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REVIEW

Formation of Plant Cell Wall Supramolecular Structure

T. A. Gorshkova*, P. V. Mikshina, O. P. Gurjanov, and S. B. Chemikosova

Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences, ul. Lobachevskogo 2/31, 420111 Kazan, Russia; fax: (843) 292-7341; E-mail: gorshkova@mail.knc.ru

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Abstract—Plant cell wall is an example of a widespread natural supramolecular structure: its components are considered to be the most abundant organic compounds renewable by living organisms. Plant cell wall includes numerous components, mainly polysaccharidic; its formation is largely based on carbohydrate–carbohydrate interactions. In contrast to the extracellular matrix of most other organisms, the plant cell compartment located outside the plasma membrane is so structured that has been named "wall". The present review summarizes data on the mechanisms of formation of this supramolecular structure and considers major difficulties and results of research. Existing approaches to the study of interactions between polysaccharides during plant cell wall formation have been analyzed, including: (i) characterization of the structure of natural polysaccharide complexes obtained during cell wall fractionation; (ii) analysis of the interactions between polysaccharides "at mixing in a tube"; (iii) study of the interactions between isolated individual plant cell wall matrix polysaccharides and microfibrils formed by cellulose-synthesizing microorganisms; and (iv) investigation of cell wall formation and modification directly in plant objects. The key stages in formation of plant cell wall supramolecular structure are defined and characterized as follows: (i) formation of cellulose microfibrils; (ii) interactions between matrix polysaccharides within Golgi apparatus substructures; (iii) interaction between matrix polysaccharides, newly secreted outside the plasma membrane, and cellulose microfibrils during formation of the latter; (iv) packaging of the formed complexes and individual polysaccharides in cell wall layers; and (v) modification of deposited cell wall layers.

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Cell wall is a multifunctional, exceptionally important part of a plant cell and whole plant organism, which determines its biological features in many respects. Cell wall properties and characteristics are a basis for or have to be taken into consideration in practically all fields of application and processing of plant raw materials. Therefore, the study of formation of cell wall supramolecular structure is extremely important, especially in view of the current attention to the study of nanostructures. However, in spite of the demand, works in this field are few and specialized reviews are practically absent. First of all, this is due to the complexity of the system under study.

Many problems concerning the biosynthesis of individual polymers still remain unsolved; as before, cell wall serves as a real "stumbling stone" for molecular biologists trying to identify the genes that encode enzymes for the synthesis of separate bonds in a polymer [1]. The absence of understanding of how individual polysaccharides are synthesized impedes the study of their interactions during supramolecular structure formation. The diversity of bond types in plant cell wall polysaccharides is a separate problem extremely complicating their analysis. Besides, the key interactions during plant cell wall formation are of the carbohydrate–carbohydrate type, one of the least studied bond types [2, 3]. The existence of such complexes as plant cell wall is clear evidence of the importance and large scale of carbohydrate–carbohydrate interactions in nature. This review analyzes the existing approaches to the study of interactions between polysaccharides during the formation of plant cell wall, defines the key components of this process, and generalizes the findings.

Abbreviations: CP/MAS ^{13}C-NMR, cross-polarization NMR spectroscopy of ^{13}C nuclei with magic angle rotation; FTIR spectroscopy, Fourier transform infrared spectroscopy; GalA, galacturonic acid; GlcA, glucuronic acid; Rha, rhamnose; SP/MAS ^{13}C-NMR, single-pulse sequence in NMR spectroscopy of ^{13}C nuclei with magic angle rotation.

* To whom correspondence should be addressed.

PLANT CELL WALL AS A SUPRAMOLECULAR FORMATION

Plant cell wall is considered to be a composite material, where crystalline components (cellulose microfibrils) interact with an amorphous matrix, the major components of which are polysaccharides [4-6]. Cellulose is a linear polymer consisting of glucose residues linked by β-(1→4) bonds; individual chains are connected into a supramolecular formation, i.e. microfibril. Cellulose is a polymorphous compound; six polymorphs of this polysaccharide have been described. The most widespread of these, cellulose I, is present in plants; it is characterized by the parallel location of individual chains (all reducing ends are uniformly directed).

Natural cellulose usually occurs in amorphous-crystal state with a few types of polysaccharide organization: crystal structure, amorphous state, surface cellulose of crystal domains, and paracrystalline cellulose. Cellulose I crystal structure consists of the two modifications: I_α and I_β. Phases I_α and I_β are represented by triclinic and monoclinic unit cell, respectively. Phase I_α is metastable because it can be converted into the more stable I_β. Both crystal cellulose phases are present in all cellulose sources but in different proportions.

Non-cellulose polysaccharides are divided into two groups. The first includes pectin substances – acid polysaccharides containing galacturonic acid. The major ones are as follows: (i) polygalacturonic acid, a homopolymer of galacturonic acid residues in the pyranose form linked by α-(1→4) bonds; (ii) rhamnogalacturonan I; its backbone is constructed of dimers [→4)-α-D-Gal*A*p-(1→2)-α-L-Rha*p*(1→], side chains are structurally diverse (different length and degree of branching) and may consist of β-D-(1→4)-galactans, α-L-(1→5)-arabinans, or arabinogalactans, where arabinose is usually terminal and galactose links can be connected through C-4, C-3, or C-6; and (iii) rhamnogalacturonan II, comparatively small (with backbone containing only nine α-(1→4)-D-Gal*A*p links) but with an exceptionally complex structure; this polysaccharide contains 12 different monosaccharides at polymerization degree of about 60, and the diversity of bonds between the monomers can be as much as 20 variants.

The second group has not so evident definition as the first. Previously, it was named "hemicelluloses", and now it is called "cross-linking glycans" because the latter can be bound to cellulose microfibrils; it is supposed to be a basis of their functional role. Most of them are neutral molecules, but sometimes glucuronic acid is found. It should be noted that the ability to be bound to cellulose has been revealed also in some pectin substances [7, 8].

The best known cross-linking glycan is xyloglucan. Its backbone is a β-D-(1→4)-glucan, which branches by means of binding xylose to the C-6 atoms of glucose. In turn, xylose residues can be bound to galactose or arabinose residues and galactose can be bound to fucose. The structure of side chains is recorded, respectively, as D-Xyl*p*-α-(1→-backbone, or D-Gal*p*-β-(1→2)-D-Xyl*p*-α-(1→-backbone, or L-Fuc*p*-α-(1→2)-D-Gal*p*-β-(1→2)-D-Xyl*p*-α-(1→-backbone, or L-Ara*f*-α-(1→2)-D-Xyl*p*-α-(1→-backbone.

The widespread cross-linking glycans include also β-D-(1→4)-xylans, including glucuronoarabinoxylan. The C-2 or C-3 xylose residues can have branches containing a single (or in short chains) α-L-Ara*f*, as well as a single α-D-GlcA*p*. In the cell walls of cereals, one of the major polymers is glucan with mixed-type bonds: a linear molecule, where glucose residues linked by β-(1→4) bonds, like in the cellulose molecule, are alternated with β-(1→3) bonds. Finally, the cross-linking glycans include mannose-containing polymers (mannans, glucomannans, galactomannans, glucogalactomannans), which are usually present in minor amounts.

