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The ER Within Plasmodesmata

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Abstract The endoplasmic reticulum (ER) is an essential component of plasmodesmata, the membrane-lined pores that interconnect plant cells. The desmotubule which traverses the centre of a plasmodesma is formed from, and continuous with, the cortical ER. Whilst the exact role of the ER is only now being characterised, it is recognised that the ER is intimately involved in the transfer of molecules to and through plasmodesmata, providing a number of pathways for movement between cells as well as being implicated in the mechanisms that control transport. It is believed that molecules may be transported by passive flow within the desmotubule lumen, by diffusion along the inner desmotubule membranes or by specific attachment to the cytoplasmic face of the desmotubule followed by facilitated transport through the cytoplasmic sleeve. The ER is also involved in the formation of plasmodesmata either during cell division or when formed de novo across non-division walls. This chapter focusses on the role of the ER in plasmodesmatal formation and function.

1 Introduction

Plasmodesmata (singular plasmodesma) are membrane-lined pores that interconnect plant cells to form a functional cytoplasmic continuum known as the symplasm. Although first described in the context of cell-to-cell communication by Eduard Tangl in 1879 (cited by Carr 1976), it was not until relatively recently that the importance of the endoplasmic reticulum (ER) within plasmodesmata became appreciated. Whilst the exact role of the ER is only now being characterised, it is recognised that the ER is an essential component of the structure and function of plasmodesmata.

Neighbouring cells within all multicellular eukaryotes must communicate to allow coordinated differentiation (Hashimoto and Inze 2003). From recent ultrastructural, physiological, biochemical and molecular studies it is now clear that plasmodesmata are not merely static channels that allow the movement of low molecular weight solutes, but rather function as flexible structures that are able selectively to control the cell-to-cell movement of proteins and RNAs (Lucas et al. 1993; Blackman and Overall 2001; Ehlers and Kollmann 2001; Roberts and Oparka 2003; Ding et al. 2003). As will be described, the ER forms an essential component of plasmodesmata, and can also determine where they are formed within the cell wall. The ER is inti-

mately involved in the transfer of molecules to and through plasmodesmata, providing a number of pathways for movement between cells, and has been implicated in the mechanisms that control transport. A number of reviews have appeared recently providing a comprehensive description of plasmodesmata and their function, and the reader is referred to these for more detailed discussions (Haywood et al. 2002; Roberts and Oparka 2003; Oparka 2004; Ruiz-Medrano et al. 2004; Heinlein and Epel 2004; Gillespie and Oparka 2005; Roberts 2005). In this chapter we will focus on the ER and its role in plasmodesmatal formation and function.

2
Structure

A simple plasmodesma comprises a single pore, lined by the plasma membrane and traversed in its centre by a structure known as the desmotubule. The desmotubule is formed by the cortical ER which remains continuous between cells via the desmotubule. The space between the plasma membrane and the desmotubule is called the cytoplasmic sleeve and is believed to be the principal route for the movement of small molecules between cells. Each plasmodesma is surrounded by an electron-lucent sleeve and at each end are neck regions, one or both of which may be constricted (Fig. 1 and Fig. 2a).

Structural models of plasmodesmata are based predominantly on data from transmission electron microscopy. On the basis of ultrastructure alone, plasmodesmata are classified into two basic types: simple and branched. The majority of models describe the structure of simple plasmodesmata, and interpretations have been influenced by the effects of the various fixation and staining procedures on plasmodesmata (Gunning and Robards 1976; Robards and Lucas 1990; Beebe and Turgeon 1991; Tilney et al. 1991; Ding et al. 1992b; Botha et al. 1993; Turner et al. 1994; White et al. 1994; Overall and Blackman 1996; Ding 1997, 1999; Waigmann et al. 1997; Radford et al. 1998; Overall 1999). Although there is consensus that all plasmodesmata have the same basic configuration, there is still much debate about their fine structure.

2.1
The ER Lumen

The desmotubule is now accepted to be a tightly furled membrane, running through the plasmodesma, in many cases appearing as an electron-dense rod (Lopez-Saez et al. 1966; Gunning and Hughes 1976; Overall et al. 1982; Olesen 1979; Gunning and Overall 1983; Robinson-Beers and Evert 1991; Ding et al. 1992b; Waigmann et al. 1997). There is a layer of proteinaceous material either integral to, or closely associated with, the desmotubule. Ding and co-workers (1992b) have shown that the wall of the desmotubule is com-

posed of up to ten electron-dense particles about 3 nm in diameter, embedded in the desmotubule membrane, possibly in a helical arrangement with a pitch of 20–30°. In contrast, Overall and Blackman (1996) suggest that the proteinaceous material appears as negatively stained, electron-lucent particles, these being interpreted by Ding and co-workers (1992b) as spaces between the electron-dense particles. In transverse views, at the centre of the desmotubule is an electron-dense rod of about 3 nm in diameter (Ding et al. 1992b) (Fig. 2a). Fine filamentous structures 1–1.5 nm in diameter may extend between this central rod and the desmotubule wall. In material treated without tannic acid or uranyl acetate/osmium tetroxide, most of the desmotubule particles, including the central rod particles, are absent (Ding et al. 1992b). These central rod particles, along with the proteins embedded in the outer membrane of the desmotubule cylinder, are believed to occlude the desmotubule lumen (Overall et al. 1982). However, this may be due to the fixation protocol employed, since other workers have estimated that the space between the central particles is between 2.5 and 3 nm (Botha et al. 1993).

The presence or absence of a desmotubule lumen may be tissue specific. In certain trichomes, instead of the desmotubule appearing as an electron-dense solid rod, the desmotubule may have an "open" electron-lucent lumen (Eleftheriou and Hall 1983; Waigmann et al. 1997).

2.2 The Cytoplasmic Sleeve

The plasma membrane within a plasmodesma is lined on its cytoplasmic face with electron-dense structures (Botha et al. 1993; Ding et al. 1992b; Tilney et al. 1991). These globular particles are connected by elongated spokes to the particles embedded in the wall of the desmotubule (Fig. 1) and may regulate transport by expanding or contracting the cytoplasmic sleeve (Ding et al. 1992b; Overall and Blackman 1996). Although the diameter of a plasmodesma channel is 20–50 nm and the desmotubule is about 15 nm in diameter (Ehlers and Kollmann 2001), the helical arrangement of the embedded particles produces spiralling channels that may reduce the functional diameter of the cytoplasmic sleeve (Zee 1969; Robards 1976; Olesen 1979; Overall et al. 1982; Wolf et al. 1989; Olesen and Robards 1990; Robards and Lucas 1990; Ding et al. 1991; Robinson-Beers and Evert 1991; Lucas and Wolf 1993; Lucas et al. 1993; Overall and Blackman 1996; Waigmann et al. 1997). Various estimates give the diameter of the resulting microchannels between 2.5 and 6 nm (Overall et al. 1982; Ding et al. 1992b; Botha et al. 1993).

