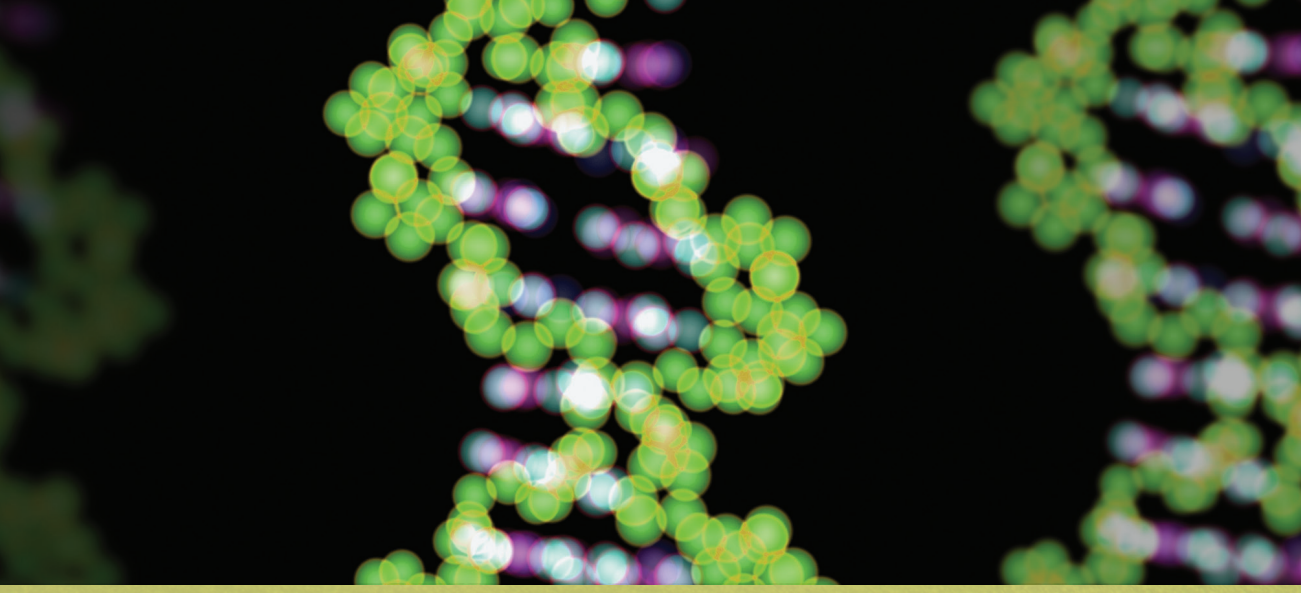

Root Genomics and Soil Interactions

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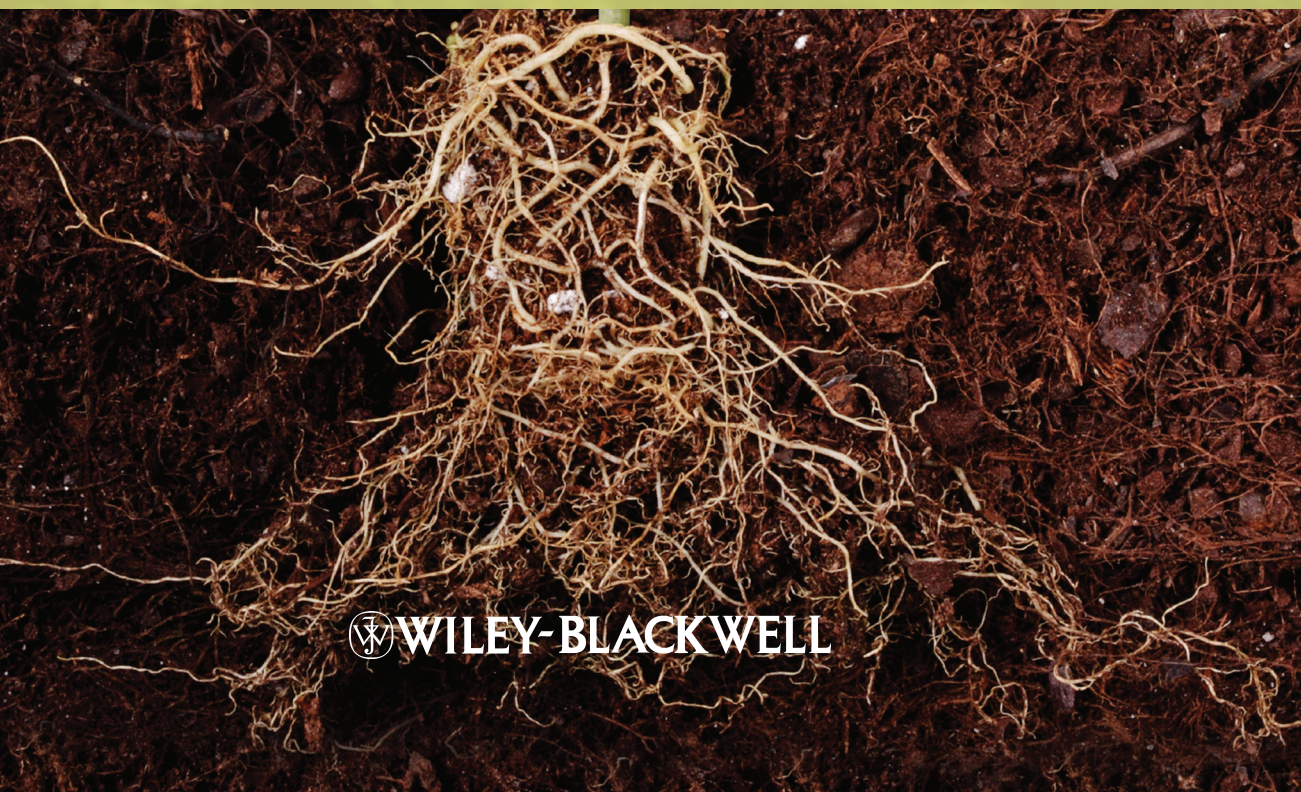

