

How the Cell Wall Acquired a Cellular Context

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It seems that 25 years ago plant cell walls were different than they are now. I don't mean physically different, of course—they were made of the same old stuff—I mean conceptually different. Let me try to explain. Much of the plant body (and in large plants the bulk of it) is comprised of cell wall material. It forms a tough yet extensible extracellular matrix of polysaccharides for young and growing cells (the primary cell wall), and a strong, thicker, and sometimes lignin-impregnated structure in secondary tissues (the secondary cell wall). The composition, architecture, and mechanical properties of these walls are ideally suited to the functions they perform, and so for many years plant biologists have been asking the same obvious yet important questions about cell walls: What are the structures of the key cell wall polymers? How are they made and deposited? How do they function in cell growth and differentiation? What is the relationship between extracellular events in the wall and intracellular events? How is all this regulated? In 1975, the main tools available for tackling these questions were still biochemistry and electron microscopy, and the conceptual picture of the wall that emerged from these approaches was understandably structural and static. Nevertheless, the broad framework was available for describing the three main classes of structural polysaccharides in the wall (cellulose, pectin, and the so-called hemicelluloses or cross-linking glycans), the proteins and lignin, and influential models already existed suggesting how they might all be put together (7). The problem was that most of this work started with large preparations of purified cell walls, commonly from whole-plant organs or from cell cultures, and this was hard to relate back to the obvious fact that the plant body itself contains numerous different cell types, most with clearly distinguishable cell walls. The biggest single shift in the next 25 years was to be from the chemistry of isolated and homogenized cell walls to an appreciation of the subtle, changing, functional complexity of individual walls around individual cells within the plant; in other words, a conceptual shift toward understanding walls in their cellular context. I shall chart this shift by looking at six areas where the cellular context has extended and changed our understanding of cell wall biology. In many cases, it is not surprising that these conceptual shifts have been driven by technical innovations in

microscopies, spectroscopies, model systems, and molecular genetics, as well as by valuable lessons learned from bacterial, animal, and fungal systems.

THE INCREASING COMPLEXITY OF WALL POLYMERS

Both classes of polymer in the primary wall, polysaccharide and protein, have been shown to harbor remarkable complexity. A combination of chemical and physical techniques that include HPLC, gas chromatography-mass spectrometry, and NMR, coupled with isotope labeling, linkage analysis, and the use of pure degradative enzymes with defined specificity, have slowly revealed the remarkable chemical heterogeneity of polysaccharides such as xyloglucan and the baroque complexity of the pectic polysaccharide rhamnogalacturonan II. The groups associated with Fons Voragen (Wageningen, The Netherlands) and Peter Albersheim (Athens, GA) have made major contributions in this area, the latter group exploiting the use of uniform cell suspension cultures (7). There was also a growing appreciation that the cell walls of the grasses differed in several important respects from those of other flowering plants, particularly in the non-cellulosic polysaccharides. This was highlighted by Bruce Stone's work at LaTrobe (Australia) and by Nick Carpita's work at Purdue (West Lafayette, IN; 3). There has been a similar growth in our understanding of the families of cell wall proteins. After Derek T.A. Lamport, working in Don Northcote's lab (Cambridge, UK), identified the unusual amino acid hydroxy-Pro in cell wall proteins, both polypeptide sequencing and gene cloning have allowed large families of wall proteins to be classified, both with and without this unusual amino acid. These now include Pro-rich proteins, Gly-rich proteins, and arabinogalactan proteins. It is sad that less progress has been made in attributing secure biological functions to these polymers (17), although functional genomics approaches in Arabidopsis should soon change this situation.

THE IMPORTANCE OF WALL ARCHITECTURE

The mechanical and functional properties of the cell wall are defined by its detailed molecular architecture as a fiber composite (17). Our current picture of wall architecture has been transformed in the last 20 years by the information provided by high-

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resolution electron microscopy and the use of polymer-specific probes in particular specific antibodies.

Electron microscopy has a distinguished history in wall research, and high resolution images of replicas (10) led to a new spate of model building based on structure rather than the biochemistry that had underpinned older models (7). A consensus emerged that walls were built of two independent networks, cross-linked cellulose microfibrils and cross-linked pectin, each with distinct physical and mechanical properties (3). The nature of the cross-links in both cases has been slow to emerge but most contributions here have come from Stephen Fry's (Edinburgh University, Scotland) thorough biochemical approach (18). The relative contributions of each network to the mechanical properties of the wall have been greatly clarified by the exploitation of a beautiful *in vitro* system that uses *Acetobacter xylinum* as a source of cellulose microfibrils to mimic the self-assembly of the higher plant cell wall (19).

