Primer and Interviews: Gene Regulation in *Arabidopsis thaliana*

Julie C. Kiefer*

The animal and plant kingdoms use many of the same molecular tools to build decidedly different multicellular organisms. Learning how plants approach challenges common to both kingdoms can inspire new ways of thinking in the animal biologist. This primer introduces how a weed from the mustard family, *Arabidopsis thaliana*, has been used to work through developmental problems. It also compares and contrasts gene regulation tools in animals and plants. Accompanying the primer is a discussion of current topics in root development with *Arabidopsis* researchers Philip N. Benfey, Ph.D., and Kenneth D. Birnbaum, Ph.D. *Developmental Dynamics 238:2449–2458, 2009.* © 2009 Wiley-Liss, Inc.

Key words: *Arabidopsis thaliana*; plant models; root development; genome

Accepted 17 June 2009

THE JOURNEY FROM WALLFLOWER TO SUPERSTAR

Arabidopsis thaliana, common name mouse-ear cress, is the genomic model system of choice for plant researchers (Figs. 1, 2; Table 1). The spindly weed from the mustard family was first proposed as a model system in the 1940s based on biological attributes that make it genetically tractable and an ideal lab cultivar. At approximately 20 cm in height it is small in stature, it has a rapid, 6-week life cycle, it self-fertilizes, produces thousands of progeny, and has only five chromosomes. Another useful feature is that seedlings and roots are translucent, facilitating microscopy analysis. In subsequent decades, the plant proved its utility as studies of *Arabidopsis* mutants shed light on physiological, biochemical, and developmental processes.

Two breakthrough advances in the 1980s positioned *Arabidopsis* as the model that would launch plant biology into the emerging genomic age. The first transformation protocols were published, as was critical information about the plant's genome. The genome is small (125 Mbp) compared with crop species like maize (2,300 Mbp), and other prominent models of the time, like tobacco (4,500 Mbp). Just as important, *Arabidopsis* molecular pathways are conserved, making it a good representative for the 250,000 species of flowering plants. These discoveries led to the creation of the *Arabidopsis* Genome Initiative, bringing together international resources with the common goal of sequencing the genome.

In 2000, *Arabidopsis* joined the ranks of model organism powerhouses *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans*, to become the fourth eukaryote with its genome completely sequenced. Research on the unassuming weed has since blossomed. New genomic tools, including structural and functional genome annotation and genome-wide insertional mutagenesis, make accessible nearly any biological mechanism of interest, including numerous developmental pathways (Table 2). Following in the footsteps of *Arabidopsis*, the genomes of many other plant species have been or are in the process of being sequenced. The plant models in Figure 1 and Table 1 each have specific attributes that are of particular interest to developmental biologists.

Arabidopsis will again play a leading role in bringing molecular research to the next age. The Multinational Coordinated *Arabidopsis* Functional Genomics Project was formed in 2000 with

Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, Utah
***Correspondence to: Julie C. Kiefer, Department of Neurobiology and Anatomy, 20 North 1900 East, 401 MREB, University of Utah, Salt Lake City, UT 84132. E-mail: jkiefer@neuro.utah.edu**

DOI 10.1002/dvdy.22042
Published online 3 August 2009 in Wiley InterScience (www.interscience.wiley.com).

