
Cellular basis of plant growth

OM8.1 CATEGORIES OF TRANSPORT PROTEINS IN THE PLASMA MEMBRANE

Transport proteins – often referred to by the collective term “transporters” – facilitate movement of substances across the plasma membrane. They fall into three general categories: pumps, carriers, and channels.

Pumps move molecules across the membrane against their concentration or electrochemical gradients, using energy from hydrolysis of ATP (**Figure OM8.1-1A**). By far the major type of pump in the plasma membrane is the proton pump, which uses energy from ATP hydrolysis to move protons against their electrochemical gradient from inside the cell into the apoplast, thereby creating a proton motive force across the membrane. The proton motive force provides energy for the movement of substances across the membrane via carrier proteins. Two other pumps in the plasma membrane move substances out of the cell using energy from direct ATP hydrolysis. Ca^{2+} pumps form part of the diverse mechanisms by which Ca^{2+} concentrations inside the cell are modulated (see Online Material 9.1). **ABC (ATP binding cassette) transporters** are pumps that move very diverse substances out of the cell. These include components of the cuticle (see Section 6.3), some hormones including auxin (see Section 9.2), toxins that act on fungal pathogens (see Section 15.1), exudates that modulate the microbial population of the rhizosphere (see Section 16.4), and the toxic heavy metals cadmium and lead.

Carrier proteins bind molecules on one side of the membrane and release them on the other side (**Figure OM8.1-1B**). Many carrier proteins are involved in active transport: they move substances against their concentration or electrochemical gradients. Energy for this process is provided by the proton motive force across the membrane: carrier proteins allow the diffusion of protons down their electrochemical gradient from the apoplast into the cell, and use this proton flux to drive the movement of other solutes against their concentration or electrochemical gradients. A specific carrier may allow movement of a solute in the same direction as the passive inward flux of protons (in which case the carrier is referred to as a symporter) or in the opposite direction to the proton flux (in which case the carrier is referred to as an antiporter).

The plasma membrane contains numerous different channel proteins that allow passive fluxes of cations or anions across the plasma membrane. Channels have diverse specificities and responses to changes in their environments, permitting rapid and sensitive control of the ion content and composition of the cell. They may be specific for one or several different ions. Flux through a given channel is variable: it may be controlled by several different factors including the membrane potential, the ion concentration gradient across the membrane, post-translational modification of the channel protein (for example phosphorylation by specific protein kinases), and binding to other substances including hormones. This type of control is called “gating.” Voltage gating – control of channel flux by the membrane potential – is widespread and important. In the steady state many voltage-gated channels permit no or low fluxes. Changes in membrane potential can result in opening of channels to allow ion fluxes that establish a new steady state. Other types of channels are gated by specific metabolites (called ligand-gated channels; a **ligand** is any molecule that binds to another to bring about a specific biochemical change). Arabidopsis genes encode about 40 ligand-gated cation channels that open to allow influx of Na^+ , K^+ , and Ca^{2+} in response to elevation of the concentration of either glutamate or cyclic nucleotides (e.g. cyclic GMP). Channels may also be gated by changes in the mechanical properties of the plasma membrane. These

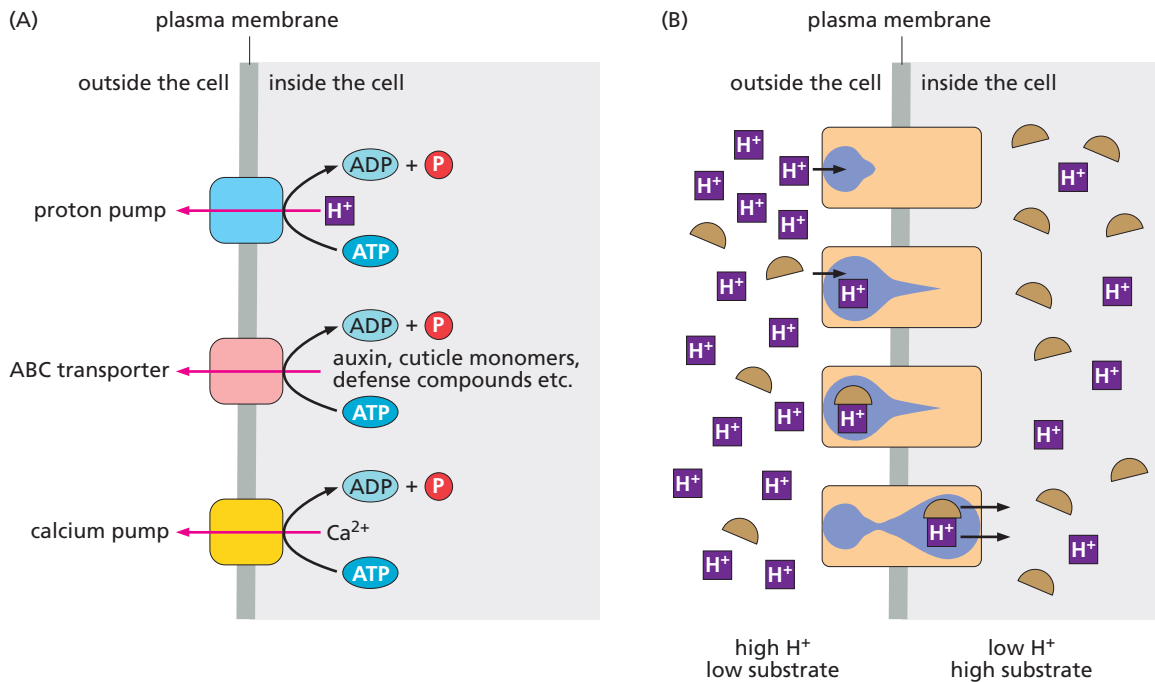


Figure OM8.1-1 Pumps and carriers in the plasma membrane. (A) Some transporters – termed active transporters or pumps – move solutes across the membrane against their concentration gradients using energy from the hydrolysis of ATP. These include the proton pump that moves protons from the inside to the outside of the cell, ABC transporters that move a wide range of different solutes and have differing solute preferences, and Ca²⁺ pumps that form part of the complex mechanisms of Ca²⁺ signaling in the cell (see Online Material 9.1). (B) A class of transporters termed carriers uses the electrochemical gradient set up by the proton pump to drive the movement of other solutes into or out of the cell against their concentration gradients. The cartoon shows the proposed successive stages in the operation of a hypothetical carrier (a symporter) that moves a specific solute (*brown*) into the cell from the apoplast. From *top* to *bottom*, the carrier protein is initially open to the outside of the cell, where it binds a proton. This binding enables binding of the solute. The proton moves through the carrier down its concentration gradient, allowing inwards movement of the solute and its discharge to the interior of the cell.

mechanosensitive channels open in response to increased membrane tension, allowing ion fluxes across the plasma membrane. The functions of mechanically induced fluxes are not fully understood, but one function may be to act as signals that promote changes in response to membrane stress caused by, for example, plasmolysis or pathogen invasion.

Two further categories of plasma membrane protein facilitate diffusion of specific uncharged substances down concentration gradients. SWEET proteins permit movement of sugars across the plasma membrane (discussed in Section 5.1, and in Section 14.2 in the context of sugar movement from host plants to pathogens), and aquaporins facilitate movement of water (discussed in Sections 7.1 and 8.1).

OM8.2 STOMATA: AN OVERVIEW OF STRUCTURE AND FUNCTION

Stomata are the pores on the surface of leaves that permit exchange of CO₂ and water vapor between the atmosphere and the intercellular spaces of the leaf. The cuticle that covers the leaf surface is relatively impermeable to both of these gases so almost all of the CO₂ consumed in photosynthesis and the water vapor lost in the transpiration stream that supplies water from the soil to the aerial parts of the plant passes through stomata. The size of the stomatal aperture is controlled by mechanisms responsive to both CO₂ consumption by photosynthesis and leaf water potential. The balance between these two processes is subject to environmental conditions including water availability, temperature, and light, all of which undergo diel changes and in natural conditions fluctuate unpredictably on longer and shorter timescales. The mechanisms controlling aperture must therefore enable dynamic changes that integrate multiple internal (intercellular CO₂ and water vapor concentration) and external signals to maximize carbon gain while avoiding excessive water loss. Some examples to illustrate the complexity of the “balancing act” involved in the control of stomatal aperture are as follows.

