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Primary and secondary plasmodesmata: structure, origin, and functioning

Review article

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Dedicated to the memory of the late Professor Dr. A. L. Kursanov

Summary. In the multicellular organisms of higher plants, plasmodesmata provide pathways for intimate symplasmic communication between neighboring cells. The arguments summarized in the present review demonstrate that plasmodesmata are diverse and highly dynamic structures. Differences in the plasmodesmal origin and modifications of the plasmodesmal structure and functioning at the various cell interfaces are the basic means which give rise to a complicated and flexibile symplasmic network. This complex communication system is discussed to serve a significant role in the coordinated development and in the concerted physiological functioning of the cells within the plant tissues, organs, and organisms.

Keywords: Cell communication; Development; Primary plasmodesma; Secondary plasmodesma; Symplasm; Symplasmic domain.

Abbreviations: ER endoplasmic reticulum; GFP green-fluorescent protein; MP movement protein; SEL size exclusion limit.

1 Introduction

Plasmodesmata are the complex plant cell connections which join the protoplasts of the cells across the cell walls to a symplasmic continuum (for reviews, see Gunning and Robards 1976, Robards and Lucas 1990, Lucas et al. 1993, van Bel and van Kesteren 1999). Plasmodesmata offer a unique pathway for symplasmic cell-to-cell communication, including electrical signalling, diffusion of lipids and small soluble molecules of up to 1 kDa, and even the exchange of macromolecules like proteins and nucleic acids. The intercellular communication via plasmodesmata appears to play an essential role in the non-cell-autonomous programming of plant development, and in the coordination of the physiological functioning of the mature plant (for reviews, see Oparka 1993; Epel 1994; Lucas 1995, 1999; Mezitt and Lucas 1996; Ghoshroy et al. 1997; Jackson and Hake 1997; McLean et al. 1997; Ding 1998; Kragler et al. 1998a; Crawford and Zambryski 1999; Ding et al. 1999; Ehlers and van Bel 1999; Pickard and Beachy 1999; van der Schoot and Rinne 1999a, b).

A plant organism does certainly not represent a single symplasmic continuum with unlimited intercellular communication as proposed by Münch (1930). In order to guarantee the differential functioning of cell types and tissues, the plant symplasm presumably functions as a system of operational subunits, termed symplasmic domains (Erwee and Goodwin 1985; for reviews, see Robards and Lucas 1990, Lucas et al. 1993, McLean et al. 1997, Ding et al. 1999, Ehlers and van Bel 1999, Lucas 1999). Therefore, not all the cells within the plant tissues can be expected to be likewise interconnected by plasmodesmata. Plasmodesmal connectivity at the different cell interfaces is rather assumed to be precisely controlled. Plasmodesmata now appear to be highly dynamic structures whose number, structure, and functioning can be modified in

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order to correspond with the particular functional demands.

As will be shown in the present review, different mechanisms have evolved in higher plants to regulate the plasmodesmal connectivity at the distinct cell interfaces. The primary plasmodesmata developing in the cell plate during cytokinesis may undergo various modifications in structure, distribution, and functioning in the course of plant development. Moreover, new plasmodesmata can develop independently of cytokinesis, completely de novo across existing cell walls, forming so-called secondary plasmodesmata, which may possess special structural and functional properties (Fig. 1). Existing primary and secondary plasmodesmata can also be degraded or sealed off at certain cell interfaces, in order to create symplasmic barriers within the plant tissues. Apart from these predominantly permanent modifications of plasmodesmal connectivity, transient changes in the functioning of primary and secondary plasmodesmata have been described which are known as plasmodesmal "gating" (for a recent review, see Schulz 1999).

2 Structure of plasmodesmata

Even a brief view of the plant tissues shows that there occur several plasmodesmal morphotypes. Besides simple unbranched plasmodesmata (Fig. 2A) traversing the cell walls as straight tubes, different types of branched cell connections can be observed (Fig. 2B–D). In contrast to a few reports in which each plasmodesmal strand of these branched cell connections has been referred to as a plasmodesma (e.g., Warmbrodt and VanDerWoude 1990), the sum of all branches is usually considered to represent one single branched plasmodesma. With a complex branched plasmodesma, several plasmodesmal strands on either side of the wall merge in a median branching plane forming a so-called central or median cavity in the region of the middle lamella (Fig. 2B, D). Other types

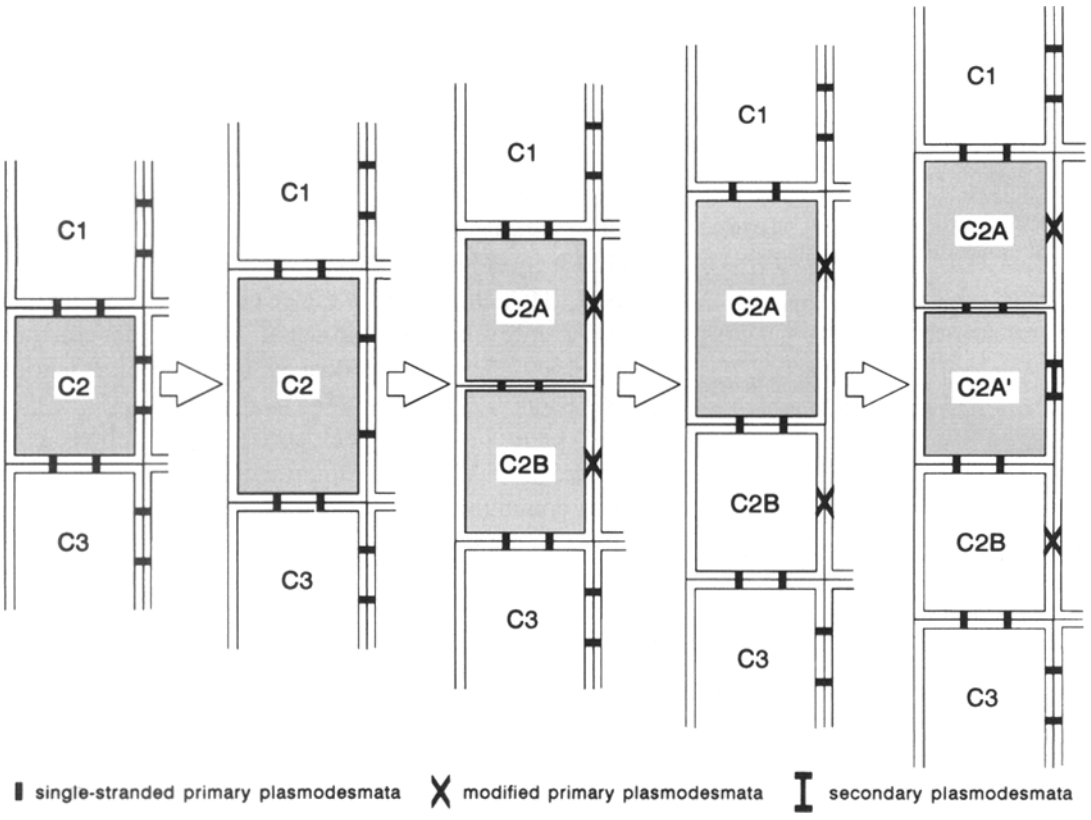


Fig. 1. Changes in plasmodesmal connectivity during cell growth. In the expanding longitudinal cell walls, the density of primary plasmodesmata progressively decreases during cell growth and cell division. Dilution in number of primary plasmodesmata is compensated for by modifications of simple primary plasmodesmata leading to the formation of branched primary plasmodesmata (*C2A* and *C2B*), and by de novo formation of secondary plasmodesmata (*C2A'*). In the less-expanding transverse cell walls, structure and distribution of the simple primary plasmodesmata remain unchanged. From Kollmann and Glockmann (1999)

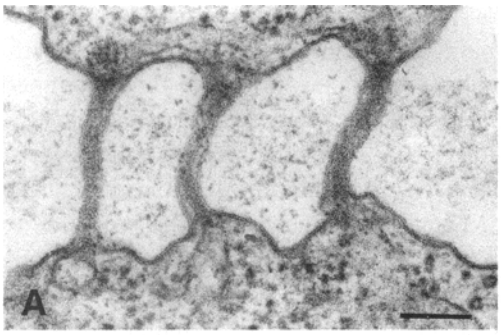
of branched plasmodesmata lack central cavities but show branching planes in the newly formed layers of the cell wall, closer to the plasma membrane of an adjacent cell (Fig. 2C). Branchings in the younger wall layers may also occur in addition to median branching planes with the complex plasmodesmata (Fig. 2D).

In spite of their distinct morphology, all higher-plant plasmodesmata show an identical fine-structural architecture (Fig. 2E–I) (see, e.g., Overall et al. 1982; Ding et al. 1992b, 1999; Botha et al. 1993; Lucas et al. 1993; Overall and Blackman 1996; Overall 1999). In both, simple and branched cell connections, the plasmodesmal channels are 20–50 nm in diameter. Often, they are narrowed at their orifices forming so-called neck constrictions supposed to represent the bottlenecks for symplasmic transport (e.g., Overall et al. 1982, Robards and Lucas 1990, Ding et al. 1992b, Schulz 1995, Botha and Cross 2000). However, the neck constrictions have also been discussed to be artifacts caused by the deposition of callose in response to preparation for electron microscopy (Radford et al. 1998; see the discussions in Overall 1999, Schulz 1999, Botha and Cross 2000).

Each plasmodesma is lined by the plasmalemma that is continuous from one cell to the other (Fig. 2E, G). Yet the plasmalemma appears to be modified in the plasmodesmata and fails to allow diffusion of lipids between neighboring cells (Grabski et al. 1993). In contrast, lipid diffusion may occur via the desmotubule (Grabski et al. 1993), i.e., the central axial substructure of plasmodesmata (Fig. 2E, G). The desmotubule is about 15 nm in diameter and is thought to be a tightly appressed cylinder of the endoplasmic reticulum (ER) continuous with cytoplasmic ER cisternae of the adjoining cells. In particular plasmodesmata, the desmotubule appears to possess an open lumen (Botha et al. 1993, Gamalei et al. 1994, Waigmann et al. 1997) that may function as a pathway for intercellular transport (Lazzaro and Thomson 1996). Open portions of the desmotubules may also occur in the expanded median parts of other cell connections (e.g., Robinson-Beers and Evert 1991a, b; Glockmann and Kollmann 1996). In most transverse sections of plasmodesmata, however, an electron-dense dot was observed in the center of the desmotubule ("central rod") (Fig. 2F, H, I). It is therefore believed that, with a few exceptions, the plasmodesmal ER cylinders are tightly appressed (for a recent review, see Overall 1999) and do not form lumina open for cell-to-cell transport (e.g., Oparka et al. 1999).

The major pathway for symplasmic transport via plasmodesmata is thought to be the so-called cytoplasmic annulus or cytoplasmic sleeve that is defined as the space between the plasmalemma and the desmotubule (Fig. 2F–I). Globular and spokelike particles regularly occur within the cytoplasmic sleeve (Fig. 2E–I), some of which may be related to cytoskeletal proteins like actin and myosin, according to immunocytochemical investigations (White et al. 1994, Radford and White 1998, Blackman et al. 1999). Although details of dimensions, geometry, and arrangement of these plasmodesmal substructures are still a matter of debate (Overall 1999), it is commonly accepted that the particles in the cytoplasmic sleeve strongly reduce the pathway available for symplasmic transport. Supposedly, transport actually takes place via small microchannels with a diameter of about 2.5 nm to 4 nm kept free between the particles (Fig. 2F–I) (Overall et al. 1982, Ding et al. 1992b, Botha et al. 1993, Fisher 1999).

With particular plasmodesmata, further structural details have been observed. Specializations of the cell wall sleeve surrounding the cell connections, special wall-to-membrane linkers, and so-called external sphincters with putative regulatory function, have been recently reviewed by Overall (1999). Detailed studies on grass leaves demonstrated that such structural peculiarities of plasmodesmata show a cell-specific distribution (e.g., Robinson-Beers and Evert 1991a, Botha 1992, Botha et al. 1993; for a review, see Beebe and Russin 1999). In mature leaves of sugarcane (Robinson-Beers and Evert 1991a), for example, plasmodesmata between the vascular parenchyma cells represent the "normal" type, as they have neck constrictions at both orifices and an entirely constricted desmotubule. Those plasmodesmata interconnecting mesophyll cells possess sphincters at both orifices and desmotubule constrictions in these regions only. Yet plasmodesmata between bundle-sheath cells, and at the interface between (Kranz) mesophyll cells and bundle-sheath cells have sphincters with desmotubule constrictions at their orifices in addition to an extra constriction of the plasmodesmal sleeve and the desmotubule in the median layer of the cell wall, where a suberin lamella is deposited. The structurally different plasmodesmata at the various cell interfaces of grass leaves have been discussed to have distinct control functions in the symplasmic transport of assimilates from the mesophyll to the vascular tissue (for a review, see Beebe and Russin 1999).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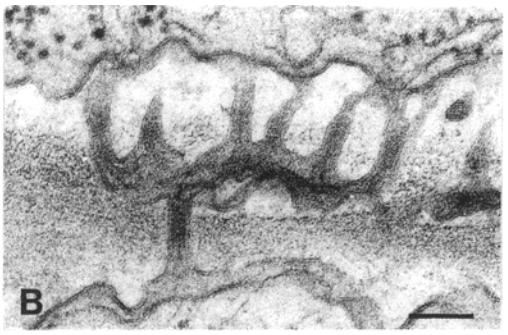
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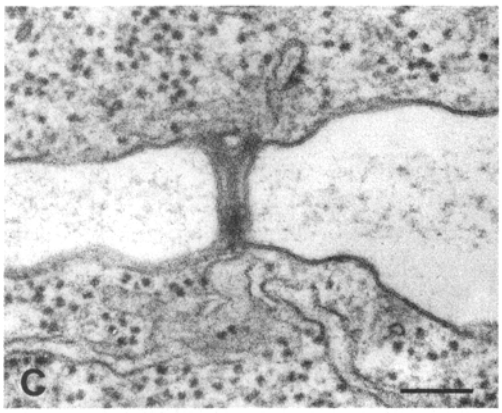
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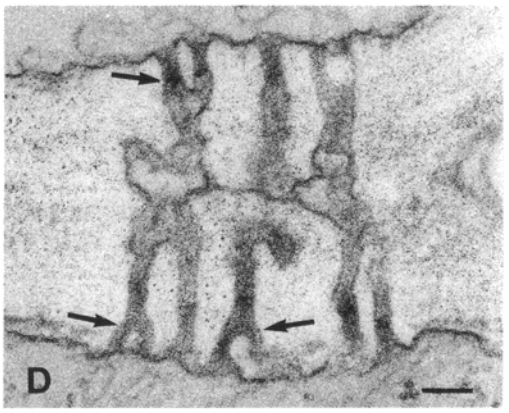
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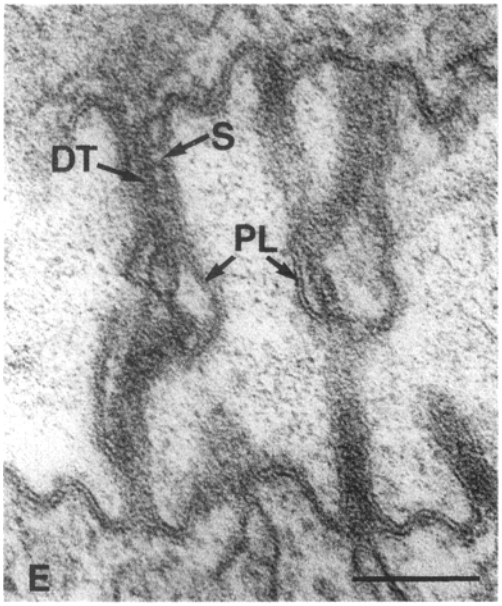
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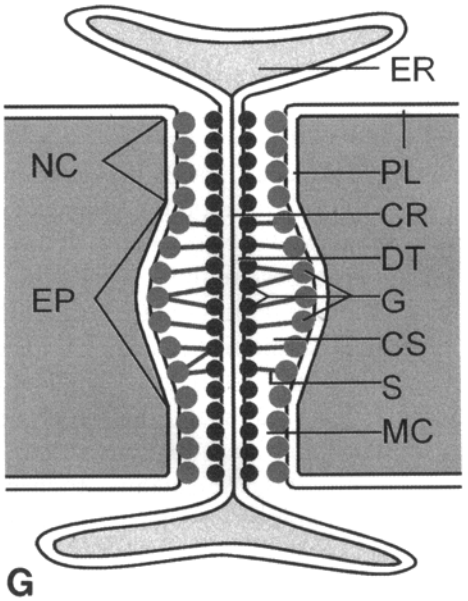
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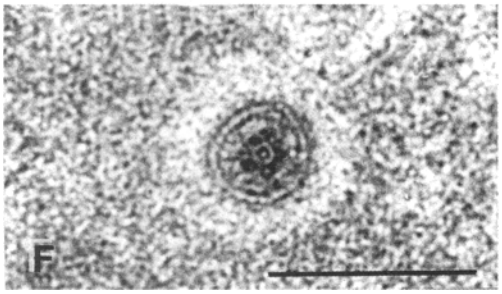
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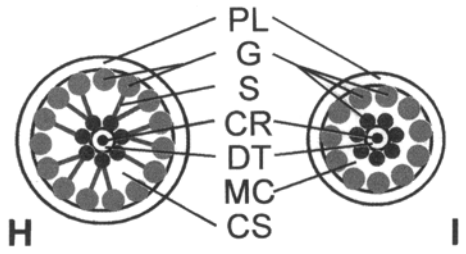
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3 Origin of plasmodesmata

