New Phytologist

Review

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50 years of Arabidopsis research: highlights and future directions

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Received: *26 June 2015*
Accepted: *24 August 2015*

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Contents

New Phytologist

New Phytologist (2016) **209:** 921–944
doi: 10.1111/nph.13687

Key words: Arabidopsis, development, epigenetics, genome, model system, reverse genetics, signaling, synthetic biology.

Summary

The year 2014 marked the 25th International Conference on Arabidopsis Research. In the 50 yr since the first International Conference on Arabidopsis Research, held in 1965 in Göttingen, Germany, > 54 000 papers that mention *Arabidopsis thaliana* in the title, abstract or keywords have been published. We present herein a citational network analysis of these papers, and touch on some of the important discoveries in plant biology that have been made in this powerful model system, and highlight how these discoveries have then had an impact in crop species. We also look to the future, highlighting some outstanding questions that can be readily addressed in Arabidopsis. Topics that are discussed include Arabidopsis reverse genetic resources, stock centers, databases and online tools, cell biology, development, hormones, plant immunity, signaling in response to abiotic stress, transporters, biosynthesis of cells walls and macromolecules such as starch and lipids, epigenetics and epigenomics, genome-wide association studies and natural variation, gene regulatory networks, modeling and systems biology, and synthetic biology.

I. Introduction and a brief survey of 54 033 Arabidopsis publications

The year 2015 marks the 50th anniversary of the first conference on Arabidopsis research, held in Göttingen, Germany, in 1965. Although Friedrich Laibach (1885–1967) proposed using Arabidopsis as a genetic model organism almost 75 yr ago, it was not until the 1980s after intense discussions among early Arabidopsis proponents that it was widely adopted as such (Laibach, 1943; Meyerowitz, 2001, which lists these Arabidopsis pioneers). Factors such as small genome size, short generation time, ease of crossing, fecundity, and the ability to do mutational screens to saturation in the laboratory have all led to a huge increase in the volume of Arabidopsis research. In the past 50 yr, 54 033 Arabidopsis papers – defined as having Arabidopsis in the title, abstract or keywords in Thomson Reuter's BIOSIS database – covering 406 different biological fields have been published, most in the second half of this time frame (see Fig. 1 for the distribution of some of the more general research areas). The Arabidopsis publications and their citations may be explored interactively at http://bar.utoronto.ca/50YearsOfArabidopsis/.

The average Arabidopsis paper from the past 50 yr has been cited 33.8 times, and the number of citations follows a power law distribution, with just a few papers having been cited many times. The most frequently cited Arabidopsis paper describes transforming Arabidopsis by the floral dip method (Clough & Bent, 1998). According to BIOSIS, this paper has been cited 7195 times. Importantly, this collection of Arabidopsis papers has been widely cited outside of the Arabidopsis community (see Fig. 2). In 37 of the past 50 yr > 50% of the cited Arabidopsis papers published each year have been referenced by papers where Arabidopsis was not the focus of the research, as determined by the absence of this species in the taxonomic data available for each paper in the BIOSIS database. A maximum of 88% of cited Arabidopsis papers in 1989 have been referenced by non-Arabidopsis papers. Of 41 682 Arabidopsis papers published in the past 50 yr that have been cited one or more times, 15 388 of these have been cited by a non-Arabidopsis-focused paper.

We now review what we have learned about aspects of plant biology from this small plant that Laibach suggested using as a model in 1943. Many 'firsts' were discovered in Arabidopsis (of course, we cannot cover everything, owing to space limitations – our apologies to the exciting discoveries that we have not included here). But even when initial breakthroughs were made in another species, Arabidopsis research often helped to illuminate their fundamental workings. Fig. 3 highlights some of the discoveries made in Arabidopsis. The first four sections of this review cover resources and methods that have enabled much of this research. The final four sections cover newer research directions that will move plant biology forward in the coming years. The decision to grow and focus a research community around Arabidopsis as a model revolutionized our understanding of plants and, indeed, of all biology (see, e.g. Jones *et al.*, 2008 for a review of how Arabidopsis has had an impact on human health).

II. Arabidopsis reverse genetics: paving the way for gene function studies

Before the genome era, classical 'forward' genetics was the preferred strategy to establish causal relationships between a genotype and a phenotype. This consisted of the generation of large randomly mutagenized populations, the identification among them of individuals with the desired phenotype, mapping and fine mapping the mutations causing the phenotype, followed by sequencing of the corresponding genomic regions and subsequent complementation testing to confirm causality (Alonso & Ecker, 2006). The small genome of Arabidopsis, the inbred nature of the different laboratory strains, and the relative ease of developing genetic and molecular markers made the routine use of these powerful, albeit somewhat laborious, genetic strategies possible in this plant system. With the progression of the Arabidopsis genome project during the late 1990s, thousands of 'interesting' genes were discovered at an unprecedented pace, fueling the desire by plant researchers to test their favorite hypothesis about these genes' potential functions. To be able to do that, however, plants harboring disruptive

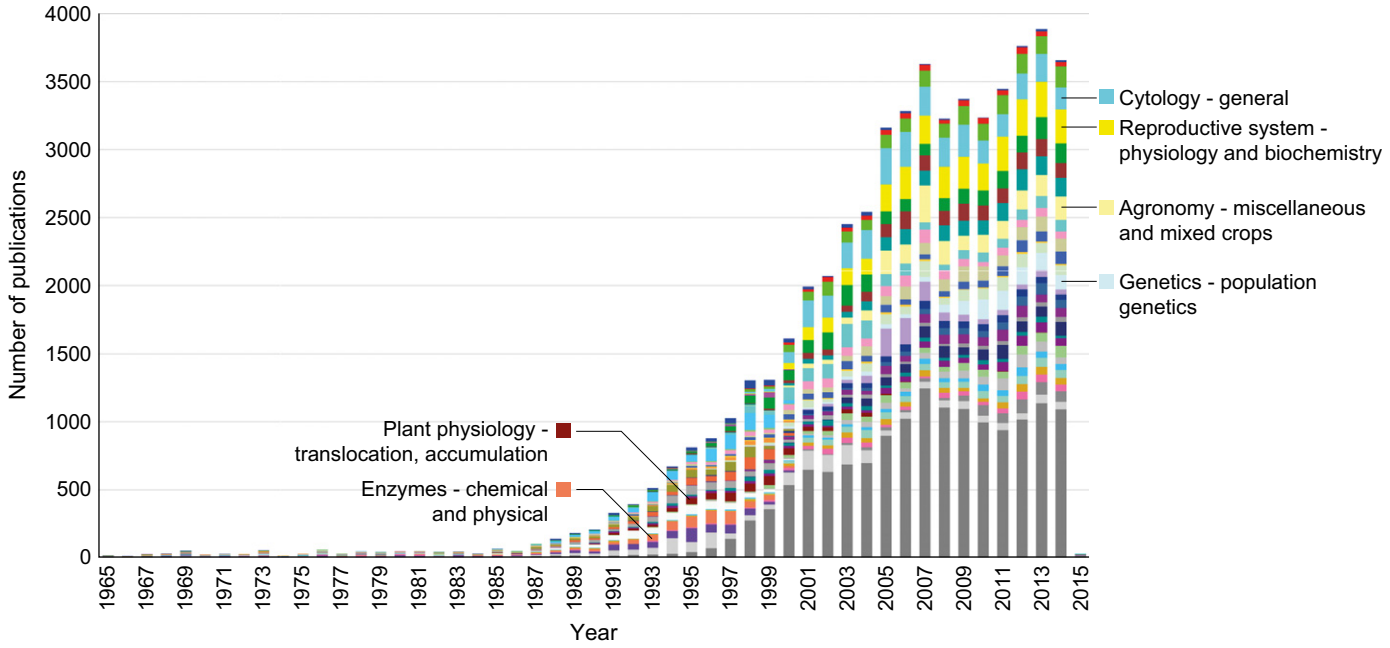

Fig. 1 Breakdown of 54 033 Arabidopsis publications by research area, from 1965 to 2015 (partial data for 2015). BIOSIS concept codes were used to flag papers in the 54 033 set. If a paper had been assigned multiple codes, the least abundant code was used for this display, out of 46 that were chosen to provide a balance between not being too general (75% of the papers were tagged with '03504': Genetics – Plant) and not being too specific (just one paper was tagged with '64500: Paleobiology'). Concept code terms ranged in prevalence from 11.6% for '51512: Plant physiology – Reproduction' to 1.04% for '13014: Metabolism – Nucleic acids, purines and pyrimidines'. Selected categories that have waxed and waned as research areas over the years are highlighted. All categories and publications may be explored online at http://bar.utoronto.ca/50YearsOfArabidopsis/.

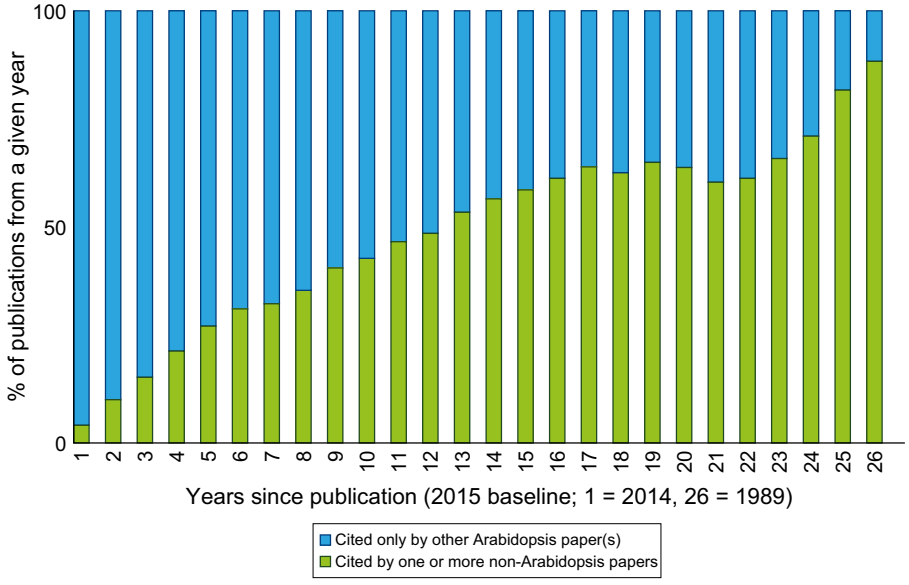

Fig. 2 Number of Arabidopsis publications per year, categorized by whether or not they have been cited by a paper that was focused on a species other than Arabidopsis, as determined by the taxonomic data provided in the BIOSIS database. Eighty-eight per cent of cited Arabidopsis papers in 1989 have been referenced by non-Arabidopsis papers.

mutations in these genes were urgently needed. Once again, the vibrant Arabidopsis research community accepted the new challenge and, soon after, the first large collection of T-DNA mutants was created (Feldmann & Marks, 1987), although initially the idea was that these collections of mutants would simplify the sometimes tedious cloning of the mutated gene in 'forward' genetic screens. The nature of the mutagenic agent in these collections, an Agrobacterial plasmid T-DNA of known sequence, also enabled the rapid screening of hundreds of mutant lines not based on their phenotype, but on their sequence (Alonso & Ecker, 2006). This initial success of the so-called 'reverse genetics', together with the development of extremely efficient methods to generate insertional mutants (T-DNA transformation via vacuum infiltration and later via 'floral dip': Bechtold *et al.*, 1993; Clough & Bent, 1998) and transposon-based mutants (Altmann *et al.*, 1992; Bancroft *et al.*, 1992), resulted in the generation of larger and larger T-DNA and transposon collections that made it possible to find mutations in practically any gene. Mutant identification, however, still required the tedious process of testing large pools of mutants

