CHAPTER THREE

The Protoplast: Endomembrane System, Secretory Pathways, Cytoskeleton, and Stored Compounds

ENDOMEMBRANE SYSTEM

In the previous chapter various components of the protoplast were considered in isolation. With the exception of mitochondrial, plastid, and peroxisomal membranes, however, all cellular membranes—including plasma membrane, nuclear envelope, endoplasmic reticulum (ER), Golgi apparatus, tonoplast (vacuolar membrane), and various kinds of vesicles—constitute a continuous, interconnected system. This system is known as the **endomembrane system** (Fig. 3.1), whose ER is the initial source of membranes (Morré and Mollenhauer, 1974; Mollenhauer and Morré, 1980). Transition vesicles derived from the ER transport new membrane material to the Golgi apparatus, and secretory vesicles derived from the Golgi apparatus contribute to the plasma membrane. The ER and Golgi apparatus therefore constitute a functional unit, in which the Golgi apparatus serves as the main vehicle for the transformation of ER-like membranes into plasma membrane-like membranes.

Transition vesicles budding off ER membranes close to Golgi bodies are only rarely encountered because of the low volume of protein transport between the ER and the Golgi bodies in most plant cells. Transition vesicles are commonly encountered, however, in cells that produce large quantities of globulin-type storage proteins (as in legumes) or secretory proteins. In such cells proteins travel via vesicle budding with subsequent fusion from the ER through the Golgi apparatus to arrive at the storage vacuoles or at the surface of the plasma membrane (Staehelin, 1997; Vitale and Denecke, 1999).

The Endoplasmic Reticulum Is a Continuous, Three-dimensional Membrane System That Permeates the Entire Cytosol

In profile the ER appears as two parallel membranes with a narrow space, or lumen, between them. This profile of ER should not be confused with a single unit membrane. Each of the parallel ER membranes is itself a unit membrane. The form and abundance of the ER varies greatly from cell to cell, depending on the cell type, its metabolic activity, and its stage of development. For example, cells that store or secrete large quantities of proteins have abundant **rough ER**, which consists of

Esau's Plant Anatomy, Third Edition, By Ray F. Evert.

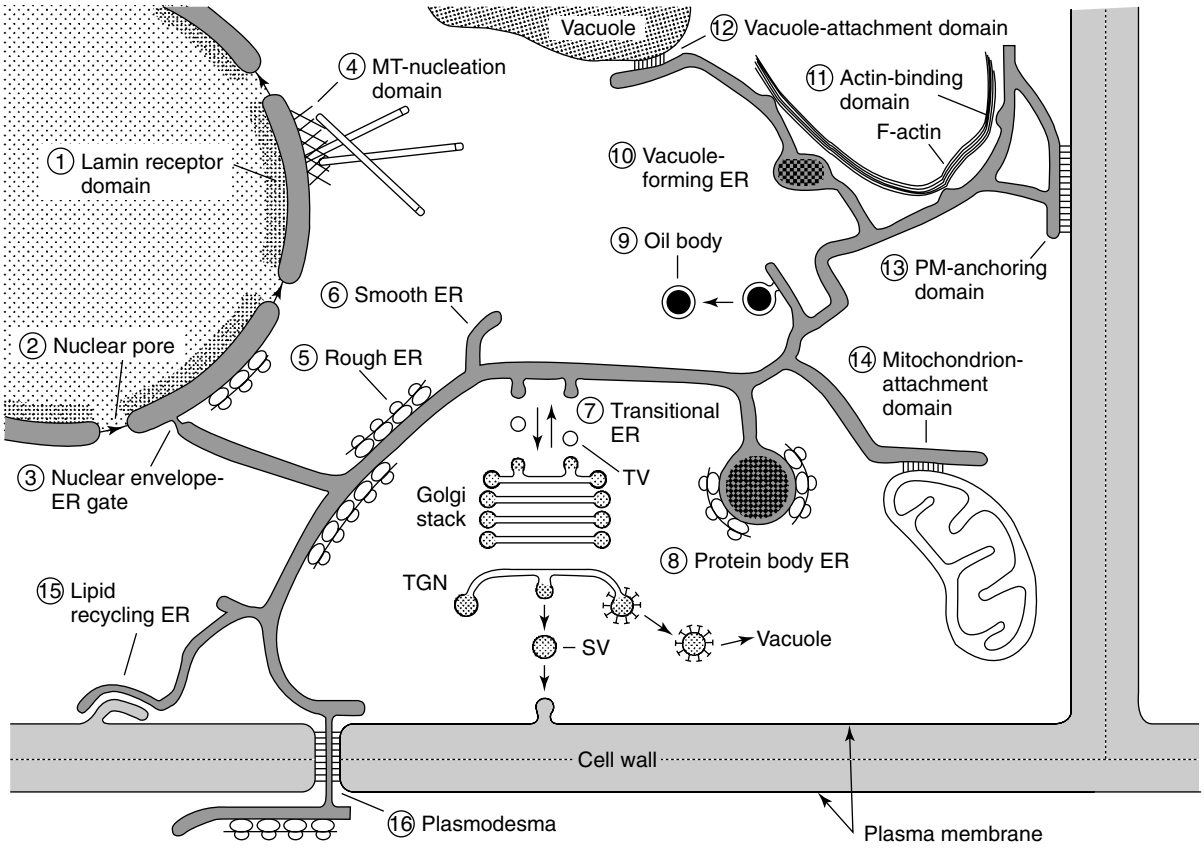

FIGURE 3.1

A diagrammatic representation of the endomembrane system, which includes all membranes except mitochondrial, plastid, and peroxisomal membranes. This drawing depicts 16 types of endoplasmic reticulum (ER) domains. Note the secretory pathway depicted here, involving the endoplasmic reticulum, the Golgi stack, and *trans*-Golgi network (TGN). Other details: TV, transport vesicle; SV, secretory vesicle. (From Staehelin, 1997. © Blackwell Publishing.)

flattened sacs, or ***cisternae*** (singular: ***cisterna***), with numerous ribosomes on their outer surface. In contrast, cells that produce large quantities of lipidic compounds have extensive systems of **smooth ER**, which lacks ribosomes and is largely tubular in form. Both rough and smooth forms of ER occur within the same cell and are physically continuous. Rough and smooth ER are illustrated in Fig. 3.2A and B, respectively.

The ER is a multifunctional membrane system. Staehelin (1997) recognized 16 types of functional ER domains, or subregions, in plant cells (Fig. 3.1). Among those domains are the nuclear pores; the nuclear envelope–ER gates (connections); the transitional ER domain in the vicinity of Golgi bodies; a rough ER domain that acts as the port of entry of proteins into the secretory pathway; a smooth ER domain involved with the synthesis of lipidic molecules, including glycerolipids, isoprenoids, and flavonoids; protein body-forming and oil body-forming domains; a vacuole-forming domain; and the plasmodesmata (Fig. 3.2B), which traverse the common walls between cells and play an important role in cell-to-cell communication (Chapter 4). This list will continue to expand as more cells are investigated by advanced techniques. In 2001 two more domains were added to Staehelin's list, a ricinosome-forming domain (Gietl and Schmid, 2001) and the "nodal ER" domain, which is unique to gravisensing columella rootcap cells (Zheng and Staehelin, 2001). Discovered in senescing endosperm of germinating castor bean (*Ricinus communis*) seeds, the **ricinosomes** bud off the ER at the beginning of programmed cell death and deliver large amounts of a papin-type cysteine endopeptidase to the cytosol in the final stages of cellular disintegration.

