

Review

What Is Phloem Unloading?

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ABSTRACT

Several studies of phloem unloading have failed to distinguish between transport events occurring at the sieve element/companion cell boundary and subsequent short-distance transport through parenchyma cells. Indirect evidence has been obtained for symplastic unloading in storage and utilization sinks. In other sinks transfer to the apoplast may occur, but not necessarily at the sieve element/companion cell complex, and the evidence for apoplastic phloem unloading is equivocal, as is the role of apoplastic acid invertase in this process. The ability of several types of sink cells to accumulate sugars from the apoplast is discussed in the conflicting light of functional symplastic continuity between sink cells. Attention is drawn to the complexity of the postunloading pathway in many sinks and the difficulty of determining the exact sites of symplast/apoplast solute exchange. Potential future areas for study in the field are highlighted.

Following the initial surge of interest in the mechanism of phloem loading in leaves, a number of scientists turned their attention to sink regions of the plant and a study of the pathways and mechanisms of phloem unloading. It is now well accepted that sinks exert a considerable influence in determining the pattern of assimilate distribution throughout the whole plant (1, 11, 16), and as a result of several studies the rather broad term 'unloading' has become commonplace in the plant physiology literature. It is apparent, however, that the term has given rise to a number of misconceptions concerning the nature of phloem unloading in sink tissues. The reasons for this are manifold. Sieve tubes constitute a highly pressurized transport system which is remarkably sensitive to mechanical disturbance. Furthermore, the small size of the component SE¹ and their inaccessibility within sink tissues has made it difficult to separate, both experimentally and conceptually, transport events at the SE/CC boundary (the true unloading step) from subsequent short-distance transport (and metabolism) by sink parenchyma cells.

In the last decade, a number of comprehensive reviews have appeared on the subject of phloem unloading (1, 5, 11, 13), and for further details of individual investigations the reader is referred to these. The aim of this article is to attempt to

¹ Abbreviations: SE, sieve element; CC, companion cell; PCMB, parachloromercuribenzenesulfonic acid; CCCP, carbonyl cyanide 3-chlorophenylhydrazone.

clarify some of the more commonly used terminology in the field and to question some of the interpretations which have become prevalent in the literature.

SE UNLOADING

Following long-distance transport in the phloem the exit of assimilates from the sieve element is the first step in a complex series of short-distance transport events. Two distinct approaches are available to study unloading from the sieve element: anatomical and physiological. The most comprehensive studies are those that have utilized both approaches simultaneously, although it should be noted that the two approaches have sometimes produced conflicting interpretations concerning the nature of the unloading pathway (7, 10). Available studies indicate that SE are connected to CC by abundant plasmodesmata (7, 11), and so the SE/CC complex might be regarded as a functional unit, as it is in the leaf.

Assimilates may leave the SE/CC complex by two potential pathways: through plasmodesmata into vascular parenchyma elements (symplastic unloading) or directly across the plasmalemma into the apoplast (apoplastic unloading). It is conceivable that both pathways operate simultaneously, although this is seldom considered. Movement out of the SE/CC complex has not been unequivocally studied, largely due to the inaccessibility of the phloem and a lack of experimental techniques for direct visualization of the unloading step.

SYMPLASTIC UNLOADING

In several species the SE/CC complexes are connected directly to vascular parenchyma by plasmodesmata. To date, no evidence has been forwarded for symplastic isolation of SE/CC complexes in sinks, as reported for the minor veins in mature leaves of some species (5). Exit of assimilates from the SE/CC complex by the symplast is the pathway of least resistance and symplastic unloading may be prevalent in several types of sink in which a concentration gradient is maintained between the sieve elements and the sink cells by polymer formation (storage sinks) or by utilization of the assimilates for growth (utilization sinks) (11).

A body of evidence, albeit indirect, has accumulated to support symplastic unloading in many storage and utilization sinks. Symplastic unloading has been inferred from studies of plasmodesmatal frequencies (7), the inhibition of solute transport by plasmolysis of cells surrounding the phloem (8, 11),

and the use of inhibitors such as PCMBS, a sulfhydryl compound thought to block the action of the sucrose carrier (2, 5, 11). PCMBS, when applied to the apoplast of some importing sinks, *e.g.* developing leaves, has been found to have no effect on assimilate import, suggesting the absence of transmembrane transport during the exit of assimilates from the phloem (2).

