

BOT 4503

3.5 hour

Plant-cell-wall metabolism and symplastic-intercellular transport.

Objectives

1. Name some general functions of cell walls and give examples if you can.
2. Briefly describe walls of Gram-negative and Gram-positive bacteria. In general, what is a major structural difference between the walls of prokaryotes and eukaryotes?
3. Describe a eukaryotic-cell wall by analogy to a “reinforced concrete construction” model.
4. What is the middle lamella? What is its function? Where is it found? Where is it formed? What is it made of? How does its composition relate to its structural function? How is calcium involved?
5. Describe pectin. Give an everyday example of pectin. How would you isolate pectin? What is the importance of the negatively charged functional groups? Name the monomer that confers the negative charges. What does chelate mean? Is pectin chemically complex? Compare its complexity to cellulose.
6. With reference to the previous question, how might you plan to isolate individual plant cells?
7. Name one aspect of fruit softening during fruit ripening. How might some pathogenic organisms attack the plant cell?
8. How thick are plant-cell walls? Remark on the heterogeneity of cell-wall layers, of walls of different cells.
9. Describe cytokinesis. How are Golgi-derived vesicles involved? What is the cell plate? How is it related to the middle lamella? What is the phragmoplast? Is it distinct from spindle fibers?
10. Give the standard textbook-labelled diagram of a plasmodesma. Identify the two potential pathways for intercellular transport. What is the normal exclusion limit of a plasmodesma, in terms of physical size and molecular mass? Describe how this fact was determined. Would you “expect” that sugars, hormones, ions could pass through the cytoplasmic sleeve of the plasmodesma?
11. Name two cells that are not connected to adjacent cells by plasmodesmata? What special attribute do they share?
12. Describe an experiment that showed that plasmodesmata can be closed.
13. Describe, draw, and label the plasmodesma in all its complexity. Based on this model, identify the path for solution transport. How can it be occluded?
14. How large are whole virus particles? How large is the nucleic acid component if it is in a spheroid configuration? Is the size consistent with passage through plasmodesmata? Do viruses move intercellularly in plants?

15. What is a “movement” protein? Is it of viral or plant origin? Describe a postulated role as a molecular chaperone.
16. Describe how the TMV movement protein interacts with plasmodesmal components to increase the permeability of the plasmodesma. Is this the universal mechanism for movement proteins? If not, give a counter example.
17. Can “naked” proteins move through plasmodesmata? Give an experimental example.
18. Can organelles move between eukaryotic walled cells? Give examples. What about plants? Compare the path diameter required to that of a “normal” plasmodesma.
19. What is cellulose? What is the repeating unit in cellulose? How long are the chains of cellulose? Is DP variable? Contrast the structure of cellulose to all other wall components. How abundant is cellulose? Is it in “all” wall layers? What are microfibrils? How many chains of cellulose are packaged in each microfibril? What is a typical diameter of a microfibril?
20. What are the requirements for the synthesis and assembly of such a complicated and ordered structure as a microfibril?
21. What is the precursor for cellulose biosynthesis? Briefly describe the cellulose-synthesizing rosette by use of a diagram. Is the rosette stationary in the membrane? If no, why not?
22. Describe the spatial relationship between the associated cortical microtubules, the nascent microfibril, and the axis of cellular elongation. Describe evidence that correlates microtubule orientation and microfibril orientation. How else might this evidence be interpreted?
23. What is hemicellulose? Do some hemicellulose components have a backbone similar to cellulose? Is hemicellulose amorphous or crystalline? How does hemicellulose fit into the reinforced concrete analogy of eukaryotic-cell walls? Which cellular organelle or system is involved in the synthesis of hemicellulose? Is the synthesis of hemicellulose analogous to the synthesis of cellulose?
24. Give an overview of some of the attributes of general-structural proteins. Which amino acids are common? Which aspects of their structures confer special properties that are important to incorporation into structural proteins? Name a non-DNA-encoded “amino” acid that is common in structural proteins. Give some idea of the state of our knowledge of plant-cell-wall structural proteins. Name four classes of plant-cell-wall proteins. Which is known best? Briefly, are cell-wall proteins equally expressed among cells, wall layers, and developmental stages? Can their synthesis be induced?
25. What is lignin? Where is it preferentially localized? Compare its strength to that of cellulose, showing that these cell-wall components complement each other. What are the monomeric units of lignin? How abundant is lignin? Describe the specificity (or lack thereof) of its synthesis. How is peroxide involved?
26. Draw a comprehensive model of a primary-plant-cell wall and show the interactions among the various components.
27. What are oligosaccharins? What is their origin? Give examples of effects on plants. Speculate on how one might be involved in averting pathogen attack.