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Edited by
MARTIN CRESPI

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Preface

Roots and Their Soil Interactions: What We Can Learn from Genomics

Developmental plasticity allows higher organisms to adapt to their environment. In contrast to animals, plants exhibit a remarkable flexibility in their architecture and growth pattern in response to external conditions, due to the continuously active shoot and root meristems and their capability to generate new organs after embryogenesis. External cues influence plant growth by modulating hormone levels and signaling. The root architecture of the plant constitutes an important model to study how developmental plasticity is translated into growth responses under different soil conditions and plays an important role in water and nutrient acquisition. Indeed, primary root development and the formation of *de novo* meristems to generate lateral roots are conditioned by the soil environment. Lateral root growth and development is the main determinant of the shape of the root system, a trait controlled by internal cues and external factors. In addition to Arabidopsis, there are other relevant models where genomic information is becoming available, notably cereals and legumes. Both plants are able to develop symbiotic interactions with soil organisms, namely, mycorrhizal fungi and, for legumes, soil rhizobia. These interactions lead to further adaptation of root growth, the so-called mycorrhizal roots, and even to the formation of new organs, distinct from lateral roots, the nitrogen-fixing root nodules.

The diversity of root responses to biotic and abiotic stresses as well as symbiotic interaction can be analyzed at a genome-wide scale using transcriptomic and proteomic approaches. The advent of genomic technologies will open new perspectives for the analysis of how roots adapt to the soil environment. This work, mainly done in model systems such as Arabidopsis, uncover diverse regulatory genes (e.g., environmental sensors, protein kinases, transcription factors, and more recently, small regulatory RNAs) that participate in genetic programs, regulating root growth and architecture. Integration of these data with genomic approaches on different genetic backgrounds has already revealed, and will continue to reveal, critical regulatory networks and molecular hubs, whose orthologs could then be analyzed in crop plants to establish the generality of these mechanisms and impact agricultural practices.

This book contains 13 chapters from recognized experts in the field, which provide a comprehensive and integrated view of how root genomics can open new perspectives for root physiology and agriculture. The first six chapters deal with various novel areas where genomics, in combination with modeling, physiology, in-depth analysis of the transcriptome, and epigenetics, have revealed several regulatory networks controlling diverse aspects of root growth and development. Then, the remaining chapters describe genomic approaches being applied for the analysis of root responses to the soil environment, such as abiotic stresses, symbiotic interactions, or pathogenic nematode infections. The final chapter focuses on translational genomics and how genomics can guide crop

improvement. I hope that this book will serve many, from plant researchers to plant and crop physiologists, breeders, graduate students, and their professors who want to have an overview of the highlights in root genomics and how this information could be screened and integrated without having considerable expertise in bio-statistics. While reading this book, the reader will realize how fascinating the actual global view of the genome is and how many complex mechanisms remain to be discovered to understand root growth and development. There are exciting agricultural challenges, such as the modulation of root architecture or drought adaptation, which may derive from the application of this new fundamental understanding of life principles to the control of major root traits.

Martin Crespi

Root Genomics and Soil Interactions

1 Genomics of Root Development

Boris Parizot and Tom Beeckman

Introduction

Roots: Rising from the Underground

Because of the different roles the root system plays in overall plant growth, root architecture is a fundamental aspect of plant growth and development. The root system especially acquires water and nutrients from the soil, anchors the plant in the substrate, synthesizes hormones and metabolites, interacts with symbiotic microorganisms, and insures storage functions. In light of these characteristics, more and more breeders turn their attention to this underground organ in order to increase yield. This requires a better understanding of the relation of this part of the plant with the environment and of its highly adaptive behavior (Lynch 2007; Gewin 2010; Den Herder et al. 2010).

Within the angiosperms, major differences in root architecture between dicotyledonous and monocotyledonous plants exist. Dicots develop a tap root system composed of a main primary root, already formed during embryogenesis, which grows vertically into the soil and gives rise to the emergence of numerous lateral roots extending the surface area. Monocots have a fibrous root system in which the embryonic primary root is only important for the early development of the plant (Feix et al. 2002) and in which an extensive postembryonic shoot-born root system is formed later on. Very little is known about the genetic and molecular mechanisms involved in the development and architecture of the root system in major crop species, generally monocotyledonous plants. Lack of insight is certainly a consequence of the difficulty to access and observe this organ in its natural habitat, namely the soil. Moreover, and probably because of this hidden character, the root has been neglected for a long time in crop improvement and in agricultural approaches aiming at increasing shoot biomass. Nevertheless, while most of the work has been done on *Arabidopsis thaliana*, the awareness of the importance of the root system in modulating plant growth, together with progress in sequencing and new molecular techniques, has caused renewed interest in understanding molecular mechanisms in crop species (Hochholdinger and Zimmermann 2009; Coudert et al. 2010).