In C₃ plants in the light with a good water supply, the stomatal aperture is large. This ensures a high rate of entry of CO₂, accompanied by a high rate of water loss, hence a fast transpiration stream. If the rate of photosynthesis declines – for example because of external factors such as a fall in temperature or light level, or internal factors including reduced demand for photosynthetic products – the stomatal aperture is reduced, limiting both CO₂ and water loss. If the availability of water to the plant declines because of reduced soil water potential (see Section 7.2), stomatal aperture is reduced, limiting water loss at the expense of a decrease in CO₂ entry and hence in the rate of photosynthesis. Figure 4.33 illustrates how stomatal closure due to water deficit impairs or prevents carbon gain through photosynthesis.

Stomatal aperture is adjusted by changes in volume of the guard cells

Opening and closure of stomata is brought about by changes in the volume and hence the shape of two cells that flank the aperture – the guard cells. These two cells are symplastically isolated from other epidermal cells and are joined at either end of the stomatal pore. Their development in the epidermis of dicot plants is described in Section 10.4. Each dicot guard cell is approximately kidney-shaped, whereas monocot guard cells are usually dumbbell-shaped. The inner walls of the guard cells that line the pore are extensively thickened and inextensible relative to the outer walls that are adjacent to other epidermal cells (**Figure OM8.2-1A**). Opening of the stomatal pore is brought about by increased solute concentration in the guard cells, followed by passive uptake of water. These changes increase guard cell turgor pressure, stretching the thin outer cell walls of the guard cells and resulting in concave bending of the inner walls of the cells, thus opening the pore. The change in shape of the guard cell is facilitated by the radial arrangement of cellulose microfibrils in cell walls. Closure of the pore is a reverse of this process. Solutes and hence water are lost from guard cells, causing a decrease in volume and hence relaxation of the bending of the inner wall.

Two mechanisms bring about increased solute concentration and hence increased volume in guard cells during stomatal opening: import of solutes from the apoplast, and generation of solutes by guard cell metabolism (**Figure OM8.2-1B**). The more important mechanism in most circumstances is the import of solutes, in particular K⁺, Cl⁻, NO₃⁻, malate, and sometimes sucrose across the plasma membrane from the apoplast. When opening is triggered, a proton pump (proton ATPase) exports protons to the apoplast. The plasma membrane becomes **hyperpolarized**, activating voltage-gated inwardly rectifying K⁺ channels (see Online Material 8.1) and thus causing a several-fold increase in the concentration of K⁺ in the cell. The influx of K⁺ is accompanied by influx of anions including Cl⁻, NO₃⁻, and malate. Solute concentration may also be increased by import of sucrose and hexoses via plasma membrane antiporters of the SUC family, although at least some imported sugar is used in guard cell metabolism to provide the substrate for synthesis of ATP needed for proton extrusion. The increase in solute concentration in the guard cell causes a passive influx of water via activated aquaporin channels, resulting in an increase in cell volume.

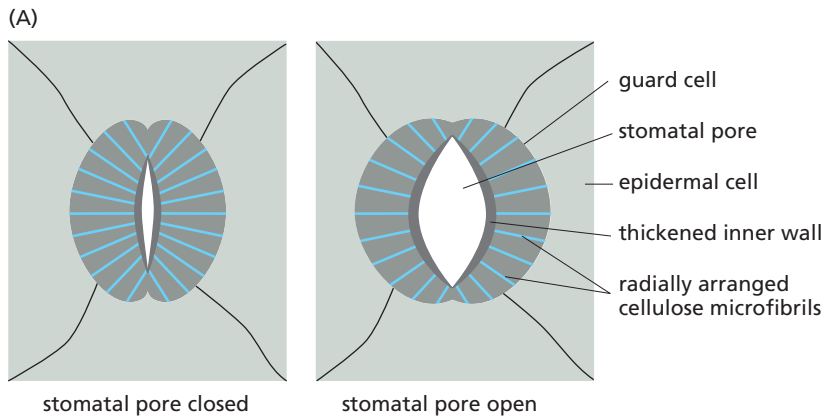
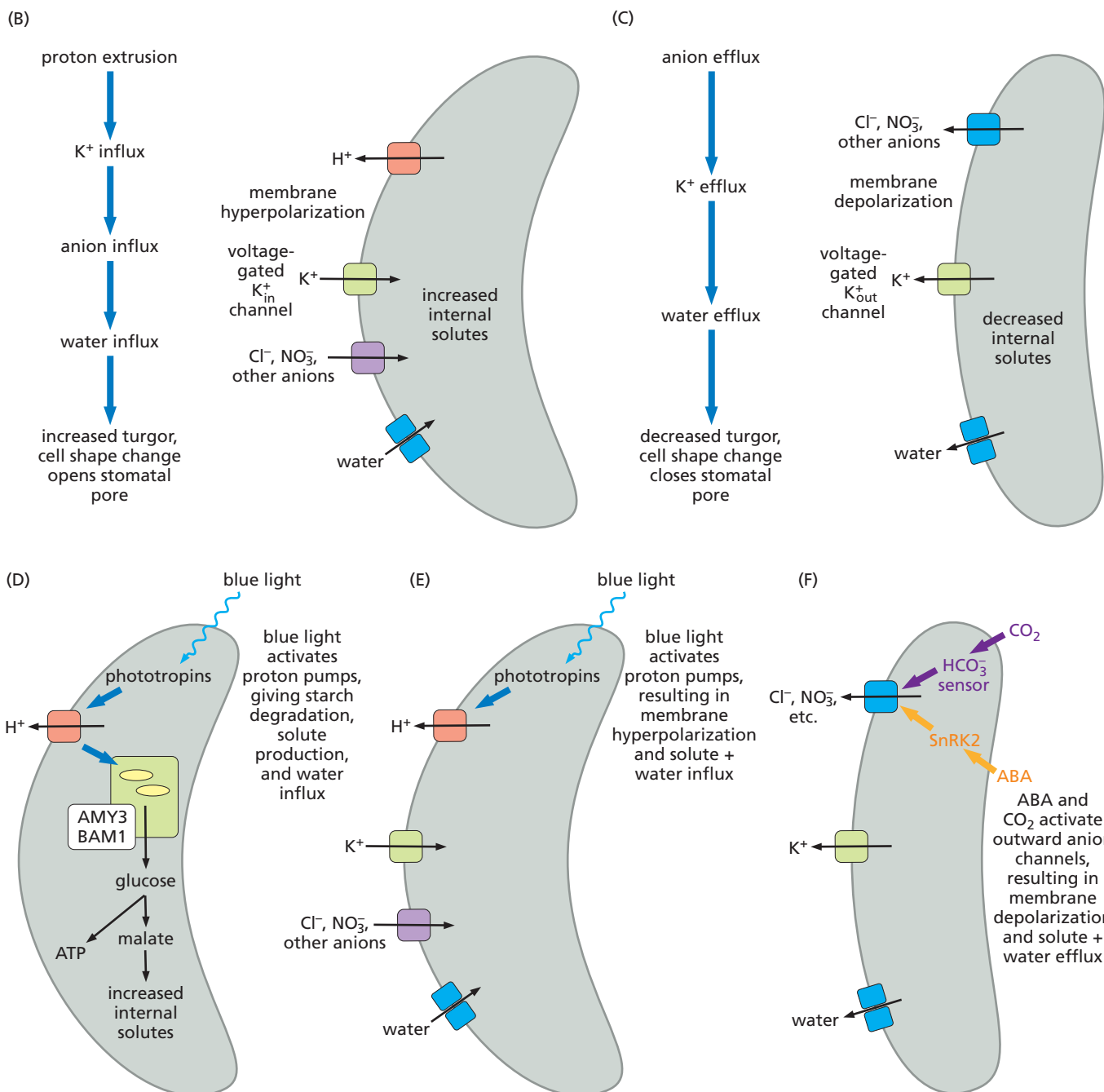