An extensive two-dimensional network of ER, consisting of interconnected cisternae and tubules, is located just inside the plasma membrane in the peripheral, or cortical, cytoplasm (Fig. 3.3; Hepler et al., 1990; Knebel et al., 1990; Lichtscheidl and Hepler, 1996; Ridge et al., 1999). The membranes of this **cortical ER** are continuous with those of the ER lying deeper within the

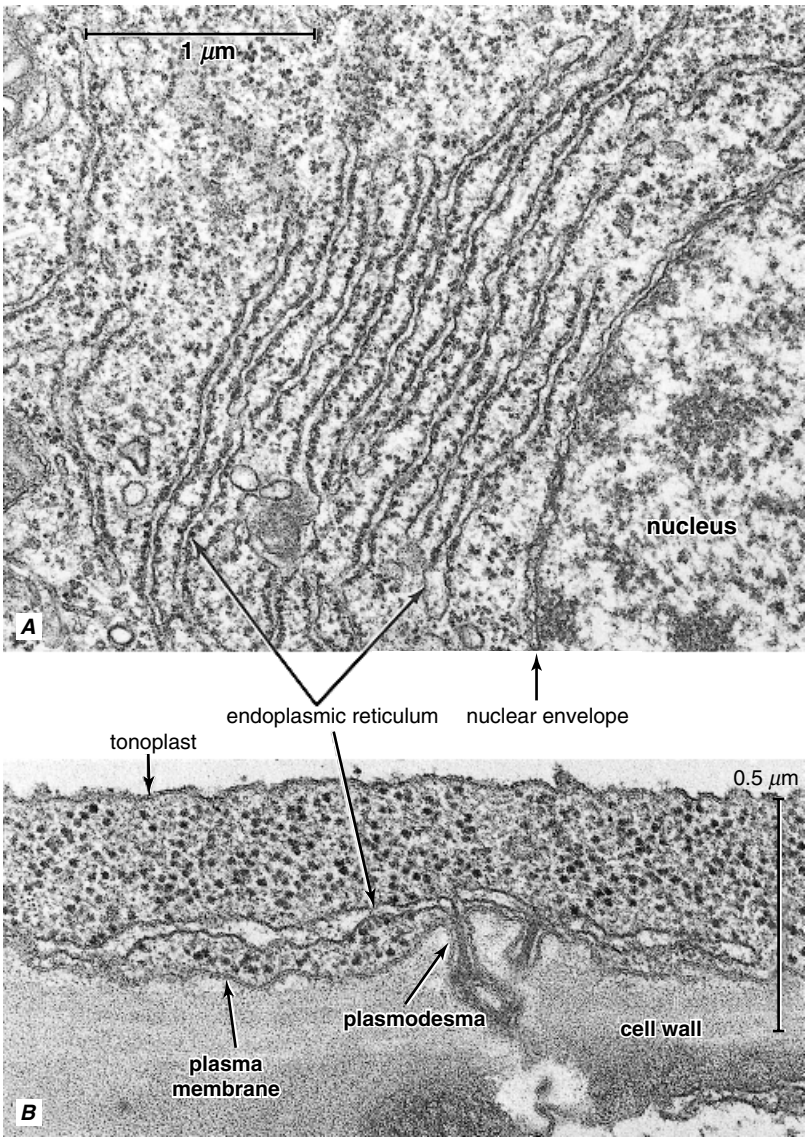

FIGURE 3.2

Endoplasmic reticulum (ER) seen in profile in leaf cells of tobacco (*Nicotiana tabacum*, **A**) and sugar beet (*Beta vulgaris*, **B**). The ER is associated with numerous ribosomes (rough ER) in **A**, with fewer in **B**. The largely smooth ER in **B** is connected to the electron dense cores (desmotubules) of plasmodesmata (seen only in part). Plasma membrane lines the plasmodesmatal canals. Note the three-layered appearance of tonoplast and plasma membrane in **B**. (From Esau, 1977.)

cytosol, including those in the transvacuolar strands of highly vacuolated cells. As mentioned previously, the outer nuclear membrane is also continuous with the ER. Thus the rough and smooth ER along with the nuclear envelope form a membrane continuum that encloses a single lumen and pervades the entire cytosol.

It has been suggested that the network of cortical ER serves as a structural element that stabilizes or anchors the cytoskeleton of the cell (Lichtscheidl et al., 1990). The cortical ER may function in Ca^{2+} regulation; if so, it could play a profound role in a host of developmental and physiological processes (Hepler and Wayne, 1985; Hepler et al., 1990; Lichtscheidl and Hepler, 1996).

Insights into the dynamic nature of the ER have come from studies of living cells, utilizing vital fluorescent dyes such as dihexyloxacarbocyanine iodide (DiOC) (Quader and Schnepf, 1986; Quader et al., 1989; Knebel et al., 1990), which stains endomembranes and, more recently, constructs delivering green fluorescent protein to the ER (Ridge et al., 1999). These studies have revealed that the ER membranes are in continuous motion and are constantly changing their shape and distribution

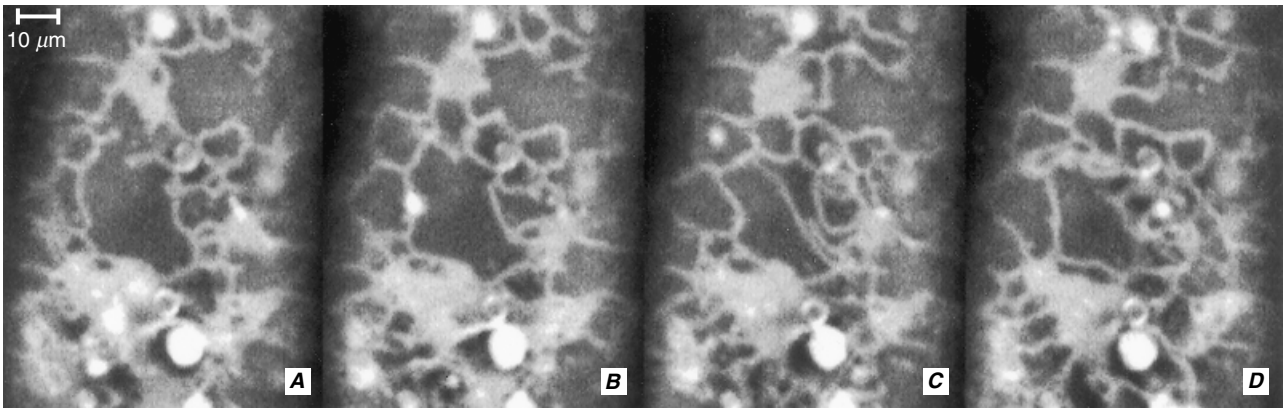

FIGURE 3.3

Four confocal scanning light micrographs of cortical ER membranes of tobacco BY-2 cells. The cells were grown and imaged in suspension culture in the presence of 10 μg of rhodamine 123 per ml. These micrographs, taken at 1 minute intervals, illustrate the changes that have taken place to the organization of the ER during this period of time. (From Hepler and Gunning, 1998.)

(Fig. 3.3). The ER deeper in the cell moves more actively than the cortical ER, which although constantly restructured, does not move with the rest of the ER or the organelles of the deeper streaming cytoplasm. The mobility of the cortical ER is limited by its presumed anchorage at plasmodesmata and by its adhesion to the plasma membrane (Lichtscheidl and Hepler, 1996).

The Golgi Apparatus Is a Highly Polarized Membrane System Involved in Secretion

The term **Golgi apparatus** refers collectively to all of the Golgi body–*trans*-Golgi network complexes of a cell. Golgi bodies are also called ***dictyosomes*** or simply ***Golgi stacks***.

Each **Golgi body** consists of five to eight stacks of flattened cisternae, which often have bulbous and fenestrated margins (Fig. 3.4). The Golgi stacks are polarized structures. The opposite surfaces or poles of a stack are referred to as *cis*- and *trans*-faces. Three morphologically distinct cisternae may be recognized across the stack: *cis*-, medial-, and *trans*-cisternae, which differ from one another both structurally and biochemically (Driouich and Staehelin, 1997; Andreeva et al., 1998). The ***trans*-Golgi network (TGN)**, a tubular reticulum with clathrin-coated and noncoated budding vesicles, is associated with the *trans*-face of the Golgi stack (Fig. 3.1). Each Golgi-TGN complex is embedded in and surrounded by a ribosome-free zone called the ***Golgi matrix***.

Unlike the centralized Golgi of mammalian cells, the Golgi apparatus of plant cells consists of many separate stacks that remain functionally active during mitosis and cytokinesis (Andreeva et al., 1998; Dupree and

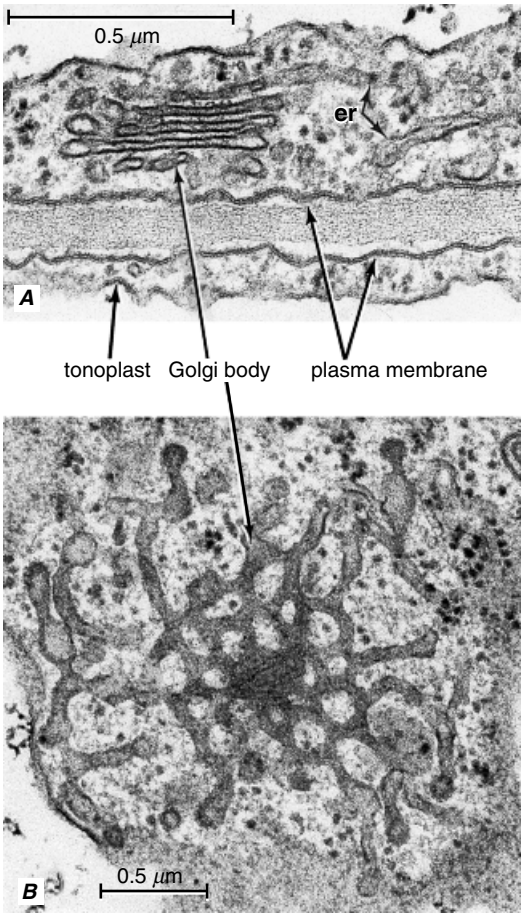

FIGURE 3.4

Golgi bodies from a tobacco (*Nicotiana tabacum*) leaf. **A**, Golgi body in profile with the fenestrated *trans*-face toward the cell wall. **B**, Golgi body is seen from its fenestrated *trans*-face. Some of the vesicles to be pinched off are coated. Detail: er, endoplasmic reticulum. (From Esau, 1977.)

