Plasma Membrane-Cell Wall Contacts[1]

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In the simplest sense, cell walls are a carbohydrate and protein structures that surround and separate cells. Perhaps the most familiar cell wall structures are cork and wood, where the cell wall remains after the cells within have degraded. Cotton, the fiber that keeps some of us clothed in our complex world, is also a type of cell wall. These are specialized examples, and it is clear that living cells have a dynamic interaction with their surrounding wall and with each other, perhaps through the wall. This extracellular matrix (ECM) has the potential to influence almost every aspect of cell function simply because of its position and physical properties. For this reason many have speculated on its role in a plant's development and response to the outside world. Cell growth can occur in many dimensions, such as the polarized expansion of a pollen tube tip, the creation of elongated cells characteristic of many vegetative tissues, or even the jig saw-like arrangement of cells at the leaf surface. As these cells expand in a regulated fashion they must necessarily modify and enlarge their ECM to permit the subsequent increase in volume, but it is thought that the cell wall laid down by the same or an adjacent cell itself might also influence this process. Currently our understanding of these processes is quite limited.

Although there are as yet no direct answers to how the cell wall functions in development and plant responses, there have been some recent advances in understanding what molecules might be involved, and how they might interact with each other and the cells. For cell walls and cells to influence each other, there must necessarily be contact, and although there are numerous potential interactions at the plasma membrane-cell wall interface, this essay will focus on the direct physical connections that are known to occur between the plasma membrane and the ECM in angiosperms. Recent reviews describe other cell wall components and their role in expansion and plant development and the role of chemical modifications in response to environmental influences (Carpita and Gibeaut, 1993; Showalter, 1993; Reiter, 1994; Cosgrove, 1997). What emerges is the idea that the angiosperm cell wall is more than an exoskeleton; it is also a dynamic substrate for interacting cells.

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WHAT IS THE CELL WALL?

The cell wall has been described as a complex of carbohydrate and protein that is secreted by the cell and appears to be a continuous matrix that forms a scaffold and substrate for cells within a tissue (Roberts, 1990). The simplest interpretation from many studies of the wall predicts an ordered array of cellulose microfibrils that are coated with hemicellulose. This matrix is embedded in a gel of pectin, and somehow within this arrangement are proteins with varying amounts of linked carbohydrates (Reiter, 1994; Cosgrove, 1997; Fig. 1). Lignins and other organic compounds can be laid down on this matrix to impart mechanical strength and rigidity (Reiter, 1994).

Cell walls have been classified as primary or secondary. The primary wall is laid down during cell division and expansion, and material deposited on the primary wall once growth has ceased is termed the secondary wall (Cosgrove, 1997). For simplicity, and since many dynamic interactions are found in expanding cells, the discussion of the plasma membrane-wall interface will be restricted to the primary cell wall.

Many have equated the plant cell wall with the ECM of metazoans, and indeed the terms are often used interchangeably (Roberts, 1990; Carpita and Gibeaut, 1993). Although the cell wall has a radically different composition from the metazoan ECM, they do play similar roles; one could equate wood with cartilage at one extreme, and primary cell walls with basement membranes. Defining the cell wall is a battle with semantics, and raises some important points. Until recently the cell wall has been considered in the most part an exoskeleton of protein and carbohydrate that is secreted by its own caged or adjacent cell. In this way the cell defines its immediate environment and shape. The metazoan ECM is approached more from the view that the ECM is a carbohydrate substrate that influences the behavior of the surroundings through receptors and modifying proteins (Bissell and Nelson, 1999). Perhaps the most parsimonious view of the cell wall would encompass both views (Roberts, 1990). An easy working definition for the cell wall would see it as a carbohydrate matrix that provides a dynamic scaffold with which a variety of other carbohydrates and proteins associate. Whether these carbohydrates and proteins are "cell wall " components is only a matter

