Biochemical and Genetic Determinants of Cell Wall Disassembly in Ripening Fruit: A General Model

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Fruit ripening is accompanied by textural changes that are derived, at least in part, from structural changes in the cell wall. These textural changes collectively contribute to fruit softening, to increased pathogen susceptibility, and ultimately to deterioration of fruit tissue. Blocking ethylene production or perception has been shown to maintain fruit firmness and to greatly extend the shelf life of fruit (Klee, 1993; Murray et al., 1993; Picton et al., 1993). However, ethylene control also retards ripening-associated biochemical processes that contribute to the ultimate quality of the fruit. Thus, there is a continuing need to dissect the molecular mechanisms that underlie fruit softening so that these processes can be regulated independently of other aspects of fruit ripening.

Structural models of the plant cell wall remain controversial but can be viewed in the simplest form as a core structure of cellulose microfibrils embedded in two coextensive networks of pectin and hemicellulose (Carpita and Gibeaut, 1993). Recent cell wall models emphasize the degree of non-covalent associations between polymers, such as the extensive hydrogen bonding between cellulose and xyloglucan that occurs in dicot primary cell walls. This cell wall matrix provides structural support to the plant body but also must respond dynamically to developmental processes that require changes in cell shape and, in some cases, cell separation (Rose and Bennett, 1999). Thus, highly regulated disassembly of cell wall components is a critical component of plant development, and one of its most dramatic manifestations is in ripening fruit. In most cases, cellulose microfibrils are not disassembled during normal plant developmental processes, including fruit ripening. However, there is extensive evidence that both of the major matrix polymers, pectins and hemicellulose, are extensively disassembled during fruit ripening (Crookes and Grierson, 1983; Maclachlan and Brady, 1994; McCollum et al., 1989; Sakurai and Nevins, 1993). This paper reviews recent data, which suggests a general model for ripening-associated cell wall disassembly that provides experimental guidance to regulate fruit softening as a strategy to improve fruit quality.

RESOLUTION OF TWO SEQUENTIAL STAGES OF CELL WALL DISASSEMBLY

It has been recognized that fruit softening and the underlying cell wall structural changes must be complex (Fischer and Bennett, 1991). The complex patterns of cell wall disassembly may reflect cooperative biochemical actions that collectively contribute to the overall textural changes that occur in ripening fruit. Alternatively, these complex patterns of cell wall disassembly may reflect relatively distinct events that occur sequentially with each event contributing to separate and distinct aspects of fruit softening. Experiments attempting to resolve temporal events in cell wall disassembly were carried out in ripening ‘Charentais’ melons to assess the possibility that cell wall disassembly could be resolved into distinct sequential events (Rose et al., 1998). These experiments took advantage of the rapid ripening of climacteric ‘Charentais’ melon and the ability to sample fruit at specific points in

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the ripening process based on measurements of ethylene production and texture.

Cell wall fractions were isolated by sequential chemical extraction and size fractionated to assess their degree of polymerization. At the earliest ripening stage, characterized by 25% of total softening, the only cell wall fraction exhibiting any disassembly was a fraction of tightly bound hemicellulose, comprised almost exclusively of xyloglucan (Rose et al., 1998). This indicated that depolymerization of xyloglucan, which is tightly bound to the cellulose surface, was the earliest event in ripening-associated cell wall disassembly. In addition, this change in xyloglucan structure was the only cell wall change that correlated with the early stage of fruit softening. Xyloglucan disassembly has been observed in a large number of ripening fruit (Maclachlan and Brady, 1994; Tong and Gross, 1988), and characterization of ripening in ‘Charentais’ melon suggested that this is one of the earliest events in a sequence of cell wall disassembly.

In contrast to the early stages of fruit softening, late ripening and overripening of ‘Charentais’ melon fruit was characterized by progressive disassembly of the pectin network (Rose et al., 1998). Indeed, during the early stages of fruit ripening, the chelator-soluble pectins actually increased slightly in molecular size followed by a progressive decrease in average molecular mass in the final ripening and overripe stages. Analysis of molecular changes in all the pectic fractions suggested a model of pectin disassembly in ‘Charentais’ melon in which the loss of galactose, presumably from pectin-associated galactan side chains, contributes to the solubilization of covalently linked proteins that interact with the cellulose/hemicellulose association and may contribute to changes in this region of the cell wall, potentially increasing the accessibility of xyloglucan at this interface for enzymatic attack.

β-1,4-Galactanases (EGases or cellulases) provide the simplest enzymic mechanism for xyloglucan cleavage and most likely play this role in a number of physiological contexts, such as cell expansion, abscission, and fruit ripening (Shani et al., 1997). However, the action of EGase alone would lead to an irreversible loss of cell wall integrity and it is likely that the action of EGases is closely coordinated with cell wall enzymes to maintain a dynamic equilibrium between processes of cell wall assembly and disassembly. Interestingly, a membrane-bound EGase has been proposed to play a role in cell wall synthesis (Brummell et al., 1997) and a mutation in this gene in Arabidopsis appears to disrupt cell wall assembly (Nicol et al., 1998). Thus, members of the EGase gene family may be responsible both for establishing cell wall architecture as well as catalyzing its degradation.