APOPLASTIC UNLOADING

Direct transfer of assimilates across the SE/CC plasmalemma has been argued for a number of species, and apoplastic unloading would appear to be prevalent in stem tissues (11). It should be stressed, however, that apoplastic unloading is extremely difficult to validate and several investigators have argued the case for apoplastic unloading in the absence of corroboratory data regarding plasmodesmatal frequencies between the SE/CC complexes and contiguous tissues.

The terms 'apoplastic unloading' and 'apoplastic unloading sink' have frequently been misinterpreted in the literature and have been used to describe phloem unloading in a wide range of sinks (5). The latter term, when applied to seeds, is an unfortunate misnomer which has led to some confusion. In most seeds which have been examined to date, including the much studied legume seed coat (6, 11, 13, 16), the phloem unloading step has been found to be symplastic, with a subsequent transfer to the apoplast at some point removed from the SE/CC complexes. In many reproductive structures, *e.g.* cereal caryopses, the exact site of assimilate transfer to the apoplast remains to be demonstrated (see below). In legume seed coats, however, the branch parenchyma have been forwarded as a likely candidate for the site of symplast/apoplast exchange (11).

A ROLE FOR INVERTASE?

The enzyme acid invertase has frequently been implicated in apoplastic phloem unloading, a popular view being that sucrose unloaded to the apoplast is converted to hexoses, thereby allowing the continued exit of sucrose from the phloem (1). The hexoses may then enter sink cells where they are reconverted to sucrose. Such a mechanism may operate in several sugar-storing sinks (1, 11). Patrick (11) has suggested recently that apoplastic unloading might be confined to axial (stem) unloading and to those sinks in which symplastic unloading is incompatible with sink function, such as in sugar-storing sinks where osmotically active solutes are stored against a concentration gradient.

While many sinks clearly possess apoplastic invertase activity, there is no direct evidence that the inversion of sucrose occurs in the apoplast surrounding the SE/CC complexes. Furthermore, recent studies utilizing asymmetrically labeled sucrose (4) and the sucrose analog, fluorosucrose (3), a substrate poorly hydrolyzed by invertase, have provided evidence that sucrose may be taken up by both sugarcane cells and maize endosperm cells, respectively, without prior hydrolysis. Thus, although apoplastic invertase is prevalent in many sink tissues, sucrose hydrolysis may not be a prerequisite for the unloading and subsequent import of sugar into the sink cells.

SUGAR UPTAKE BY SINK CELLS

Many sink cells possess the capability to accumulate solutes which have been exogenously supplied to the apoplast (1, 5, 10, 11). This has been interpreted in some studies as evidence that unloading occurred first to the apoplast, followed by uptake into the individual sink cells across the plasmalemma. However, it is apparent that many types of sink cells are connected by abundant plasmodesmata and this feature makes it difficult to determine the precise contribution of apoplast versus symplast *in vivo*. For example, in potato tubers ultrastructural (7) and physiological (8, 9) evidence favors an entirely symplastic unloading pathway between the SE and the large starch-storage parenchyma of the perimedulla. Functional symplastic continuity between storage cells has been demonstrated indirectly using plasmolysis procedures (8) and directly by microinjection of the membrane-impermeant fluorescent probe Lucifer Yellow CH (9). However, the storage cells also possess an effective mechanism for the uptake of sucrose from the apoplast, a process which is acutely sensitive to changes in cell turgor pressure (10).

The presence of functional plasmodesmata between storage cells would appear at first to be contradictory to a demonstrated mechanism for apoplastic sucrose uptake. However, the above observations are reconcilable if one considers the latter as a retrieval mechanism for sucrose which was originally moving via the symplast (9, 10) and not as an integral part of an unloading mechanism. Such a transport mechanism on the plasmalemma might function to prevent the escape of sucrose from the storage cells as well as providing a means of sucrose retrieval. The sensitivity of sucrose uptake to turgor (10, 11) would provide a means of regulating such a mechanism.

Recently, it has been suggested (5) that the active apoplastic loading step in leaves might not be occurring at the SE/CC complexes but rather in mesophyll cells, followed subsequently by symplastic transfer of sucrose to the minor veins. By a similar token, in sink regions of the plant it may prove misleading to consider the apoplastic uptake of solutes by sink cells as evidence for unloading to the apoplast. It now appears that sucrose retrieval is a ubiquitous feature of non-vascular elements in sinks (10, 11) as well as in sources (5).

