Female Gametophyte Development and Auxin Regulation in *Aquilegia*

Rebecca Povilus* and William Friedman

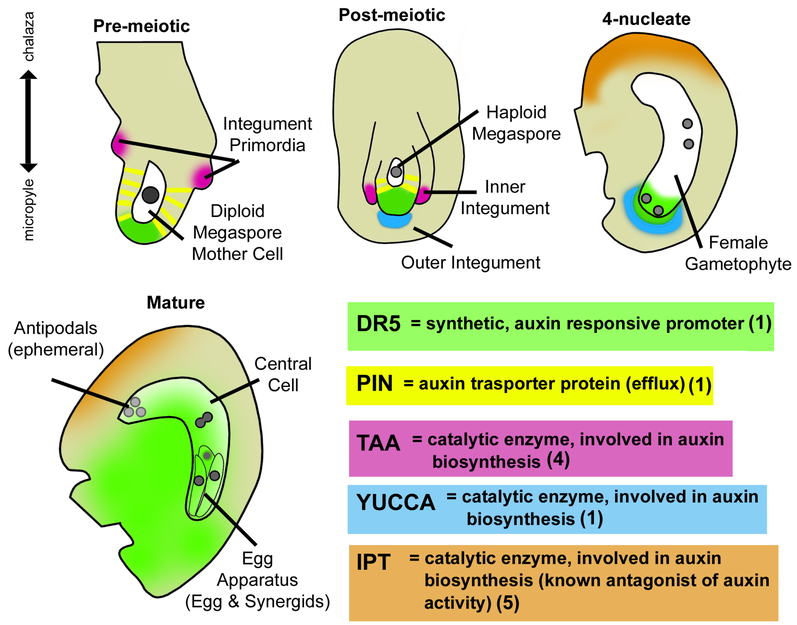
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138. *Corresponding author: rpovilus@fas.harvard.edu

Introduction

Buried deeply within each angiosperm ovule is a haploid, egg-producing structure known as the female gametophyte. This structure consists of a specific arrangement of cell types along a longitudinal micropylar-chalazal axis, and it has been shown that auxin is essential to setting up this patterning. In *Arabidopsis thaliana*, local auxin synthesis at the micropylar end of a developing ovule results in establishment of the egg apparatus, whereas low auxin concentration at the chalazal end correlates to the formation of sterile cells (1). **Whether our understanding of the molecular framework underlying this developmental process in a single, highly derived model organism can be extended to phylogenetically distant taxa with similar features, let alone be used to explain production of morphological diversity, remains unknown.** Species in *Aquilegia* share the same type of female gametophyte as *Arabidopsis thaliana* (2) and, as part of the early diverging eudicot family Ranunculaceae, provide an excellent opportunity to address this question in an evolutionarily meaningful context.

In order to obtain a complete ontogeny of female gametophyte development in Aquilegia origami and to infer the dynamics of local auxin regulation during carpel and ovule development in Aquilegia, 1) I adapted whole mount confocal microscopy methods to study ovule and female gametophyte development in *Aquilegia origami* and, 2) I identified from within the genome of *Aquilegia coerulea* (www.phytozome.net) putative members of gene families known from *Arabidopsis thaliana* to be involved in auxin biosynthesis, transport, and antagonism. I distinguish which copies are specific to early carpel and ovule development, and thus are good candidates for future analysis via mRNA in situ hybridization.

Auxin Regulation in *Arabidopsis* Ovules



DR5 = synthetic, auxin responsive promoter (1)

PIN = auxin trasporter protein (efflux) (1)

TAA = catalytic enzyme, involved in auxin biosynthesis (4)

YUCCA = catalytic enzyme, involved in auxin biosynthesis (1)