Following the localisation of actin to plasmodesmata (White et al. 1994), Overall and Blackman (1996) proposed a model, in which a cable of actin runs through the centre of the pore, closely associated with the desmotubule. It has been suggested that the spokes may be composed of myosin and con-

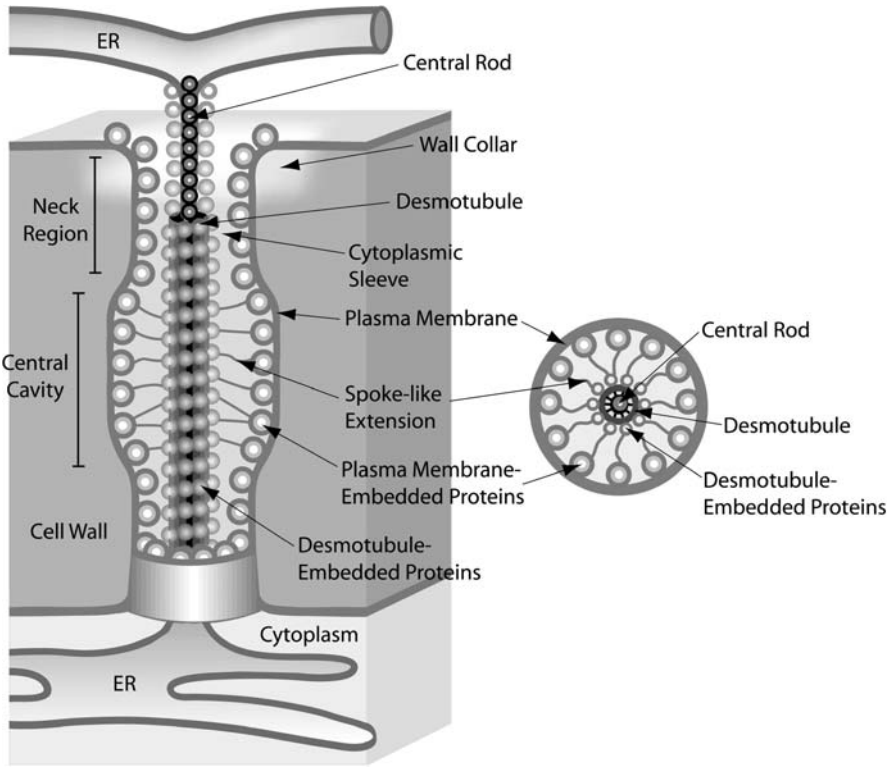

Fig. 1 Diagrammatic representation of the structure of a simple plasmodesma including all sub-structural components that have been confirmed to date. A longitudinal section through the pore is shown to the *left*, while a transverse section through the central cavity region is shown to the *right*. Based primarily on Ding (1992b) and reproduced with permission from Roberts (2005)

nect proteins embedded in the plasma membrane to the actin cable at the centre of the pore, forming an important regulatory structure that can alter the size exclusion limit of plasmodesmata (Oparka 2004). Additional support for this model is provided by the identification of myosin within higher-plant plasmodesmata (Radford and White 1998) and the green alga *Chara corallina* (Blackman and Overall 1998).

The neck region at each end of a plasmodesma may appear constricted, bringing the plasma membrane into close proximity with the desmotubule. This constriction may be the result of callose deposition since treatment with inhibitors of callose synthesis result in funnel-shaped rather than constricted plasmodesmata (Radford et al. 1998). Since callose deposition can be triggered as a response to wounding or chemical fixation, it remains to be determined whether the constricted state is an artefact of fixation or whether callose has a regulatory role in the control of plasmod-

esmal aperture (for discussions, see Overall 1999; Schulz 1999; Botha and Cross 2000).

2.3 Modifications to Plasmodesmatal Structure

In importing tobacco leaf tissue, plasmodesmata between most cell types are simple and lack a central cavity (Ding et al. 1992b). However, occasionally,

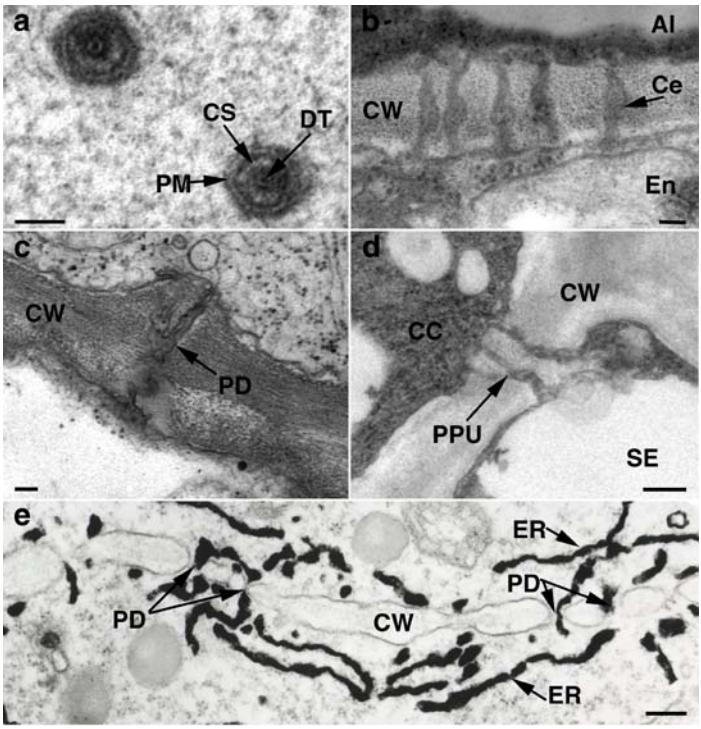

Fig. 2 **a** Transverse section through simple plasmodesmata connecting potato tuber parenchyma cells, showing the desmotubule (DT) with a central, electron-dense rod, the cytoplasmic sleeve (CS) and the plasma membrane (PM). Bar = 20 nm. **b** Longitudinal section through simple plasmodesmata between the aleurone layer (Al) and the starchy endosperm layer (En) in rice, showing the wide central cavity (Ce) within the cell wall (CW). Bar = 50 nm. **c** Longitudinal section through a branched plasmodesma (PD) connecting cells of *Narcissus* tuber. Bar = 100 nm. **d** Longitudinal section through a pore plasmodesma unit (PPU) connecting a companion cell (CC) and a sieve element (SE) in an *Arabidopsis* root. Bar = 25 nm. **e** Entrapment of the endoplasmic reticulum (ER) during cytokinesis in the root tip of *Zea mays*, resulting in the formation of primary plasmodesmata (PD) within the cell wall (CW). Bar = 50 nm. Image courtesy of C. Hawes, Oxford Brookes University. Images **a**–**d** courtesy of the Electron Microscopy Laboratory, SCRI