The most often cited concepts of the supramolecular structure of plant cell wall concern the primary wall (the wall of cells capable of growth, 100-200 nm thick, corresponding to 3-4 microfibril layers) and describe it as three interweaving but relatively independent polymer networks: (i) the network of cellulose microfibrils linked with each other by different glycans (mainly through hydrogen bonds); (ii) the gel-like network of pectins linked by calcium ion bridges; and (iii) the network of structural proteins covalently bound to each other and probably to other cell wall components [9, 10].

The composition and structure of cell walls are varied not only in different tissues but also in separate layers and regions of an individual cell wall. This is most clearly demonstrated during immunochemical staining with antibodies – an exceptionally fruitful and extensively applied method [11]. Nevertheless, the four main types of cell wall combine, after deduction of nuances, nearly all variants – two types of the primary cell wall and two types of the secondary wall formed after cell extension has stopped (its thickness can be up to 15,000 nm). In all cell wall types, the backbone consists of cellulose microfibrils and the differences are observed first of all in the composition of matrix polysaccharides, in particular major cross-linking glycans. This is xyloglucan in the primary cell wall of type I; β-D-glucan with the mixed bond type and arabinoxylan in type II [10]. Xylan is almost the only non-cellulose polysaccharide in the secondary cell wall of type I; galactose-containing polymers are for type II [12]. Although cell wall types can be different also in the presence of non-carbohydrate components (e.g. proteins and lignin), which are not considered in this review, classification just on the basis of cross-linking glycans is characteristic in itself as it underlines their key role.

The principal interaction in plant cell wall is formation of a cellulose–xyloglucan complex. Both polysaccharides are β-D-(1→4)-glucan chains. In cellulose, these chains are linked by hydrogen bonds into microfib-

rils; self-association of xyloglucan molecules is prevented by a large number of side chains [13]. Xyloglucan is present in all types of primary cell walls [13, 14] and has been noted in some layers of secondary walls [15]. It is the study of interactions between these polysaccharides that has attracted the greatest attention. At the same time, new approaches have been developed in recent years for the analysis of participation of all key polymers of plant cell walls in formation of their supramolecular structure.

APPROACHES TO STUDY OF INTERACTIONS BETWEEN POLYSACCHARIDES DURING PLANT CELL WALL FORMATION

Several approaches give insight into the mechanisms of formation of cell wall supramolecular structure (table). Let us consider the capabilities and drawbacks of each method.

Study of structure of natural supramolecular polysaccharide complexes. Different methods for fractionation and study of polymer complexes isolated from cell wall give important information about its supramolecular structure. All cell wall polysaccharides except for cellulose are water-soluble compounds; they are fixed in the cell wall by the bonds of different types [4]. Some bonds are individually weak (e.g. hydrogen) but strongly bind polymers due to their considerable quantity; other (covalent) bonds, being relatively few, provide binding due to the strength of a single bond.

The character of key bonds between polymers can be studied by methods of cell wall fractionation, including treatment of isolated cell walls first by chelating agents (ammonium oxalate, EDTA, etc.), which bind calcium and release the major portion of pectin substances, and then by extraction with chaotropic agents (usually alkaline solutions, up to 6 M), which solubilize many cross-linking glycans by means of breaking their hydrogen bonds with cellulose.

After these treatments, some of the molecules of some polymers remain bound to cellulose and are released only during microfibril destruction. Xyloglucan [13, 16-18], galactan [12], and polygalacturonic acid [19] have been found among such polymers in cell walls of different types. It is supposed that these polysaccharides are physically captured by the formed cellulose microfibrils. They are extracted by decomposition with cellulase [18] or, when it is inefficient, dissolution of microfibrils in, e.g. lithium chloride solution in N,N-dimethyl acetamide [20], dimethyl sulfoxide solution containing tetrabutylammonium fluoride [21], or methylmorpholine-N-oxide solution [4, 22].

Each of these fractions, even on application of step-by-step fractional extraction, contains different polysaccharides. Moreover, the same polysaccharide can be found in different fractions. The differences in extractability make it possible to divide xyloglucan molecules into three fractions: physically interwoven into cell wall (easily extractable by treatment with xyloglucan endoglucanase); forming hydrogen bonds with polymers (extracted by alkali); and tightly associated with microfibrils, probably captured by them (extracted by treatment with cellulase) [18].

Step-by-step enzymatic and/or chemical hydrolysis of cell walls and their separate fractions reveals different extractability of pectin substances. This approach has shown that the most arabinan and galactan chains in the cell walls of sugar beet and potato are rather tightly associated with cellulose and not hydrolyzed by specific enzymes [7, 8].

Obviously, such "smearing" over the fractions is evidence of the different roles of groups of molecules of individual polysaccharide in the maintenance of the supramolecular structure. These features can be associated with (i) structural nuances of a certain group of molecules (e.g. polymerization degree, side chain structures, methylation and/or acetylation degree, localization of modified groups), (ii) appearance in some of polysaccharide molecules of covalent bonds with other polymers, and (iii) probabilistic character of noncovalent interaction with cellulose microfibrils. Most evidence has been collected for the second assumption; the arguments for the two other assumptions are mainly logical, though arabinoxylan of monocotyledons has become an example of peculiar composition of a polymer extracted by increasing concentrations of alkali [23, 24].

There is much evidence of the presence of covalent bonds between homologous and heterologous cell wall polymers. In many cases these bonds have been biochemically identified, while in other cases it is assumed on the basis of co-elution of two polymers with different properties. The latter is exemplified by elution on an ion-exchange column, together with rhamnogalacturonan I of xyloglucan [25] or glucomannan [12].

The identified covalent bonds between cell wall polysaccharides include ester bonds through a boron atom between apiose residues (OH-2 and OH-3) of two rhamnogalacturonan II molecules with the formation of dimers [26, 27] and a cross-link between cell wall polymers through phenolic compounds – hydroxycinnamic acids attached to polysaccharides and aromatic amino acids being components of proteins, first of all tyrosine. The existence of bridges of aromatic components between two polysaccharides (diferulic bridges) has been proved biochemically. Besides, numerous isomers can be formed, because different atoms of the phenol ring and propanoid chain can participate in formation of this bond [28]. Not only dimers but also tri- and tetramers of ferulic acid have been isolated from cell wall [29-31]. This means that up to four individual polysaccharides can be potentially bound together.