According to the classical definitions, distinct types of plasmodesmata can be distinguished on the basis of their origins (e.g., Gunning and Robards 1976, Robards and Lucas 1990). Primary plasmodesmata are formed during cytokinesis in the growing cell plate, whereas secondary plasmodesmata develop independently of cytokinesis, entirely de novo across existing cell walls. The mechanisms leading to the formation of primary and secondary plasmodesmata are discussed in the following sections.

3.1 Primary plasmodesmata

Formation of primary plasmodesmata

Primary plasmodesmata develop during cytokinesis at sites where ER tubules cross the phragmoplast of a dividing cell (Hepler 1982, Staehelin and Hepler 1996). Cytoplasmic strands enclosing the ER tubules are trapped among the fusing Golgi vesicles delivering the wall material of the growing cell plate (Fig. 3A). The cytoplasmic strands become increasingly constricted during cell plate growth (Fig. 3B, C) and develop into plasmodesmal strands lined by the plasmalemma, which has originally been derived from the Golgi membrane. The enclosed ER tubules are transformed into the plasmodesmal desmotubules, but remain connected to the ER systems of the adjacent cells. Generally, the arising primary plasmodesmata are simple, unbranched strands, which are randomly distributed in the young cell wall, and interconnect the sister cells from the very beginning of cell division on.

Modification of primary plasmodesmata

In the course of cell growth and differentiation, the primary plasmodesmata may undergo postcytokinetic structural modifications. The most obvious one is the formation of distinct types of branched primary plasmodesmata which develop from simple primary plasmodesmata by different modes (for reviews, see Ding and Lucas 1996, Ding et al. 1999, Kollmann and Glockmann 1999).

One mechanism of plasmodesmal branching, showing striking similarities to the mode of plasmodesmata formation in the cell plate, has been elucidated with the primary plasmodesmata in the division walls of protoplast-derived microcalluses (Ehlers and Kollmann 1996a). In order to maintain intercellular communication during thickening growth of the division walls, the simple primary plasmodesmata have to be elongated. Thereby, cytoplasmic strands, enclosing straight or branched cytoplasmic ER cisternae which are connected to the desmotubule, are trapped among the Golgi-derived vesicles with wall material fusing with the plasmalemma (Fig. 4A). The shape of the entrapped ER cisternae elongating the desmotubule determines the shape of the new parts of the plasmodesma (Fig. 4A, B). The elongated primary plasmodesmata may belong to the simple morphotype or show branching planes in the young wall layers close to the plasmalemma of an adjacent cell (Fig. 4B). Multiple branchings may occur with the elongated primary plasmodesmata (Fig. 4B).

Simple primary plasmodesmata may also develop into complex plasmodesmal morphotypes with median branching planes and central cavities following a mechanism shown in Fig. 5A, C, E. According to this model, the simple primary plasmodesmata established in the cell plate become considerably extended in width during cell expansion growth and thinning of the young cell wall. The dilated primary plasmodesmata form the median plasmodesmal parts in the middle lamella which are called the central cavities later on. An uneven wall stretching caused by unequal

Fig. 2A–I. Structure of plasmodesmata. **A–D** Plasmodesmal morphotypes. Simple plasmodesmata (**A**) can be distinguished from different types of branched plasmodesmata (**B–D**). With complex branched plasmodesmata (**B** and **D**) several plasmodesmal strands on either side of the wall merge in a median branching plane, which mostly is dilated to form a central cavity. Other branched plasmodesmata (**C**) lack central cavities but show branching planes in the younger wall layers. Branchings in the younger wall layers may also occur additionally to central cavities with the complex plasmodesmata (**D**, arrows). **E–I** Plasmodesmal substructure in longitudinal view (**E** and **G**) and in transverse view (**F**, **H**, and **I**) of the expanded part of the plasmodesma (**F** and **H**; cf. *EP* in **G**) and of the neck constriction (**I**; cf. *NC* in **G**). Details of the structures shown in the model (**G–I**) can be recognized in the electron micrographs (**E** and **F**). **G–I** The plasmalemma (*PL*) is continuous between the adjoining cells and lines the plasmodesma. The ER of the adjacent cells is connected to the desmotubule (*DT*), which is tightly appressed forming a central rod (*CR*). Globular (*G*) and spokelike (*S*) particles reduce the space of the cytoplasmic sleeve (*CS*) to small microchannels (*MC*). *EP* Expanded part of plasmodesma, *NC* neck constriction **A** and **C** Primary plasmodesmata in cultured cells of *Solanum nigrum* (Ehlers and Kollmann 1996a). **B** Secondary plasmodesmata in the graft union of *Vicia faba* on *Helianthus annuus* (Kollmann and Glockmann 1985). **D** *Vicia faba* pith cells (original). **E** *Cuscuta odorata* haustorial cells (original). **F** *Metasequoia glyptostroboides* phloem parenchyma cells (original). **G–I** Diagrams modified from Ding et al. (1992b). Bars: 0.1 μm

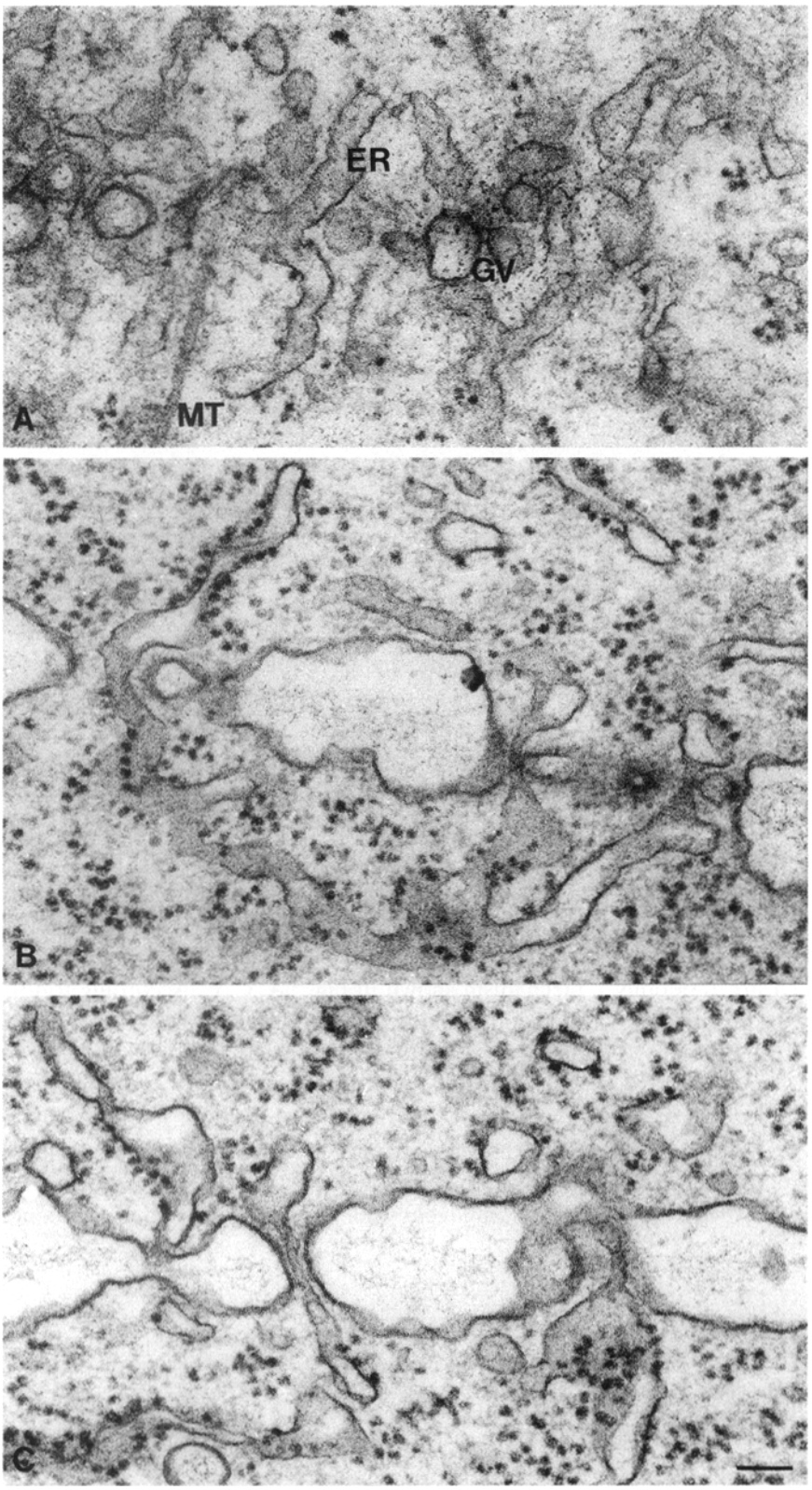


Fig. 3. Formation of primary plasmodesmata in growing cell plates of a *Solanum nigrum* microcallus (**A**) and *Metasequoia glyptostroboides* Strasburger cells (**B** and **C**). **A** Golgi vesicles (*GV*) of the phragmoplast accumulate between ER tubules and microtubules (*MT*) crossing the equatorial plane. **B** ER tubules crossing the growing cell plate determine the sites where primary plasmodesmata are formed. **C** Developing primary plasmodesmata become increasingly constricted during cell plate growth. The enclosed ER cisternae develop into desmotubules. From Kollmann and Glockmann (1999). Bar in C for A–C: 0.1 μm

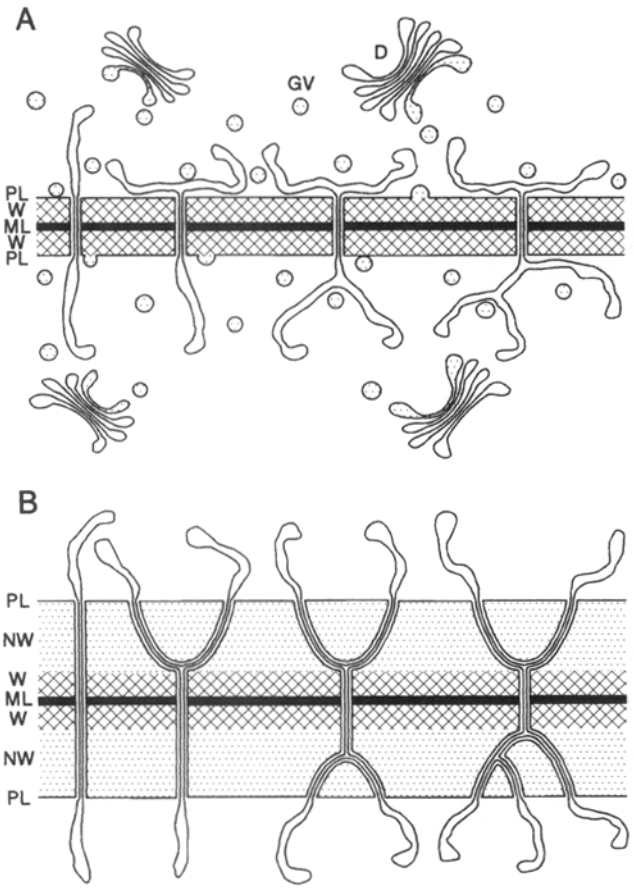


Fig. 4. Modification of primary plasmodesmata during plasmodesmal elongation in the course of thickening growth of the wall. Branches develop from the enclosure of branched ER tubules within Golgi vesicle-derived wall material. *PL* Plasmalemma; *W* first-formed wall layers; *ML* middle lamella; *NW* subsequently formed new wall layers; *D* dictyosomes; *GV* Golgi vesicles. From Ehlers and Kollmann (1996a)