Complexity has been mapped onto this simple model through the use of antibodies. Over the last 10

years we have learned that not only do the walls of different cells have different compositions, but that different wall polymers may be concentrated in different wall layers, and even that different domains or facets of a single cell wall may have very different compositions. This is true, not only for wall polysaccharides (8), but also for wall-associated proteins (14). This insight has focussed attention on the relationship between activities within the cell, in particular the cytoskeleton and targeted secretion, and the ordered structure of the wall outside; in other words, on cell biology (Fig. 1).

THE WALL OF THE SINGLE CELL

Local variation in wall thickness and composition is now seen as an integral part of a cell's differentiation process in relation to its cellular neighbors. This extends from the birth of the wall during cytokinesis, through cell expansion to local thickening (collenchyma, xylem, vessel elements, and epidermal cells), and wall dissolution (vessel elements and sieve tube

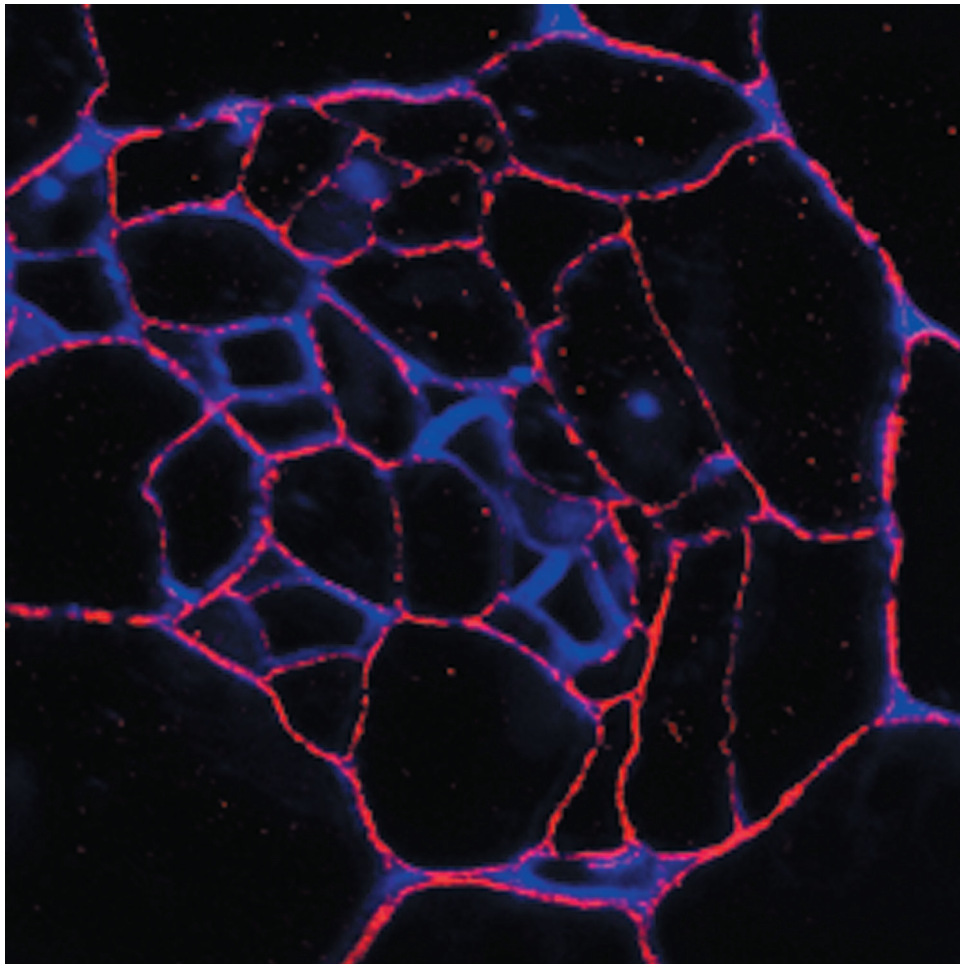


Figure 1. A section through part of a potato tuber stained with calcofluor (blue) to show the cellulose in the cell walls, together with the monoclonal antibody LM5 (red) that reveals the nonuniform distribution of 1,4-galactan in the walls. (Photo courtesy of Max Bush and Grant Calder [John Innes Centre].)

elements). The dynamic nature of the wall during the life of a cell is well illustrated by the appearance and rearrangements of the plasmodesmata that penetrate the wall and provide cytoplasmic connectivity between cells.

