RESEARCH ESSAY

Identifying Proteins Involved in Plant Cell Wall Modification:
How Forward Genetics Can Be Used to Study Biology

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Introduction

Have you ever made jam or watched someone else do it? If you have, then you know that the berries initially form a liquid as they cook, but as the liquid cools, it becomes gelatinous. The substance that turns berry juice into a thick spread is called **pectin**. Pectin is a complex carbohydrate that is an important component of plant cell walls. Plant cells make pectin and secrete it to the wall, where it acts as an adhesive, keeping cells glued together. When plants need to shed organs, such as when trees lose their leaves or floral organs are dropped, or to loosen tissue during fruit ripening ~~(abscission)~~, the pectin between the cells must be dissolved with pectin-degrading enzymes. In addition to its role as an adhesive, pectin forms a hydrophilic (absorbs water) barrier or filter between the plant cell and the external environment. Because of its importance, scientists would like to know more about the cellular proteins that are involved in pectin synthesis and modification. However, progress in this area has been slow, in part because pectin is a large, complex, and heterogeneous molecule that can vary with cell types.

Because all proteins are encoded by genes, one approach to identifying and studying proteins involved in pectin formation and deposition is to use genetics. Mutants with defective pectin will typically have mutations in gene encoding proteins needed for pectin synthesis or modification. Mapping the mutations to a specific position on a chromosome allows scientists to isolate the mutated gene (**positional cloning**) and identify the protein that it encodes. Further, the specific defect observed in the mutant provides information concerning what the protein does in the plant. But how can scientists find pectin mutants in the first place? Although many strategies exist, one way is to exploit the seed coat because in some plant species, like the model organism for plant genetics, *Arabidopsis thaliana*, the seed coat epidermal cells make large amounts of pectin (**mucilage**) that extrudes from the seed when it is exposed to water (Photo A).

KEY CONCEPTS

- Mutants can be used to identify genes that encode the proteins involved in various biological processes.
- A mutant phenotype provides information concerning when, where, and how proteins function in an organism.
- Pectins are important components in plant cell walls.

Research Question
Can seed coat mucilage extrusion be used as a phenotype to identify mutants defective in genes needed for pectin biology?

Mutants unable to extrude mucilage can be easily identified by screening seeds produced by plants from a mutagenized population. When such a screen was carried out by researchers in my laboratory at the University of British Columbia, several

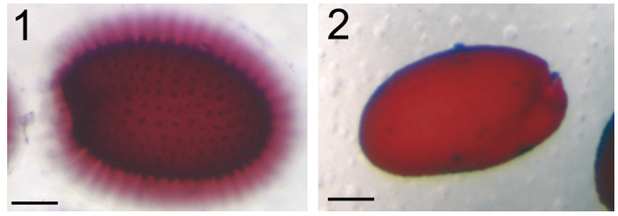

PHOTO A. Normal and mutant arabidopsis seeds following exposure to water.
A1. Ruthenium red, a dye that stains pectin, allows the seed coat mucilage that extrudes when seeds are placed in water to be easily observed. A2. Mutants producing seeds with defects in mucilage extrusion, such as this Mum2 seed, can be identified by using ruthenium red staining. The scale bars in A1 and A2 represent 100 μm.
Source: Photo from Dean et al., 2007.

mucilage-deficient mutants were identified. Failure to extrude mucilage can occur (1) because the mutant does not make enough pectin in the seed coat or (2) because the pectin produced is abnormal and does not swell when exposed to water. One mucilage extrusion mutant, called mucilage modified 2 (Mum2 in Photo A2), has seeds with a normal amount of mucilage, suggesting that the Mum2 mutant has mucilage with a modified structure. The *MUM2* gene was cloned by position and sequenced, and the protein it encodes was deduced. MUM2 protein was found to have similarity to β-galactosidases, enzymes that remove galactose sugar molecules from complex polysaccharides, such as pectin. In addition, the MUM2 protein was predicted to have a signal sequence (a sequence of amino acids that directs the movement of a protein), suggesting that it is secreted from the cell.

Hypothesis

The MUM2 protein is an enzyme that is made and secreted from the seed coat epidermal cells, where it modifies the structure of pectin to allow it to expand on exposure to water.

Methods and Results

Four testable predictions arise from the hypothesis. First, MUM2 protein should be present in the seed coat and, if it is, its mRNA is likely to be detectable in seed coat epidermal cells. To test this prediction, the amount of MUM2 mRNA was measured at different times during seed coat development by using a technique called quantitative reverse transcriptase polymerase chain reaction. It was found that MUM2 mRNA was detectable in the seed coat and reached its highest level at the precise time when mucilage was being produced.