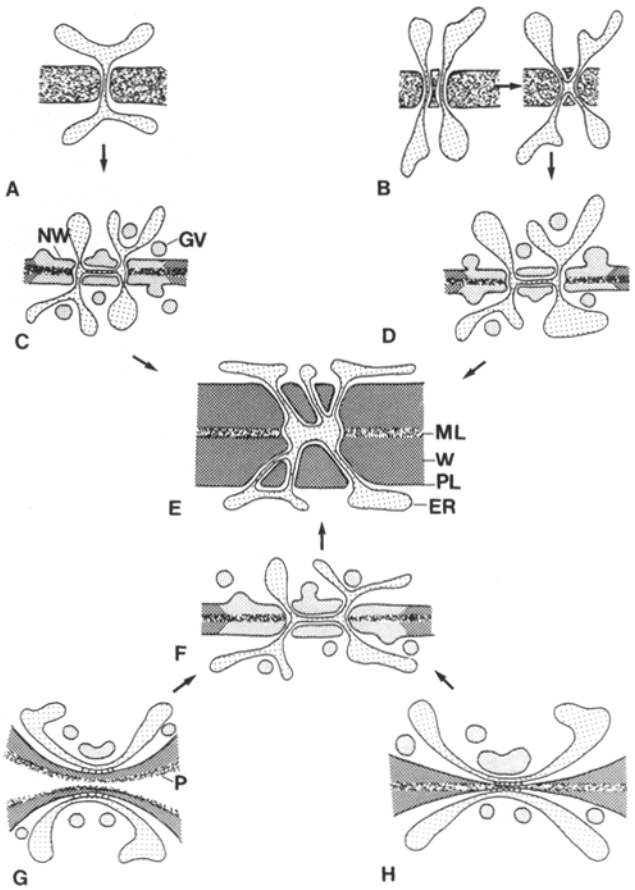


Fig. 5. Formation of complex branched plasmodesmal morphotypes (**E**) by modification of primary plasmodesmata (**A–D**) or by secondary-plasmodesma formation (**G**, **H**, and **F**). During cell expansion growth, central cavities are formed by dilation (**A** and **C**) or fusion (**B** and **D**) of simple primary plasmodesmata. Further strands can be added to the primary plasmodesmata by passive enclosure of branched ER tubules during wall thickening (**C**, **D**, and **E**). Secondary plasmodesmata develop from the fusion of opposite plasmodesmal halves, established in fusion walls (**G**) or in locally thinned areas of existing walls (**H**), each giving rise to the formation of central cavities (**F**). Further strands are added to the secondary plasmodesmata by passive enclosure of ER tubules (**F** and **E**). At the final stage of development (**E**) primary or secondary complex plasmodesmata show an identical morphology and cannot be distinguished any further. *GV* Golgi vesicles; *NW* new wall layers; *ML* middle lamella and primary wall; *W* wall layers; *PL* plasmalemma; *P* pectic layer. From Kollmann and Glockmann (1999)

expansion of the adjacent cells may also contribute to the formation of dilated central cavities (cf. van der Schoot and Rinne 1999a). Subsequently, during thickening growth of the cell wall by apposition of additional wall layers, several plasmodesmal branches extending from the central cavities are entrapped within the new wall material at one or both sides of the middle lamella (Fig. 5C, E; cf. Fig. 4). Complex, multiple-branched plasmodesmata with dilated central cavities are formed by this mechanism, which is in accordance with the model of Krull (1960) on the basis of light microscopical observations.

According to another model, neighboring simple primary plasmodesmata fuse laterally in the region of the middle lamellae (Figs. 5B, D and 6A–C), forming H-shaped plasmodesmata which may possess dilated central cavities (Ding et al. 1992a, 1993; Glockmann and Kollmann 1996; Volk et al. 1996; Itaya et al. 1998; Kollmann and Glockmann 1999; Oparka et al. 1999). The fusion process has not been elucidated conclusively, but it may be facilitated by the disintegration of the oldest, median wall layers in the course of expansion growth. Subsequently, further branches can be added to the fused primary plasmodesmata by ER entrapment, giving rise to complex, multiple-branched plasmodesmata (Figs. 5D, E and 6D, E) (Ehlers and

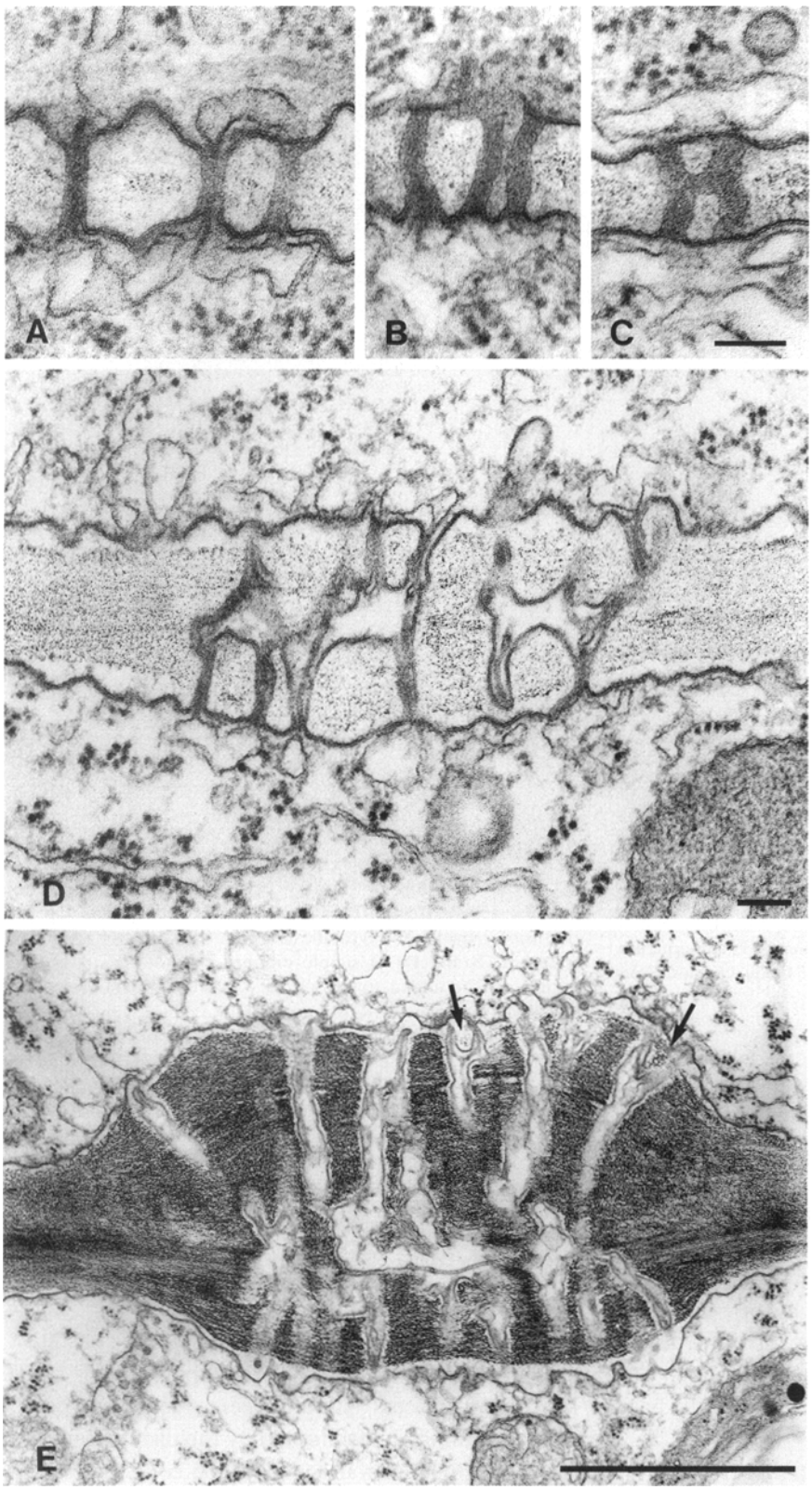


Fig. 6 A–E. Modification of primary plasmodesmata during differentiation of Strasburger cells in the needle of *Metasequoia glyptostoboides*. **A** Simple primary plasmodesmata. **B** and **C** H-shaped plasmodesmata in the young cell walls have possibly developed from the lateral fusion of two adjacent simple plasmodesmata. **D** Complex branched plasmodesmata with central cavities at early stages of development. **E** Complex branched plasmodesmata in thickened-wall areas between mature Strasburger cells. Arrows point to additional branchings in the younger wall layers. From Kollmann and Glockmann (1999). Bars: in C for A–C, 0.1 μm; D, 0.1 μm; E, 1 μm

Kollmann 1996a, Glockmann and Kollmann 1996, Kollmann and Glockmann 1999). Alternatively, it has been supposed that a local, enzymatic digestion of existing cell wall parts allows the penetration of the additional plasmodesmal strands (Jones 1976; Ding et al. 1992a, 1993, 1999; Lucas et al. 1993; Lucas and Gilbertson 1994; Ding and Lucas 1996; Itaya et al. 1998).

3.2 Secondary plasmodesmata

In the course of plant development, secondary plasmodesmata develop independently of cytokinesis, entirely de novo in existing cell walls (for reviews, see Gunning and Robards 1976, Jones 1976, Robards and Lucas 1990, Lucas et al. 1993, Ding et al. 1999, Kollmann and Glockmann 1999). It has been suggested by Strasburger (1901) that the formation of secondary plasmodesmata is required in all nondivision walls between plant cells and tissues of various origin which necessarily lack primary plasmodesmata. Moreover, secondary plasmodesmata can be expected to be formed in all those longitudinal cell walls which undergo intensive extension growth, in order to compensate for the progressive dilution in number of the possibly preexisting primary plasmodesmata (see Fig. 1) (Schnepf and Sych 1983, Seagull 1983).

However, as will be shown in the following sections, in most cases secondary plasmodesmata cannot unambiguously be distinguished from primary plasmodesmata on the basis of structural criteria alone. A direct proof for the secondary origin of plasmodesmata requires either meticulous developmental analyses or special prerequisites as for the selection of the objects to be studied. Many studies pertain to the occurrence of interspecific plasmodesmata formed in the nondivision walls between heterotypic cells, since these plasmodesmata are unequivocally of secondary origin.

Secondary plasmodesmata in protoplast-derived cell cultures

During regeneration of their outer cell walls, protoplast-derived cultured cells form half, branched plasmodesmata by a passive entrapment mechanism which resembles the formation of primary plasmodesmata (Monzer 1990, 1991; Ehlers and Kollmann 1996a). Cytoplasmic ER cisternae become closely associated with the plasmalemma (Fig. 7A), and cytoplasmic strands enclosing these ER cisternae are trapped among the Golgi-derived vesicles with cell wall material fusing with the plasmalemma (Monzer 1990, 1991). The arising half plasmodesmata (Fig. 7B) were termed outer-wall plasmodesmata (Ehlers and Kollmann 1996a), as they are scattered over the cell surface, and are exposed only to the culture medium.

When two cultured cells come into intimate contact with one another, opposite half outer-wall plasmodesmata may fuse, giving rise to a continuous plasmodesma interconnecting the secondarily associated cells (Fig. 5G, F, E). The continuous, intra- or interspecific plasmodesmata formed in this way in the fusion walls represent truly secondary plasmodesmata which usually have a complex branched morphology and possess dilated central cavities in the median fusion plane (Fig. 5F, E) (Monzer 1990, 1991; Ehlers and Kollmann 1996a, b). The diameter and the fine-structural architecture of the secondary plasmodesmata resemble those of primary plasmodesmata.

Secondary plasmodesmata in graft unions

Heterografts of plants with species-specific subcellular markers were used to identify interspecific secondary plasmodesmata between heterotypic cells in graft unions (Jeffree and Yeoman 1983, Kollmann and Glockmann 1985, Kollmann et al. 1985) and to elucidate the mechanism of secondary-plasmodesma formation at the graft interfaces (Kollmann and Glockmann 1991, 1999), which essentially corresponds to the formation of secondary plasmodesmata in cultured cells.

A prerequisite for the establishment of secondary plasmodesmata in graft unions is the local thinning and loosening of the fusion walls between the cells of the graft partners (Figs. 5G, 7C, and 8C, D). Thereby, parts of the plasma membranes of the adjacent cells, which are associated with ER cisternae, come into close contact, enabling membrane fusions (Fig. 8E). During the subsequent reconstruction of the modified wall parts by wall material secreted from Golgi vesicles, cytoplasmic strands enclosing ER cisternae are entrapped in the growing wall on either side of the graft interface (Figs. 7D, E and 8E–G). The arising interspecific, continuous secondary plasmodesmata may be simple strands, but they usually belong to the complex branched plasmodesmal morphotype and possess dilated central cavities in the median fusion plane (Figs. 5F, E, 7F, and 8G). Intraspecific secondary

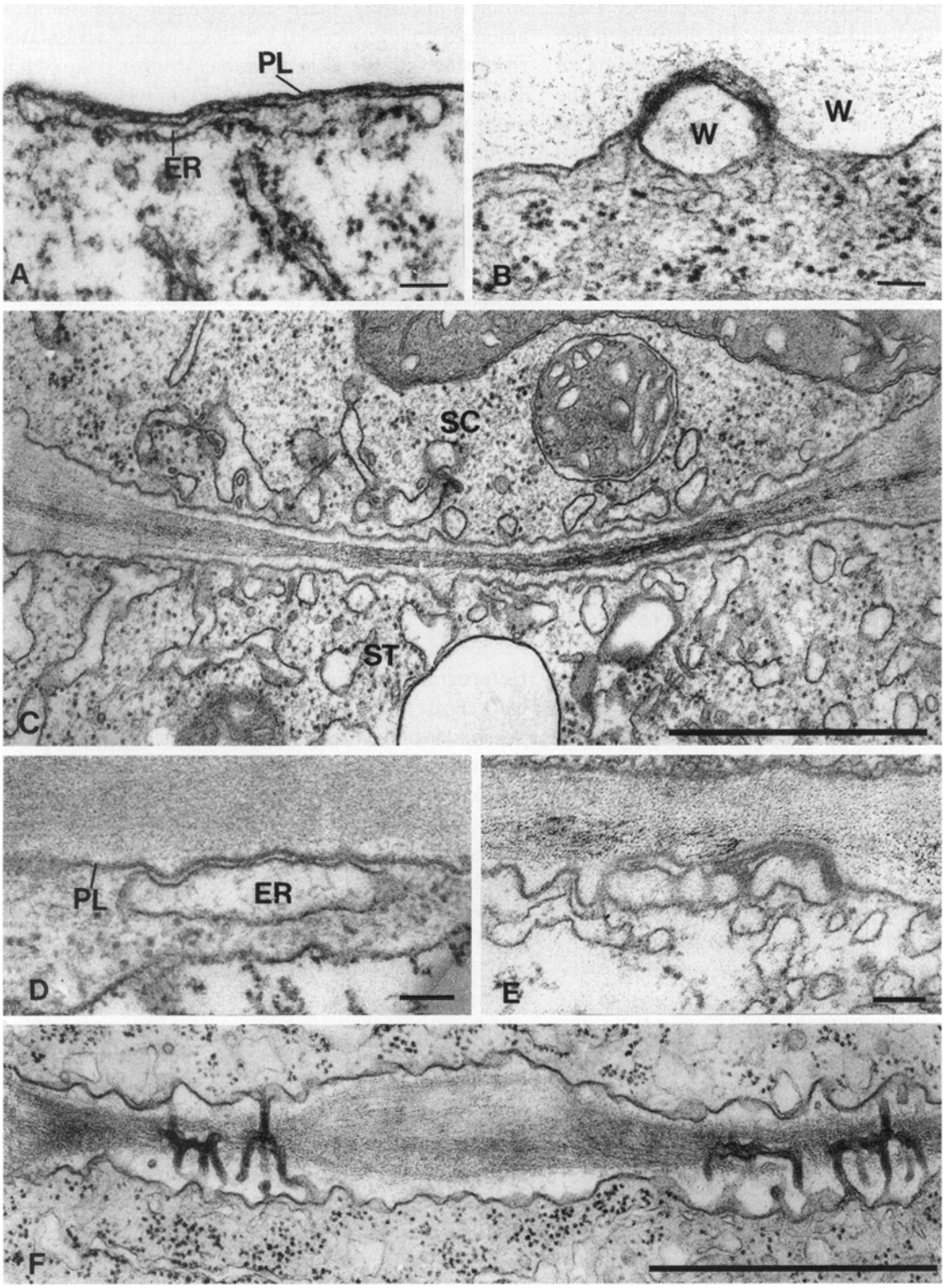


Fig. 7 A–F. Half plasmodesmata, the first stages of secondary plasmodesmata formation. **A** and **B** Outer-wall plasmodesmata (**B**) develop at places of ER–plasmalemma (*PL*) contacts (**A**) by entrapment of ER tubules within Golgi vesicle-derived wall material (*W*) during cell wall regeneration of protoplast-derived cultured cells. **C–E** At graft interfaces, half plasmodesmata (**E**) are formed during wall reconstruction in areas where the fusion walls between scion (*SC*) and stock (*ST*) has become thinned (**C**) and the ER is in close contact with the plasmalemma (*PL*) (**D**). **F** Continuous secondary plasmodesmata are established by fusion of opposite half plasmodesmata. **A** and **B** Cultured cells of *Solanum nigrum* (Ehlers and Kollmann 1996a). **C–F** Graft union of *Vicia faba* on *Helianthus annuus* (Kollmann and Glockmann 1991). Bars: A, B, D, and E, 0.1 μm; C and F, 1 μm