Lecture

Everywhere we look, we find walled cells—fungi, bacteria, protists, and plants. The variety and abundance of these walled organisms attest to the evolutionary success of the presence of a wall. In fact, cellulose—a constituent of the walls of plants and some photosynthetic as well as non-photosynthetic protists—is overall only a fraction of the total cell-wall substances, and yet it is the one of the most abundant biomolecules on earth. Lignin, a major component of plant secondary cell walls, is also among the most abundant molecules on Earth. How much energy must flow through the living world just to make walls! Taking the liberty of an introductory essay sprinkled with speculation (and borrowing unabashedly from Bartnicki-Garcia), let us explore the uses to which walls are put. (1) Perhaps the most recognized use is its rather passive function of providing a protective enclosure for the cell. Thus, many organisms are pathogenic only because they secrete cell-wall-degrading enzymes. (2) Cell walls provide for form, i.e., they provide for structural support. As we mentioned earlier, plants are not motile in the general sense, and they, therefore, either must cope with a stress, or they must grow in a way to maximize their competitiveness. Any cursory examination of a natural plant community will reveal that plants grow toward light, and that this form is a result of walls. (3) Walls prevent the distention of the protoplast. Plant and other walled cells have a high content of small solutes, which favors osmotic water influx. One notion is that high internal pressure concomitant with high concentrations of solutes permits high metabolic rates and rapid rates of growth. (4) Walls can prevent desiccation because they can be impregnated with or covered with hydrophobic layers, as we mentioned in our discussion of the shoot cuticle. As an incidental point, the fatty acids used in the synthesis of cuticular waxes are themselves synthesized in plastids. (This point is interjected because of the focus in the last unit on the endomembrane system as a synthesis/delivery system for secreted substances.) (5) Walls provide for an external metabolic compartment. Recall that sucrose is the prototypic transport carbohydrate. Some plants do have mechanisms for the uptake of sucrose, whereas others secrete the sucrose-hydrolyzing enzyme invertase into the wall. Thus, arriving sucrose is degraded to the constituent hexoses, one of which is the molecule favored for uptake. (6) Walls provide for water movement through plants. We might break this phenomenon into two parts. First, within any organ, e.g., a leaf, water movement through the apoplast is a less resistant pathway than through the symplast. Thus, water moves along one cell wall, to the neighboring cell, and so forth. The second aspect involves long distance transport of water. As you will see later, the only universal mechanism that can account for the ascent of sap involves the maintenance of large negative pressure (“suction”) in the

tracheary elements¹. These cells, recall, are dead and have very thick cell walls. Without this small strong “pipe,” plants would not be able to grow more than a few feet tall. As we discussed earlier also, many substances—e.g., minerals, plant-growth regulators—are swept along the transpiration stream, so as we say “water” movement, we are referring to “solution” movement, but we recognize that the cell-wall path (in contrast to the tracheary-element path) is governed by the differing porosities of the different cell walls.

Walls owe their physical properties (hydrophilicity, strength, and elasticity) to the nature and the organization of the chemical constituents. Eubacterial cells typically have a cell wall and it lies external to the plasma membrane, as it does in plants. The eubacterial-cell wall is composed of a single molecule of peptidoglycan², which is a matrix of substituted disaccharide crosslinked by short chains of amino acids. Gram-positive bacteria have no additional wall materials. Gram-negative bacteria have additional layers external to the peptidoglycan wall that is made of large molecules of lipopolysaccharides. This outer “membrane” is studded with pore-forming proteins that permit entry of solutes into a so-called periplasmic space. (From the inside: plasma membrane, wall, periplasmic space, outer membrane.) [Archaeobacteria, the other prokaryotic taxon, have various kinds of cell walls and will not be discussed.] There are two points to be drawn from the previous information: (1) All eukaryotic-cell walls have a microfibrillar nature, and, thus, differ fundamentally from general the wall structure of prokaryotes. We can think of a reinforced concrete model for eukaryotic cell walls³. The interwoven microfibrillar material in plants is (almost always) cellulose, which can be thought of as analogous to rebar (reinforcing bars of metal, as you see in the construction of large buildings). Various substances, prototypically hemicelluloses in primary walls of plants, contribute to the amorphous matrix, which is analogous to the aggregate/cement mixture in reinforced concrete. The chemical nature of these substances will be revealed later. (2) Plant cells, without exception, have only a single external membrane. In a few cases (particularly in the gametophytic generations), plants may be “transiently” coenocytic (or syncytial), but in the current context, these exceptions do not merit discussion.