plasmodesmata between bundle-sheath cells and phloem-parenchyma cells contain extensive central cavities. The presence of a central cavity appears to be related to leaf development, since in non-importing tissue all plasmodesmata contain central cavities whilst retaining the neck constriction (Ding et al. 1992b). Simple plasmodesmata predominate in immature plant tissues (Oparka et al. 1999) and may be grouped together in primary pit fields. They are common in algae and mosses (Franceschi et al. 1994; Cook et al. 1997) and appear to represent a less evolutionarily advanced structure compared to branched plasmodesmata. Branched plasmodesmata comprise two or more channels on either side of the middle lamella, often joined by a central cavity (Fig. 2b–d), and are found extensively in mature tissues. Some simple plasmodesmata are gradually converted to branched plasmodesmata during the sink–source transition that occurs in leaves, by a mechanism that involves the formation of bridges between adjoining simple pores (Oparka et al. 1999; Roberts et al. 2001). The remaining simple plasmodesmata are lost during the phase of rapid leaf expansion, being ripped apart as intercellular air spaces form in the mesophyll (Roberts et al. 2001).

3
Role of ER in the Formation of Plasmodesmata

In order to allow the differential functioning of cell types and tissues, the plant symplasm behaves 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). It is now recognised that the amount of symplasmic connectivity at different cell interfaces, although not identical, is precisely controlled (Ehlers and van Bel 1999) with the degree of branching of plasmodesmata being genetically controlled, and specific for each cell type (Ehlers et al. 2004). Cells with common walls created during cell division may be connected by primary plasmodesmata that form during cytokinesis, whereas secondary plasmodesmata, that form de novo, are required to connect cells across non-division walls (Kollmann and Glockmann 1999; Ehlers and Kollmann 2001).

3.1
Primary Plasmodesmata

Based on where and how they are formed, plasmodesmata are categorised as either primary or secondary. Primary plasmodesmata form during cytokinesis at sites where ER tubules cross the phragmoplast of a dividing cell (Fig. 2e) (Hepler 1982; Staehelin and Hepler 1996). ER tubules, surrounded by cytoplasm, are trapped among the fusing Golgi vesicles that deliver wall

material to the developing cell plate (Fig. 3a). As the cytoplasmic bridges become increasingly constricted they develop into plasmodesmal strands that are lined with plasma membrane derived from the Golgi membrane (Fig. 3b). Although transformed into the desmotubule, the ER tubules remain connected with the cortical ER network of the adjacent cells. When formed these plasmodesmata are simple in structure, but during the course of growth and differentiation may be modified into branched forms (for reviews, see Ding and Lucas 1996; Kollmann and Glockmann 1999; Ehlers and Kollmann 2001).

One mechanism of plasmodesmal branching, observed in the division walls of protoplast-derived calluses (Ehlers and Kollmann 1996), is similar to the mode of formation of primary plasmodesmata. As the cell walls thicken the simple primary plasmodesmata have to elongate to maintain intercellular communication. Golgi-derived vesicles carrying wall material fuse with the plasma membrane and trap cytoplasmic strands, enclosing straight or branched cytoplasmic ER cisternae which are continuous with the desmotubule (Fig. 3c). The shape of the entrapped ER determines the shape of the resulting branched plasmodesmata (Ehlers and Kollmann 1996, 2001).

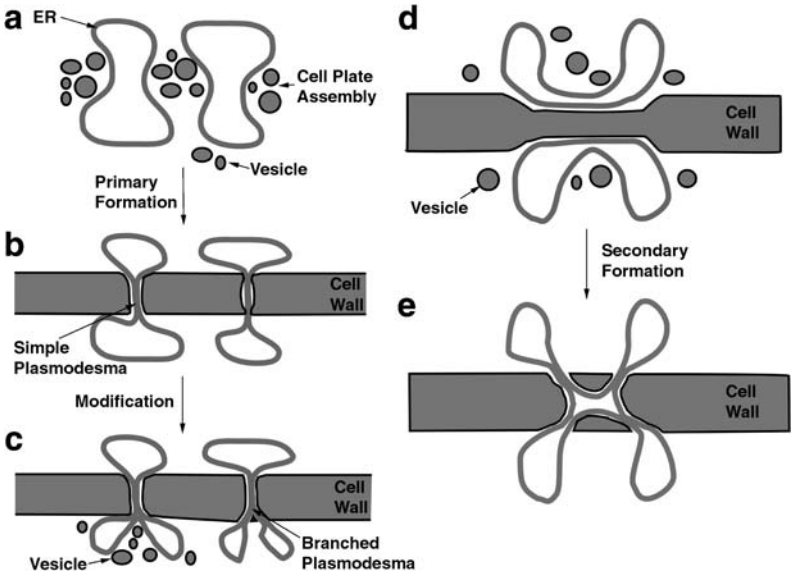

Fig. 3 Diagrammatic representation of plasmodesma biogenesis. Primary plasmodesmata (*left*) are formed during cytokinesis at sites where the ER tubules cross the phragmoplast (**a**). ER and cytoplasm are trapped among the fusing Golgi vesicles forming the desmotubule and cytoplasmic sleeve (**b**). These simple plasmodesmata may be modified into branched forms by the entrapment of additional ER strands during wall thickening (**c**). Secondary plasmodesmata (*right*) form in existing cell walls by the fusion of ER across thinned wall areas (**d**), establishing usually branched connections (**e**) (Modified from Kragler et al. 1998)

Similar entrapment of ER cisternae during cell wall deposition also results in the conversion of simple, primary plasmodesmata into complex plasmodesmatal morphotypes with median branching planes and central cavities. Primary plasmodesmata may become considerably extended in width during cell expansion growth and thinning of the cell wall. In this case, the dilated primary plasmodesmata form the median plasmodesmal parts in the middle lamella which later become the central cavities (for details, see Kollmann and Glockmann 1999; Ehlers and Kollmann 2001), with uneven wall expansion of the adjacent cells resulting in the formation of dilated central cavities (van der Schoot and Rinne 1999).

The lateral fusion of neighbouring simple primary plasmodesmata to form 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), may involve local enzymatic digestion of existing cell wall to allow penetration of additional plasmodesmatal 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), or the formation of further branches by ER entrapment (Ehlers and Kollmann 1996, 2001; Glockmann and Kollmann 1996; Kollmann and Glockmann 1999).

