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Preface

The exchange of small solutes and macromolecules between cells is a crucial process for system integration in any multicellular organism. Animals and plants solve the problem of cell-to-cell transport in different ways. In animals transport across the cell membrane is the only, or at least the main, pathway for molecules of different kinds to travel between cells. In plants, however, aside from transmembrane transport, a second (and apparently the most important) transport mode exists, i.e., molecule movement through plasmodesmata—the numerous thin channels connecting living protoplasts. Plasmodesmata allow plant cells to communicate in spite of the cell wall, a more or less rigid layer, surrounding every living plant protoplast. Its presence is responsible for the existence of two different systems—symplasm (protoplasts connected by plasmodesmata) and apoplasm (cell walls and intercellular spaces)—that build every plant organism. Plasmodesmal connections appear as a highly dynamic network, responsible not only for cell-to-cell exchange of organic compounds of a different nature, e.g., carbohydrates and amino acids, but also for movement of signaling macromolecules involved in plant development, such as transcription factors and nucleic acids. Symplasmic transport in plants also regards the movement of solutes and macromolecules over a distance of several meters or even more, by using specialized cells, sieve cells or sieve elements; however, the mechanism of such long-distance transport differs from that of cell-to-cell transport. Hence, symplasmic transport (being responsible for the exchange of solutes and signal macromolecules between cells, tissues, and organs) integrates the plant as the unit.

In the presence of many outstanding papers and books on the processes of transport, the symplasmic transport of molecules in plants seems to have been left aside. In this book we would like to emphasize what an important role symplasmic communication plays in plants. Herein, we would like to concentrate on symplasmic transport of small molecules, although the cell-to-cell transport of macromolecules will also be discussed. We are going to characterize the efficiency of symplasmic transport, mechanisms of molecule passage via plasmodesmata, and the external and internal factors that regulate plasmodesmatal conductivity. In this context, we will concern ourselves with the role of symplasmic domains in plant development, as well as the influence of environmental stresses on the plasmodesmata.

Besides cell-to-cell symplasmic transport, the significance of long-distance symplasmic transport of solutes in phloem elements will likewise be reviewed. We intend to present the mechanism of phloem transport, the processes of symplasmic loading and unloading, as well as the role of pre- and post-phloem transport, with special attention paid to symplasmic transport in wood. Finally, the relevance of the spread of both macromolecules and viruses, via plasmodesmata and phloem, will be presented.

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Chapter 1
Characteristics of Symplasmic Transport

Paweł Sowiński

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

		Plasmodesmata		Conducting cells	
Kingdom	Phylum	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

		Plasmodesmata		Conducting cells	
Kingdom	Phylum	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

		Plasmodesmata		Conducting cells	
Kingdom	Phylum	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		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	Plugging or disappearance at some developmental stages	Giant cells, intercellular connection by PD	Cytoplasmic streaming by means of actin-myosin mechanism

Table 1.4 Symplasmic pathway in lower vascular plants

		Plasmodesmata		Conducting cells	
Kingdom	Phylum	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

		Plasmodesmata		Conducting cells	
Kingdom	Phylum	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

	Plasmodesmata		Conducting cells	
Phylum	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