Given the assumption that fibrillar walls are superior, one wonders why, in general, prokaryotes did not evolve them, since many prokaryotes can synthesize cellulose and even make microfibrils. The answer seems to lie in the complexity of the organization required to assemble the microfibrils. Chitin (in addition to cellulose, the other major fibrillar component of eukaryotic cell walls) is synthesized and assembled on special structures, chitosomes, that are 40 to 70 nm in diameter. As we will discuss, less is known about

¹ This idea will be developed more fully later

² With one after another taxon, we have learned what is probably general: prokaryotes, even the garden variety *E. coli*, are capable of synthesizing cellulose. Therefore, as in all cases with these notes, please accept the generality of my statements.

³ Fiberglass, with fibers and filler materials, also serve as a useful model.

the synthesis of cellulose and its assembly into the cell wall, but we do know that a great deal of organization is involved, e.g., in plants and some algae, multisubunit cellulose-synthesizing rosettes float in the membrane and extrude cellulose. As a further complication, it appears that in some extreme cases, different rosettes congregate to synthesize especially large microfibrils.

A good place to start a discussion of a plant cell wall is to examine it visually. As we have mentioned, cell walls of adjacent cells touch each other—sometimes the walls are virtually completely appressed, and in other situations (like the mesophyll attachment to epidermal cells), the points of contact represent only a small fraction of the total cell-wall surface. The region of union between two adjacent cells is called the middle lamella (literally, middle leaf, in the sense of “leaf of paper”). The middle lamella is the glue that holds the adjacent cells together. By various means, it has been discerned that it is pectic in nature. Pectin—just like you buy in the supermarket to make jelly, or extract from fruits with hot water—is hydrophilic, as your experience indicates. By scientific definition, pectin is the water-soluble, weak-alkali-soluble, or chelate-solution soluble fraction of the cell wall. Of all such fractions, it is the most complex class. At first, think of pectin as being a linear chain of galacturonic acid residues. (Galactose is an isomer of the hexose glucose and galacturonic acid is a simple modification of galactose caused by the addition of a carboxylic acid residue.) Thus, pectin is a negatively charged polymer, which causes it to associate (or chelate) with Ca^{2+} . Chelation by (divalent) Ca^{2+} with two proximal polymers effectively stitches the polymers together. As mentioned, pectin is complicated, and certain sugars are interspersed as part of the galacturonic-acid-backbone chain. Further, other polysaccharides and functional groups modify the predominately galacturonic-acid backbone. As an example, the carboxylic acid residue is predominately esterified (and thus, can not bind Ca^{2+}) when it is delivered to the cell wall and certain enzymes de-esterify portions of the chain. Plant cells can be dissociated by tissue incubation with pectinases—enzymes that degrade pectin. In some cases, this process can be improved by the inclusion of a chemical such as EGTA that binds Ca^{2+} more tightly than does pectin. As a side point, pectinases are widely distributed in nature. Pathogenic organisms produce and secrete them; saprophytic organisms do likewise. Perhaps surprisingly to you, plants do too. Indeed, senescence in plants is only another phase of development; a familiar example to you is the softening of a tomato fruit, which is part of the ripening syndrome. The softening is programmed, is initiated by the plant-growth regulator ethylene, and is caused, in part, by the plant production of pectinases.

The middle lamella is the external boundary of the **primary wall**, which is the layer that is laid down before final cell shape has been determined. As we discussed in connection with collenchyma cells, it may be the only layer that is laid down. It can be very thin, as little as 0.2 μm . On the other hand, there is tremendous variability in plant-cell-wall structure. Some walls may be as thick as 15 μm , and have not

only secondary layers, but also tertiary layers. At this point, two facts should be recognized: (1) the walls of one plant cell may be very different from the walls of the adjacent cell. Even the wall on one side of the cell may be different from the wall on the other side of the same cell! (2) the wall layers differ tremendously in chemical composition. Both of these facts contribute to the difficulty of studying plant-cell wall chemistry. When the plant biochemist grinds a tissue, or differentially extracts it, obviously the chemicals of the different cell walls and wall layers are mixed.