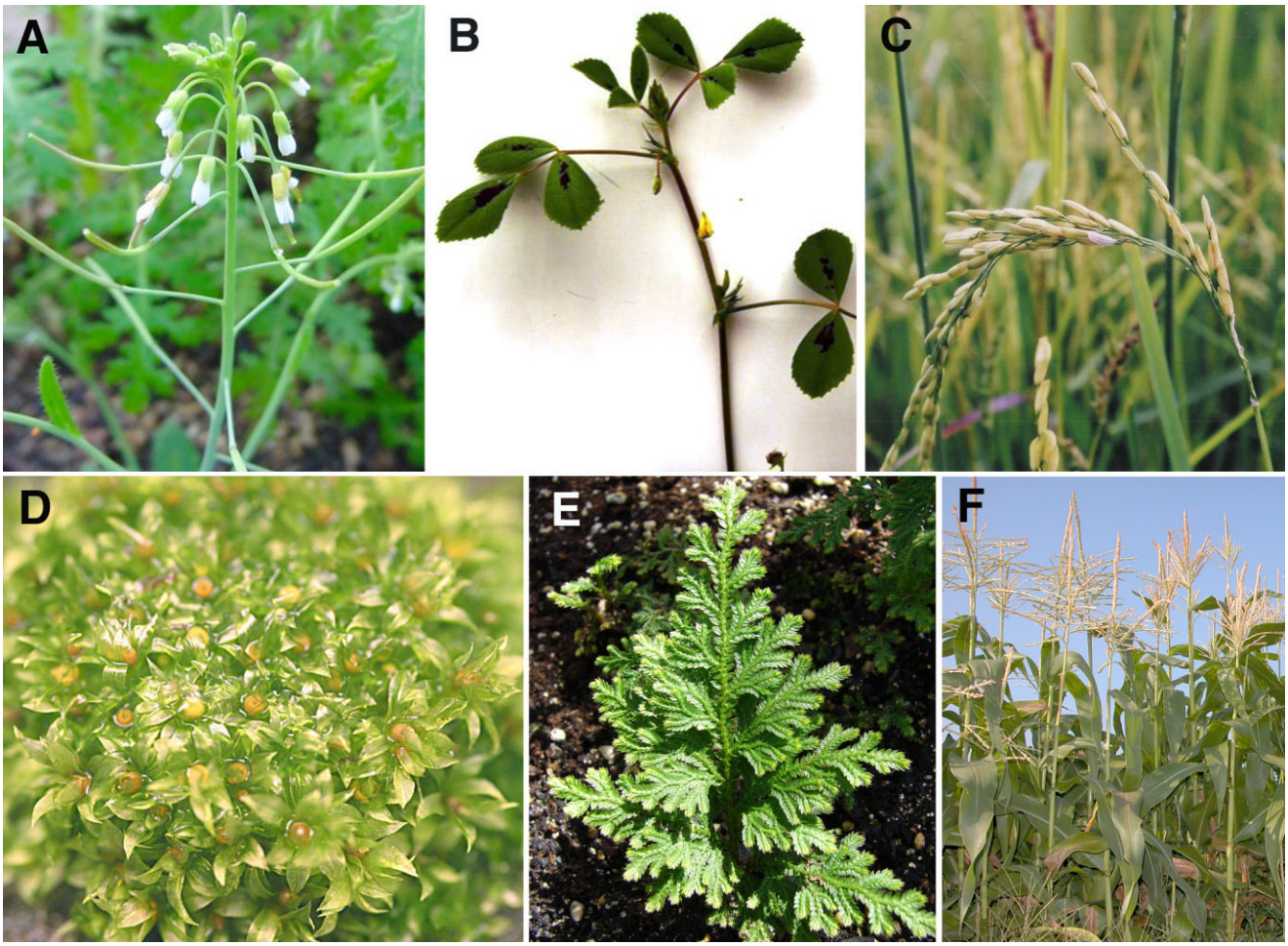

Fig. 1. Plant model organisms. (A) *Arabidopsis thaliana*, (B) *Medicago truncatula*, (C) *Oryza sativa*, (D) *Physcomitrella patens*, (E) *Selaginella moellendorffii*, and (F) *Zea mays*. Photo credits [attribution licenses] (A) Michael Charters, Califlora.net; (B) Jean-Michel Ane, University of Wisconsin; (C) World Resources Institute staff; (D) David Cove, University of Leeds; (E) Jing-Ke Wang, Purdue University; (F) Carl E. Lewis.

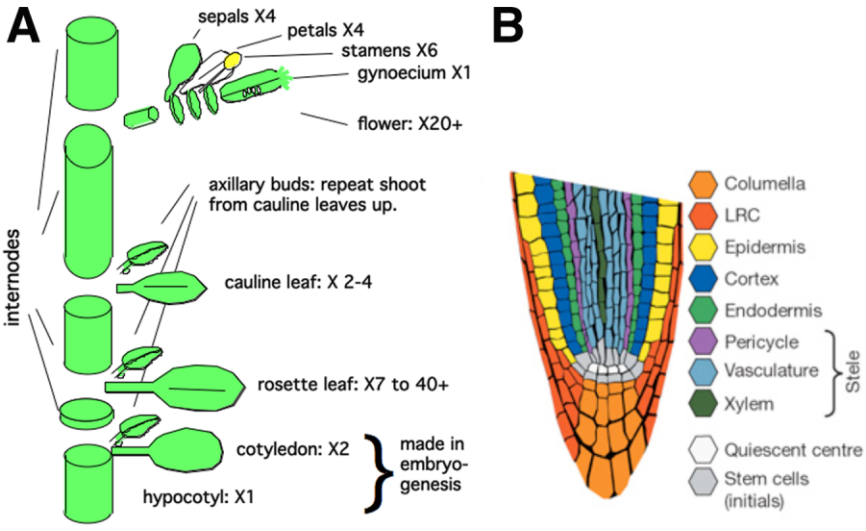

Fig. 2. *Arabidopsis* anatomy. (A) Basic shoot anatomy. (B) Root anatomy. Credits (A) Kathryn Barton, Stanford University; (B) Adapted by permission from Kenneth Birnbaum and Macmillan Publishers Ltd: Nature 457:1150–1154, copyright 2009.

Fig. 3. **Left:** Philip N. Benfey, PhD, Paul Kramer Distinguished Professor of Biology and Director, Duke Center for Systems Biology, Biology Department and Institute for Genome Science and Policy, Duke University, North Carolina. **Right:** Kenneth D. Birnbaum, PhD, Assistant Professor, Biology Department and Center for Genomics and Systems Biology, New York University, New York.

Developmental Dynamics

TABLE 1. Plant Model Systems for the Genomic Age

Latin name	Common name	Classification	Genome size	Sequence status	Biological attributes	Research strengths
Arabidopsis thaliana	Mouse-ear cress	Flowering eudicot; mustard family	125 Mbp, 27,000 genes	Complete	Small genome, ease of transformation, amenable to lab cultivation	[development topics highlighted] Organogenesis, differentiation, cell fate specification, morphogenesis, polarity, patterning, regeneration, environmental response
Medicago truncatula	Barrel medic	Flowering eudicot; pea family	500 Mbp, 40,000 genes	In progress	Symbiosis with fungi and nitrogen fixing bacteria, ease of transformation, amenable to lab cultivation	Organogenesis, stress response, disease/ defense mechanisms, symbiotic nitrogen fixation and mycorrhization
Oryza sativa	Rice	Flowering monocot; grass family	390 Mbp, 38,000 genes	Complete	Genes and synteny conserved among cereal (grass family) crops	Molecular evolution, comparative genomics
Physcomitrella patens	Physcomitrella moss	Nonvascular; true moss	480 Mbp, 36,000 genes	Complete	Nonvascular plant, homologous recombination, amenable to lab cultivation	Molecular evolution, comparative genomics, organogenesis, morphogenesis, stress response
Selaginella moellendorffii	Gemmiferous spikemoss	Vascular; spike-moss family	110 Mbp, 22,000 genes	Complete	Closest living relative of earliest vascular plants, small genome, amenable to lab cultivation	Molecular evolution, comparative genomics
Zea mays	Corn, maize	Flowering monocot; grass family	2300 Mbp, 50,000 genes	Complete	Large chromosomes (1500cM) facilitate cytogenetics	Developmental topics listed under *Arabidopsis*, imprinting, gene silencing, transposons, molecular evolution

TABLE 2. Sampling of *Arabidopsis* Research Topics

Topics	Mechanisms under research
Organogenesis	Flower, fruit, ovule, shoot, root
Cell fate specification	Epidermis (hair, vs. non-hair cells), shoot apical meristem (meristem vs. leaf), seed (root vs. shoot)
Differentiation	Stomata, epidermis, vasculature, hair cells (trichome)
Morphogenesis	Leaf, petal, hair cells, epidermis, embryo, seedling
Polarity	Stomata (epidermal pores), pollen tube, root hair cells, epidermal cells, fruit, ovule
Patterning	Vascular tissue, epidermis, petal, flower, leaf
Environmental response	Tropisms, biotic and abiotic stress response

the ambitious goal of understanding every molecular interaction in each cell throughout the plant's lifecycle. Reminiscent of something out of *Star Trek*, the information will go toward building a computerized virtual plant that can be used to understand biochemical and physiological responses to simulated experimentation. The once-incon-

spicuous wallflower has successfully captured the imagination of researchers worldwide.