Approaches to study of interaction between polysaccharides during formation and modification of plant cell wall supramolecular structure

Approach		Results obtained and possibilities available	Disadvantages
Survey of structure of natural supramolecular complexes of polysaccharides obtained during cell wall fractionation		Revealing character of key bonds between cell wall polysaccharides	1. Occurrence of a series of different polysaccharides in the same fraction 2. Occurrence of the same polysaccharide in different fractions 3. Absence of information about time of bond appearance, i.e. about steps of formation or modification of cell wall supramolecular structure where this bond is employed
Analysis of interaction between polysaccharides "at mixing in a tube"		Demonstration of possibility of spontaneous interaction of polysaccharides between each other and revealing of factors facilitating or preventing this interaction	1. Difference of conditions *in vitro* from conditions *in vivo* 2. Exclusion of interaction between polymers and cellulose microfibrils at moment of their assembly 3. Difference of used polysaccharides subject to modifications during isolation from native ones 4. Absence of information about steps of formation or modification of cell wall supramolecular structure, to which revealed interaction between polymers is coupled 5. Difficulties of registration of weak interactions
Study of interaction between individual isolated matrix polysaccharides of plant cell wall and microfibrils formed by cellulose-synthesizing bacteria		Possibility of analysis of interactions between matrix polysaccharides and cellulose microfibrils during their crystallization	1. Difference of used polysaccharides subject to modifications during isolation from native ones 2. Irreproducibility in a bacterial system of complex of conditions existing in a plant cell
Study of cell wall formation and modification directly in plant objects	a) application of suspension cultures (also in pulse–chase experiments)	Possibility of studying processes *in vivo*, relative homogeneity of plant material, simplicity of introduction of exogenous substrates and regulators	1. Absence of intercellular contacts and organismal level of organization 2. Difference (from cell in tissue) of balance between cell (cell wall) volume and ambient solution 3. Washing of substantial part of cell wall polymers into cultivation medium 4. Application of exogenous labeled compounds, which never get into plant cell from the environment *in vivo*
	b) analysis of label redistribution in intact plant, including analysis of tissue- and stage-specific polysaccharides in pulse–chase experiments	Possibility of studying processes *in vivo* in an intact system, without disturbance of the course of natural processes In rare cases, possibility of analyzing metabolism of individual polysaccharide in a particular type of cells at a particular stage of their development	1. Necessary application of substantial quantities of label (entry of label into all compounds of a cell and decrease in specific radioactivity of individual compounds) 2. Difficulties with clear characterization of time of pulse period due to prolonged transport of assimilates and their repeated reutilization 3. Heterogeneity of tissues and complexity of isolation of individual polysaccharides (in most cases)

Hydroxycinnamic acids (mainly ferulic acid) are attached only to some of the polysaccharides and, inside the latter, only to some of the -OH groups of certain monomers. Partial enzymatic hydrolysis of cell wall followed by chromatography and identification of compounds has shown the linkage of hydroxycinnamic acids with the terminal arabinose residues (O-5) in glucuronoarabinoxylan [32, 33], with the terminal xylose residue in xyloglucan [34], and with the side chains of rhamnogalacturonan I: galactose (in position O-6 in the backbone of β-(1→4)-galactan) and arabinose (in positions O-2 and O-5 in the backbone of α-(1→5)-arabinan) [35]. In these polysaccharides the ratio of the above monosaccharides and hydroxycinnamic acids is several tens [36, 37]; the total content of phenolic acids in the cell wall is not more than a few percent and, nevertheless, they can substantially affect its properties [38].

It is supposed that there are bridges of a single ferulic acid between a polysaccharide and a protein [39, 40] and between polysaccharides [41]. In this case, the same molecule of hydroxycinnamic acid forms an ester bond with a polysaccharide and an ether bond with a protein or another polysaccharide. However, no components forming such bonds have been revealed as yet. Additional bonds appearing in the lignified cell wall [28, 42] are not considered in this review.

Ionic bonds play an important role in maintenance of the supramolecular structure of plant cell wall. They include Ca^{2+}-mediated interactions between uronic acid residues with the formation of so-called "egg-box" structure [4, 43]. Steric limitations allow such structures to be formed only when the length of non-methylated region of polygalacturonic acid is no less than 10-15 monosaccharide links. Ionic bonds can appear between alkaline cell wall proteins and uronic acids [44].

Isolation of interacting polymers from the cell wall is indispensable evidence for the actual presence of interaction *in vivo*. However, it is difficult to study the mechanisms of interaction in a multicomponent system such as plant cell wall; besides, detection of one or another bond gives no information about the time of its appearance and, consequently, the steps of formation or modification of the cell wall supramolecular structure where this bond is employed. These problems are solved by using other approaches.

Analysis of interaction between polysaccharides "at mixing in a tube". The simplest and most evident type of experiments concerning the interaction between polysaccharides is mixing cell wall components or fragments *in vitro* and revealing the character of interactions between them. This approach is reported in quite a number of works, in particular, related to the interaction between cellulose and xyloglucan or its fragments [18, 45-47] and the interaction between xyloglucan and pectin substances [48-50], pectin side chains and cellulose [7, 51], or the binding of cell wall components to whole tissue fragments, cells, and cell walls [52]. At the same time, the conditions *in vitro* were varied, both in the presence of different additives and in other parameters, in attempts of reproducing some conditions *in vivo*.

If the assumption about the interaction between polymers during cell wall fractionation is based on their presence in the same fraction, registration of polymer interaction "at mixing in a tube" requires special techniques. The problem in the latter case is the weakness of interactions, the complexity of polysaccharide structure, and heterogeneity of molecules of individual polysaccharide (by weight, structure and length of side chains, presence of substituents, etc.). The strength of molecular interactions is quantitatively assessed by dissociation equilibrium constant (K_d); it is as higher as the strength of these interactions is less. The interaction is considered as strong at $K_d < 100$ nM, medium at K_d of 100 nM to 10 μM, weak at $K_d > 10$ μM, and absent at $K_d > 3000$ μM [53]. Biologically significant interactions are often within the range of tens and hundreds of micromoles; therefore, registration of such interactions is difficult.

Among the methods for studying interactions between cell wall polysaccharides, there are practically no methods extensively used in the analysis of carbohydrate–carbohydrate interactions in microorganisms and animals that are based on immobilization (including selective immobilization) of carbohydrate-containing molecules [2, 54]. This is partly associated with different tasks, because the above experiments most often are carried out to study the processes of recognition, which are highly selective and provided by short carbohydrate chains; in the study of formation of plant cell wall supramolecular structure, emphasis is placed on basic interactions of large polysaccharides, each of them having a certain degree of heterogeneity. The interaction between plant cell wall polysaccharides is characterized by using the following approaches:

– determination of the total content of carbohydrates in solution before and after the interaction (this approach is realistic provided that the interaction product is precipitated or can be separated from the solution in some other way); at the same time, the sensitivity or information value of the method can be enhanced by introducing respective labels (radioactive, fluorescent, etc.) into analyzed polymers [7, 17, 18, 45, 51];

– the analysis of variations in the size and/or molecular weight by chromatography [17, 55, 56] and multiangular laser light scattering (MALLS) [53, 57], or determination of sedimentation coefficient in an analytical centrifuge [53];

– revealing and characterization of the formed complexes using different types of microscopy, including electron microscopy; optical analysis is also performed with different dyes and labels [17, 46]; recently atomic-force microscopy has been often used [56];

– viscosimetric method; viscosity of the solution differs from the sum of viscosities of mixed components [17, 46].