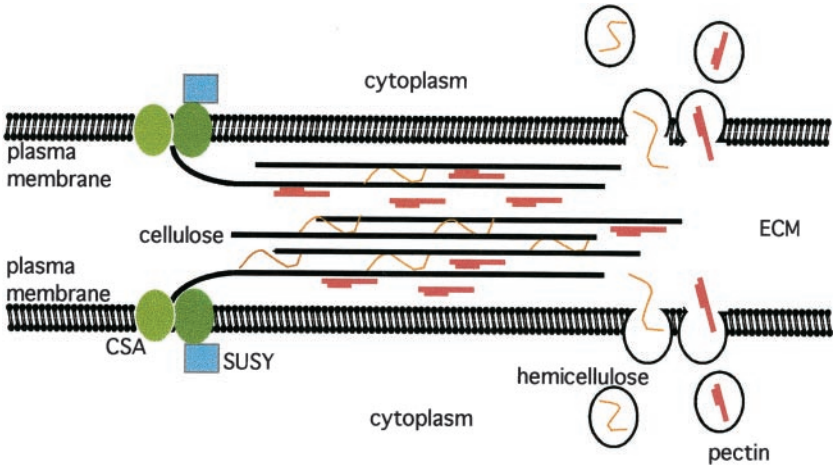

Figure 1. Diagramatic representation of primary cell wall assembly and structure between two adjacent cells. Cellulose is synthesized and secreted by a complex of cellulose synthases (CSAs) on the plasma membrane, and forms a bundle of crystalline microfibrils (black cables; cellulose). Cellulose synthase may be associated with cytoplasmic sucrose synthase (SUSY) that provides the sugars for polymer synthesis. Pectin (red bars) and hemicellulose (orange wiggles) are synthesized in and secreted through the endomembrane system and are complexed with cellulose in the ECM. How the synthesis of the wall is coordinated between two adjacent cells and between the cellulose synthase and the endomembrane system is not known.

of definition, and it is often hard to distinguish whether they are structural or regulatory or both.

CELL WALL COMPONENTS

Carbohydrates

The primary cell wall of angiosperms is in part laid down through the ordered secretion of 1-4-linked β-D-glucose polymers by plasma membrane-associated cellulose synthases (Amor et al., 1995; Pear et al., 1996). These polymers are woven together into linear bundles of cellulose fibers that have an average diameter of 7 nm and are thought to form a liquid crystalline array.

Hemicellulose is a term used to describe a family of polymers rich in glucose, xylose, or arabinose that, unlike cellulose, have extensive side chains often including xylose, galactose, and fucose. The dicots and monocots differ substantially in their hemicellulose composition and comprehensive descriptions can be found in a number of reviews (Carpita and Gibeaut, 1993; Reiter, 1994; Cosgrove, 1997). The hemicellulose structure permits these complex sugars to lie along the surface of, and perhaps intercalate within the cellulose bundles, providing a linked matrix. The hemicelluloses are secreted through the endomembrane system (Fig. 1). How the secretion of hemicellulose and the synthesis of cellulose are coordinated is not known but this may be important in defining localized wall architecture and its interface with the cell.

Pectins are a family of polygalacturonic acids that can vary in their side chains, usually arabinose, galactose, or a complex branched arrangement of monosaccharides (Cosgrove, 1997). The pectins are also secreted through the endomembrane system such that they may form a jelly like matrix that is intercalated with the cellulose/hemicellulose structure (Carpita and Gibeaut, 1993). The abundance of negative charges on pectins allows Ca^{2+}-mediated cross-linking that may be regulated by the masking of pectic negative charges through the addition of methyl esters. Antibodies directed to either pectin or methyl-esterifed pectin detect epitopes that are distributed unevenly in a variety of tissues, including pollen tubes, providing evidence that this modification could have a regulatory function (Knox, 1997). When pollen contacts the stigma there is a rapid expansion of membrane at the pollen tip and the continued tip growth has been correlated with the de-esterification and Ca^{2+} cross-linking of pectins peripheral to the growing tip. The cross-linking leads to an increased ridgidity of the lateral pectin matrix of the pollen tube thereby permitting only tip expansion (Yang, 1999). Similar models are proposed for root hair growth (Wen et al., 1999). Nothing is known of how the synthesis of cellulose and the secretion of pectins are coordinated although their respective matrices can exist independently (Roberts, 1990).