Sherrier, 1998). In living cells, stacks tagged with green fluorescent protein can be observed along bundles of actin filaments that match precisely the architecture of the ER network (Boevink et al., 1998). The stacks have been observed undergoing stop-and-go movements, oscillating rapidly between directed movement and random "wiggling." Nebenführ et al. (1999) have postulated that the stop-and-go motion of the Golgi-TGN complexes is regulated by "stop signals" produced by ER export sites and locally expanding cell wall domains to optimize ER to Golgi and Golgi to cell wall trafficking. During mitosis and cytokinesis, the Golgi stacks redistribute to specific locations as cytoplasmic streaming stops (Chapter 4; Nebenführ et al., 2000). Just prior to mitosis, the number of Golgi stacks doubles by cisternal fission, which takes place in a *cis*-to-*trans* direction (Garcia-Herdugo et al., 1988).

In most plant cells the Golgi apparatus serves two major functions: the synthesis of noncellulosic cell wall polysaccharides (hemicelluloses and pectins; Chapter 4) and protein glycosylation. Evidence obtained through the use of polyclonal antibodies indicates that different steps in polysaccharide synthesis occur in different cisternae of the Golgi body (Moore et al., 1991; Zhang and Staehelin, 1992; Driouich et al., 1993). The different polysaccharides are packaged in secretory vesicles, which migrate to and fuse with the plasma membrane (exocytosis). The vesicles then discharge their contents and the polysaccharides become part of the cell wall. In enlarging cells the vesicles contribute to growth of the plasma membrane.

The initial stage of protein glycosylation occurs in the rough ER. These glycoproteins then are transferred from the ER to the *cis*-face of the Golgi body via transition vesicles (Bednarek and Raikhel, 1992; Holtzman, 1992; Schnepf, 1993). The glycoproteins proceed stepwise across the stack to the *trans*-face and then are sorted in the TGN for delivery to the vacuole or for secretion at the cell surface. Polysaccharides destined for secretion at the cell surface are also packaged into vesicles at the TGN. A given Golgi body can process polysaccharides and glycoproteins simultaneously.

Glycoproteins and complex polysaccharides destined for secretion into the cell wall are packaged in noncoated, or smooth-surfaced, vesicles, whereas hydrolytic enzymes and storage proteins (water-soluble globulins) destined for vacuoles are packaged at the TGN into clathrin-coated vesicles and smooth, electron-dense vesicles, respectively (Herman and Larkins, 1999; Miller and Anderson, 1999; Chrispeels and Herman, 2000). The formation of Golgi-derived **dense vesicles** is not restricted to the TGN, but may also occur in the *cis*-cisternae (Hillmer et al., 2001).

Some types of storage proteins (alcohol-soluble prolamins) form aggregates and are packaged into vesicles in the ER from where they are transported directly to the protein storage vacuoles, bypassing the Golgi (Matsuoka and Bednarek, 1998; Herman and Larkins, 1999). In wheat, for example, a considerable amount of the prolamin aggregates directly into protein bodies (aleurone grains) within the rough ER, and then the protein bodies are transported intact to the vacuoles without Golgi involvement (Levanony et al., 1992). In maize, sorghum, and rice similarly formed protein bodies remain within the ER and are bounded by ER membranes (Vitale et al., 1993).

The delivery of secretory vesicles to the plasma membrane by exocytosis must be balanced by the equivalent recycling of membranes from the plasma membrane by clathrin-mediated endocytosis (Battey et al., 1999; Marty, 1999; Sanderfoot and Raikhel, 1999). Recycling is essential to support a functional endomembrane system (Battey et al., 1999).

CYTOSKELETON

The **cytoskeleton** is a dynamic, three-dimensional network of protein filaments that extends throughout the cytosol and is intimately involved in many cellular processes, including mitosis and cytokinesis, cell expansion and differentiation, cell-to-cell communication, and the movement of organelles and other cytoplasmic components from one location to another within the cell (Seagull, 1989; Derksen et al., 1990; Goddard et al., 1994; Kost et al., 1999; Brown and Lemmon, 2001; Kost and Chua, 2002; Sheahan et al., 2004). In plant cells it consists of at least two types of protein filaments: microtubules and actin filaments. The presence of intermediate filaments, which occur in animal cells, has not been unequivocally demonstrated in plant cells. Immunofluorescence microscopy and, more recently, the use of green fluorescent protein tags to cytoskeletal proteins and confocal microscopy, have made it possible to examine the three-dimensional organization of the cytoskeleton in both fixed and living cells, and have contributed greatly to our understanding of both cytoskeletal structure and function (Lloyd, 1987; Staiger and Schliwa, 1987; Flanders et al., 1990; Marc, 1997; Collings et al., 1998; Kost et al., 1999; Kost et al., 2000).

Microtubules Are Cylindrical Structures Composed of Tubulin Subunits

Microtubules are cylindrical structures about 24 nanometers in diameter and of varying lengths (Fig. 3.5). The lengths of cortical microtubules, that is, of microtubules located in the peripheral cytoplasm just inside the plasma membrane, generally correspond to the cross-sectional width of the cell facet with which they are associated (Barlow and Baluška, 2000). Each microtubule is composed of two different types of protein molecules, alpha (α) tubulin and beta (β) tubulin. These

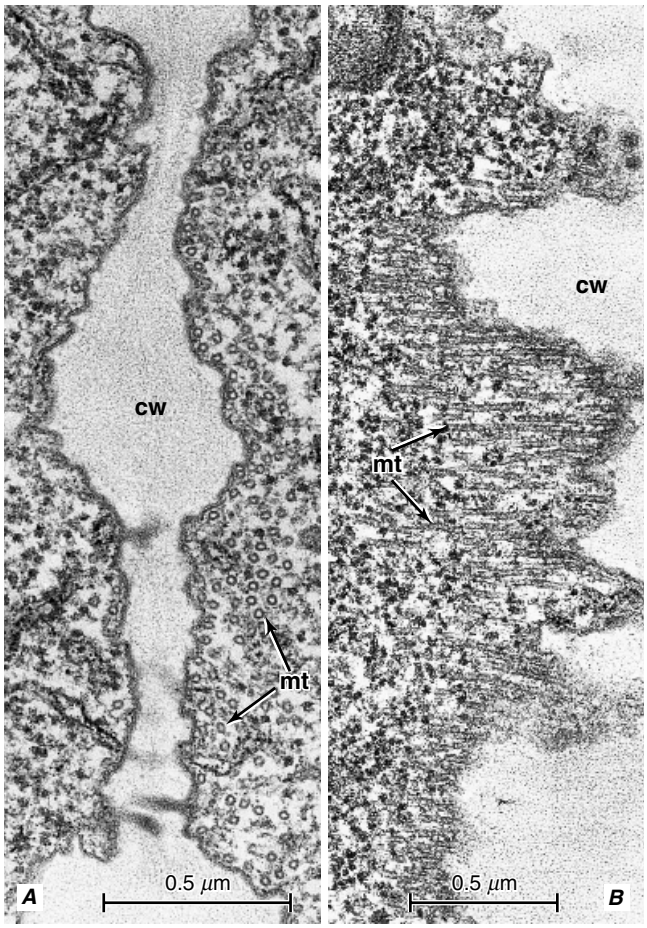

FIGURE 3.5

Cortical microtubules (mt) in *Allium cepa* root tip cells seen in transverse (**A**) and longitudinal (**B**) views. Other detail: cw, cell wall.

subunits come together to form soluble dimers ("two parts"), which then self-assemble into insoluble tubules. The subunits are arranged in a helix to form 13 rows, or "protofilaments," around the core of lightly contrasted material. Within each protofilament the subunits are oriented in the same direction, and all of the protofilaments are aligned in parallel with the same polarity; consequently the microtubule is a polar structure for which there can be designated plus and minus ends. The plus ends grow faster than the minus ends, and the ends of the microtubules can alternate between growing and shrinking states, a behavior called **dynamic instability** (Cassimeris et al., 1987). Indeed microtubules are dynamic structures that undergo regular sequences of breakdown, re-formation, and rearrangement into new configurations, or arrays, at specific points in the cell cycle and during differentiation (Hush et al., 1994; Vantard et al., 2000; Azimzadeh et al., 2001). The most prominent cell-cycle arrays are the interphase cortical array, the preprophase band, the mitotic spindle, and the phragmoplast, which is located between the two newly formed daughter nuclei (Fig. 3.6; Chapter 4; Baskin and Cande, 1990; Barlow and Baluška, 2000; Kumagai and Hasezawa, 2001).