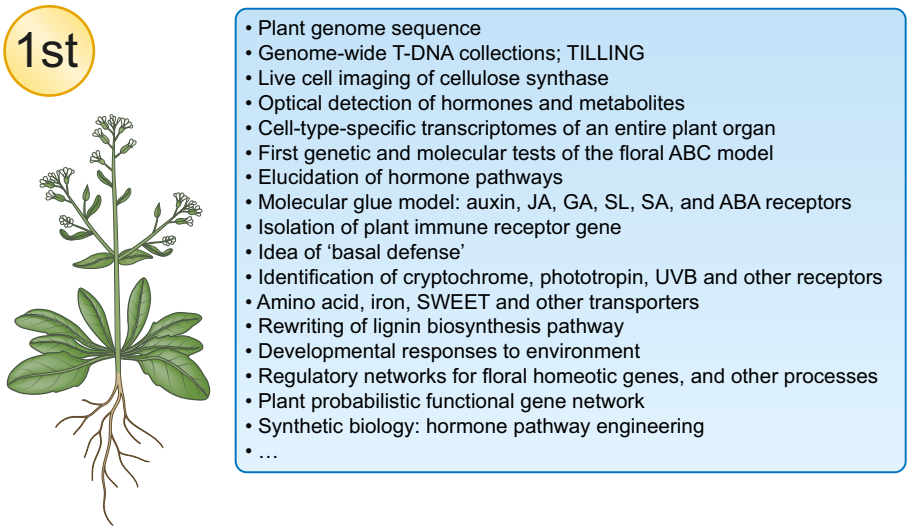

Fig. 3 Arabidopsis 'firsts' (highlights), ordered according to their mention in this review. ABA, abscisic acid; JA, jasmonic acid; SL, strigolactones; SA, salicyclic acid.

one gene at a time (Krysan *et al.*, 1996). This laborious stage in Arabidopsis reverse genetics ended a few years later when new high-throughput ways of sequencing the insertional sites in these collections were established (Sessions *et al.*, 2002), and searchable databases and seeds stocks from individual mutants lines were generated and made publicly available (Alonso *et al.*, 2003). Together, these advances allowed for access to a mutant T-DNA line in the gene of interest by simply ordering the corresponding seeds from a stock center. The ease with which one could obtain the desired mutants had a dramatic impact on the way gene function was approached not only in Arabidopsis, but also in other plant species where the analysis of an Arabidopsis mutant has become a routine and quick method for shedding light on the function of the orthologous gene of interest in less experimentally tractable plant species. Nevertheless, because of the random way in which T-DNA mutants were generated and the insertion sites sequenced, this approach became less and less effective as the number of sequenced lines approached genome saturation. The recent advent of ultra-efficient next-generation sequencing-based approaches is allowing the rapid identification of tens of thousands of new insertional or other kinds of alleles in pre-existing mutant collections (J. Ecker, pers. comm.). As with all the approaches initially developed and tested in Arabidopsis, insertional mutagenesis-based reverse genetics strategies were soon implemented in other plant species and became a fundamental research tool in important crop plants such as rice and maize (reviewed in Jung *et al.*, 2008; Nannas & Dawe, 2015).

Although gene-indexed insertional collections are important pillars of reverse genetics approaches, they have some obvious limitations. For example, a majority of the lines in these collections represent null or hypomorphic alleles and require the stable integration of foreign DNA in the genome of the target plant. Thus, other means of altering the activity of desired genes were developed and, as before, used in Arabidopsis for quick testing and protocol optimization. Large collections of chemically induced mutants were generated and screened using a technology pioneered in Arabidopsis, TILLING (Targeting Induced Local Lesions in Genomes; Till *et al.*, 2003), and then exported to other plant and animal systems (Till *et al.*, 2006). Since TILLING mutants usually contain a single nucleotide change, a wider array of allelic series could be obtained. Furthermore, because this technology does not rely on plant transformation, it was possible to rapidly translate the protocols developed for Arabidopsis to other plant and animal species that are not easily transformable. As in the case of insertional mutagenesis, the advent of next-generation sequencing has also had an important impact on the throughput of TILLING screening, allowing for the rapid testing of thousands of lines in order to find more and potentially rare mutant alleles (Till *et al.*, 2003). One of the limitations of both insertional mutagenesis and TILLING is the difficulty in combining linked mutations, which represents a significant problem in Arabidopsis and other plant species in which recently duplicated (and often functionally redundant) genes are found in adjacent locations in the genome. This and other caveats, such as the limited diversity of genetic backgrounds available and the inability to manipulate the spatial and temporal effects of the targeted gene's activity, provided a fertile ground for the development of RNA silencing-based approaches such as RNAi and artificial microRNAs (Hilson *et al.*, 2004; Schwab *et al.*, 2006). Once more, the Arabidopsis community played a key role in the implementation of these approaches in other plant systems by setting up the pipelines, generating the expertise and defining the basic rules affecting the efficiency of these reverse genetics approaches.

Finally, new genome editing technologies based on a variety of engineered endonucleases, such as homing endonucleases (Antunes *et al.*, 2012), zing-finger nucleases (Wright *et al.*, 2005), TALENs (Bedell *et al.*, 2012) and, in recent years, CRISPR-Cas9 (Feng *et al.*, 2014; Li *et al.*, 2014a), have been added to the plant reverse genetics toolbox. With these new technologies, Arabidopsis research continues to play a critical role in the implementation and optimization of the experimental protocols.

III. Arabidopsis stock centers

1. Pre-stock center era

The need for a well organized Arabidopsis stock collection was recognized long before the Arabidopsis Biological Resource Center (ABRC) and the Nottingham Arabidopsis Stock Centre (NASC) were established in the early 1990s. The Arabidopsis Information Service (AIS) newsletter, initiated in 1964, represented the first version of a curated Arabidopsis stock collection (Meyerowitz, 2001). The AIS newsletter volume 24, issued in 1987, and the 'Green Book' (Meyerowitz & Pruitt, 1984) compiled the data for the existing set of stocks for the first time. The collection consisted of *c.* 1000 stocks, mostly natural accessions and individual mutant lines generated by a handful of Arabidopsis researchers, including Friedrich Laibach, Gerhard Röbbelen, Maarten Koornneef, and Albert Kranz. An additional 350 stocks (mostly mutants with a few natural accessions) were contributed by George Rédei. According to the Green Book, *c.* 1600 stocks were exchanged among researchers between 1974 and 1987, illustrating that the pioneers of Arabidopsis research were well aware of the crucial role of sharing materials and data, which helped set the stage for all future community efforts.

2. Stock center history and data

The vision of establishing two stock centers came from the Multinational Arabidopsis Steering Committee (MASC) and was driven both by a number of new resources generated as a result of the development of efficient transformation procedures and by a push to develop a new nonhuman model organism for functional studies (Somerville & Koornneef, 2002; Koornneef & Meinke, 2010). Since their inception in 1991 and 1992, respectively, both the NASC and the ABRC have been supported by their respective national funding agencies, as well as by stock contributions from the Arabidopsis community. The policy of sharing donated resources between the two stock centers remains one of the most important strengths of Arabidopsis research and one of the main factors contributing to its success. The initial stock center collection comprised seed stocks donated by Albert Kranz (> 1000), George Rédei (< 1000), Chris Somerville (200) and Maarten Koornneef (150). The donation of 4900 Arabidopsis T-DNA insertion lines by Kenneth Feldmann in 1992, and of *c.* 18 000 expressed sequence tags (ESTs) by Chris Somerville and Thomas Newman in 1994–1995, represented the first large seed and DNA donations, respectively (Meinke & Scholl, 2003). Many other large donations followed, contributing to the current ABRC holdings of 959 000 stocks. This 'sharing community' has encompassed > 27 000 people in 11 000 laboratories to date. Additional regional centers (INRA, France; GABI, Germany; and RIKEN BRC, Japan) also participate in the distribution of Arabidopsis resources, some of which are shared and some of which are unique.

The overall ABRC stock distribution has increased from *c.* 8000 stocks sent in 1992 to > 180 000 in 2014, including large sets (NASC shipped 109 311 stocks in 2014; S. May, pers. comm.). With *c.* 80% of the total distribution, T-DNA pools and individual lines remain the most distributed stock category (Brkljacic *et al.*, 2011), illustrating a high demand for stocks that enable functional analyses. Natural accessions and mapping populations derived from accession crosses represent the second most ordered stock category, with *c.* 10% of the total distribution. The Salk uni-mutant collection of homozygous T-DNA insertion lines donated by Joseph Ecker (O'Malley & Ecker, 2010) permits genome-wide genetic screens to be carried out and represents a highly popular stock that lends itself to high-throughput experiments.

3. Order trends and projections

Stock centers have always closely followed new research trends by soliciting and accepting donations for new types of stocks. An explosion of publications using high-throughput technologies has been followed by the donation of resulting resources and their subsequent release. New additions have included new T-DNA lines and targeted mutagenesis lines as described in the previous section. A set of 1135 natural accessions sequenced by the 1001 Genomes project (Cao *et al.*, 2011; Long *et al.*, 2013; Schmitz *et al.*, 2013) has been available since the summer of 2015, enabling further analyses of the genetic and epigenetic variation of Arabidopsis. Based on the statistics showing that the number of orders is tightly linked with the number of papers published on Arabidopsis (Fig. 4), it is predicted that the overall number of orders will remain stable, as long as the newly published research resources become available shortly after publication (and researchers continue to use Arabidopsis!) As Arabidopsis stock collections are expected to grow, an expandable and robust Stock Center Database is envisioned that will be fully integrated within the larger Arabidopsis Information Portal framework at Araport.org (Krishnakumar *et al.*, 2015) to help support stock searching and ordering.

IV. Databases and online tools

The Arabidopsis community has a long history of organizing genomic and other data in publicly accessible portals. AtDB (Flanders *et al.*, 1998) and its successor, TAIR (Huala *et al.*, 2001), along with other, more specialized portals, initially served to organize sequence, bacterial artificial chromosome, physical maps, and other kinds of data necessary for and generated by the Arabidopsis Genome Initiative (Arabidopsis Genome Initiative, 2000). Over the following 15 yr, these portals and a collection of other online tools and databases have considerably altered the way that researchers design and interpret their experiments. Notably, the AtGenExpress project, a multinational initiative to document the transcriptome of Arabidopsis, generated thousands of gene expression data sets (Schmid *et al.*, 2005; Kilian *et al.*, 2007). These and many other expression data sets from individual researchers have not only helped to understand Arabidopsis's response during development and to different environmental conditions, but have also enabled 'electronic northerns' to be performed with tools such as the Bio-Analytic Resource (Toufighi *et al.*, 2005) or Genevestigator (Zimmermann *et al.*, 2004). Coexpression analysis –