3.2
Secondary Plasmodesmata

By definition, secondary plasmodesmata are formed de novo across existing cell walls (Ehlers and Kollmann 2001). They cannot be distinguished from primary plasmodesmata on the basis solely of their structure, since they also may display simple or branched architectures (Ding et al. 1992b), and therefore can only be identified unambiguously by their location. At the time of their formation some secondary plasmodesmata, like primary plasmodesmata, have a simple structure that may subsequently be modified. However, other secondary plasmodesmata are branched when formed, and ultimately the majority of secondary plasmodesmata are modified to have a branched morphology (Lucas et al. 1993).

Although secondary plasmodesmata are formed during normal plant development (Jones 1976; Robards and Lucas 1990; Lucas et al. 1993; Ding et al. 1999; Kollmann and Glockmann 1999), detailed studies in a number of systems, including protoplast fusion, graft unions, plant chimeras and parasite–host interactions, have provided evidence for the de novo formation of plasmodesmata across cell types from different developmental origins. In all cases, the ER plays a central role in the formation of these secondary plasmodesmata (for reviews, see Jones 1976; Robards and Lucas 1990; Lucas et al. 1993; Kollmann and Glockmann 1999; Ehlers and Kollmann 2001).

As the outer cell walls of protoplast-derived cultured cells regenerate, half, branched plasmodesmata form by a passive entrapment mechanism resem-

bling primary plasmodesmata formation (Monzer 1990, 1991; Ehlers and Kollmann 1996). Cytoplasmic ER cisternae become closely associated with the plasma membrane and, along with the enclosing cytoplasmic strands, are trapped by Golgi-derived vesicles carrying cell wall material and fusing with the plasma membrane (Monzer 1990, 1991). The resulting half plasmodesmata, known as "outer-wall plasmodesmata" (Ehlers and Kollmann 1996), are scattered over the cell surface and when cultured cells come into intimate contact, opposite half plasmodesmata may fuse to form continuous, usually branched, plasmodesmata that connect the two cells (Monzer 1990, 1991; Ehlers and Kollmann 1996).

Heterografts of plants with species-specific subcellular markers have been used to identify the mechanism of formation of interspecific secondary plasmodesmata (Jeffree and Yeoman 1983; Kollmann and Glockmann 1985, 1991, 1999; Kollmann et al. 1985). This process involves the local thinning and loosening of the fusion walls between the cells of the graft partners followed by fusion of the plasma membranes which are associated with ER cisternae (Fig. 3d). Once again, during reconstruction of the modified wall parts, cytoplasm and ER cisternae become entrapped on either side of the graft interface (Fig. 3e). Although the resulting interspecific continuous secondary plasmodesmata may be simple strands, they are usually of a complex branched morphology with dilated central cavities in the median fusion plane. An exchange of information signals appears to be involved in this mechanism (Jeffree and Yeoman 1983; Kollmann and Glockmann 1991, 1999), and it has been shown that lack of cooperation between cell partners results in the formation of mismatching half plasmodesmata at graft interfaces of incompatible heterografts, between different cell types and between cells at different stages of differentiation (Kollmann et al. 1985).

Although not investigated in detail, it appears that the mechanism of formation of secondary plasmodesmata within chimeras and at host/parasite interfaces is essentially similar to that occurring at graft interfaces (for reviews, see Jones 1976; Robards and Lucas 1990; Lucas et al. 1993; Kollmann and Glockmann 1999; Ehlers and Kollmann 2001). Indeed, it is likely that a common mechanism is involved in the establishment and modification of primary plasmodesmata, as well as the de novo formation and modification of secondary plasmodesmata (Kollmann and Glockmann 1991, 1999; Kragler et al. 1998; Ehlers and Kollmann 1996, 2001).

4
Movement of Molecules Through Plasmodesmata

The architecture of a plasmodesma provides three possible routes for the movement of molecules from cell to cell: the ER, the cytoplasmic sleeve and the plasma membrane. Of these routes, movement within or along the ER

is the most contentious, while movement via the cytoplasmic sleeve is generally accepted for small solutes. It is a matter of debate as to whether all three of these pathways are available as transport routes within plasmodesmata, or whether the route via the desmotubule lumen is only available in plasmodesmata that have an "open" desmotubule configuration.

4.1 Transport Through Plasmodesmata via the Endoplasmic Reticulum

The ER–desmotubule–ER continuum provides three potential routes for cell-to-cell transport via plasmodesmata: (1) by passive flow within the desmotubule lumen; (2) by diffusion along the inner desmotubule membranes; or (3) by specific attachment of molecules to the cytoplasmic face of the desmotubule, followed by facilitated transport through the cytoplasmic sleeve. The last of these will be covered in Sect. 4.2.3, dealing with selective transport between sieve element and companion cell.

4.1.1 Transport Within the Desmotubule Lumen

In many cases plasmodesmata have been observed to be occluded by the proteins embedded in the desmotubule membrane, so it is envisaged that only a single water molecule could pass (Overall et al. 1982). However, other plasmodesmata have been observed to have an open desmotubule lumen. It appears that the open lumen in plasmodesmata of some trichomes may be a particular adaptation to their secretory role. In chickpea (*Cicer arietinum*) trichomes there appears to be a specialised system of vacuoles and tubules belonging to the ER system. Following the endocytic uptake of Lucifer Yellow carbohydrazide (LYCH) into this system the fluorescent probe was able to move, apparently within the ER network, through plasmodesmata (Lazzaro and Thomson 1996). Although not conclusively demonstrated, it has been proposed that the transport of pre-nectar in cotton papillae takes place through the open desmotubule lumen, followed by budding of pre-nectar filled vesicles from the ER and secretion of their contents via reverse pinocytosis (Eleftheriou and Hall 1983; Waigmann and Zambryski 2000). Movement of fluorescent probes through the desmotubule lumen has also been demonstrated. Fluorescently labelled dextrans with a molecular weight of 10 000 were able to move from cell to cell in *Torenia fournieri* stem explants following microinjection into the ER but not after injection into the cytoplasm, although it was not demonstrated whether the plasmodesmata involved had an open or closed lumen (Cantrill et al. 1999).

Although some fluorescent probes have been observed to move from cell to cell presumably via the ER lumen, ER-targeted green fluorescent protein (GFP) does not. When expressed under the AtSUC2 promoter, GFP targeted to the ER

lumen of companion cells was not able to move through the desmotubules into the neighbouring sieve elements (Martens et al. 2001; Wright et al. 2003).

