et al. 1990, Jauneau et al. 1994, 1997), and are believed to cement the adjacent cells and prevent cell separation. Acidic pectins in the middle lamellae of early-forming flax fibers are likely to serve similar purposes, thereby maintaining their cohesion and stability during the process of intrusive growth. This is supported by the fact that, in late development, fibers are tightly cemented and the separation of fiber bundles during retting is associated with solubilization of acidic pectins of surrounding tissues (Morvan et al. 1989, 1990; Van Sumere 1992; Girault et al. 1997). Secondary walls of young flax fibers are also rich in arabinans and galactans that could be linked to RG-I. However, free 4-galactan molecules are also found in retted flax fibers (Girault et al. 1997) and may originate from hydrolysis of RG-I during further development and/or during retting. The direct detection of arabinans in the secondary walls of young fibers (Fig. 4) is consistent with the biochemical identification of these polymers from fiber-rich strips, which also contain other tissues (Gorshkova et al. 1996).

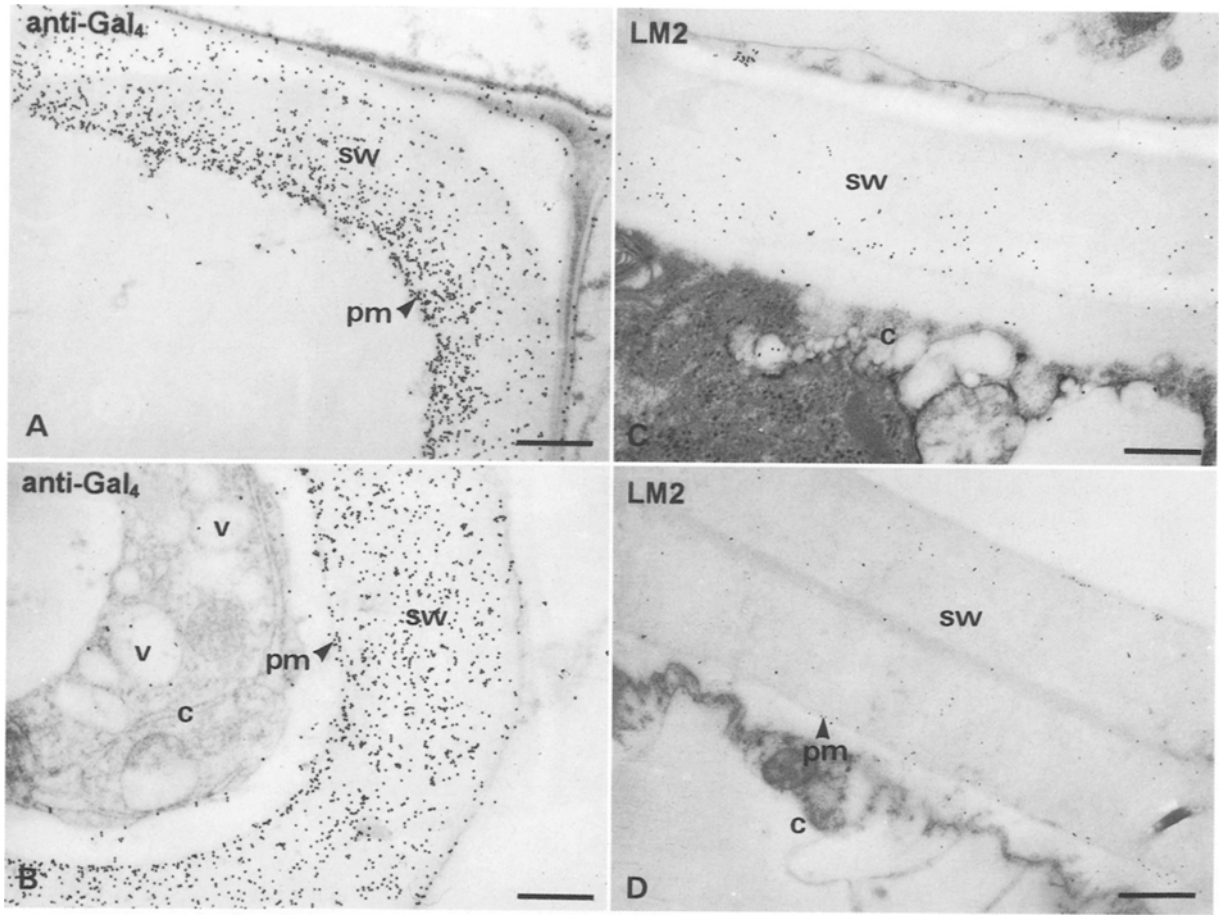

Fig. 5. Immunogold labeling of fiber cell walls with anti-β-(1→6) galactan (**A** and **B**) and LM2 (**C** and **D**) antibodies which recognize carbohydrate epitopes of AGPs. Note the strong labeling of fiber secondary walls with anti-β-(1→6) galactan compared to LM2 labeling. Note also the almost complete absence of LM2 labeling in 2-week-old plants (**D**). *c* Cytoplasm; *pm* plasma membrane; *sw* secondary wall; *v* secretory vesicles. Bars: A and C, 0.70 μm; B, 0.65 μm; D, 1.00 μm