Proteins

Traditionally "cell wall" proteins have been classified by their association with one or more of the complex carbohydrates secreted by plant cells. These include the abundant hydroxy-Pro-rich glycoproteins (HRGPs; Showalter, 1993), Pro-rich proteins (Showalter, 1993), Gly-rich proteins (GRPs, Keller 1993), arabinogalactan proteins (AGPs; Oxley and Bacic, 1999; Majewska-Sawka and Nothnagel, 2000), wall-associated kinases (WAKs; He et al., 1996, 1999), lectins (Herve et al., 1999), and expansins (Cosgrove, 1997). But the list is far more extensive and includes peroxidases, methyltransferases, galactosidases, glycanases, and proteases to name just a few (Showalter, 1993). Analysis of genome information and detailed gel analysis (Robertson et al., 1997) will likely provide an exhaustive list of additional cell wall proteins. It may not be a useful exercise to anoint a protein the honor of being a "cell wall" component, but rather deal with this large class of secreted proteins from a functional standpoint. Indeed perhaps

the best example is provided by the protein ligand SCR for the receptor kinases that regulate self-incompatibility in *Brassica* sp. (Schopfer et al., 1999). SCR is secreted by the pollen grain and resides on its surface to be presented to its receptor on the plasma membrane of stigma cells. SCR is on the surface of the pollen and thus is in direct contact with and part of the pollen cell wall, but is it a "cell wall" protein? It is also important to remember that recent rapid freezing methods show the distances between the plasma membrane and the ECM are in fact smaller than previously observed (Roberts, 1990), such that it is possible for proteins to extend well into the carbohydrate matrix and perhaps even contact proteins or carbohydrates on another cell surface. One could also include in a discussion of cell walls the numerous receptor kinases on the plasma membrane (Kohorn, 1999). An example would be the CLAVATA 1 receptor (Trotochaud et al., 1999) on the lower meristem layer that influences cell identity and proliferation. The CLAVATA 3 protein is secreted by the uppermost meristem layer (Fletcher et al., 1999) and is postulated to bind CLAVATA 1 and serve as a ligand.

To avoid the exclusion of many interesting proteins, it might be best to refer to the carbohydrates as the cell wall and to view the proteins as influential visitors. This indeed seems to be the view taken for the study of most other kingdoms (Bissell and Nelson, 1999). The question pertinent here then becomes which visitors have an influence that requires contact with both the plasma membrane and the extracellular carbohydrate.

PLASMA MEMBRANE-WALL INTERFACE

Physical connections between the cell wall and the plasma membrane have been observed in a number of ways. Most electron micrographs show that the plasma membrane is appressed against the extracellular material, and thus they are apparently in direct contact (Roberts, 1990). It is assumed that turgor pressure is responsible for this appression, because disruption of the turgor by osmotic shock induces plasmolysis and results in the separation of the membrane from the cell wall. In most cells this separation is quite complete, although appressed regions do remain and can be enhanced in frequency in salt-adapted cultured cells (Carpita and Gibeaut, 1993). Plasmolyzed cells have thin lingering strands of membrane that extend from the collapsed plasma membrane to the cell wall which have been termed Hechtian strands (Roberts, 1990). It remains to be determined if these are in fact sites of plasmodesmata that form cytoplasmic passages between cells (see below; Crawford and Zambryski, 1999), but the fact that they occur on the outer walls of the epidermis makes this less likely. The nature of the contact sites in either the Hechtian strands or the salt induced contacts is unknown, although they have been called "adhesion sites." The term adhesion invokes homology with similar sites in metazoan cells, where integrins and similar receptors bind the ECM. These adhesion sites are clustered into islands that are associated with regulatory kinases, their ligands, and the cytoskeleton (Bissell and Nelson, 1999). Convincing evidence for such islands is still lacking in angiosperms, despite numerous attempts to identify such sites (Carpita and Gibeaut, 1993; Canut et al., 1998; Laval et al., 1999). Ironic and perhaps most pertinent is that their abundance in metazoans is greatly exaggerated in cultured cells and quite diffuse if not rare in real tissues (Bissell and Nelson, 1999). The plant cytoskeleton may have a role in defining contact sites between the plasma membrane and cell wall as it is clear that both actin and tubulin play essential roles in plant morphogenesis (Kost et al., 1999). It remains to be established, however, if angiosperm cells have true adhesion sites in the sense that there are locations on the membrane whose major role is to anchor the cell to the cell wall.