Figure OM8.2-1 (A) Two guard cells with thickened inner walls flank the stomatal pore. Because of the pattern of wall thickening and the orientation of the cellulose microfibrils, an increase in cell turgor causes the cells to swell and bulge outwards, opening the stomatal pore (right). Loss of turgor relaxes the cell shape, causing closure of the stomatal pore (left). (B) The succession of events that lead to guard cell swelling and opening of the stomatal pore. (C) The succession of events leading to guard cell relaxation and closure of the stomatal pore. (D, E) Blue light activation of phototropins results in activation of proton pumps. The resulting proton gradient drives starch degradation via amylases AMY3 and BAM1 and generation of solutes and ATP at the beginning of the day (D), and the import of solutes from the apoplast (E). Solute accumulation is followed by influx of water, resulting in increased turgor and cell swelling. (F) CO_2 concentration in the chloroplast is monitored by a bicarbonate sensor. High CO_2 triggers stomatal closure through activation of outward anion pumps, which is followed by cation efflux. The resultant water efflux reduces turgor and thus brings about closure of the stomatal pore.



Stomatal closure is driven by activation of anion channels, which export anions including Cl^- , NO_3^- , and malate across the plasma membrane into the apoplast (**Figure OM8.2-1C**). The loss of anions reduces the membrane potential (depolarization), resulting in the export of K^+ via voltage-gated outwardly rectifying K^+ channels. Solute concentration in the guard cells is reduced, causing an efflux of water and lowered turgor. Cell shape is relaxed, occluding the stomatal pore.

Changes in solute concentration in the guard cells during opening and closing of stomata occur in both the cytosol and the vacuole. The vacuole occupies a large fraction of the volume of the guard cell. The tonoplast membrane has a suite of channels and transporters, including aquaporins, that mirror the transport capacity of the plasma membrane. Changes in concentration of ions and other solutes in the cell take place in both the cytosol and the vacuole.

The second mechanism that can bring about increased solute concentration and hence increased volume in guard cells is conversion of starch stored in the plastids of these cells to glucose (**Figure OM8.2-1D**). Glucose formation from starch appears to be important for rapid stomatal opening when plants return to light at the end of the night. Whereas starch is an osmotically inert polymer, glucose derived from it is an osmotically active solute: starch breakdown effectively increases the solute concentration in the cell. Glucose metabolism also fuels synthesis of the malate ion, and of ATP via the tricarboxylic acid cycle, contributing to control of solute concentration, pH, and membrane potential in the guard cell.

It is important to note that the precise means by which solute concentration in guard cells is changed to bring about stomatal opening and closing varies considerably between species, with environmental conditions, and with time of day.

Numerous internal and external factors have been reported to control changes in stomatal aperture. It seems likely that guard cell volume and hence aperture is controlled by a web of interlinked signal transduction pathways that act on the solute-adjusting processes just described, rather than by a few discrete, linear pathways emanating from easy-to-define signals. To provide some insight into this complex subject, the following is an overview of the roles of three important signals – blue light (triggering stomatal opening), CO_2 concentration inside the leaf, and water deficit (triggering stomatal closure).

Blue light triggers stomatal opening through activation of the proton pump

It has long been known that stomatal opening is triggered by light, but our understanding of the mechanisms is still incomplete. It is clear that while either blue or red light can trigger opening, they do so in different ways. Triggering by red light occurs at light levels sufficient for photosynthesis, and it seems likely that the operation of photosynthesis and the consequent reduction in internal CO_2 concentration, rather than a direct effect of red light on guard cells, is largely responsible for red-light-induced opening. Blue light, by contrast, causes stomatal opening at light levels below those required for photosynthesis. It can thus promote stomatal opening at dawn, ensuring adequate CO_2 levels inside the leaf as photosynthesis resumes. Blue light activates two blue-light photoreceptors, **phototropins** 1 and 2 (see Section 12.1). These have modes of action that ultimately increase solute concentration inside the guard cells via various protein kinase-mediated signaling cascades (**Figure OM8.2-1D, E**). Activated phototropins phosphorylate and thus activate a guard cell-specific protein kinase, BLUS1 (BLUE LIGHT SIGNALING 1), which initiates a phosphorylation cascade that ultimately phosphorylates and thus activates the plasma membrane proton pump, energizing the process of solute import. Activation of proton pumps by blue light also promotes starch degradation in guard cells (Figure OM8.2-1D). The link between proton pumps on the plasma membrane and the triggering of chloroplastic starch degradation is not yet fully understood. Interestingly, starch degradation in guard cells proceeds via a different pathway from this process in mesophyll cells (described in Section 5.3). Specific isoforms of both β -amylase and α -amylase (BAM1 and AMY3, respectively) are essential for the process in guard cells but not in mesophyll cells. It appears that activities of these enzymes are increased at translational and/or post-translational levels in response to blue light. There is increasing evidence that blue light can also activate stomatal opening by targeting solute transport processes other than the proton pump. Signaling from phototropins is reported to inhibit anion channels important in stomatal closure, and to activate inwardly directed K^+ channels (Figure OM8.2-1E).

Stomatal aperture is adjusted to intercellular CO₂ concentration by a guard-cell bicarbonate sensor

Stomata open in response to an increase in the rate of photosynthesis, and close as the rate drops. The mechanism that links stomatal aperture to photosynthesis involves sensing by guard cells of the CO₂ concentration in the intercellular spaces of the leaf. Low intercellular CO₂ is indicative of its rapid consumption in photosynthesis, triggering stomatal opening to allow more CO₂ into the leaf. High intercellular CO₂ is indicative of a low rate of photosynthesis, triggering reduced stomatal aperture. The CO₂-sensing mechanism in the guard cells is responsive to bicarbonate, rather than CO₂ concentration inside the cells. CO₂ entering the cells reaches an equilibrium with bicarbonate and protons, mediated in part by carbonic anhydrases. Since the plasma membrane is permeable to CO₂ but not HCO₃⁻, bicarbonate levels inside the guard cell reflect the CO₂ concentration in the intercellular space. Bicarbonate forms a complex with two mitogen-activated protein (MAP) kinases (see Online Material 9.1) required for stomatal opening and a kinase HT1 (HIGH TEMPERATURE 1) required for stomatal closure. This HCO₃⁻-mediated complex initiates a signal transduction pathway that adjusts stomatal aperture according to the intercellular CO₂ concentration (**Figure OM8.2-1F**).

The fact that stomata tend to shut when intercellular CO₂ concentrations are high raises concerns about how crop productivity will be affected by the rapidly rising levels of CO₂ in the present atmosphere. Although higher CO₂ levels might be expected to increase photosynthesis and hence the productivity of C₃ crops (see Online Material 4.6), future yield increases may be limited by lower stomatal aperture caused by higher CO₂ levels.

Abscisic acid mediates stomatal closure in response to water deficit

The central role of ABA in plant responses to water deficit is described in Section 7.2 and Figure 7.8. Elevation of ABA in guard cells in response to water deficit activates the protein kinase SnRK2.6 (OST1), which in turn activates outward anion channels, causing an efflux of solutes and water from the guard cell and hence stomatal closure (see Figure OM8.2-1F). It seems likely that active SnRK2 phosphorylates components of a downstream signal transduction network that is shared by other external signaling pathways including light and CO₂ sensing.

Several aspects of stomatal control of leaf gas exchange are not covered in this overview, including the importance of stomatal location and density on the leaf surface, the proposed roles of the subsidiary cells that surround the guard cells in some groups of plants, and the potential applications of improved understanding of guard cell function in development of drought- and high CO₂-tolerant crops. Reviews that provide further information are provided in "Further Reading."

