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Functional genomics of root growth and development in Arabidopsis

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Summary

Roots are vital for the uptake of water and nutrients, and for anchorage in the soil. They are highly plastic, able to adapt developmentally and physiologically to changing environmental conditions. Understanding the molecular mechanisms behind this growth and development requires knowledge of root transcriptomics, proteomics and metabolomics. Genomics approaches, including the recent publication of a root expression map, root proteome, and environment-specific root expression studies, are uncovering complex transcriptional and post-transcriptional networks underlying root development. The challenge is in further capitalizing on the information in these datasets to understand the fundamental principles of root growth and development. In this review, we highlight progress researchers have made toward this goal.

Introduction

The simple structure of the Arabidopsis root makes it ideal for studying root development. The root is composed of 15 distinct cell types (Figure 1), with the outer cell types arranged in concentric cylinders around the radial axis. Root cells are produced from stem cells in the meristematic zone at the tip of the root. The stem cells are kept in their undifferentiated state by the quiescent center (QC), a mitotically less active region composed of 4–7 cells in Arabidopsis. As new cells are produced from stem cells, older cells are displaced into single files toward the shoot, passing through the elongation and maturation zones as they age. Thus the root can be thought of as a developmental timeline, with young cells at the tip and differentiated cells more distal. In the past, most work on root development focused on single mutant analysis. Using this approach, researchers have defined many of the processes that pattern the different cell types within the root (recently reviewed in [1*]). Although this approach has been fruitful, it only begins to reveal the myriad interactions underlying the global complexity of root growth and development.

Recent publication of several genome-wide data sets of the root provide researchers the needed tools to understand this complexity. Already, these data are unveiling complex transcriptional and posttranscriptional pathways within the root. Major challenges in future years include moving past purely descriptive studies to understand the principles driving these pathways, integrating these large-scale datasets, and transferring what we learn from model plant systems to agriculturally relevant crop species. In this review we focus on analyses characterizing root

development with genome-wide approaches, and discuss examples in which researchers identified genes or underlying pathways involved in root development.

The Arabidopsis “Root Map”

Transcriptomics

Understanding the underlying biological principles guiding development requires knowledge of transcriptional, proteomic, and metabolic responses occurring in multiple cell types and developmental stages of an organ. Several recent studies are moving us towards this understanding [2,3**, 4–6]. Cell-type specific profiling reveals more transcriptional complexity than whole organ profiling, due to dilution of cell-type specific genes in whole organ experiments. Transcriptional profiling of nearly all cell types in the root coupled with 13 longitudinal sections created a complete spatiotemporal map of the root [3**]. The analysis revealed dominant expression patterns between ontologically unrelated cell types and identified those that fluctuate in developmental time. Examination of enriched *cis*-elements in these groups of coexpressed genes enabled prediction of new transcription factor targets. One pattern with enrichment of auxin biosynthesis functions and MYB-binding sites contained a single member of the MYB transcription factor family, *Altered Tryptophan Regulation 1* (*ATR1*). Of the genes within this pattern containing MYB binding sites, several had known functions in auxin biosynthesis, and all provide potential targets for *ATR1* regulation. Understanding the functional importance of these dominant expression patterns will contribute to our understanding of the networks that guide root development in space and time.

Another set of studies has examined the transcriptome of different organs and developmental stages of Arabidopsis, including the root, in response to over 40 conditions [4–6]. While not at cell-type specific resolution, the AtGenExpress data provide a powerful tool for coexpression analyses, reverse genetics, and functional genomics approaches.

Proteomics and Metabolomics

In addition to gene expression, proteins and metabolites contribute to an organism’s molecular phenotype. A recent global analysis of the Arabidopsis whole root proteome identified approximately 5159 proteins in 10 day old roots, and 4466 in 23 day old roots [7**]. Proteins in GO categories for intracellular protein transport, response to oxidative stress, and toxin catabolic process were overrepresented in their analyses.

Several recent reports analyze the effects of stress, nutrients, or genotype on the Arabidopsis metabolome [8–12], but these have focused primarily on aerial organs or whole plants. Cell-type specific proteomic and metabolic data are currently in development (Benfey lab, unpublished data). The combination of transcriptional, proteomic and metabolomic cell-type specific data will facilitate not only the functional identification of networks controlling root growth and development, but an understanding of how these networks interact with each other and the emergent properties of the root.