Microtubules have many functions (Wasteneys, 2004). In enlarging and differentiating cells, the cortical microtubules control the alignment of cellulose microfibrils that are being added to the wall, and the direction of cell expansion is governed, in turn, by this alignment of cellulose microfibrils in the wall (Chapter 4; Mathur and Hülskamp, 2002). In addition microtubules that make up the fibers of the mitotic spindle play a role in chromosome movement, and those forming the phragmoplast, probably with the help of kinesin-like motor proteins (Otegui et al., 2001), are involved it the formation of the cell plate (the initial partition between dividing cells).

During most of the cell cycle (interphase) microtubules radiate from all over the nuclear surface, which is the primary "nucleating site," or **microtubular organizing center (MTOC)** in the plant cell. Secondary MTOCs are located at the plasma membrane where they organize arrays of cortical microtubules, which are essential for ordered cell wall synthesis and hence for cellular morphogenesis (Wymer and Lloyd, 1996; Wymer et al., 1996). It has been suggested that the material comprising the secondary MTOCs is translocated to the cell periphery by the microtubules organized and radiating from the nuclear surface (the primary MTOC) (Baluška et al., 1997b, 1998). γ-Tubulin, which is found in all MTOCs, is believed to be essential for microtubule nucleation (Marc, 1997).

Actin Filaments Consist of Two Linear Chains of Actin Molecules in the Form of a Helix

Actin filaments, also called **microfilaments** and **filamentous actin (*F actin*)**, are, like microtubules, polar structures with distinct plus and minus ends. They are composed of actin monomers that self-assemble into filaments and resemble a double-stranded helix, with an average diameter of 7 nanometers (Meagher et al., 1999; Staiger, 2000). Actin filaments occur singly and in bundles (Fig. 3.7). Actin filaments constitute a cytoskeleton system that can assemble and function independently of microtubules (e.g., actin filaments drive cytoplasmic streaming and Golgi dynamics). However, in some instances actin and microtubules can work together to perform specific functions. Some actin filaments are associated spatially with microtubules and, like microtubules, form new configurations, or arrays, at specific points in the cell cycle (Staiger and Schliwa, 1987; Lloyd, 1988; Baluška et al., 1997a; Collings et al., 1998). In cells of the transition region—a postmitotic zone interpolated between the meristem and the rapidly

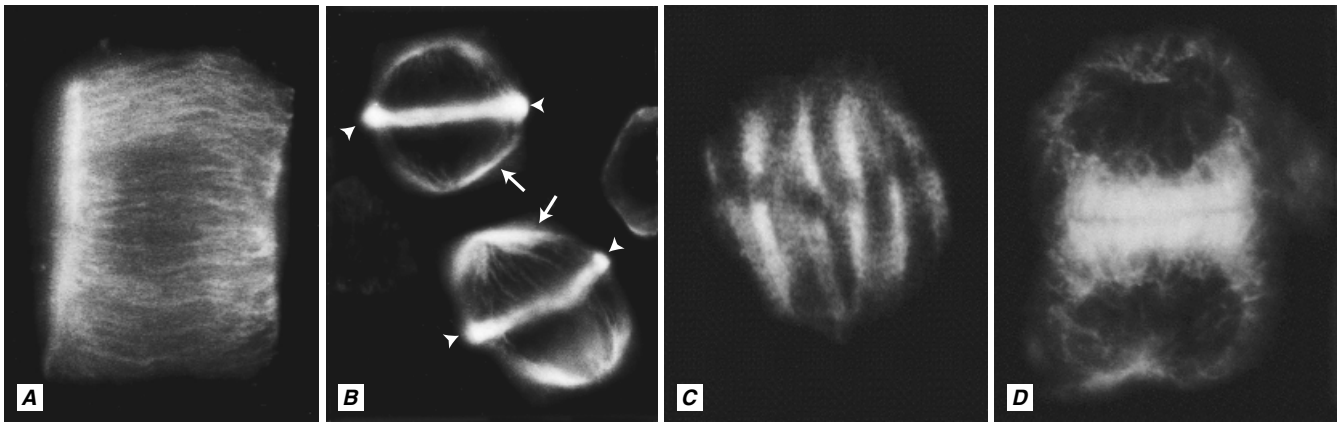

FIGURE 3.6

Fluorescence micrographs of microtubular arrangements, or arrays, in root tips of onion (*Allium cepa*). **A**, interphase cortical array. The microtubules lie just beneath the plasma membrane. **B**, a preprophase band of microtubules (arrowheads) encircles the nucleus at the site of the future cell plate. The prophase spindle, comprised of other microtubules (arrows), outlines the nuclear envelope (not visible). The lower cell is at a later stage than the upper one. **C**, the mitotic spindle at metaphase. **D**, during telophase new microtubules form a phragmoplast, which is involved with cell plate formation. (Reprinted with permission from Goddard et al., 1994. © American Society of Plant Biologists.)

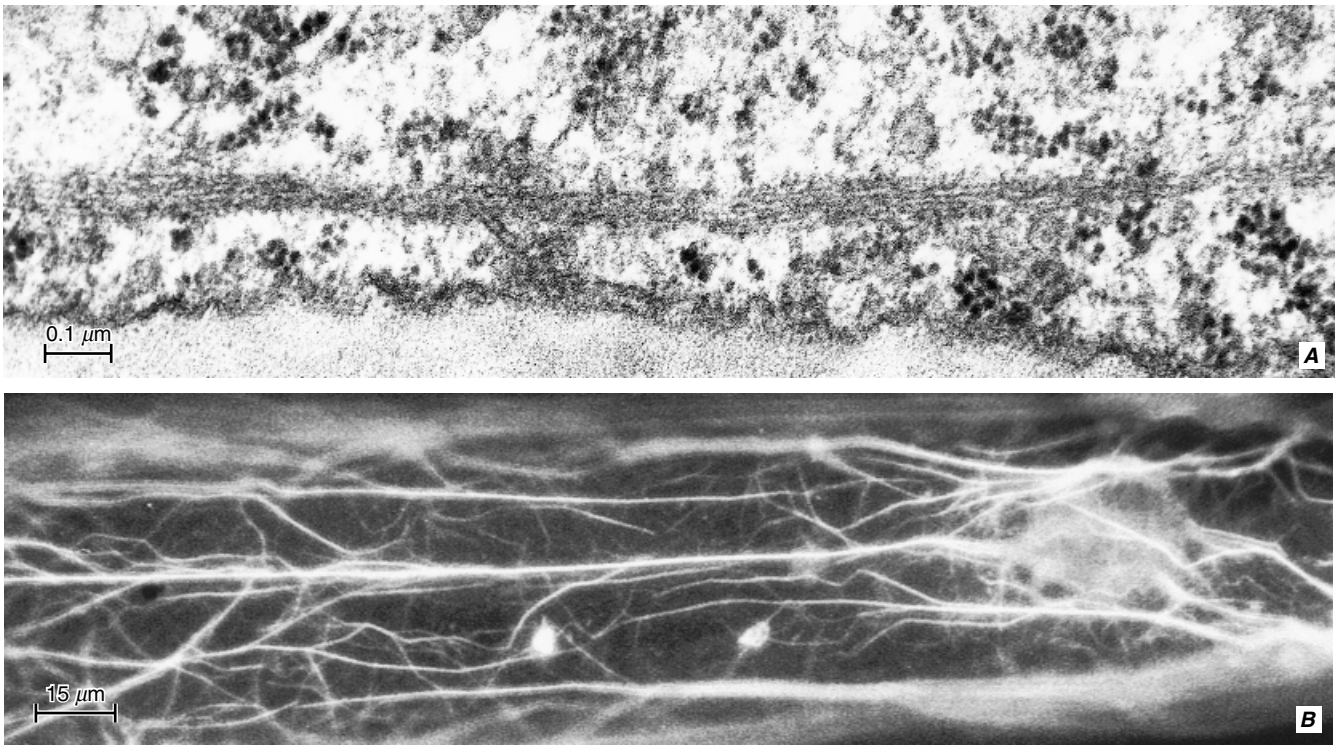

FIGURE 3.7

Actin filaments. **A**, a bundle of actin filaments as revealed in an electron micrograph of a leaf cell of maize (*Zea mays*). **B**, several bundles of actin filaments as revealed in a fluorescence micrograph of a stem hair of tomato (*Solanum lycopersicum*). (**B**, from Parthasarathy et al., 1985.)

elongating region—of growing maize root tips, the nuclear surface and the cortical cytoplasm associated with the two end walls have been identified as the principal organizing regions of bundles of actin filaments (Baluška et al., 1997a).