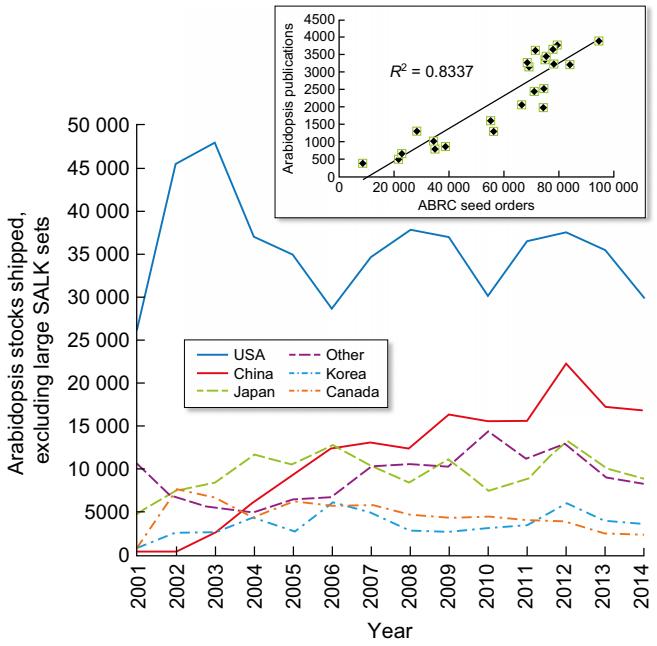

Fig. 4 Number of seed stock orders from the Arabidopsis Biological Resource Center (ABRC) from 2001 to 2015 from the five countries that ordered the highest number of seed stocks. The number of orders per year is tabulated by the ABRC from 1 April of the indicated year to 31 March of the following year. The declining number of orders from the USA is being offset by increasing orders from China. The number of seed stocks ordered correlates strongly with the number of Arabidopsis publications per year, from the BIOSIS data described earlier in this review (inset). Since the graph shows only orders from the ABRC, countries ordering exclusively from the Nottingham Arabidopsis Stock Centre (e.g. those in Europe) or from other stock centers are not represented.

identifying genes with similar patterns of expression in a compendium of gene expression data sets – has emerged as a new kind of *in silico* screen to identify genes associated with a particular biological pathway (Usadel *et al.*, 2009). The ability to explore predicted (Geisler-Lee *et al.*, 2007) and documented (Dreze *et al.*, 2011; Mukhtar *et al.*, 2011 and many other smaller studies) protein–protein interactions is also another method for hypothesis generation. Further online and standalone computer tools can be used to help make sense of the large amount of data coming from -omics experiments, such as those for Gene Ontology (GO; Ashburner *et al.*, 2000) enrichment analysis. Tools for doing these and additional kinds of analyses are described by Brady & Provart (2009) and de De Lucas *et al.* (2014). Other useful software tools permit the easy mapping of causal mutations based on next-generation sequencing of pooled segregants (Schneeberger *et al.*, 2009; Austin *et al.*, 2011). Recently, after considerable community discussion and brain-storming mediated by the North American Arabidopsis Steering Committee (NAASC) and its international counterpart MASC, the Arabidopsis Information Portal Araport.org (Krishnakumar *et al.*, 2015; International Arabidopsis Informatics Consortium, 2010, 2012) was launched, whose goal it is to integrate the large number of data sets generated in the past decade to further facilitate hypothesis generation at the click of a mouse.

V. Cell biology

For many of the same reasons that Arabidopsis has been a powerful model system to accelerate discovery in other fields of plant biology, Arabidopsis has also transformed plant cell biology. Cell biology can encompass a wide range of studies. Since many subjects that might be considered a part of cell biology, such as signal transduction, transport and the biology of cell walls, are covered elsewhere in this overview, here we focus on how Arabidopsis has contributed to studies of plant cell organization, featuring a handful of highlights.

A central tool in cell biology is microscopy, the technology that literally opened our eyes to the fine structure of life. Plants have a special place in this history, with the term 'cell' being credited to Robert Hooke when he applied his lens to cork, the box-like walls reminding him of the rooms occupied by monks, known as cells (Hooke, 1665). Nearly 200 yr later, the botanist Mattias Schleiden concluded that all plant life was in fact composed of cells (Schleiden, 1839), and together with Theodor Schwann articulated the foundational idea that cells were fundamental units of life (Schwann & Schleiden, 1847).

The confluence of Arabidopsis as a molecular genetic model, the discovery of intrinsically fluorescent proteins in jellyfish and other invertebrates, and advances in live cell microscopy addressed the limitations of then state-of-the art immunocytochemistry and enabled a new era of cellular discovery in plant science based on real-time, *in planta* observations.

The power of genetic analysis in Arabidopsis alone was revolutionary for plant cell biology. Prominent examples include screens for cell development and pattern using root hairs, trichomes, pollen, guard cells, roots and hypocotyl cells as models. These screens uncovered or helped to reveal important new insights into the function of essential players in cellular growth and morphology, such as cell wall biosynthetic enzymes and regulators (Arioli *et al.*, 1998; Nicol *et al.*, 1998; Favery *et al.*, 2001), the arp2/3 complex (Li *et al.*, 1999; Le *et al.*, 2003; Mathur *et al.*, 2003), plant-specific microtubule-associated proteins (Shoji *et al.*, 2004), and insights into the molecular basis of plant cell polarity, including novel cell polarity factors (Friml *et al.*, 2004; Dong *et al.*, 2009).

As powerful as genetic analysis can be, what can be learned is defined and limited by the phenotypes that can be observed and measured. Here, advances in microscopy have combined with genetics to push cell biology forward. An early example in plant biology was the immunocytochemical visualization of the interphase microtubule cytoskeleton as a phenotype in Arabidopsis, a screen that yielded the essential cytoskeletal protein MOR1 (Whittington *et al.*, 2001). This was a heroic screen that was not repeated owing to its difficulty. Genetic tagging with fluorescent proteins greatly simplified the marking of specific proteins and cellular structures. Protein localization alone could now be used as a phenotype, allowing for identification of peptide sequences that allow for subcellular targeting and the creation of useful subcellular markers (Cutler *et al.*, 2000). The distribution of such subcellular markers has since been used in direct microscopy screens to identify mutations or small molecules that perturb subcellular morphology and function (Hicks & Raikhel, 2012; Renna *et al.*, 2013). We can

anticipate more such screens in the future, especially as platforms for automated imaging and image analysis are developed further.

Fresh insights are being made into the age-old mystery of cytoskeletal organization, with the interphase microtubule cytoskeleton emerging as a self-organizing system (Deinum & Mulder, 2013) that may be directed by a variety of mechanisms including localized regulation of polymer dynamics (Ambrose *et al.*, 2011) and regulation of essential activities such as microtubule severing by states of polymer interaction. New plant-specific mechanisms for basic cellular processes are being discovered, including discovery of novel pathways for cellular polarity (Dong *et al.*, 2009), endocytosis (Gadeyne *et al.*, 2014), and vacuolar trafficking (Sanmartín *et al.*, 2007).

Arabidopsis is now coming into its own as a model for cell biological processes that are shared across a wide range of biological diversity. This is in part because cells can be easily observed *in situ*, permitting investigation of cellular behavior in a native developmental context. The architecture of plant cells is also helpful. The large central vacuole pushes cytosol and organelles into a thin shell at the cell periphery, producing a kind of natural optical section that increases signal to background, aiding detection of labeled single molecular complexes. Finally, plant cells display variations on basic cellular processes that allow for new experimental opportunities. An example is the architecture of cytokinesis, where the large phragmoplast is much more easily studied than in the more obscure midbody, which is thought to pay a similar role as the phragmoplast in completing cytokinesis in animal cells.

In looking to the future, new experimental opportunities will be opened by the invention of methods to manipulate cells and their molecular components in time and space, as exemplified by the recent development of tools to regulate protein localization and activity by light in neuronal systems (Fenno *et al.*, 2011), and the continued innovation of imaging methods and tools to observe dynamic biological processes across greater ranges of time and space and with an expanding range of molecular and material measurements, including optical detection of metabolites, hormones and protein activity (Okumoto *et al.*, 2012; Michele *et al.*, 2013).

VI. Development

The study of plant development goes back centuries (Wolff, 1759), but a plausible case could be made that most of what we know now about the mechanisms of cellular differentiation, pattern formation, control of cell division, and the relationships between environment and plant development has been learned from experiments, largely with *Arabidopsis thaliana,* that have been done since the early 1990s.

The current systems biological approach to development has leveraged the power of Arabidopsis as a genetic model system (Rédei, 1975; Meyerowitz & Pruitt, 1985) through the addition of numerous tools built and shared among members of the Arabidopsis research community and our discovery and appreciation of other, more hidden attributes of this plant; some of these discoveries arose from the completion of the annotated genome sequence in 2000 (Arabidopsis Genome Initiative, 2000). For example, the complementarity between small RNAs and their target genes enabled a revolution in understanding how miRNAs regulate spatial and temporal aspects of development. The particular history of genome duplications and losses simplified some regulatory systems (e.g. RETINOBLASTOMA-RELATED proteins and heterotrimeric G proteins, notwithstanding three recently discovered XLGs: Chakravorty *et al.*, 2015) while expanding others (like Polycomb complexes), allowing the roles of broadly conserved genes and processes to be approached in ways that are impossible in animals.

Our concept of development now is both more finely dissected and more integrated than it was 25 yr ago. Today, instead of monitoring gene expression of a few key regulators, we characterize transcriptional responses of entire genomes, and can do so within individual cells (Brady *et al.*, 2007; Yadav *et al.*, 2009; Adrian *et al.*, 2015; Efroni *et al.*, 2015). We are now modeling gene regulatory networks within cells and tissues in real time with fluorescent reporters and new methods of microscopy (Roeder *et al.*, 2010; Robinson *et al.*, 2011), and integrating multiple signaling modes (particularly hormone signaling) into these transcriptional networks.

Signaling, especially cell-to-cell communication, is a topic of long-term interest that has undergone conceptual revisions, often as a result of technical advances. We now appreciate that such communication is mediated not only by classical plant hormones, but also by newly discovered ones such as brassinosteroids and strigolactones, and that the classical hormone responses have considerable degrees of crosstalk (see the Hormones section). The diversity of signaling molecules, their modes of transport, and the degree to which they control each other are far greater than we imagined 25 yr ago. For example, investigating cell fate and patterning in the Arabidopsis root led to the identification of small regulatory RNAs and mobile transcription factors, whose movement and interaction dictate radial tissue organization (Nakajima *et al.*, 2001; Carlsbecker *et al.*, 2010). Peptides of a growing number of families (Katsir *et al.*, 2011) interact with numerous cell-surface receptors, and the resulting landscape demands new ways of understanding crosstalk and specificity. Beyond these chemical signals, we are also reappreciating the role of mechanical forces as a critical feedback leading to morphogenesis in developing plants, but now with the physical tools to manipulate forces and computational tools to understand their contributions (Kierzkowski *et al.*, 2012; Sampathkumar *et al.*, 2014).

An example of how technical advance and community effort have led to conceptual change in plant development is in the understanding of flower development. In 1980 there was no model of regulatory gene interaction to specify floral organs, but with the conceptual elegance of the ABC model and the cloning of the first ABC genes (in 1990, in Antirrhinum as well as Arabidopsis; Sommer *et al.*, 1990; Yanofsky *et al.*, 1990), it became clear that organ specification is a transcriptionally regulated process, and that combinatorial action of transcription factors leads to specific cellular differentiation (Bowman *et al.*, 1991; Coen & Meyerowitz, 1991). Work on auxin transport, cell division, and cell–cell communication then showed that these functions interact to create organs with patterned cells (Lampugnani *et al.*, 2013) in particular organ shapes and sizes. The addition of the integration of different

signaling modalities – hormone interactions, peptide signals, and mechanical signals, all feeding back on each other – has led to new hypotheses and computational models with predictive capabilities (Heisler *et al.*, 2010; Sahlin *et al.*, 2011), and a real understanding of the mechanisms and integrated systems that result in flowers (Smyth & Banks, 2014).