THE MOLECULAR TOOLKIT OF GENE REGULATION

The Genome

Although *Arabidopsis* has a relatively small genome, owing to compact gene structure and abbreviated stretches of noncoding sequence, approximately 70% of it has been duplicated. There are over 27,000 genes in total, but only about 15,000 different genes.

At a basic level, plants and animals are not so different. A high percentage of proteins involved in protein synthesis, basal transcription, DNA repair, vesicle trafficking, and other basal cellular processes are conserved (*Arabidopsis* Genome Initiative, 2000). Notably, plants harbor counterparts to human disease genes that also regulate such fundamental pathways. These include the DNA repair and Xeraderma pigmentosum disease gene (OMIM 278730), D-XPD, the ABC transporter and Wilson disease gene (OMIM 277900), ATP7B, and the H+ transporting ATPase and Renal tubule acidosis gene (OMIM 267300), ATP6B1. Indeed, *Arabidopsis* research has informed mechanisms behind human inflammatory responses, cancer, and Alzheimer's disease (Jones et al., 2008).

Beyond basal mechanisms, the plant genome reflects the fact that plants, unlike most animals, are sessile, a theme reiterated in the gene regulation mechanisms described below. For example compared with animals, there are 10 times as many peptide-hormone transporters that, in plants, can act as second messengers to environmental cues. Given the vast differences in kingdom lifestyles, it is not surprising that plants have 150 unique protein families. Many plant-specific genes are devoted to mechanisms like pathogen resistance, photosynthesis and secondary metabolism, which help them respond to an ever-changing local environment.

Transcription Factors

Plants have evolved kingdom-specific transcription factor families to help fill their distinctive needs. A total of 45% of *Arabidopsis*' ~1,500 transcription factors are lineage specific, the largest families of which are the AP2-EREBP, WRKY, and NAC families (Riechmann et al., 2000). Several WRKY proteins are important for defense response, and the ethylene response element binding protein (EREBP) subfamily regulates hormone, stress, and defense responses. The NAC family contains 13 membrane-bound transcription factors (MTFs), some of which are implicated in salt, osmotic, and cold stress responses. Tethering to membranes is a structural strategy that, by means of proteolytic cleavage, allows for quick reaction to stimuli. In fact, a predicted 10% of *Arabidopsis* transcription factors are MTFs, suggesting that this is a successful regulatory strategy for an immobile organism.

Notable developmental regulators that shape animals, Forkhead, SOX, POU, and nuclear hormone receptors, are absent from plants. Some of these developmental requirements are instead met by plant-specific factors. For instance members of the AP2 transcription factor family regulate developmental processes such as flower and seed development, while NAC family members control establishment of cotyledon boundary, shoot apical meristem formation, and apical patterning of the embryo.

Arabidopsis also harbors developmental regulators that are common to both plant and animal kingdoms; the largest are MYB, bHLH, C2H2 zinc-finger, and MADS families. A comparison of conserved families at the structural level finds that there is little homology beyond the DNA binding domain. At the functional level, roles can be swapped between transcription factor families (Meyerowitz, 2002). For example, an overlapping series of Hox homeobox genes regionalize axis patterning of tissues and organs in animals. In *Arabidopsis*, this role has been adopted by overlapping MADS genes that regionalize the radial patterning of floral organs. Plant homeobox genes are involved in development, but to date mutations in this gene family do not confer a homeotic-like phenotype. These examples illustrate that plants use some of the same tools as animals, but the tools have been repurposed.

Signal Transduction

Upon perusing the list of plant signaling pathways, the animal biologist may feel disoriented. How can these complex organisms pattern tissues and build organs without Wnt, Sonic Hedgehog, Notch, JAK/STAT, Ras, and TGF-β? In their stead are pathways headed by the largest class of transmembrane sensors, the ~350 receptor-like kinases (RLKs), the ligands of which are mostly unidentified (Initiative, 2000). Many RLK subfamilies lack animal counterparts, like the ~50 member light repressible receptor family, whose mRNA is expressed in the absence of light. The leucine-rich repeat (LRR) RLKs, on the other hand, are similar to animal LRR domain receptors, except the latter are not coupled to kinase domains. Other identified receptor classes are the animal-like G-protein coupled receptors, and a plant-specific mildew resistance class of 7-transmembrane receptors, both of whose downstream pathways have yet to be teased out.

Plant signal transduction pathways also contain bacterial genes, which oozed into the plant genome by means of horizontal transfer. The bacterial two-component signaling system, a histidine-to-aspargine phosphorelay lies downstream of cytokinin hormone and other receptors (To and Kieber, 2008). The phosphorelay travels from *Arabidopsis* histidine kinase (AHK) receptors to *Arabidopsis* histidine phosphotransfers (AHPs) to *Arabidopsis* response regulators (ARRs), culminating in target gene activation. The cytokinin pathway plays roles in germination, root and shoot development, and circadian rhythms. For the most part, signaling pathways appear to have evolved separately in animals and plants.

