Chapter 1
Characteristics of Symplasmic Transport

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Abstract Symplasmic transport is possible in organisms of plants, fungi, and even in animals and some prokaryotes, where cell-to-cell protoplasmic junctions are present. However, a spectacular evolution of the symplasm was limited to plants, where highly efficient long-distance transport occurring inside the cells is responsible for the spread of molecules of different nature along the plant body of length up to tens of meters. Several aspects of symplasmic transport are considered in this chapter. A short review of the history of this research is presented with particular attention to old but still inspiring ideas and unanswered questions. Ultrastructure, phylogeny, and ontogeny of the symplasm as well as different mechanisms that allow symplasmic transport (diffusion, cytoplasmic streaming, and mass flow) are discussed thoroughly. Examples of tissues where symplasmic transport covers the distance of several or even more cells without participation of sieve tubes are also discussed, besides the strictly local cell-to-cell symplasmic transport and long-distance transport in phloem.

Keywords Apoplasm • Cytoplasmic streaming • Diffusion • Long-distance transport • Mass flow • Ontogeny • Plasmodesmata • Phloem • Phylogeny • Short-distance transport • Symplasm

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Abbreviations

BS	Bundle sheath
EBS	Extended bundle sheath
KMS	Kranz mesophyll
PCA	Primary carbon assimilation
PCR	Primary carbon reduction
PD	Plasmodesma/plasmodesmata
PVM	Paraveinal mesophyll
SEL	Size exclusion limit
VP	Vascular parenchyma

1.1   Introduction

Life is a flow. Even an immobile single cell demonstrates movement of organelles, vesicles, and cytoplasm streaming. It also exchanges solutes with the environment. The movement of diverse particles and molecular forms is crucial to cooperation between cells in multicellular organisms. To fulfill this demand, a system of cell-to-cell transport has evolved in multicellular organisms. The system comprises protoplasts and cytoplasmic channels bridging neighboring cells—plasmodesmata. They are particularly important for cells enclosed by a cell wall, such as fungi, algae, and plants, but cytoplasmic bridges do connect also animal cells. Plasmodesmata allow not only exchange of small solutes but also of macromolecules such as proteins and nucleic acids, thus forming a versatile system of cell-to-cell communication. The entire system of protoplasts interconnected by plasmodesmata is called the symplasm. It forms the plant body together with the apoplasm comprising cell walls and intercellular spaces. Accordingly, the transport inside and outside cells is called, respectively, symplasmic or apoplasmic. The symplasmic transport system has evolved further in telomic plants parallelly with their increasing size. The corollary was the phloem present in vascular plants. The conducting elements in this system are sieve elements forming sieve tubes transporting phloem sap from leaves to other plant organs. The key feature of this long-distance transport system is that the movement of solutes occurs inside the cells, unlike in the conducting systems functioning in animals, where diverse liquids (e.g., blood, lymph, and food) are transported inside hollow tubes—vessels whose walls are built from cells. Another fundamental difference between those two modes of long-distance transport is that in plants it is not powered by any contracting elements corresponding to the animal heart but solely by hydrostatic gradient along the sieve tubes. Despite its apparent simplicity, the long-distance transport in plants is astonishingly efficient: it allows transport of high amounts of solutes for a distance of several dozen meters in case of some trees. Additionally, it is a pathway for signals of different nature: biochemical, such as

hormones, nucleic acids, and proteins, and biophysical, such as the action and water potentials. This book is focused on the symplasmic transport, but some aspects of the apoplasmic ones will be presented as well.

1.2   Research on the Symplasmic Transport: Milestones

Virtually all reviews on the history of botany begin from Aristotle (384–322 bc); however, the true foundation of modern science is Francis Bacon's (1561–1626) scientific method based on experiment. Probably, the first researchers to contribute substantially to the study of the transport in plants were the inventor of the light microscope, Anton van Leeuwenhoek (1632–1723), who described xylem vessels (after Pardos 2005), and Marcello Malpighi (1628–1694), who showed upstair transport of water in the wood and the downstair transport in bark (after Kursanov 1984). Studies on water movement and transpiration in plants have continued since then (Pardos 2005). The first to study the transport phenomena in plants systematically was Henri-Louis Duhamel du Monceau (1700–1782), considered by many to be the founder of modern plant physiology. At the same time (1774), Bonaventura Corti observed cytoplasmic streaming in plant cells (after Verchot-Lubicz and Goldstein 2010).

According to Zimmermann (1974), intensive studies on transport began in middle of 1800s with the discovery of sieve tubes and of exudation from both phloem and xylem by Theodor Hartig (1805–1880) in 1837. The other ultrastructural component of symplasmic transport, plasmodesmata (PD), was first described by Eduard Tangl (1848–1905), who observed strands of cytoplasm connecting cells in the cotyledon of *Strychnos nux-vomica* (Tangl 1880; after Köhler and Carr 2006a). That discovery attracted soon the interest of numerous investigators (Meeuse 1941). The term *Plasmodesmen* (Germ.) was introduced by Strasburger (1844–1912) in his review (Strasburger 1901). The term "symplasm" was introduced much later by Münch (1930), but yet Tangl noticed that "the connecting ducts unite them (the cells) to an entity of higher order" (after Köhler and Carr 2006b).

Studies at the beginning of the twentieth century added much to the understanding of transport phenomena in plants and, in fact, put forward most of relevant concepts discussed and developed until present. Concerning the plasmodesmata, Meeuse stated in his review (1941) that soon after the Tangl's discovery, the general presence of plasmodesmata throughout the plant kingdom and in all living tissues was accepted. Even animal cells were postulated to contain plasmodesmata (ibid.), but only recently important data in that field have been obtained (Wade et al. 1986; Nicholson 2003; Rustom et al. 2004). The problem of how plasmodesmata develop was discussed already at the beginning of twentieth century, concentrating on the origin of primary (the term not used then) plasmodesmata during the formation of cell walls after mitosis and the secondary (the term used by Meeuse in 1941) ones crossing existing cell walls. At those primordial stages of research on

plasmodesmata, their role in the cell-to-cell transport as well as the origin of sieve pores from plasmodesmata were first postulated. Although there was much concern regarding possible artifacts of specimen fixation, the protoplasmic nature of the plasmodesmata was generally accepted. The most convincing piece of evidence was that "there is a translocation of viruses from cell to cell" (ibid.), a phenomenon being the research area studied to date.

In the case of long-distance transport, most of the early studies concentrated on the ascent transport of water and some modern ideas on phloem transport were formulated as well. In particular, Dixon, who proposed the cohesion theory of water transport above the barometric height (Dixon 1914), neglected diffusion as a mechanism of transport of organic compounds from leaves to other organs (Dixon and Ball 1922). Those authors calculated that, even if the transport of sucrose solution was accelerated by protoplasmic streaming, the diffusion rate was too low to account for the actual rate of transport of carbohydrates in plants. Later on Münch (1930) formulated his concept of pressure flow, fully accepted only recently, to explain the mechanism of long-distance transport. He also coined the terms "symplasm" and "apoplasm."

Any further progress in the research on the symplasmic path components was crucially dependent on the developments of experimental techniques. Systematic studies were performed on the mechanism of cytoplasmic streaming (Kamiya and Kuroda 1956; Kamiya 1981 and citations therein). At the same time, the velocity of organelles' movement was measured at 2–5 µm s⁻¹ and 5–6 µm s⁻¹ for chloroplasts and vesicles, respectively (Zubrzycki 1951). One cannot but admire the accuracy of those estimates obtained with rather crude tools: the most recent measurement of the velocity of small organelles using GFP fused to peroxisome targeting signal 1 (PTS1) and time-lapse laser scanning confocal microscopy reported an almost identical value of 10 µm s⁻¹ (Jedd and Chua 2002).

Regarding the mechanism of long-distance phloem transport, several hypotheses were formulated besides the pressure flow theory of Münch, even though the finding of Mittler (1957) that the turgor pressure in sieve elements was high enough to explain the observed velocity of phloem transport spoke eloquently in favor of the latter. Those challenging the Münch theory claimed that sieve pores were always occluded by a dense material which precluded an efficient mass flow of solutes under pressure. On the basis of that observation, Spanner proposed his concept of electroosmosis (Spanner 1975) as an alternative for the pressure flow theory. It was argued that if sieve pores were indeed narrowed by occludes, electrical phenomena would be more efficient in powering of the phloem transport than pressure flow. In several other hypotheses, described in reviews of Canny (1975) and Kursanov (1984), pulsations of microstructures in the sieve tubes were proposed as the motive force for transport. Another proposition was the movement in monolayer, i.e., sliding of molecules along the phase boundaries due to uneven distribution of molecular forces (ibid.). Additionally, in many electron microscopic studies, P-proteins were found forming strands along the sieve tubes. These observations prompted hypotheses on the participation of P-proteins in longitudinal transport despite their scarcity or even absence in many plants, e.g., maize and

barley (Evans 1976). One of them proposed P-proteins as ducts for electric waves powering the longitudinal transport in sieve tubes (Hejnowicz 1970). In the 1980s and 1900s, most of those hypotheses were discredited as either unrealistic or based on artifacts.