In the scope of root development and its interaction with the soil, in this chapter, we propose to focus on the mechanisms involved in root branching, which is a major determinant of root system architecture. The plasticity of the root system represents indeed an important potential for plants, being sessile organisms, to adapt to the heterogeneity of their environment. The soil is a complex mixture of solid, gaseous, and liquid phases, wherein nutrients are unequally distributed. Plants have therefore developed a highly sophisticated regulatory system to control their root architecture, in

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response to environmental cues, by modulating intrinsic pathways to optimize their root distribution in the soil and consequently guarantee an optimal uptake of nutrients necessary for growth and development (reviewed in Croft et al. 2012).

Primary Root Structure and Development: Lessons from the Arabidopsis Model

Branching of roots occurs through the development of new meristems inside the primary parent root. We therefore first discuss briefly the structure and development of the primary root in *Arabidopsis*, the model species in which major insights were obtained, thanks to its simple root architecture (Dolan et al. 1993; Malamy and Ryan 2001; Scheres et al. 2002; Casimiro et al. 2003; Casson and Lindsey 2003; Ueda et al. 2005; Iyer-Pascuzzi et al. 2009; Peret et al. 2009).

The root can be divided in three main zones. The most distal, at the tip of the root, is the meristematic zone, where the so-called initial cells give rise to the tissues constituting the root. The initial cells are kept in an undifferentiated state by the neighboring quiescent center (Van den Berg et al. 1997), a mitotically less active region, composed of few central cells in *Arabidopsis*. Higher up, in the elongation zone, cells progressively stop dividing and start to expand longitudinally. Finally, cells differentiate and acquire their final cell fate in the maturation zone (Truernit et al. 2006), which can be recognized by the appearance of the anatomical structures of the vascular tissues.

Distinct cell types are then composing the mature root (Figure 1.1). The outer layers, endodermis, cortex, and epidermis are organized in concentric layers and present a radial organization toward the longitudinal axis of the primary root (Dolan et al. 1993). The epidermis, which is the outermost layer of the root, is in direct contact with the soil and is often designated as rhizodermis. It is composed of two populations of cells: one producing root hairs and the other nonhair cells (Schneider et al. 1997). The root hairs are responsible for the major part of the nutrient uptake from the soil (Muller and Schmidt 2004) and also play other important roles such as the initial contact with certain symbiotic partners (Gilroy and Jones 2000; Perrine-Walker et al. 2011). Cortex and endodermis constitute the ground tissue and are derived from one single initial cell in *Arabidopsis* (Dolan et al. 1993; Scheres et al. 1994). The stele is situated internal to these layers and comprises the vascular cylinder, consisting of two bilateral poles of xylem alternating with two bilateral poles of phloem separated by procambium cells (Dolan et al. 1993). The stele also contains a heterogeneous layer, the pericycle, interfacing the vascular cylinder and the outer layers, playing a predominant role in root architecture and root branching (Parizot et al. 2008).

Root Branching

In dicotyledonous plants, such as *Arabidopsis*, elaboration of the root system occurs postembryonically by the formation of numerous secondary roots from the primary root that was formed during embryogenesis. These new roots are comparable to the primary root in structure and will be able to reiterate the branching process by in turn initiating tertiary roots. Roots of second, third, and higher order are defined as lateral roots. The plant can also produce adventitious roots, which initiate mostly at the base of the hypocotyl. Different markers related with cell identity show a similar pattern in the primary and lateral roots (Malamy and Benfey 1997b; Laplaze et al. 2005), indicating the possibility of a common developmental pathway. This hypothesis is supported by a high number of mutants affected in genes involved in root patterning, such as *SHORTROOT*, *SCARECROW*, and *LONESOME HIGHWAY*, showing similar defects in the primary and lateral roots (Helariutta