If adhesion sites are not required to maintain cell shape, they may have a function in keeping a cell from rotating within a cell wall frame. This appears to be unnecessary in most cells due to the presence of plasmodesmata. Plasmodesmata are membrane filled channels that connect adjacent cells in defined locations, are laid down during cell division, and may indeed provide sufficient structural force to fix cells in position (Crawford and Zambryski, 1999). If plants do not have strong adhesion sites, they might have sites of weaker contact that are of sufficiently low affinity not to be detected in abundance during plasmolysis; these might be involved in signaling and cell wall synthesis. This appears to be the emerging principle in a survey of the known proteins that are both in the plasma membrane and the cell wall, as discussed below.

PROTEINS AT THE INTERFACE

There are several classes of proteins that have been put forward as defining or regulating the cell wall-plasma membrane interface, and these include the AGPs (Oxley and Bacic, 1999; Majewska-Sawka and Nothnagel, 2000), cellulose synthases (Pear et al., 1996), a hydrolytic enzyme (Nicol et al., 1998), and the WAKs (He et al., 1996, 1999; Fig. 2). All of these proteins are bound to both the plasma membrane and the extracellular carbohydrate. Other protein families such as HRGPs (Showalter, 1993), expansins that facilitate cell wall loosening during cell expansion (Cosgrove, 1997), and proteins that are completely secreted by the cell in a polar or time-regulated fashion and that modify the wall (Cosgrove, 1997) will not be considered here despite their importance.

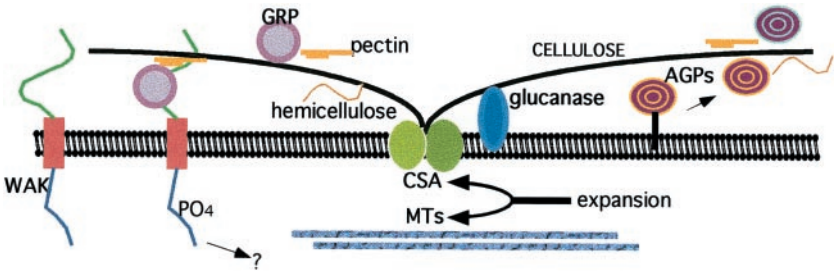

Figure 2. Speculative representation of proteins known to contact both the plasma membrane and the extracellular carbohydrate. Cellulose synthase (CSA) forms a rosette complex in the membrane and secretes cellulose. Signals that direct cell expansion and growth influence the cytoplasmic microtubules (MTs in blue) and the cellulose microfibrils in similar ways such that they have similar orientations. Ambinogabactus protein (AGPs) are composed primarily of carbohydrate linked to a smaller protein core. Some types of AGPs (red whorl) can be found anchored to the membrane via a carboxyl-terminal GPI which can be cleaved on the cell surface to produce a fully secreted form. Some AGPs are directly secreted in the absence of a membrane anchor (purple whorl). Both the membrane-bound form and the secreted AGPs have been found to associate with a variety of cell wall components and have been suggested to provide adhesive or perhaps positional cues. Several enzymes (such as glucanase in blue) that modify or cleave the carbohydrate are also membrane bound and may help to modify the matrix once it is laid down. A family of cell wall-associated kinases (WAK) has cytoplasmic Ser/Thr kinase domains and span the membrane to have an extracellular domain in the ECM. Their activity is associated with development, pathogen infection and wounding. A small population of WAK is not bound to the ECM, but most is covalently linked to pectin. WAK1 is also associated with a secreted GRP. WAK that is associated with both pectin and GRP is phosphorylated, suggesting a functional relationship. These proteins, in combination with fully secreted proteins such as HRGPs, lectin like proteins, expansins, and likely a cadre of yet to be identified proteins, somehow shape and interact with the ECM to effect cell growth and determine developmental programs and environmental responses.