Transport physiologists have traditionally challenged symplastic transport events by introducing experimental treatments to the apoplast. This is simply because the latter is more readily accessible to experimental manipulation. The above considerations, however, point to an urgent need to determine, directly, the pathway taken by assimilates *in vivo*, and in this respect the symplastic pathway has been sadly neglected.

POSTUNLOADING PATHWAY

It is apparent that events at the SE/CC complex are inextricably linked with subsequent transport events. In most cases, a rather tortuous pathway separates the SE from the eventual sites of solute accumulation. In seeds, several specialized parenchyma elements are involved in short-distance assimilate transfer from the phloem (13). Little is known of

the function of these cells or the exact site(s) at which assimilates are transferred to the apoplast. An absence of plasmodesmata occurs between maternal and filial generations of seeds, *e.g.* cereals (13), and this discontinuity may provide a potential regulatory site at which several symplastically mobile agents, including viruses, could be prevented from entering the seed. Thus, only compounds with the ability to exchange (or be carried) from symplast to apoplast are likely to be taken up into the developing endosperm. However, it remains to be demonstrated as to whether the nucellus/aleurone discontinuity is the only site of symplast/apoplast solute exchange in cereal seeds and further studies of the postunloading pathway are required.

The presence of numerous cell types with potentially different roles in solute transport has made experimental challenges to the unloading process particularly difficult to interpret. Thus, the site of action of transport inhibitors such as PCMBs and CCCP, and treatments such as anoxia, altered pH and solution osmolality have proved particularly difficult to determine (11, 13, 16).

SEED-COAT UNLOADING

One of the major aims of studies of phloem unloading has been to get as close to the unloading site as possible without impairing the unloading mechanism. For the reasons stated above this has proven difficult. In legumes, however, the empty seed coat technique has become a valuable tool with which to study transport processes which culminate in the release of assimilates to the developing seed. In the above method the seed is removed through a window made in the pod wall. The space vacated is then filled with a solution designed to influence assimilate transport. The method utilizes the natural symplastic discontinuity between the maternal and filial generations of the seed and has the additional advantage that the transport pathway is not disrupted by the surgical treatment used. The empty seed coat technique has now been applied successfully to other nonlegume species (16) and a modification of the method has been used to study solute transport in vegetative sinks, such as the potato tuber (8).

There is little doubt that, using the above method, much has been learned concerning the factors which influence assimilate transport into seeds, particularly the role of the osmotic environment (16). However, it should be stressed that even in this simplified experimental system the SE/CC complexes are several cells away from the collecting solutions, giving rise to several differences of opinion concerning the pathways and mechanisms of assimilate transport through the seed coat (11, 13, 16). Unfortunately, in many published articles the term seed-coat unloading (an apoplastic event) has become synonymous with phloem unloading (a symplastic event).

Although the above technique is now in widespread use, recent studies using the short-lived isotope ^{14}C have demonstrated that the import of assimilates into seed coats declines markedly after 200 min following seed detachment (6). These observations are likely to limit the use of the technique to short-term studies of assimilate transport.

PATHWAY UNLOADING

In addition to 'terminal' unloading of the type found in seeds, lateral transport of assimilates occurs along the entire transport pathway from source to sink. The most popular hypothesis to explain lateral solute transfer has been the 'pump-leak' hypothesis. According to this hypothesis assimilates, such as sucrose, constantly leak to the apoplast due to the large concentration difference between the sieve elements and surrounding apoplast. Sucrose is then retrieved across the plasmalemma by energized sucrose/proton cotransport. Within the framework of this scheme, enhanced sucrose leakage (unloading) to the apoplast could occur by localized inhibitions of the sucrose retrieval mechanism (11).

ACTIVE VERSUS PASSIVE PHLOEM UNLOADING

Early suggestions that phloem unloading could occur by an ATPase-driven sucrose-proton antiport system have found little support. ATPase activity, localized cytochemically, has been found to be associated with sink phloem (7), but no direct evidence has been obtained that this enzyme is involved in the unloading process. Proton extrusion activity has been shown to occur in legume seed coats and assimilate transport in sink regions is influenced by a wide range of metabolic inhibitors (11, 13). However, as stated above, the precise site of action of these agents requires to be demonstrated.

