Pablo D. Jenik

Pattern formation and morphogenesis during plant embryogenesis

Past Research Progress

I am working on some of the most fundamental questions in developmental biology, the processes that pattern and shape embryos. My research has focused on a suite of mutants whose analysis will identify new genes and pathways for regulating embryogenesis in plants. Furthermore, one of these mutants has opened up exciting avenues to explore the connections between the cell cycle, patterning and morphogenesis.

In plants patterning occurs during the early stages of embryogenesis, and it establishes the basic body plan and the meristems (shoot and root) that will be responsible for all post-embryonic development. The end product of patterning is an embryo with particular tissue types organized along apical-basal (top-bottom) and radial (inside-outside) axes (Fig.1A). This involves the coordination of cell division rates and planes with the spatial acquisition of specific cell fates. After all the elements of pattern are set up, the embryonic organs grow to their final sizes and shapes (morphogenesis) to form a mature embryo.

We are just starting to learn how patterning and morphogenesis are controlled in plant embryogenesis (Laux et al., 2004). Much more research is needed to understand the coordination between cell division, patterning and morphogenesis. The local and long range signals that pattern the embryo, and how most cell identities are specified and maintained throughout development are also not well understood. As a post-doctoral researcher I have been analyzing several very interesting mutants of *Arabidopsis thaliana* that are involved in important developmental processes during embryogenesis. My recent studies have focused on a viable allele of the gene *tilted1* (*til1*) (Jenik et al., in press). I have shown that the mutation responsible for the *til1* phenotype is a missense mutation in the catalytic subunit of DNA polymerase epsilon (DNA pol ε). DNA pol ε has a role in chromosomal DNA replication during the S-phase, as well as in associated processes including DNA repair and subtelomeric silencing (Pospiech and Syväoja, 2003). To my knowledge, this is the only existing viable mutation in a replicative DNA polymerase (δ or ε) from a multicellular organism. The primary defect of *til1* mutants is longer cell cycles. The longer cell cycles in turn cause abnormal cell divisions mostly in the top-most cell of the suspensor (hypophysis). This leads to a displacement of the root pole end of the shoot-root (or apical-basal) axis from its normal position on top of the suspensor (Fig. 1B).

One important conclusion from my studies is that embryonic development is surprisingly robust and can tolerate, to a certain degree, alterations in cell cycle length. My results also suggest that the patterning of some tissues is more sensitive than others to these changes. In the case of the hypophysis, it is possible that this cell is competent to respond to patterning signals from the rest of embryo only at a certain point in the cell cycle. Changes in cell cycle length may affect this signaling process, leading to abnormal cell divisions.

Another intriguing aspect of the development of *til1* embryos is that, unlike wild type embryos, they pause for several hours at the late globular stage, before moving on to the heart stage. One hypothesis is that *til1* uncovers a previously undescribed embryonic "checkpoint", where the embryos assess their developmental status before they are allowed to continue to the next stage.

As a faculty member I plan to continue working on this suite of mutants and the pathways they affect. In addition I will continue my studies on the interactions between the cell cycle and embryonic development, and on the conservation of all these mechanisms across the plant kingdom.

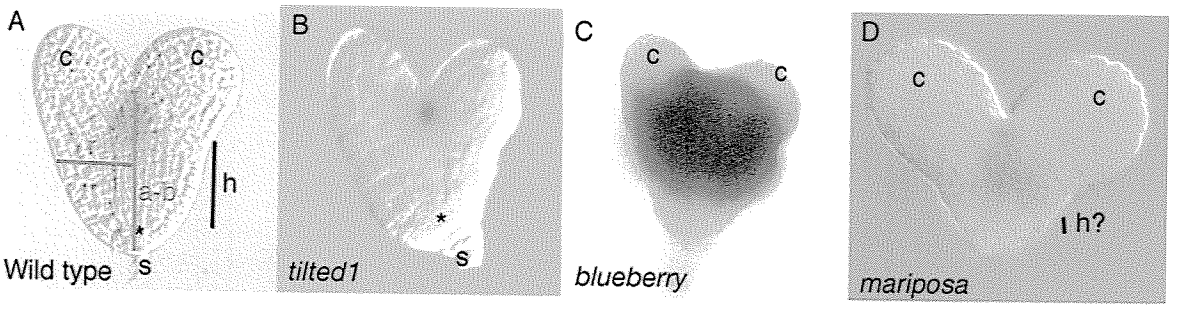

Figure 1. *Arabidopsis* embryos at the heart stage. The blue color indicates the expression of the *STM-GUS* apical marker. (a-b) apical-basal axis, (r) radial axis, (c) cotyledons, (h) hypocotyl, (s) suspensor. (A) Wild type: notice the center of the root pole (asterisk) right above the suspensor. (B) *tilted1*: the center of the root pole (asterisk) is displaced to the side with respect to the suspensor. (C) *blueberry*: *STM-GUS* is expressed throughout the apical and central portions of the embryo. (D) *mariposa*: the hypocotyl (h) has not developed.