Second, if MUM2 protein is a β,-galactosidase enzyme, it should be able to remove galactose molecules from carbohydrates in vitro (independent of the cell). To isolate purified MUM2 protein, a **recombinant** *MUM2* gene was engineered that could be expressed in yeast and easily purified from a mixture of yeast cellular proteins. A recombinant gene combines parts of different genes from two or more sources. In this case, the new *MUM2* recombinant gene included three parts: (1) transcriptional regulatory sequences from yeast, (2) the protein-coding region from the *MUM2* gene, and (3) an extension to the MUM2 coding region. The translation of the extension to the coding regions results in a small number of amino acids (**epitope**) that can be recognized by a commercially available antibody. The antibody is used to recognize and purify the recombinant MUM2 protein from all other proteins in yeast. This recombinant *MUM2* gene was transformed into yeast. Large numbers of yeast cells were grown, the

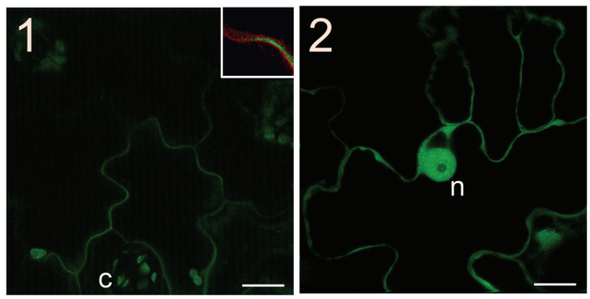

PHOTO B. The MUM4-GFP fusion protein is secreted from the cell, while GFP alone is not.
B1. GFP fluorescence is associated with only the cell boundaries of tobacco mesophyll cells containing the *MUM2-GFP* fusion gene, although some autoflurescence is observed in chloroplasts (c). The fluorescence is located outside the cell membrane, stained red with a dye (see the inset photo). B2. In cells containing the *GFP* gene alone, GFP fluorescence is found throughout the cytoplasm, including the region surrounding the nucleus (n). The scale bars in both B1 and B2 represent 30 μm.
Source: Photo from Dean et al., 2007.

cells were broken open, and the MUM2 protein purified. When MUM2 protein was mixed with a carbohydrate containing galactose, MUM2 was able to remove the galactose, just like other known β-galactosidases.

Third, if MUM2 is required for the removal of galactose from the pectin of mucilage, then the mucilage extracted from Mum2 mutant seeds (those that lack functional MUM2 protein) should have more galactose than the mucilage of normal (wild-type) seeds. Mucilage from both wild-type and normal seeds was extracted and degraded into single sugar molecules, and the amount of each sugar was determined by high-performance liquid chromatography. As predicted, Mum2 mutant mucilage contained a higher percentage of galactose than the mucilage of normal seeds.

Fourth, if the MUM2 protein is actually secreted from the cell, it should be detectable outside the cell membrane. To test this prediction, a recombinant gene was made by fusing the *MUM2* coding region with that of a green fluorescent protein (GFP). The GFP is a small protein, derived from jellyfish, that fluoresces when exposed to blue light. The recombinant *MUM2-GFP* gene, when translated, produces a fusion protein. Its location in the cell can be followed by using a fluorescence microscope. When the *MUM2-GFP* gene was introduced into tobacco, the MUM2-GFP protein was found to be located outside the cell in the cell wall, whereas GFP alone was found in the cytoplasm (Photo B). These data confirmed that the MUM2 protein contains signals that result in its secretion from the cell.

Conclusions

The evidence supports the major predictions of the hypothesis. The *MUM2* gene is transcribed in the seed coat and encodes a β-galactosidase enzyme. The enzyme is secreted outside the cell, where it removes galactose molecules from mucilage, thus altering the mucilage's properties and allowing it to be extruded from the cell.

Future Directions

An important question remains concerning the function of MUM2: How does the removal of the galactose molecules change the mucilage pectin properties to allow it to be extruded from the seed coat? To answer it, the precise structure of mucilage pectin in both normal and mutant seeds needs to be determined. The question is an important one because it is likely that extrusion is dependent on the biophysical properties of pectin or

its interactions with other types of carbohydrates. Knowing the answer may allow the horticultural industry to manipulate pectin's adhesive properties and prevent abscission of floral organs and it may allow the food industry to manipulate the softening of ripening fruit and to create new types of pectin for use as gelling agents.

Critical Thinking Questions

1. What kind of a mutant phenotype would you look for if you wanted to find a plant with a mutation in a gene encoding an enzyme that dissolves pectin during abscission?
2. When making MUM2 protein, why was the plant *MUM2* gene introduced into yeast and not into a plant?
3. Why was it necessary to test in vitro that the MUM2 protein functions as a β-galactosidase when its predicted protein sequence already indicated that MUM4 was very similar in sequence to such enzymes?

Further Research Question

Pectin is an important component of all primary plant cell walls. Where in the cell is it made and how does it get outside the cell?

References

Dean, GH, Zheng H, Tewari J, Huang J, Young DS, Hwang YT, Western TL, Carpita NC, McCann, MC Mansfield SD, et al. 2007. The arabidopsis *MUM2* gene encodes a β-galactosidase required for the production of seed coat mucilage with correct hydration properties. Plant Cell. 19:4007–4021.

Haughn G, Chaudhury A. 2005. Genetic analysis of seed coat development in arabidopsis. Trends Plant Sci. 10:472–477.

Sila DN, Van Buggenhout S, Duvetter T, Fraeye I, De Roeck, Van Loey A, Hendricks M. 2009. Pectins in processed fruits and vegetables: part II—structure–function relationships. Compre. Rev. Food Sci. Food Saf. 8:86–104.