Further reading

- Blatt MR, Jezek M, Lew VL, Hills A (2021) What can mechanistic models tell us about guard cells, photosynthesis, and water use efficiency? *Trends Plant Sci* 27:166–179.
- Lawson T, Matthews J (2020) Guard cell metabolism and stomatal function. *Annu Rev Plant Biol* 71:273–302.
- Nguyen TBA, Lefoulon C, Nguyen TH, Blatt MR, Carroll W (2023) Engineering stomata for enhanced carbon capture and water-use efficiency. *Trends Plant Sci* 28:1290–1309.

OM8.3 AQUAPORINS AND RAPID CELL AND ORGAN MOVEMENTS

Aquaporins allow very high fluxes of water across the membranes of specialized cell types that swell and contract rapidly in response to environmental stimuli. These include stomatal guard cells (see Online Material 8.2) and cells associated with the rapid movements of whole-plant organs. For example, closure of the trap of the Venus flytrap (see Figure 13.27), closure and drooping of leaves of the sensitive plant *Mimosa pudica*, and the springing apart of stamens of *Sparmannia* in response to touch are all brought about by sudden changes in turgor in “motor cells” in these organs, involving very rapid efflux of water via aquaporins. Touch stimuli to the leaves or stamens trigger collapse of the membrane potential in the motor cells (depolarization); potassium and chloride ions rapidly leave the cell and loss of water follows, facilitated by aquaporins. Cell turgor drops dramatically within seconds and the thin-walled motor cells collapse, resulting in rapid movement of the whole organ.

OM8.4 THE CYTOSKELETON

The division, growth, and development of plant cells are dependent on the cytoskeleton, a system of filaments and microtubules in the cytosol that is involved in many of the processes described in Chapter 8. The cytoskeleton forms the spindle on which the chromosomes separate at mitosis, controls the movement of organelles within the cell, guides the synthesis of the cell wall, and determines the direction of cell expansion.

The main components of the plant cell cytoskeleton are actin filaments and microtubules (Figure OM8.4-1A, B). Actin filaments are linear polymers of the protein actin, which is encoded by a multigene family. The number of genes in the actin family differs greatly from one species to another: Arabidopsis has ten actin genes, whereas petunia has more than 100. Different members of the gene family are expressed in different parts of a plant, and in response to different environmental and developmental stimuli. Microtubules consist of subunits that are dimers of two proteins, α -tubulin and β -tubulin; the subunits polymerize to form a hollow cylinder. Tubulins are also encoded by a multigene family: there are 19 tubulin genes in the Arabidopsis genome.

The two ends of an actin filament or a microtubule are not identical. Polymerization can occur much faster at the “plus end” than at the “minus end” (Figure OM8.4-1C). Motor proteins, which move along filaments and microtubules, usually move in only one direction. Some move only from plus to minus ends, and others only from minus to plus ends.

Actin filaments and microtubules are highly dynamic structures, undergoing continuous polymerization and depolymerization. Sometimes this involves net loss or gain of these cytoskeletal structures, such as during the formation and disassembly of the spindle during mitosis. However, loss and gain of subunits at either end of filaments and microtubules sometimes occurs without any net change in their length. Actin filaments undergo “treadmilling,” in which a net gain of actin proteins at the plus end is balanced by an equal net loss at the minus end (Figure OM8.4-1D). Microtubules can alternate rapidly between growth and shrinkage, a phenomenon known as “dynamic instability” (Figure OM8.4-1E). When subunits are added to a microtubule, they are

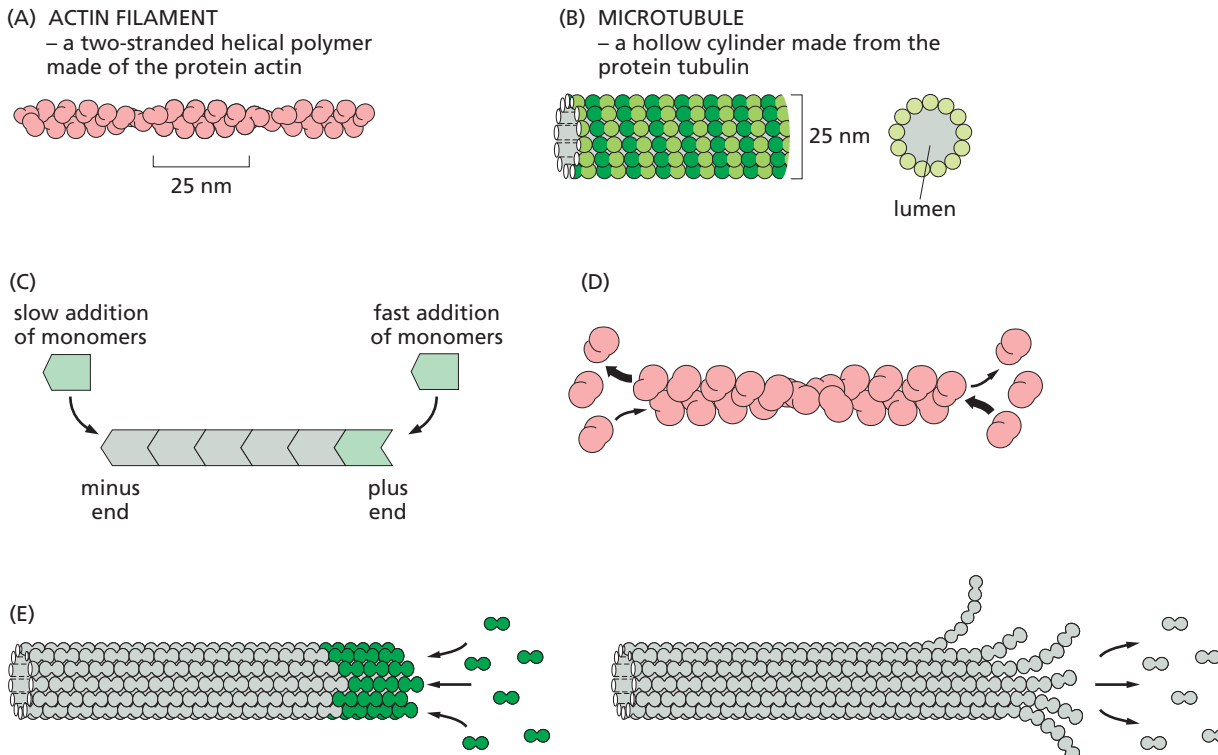


Figure OM8.4-1 The cytoskeleton. (A) Structure of an actin filament. (B) Structure of a microtubule; note that tubulin is a heterodimer of α and β subunits (indicated in different shades of green). (C) Polymerization of cytoskeletal components. (D) Treadmilling of an actin filament: net gain at the plus end is matched by net loss at the minus end. (E) Dynamic instability of a microtubule. Tubulin is added in the form of GTP-tubulin (green). After addition, the GTP is hydrolyzed to GDP-tubulin (gray). If the rate of addition of subunits slows down so that newly added subunits are immediately converted to GDP-tubulin, the presence of GDP-tubulin at the end of the microtubule promotes a switch from polymerization to depolymerization (right).

bound to the energy-rich molecule GTP; after addition, the GTP is hydrolyzed to GDP. The presence of GTP-tubulin at the end of a microtubule favors addition of subunits, whereas the presence of GDP-tubulin favors loss of subunits. When polymerization is fast, the rate of hydrolysis of GTP to GDP on the newly added subunits lags behind, and the subunits at the newly forming end are GTP-tubulin rather than GDP-tubulin. This favors further polymerization. If polymerization slows down, hydrolysis of GTP on newly added subunits can catch up, and subunits at the newly formed end are GDP-tubulin. This promotes a switch from polymerization (growth of the microtubule) to depolymerization (shrinkage of the microtubule).