The above approaches have demonstrated that cell wall polysaccharides can really interact with each other, and these interactions are influenced by a number of factors. So, it has been shown that dissolved cross-linking glycans during precipitation can form ordered structures similar in ultrastructure to the parts of the cell wall [58]. The study of interaction between xyloglucan and cellulose *in vitro* showed an association of these polymers in solution; it depended on pH, assuming the involvement of hydrogen bonds [46]. Xyloglucan fragments, the backbones of which are less than five glucose residues in length, do not interact with cellulose [45, 59]. The character of interaction between xyloglucan and cellulose through hydrogen bonds depends on the degree of crystallinity of microfibrils [60] and on the molecular weight of xyloglucan and composition of its side chains [61, 62]. Among the monomers of xyloglucan side chains, galactose has the greatest effect on its interaction with cellulose: in its absence, xyloglucan molecules aggregate with each other; fucose has comparatively little effect [59, 63]. Computer modeling of xyloglucan–cellulose interaction shows that the electrostatic interaction and van der Waals forces make a significant contribution to adsorption stabilization, in addition to hydrogen bonds [62].

The cross-linking glycans constructed on the xylan backbone are capable of self-association and interaction with cellulose; at the same time, the key factor is degree of substitution – the fewer are side chains, the stronger is the interaction [57, 64]. The first work where the ability for self-association in a non-cellulose plant cell wall polymer (arabinoxylan) was assessed quantitatively showed the K_d value of 340 μM, i.e. the level of interaction was within the same range as for some of the protein interactions known for their biological significance [53]. The character of temperature dependence is evidence of substantial contribution of hydrophobic interactions to self-association of arabinoxylan molecules. It is considered that the driving force of hydrophobic interaction between carbohydrate chains is the amphiphilic nature of these compounds possessing both hydrophobic (C/H) and hydrophilic (C/O) surfaces [65]. It should be noted that the polysaccharides used in that study were slightly different from native polysaccharides as they had been isolated from plant material by concentrated alkali.

In a series of experiments *in vitro*, M. C. Ralet and J. F. Thibault investigated the potentials for interaction between pectins and cellulose. In some of these experiments, they made the conditions "in a tube" closer to the conditions *in vivo* by using a press to produce a pressure and obtain the high concentration of interacting polymers available in the periplasm of plants [7, 51]. The analysis of interaction between cellulose and pectins or their structural domains (homogalacturonan, rhamnogalacturonan I, side chains) in such experiments showed that arabinan- and galactan-enriched pectins, just as isolated arabinan and galactan side chains, bind to cellulose microfibrils, in contrast to commercial pectin preparations and pectin backbone regions lacking side chains. Transmission electron microscopy and X-ray analysis have shown that only arabinan and galactan side chains can interact with cellulose. A hypothesis has been made that pectins can serve as a "double cohesive phase", interacting through homogalacturonan regions with the major proteins and polypeptides and through side chains with cellulose [51].

The interactions of matrix polysaccharides not only with cellulose but also with each other have been analyzed. The literature on interactions between pectin substances is voluminous due to their extensive application, first of all in food industry. Indeed, many experiments were oriented towards process technology; their conditions were non-physiological with some exceptions [66-69].

The principal result of experiments *in vitro* was demonstration of the possibility of spontaneous interaction of polysaccharides with each other and identification of some factors favoring or interfering with this interaction. However, the conditions *in vitro* are significantly different from those in plant periplasm. It has been shown, for example, that pectin concentrations within cell wall are up to ~20%; however, in solution these polymers have the form of a highly hydrated gel and their concentration is no more than 5% [70]. Xyloglucan–cellulose complexes formed *in vitro* are less stable than those isolated from the cell wall [18]; moreover, the content of xyloglucan in these complexes is only 7% of its content in native complexes [71].

It is obvious that the experiments *in vitro* reveal only possibilities (and not all of them) for the interaction between polymers, not allowing the assessment of whether this interaction actually occurs, on what scale, and when exactly during the formation of the real cell wall. Besides, some of the interactions seem to take place in the course of microfibril assembly, which is practically impossible to reproduce *in vitro*. So, these experiments are carried out with commercially available cellulose preparations obtained from the formed cell walls; the volume/surface ratio in cellulose molecules from such samples is far from the value during microfibril synthesis *in vivo* [70]. In this respect, the microorganism *Acetobacter xylinum* synthesizing practically pure cellulose is a very interesting model system. One can study the changes in the structure and properties of formed cellulose by adding different plant cell wall polymers to the cultivation medium of *A. xylinum*. This model system reflects the situation in a plant cell much more adequately than experiments *in vitro*, because the interaction between polymers can occur not only with the already formed microfibrils but also during their crystallization.

Study of interaction of polysaccharides of plant cell wall matrix with microfibrils formed by cellulose-synthesizing bacteria. The ability to form cellulose microfibrils is inherent not only in plants, but also in some other types of organisms. An interesting model system for the study of polysaccharide interaction has been developed on the basis of the microorganism *A. xylinum* synthesizing practically pure cellulose characterized, like plant cellulose, by the high crystallinity and strength [72]. Each bacterial cell forms a single long cellulose microfibril; in the culture medium, these microfibrils repeatedly interlace to form a film. By adding different plant cell wall components to the *A. xylinum* culture medium, one can follow the changes in the structure and properties of the formed cellulose [71-75]. This model system has been used in a series of works assessing the effect of nearly all key polysaccharides of the plant cell wall, including xyloglucan, glucomannan, arabinoglucuronoxylan, and arabinogalactan [72].

It has been shown that the family of glucomannan polysaccharides has the highest affinity to *A. xylinum* cellulose. X-Ray structure analysis, CP/MAS ^{13}C-NMR, FTIR-spectroscopy, and Raman scattering spectroscopy have shown the effect of water-soluble β-1,4-bound polysaccharides (mannan, glucomannan, xylan, and xyloglucan) on aggregation of bacterial cellulose [76-79]. It has been shown that the nature of xyloglucan binding to *A. xylinum* cellulose is similar to the one observed in the plant cell wall and that the binding occurs through formation of cross bridges between microfibrils [71].

It has been shown that bacterial cellulose produced in the presence of cross-linking glycans changes its crystallinity. So, mannan reduces the degree of cellulose crystallinity by decreasing the size of crystallites, whereas xylan and xyloglucan are co-crystallized with cellulose, altering the structure of its crystal lattice [78] and the Iα/Iβ cellulose ratio [77, 79]. This effect is not observed during cultivation of microorganisms on a medium with pectin [80].

This approach has not only revealed some factors determining the effects of different plant cell wall polymers on cellulose structure but also clearly demonstrated that the interaction between polymers can happen directly during the assembly of cellulose microfibrils. Upon addition of xyloglucan to the *A. xylinum* cultivation medium, the content of xyloglucan bound to cellulose was tenfold higher than in the case of mixing the commercial preparations of these polymers *in vitro* [71].