The final argument for the Münch's pressure flow was found at the very end of the twentieth century, when Ehlers et al. (2000) showed that carefully fixed sieve tubes did not show any occlusions at the sieve pores and the lumen of sieve tubes was clear. It seems that most of the artifacts found in sieve tube preparations were related to the sample preparation (ibid. and references there) and induction of mechanisms preventing the leakage of the phloem sap from injured sieve tube. The response seems to be particularly sensitive (Knoblauch and van Bel 1998) to even delicate mechanical stress.

Currently, the pressure flow theory of long-distance transport in the phloem seems to be widely accepted. Nevertheless, three ideas concerning phloem transport formulated in the twentieth century, outside the mainstream considerations in this area, seem worth mentioning.

The first question concerns the problem of bidirectional transport in the phloem. The movement of different molecules in opposite directions in individual sieve tubes was assumed as a strong argument against mass flow mechanism (for reviews, see Evans 1976; Kursanov 1984). It is, however, possible that phloem transport in opposite directions occurs in separate sieve tubes. Additionally, modeling the dynamics of solute transport (Henton et al. 2002) has demonstrated that the solute can move in opposite directions along the single tube. No experimental current data on the problem are accessible, beside of technical progress and development of methods.

The second problem was formulated by Romberger et al. (1993) and concerned the mechanism of phloem transport. Basing on their calculations, those authors stated that the sieve pores were too wide for efficient electroosmosis, which neglected Spanner's electroosmosis theory, yet they were too narrow for efficient mass flow of solutes which excluded the Münch's pressure flow theory. In conclusion, the authors proposed that if pressure flow was accelerated by electroosmosis, the pore diameter would be optimal for the transport. The concept has not been developed further.

The third idea concerns the so-called vacuome, i.e., a system of connections between vacuoles crossing the plasmodesmata as desmotubules and involving also the sieve tube lumen (Gamalei and Pakhomova 2002; Velikanov et al. 2005). Already Esau (1971) reviewed the suggestions of several researchers that a membrane (tonoplast) could separate the parietal cytoplasm from the central cavity in sieve tubes. Against such a hypothesis were reports describing the disappearance of the tonoplast in mature sieve elements (for review, see Kursanov 1984), although other authors argued for an extreme sensitivity of tonoplast to preparation and fixation (Esau 1971). Contacts between vacuoles and plasmodesmata were shown by some authors. Rinne et al. (2001) demonstrated that spherosome-like vacuoles became displaced toward plasmalemma near plasmodesmata during the releasing from dormancy in the apical meristems of *Betula pubescens*. The postulated role of

the movement was limited to the transient delivery of β-1,3-glucanase to the plasmodesmata. A vacuolar-tubular continuum was also reported in trichomes of *Cicer arietinum* (Lazzaro and Thomson 1996). The idea of participation of vacuome in assimilate transport from mesophyll chloroplasts to sieve tubes and then to other organs, presented by Gamalei (2007), is based almost exclusively on the results of Gamalei, and his collaborators and therefore a critical discussion by others would be desirable to add credence to it.

1.3 Ultrastructure, Ontogeny, and Phylogeny of Symplasm

In higher plants, the symplasm consists of protoplasts linked by plasmodesmata and sieve elements involved, respectively, in cell-to-cell and long-distance transport. The ultrastructural details of plasmodesmata and sieve tubes are discussed in other chapters of this book. Therefore, only basic information is provided here.

1.3.1 Ultrastructure and Ontogeny

1.3.1.1 Plasmodesmata

Plasmodesmata in higher plants are cytoplasmic channels penetrating cell walls with the plasmalemma as the outer border and a desmotubule in the center inside the channel. The diameter of plasmodesmata is 20–50 nm (Ehlers and Kollmann 2001), and their length reflects the cell wall thickness. However, depending on the tissue and developmental stage, plasmodesmata may differ strongly in shape and form (Robinson-Beers and Evert 1991; Ehlers and Kollmann 2001; Botha 2005; Burch-Smith et al. 2011). They can be simple or branched, with or without constrictions in the neck and/or central regions. The desmotubule is approximately 15 nm in diameter (Ehlers and Kollmann 2001), occupies the center of the plasmodesma channel, and is a tubular process of the endoplasmic reticulum membrane connecting the ER systems of the neighboring cells. It is used to be also called the "central rod" due to its apparently solid structure on most of plasmodesmata microphotographs (Gunning and Overall 1983; Tilney et al. 1991; Botha et al. 1993; Overall and Blackman 1996; Ding 1998). Recent data show, however, that the desmotubule is a membranous tubule composed of lipids and proteins, the latter allowing an extremely strong contraction of the tube (Tilsner et al. 2011). The desmotubule is surrounded by a cytoplasmic sleeve penetrated by spoke-like proteinaceous extensions linking the desmotubule with the plasmalemma. In principle, the transport routes through plasmodesmata could involve the cytoplasmic sleeve, the desmotubule membrane, and the desmotubule lumen (Evert et al. 1977; Waigmann et al. 1997; Cantrill et al. 1999; Roberts and Oparka 2003; Sowiński et al. 2008; Barton et al. 2011), but only the first one is widely accepted in the literature.

Two main types of plasmodesmata are discussed in the literature: primary and secondary ones (Ehlers and Kollmann 2001; Burch-Smith et al. 2011). It is widely accepted that primary plasmodesmata are those forming during cell division. The case of secondary plasmodesmata is less clear-cut, since to some authors secondary PD are only those formed across preexisting cell walls, while others use the term also for PD formed by modification of primary PD, such as PD twinning. To avoid misunderstanding, some authors speak of "twinned secondary plasmodesmata" and "de novo secondary plasmodesmata" (Burch-Smith et al. 2011).

In general, plasmodesmata can undergo distinct modifications during plant development. Between some cells, plasmodesmata can be eliminated, which leads to symplasmic isolation of the symplasmic domains (Rinne and van der Schoot 2003), an important step in plant development (see Chap. 2). At other locations, the plasmodesmata become branched at certain developmental stages, thereby providing an improved symplasmic transport path; examples are the plasmodesmata linking intermediary cells (a form of companion cells) with sieve elements in symplasmic phloem loaders (Volk et al. 1996, see Chap. 5). A unique plasmodesma modification is its conversion into a sieve pore, occurring during the development of sieve elements (Sjölund 1997). This modification involves widening of the pores up to 200–400 nm, thus allowing almost unimpeded symplasmic transport in sieve tubes by means of the pressure flow mechanism.

1.3.1.2 Phloem

Mature sieve tubes in angiosperms are columns of elongated cells, sieve elements, up to 20 µm in diameter and 250 µm in length (Sjölund 1997). They contact one another in the file through sieve plates massively penetrated by sieve pores. The sieve elements contain no nucleus, vacuoles, ribosomes, Golgi bodies, microfibrils, or microtubules (van Bel and Knoblauch 2000). Their only structural components are the modified ER, mitochondria, and plastids (ibid.). The latter are either P-plastids or S-plastids, containing proteinaceous or starch inclusions, respectively (ibid.). The presence of P-plastids and S-plastids is family specific (Behnke 1991). The elimination of the other organelles occurs during sieve element maturation and is accompanied by cell wall thickening and conversion of "ordinary" plastids into the S- or P-plastids. The cytoplasm is apparently present only at the cell periphery with the organelles linked to the plasmalemma by clamps (Ehlers et al. 2000). Thus, the central part of the sieve element is empty, allowing efficient transport of the phloem sap.

The sieve elements are joined to companion cells, both structurally (numerous plasmodesmata branching at the companion cell side) and functionally (the companion cells provide proteins for the sieve elements and may also participate in phloem loading; see Chap. 5). Thus, the cells of both types are often treated as a unit: a companion cell/sieve element complex. Besides their structural and functional cooperation, both sieve elements and companion cells originate from phloem mother cells; one sieve element and several companion cells form one mother cell (van Bel 2003), which differentiates them from the sieve cells of other vascular plants.

Table 1.1 Symplasmic pathway in prokaryotes and animals

Kingdom	Phylum	Plasmodesmata		Conducting cells	
		Structural aspects	Functional aspects	Structural aspects	Functional aspects
Eubacteria	Cyanophyta	Microplasmodesmata	Rapid intra-filament movement of metabo-lites, possibly transfer of signals	No special-ized cells regarded	
Animalia		Gap junctions, <4 nm in diameter, composed of connexins (vertebrates) or innexins (invertebrates)	Transport of solutes of low molecular weight (<1.7 kDa)		
		Tunneling nanotubes (found in mammals) cytoplasmic channels (possible transient), 50–200 nm in diameter and length of tens of μm, enriched with actin filaments but lacking microtubules	Transfer of endosome-based vesicles, organelles, viruses	No special-ized cells regarded	

1.3.2 Phylogeny

For decades, a dogma was in force that the symplasm and symplasmic transport were limited to plants and some fungi. The challenge of this dogma has come only recently with the discovery of tunneling nanotubes (Rustom et al. 2004) in animals, although even much before that gap junctions linking animal cells were widely known (Wade et al. 1986; Nicholson 2003), but their existence seemed not to violate the view of the animal tissue as a sum of isolated cells exchanging "signals" and molecules only via specialized relay and channel proteins. Nowadays, it is commonly accepted that all multicellular organisms demonstrate direct cell connections (Baluška et al. 2004; Gerdes et al. 2007; Rustom 2009), albeit the extent of such "symplasmic" network varies greatly among kingdoms.