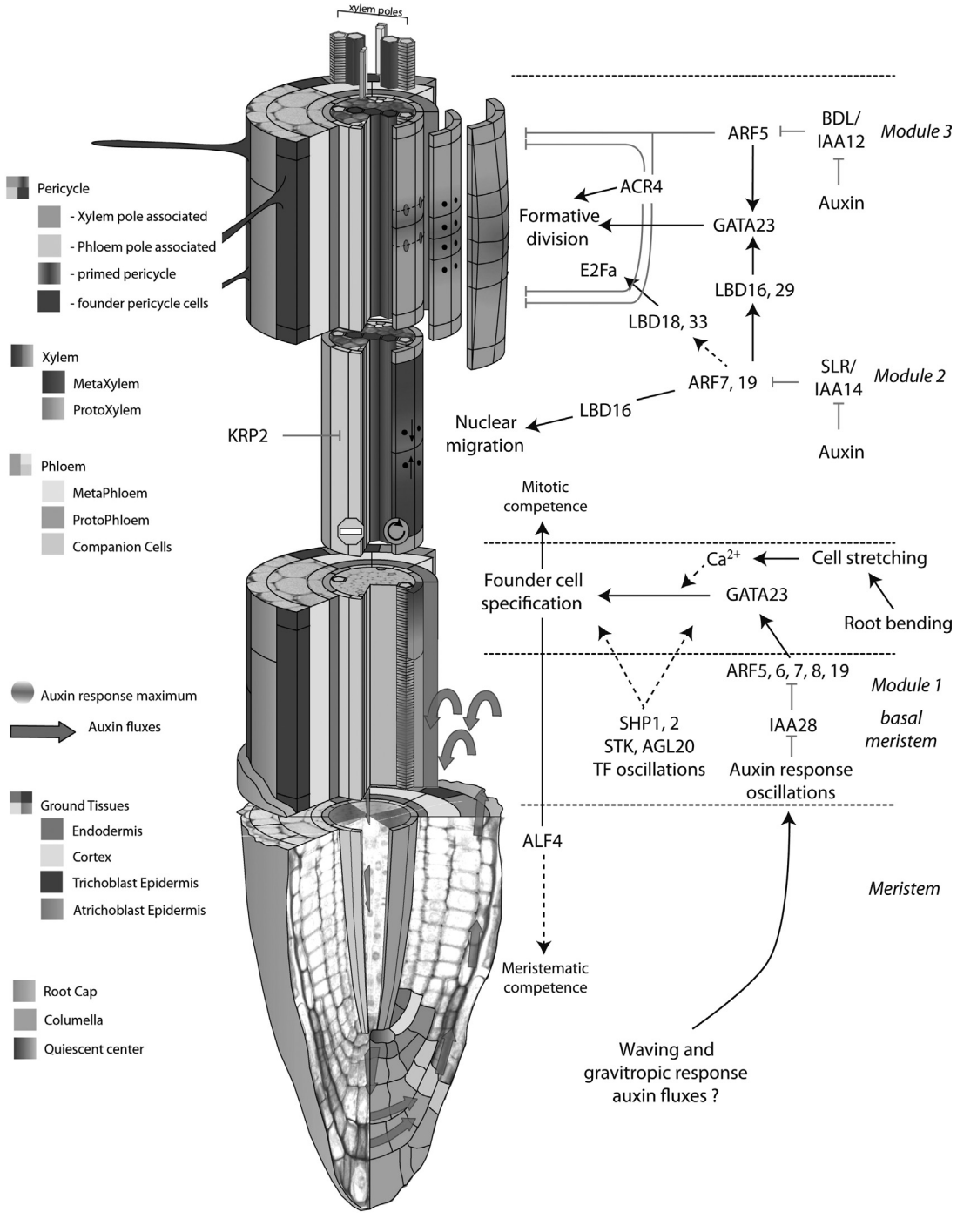


Figure 1.1 Structure of the primary root and different steps of lateral root initiation. See the text for detailed description. (For a color version of this figure, see the color plate section.)

et al. 2000; Wysocka-Diller et al. 2000; Parizot et al. 2008; Lucas et al. 2011). However, some differences can be observed in the behavior of the primary and the lateral roots toward external cues such as gravity and substrate nutrient concentrations (Zhang and Forde 1998; Mullen and Hangarter 2003; Bai and Wolverton 2011). A mutation in the gene *MONOPTEROS* impairs the apical–basal pattern formation of the embryo and leads to plants lacking a primary root, but that are still able to generate adventitious roots (Berleth and Jurgens 1993; Przemeck et al. 1996), indicating that early pathway(s) required for the embryonic formation of a root meristem are not required postembryonically. Also, a mutation in the gene *WOODEN LEG* has a major effect on the primary root development, with the suppression of the phloem elements and a drastic reduction in lateral root initiation (LRI), but does not affect the formation and branching of adventitious roots (Kuroha et al. 2006).

The monocots, such as maize, form different types of roots: primary, seminal, and adventitious roots, which can all form lateral roots. These root types also present similarities in their structures. However, mutants missing only a subset of these root types have been isolated, indicating that at least a part of the genetic program necessary for their formation is root-type specific (Woll et al. 2005; Hochholdinger and Tuberosa 2009).

Lateral Root Initiation

In *Arabidopsis* and most other dicotyledonous plants, lateral roots are formed from a restricted number of pericycle cells located in front of the xylem poles (Figure 1.1). The pericycle is a heterogeneous tissue composed of quiescent cells adjacent to the phloem poles and cells competent for LRI in front of the xylem poles (Beeckman et al. 2001; Parizot et al. 2008). Therefore, this layer presents a radial bilateral symmetry along the primary root, which reflects the diarch symmetry of the more internal vascular bundle as compared to the surrounding concentric radial layers of the outer tissues. The subpopulation of pericycle cells adjacent to the xylem poles can be considered as an extended meristem, as they conserve the ability to divide after leaving the root apical meristem (in contrast to the cells in front of the phloem poles), and give rise to the formation of a new organ (Beeckman et al. 2001; Casimiro et al. 2003). Although up to three adjacent pericycle cell files associated with each xylem pole are dividing during lateral root formation, cell lineage experiments have shown that only the central cell file will contribute significantly to the formation of the lateral root primordium (Kurup et al. 2005).

The first pericycle cell divisions that will give rise to a lateral root (i.e., formative divisions) can only be detected several millimeters above the primary root meristem, whereas in the lower part of a region named developmental window (Dubrovsky et al. 2006), it has been demonstrated that a subset of pericycle cells is already specified for LRI in a zone situated immediately above the primary root apical meristem, the basal meristem (De Smet et al. 2007; De Rybel et al. 2010b). The phytohormone auxin is most likely the signal triggering this priming, as auxin response recorded using the auxin response marker DR5 shows pulsations in the protoxylem cells of the basal meristem with a periodicity that can be correlated with the initiation of new lateral roots (Ulmasov et al. 1997; De Smet et al. 2007; De Rybel et al. 2010b; Moreno-Risueno et al. 2010). Up to now, different hypotheses have been proposed to explain the origin of these oscillating auxin response maxima in the protoxylem cells, and no consensus has been reached yet. Also the mechanism by which this auxin signal in the protoxylem cells is translated into the specification of founder cell identity in the neighboring pericycle cells is still unknown. Nevertheless, this intrinsic mechanism can

be overruled, as the application of auxin on mature parts of the root above the basal meristem is still able to trigger LRI (Himanen et al. 2002), further reflecting the high plasticity of the root system.