In spite of indisputable success of the studies with application of cellulose-synthesizing bacteria, the constraints of this experimental system are evident. First, the composition of plant cell wall polymers concurrently present during the assembly of cell wall supramolecular structure is diverse compared to individual polysaccharides, which might correct their interactions; the composite mixtures of such type have not been studied in the works with *A. xylinum*, and it is extremely difficult to adequately assess their composition in a plant cell. Second, the polysaccharides used in the experiments had been isolated from the cell wall, so they could differ from the native ones (in the course of isolation, they are often subjected to rather severe treatments resulting in modifications of extracted polymers). For instance, alkaline extraction used for the isolation of cross-linking glycans leads to deacetylation of polysaccharides. Moreover, the initial polysaccharide structure formed in the Golgi apparatus, which after secretion of a polymer determines its interaction with cellulose microfibrils during their formation, might substantially differ from the structure of the same polysaccharide in the cell wall [20]. Finally, the plant cell has a complex of conditions that are difficult and sometimes impossible for reproduction in a bacterial system. All the above necessitates the study of cell wall formation directly in a plant.

Study of cell wall formation and modification directly in plant materials. The analysis of processes that take place during the formation of cell wall supramolecular structure is coupled with some difficulties. In addition to methodical problems concerning the isolation and analysis of complex polysaccharides, they include, in particular, tissue heterogeneity of the samples. Cells of different tissues have different composition and different dynamics of cell wall deposition. Besides, cells of the same tissue can be in different stages of development; it is not easy to reveal the key processes and particularly their nuances in different cell wall types by analyzing an averaged sample. One more problem is that the cell wall polymers are accumulated during the lifetime of a cell and some of them are never subject to hydrolysis; against this constant background, it is difficult to single out the share of polymers that have been deposited or modified during a certain time. Besides, the necessary "reference point" in the study of processes that happen with a polysaccharide during its incorporation into cell wall is the detailed characterization of initial polymer structure created in the Golgi apparatus; however, polysaccharides present in the Golgi apparatus seldom are accumulated in amounts sufficient for analysis.

None of the existing model systems or approaches would avoid all of these problems without creating new ones. Nevertheless, an advance in research has been made owing to (i) application of suspension cell cultures; (ii) pulse–chase experiments for the study of label redistribution; and (iii) analysis of tissue- and stage-specific polysaccharides in the rare cases when they are available.

The advantages of plant cell suspension cultures, including relative physiological homogeneity of a sample (though genetic heterogeneity is generally significant) and simplicity of introduction of exogenous labeled substrates (they are added to cultivation medium), were realized in a series of works from S. Fry's laboratory [25, 81-86]. In particular, they analyzed the processes taking place during xyloglucan incorporation into cell wall.

Xyloglucan was not purified to an individual compound, and its presence in various fractions was assessed upon the action of Driselase enzyme preparation (a complex of identified endo- and exoglycanases). This preparation decomposes various polysaccharides with the formation of diagnostic disaccharide D-Xyl*p*-α-(1→6)-D-Glc (isoprimeverose) from xyloglucan; this disaccharide is used for detection and quantitative assessment of the presence of this polysaccharide in a complex mixture of polymers. The changed weight of xyloglucan was registered by gel filtration of separate fractions, and its association with acid polysaccharides was registered by anion-exchange chromatography.

In the course of experiments, radioactive exogenous substrates (in small amounts but with high specific radioactivity) were introduced into the cultivation medium of plant cells; the fractions where the labeled xyloglucan penetrated and the time of this event were registered. This is an example of the so-called pulse–chase technique, the essence of which is that after the completion of exposure to labeled substrate (pulse period) the plant material is sustained in its absence (chase period). The ^{14}C- or ^{3}H-monosaccharides are commonly used as exogenous labeled compounds in the analysis of cell wall formation and in the work with suspension cultures; double labeling with $^{14}C/^{3}H$ [82, 87] and other substrates is used in some cases, e.g. in the study of the course of formation of cross-links between polymers through phenolic compounds: ^{14}C-labeled cinnamate [29, 88] or ^{14}C-labeled ferulate [89]. The choice of labeled substrate, its concentration, specific radioactivity, and time of introduction are the key experimental parameters that need thorough elaboration.

This approach has shown that most newly synthesized ^{3}H-labeled xyloglucan is bound to the cell wall almost immediately after the secretion [90]. Incorporation into the cell wall is accompanied by a drastic increase in polysaccharide molecular weight by means of formation of covalent bonds catalyzed by xyloglucan endotransglycosylase (XET) [91]; the essential role of this enzyme in formation of cell wall supramolecular structure has been demonstrated using labeled xyloglucan-derived nonasaccharides [81].

The dynamics of appearance of cross-links between polysaccharides due to formation of diferulic acid has been characterized using the cells from suspension cultures; it has been found out which isomers of this acid are formed within the Golgi apparatus and directly in the cell wall [88]. The complexes of different polymers were found; the dynamics of their appearance and duration of existence were characterized [25]. Pulse-chase experiments with the suspension cultures of monocotyledonous cells showed substantial modification of the structure of deposited cell wall polysaccharides during cell extension [92].

At the same time, the studies with suspension cultures have a number of constraints. First, because of the absence of cell–cell contacts and the organismal level of organization, many processes occurring in a whole plant cannot be reproduced. Second, suspension cultures have quite different balance between cell (cell wall) volume and ambient solution compared to a cell as a component of tissue. A considerable part of cell wall polymers are washed out into the cultivation medium; on one hand, this makes it possible to analyze the processes probably taking place in a plant but, on the other hand, it may significantly change the process of polymer interaction, e.g. due to variation in their concentrations in the cell wall or periplasm. Third, application of exogenous labeled compounds, which can never enter plant cells *in vivo*, always poses the question about the natural course of the analyzed process, which is thoroughly considered in only a few works.

Experiments on label redistribution in an intact plant most fully reproduce the real processes that occur during the formation and further modification of cell wall supramolecular structure. The exogenous substrate in this case can be $^{14}CO_2$; photosynthesis in a $^{14}CO_2$-containing atmosphere provides natural influx of the substrate, the effective entry of the label into different organs and cell wall components, the absence of any damage to the plant, and the possibility of prolonged chase periods make it possible to follow the processes during different stages of development. However, this approach also has some drawbacks. The label enters all of the cell compounds; consequently, specific radioactivity of individual compounds decreases and, therefore, it is necessary to use considerable amounts of the label, which is always difficult during work with radioactive compounds. It is not always possible to clearly characterize the time of the pulse period because the label continues "walking" over the plant, getting into tissues (especially heterotrophic) not only during the photosynthesis with $^{14}CO_2$ but also after it. Besides, the above problems associated with heterogeneity of tissues and with the difficulty of isolating individual cell wall polysaccharides are important in work with plants.