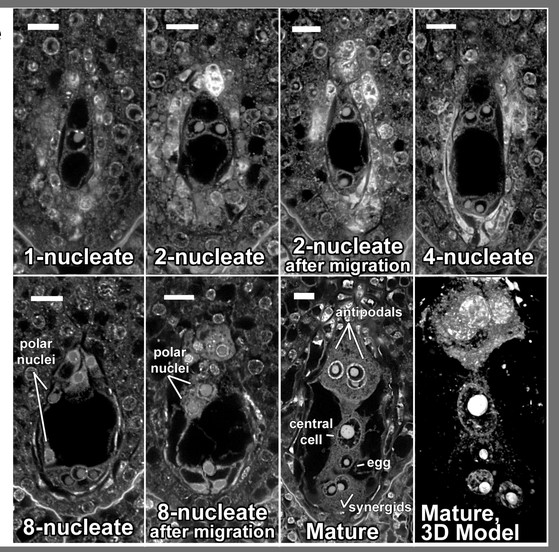
IPT = catalytic enzyme, involved in auxin biosynthesis (known antagonist of auxin activity) (5)

Information from *A. thaliana* suggests that auxin biosynthesis, transport, and antagonism all play a role in establishment and maintenance of high auxin concentrations exclusively at the micropylar ends of developing ovules and female gametophytes. This pattern is essential for proper development of the egg apparatus, and disappears by the time the female gametophyte is mature. (1, 3)

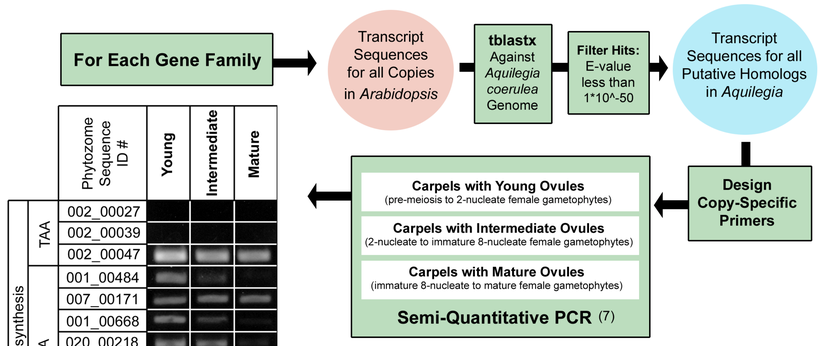
Female Gametophyte Development in *Aquilegia*

Whole mount, confocal microscopy of Feulgen-stained, cleared *Aquilegia* ovules (procedure modified from (6)) confirms the Polygonum-type development of *Aquilegia* female gametophytes and allows for 3D-model construction. Streamlined processing means that samples can be imaged within 48 hours of collection.

Micropyle is towards the bottom of all pictures. Scale bars are 10 µm. ("2- nucleate" through "Mature") are composite images of several optical sections.



Auxin Regulation Genes in *Aquilegia* Carpels



Potential homologs in *Aquilegia* were identified for all gene families analyzed. At least one copy within each family showed expression during carpel development (further analysis is required to determine if those copies are expressed specifically in ovules and female gametophytes). Most auxin biosynthesis and transport homologs showed stronger expression in carpels with young female gametophytes, while auxin antagonist copies showed stronger expression in carpels with either young or mature female gametophytes. This is similar to the expected expression patterns assuming that auxin patterning is similar to the situation in *Arabidopsis*.

References and Acknowledgements

(1) Pagnussat, G., M. Alandete-Saez, et al. (2009). "Auxin-Dependent Patterning and Gamete Specification in the *Arabidopsis* Female Gametophyte." Science 324: 1684-1689.

(2) Mottier, D. (1895). "Contributions to the Embryology of the Ranunculaceae." Botanical Gazette 20(6): 241-248.

(3) Bencivenga, S., L. Colombo, et al. (2011). "Cross talk between the sporophyte and the megagametophytes during ovule development." Sex Plant Reprod.

(4) Nole-Wilson, S., S. Ashakanandam, et al. (2010). "Polar auxin transport together with AINTEGUMENTA and REVOLUTA coordinate early *Arabidopsis* gynoecium development." Developmental Biology 346: 181-195.

(5) Miyawaki, K., M. Matsumoto-Kitano, et al. (2004). "Expression of cytokinin biosynthetic isopentenyltransferase genes in *Arabidopsis*: tissue specificity and regulation by auxin, cytokinin, and nitrate." The Plant Journal 37: 128-138.