An exception to this rule is the heavily conserved innate immunity pathway. Pathogen-associated-molecular-pathogens (PAMPs) are molecular signatures expressed on infectious agents but not in the host organism. In *Arabidopsis*, the LRR-RLK, FLS2, is a serine/threonine kinase activated by a PAMP recognized by plants and animals, bacterial flagellin. FLS2 triggers a MAP kinase cascade that activates transcription of immune response genes by means of plant-

specific WRKY transcription factors, WRKY22 and 29 (Asai et al., 2002). The pathway need be only tweaked a little to get a vertebrate immunity pathway. In vertebrates, the LRR domain receptors, Toll-like receptors (TLR), recognize flagellin and mediate activation of a MAPK cascade, which triggers cytokine transcription through transcription factors NF-κB and Jun/Fos. Conservation of this pathway is a testament to its longstanding success in recognizing and combating a foe common to both animals and plants.

Chromatin Remodelers

Although plants and animals evolutionarily branched from one another 1.6 billion years ago, chromatin remodeling, largely accomplished by posttranslational histone modification, is an important mode of gene regulation in both kingdoms. In *Arabidopsis*, the requisite protein classes have answered the call of duty, including histone acetylases (HATs), histone deacetylases (HDACs), Swi2/Snf2 proteins, and SET domain proteins (Riechmann, 2002). These families share functional but not necessarily structural integrity with their counterparts in other eukaryotes, and some plant-specific subfamilies do exist. Chromatin remodelers in plants experience a fair degree of domain shuffling, accretion, and deletion, but pertinent protein domains are conserved, the ring, bromodomain, chromodomain, and PHD finger.

In contrast to animal models, many chromatin regulation mutants are viable, allowing for their study throughout the lifespan of the plant. As a result, genetics has helped to demonstrate that chromatin remodeling is integral to many facets of development. Mutations in just one class of chromatin remodelers, HDACs, affect a bevy of development events: root and embryonic development, leaf polarity, floral organ identity, vascularization, and leaf senescence, to name a few. The observation that chromatin regulators have a finger in so many developmental processes gave rise to the idea that chromatin states are more dynamic in plants than animals; perhaps yet another mechanism to ensure their survival as a sessile organism.

Small RNAs

Another conserved facet of gene regulation are small RNAs, 21–24 nucleotide noncoding transcripts that guide cleavage or block translation of their target transcripts (Vaucheret, 2006). Small interfering RNAs (siRNAs), which were first discovered in plants, are generated from cleaved dsRNA. The most prevalent class, also found in protists, fungi, and animals, is RNA-dependent RNA polymerase-2 (RDR-2) -dependent siRNAs. By directing cleavage or blocking translation of target transcripts, these tiny workhorses perform varied duties: they control gene expression, regulate movement of transposable elements, and protect against viral infection. Their mRNA targets typically have perfect complementarity, as a result, they frequently regulate the loci from which they are derived, and are rarely conserved between organisms.

Plants also harbor two novel siRNA classes. Natural antisense siRNAs (nat-siRNAs) are produced from overlapping antisense transcripts, the best studied of which act in stress response. The *trans*-acting siRNAs (ta-siRNAs) require miRNA activity for their genesis and direct cleavage of nonidentical transcripts. One family of ta-siRNAs acts as suppressors of auxin response factors (ARFs), through which they affect vegetative developmental timing and polarity of lateral organs. Although definitive proof awaits, ta-siRNAs may be the source of observed small RNA non–cell-autonomous silencing (Garcia et al., 2006).

Mature microRNAs (miRNAs) may look like their siRNA cousins, but there are many differences between them. Unlike siRNAs, miRNAs are derived from large single-stranded precursor RNAs with hairpin structures, and usually share imperfect complementarity with their mRNA targets. In contrast to siRNAs, miRNA families are often conserved between organisms. At least 14 miRNA families are conserved in plants from flowering eudicots to mosses (see also below), and two miRNAs that target RNA binding proteins, are conserved in plants and animals. Approximately two-thirds of *Arabidopsis* miRNA targets are transcription factors that regulate development. This differs from human miRNA targets, which are widespread in the biological processes they inhabit. To respond to sudden changes in local environment, *Arabidopsis* may have evolved to use miRNAs as a way to purge existing transcripts from cells, allowing for rapid onset of a new expression program.

PERSPECTIVES

Beyond understanding developmental processes for their own sake, comparative research between *Arabidopsis* and other species has exposed developmental trends that shape plantkind. For example, as in *Arabidopsis*, MADS-box factors pattern the flower in such diverse plants as rice, peach and orchid. This observation gave birth to the hypothesis that MADS transcription factors were the driving force behind flower diversification during the evolutionary radiation of flowering plants (Yamaguchi and Hirano, 2006). Similarly, comparative work with *Physcomitrella patens* (Fig. 1; Table 1), a nonvascular moss that colonized the land 450 million years ago, shows that at least 14 miRNA families are conserved in *Arabidopsis* and in plants that span the evolutionary distance between them (Fig. 1; Table 1; Axtell et al., 2007). Targets of these broadly conserved miRNAs are mainly developmental genes, suggesting that these gene cassettes have been essential for species expansion of land plants.

Arabidopsis research also has a more immediate purpose: informing studies with agricultural importance. Findings translated to crop models like corn (*Zea mays*), rice (*Oryza sativa*), and the legume, *Medicago truncatula* (Fig. 1; Table 1) are indispensable for adapting commercial crops to survive in varied growing conditions, improve crop yields, and make them more nutritive or otherwise useful. The work will prove to be invaluable to feed a world experiencing increasing population densities, and a global environment in flux.

SPOTLIGHT ON ROOT DEVELOPMENT

A Conversation With Systems Biologist, Philip Benfey, PhD

Developmental Dynamics: What initially provoked your interest in root development?

Philip Benfey: My scientific interests have always been on questions of developmental biology. My graduate work was with Phil Leder, and I was involved in cloning the IgE receptor as well as other genes involved in the allergic response. When it came time to look for a post-doc, I knew that I wanted to work on a developmental system. I also was looking for a genetically tractable organism. I was originally attracted to plants in large part due to the ease of introducing genes into the genome. The transition to plant research was quite straightforward. DNA is DNA, although it took me a while to master some of the terminology. I remember referring to the vascular cell types at one point as the "xylem and phylem" as opposed to "xylem and phloem."

