Plant cell adhesion: A bioassay facilitates discovery of the first pectin biosynthetic gene

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As in all multicellular organisms, plants have adhesion molecules in their extracellular matrices (ECMs) that serve to maintain the integrity of the organism and to provide a scaffold for cell/cell communication. In flowering plants, adhesion molecules in somatic cells are produced by the secretory system at the cell plate during cell division (1) and in reproductive tissues, in the transmitting tract of the pistil that is formed from epidermal cells of the carpels (2). Until recently, no adhesion molecules were known for plants. The plant cell wall or ECM contains many polymers. The most abundant and best known is cellulose, but this well known polymer is embedded in a matrix of molecules called hemicelluloses and pectins. Pectins are likely to be implicated in adhesion because they occur in the middle lamella, the site of cell adhesion, and they are secreted from epidermal walls when carpel fusion occurs in formation of the transmitting tract. Both antibody localization data and genetic approaches have revealed only circumstantial evidence that pectins are adhesion molecules. *De novo* adhesion occurs in reproduction when pollen grains land on a stigma, when pollen tubes traverse the transmitting tract of the style, and when a sperm cell meets the egg cell during fertilization. An *in vitro* functional adhesion assay, produced by applying pollen tubes to the ECM molecules of the stylar transmitting tract (3), was used to isolate the adhesion molecules from the lily style. The matrix polymer identified as an adhesion molecule was a pectic polysaccharide (4). In this issue of PNAS, Iwai *et al.* (5) make use of an *in vitro* biological assay for adhesion to prove that pectin is the adhesion molecule binding somatic cells and that a previously uncharacterized pectin biosynthetic enzyme (a glucuronyltransferase) is a primary player in this adhesion event. This work is a major advance for plant cell biology for two reasons: it firmly establishes pectin as an adhesion molecule, and it provides an inroad into pectin biosynthesis.

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Whereas cellulose polymerization occurs at the plasma membrane, pectin and hemicellulose biosynthesis takes place in the Golgi. Pectins, one of the main components of the plant primary cell wall, are complex polysaccharides containing homogalacturonan (HG), and rhamnogalacturonans I (RG-I) and II (RG-II) regions. Xylogalacturonan (XGA) and apiogalacturonan can also be found in particular tissues or species (6). HG, RG-II, XGA, and apiogalacturonan have a (1, 4)-α-D-galacturonic acid backbone, whereas RG-I is built up with the repeating disaccharide (1, 4)-α-D-galacturonic acid-(1, 2)-α-L-rhamnose, in which 20–80% of the rhamnosyl residues can be substituted with (1, 5)-α-L-arabinan, (1, 4)-β-D-galactan, and/or arabinogalactans. The existence of covalent linkages between these different regions (HG, RG-I, and RG-II) is often assumed because endopolygalacturonase treatment of an insoluble wall fraction can release RG-I, RG-II, and oligouronides. It is well accepted that HG is synthesized in the Golgi in a highly esterified form and, after deposition into the wall, can become partially de-esterified by the actions of pectin methyl esterases that would allow calcium crosslinks between several unesterified HG blocks. RG-II contains four well defined oligosaccharides (two different disaccharides, one octasaccharide, and one nonasaccharide), with sugars such as glucuronic acid, galactose, apiose, rhamnose, aceric acid, KDO, 2-O-methyl xylose, and others, that are linked to the galacturonan backbone. It has been shown that RG-II exists as a dimer in the primary cell wall (7, 8) by means of borate diester crosslinks between two apiosyl residues of two octasaccharides (9). There are an estimated 51 enzymes necessary for the synthesis of pectic polysaccharides, 41 of which are unique. At least 20 of them may be required for RGII elaboration, but none have been purified yet (10). One of the difficulties has been that screens for mutations in cell wall biosynthetic genes have proven laborious, with lethality and redundancy serious impediments. In mutants where cell wall biosynthetic genes were described, few have been in the pectin biosynthetic pathway. Progress in the field of pectin biosynthesis has been slow (11). Only recently have the first glycosyltransferases been characterized for the biosynthesis of wall matrix molecules other than cellulose by using both biochemical and genetic approaches (12–14). These glycosyltransferases are enzymes that attach a sugar molecule to a specific acceptor, thus creating a glycosidic bond. Such enzymes are particularly important in plants, as evidenced by the *Arabidopsis* genome estimate of hundreds of putative glycosyltransferase genes (15).