Future Research Plans

I am interested in understanding the patterning and morphogenesis of the plant embryo, the processes and pathways involved in growing from a single cell to a mature embryo, with a specific arrangement of tissue types along two axes. *Arabidopsis thaliana* is an excellent model system to address these issues. Embryogenesis in *Arabidopsis* is characterized by extremely regular cell divisions and patterning decisions, which make it easy to find mutants that affect these events. Combined with this is the demonstrated power of forward and reverse genetics approaches in this plant, a fully sequenced genome, the availability of full-genome microarray chips and a broad and supportive research community. The four specific questions I would like to answer are:

- **Which are the genetic pathways involved in *Arabidopsis* embryo patterning and morphogenesis?**
- **What are the connections between cell cycle regulation and patterning and morphogenesis during embryogenesis?**
- **How are cell number, cell size and organ size regulated?**
- **Are the genetic pathways that regulate embryogenesis conserved across the plant kingdom?**

(1) Which are the genetic pathways involved in *Arabidopsis* embryo patterning and morphogenesis?

In the past ten years we have begun to learn about the mechanisms involved in embryo patterning and morphogenesis. Most of what we know comes from genetic screens for embryo- or seedling-defects and, more recently, from reverse genetic approaches. Every new phenotype described and gene identified has broadened our view of the pathways involved in embryogenesis. These pathways now include auxin flow and response, MAP kinase signaling, protein trafficking, tissue specific transcription factors and microRNAs (Laux et al., 2004;

Tzafrir et al., 2004). However, in many cases defective embryos are very abnormal and therefore the phenotypes are hard to interpret. In addition, many of the embyo lethal phenotypes are the result of mutations in housekeeping genes (Tzafrir et al., 2004). One way to pre-select for mutants that may affect patterning and morphogenesis is to screen for embryo defects that also affect the pattern of expression of a tissue specific marker. Dr Kathy Barton followed this approach by conducting a screen looking for the misexpression of the shoot meristem marker *STM-GUS* (Fig. 1A). I found ten of the mutants identified in this screen particularly interesting. In two of the mutants early embryonic patterning is likely affected, since in both cases the domain of expression of *STM-GUS* in the mutant embryos is greatly expanded (Fig. 1C). In at least one of these mutants (*blueberry*) the root meristem/endodermis marker *SCR-GFP* is ectopically expressed throughout the embryo. Other mutants affect the morphogenesis of particular organs: I have identified three independent loci that are involved in cell division and expansion of the hypocotyl (the central domain of the embryo) (e.g. *mariposa*, Fig. 1D). I have also isolated two loci required for the development of the cotyledons (embryonic leaves). I am also studying a mutant where pigments that are normally produced in late embryogenesis appear very early, and this phenomenon is correlated with an apparent lack of tissue differentiation. By determining which genes are responsible for the mutant phenotypes I will be able to identify new pathways implicated in embryo development. First I plan to more thoroughly characterize the mutant phenotypes, by analyzing their morphology and the expression pattern of a variety of stage and region specific markers. The nature of the protein encoded by the mutant loci will then suggest the type of experiments required to explore its function during development. Generation of weaker, viable alleles will provide suitable material for modifier screens in order to identify more components of these genetic pathways.

(2) What are the connections between cell cycle regulation and patterning and morphogenesis during embryogenesis?

The unique weak allele of *till*, which slows down the cell cycle and uncovers an embryonic "checkpoint", offers a good starting point to examine the connections between cell cycle regulation, patterning, and morphogenesis. First, expressing *TIL1* in different regions of a *till* embryo using tissue and/or stage specific promoters can reveal local or long ranging coordination of cell divisions and morphogenesis. These experiments will also allow me to study the cell autonomy of the root pole defects observed in *till* embryos. Second, *till* homozygous plants appear wild type, although they show reduced fertility due to abnormal ovule development. Taking advantage of this latter phenotype, I am screening for suppressors (increased fertility), to identify mutations that compensate for the cell cycle defect of *till*, resulting in normal ovules. The effects of these mutations will then be evaluated during embryo development, using strategies outlined above. So far I have isolated ten suppressors, which I am in the process of confirming. Finally, I am also particularly interested in probing the existence of the putative embryonic "checkpoint" by testing whether other previously described embryo mutants exhibit this pause at the late globular stage.