Another area where the understanding of mechanism has created a revolution is in the developmental responses of plants to their environments. As sessile organisms, plants use control of development to adapt, and plant size, branching, growth rate and time of flowering have long been known to depend on the abiotic environment. The past quarter-century has seen explanations for the mechanism of flowering time calculation (Jaeger *et al.*, 2013), including chromatin-based vernalization (described in more detail in the Epigenetics and epigenomics section) in response to temperature (Song *et al.*, 2013), the discovery of the long-known graft-transmissible flowering signal (Wigge *et al.*, 2005; Corbesier *et al.*, 2007; tomato FT (SFT) shown to be mobile signal: Lifschitz *et al.*, 2006; rice FT (Hd3a) shown to be mobile signal at same time as Arabidopsis FT: Tamaki *et al.*, 2007), and of the mechanisms by which day-length is measured to allow for seasonal flowering (Suárez-López *et al.*, 2001), all from work on Arabidopsis that was initially based on studies of single mutants and the genes responsible, and then progressed to studies of networks of interacting genes, cells and regulatory feedback loops, which have since been combined into a predictive set of models.

VII. Hormones

Phytohormones are a collection of structurally diverse small molecules that act at a distance to control plant growth, development and environmental responses. The 20th century was a golden age for phytohormone discovery that exploited sensitive bioassays coupled with purification to identify and elucidate the chemical structures of the major phytohormones. Further, biosynthetic pathways have been fully decoded, often using Arabidopsis (summarized in Davies, 2004; Davies writes in the preface to the 3rd edition that Arabidopsis has gone from a mention in the 1995 2nd edition to centre-stage in every chapter of the 3rd edition). Unsuccessful 'pray and spray' approaches to biochemically dissect mechanisms of action have given way to saturating mutational screens in Arabidopsis, which, quite remarkably, have revealed that most phytohormones signal through a limited number of mechanisms (see Fig. 5).

Early ideas on plant hormone signaling were heavily influenced by animal models of plasma membrane receptors that signal through cytoplasmic proteins which then transduce information to nuclear transcription factors. Surprisingly, this paradigm appears to hold only for the hormone brassinosteroid (BR). The major components of the BR pathway were defined in Arabidopsis, capitalizing on the characteristic dwarf phenotype of BR biosynthetic and signaling mutants (Li & Chory, 1997). Loss of function *BRI1* (*Brassinosteroid Insensitive 1*) alleles are BR-insensitive, and

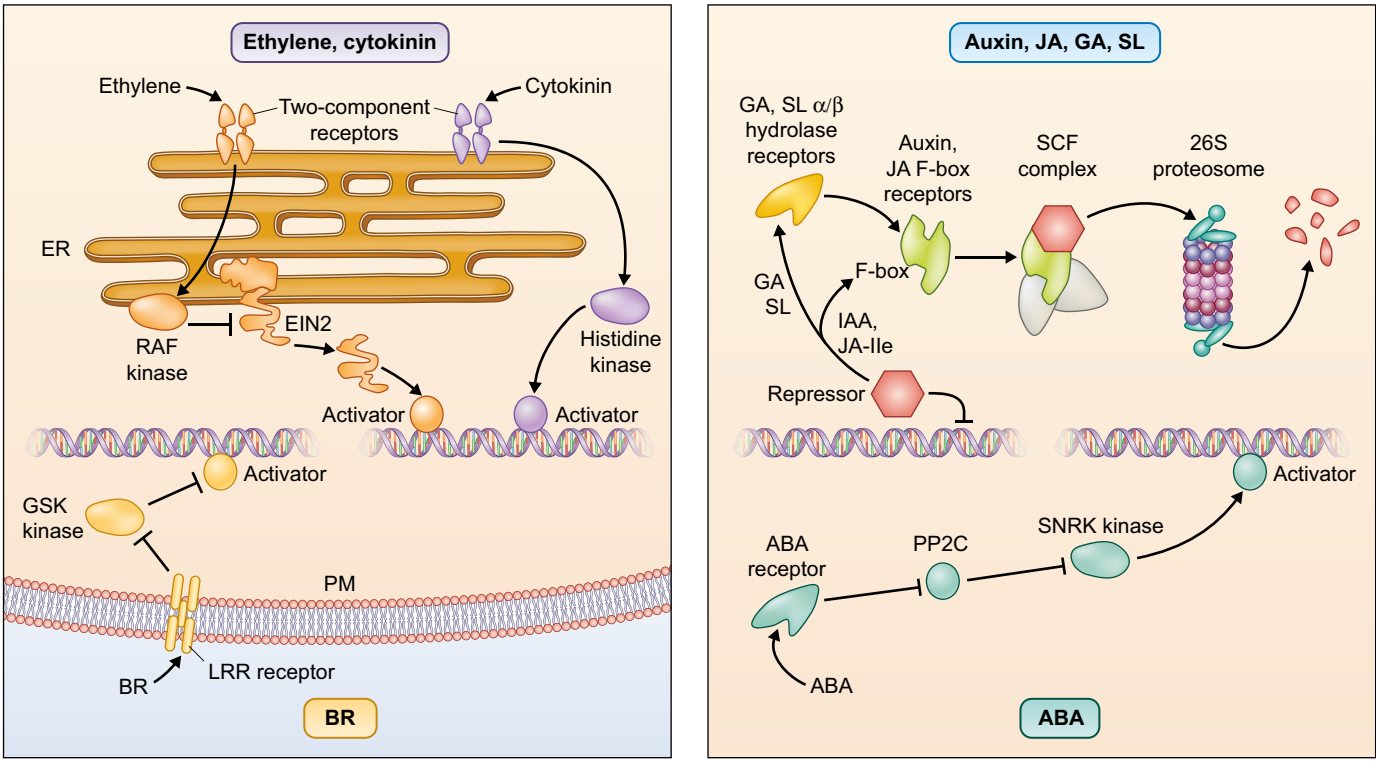

Fig. 5 Abridged mechanisms of hormone signaling as elucidated in Arabidopsis. ER, endoplasmic reticulum; RAF K, RAPIDLY ACCELERATED FIBROSARCOMA; EIN2, ETHYLENE INSENSITIVE 2; GSK, GLYCOGEN SYNTAHASE KINASE; LRR, leucine rich repeat; PM, plasma membrane; BR, brassinosteroid; JA, jasmonic acid; GA, gibberellic acid; SL, strigolactone; IAA, indole-3-acetic acid; JA-Ile, jasmonic acid-isoleucine conjugate; F-box receptor, F-box domain-containing receptor; SCF, SKP/CULLIN/F-box-containing complex; PP2C, PROTEIN PHOSPHATASE 2C; ABA, abscisic acid; SnRK, SUCROSE NONFERMENTING-1 (SNF1)-RELATED KINASE.

extensive analyses established that BRI1 is a plasma membrane-anchored LRR-kinase that binds BR along with a coreceptor BAK1 (Chang *et al.*, 1993; Li *et al.*, 2002). Activation of the BR receptor complex triggers a cascade of phosphorylation events that control transcription. Although the BR response pathway is the most 'animal'-like of the plant pathways, curiously animals perceive steroids primarily through soluble nuclear hormone receptors, which lack homologs in plants; Arabidopsis has therefore provided a window into alternate mechanisms of steroid action.

A second mechanism of hormone signaling derives from bacterial signaling modules and probably originated from the chloroplast genomes that were internalized early during plant evolution. Both ethylene (ET) and cytokinins (CKs) are perceived by endoplasmic reticulum (ER) membrane-anchored receptors that are homologous to bacterial two-component histidine kinases (Chang *et al.*, 1993; Inoue *et al.*, 2001). To identify mutations in these and other pathway components, Arabidopsis researchers took advantage of two classic physiological responses: the ET-controlled triple response of etiolated seedlings and the CK-induced greening of callus tissue (Bleecker *et al.*, 1988; Inoue *et al.*, 2001). For CK signaling, the pathway appears to be more like those seen in bacteria, which use a phosphorelay system from receptor to gene expression. In the case of ET, however, the pathway is novel, involving an 'animal'-like RAF kinase (CTR1) and an ER membrane-localized Nramp homolog (EIN2) between the 'bacterial'-like receptor (ETR family) and plant-specific transcription factors (EIN3 family) (Guzmán & Ecker, 1990; Roman *et al.*, 1995). Aside from defining the core components in ET signaling, Arabidopsis enabled rapid construction of double mutant lines and epistasis tests, which unequivocally ordered the steps of the pathway (Kieber *et al.*, 1993). Moreover, Arabidopsis enables quick construction of complex genotypes, which is onerous in many plants; this was essential to the rapid progress, as it allowed investigators to make functional insights despite the high redundancy that is typical of plant genomes. In this context, the ability to screen large numbers facilitated the isolation of rare dominant mutations that reveal phenotypes in spite of redundancy (Bleecker *et al.*, 1988; Inoue *et al.*, 2001).

The third and largest mechanism of plant hormone signaling involves soluble cytoplasmic receptors that modulate protein–protein interactions. Small molecule control of protein–protein interactions had previously been described with immunosuppressant drugs, but its role as a general signaling mechanism emerged from Arabidopsis. This 'plant'-like mechanism is used in the perception of auxin, jasmonates (JA), GAs, strigolactones (SL), salicylic acid (SA) and ABA (Lumba *et al.*, 2010). Auxin-regulated transcription is controlled by the AUX/IAA family of small corepressor proteins, whose stability is controlled by ubiquitin-mediated proteolysis via a canonical SKP/CULLIN/F-box-containing complex (SCF) mechanism, which itself turns out to play a hugely important role in a wide variety of plant processes, such as photomorphogenesis, circadian response and flower development (reviewed in Smalle & Vierstra, 2004). Auxin binds directly to the SCF's F-box, TIR1 or related Auxin-signaling F-Boxes, and creates a modified binding surface that stabilizes AUX/IAA binding, acting as a 'molecular glue' (Dharmasiri *et al.*, 2005; Kepinski & Leyser, 2005; Tan *et al.*, 2007). This promotes AUX/IAA ubiquitylation by the SCF and subsequent degradation. Although the molecular details differ from hormone to hormone, auxin illustrates a general mechanism: plant hormones directly stabilize protein–protein interactions that are coupled to control of enzyme action. Auxin, JA and SA control E3-ligase action and repressor degradation. GA and, most likely, SL also control degradation, although not by direct binding to an SCF, but by stabilizing complexes that in turn trigger degradation of repressors (Dill *et al.*, 2001; Stirnberg *et al.*, 2002). Although ABA does not signal through ubiquitin-mediated proteolysis, it still regulates phosphatase activity by stabilizing protein complexes between receptors and clade A PP2Cs (Ma *et al.*, 2009; Park *et al.*, 2009). It is noteworthy that all the receptors, with the exception of BR, are encoded by more than a single gene, and redundancy therefore complicated their genetic identification. While classic ABA-insensitive screens defined key signaling components, *bona fide* receptors never emerged from genetic analyses because of redundancy (Koornneef *et al.*, 1984; Merlot *et al.*, 2002). The identification of a receptor-selective agonist illustrated how chemical probes can combat this redundancy when combined with the power of genetic analysis of mechanism (Park *et al.*, 2009). The SL, ABA and SCF-type receptors all belong to large gene families that hint at the existence of undiscovered hormones. If true, clever genetic screens will help define these functions.

VIII. The plant immune system and Arabidopsis research

Plant breeders have, since days that were fast on the heels of the rediscovery of Mendel's laws (Biffen 1905), been remarkably successful in keeping crops one step ahead of pathogen evolution, which follows a classic 'arms race' model. Furthermore, the efforts of H. H. Flor and others provided a genetic concept for how recognition of pathogens required specific molecules encoded by the pathogen acting as single triggers of disease resistance gene products (Flor, 1971). However, the relative speed by which microbes and insects with short generation times can evolve to avoid plant immune system detection, combined with large-scale monoculture agricultural practices, gives pathogens and pests a never-ending advantage in this game of immunological hide and seek.