The use of monoclonal antibodies, notably by Mike Hahn (Athens, GA), Andrew Staehelin (Boulder, CA), J. Paul Knox (Leeds, UK), and Roger I. Pennell (Ceres Inc., CA), has been crucial in enabling us to appreciate just how complex and dynamic the plant extracellular matrix can be (8, 14).

Cell suspension cultures offer ready access to the primary cell walls of a single cell type (7) and the single greatest contribution to our understanding of secondary cell walls is also likely to come from a single cell model for tracheary element differentiation, derived from *Zinnia elegans* mesophyll cells (4).

THE BIOSYNTHESIS, DEPOSITION, AND GROWTH OF THE WALL

Although the precursors for wall polymer biosynthesis were identified long ago, the biosynthetic enzymes, the glycosyl transferases, that used them remained doggedly resistant to discovery until only about 5 years ago. Cellulose, long known to be made at the plasma membrane by large "protein machines" called rosettes (12), is now known to be made in plants by enzymes that have some homology to their bacterial counterparts (13). It emerges that the cellulose synthase and cellulose synthase-like gene families are enormous and are now the focus of a major research effort. Related genes are being uncovered, and those encoding enzymes that make matrix polysaccharides also are being revealed.

The ordered deposition of cellulose microfibrils has long been known to involve the plant cytoskeleton. Although a straightforward causal effect, from microtubule orientation to cellulose orientation, probably operates in some cases (9), the situation is far from simple (1), and the cellular context suggests that extensive cross talk exists between the extracellular matrix and the cytoskeleton (16, 20). Microinjection of fluorescent analogs and the use of green fluorescent protein-tagged proteins, coupled with confocal microscopy, have vastly increased our understanding of cytoskeletal dynamics in plant cells (6), and this is likely to expand into time-resolved studies of the wall itself.

Along with wall deposition have come some initial steps in clarifying how the wall architecture of growing cells is remodeled during the incorporation of new material. Several new enzymes have been characterized, including cut-and-paste enzymes for remodeling cross-linking xyloglucan, as well as enzymes that appear to regulate both the yield threshold of the wall and its extensibility (11). A comprehensive and synthetic picture of the molecular basis of wall extension and its cellular con-

trols remains a major problem for the future. Recent progress in this field is reviewed in this issue in an article by Dan Cosgrove (Pennsylvania State University).

THE IMPACT OF MOLECULAR GENETICS

Partly because wall polysaccharides are not themselves direct gene products, and partly because screens were hard to devise, the power of the tools of molecular genetics has been slower to impinge on the wall field than on many others. It's now clear from expressed sequence tag and cDNA databases and the Arabidopsis genome project that at least 1,000 genes are involved in making, assembling, and remodeling the cell wall and endowing it with particular properties; however, functional analysis of most of them remains a project for the future. Forward genetic screens have now begun to generate mutants with altered walls, largely as a result of the initiatives of Chris Somerville's group (Carnegie Institute, Stanford, CA; 15) and Richard Williamson's group (Canberra, Australia). The cloning of the genes involved in several characterized cell wall mutants is the first notable step in this field, and it's likely that analysis of insertional mutants and mutants in sensitized backgrounds will move the field along quickly. Gene sequencing projects have highlighted the large size of many families of wall-related genes; for example, pectin-mobilizing enzymes.

THE WALL TALKS BACK

In this necessarily brief account I have placed emphasis on the cellular context of the wall. Nowhere is that more obviously important than in the slow realization that the wall, far from being inert and silent stuff outside the cell, is instead intimately involved in extensive conversations with the cell, sometimes even with a managerial tone! The plant cell senses numerous signals through the wall via its connections to integral plasma membrane proteins. Turgor, osmosensing, mechanical stresses, and strains all are mediated through the wall, and I have already mentioned the wall's talk back to the cytoskeleton (16, 20). In addition, the wall seems to have homeostatic properties because there are many cases now where the lowering of one wall component appears to be compensated for by the rise in another. Two examples suffice to show how the wall has emerged as a repository for important signals or instructions to the cell within. These signals can act during the course of normal development to influence local cell fate decisions, as was elegantly shown by Berger et al. (2), or they can act in response to external events such as pathogen attack. In the latter case, small fragments of structural wall polysaccharides, when released by degradative enzymes, act back as signaling ligands to alert the cell to mount a defense response (5).

The last 25 years have seen a progressive shift in our perception of the wall toward a structure that is unique to the cell within, locally deposited and re-modeled, dynamic and developmentally regulated, complex, and able to respond not only to cellular events, but to influence them in return. This is a great platform for the boom time that functional genomics and the new cell biology promise.

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