The first morphological event preceding the division of two adjacent pericycle founder cells is the simultaneous migration of their nuclei to their common cell wall (De Smet et al. 2007). This migration is followed by an asymmetric anticlinal division of the pericycle cells, resulting in the formation of a core of small daughter cells flanked by larger cells (Dubrovsky et al. 2000). Successive anticlinal and periclinal divisions give rise to a lateral root primordium. Further divisions and elongation of the primordium cells result in the formation of a fully autonomous root, with a meristem similar to that of the primary root (Malamy and Benfey 1997b; Dubrovsky et al. 2001). Although the place of LRI differs between plant species, early patterning of the primordium is quite conserved (Casero et al. 1995; Malamy and Benfey 1997bb). The frequency of LRI in the *Arabidopsis* primary root can fluctuate in response to tropic and/or mechanical stimuli (De Smet et al. 2007; Ditengou et al. 2008; Laskowski et al. 2008; Lucas et al. 2008a). For example, a gravitropic stimulus applied to seedlings induces a lateral root at the place where the root bends to recover its normal growth angle (Lucas et al. 2008a).

Genomics of LRI

Most of the work on root development focused on the analysis of single mutants and allowed the discovery of many processes involved in the patterning of the different cell types within the primary root and in LRI. These studies show that root growth and development are complex processes with intricate pathways dealing with hormone biosynthesis, transport and signaling, tissue differentiation and dedifferentiation, nutrient sensing, cell divisions, and others (Iyer-Pascuzzi and Benfey 2009; Orman et al. 2011).

LRI has been increasingly studied over the last decade in the light of transcriptomics and proteomics. Material extraction for these analyses evolved from simple global root harvesting to more elaborated sampling techniques allowing a specific access to the tissues involved, such as laser capture microscopy (LCM; Woll et al. 2005) or cell sorting (De Smet et al. 2008). Also, the possibility to synchronize LRI circumvented the problems due to the discreteness of this event in plants grown in natural conditions (Himanen et al. 2002; Himanen et al. 2004). Different large-scale transcriptome and proteome studies have therefore been realized in different species, mainly *Arabidopsis* and maize, yielding information on various aspects of this de novo organogenesis: auxin response, asymmetric cell division, and pericycle tissue involvement. While these studies focused initially on the onset of lateral root development, a new era initiates now with the study of the formation and the patterning of the primordium after LRI and the emergence of the primordia from the parent root. Moreover, many other experiments are dealing with mechanisms related to LRI, such as meristem function, pericycle identity, and hormone treatment, and bring useful novel information, shedding light on this process. A list of omics experiments, directly or indirectly related to LRI is displayed in Table 1.1. A challenging task for the community will be to handle this wealth of data and search for appropriate system biology strategies to better understand the LRI process at the molecular level. To address this, a common effort of the biologists and the bioinformaticians is needed to design better experiments, rationalize and interpret the data, and make it accessible and understandable for the community. The most often characterized process in relation to LRI is the response to the hormone auxin.

Table 1.1 Omics Experiments dealing directly or indirectly with lateral root initiation. Publication year and reference, species, technique and experimental design, platform, tissues and preparation, pathways, treatments, and the biological process questioned by the experiment.

Year	Reference	Species	Omics technique & Experimental design		Platform / Technique
2003	**Birnbaum et al.**	Ath	Ts	Transcriptome atlas using FACS and GFP-reporter lines	Affymetrix ATH1
	Imin et al.	Mtr	Pr	Protein expression at the globular stages of somatic embryogenesis	Two-dimensional electrophoresis + MALDI-TOF-MS and MS/MS
	Menges et al.	Ath	Ts	Gene expression profiles during normal culture growth, synchronous cell cycle re-entry and synchronous cell cycle progression	Affymetrix ATH1
2004	**Brinker et al.**	Pta	Ts	Gene expression during adventitious root development by treating hypocotyls with auxin	Custom Pinus taeda microarray (2,178 cDNAs)
	Himanem et al.	Ath	Ts	Gene expression during lateral root inducible system (LRIS)	Spotted cDNA microarray (4k, Incyte Microarray Systems, Fremont, CA)
	Hochholdinger et al.	Zma	Pr	Comparison of protein accumulation between lrt1 mutant and wild type	Two-dimensional separation of proteins and MALDI-TOF mass spectrometry
	Nemhauser et al.	Ath	Ts	Gene expression upon treatment with Auxin or Brassinolide	Affymetrix ATH1
2005	**Casson et al.**	Ath	Ts	Gene expression in the apical and basal domains of the embryo at globular and heart stage	Affymetrix ATH1
	Nawy et al.	Ath	Ts	Tissue layer specific gene expression using FACS and GFP-reporter lines of the quiescent center and columella root cap	Affymetrix ATH1
	Okushima et al.	Ath	Ts	Comparison of gene expression upon auxin treatment between *arf7* , *arf19* , and *ar7 arf19* mutants and wild type	Affymetrix ATH1
	Vanneste et al.	Ath	Ts	Comparison of gene expression upon lateral root inducible system (LRIS) between *slr* mutant and wild type	Affymetrix ATH1
	Woll et al.	Zma	Ts	Comparison of gene expression between *rum1* mutant and wild type pericycle cells	Spotted cDNA microarray (12k , Generation II, Version A Iowa State MicroArray Facility)
2006	**Che et al.**	Ath	Ts	Gene expression during shoot, root and callus development in Arabidopsis tissue culture	Affymetrix ATH1
	Druka et al.	Hvu	Ts	Transcriptome atlas	Affymetrix Barley1 GeneChip
	Jiang et al.	Zma	Ts	Gene expression in the root cap, compared to the primary meristem and the quiescent center	Affymetrix Heterologous rice GeneChips
	Laskowski et al.	Ath	Ts	Gene expression during auxin treatment of seedlings roots	Custom Arabidopsis microarrays
	Lee et al.	Ath	Ts	Tissue layer specific gene expression using FACS and GFP-reporter lines of the phloem, xylem, and cortex	Affymetrix ATH1
	Levesque et al.	Ath	Ts	Comparison of tissue layer specific gene expression in *shr* mutant in comparison with wild type	Affymetrix ATH1
	Liu et al.	Zma	Pr	Comparison of protein accumulation between the mutant *rum1* and wild type	Two-dimensional electrophoresis + nanoHPLC-ESI-MS/MS massspectrometry
	Mouchel et al.	Ath	Ts	Comparison of gene expression in a natural allele variant of BRX gene with wild type, upon treatment with Auxin or Brassinolide	Affymetrix ATH1
2007	**Brady et al.**	Ath	Ts	Transcriptome atlas using FACS and GFP-reporter lines	Affymetrix ATH1
	Dembinsky et al.	Zma	TsPr	Comparison of gene expression and protein accumulation between the stele, the pericycle and the ground tissues	Custom 12K microarray (GenII vA: GPL 4876) and 2D electrophoresis + nanoHPLC-ESI-MS/MS
	van Noorden et al.	Mtr	Pr	Comparison of protein accumulation between nodule initiation and auxin treatment in Medicago truncatula	Two-dimensional DIGE electrophoresis + MALDI-TOF/TOF MS