In tissues in which the phloem unloading step is symplastic, unloading could occur passively down a concentration gradient maintained by the continuing conversion and/or compartmentation of assimilates in the sink cells. Transport through plasmodesmata most likely occurs by diffusion and it has recently been suggested that plasmodesmata might operate as pressure-sensitive valves, providing a potential link between the cell turgor of sink cells and control of the symplastic transport pathway (11).

Continuous symplastic continuity between sieve elements and sink cells, such as that found in potato tubers (7-9), would suggest that metabolic events in the cytoplasm of sink cells, particularly the initial entry of sucrose into glycolysis, might be expected to influence unloading from the SE/CC complexes. It is, therefore, significant that the enzyme sucrose synthase has recently been shown to play a major role in determining the sink strength of storage organs which accumulate starch as a major reserve (12).

CONCLUSIONS AND FUTURE PROSPECTS

Despite a decade of research into phloem unloading, a universal mechanism has not emerged regarding the pathways and mechanism by which assimilates exit the phloem. It is apparent that species differ remarkably in the pathways utilized in phloem unloading and that any given pathway is closely linked to the particular function of that sink and the nature of its final storage product. Despite several efforts, the specific study of transport events at the SE/CC complex has remained an elusive goal. Clearly, much has been learned concerning transport processes in sink tissues, but the small size and inaccessibility of the SE/CC complexes, coupled to

the complexity of the postunloading pathway, has hindered attempts to isolate physiologically the phloem unloading step.

Many of the above arguments concerning the pathway of phloem unloading have centered on the role of symplast versus apoplast in solute transport. Recent developments in microinjection procedures have considerably advanced our knowledge of symplastic transport in leaf tissues and have brought into question the apoplastic concept of phloem loading (5). The transfer of such techniques to sink tissues has already begun (9) and is likely to provide valuable information on postunloading pathways. Increasing evidence is accumulating that some sinks may switch from an apoplastic to a symplastic pathway depending on the physiological status of the tissue (11). The factors regulating such major changes in transport pathways have still to be determined but clearly require further investigation.

Direct visualization of the phloem unloading step, using microinjection techniques, has not yet occurred. Phloem-mobile fluorochromes, such as fluorescein, have found considerable use in unloading studies but are not sufficiently membrane-impermeant to remain confined to the symplast following their movement out of the phloem. The direct introduction of membrane-impermeant probes into pathway sieve elements, followed by visualization of their symplastic exit in sinks, offers a technically demanding challenge for the future and a potential means of studying symplastic unloading without the interference of cellular metabolism and intracellular compartmentation.

The factors controlling plasmodesmatal aperture will undoubtedly prove central to understanding intercellular transport. Recently, a major achievement has been made in transforming tobacco plants with a viral movement protein (15). Plasmodesmata in the mesophyll cells of these plants have been found to allow the intercellular passage of fluorescent probes of much higher mol wt (9400) than control plants (700–800). Such genetically transformed plants are likely to become invaluable tools in the study of basic transport processes in both source and sink tissues.

Future studies of apoplastic phloem unloading and on symplast/apoplast exchange are likely to benefit from the isolation and characterization of membrane-bound carrier proteins, such as the putative sucrose carrier isolated from soybean cotyledons and shown in spinach, by immunogold labeling, to be localized on the sieve-element plasmalemma at the onset of export in source leaves (14). Further elucidation of the role of other solute carrier proteins in sink tissues and their precise localizations are likely to help pinpoint the exact site(s) of assimilate transfer between symplast and apoplast, although not necessarily the direction of transport.

In summary, the term 'phloem unloading' has been used in the literature to cover a multitude of transport processes in

sink tissues, beginning with phloem unloading and ending with the accumulation of solutes inside sink cells. Crucial for further understanding of the unloading pathway will be a determination of the precise roles played by the apoplast and symplast in short-distance solute transfer. In this respect, the development of several new microtechniques is likely to be of great benefit in determining the pathway taken by solutes out of the phloem. In future, a clearer distinction between unloading (at the SE/CC complex) and the postunloading pathway will help to clarify the nature of the investigations being conducted and the conclusions subsequently drawn from them.

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