4.1.2 Diffusion Within ER Membranes

A second route via the desmotubule potentially involves diffusion along the ER membrane. Hydrophilic fluorescent lipids partitioned within the ER membrane were demonstrated to be able to move from cell to cell following photobleaching, in contrast to lipids inserted within the plasma membrane (Grabski et al. 1993). Similarly, the fluorescent probe DiOC6 was observed to move from cell to cell, presumably via the membrane of the desmotubule, in pea root cortex tissue (Schulz 1999). It appears that the plasma membrane, but not the desmotubule, within plasmodesmata is modified in such a way that it forms a barrier to the diffusion of lipids across plasmodesmata (Grabski et al. 1993).

4.2 Transport Through the Cytoplasmic Sleeve

It is now generally accepted that the majority of molecular transport through plasmodesmata takes place via the cytoplasmic sleeve, through the helical channels between the plasma membrane and the desmotubule. This of course includes molecules moving in association with the ER. In many cases it is assumed that molecules are moving through the cytoplasmic sleeve without requiring any specific interaction with components of the pore, i.e. non-selectively (Schulz 1999; Zambryski and Crawford 2000; Oparka 2004).

The conductive properties of plasmodesmata have been established using a combination of techniques including monitoring of cell-to-cell transfer of radiolabelled substances (Arisz 1969), electrical coupling experiments (Overall and Gunning 1982) and dye coupling (Tucker 1982; Erwee and Goodwin 1983, 1984, 1985; Goodwin 1983; Palevitz and Hepler 1985; Tucker and Spanswick 1985; Madore et al. 1986; van der Schoot and van Bel 1989). Fluorescent probes in the form of fluorescein were first used to investigate short-distance transport through plasmodesmata in trichomes by Schumacher (1936), but it was not until 40 years later that fluorescent probes started to make a significant impact on the study of cell-to-cell communication.

4.2.1 Molecular (Size) Exclusion Limit

Open plasmodesmata allow the diffusive exchange of metabolites and small molecules (Tyree 1970; Tucker et al. 1989). The size of molecules able to pass through plasmodesmata determines the size exclusion limit (SEL) of the pore

but should more accurately be referred to as the molecular exclusion limit (MEL) when it is measured in terms of the mass, rather than the physical dimensions, of the molecules involved. On the basis of investigations using predominantly fluorescently labelled dextrans and other conjugates, the MEL for such movement through many plasmodesmata has been determined to be in the order of 850–900 Da. However, it is not the mass of the molecule that determines its ability to move through plasmodesmata but rather its physical size, which is usually measured in terms of its hydrodynamic radius or Stokes radius (R_s) (Terry and Robards 1987). Many open plasmodesmata allow the diffusion of molecules with an R_s of 0.75 nm. Predictions of the diameter of pores within plasmodesmata, determined using fluorescent dextrans or peptides, fall in the region of 3–4 nm (Terry and Robards 1987; Fisher 1999), which corresponds well with the measurements obtained from electron micrographs (2.5–6 nm) (Ding et al. 1992b; Overall and Blackman 1996; Overall et al. 1982). It should be noted that the relationship between molecular mass and size for dextrans differs greatly from that for proteins. For example, a 27-kDa dextran has the same R_s value (3.5 nm) as a 67-kDa protein (Jørgensen and Møller 1979; Le Maire et al. 1986; Fisher 2000). This becomes of particular relevance in considering more recent studies investigating the movement of the fluorescent protein GFP, which has a molecular mass of 27 kDa and an R_s of 2.82 nm (Terry et al. 1995).

As described previously, a plant is divided into a number of functional symplasmic domains (Erwee and Goodwin 1985; Robards and Lucas 1990; Lucas et al. 1993; McLean et al. 1997; Ding et al. 1999; Ehlers and van Bel 1999; Lucas 1999). In this context, it has been demonstrated that plasmodesmata do not all have the same SEL. Whilst leaf mesophyll cells are frequently quoted to have an MEL for dextrans in the region of 1 kDa, some cell types including epidermal cells have a lower MEL of 370 Da, as assessed by the movement of carboxyfluorescein (Erwee and Goodwin 1985; Duckett et al. 1994), while others have a higher $MEL_{(dextran)}$, including tobacco trichomes at 7 kDa (R_s 1.6 nm, estimated from Jørgensen and Møller 1979; Waigmann and Zambryski 1995).

Simple plasmodesmata have a much larger SEL than branched plasmodesmata (Oparka et al. 1999). In sink tobacco leaf epidermis, simple plasmodesmata predominate and allow the passage of GFP fusion proteins up to 47 kDa. However, after the sink–source transition, during which the simple plasmodesmata are replaced or modified to the branched morphotype, the SEL decreases so that GFP is able to move only from the bombarded cell to its immediate neighbours (Oparka et al. 1999).

4.2.2
Role of ER in the Regulation of SEL

The SEL of plasmodesmata is clearly highly regulated at both a tissue and developmental level, and a number of mechanisms have been proposed to

be involved. Plasmodesmata may be considered to have two potential functional states: closed or open (Lucas et al. 1993; Schulz 1999; Zambryski and Crawford 2000). Closed plasmodesmata are characterised by a lack of all intercellular exchange (Zambryski and Crawford 2000), but since this has often been determined using lower molecular weight fluorescent probes (340 Da) it has not been possible to prove that they are "watertight" (Schulz 1999). Closure of plasmodesmata may be temporary or more permanent, involving the breakdown and removal of plasmodesmata from the cell wall (Duckett et al. 1994; Oparka et al. 1995; Palevitz and Hepler 1985). The deposition of callose within the neck region of plasmodesmata has been implicated in both the transient closure and also in the fine regulation of SEL (Lucas et al. 1993; Roberts and Oparka 2003; Ruan et al. 2004).

Another possibility for regulation of plasmodesmata is that the central ER-derived desmotubule functions as an actin-clad scaffold, which is linked to the plasma membrane by myosin molecules that span the space between the desmotubule and the plasma membrane. In this model, myosin may provide a contractile mechanism for closing the pore aperture, in a similar way to which myosin VIII constricts microvilli in mammalian cells (Baluška et al. 2004; Oparka 2004). Myosin VIII has been detected within plasmodesmata, and it has been suggested that the spokes that radiate from the desmotubule to the plasma membrane may be myosin molecules (Overall and Blackman 1996). Myosin VIII has also been implicated as a structural support of the cortical ER elements tightly underlying the plasma membrane both outside and within plasmodesmata (Volkmann et al. 2003).