The actin cytoskeleton has been implicated in a variety of roles in plant cells, in addition to the causative role it plays—in association with myosin motor proteins (Shimmen et al., 2000)—in cytoplasmic streaming and in the movement of plastids, vesicles (Jeng and Welch, 2001), and other cytoplasmic components. Other demonstrated or proposed roles include establishing cell polarity, division plane determination (by positioning the preprophase band), cell signaling (Drøbak et al., 2004), tip growth of pollen tubes and root hairs (Kropf et al., 1998), control of plasmodesmal transport (White et al., 1994; Ding et al., 1996; Aaziz et al., 2001), and mechanosensation processes such as touch responses of leaves (Xu et al., 1996) and the grasping of support-gyrating tendrils (Engelberth et al., 1995).

STORED COMPOUNDS

All compounds stored by plants are products of metabolism. Sometimes collectively referred to as ergastic substances, these compounds may appear, disappear, and reappear at different times in the life of a cell. Most are storage products, some are involved in plant defenses, and a few have been characterized as waste products. In most instances they form structures that are visible in light and/or electron microscopes, including starch grains, protein bodies, oil bodies, tannin-filled vacuoles, and mineral matter in the form of crystals. These substances are found in the cell wall, in the cytosol, and in organelles, including vacuoles.

Starch Develops in the Form of Grains in Plastids

Next to cellulose, **starch** is the most abundant carbohydrate in the plant world. Moreover it is the principal storage polysaccharide in plants. During photosynthesis assimilatory starch is formed in chloroplasts (Fig. 3.8). Later it is broken down into sugars, transported to storage cells, and resynthesized as storage starch in amyloplasts (Fig. 3.9). As mentioned previously, an amyloplast may contain one (simple) or more (compound) starch grains. If several starch grains develop together, they may become enclosed in common outer layers, forming a complex starch grain (Ferri, 1974).

Starch grains, or granules, are varied in shape and size and commonly show layering around a point, the ***hilum***, which may be the center of the grain or to one side (Fig. 3.9A). Fractures, often radiating from the hilum, appear during dehydration of the grains. All grains consist of two types of molecules, unbranched amylose chains and branched amylopectin molecules

FIGURE 3.8

A chloroplast containing assimilatory starch (s), from a mesophyll cell of the pigweed (*Amaranthus retroflexus*) leaf. During periods of intense photosynthesis some of the carbohydrate is stored temporarily in the chloroplast as grains of assimilatory starch. At night sucrose is produced from the starch and exported from the leaf to other parts of the plant, where it is eventually used for the manufacture of other molecules needed by the plant. (From Fisher and Evert, 1982. © 1982 by The University of Chicago. All rights reserved.)

(Martin and Smith, 1995). The layering of starch grains is attributed to an alternation of these two polysaccharide molecules. The layering is accentuated when the starch grain is placed in water because of differential swelling of the two substances: amylose is soluble in water, and amylopectin is not. Amylose appears to be the predominant component of starch found in the leaves of sorghum (*Sorghum bicolor*) and maize (*Zea*

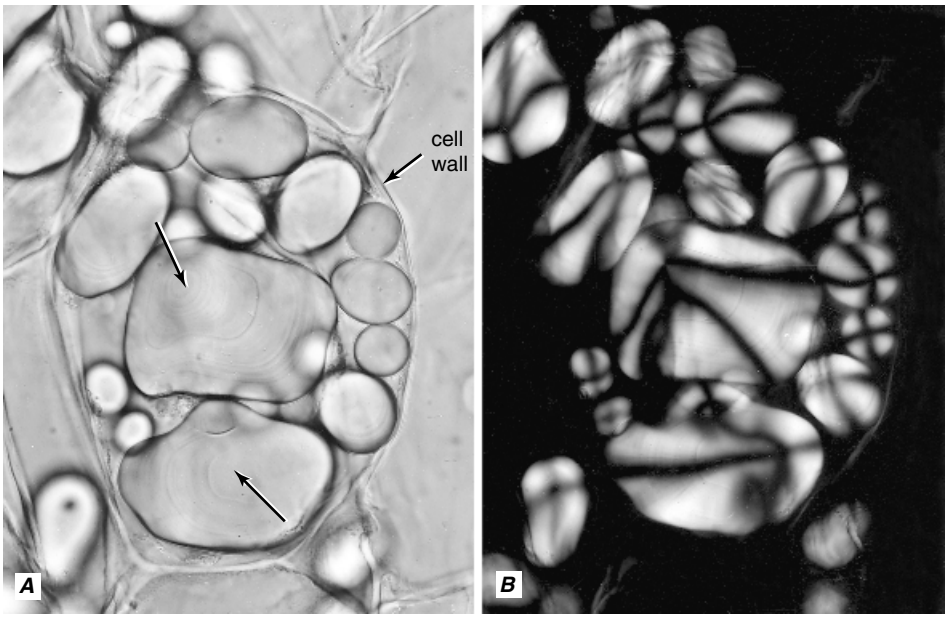

FIGURE 3.9

Starch grains of potato (*Solanum tuberosum*) tuber photographed with ordinary light (**A**) and with polarized light (**B**). Arrows point to the hilum of some starch grains in **A**. In **B** the starch grains show the figure of a Maltese cross. The amyloplasts in potato each contain a single starch grain. (A, B, ×620.)

mays), whereas the seeds contain 70% to 90% amylopectin (Vickery and Vickery, 1981). In potato tuber starch their proportion is 22% amylose and 78% amylopectin (Frey-Wyssling, 1980). Starch grains are composed of amorphous and crystalline regions, whose chains are held together by hydrogen bonds. Under polarized light, starch grains show a figure of a Maltese cross (Fig. 3.9B) (Varriano-Marston, 1983). Starch commonly stains bluish-black with iodine in potassium iodide (I_2KI).

Storage starch occurs widely in the plant body. It is found in parenchyma cells of the cortex, pith, and vascular tissues of roots and stems; in parenchyma cells of fleshy leaves (bulb scales), rhizomes, tubers, corms, fruits, and cotyledons; and in the endosperm of seeds. Commercial starches are obtained from various sources as, for example, the endosperm of cereals, fleshy roots of the tropical *Manihot esculenta* (tapioca starch), tubers of potato, tuberous rhizomes of *Maranta arundinacea* (arrowroot starch), and stems of *Metroxylon sagu* (sago starch).

The Site of Protein Body Assembly Depends on Protein Composition

Storage proteins may be formed in different ways, depending in part upon whether they are composed of salt-soluble globulins or alcohol-soluble prolamins (Chrispeels, 1991; Herman and Larkins, 1999; Chrispeels and Herman, 2000). Globulins are the major storage proteins in legumes, and prolamins in most cereals. Typically globulins aggregate in protein storage vacuoles after having been transported there from the rough ER via the Golgi apparatus. However, as indicated previously, the Golgi apparatus is not necessarily involved with prolamin transport to the vacuoles in cereals. In wheat, for example, a considerable part of the prolamins aggregate directly into **protein bodies** (aleurone grains) within the rough ER and then are transported in distinct vesicles to the vacuoles without Golgi involvement (Levanony et al., 1992). In other cereals, such as maize, sorghum, and rice, similarly formed protein bodies are not transported to vacuoles, but remain within the rough ER and bounded by ER membranes (ER domain 8, Fig. 3.1) (Vitale et al., 1993). Upon germination the stored proteins are mobilized by hydrolysis to provide energy, nitrogenous compounds, and minerals needed by the growing seedling. At the same time the protein storage vacuoles may function as lysosomal compartments, or autophagic organelles (Herman et al., 1981), taking up and digesting portions of the cytoplasm. As germination continues, the numerous small vacuoles may fuse to form one large vacuole. Although protein bodies are most abundant in seeds, they also occur in roots, stems, leaves, flowers, and fruits.