The current state of our understanding of the symplasmic connection at the cell and organismal levels is summarized in Tables 1.1, 1.2, 1.3, 1.4, 1.5, and 1.6, compiling of data from many original papers, reviews, and monographs concerning animals (Wade et al. 1986; Nicholson 2003; Rustom et al. 2004), fungi (Kirk and Sinclair 1966; Powell 1974; Marchant 1976; Taylor and Fuller 1980; Cook and Graham 1999; Müller et al. 1999), nonvascular plants (Schmitz and Srivastava

Table 1.2 Symplasmic pathway in fungi

Kingdom	Phylum	Plasmodesmata		Conducting cells	
		Structural aspects	Functional aspects	Structural aspects	Functional aspects
Fungi	Basidiomycota	Simple plasmodesmata		Central pore in septa	Cytoplasmic streaming, communication between cells, flow of cytoplasm and organelles, dead cells in hypha isolated by plugging of the central pore
	Ascomycota	Simple PD, 60 nm in diameter with desmotubule (10 nm in diameter) linked to ER		Central pore in septa	Cytoplasmic streaming, communication between cells, flow of cytoplasm and organelles
	Chytridiomycota	Simple PD (23 nm in diameter) with dense core			
	Zygomycota	Simple PD linked to ER			

1974; Marchant 1976; Cook et al. 1997; Cook and Graham 1999; Kwiatkowska 1999, 2003), lower vascular plants (Mueller 1972; Evert and Eichhorn 1976; Warmbrodt and Evert 1979; Smoot 1985; Evert et al. 1989; van Bel 1999; Cooke et al. 2000; Raven 2003; Dong et al. 2004; Halarewicz and Gabryś 2012), and higher vascular plants (Glockmann and Kollmann 1996; Cook and Graham 1999; van Bel 1999; Beck 2010).

1.3.2.1 Plasmodesmata

The lowest common denominator of the symplasmic contacts in all eukaryonts and some prokaryotes is cytoplasmic channel of 20–30 nm in diameter, with the exception of narrower gap junctions in animals and microplasmodesmata in *Cyanophyta* (Table 1.1). Since the discovery of tunneling nanotubes is fairly recent, comprehensive study is still to come. Only then will comparative analysis of cell-to-cell connections throughout the kingdoms become meaningful. For now, one may conclude that the appearance of complex structures in the channels connecting cells is unique to the plants. The desmotubule and the ability to form de novo secondary plasmodesmata are of particular interest here.

Table 1.3 Symplasmic pathway in algae

Kingdom	Phylum	Plasmodesmata		Conducting cells	
		Structural aspects	Functional aspects	Structural aspects	Functional aspects
Plantae	Rhodophyta			Elongated cells with cross walls with pit plugs	Possibly mass flow
	Phaeophyta	Simple PD, some authors report desmotubules		Conducting filaments, length of 1 mm and more, numerous pores in cross walls, small vacuoles, many mitochondria and plastids, nucleus degenerates at maturity, ER, ribosomes, and coated vesicles	Possible mass flow, assimilate transport, accumulation of callose with age and degeneration of some organelles
	Chlorophyta	Simple PD, some authors report ER inside plasmodesma	Plugging or disappearance at some developmental stages	Giant cells, intercellular connection by PD	Cytoplasmic streaming by means of actin-myosin mechanism
	Charophyta	Simple PD with uncompressed ER inside at some developmental stages; possible secondary PD		Giant cells, intercellular connection by PD	Cytoplasmic streaming by means of actin-myosin mechanism

Table 1.4 Symplasmic pathway in lower vascular plants

Kingdom	Phylum	Plasmodesmata		Conducting cells	
		Structural aspects	Functional aspects	Structural aspects	Functional aspects
Plantae	Marchantiophyta	Simple PD with desmotubules		Elongated cells in gametophyte, vacuoles disappeared at maturity, numerous PD in end walls, ER, other organelles present. Absent in sporophytes	Cell polarization
	Anthocerotophyta	Simple PD with desmotubules		Absent in gametophytes and sporophytes	
	Bryophyta	Cytoplasmic bridges, similar to PD, no desmotubule. Single or branched plasmodesmata with desmotubules	Possible role during spermatogenesis	Conductive parenchyma cells; in *Polytrichaceae*: leptoides in gametophytes, dimension 500×20 μm, frequent PD of 120–250 nm in diameter in end walls, nucleus degradation, lack of large vacuoles, ER, different organelles. In sporophyte absent or similar to those in gametophyte	Cell polarization

Table 1.5 Symplasmic pathway in lower vascular plants

Kingdom	Phylum	Plasmodesmata		Conducting cells	
		Structural aspects	Functional aspects	Structural aspects	Functional aspects
Plantae	Lycopodiophyta	Primary PD		Long, narrow sieve cells, nuclear remnants, no vacuole, mitochondria and plastids, tubular network of ER, no difference between pore sizes in end and lateral walls	Adjoining parenchyma cells linked by numerous PD
	Polypodiophyta	Primary PD		Sieve cells with nuclear remnants, no vacuole, smooth ER, mitochondria, lack of plastid grana in some species, no difference between pore sizes in end and lateral walls, considerable variation in the size of cytoplasmic connections among different fern species (0.06–0.7 μm)	Adjoining parenchyma cells linked by numerous PD

The desmotubule was reported as a plasmodesma constituent for vascular plants (Cook et al. 1997; Table 1.4). However, some authors reported the presence of ER protrusions into plasmodesmata in the less evolutionarily advanced plants, *Phaeophyta, Chlorophyta,* and *Charophyta* (Cook and Graham 1999; Table 1.3). Interestingly, desmotubule was found in one division of the fungi, the *Ascomycota* (Marchant 1976; Table 1.2). It is, however, generally accepted that the desmotubule is an "invention" of vascular plants; similarly, the ability to form de novo secondary plasmodesmata seems to have developed in higher plants (Table 1.6). Cooke et al. (2000) went even as far as to propose that this trait is limited to angiosperms. However, one should note two reports (Wetherbee et al. 1984; Franceschi et al. 1994) that some *Charophyta* and *Rhodophyta* divisions are also able to form secondary plasmodesmata. For a thorough discussion on secondary plasmodesmata formation in nonvascular plants, the reader is referred to the review of Kwiatkowska (1999). Although over a decade old, it is, to my knowledge, the most recent comprehensive analysis on the subject. Also, one should note that secondary plasmodesmata are formed in gymnosperms, at least in conifers. Bearing in mind that in conifers the sieve cells and Strasburger cells do not originate from common mother cells as do sieve elements and companion cells in angiosperms (van Bel 1999), plasmodesmata linking the sieve cells and the Strasburger cells should by definition

Table 1.6 Symplasmic pathway in higher vascular plants

Phylum	Plasmodesmata		Conducting cells	
	Structural aspects	Functional aspects	Structural aspects	Functional aspects
Pinophyta	Simple or branched PD with desmotubules		Very long sieve cells with overlapping ends, nuclear remnants, modified plastids, smooth ER, cell components located peripherally	Strasburger (albuminous) cells = modified parenchymatic cells (no true companion cells)
Magnoliophyta	Simple or branched PD, primary or secondary with desmotubules	Transport of low molecular weight solutes (<1 kDa), macromolecules (proteins, RNA); transfer of viruses, viroids; possible transfer of whole genomes of mitochondria and chloroplasts	Sieve elements, no nucleus, modified plastids, some mitochondria, smooth ER, cell components located peripherally, sieve pores in transverse walls, branched PD at companion cell side	True companion cells (same cell line as sieve elements). In grasses, two forms of sieve elements: thin walled (similar to "obscure" sieve elements, connected to companion cells) participating in long-distance transport; thick walled of unknown function, lacking of companion cells, linked by PD to adjoining parenchyma cell

be treated as secondary ones. Additionally, since some conifer species can be grafted, one should expect formation of secondary plasmodesmata between cells of the scion and stock.