Table 1.1 *(Continued)*

Tissues	Tissue preparation					Pathways		Treatment							Biological process									
	Organ isolation	Micro dissection	LCM micro dissection	Cell Sorting	Other	Mutants	Biotic, abiotic factors	Auxin	NPA	Cytokinin	Brassinolide	Gibberellin	Stress	Biotic	Other	Lateral Root Priming	Lateral Root Initiation	Lateral Root Patterning	Root organogenesis	Embryo & Primary root	Pericycle	Primary root meristem	Callus & Regeneration	Tissue identity	Cell Cycle
Primary root sections and root cell layers																				D		D		D	
Embryonic cultures																			i	D					
Cell suspension															i		i								D
Hypocotyl segments								i									i	i	D						
Root section								i	i								D								
Primary roots						*lrt1*											D					i			
Entire seedlings								D			D						i								
Embryo apical and basal domains																			i	D		i			
Quiescent center and columella root cap																						i		D	
Entire seedlings						*arf7, arf19*		D									i								
Root segments						*slr*		i	i								D								
Pericycle cells						*rum1*											D				D				
Root segments								i		i							i	i				i	D		
Primary, seminal and crown roots																				i				D	
Primary meristem, quiescent center, root cap																						D		D	
Whole roots								D									D								
Phloem, xylem, and cortex																	i							D	
shr -2, pSHR::SHR:GR, *shr* root tips, sorted pWER::SHR:GFP						*shr*															D				
Primary roots						*rum1*											D								
Roots						BRX variant		D			D						i								
Primary root sections and root cell layers																	i	i		D	D	D		D	
Stele, Pericycle and ground tissues																	i				D			D	
Root segments						*sunn*	Nodulation	D						D			i	i	i						

Dicotyledonous species	
Ath	Arabidopsis thaliana
Mtr	Medicago truncatula
Gma	Glycine max
Pta	Pinus taeda
Ptr	Populus trichocarpa