This approach has particular prospects in the rare situations of studying a tissue- and stage-specific polymer like, e.g. the particular type of rhamnogalacturonan I (with the specific structure of side chains of galactose), which is synthesized in sclerenchyma fibers during the formation of secondary cell wall [93, 94]. This polysaccharide accumulates in substructures of Golgi apparatus before incorporation into the cell wall [95], so that it can be extracted in amounts sufficient for structural analysis. In the cell wall this polymer is concentrated in the fraction of polysaccharides that are exceptionally tightly bound to cellulose; a special method has been developed recently for extraction of such polymers [20]. Pulse–chase experiments with such model system make it possible to study in intact plants the processes that take place during and after incorporation of individual polysaccharides into the cell wall of a certain type of cells at a certain stage of development.

Relevant information about processes of cell wall supramolecular structure formation in plant cells has been obtained by different methods of microscopy. In particular, the presence and localization of some of the enzymes has been established by immunolocalization; their activity *in vivo* has been demonstrated using, e.g. fluorogenic substrates [96].

KEY PROCESSES DURING FORMATION OF PLANT CELL WALL SUPRAMOLECULAR STRUCTURE

The interaction of polysaccharides between each other begins immediately after completion of their synthesis or even during the course of it. Cellulose is synthesized by a multienzyme complex located on the plasmalemma, and polysaccharides are synthesized in the Golgi apparatus [97]. With allowance for such localization, several key stages in the formation of cell wall supramolecular structure (figure) can be defined (to a certain extent conditionally):

1) formation of cellulose microfibrils, i.e. supramolecular structures with several tens of cellulose molecules in each section;

2) interaction between matrix polysaccharides within the Golgi apparatus substructures;

3) interaction between newly secreted matrix polysaccharides and the being formed cellulose microfibrils after exit of the contents of Golgi vesicles out of the plasmalemma;

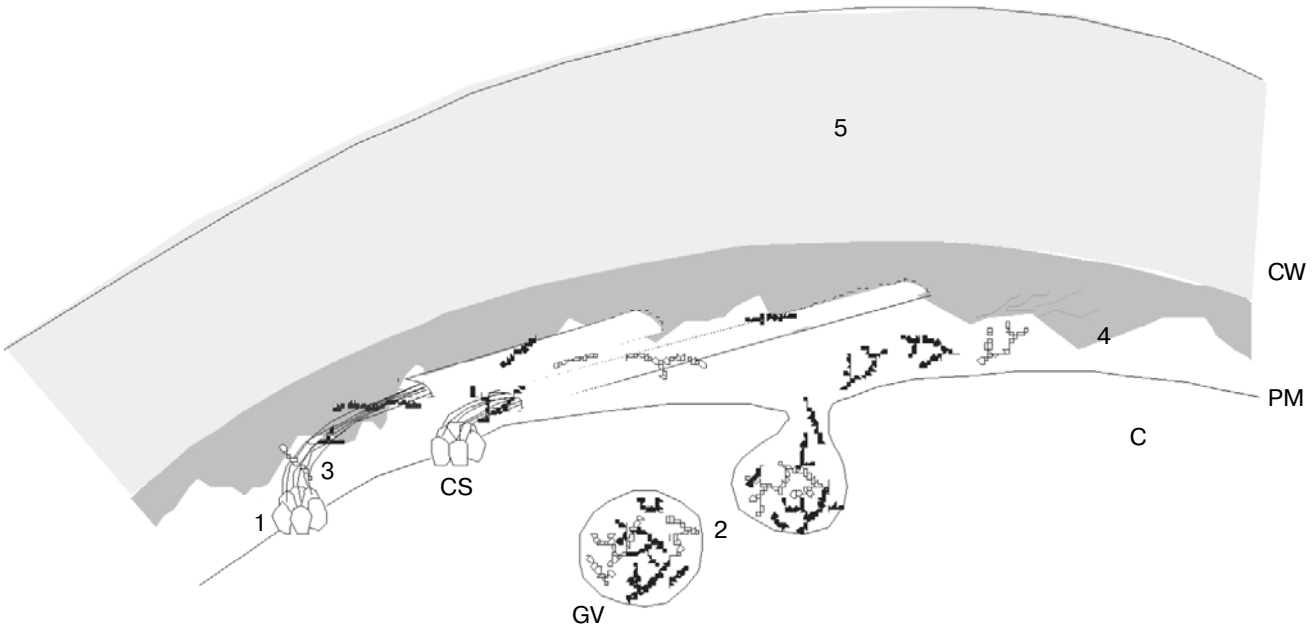
4) packaging of formed complexes and individual polysaccharides in cell wall layers;

5) modification of deposited cell wall layers (also during cell growth and development).

Formation of cellulose microfibrils. Cellulose I formed by plants differs from thermodynamically more stable cellulose II, which is formed, e.g. *in vitro* during precipitation of dissolved cellulose, in a parallel arrangement of all individual polymer chains in a microfibril [98].

The section of a microfibril from higher plant cell walls has always an approximately equal number (several tens) of individual chains. Cellulose molecules within a microfibril can begin and end in different places; their total length reaches several micrometers. Such quantity and arrangement of molecules in a plant microfibril is explained by the mechanism of cellulose synthesis by a multienzyme complex that contains several tens of catalytic subunits, each synthesizing a single chain [97]. Enzymes of this complex are integral proteins of the plasma membrane; the substrate for the synthesis comes in from the cytoplasmic side and the formed product comes out on the opposite side of the membrane. Cellulose microfibrils are formed on the basis of hydrogen bonds appearing between single polymer molecules. Crystallization takes place immediately after the formation of cellulose chains and is considered as a process that limits the rate of synthesis [99].

Cellulose from different sources varies in the size of crystalline regions; in most cases it is 2-3 nm. The largest crystalline regions have been found in the cellulose of the alga *Valonia* (up to 20 nm); in the fibrils of cotton seeds



Scheme of key processes during formation of plant cell wall supramolecular structure. 1) Formation of cellulose microfibrils; 2) interaction of matrix polysaccharides within Golgi apparatus substructures; 3) interaction between secreted matrix polysaccharides and formed cellulose microfibrils; 4) packaging of the formed complexes and individual polysaccharides in cell wall layers; 5) modification of deposited cell wall layers. GV, Golgi vesicles; CW, cell wall; PM, plasma membrane; C, cytoplasm; CS, cellulose synthase

and some plant fibers, the size of these regions is 5-6 nm [100, 101].

The structure of cellulose on the surface of crystalline regions (surface cellulose) differs from the structure of cellulose within them. In the CP/MAS ^{13}C-NMR spectra, they are discriminated by C-4 signals: the signals of surface and crystal celluloses are in the regions of 84 and 89 ppm, respectively. For the surface cellulose, it is supposed that the O6–O2′ hydrogen bonds break up and the O3′–O5 hydrogen bonds weaken; the planes of neighboring glucose residues are turned by about 20° relative to each other. The C-6 atom becomes more mobile and can move from one conformation to another, which is clearly demonstrated by the SP/MAS ^{13}C-NMR spectra recorded with different intervals between the impulses [101]. The surface cellulose structure depends neither on the size of crystallite nor on the phase of crystalline domain directly adjoining the surface cellulose. Proportion of the surface cellulose is inversely related to the size of crystalline domain.