Towards Rice and Maize Root Maps

In contrast to the dicot root system of Arabidopsis, the monocot root system is composed of several different root structures with different functions. Genome-wide analyses of different root types and specific cell types in roots of grasses are important for understanding the differences between monocot and dicot root development. Laser capture microdissection (LCM) is frequently the method of choice for cell type specific analyses in species where GFP-marked lines are not readily available. A LCM transcriptome atlas for rice contains 40 cell types, including 13 from the root [13**]. As with Arabidopsis, GO category enrichment in specific cell types correlated with known biological functions, and new functions could be

predicted. Comparisons between Arabidopsis and rice roots showed interesting parallels and dissimilarities. This will be an exciting area for future research.

Though a comprehensive study examining all cell types within the maize root system has yet to emerge, several groups have begun to profile the transcriptome or proteome of different developmental sections of the primary root or different types of roots within the system [14–21]. Together these studies demonstrate differences in gene and protein expression between dissimilar root structures and cell types and highlight the need for additional work in this area.

Bioinformatics

One of the major challenges now facing biologists is how to capitalize on the wealth of information currently available. Integrating these ‘omics-based’ data sets in a systems biology approach is necessary for a complete understanding of the root as a whole. A full evaluation of these tools and approaches is out of the scope of this review, but for recent discussions see [22–25*].

Meta-analyses, in which many large-scale data sets are integrated to improve statistical confidence, have revealed global expression views of several root growth responses, including root patterning [26*] and those related to stress [27–29]. Using a meta-analysis of three microarray datasets, Levesque et al. [26*] identified 8 putative direct targets and nearly 500 indirect targets of SHORTROOT, a TF important for the developmental pathway regulating root patterning [30,31]. The authors suggest a model in which SHR regulation of these targets and the interactions between them determines the outcome of the SHR pathway in root development [26*].

Several studies have combined meta-analyses or data integration analyses with reverse genetics to identify the function of unknown genes [32*–36]. Although these studies are not root-focused, as more high-resolution root-specific datasets are created, similar approaches promise to lead to comparable results. Below, we highlight studies that have revealed novel genes and networks using transcriptomics or proteomics-based datasets in the root. Since a comprehensive review is not possible here, we focus on important examples of the use of genomic approaches to identify genes involved in the modulation of root growth and development by nutrient availability.

Dissecting root growth and development through functional genomics

Nutritional responses at whole root resolution

Soil nutrient deficiencies are one of the most important constraints for plant growth and development. Because nutrients are not evenly distributed in soils, changes in root architecture can profoundly affect the nutrient uptake capacity of plants. The availability of nutrients such as phosphate, nitrate and iron modulates lateral root (LR) and root hair (RH) formation and elongation [37]. Functional genomic analysis of plant responses to nutrient deficiency has been mainly based on transcriptional profiling coupled with the use of T-DNA mutants, overexpression, and RNAi strategies.

Pi starvation led to the differential expression of several hundred genes, of which 40 to 50% were repressed when Pi was re-supplied [38–40]. *PLDZ1* and *PLDZ2* are strongly induced in Pi deprived plants, and mutational analysis showed that the encoded phospholipases release Pi for other cellular activities and regulation of primary root growth by degrading phospholipids. This may be due to reduced Pi recycling or because the biosynthesis of phospholipid-derived signaling molecules is compromised [41,42]. In addition to genes involved in metabolic processes, four TFs induced by Pi deprivation were shown to play roles in root architecture

and/or root hair formation. Overexpression of *AtZAT6* altered root architecture [43], while mutant analysis of *AtbHLH32* reduced RH number and resulted in higher anthocyanin and Pi content [44]. RNAi suppression of *AtWRKY75* resulted in an increase in LR and RH number and length [45]. Finally, overexpression of a rice bHLH TF, *OsPTF1*, led to an increase in root biomass and a concomitant increase in tiller number and Pi content [46*]. It remains to be determined how these transcription factors act to coordinate Pi deprivation responses, however, these data show that genomic analysis can lead to the discovery of genes that could be used to enhance plant productivity in low Pi soil. Additionally, comparative proteome analysis of Pi-responses in maize, indicates that organic acid secretion, sugar metabolism and root-cell proliferation are important components of high tolerance to low Pi conditions [47].