Cell adhesion and morphogenesis are correlated in many biological systems, and the adhesion molecules involved in animal development are well known to be crucial for signaling throughout the life of the organism (16). Less attention has been paid to this role for adhesion in plants. By using a variety of approaches, genetic studies have implicated pectins in adhesion (17–21), but none are as definitive as the Iwai *et al.* 2002 study (5). A very recent report (22) of two dwarf *Arabidopsis* mutants that have a 25% reduction in galacturonic acid levels is the most intriguing. The mutants carry a T-DNA insertion in a gene that encodes a putative glycosyltransferase, and they show disruptions in cell adhesion in their organs. Another approach is to study mutants that show inappropriate adhesion between organs like leaves, but these have been found to be caused by defective cuticle biosynthesis, proving a role for cutin in maintaining the surface integrity of organs (23). Good biological assay systems such as the one developed to study tracheary cell differentiation

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(24) have been lacking in the study of adhesion. Satoh's group (25) developed a clever *in vitro* assay designed to determine matrix polymers responsible for somatic cell/cell adhesion. They took advantage of the ability of plant cells, in the presence of hormones, to regenerate shoots in culture. Leaf discs placed *in vitro* will often form clusters of dedifferentiated cells called callus from which organized shoot apical meristems will form and produce whole-shoot systems. They developed lines of callus cultures made from haploid *Nicotiana plumbaginifolia* (a tobacco relative) transformed with T-DNA. A small percentage of these were "nonorganogenic with loosely attached cells," called *nolac* for short. Callus from *nolac* cultures has defects in cell adhesion and in the capability of producing shoots. The *nolac* cultures have proven useful for confirming that pectin is an adhesion molecule and for providing a way to screen for cell wall biosynthetic mutants (5). The use of haploids allows for direct observation of a mutation caused by T-DNA insertion, and if the mutation proves lethal in terms of morphogenesis, the callus-cultured cells can still be maintained for cell wall composition studies. By using the *nolac-18* line, the authors isolated a single-copy gene called *NpGUT1* that has sequence similarity to an animal glycosyltransferase, *EXT2*. *EXT2* is a transmembrane, Golgi-localized glucuronyltransferase involved in heparin sulfate synthesis. *In vitro*, *EXT2* is able to add single D-glucuronic acid and *N*-acetylglucosamine to an artificial substrate (26). Although Satoh's group has not shown enzymatic activity for the *NpGUT1* gene product as yet, they were able to complement the mutation, getting 80% of the transformed *nolac-18* callus clusters to regenerate shoots, and an antisense construct introduced into normal leaf discs showed crumpled shoots when they formed at all. The sugar composition of the pectic fraction of the mutant has a decreased level of glucuronic acid (GlcA). This sugar is a component of the RG-II pectic polysaccharide, so the authors focused on this fraction and found that the RG-II of *nolac-18* has no GlcA at all and only half the level of galactose (Gal) compared with the normal callus. RG-II from the mutant is less capable of forming borate diester crosslinks. They postulate that the disaccharide (Gal-GlcA), at the terminus of the RG-II octasaccharide side chain that forms the crosslinks with another RG-II molecule, is missing in the mutant. The lessening of adhesion between cells in *nolac-18* implicates the RG-II dimer in cell/cell adhesion.

The pectic polysaccharide RG-II occurs in all primary cell walls studied to date (6). RG-II crosslinking can control pore size in the wall and so may play a role in establishing a scaffold of pectic polymers to which other molecules in the wall can bind (27). Boron is an essential element in plant growth (28). It is taken up by the roots but accumulates at the growing points where it enters the cell wall. Up to 90% of the boron in plants is in the walls, and RG-II may be the only polymer it binds. An *Arabidopsis* mutant, *Mur1,* is fucose deficient and has a dwarfed phenotype. The mutation affects the ability of RG-II to form a dimer, just as does the *NpGUT1* mutation. The addition of boron to the roots compensates for the defect in the *Mur1* mutant (29), but not in the *nolac-18* line (5). It is interesting that neither the *NpGUT1* nor the *Mur1* mutation disrupts the apiose residue that actually binds the boron in the covalent crosslink of two RG-IIs. In both cases, disruption of nearby sugar residues on the side chain is enough to cause instability of the RG-II dimer. It would be interesting to see how the *Mur1* mutant tissue behaved in cell culture. A universal trait for boron deficiency in plants is sterility. Boron is essential for pollen tube growth, and it is known to be incorporated into the pectic walls of pollen that contain RG-II (30). The transmitting tract has abundant boron that is presumed to be the source for pollen tube growth (28). The adhesive pectic matrix in lily contains RG-II (J.-C.M. and E.M.L., unpublished data), so it is possible that the pollen tube walls bind the stylar matrix by means of these RG-II borate crosslinks. It would be interesting to know the level of expression of *NpGUT1* in reproductive tissues.