Cellulose Synthases

One cannot ignore that the most obvious connection between the plasma membrane and the cell wall is the enzyme that synthesizes cellulose. Cellulose synthase can form a rosette of multiple protein subunits in the plasma membrane and is thought to associate with sucrose synthase (Susy) on the cytoplasmic face of the plasma membrane (Amor et al., 1995; Pear et al., 1996). The association of the rosette with Susy could allow the transfer of glucose from sucrose (via UDP-glucose) to a growing cellulose chain. Since the enzyme complex is coupled to Susy it allows the possibility of linking cytoplasmic metabolism to the establishment of cell wall architecture. Cellulose synthase is encoded by a large gene family in angiosperms and it is possible that the different encoded isoforms have distinct functions. A null mutation, rsw1, in Arabidopsis lies in one synthase isoform and leads to a reduction in crystalline cellulose and the elimination of cell surface rosette structures (Arioli et al., 1998). However, cellulose is still synthesized, so it is likely that the rosette is involved in the bundling of cellulose fibers, and other synthases can still extrude 1-4-linked β-D-Glc polymers in the absence of a plasma membrane rosette. It is quite possible that the rosette itself is composed of multiple isoforms whose representation within the membrane can be altered so as to modulate the cellulose composition in the cell wall. Other mutations such as irx3 (Taylor et al., 1999) describe cellulose synthase-like genes involved in depositions in existing primary cell walls (hence secondary wall formation), providing more evidence that the cellulose synthase gene family encodes proteins of diverse function, whose study may reveal how localized synthesis at the plasma membrane can control the architecture of the wall.

Not only can the composition of the cellulose synthase rosette complex have profound influences on the synthesis and makeup of the cellulose matrix, but it is also clear that the rosette is somehow associated with the cytoskeleton. It has long been observed that the microtubules lining the plasma membrane and the cellulose fibrils are both transverse to the direction of cell elongation. This has led to the widely accepted idea that the rosettes follow the cytoskeletal arrays on the cytoplasmic face of the plasma membrane as the rosettes move in the membrane during cellulose synthesis (Kost et al., 1999). In support of this, a number of microtubule inhibitors do alter the orientation of the cellulose fibrils. However, some reports suggest instead that it is the process that drives cell expansion that determines the orientation of both microtubules and cellulose fibrils independently, but in the same direction. Genetic analysis that separates elongation from cellulose deposition supports the latter model, although there are differing interpretations (Baskin et al., 1999; Fisher and Cyr, 1998). There is potential in either scenario for cellular processes, be it cytoskeletal orientation or elongation mechanisms, to influence cell wall architecture, and the mechanism may rely upon plasma membrane contacts with the cell wall. Unlike many vegetative cell types, both root hairs (Kost et al., 1999) and pollen tubes (Yang, 1998) grow by tip extension. In these specialized cells it is clear that the

cytoskeleton and the activity of small GTPases are coordinated with the deposition of new extracellular carbohydrate, and it will be of interest to see if the paradigms derived from roots and pollen tubes can be extended to other cell types.

Other Plasma Membrane-Bound Enzymes

Endo-1-4-β-D-glucanases (EGases) hydrolyze β-1,4-linkages at unsubstituted glucose residues and are encoded by a large family in angiosperms. Many EGases are completely secreted from the cell to modify the carbohydrate matrix. One class of EGase is integral to the plasma membrane and a mutation in one of these, encoded by the Korrigan gene, disrupts the correct assembly of the cellulose-hemicellulose matrix and cell expansion in non-tip growing cells (Nicol et al., 1998). The placement of this EGase in the membrane may allow coordination of its activity with the assembly of the cellulose synthase complex, and perhaps provide a direct link to cellular physiology. It is likely that as newly sequenced genomes are analyzed and proteins identified, a number of membrane-linked hydrolytic and synthetic enzymes will appear, and our view of how the surface of the cell acts as an organizing surface for the cell wall will mature.

AGPs

AGPs are represented by a large gene family in a variety of angiosperms. AGPs are heavily glycosylated in the endomembrane system, and some contain signals for the addition of a carboxy-terminal glycosyl phosphatidyl inositol (GPI) anchor such that upon secretion AGPs remain on the plasma membrane exposed to the cell wall (Majewska-Sawka and Nothnagel, 2000; Oxley and Bacic,1999). Up to 90% of the mass of an individual AGP can be carbohydrate that is added in the endomembrane system. The structure has great potential to bind to components of the cell wall, and numerous reports demonstrate that AGPs purify with cell wall preparations (Showalter, 1993; Cosgrove, 1997). Different family members can be expressed in tissue specific patterns, leading many to speculate that AGPs play crucial roles in plant growth and development. The GPI anchor can be cleaved at the cell surface (Oxley and Bacic, 1999), much as in yeast cells where cell wall composition is modulated by the enzymatic release of lipid-anchored glycoproteins (Kapteyn et al., 1999). It is easy to speculate that AGPs can reversibly link the carbohydrate of the cell wall to the cell.