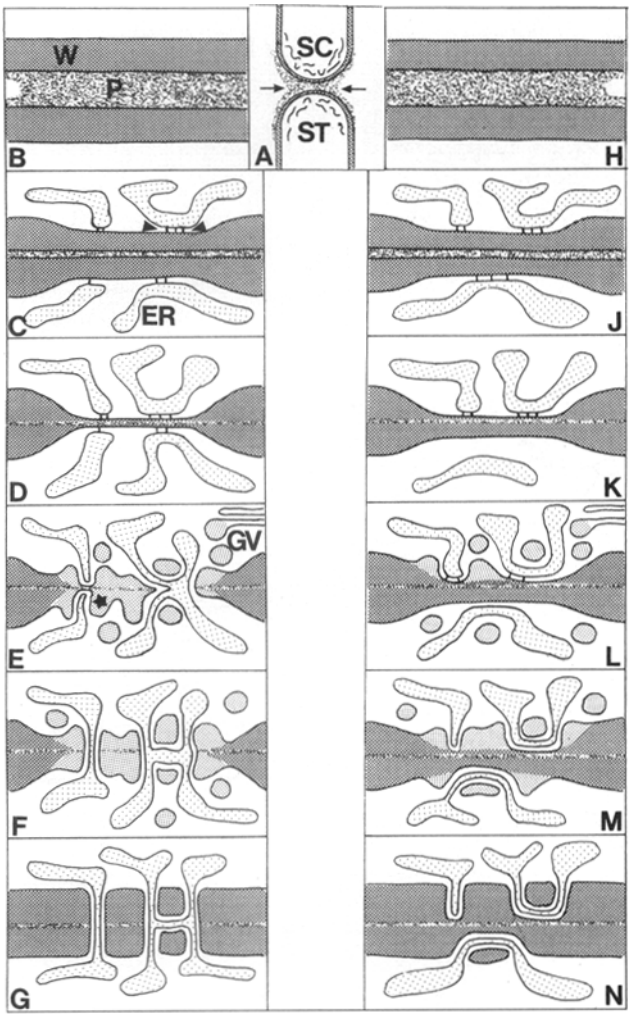


Fig. 8 A–N. Formation of secondary plasmodesmata at graft interfaces. **A** Approaching callus cells of scion (*SC*) and stock (*ST*). Secondary plasmodesmata develop in the fusion walls in the contact region (arrows). **B** and **H** Details of **A** showing the contact walls (*W*) and the pectic layer (*P*) in between. **C–G** At sites where opposite parts of the contact walls are synchronously thinned (**C** and **D**), continuous secondary plasmodesmata develop from the fusion of the plasmalemma and the ER of the partner cells (**E**). During rebuilding of the contact walls, branched or simple plasmodesmata are enclosed within the Golgi vesicle-derived wall material (asterisk in **E**). **I–N** Formation of discontinuous, half plasmodesmata occurs at sites where opposite wall parts are asynchronously thinned (**K** and **L**). *GV* Golgi vesicle; arrowheads in **C** point to unidentified 5 nm particles interconnecting ER and plasmalemma. From Kollmann and Glockmann (1991)

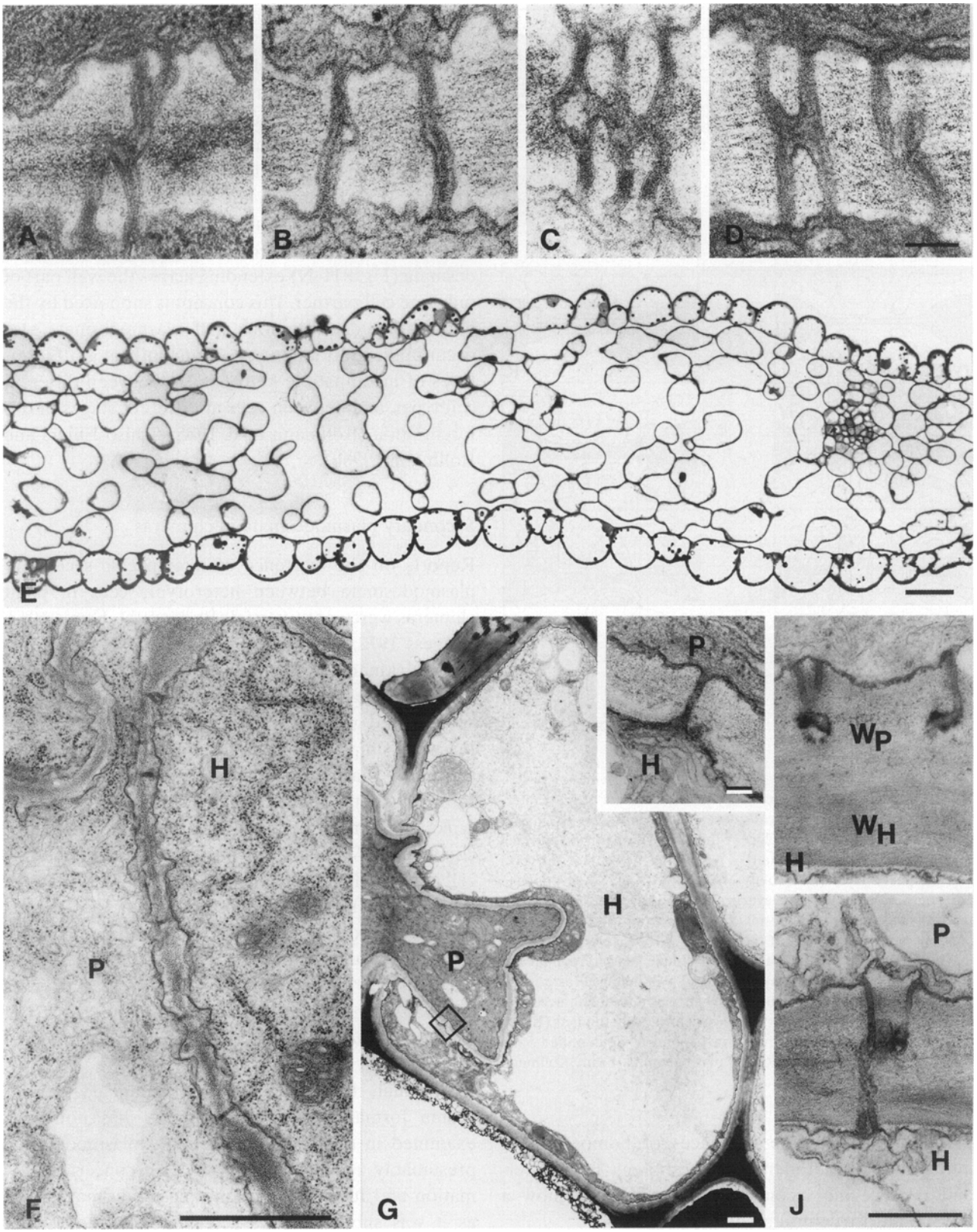
plasmodesmata at the interfaces of homografts or autografts have an identical morphology. Both, inter- and intraspecific secondary plasmodesmata show a normal ultrastructural architecture.

The formation of continuous secondary plasmodesmata following this mode can hardly be understood without an exact cooperation of both cell partners in the synchronous wall thinning and in the precise positioning of the opposite ER–plasmalemma contacts which develop into the plasmodesmal halves (Fig. 8 C, D). Since these processes are initiated before any symplasmic cell-to-cell contact is established (Fig. 8 A–D), the exchange of informational signals across the cell wall may be involved in the coordination (Jeffree and Yeoman 1983; Kollmann and Glockmann 1991, 1999). An insufficient cooperation of the cell partners likely results in the formation of mismatching, half plasmodesmata (Fig. 8 H–N) extending across the wall part of only one cell partner. This concept is supported by the finding that discontinuous, half plasmodesmata also occur in the graft unions, predominantly at graft interfaces of incompatible heterografts, between different cell types, and between cells at different stages of differentiation (Kollmann et al. 1985; cf. also Ehlers and Kollmann 1996b).

Secondary plasmodesmata in chimeras

Reports on the existence of interspecific secondary plasmodesmata between heterotypic cells in plant chimeras were controversial (Buder 1911, Hume 1913, Burgess 1972; for reviews, see Jones 1976, Kollmann and Glockmann 1999), until the decisive cell interfaces have unambiguously been identified with the help of species- and genotype-specific subcellular markers. In this way, simple and complex branched interspecific secondary plasmodesmata with a normal ultrastructural architecture have been detected between heterotypic cells in periclinal, sectorial, and mericlinal chimeras of *Solanum nigrum* genotypes obtained by cell grafting (Fig. 9 A) (Binding et al. 1987). Moreover, interspecific secondary plasmodesmata have been proven to exist in the monekto-periklinal chimera *Laburnocytisus adamii* (Fig. 9 B, C) (Steinberg and Kollmann 1994), which is composed of a *Cytisus purpureus* epidermis (L1) overlying the tissues of *Laburnum anagyroides* (L2 and L3) (Fig. 9 E) (Tilney-Bassett 1986).

Although the mechanism of secondary-plasmodesma formation in plant chimeras has not been examined in detail, these secondary plasmodesmata presumably also develop from the concerted formation and fusion of opposite plasmodesmal halves, as it was shown for the secondary plasmodesmata in graft unions (Fig. 5 G, H, F, E). The sporadic occurrence of half plasmodesmata at the interface between the heterotypic L1- and L2-cells in the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Laburnocytisus chimeras point to this mode of secondary-plasmodesma formation (Steinberg and Kollmann 1994). Moreover, in contrast to the intraspecific plasmodesmata between L1 and L2 in the parental species *Cytisus* (Fig. 9D) and *Laburnum*, the continuous interspecific plasmodesmata between the heterotypic cells in the Laburnocytisus chimera were frequently found to be asymmetrically branched (Steinberg and Kollmann 1994). This finding suggests an autonomous origin of the two plasmodesmal halves and supports the concept that both heterotypic cell partners take an active part in the formation of the continuous, interspecific secondary plasmodesmata.

Secondary plasmodesmata at host–parasite interfaces

Interspecific secondary plasmodesmata have also been reported to occur between the heterotypic cells of parasitic flowering plants and their hosts (for reviews, see Dörr 1969a, b; Kollmann and Glockmann 1999). The identification of interspecific secondary plasmodesmata at the interfaces between the dwarf mistletoes *Arceuthobium pusillum* on *Picea mariana* (Tainter 1971) and *A. occidentale* on *Pinus sabiniana* (Alosi and Calvin 1985) remains uncertain, since the cells of the parasite and the host have not been unequivocally distinguished. In other host–parasite systems, however, the discrimination of the heterotypic partner cells was accomplished with species-specific subcellular markers. In this way, interspecific secondary plasmodesmata, which predominantly belong to the simple morphotype, have been proven to exist between the intermingling, undifferentiated heterotypic cells of *Pilostyles hamiltonii* (Rafflesiaceae) on *Daviesia preisii* (Dell et al. 1982), and *Striga gesneroides* on *Pisum sativum* (Dörr 1996a, b). Remarkably, all simple or branched interspecific secondary plasmodesmata have been localized in thinned-wall areas of the contact walls between host and parasite (Fig. 9F), which corresponds to the observations on graft unions and likely points to a similar mechanism of secondary-plasmodesma formation (Fig. 5G, F, E).

With respect to the formation of secondary plasmodesmata, a special situation appears to exist with the shoot parasite *Cuscuta odorata* (Dörr 1968, 1969, 1987; Dawson et al. 1994). This parasite forms a special intrusive organ, the haustorium, growing deeply into the host's tissues. So-called searching hyphae grow out from the haustorium apex and penetrate the host's tissues either inter- or intracellularly up to the vascular bundle. Even the "intracellularly" growing hyphae do not actually break through the plasmalemma of the host cells but remain surrounded by this membrane. At the tips of the growing searching hyphae, outer-wall plasmodesmata are continuously formed in the thin walls of the parasitic cells (Fig. 9G). With the intercellularly growing hyphae, these outer-wall plasmodesmata fail to establish symplasmic contacts to the host cells. In contrast, the outer-wall plasmodesmata of the intracellulary growing hyphae may fuse with the host cell's plasmalemma, forming true interspecific symplasmic cell connections (Fig. 9G). In older parts of the intracellularly growing hyphae, however, most of these interspecific cell connections become sealed off with wall material deposited by the host cell (Fig. 9H) (cf. also Sect. 4.2). Only a few cell connections become elongated in the host's cell wall, forming complete interspecific secondary plasmodesmata (Fig. 9I).