In addition to actin filaments and microtubules, the plant cytoskeleton contains many other protein components. These include motor proteins (e.g. myosin) that drive the movement of subcellular structures such as organelles and paired chromosomes along filaments and microtubules; proteins that bind filaments and microtubules together into bundles; and proteins that regulate the dynamics of polymerization and depolymerization of these cytoskeletal structures. An interesting example in the latter category is **profilin**, a protein that binds to actin monomers and so determines their availability for polymerization into filaments. Much of the actin in pollen, for example, is bound in complexes with profilin. In fact, profilin is one of the major allergens causing hay fever.

OM8.5 SECRETED PROTEINS HAVE IMPORTANT FUNCTIONS IN THE CELL WALL

In addition to its polysaccharide components, the plant cell wall also contains proteins that play structural roles and enzymes that metabolize the carbohydrate polymers to regulate cell wall extension.

Most of the structural proteins have large polysaccharide side chains, and the protein may be only a small component (as little as 5%) of the whole molecule. Like the polysaccharide components of the wall matrix, these glycoproteins are synthesized in the endomembrane system and transferred to the wall via vesicles. Both the proteins and their carbohydrate side chains are extremely variable: the nature of the cell wall glycoproteins differs from one type of tissue to another within the plant. The functions of these glycoproteins are not fully understood, but they may be involved in the cross-linking of carbohydrates in the wall. The structure of one glycoprotein, extensin, is shown in **Figure OM8.5-1**.

Other proteins that are secreted to the cell wall include enzymes that modify cell wall components. Some of these enzymes loosen the matrix by cleaving specific bonds in the cell wall polysaccharides, allowing the wall to extend and new polysaccharides to be inserted as the cell grows. The xyloglucan endotransglycosylases, for example, break and re-form xyloglucan chains in the wall during cell elongation. Their action may allow transient slippage of cellulose microfibrils as the cell expands.

Other enzymes change cell wall properties in ways that facilitate or restrict cell expansion. One example is the expansins, which break the hydrogen bonds between cellulose microfibrils and xyloglucans and thus allow the cell wall to expand. Expansins are active at acidic pH, so their activity, and consequently cell wall extension, is regulated by changes in apoplastic pH. Another secreted enzyme is pectin methylesterase, which cleaves methyl groups of homogalacturonan, modifying Ca^{2+} -mediated cross-linking and consequently the accessibility of pectin to other cell wall-modifying proteins. The ability to relax or rigidify the walls of different cells is important for the differential growth that shapes plant tissues and organs. This is demonstrated, for example, in transgenic plants in which expression of an inhibitor of pectin methylesterase can be induced in the developing shoot apex; without pectin methylesterase activity these plants cannot initiate floral buds (**Figure OM8.5-2**).

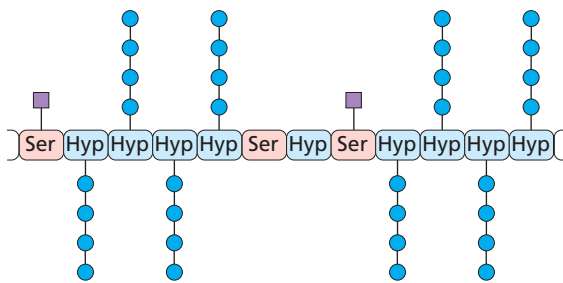


Figure OM8.5-1 O-glycosylation in a portion of the protein extensin. This cell wall protein contains many repeats of the amino acid sequence Hyp-Hyp-Hyp-Hyp-Ser. In the Golgi apparatus, single sugars or short oligosaccharides are added to the hydroxyl groups of hydroxyproline (Hyp) and serine (Ser) residues – hence the term *O*-glycosylation. Typically, a single galactose residue (*purple square*) is added to serine, and one to four arabinose residues (*blue circle*) are added to hydroxyproline.

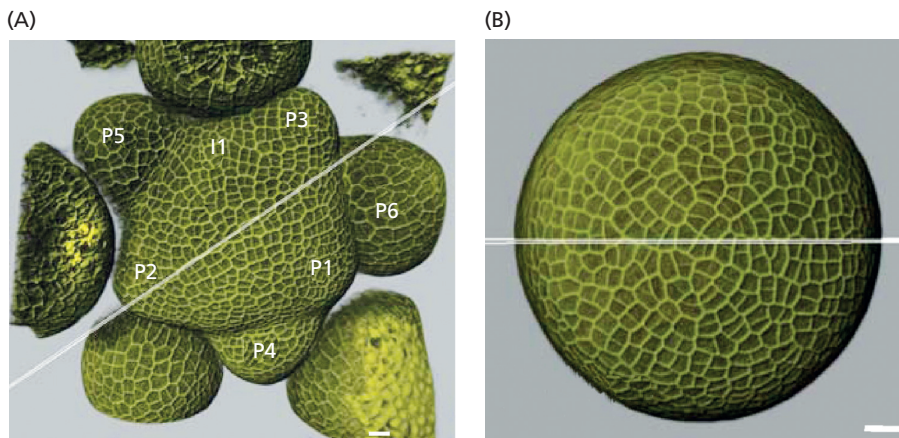


Figure OM8.5-2 Altered cell growth caused by reduced pectin methylesterase activity. The images show a top view of the inflorescence apex of *Arabidopsis*, comparing the wild-type (**A**) with a transgenic plant that expresses a pectin methylesterase inhibitor at the shoot apex (**B**). Differential cell growth gives rise to floral buds in the wild-type plant; in the plant without pectin methylesterase activity, all cells grow equally and new buds cannot emerge from the shoot apex. (From Peaucelle A *et al.* (2011) *Curr. Biol* 21:1720–1726. With permission from Elsevier.)

OM8.6 TRICHOME DEVELOPMENT

The trichomes of *Arabidopsis* leaves have been used to study how the coordination of elements of the cytoskeleton determines final cell shape. Trichomes are outgrowing epidermal cells with a distinctive branched morphology. Because of their surface location, their development can be studied relatively easily. Epidermal cells that develop into trichomes become larger than the surrounding epidermal cells, eventually forming a spike that elongates away from the surface of the epidermis. Branches grow out from this spike by expansion of localized regions of its cell wall (Figure OM8.6-1A, B, see also Figure 8.41 showing the importance of endoreduplication in trichome formation). During this process, microtubules control the initiation and formation of branches, while actin filaments ensure deposition of new material at appropriate places on the cell surface.

The involvement of microtubules in the initiation and formation of trichome branches is revealed by the study of mutant plants with abnormal trichomes. Some of these mutants are defective in proteins that modulate microtubule organization (Figure OM8.6-1C). For example, *zwichel* mutants of *Arabidopsis* lack a motor protein (a kinesin) that uses energy to drive microtubule reorganization. (Motor proteins use energy provided by the hydrolysis of ATP to generate mechanical energy or force; the kinesin class of motor proteins is also involved in spindle and phragmoplast formation; see Section 8.2.) The trichomes of these mutants have reduced or no branching. As well as a general role in determining the direction of cell expansion, the protein **katanin** is important in branch formation in trichomes. In *katanin* mutants, epidermal cells undergo the initial stages of trichome formation (swelling) but rarely elongate to form a spike and do not form branches.

The actin component of the cytoskeleton also plays an essential role in cell wall deposition. New material is delivered to the surface of growing parts of the trichome in vesicles derived from the endomembrane system, which track along actin filaments to the plasma membrane where they discharge their cargoes into the wall. Filaments are present at the growing regions of the trichome cells in the early stages of growth and accumulate at branch sites where new growth is initiated. Defects in the polymerization of actin filaments – for example in the *itb1* (*irregular trichome branch 1*) mutant – demonstrate the importance of the regulated deposition of new cell surface material for determining cell shape (see Figure OM8.6-1C).