1.3.2.2 Conducting Elements

A spectacular evolution of the symplasm was limited to plants. It involved elongation of some cells and their specialization in transport of organic molecules at longer distances. Actually, also in the fungi divisions of *Ascomycota* and *Basidiomycota*, where cells are separated by septa with septal central pores, a sort of long-distance transport driven by cytoplasmic streaming (as in green algae) may function since septal pore allows movement of cytoplasm and organelles (Müller et al. 1999). The septal pores resemble the pit plugs of red algae, apparently an example of convergent evolution (Cook and Graham 1999). The symplasmic transport system developed fully in vascular plants (Table 1.6). The increase of the size of land plants according to the Cope's rule stating that the average body size in a population tends to increase over evolutionary time (after Enquist 2003) puts a strong selection pressure on the development of efficient long-distance transport systems. In the case of xylem, selection resulted in minimization of resistance within xylem vessels (ibid.) by removing all their contents and widening the pores in transverse cell walls. Apparently, the same concerned the sieve cells, since the general evolutionary trend here was the elimination of organelles and cytoplasm content and the formation of the sieve pores in transverse cell walls. Despite these similarities, a crucial difference between xylem vessels and sieve cells remained, since the latter are live cells which greatly increases the resistance to flow. The reason for that could be that the transport in sieve tubes is often against the air-soil water potential gradient; therefore, a positive water pressure must be imposed on the water column in the pipe. Another plausible reason is that only living cells can offer efficient mechanisms of defense against leakage of the precious in case of wounding or herbivore attack. Indeed, another trend in the evolution of sieve cells was the development of such defense mechanisms, e.g., callose accumulation at the sieve plate and other mechanisms of sieve cells sealing.

1.4 What, How, and How Fast: Mechanisms and Efficiency of Symplasmic Transport

1.4.1 What Is Transported in Symplasm?

Myriads of molecules are transported every second in a living plant. Roughly, they can be divided into the low molecular weight and high molecular weight ones. Inorganic ions and organic compounds of different nature of a mass below ca. 1 kDa belong to the first class, while molecules of several kDa and heavier belong to the

second one. In general, the transporting limit of plasmodesmata is called size exclusion limit (SEL), which usually is ca. 900 Da (Crawford and Zambryski 1999). Actually, the better measure is a molecule's Stokes radius, i.e., the radius of a sphere whose hydrodynamic properties mimic those of the molecule. Some other restrictions are related to the physicochemical properties of the molecules to be transported. Thus, several types of molecules are not transported through plasmodesmata, like auxins (Drake and Carr 1978), small hydrophilic molecules of charge in range −4 to −2; molecules containing either Phe, Try, Met, or His groups (Tucker and Tucker 1993); and aromatic amino acids (Tucker 1982; Erwee and Goodwin 1984). One should note, however, some discrepancies concerning the transport of aromatic amino acids either polar or hydrophobic types (Terry and Robards 1987). Possible, plasmodesmata from different tissues differ in their properties (see below, Sect. 1.5.1).

SEL may change under some circumstances to allow transport of high molecular weight molecules through plasmodesmata. Proteins and nucleic acids, as well as bigger complexes, as viruses are now known to be transported in plants through plasmodesmata (Lucas et al. 2009; Maule et al. 2011; Xu and Jackson 2010). For details the reader is referred to Chap. 7. Here, however, it is worth noting that even whole organelles seem to be transported in symplasm, at least through plasmodesmata. Recently, two laboratories independently have presented evidence for the cell-to-cell movement of an entire plastid genome from one plant to another, possibly in an intact organelle (Stegemann et al. 2012; Thyssen et al. 2012). This finding explains former observations of horizontal gene transfer between stock and graft in some grafting experiments (Stegemann and Bock 2009; Talianova and Janousek 2011). Transfer of entire organelles between cells is possible in fungi, nonvascular plants, and lower vascular plants (Tables 1.2, 1.3, and 1.4). The possibility of movement of plastids and mitochondria opens a new area of research on the mechanism of transport of such big organelles. The finding is also intriguing in the context of discussion on the role of horizontal gene transfer in plant evolution as well as its consequences for the safety of GMO in environment.

In the phloem, sucrose and other oligosaccharides are main solutes at concentrations depending on the phloem loading mode (see Chap. 5), while reducing carbohydrates, such as fructose, are absent (Kursanov 1984). Besides carbohydrates, amino acids produced in leaves are transported from leaves to other organs as well (see Chap. 6). The phloem sap also contains inorganic ions at pretty high concentrations, except magnesium, calcium, and boron, which are practically absent (Marschner et al. 1996; Brown and Hu 1996; Atkins 1999). It is worth noting that also heavy metal ions of no apparent use to the plant can translocate through the phloem (for review, see Antosiewicz et al. 2008). Phloem sap contains many other endogenous substances, e.g., secondary metabolites of diverse biological activities such as alkaloids (Kitamura et al. 1993), glucosinolates (Brudenell et al. 1999; Chen et al. 2001), hormones (gibberellins) (Hoad et al. 1993), ABA (Zhong et al. 1996), and precursor of ethylene ACC (Morris and Larcombe 1995). Also auxins and cytokinins are detected in the phloem sap at concentrations sufficient for their biological activity (Baker 2000a, b), even though auxins are generally transported in the cell-to-cell manner outside the vascular systems and cytokinins are synthesized in roots. Also diverse important constituents of the phloem sap are proteins and nucleic acids.

Apart from endogenous substances, phloem translocates also foreign molecules of neutral or negative influence on plant, such as many xenobiotics of herbicide activity (for review, see Brudenell et al. 1995). They are loaded into phloem by carrier-mediated mechanism (Delétage-Grandon et al. 2001) or by diffusion through plasmalemma depending on the physicochemical and structural properties of a given herbicide. Thus, e.g., the non-ionized, monobasic weak acids diffuse easily (Bromilow and Chamberlain 2000). Phloem is also the route of dispersal of viroids (Palukaitis 1987; Zhu et al. 2002), viruses (Esau 1956; Lucas 2006), and bacteria (Rudzińska-Langwald and Kamińska 1999; Moran 2001). The illustration of bacteria presence in sieve tubes is shown on Fig. 1.1. Thus, in addition to its crucial role in the transport and distribution of photosynthates and in systemic signaling, phloem is also plant's Achilles' heel, since it allows invasion by pathogens as well as facilitates the action of herbicides.

1.4.2   Mechanisms of Symplasmic Transport

As already mentioned, most of the transport in plants take place in the symplasm. Three main transport mechanisms have been proposed to explain the movement of molecules in the symplasm: diffusion, cytoplasmic streaming, and mass (bulk) flow. Diffusion is a motion of molecules in a solvent (fluid, gas) according to concentration gradient and is required for numerous cellular processes (Verkman 2002). Cytoplasmic streaming has been classified in physical terms as a form of convection (Pickard 2003), i.e., a movement of distinct zones of fluid. However, the term "advection" seems to be more proper for cytoplasmic streaming (Verchot-Lubicz and Goldstein 2010). Advection is a transport mechanism of a dispersed substance (molecules, particles, etc.) by a fluid due to the fluid's bulk motion. Formally, the term convection is used to refer to the sum of advective and diffusive transfer. The third mechanism could be defined as the movement of a solute molecule together with solvent molecules according to the gradient of a physical force (e.g., water pressure gradient). All three mechanisms have been the subject of numerous, both experimental and theoretical, modeling.

1.4.2.1   Diffusion

Diffusion is assumed to be an important mechanism for intra- and intercellular movement of solutes. According to Tucker (1990), cell and plasmodesmata should be treated as separate diffusion systems. As measured *in vivo*, the velocity of diffusion through plasmodesmata is in the range of 2.8–17 μm s^{-1} (after Anisimov and Egorov 2002), while between cells in a file only 1.1–8.5 μm s^{-1} (Rutschow et al. 2011 and citations there). The diffusion coefficient calculated for plasmodesmata (ibid.) was 2–20 times higher than across the corresponding cell walls (Kramer et al. 2007). Both studies measured the transport of carboxyfluorescein

Fig. 1.1 Bacteria (spheroidal structures) in immature sieve tubes of shoot apex of *Catharanthus roseus* plants, bar = 2μm (Courtesy of Dr. Anna Rudzińska-Langwald, Department of Botany, Faculty of Agriculture, Warsaw Agricultural University)
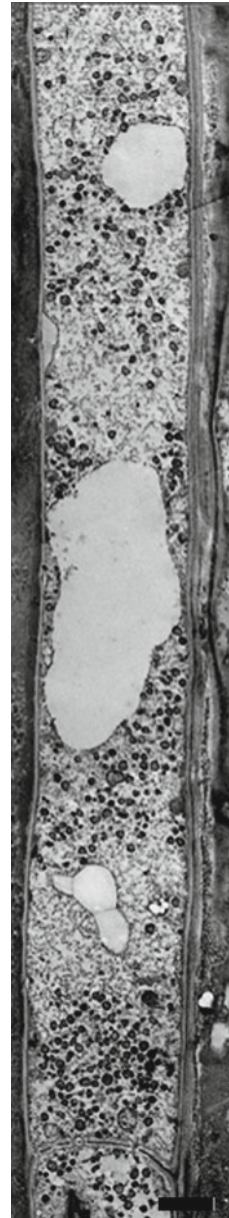
(CF) in *Arabidopsis* roots. Additionally, apoplasmic transport may be limited by the kinetic properties of a given carrier (affinity, capacity). A comparison of rates of symplasmic and apoplasmic modes of transport (Patrick and Offler 1996) showed an even bigger advantage of symplasmic transport, possibly related to a membrane transport component. Hence, symplasmic transport by means of

diffusion between cells seems to be the most efficient mode of transport. Some authors postulated, however, that transport through plasmodesmata occurs by means of mass flow (Patrick and Offler 1996; Voitsekhovskaja et al. 2006) involving a parallel flow of solvent and solutes. This would require permanent flow of water through plasmodesmata.