Secondary plasmodesmata formed during plant development

As mentioned above, the formation of secondary plasmodesmata is presumably a general event in plant growth and development (Fig. 1) (for reviews, see Strasburger 1901, Gunning and Robards 1976, Jones 1976, Robards and Lucas 1990, Lucas et al. 1993, Ding et al. 1999, Kollmann and Glockmann 1999). Analyses of developing leaflets of the moss *Sphagnum palustre* (Schnepf and Sych 1983) and of growing roots of

Fig. 9A–J. Secondary plasmodesmata. **A** Branched secondary plasmodesma between heterotypic leaf mesophyll cells of the sectorial chimera *Solanum nigrum* L.-F (+) S.n.-F-w2. **B** and **C** Simple and branched interspecific secondary plasmodesmata between epidermis (above) and mesophyll of the petal of the periclinal chimera *Laburnocytisus adamii*. **D** Branched intraspecific secondary plasmodesmata between epidermis (above) and mesophyll of the petal of *Cytisus purpureus*. **E** Transverse section of the petal of the chimera *Laburnocytisus adamii* with *Cytisus purpureus* epidermis and *Laburnum anagyroides* mesophyll. **F** Interspecific secondary plasmodesmata in the host–parasite interface between *Striga gesneroides* (*P*) and its host *Vigna unguiculata* (*H*). **G** Interspecific outer-wall plasmodesma in the wall of the hyphae of *Cuscuta odorata* (*P*) growing into the host cell of *Pelargonium zonale* (*H*). **Inset** Enlarged plasmodesma of the framed area. **H** Outer-wall plasmodesmata in the *C. odorata* wall (*WP*) truncated by the host cell wall (*WH*). **I** Continuous branched interspecific plasmodesmata between *C. odorata* hyphae (*P*) and *P. zonale* cell (*H*). **A** Original; **B–E** from Steinberg and Kollmann (1994); **F–I** originals, kindly provided by I. Dörr. Bars: in D for A–D and in inset in G, 0.1 μm; F and G, 1 μm; in I for H and I, 0.5 μm; E, 100 μm

several higher-plant species (Seagull 1983) demonstrated that the number of plasmodesmata per unit wall area does not decline despite cell expansion growth. These findings strongly suggest that new plasmodesmata must have been formed secondarily to compensate for the dilution of the number of primary plasmodesmata (see also Fig. 1). However, intraspecific secondary plasmodesmata occurring beside (modified) primary plasmodesmata in the division walls of a plant organism can hardly be discriminated (cf. Ehlers and Kollmann 1996a, Kollmann and Glockmann 1999). Unequivocal evidence for the formation of intraspecific secondary plasmodesmata during plant development comes from studies on nondivision walls between ontogenically unrelated cells within the plant body, particularly from those contact walls which become secondarily associated and fuse postgenital (see Fig. 5 G, F, E).

A well-known example of secondary-plasmodesma formation during postgenital cell fusion occurs in the course of gynoecium development of flowering plants, when carpel primordia coalesce (Baum 1948; Boeke 1971, 1973a, b; Boeke and van Vliet 1979; van der Schoot et al. 1995). In *Catharanthus roseus*, diffusable factors have been shown to induce the redifferentiation of the closely attached carpel epidermal cells into parenchymatous cells (Verbeke and Walker 1985, Siegel and Verbeke 1989). Functional secondary plasmodesmata with a simple or complex branched morphology are formed in the fusion walls between the secondarily contacting carpel epidermal cells at an early stage of redifferentiation (van der Schoot et al. 1995).

Another example of postgenital cell fusion within a plant body is the formation of tyloses, i.e., proliferations of vascular parenchyma cells extending into the lumina of xylem vessel elements (Esau 1948). The exact location of the fusion walls between the tyloses can hardly be identified, except for the sites where the tyloses contact other vascular parenchyma cells across pit membranes. At these sites, complex branched secondary plasmodesmata with large central cavities have been detected in the secondarily fused contact walls of tyloses of *Daucus carota* (Czaninski et al. 1974).

Further evidence for the formation of intraspecific secondary plasmodesmata during plant development has been given in detailed developmental studies, in which nondivision walls have been proven to occur within the plant tissues.

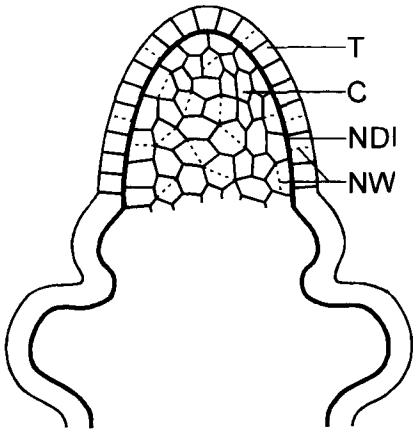

Fig. 10. Duplex shoot apical meristem of angiosperms. The outer tunica layer (*T*) undergoes anticlinal cell divisions. The underlying corpus cells (*C*) divide in all planes. As no cell divisions occur between tunica and corpus, they are separated by a nondivision wall which expands continuously forming the interface between epidermis and subepidermal tissues (*NDI*). Almost all plasmodesmata at this interface must be of secondary origin. *NW* New cell wall

In the duplex shoot apical meristems of angiosperms, controlled divisions of the initial cells and their derivatives lead to specific cell lineage patterns (Fig. 10) (for a recent review, see van der Schoot and Rinne 1999a). The cells in the outermost tunica layer (L1) always divide anticlinally, giving rise to a two-dimensional cell sheet that expands continuously and develops into the epidermis. The tunica overlays the corpus cells (L2 and L3), which divide in all planes and develop into the subepidermal and central tissues of the shoot. Since there are, as a rule, no cell divisions between the L1- and L2-cell layers, they are separated by a nondivision wall which is continuously stretched to form the interface between the epidermis and the subepidermal tissue (Fig. 10). The numerous cell connections at this particular nondivision interface must be of secondary origin (cf., e.g., Strasburger 1901, Steinberg and Kollmann 1994, Cooke et al. 1996, Ding and Lucas 1996, Bergmans et al. 1997, van der Schoot and Rinne 1999a). In the apical meristems, the intraspecific secondary plasmodesmata between the L1- and L2-cells mostly have a simple morphology (for a review, see van der Schoot and Rinne 1999a), whereas in mature tissues, complex branched secondary plasmodesmata predominate, and half plasmodesmata were occasionally observed at the interface between the epidermis and the subepidermal tissue (e.g., Steinberg and Kollmann 1994). These findings may indicate that secondary plasmodesmata

belonging to distinct morphotypes are continuously formed during extension growth. On the other hand, at least some of the complex branched plasmodesmata at the decisive interface in the mature tissues may also have developed from the structural modification of simple secondary plasmodesmata, following similar mechanisms as the modification of simple primary plasmodesmata (cf. Sect. 3.1; Figs. 4 and 5 A–E).

In root apex meristems with a closed organization, such as found in *Arabidopsis thaliana*, three tiers of initial cells give rise to longitudinal cell file lineages which develop into the concentric tissue cylinders of the mature root, i.e., the vascular tissue and the pericyle, the cortex including the endodermis, and the rhizodermis (Dolan et al. 1993; Rost et al. 1996; Zhu et al. 1998a, b). The identification of true nondivision walls in developing roots is more difficult than in shoot apices, since the cells in the immature portion of the root apex undergo some periclinal cell divisions to form additional cell layers, and a limited number of cell divisions also occurs in the radial plane (Rost et al. 1996, Zhu et al. 1998a). However, the majority of the longitudinal walls which interconnect cell files and neighboring tissues of the roots can be expected to be nondivision walls, and the plasmodesmata therein are most likely of secondary origin (Zhu et al. 1998a, b).

Nondivision walls, in which intraspecific secondary plasmodesmata are expected to exist, can generally be found in the plant organs between cells of ontogenetically different origin. Apart from the nondivision walls connecting the epidermis and the subepidermal tissue, another decisive interface occurs between the innermost cell layer developing from the ground meristem (i.e., usually the bundle sheath) and the vascular cells which derive from the procambial strands. The exact location of this nondivision wall has been identified in thorough investigations on the leaf development in several plant species (e.g., Dengler et al. 1985, Bosabalidis et al. 1994; see also the references in Ding and Lucas 1996, Beebe and Russin 1999). In leaves of grasses which possess an inner mestome sheath in addition to the outer parenchymatous bundle sheath, the nondivision wall has consistently been localized at the interface between the two bundle sheaths (e.g., Dengler et al. 1985, Trivett and Evert 1998). Data on the frequency and structure of the intraspecific secondary plasmodesmata in the nondivision walls of leaves have recently been reviewed by Beebe and Russin (1999).

Radial walls of cambium descendants are other nondivision walls which undergo considerable extension growth during cell differentiation (Strasburger 1901). Barnett (1987a, b) disclosed complex branched plasmodesmata which must have been formed secondarily in pit membranes of the radial walls of developing fiber tracheids.

3.3 Comparison of primary- and secondary-plasmodesma formation

Mechanistic similarity of formation processes

The formation of primary and secondary plasmodesmata has previously been considered to be entirely different processes (e.g., Jones 1976). Today, however, there is a growing body of evidence suggesting that a common mechanism is involved in the establishment and modification of primary plasmodesmata as well as in the de novo formation and modification of secondary plasmodesmata (see Figs. 4 and 5) (Kollmann and Glockmann 1991, 1999; Ehlers and Kollmann 1996a; Kragler et al. 1998a). Summarizing the information discussed in the previous sections the following can be stated.

(1) Primary and secondary plasmodesmal strands are formed during cell wall growth. Possibly preexisting cell walls are locally loosened and thinned before they are subsequently reconstructed. (2) ER cisternae, which are passively trapped among fusing Golgi vesicles delivering the new wall material, determine the location and the shape of the developing plasmodesmal strands. (3) The membranes of the fusing Golgi vesicles form the plasmalemma which lines the plasmodesmal strands. The entrapped ER cisternae develop into the plasmodesmal desmotubules. (4) The plasmodesmal strands enclosing the desmotubules become increasingly constricted during wall growth. At their final stage of development all primarily and secondarily formed plasmodesmata have almost identical dimensions and an identical architecture and cannot be discriminated any further. (5) Young, primary plasmodesmata are usually simple strands, whereas secondary plasmodesmata mostly have a complex branched morphology even at early stages of development. However, both primary and secondary plasmodesmata may belong either to the simple or to the branched morphotype and cannot unambiguously be distinguished on the basis of structural criteria alone.

Nomenclature

The morphological similarity of primary and secondary plasmodesmata was the reason for a terminological discussion. Particularly, the nomenclature of the complex, multiple-branched plasmodesmata with central cavities originating from the modification of simple, unbranched primary plasmodesmata (Fig. 5A–E) has been a matter of debate. According to the classical terminology (e.g., Gunning and Robards 1976, Robards and Lucas 1990), these complex branched plasmodesmata should be classified as "modified primary plasmodesmata" or "branched primary plasmodesmata" because of their primary origin (Ehlers and Kollmann 1996a, Glockmann and Kollmann 1996, Kollmann and Glockmann 1999). However, Ding and colleagues (Ding et al. 1992a, 1993, 1999; Lucas et al. 1993; Lucas and Gilbertson 1994; Ding and Lucas 1996; Volk et al. 1996; Itaya et al. 1998) suggested the terms "secondary plasmodesmata" or "complex secondary plasmodesmata" to describe the complex branched cell connections because of their morphological similarity to truly secondary plasmodesmata (Fig. 5E), and they opened the discussion on a "primary-to-secondary transformation" of plasmodesmata. Yet, as stated by the authors themselves, the terminology proposed by Ding et al. (1992a) is not consistent with the classical definitions of primary and secondary plasmodesmata, which strictly refer to the plasmodesmal origin. According to the classical nomenclature, a primary plasmodesma remains primary in origin, whatever modifications it may undergo. Generally, a primary plasmodesma originating during cytokinesis will never be transformed into a secondary plasmodesma developing postcytokinetically across existing cell walls (Ehlers and Kollmann 1996a).

Ding et al. (1993, 1999) argued that the classical definition of secondary plasmodesmata needs to be extended to the complex branched plasmodesmal morphotypes of primary origin, as their formation includes the addition of new, de novo formed plasmodesmal branches (Jones 1976; Ding et al. 1992a, 1993). This proposal evokes other nomenclatory inconsistencies, though. Certainly, the new plasmodesmal branches of the complex plasmodesmata can be regarded as "secondary" parts of the preexisting primary plasmodesmata since they develop postcytokinetically (cf. Ehlers and Kollmann 1996a; also van der Schoot and Rinne 1999a). However, as shown above (Fig. 4), new parts are added postcytokinetically to any modified primary plasmodesma which is elongated during thickening growth of the cell wall. This does obviously not justify to call every elongated primary plasmodesma a secondary plasmodesma (Ehlers and Kollmann 1996a; also Ding et al. 1999).

In an attempt to discriminate between the different modes of plasmodesmal modification on the basis of their mechanisms, Ding et al. (1999) further emphasized that the new parts of the elongated primary plasmodesmata develop during continuing cell wall growth, by a passive entrapment mechanism which mimics the formation of primary plasmodesmata (Fig. 4). In contrast, the new "secondary" branches of the complex plasmodesmata are possibly formed independently of wall growth processes by active penetration of existing walls (cf. Jones 1976). According to Ding et al. (1999), this possible mechanistic difference, which remains to be proven, should be a valid criterion to differentiate between "modified primary" and "(complex) secondary" plasmodesmata. However, on the basis of this criterion, many of the truly secondary plasmodesmata described in the previous sections cannot be termed "secondary" plasmodesmata any longer, since they are not formed by active penetration of existing walls but by a passive entrapment mechanism which resembles the formation of primary plasmodesmata and includes cell wall alterations and cell wall growth (Fig. 5G, H, F, E) (Monzer 1990, 1991; Kollmann and Glockmann 1991) (see Sect. 3.2). The nomenclatory proposal of Ding et al. (1999) clearly contradicts these findings.

In conclusion, in order to avoid further nomenclatory inconsistencies, we suggest the following terminology: In accordance with the classical definition, all branched plasmodesmal morphotypes developing from simple primary plasmodesmata should be classified as "modified primary plasmodesmata" or "(complex) branched primary plasmodesmata" (Figs. 4 and 5A–E). The term "secondary plasmodesmata" should be exclusively used to describe those plasmodesmata which are formed independently of cytokinesis, entirely de novo across existing cell walls, at sites where there has not been a plasmodesma before (Fig. 5G, H, F, E). Furthermore, the developmental classifications "primary" and "secondary" plasmodesmata should be avoided, unless the origin of a particular plasmodesma has unequivocally been proven. For many purposes it is sufficient to use a terminology which simply describes the plasmodesmal morphology, e.g., "unbranched" and "branched" or "continuous"

and "half" plasmodesmata (Ehlers and Kollmann 1996a, Kollmann and Glockmann 1999).