(6) Barrell, P. and U. Grossniklaus (2005). "Confocal microscopy of whole ovules for analysis of reproductive development: the *elongate1* mutant affects meiosis II." The Plant Journal 43: 309-320.

(7) Kramer, E., L. Holappa, et al. (2007). "Elaboration of B function to include the identity of novel floral organs in the lower eudicot *Aquilegia*." The Plant Cell 19: 750-766.

(8) Křeček, P., P. Skůpa, et al. (2009). "The PIN-FORMED (PIN) protein family of auxin transporters." Genome Biology 10(249): 1-11.

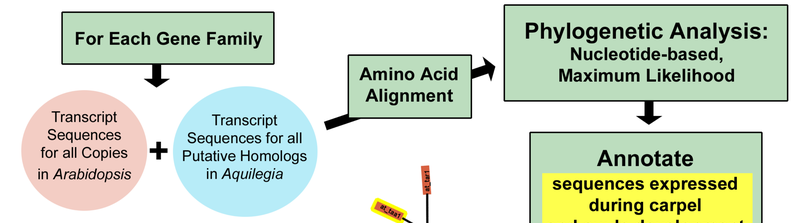
(9) Yamamoto, Y., N. Kamiya, et al. (2007). "Auxin Biosynthesis by the YUCCA genes in Rice." Plant Physiology 143: 1362-1371.

(10) Garcia-Aguilar, M., A. Dorantes-Acosta, et al. (2005). "Whole-Mount In Situ mRNA Localization in Developing Ovules and Seeds of *Arabidopsis*." Plant Molecular Biology Reporter 23: 279-289.

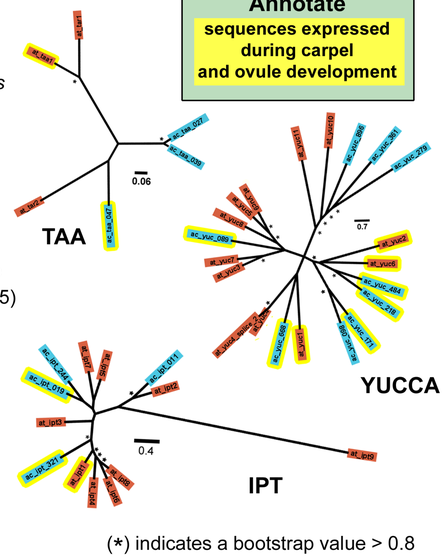
(11) Friedman, W. (2009). "Auxin at the Evo-Devo Intersection." Science 324: 1652-1653.

Special thanks to Elena Kramer and members of the Kramer lab for plant material, and to Faye Rosin for technical advice.

Comparison of Auxin Regulation Genes in *Aquilegia* and *Arabidopsis*



For all gene famlies, the *Arabidopsis* and *Aquilegia* copies expressed during ovule/carpel development were not necessarily closely related.This means that coding sequence similarity is not sufficient to assign functional similiarty. However, relationships of *Arabidopsis* sequences agreed with published gene trees for PIN (8), YUCCA (9), and IPT (5) at high confidence nodes (*).



(*) indicates a bootstrap value > 0.8

Future Directions

Detailed Expression Analysis with in situ hybridization: For each of the *Aquilegia* auxin-regulation genes that show expression during carpel development, I will further investigate the spatial/temporal expression patterns with in situ hybridization. This technique has already been developed for analysis of MADS-box gene homologs in floral bud tissue of *Aquilegia* (7). In addition, whole-mount in situ hybridization protocols have been developed for *Arabidopsis* ovules (10). I am adapting these techniques for use with whole-mount *Aquilegia* ovules, using confocal microscopy if possible.

The Role of Auxin During the Development of Other Types of Female Gametophytes: While *Aquilegia* and *Arabidopsis* share the same type of female gametophyte, other types exist among angiosperms. Across this diversity, the presence and micropylar position of the egg apparatus is conserved in all functional female gametophytes. The development of the egg apparatus has been shown to be controlled by high local auxin concentrations in *Arabidopsis thaliana* (1). By characterizing the expression of auxin-regulation genes throughout ovule development in a phylogenetically broad and morphologically diverse sample of taxa, I will be able to determine how conserved the role of auxin is during female gametophyte development in angoisperms. (11)