Arabidopsis research has been the key instrument in the remarkable progress that has been made in our understanding of the molecular details of the plant immune system. Arabidopsis research has also been an important cornerstone in the bid to describe how plants respond to chewing pests. A few milestones are outlined here, focusing mostly on genetics and older breakthroughs. A detailed review of this history was published in 2010 (Nishimura & Dangl, 2010). Currently, Arabidopsis remains the primary organism in which to derive new principles regarding how plants and microbes interact. This was not always the case. In the mid-to-late 1980s, several of the initial grant proposals from those looking to apply Arabidopsis as a model for plant–pathogen interactions were met with skepticism that a noncrop plant with such a short generation time would not have real pathogens.

Pioneering work from Shauna Somerville (Tsuji & Somerville, 1988), Alan Slusarenko (Koch & Slusarenko, 1990) and Eric Holub (summarized in Holub & Beynon, 1996) laid this shortsightedness to rest. By 1991, the first small workshop was organized at the Max-Planck Institute for Plant Breeding Research in Cologne, Germany, to discuss progress to date and to collaborate around shared bacterial and oomycete pathogen strains and standard protocols between labs *well before the submission of papers*. Attendees at that workshop agreed that the very open flow of ideas, reagents, detailed protocols and people among laboratories that had already been established in this subdiscipline was instrumental in driving further rapid progress. By 1993 there was significant momentum in the field and progress was made in establishing useful pathosystems with which to look for Arabidopsis mutants altered in their responses to infection (Dangl, 1993).

Important milestones (by no means an exhaustive list) were quickly achieved: isolation of the first intracellular immune system receptors, including several from Arabidopsis using both mutagenesis (Bent *et al.*, 1994; Mindrinos *et al.*, 1994) and natural variation (Grant *et al.*, 1995), revealed that the ability to respond to and stop infection was mediated by a superfamily of related proteins, now called NLR proteins after their nucleotide-binding, leucine-rich repeat domains. Subsequently, it became apparent that NLRs were often encoded at loci that had been the object of plant breeding for many decades, the so-called disease resistance or R genes.

The notion that a susceptible parent could give rise to mutants that were even more susceptible defined the concept of 'basal defense' and led to the identification of many key immune system cornerstones (Glazebrook *et al.*, 1997). Equally compelling was the isolation of mutants defining host loci required for successful systemic acquired resistance (Cao *et al.*, 1994) and host loci required by pathogens for successful pathogen colonization (Vogel & Somerville, 2000). A critical breakthrough that unified disparate thinking about microbe- or plant-derived small signal molecules that triggered very rapid plant cell biological responses was the definition and isolation of the flagellin receptor (Gómez-Gómez & Boller, 2000). Equally important was the finding that many pathogens deliver virulence effectors into plant cells to disrupt immune system signaling (Gopalan *et al.*, 1996; Leister *et al.*, 1996). A reasonably clear conceptual view of the plant immune system was at hand (Dangl & Jones, 2001; Jones & Dangl, 2006).

Breathtaking progress continues. Plant immunology has expanded beyond binary interactions of one pathogen and the plant to encompass the plant's above- and below-ground microbiota (Vorholt, 2012; Bulgarelli *et al.*, 2013). Similarly, progress in plant immunology has overlapped with the dissection of the molecular switches guiding normal growth and development (Belkhadir *et al.*, 2014). There is still no better test bed for the study of the plant immune system than Arabidopsis, once thought a useless little weed with no pathogens worthy of study.

IX. Signaling in response to abiotic stress

Counted among abiotic stresses are nonoptimal light environments, sub- or supraoptimal temperatures, drought, and nonideal soil conditions caused by nutrient limitation, salinity, or heavy metal content. Other abiotic parameters that affect plant growth are those related to atmospheric constituents, including soil O_2 profiles, ambient humidity and CO_2 concentrations, and atmospheric pollutants such as ozone. Although impacts of abiotic stresses on plant physiological attributes have been studied for many decades, it was not until the development of Arabidopsis as a model system that the mechanistic underpinnings of many abiotic stress sensing and response pathways began to be uncovered. It is impossible to do justice to the countless discoveries made in this area based on the study of this small yet mighty weed. Instead, a small sampling of noteworthy findings that exemplify the essentiality of key Arabidopsis-based tools is presented.

The ease of forward genetic screens in Arabidopsis enabled the identification of the cryptochrome, phototropin and Zeitlupe blue light sensors (Ahmad & Cashmore, 1993; Christie *et al.*, 1998; Kim *et al.*, 2007b; note that Koornneef *et al.*, 1980 also identified *cry1* (which they called *hy4*) in an Arabidopsis genetic screen for light-inhibited hypocotyl elongation mutants); in all three cases, classical genetics approaches were central to definitive photoreceptor identification and to discrimination of which light responses are governed by which photoreceptor (Briggs, 2014). The 'new(est) kid on the block', the UV-B photoreceptor UVR8, essential in plant protective responses to damaging UV stress, was also first identified in Arabidopsis, starting with screens for mutants exhibiting UV-B hypersensitivity (Kliebenstein *et al.*, 2002; Rizzini *et al.*, 2011).

Another major plant photoreceptor, the R/FR sensor phytochrome, was first identified in lettuce (Borthwick *et al.*, 1952) as the sensing agent and cloned from oats (Hershey *et al.*, 1985), followed by work in tobacco identifying it as a photoreceptor that controls flowering in response to day-length. However, whole-genome sequencing in Arabidopsis confirmed the presence of five phytochrome (*PHY*) genes, and a wealth of studies on induced, natural and insertional *phy* mutations has allowed partitioning of the multitude of plant R/FR responses between and among the five *PHY* gene products (Franklin & Quail, 2010). The complete genome sequence of Arabidopsis also ushered in the -omics era. There is a plethora of publications on Arabidopsis transcriptome and proteome responses to abiotic stressors. More recently, impacts of stress on the metabolome (Weckwerth, 2011), and Barbara McClintock's prescient speculation that the epigenome is stress-regulated (McClintock, 1984) have also been experimentally evaluated in Arabidopsis (Chinnusamy & Zhu, 2009; Iwasaki & Paszkowski, 2014).

One example of the power of transcriptome analysis is seminal work demonstrating the importance of the CBF regulon in cold acclimation (Thomashow, 2010). The identification of CBF target genes and gene cascades in Arabidopsis illustrates the value of a model species in which extensive annotation of promoter and other regulatory elements can enhance the comprehensive elucidation of transcriptional networks containing hundreds of individual elements. As a complement to transcriptome measures of RNA abundance, methods have recently been developed, initially in Arabidopsis, to elucidate RNA structures *in vivo* on a genome-wide basis (Ding *et al.*, 2014). As many abiotic stresses (e.g. temperature, heavy metals) are known to affect folding of RNA *in vitro*, it will be

of interest to determine *in vivo* stress-regulated RNA 'structure-omes'. Yet another -ome related to RNA is the translatome, defined as the set of mRNAs undergoing active translation. Low oxygen titer, as can occur in the soil during flooding stress, has wholesale impacts on cellular physiology as a result of its detrimental effect on cellular energy charge, and this has been illustrated by analysis of low O_2 effects on the translatome (Branco-Price *et al.*, 2008).

Also of particular note are targeted approaches first developed in Arabidopsis that allow -omes to be analyzed in single cell types (Dinneny *et al.*, 2008; Misra *et al.*, 2014). With regard to ABA signaling and drought stress, for example, the single most analyzed cell type is the guard cell. To date, -omics analyses of guard cells have been largely conducted in Arabidopsis and, to a lesser extent, in its brassicaceous relative, *Brassica napus*. And yet another -omics approach, chemical genomics, facilitated identification of the RCAR/PYR1/PYL soluble receptors for ABA (Cutler *et al.*, 2010; Raghavendra *et al.*, 2010). Indeed, identification of receptors for multiple plant hormones is one of the crowning achievements of Arabidopsis research (see the Hormones section).

While low soil water potentials initiate ABA and drought responses, nonoptimal soil mineral profiles also impact plant growth. Elegant biochemical genetic experiments in Arabidopsis have revealed that some inorganic nutrients, such as NO_3^-, are sensed by the membrane protein that also transports them (Ho *et al.*, 2009). On the other hand, Na^+ and heavy metals are toxic ions for which dedicated receptors have not been identified. Nevertheless, the proteins that bind and either extrude or sequester these toxic ions are essential to the survival of sessile plants subjected to adverse edaphic conditions. Our understanding of mechanisms underlying tolerance of such unfavorable conditions has particularly benefited from one of the more recent tools in the Arabidopsis arsenal: genomes from the Arabidopsis 1001 Genomes project, accompanied by extensive information on environmental conditions at the collection sites of these Arabidopsis accessions (Hancock *et al.*, 2011). This information, combined with development of statistical methods for genome-wide association study (GWAS) analysis (Segura *et al.*, 2012), is providing an unsurpassed resource for elucidation of the allelic diversity that can result from selective pressure exerted by environmental conditions (Assmann, 2013). One compelling illustration of the power of this approach comes from analysis of allelic diversity at the *AtHKT1* locus. The AtHKT1 transporter reduces Na^+ toxicity in the shoot via recovery of Na^+ from the xylem sap. GWAS and functional analyses have shown that a particular allele of *AtHKT1* confers improved salinity tolerance and is prevalent in accessions found in saline environments (Rus *et al.*, 2006; Baxter *et al.*, 2010).

Finally, it is crucial to note that mechanisms of stress tolerance first identified in Arabidopsis have been introduced into crop species with resulting agronomic improvements. For example, overexpression of either native or Arabidopsis CBF transcription factors in a number of crops has been shown to improve cold tolerance, and improved salinity tolerance is conferred in crops upon expression of Arabidopsis or native transporters that promote Na^+ extrusion or seclusion (Zhang *et al.*, 2004; Reguera *et al.*, 2012).

X. Pumps, channels, transporters and the like

The molecular nature of transporters was largely a mystery in plants before Arabidopsis started to be used as a model system. Most of what was known in the late 1980s was based on radiotracer studies and electrophysiology. Classical biochemistry led to the identification of a few transporters, the prime example of which is the plastidic triose phosphate translocator originally identified from spinach (Flügge *et al.*, 1989). But Arabidopsis rapidly took over as the key species in which transporter genes have been first identified. The proton ATPase protein was identified from oat and subsequently cloned simultaneously from *Nicotiana plumbaginifolia* and Arabidopsis (Schaller & Sussman, 1988; Boutry *et al.*, 1989; Harper *et al.*, 1989; Pardo & Serrano, 1989). At the same time, Sauer & Tanner (1989) identified the first sugar transporters from *Chlorella*. Just 1 yr later they had the homolog from Arabidopsis (Sauer *et al.*, 1990). A genetic trick rapidly changed the landscape: suppression cloning in yeast mutants. In 1992, two groups identified KAT1 and AKT1 potassium channels from Arabidopsis using yeast mutants (Anderson *et al.*, 1992; Sentenac *et al.*, 1992). In the same year, the first sucrose transporter was identified from spinach, followed by one from potato in 1993, and then in 1994 the Arabidopsis genes (Riesmeier *et al.*, 1992, 1993; Sauer & Stolz, 1994). Amino acid, iron, auxin, and many other transporters were then all identified first from Arabidopsis (Frommer *et al.*, 1993; Hsu *et al.*, 1993; Bennett *et al.*, 1996; Eide *et al.*, 1996; Gälweiler *et al.*, 1998). A unique exception was the identification of the sulfate transporter from a tropical grass *Stylosanthes* (Smith *et al.*, 1995). Arabidopsis became the most efficient system to clone transporter genes, and to study the localization, regulation and physiology by efficient access to mutants. Some of these important proteins were also studied in crops, yet discovery of new transporters is still occurring in Arabidopsis. And the value of a transporter identified from Arabidopsis is immediate – it explains quantitative trait loci (QTLs), and physiological roles discovered in Arabidopsis can be used to bring crops with improved properties to the field.