Control of formation processes

At present, there is little information available on the questions if and how the formation and modification of primary and secondary plasmodesmata are controlled (for reviews, see Ding et al. 1999, Overall 1999, Kollmann and Glockmann 1999). In some systems, like in growing roots of the aquatic fern *Azolla pinnata*, the number of primary plasmodesmata formed in a particular division wall appears to be strictly determined (Gunning 1978). As the root apical cell undergoes progressive divisions, the plasmodesmal density becomes reduced in each of the new division walls, leading to a gradual symplasmic isolation of the apical cell which was thought to be the regulatory factor for the limited growth of this plant organ (Gunning 1978). With respect to the development of secondary plasmodesmata, the formation of outer-wall plasmodesmata rather appears to be an autonomous function, on the basis of observations on cell cultures (Monzer 1990, 1991; Ehlers and Kollmann 1996a, b), graft unions (Kollmann and Glockmann 1985, 1991; Kollmann et al. 1985), and searching hyphae of the parasite *Cuscuta odorata* (Dörr 1968, 1969, 1987). However, the successful establishment of continuous secondary cell connections from two opposite plasmodesmal halves most likely requires a similar, dedifferentiated status and an exact cooperation of the two partner cells involved (Kollmann et al. 1985, Kollmann and Glockmann 1999). It can be speculated that the processes of wall thinning, ER positioning, vesicle targeting, and membrane fusion are coordinated by the exchange of informational signals across the cell walls during initial secondary-plasmodesma formation between ontogenetically unrelated cells. On the other hand, cell cooperation may also be controlled by symplasmic communication. Thus, the coordinated formation of continuous secondary plasmodesmata would be facilitated in division walls, where primary plasmodesmata already exist, and in nondivision walls, as soon as the first continuous secondary plasmodesmata have been formed by chance.

4 Functioning of plasmodesmata

Investigations on the number, structure, and origin of plasmodesmata at the different cell interfaces yield only limited insights in the symplasmic organization of the plant organism (for reviews, see van Bel and Oparka 1995, Ehlers and van Bel 1999). With respect to the developmental and physiological functioning of the plant, it is of major importance to characterize the functional properties of plasmodesmata, as well. A complex symplasmic organization may be achieved by a quantitative and qualitative variability in the plasmodesmal transport capacity, pertaining to the amount and nature of molecules which are transported through plasmodesmata between specific cells and at specific developmental stages. Examples illustrating the diverse functional properties of plasmodesmata and the potential significance for plant development and plant physiology will be discussed in the following sections (for reviews, see Lucas et al. 1993; Lucas 1995, 1999; Mezitt and Lucas 1996; McLean et al. 1997; Kragler et al. 1998a; Crawford and Zambryski 1999; Ding et al. 1999; Ehlers and van Bel 1999; Pickard and Beachy 1999; Schulz 1999; van Bel et al. 1999; van der Schoot and Rinne 1999a, b).

4.1 Differential functional properties of plasmodesmata

Functional diversity in the mature plant

The functional properties of plasmodesmata have usually been studied with the help of dye-coupling experiments, in which fluorochromes with different molecular sizes were introduced into single cells in order to determine the diffusion permeability of their cell connections (size exclusion limit; SEL). In early studies, a uniform SEL for molecules of about 1 kDa has consistently been determined for many plasmodesmata, which corresponds to the calculated size of the plasmodesmal microchannels (see, e.g., Terry and Robards 1987, Fisher 1999). Yet it has also been reported that the actual transport capacity depends on the shape, the charge, and the biochemical nature of the tracer molecules (for reviews, see Robards and Lucas 1990, Ding et al. 1999, Goodwin and Cantril 1999, Schulz 1999).

A diversity in plasmodesmal functioning has initially been demonstrated in the extrastelar tissues of *Egeria densa* (Erwee and Goodwin 1985), where the basal plasmodesmal SEL varies slightly between different plant organs and tissues in the range of about 0.4 and 0.7 kDa. Moreover, functional transport barriers show up at five defined cell interfaces in *Egeria*

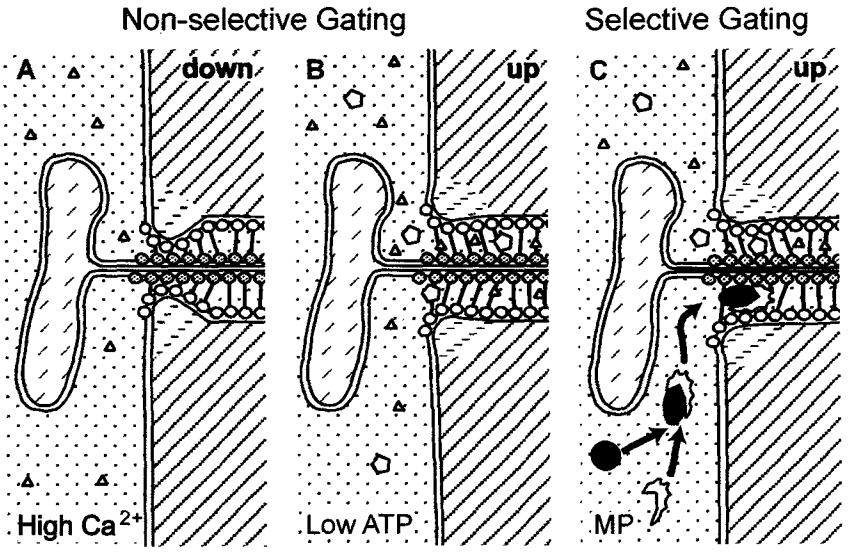

Fig. 11. Up- and downregulation of plasmodesmal transport in nonselective (**A** and **B**) and selective (**C**) gating. **A** and **B** Physiological changes lead to a decrease or an increase of the plasmodesmal SEL. **C** Movement proteins (*MP*) traffic selectively through plasmodesmata and chaperon the transport of other macromolecules, which is often accompanied by an increase in the plasmodesmal SEL. Reprinted with permission from Schulz (1999)

densa, although apparently normal plasmodesmata occur in these cell walls (Erwee and Goodwin 1985). Similar symplasmic barriers at sites were plasmodesmata are present have also been identified, e.g., in tomato stem tissues (van der Schoot and van Bel 1989, 1990) and between the basal cell of the nectary trichome of *Abutilon striatum* and the cells of the underlying nectary (Gunning and Hughes 1976, Terry and Robards 1987). On the other hand, a basal SEL well above the normal size has been reported, e.g., for the plasmodesmata of vascular parenchyma cells in the crease regions of wheat grain (10 kDa, Wang and Fisher 1994), for the plasmodesmata of tobacco trichome cells (7–9 kDa, Waigmann and Zambryski 1995), and for the complex pore-plasmodesma units between sieve elements and companion cells in the phloem tissue of angiosperms (>10 kDa, Kempers and van Bel 1997; >26 kDa, Golecki et al. 1999; >27 kDa, Imlau et al. 1999; for reviews, see van Bel and Kempers 1996, Beebe and Russin 1999, Lucas 1999, Thompson 1999). Thus, in contrast to the initial presumption, the functional properties of the plasmodesmata in the mature tissues appear to depend on the specific nature of the plasmodesmal types and range from the nonfunctional to the highly permeable state. Supposedly, the functional diversity of the plasmodesmata at the different cell interfaces represents adaptations to the various physiological tasks of the cell types and tissues and reflects the complexity of the symplasmic organization of the mature plant (for reviews, see Ding et al. 1999, Ehlers and van Bel 1999, Goodwin and Cantril 1999, Schulz 1999, van Bel et al. 1999).

Moreover, the symplasmic network of the mature tissues appears to be variable. Temporary modifications of the plasmodesmal functioning have been observed in response to environmental changes and physiological alterations, which lead either to an increase or to a decrease of the basal plasmodesmal SEL (Fig. 11) (for reviews, see Ding et al. 1999, Schulz 1999). These functional modifications partly correlate to structural alterations of the plasmodesmal architecture (Schulz 1995, 1999, Botha and Cross 2000), but they may also be achieved by a structurally inconspicuous plasmodesmal "gating", which likely involves changes in the molecular configurations of plasmodesmal components (for a review, see Schulz 1999).

Functional modification during leaf development

The probably best-studied examples of modifications of plasmodesmal functioning occur during leaf development, while the leaves undergo a progressive transition from a sink (i.e., net carbon importing) to a source (i.e., net carbon exporting) organ (e.g., Turgeon 1989). Since this physiological switch is accompanied by the modification of simple plasmodesmata into complex branched morphotypes (e.g., Robinson-Beers and Evert 1991a, b; Moore et al. 1992; Ding et al. 1992a, 1993; Evert et al. 1996; Gagnon and Beebe 1996; Glockmann and Kollmann 1996; Volk et al. 1996; Itaya et al. 1998; Oparka et al. 1999; cf. also the references in Beebe and Russin 1999), a causal connection has been suggested (but see Gagnon and Beebe 1996). The formation of branched plasmodesmata during leaf devel-

opment may be a means to enhance the quantitative capacity for symplasmic transport in the developing phloem-loading pathway by increasing the total plasmodesmal cross-sectional area. Furthermore, evidence has been given that the branched plasmodesmata differ from the simple plasmodesmata in having special qualitative abilities.

A distinct permeability of the simple and branched plasmodesmata in developing leaves has convincingly been demonstrated by Oparka et al. (1999) (see also Imlau et al. 1999). In transgenic tobacco plants, expressing green-fluorescent protein (GFP) (27 kDa) in the companion cells of source leaves under control of the promotor of the sucrose transporter, GFP enters the sieve elements via the pore-plasmodesma units and is translocated to the sink tissues (Imlau et al. 1999). In the source leaves, the GFP is restricted to the phloem. In sink leaves, however, the large GFP spreads symplasmically from the phloem in the major veins throughout the mesophyll and the epidermal cells, which indicates an extremely high basal plasmodesmal SEL at all cell interfaces in the immature leaves (Oparka et al. 1999). Similar results have been obtained with wild-type tobacco plants when GFP-fusion proteins have been introduced into single leaf-epidermis cells either by microinjection or by biolistic bombardment with plasmids encoding GFP constructs (Oparka et al. 1999). In wild-type sink leaves, the plasmodesmata of the epidermal cells allow the unrestricted diffusion of GFP-fusion proteins of up to 47 kDa into neighboring epidermal and mesophyll cells. In contrast, the proteins fail to diffuse through the branched epidermal-cell plasmodesmata of wild-type source leaves. Collectively, these findings illustrate that a massive decrease in the basal plasmodesmal SEL occurs during the sink–source transition of the tobacco leaves, which correlates to the structural alterations of the plasmodesmata in the maturing leaf tissues. Obviously, this developmental modulation of plasmodesmal functioning occurs with the primary plasmodesmata at the various cell interfaces, as well as with the secondary plasmodesmata at the nondivision interfaces between vascular cells and bundle-sheath cells and between mesophyll and epidermal cells (cf. Sect. 3.2).

Beside these changes in their basal SEL, the simple and branched plasmodesmata in the developing leaves have also been discussed to differ in their capacity for selective macromolecule transport. Evidence for this concept comes from studies on transgenic tobacco plants constitutively expressing movement proteins (MPs) of plant viruses, which are capable of increasing the plasmodesmal SEL, to move from cell to cell, and to act as chaperons in the trafficking of viral nucleic acids (Fig. 11) (for reviews, see Lucas and Gilbertson 1994, Goshroy et al. 1997, McLean et al. 1997, Ding 1998, Ding et al. 1999, Lucas 1999, van Bel et al. 1999). Immunocytochemical and functional studies revealed comprehensive results, implying that the MPs target to the branched plasmodesmata of the nonvascular tissues in mature transgenic leaves, and increase the plasmodesmal SEL of mesophyll cells and bundle-sheath cells to 9.4 kDa (Ding et al. 1992a; also Wolf et al. 1989, Deom et al. 1990, Moore et al. 1992, Itaya et al. 1998). In contrast, although the MPs are also present in young transgenic leaves, they have not been found to be targeted to any immature simple plasmodesma and do not influence the plasmodesmal SEL, which has been determined to be 0.8 kDa (Deom et al. 1990, Ding et al. 1992a). On the basis of these findings on transgenic plants, it has been hypothesized that the two plasmodesmal morphotypes in developing leaves may have different biochemical compositions (for a review, see Epel 1994), so that only the mature branched plasmodesmata possess the appropriate molecular constituents for binding and trafficking MPs and other macromolecules (Fig. 11) (for reviews and detailed discussions, see Lucas 1995, 1999; Mezitt and Lucas 1996; Goshroy et al. 1997; Itaya et al. 1998; Kragler et al. 1998a, b; Ding et al. 1999; Oparka et al. 1999; Schulz 1999). Alternatively, there may be a specific cytosolic receptor involved in the macromolecule targeting, which is only present at the later developmental stages of the leaf cells (for reviews, see Lucas 1995, 1999; Mezitt and Lucas 1996; Goshroy et al. 1997; Kragler et al. 1998a; Ding et al. 1999).

However, the general validity of the concept on a different macromolecule-trafficking capacity of immature simple and mature branched leaf plasmodesmata is called in question, as studies on nontransgenic tobacco plants do not add up with a comprehensive picture. In accordance with the hypothesis, Itaya et al. (1998) demonstrated that a 57 kDa MP:GFP fusion protein, expressed in single epidermal cells of wild-type tobacco leaves after biolistic bombardment, neither targets to nor traffics through the simple plasmodesmata between young-leaf epidermal cells but moves through the branched plasmodesmata interconnecting the epidermal cells of mature leaves. This selective cell-to-cell transport appears to be medi-

ated by the MP, as GFP alone failed to traffic through either simple or branched plasmodesmata of the wild-type leaves. However, these results contradict the very high SEL of 47 kDa determined for the immature plasmodesmata of wild-type tobacco leaves by Oparka et al. (1999) and do not match with the findings of Waigmann et al. (1994), who demonstrated that 10 kDa dextran coinjected with a 30 kDa MP into mesophyll cells of young wild-type tobacco leaves diffuses extensively between the cells. According to Oparka et al. (1999), these discrepancies may be explained by the fact that the exact position of the sink–source transition in the examined tobacco leaves has not always been carefully determined.