One of my post-doctoral projects with Nam-Hai Chua involved characterizing a complex promoter, the CaMV 35S promoter. My approach was to cut it up into subdomains and fuse each of them to a reporter gene. While analyzing the cellular-specificity of the expression patterns conferred by the subdomains, I came to appreciate the simplicity of root development.

Root development originates with stem cells at the root tip whose progeny remain in cell files emanating from specific stem cells (Fig. 2B). Thus the age and provenance of every cell is easily ascertained. Moreover, all developmental stages are present in the root at any time. One further simplification—the outer four cell layers are organized as concentric circles around the central vasculature. Taken together, these features reduce the developmental parameters from four dimensions to two dimensions

Dev Dyn: What is your lab's research focus today?

PB: The focus of our work is on root development, primarily in *Arabidopsis*. We use a combination of molecular genetics, genomics and systems biology approaches to address fundamental questions in developmental biology. We began our work on root development with a genetic screen for short or fat root mutants. This was really a matter of expediency as it was easier to screen for these than for long or thin mutants. Among the first mutants we characterized was one we not very imaginatively called "short root". In addition to the feature for which it was named, it turned out to be entirely missing one of the four cell layers that surround the root vascular cylinder. Shortly after finding shortroot we found another mutant missing a cell layer. By that time we became a bit more whimsical and called it "scarecrow" after the Wizard of Oz character who was missing a brain. We cloned both genes and found that they encode related transcription factors (Di Laurenzio et al., 1996; Helariutta et al., 2000).

The big surprise came when we performed expression studies. The SHORT-ROOT mRNA is made only in the vascular tissue, but its protein is found in the vascular tissue and the adjacent endodermal cell layer. Although this was not the first instance of a moving transcription factor in plants, it was the first case in which movement was closely tied to function. When SHORT-ROOT moves from the stele it physically interacts with SCARECROW in the endodermis. This interaction sequesters SHORT-ROOT in the nucleus and prevents further movement (Cui et al., 2007). The interaction also results in the activation of downstream target genes. To identify and characterize the downstream targets we have been employing genomics approaches. What is becoming clear is that SHORT-ROOT and SCARECROW not only interact with each other, but they also regulate a large number of transcription factors, which in turn regulate other transcription factors. Mapping these gene regulatory networks is a major aim of our current work.

Dev Dyn: What is your approach to mapping gene regulatory networks?

PB: Given the simplicity of the root, we realized that if we could profile expression in each of the cell types as well as sample all the developmental stages we could get a fairly complete picture of gene expression in an entire organ (Brady et al., 2007). To profile the cell types, we used GFP lines that were specific to individual cell lineages. We enzymatically digested the cell walls then passed the cells through a fluorescence activated cell sorter (FACS). We used the RNA from the sorted cells to hybridize to Affymetrix microarrays. Somewhat surprisingly we found that when the entire procedure was performed rapidly there was little change in the transcriptional profiles induced by these manipulations. For the developmental stages, we microdissected individual roots into 13 sections and used that material for microarray analysis. Recently, we have been able to use a mathematical approach to combine these datasets allowing us to assign expression levels to each of the cell types at different stages of development. We are now performing ChIP/chip and ChIP/seq in an effort to reconstruct the transcriptional networks that link the stem cells at the tip of the root to their differentiated progeny,

As we identify the regulatory networks, we are making use of the nearly complete library of insertional mutants in *Arabidopsis* as well as our extensive collection of tissue-specific promoters to determine the effects on cell specification, or loss-of-function and gain-of-function of key genes in the network.

Dev Dyn: Which papers have most impacted your research?

PB: (Coen and Meyerowitz, 1991) This study describes a remarkably elegant model for how floral organs are specified. It was derived entirely from genetics and has, by and large, stood the test of time.

(Lee et al., 2002) One of the first papers to combine microarray expression analysis with ChIP/chip, they were able to identify a large number of known and many unknown connections between transcription factors and target genes. This was a key early paper in the field of systems biology.

Dev Dyn: How might your work impact animal developmental biology research?

PB: It is important to realize that development in multicellular organisms only evolved twice. By comparing plant and animal development one can compare and contrast mechanisms and begin to understand the

underlying constraints. How many different ways are there to solve a particular biological problem? If we see the same solution being used in independently evolved organisms, this could point to a small number or perhaps even a single possible solution. Understanding why the search space of solutions is limited could reveal fundamental constants and/or laws.

More specifically, our work points to the need to perform genome-wide expression analyses at the level of individual cell types. We have shown that response to environmental stimuli can differ dramatically among cell types within an organ. In our initial study, we subjected plants to salt stress and to growth on iron deficient media. We then sorted cells and analyzed expression in different cell types. In both cases we found a remarkable amount of cell-type specific response to these environmental stimuli. Most of these responses had been missed in previous analyses, presumably because they were diluted out when the assay was performed on the entire plant or on an entire organ such as a leaf or root. There may well be a corollary within animals that goes beyond the obvious cellular specializations such as nerve cells.

Dev Dyn: What are some important questions in your field that remain to be answered?

PB: A question that we are particularly interested in is, "What happens as cells progress along a developmental pathway?" Are there abrupt changes in regulatory networks that mark developmental changes or is development more a series of incremental steps?

Dev Dyn: What exciting ideas are emerging in your field?

PB: Plants are playing a leading role in efforts to link phenotype and genotype. It is striking that we still have such a poor ability to predict phenotype from genotype, except in the case of a small number of Mendelian mutants. What we lack is a good knowledge of how different alleles interact to produce traits. In humans, most of the work is directed toward understanding disease states. In plants we can focus primarily on adaptive traits. Plants also have the advantage of being nonmobile and we can control their interactions both among themselves and with the environment. With high throughput automated phenotyping and next-generation sequencing there is a real opportunity to use natural variation to understand the regulatory networks controlling plant development. The basic idea is that by understanding the way different alleles interact to refine traits will provide insight into the way regulatory networks are tuned.