Monocotyledonous species	
Zma	Zea maize
Osa	Oriza sativa
Hvu	Hordeum vulgare

Omics type	
Ts	Transcriptome
Tl	Translatome
Pr	Proteome
Mb	Metabolome

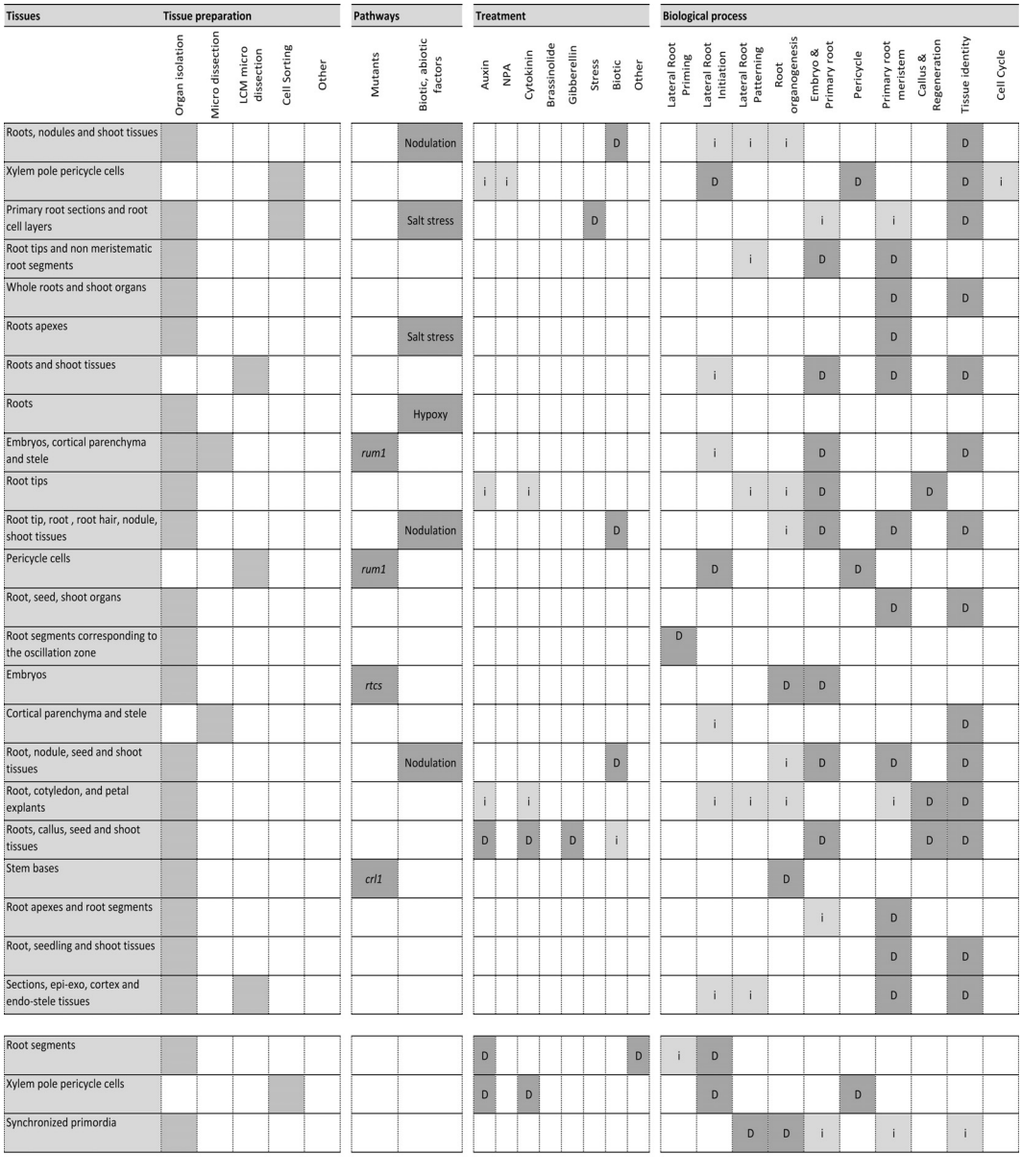
Information	
D	Direct
i	Indirect

(Continued)

Table 1.1 *(Continued)*

Year	Reference	Species	Omics technique & Experimental design		Platform / Technique
2008	**Benedito et al.**	Mtr	Ts	Transcriptome atlas	Affymetrix Medicago GeneChip
	De Smet et al.	Ath	Ts	Pericycle layer specific gene expression upon lateral root inducible system (LRIS)	Affymetrix ATH1
	Dineny et al.	Ath	Ts	Transcriptome atlas using FACS and GFP-reporter lines upon salt stress	Affymetrix ATH1
	Holmes et al.	Mtr	Ts	Comparison of gene expression between meristematic and non-meristematic root tissues	Affymetrix Medicago GeneChip
	Quesada et al.,	Ptr	Ts	Comparative analysis of the transcriptomes of Populus trichocarpa and Arabidopsis	42346 K 60-mer custom made microarray
2009	**Gruber et al.**	Mtr	Ts	Gene expression during salt-stress in root apexes	M. truncatula 16K+ microarrays (Mt16KOLI1)
	Jiao et al.	Osa	Ts	Transcriptome atlas using laser microdissection	70-mer custom made microarray (Qiagen/Operon)
	Mustroph et al.	Ath	Tl	Translatome atlas upon salt stress	Affymetrix ATH1
	Saleem et al.	Zma	Pr	Comparison of protein accumulation in immature embryo, cortical parenchyma and stele tissues between the mutant *rum1* and wild type	Two-dimensional electrophoresis + nanoHPLC-ESI-MS/MS massspectrometry
	Sena et al.	Ath	Ts	Gene expression during recovery of cell fates after cutting the root tip, and during the regeneration process	Affymetrix ATH-121501 full genome Arabidopsis microarray
2010	**Libault et al**	Gma	Ts	Transcriptome atlas using RNA-Seq	Illumina Solexa deep sequencing
	Liu et al.	Zma	Pr	Comparison of the pericycle layer specific protein accumulation between the mutant *rum1* and the wild type	Two-dimensional electrophoresis + nanoHPLC-ESI-MS/MS massspectrometry
	Matsuda et al.	Ath	Mb	Transcriptome atlas using metabolome data	LC-ESI-MS
	Moreno-Risueno et al.	Ath	Ts	Gene expression oscillations in the root tip	Affymetrix ATH1
	Muthreich et al.	Zma	Pr	Comparison of protein accumulation between the *rtcs* mutant and wild type immature embryos	Two-dimensional electrophoresis + nanoHPLC-ESI-MS/MS massspectrometry
	Saleem et al.	Zma	Pr	Protein accumulation in the cortical parenchyma and the stele of primary roots	Two-dimensional electrophoresis + nanoHPLC-ESI-MS/MS massspectrometry
	Severin et al.	Gma	Ts	Transcriptome atlas using RNA-Seq	Illumina deep sequencing
	Sugimoto et al.	Ath	Ts	Gene expression during in vitro callus induction	Operon Arabidopsis Genome Oligo Set Version 1.0 (Operon, Alameda, CA)
	Wang et al.	Osa	Ts	Transcriptome atlas on two different varieties	Affymetrix Rice GeneChip Genome Array
2011	**Coudert et al.**	Osa	Ts	Comparison of gene expression between *crl1* mutant and wild type stem bases	Affymetrix Rice GeneChip Genome Array
	Mathesius et al.	Gma	Pr	Comparison of protein accumulation between root apexes and root segments	Two-dimensional electrophoresis + MALDI-TOF/TOF MS
	Sekhon, R. S. et al.	Zma	Ts	Transcriptome atlas	Custom made microarray
	Takehisa et al.	Osa	Ts	Transcriptome atlas using laser microdissection	Agilent 4x44K microarray RAP-DB (G2519F#15241)
Unpublished	**De Rybel et al.**	Ath	Ts	Gene expression during lateral root initiation synchronization using a new chemical molecule, naxillin	Affymetrix ATH1
	Duclercq et al.	Ath	Ts	Auxin Cytokinin crosstalk and lateral root initiation	Affymetrix Tilling Microarray
	Middleton et al.	Ath	Ts	Gene expression during lateral root formation and emergence after synchronous induction of lateral root initiation	Affymetrix ATH1