Iwai *et al.* suggest that the lack of RG-II crosslinks in the mutant disrupts adhesion enough to break plasmodesmatal connections.

NpGUT1 expression is at the meristems in *Nicotiana plumbaginifolia* where cell division is centered and new cell walls are laid down between daughter cells. It is at the cell plate that the initial cell adhesion events take place that result in formation of the primary wall between cells and the cementing middle lamella (1). The *nolac-18* callus clusters show some adhesion, but the adhesion is loose and the cells are vacuolate, unlike those of the organogenic callus and those of apical meristems in general. Here, cells are tightly appressed with no intercellular space, and they are densely cytoplasmic. Iwai *et al.* (5) suggest that the lack of RG-II crosslinks in the mutant disrupts adhesion enough to break plasmodesmatal connections between cells, and this prevents the cell/cell communication essential for shoot morphogenesis. Although there is good evidence that such cytoplasmic connections allow for signaling between cells at the meristem and elsewhere (31), there is equally good evidence that signaling molecules travel through the cell wall to their target receptors in the plasma membranes of neighboring cells. The *Arabidopsis* genome shows plants to have >500 receptor-like kinase genes, so receptors and their ligands will no doubt play a major role in morphogenesis (32). We are only beginning to identify those molecules active in meristems, like *Clavata 3*, which is secreted from the stem cells in the shoot apex into the ECM and then interacts with its receptor in the plasma membrane of underlying cells (33). If RG-II dimers control pore size in the pectin matrix, then they may have a real influence on protein "commerce" in the wall. There is evidence that a wall-associated kinase (WAK) in the plasma membrane has an extracellular matrix connection with pectin (34), so membrane wall connections may also involve pectins. In contrast to an old perception that the cell wall is an impediment to signaling, it is now perceived to play a major role. Heparin sulfate in animal ECMs is essential for the ionic binding of a variety of growth factors that are ligands for receptors in the membrane. It forms a scaffold to "juxtapose components of the signal transduction pathway" (35). Pectins also may play such a role. According to antibody localization and enzyme data, low esterified HG and the arabinan and galactan side chains of RG-I are also involved in adhesion at the middle lamella (36). In fact, antibodies to RG-II localize it and its dimer in the primary wall next to the plasma membrane and not to the cementing middle lamella (30, 37). Adhesion of the plasma membrane to the newly secreted matrix materials in the primary wall is also an important adhesion event in the meristem. One can imagine a cascade of adhesion events during the formation of the primary wall at the meristems, and the RG-II dimer formation could be one of the first of these crosslinking events, and is therefore essential. Another possibility is that RG-II crosslinking provides a scaffold of pectin that is a prerequisite for assembly of additional molecules in the site of adhesion. In the pollen tube adhesion assay where tube growth is necessary for adhesion to the *in vitro* stylar matrix, adhesion is progressive (3). Although pectin is the major matrix component respon-

sible for this *de novo* adhesion event, another molecule is involved as well: a small protein called SCA that binds to the pectin ionically (38).

Localization data for the RG-II dimer during cell plate formation and the enzymes involved in forming the necessary side chains will help to establish its role in the early stages of wall adhesion. Localization of *NpGUT1* protein in the secretory system is also important, as is a demonstration of its enzymatic activity. This task will not be easy, as the methods to generate specific acceptor molecules are generally lacking. With this discovery of the first pectin biosynthetic enzyme, the pace of progress in the pectin field will pick up. We also hope it will encourage the use of more *in vitro* biological assays in creative screens to decipher the three-dimensional complexities of the plant extracellular matrix and the biological roles for its many fascinating components.

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