Overhead 1: Cytokinesis in plants

Continuing with a perspective gained from simple visual examination of cell walls, let us **briefly** turn our attention to cell division (cytokinesis, which is to be distinguished from karyokinesis, which is nuclear division). (Because of time constraints, many interesting and relevant aspects of plant biology have to be covered in a cursory fashion.) This overhead shows that plant cells do not divide as other cells do. Other cells divide by furrowing, a process that you can visualize by thinking of squeezing a sausage into two pieces by a constricting noose. Plant cells lay down by coalescence of Golgi-derived vesicular materials a cell plate across the mother cell. Typically, the cell plate is first deposited as a disc, more-or-less centered between the nascent daughter cells. (The cell plate is actually the initiation of the middle lamella, but it also characteristically contains callose, a β 1 \rightarrow 3 glucan.) The positioning of the cell plate is guided by an assemblage of microtubules called the phragmoplast. (This assemblage is distinct from the spindle fibers that guide chromosome allocation between the daughter cells.)

We see that plant-cell walls are uneven. In certain places, there are discontinuities in secondary wall deposition. (Aside from the discussion that follows, the absence of a secondary wall generally implies the absence of lignin, and thus, these areas of wall are hydrophilic.) These areas—where even the primary wall is thin and is called (unfortunately) a membrane—are called pits, and if there is matching pit in the adjacent cell wall, the area is a pit pair. As a matter of perspective, a pit may be large, say 10 μ m. Pit fields comprise numerous pit pairs that are punctuated by intercellular connections, plasmodesmata (singular, plasmodesma), that are analogous to gap junctions that connect animal cells. Plasmodesmata may traverse the wall in groups—as in a pit field—or they may be more-or-less isolated. We will return for a full discussion of them. Pits can be elaborate as exemplified by the bordered pit found between adjacent tracheids of pine. The secondary wall of bordered pits overarches the “membrane” and nearly occludes the opening on either side of the pit. In the case of pine, the so-called membrane comprises a thickened disc in

the center formed by heavy deposition of primary wall there and the gossamer-like strands of primary wall in which the disc is suspended. (The porosity of the wall in these thin regions is high, up to 400 nm, compared with a typical value in parenchyma of 7 nm.) If the pressures on either side of the bordered pit are equal, the disc or torus floats in the center, as implied. However, if there is a pressure differential (formed, e.g., by a break in the stem or a freeze-thaw cycle), the torus is slammed against the overarching secondary wall. Thus, the torus serves as a two-way valve. (As a matter of caution, the vocabulary that I used above is the most common, but it is not that exclusively used. When one is reading generally, she is advised to use caution and to infer exact meaning from context. Note, especially, that “pit” is used for living-cell connections as well as dead-cell connections.)

Overhead 2: Simple view of plasmodesmal structure.

We will start a description of plasmodesmata with the standard textbook description, as this overhead depicts. A plasmodesma is a two-fold connection between adjacent (living) cells. Simply put, as Don Fisher said, the basic plasmodesmal structure is a tube of plasma membrane surrounding a strand of modified endoplasmic reticulum, with particulate material between them. There is a small pore in the wall, and the plasma membranes of adjacent cells are continuous. I.e., movement from one cell to the next does not involve crossing a membrane. Plasmodesmata form during cell division⁴, and you may think of this process as incomplete division of the cell in which a tubule of ER is “trapped.” More complicated configurations (branched plasmodesmata, sometime with a shared central cavity in the cell wall) are derived from simple, or primary, plasmodesmata that form during cell division. Through this membrane-lined pore passes a tube of membrane, the desmotubule, that is continuous with the ER. (Sometimes, the desmotubule is called “appressed ER.”) Thus, potentially two pathways connect the contiguous cells. First, a solute in the lumen of the ER of Cell 1 could pass through the desmotubule into the ER of Cell 2. Second, materials may pass through the cytoplasmic sleeve from the cytosol of Cell 1 to the cytosol of Cell 2. Evidence indicates that this latter cytoplasmic connection is the predominate or sole operating one. Obviously, the first question is, “What actually passes through the pore?” The possibilities are endless—e.g., hormones, electrical wave variations (caused by ion redistributions), nutrients, “intracellular” signal substances. The question can not be answered definitively yet, although we are

⁴ Plasmodesmata are also found between cells that do not share a common mother—even between cells of different genera (!) that have formed successful graft unions. Time precludes a complete coverage of any one topic, so I restrict myself to the core.