The Yariv reagent, which specifically binds the carbohydrate of AGP, has been used to dissect AGP function. Yariv reagent clearly has major inhibitory effects on plant development (Willats and Knox, 1996), cell expansion in roots (Yang, 1998), pollen tube tip growth (Roy et al., 1998), and cell growth in tissue culture (Gao and Showalter, 1999). There is also evidence that an AGP can direct pollen tube growth (Wu et al., 1995). It is unclear what the relationship is between the lipid-anchored AGPs and those completely secreted, and which AGP is most affected by Yariv reagent. Because different AGPs have different compositions and thus perhaps different wall binding capacities, and since they are expressed in a variety of cells, it is tempting to speculate that AGPs help to define cell location (Roberts, 1990; Showalter, 1993; Oxley and Bacic, 1999). This model remains to be tested, although it provides a context where the cell wall should be treated as a substrate for developmentally defined cell surfaces.

WAKs

There are five cell WAKs in Arabidopsis and representatives in other angiosperm families. WAKs each have a cytoplasmic Ser/Thr protein kinase domain, span the plasma membrane and extend a domain into the cell wall (He et al., 1996, 1999). WAKs, like GPI-anchored AGPs, physically link the plasma membrane to the carbohydrate matrix but WAKs are unique in that they have the potential to directly signal cellular events through their kinase domain. The WAK extracellular domain is variable between the five isoforms, and collectively the family is expressed in all organs. *WAK1* and *WAK2* are the most ubiquitously and abundantly expressed of the five tandemly arrayed genes, and their messages are present in vegetative meristems, junctions of organ types, and areas of cell expansion. They are also induced by pathogen infection and wounding (Wagner et al., 1999).

Mutations in WAKs demonstrate that they are essential for plant development and required during the pathogen response (He et al., 1998, 1999; Wagner et al., 1999) The WAK1 but not WAK2 cell wall domain binds to a GRP of the cell wall in vitro assays. WAK1 and GRP can be co-immunoprecipitated from leaf or seedling extracts, and this WAK is phosphorylated (A.R. Park, U. Yun, S.K. Cho, Y.S. Kim, M.Y. Jin, S.H. Lee, B. Oh, G. Sachetto-Martins, B.D. Kohorn, and O.K. Park, unpublished data). A large amount of WAK is also covalently linked to pectin and most of WAK that is bound to pectin is also phosphorylated. There is a small population of WAK that is not bound to pectin or any cell wall carbohydrate and this can be extracted with detergent. The data support a model where WAK1 becomes bound to GRP as a phosphorylated kinase, and then binds to pectin (Fig. 2). How WAKs are involved in signaling from the pectin matrix in coordination with GRPs will be key to our understanding of the cell wall's role in cell expansion and development.

Metazoan-Like ECM Receptors?

Attempts to identify metazoan-like ECM plasma membrane receptors have not provided convincing evidence that these molecules exist in angiosperms. Integrins bind the RGD protein motif of fibronectin, which is bound to the metazoan ECM (Bissell and Nelson, 1999). A number of studies have identified RGD binding activities in the angiosperm plasma membrane, but none have provided evidence for proteins with amino acid similarity to integrins (Canut et al., 1998; Laval et al., 1999). Antiserum to a series of metazoan proteins that are known to link the ECM to the cell have identified cross-reactive material in angiosperms (for review, see Canut et al., 1998). However, reports that identify these proteins or describe their genes demonstrate that the identified plant epitopes are not present in proteins involved in extracellular linkages (Wang et al., 1996). This may not be surprising given the differences in carbohydrates between the two kingdoms, and a more likely similarity may be sought in the cytoplasmic domains of ECM receptors where cellular processes have a greater likelihood of being conserved.