Unlike RG-I, the presence of RG-II in flax fibers has never been reported from biochemical studies (Davis et al. 1990). Using the polyclonal antibodies raised recently against the borate-RG-II complex (Matoh et al. 1998), we show that RG-II-like molecules containing borate esters are detected and uniformly distributed within the secondary wall of young flax fibers. To our knowledge, this is the first demonstration of the occurrence of the RG-II polysaccharide in the secondary walls of flax fibers. This finding, together with the observation that boron is present in the walls of flax fibers (Pariot et al. 1994), suggets that the boron-RG-II complex is important for the structural organization of fiber walls.

Occurrence of AGP/AG in young flax fibers

Anti-β-(1→6) galactan and LM2 antibodies have been shown to recognize carbohydrate epitopes of plant AGPs (Smallwood et al. 1996, Kikuchi et al. 1993, Vicré et al. 1998). On immunodot-blot assays, anti-β-(1→6) galactan antibodies also bind to a Yariv-reactive polysaccharide fraction from flax fibers and react strongly with AGPs purified from red wine (data not shown) (Pellerin et al. 1995). In the secondary walls of fibers, LM2 epitopes are less abundant than β-(1→6) galactan epitopes (Fig. 5), suggesting a low level of synthesis and/or secretion of the corresponding AGP/AG at that stage of development. However, the most striking observation is that LM2 epitopes are not detected in 2- or 4-week-old fibers (Fig. 5D and data not shown). This may result from an in muro degradation and/or masking of the epitopes due to structural and/or conformational changes of wall polysaccharides during development. An alternative explanation is that the synthesis and secretion of LM2 epitope-containing molecules is developmentally regulated by fiber cells during their differentiation.