Diffusion has some limitations as a mechanism of solute movement in the cytoplasm. They are related to fluid-phase viscosity, interactions of the solute molecules with other components of the cytoplasm, and the resistance of the diffusion medium due to accumulation of intracellular components, i.e., molecular crowding (Verkman 2002). The effect of fluid-phase viscosity on solute diffusion in the cytoplasm is neglected by some authors (Fushimi and Verkman 1991; Luby-Phelps et al. 1993) showing experimentally that the viscosity of the cytoplasm is just 10–30 % higher than that of water. On the other hand, *in vivo* measurements of GFP (27 kDa) movement in *Escherichia coli* cells found cytoplasm ten times more viscous than water (Sear 2005). Also the binding of solute molecules to other components of the cytoplasm seems to have little impact on diffusion (Verkman 2002); one should note, however, that this conclusion was arrived at the basis of measurements for a fluorescent probe; BCECF and the binding effect for endogenous molecules of different character could be more pronounced. The strongest negative effect on diffusion was related to the crowding, for both small solutes and macromolecules (ibid.). Some other limitations for diffusion as the means of solute transport in the cytoplasm came from theoretical considerations regarding the nature of diffusion (Pickard 2003). An example is the randomness of diffusion which makes directional transport of rare metabolites highly inefficient (ibid.). Also diffusion through plasmodesmata has its limits (Sowiński et al. 2008) due to the transport channels being not much wider than the Stokes radii of the solutes (Cui 2005; Liu et al. 2004), the tortuosity of these channels (Malek and Coppens 2003), and binding of solutes by the channel walls (Cui 2005; Valiullin et al. 2004). The constraints to molecule diffusion in plasmodesmata are shown on Fig. 1.2.

One should note, however, that our knowledge on the architecture of such delicate structures as plasmodesmata seems to be far from complete, so the modeling of solute flow along these structures can only suggest new research approaches rather than unequivocally explain the mechanism of flow transport.

1.4.2.2 Cytoplasmic Streaming

Most of the aforementioned problems concerning diffusion in the cytoplasm do not apply if one considers the role of cytoplasmic streaming in moving the cytoplasm components (Pickard 2003). Cytoplasmic streaming takes different shapes: unidirectional, circular, fountain, or rotational (Fig. 1.3). The velocity of cytoplasmic streaming was studied mostly in the alga *Chara* sp. because of its large cells. The use of different methods including direct observations under light microscope (Kamiya 1959) and modern, sophisticated methods as laser-Doppler velocimetry (Ackers et al. 1994) and magnetic resonance velocimetry (van de Meent et al. 2010)
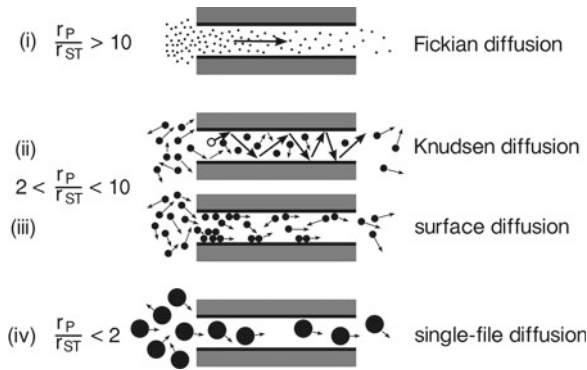
Fig. 1.2 Types of diffusion in narrow tubes. (i) Fickian diffusion (no constriction to diffusion), pore diameter is over 10 times more than that of transported molecule, (ii) Knudsen's diffusion (constriction to diffusion due to molecule collisions with pore walls), (iii) surface diffusion (constriction to diffusion due to molecule adhesion with pore walls), pore to molecule diameter 2–10, and (iv) single-file diffusion (the strongest constriction to diffusion), pore diameter smaller than two molecular diameters. r_p pore radius, r_{ST} Stoke's radius of transported molecule (Courtesy of Dr. Jarosław Szczepanik, Department of Plant Molecular Ecophysiology, Institute of Plant Experimental Biology and Biotechnology, Faculty of Biology, University of Warsaw)

showed that the velocity of cytoplasmic streaming in internodal cells is rather high, in the range of 40–100 μm s^{-1}. A similar high speed of cytoplasmic streaming was also observed in the plasmodia of myxomycetes, another classic material for studying of the phenomenon (Kamiya 1981). In contrast, the velocity of cytoplasmic streaming in higher plants was reported as severalfold lower than in *Charophyta* (Kamiya 1959).

Cytoplasmic streaming seems to be driven indirectly by organelles and diverse vesicles bound to myosin sliding along actin microfilaments (Shimmen and Yokota 2004). The streaming of the cytoplasm speeds up distribution of solutes along the cell immensely. In a recent review on the topic (Verchot-Lubicz and Goldstein 2010), the authors noticed that fluid would move across a giant *Chara* cell in 17 min by means of cytoplasmic streaming but would require almost 3 months to achieve it by diffusion. Cytoplasmic streaming as a transport mechanism has its own limitations, however. Since cytoplasm is a medium of rather high viscosity and low Reynolds number, laminar flow takes place instead of turbulent flow typical for media of high Reynolds numbers. A consequence of laminar flow is little mixing of the medium layers. Thus, for efficient pole-to-pole delivery of solutes and vesicles in the cell, cytoplasmic streaming and diffusion mechanisms should work in concert.

Cytoplasmic streaming is commonly assumed to be limited to intracellular transport. One should note, however, that it could also participate in long-distance transport in some organisms (Tables 1.2 and 1.3), i.e., *Basidiomycota, Ascomycota* fungi, and *Charophyta* and *Chlorophyta* algae. In these organisms, cytoplasmic streaming
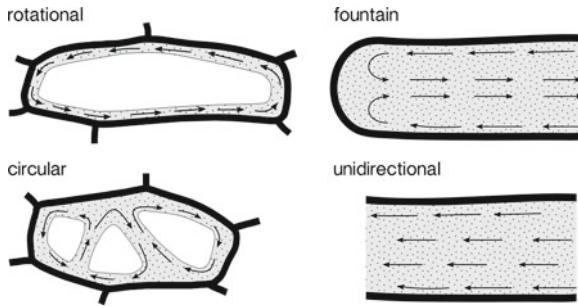
Fig. 1.3 Different shapes of cytoplasmic streaming. Rotational and circular cytoplasmic streaming typical for plant cells. Fountain and unidirectional cytoplasmic streaming typical for fungi hyphae (Courtesy of Dr. Jarosław Szczepanik, Department of Plant Molecular Ecophysiology, Institute of Plant Experimental Biology and Biotechnology, Faculty of Biology, University of Warsaw)

may participate in long-distance transport of solutes, vesicles, and even organelles. In higher plants, cytoplasmic streaming was postulated to occur also in the phloem (Jedd and Chua 2002) to explain the observed movement in sieve tubes. However, the mechanism of such a movement would be difficult to conceive since sieve elements lack a cytoskeleton (van Bel and Knoblauch 2000).

1.4.2.3 Mass Flow

The phloem translocation in higher vascular plants is highly effective. The high velocity of phloem transport has fascinated researchers since the rise of plant physiology. Diverse methods were used to measure it, including calculation of linear speed based on estimates of mass transfer between source and sink (Zimmermann 1974) or the rate of phloem sap leakage from sieve tubes (Mittler 1957), direct *in vivo* measurements of transport speed of photosynthates labeled with radioactive carbon isotopes, ^{11}C (Fensom et al. 1977; Magnuson et al. 1982; Jahnke et al. 1998) or ^{14}C (Sowiński et al. 1990, 2007; Black et al. 2012), as well as of the water flow along phloem measured by means of NMR (Rokitta et al. 1999). The velocity of phloem transport may reach 1 m h^{-1} (ca., 300 μm s^{-1}) or even more. Thus, it exceeds maximal values of velocity of cytoplasmic streaming (up to 100 μm s^{-1}) and diffusion (up to 10 μm s^{-1}) 3 and 30 times, respectively. To underline the efficiency of phloem translocation, Sjölund (1997) has expressively compared the phloem sap flow in sieve tubes to a river of 20 m wide flowing at speed 400 km h^{-1}. However, a comparison of sieve tubes with animal blood vessels of a similar diameter seems more informative. Velocity of blood flow in conjunctival vessels 20–50 μm in diameter was 300–1,500 μm s^{-1} (Mayrovitz et al. 1981), i.e., very close to the linear speed of translocation in 20 μm sieve tubes. Thus, despite the distinct mechanism that drives the fluid in sieve tubes of higher plants (no mechanical pumps) as well as the different nature of the vein (transport inside the cell files), the efficiency of the

transport system in supporting sink organs often tens of meters away from the source leaf with photosynthates and other organic compounds is as high as in vertebrates; a highly effective system of transporting molecules throughout the body was pivotal in allowing plants to colonize land.