learning a great deal, and what we are learning is exciting. The first generality is that a “normal” plasmodesma has an exclusion limit of approximately 800 daltons, as inferred from the conductance of differently sized dextrans. (Please note, however, that the basal exclusion limit varies with type of cells and is often high (~20 kD) where there is large symplastic flux as in the unloading zone of sinks.). Thus, it would seem that most plant signal substances and metabolites could potentially pass from cell to cell. The second generality is that materials do, indeed, move from cell to cell. For instance, if the low-molecular-weight dye, Lucifer Yellow, is injected into a cell by use of a micropipette, the dye moves into other cells with which the injected dye is connected through plasmodesmata. The third generality is that most cells are connected to adjacent cells by a variable number of plasmodesmata. General exceptions are mature guard cells and the companion-cell/sieve tube element complex⁵. (The guard cells of a stomatal guard-cell pair are connected, and companion cells are connected to the adjacent sieve-tube element.) Interesting, both these exceptions are cells that always or sometimes have much higher internal pressure than do the surrounding cells. Significant pressure differential (about two atmospheres) between adjacent cells impedes intercellular transport by plasmodesmata. The diminution of transport occurs regardless whether the pressure differential is brought about by release of pressure in one cell or increase in pressure of another cell. Moreover, even transiently applied pressure differentials continue to have a lasting effect (after the imposed pressure differential is relaxed). In brief summary, the foregoing provides evidence that plasmodesmata may close down from a “normal” size.

Overhead 3: Complicated view of plasmodesmal structure

Plasmodesmal structure is complicated. This overhead shows that there are particular proteins associated with the inner leaflet of the including plasma membrane. Other proteins are associated with the outer leaflet of the ER channel (the desmotubule⁶); inside the ER channel is yet another kind of protein particle. Filaments anchor these proteins. In contrast to the situation with (animal) gap junctions in which we know a great deal about the proteins, our biochemical knowledge of plasmodesmal proteins is limited. As the two cross-sections show, the aqueous-permeable space is the cytoplasmic sleeve, and rearrangement of the protein particles on the outer leaflet of the ER channel causes occlusion. Thus, this model for closure

⁵ The generality about guard cells is fine. The generality about the CC/STE with other cells has some holes. It is generally true in the path region, and it is true in so-called apoplastic phloem loaders in the source (which includes most herbaceous plants).

⁶ The core of the desmotubule is filled with lipid. It does not permit aqueous transport, but movement of ER lipids from one to the adjacent cell can occur through this pathway.

complements the observations made above. You may note, however, that the interpretations are subject to refinement or rejection, as visualization of the internal structure of plasmodesmata is difficult. Note also that cytoskeletal elements (microtubules, and actin and myosin) interact with the plasmodesmata.

The “normal” exclusion limit of plasmodesmata (800 daltons) is consistent with the passage of a globular 2-nm particle on structural grounds⁷. However, it is long-standing observation that plant viruses move from cell to cell, and this observation has led to a great deal of work, both because of its importance for intrinsic “basic science” reasons and because of the huge agronomic impact of plant viruses. Whole-plant-virus particles range from approximately 10 to 110 nm, and free viral nucleic acid in a spheroid configuration is about 10 nm. The explanation of this apparent paradox can be divided into two parts. First, viruses encode so-called movement proteins, which are proposed to act as molecular chaperones during transport. In brief, they are suggested to bind to the viral nucleic acid and cause it to unfold from its bulky and collapsed structure. Stretched out, the nucleic acid is only about 2 nm, and this size is compatible with its passage through plasmodesmata. As an additional point, it is thought that the binding protein also protects the viral nucleic acid from cellular nucleases, enzymes that degrade nucleic acids. Finally, movement protein facilitates movement of the virus, along the plant cytoskeleton from the cytosol to the plasmodesma. Second, and more remarkable, this movement protein interacts with plasmodesmal components, an event that increases permeability and, thus, promotes transport.

Overhead 4: Transport of macromolecules through plasmodesmata.

Injection of the purified movement protein along with sized dextrans (see above) showed that the path diameter was increased to as much as 9 nm. Other experiments showed that the movement protein expressed in transgenic plants increased the path diameter to as much as 6 nm. The above descriptions pertain to the movement protein of TMV (tobacco mosaic virus, which infects a range of plants). In this case, the increased path diameter is caused by an asymmetric redistribution of the protein particles that are associated with the plasma membrane of the plasmodesma. Instead of being equally spaced, they are bunched up on one side. The desmotubule then migrates into the created empty space, which leaves a large vacant spot, more-or-less in the center of the plasmodesma. Interestingly, the movement protein of TMV binds viral single-stranded nucleic acid (DNA or RNA) non-specifically. In other words, the movement protein of TMV facilitates the cell-to-cell transfer of viruses that do not ordinarily move through

⁷ The relationship between mass and molecular radius is not too good. Use caution.

plasmodesmata. The mechanism described and consequences for TMV are not universal. The cowpea-mosaic-virus movement protein acts by causing a loss of the desmotubule. It is replaced by a membranous tubule that terminates bluntly in each cell. The virus is transported through the lumen of the tubule. In another case, the 9.4 kD movement protein of red-clover-necrotic-mosaic dianthovirus itself, even in the absence of the viral nucleic acid, has been shown to cross the plasmodesma. Thus, the plasmodesma—like the nuclear pore—facilitates the bi-directional flow of two types of macromolecules (i.e., nucleic acid and protein.).