The relationship between EGase gene expression and fruit ripening has been extensively characterized, and indeed avoado EGase was the first ripening-regulated gene cloned from a plant (Christofersen et al., 1994). It has nevertheless been difficult to firmly establish a role for ripening-regulated EGases in fruit softening. Recently, antisense suppression of expression of several EGase genes significantly suppressed tomato flower or fruit abscission but had no effect on fruit softening (Brummell et al., 1999a; Laslborsh et al., 1998). This result suggests that, while EGases may play a role in xyloglucan disassembly, single EGases do not regulate early fruit softening.

The second enzymic mechanism for xyloglucan disassembly was suggested by the discovery of a novel enzyme, xyloglucan endotransglycosylase (XET; Fry et al., 1992). XETs catalyze the cleavage and reorientation of xyloglucan molecules. This activity could result in progressive xyloglucan disassembly, particularly when acting in concert with EGase activity to provide low molecular size xyloglucan acceptor molecules. Ripening-regulated XETs have been identified in tomato, kiwifruit, and melon (Arrowsmith and desVille, 1995; Schroder et al., 1998; Rose and Bennett, unpublished) and thus may play a role in xyloglucan disassembly during this developmental transition. However, it is unlikely that XETs regulate the early ripening onset of xyloglucan disassembly because XET activity is present and detectable in fruit prior to the onset of ripening (Catala et al., 2000).

Expansins are a class of proteins that have been proposed to disrupt hydrogen bonds at the cellulose/hemicellulose interface and to allow “cell wall creep” in expanding cells (Cosgrove, 1997). Expansin proteins contain a series of conserved tryptophan residues that exhibit similarity to the spacing of tryptophan in the cellulose binding domain of microbial cellulases and bind tightly to cellulose-enriched cell wall fractions (Cosgrove, 1997). In addition, expansins have been reported to “weaken” paper in the absence of detectable cellulose hydrolysis, suggesting that these proteins act by disrupting hydrogen bonds between cell wall polymers (McQueen-Mason and Cosgrove, 1994). Although expansins have been primarily characterized in relation to cell expansion, the identification of a phylogenetically divergent subclass of expansins expressed specifically in fully expanded and ripening fruit suggests that they may act in a broader physiological context (Civello et al., 1999; Rose et al., 1997).

Expression of a tomato ripening-regulated expansin gene, LeExp1, parallels the pattern of xyloglucan disassembly and early fruit softening. The LeExp1 mRNA accumulates early in fruit ripening, is suppressed in transgenic fruit with low ethylene production, and is suppressed in tomato fruit carrying the rin or nor mutations that prevent normal fruit ripening and softening (Rose et al., 1997). Recently, the role of the ripening-specific Exp1 protein was investigated by suppression and overexpression of Exp1 in transgenic tomato plants. Fruit in which Exp1 protein accumulation was suppressed to 3% that of wild-type levels were firmer than controls throughout ripening, and fruit overexpressing high levels of recombinant Exp1 protein were much softer than controls, even in mature green fruit before ripening commenced (Brummell et al., 1999b). The softening of mature green fruit overexpressing Exp1 was suppressed to a precocious and extensive depolymerization of structural hemicelluloses, whereas polyuronide depolymerization was not altered. In addition, the expression of a functional chimeric LeExp1 gene in rin tomato fruit partially restores fruit softening in this ripening-impaired genetic background (Powell, Gurrieri, and Bennett, unpublished results). Taken together, these results indicate that ripening-regulated expansin protein play a significant role in early fruit softening. The mechanism of expansin action is not clear but it seems likely that expansins act cooperatively with cell wall hydrolases, such as EGases and/or XETs, to bring about a coordinated disassembly of cell wall hemicellulose (Rose and Bennett, 1999). However, the observation that suppression of LeExp1 gene expression inhibits early fruit softening indicates that expansin action is a key component of this process and may represent the primary regulator of early events in cell wall disassembly.

### BIOCHEMICAL AND GENETIC REGULATION OF XYLOGLUCAN DISASSEMBLY

Mechanisms regulating xyloglucan disassembly have remained elusive in spite of the recognition of a number of enzymes capable of cleaving the β-1,4-glucan backbone of the polymer. At least two classes of enzymes can cleave the xyloglucan backbone, endo-β-1,4-glucanase (EGase or cellulase) and xyloglucan endotransglycosylase (XET) (Rose and Bennett, 1999). In addition, expansins are a class of proteins that interact with the cellulose/hemicellulose association and may contribute to changes in this region of the cell wall, potentially increasing the accessibility of xyloglucan at this interface for enzymatic attack.

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