The long-distance transport in the phloem is driven by pressure flow. This simple statement by Münch (1930) used to be challenged periodically by many during the last 100 years (see above, Sect. 1.2) but has finally been accepted. Pressure flow is the effect of a water pressure gradient between the source (adult leaves in most cases) and sinks (other organs), caused mainly by two processes, phloem loading in collection phloem and phloem unloading in release phloem, the former responsible for the delivery of carbohydrates into and the latter out of sieve tubes. Carbohydrates are osmotically active; thus, in the phloem loading zone, water is sucked into sieve tubes, either by osmosis or through aquaporins, building up the water pressure inside the sieve tube. In the phloem unloading zone, the opposite process takes place. The resulting gradient of water pressure drives the phloem sap flow in sieve tubes along the plant. It is worth noting that the osmotic potential of the phloem sap may depend not only on sucrose concentration but also to level of potassium ions (Grange and Peel 1978; Lang 1983). The mechanism described above assumes a passive role of sieve tubes in long-distance transport. Actually, sieve tubes do participate in the transport albeit indirectly, by reloading the sucrose leaking from the sieve tubes in transport phloem (Minchin et al. 1984; Minchin and Thorpe 1987; van Bel 2003; Ayre 2011).

The above mechanism describes well the long-distance transport of solutes of low molecular weight. However, phloem transport of macromolecules such as proteins and nucleic acids seems to be different from the former, since endogenous macromolecules are often targeted to a given organ or tissue, while the mass flow mechanism assumes an even distribution of phloem sap to sinks depending only on a sink's strength. The destination-selective movement of CmPP16 (*Cucurbita maxima* phloem protein 16) into roots (Aoki et al. 2005) and directed transport of PSTVd (potato spindle tuber viroid) into sepals (Zhu et al. 2002) are both examples of macromolecule long-distance transport which, at first sight, cannot be explained in terms of the mass flow mechanism. The simplest explanation is that unloading of some macromolecules in sink tissues could be strictly controlled (Chen and Kim 2006). It is also possible that sieve tubes are not continuous files of sieve elements but rather a series of sieve tubes divided by zones of unloading and reloading into subsequent sieve tube. These zones could serve as control points to redirect some macromolecules. The idea that sieve tubes could be discontinued at some regions came from modeling. Theoretical considerations demonstrated that sieve tubes longer than several meters could not support the observed flow rates. To overcome the problem, Lang (1979) proposed that long-distance transport goes along a series of shorter sieve tube files. The idea was revitalized recently (Thompson and Holbrook 2003) basing on theoretical considerations. The very existence of discontinuous sieve tubes has not been verified experimentally, but their possible biological importance is unquestionable in the light of destination-selective long-distance transport of macromolecules. One may

hypothesize that zones of phloem unloading/reloading could serve as transshipment stations redirecting macromolecules to their individual destinations.

1.5 Short-Distance Symplasmic Transport in Selected Plant Tissues

The cell-to-cell symplasmic transport is commonly assumed to be strictly local, with any transport at distances exceeding a pair of cells being only possible through sieve tubes. There are, however, many examples of tissues where symplasmic transport covers the distance of several or even more cells without participation of sieve tubes and therefore could justifiably be dubbed short-distance transport and its specific mechanisms. As it was discussed above (see Sect. 1.4.2.3), the diffusive cell-to-cell transport is much less effective than the mass flow in sieve tubes. Therefore, the question arises on the adaptations of some tissues to short-distance transport. Three cases of tissues employing the short-distance transport are presented below shortly: secretory structures, the photosynthate path to the vein in C4 plants, and paraveinal mesophyll. Separate chapters consider symplasmic communication functioning in xylem rays (Chap. 4) as well as phloem loading and unloading (Chap. 5).

1.5.1 External Secretory Structures

Plants secrete diverse substances outside their body. This may simply be a means of dealing with unfavorable external conditions, as when salt is excreted by plants growing on salt marshes, but often is an important aspect of normal physiology, as in examples below. Owing to their toxicity, some secreted substances may be involved in the protection against herbivores (Adler 2000). Other compounds are attractive for pollinators or protecting insects such as ants (Wist and Davis 2006). To secrete these substances, plants have evolved specialized structures—salt glands, salt trichomes, and glandular trichomes. Also staminal hairs may secrete attractive substances apart from other functions they play (Naidoo et al. 2011). All these secretory organs of different structure (Beck 2010) carry out intensive transport, often of symplasmic character. The unique properties of the secretory structures as a model for symplasmic transport studies have been appreciated by many authors (for review, see Waigmann and Zambryski 2000). These structures are easy to handle, including preparation, *in vivo* observations, and microinjection. Hence, a major part of our current knowledge on the topic has come from research on secretory structures.

Salt glands, best known in *Tamarisk aphylla,* are borne on leaves and stems and sunken in the epidermis. They consist of large secretory cells attached to basal collecting cells (Beck 2010). Since the salt glands are covered by a cutinized cell wall, salt has to be transported from the mesophyll to collecting cells symplasmically.

The further transport to the secretory cell is by the same mode (ibid.). Salt secretion to the apoplasm and thus outside the leaf is enabled by secretory cell ingrowth, typical for transfer cells (ibid.). Salt trichomes sit on buds, young green stems, and leaves in many species of *Atriplex* (Smaoui et al. 2011). A salt trichome consists of stalk cells embedded in epidermal cells and a large bladder cell. The latter accumulates salt in the central vacuole and releases its content by cell rupture, not by secretion into the apoplasm as is the case with salt glands (ibid.). The salt accumulation in secretory cells seems to be supported by an active salt-concentration step possibly performed by the tonoplast (Echeverria 2000). Participation of endocytosis and release of the vesicle content to the vacuole has been suggested recently as well (Smaoui et al. 2011). The difference in the salt release mechanism between salt glands and salt trichomes notwithstanding the mode of salt transport into and throughout the secretory structure of both types is symplasmic. This mode of transport is imposed by the highly cutinized cell walls separating the gland from the epidermis and mesophyll (Beck 2010).

Nectary trichomes represent another type of secretory glands. Nectaries are highly variable in morphology, position on the plant body, and the role they play. The transport of pre-nectar to the nectaries may be apoplasmic (eccrinous), like in the floral nectaries of *Digitalis purpurea,* rather large structures of 40–50 cells containing phloem unloaded apoplasmically (Gaffal et al. 2007) or symplasmic (granulocrinous) (ibid.) like in nectary trichomes where symplasmic transport dominates (for review, see Waigmann and Zambryski 2000). In nectary trichomes, several adaptations are observed likely to facilitate efficient symplasmic transport, including a high abundance of plasmodesmata linking the cells, from the basal, through stalk to secretory ones (ibid.). The high plasmodesmal frequency found in leaf trichomes of different types might be related to the formation of secondary plasmodesmata, as found for leaf trichomes in tobacco during the sink/source transition (Roberts et al. 2001). Thus, the high density of plasmodesmata seems to be the most important feature of tissues carrying out intensive symplasmic transport. It is in line with theoretical considerations on the mechanism of control of phloem unloading in sinks (Patrick 1997), which indicate that the number of plasmodesmata is the determinant of efficiency of symplasmic transport.