Calreticulin is a highly conserved calcium sequestering protein that resides in the ER lumen and is the first ER-resident protein to have been localised within plasmodesmata (Baluska et al. 1999, 2001). It may be involved in regulating plasmodesmatal transport in association with centrin, a calcium-binding contractile protein, localised to the neck region of plasmodesmata (Blackman et al. 1999). An increase in the concentration of cytoplasmic calcium causes a decrease in the phosphorylation of this protein, causing the centrin nanofilaments to contract (Martindale and Salisbury 1990; Blackman et al. 1999). In support of this model, increased levels of calcium have been shown to lead to plasmodesmal closure (Erwee and Goodwin 1983; Tucker 1990; Holdaway-Clarke et al. 2000), and two protein kinases, one calcium-dependent, have been localised to plant cell walls or plasmodesmata (Citovsky and Zambryski 1993; Yahalom et al. 1998).

4.2.3
The Specialised Case of Pore–Plasmodesma Units

Plasmodesmata connecting companion cells (CC) to sieve elements (SE) are specifically modified to form specialised structures known as pore–plasmodesma units (PPU) (van Bel 1996) (Fig. 2d). The ER within these PPU

is believed to play a vital role in the survival of SE and functioning of the phloem. The SE–CC complexes form the functional units of phloem and are specialised for the long-distance flow of solutes. Both cells originate from an unequal division of the SE–CC mother cell. Soon after division, the plasmodesmata on the CC side become highly branched, with up to 100 branches in some species (Evert 1990), coalescing in a central cavity that connects to a wider symplasmic pore at the SE side. It was previously reported that the ER was not continuous through the PPU, but it is now accepted that ER tubules traverse the PPU (Ding et al. 1993), although the ultrastructure of the central cavity has not as yet been resolved (van Bel and Kempers 1997). It has been suggested that branching of plasmodesmata occurs wherever the demand for symplasmic contact increases during development (Kollmann and Glockmann 1999; Ehlers and Kollmann 2001). If so, the asymmetric branching of the PPU implies the difference in permeability of the plasmodesmata on both sides of the cell wall (Schulz 2005).

During differentiation, selective autophagy results in the disintegration of most of the cellular components of the SE including the nucleus and vacuole. What remains at maturity is the plasma membrane, a thin layer of parietal cytoplasm composed of stacked ER or fenestrated ER (Thorsch and Esau 1981a,b; Sjolund and Shih 1983) and a few dilated mitochondria (Evert 1990). Phloem-specific plastids of two types, with proteinaceous (P-type plastids) or starch (S-type plastids) inclusions (Esau 1969; Behnke 1991a; van Bel and Knoblauch 2000; van Bel 2003), and SE phloem proteins (Eleftheriou 1990; Evert 1990; Cronshaw and Sabnis 1990; Behnke 1991b; Iqbal 1995) are also conspicuous in the SE. To prevent these organelles being dragged along in the turbulent mass flow within the SE, 7-nm-long macromolecular extensions anchor the ER, mitochondria and plastids to each other and to the plasma membrane (Ehlers et al. 2000). The resulting mature SE has been described as almost "clinically dead", and in many cases has to be maintained by the adjacent CC. The ER that is continuous from CC to SE is thought to provide a major "lifeline" for the trafficking of macromolecules that are essential to maintain the integrity of the SE (Oparka and Turgeon 1999; van Bel and Knoblauch 2000).

Compared to other organelles within the SE, the ER is well preserved in the form of nets or stacks (Thorsch and Esau 1981a,b; Sjolund and Shih 1983). The parietal fenestrated ER, which shares some traits with the cortical ER (Staehelin 1997), has been postulated to be a structural necessity for ATP-fuelled retrieval (Sjolund and Shih 1983), and may thus be essential for sucrose uptake and retrieval by the SE (van Bel 1996).

Attachment of macromolecules to the cytoplasmic face of the desmotubule has been proposed to allow the movement of proteins through the PPU connecting SE and CC (see Sect. 4.1 and Fig. 4). Due to the enucleate nature of SE and their lack of rough ER, it has been recognised that SE are dependent on CC for their functioning, in some cases for decades (Raven 1991). The flow

rate within sieve tubes has been estimated to exceed 40 cm h^{-1} (Fisher 1990). If proteins or mRNA entering the SE via the PPU were simply "dumped" into the translocation stream, they would be swept rapidly away to sinks. This might be the case for some macromolecules, but it is likely that others are in some way anchored, particularly those required for the maintenance of the SE. It has been suggested that proteins or mRNA trafficked across a PPU remain bound to the desmotubule and subsequently move in association with the ER (Fig. 4). Such a process may occur following the targeting of the movement protein (MP) of *Cucumber mosaic virus* (CMV), fused to GFP, to PPUs.

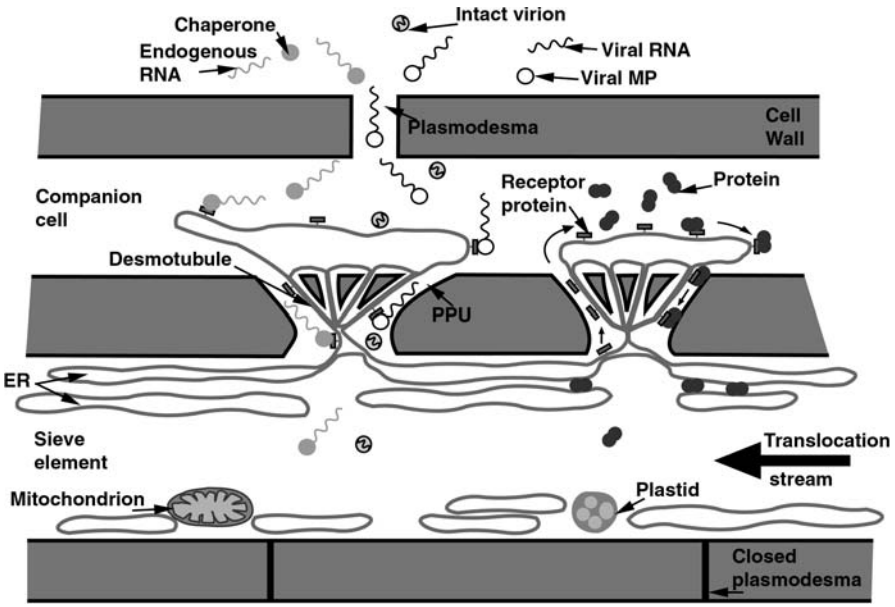

Fig. 4 Schematic model depicting molecular trafficking between the CC and the SE. *Left*: Endogenous RNA molecules originating in the mesophyll are trafficked by specific molecular chaperones into the CC. At the PPU, the chaperone interacts with a receptor protein located on the desmotubule. In the case of some systemic RNA viruses, the viral RNA is trafficked into the CC by a specific viral movement protein (MP). As in the case of endogenous RNA chaperones, the MP interacts with the desmotubule to facilitate RNA movement into the SE. Other systemic viruses may traffic across the PPU as intact virions, without the need for disassembly in the CC. *Right*: Selective protein trafficking between CC and SE. Proteins synthesized within the CC and destined for the SE parietal layer are trafficked across the PPU by an interaction with receptor proteins located on the desmotubule. Such receptors ensure that the proteins are delivered along the ER to their target sites without loss to the translocation stream. After delivery, the receptor proteins may be recycled back into the CC to collect further cargo (*arrows*). Other low molecular weight proteins may enter the SE from the CC by diffusion. Such proteins may not possess "retention signals" for the ER, and may be translocated and unloaded in sink regions of the plant. (Adapted from Oparka and Turgeon 1999)

Upon entry into the SE, the MP trafficked along a reticular structure within the SE parietal layer (Blackman et al. 1998).