Movement proteins were discovered as components of viruses, but it was never believed that plants evolved plasmodesmata of adjustable aperture size to accommodate viral intrusion. (I.e., viruses must have commandeered a normal plant process.) As background, we know that mature sieve-tube elements may function for months or years and they lack a nucleus and ribosomes, implying that their proteins were synthesized in the companion cell and translocated into the sieve-tube element via plasmodesmata. As expected, a movement protein of plant origin has been discovered. In this case, a particular mRNA appears in one cell layer, but the product appears in another cell layer as well. This protein moves between cells and, like movement proteins of viral origin, facilitates movement of large dextrans and protein. There are some puzzling aspects of how this protein works, and details await clarification.

It has been known and widely appreciated that there is a great deal of intercellular traffic through walled cells of certain organisms. The cellular fungi (Ascomycetes and Basidiomycetes) and derivatives (Rhodophyta) are remarkable in having large wall perforations that allow for the passage of organelles, even the nucleus. (Note that the discussion has shifted from 0.01 μm to, say, 0.5 μm for a constricted region.) Plant-cell biologists have “known” that such transport through plasmodesmata is forbidden. Actually, it is not! For many years—but not known in the West—Beijing scientists have studied transport of organelles through plasmodesmata in specialized organs. Under some circumstances, the plasmodesmata are modified to provide an opening of 100 to 400 nm, through which the organelles are squeezed, albeit deformed in the process, but recovering to a typical shape after passage. Apparently, the cytosol is not in bulk “pumped” through the pore; instead, there is selectivity. This observation and those that show that the intracellular messenger Ca^{2+} reduces conductivity is an additional basis for the belief that there are natural plant-movement proteins, and that regulating intercellular transport through plasmodesmata is an important physiological function that awaits elucidation. Finally, in this wrap-up paragraph, I note that some cell-wall proteins (discussed later) may be involved in regulation of plasmodesmata.

We return now to the wall *per se*, begin with the primary cell wall and shift our focus more toward chemistry and the fine ultrastructure of walls. It was mentioned that eukaryotic-cell walls are built

like reinforced concrete construction. As also mentioned, in plants and some other organisms, the microfibrillar component is all or mostly cellulose, which is a β 1,4 glucan. (Some “cellulose” may contain a small (artefactual?) amount of non-glucose monomer.) The repeating unit in cellulose is the dimer of glucose, however, since alternate glucose molecules are “flipped” along the axis of the microfibril. (This alternating bond provides for a linear flat glucose chain.) The amount of cellulose in a wall varies considerably, from a low of about 3% by mass to nearly 100%, as in the cotton “fiber” of commerce. A typical situation—obtained from suspension cells of *Acer*, which produces only primary walls—is 25% cellulose, which is to be compared with two pectic fractions, which each represents slightly less than 20%. The following overhead depicts in more detail the arrangement of microfibrillar cellulose.

Overhead 5: Cellulose microfibrils

Individual chains of cellulose (DP = 2 000 to 6 000 in primary walls, nominally 14 000 in secondary walls) are assembled into microfibrils. (For perspective, each glucosyl moiety is approximately 1 nm “long,” or about 1/10 the thickness of a biological membrane.) As I have indicated on the overhead, the number of chains of cellulose is variable, with 40 (or fewer) to 70 chains per microfibril being typical for plants, but algae may assemble 1000 cellulose chains into a single microfibril. Individual cellulose molecules begin and end at different places along the microfibril, and parts of the microfibril may be crystalline and other parts amorphous. The microfibril, 3-15 nm in diameter and as much as 5 μ m in length, can be visualized with the electron microscope. (In some systems, microfibrils are further assembled into macrofibrils, but we will not discuss these specialized cases.) As alluded to, the requirements for the synthesis of such a complicated assembly are many-fold.

Overhead 6: EM: Cellulose-synthesizing rosette

Overhead 7: Model: Cellulose-synthesizing rosette

This overhead would imply that we “understand” cellulose biosynthesis but unfortunately, that is not the case⁸. The precursor is a nucleoside diphosphate glucose, as indicated by *in vivo* labeling experiments, by analogy with other precursors for polysaccharide synthesis (e.g., the precursor for starch in ADP-glucose), and by the very strong conservation of XDP-sugar-binding motifs in cellulose synthesizing enzymes from an array of organisms. The model calls for an assemblage of nominally six cellulose-synthesizing complexes to form a rosette that is embedded in the membrane. In turn, each cellulose-synthesizing complex comprises several multi-subunit components, each of which extrudes a cellulose chain. The many chains of an individual microfibril are thus simultaneously polymerized in a parallel orientation. As the microfibril is extruded, it is stationary; the rosette is pushed through the liquid membrane.