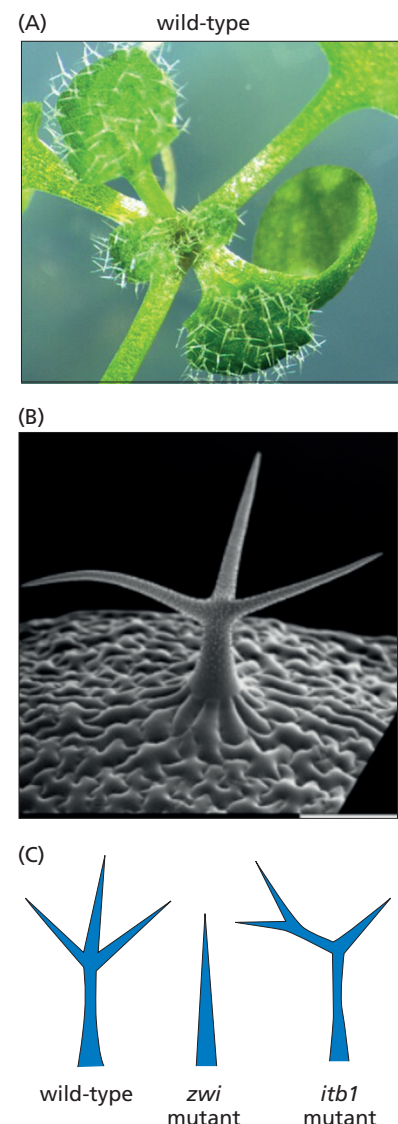


Figure OM8.6-1 Abnormal trichome development caused by cytoskeletal defects. (A) Photograph and (B) scanning electron micrograph of the leaf surface of wild-type *Arabidopsis*. The trichome cells have three branches. (C) Drawings of (left) a wild-type trichome, (center) a trichome of a *zwi* (*zwichel*) mutant, and (right) a trichome of an *itb1* mutant. The *zwi* mutant is defective in the organization of microtubules, resulting in a failure to initiate trichome branches. The *itb1* mutant is defective in a protein required for organization of actin filaments. It can initiate branches, but their growth is distorted. (A, from “Trichomes in *Arabidopsis thaliana* leaves” by Alena Kravchenko. Licensed under CC BY SA 4.0 via Wikimedia Commons. B, from Marks MD *et al.* (2009) *Mol Plant* 2:803–822. With permission from Elsevier.)

OM8.7 FORMATION OF NEW CELLS IN THE ENDOSPERM OF SEEDS

In the early stages of endosperm development in many seeds, nuclear division and cell division occur independently rather than together. This process has been extensively studied in the endosperms of brassicas and cereal seeds. Large amounts of starch are subsequently deposited in cereal endosperms whereas the endosperm usually occupies a tiny fraction of the mature seed in brassicas. However, the initial stages of endosperm development – the transition from a multinucleated structure to a cellularized tissue – is similar in brassicas and cereals.

After fertilization, the nucleus that will give rise to the endosperm (see Section 11.3) undergoes several rounds of nuclear division without any cell division. This produces a single, large cell with many nuclei arranged around its periphery (a structure called a **coenocyte**). Cell walls then develop perpendicular to the outer edges of this single cell to create compartments, each containing a single nucleus.

The formation of anticlinal walls between the nuclei of the coenocyte originates with a phragmoplast formed between bundles of microtubules and actin filaments that radiate from the membrane of each nucleus. Further nuclear division accompanied by cytokinesis then forms the inner, periclinal, wall of these cells, and gives rise to nuclei that will form the next layer of cells within the original single cell. Eventually, the entire space in the original cell may become cellularized in this way (**Figure OM8.7-1**).

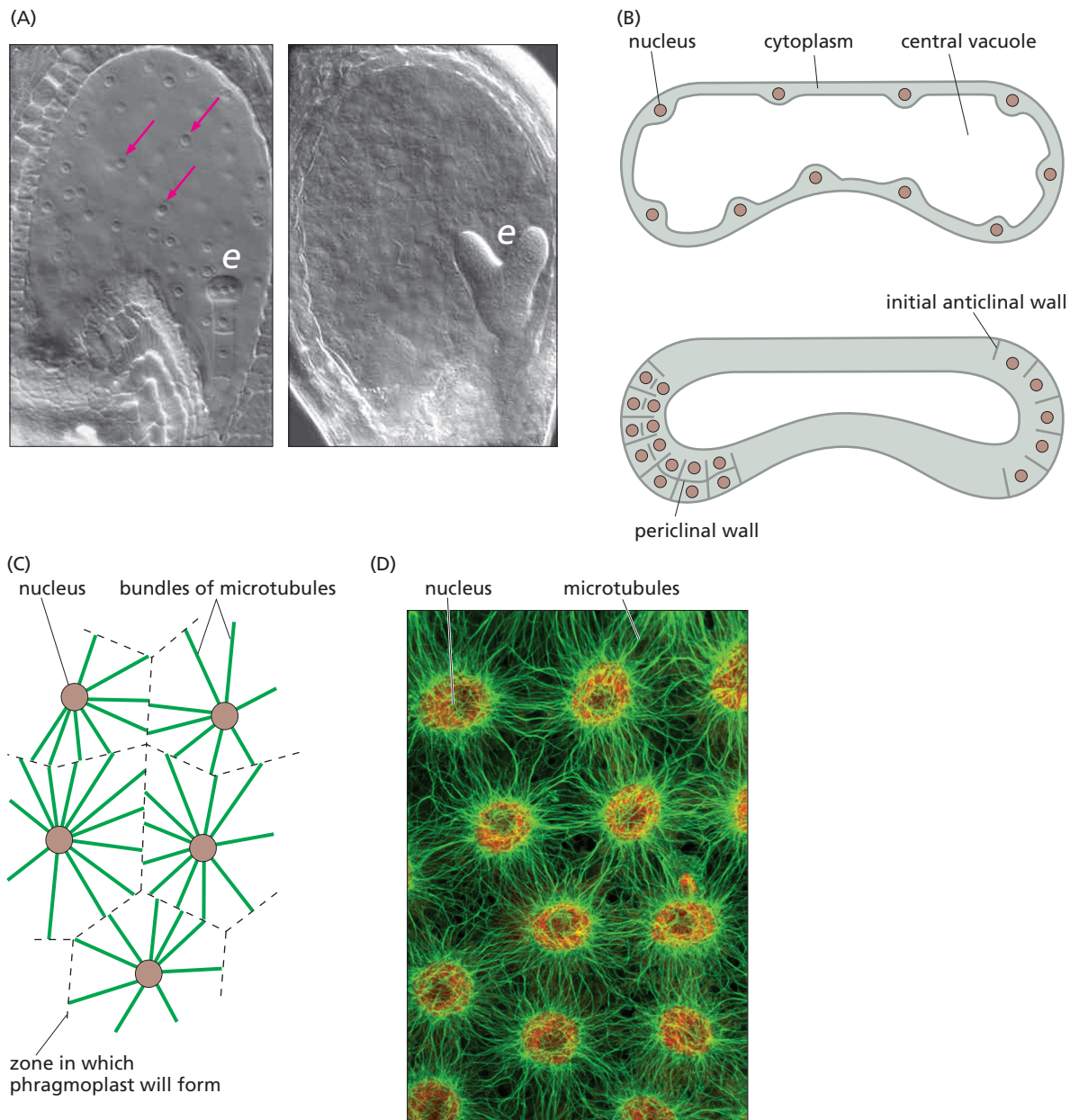


Figure OM8.7-1 Cellularization of the endosperm. (A) Sections of developing *Arabidopsis* seed. (Left) Embryo (e) at the pre-globular stage (see Section 10.2 for a description of stages of embryo development). The endosperm is syncytial; nuclei are indicated with arrows. (Right) Embryo at late heart stage. The endosperm is cellular. (B) Development of the endosperm in a cereal seed. (Top) Early development. The endosperm is a single cell, with multiple nuclei in cytoplasm lining a central vacuole. (Bottom) Cellularization, showing formation of the first anticlinal wall (right end), and formation of periclinal walls and further anticlinal wall deposition (left end). (C) Diagram showing nuclei and radiating microtubule bundles lining the periphery of the single-celled endosperm, at the early stage shown in (B). The view is from the inside of the cell toward the wall. (D) Confocal laser scanning microscopy of nuclei and radiating microtubules early in the development of the endosperm of *Coronopus didymus* (Brassicaceae), at an early stage equivalent to (A, left). Microtubules are stained green; nuclei are orange-red. (A, from Boissard-Lorig C *et al.* (2001) *Plant Cell* 13:495–509. Reprinted by permission of Oxford University Press on behalf of the American Society of Plant Biologists, courtesy of Frederic Berger. D, courtesy of Roy Brown.)

