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Pectate chemistry links cell expansion to wall deposition in *Chara corallina*

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Pectate (polygalacturonic acid) acts as a chelator to bind calcium and form cross-links that hold adjacent pectate polymers and thus plant cell walls together. When under tension from turgor pressure in the cell, the cross-links appear to distort and weaken. New pectate supplied by the cytoplasm is undistorted and removes wall calcium preferentially from the weakened bonds, loosening the wall and accelerating cell expansion. The new pectate now containing the removed calcium can bind to the wall, strengthening it and linking expansion to wall deposition. But new calcium needs to be added as well to replenish the calcium lost from the vacated wall pectate. A recent report demonstrated that growth was disrupted if new calcium was unavailable. The present addendum highlights this conclusion by reviewing an experiment from before the chelation chemistry was understood. Using cell wall labeling, a direct link appeared between wall expansion and wall deposition. Together, these experiments support the concept that newly supplied pectate has growth activity on its way to deposition in the wall. Growth rate is thus controlled by signals affecting the rate of pectate release. After release, the coordination of expansion and deposition arises naturally from chelation chemistry when polymers are under tension from turgor pressure.

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It was recently reported that calcium needs to be added to growing cell walls in order to keep them growing.[1] It is well known that most cell calcium is in the wall, but the experiment was conducted because earlier work[2,3] implicated calcium chemistry as a key feature of cell enlargement. It was predicted that growth rates were controlled by calcium cross-linking to pectates in the wall, and the experiment was a test of the prediction. The results were supportive and added to other tests already described.[4]

Some background for the earlier work may be needed. Most plant growth involves increases in cell size while the cytoplasm is encased in a tough cell wall. During the process, the wall stretches to a larger size irreversibly while incorporating new wall materials that maintain wall strength. Without the incorporation, the wall would become progressively thinner and unable to withstand tensions normally developed by turgor pressure. Importantly, this same pressure is required for wall stretching and is involved in depositing new wall material.[4] In fact, pressure can force new polymers into the wall that otherwise would be too large to enter.[5]

In order to investigate these processes, it has been convenient to use a large-celled alga such as *Chara corallina*, which is closely related to the progenitor of terrestrial plants.[6-9] Not only do the walls resemble those of terrestrial plants[10-13] but the large cells can be separated from the plant without any other adjoining cells. This enables turgor pressure to be measured and controlled while simultaneously monitoring the wall stretching and the depositing of new material.[4] From these cells, the walls are easily isolated without removing them from the medium in which the plants were grown, so processes can be studied with or without cytoplasm. Also, various molecules can be microinjected or supplied to the external medium.[5]

The net result was the discovery that pectin stimulates cell wall enlargement.[2,3] Because pectins are produced in the cytoplasm and deposited in the wall, the discovery links the enlargement of the cell to the deposition of new wall material. In effect, pectin accelerates growth while on the way to being deposited in the wall. Once deposited, the wall returns to its thickness before the acceleration, thus preventing thinning. This dual action occurs at a rate apparently depending on how fast the pectin is supplied by the cytoplasm.

Proseus and Boyer[14] developed a theory to explain this expansion and deposition and termed it the pressure-dependent calcium pectate cycle. The cycle resides in the wall and is strictly chemical. It begins when turgor pressure puts wall polymers under tension. Many of the polymers are pectins (in this situation, pectates with reactive carboxyl groups) that are cross-linked by calcium. A few of them bear the load of the wall tension and are distorted by it, weakening the coordination bonding of the cross-links. As new pectin is released to the wall by the cytoplasm, its non-distorted conformation allows it to remove calcium preferentially from these distorted, load-bearing cross-links, loosening the wall and accelerating its expansion. The new pectin now contains the removed calcium and can bind to the wall. With additional calcium plus the new pectin, the wall is maintained at its original thickness and strength. The acceleration, binding and added calcium probably occur as a continuum seen as a link between wall expansion and wall thickness.

These sub-reactions were somewhat unexpected and required the cycle to be given rigorous tests. Among them was the recent one withholding calcium.[1] The prediction is that, without additional calcium, the number of cross-links would not change as the wall is stretched to a larger size, the cross-linking would be spread over larger areas, and the wall would eventually weaken sufficiently to disrupt growth at the normal pressures in the cell. This is in fact what was observed.

Another equally stringent test would be to establish whether expansion and deposition of new wall material are in fact linked. According to the hypothesis, the accelerating action of new pectin should lead to more rapid deposition of new wall material. Conversely because the cycle is pressure-dependent, decreasing the pressure and thus the rate of wall expansion would decrease the rate of wall deposition.

There is evidence that this linkage occurs. In an experiment done before the pectate cycle model was developed, Proseus and Boyer[15] used live Chara cells to which ^{13}C bicarbonate was supplied. The cells are photosynthetic and incorporate the ^{13}C into wall structure that can be isolated and analyzed. The pools of metabolic intermediates took about six hours to reach the steady-state and thereafter sent label to the wall steadily while growth occurred. The rapid wall deposition in young growing cells was absent in mature non-growing ones at normal turgor pressure. Importantly, dropping the turgor pressure 0.1 MPa below normal in the growing cells decreased the expansion rate but also the deposition rate for the wall. Although these experiments were conducted before the pectate cycle had been conceived, this test supports it and illustrates the link between wall expansion and wall deposition. Interestingly, no coordinated signaling is required for this mechanism. The coordination comes automatically as a simple consequence of the chemistry of the pectate with calcium.

In support of this concept, it may be noted that Cleland[16] reported a turgor-dependent link between auxin-induced expansion and the deposition of new wall material in multicellular tissue. Also, Baker and Ray[17] found that cell expansion accelerated by auxin was accompanied by enhanced deposition of new wall matrix materials, pectin included. But the growth activity of pectin was unknown at the time, and it was unclear why these linkages occurred.

Pectates are some of the most evolutionarily conserved wall constituents in green plants and serve to hold cells together, making multicellular tissues possible.[18] Therefore, these findings in Chara may have wide application in terrestrial plants. The chemistry of pectate should be the same regardless of the plant, and there is considerable evidence that pectins might contribute to growth in terrestrial plants.[19-23] But other growth mechanisms also exist,[24-26] and these may serve to expand cells even faster than with pectates alone. Evolutionarily, the terrestrial environment may give competitive advantage to plants with these additional mechanisms during competition for resources such as light. Further understanding of wall chemistry and its evolution could be revealing.

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