Note that the cellulose-synthesizing enzymes have two active sites each that bind to UDP-glucose. Apparently, two glucosyl moieties are added to the growing chain at each turn over of enzyme, which would explain the cellobiose repeating unit in cellulose (which is marked on the overhead). A second point is the presence of membrane-associated sucrose synthase. (As discussed later, this enzyme provides a means of degrading sucrose to UDP-glucose and fructose, despite its name.) One model calls for this enzyme to “deliver” the substrate to the cellulose-synthesizing enzyme.

As we mentioned during our review on anatomy, orientation of the cellulose microfibrils is critical. Cell expansion is constrained by the microfibrils, which have high tensile strength. A cell can not expand in the direction of the long axis of the microfibrils. The generally held belief is that microtubules play a necessary role in the orientation of the microfibrils during microfibril deposition. The reasons, accumulated over 30 years, are based primarily on the fact that normally (but with exceptions) cortical microtubules in the cell are aligned parallel to the deposition of microfibrils and perpendicular to the axis of cell elongation. If the microtubules are depolymerized, e.g., by treatment with colchicine or cremart, the microfibril orientation is lost. Moreover, experiments with plant-growth regulators seem to support this belief. E.g., treatment of maize coleoptiles with auxin⁹, which stimulates growth, causes the reorientation of both microtubules and microfibrils. Similarly, treatment of pea stems with ethylene causes cell expansion transverse to the stem. This new growth direction is accompanied by a reorientation of the microtubules to be perpendicular to the axis of expansion. Nevertheless, there are objections. Perhaps the hypothesis can only be applied to some (most?) cells, or maybe the same unidentified force directs the

⁸ At present, there are no cell-free systems that synthesize appreciable amounts of cellulose.

⁹ Auxin also causes an acidification of the wall through its activation of the H⁺-extruding ATPase. It is thought, but not proven, that the change in pH activates enzymes that “disconnect” the adjacent microfibrils.

positioning of both microtubules and microfibrils. (The expansion of the cell also implies concomitant wall synthesis; otherwise, the wall would become thin as it covered a larger area, eventually causing a rupture.)

Finally, following a historical classification based on solubilities, we turn to hemicellulose, another and very heterogeneous class of cell-wall polysaccharides. (Note that some current authors disparage the term hemicellulose and identify the different polysaccharides by the major monomeric component, for example, xyloglycan.) Hemicelluloses are polymers of pentoses and hexoses, represent perhaps 20-to-40 % of the primary wall (i.e., similar to cellulose), and are the substances that can be extracted by harsh alkali treatments (after pectin has been extracted by more mild procedures). At the expense of redundancy, only cellulose has a (para)crystalline structure and is orderly; thus, hemicelluloses are “fill-in” materials, analogous to the aggregate-cement phase of reinforced concrete construction. (However, because hemicelluloses are oriented with cellulose microfibrils, they may have regions of structure.) Also unlike cellulose, hemicelluloses are not polymers of a single sugar, they may be branched, and the sugars may be modified by additional of a functional group, even aromatic moieties. Hemicellulose synthesis occurs in the Golgi apparatus and is delivered to the cell wall by vesicles of the endomembrane system.

(Please refer back to the discussion on the middle lamella for information on pectin, which is abundant throughout the primary wall.)

Primary-cell walls contain a fair amount of structural protein (10-15% by mass) and secondary cell walls contain less. Some of these proteins are probably simply structural and others have a physiological role in temporarily “relaxing” the wall in response to a physiological stimulus. (In addition, cell walls contain a number of enzymes, such as invertase.) A common feature of diverse structural proteins (e.g., in skin, hair, spider webs, silk cocoons, connective tissue) is the abundance of glycine and proline (and the post-translational hydroxylation product of this imino acid, OH-proline). Both glycine and proline are special: glycine has an R group a H atom, and, thus, glycine, is uniquely symmetrical around the α -carbon; proline always causes a kink in the peptide chain. Both are well suited for structural roles. Collagen, the most abundant protein in the animal kingdom, consists of at least twelve classes. The basic structural unit of collagen is the triple alpha helix—every third amino acid is glycine, presumably because only glycine, being the smallest amino acid, can fit into the structure. A common motif in collagen is GLY-PRO-X, with X being any amino acid. Somewhat arbitrarily, the most abundant plant-cell-wall proteins, some known only from cDNA sequences, are divided into four classes: (1) The best known are hydroxyproline-rich glycoproteins (HRGPs). As the name implies, these proteins are modified by the addition of carbohydrate, specifically, arabinose (a pentose) and galactose. A common motif is SER-(HYP)₄. The abundance of lysine makes these proteins basic and it has been speculated that this charge would favor their interaction with pectin, which, recall, is acidic (if not esterified). Some HRGPs are members of the multigene family,