Dev Dyn: Do you think there is a lack of communication between plant and animal biologists? If so, what are your ideas for bridging the gap?

PB: I think this varies greatly. There certainly are some animal scientists who feel that they can ignore findings in plant biology. This is less true for those working on animal model organisms. With new imperatives of food and energy security, it seems all the more short sighted for animal biologists to think that results from plants are not relevant. At the same time, I think it is incumbent upon plant scientists to be as clear as possible in describing our work so that it is comprehensible to scientists from all backgrounds.

I would note that we have an ongoing and very pleasant collaboration with Olivier Pourquie whose aim is to compare developmental processes in plant root and vertebrate tail development. In particular we are comparing the repetitive processes that result in somites in the tail and lateral root formation in the root. To date, we have found some very intriguing similarities that suggest that there might be biological constraints that have led to the use of similar processes in both cases.

A Conversation With Regeneration Biologist, Kenneth Birnbaum, Ph.D.

Dev Dyn: What is your lab's research focus?

Kenneth Birnbaum: We are interested in the question of self-organization during regeneration and what gives certain cells the ability to reshape their fates during regeneration. We recently used genomic techniques together with live imaging and genetics to show that the root tip can regenerate without an active stem cell niche (Sena et al., 2009). We also used cell-specific profiling techniques to examine the genetic circuitry of specific cell types exhibiting developmental plasticity (Gifford et al., 2008).

Dev Dyn: What initially provoked your interest in this field?

KB: In my graduate work, I worked on the population genetics of avocados. My work now is quite different, but it all evolved in a few logical steps. My shift to *Arabidopsis* came from a gradual realization during graduate work that a powerful system was needed for the question in which I was most interested—What was the connection between the molecular attributes (which were simply markers for me at the time) and the tangible traits that gave a plant its specific attributes? *Arabidopsis* was just the most powerful system. If there was one "a-ha" moment in that transition, I would probably attribute it to my first glimpse of organisms expressing green fluorescent protein (GFP). These images really demonstrated to me that it was becoming possible to make the direct, live connection between the molecular level and the morphology of the organism. It is an incredibly versatile tool and remains an important part of our current arsenal in my lab. After my avocado work, I then shifted gears, doing my postdoc with Philip Benfey and using GFP techniques to take a genomics approach to development.

My interest in regeneration grew out of that moment when you start your own lab and realize you can work on anything you want. I recall one influential conversation with a physicist colleague who posed the question simply, what is so special about plant development? Regeneration was a good fit on many levels.

Regeneration captures many of the central questions in development similar to the problem of creating patterns during embryogenesis. But, in addition, unlike embryogenesis or adult organogenesis in plants, this pattern reestablishes itself from an unpredictable starting point, typically a damaged piece of tissue. So, regeneration is a great experimental system to examine the feedback mechanisms behind a self-organizing developmental system that can adapt to a different cellular layout or body plan for

each "repair job." Plants are particularly adept at regeneration so they are a nice system to unravel some of the basic principles that guide regeneration.

I also felt we had some new tools to attack this classic question. Those came largely from the work we had done in isolating specific cells for global transcriptional profiling. The premise was that a global view of regeneration at the cellular level would not only point us to the genetic players but also reveal some fundamental properties about the order of events and the return of cell identities during regeneration. The *Arabidopsis* root is also a nice system for imaging because of its transparency, highly ordered cellular arrangement, and array of available GFP markers. This gives us an opportunity to examine the coordination between molecular and morphological events at high resolution during regeneration.

Dev Dyn: Which papers have most impacted your research?

KB: (Skoog and Miller, 1957). This was seminal work on a few levels. First, it showed one mechanism by which plants can create their basic polarity, roots vs. shoots, by varying a ratio between the phytohormones auxin and cytokinin. In a sense, this was an early illustration of how organisms use combinatorial signals to lock in specific developmental trajectories. Skoog and Miller are not directly credited with demonstrating the totipotency of plant cells but their finding was one of the critical pieces of knowledge that enabled several other groups to accomplish that feat and I would argue one of the most critical puzzle pieces of the decades long quest.

(van den Berg et al., 1997) and (Kidner et al., 2000). I put these two together because they use relatively simple tools, cell ablation and lineage marking, to provide firm evidence on the role of positional information in the root where the highly organized tissue patterning makes these techniques particularly informative. From my perspective, we are still exploring the implications of the van den Berg et al. experiments, which provided evidence of a stem cell niche in which quiescent center cells prevented differentiation of surrounding cells. This was important, timely work that continues to frame our view of root patterning and growth.

(Benkova et al., 2003) and (Nakajima et al., 2001). These papers provided critical examples on the ground rules for tissue organization at different scales. On a global scale, the Friml lab, in a series of elegant papers, has essentially revealed the plant's hidden transport system for auxin, which feeds back to shape its own transport and influences tissue development. On a local scale, the Nakajima et al. paper was the definitive work that showed how plants use the movement of transcription factors to establish pattern by communicating cell fate decisions from an inner to an outer tissue.

Dev Dyn: Your group recently reported that *Arabidopsis* organ regeneration can occur without a functional stem cell niche. You also show that young, but not older stage leaves can regenerate. Do you think there are dispersed specialized cells that are called to action? Or rather does any young cell have the capacity to fill the void?

KB: Other groups have shown that, in mature tissue, certain pericycle cells, which exhibit stem-cell like properties during lateral root formation, are the likely origin of callus, which is a blastema-like structure capable of regenerating the entire plant. So, in regeneration from callus, pericycle does seem to act like a stem cell reserve that can regenerate organs at both poles. In our leaf and root experiments, it looks like any young cell has the capacity to reshape its fate according to the demands of the new, regenerating organ coordinate system. This may tell us that part of the plant's high capacity to regenerate may be tied to the pluripotency of at least two classes of cells, those that are poised to exhibit developmental plasticity in response to the environment like pericycle cells and those that are developmentally young or kept that way like the meristematic cells in our experiments.