It is interesting to note that the cortical ER of higher plants is a highly dynamic system of tubules and sheets, and that this ER flow is probably driven by a close association with an underlying actin –myosin network (Boevink et al. 1998; Brandizzi et al. 2002). This raises the possibility that macromolecules anchored on the cytoplasmic face of the cortical ER network might be delivered to the vicinity of plasmodesmata simply by random flow along ER membranes. Although the desmotubule is thought to be inserted into plasmodesmata from their inception, it is intriguing to speculate that in some circumstances the cortical ER might be able to physically move along actin filaments that traverse the plasmodesmal pore. In such a scenario, macromolecules (including viral ribonucleoprotein complexes) might be able to "hitch a ride" on the mobile cortical ER system (Oparka 2004).

5
Macromolecular Transport via Plasmodesmata

There is now a considerable body of evidence supporting the concept that plasmodesmata can mediate the cell-to-cell trafficking of numerous endogenous macromolecules including transcription factors, plant defence-related proteins and RNA (Mezitt and Lucas 1996; Ghoshroy et al. 1997; McLean et al. 1997; Haywood et al. 2002; Heinlein and Epel 2004). Intercellular trafficking through plasmodesmata is now thought to be an important means of regulating plant development, physiology and defence (Ding 1998). Two main mechanisms are now considered relevant to macromolecular transfer: non-selective transport and selective trafficking.

5.1
Non-selective Transport Through Plasmodesmata

It has been suggested that, given an adequate exclusion limit, non-cell autonomous proteins (NCAP) and other macromolecules diffuse from cell to cell via the cytoplasmic sleeve (Crawford and Zambryski 2000; Oparka 2004). According to this model, molecules are retained by cells by targeting to specific subcellular compartments, e.g. nucleus. This would explain the extensive cell-to-cell movement of GFP within epidermal cells of sink tissues (simple plasmodesmata and high exclusion limit) and the rarer movement in source tissues. In the case of the SE–CC complex, it appears that unless a protein has a retention signal for the CC or a targeting signal that directs it to the SE ER, it will be exported in the translocation stream (Oparka and Turgeon 1999). PPU connecting the CC and SE have been shown to have a large SEL for the non-selective movement of molecules. Using fluorescently tagged dex-

trans, the MEL has been demonstrated to be in excess of 10 kDa (Kempers and van Bel 1997) (R_s 2 nm, estimated from Jørgensen and Møller 1979), and proteins in excess of 67 kDa (R_s 3.3 nm, estimated from Jørgensen and Møller 1979) have recently been shown to move from the CC into the SE of *Arabidopsis* roots (Stadler et al. 2005). It is beyond the scope of this chapter to describe the wide range of macromolecules that pass through plasmodesmata, and the reader is referred to recent reviews on this topic (Haywood et al. 2002; Roberts and Oparka 2003; Oparka 2004; Ruiz-Medrano et al. 2004; Heinlein and Epel 2004; Gillespie and Oparka 2005).

5.2 Selective Trafficking Through Plasmodesmata

In contrast to non-selective movement, the selective or targeted movement of molecules requires conformational changes in the architecture of the plasmodesmal pore, and possibly conformational changes to the molecule being trafficked (Schulz 1999; Zambryski and Crawford 2000; Oparka 2004). Of the many proteins identified to date, many appear to show selective transport through plasmodesmata, and many also increase the exclusion limit of the pore.

5.2.1 Role of the ER in Macromolecular Trafficking from CC to SE

Due to the wealth of data provided by analysis of sieve tube exudates, much of the information regarding the mechanisms of macromolecular transport through plasmodesmata has come from studies of the PPU that connect CC and SE. A number of NCAPs have been shown to be synthesised in the CC before transfer to the SE. In cucurbits, the genes encoding the 25-kDa phloem lectin (phloem protein 2, PP2) and the 96-kDa phloem filament protein (phloem protein 1, PP1) are expressed in CC. Whilst the mRNA has been localised by in situ hybridisation to the CC, the proteins are the principal components of the P-protein aggregates in SE (Bostwick et al. 1992; Clark et al. 1997; Dannenhoffer et al. 1997). The phloem-specific thioredoxin h RPP13-1, a 13-kDa protein first identified in rice phloem sap (Ishiwatari et al. 1995) and subsequently shown to be present in vascular saps from a wide range of species (Schobert et al. 1998), has a similar localisation pattern with transcripts again accumulating specifically in CC (Ishiwatari et al. 1998).

In considering the major role of the phloem in sugar transport it is interesting to note that although some of the transporters involved in phloem loading, for example the SUC2 transporter (Stadler et al. 1995) and the proton pump (H^+-ATPase) (DeWitt and Sussman 1995), are located on the plasma membrane of the CC, the leaf sucrose transporter SUT1 is located only on the plasma membrane of the SE (Kühn et al. 1997; Lalonde et al. 1999). In this

case the mRNA has been localised to the SE as well as the CC, being preferentially associated with the PPU (Kühn et al. 1997). This raises the question as to why the *SUT1* mRNA is apparently trafficked into the SE. It has been proposed that *SUT1* transcript is translated in the CC followed by translocation of the SUT1 protein into the SE in association with the desmotubule and the ER of the SE, as described above (see Sect. 4.1) (Kühn et al. 1997). There has been some debate as to whether it is possible for *SUT1* mRNA to be translated in the SE (Thompson and Schulz 1999) but it has recently been postulated that the *SUT1* mRNA, which moves within the phloem translocation stream, is acting as a long-distance signalling molecule (Haywood et al. 2002).