The use of Arabidopsis as a discovery tool continues to be productive: recently, a whole suite of novel sugar transporters, the SWEETs, has been identified in Arabidopsis. These have been shown to play roles across dicots in nectar secretion, in phloem loading, pollen nutrition and seed filling (Chen *et al.*, 2010, 2012, 2015; Lin *et al.*, 2014; Eom *et al.*, 2015). The application potential in the context of crop yield here is apparent, but surprisingly SWEETs also serve as pathogen susceptibility loci in rice, opening up the possibility of engineering resistance via TALENs or CRISPRs (Chen *et al.*, 2010; Li *et al.*, 2012). Vacuolar sugar storage is another example where Arabidopsis research lay the basis for discoveries with major biotechnological relevance: the sugar we eat is mainly derived from three sources: vacuole-stored sugars from sugarcane and sugarbeet or from fermented corn starch. The fermentation for winemaking critically depends on vacuolar sugars in grapes. Little was known about the transporters involved in vacuolar storage of sugars. A couple of years ago, Neuhaus's group identified a vacuolar monosaccharide transporter named TMT. TMT served as bait to identify a sucrose-transporting homolog (TST) in sugarbeet that is important for sugar accumulation in tap roots (Jung *et al.*, 2015).

In summary, Arabidopsis has been the workhorse that has not only served as an ideal system for identifying transporters, but also allowed for rapid identification of the role of the transporters and channels *in planta*. Importantly, the broad knowledge base derived from Arabidopsis has laid the groundwork for biotech applications, as summarized in detail elsewhere (Schroeder *et al.*, 2013).

XI. Cell walls, starch and lipids

The vast majority of what humans obtain from plants consists of cell walls, starch, sugar or lipids. Thus, many of the discoveries from Arabidopsis research have concerned synthesis, structure and function of these components.

1. Starch

Starch synthesis in Arabidopsis is similar to that in other plants. Arabidopsis has been important in identifying and characterizing enzymes involved in the synthesis of amylopectin and amylose, and the trimming and modification of nascent glucans to allow them to crystallize into a granule, and in identifying genes that control the initiation and number of starch granule per plastid (Crumpton-Taylor *et al.*, 2012; Seung *et al.*, 2015). By contrast, the pathway for degradation of starch in Arabidopsis is very different from that in germinating cereals, which is based on α-amylases (Smith, 2012). Screens for *starch excess* (*sex*) mutants in combination with reverse genetics revealed that solubilization of glucans at the granule surface is triggered by a unique cycle of phosphorylation and dephosphorylation (catalyzed by *SEX1*/*GWD* and *PWD*, and *PIPKIS1*, *LSF1* and *LSF2*, respectively), followed by a β-amylolytic attack to release maltose that is exported to the cytosol via a maltose exporter and converted to sucrose.

Source-limited plants regulate the rate of starch degradation to match the length of the night, thereby avoiding deleterious periods of carbon starvation at the end of the night (Stitt & Zeeman, 2012). Wild-type plants adjust the rates of both starch synthesis and degradation to maintain this pattern of starch turnover across a wide range of conditions. Control of synthesis has been attributed to allosteric and thioredoxin-dependent redox regulation of ADP-glucose pyrophosphorylase (Mugford *et al.*, 2014). The rate of starch degradation is regulated by the biological clock, which sets a maximum rate to ensure that starch is not exhausted before dawn (Smith, 2012; Stitt & Zeeman, 2012). These insights are leading to the development of new whole-plant growth models in which assumptions or rigid parameterization are replaced by modules that predict carbon allocation on the basis of the underlying regulatory pathways (Chew *et al.*, 2014).

As noted by Smith (2012), information from Arabidopsis cannot always simply be extrapolated to crop plants. However, the depth of understanding of Arabidopsis starch metabolism now informs and guides research in many crop plants.

2. Cell wall polysaccharides

Mutants in cell wall polysaccharide and proteoglycan composition have been isolated by screening for alterations in sugar composition of the polysaccharides, but reverse genetics is the preferred approach because many of the proteins of interest can be identified by sequence similarity to related proteins (Velasquez *et al.*, 2011). The mutants have been essential in deciphering the biochemical mechanisms by which cell walls are synthesized and deposited. An example of the types of perplexing observations from mutant analysis that reflect the incompletely understood complexity of cell walls was the surprising discovery that the major hemicellulose, xyloglucan, is dispensable (Zabotina *et al.*, 2012). However, mutations that alter the structure of xyloglucan are deleterious (Kong *et al.*, 2015).

For many proteins implicated in cell wall synthesis and assembly, the purified proteins cannot be assayed by conventional biochemical assays, but the function can be convincingly tested or observed in mutants or transgenic plants. For example, cellulose synthase cannot be reliably assayed in cell-free extracts, but the development of transgenic plants that express fluorescently labeled protein allows the individual complexes to be observed while synthesizing cellulose in live cells (Paredez *et al.*, 2006). Live-cell imaging revealed that the pattern of cellulose deposition is controlled by association of the cellulose synthase complexes with microtubules and also revealed that this process can be disrupted by several herbicides and by mutations in a number of genes, such as *CSI1*/*POM2*, *KOR* and *COB*, that appear to participate in the overall process. Thus, although many questions remain, such as what controls the amount of cellulose or the properties of the microfibrils, there is steady progress (McFarlane *et al.*, 2014).

Cell wall synthesis and deposition is part of the plant cell cycle, the response to pathogenesis and herbivory, and many aspects of morphogenesis. Several proteins that participate in the regulation of cell wall structure and function have been identified (Wolf *et al.*, 2012), but this aspect of plant biology is in its infancy.

3. Lignin

Lignification mutants of Arabidopsis were identified primarily by anatomical/histochemical identification of plants with reduced lignin deposition and screens for plants with reduced concentrations of the easily scored leaf metabolite, sinapoylmalate, the synthesis of which shares many steps in common with lignin (Vanholme *et al.*, 2010). Reverse genetic approaches have also contributed substantially, particularly for those enzymes encoded by multigene families, such as PAL and CAD, and where coexpression analyses (see the Databases and online tools section) suggested a potential role for previously unidentified catalysts, such as CSE. Along with other studies, the analysis of these mutants resulted in an almost total rewriting of a pathway that was thought to be well understood (Vanholme *et al.*, 2010).

Some mutants defective in lignin biosynthesis exhibit dwarfism and a collapsed xylem phenotype indicative of the importance of the polymer to cell wall rigidification and overall plant growth (Bonawitz *et al.*, 2014). Others, such as *cadc, cadd*, *fah1* and *comt1*, lead only to minor perturbations in development, even though, in these plants, lignin monomer composition is substantially altered, in some cases including noncanonical lignin subunits. Analysis of these mutants and other experiments have demonstrated the plasticity of lignin

composition that is an outcome of the nontemplate-mediated mode of synthesis of this biopolymer and have provided important insights into how lignin modification can be used to enhance the conversion of biomass in biofuel production scenarios.

Recent studies have begun to describe the complex regulatory machinery that orchestrates lignin deposition and maintains phenylpropanoid homeostasis (Bonawitz *et al.*, 2014; Taylor-Teeples *et al.*, 2015). The recent discovery that inactivation of three laccases leads to loss of lignin (Zhao *et al.*, 2013) has stimulated a reanalysis of the pathway of polymerization.

4. Lipids

Forward genetic approaches based on 'brute force' screens identified mutants with altered fatty acid, glycerolipid and wax compositions. These mutants facilitated studies directed at resolving uncertainties in some aspects of the pathways of fatty acid and lipid modification and facilitated identification and cloning of the corresponding genes. More importantly, mutant lines provided new insights into membrane structure and function (Wallis & Browse, 2010). The identification of mutants that globally affect oil biosynthesis led to the identification of critical transcription factors such as WRINKLED1 and LEC2 (Baud & Lepiniec, 2010). By contrast, discoveries in sphingolipid metabolism and function leveraged reverse genetics. Completion of the Arabidopsis genome sequence allowed comprehensive annotation of putative lipid-metabolism genes (Li-Beisson *et al.*, 2013), including homologs of yeast genes encoding enzymes of sphingolipid synthesis that were tested genetically by identifying T-DNA insertion mutants and biochemically by yeast expression (Markham *et al.*, 2013).

Transgenic approaches have been useful in many areas of lipid biology, particularly in seed lipid metabolism through experiments that engineer the fatty acid composition of vegetable oils. Efforts to increase the nutritional value of food oils and, alternatively, to produce unusual, industrially useful oil components have contributed to basic scientific knowledge (Nguyen *et al.*, 2010; Lu *et al.*, 2011). Recent studies highlighted differences between leaf and seed lipid metabolism that point the way to converting vegetative tissues of crop plants to accumulate oil (Fan *et al.*, 2014).

XII. Epigenetics and epigenomics: from genotype to phenotype

Compared with humans or other model systems, Arabidopsis has an unprecedented repertoire of chromatin regulatory proteins for methylating DNA, chemically modifying histones, or altering nucleosome spacing to control DNA accessibility to DNA-binding proteins (Pikaard & Mittelsten Scheid, 2014). Having multiple paralogous genes with partially overlapping functions presumably allows for fine-tuning of transcriptional programs that bring about physiological or developmental responses in a changeable environment. A consequence of having such a rich integrated network of partially redundant and overlapping functions is that null mutants in Arabidopsis chromatin regulatory protein genes are typically viable, allowing them to be studied. By contrast, corresponding mutations in metazoans are often embryo-lethal.

Genetic screens in Arabidopsis have uncovered biological roles of chromatin regulatory proteins in the control of developmental phase transitions, in cell identity switches and in activation of stress responses. One paradigm that emerged is that opposing types of chromatin regulators – those that inhibit and those that promote access to critical regions in the genomic DNA – together ensure that transcriptional activation of master regulatory proteins occurs at the correct stage and in the correct cell type and environment. Examples include activation of the floral homeotic genes in the developing flower, or silencing of the flowering time repressor *FLC* after prolonged cold (Goodrich *et al.*, 1997; Bastow *et al.*, 2004; Carles *et al.*, 2005; Aichinger *et al.*, 2009; Wu *et al.*, 2012; Hepworth & Dean, 2015, see this review for an overview of 'lessons' from FLC). In this manner, epigenetic mechanisms prevent 'unlicensed' gene activation that could be detrimental for growth or reproductive success.