Structurally the simplest protein bodies consist of an amorphous proteinaceous matrix surrounded by a bounding membrane. Other protein bodies may contain

one or more nonproteinaceous globoids (Fig. 3.10) or one or more globoids and one or more protein crystalloids, in addition to the proteinaceous matrix. Protein bodies also contain a large number of enzymes and fair amounts of phytic acid, a cation salt of myo-inositol hexaphosphoric acid, which usually is stored in the globoids. Phytic acid is an important source of phosphorous during seedling development. Some protein bodies contain calcium oxalate crystals (Apiaceae).

Proteins may occur in the form of crystalloids in the cytosol as, for example, in parenchyma cells of the potato tuber, among starch grains of *Musa*, and in the fruit parenchyma of *Capsicum*. In the potato tuber the cuboidal protein crystals typically are found in subphellogen cells. The crystals apparently are formed within vesicles from which they may or may not be released into the cytosol at maturity (Marinos, 1965; Lyshede, 1980). Proteinaceous crystalloids also occur in the nuclei. Such nuclear inclusions are widespread in occurrence among vascular plants (Wergin et al., 1970).

Oil Bodies Bud from Smooth ER Membranes by an Oleosin-mediated Process

Oil bodies are more or less spherical structures that impart a granular appearance to the cytoplasm of a plant cell when viewed with the light microscope. In electron micrographs the oil bodies have an amorphous appearance (Fig. 3.10). Oil bodies are widely distributed throughout the plant body but are most abundant in fruits and seeds. Approximately 45% of the weight of sunflower, peanut, flax, and sesame seed is composed of oil (Somerville and Browse, 1991). The oil provides energy and a source of carbon to the developing seedling.

Oil bodies, also known as spherosomes or oleosomes, arise by the accumulation of ***triacylglycerol molecules*** at specific sites (ER domain 9, Fig. 3.1) in the interior of the ER lipid bilayer (Wanner and Thelmer, 1978; Ohlrogge and Browse, 1995). These lipid-accumulation sites are defined by the presence of 16 to 25 kDa integral membrane proteins known as ***oleosins***, thumbtack-like

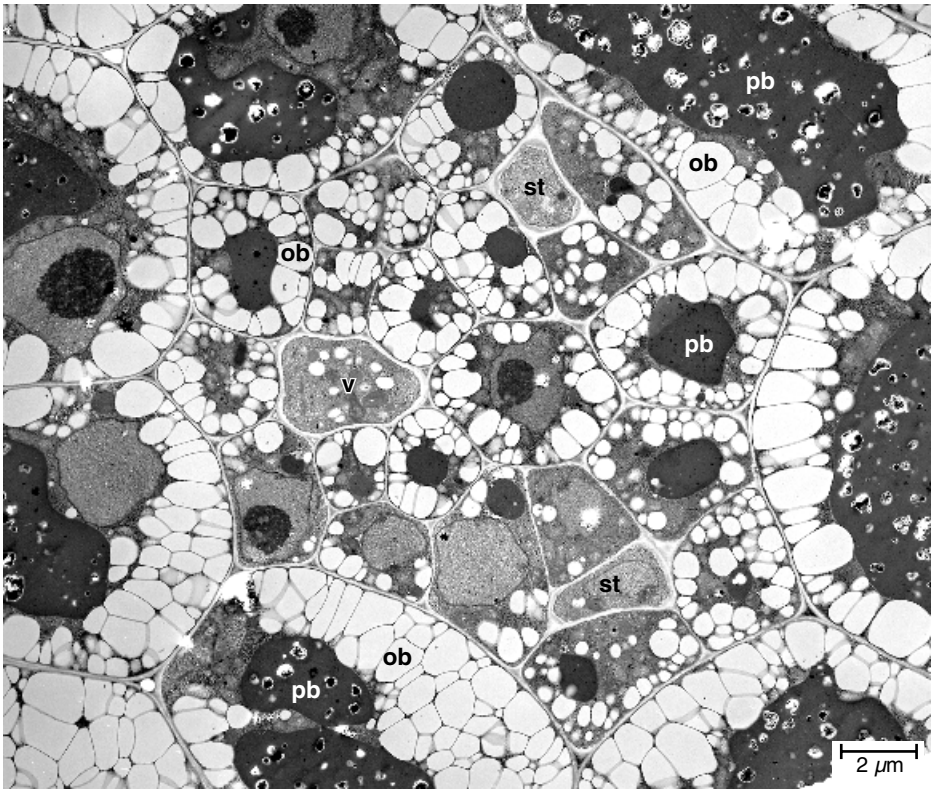

FIGURE 3.10

Immature vascular bundle, surrounded by storage parenchyma cells, in cotyledon of *Arabidopsis thaliana* embryo. Oil bodies (ob) and globoid-containing protein bodies (pb) occupy most of the volume of the procambial cells and storage parenchyma cells. Other details: st, immature sieve tube; v, immature vessel. (From Busse and Evert, 1999. © 1999 by The University of Chicago. All rights reserved.)

molecules that cause the oil bodies to bud into the cytosol (Huang, 1996). Each oil body is bounded by a phospholipid monolayer in which the oleosins are embedded (Sommerville and Browse, 1991; Loer and Herman, 1993). The oleosins and phospholipids stabilize the oil bodies and prevent them from coalescing (Tzen and Huang, 1992; Cummins et al., 1993). Maintaining the oil bodies as small entities ensures ample surface area for the attachment of lipases and rapid mobilization of the triacylglycerols when necessary.

Storage lipids occur in all plant taxa and are probably present in every cell, at least in small amounts (Küster, 1956). Usually they are found in liquid form as oil bodies. Crystalline forms are rare. An example was reported for the endosperm of the palm *Elais*, in which the cells were filled with short needle-shaped crystals of fat (Küster, 1956). (The distinction between fats and oils is primarily physical, fats being solid at room temperature and oils liquid.) So-called essential oils are volatile oils that contribute to the essence, or odor, of plants. They are made by special cells and excreted into intercellular cavities (Chapter 17). Oils and fats may be identified by a reddish color when they are treated with Sudan III or IV.

Mention should be made of **waxes**, long-chain lipid compounds, that occur as part of the protective coating (cuticle) on the epidermis of the aerial parts of the primary plant body and on the inner surface of the primary wall of cork cells in woody roots and stems. These waxes constitute a major barrier to water loss from the surface of the plant (Chapter 9). By reducing the wetability of leaves, they also reduce the ability of fungal spores to germinate and of bacteria to grow, thereby reducing the possibility of these agents to cause disease. Most plants contain too little wax to be valuable for commercial use. Exceptions are the palm *Copernicia cerifera*, which yields the carnauba wax of commerce, and *Simmondia chinensis* (jojoba), the cotyledons of which contain a liquid wax similar in quality to the oil of the sperm whale (Rost et al., 1977; Rost and Paterson, 1978).

Tannins Typically Occur in Vacuoles but Also Are Found in Cell Walls

Tannins are a heterogeneous group of polyphenolic substances, important secondary metabolites, with an astringent taste and an ability to tan leather. They usually are divided into two categories, hydrolyzable and condensed. The hydrolyzable tannins can be hydrolyzed with hot, dilute acid to form carbohydrates (mainly glucose) and phenolic acids. The condensed tannins cannot be hydrolyzed. In some of their forms, tannins are quite conspicuous in sectioned material. They appear as coarsely or finely granular material or as bodies of various sizes colored yellow, red, or brown. No tissue appears to lack tannins completely. Tannins are abundant in leaves of many plants, in vascular tissues, in the periderm, in unripe fruits, in seed coats, and in pathologic growths like galls (Küster, 1956). Typically they occur in the vacuole (Fig. 2.21) but apparently originate in the ER (Zobel, 1985; Rao, 1988). Tannins may be present in many cells of a given tissue or isolated in specialized cells (tannin idioblasts) scattered throughout the tissue (Gonzalez, 1996; Yonemori et al., 1997). In addition they may be located in much enlarged cells called tannin sacs or in tube-like cells (Chapter 17).