Dev Dyn: Are there certain molecular mechanisms in place that might enable young organs to regenerate?

KB: This is a critical question for us that goes to the core of what enables some tissues to regenerate and others not. Many developmental transitions occur close to the zone where cells lose their competence to regenerate a root tip. The number of dividing cells decreases along a sharp gradient as cells enter endoreduplication cycles and begin to display morphological signs of differentiation, including rapid elongation. We also have indirect evidence that chromatin structure is also changing along this gradient. And, recently, there has been some nice work in the root showing the role of hormones, in particular cytokinin, auxin, and ethylene in the transition from dividing to elongating cells. Interestingly, different treatments can affect different aspects of the developmental transition. We are trying to disentangle which of these different developmental transitions is most closely correlated with pluripotency and the competence to regenerate. We are hoping that this will give us clues to the specific phenomena, including signaling mechanisms that control a cell's ability to alter its fate.

Dev Dyn: Where do you go from here?

KB: Beside our work on the mechanisms that regulate competency in young cells, much of our efforts are focused on a better understanding of the mechanisms that restore pattern and order to the regenerating root. We believe that our demonstration of regeneration without an active stem cell niche told us something important about the source of signals that generate new patterns during regeneration and perhaps normal adult organogenesis. We would have thought previously that cell identities were specified at the reconstituted niche and maintained by other mechanisms. Our thinking has now shifted to the possibility that patterning mechanisms could in fact be controlled by a less centralized and more dispersed organizing system. There may not be a distinction between establishment and maintenance of pattern but rather one system that elaborates the entire pattern of the root, including the stem cell niche, at once. This is analogous to the difference between a perpetuating mechanism like a pebble thrown in a pond from which a ripple pattern emanates vs. a template mechanism like a stencil that elaborates the complete pattern at one

time. In the template model, the stem cell niche, which we still believe is a critical regulator of growth, is still just one part of the entire pattern. It's a hypothesis that we want to address.

So, to me, this changes the kind of signals we may be looking for. One straightforward way to look for those signals is among the factors induced in the early stages of regeneration in our global analysis of regenerating roots. Some have roles in embryonic patterning and this makes them good candidates. We are also developing new techniques to ask about the role of cell–cell communication during regeneration, not just within the stem cell niche but among the tissues of the root. Regeneration looks like a process of sequential refinement so we hypothesize that there is extensive communication between tissues during the process. We are also adapting techniques that assay how the global transcriptional status of one cell type depends upon other cell types during regeneration.

We are also developing more high throughput methods to test gene function given the high level of redundancy of plants. One area where we are currently at the exploration phase is in cell-based assays that we can adapt to report on critical events during regeneration.

Dev Dyn: How might your work impact animal developmental biology research?

KB: Since plants and animals seem to have evolved multicellularity separately, the mechanisms of regeneration are likely to be quite divergent. However, I think there are two fertile areas where mechanisms or organizing principles may be shared across Kingdoms (Birnbaum and Sanchez Alvarado, 2008). First, regeneration calls on some basic cellular machinery like reactivation of the cell cycle and the likely restructuring of chromatin in order to reshape cell fates. These are among the mechanisms most conserved in Eukaryotes. For example, we know already that the homolog of RETINOBLASTOMA in plants has an analogous role to that in humans, regulating developmental transitions and cell cycle control. What's also intriguing to me is the potential for common organizing principles in regeneration. In a general sense, developing tissues may have certain design constraints that force plants and animals to converge on similar solutions. For example, in general tissue growth, the existence of a stem cell niche to control the growth of tissue appears to be a convergent characteristic that operates across Kingdoms. In regeneration, the task of repatterning an existing organ from a damaged piece of tissue may be another problem whose solution is dictated by some shared constraints. Our questions about the existence of a perpetuating vs. template mechanism may be relevant in certain regeneration systems in animals. One can think of some basic mechanical problems in regeneration that are common across Kingdoms like sensing the edges of the new damaged tissue and re-establishing tissue symmetry. I think that if we keep an open mind and not always look to specific mechanisms in heterologous systems, we could benefit from thinking about how models of regulation and design principles from one Kingdom fit in the other Kingdom. This might give us ideas about how we design experiments in regeneration and interpret mutant phenotypes.

Dev Dyn: What are some important questions in your field that remain to be answered?

KB: I think some critical questions in development are centered around knowing the details of the dynamic trajectories of specific cells during development. That is especially true of cells that switch their fates, and intermediate states of regenerating cells. Some motivating questions concern what happens inside the cell to allow this transition from one fate to another to take place. Do these cells pass through states that resemble stem cells in some ways?

Another set of questions revolves around the process of repatterning damaged tissue. It has become apparent that we have an increasing ability to control the pluripotency of cells in plants and animals. Our next and perhaps more challenging task is to understand how to invoke the developmental programs of specific organs or tissues, without completely reverting back to embryonic states. In plants, the Skoog and Miller experiments showed how hormone ratios could control the master regulatory switches for the two poles of the plants. A great deal of elegant work in the past 20 years has uncovered critical aspects of pattern formation in the root. And, we do have some mechanisms that link hormones to patterning circuits. But, I believe that one of our challenges is to provide models of how we get from hormone signaling to the backbone circuitry that can lay out an entire organ.

Dev Dyn: What exciting ideas are emerging in your field?

KB: First, the unfolding details of auxin transport, the ability of auxin to trigger specific cell fates, and its ability to shape its own transport has all the trappings of at least one major component of the self-organizing system shaping organ development in plants. There are several models of how auxin can elicit cell fate changes, for example, as a morphogen or a threshold trigger. These ideas have the potential to provide a mechanism that connects broad cues to local cell fate decisions.

Second, in the field of stem cells and regeneration, recent work that has shown the ability of tissues and organ-like structures to form outside of the classical niche. This work has parallels to our own system. These experiments could help us think broadly about the question of where the patterning information for specific organs comes from.