Paracrystalline cellulose is practically unstudied; there are no direct methods for its study, and it is judged only by the CP/MAS ^{13}C-NMR spectrum analyzed by special mathematical processing [102]. By the time of longitudinal relaxation on NMR spectra (by the mobility of cellulose molecule), the degree of orderliness of a paracrystalline cellulose molecule has intermediate values between its crystalline and amorphous forms [102]. It is supposed that paracrystalline cellulose is concentrated within the crystalline domain, but there is no experimental evidence of its localization in the three-dimensional cellulose structure.

In the amorphous regions of cellulose microfibrils, the harmonious three-dimensional order is disturbed and only the total directedness of chains is maintained. In accordance with the amorphous–crystalline model, there are no distinct boundaries between crystalline and amorphous regions and the transition between them is gradual [98]. In spite of the great significance of amorphous cellulose, its structure and role are practically unknown. The reactions of interaction between cellulose and other substances can occur more readily in the amorphous regions of cellulose microfibrils, and it can facilitate the formation of a complex between cellulose molecules and matrix polysaccharides during cell wall formation.

Interaction of matrix polysaccharides within Golgi apparatus substructures. The cell wall matrix polysaccharides are synthesized in the Golgi apparatus; the interaction between polymers begins in its substructures where conditions for its regulation are created. These conditions include modification of polysaccharides, preventing or facilitating formation of a complex of individual molecules. Thus, uronic acids are subject to methylation in the Golgi apparatus, which substantially influences their ability to form gels and provide homo- and heterologous interactions [103]. The methylation substrate is S-adenosylmethionine [104], and the enzyme is pectin methyltransferase [105].

During the synthesis in the Golgi apparatus, some of the polysaccharides are acetylated by means of acetyl-CoA and acetyltransferase [106]. Acetylation can be very intensive, but its functional role has not yet been elucidated. Hydroxycinnamic acid residues [107, 108] providing covalent cross-links between cell wall polymers are attached to polysaccharides also in the Golgi apparatus. The transfer of ferulic acid residues from feruloyl-CoA to arabinoxylan-modeling trisaccharide has been demonstrated *in vitro* [109], but the enzyme performing this reaction has not yet been isolated. Formation of cross-links between polysaccharides with the involvement of ferulic acid begins already in the Golgi apparatus with formation of different diferulate isomers [88].

In some cases the presence of covalent bonds between individual polysaccharides of the Golgi apparatus has been demonstrated, but their nature is still unclear. Thus, experiments in corn cell culture with labeled arabinose have shown the ability of xylan and xyloglucan molecules to interact in the Golgi apparatus with each other or with other polymers [84]. Galactan synthesized by the microsomal fraction of pea epicotyls is included in a high-molecular-weight complex containing polygalacturonic acid, rhamnogalacturonan I, and xyloglucan, probably linked by covalent bonds [49]. Xyloglucan molecules can bind to each other in the Golgi apparatus, and their weight increases from 300 to 2000 kDa [84]. This probably occurs through transglycosylation; the enzyme that performs this reaction, xyloglucan endotransglycosylase, has been found in the Golgi apparatus [110].

The previously assumed existence of covalent bonds between pectin substances and xyloglucan has been proved recently, and this complex is formed not in the cell wall but in the Golgi apparatus [25]. Monosaccharides, especially their particular atoms participating in bond formation, have not yet been revealed; however, some arguments favor the idea that the bond is formed not through a transglycosylation reaction but by using a pectin molecule as a primer for xyloglucan synthesis. Using labeled xyloglucan in the cell culture of *Arabidopsis thaliana*, it has been shown that ~50% of xyloglucan is synthesized *de novo* on pectin primer (the side chain of rhamnogalacturonan I) in the protoplast and then enters the apoplast and facilitates the assembly of cell wall supramolecular structure, being stably retained in the latter [25]. These results somewhat dilute the term of "individual polysaccharide". Therein, one can consider the question that has been debated up to now: whether the components of pectin substances (polygalacturonic acid, rhamnogalacturonan I, and rhamnogalacturonan II) and separate molecules are synthesized as a single complex [103, 111].

It should be noted that in most of the above works Golgi apparatus was not isolated, but the polysaccharides

present in clarified tissue homogenate were analyzed. This approach is substantiated, besides the early works where division into subcellular structures was really performed [112], by the dynamics of label redistribution among different fractions in pulse–chase experiments. The label as a component of polysaccharides is found exactly in this protoplasmic fraction (and in this fraction only) already in the very first minutes after adding the labeled polysaccharide to the medium. This is most obvious with labeled arabinose, a monosaccharide incorporated only into cell wall polymers. The dynamics of a label suggests that secretion of polysaccharides takes no less than 5 min; usually it is estimated as taking 20-25 min [113].

The interaction of matrix polysaccharides with each other in Golgi apparatus confines their distribution over the inner cell wall layer after secretion. In some cases this has been registered by electron microscopy. The matrix polysaccharides secreted during the formation of gelatinous cell wall of flax fibers have gel-like consistence [94]. Thus, the contents of Golgi vesicles during secretion out of the plasmalemma seem to be a macromolecular conglomerate (coagulum, as stated by Fry [113]) rather than a solution of individual polysaccharides.

Interaction of secreted matrix polysaccharides with the cellulose microfibrils being formed. When the contents of Golgi vesicles are splashed out of the plasmalemma, they interact with cellulose microfibrils on the basis of (i) spontaneous interaction between polysaccharides and (ii) the capture of some of the matrix polysaccharides by being formed microfibrils. The formation of cell wall supramolecular structure needs special conditions, which can be provided by specific concentrations of interacting substances, spatial restrictions, presence of cofactors, and involvement of yet unidentified factors. There is scanty information about possible participation of yet uncharacterized proteins in this process [48].

Upon fusion of a Golgi vesicle with plasmalemma, its contents lie flat over the inner cell wall surface. As a result of turgor pressure of the protoplast, the secreted polysaccharides are concentrated, because the water from the vesicles "flows" to distant cell wall layers, i.e. ultrafiltration takes place [113-115].

Enhanced concentration of polysaccharides creates conditions for their marked spontaneous interactions (i.e. conditioned thermodynamically rather than mediated by enzymes), which seem to play the key role in supramolecular structure formation. The size of a "mesh" (i.e. the distance between microfibrils) in the xyloglucan–cellulose network is the same in the systems *in vitro* and *in vivo* [46]. It is believed that xyloglucan forms a monolayer on the surface of microfibrils [59]; on average, each glucose residue in the xyloglucan backbone corresponds to one hydrogen bond with cellulose [116]. The molecules of cross-linking glycans during the interaction with cellulose microfibrils change their conformation [63, 71, 117].