Another example of secretory structures where symplasmic transport was studied thoroughly is the staminal hair of *Setcreasea purpurea* (Tucker 1982, 1987; Tucker et al. 1989; Tucker and Tucker 1993). Staminal hairs are filamentous files of cells differing in length, shape, and other anatomical traits; they aid pollination by attracting pollinators visually or chemically, by excretion of attractants. Studies on the staminal hairs of *S. purpurea* showed that the main mode of transport of low molecular weight solutes through plasmodesmata is diffusion (Tucker et al. 1989) and cytoplasmic streaming plays no role in cell-to-cell transport (Tucker 1987). The data regarding symplasmic transport obtained by studying staminal hairs should not be viewed as representative for other secretory structures, as they differ from each other in many important details. The diffusion coefficient for movement through plasmodesmata in staminal hairs was found not to depend on the direction (Tucker et al. 1989). However, unidirectional transport from leaf cells to the

trichome and from the basal trichome cell to the distal cell was observed, respectively, in *Abutilon* nectaries (Terry and Robards 1987) and tobacco trichomes (Christensen et al. 2009). This shows that secretory structures may differ in the mechanism of transport and/or its regulation. Another example of a specific symplasmic transport mechanism in secretory structures is the cell-to-cell transport with participation of desmotubules in cotton extrafloral nectary trichomes (Waigmann et al. 1997). This is one of the several examples of "open" desmotubules countering the claim of numerous authors, founded on their observations, that the desmotubules are static, appressed structures not engaged for transport through the plasmodesma (Gunning and Overall 1983; Tilney et al. 1991; Botha et al. 1993; Overall and Blackman 1996; Ding 1998). In that context, one should mention the discovery of Lazzaro and Thomson (1996) on a vacuolar-tubular continuum from base to tip of chickpea trichomes. According to those authors, the vacuolar-tubular continuum could represent the path for solute diffusion through plasmodesmata via a channel in the center of the axial component. In the light of the above observations, it seems likely that the desmotubule could act as a pathway for low molecular weight solutes, at least in some types of secretory structures. Far from being uniform, plasmodesmata exhibit features unique to only a certain type of secretory structures. For instance, the plasmodesmata linking cells of staminal hairs do not transport aromatic amino acids and are even blocked by them (Tucker 1982), while their transport through nectary plasmodesmata is efficient (Terry and Robards 1987). Trichome plasmodesmata in *Nicotiana clevelandii* differ much in ultrastructure (Waigmann et al. 1997) and protein transport ability (Waigmann and Zambryski 1995) from those in the mesophyll. These data show that the only common feature of secretory structures of different types specialized in efficient symplasmic transport is just a high frequency of plasmodesmata, while other may differ.

1.5.2   Photosynthate Path to Vein in C4 Plants

The C4 pathway enhances photosynthetic efficiency, particularly under conditions promoting closing of stomata, i.e., in open, dry, and hot habitats, when both low internal carbon dioxide concentration and high temperature would promote photorespiration in C3 plants (Pearcy and Ehleringer 1984; Sage and Pearcy 2000). This is realized by increasing CO_2 concentration at the site of the dual-activity enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, which tips the balance between ribulose-1,5-bisphosphate carboxylation and oxygenation in favor of the former. The C4 pathway is distributed between two types of cells: Kranz mesophyll (KMS) and chlorenchymatous bundle sheath (BS). The former tissue is the site of primary carbon assimilation (PCA) and the latter of primary carbon reduction (PCR). PCA is the carboxylation of phosphoenolpyruvate catalyzed by phosphoenolpyruvate carboxylase resulting in the formation of C4 organic acids. These acids are then decarboxylated, and the released CO_2 is consumed again by RuBisCO, both reactions in BS cells, thereby introducing carbon to the Calvin cycle (Furbank and

Foyer 1988). The apparently futile cycle of carboxylation-decarboxylation split between KMS and BS cells is the crucial component of the C4 mode as it allows building in BS cells CO_2 concentration severalfold higher than the ambient one. To aid the metabolic "pumping" of CO_2 into BS cells, specialized physical barriers prevent its leakage from the BS. This is achieved by either suberinization of the BS cell walls or maximizing the distance between the KMS and BS chloroplasts (Leegood 2000). There are three main subtypes of C4 photosynthesis: NADP-ME and NAD in dicots and monocots and PEP-CK, only in monocots. Their names derive from the type of enzyme catalyzing malate decarboxylation. For details, the reader should consult other reviews (Furbank and Foyer 1988; Leegood 2000; Sage and Pearcy 2000; Griffiths et al. 2013).

The basic architecture of KMS and BS tissues in relation to each other is rather simple: the two photosynthetic tissues form concentric cylinders—the bundle sheath is located around the veins and is then encircled by a layer of Kranz mesophyll cells (Botha 2005). In some species, an additional layer of mestome sheath can be located between the bundle sheath layer and the vein (ibid.). Beside its role in C4 photosynthesis, such tissue architecture aids efficient exchange of photosynthetic intermediates between KMS and BS cells as well as facilitates the delivery of sucrose to companion cell/sieve element complex. Here, the Haberlandt's principle of expeditious translocation is realized as the transport of synthetic product follows the shortest and most efficient route (after Cittadino 1990).

The transport of C4 intermediates is likely to proceed exclusively through the numerous plasmodesmata crossing the KMS/BS cell interface, since the suberin lamellae often (but not always) decorating the cell walls of BS cells in C4 plants should exclude the apoplasmic mode of transport (Hattersley 1987; Hattersley and Browning 1981, but see Eastman et al. 1988a, b). These plasmodesmata are often simple ones, grouped in pit fields. The KMS/BS plasmodesmata in C4 plants are generally more numerous than their analogues in C3 plants (Botha 1992; Botha and van Bel 1992; Cooke et al. 2000), which positively correlates with the high photosynthesis rate of C4 plants (Botha 1992). Some authors insist that the KMS/BS plasmodesmata are of secondary origin since these two types of cells represent distinct lineages (Dengler and Taylor 2000). Cooke et al. (2000) even argued that the evolution of the C4 type of photosynthesis was only possible in angiosperms because of their unique ability to form secondary plasmodesmata (however, for arguments for secondary plasmodesmata formation in gymnosperms, see above, Sect. 1.3). Others (Botha 2005), however, pointed out that the KMS/BS plasmodesmata are only secondarily modified ones. The frequency of the KMS/BS plasmodesmata in C4 plants shows some adaptability, correlating positively with the demand for more efficient transport through the cell interface: it increases when plants are grown at high light intensities (Sowiński et al. 2007) and at low temperatures (Sowiński et al. 2003). Regardless of the mechanism of formation of KMS/BS plasmodesmata in C4 plants, the high frequency of these plasmodesmata seems to be the determining factor of the high fluxes of photosynthates through the corresponding cell interface and hence the high photosynthetic rate observed in C4 plants.

For decades, simple diffusion was widely accepted as the mode of transport of photosynthetic intermediates through the KMS/BS plasmodesmata. This mechanism was recently questioned on theoretical grounds as not efficient enough for the observed transport fluxes (Sowiński et al. 2008) through the cell interface. Simple calculations using experimentally derived parameters, such as solute concentrations and plasmodesma frequency and dimensions, gave maximal allowable flow rates orders of magnitude below the real ones. Paradoxically, none of the possible alternative transport mechanisms (ibid.), apoplasmic, vesicular, or bulk flow within the vacuome (see Sect. 1.2, above), have been found to be involved in the exchange of photosynthetic intermediates between KMS and BS cells (Szczepanik and Sowiński, unpublished data). This discrepancy indicates that some as yet unknown factors must be in action. One possibility is that the diffusive transport through the KMS/BS interface is facilitated by local accumulation of the photosynthetic intermediates near the pit fields. A possible role of peripheral reticulum concentrated in chloroplast protrusions in directing photosynthates toward plasmodesmata was discussed already by Kursanov (1984). Alternatively, the transport could be powered by superdiffusion involving active cellular processes. A conveyer-belt-like movement of the ER membrane along the plasmodesmata and cytoskeleton elements could enhance the cell-to-cell transport (Roberts 2005). In that context, one should note a recent report of Kramer et al. (2007) who found cell-to-cell movement of low molecular weight solutes much faster than estimated before. Apparently, our understanding of the mechanism of transport through plasmodesmata is far from comprehensive.

The KMS/BS plasmodesmata apparently do not stand out in functional properties, as their SEL is around 850 Da (Weiner et al. 1988), similar to other plasmodesmata. They often have sphincters located at neck regions (Evert et al. 1977; Olesen 1979; Robinson-Beers and Evert 1991; Botha 2005; Sowiński et al. 2007). The sphincters can be located externally or internally of the plasmodesma at either one or both sides. Their role is not clear since relevant experimental data are scarce. A role has been reported for external sphincters in *Betula pubescens* where their strong swelling led to the inactivation of shoot apical meristem by abrogation of cell-to-cell communication (Rinne and van der Schoot 1998). Swelling of internal sphincters at the KMS/BS boundary in cold-treated maize was accompanied by a decrease of photosynthetic rate and less efficient transfer of ^{14}C-photosynthates to the phloem (Bilska and Sowiński 2010). That was is in line with the theoretical considerations of Anisimov and Egorov (2002) who proposed that the degree of neck region opening determines the flow rate of molecules through plasmodesmata. Also, Botha (2005) was of the opinion that internal sphincters were responsible for controlling the flow rates through the KMS/BS cell interface. More experimental data are badly needed to fully uncover the role of sphincters in the regulation of exchange of C4 photosynthetic intermediates between the KMS and BS cells.

The pre-phloem loading transport of sucrose in C4 plants is also symplasmic and comprises the route from the site of its synthesis, KMS, through BS to the vascular parenchyma (VP) cells. The BS/VP plasmodesmata are less numerous than the KMS/BS ones (Botha 1992; Botha and van Bel 1992; Sowiński et al. 2007).

