The molecular basis for the initiation of fruit development and parthenocarpy

by

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Abstract

Parthenocarpy, or seedless fruit development, has an agronomic importance in many horticultural crops. In most fruit, fertilization or seed set usually determines whether fruit growth is sustained. Naturally occurring parthenocarpy results from a genetic lesion that permits fruit to develop in the absence of fertilization and seed development. Parthenocarpy can also be induced artificially with cytokinin, gibberellin or auxin plant growth regulators applied to anthesis pistils. This thesis describes genetic research using Arabidopsis as a model plant to identify integral mechanisms that control parthenocarpy and the initiation of fruit development.

The growth and structure of the Arabidopsis pistil was determined post-fertilization. Experiments were designed to understand how plant growth regulators induce Arabidopsis silique (fruit) development in emasculated anthesis stage pistils. Exogenous gibberellin (GA₃) induced growth and cellular differentiation most comparable to pollinated pistils. Dependencies on gibberellins during silique development were examined in mutants defective for gibberellin biosynthesis (ga1, ga4-1, ga5-1) or perception (spy-4, gai-1). Although exogenous GAs are effective at inducing parthenocarpy, mutant studies concluded that GAs are not the sole cue for fruit development in Arabidopsis. Mutants blocked in GA perception could develop siliques in response to pollination, auxin, cytokinin but not to exogenously applied gibberellins. Silique structure in pollinated gai-1 and ga5-1 provided strong evidence for a model supporting evidence of an auxin-like signal regulating structural development and that GAs limit anticlinal cellular division. A specialized function for GAI and related GRAS family members in controlling cellular division during fruit development was uncovered.
A mutant that forms parthenocarpic siliques without fertilization (*fwf*), was also characterized. The presence of surrounding floral whorls reduced the extent of parthenocarpic silique formation in *fwf*. Silique growth in the *fwf* background was examined when hormone perception, ovule and carpel identity functions were removed genetically. This established that *FWF* functions independent of *GAI*-mediated GA perception. Carpel identity conferred by *FUL* was critical for parthenocarpic silique elongation and ovule development beyond integument initiation, nucellar specification and subsequent morphogenesis, was essential for parthenocarpic silique development in *fwf*. Silique elongation occurs over a four-day period post-pollination or post-anthesis. This coincides with a similar time period in which *fwf* ovules remained receptive to fertilization. These observations are congruent with the hypothesis that *FWF* potentially represses a signal transduction process initiated within the ovule that mediates subsequent transition from carpel to silique development. Further analysis revealed that *aberrant testa shape* (*ats*) a mutant defective in integument formation enhanced parthenocarpic development in *fwf*, indicating that an ovule located repressor other than *fwf* can function to affect silique formation.

Other studies have shown that ethylene can modulate auxin-dependent growth in both aerial and root tissues by altering both polar and lateral auxin transport. The contribution of ethylene perception to signal transduction between ovule and carpel was also genetically assessed. Constitutive ethylene responses, conferred by *ctr1-1*, enhanced cellular expansion in *fwf* and also the autonomous silique development in *fis-2*, which develops autonomous endosperm. *ats ctr1-1* and *ino ctr1-1* double mutants were also found to be parthenocarpic. This indicates that ethylene perception and integumentary structure play an important role in autonomous silique development, conceivably by
changing the polar and lateral movement of an auxin-like signal within the integumentary tissues of the ovule.

fwf and ats were fine mapped on chromosome 5 of Arabidopsis. Candidate genes were identified corresponding to both mutations but only the identity of FWF was established. Auxin Response Factor 8 (ARF8) was cloned and sequenced from the fwf mutant background. The gene encodes a protein with an amino-terminal DNA binding domain and a carboxy-terminal protein binding domain which homo- and hetero-dimerizes with other ARF or Aux / IAA class proteins. ARF8 sequence from fwf mutants encoded a mutation in the translation start site. Complementation of fwf plants by the transformation of wild type copies of ARF8 into fwf plants was hampered by reduced transformation efficiency. However wild type L.er and No.O plants transformed with mutant copies of ARF8 were obtained in higher frequency, and these formed parthenocarpic siliques when primary transformants were emasculated. This indicated that an interfering protein is produced from the mutated ARF8 gene that has altered regulatory activity. Sequence analysis indicated this and found that interference resulted from functional activity of the Q-rich and carboxy-terminal domains of the ARF8 protein. This inference is consistent with other published molecular data, which has demonstrated that the carboxy-terminal domain, together with the Q-rich region of selected ARF members, can activate auxin-responsive. Thus the FWF / ARF8 protein may have a dual role, repressing carpel growth development through the DNA binding domain and then ensuring activation of silique development through the carboxy-terminal domain.

The combined molecular and genetic data has been used to construct models concerning the genetic control of silique development. The first model considers the role of plant hormones and how signals from floral whorls surrounding the carpel and from within the ovule control silique growth. A model is also presented for the control of adaxial
growth and development of the outer integument by the *INNER NO OUTER* gene. Finally the role of *FWF* and *SPY* in controlling floral tissue identity and boundary tissue specification is considered in a third model. Modification of the *FWF / ARF8* gene could be used as a tool to improve fruit set and retention in horticultural crops, in addition to creating seedless parthenocarpic fruit.
Declaration

I declare that this work contains no material, which has been accepted for the award of any other degree or diploma in any University or any other tertiary institution. To the best of my knowledge and belief this thesis is original and contains no material previously written or published by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University library, being available for loan and for photocopying. A twelve-month embargo effective from the 1st day of November, 2000 was placed on this thesis.

Adam Vivian-Smith

November, 2000
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I thank Dr Kay Schneitz for providing *ant* and *ino* alleles, Dr Steve Swain for *spy-4*, Jason Walker and Prof John Larkins for molecular markers, and the *Arabidopsis* Biological Resource Center for seed. Dr Lyn Waterhouse at CEMMSA provided help with SEM microscopy facilities.
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## Abbreviations

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<th>Definition</th>
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<tr>
<td>A</td>
<td>adenine</td>
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<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphism</td>
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<td>ARF</td>
<td>auxin response factor</td>
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<tr>
<td>BA</td>
<td>benzyl adenine</td>
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<tr>
<td>bp, kbp, mbp</td>
<td>nucleotide base pairs, kilobase pairs, megabase pairs</td>
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<td>CAPS</td>
<td>cleaved amplified polymorphic sequence</td>
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<tr>
<td>C</td>
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<td>cm, mm, µm</td>
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<td><em>Arabidopsis</em> Columbia ecotype</td>
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<td>DPA</td>
<td>days post-anthesis</td>
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<td>g, mg, µg</td>
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<td>IAA</td>
<td>indole acetic acid</td>
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<td>L.eR, L.ER</td>
<td><em>Arabidopsis</em> Landsberg <em>erecta</em>, <em>Arabidopsis</em> Landsberg ERECTA ecotypes</td>
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<tr>
<td>L, ml, µl</td>
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<td>PGR</td>
<td>plant growth regulator</td>
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