EMERGING CONNECTIONS

The extent of our knowledge of the interface between the plasma membrane and cell wall is clearly increasing at a rapid rate. With more and more examples of proteins that appear to be in the plasma membrane and either directly or indirectly contact the extracellular carbohydrate, the interface becomes more complex. This review has concentrated on only those components that have been clearly demonstrated to reside in the plasma membrane and cell wall, but in doing so, has ignored some extremely important areas that may soon need to be included. For example, one would predict that membrane-cell wall contacts would be important in the formation, regulation of the timing, and in the orientation of the cell plate during cell division. Some of the proteins mentioned here may be involved in this process, and genetic analysis of cell division (Kost et al., 1999) will identify additional components that could lie at the interface of the wall and plasma membrane. The study of pollen tube growth may also provide insights into cell wall-membrane connections.

LTPs

Pollen tubes adhere to the stylar ECMs in lily via proteins that have sequence similarity to plant lipid transfer proteins (LTP; Park et al., 2000). The name of these peptides is misleading since plant LTPs are probably not acting in lipid transfer in the same way as animal LTPs. The LTP mediated adhesion in lily requires a large carbohydrate also found in the stylar ECM for activity, and it will be important to determine whether this LTP complex binds to the plasma membrane or wall components in the pollen tube to signal the cytoplasm of an appropriate adhesion event. Plant LTPs are encoded by a diverse family of genes that are expressed in a variety of tissues, and so have the potential to define spatially distinct substrates for other types of cells.

Other Candidates

There are a variety of other cell wall proteins that also have great potential to mediate membrane interactions, but as yet there is no clear evidence that establishes this. These include the diverse family of HRGPs (Showalter, 1993), and cDNAs that predict membrane-associated lectin binding proteins (Herve et al., 1999). Many believe these proteins to be important in aspects of development.

CONCLUDING REMARKS

In this review the cell wall has been divided into two components; large structural carbohydrate complexes and regulatory proteins. While this separation has helped in organization, this classification may be too stark as we know too little to make a clear distinction between structural and regulatory components. Indeed some smaller carbohydrates have been implicated in signaling (Cosgrove, 1997), and secreted proteins can form structural extracellular matrices (Keller, 1993). It is clear nevertheless that the cellulose/hemicellulose/pectin matrix not only provides structural integrity but also serves as a substrate for cells to mark their location and identity through protein interaction.

This essay has highlighted AGPs, WAKs, and cellulose synthase, as these are proteins known to directly contact the lipid bilayer and the carbohydrate complex. There is a suggestion that AGPs and perhaps WAKs define location and signal cell wall architecture. Along with cellulose synthase their regulated and specific association with the carbohydrate matrix may also confer a generalized low affinity binding that in combination with turgor fixes the cell in position.

We know of only a few cell wall-membrane contacts, as described here, but there are likely more to be discovered. Of those that have been defined, such as WAKs and AGPs, it remains to be determined how these molecules, among others, achieve an interaction that determines cell shape, size, and form.

Although some interactions between the wall and cell may be dynamic, there is a vast reservoir of extracellular protein and carbohydrate that is cross-linked into an insoluble, biochemically intractable matrix (Cosgrove, 1997). It is very likely that this matrix serves a structural role, and its integrity can be regulated both developmentally and environmentally. Indeed, pathogens and wounding can cause extensive cross-linking between carbohydrate and

protein in this matrix. Complexes that evolve reactive oxygen species in the extracellular space are likely responsible for some of the cross-linking that is seen, and an understanding of how these proteins are regulated may be key to determining how cells modulate their external environment. But one must also ask about the relationship between this extensive cross-linked material and the membrane proteins, such as AGPs, WAKs, and cellulose synthase that at certain times have a dynamic interactions with the cell wall. Although this relationship is not clear, it is possible that components active at the membrane-wall interface during cell expansion may well lose their initial function once the primary wall has been synthesized and be transferred to the cross-linked extracellular graveyard to serve as structural elements of a system that is continuous throughout a given plant organ. In this sense the proteins at the wall-membrane interface might serve two functions, first in communication and subsequently in a structural and perhaps less specific role.

Finally, it will be important to include in our concept of the cell wall the mechanical force that it provides. A number of studies demonstrate that force vectors generated by the wall have a role in determining cell development and fate (Lynch and Lintilhac, 1997). Whether these signals are transmitted directly to the cytoskeleton or via cell surface receptors remains to be established.

In the next few years it is likely that the small space that separates the plasma membrane from the carbohydrate matrix will be filled with additional fascinating molecules whose interactions will answer some of the questions raised in this essay.

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