OM8.8 MUTANTS LACKING COMPONENTS OF THE VESICLE-MEMBRANE FUSION APPARATUS

The importance of interactions between v- and t-SNAREs for the correct formation of cell plates is illustrated by phenotypes of *Arabidopsis* mutants that lack components of the membrane-fusion apparatus (**Figure OM8.8-1**). The *knolle* and *keule* mutants are unable to form normal cell plates. Embryos homozygous for these mutations have multinucleate cells with incomplete and abnormal cross-walls, and seedlings die soon after germination. The KNOLLE protein is a type of t-SNARE called a syntaxin. The KEULE protein regulates the ability of the t-SNAREs to form receptor complexes for the v-SNAREs. Thus, loss of either protein reduces the delivery of new material to the cell plate, resulting in incomplete cytokinesis.

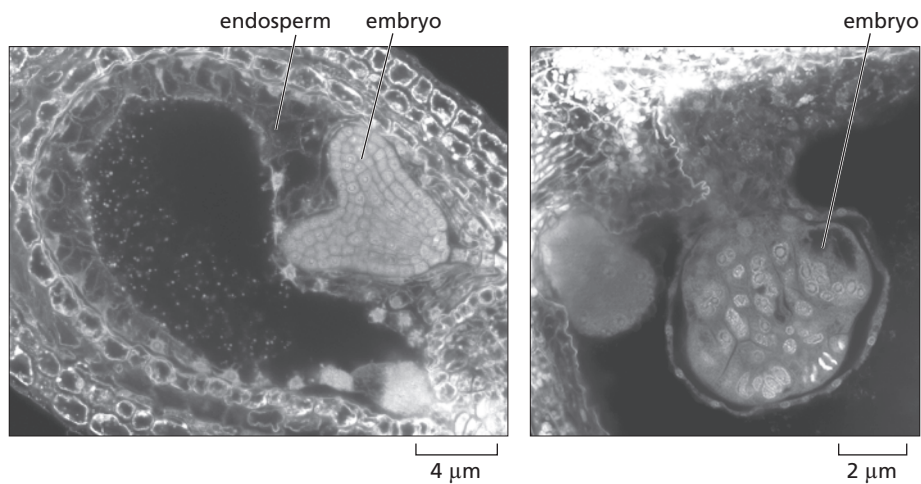


Figure OM8.8-1 Wild-type and *knolle* mutant embryos in developing *Arabidopsis* seeds. In the wild-type seed (*left*) the embryo has reached the late heart stage (see Section 10.2 for a description of stages of embryo development). At the same stage in the *knolle* mutant (*right*), cells are enlarged and have multiple nuclei. In the absence of the KNOLLE protein, cell plate formation and hence normal cytokinesis is limited. (Courtesy of Frederic Berger.)

OM8.9 PERMEABILITY OF PLASMODESMATA

The cytoplasmic sleeve is not freely permeable to all molecules in the cytoplasm. By injecting individual cells with fluorescently tagged molecules of known size, researchers can ascertain the maximum size (mass) of molecules that can move freely through plasmodesmata. For most plasmodesmata, this mass is about 1 kDa (**kilodalton**): this is called the **size exclusion limit** of the plasmodesmata. Primary metabolites – for example, sugar phosphates, ATP, and NAD – and hormones have molecular masses of less than 1 kDa whereas proteins generally have much greater masses: usually tens of kilodaltons. The exclusion limit can, however, be regulated to allow movement of much larger molecules, including proteins and RNA. Examples include transcription factors responsible for the coordination of expression of genes involved in plant development. Some types of developmental transcription factors move from the cell in which they are synthesized into adjacent cells in which they regulate developmental processes. This process is explained in more detail in Section 10.3. The size exclusion limit of the plasmodesmata may also increase or decrease when a plant is infected with viruses. Virus-encoded **movement proteins** increase the size exclusion limit of plasmodesmata and thus facilitate the movement of viral nucleic acid or entire viral particles, promoting the spread of infection from cell to cell (see Section 14.4). When plant tissues are wounded or infected with pathogens, signals from these events may trigger the closure of plasmodesmata. This is brought about by the deposition of a constricting ring of the carbohydrate polymer callose in the wall adjacent to the plasmodesmatal opening.

OM8.10 CROP AND HORTICULTURAL SPECIES WITH HIGH LEVELS OF PLOIDY

Some higher levels of ploidy are exploited in agriculture or horticulture. Seedless watermelons (*Citrullus lanatus*) are triploid ($3n$) and consequently sterile as they only produce unbalanced gametes. Triploids are generated by crossing an autotetraploid parent ($4n$, that produces $2n$ gametes) with a diploid parent ($2n$, that produces n gametes). At meiosis, three copies of each chromosome are present, which may result in formation of either a trivalent (six chromatids) or a normal bivalent plus one univalent. The trivalent cannot be partitioned in a balanced way at division I, and in the second scenario half the gametes produced will contain two homologs and the other half a single homolog. Overall, the cross will produce gametes with variable chromosome copy numbers that result in non-viable zygotes following fertilization. Normal fruits develop after fertilization but no viable seeds are produced, creating commercially attractive fleshy, seedless watermelons. Another important triploid crop is the Cavendish banana. As for watermelon, these plants cannot produce balanced gametes and are sterile as a consequence: the plants yield commercially attractive, seedless fruits. A disadvantage of this situation is that viable plants cannot be grown from seed, so Cavendish bananas are propagated vegetatively. The clonal, genetically identical nature of these plants makes them particularly vulnerable to disease epidemics.

An understanding of the origins of polyploid crop plants has important practical applications in crop improvement. Plant breeders are able to “resynthesize” these polyploids (creating “synthetics”) from their genetically diverse diploid progenitors, thereby introducing novel genetic variation into modern breeding programs.

OM8.11 SPECIALIZED FUNCTIONS OF SECONDARY CELL WALLS

In addition to cellulose, many secondary cell walls contain other types of polymers that confer specialized properties on the wall. For example, the secondary walls in some seeds contain large deposits of polysaccharides that serve as a reserve material. Mixed-linkage β -glucans accumulate in the cell walls of cereal seeds and are degraded to provide sugars during germination (**Figure OM8.11-1A**). Mannans – polymers of the sugar mannose, which may also contain glucose and galactose residues – are deposited in the secondary cell walls of seeds of many species, then degraded to provide sugars during seed germination. The cell walls of the endosperm of lettuce seeds are 70% mannans. **Figure OM8.11-1B** shows the hard white nuts of the palm *Phytelephas seemannii*, which have an extremely high mannann content. The secondary walls of some cell types contain a hydrophobic (water-repellant) polymer called **suberin**, which restricts the movement of water through the wall. Suberin is a principal component of the cell walls of cork, which forms the outer bark of woody plants (**Figure OM8.11-1C**).