The studies on the interaction between tobacco leaf plasmodesmata and viral MPs reveal another important aspect which has not been emphasized so far. Despite their similar complex branched morphology, there appear to be differences in the capacity for selective macromolecule trafficking with mature tobacco leaf plasmodesmata of different developmental origin. Apparently, MP trafficking in mature tobacco leaves only occurs with the branched plasmodesmata between epidermal cells, mesophyll cells, and bundle-sheath cells, which at least mostly have developed from the modification of simple primary plasmodesmata (cf. Sect. 3.1), but not with the branched plasmodesmata of truly secondary origin at the nondivision interfaces between vascular parenchyma and bundle-sheath cells and between mesophyll and epidermal cells (cf. Sect. 3.2). In mature transgenic leaves, constitutively expressing tobacco mosaic virus MP, the MP targets to both branched plasmodesmal types, but in contrast to the modified primary plasmodesmata, the SEL of the truly secondary plasmodesmata at the bundle sheath–vascular parenchyma interface is not increased by the MP (Ding et al. 1992a). Although MP transport is not necessarily accompanied by an increase in the plasmodesmal SEL, particularly with those plasmodesmata which have a high basal SEL (Waigmann and Zambryski 1995), this finding likely indicates that the MP is not able to traffic through the truly secondary plasmodesmata between bundle sheath and vascular parenchyma cells. The well-known fact that this special interface is a significant barrier for several plant viruses (see Nelson and van Bel 1998, Derrick and Nelson 1999) matches with this interpretation. Furthermore, despite the lateral transport of a 57 kDa MP:GFP fusion protein from a transformed epidermis cell into neighboring epidermal cells of mature wild-type leaves via modified primary plasmodesmata (Itaya et al. 1998), the protein has not been demonstrated to move through the truly secondary plasmodesmata at the tangential nondivision interface into adjacent mesophyll cells (cf. Itaya et al. 1998) (Fig. 10). On the basis of these data it can be speculated that the modified primary and secondary plasmodesmata of mature leaves generally differ in their capacity for selective macromolecule trafficking, which may be due to a different biochemical equipment of the two plasmodesmal types (for reviews, see Lucas 1995, 1999; Mezitt and Lucas 1996; Goshroy et al. 1997; Ding et al. 1999; Schulz 1999).

In summary, it has been suggested that different plasmodesmal types with distinct functional properties can be distinguished in developing tobacco leaves. The primary and secondary plasmodesmata in young leaves may be characterized as channels for nonspecific (macro)molecule trafficking. They have a high SEL allowing the unhindered diffusion of nutrients and presumably even of small informational macromolecules of up to 50 kDa (cf. Oparka et al. 1999), but possibly they do not mediate the selective transport of larger macromolecules (Ding et al. 1992a, Itaya et al. 1998; but see Waigmann et al. 1994). In mature leaves, the primary and secondary plasmodesmata are modified, and they are apparently more advanced, since they have more selective transport properties. Due to the low SEL of all mature plasmodesmata, the nonspecific diffusion is strongly restricted to small molecules of about 1 kDa (Wolf et al. 1989, Oparka et al. 1999). Distinct types of mature plasmodesmata may have different capacities for selective macromolecular trafficking (Ding et al. 1992a), as can be assumed for the modified primary plasmodesmata and the truly secondary plasmodesmata in mature tobacco leaves.

Functional modification during plant development

The formation of distinct plasmodesmal types with differential functional properties observed in the course of leaf development may exemplify a general developmental event. Complex plasmodesmal networks are obviously established very early in the development of every plant organ, as has been demonstrated in structural and functional studies on shoot and root apical meristems (e.g., Bergmans et al. 1993, 1997; Duckett et al. 1994; Oparka et al. 1994, 1995; Rinne and van der

Schoot 1998; Zhu et al. 1998a, b; for recent reviews, see Crawford and Zambryski 1999; Pickard and Beachy 1999; van der Schoot and Rinne 1999a, b). Moreover, morphogenetic switches, leading to a changing developmental patterning and growth behavior, have often been shown to be accompanied by alterations of the plasmodesmal network, e.g., in developing antheridia of *Chara vulgaris* (Kwiatkowska and Maszewski 1976, 1985, 1986; Kwiatkowska 1999), in fern gametophytes of *Onoclea sensibilis* developing from protonemata (Tilney et al. 1990), and in shoot apical meristems after induction of flowering (Bergmans et al. 1993, 1997; Santiago and Goodwin 1988; Gisel et al. 1999; Ormenese et al. 2000) or dormancy (Jian et al. 1997, Rinne and van der Schoot 1998). These findings may reflect the universal need to reorganize the plant's symplasm during developmental processes and to adjust the available symplasmic pathways to the changing demands.

Apart from alterations of the plasmodesmal frequency and structure, modulations of the plasmodesmal functioning, pertaining either to the diffusion permeability (SEL) or to the capacity for selective macromolecule trafficking, may be effective means to establish complex symplasmic networks with preferential pathways and barriers for the symplasmic transport of certain molecules. Thus, the plant may precisely control the symplasmic exchange of nutrients (e.g., Duckett et al. 1994; Oparka et al. 1994, 1995; Zhu et al. 1998a, b), plant hormones (e.g., Epel et al. 1992, Kwiatkowska 1991), and informational macromolecules like morphogenes and position-dependent signals. Particularly in the view of plasmodesma-permeant "supracellular control proteins" (Mezitt and Lucas 1996), which have been supposed to exert non-cell-autonomous control over differential gene expression in the course of developmental processes (e.g., Carpenter and Coen 1995, Hantke et al. 1995, Lucas et al. 1995, Bouhidel and Irish 1996, Perbal et al. 1996), the complex plasmodesmal networks of the developing tissues appear to be of major importance. Preferential symplasmic pathways and symplasmic barriers may control the translocation and sorting of such developmental signals and may guarantee that the effects of "supracellular control proteins" can be restricted to individual target cells or specific target cell domains, respectively (for reviews, see Lucas et al. 1993; Lucas 1995, 1999; Mezitt and Lucas 1996; Jackson and Hake 1997; McLean et al. 1997; Ding 1998; Kragler et al. 1998a; Crawford and Zambryski 1999; Ding et al. 1999; Ehlers and van Bel 1999; Pickard and Beachy 1999; van der Schoot and Rinne 1999a, b).

4.2 Symplasmic barriers and symplasmic domains

Formation of symplasmic barriers

The controlled degradation or the sealing of existing primary and secondary plasmodesmata at certain cell interfaces are effective means to establish symplasmic barriers within the plant tissues, leading to the complete symplasmic disconnection of adjacent cells (for reviews, see Gunning and Robards 1976, Robards and Lucas 1990, Lucas et al. 1993, Ding et al. 1999, Ehlers and van Bel 1999). The mechanism of plasmodesmal degradation, which represents an inverse way of their formation, has been studied in detail with protoplast-derived microcalluses (Ehlers et al. 1996). In this system, most of the outer-wall plasmodesmata formed in early culture stages (Monzer 1990, 1991; Ehlers and Kollmann 1996a) (cf. Sect. 3.2) fail to establish secondary contacts to neighboring cells and become selectively degraded in the course of cell growth. Due to the loosening and expansion of the cell wall during cell extension, the outer-wall plasmodesmata dilate and become stretched, and the plasmodesmal membranes closely approach the plasmalemma. Eventually, the two membranes fuse, so that the plasmodesmata reintegrate into the cytoplasm (Fig. 12) (Ehlers et al. 1996). Ubiquitination of plasmodesmal proteins, which is well known as a signal for protein degradation, appears to control the selective degradation of outer-wall plasmodesmata, according to immunocytochemical studies (Ehlers et al. 1996).

A selective sealing of plasmodesmata can be performed by different mechanisms. The apposition of cell wall material across the plasmodesmal orifices causes a permanent sealing of the plasmodesmata (e.g., Dörr 1969, Wille and Lucas 1984, Barnett 1987a, Schnepf and Sawidis 1991, Lachaud and Maurousset 1996). Occluding the plasmodesmal microchannels with an impermeable electron-dense material of unknown chemical nature presumably represents a reversible mode of plasmodesmal sealing (Fig. 13 D–F) (Kwiatkowska and Maszewski 1976, 1985, 1986; Moore-Gordon et al. 1998; Ehlers et al. 1999). Moreover, reversible sealing of plasmodesmata can also be achieved by plasmodesmal "gating", so that no struc-

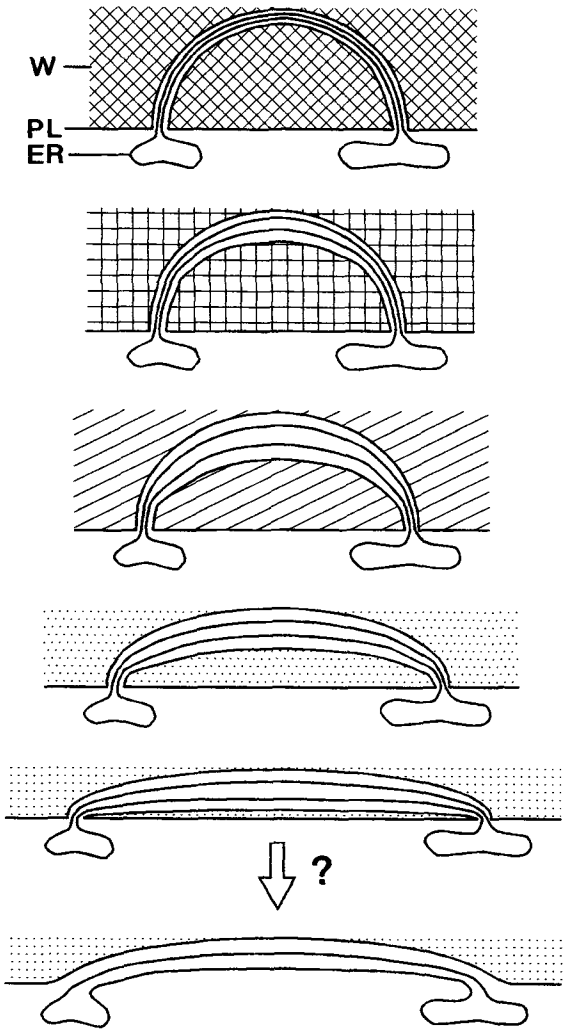

Fig. 12. Degradation of an outer-wall plasmodesma during growth of protoplast-derived cultured cells. During loosening of the cell wall matrix, the half plasmodesma becomes stretched and reintegrates into the protoplast. *W* Wall; *PL* plasmalemma. From Ehlers et al. (1996)

turally apparent peculiarities need to occur with the nonfunctional plasmodesmata (e.g., Palevitz and Hepler 1985, Duckett et al. 1994, Ehlers and Kollmann 2000).

Symplasmic barriers and domains in the mature plant

The occurrence of symplasmic barriers within the mature plant tissues has been supposed to have far-reaching physiological consequences, since a symplasmic barrier necessarily forces a carrier-mediated transmembrane step in the exchange of substances across a particular cell interface. In several plant species, for example, a continuous symplasmic transport pathway with numerous plasmodesmata at the various cell interfaces exists in the phloem-loading zone of source leaves, where assimilates are transported from the photosynthesizing mesophyll into the sieve elements of the minor veins. With other plant species, however, very low plasmodesmal frequencies have been observed between the sieve element–companion cell complexes in the minor veins and the adjacent phloem parenchyma or bundle-sheath cells (<0.1 plasmodesmata per square micrometer of interface; Gamalei 1985, 1989), suggesting that a symplasmic barrier occurs at this cell interface. Consequently, in the latter species, an apoplasmic, carrier-mediated step is required in the transport of photosynthates. The existence of a symplasmic and an apoplasmic mode of phloem loading has been confirmed in functional studies (for reviews, see van Bel 1992, 1993a, 1996; Beebe and Russin 1999; Ehlers and van Bel 1999).

Certain specialized cell types within the mature tissues are apparently entirely disconnected from the adjacent cells, as has been shown in structural and functional studies. These cells function as independent symplasmic domains, which has been assumed to be indispensable to their particular physiological tasks (for reviews, see Jones 1976, Robards and Lucas 1990, Lucas et al. 1993, Ding et al. 1999, Ehlers and van Bel 1999). In the transport phloem of stems and roots, for example, adjoining sieve element–companion cell complexes form a multicellular symplasmic domain that is isolated from the surrounding parenchyma cells, despite the sporadic occurrence of apparently normal plasmodesmata at the domain border (e.g., Hayes et al. 1985; van Bel and Kempers 1990; van Bel and van Rijen 1994; Oparka et al. 1994, 1995; Wright and Oparka 1997; Kempers et al. 1998; Knoblauch and van Bel 1998). The functional symplasmic isolation of the sieve element–companion cell domain has been discussed to play a key role in regulating the balance between sugar release and carrier-mediated retrieval of photosynthates along the phloem pathway. Gating of the existing plasmodemata at the domain border may additionally be involved in controlling these processes (e.g., Patrick and Offler 1996, Wright and Oparka 1997; for detailed discussions, see van Bel 1993b, 1996; van Bel and Ehlers 2000). Moreover, the full symplasmic isolation of mature guard cell pairs from the surrounding epidermal or subsidiary cells (Wille and Lucas 1984, Erwee et al. 1985, Palevitz and Hepler 1985, Lucas et al. 1993) may be essential for the

controlled membrane transport processes associated with the turgor regulation of the guard cells, which is responsible for adjusting the stomatal pore aperture. However, as symplasmic isolation has also been reported for the modified stomata of the floral nectary of *Vicia faba* which cannot regulate the pores (Davis and Gunning 1992), the symplasmic separation of stomatal cells appears to be a programmed developmental event rather than a physiologically controlled adaptation.

Symplasmic barriers and domains formed during plant development

The selective sealing of plasmodesmata leading to the formation of symplasmic barriers and to the segregation of symplasmic domains apparently also contributes to the control of developmental processes (for recent reviews, see Crawford and Zambryski 1999, Ding et al. 1999, Ehlers and van Bel 1999, Pickard and Beachy 1999). Evidence has been given that plasmodesmal sealing is a universal phenomenon involved in apoptosis and senescence. In ageing protonemata of the moss *Funaria hygometrica*, short-lived so-called tmema cells are formed which become symplasmically isolated from the adjacent cells by blocking the plasmodesmata with wall material. Subsequently, the tmema cells are decaying, leading to a fragmentation of the protonemata (Schnepf and Sawidis 1991). When developing xylem vessels and tracheids enter the final stage of programmed cell death, the numerous plasmodesmata at the interface with neighboring parenchyma cells also become sealed off by cell wall apposition (Barnett 1987a, Lachaud and Maurousset 1996). Moreover, in the seed coat and the mesocarp of developing avocado fruits, the reversible plugging of plasmodesmata, which is regulated by endogenous phytohormone ratios, results in seed coat senescence and retardation of fruit growth (Moore-Gordon et al. 1998).

Besides, controlled symplasmic isolation achieved by distinct modes of plasmodesmal sealing has often been reported to occur simultaneously or even prior to differentiation processes and may therefore represent a universal prerequisite for cell differentiation (for reviews, see Crawford and Zambryski 1999, Ding et al. 1999, Ehlers and van Bel 1999, Pickard and Beachy 1999). In the course of guard cell development, the functional plasmodesmata between the stomatal precursors and the surrounding cells are selectively downregulated and become permanently sealed by wall material, well before the stomatal pore eventually opens (e.g., Wille and Lucas 1984, Erwee et al. 1985, Palevitz and Hepler 1985). Duckett et al. (1994) demonstrated by dye-coupling experiments that the symplasmic continuity between all rhizodermis cells in the meristematic and elongation zone of Arabidopsis roots diminishes as the cells differentiate. By the time that root hair growth becomes visible, individual rhizodermal cells are symplasmically isolated from each other, despite the occurrence of apparently normal plasmodesmata in their common cells walls. Similarly, the plasmodesmata of the sieve element–companion cell precursors in the stem cambium are gradually shut off at all interfaces before the cells differentiate (van Bel and van Rijen 1994). After maturation, cell connections open up in the transverse walls, but symplasmic discontinuity is maintained at the longitudinal interfaces with adjacent parenchyma cells. Moreover, in the developing seed coat of *Ranunculus scleratus*, the plasmodesmata between the three cell layers gradually disappear, before the cells of the distinct layers undergo a different developmental fate (Xuhan and van Lammeren 1994).