Most of the vegetable extracts used for tanning come from a few eudicotyledonous plants, in particular, from the wood, bark, leaves, and/or fruit of species in the Anacardiaceae, Fabaceae, and Fagaceae (Haslam, 1981). Apparently the primary function of tannins is protective, their astringency serving as a repellent to predators and an impediment to the invasion of parasitic organisms by immobilizing extracellular enzymes. Plants that produce and secrete substantial quantities of polyphenols, including tannins, may exclude other plant species from growing under them or in their near vicinity, a phenomenon known as **allelopathy**. Tannins released from leaves decaying in water are known to be harmful against some insects (Ayres et al., 1997), including phytophagous lepidopteran larvae (Barbehenn and Martin, 1994). They apparently play an important role in habitat selection among mosquito communities from Alpine hydrosystems (Rey et al., 2000).

Phenolic compounds, mainly tannins, were synthesized in increased amounts in the leaves of beech trees (*Fagus sylvatica*) in response to environmental stress (Bussotti et al., 1998). They initially accumulated in the vacuoles, especially in those of the upper epidermis and palisade parenchyma. At a later stage the tannins appeared to be solubilized in the cytosol and retranslocated, eventually impregnating the outer epidermal cell walls. The impregnation of the walls by the tannins has been interpreted as an impermeabilization mechanism associated with a reduction in cuticular transpiration. The browning associated with growing jack pine (*Pinus banksiana*) and eucalypt (*Eucalyptus pilularis*) roots has been shown to be due to the deposition of condensed tannins in the walls of all cells external to the vascular cylinder (McKenzie and Peterson, 1995a, b). The epidermal and cortical cells in the brown "tannin zone" of the roots are dead. Condensed tannins also have been found in the *phi* thickenings of *Ceratonia siliqua* roots (Pratikakis et al., 1998). *Phi* thickenings are reticulate or band-like wall thickenings on cortical cells of certain gymnosperms (Ginkgoaceae, Araucariaceae, Taxaceae, and Cupressaceae; Gerrath et al., 2002) and a few species of angiosperms such as *Ceratonia siliqua, Pyrus malus (Malus domestica),* and *Pelargonium hortorum* (Peterson et al., 1981).

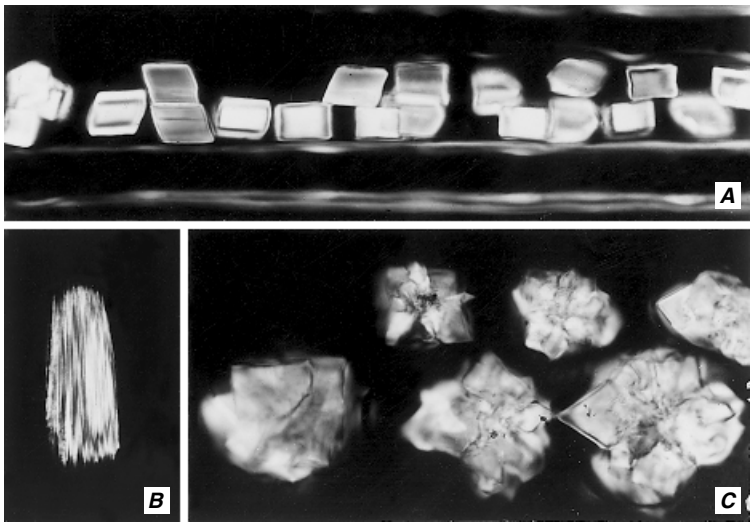

FIGURE 3.11

Calcium oxalate crystals seen in polarized light. **A**, prismatic crystals in phloem parenchyma of root of *Abies*. **B**, raphides in leaf of *Vitis*. **C**, druses in cortex of stem of *Tilia*. (A, ×500; B, C, ×750.)

Crystals of Calcium Oxalate Usually Develop in Vacuoles but Also Are Found in the Cell Wall and Cuticle

Inorganic deposits in plants consist mostly of calcium salts and anhydrides of silica. Among the calcium salts, the most common is **calcium oxalate,** which occurs in the majority of plant families, notable exceptions being the Cucurbitaceae and some families of Liliales, Poales, and all Alismatidae (Prychid and Rudall, 1999). Calcium oxalate occurs as mono- and dihydrate salts in many crystalline forms. The monohydrate is the more stable and is more commonly found in plants than is the dihydrate. The most common forms of calcium oxalate crystals are (1) **prismatic crystals** (Fig. 3.11A), variously shaped prisms, usually one per cell; (2) **raphides** (Figs. 3.11B and 3.12A), needle-shaped crystals that occur in bundles; (3) **druses** (Figs. 3.11C and 3.12B), spherical aggregates of prismatic crystals; (4) **styloids,** elongated crystals with pointed or ridged ends, one or two to a cell; and (5) **crystal sand,** very small crystals, usually in masses. In some tissues calcium oxalate crystals arise in cells that resemble adjacent, crystal-free cells. In others, the crystals are formed in cells—**crystal idioblasts**—specialized to produce crystals. Crystal idioblasts contain an abundance of ER and Golgi bodies. Most crystal cells are probably alive at maturity. The location and type of calcium oxalate crystals within a given taxon may be very consistent and, hence, useful in taxonomic classification (Küster, 1956; Prychid and Rudall, 1999; Pennisi and McConnell, 2001).

Calcium oxalate crystals usually develop in vacuoles. The period of crystal cell differentiation may

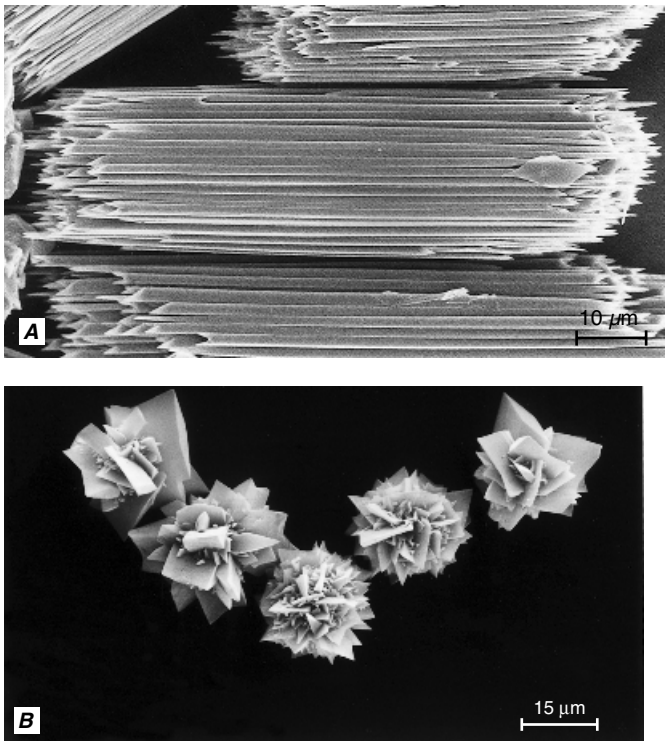

FIGURE 3.12

Scanning electron micrographs (**A**) of raphide bundle isolated from grape (*Vitis mustangensis*) fruit and (**B**) druses from *Cercis canadensis* epidermal cells. (**A**, from Arnott and Webb, 2000. © 2000 by The University of Chicago. All rights reserved.; **B**, courtesy of Mary Alice Webb.)

correspond to that of neighboring cells, precede that of neighboring cells, or occur belatedly. The latter phenomenon is common in the nonconducting phloem in the bark of many trees and is associated with belated sclerification of many of the same cells (Chapter 14). Crystal formation commonly is preceded by the formation of some type of membrane system, or complex, that arises de novo in the vacuole and forms one or more crystal chambers (Franceschi and Horner, 1980; Arnott, 1982; Webb, 1999; Mazen et al., 2003). In raphide cells each crystal is included in an individual chamber (Fig. 3.13; Kausch and Horner, 1984; Webb et al., 1995). In addition to the crystals the vacuoles may contain mucilage (Kausch and Horner, 1983; Wang et al., 1994; Webb et al., 1995). A further stage of development may involve the deposition of a cell wall around the crystal, completely isolating the crystal from the protoplast (Ilarslan et al., 2001).

Horner and Wagner (1995) recognized two general systems of vacuolar crystal formation based in part on the presence or absence of membrane complexes in the vacuoles. System I, which is exemplified by the druses in *Capsicum* and *Vitis*, the raphides in *Psychotria*, and crystal sand in *Beta*, all eudicotyledons, is characterized by the presence of vacuolar membrane complexes and of organic paracrystalline bodies that display subunits with large periodicity. System II, which is characterized by the absence of vacuolar membrane complexes and the presence of paracrystalline bodies with closely spaced subunits, is exemplified by the raphide crystal idioblasts in *Typha, Vanilla, Yucca* (Horner and Wagner, 1995), and *Dracaena* (Pennisi et al., 2001b), all monocots.