Genomic analysis also led to the discovery of microRNAs involved in Pi homeostasis. Pi starvation rapidly induces the expression of microRNA399 (miR399), which mediates the degradation of the mRNA of *PHO2*. Arabidopsis *pho2* mutants accumulate excessive amounts of Pi in the shoot; suppression of *PHO2* by the transcriptional activation of miR399 leads to increased Pi acquisition and translocation to the shoot. miR399 acts as a systemic signal since it undergoes long distance movement from shoot to roots [48,49**].

Low postassium (K) availability also regulates root architecture, and leads to a reduction in the number and length of LRs. Microarray analysis showed that genes related to reactive oxygen species (ROS) are activated, while the TF *AtMYB77* is involved in K repressed in K limiting conditions. Further analysis revealed that H₂O₂ signaling and leads to increased K uptake [50] and that MBY77 modulates auxin sensitivity and LR formation by interacting with Auxin Responsive Factors, suggesting that reduction of LR formation under low K availability may be due to a reduction in auxin response [51*].

In addition to the nutrients discussed above, genome-wide nitrogen (N) responses have been examined in Arabidopsis, maize and *Medicago trunculata*. Over one thousand genes were found to be responsive in the three plant species, and responses to N deprivation were more rapid than for other nutrients [52,53]. Systemic signals were also shown to trigger specific transcriptome responses depending upon the nitrogen source available to the plants [54]. Although transcription profiling identified many N responsive genes, including several dozen TFs, to date the functional characterization of genes responsive to local or systemic N signals is still lacking.

Environmental responses at cell type resolution

Two recent studies give us the first look at environmental effects on the transcription network in specific cell types. Dinneny, Long and colleagues [55**] examined the response to high salt and iron deficiency in specific cell types and developmental stages of the root. Most responses to salt and iron were cell-type specific and were dependent on specific environmental conditions. The 244 genes unaffected by either stress were enriched for genes functioning in cell-type specification, suggesting that genes necessary for cell identity are environment-independent. Unexpectedly, genes co-regulated by salt and iron are not ubiquitously expressed. This indicates that cell-type specific genes may be targets for stress regulation. The level of conservation among biological functions enriched in cell types under high salt and low iron conditions varied according to cell type and stress, highlighting the need for additional studies in this area.

A similar study illustrates the power of these datasets to reveal novel connections in the root. Gifford et al. [56**] examined the effect of adding back nitrogen (N) on the transcriptional profile of different cell types in N-depleted roots. Their analysis revealed that miR167a,b and its target ARF8 regulate lateral root development in response to nitrogen. The authors identified 126 putative targets of ARF8 and showed that N status coordinately regulates these genes.

Conclusions

We are at an exciting time in root biology. The advent of large-scale, high resolution datasets provides the means to deepen our understanding of single genes and their phenotypic effects. There is a need for cell-type specific proteomic and metabolic data, as well as more high-resolution information regarding the root's response to different environmental stresses. Integration of these datasets coupled with reverse genetics and cell biology techniques will allow elucidation of root growth and developmental pathways at the systems level (Figure 3). Though much work remains to be done, we are now beginning to identify the complex transcriptional and post-transcriptional circuits that govern root developmental processes, both in standard and changing environmental conditions. Comparisons of Arabidopsis and rice cell-type specific root transcriptomes will reveal basic differences between dicot and monocot root systems. The challenges now include capitalizing on these datasets to extract the underlying biological meaning, translating this information to crop plants for which data are not available, and applying this information to crop improvement. Ultimately, these data will be a valuable tool for understanding root development across many plant species.

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Figure 1.

(A) Diagram of cell types in the Arabidopsis root meristem. The stele is a heterogenous tissue composed of multiple cell types, three of which are shown in the transverse section of the root in (B).

Figure 2.

Phosphate sensing and signaling in Arabidopsis. Phosphate levels are perceived both by local and systemic sensing systems. Phosphate starvation alters gene expression leading to alterations in primary root length, LR initiation and elongation, and root hair formation and elongation.

Figure 3.

Functional '-Omic' approaches for root growth and development: Isolation of specific cell types by FACS or LCM followed by RNA, protein, or metabolite extraction, coexpression analyses, and reverse genetics can identify factors in root growth and developmental processes.