The neutral side chains of rhamnogalacturonan I consisting of arabinans and galactans can also spontaneously interact with cellulose. However, these interactions are less advantageous energetically than in the case of xyloglucan and become less marked in the presence of this polysaccharide in the system [51].

As a result of spontaneous interactions, matrix polysaccharides are not only localized on the surface of a microfibril, but some of them penetrate it. This is possible only in the period that precedes crystallization of the microfibril, i.e. matrix polysaccharides must be in immediate proximity to the cellulose–synthase complex. It seems that the formation of a cellulose microfibril is accompanied by physical capture of non-cellulose polysaccharide molecules. Xyloglucan is often mentioned as a captured polysaccharide [13, 18]. Besides, there can be other variants: in gelatinous cell wall, the major matrix polymer in the fraction of polysaccharides tightly bound to cellulose and extracted only under microfibril structure disturbance is rhamnogalacturonan I with long galactan chains [20]. The presence of galacturonic acid and the absence of rhamnose in some of the systems suggest that this fraction contains also polygalacturonic acid [118]. The captured polysaccharides can change the properties of microfibrils and facilitate their interaction with the matrix [119].

Packaging of polysaccharides in cell wall layers. Autoradiography and electron microscopy of deposition of polysaccharides has shown that the synthesized polysaccharides are deposited on the inner side of cell wall adjoining the plasmalemma [120]; as a rule, the mobility of polymers in the cell wall is substantially limited. In cells cultured *in vitro*, some of the cell wall polysaccharides pass through the cell wall and are washed out into the medium; this means that the polymers can penetrate through the cell wall (at least the primary one). Let us consider the processes providing fixation of the polymers in the cell wall, which happens rather quickly: secreted xyloglucan is incorporated into the cell wall in less than 1 min [113].

Physical contact is achieved largely due to the turgor pressure minimizing the gap between plasmalemma and inner cell wall layers. The turgor pressure also facilitates penetration of certain regions of some of the "new" polymers to already deposited layers [115].

One of the important factors determining cell wall structure is layer-by-layer deposition of microfibrils due to the parallel movement of cellulose–synthase complexes over the plasmalemma surface. The direction of microfibril deposition relative to the cell axis can vary in time (e.g. during deposition of the secondary cell wall), the orientation of microfibrils can be modified during cell extension, but the microfibrils being formed are located parallel to each other during deposition. The mechanism of control of microfibril orientation is still debated [121-123], but the overwhelming majority of authors think that

it is implemented by cortical microtubes [5]. Exceptional extension of microfibrils up to micrometers provides the "anchoring" of new portions of cell wall material in the already packed layers.

Transglycosylase reactions with participation of polysaccharide molecules already incorporated into the cell wall play a substantial role in fixation of secreted polysaccharides in the cell wall. The best known of such enzymes is xyloglucan endotransglycosylase. This enzyme uses xyloglucan molecules both as a donor and as an acceptor; it cleaves some part of the chain in one xyloglucan molecule and transfers it to another molecule [124]. Fixation of new xyloglucan molecules in the cell wall is one of the most important functions of xyloglucan endotransglycosylase [125]. This function is argued by the fact that the inhibition of cellulose formation by dichlobenil does not eliminate xyloglucan integration into the cell wall, at least during 24 h [126]. Other transglycosylases, although less effective and widespread than xyloglucan endotransglycosylase, can provide incorporation into the cell wall of other polymers, e.g. mannan [127].

The cross-links of polymers with involvement of ferulic acid play an essential role in polysaccharide anchoring in the cell wall. In experiments with the suspension culture with ^{14}C-labeled cinnamate, peroxidase blockers (iodide, dithiothreitol, cysteine) inhibited the formation of active oxygen forms, facilitating the formation of bonds between phenolic compounds, and thus increased withdrawal of labeled polysaccharides not fixed in the cell wall into the cultivation medium [29, 88].

Formation of the bonds between polysaccharides is influenced by variation of the character of their methylation and acetylation. Formation of ionic bonds, the most typical of pectin substances, directly depends on the methylation of uronic acids [128]. Carboxylic groups of uronic acids are methylated during the synthesis of pectins in Golgi apparatus. The cell wall contains an enzyme (pectin methyl esterase) splitting off methyl groups. Acetylation has been shown to occur along with methylation in Golgi apparatus during the synthesis of polysaccharides [129]. For instance, acetyl groups are present in 80% of galactosyl residues in the trisaccharide side chains of xyloglucan washed out into the cultivation medium of maple cells [130]. The cell wall contains pectin acetyl esterases; this fact argues for the possibility of deacetylation of polysaccharides after their incorporation into cell wall, but neither deacetylation nor its functional role has been studied in detail.

Deposited cell wall layers can be subject to subsequent restructuring due to post-synthetic modifications of the polymers.

Modification of formed cell wall structure. The types of restructuring of deposited cell wall layers can be divided into two groups. The first ensures cell wall modification during various physiological processes, e.g. growth through extension, fruit softening, exfoliation, formation of intercellular spaces and aerenchyma, etc.; the second is associated properly with the mechanism of formation of cell wall supramolecular structure of a certain type. Modifications of the first group are beyond the framework of this review, the more so that the available information is completely enough generalized in a series of publications [6, 131-133]. We will dwell on the processes related to the second group; at the same time, cell wall lignification will be left beyond consideration: this is a vast subject but we are interested first of all in the carbohydrate–carbohydrate interactions.

One of the examples of restructuring of deposited cell wall layers is transformation of a cell plate formed during cytokinesis into a middle lamella (the outermost cell wall layer). This process is accompanied by the hydrolysis of callose, an amorphous β-(1$\rightarrow$3)-glucan forming the great bulk of the cell plate, and by demethylation of polygalacturonic acid resulting in the formation of Ca^{2+}-pectate gel, through which plant cells are linked with each other [128].

The restructuring of deposited cell wall "portions" is most pronounced in gelatinous cell wall layers typical of many plant fibrils [12, 134]. On electron-microscopic photographs, the new (closest to plasmalemma) cell wall layers have a typical "striped" structure with alternating dark and light regions; such structure is subsequently transformed into a homogenous structure. There are reasons to believe that this process is associated with partial hydrolysis and modification of the structure of tissue- and stage-specific galactan; however, the essence of these changes still has to be elucidated.

In conclusion of the survey of formation of cell wall supramolecular structure, we should note that the extent of comprehension of the studied processes does not correspond to their scale. Even the seemingly simplest polysaccharide such as cellulose still remains insufficiently characterized by the types of three-dimensional structure and the role of its separate components in interaction with other cell wall polymers. The peculiarities of cell wall supramolecular structure determined by the diversity of matrix polysaccharides have not been identified. The more general problems concerning, e.g. the basic principles of carbohydrate–carbohydrate interactions or the bases of formation of the secondary and tertiary polysaccharide structure also need to be solved (sometimes, even the existence of such concepts is rejected). Particular consideration given recently to complex supramolecular structures inspires hope for the finding of adequate approaches to cell wall studies.

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