Third, I find intriguing models that examine cell identity as trajectories defined by expression of all genes in cell. In these models, there is the potential that cells have some freedom to embark on different fate trajectories but their potential is limited to specific paths. In one manifestation of these ideas, cell fates adhere to models of dynamic systems where small fluctuations can influence their ultimate fate or, in terms of dynamic systems, attractor state (Kashiwagi et al., 2006). I am still not entirely sure how well cells undergoing development will fit these models but I believe we will gain a new perspective by examining cell fate on a global level and asking whether the total contents of a cell are relevant to its potential. For example, cells may be able to reach certain states through multiple developmental routes and thus the concept

of master regulator may be context dependent.

Dev Dyn: Do you think there is a lack of communication between plant and animal biologists?

KB: I think we would benefit from more communication between plant and animal biologists, largely because I find personally that I can make the most progress on tough problems by approaching them from a new perspective. It has been said by others that plants and animals represent nature's two independent experiments in multicellularity. The comparison of these two experiments represents a great opportunity to think about how developmental systems are designed as we dissect their mechanics. Our fields have influenced each other but our techniques and approaches have also developed independently to some extent. I think that those light bulb ideas often come by combining little tricks or even big concepts from other systems with familiar ideas to create entirely new approaches.

I think the most natural way to increase exposure to each other's ideas is through our current avenues of sharing information through meetings and journals. Such mixing already happens at many meetings and I know firsthand that journal editors often invite plant–animal comparative reviews. But we could probably increase these opportunities. Editors of broader journals are a critical resource for meaningful comparisons because they have an excellent view of the breadth of research in a field among many different model organisms. They are probably the first ones to recognize common themes across fields. In the specialty journals, even those specifically geared toward plants or animals could invite more comparative reviews on perhaps very specific topics, which the wider interest journals might not be likely to pursue.

ACKNOWLEDGMENTS

Many thanks to Susan Singer for critically evaluating the manuscript and to Philip Benfey and Kenneth Birnbaum for their insightful dialog. I also extend my gratitude to those acknowledged in the photo and figure credits for sharing their beautiful works.

REFERENCES

Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408:796–815.

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J. 2002. MAP kinase signalling cascade in Arabidopsis innate immunity. Nature 415:977–983.

Axtell MJ, Snyder JA, Bartel DP. 2007. Common functions for diverse small RNAs of land plants. Plant Cell 19:1750–1769.

Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, Jurgens G, Friml J. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115:591–602.

Birnbaum KD, Sanchez Alvarado A. 2008. Slicing across kingdoms: regeneration in plants and animals. Cell 132:697–710.

Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR, Mace D, Ohler U, Benfey PN. 2007. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science 318:801–806.

Coen ES, Meyerowitz EM. 1991. The war of the whorls: genetic interactions controlling flower development. Nature 353:31–37.

Cui H, Levesque MP, Vernoux T, Jung JW, Paquette AJ, Gallagher KL, Wang JY, Blilou I, Scheres B, Benfey PN. 2007. An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. Science 316:421–425.

Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN. 1996. The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root. Cell 86:423–433.

Garcia D, Collier SA, Byrne ME, Martienssen RA. 2006. Specification of leaf polarity in Arabidopsis via the trans-acting siRNA pathway. Curr Biol 16:933–938.

Gifford ML, Dean A, Gutierrez RA, Coruzzi GM, Birnbaum KD. 2008. Cell-specific nitrogen responses mediate developmental plasticity. Proc Natl Acad Sci U S A 105:803–808.

Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, Sena G, Hauser MT, Benfey PN. 2000. The SHORT-ROOT gene controls radial patterning of the Arabidopsis root through radial signaling. Cell 101:555–567.

Jones AM, Chory J, Dangl JL, Estelle M, Jacobsen SE, Meyerowitz EM, Nordborg M, Weigel D. 2008. The impact of Arabidopsis on human health: diversifying our portfolio. Cell 133:939–943.

Kashiwagi A, Urabe I, Kaneko K, Yomo T. 2006. Adaptive response of a gene network to environmental changes by fitness-induced attractor selection. PLoS ONE 1:e49.

Kidner C, Sundaresan V, Roberts K, Dolan L. 2000. Clonal analysis of the Arabidopsis root confirms that position, not lineage, determines cell fate. Planta 211:191–199.

Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, Gerber GK, Hannett NM, Harbison CT, Thompson CM, Simon I, Zeitlinger J, Jennings EG, Murray HL, Gordon DB, Ren B, Wyrick JJ, Tagne JB, Volkert TL, Fraenkel E, Gifford DK, Young RA. 2002. Transcriptional regulatory networks in Saccharomyces cerevisiae. Science 298:799–804.

Meyerowitz EM. 2002. Plants compared to animals: the broadest comparative study of development. Science 295:1482–1485.

Nakajima K, Sena G, Nawy T, Benfey PN. 2001. Intercellular movement of the putative transcription factor SHR in root patterning. Nature 413:307–311.

Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute of Genetic Medicine, John Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), http://www.ncbi.nlm.nih.gov/omim/ Accessed June 24, 2009.

Riechmann JL. 2002. Transcriptional regulation: a genomic overview. In: The Arabidopsis book. Rockville, MD: American Society of Plant Biologists.

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G. 2000. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science 290:2105–2110.

Sena G, Wang X, Liu HY, Hofhuis H, Birnbaum KD. 2009. Organ regeneration does not require a functional stem cell niche in plants. Nature 457:1150–1153.

Skoog F, Miller CO. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp Soc Exp Biol 54:118–130.

To JP, Kieber JJ. 2008. Cytokinin signaling: two-components and more. Trends Plant Sci 13:85–92.

van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B. 1997. Short-range control of cell differentiation in the Arabidopsis root meristem. Nature 390:287–289.

Vaucheret H. 2006. Post-transcriptional small RNA pathways in plants: mechanisms and regulations. Genes Dev 20:759–771.

Yamaguchi T, Hirano HY. 2006. Function and diversification of MADS-box genes in rice. ScientificWorldJournal 6:1923–1932.