(3) How are cell number, cell size and organ size regulated?

Little is known about how cell division and expansion are coordinated. When organs like leaves have too many or too few cells there is a compensation mechanism that leads to an increase or reduction in cell size to maintain the normal organ size (Jakoby and Schnittger, 2004). In *till* embryos and leaves the cells divide more slowly, and this is accompanied by an increase in cell size. I am currently undertaking a microarray analysis of the differences in gene expression between wild type and *till* seedlings. I will then analyze mutants in the differentially expressed

genes to identify some of the factors involved in coordinating cell division and cell expansion with organ size.

(4) Are the genetic pathways that regulate embryogenesis conserved across the plant kingdom?

In the long term I would like to investigate whether the mechanisms that regulate embryogenesis in *Arabidopsis* are conserved in other dicots in which the pattern of cell divisions is not regular. I also plan on studying these processes in monocots, which have embryos shaped unlike those of dicots. For the latter I will use rice and maize, well-established model systems with a variety of known embryonic lethal mutants and sequenced or nearly completed genomes. Using these tools, I will be able to identify orthologs of *Arabidopsis* genes and determine whether their functions are conserved among the flowering plant taxa.

I believe these studies will provide novel insights into plant embryogenesis and they will serve as the foundations of a robust and long lasting research program.

References:

Jakoby, M. and A. Schnittger (2004). Cell cycle and differentiation. *Current Opinion in Plant Biology* **7**: 661-669.

Jenik, P., R.E.J. Jurkuta and M.K. Barton. Interactions between the cell cycle and embryonic patterning in *Arabidopsis* uncovered by a mutation in DNA polymerase ε. *Plant Cell* (in press).

Laux, T., T. Würschum and H. Breuninger (2004). Genetic regulation of embryonic pattern formation. *Plant Cell* **16**: S190-S202.

Pospiech, H. and J. Syväoja (2003). DNA polymerase ε – More than a polymerase. *TheScientificWorldJournal* **3**; 87-104.

Tzafrir, I. et al. (2004). Identification of genes required for embryo development in *Arabidopsis*. *Plant Physiology* **135**: 1206-1220.

Pablo D. Jenik

Teaching philosophy and interests

I love to teach, and I relish the idea of being able to share with my students the excitement of science, and to help them become independent, critically thinking people.

I have decided to become a professor in an institution of higher learning because I believe that it is crucial to teach students to become independent critical thinkers. Being a creative and thoughtful individual is essential to be a productive and involved member of society. Science is one of the most useful vehicles to teach critical thinking. The advancement of scientific knowledge is based on the careful and objective consideration of hypotheses in the light of data and the generation of new interpretations and hypotheses that may differ from the original ones. The teaching of science also challenges and sharpens the student's problem solving abilities. All these skills can be applied to all other areas of life.

Scientific literacy is crucial to make sense of our technological world. It also helps inform our decisions, as citizens and consumers. It is important to show students, particularly those who are not science majors, that science is not just not scary, boring or incomprehensible, but that science is fascinating. I am a researcher because some of my professors inspired me by conveying the feeling that doing science is extremely exciting. This enthusiasm is what I want to transmit to my students.

I would like to teach both students with specific interests in plant and developmental biology and non-science majors. Courses at different levels would have different goals, since they would be directed to different audiences. Introductory classes present the general background of a particular area. They are a venue to introduce the bases and rationale of the scientific method, to present how interesting science is, and to give a glimpse on how it is done. Intermediate level classes address a more circumscribed area of knowledge and delve more in the details. They are also a good place to introduce the students to the primary scientific literature. Both of these levels are best complemented by a lab, where students can experience first hand doing science and applying the concepts they have learned in the lecture part of the class. Advanced seminars are directed at small groups of students and involve critically reading and analyzing the primary literature, debating the author's hypotheses and data, and allowing the students to draw their own conclusions. In all cases the classes should involve tasks to improve the student's communication and writing skills, through reports and oral presentations.

I have had fairly broad teaching experience. Both as an advanced undergraduate and as a Ph.D. student I led laboratory, problems and discussion sections. All the classes were at the introductory level, in topics ranging from molecular and cellular biology, to developmental biology, to human physiology and histology. I am interested in teaching introductory or intermediate courses in topics of molecular and cellular biology, genetics, developmental biology and plant biology. My current area of specialization is plant developmental biology. That would be a good topic for an advanced seminar.