extensions, which are thought to be important for structure. (2) proline-rich proteins, which are thought to be structural also. (3) glycine-rich protein, at least some of which may be involved in wall repair, and (4) arabinogalactan proteins, which may be more than 90 % carbohydrate. These proteins are not covalently linked to other wall components, and have a high turnover (ca. 15 minutes). They are excreted and may be involved in adhesion processes (such as pollination) and as response to wounding (gums). A current speculation (based on the turn-over and low accumulation) is that they are chaperone-type molecules that “protect” other polysaccharides while the latter are incorporated into the wall. Without belaboring the details and without knowledge of particular functions, suffice it to note that the expression of the various cell-wall structural proteins is developmentally regulated, is cell specific, and can be induced, e.g., by pathogen attack. Finally, note that protein chimeras exist (e.g., the potato lectin comprises a chitin-binding module and an evolutionarily independent hydroxyproline-rich module).

Lignin is the “final” cell-wall component. It is the second most abundant biomolecule on earth, being up to 50% of secondary walls, where it may be covalently attached to cellulose. The generality is that lignin is absent from primary-cell walls, however, it may be deposited there after cell-wall expansion is complete. Lignin confers rigidity and strength, especially compressive resistance. (Cellulose microfibrils give the walls a great deal of tensile strength, so lignin and cellulose complement each other in their properties.) In the vernacular, lignin structure is a “mess”—being composed of many monomers, connected in various ways.

Overhead 8: Partial structure of lignin

It is neither necessary nor important for you to know the exact structures involved. You should remember that the basic building blocks are variously substituted phenols that are covalently bound into branched macromolecules. (To a first approximation, lignin is the only cell wall constituent that is aromatic; however, some aromatics are esterified to cell-wall polysaccharides). Interestingly, it appeared that lignin biosynthesis was not rigorously prescribed, as virtually all biochemical reactions are. Instead, H_2O_2 formed in the cell wall generates free radicals, which then react in a more-or-less random fashion to form the various linkages. More recent evidence also implicates enzyme-directed synthesis also, which is specific.

Overhead 9: cell-wall model

This overhead provides an overview or summary of the primary-cell-wall structure of dicots and non-grass monocots¹⁰. Cellulose chains (β -1,4 glucan), packaged into parallel bundles called microfibrils during synthesis, are decorated by other polysaccharides called hemicelluloses. Hemicelluloses are a diverse group of polymers and are constructed of pentoses and hexoses, and some contain a β -1,4 glucan backbone. Pectin molecules are cross-linked with the hemicellulose molecules, and other pectin molecules are held together by Ca^{2+} bridges. Perhaps 10% of the cell wall is protein, which has special properties because it is rich in glycine, proline, and hydroxyproline. In secondary walls, lignin—a strong hydrophobic heterogeneous aromatic polymer—is deposited.

We close with cell-wall structure, metabolism, function, and so forth, by taking note of a novel role for cell-wall polysaccharides. Some cleavage products—oligosaccharides of various cell-wall fractions have biological activity at exceedingly low concentrations, in the range of 10^{-9} M. The oligosaccharides—5 to 8 monomers—that have biological activity are known as oligosaccharins. One oligosaccharin derived from the hemicellulose that is composed predominately xylose (pentose) and glucose moieties antagonizes the growth promotion induced by the plant-growth-regulator auxin. Another oligosaccharin, derived from the breakdown of a pectin rich in galacturonic acid residues, throws into action certain generalized plant defense responses. By breaking down the cell wall, do pathogens signal inadvertently to the plant their presence? Although the mode of action, the physiological function, and the importance are yet to be fleshed out, this group of cleavage products undoubtedly play important roles in organism-to-organism interactions. (In addition to the breakdown of plant-cell walls by fungal enzymes, the breakdown of fungal walls by plant enzymes also forms biologically active oligosaccharins.)

¹⁰ There is the so-called Type I wall (dicots except chenopods and monocots except some grasses). We focus on this wall. The other type, as inferred, is Type II.