Further data support the concept that the formation of symplasmic barriers and the segregation of symplasmic domains play a central role in the control of developmental processes. Selective loss of plasmodesmata generally occurs during the development of reproductive cells, and consistent symplasmic discontinuity has also been observed at the interface between maternal and embryogenic tissues (for reviews, see Gunning and Robards 1976, Ehlers and van Bel 1999). In the apical meristem of *Iris hollandica*, plasmodesmata at particular cell interfaces close selectively during the transition from the vegetative to the reproductive state. As has been demonstrated in functional studies, isolated symplasmic domains are formed which later develop into the distinct flower components (Bergmans et al. 1993). Moreover, the controlled occlusion of plasmodesmata leads to a complicated pattern of symplasmic (dis)continuity in protoplast-derived calluses of the dicotyledon *Solanum nigrum* and in globular proembryogenic structures developed from scutellar calluses of the monocotyledon *Molinia caerulea* (Fig. 13) (Ehlers and Kollmann 1996b, Ehlers et al. 1999). Certain cells or cell clusters apparently become isolated from the surrounding cells at a very early stage of development (Fig. 13B) and have been supposed to function as autonomous symplasmic

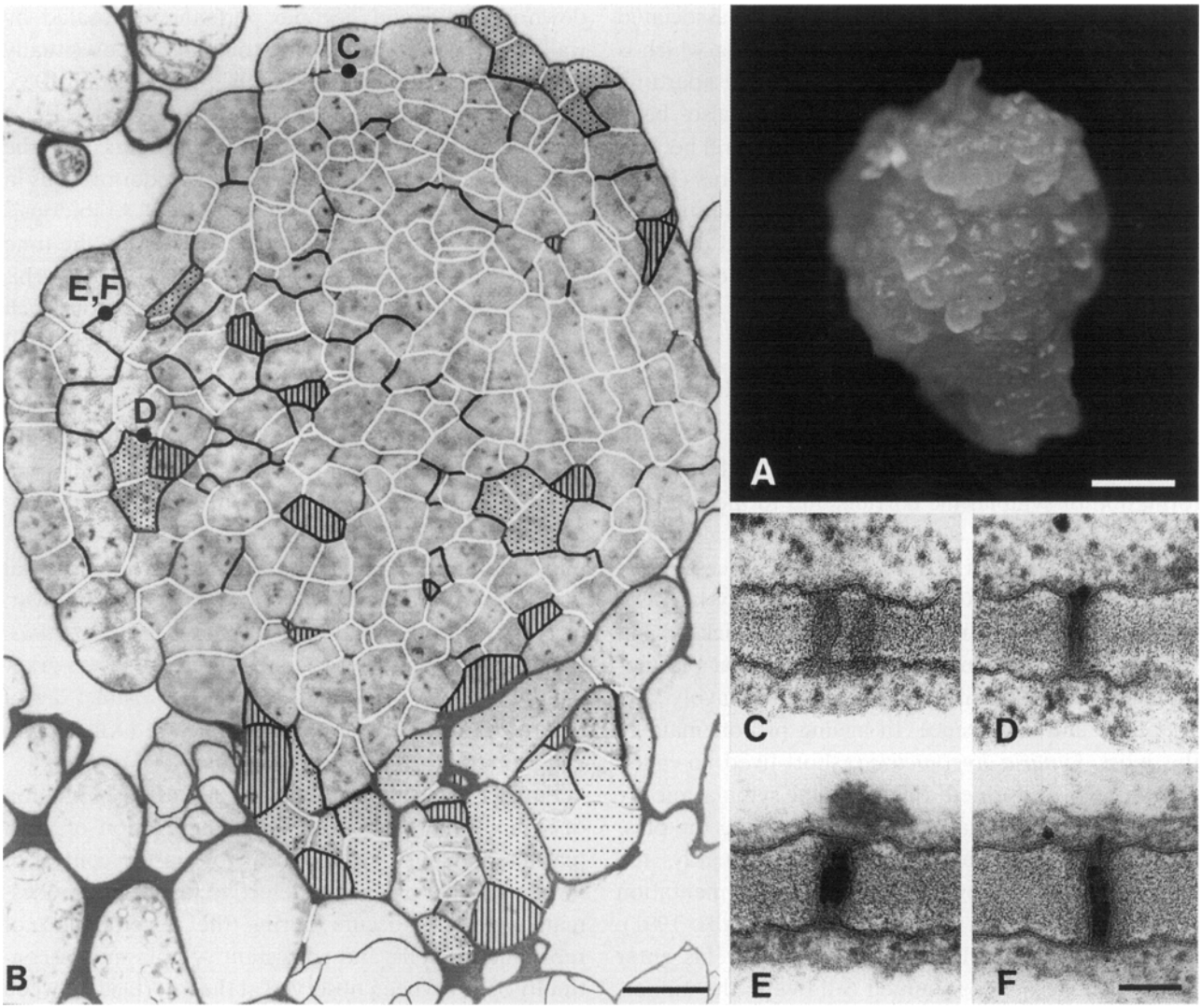

Fig. 13. Segregation of symplasmic domains during development of embryogenic calluses of *Molinia caerulea*. Plasmodesmata are sealed off by an electron-dense material (**D–F**) at particular cell interfaces, leading to the symplasmic isolation of certain cells or cell clusters (hatched and dotted areas in **B**). **A** Macroscopic view of the callus. **B** Overall view of a globular proembryogenic structure. Black lines indicate cell walls with plugged plasmodesmata; white lines mark cell walls with unplugged plasmodesmata; no plasmodesmata were observed in walls presented as gray lines. *C–F* Position of the plasmodesmata shown in panels C–F. **C** Unplugged plasmodesmata. From Ehlers et al. (1999). Bars: A, 0.5 mm; B, 20 μm; in F for C–F, 0.1 μm

domains during the subsequent cell differentiation processes in the course of organogenesis and embryogenesis (Ehlers et al. 1999). Similarly, symplasmic domains are established prior to spermatogenesis by selective loss or reversible occlusion of plasmodesmata at defined stages of the development of *Chara vulgaris* antheridial filaments (Kwiatkowska and Maszewski 1976, 1985, 1986; Maszewski and van Bel 1996; for a review, see Kwiatkowska 1999). The cells within such a domain, which remain interconnected by open plasmodesmata, are completely synchronized with respect to their mitotic activity or their advancement in cell differentiation. However, distinct types of cells, or cells of the same type at various stages of the cell cycle, are symplasmically disconnected from each other.

The findings with Chara antheridia found support by a recent study on the synchronization of mitotic activity in individual protoplast-derived microcalluses of *Solanum nigrum* (Fig. 14) (Ehlers and Kollmann 2000). According to this study, it seems to be a general rule that sister cells, which are interconnected by functional plasmodesmata allowing the diffusion of the fluorescent dye Lucifer Yellow CH (457 Da), always

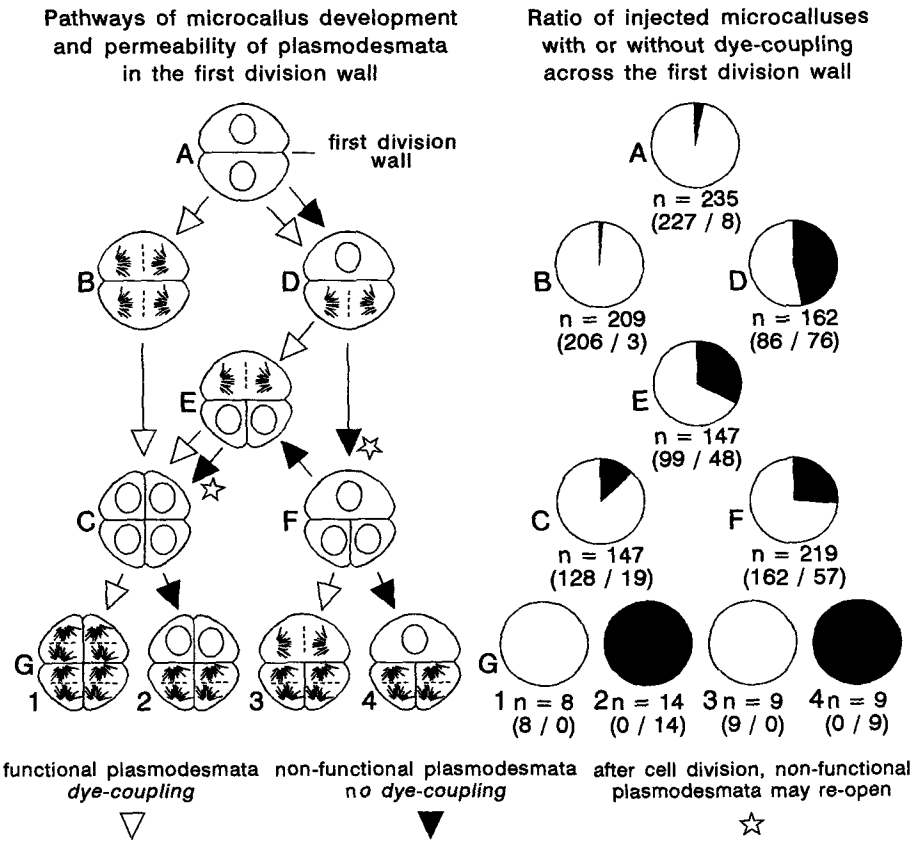

Fig. 14. Correlation between symplasmic coupling and cytokinesis in microcallus development. Just divided cell pairs (*A*) are usually interconnected by functional plasmodesmata, but differences in symplasmic continuity may occur during further development. Sister cells that remain interconnected by functional plasmodesmata and still show dye-coupling always undergo the next cytokinesis synchronously and develop into four-cell microcalluses in any case (*A*, *B*, and *C*). Functional plasmodesmata also occur between sister cells undergoing sequentially overlapping mitoses, which possibly indicates that the dividing cell triggers the mitosis of the symplasmically coupled sister cell (*A*, *D*, *E*, and *C*). Strictly independent, asynchronous cell divisions and the formation of three-cell microcalluses occur exclusively with those cell pairs which show no dye-coupling and are symplasmically isolated from each other (*A*, *D*, and *F*). Closure of plasmodesmata must be reversible because nonfunctional plasmodesmata in the first-division walls may reopen after asynchronous mitoses and dye-coupling may occur between all cells of the three-cell microcalluses (*F*). If a second asynchronous cell division takes place, the plasmodesmata remained closed (*F*, *E*, and *C*). From Ehlers and Kollmann (2000)

divide synchronously. It has been supposed that the dividing cell triggers the mitosis of the symplasmically coupled sister cell, as functional plasmodesmata also occur between cells which undergo sequentially overlapping mitoses. On the other hand, strictly asynchronous, independent cell divisions have been reported to require at least a temporary symplasmic isolation of the sister cells achieved by a reversible gating of their primary plasmodesmata (Fig. 14) (Ehlers and Kollmann 1996b, 2000). At first glance, studies on the shoot apex of *Silene coeli-rosa* (Santiago and Goodwin 1988) contradict these findings, since in Silene apices a high mitotic activity after flower induction has been observed to coincide with an overall reduction in the plasmodesmal SEL. However, individual cells have not been examined in this system, so that no information is available on the symplasmic connectivity between synchronously and asynchronously dividing Silene cells. Moreover, the plasmodesmata in the Silene apices remain open for diffusion of molecules of at least 665 Da (Santiago and Goodwin 1988) and may simply undergo a general, slight downregulation during mitotic activity of the rapidly dividing cells (see Ehlers and Kollmann 2000 for a detailed discussion).

Collectively the data on the formation of symplasmic barriers and symplasmic domains in developing tissues point to a universal principle. There appears to be a direct correlation between the extent of symplasmic (dis)continuity of the cells and the degree of their developmental synchronization, which suggests a causal connection. Supposedly, plasmodesma-permeant signals are involved in synchronizing cell

division activity and cell differentiation processes in the course of plant growth and development. Symplasmic barriers may be required to restrict the symplasmic exchange of such signals in order to allow a differential, autonomous development of neighboring cells (for reviews, see Lucas et al. 1993; Lucas 1995, 1999; Mezitt and Lucas 1996; Jackson and Hake 1997; McLean et al. 1997; Ding 1998; Kragler et al. 1998a; Crawford and Zambryski 1999; Ding et al. 1999; Ehlers and van Bel 1999; Pickard and Beachy 1999; van der Schoot and Rinne 1999a, b).

5 Concluding remarks

Today it is generally accepted that plasmodesmata play a significant role in controlling the plant's developmental and physiological processes. To fulfil these tasks, it appears reasonable that the plasmodesmal connectivity at the various cell interfaces must differ, leading to a complex symplasmic organization of the plant organism. Interesting approaches to elucidate the complicated plasmodesmal networks within the plant tissues have already been presented, but further experimental data are required to characterize the plasmodesmal connectivity between specific cells and at specific developmental stages.

Symplasmically isolated domains equipped with different types of plasmodesmata have been assumed to represent the basic developmental and physiological units of the plant symplasm. The separation of symplasmic domains may allow individual cells or groups of cells to achieve a certain degree of autonomy in order to undergo independent, synchronous developmental pathways and to fulfil special, coordinated physiological functions. Yet further experimental studies are needed to identify the types of (macro)-molecules which can be exchanged via plasmodesmata between the cells of a particular symplasmic domain and to elucidate the signal cascades by which these molecules exert a developmental synchronization or a physiological alignment of the cells within a domain.

Moreover, to guarantee the coordination of development and physiological functioning at the level of a plant organ and organism, it must also be considered that the distinct symplasmic domains somehow interact with one another. This cooperation between different domains, which may be controlled by an apoplasmic exchange of informational signals across the cell walls, has hardly been investigated so far and may also be an interesting area for future research.

The symplasmic organization of the plant organism must also be highly dynamic, to meet the changing demands in the course of development or during physiological alterations. As has been shown in the present review, modifications of the plasmodesmal network can be achieved by a set of different mechanisms which have been extensively studied in the past decades. At present, however, little is known on how these modulations of the plasmodesmal connectivity are controlled. It remains to be elucidated which cellular mechanisms regulate the diverse structural and functional modifications of the plasmodesmal network. Moreover, it will be of great interest to examine whether modulations of the plasmodesmal network are exclusively controlled by endogenous signals, or if they are also influenced by external factors.

To answer these questions is essential to our understanding of the symplasmic organization of the plant's organism.

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