Arabidopsis has been a particularly rich model system for understanding of DNA methylation and its regulation, with three cytosine methyltransferases responsible for maintaining CG, CHG and CHH methylation patterns (where H is a nucleotide other than G), and a fourth enzyme responsible for *de novo* methylation (Law & Jacobsen, 2010). Methylcytosine patterns can be perpetuated through multiple rounds of cell division, providing a basis for inheritance of epigenetic information. A fascinating aspect of *de novo* methylation is that noncoding RNAs specify the sites of DRM2 action via an elaborate process known as the RNA-directed DNA methylation pathway, whose elucidation by numerous laboratories represents a major effort over the past decade. Critical to the pathway are two plant-specific RNA polymerases, discovered in Arabidopsis and abbreviated as Pol IV and Pol V, that generate noncoding RNAs that guide the process. DNA methylation is not permanent (apart from a few cases, such as for *SUPERMAN* and *FWA,* where there does seem to be epigentic inheritance, as discussed in Henderson & Jacobsen, 2007) and can be passively removed through DNA replication or actively through enzymatic activities. Indeed, discoveries of ROS1 and DEMETER as DNA demethylases were important achievements (Choi *et al.*, 2002; Gong *et al.*, 2002). Maternal expression of *DEMETER* was shown to demethylate and derepress *MEDEA* and other methylated target genes whose maternal-specific expression was hitherto unexplained. There is crosstalk between DNA methylation and histones, and Arabidopsis has played an important role in attaining this understanding (Lindroth *et al.*, 2004; Johnson *et al.*, 2007). An important example of a mechanism for crosstalk between DNA methylation and histone methylation came from studies of the partnership between the H3K9 methyl transferase KYP/SUVH4 and the CHG methyltransferase CMT3.

Epigenomics, the genome-wide study of chromatin and DNA transactions, is a new field of research. It emerged following the advent of genomics in the late 1990s and the parallel development of DNA microarray technologies, replaced a decade later by a flurry of high-throughput DNA sequencing-based approaches. For the first time, it became possible to investigate systematically the transcriptional activity and chromatin state of genes as well as

nongenic sequences along large genomic regions or entire genomes. Indeed, pioneering work in Arabidopsis has led to major advances in our understanding of how genomes are functionally organized, notably through the first detailed molecular characterization in any eukaryote of a large region of heterochromatin, the first genome-wide mapping of cytosine methylation at single nucleotide resolution, and the integrative analysis of the genomic distribution of DNA methylation, histone modifications and histone variants (Lippman *et al.*, 2004; Cokus *et al.*, 2008; Lister *et al.*, 2008; Roudier *et al.*, 2011; Yelagandula *et al.*, 2014). Equipped with this new knowledge and with the development of methods to investigate cell type-specific epigenomes as well as the three-dimensional organization of the genome in the nucleus, attention is now turning to the dynamics of the Arabidopsis epigenome in response to endogenous or exogenous cues during development and growth.

XIII. Natural variation and GWAS

Arabidopsis is arguably unique among model organisms in that variation among so-called ecotypes was of central interest from the very beginning (Laibach, 1943). Classical linkage mapping approaches have a long history in Arabidopsis; and studies using biparental recombinant inbred line populations of Arabidopsis ecotypes have identified several genes underlying QTLs (Weinig *et al.*, 2002; Alonso-Blanco *et al.*, 2009; Keurentjes *et al.*, 2011).

Genome-wide association is rapidly becoming the default approach for studying the genetics of natural variation (Vilhjálmsson & Nordborg, 2012). *A. thaliana* was one of first nonhuman organisms for which GWAS became possible (Atwell *et al.*, 2010), and it has played (and continues to play) an important role in developing statistical methodology appropriate for heavily structured populations (Zhao *et al.*, 2007; Korte *et al.*, 2012; Segura *et al.*, 2012; Vilhjálmsson & Nordborg, 2012). There were two main reasons for picking Arabidopsis: one good and one bad. The bad reason was that, just as in humans, linkage disequilibrium was extensive enough for it to be conceivable to carry out GWAS cheaply, using sparse single nucleotide polymorphism (SNP) markers, without resequencing entire genomes (Nordborg *et al.*, 2002). And, just as in humans, rapidly decreasing sequencing costs have nullified this advantage, while the major disadvantage of extensive linkage disequilibrium remains: the difficulty of identifying the causal site(s) among many highly associated ones (Atwell *et al.*, 2010).

However, the good reason for picking Arabidopsis remains a very good one: the availability of naturally occurring inbred ('pure') lines, which make it possible to capture local (and presumably locally adapted) genotypes, and grow them with replication under a variety of conditions. The generation and characterization of genome-wide polymorphism data was a natural extension of this (Nordborg *et al.*, 2005; Clark *et al.*, 2007). At present, several hundred lines have been sequenced, in the sense of having been aligned and compared to the existing reference genome (Arabidopsis Genome Initiative, 2000; Cao *et al.*, 2011; Gan *et al.*, 2011; Long *et al.*, 2013; Schmitz *et al.*, 2013), and a publication describing a set of well over 1000 is in progress (www.1001genomes.org). When combined with the equally large collection for which dense SNP data are available (Horton *et al.*, 2012), over 2000 densely genotyped lines will be available.

The utilization of these lines is still far from ubiquitous, but is increasing. Studies involving hundreds of natural lines are time-consuming and many papers have yet to appear. Several studies have demonstrated the power of using GWAS as a tool for dissecting natural variation (Todesco *et al.*, 2010; Chao *et al.*, 2012; Karasov *et al.*, 2014), as well as for functional genomics (Todesco *et al.*, 2010; Chao *et al.*, 2012; Meijón *et al.*, 2014). GWAS is also likely to play an important role in interpreting genomic data sets, such as transcriptome or epigenome variation data (Schmitz *et al.*, 2013; Dubin *et al.*, 2015).

Where the Arabidopsis GWAS efforts have already had an impact is as a model for other studies, in particular in terms of statistical analysis. Unlike in humans, GWAS in Arabidopsis and maize were never carried out in 'case–control' settings (cases are people with a disease, and controls are a carefully selected set of healthy individuals from the same population), and it was thus immediately obvious that population structure would be a problem (Thornsberry *et al.*, 2001). The solution proposed (Yu *et al.*, 2006; Zhao *et al.*, 2007) – an adaptation of the classical 'animal model' from quantitative genetics (Henderson, 1984) – has since become the default approach in human GWAS as well as in other organisms. Indeed, GWAS in crops are now progressing much faster than in Arabidopsis, for the obvious reason that quantitative traits are of central interest in breeding (Huang *et al.*, 2011; Tian *et al.*, 2011).

The Arabidopsis germplasm collection is, of course, not only of interest for GWAS. The first effort to characterize polymorphism patterns on a large scale revealed a species-wide pattern of isolation by distance, with linkage disequilibrium decaying similarly to that in humans (Nordborg *et al.*, 2005). This work was followed by the generation of the first genome-wide SNP data (Clark *et al.*, 2007), which revealed interesting – and as yet unexplained – patterns of polymorphism. This work led directly to the generation of large, species-wide SNP data (Kim *et al.*, 2007a; Horton *et al.*, 2012) , which, in addition to GWAS, have been used to search for patterns of climate adaptation (Hancock *et al.*, 2011; Fournier-Level *et al.*, 2011). The extensive sequence data currently being generated are certain to generate new insights, about Arabidopsis and about adaptation more generally.

XIV. Gene regulatory networks

Gene expression is controlled by large protein complexes that include transcription factors and coregulators acting in a combinatorial fashion and organized into gene regulatory grids, consisting of all the possible connections between transcription factors and the corresponding target genes, in what is also known as the protein–DNA interaction (PDI) space. Gene regulatory networks provide a temporal and/or spatial manifestation of the gene regulatory grid (Mejia-Guerra *et al.*, 2012). The past decades have witnessed a complete cataloguing of Arabidopsis transcription factors, and the development of tools and approaches to investigate individual and genome-wide transcription factor–target gene

interactions. These include transcription factor-centered chromatin immunoprecipitation (ChIP)-based techniques (e.g. ChIP-chip and ChIP-Seq) and gene-centered approaches such as yeast one-hybrid. When combined with genome-wide expression experiments, for example contrasting plants that harbor a mutant or a wild-type allele for the particular transcription factor gene, these approaches provide powerful tools to probe plant gene regulatory networks (Moreno-Risueno *et al.*, 2010).

Not surprisingly, most studies so far have centered on mutants with interesting developmental phenotypes, resulting in a loosely associated collection of subnetworks that are starting to provide a first glimpse of what the Arabidopsis gene regulatory grid may look like (Heyndrickx *et al.*, 2014). For example, the control of flower development provides one of the earliest examples of how homeotic genes encoding a small set of transcription factors, primarily from the MADS-box family, combinatorially specify different floral organs, resulting in what was initially known as the ABC model (Weigel & Meyerowitz, 1994), which has since evolved into the ABC(E) model (Ó'Maoiléidigh *et al.*, 2014). Direct targets for SEPALLATA3 (SEP3) (Kaufmann *et al.*, 2009) and APETALA1 (AP1) (Kaufmann *et al.*, 2010a) have been identified, providing important insights into how plants control developmental switches (Kaufmann *et al.*, 2010b). Stomatal cell-fate specification in the Arabidopsis leaf is in part controlled by combinatorial interactions between the basic helix–loop–helix (bHLH) factors SCREAM (SCRM), SCRM2, SPEECHLESS (SPCH), MUTE and FAMA (Pillitteri & Torii, 2012). The MYB transcription factor FOUR LIPS (FLP) controls the final symmetric cellular division that results in the formation of two guard cells by directly inhibiting the expression of several cell division genes (Xie *et al.*, 2010). Cell division genes are also directly controlled by the MYB and bHLH transcription factors GL1 and GL3 in the control of another epidermal differentiation, the formation of leaf hairs, or trichomes (Morohashi & Grotewold, 2009). Modeling of root gene regulatory networks involving the SHORT ROOT transcription factor and other components is described in the Modeling, bioinformatics, and systems biology section.

The Arabidopsis gene regulatory networks studied are not limited to developmental processes. For example, a circadian clock-controlled nitrate-responsive gene regulatory network includes the bZIP1 transcription factor (Gutiérrez *et al.*, 2008). In turn, bZIP1-controlled genes include several direct targets that are transiently bound by this transcription factor (hit-and-run transcriptional control), and which could have been easily missed in conventional ChIP-Seq experiments (Para *et al.*, 2014). In contrast to transcription factor-centered approaches, genome-wide yeast one-hybrid experiments have uncovered a comprehensive gene regulatory network for secondary cell wall biosynthesis (Taylor-Teeples *et al.*, 2015), which is likely to inform our understanding of the regulation of similarly essential processes in other plants.

Resources that integrate information on experimentally validated PDIs are valuable because finding the information from the literature and from public DNA-sequence repositories is laborious. One such resource in Arabidopsis is provided by the AGRIS (http://arabidopsis.med.ohio-state.edu/) knowledge base. The AtRegNet module in AGRIS currently displays over 16 000 experimentally identified interactions between 94 transcription factors and the corresponding target genes. Clearly, while significant progress has been made, information on the targets of Arabidopsis transcription factors (estimated to be *c.* 2000) is in its infancy, but will rapidly expand with new data sets from protein-binding microarray studies (Weirauch *et al.*, 2014) and ChIP-Seq experiments with large numbers of Arabidopsis transcription factors (J. Ecker, unpublished).

XV. Modeling, bioinformatics, systems biology

The reference *A. thaliana* genome sequence and the facile nature of high-throughput gene expression profiling via microarray analyses resulted in a whole suite of data sets profiling the expression of individual genes across a range of cell, tissue and organ types. These and tools used to query them are described in the Databases and online tools section. Further functional genomic methodologies resulted in compilations of gene interactions including protein–protein interactions and protein-DNA interactions as described in the Gene regulatory networks section. In the midst of these massive data sets, however, elucidating biological novelty can still be an onerous task. In this section a subset of modeling and computational approaches that have been critical in the biological interpretation of these big data will be highlighted.