They seem to be of secondary origin as these cells develop from distinct lineages (Dengler and Taylor 2000) and their frequency increases with the growth light intensity (Sowiński et al. 2007). Studies on the SXD1 maize mutant showed that the BS/VP plasmodesmata may play a crucial role in the export of photosynthates from leaves (Russin et al. 1996; Botha et al. 2000). The mutant shows excessive accumulation of sucrose and starch in leaf blades accompanied by reduction of phloem export and has abnormal ultrastructure of BS/VP plasmodesmata (Russin et al. 1996). The observed cessation of symplasmic transport from the bundle sheath cells to the vascular parenchyma was most probably caused by accumulation of callose at the cell interface (Botha et al. 2000). Further studies showed that the *SXD1* gene encodes a protein involved in chloroplast-to-nucleus signaling (Provencher et al. 2001) and is a homolog of VTE1, whose mutation in Arabidopsis and *Synechocystis* (Porfirova et al. 2002; Sattler et al. 2003) results in tocopherol deficiency. An impairment of photosynthate export due to RNAi-induced tocopherol deficiency was also found in potato (Hofius et al. 2004). The *VTE1* mutation in Arabidopsis led to the reduction of photoassimilate export as well, but only at low temperature (Maeda et al. 2006). This is in line with the hypothesis that ROS accumulation (here caused by tocopherol deficiency in the *SXD1* or *VTE1* mutants) could activate signaling pathways leading to the closure of plasmodesmata (Benitez-Alfonso et al. 2011). In the context of C4 photosynthesis regulation, it also shows that photosynthetic processes could control the export of photosynthates by modulating of the properties of symplasmic pathway.

The last stage of pre-phloem transport is the uptake of sucrose into sieve tubes. In dicots, the vein ultrastructure has been classified by Gamalei (1989) as type 2c (low plasmodesmata frequency at the parenchyma/companion cell interface, high plasmodesmata frequency at the mesophyll/bundle sheath interface) evolved from type 2a (low plasmodesmata frequency at the parenchyma/companion cell interface) in response to dry environment. However, most data on phloem loading come from study on dicots, while C4 grasses and in fact all monocots were neglected in considerations on the evolution of the phloem loading mode. In grasses, two forms of sieve tubes can be distinguished in small and intermediate veins, i.e., those involved in phloem loading (Evert et al. 1996): thick-walled sieve tubes and thin-walled sieve tubes (Evert et al. 1978; Botha 1992; Botha and van Bel 1992). The thick-walled sieve tubes adjoin VP cells and are often connected to them by plasmodesmata, which suggests symplasmic contact. However, the functions of the thick-walled sieve tubes are not clear yet; possibly they are not involved in long-distance transport (Fritz et al. 1983) and are less functional than thin-walled tubes (Matsiliza and Botha 2002). The thin-walled sieve tubes adjoin companion cells. The phloem loading of sucrose in C4 plants seems to be apoplasmic, although some reports showed symplasmic continuity between parenchyma and companion cells, at least in C4 grasses (Botha 1992; Sowiński et al. 2001, 2003). This would allow symplasmic phloem loading, provided the plasmodesmata linking vascular parenchyma and companion cells are functional. Direct observations of symplasmic movement of fluorescent dyes to the sieve tube/companion cell complex are lacking in C4 plants. However, in barley, a C3 grass, the companion cell/sieve element complex in the third leaf was

shown not to be symplasmically isolated in some regions (Haupt et al. 2001). We have found recently in *Zea mays* spp. *indentata* (Bilska and Sowiński, unpublished data) that the KMS, BS, and VP cells plasmolyzed at a higher sorbitol concentration than did the companion cell/sieve element complex. Thus, in that maize subspecies, the sucrose transport from mesophyll to vein possibly does not need to go against a concentration gradient, similarly as in some species showing neither apoplasmic nor polymer trapping phloem loading (Turgeon and Medville 1998). The passive mode of symplasmic phloem loading has been shown to be associated with the tree growth form (Davidson et al. 2011). It seems that under conditions favoring photosynthesis in maize leaves, sucrose could be exported along its concentration gradient (Troughton and Currie 1977; Sowiński 1998). Hence, the hypothesis on the participation of symplasmic transport in phloem loading in C4 grasses needs further verification.

The mechanism of transport of C4 photosynthates and the export of sucrose from leaves in C4 plants is of particular importance in the light of recent attempts to introduce the C4 photosynthesis into C3 plants, since both the ultrastructure of the leaf and the use of particular transport mode could be additional obstacles in that effort (for review, see Langdale 2011).

1.5.3   Paraveinal Mesophyll

Paraveinal mesophyll (PVM) is a single-cell layer separating palisade and spongy mesophyll in the plane of minor veins in several legume species. The term PVM was coined by Fischer (1967) and is synonymous with the original *Mittelschicht designation* introduced by Kopff (1892, after Rutten et al. 2003). In recent years (Rutten et al. 2003; Leegood 2008), a related term EBS (extended bundle sheath) is also used which underlies the origin of the tissue. In fact, EBS is not exactly the same, since the former concerns a well-defined cell layer in legume species, while the latter refers to a range of extensions of bundle sheath cells that are widespread in nonlegume species as well (Rutten et al. 2003).

The paraveinal mesophyll is found mostly in *Fabaceae* (Fischer 1967; Brubaker and Lersten 1995; Lansing and Franceschi 2000), but similar tissue has been reported in other taxa as well (Kevekordes et al. 1988; Rutten et al. 2003). The anatomy, ultrastructure, and physiological role of PVM were studied most thoroughly in the soybean (*Glycine max*). PVM comprises large, branched cells forming a sort of a flattened lattice (Franceschi and Giaquinta 1983a, b). During a plant's development, the PVM starts to differentiate before palisade mesophyll does (Liljebjelke and Franceschi 1991). Mature PVM cells are highly vacuolated and contain dense cytoplasm rich in rough ER and dictyosomes but with few small chloroplasts with no starch grains (Franceschi and Giaquinta 1983a). PVM cells are interconnected by numerous simple plasmodesmata grouped in pit fields; they are also connected symplasmically with neighboring bundle sheath, palisade, and spongy mesophyll cells (Franceschi and Giaquinta 1983a, b; Lansing and Franceschi 2000).

Several roles proposed for the PVM have been tested experimentally. One hypothesis suggests that PVM accumulates proteins in vacuoles and is specialized in storage of nitrogenous compounds (Franceschi et al. 1983; Klauer et al. 1991; Klauer and Franceschi 1997; Voo et al. 2013). However, of utmost interest in the context of this monograph is the possibility that PVM is responsible for intermediary transfer of assimilates from the palisade mesophyll to phloem in species with the palisade mesophyll composed of cells forming multiple layers and high numbers of cells per unit area (Lansing and Franceschi 2000). This would be in line with the Haberlandt's principle of expeditious translocation (see Sect. 1.5.1) as PVM is localized between the palisade and spongy mesophyll in close contact to a phloem. In contrast, Fisher (1970) suggested that non-photosynthetic cells such as PVM actually delay the transfer of photosynthates between the photosynthetic pool and transport pool. The Haberlandt's hypothesis was confirmed by $^{14}CO_2$ pulse-chase experiments (Lansing and Franceschi 2000). That the EBS is part of the transport route to phloem was also confirmed in *Ricinus communis* (a nonlegume species) with the use of the iron-chelator nicotianamine as a marker of mass transport (Rutten et al. 2003). The presence of numerous plasmodesmata linking PVM cells with one another and with surrounding tissues speaks in favor of symplasmic transport of photosynthates via PVM to phloem. This concept could gain further support if detailed data were available on the abundance of plasmodesmata in different types of PVM cell interfaces. To the best of my knowledge, no such analysis has been reported. Also, no direct observations have been reported on the symplasmic transport of a fluorescent dye through the PVM tissue in *Glycine max*, the model species for PVM studies. According to Lansing and Franceschi (2000), cytoplasmic streaming observed in the large PVM cells should improve the efficiency of symplasmic transport in the tissue. To sum up, PVM seems likely to act both as a high-capacity symplasmic route for photosynthates and as a protein store at some developmental stage, since these functions need not be mutually exclusive.

1.6 Conclusions

Vascular plants demonstrate the highest level of symplasm integrity including the most efficient transport inside the protoplast syncytium. It seems, however, that no unique mechanism of symplasmic transport has evolved in higher plants, since also nonvascular plants use the same mechanisms of cell-to-cell, short-distance, and even long-distance transport. Despite well over a century of studies, several old and new concepts still await to be clarified, including but not limited to these: desmotubule as a transport pathway (open or closed?), mechanism of transport through plasmodesmata (diffusion, superdiffusion, other?), transport of organelles through plasmodesmata (whether and how big organelles might move through plasmodesmata?), the role of diffusion versus cytoplasmic streaming in short-distance transport, phloem continuum (a continuous sieve tube along the plant body or a file of distinct sieve tubes?), and the ability of lower plants, fungi,

and animals to modify symplasm integrity (are organisms other than higher plants able to eliminate primary plasmodesmata and form secondary plasmodesmata? Do plasmodesmata of organisms other than higher plants contain desmotubules?). Answers to these questions could change our present understanding of symplasmic transport in vascular plants.

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