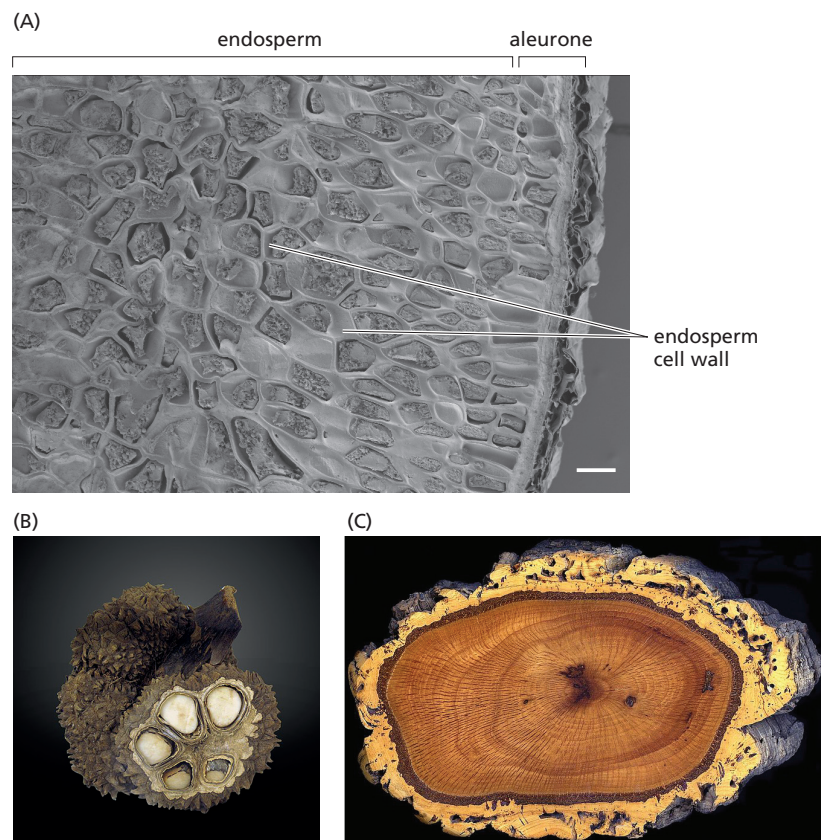


Figure OM8.11-1 Specialized secondary walls. (A) Scanning electron micrograph of a fracture face of a mature seed of the grass *Brachypodium distachyon*. The thick walls of the aleurone and endosperm cells have a high content of mixed-linkage glucans, polysaccharides that serve as storage compounds and are consumed during germination. Many cereal seeds also contain mixed-linkage glucans but their main storage material is starch; in *B. distachyon* seeds starch content is low and mixed-linkage glucans are a major storage material. (B) Fruit of the palm *Phytelephas seemannii* showing the white nuts. Their cell walls have very high contents of mannans, which act as a storage material. Mannan renders the nut very hard yet relatively easy to carve. It is known as "vegetable ivory" and used to make buttons and small decorative objects. (C) Section through the trunk of a cork oak, *Quercus suber*. The bark (outer layer) is thick and highly suberized, rendering it waterproof and light. (A, courtesy of Alexander Watson-Lazowski, John Innes Centre. B, from "Phytelephas seemannii-jardin botanique-IMG 2708-black by Rama. Licensed under CC BY SA 3.0 via Wikimedia Commons. C, from "Cork oak trunk section" by Plantsurfer at the English Wikipedia project. This work has been released into the public domain by its author.)

OM8.12 CUTICLE SYNTHESIS

Progress in understanding cuticle synthesis and assembly comes from the discovery of mutants with abnormal cuticles. Mutants with altered amounts and types of cuticular waxes (such as *eceriferum*, “without wax,” mutants) are relatively easy to identify because the reflective properties of the plant surface are changed (**Figure OM8.12-1**). Large numbers of gene loci influencing wax synthesis have been identified in some species. For example, there are more than 80 *Eceriferum* genes in barley and more than 20 discovered thus far in *Arabidopsis*. This implies that very large numbers of different gene products are necessary for the synthesis and secretion of cuticular wax.

Several *Arabidopsis* mutants with defects in cutin synthesis and assembly have severely abnormal growth patterns and reduced fertility, implying that cuticle deposition is essential for normal growth and development. In the *fiddlehead* mutant, for example, leaves and floral organs tend to fuse together at an early developmental stage. Although *fiddlehead* plants have a cuticle, the fatty acid composition of the cutin is abnormal and this seems to result in “stickiness” of the surfaces of developing organs. The gene affected in this mutant encodes an enzyme involved in fatty acid elongation. The *lacerata* mutant, which also has organ fusions and stunted growth, is defective in an enzyme that modifies fatty acids on the pathway to cutin synthesis. In these plants the cuticle is missing in the regions where organs are fused.

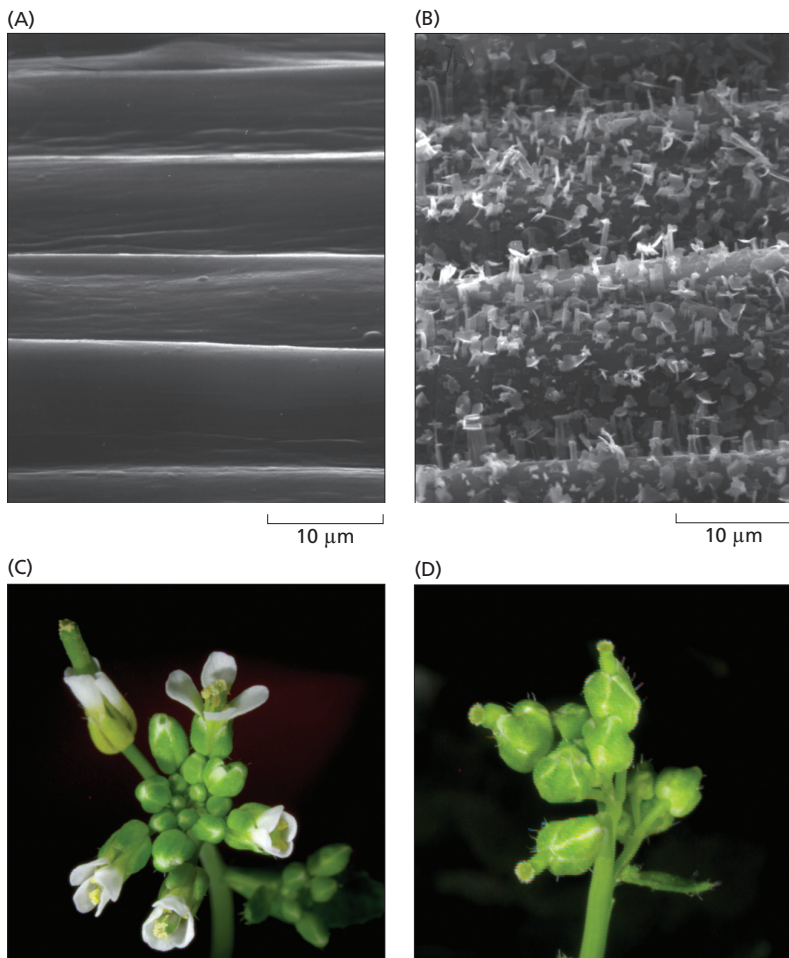


Figure OM8.12-1 Surfaces of *eceriferum* mutant and wild-type *Arabidopsis* flower stems. Scanning electron micrographs of (A) the waxless (*eceriferum*) mutant *cut1* and (B) wild-type *Arabidopsis*. The flakes on the surface of the wild-type stem are wax crystals. These are missing from *cut1*, which lacks an enzyme necessary for the synthesis of the long-chain fatty acids found in wax. (C) Wild-type and (D) *fiddlehead* mutant. The *fiddlehead* mutant lacks an enzyme necessary for synthesis of the long-chain fatty acids found in the cuticle. The floral organs stick together and fail to develop properly. (A, B, from Millar AA *et al.* (1999) *Plant Cell* 11:825–838. Reprinted by permission of Oxford University Press on behalf of the American Society of Plant Biologists, courtesy of Ljerka Kunst. C, D, from Lolle SJ *et al.* (1998) *Genetics* 149:607–619. Reprinted by permission of Oxford University Press on behalf of the Genetics Society of America, courtesy of Robert E. Pruitt.)