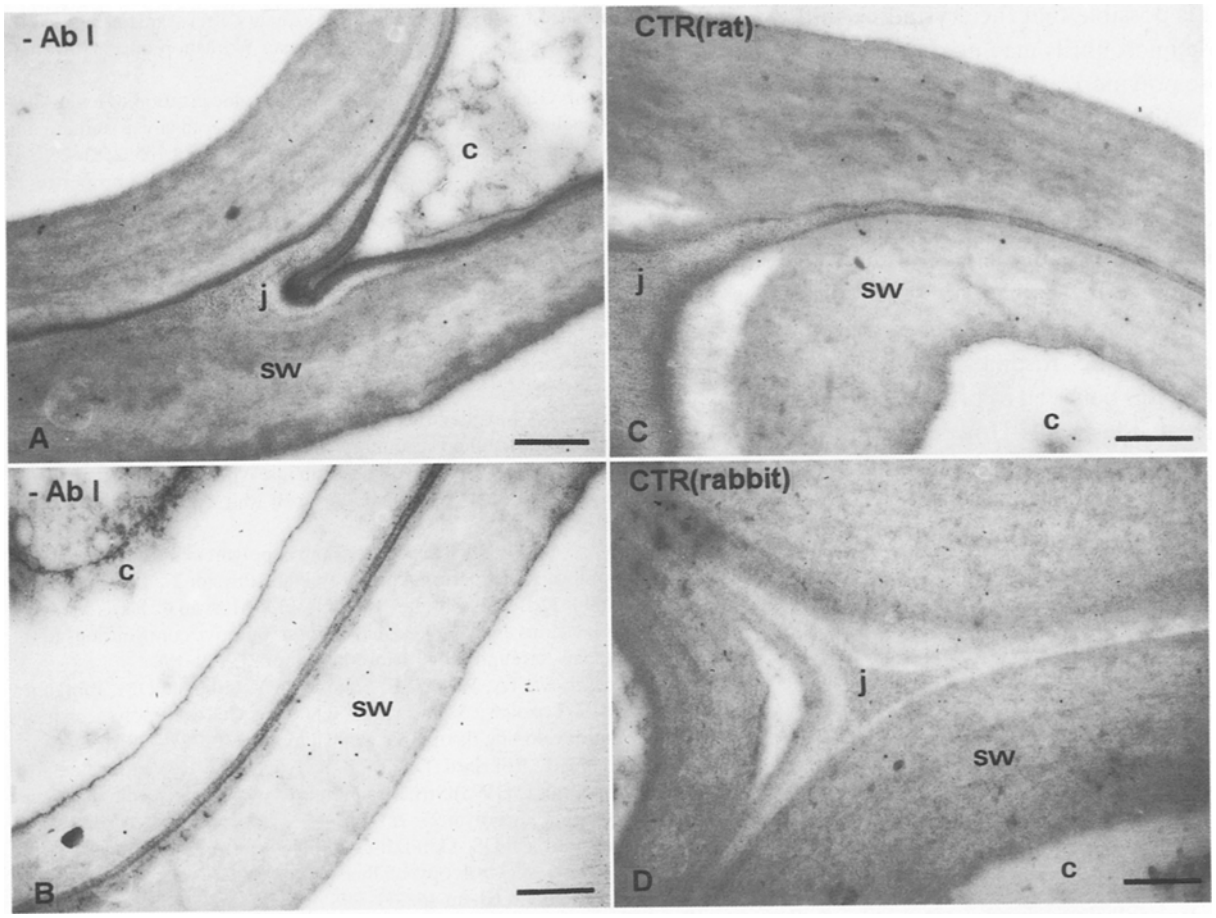

Fig. 6 A–D. Immunological controls. Few or no gold particles are observed over fiber walls of control samples in which primary polyclonal (**A**) or monoclonal (**B**) antibodies were omitted or substituted with rat (**C**) or rabbit (**D**) preimmune sera. *c* Cytoplasm; *j* cell junction; *sw* secondary wall. Bars: A and B, 0.4 μm; C and D, 0.6 μm

Another striking observation is that protophloem cells destined to differentiate into fibers are also heavily stained with anti-β-(1→6) galactan antibodies (Fig. 1E), suggesting that β-(1→6) galactan epitopes might be early markers of fiber differentiation.

Like AGPs, other cell wall structural proteins (i.e., hydroxyproline-rich glycoproteins and glycine-rich proteins) are regularly found in the secondary walls of plant cells and are believed to be essential to the final building and shaping of these walls (Cassab and Varner 1987, Keller et al. 1989, Domingo et al. 1994). The incorporation of AGPs during early stages of fiber differentiation is likely to be important for the assembly and general architecture of the secondary walls. Structural wall proteins are also known to form cross-links with pectins (Fry 1986), and the fact that fiber cell walls are rich in pectic polysaccharides suggests that AGPs could function through their interaction with pectins. Such an interaction has been shown to occur between AGPs and pectins in mature flax fibers (Girault et al. unpubl.).

Location of cellulose and mechanical strength in young flax fibers

CBH-I-gold complex is a powerful probe for cellulose detection in plants (Berg et al. 1988, Vian and Roland 1991). Using this probe, we show that labeling of cellulose is limited to that half of the secondary wall nearest the primary wall of fibers (Fig. 2). Almost no labeling is seen in the part of the wall adjacent to the plasma membrane, although significant amounts of cellulose are present in this newly deposited zone of the secondary wall as shown by the Calcofluor staining (Fig. 1C). As CBH-I is known to bind highly crystalline cellulose (Chanzy et al. 1984, Berg et al. 1988), the labeling pattern observed may reflect differences in the crystallinity of cellulose in early-forming fibers.

Thus, it is possible that the crystalline self-assembly of cellulose microfibrils may not occur immediately after cellulose polymerization and deposition near the plasmalemma in the cell wall of flax fibers. However, an alternative explanation is that cellulose microfibrils might be masked by matrix polysaccharides (e.g., pectins; see Figs. 4 and 5), making them less accessible to the CBH-I probe in the part of the wall nearest the plasmalemma. In mature fibers, cellulose has been shown to be of a highly crystalline nature by solid-state nuclear magnetic resonance methods (Love et al. 1994), and it is believed to provide tensile strength and resistance to these cells. However, although they might be less rigid than cellulose (Girault et al. 1997), noncellulosic components may also be needed, in association with cellulose, to ensure the physical properties and flexibility of mature fibers. In the generally accepted model for secondary walls of the xylem, the presence of lignin between cellulose microfibrils increases the wall resistance to compressive forces (Wardrop 1971, Frey-Wyssling 1976). Flax fiber cell walls contain no (or very low amounts) of lignin (Himmelsbach et al. 1998) but are rich in pectic substances (Davis et al. 1990, Gorshkova et al. 1996; this study) that may play a similar role as lignin in the xylem. As has been shown recently (Chanliaud and Gidley 1999) any pectin–cellulose interaction may influence the mechanical properties of the cell walls. Thus, in the secondary walls of fibers, pectins may interact with cellulose microfibrils in a manner that would increase their overall strength and flexibility. Further studies are needed to determine the contribution that individual components make to the physical properties and functions of the secondary wall of flax fibers.

Acknowledgments

We are extremely grateful to Drs. P. Knox, T. Matoh, L. A. Staehelin, Y. Tsumuraya, and B. Vian for their generous gifts of the antibody and CBH-I probes. Many thanks also go to Drs. S. Freyermuth and T. Giddings for their careful reading of the manuscript.

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