5.2.2
Trafficking of mRNA from CC to SE

The localisation of *SUT1* mRNA within the SE provides some evidence in support of the trafficking of mRNA through PPU. In characterising other sieve tube exudate proteins, further support has been given to this process by the identification of a 16-kDa *Cucurbita maxima* (pumpkin) phloem protein (CmPP16). In common with viral movement proteins, CmPP16 binds to and mediates the cell-to-cell trafficking of non-sequence-specific RNA when microinjected into mesophyll cells. CmPP16 protein localises to the periphery of SE, possibly the plasma membrane. However, both *CmPP16* mRNA and CmPP16 protein are translocated in the phloem stream through a pumpkin-to-cucumber heterograft (Xoconostle-Cazares et al. 1999). This, therefore, appears to be a protein capable of mediating the transport of its own and possibly other mRNA into the phloem. In common with the association between viral MP and RNA, it is probable that endogenous RNA must also associate with a cellular protein to mediate its trafficking through plasmodesmata.

5.2.3
NCAP–ER Interactions

As yet it has not been possible to identify any common motifs in the NCAP characterised so far that are responsible for their interaction with plasmodesmata, and it is likely that at least some NCAP interact with other cellular proteins to mediate their intercellular passage (Oparka 2004). One such protein has recently been identified and indicates a role for the ER in this process. Using CmPP16 (Xoconostle-Cazares et al. 1999) as a bait for the affinity purification of interacting proteins present within a plasmodesmata-enriched cell wall fraction, a 40-kDa protein termed NCAPP1 (non-cell-autonomous pathway protein 1) was detected (Lee et al. 2003) and immunolocalised to the cortical ER. Silencing studies suggest that NCAPP1 has a role in the selective transport of key developmental proteins, possibly shuttling NCAP to the plasmodesmata pore.

5.2.4 Cell-to-Cell Movement of Small RNAs

As well as the trafficking of proteins and large mRNA molecules, the cell-to-cell movement of the as yet unidentified systemic silencing signal, believed to be a small RNA (Mlotshwa et al. 2002), is also thought to occur via plasmodesmata (Voinnet et al. 1998; Lucas et al. 2001; Mlotshwa et al. 2002; Himber et al. 2003). The silencing signal is not able to enter meristems or stomatal guard cells that have lost their plasmodesmatal connections (Voinnet et al. 1998). There has been speculation that if these signals are small RNAs they should be able to diffuse through plasmodesmata. However, microinjection of various fluorescently labelled single-stranded or double-stranded 25-nucleotide (nt) RNAs showed no movement from the injected cell unless in the presence of phloem-purified CmPSRP1 (*C. maxima* phloem SMALL RNA BINDING PROTEIN1) (Yoo et al. 2004). This protein is believed to facilitate the specific trafficking of small single-stranded RNA, but only into neighbouring cells as expected for trafficking of RNA from a CC into a SE. It remains to be shown whether the ER plays a specific role in this process.

6 Viruses, Plasmodesmata and the ER

Until relatively recently the study of virus movement provided the only evidence available relating to macromolecular transfer via plasmodesmata. Most viruses encode movement proteins (MP) that facilitate the movement of the viral genome either as virions or some form of ribonucleoprotein complex, through plasmodesmata (Carrington et al. 1996; Ding et al. 1999; Gilbertson and Lucas 1996; Lazarowitz and Beachy 1999; Nelson and van Bel 1998; Sakuth et al. 1993). A number of viral MP bind nucleic acid (Waigmann et al. 1994), target plasmodesmata (Blackman et al. 1998; Itaya et al. 1998; Oparka et al. 1997; Ryabov et al. 1998; Santa Cruz 1999; Tomenius et al. 1987) and gate plasmodesmata to a higher than normal SEL (Itaya et al. 1998; Oparka et al. 1997; Santa Cruz 1999; Wolf et al. 1989). It has been speculated that during co-evolution with host plants, viruses "hijacked" one or more host MP for trafficking molecules through plasmodesmata. As a result many models of macromolecular trafficking are based on these viral studies (Oparka 2004; Lucas et al. 2001; Haywood et al. 2002; Ruiz-Medrano et al. 2004).

Investigations into viral movement have implicated the ER in the MP-mediated transport of viral RNA to and through plasmodesmata. The ER is recognised to be the replication site for *Tobacco mosaic virus* (TMV) RNA (Heinlein et al. 1998; Mas and Beachy 1999; see also Chap. Ritzenthaler and Elamawi, this volume). The ER has also recently been shown to be involved, along with actin, in the movement of *Potato mop-top virus* (PMTV) granules

to and through plasmodesmata (Haupt et al. 2005). PMTV encodes three proteins associated with movement. The first triple gene block protein (TGB1) is a sequence non-specific RNA binding protein with helicase activity and is believed to produce a movement competent viral ribonucleoprotein complex, while TGB2 and TGB3 function in movement to and through plasmodesmata (Heinlein and Epel 2004; Haupt et al. 2005). Early in the infection, TGB2 and TGB3 associate with the ER and co-localise in motile granules. Both proteins are able to increase the SEL of plasmodesmata and TGB3 targets plasmodesmata in the absence of TGB2. TGB3 contains a putative Tyr-based sorting motif, which when mutated abolished the ER localisation and plasmodesmatal targeting. In this study it is suggested that the TGB2/3 complex uses the ER–actin network to facilitate movement to plasmodesmata (Haupt et al. 2005). A similar conclusion was reached for the movement of *Poa semilatent hordeivirus* (PSLV) TGB3 protein (Zamyatnin Jr. et al. 2002; Gorshkova et al. 2003).

In contrast to the TGB viruses, TMV employs only one virus-encoded MP. This 30-kDa protein from TMV has been shown to target plasmodesmata, increase the molecular exclusion limit and potentiate cell-to-cell movement as a complex with viral genomic RNA (for reviews, see Haywood et al. 2002; Roberts and Oparka 2003; Heinlein and Epel 2004). TMV-MP shows an early association with the ER and later with microtubules (Heinlein et al. 1998) and it has been proposed that the MP participates in the formation of ER-associated viral ribonucleoprotein complexes, and mediates their interaction with molecular microtubule motors for movement to plasmodesmata (Heinlein and Epel 2004). However, a more direct involvement of ER and actin in TMV movement to plasmodesmata has also been suggested based on the observation that disruption of microtubules does not prevent cell-to-cell movement of TMV, while treatment with inhibitors that disrupt ER or actin reduce the targeting of TMV-MP to plasmodesmata (Gillespie et al. 2002).

7 Conclusions

From the evidence gathered so far it is clear that the selective transport of proteins and RNAs through plasmodesmata is complex and highly regulated. Many current models of cell-to-cell transport through plasmodesmata implicate the cortical ER and desmotubule in trafficking NCAP through plasmodesmata. The greatest advances at present are emerging from new discoveries at the protein level using genomic and proteomic techniques (Cutler et al. 2000; Escobar et al. 2003; Tian et al. 2004; Oparka 2004). These should allow the identification of the receptor, docking and regulatory proteins at present hypothesised to be part of the pore and increase our understanding of the role of the ER within plasmodesmata.

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