Although uncommon in flowering plants, deposition of crystals in the cell wall and cuticle rather than in vacuoles is of frequent occurrence in conifers (Evert et al., 1970; Oladele, 1982). Among the angiosperms, calcium oxalate crystals have been reported in the cuticle of *Causarina equisetifolia* (Pant et al., 1975) and of some Aizoaceae (Öztig, 1940), between the primary epidermal cell wall and the cuticle in *Dracaena* (Pennisi et al., 2001a), and between the primary and secondary walls of the astrosclereids in *Nymphaea* and *Nuphar* (Arnott and Pautard, 1970; Kuo-Huang, 1990). In both the epidermal cells of *Dracaena sanderiana* (Pennisi et al., 2001a) and the crystal-forming sclereids of the *Nymphaea tetragona* leaf each "extracellular" crystal arises in a crystal chamber bounded by a sheath initially connected with the plasma membrane (Kuo-Huang, 1992; Kuo-Huang and Chen, 1999). After the crystals are formed in the *Nymphaea* sclereids, a thick secondary wall is deposited and the crystals are embedded between the primary and secondary cell walls.

Calcium oxalate formation has been shown to be a rapid and reversible process in *Lemna minor* (Franceschi, 1989). With an increase in the exogenous calcium concentration, crystal bundles formed in cells of the root within 30 minutes of the induction stimulus. With the source of calcium limited, the recently formed crystal bundles dissolved over a period of three hours. Obviously calcium oxalate formation is not a "dead-end process." The results of this study and of others (Kostman and Franceschi, 2000; Volk et al., 2002; Mazen et al., 2003) indicate that crystal formation is a highly controlled process and may provide a mechanism for regulating calcium levels in plant organs. The raphide idioblasts of *Pistia stratiotes* have been shown to be enriched with the calcium-binding protein calreticulum, which occurs in subdomains of the ER (Quitadamo et al., 2000; Kostman et al., 2003; Nakata et al., 2003).

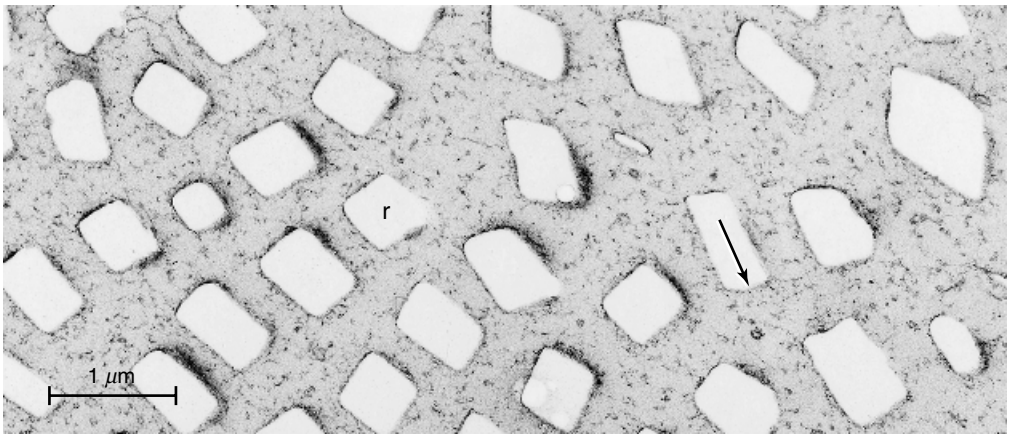

FIGURE 3.13

Crystal chambers in vacuole of a developing crystal cell in leaf of grape (*Vitis vulpina*) as seen with transmission electron microscope. The holes seen here were each occupied by a raphide (r). Each raphide is surrounded by a crystal chamber membrane (arrow). (From Webb et al., 1995. © Blackwell Publishing.)

It has been proposed that the calreticulum is involved with keeping cytosolic calcium activity low, while allowing for a rapid accumulation of calcium used for calcium oxalate formation (Mazen et al., 2003; Nakata et al., 2003). Other functions attributed to the calcification process include the removal of oxalate in plants unable to metabolize oxalate, protection against herbivory (Finley, 1999; Saltz and Ward, 2000; Molano-Flores, 2001), as a storage source of calcium (Ilarslan et al., 2001; Volk et al., 2002), the detoxification of heavy metals (see literature cited in Nakata, 2003), addition of mechanical strength, and addition of weight to the tissue. The weight added to tissue by calcium oxalate can be substantial. Eighty-five percent of the dry weight of some cacti reportedly consists of calcium oxalate (Cheavin, 1938).

Two types of raphide idioblasts occur in the leaves of *Colocasia esculenta* (taro; Sunell and Healey, 1985) and *Dieffenbachia maculata* (dumbcane; Sakai and Nagao, 1980): defensive and nondefensive. The defensive raphide idioblasts forcibly eject their "needles" through thin-walled papillae at the ends of the cells when the aroids (Araceae) are eaten or handled fresh. The nondefensive raphide idioblasts are not involved in the irritative property of aroids. The acridity of raphides from the edible aroids, including taro, may be due to the dual action of the sharp raphides puncturing soft skin and the presence of an irritant (a protease) in the raphides that causes swelling and soreness (Bradbury and Nixon, 1998). Paull et al. (1999) report, however, that the acridity is due entirely to an irritant (a 26 kDa protein, possibly a cysteine proteinase) found on the surface of the raphides.

Calcium carbonate crystals are not common in seed plants. The best known calcium carbonate formations are **cystoliths** (*kustis,* bag; *lithos,* stone), which are formed in specialized enlarged cells called **lithocysts** of the ground parenchyma and epidermis (Fig. 3.14; Chapter 9). The cystolith develops outside the plasma membrane in association with the cell wall of the lithocyst. Callose, cellulose, silica, and pectic substances also enter into the composition of cystoliths (Eschrich, 1954; Metcalfe, 1983), which are confined to a limited number (14) of plant families (Metcalfe and Chalk, 1983).

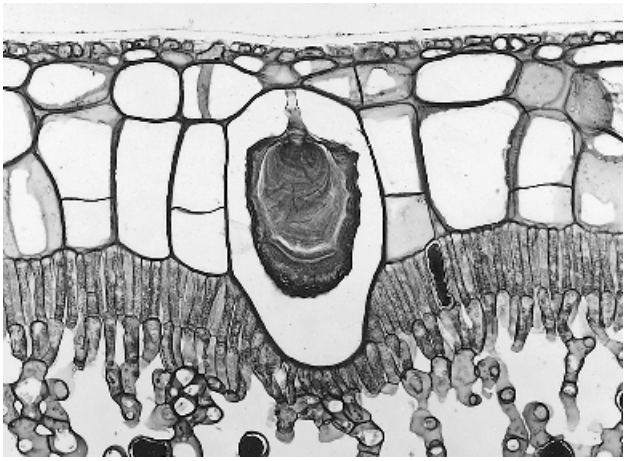

FIGURE 3.14

Calcium carbonate crystal. Transverse section of upper portion of rubber plant (*Ficus elastica*) leaf blade showing club-shaped cystolith in enlarged epidermal cell, the lithocyst. The cystolith consists mostly of calcium carbonate deposited on a cellulose stalk. (×155.)

Silica Most Commonly Is Deposited in Cell Walls

Among the seed plants the heaviest and most characteristic deposits of silica occur in the grasses (Poaceae), where they may account for 5% to 20% of the shoot's dry weight (Lewin and Reimann, 1969; Kaufman et al., 1985; Epstein, 1999). A record high silica content (41% on a dry weight basis) has been reported in the leaves of *Sasa veitchii* (Bambusoideae), which accumulate silica continuously throughout their life of about 24 months (Motomura et al., 2002). Silica deposits also occur in the roots of grasses (Sangster, 1978). In general, monocots take up and deposit more silicon than eudicots. Silicon accumulation in plants contributes to the strength of stems and provides resistance to attack by pathogenic fungi and predaceous chewing insects and other herbivores (McNaughton and Tarrants, 1983). Silica often forms bodies, termed **silica bodies** or **phytoliths,** within the lumen of the cell (Chapter 9). In the rind of *Cucurbita* fruits lignification and phytolith formation appear to be genetically linked, both being determined by a genetic locus called *hard rind (Hr)* (Piperno et al., 2002). Together with lignification of the rind, the production of phytoliths by the rind provides additional mechanical defense for the fruit.

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