1. Gene expression in space and time

The Arabidopsis root has several developmental features that enable observation of cell type development in space and time. First, the root's stem cell niche is located at the root tip. Initial or stem cells divide and give rise to daughter cells. These daughter cells successively displace slightly older cells, resulting in a continuous file of cells along the root's longitudinal axis. Furthermore, most cell types are arranged in a rotationally symmetrical manner, which allows the observation of most cell types along the root's radial axis. In order to capture cell type-specific transcriptional profiles in the Arabidopsis root, fluorescent-activated cell sorting was coupled with transcriptional profiling (Birnbaum *et al.*, 2003; Brady *et al.*, 2007). Additionally, individual sections of the Arabidopsis root were captured and gene expression profiled, with each section representing a developmental time point. The cell type-specific transcription profiles often captured expression in only a subset of developmental stages. Furthermore, each section or developmental time point contained multiple cell types. These data could be analyzed with a series of bilinear equations that describe the proportion of a given cell type in a particular longitudinal section, in addition to the amount of expression of a gene in a given cell type at a specific developmental stage (Cartwright *et al.*, 2009). Using an iterative algorithm for finding approximate roots to these systems of bilinear equations, high-resolution spatiotemporal expression patterns of each gene could be reconstructed. These data may be visualized in Bio-Analytic Resource's eFP Browser (Winter *et al.*, 2007) and are a useful resource for plant biologists to determine when and where a given gene of interest is expressed in the Arabidopsis root at high spatiotemporal resolution.

2. Modeling root development and hormone responsiveness

Plant hormones have long been known to regulate plant growth, development and response to the environment. A variety of parameters have been described for passive and active transport of these hormones. The hormone auxin, for instance, is critical for root development but is transported by distinct transporters in distinct cell types and often in distinct directions (towards or away from the root tip). Modeling approaches have facilitated our understanding of how hormones regulate developmental processes by integrating two-dimensional and three-dimensional models of roots in conjunction with particular regulatory genes. In order to determine how a nested feedback circuit involving the transcription factors SHORT-ROOT, (SHR), SCARECROW (SCR), and the cell cycle mediators RETINOBLASTOMA-RELATED (RBR) and cyclin CYCLIND6;1 (CYCD6;1) precisely control the division of just a single stem cell daughter in a longitudinal file, a series of coupled ordinary differential equations (ODEs) was overlaid on a two-dimensional multicellular model of a root (Cruz-Ramirez *et al.*, 2012). These ODEs describe the wiring of this nested feedback circuit in addition to the influence of auxin on expression of *CYCD6;1*. The two-dimensional multicellular model of the root incorporated measurements of auxin transporter distribution and dynamics. Using these, a model was developed that explains how, in the stem cell daughter, the system is switched to the 'on' state to effect an asymmetric cell division (Cruz-Ramirez *et al.*, 2012). Similarly, in order to prevent the LAX3 transporter from being transiently expressed in multiple cell files, and thus not facilitating efficient lateral root emergence, modeling predicted that the auxin transporter PIN3 must be induced first, followed by LAX3 (Peret *et al.*, 2013). Experimental validation demonstrated that the model prediction was indeed correct. Recent reviews of root systems biology include those by Hill *et al.* (2013) and Wachsman *et al.* (2015). Finally, Boolean models have proved to be very useful in terms of predicting essential components of signal transduction networks in guard cell ABA and light response, with model predictions readily evaluated with Arabidopsis mutants (Li *et al.*, 2006).

3. Mapping transcriptional regulatory cascades

Developmental genetic studies have revealed hormone-mediated signaling via transcriptional cascades to be of prime importance in plant growth and development. The plant hormone ET controls fruit ripening, cell elongation and a multitude of other processes in part via the ETHYLENE INSENSITIVE3 (EIN3) transcription factor. Identifying the downstream targets of this transcription factor and their temporal regulation was recently characterized using a combination of ChIP coupled with sequencing and temporal whole-genome expression profiling (Chang *et al.*, 2013). Importantly, using the Dynamic Regulatory Events Miner (DREM; Schulz *et al.*, 2012), a series of four distinct waves of transcriptional regulation were identified. While the first wave of gene expression was quite variable and 'noisy', subsequent waves were less variable (Chang *et al.*, 2013).

Probabilistic functional gene networks utilize and integrate multiple, different data set types. The rationale for integrating these data sets and providing measures of their likelihood of interaction will enhance model accuracy and coverage. The AraNet network was generated using such an approach by integrating mRNA coexpression patterns from microarray data sets, known protein–protein interaction data sets, protein sequence features including protein domains, and similarity of phylogenetic profiles or the genomic context of bacterial or archaebacterial homologs (Lee *et al.*, 2010). Finally, diverse gene–gene associations were incorporated from yeast, fly, worm and human genes based on orthology (Lee *et al.*, 2010). Importantly, AraNet was able to generate potential gene functions for over 7465 genes lacking functional annotation.

XVI. Synthetic biology

1. How Arabidopsis is helping plant synthetic biology bloom

The newly emerged discipline of synthetic biology aims at engineering genetic, signaling and/or metabolic pathways in a predictable manner in order to create novel functionalities (Purnick & Weiss, 2009).

Synthetic biology in Arabidopsis can be classified into two major approaches: engineering plant networks in heterologous contexts; and engineering novel networks in plants with genetic components from other organisms, as depicted in Fig. 6. These two approaches are complementary and synergistic, and together they promote a systems-level understanding of plant biology. A growing list of new functions engineered in yeast with plant proteins highlight the potential of the first approach, including small molecule-induced protein degradation (Nishimura *et al.*, 2009; Havens *et al.*, 2012) or proximity (Liang *et al.*, 2011), light-induced gene expression (Shimizu-Sato *et al.*, 2002), or protein splicing and sender–receiver communication (Tyszkiewicz & Muir, 2008). Analysis of signaling components in a heterologous setting can generate new hypotheses about pathway function in its original context, particularly where redundancy, shared signaling components and feedback play significant roles. For example, recapitulation of auxin signaling in yeast revealed that the rate of auxin-induced degradation is highly variable across the large Arabidopsis Aux/IAA family (Havens *et al.*, 2012); and that the rate of degradation of Aux/IAAs is among the most effective tuning knobs for altering downstream transcriptional dynamics (Pierre-Jerome *et al.*, 2014). This led to the

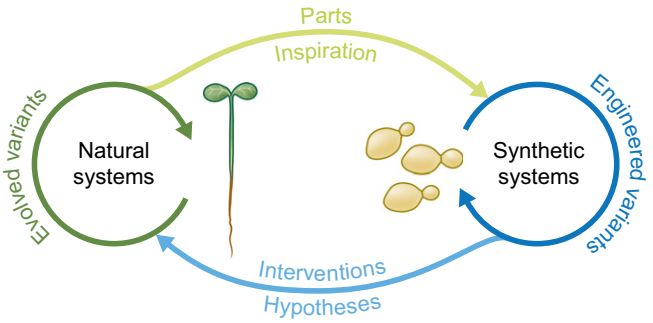

Fig. 6 The main approaches for synthetic biology in Arabidopsis.

hypothesis that the rate of auxin-induced Aux/IAA turnover was acting as a checkpoint for auxin-regulated developmental events. Indeed, when a set of degradation rate variants in IAA14 was engineered in yeast and expressed in plants, a striking correlation was discovered between the dynamics of IAA14 turnover and the dynamics of lateral root initiation (Guseman *et al.*, 2015).

Recently, there have been several compelling demonstrations of the second approach: synthetic engineering of plant networks (Sun *et al.*, 2014; Park *et al.*, 2015). One remarkable example is the recent report of the re-engineered ABA receptor (Park *et al.*, 2015). A screen of PYR1 variants led to identification of a hextuple mutant receptor with a switch in ligand recognition from ABA to an existing agrochemical compound for blight pathogens, mandipropamid (Park *et al.*, 2015). When transgenic Arabidopsis expressing the $PYR1^{MANDI}$ variant are exposed to mandipropamid, they have an ABA-like response, including transcriptional responses, stomatal closure and drought tolerance.

Synthetic biology relies on a set of tools that are increasingly becoming accessible to any molecular biologist. This could revolutionize both basic plant science and translational research for crops and energy biomass feedstock. The long history of such work in Arabidopsis lays the foundation for rapid gains in the near future.

XVII. Conclusions and outlook

Research using the model plant *A. thaliana* has clearly transformed the field of plant biology in the past 50 yr and this journey continues. As mentioned in the Databases and online tools section, new frameworks to house existing and yet-to-be-generated Arabidopsis data like Araport.org are in the works. In the fall of 2014, the NAASC developed a funding proposal to National Science Foundation (NSF) entitled 'Arabidopsis Research and Training for the 21st Century (ART-21)'. This recently funded proposal will engage community members over the next 5 yr in activities to capitalize upon the excellence in Arabidopsis research and facilitate new training directions for the next generation of diverse Arabidopsis research scientists. Collectively, the goal is to identify emerging technologies, such as network modeling (Bastien *et al.*, 2015; Dong *et al.*, 2015; Leal Valentim *et al.*, 2015) or digital image analysis (Montenegro-Johnson *et al.*, 2015; Reuille *et al.*, 2015), needed for 21st-century biology, and skills relevant to various types of organizations, not only to academia.

Criticisms have emerged with respect to the future role of Arabidopsis research in the plant sciences. The first involves the belief that 'Arabidopsis is not a real plant'; instead, we should be putting our efforts into crops. A second criticism is that many important crops will not work like Arabidopsis, hence why bother working on this 'little plant' anymore? The fault of this logic lies in the misconceived view of a model genetic system. Model systems are not created to explain everything but to give researchers a touchstone or reference for comparison. Without Arabidopsis, how would plant scientists ask, 'Is it like Arabidopsis'? For example, differences clearly exist between the way the methylome(s) function in maize vs Arabidopsis (Li *et al.*, 2014b). However, without the Arabidopsis baseline, how would we know this? But far more often than not, knowledge gained in Arabidopsis serves all of us well as a basis for follow-up experiments in other model plant species (e.g. in tomato: Piquerez *et al.*, 2014; since 2007, MASC has documented many translational examples in its annual reports, available at http://arabidopsisresearch.org). Although the NSF-funded Arabidopsis 2010 Project, which aimed to determine a function for all Arabidopsis genes, was wildly successful, 7540 Arabidopsis genes still have no GO biological process term associated with them, and 5944 have no GO molecular function term assigned (T. Berardini, TAIR, pers. comm., 21 May 2015). Determining the function of these will surely lead to a treasure-trove of new insights for plant biology in general. Interestingly, around the time of the publishing of the first draft of Arabidopsis genome, Lee Hartwell and colleagues suggested that genetic interactions would be essential to truly understanding the genotype–phenotype relationship in any organism (Hartman *et al.*, 2001). Hints of this vision are now clear from functional studies in yeast where double mutants can be systematically constructed (Tong *et al.*, 2004). It is difficult to see how parallel approaches in plants will be possible without Arabidopsis. It is ironic that, as governments around the world cut back on basic research (including Arabidopsis research) under the guise of 'cost' savings, such progress on a mechanistic understanding of how a plant or, for that matter, life works is within our reach. Perhaps with respect to Arabidopsis research funding, agencies should remember Winston Churchill's famous quote: 'This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning'.

Acknowledgements

Citation and bibliographic data for Arabidopsis publications were provided by Thomson Reuters, from their BIOSIS database, with funding for this provided by the North American Arabidopsis Steering Committee. N.J.P. would also like to thank David Guttman, Darrell Desveaux, and Zane Caplansky for helpful discussions regarding the literature analysis section of this review.

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