Table 1.1 *(Continued)*



Dicotyledonous species	
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Information	
D	Direct
i	Indirect

lrt1, lateral root 1; *slr*, solitary root 1; *arf*, auxin response factor; *rum1*, rootless with undetectable meristems 1; *shr*, short root; *wer*, werewolf; *brx*, brevis radix; *sunn*, supernumerary nodules; *rtcs*, rootless concerning crown and seminal roots; *crl1*, crown rootless 1.

IAA Proteins

Although little is known on the origin of the signals triggering the auxin maximum, which induces the priming of the pericycle cells or the migration of the nuclei leading to LRI, much more is known about the downstream auxin transduction pathways (Fukaki et al. 2007).

One of the first components of this pathway is *IAA14*/*SOLITARYROOT1* (*SLR1*), a member of the *Aux*/*IAA* gene family (Fukaki et al. 2002). Aux/IAA proteins are short-living nuclear proteins, most of which are induced early by auxin and act as active repressors of gene transcription. Accumulation of auxin causes the degradation of Aux/IAA proteins. Aux/IAA proteins are present all over the plant kingdom (Table 1.2). They are already described in monocots such as maize and rice (Jain et al. 2006; Wang et al. 2010b) and in dicots such as poplar (Kalluri et al. 2007), but are not found in animals (Riechmann et al. 2000). In the presence of auxin, Aux/IAA proteins bind to the F-box proteins TIR1, AFB1, 2, and 3 (Dharmasiri et al. 2005; Kepinski and Leyser 2005) and become targeted to the ubiquitin-dependent proteasome-degrading pathway (Zenser et al. 2001; Gray et al. 2003). Regulation of plant auxin sensitivity can be modulated by the control of TIR1 expression levels as it is the case during phosphate nutrient deprivation. Phosphate deprivation increases the expression of TIR1 and consequently causes Aux/IAA auxin response repressors to be degraded and LRI to be induced in *Arabidopsis* seedlings (Perez-Torres et al. 2008). Also, nitric oxide was recently shown to enhance TIR1–Aux/IAA interaction, which can explain how nitric oxide depletion blocks Aux/IAA protein degradation (Terrile et al. 2012).

A single point mutation in the conserved amino acid core sequence "GWPPV" in domain II of Aux/IAA proteins was shown to stabilize them (Ramos et al. 2001), leading to gain-of-function mutants. In the case of *IAA14*/*SLR1*, the resulting gain-of-function mutant *slr-1* fails to initiate formative divisions in the pericycle founder cells and consequently does not develop any lateral roots (Fukaki et al. 2002). Other gain-of-function mutants of some *Aux*/*IAA* gene family members, *IAA1*/*AXR5*, *IAA3*/*SHY2*, *IAA18*/*CRANE*, *IAA19*/*MSG2*, and *IAA28*, also show strong lateral root development phenotypes (Tian and Reed 1999; Rogg et al. 2001; Tatematsu et al. 2004; Yang et al. 2004; Uehara et al. 2008), indicating their involvement in this process. Nevertheless, none of these mutants totally block LRI as in the case of *slr-1*.

In addition, the loss-of-function *iaa14-1* mutant does not show any obvious phenotype (Okushima et al. 2005), as it is also the case for other loss-of-function *iaa* mutants (Rouse et al. 1998; Fukaki et al. 2002; Uehara et al. 2008), indicating a possible redundancy in the function of *Aux*/*IAA* genes. Recently, it was demonstrated that an OsIAA11 gain-of-function mutation caused the inhibition of lateral root development in rice (Zhu et al. 2011). Interestingly, on the basis of protein sequence, OsIAA11 is one of the closest homologs of IAA14 in *Arabidopsis* (Jain et al. 2006). Nevertheless, the mutation in OsIAA11 is semidominant for lateral root development, and the mutant phenotype differs from that of *Arabidopsis iaa14-1*, indicating that the auxin regulation pathways mediated by these two genes might be different (Zhu et al. 2011).

To unravel the pathways leading to the formative divisions downstream of SOLITARY ROOT (SLR), a comparative analysis was realized between the *slr-1* mutant and the wild type using the lateral root-inducible system (LRIS; Vanneste et al. 2005). It was observed that the mutation affected a number of cell cycle regulatory genes. The authors overexpressed the cell cycle regulator CYCD3;1 (which promotes G1 to S phase transition) in the *slr-1* background to rescue its rootless phenotype. Though inducing a few rounds of anticlinal divisions, this strategy failed in the formation of lateral root primordia, indicating that cell cycle activation in the pericycle cells of the mutant *slr-1* is not sufficient to get formative divisions and proper LRI (Vanneste et al. 2005).