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Preface

Plant growth provides the basis for life on earth and is a process that is intimately linked with human civilizations. Continuous development in agricultural practices and in plant breeding allows us to keep plant production in line with demands. Industrialization, based on an enormous input of energy, mostly fossil fuels derived from biomass produced in the past, made it possible to reach our current standard of living. However, the uncontrolled use of plants and fossil fuels is an important contributor to global warming due to the associated production of CO_2. Furthermore, the reserves of this energy source are rapidly being depleted, so alternatives need to be found. Improved plant production is seen at least as a partial solution, as photosynthesis enables the "recycling" of CO_2 and fixing of energy. Thus, in recent years, we have seen a rapidly emerging market for bio-energy as well as the production of a myriad of natural products from plants. These bio-energy and bio-product producing crops, however, compete for the available agricultural land used for the production of food and feed, which is already starting to affect market prices of these commodities. Therefore, there is a renewed pressure on plant scientists to find solutions to increase plant productivity in a sustainable way.

Plant growth is intimately connected to the capacity of source organs to produce assimilates. Light is a key energy source and environmental cue controlling development, predominantly via leaves. It is known that growth-promoting signals are perceived in mature leaves and transmitted via unknown signals to developing leaves to regulate their growth. The nature of this transmissible signal is not known, but assimilates, such as sugars are thought to play a key role. Plant hormones also provide long-distance signaling to interface environmental conditions and organ growth. At the cellular level extracellular signals are sensed, transmitted and integrated by intracellular signaling pathways, which on one hand can directly regulate metabolic enzymes and other cellular functions, while on the other hand they feed into the regulation of gene transcription, protein stability protein modifications to quantitatively fine-tune cellular components or behavior. However, little is known about the intracellular signaling pathways in plants that regulate growth or its various components. Genetic approaches are difficult when genes function in an interconnected complex network, and regulate processes that are quantitative, such as growth. Novel methods, together with systems approaches, are needed

for multiplex measurements of the outputs of signaling pathways at various complexity levels.

Growth of new organs requires a combination of cell division in or near meristems, cell growth, differentiation and cell expansion. Both developmental and environmental inputs influence organ growth by altering the pool of proliferating cells. These developmental pathways are composed of individual modules consisting of signal(s), transducers, transcriptional regulator(s) and targets. Viewed this way, plant development is a cascade of events that, by continual external and internal input, direct the orderly activation of the hierarchically arranged modules. How these processes are linked and coordinated is not understood.

To gain a systems-wide understanding of any developmental or physiological process, an increasing number of methodologies to obtain "omics" data at various levels and of computational and network-modeling techniques are available. However, a key, sometimes overlooked issue is the precise experimental approach and is the exact source of the "omics" data. To understand a system, one should be able to produce, as far as possible, a list of its parts, to introduce perturbations in the system and to monitor the behavior of the parts following the perturbation. A further source of critical information is time-resolved data, because it can be assumed that changes in concentration/activity of the regulator will inevitably precede the changes in the regulated component.

First and foremost, the sequencing of the genome of *Arabidopsis thaliana* has launched plant science into the genomics era and provided a gathering platform for plant scientists. This is now rapidly followed by the sequencing of other plant genomes with agricultural importance, including rice, poplar, grapevine, tomato and maize. The impact of having the full list of coding and regulatory sequences for understanding the behavior of plant growth is enormous, as investigators can shift their attention from gene-identification to functional analysis of these genes at the molecular, cellular and whole plant levels. Genomic sequence availability also allowed the development of profiling technologies to monitor gene expression, protein abundance, localization and modifications on a genome-wide scale under a wide range of experimental conditions and in specific cells or tissues. Our ability to simultaneously study the function of virtually all genes encoded by the plant genome, has led to a new more holistic approach to biology named systems biology. Rather than focusing on the function of a few genes in a particular pathway, the emphasis in systems biology is to understand which are the key components regulating specific processes and how such components are connected in "regulatory networks".

As outlined above, plant growth is a particularly intriguing phenomenon as it is under the control of a multitude of interacting regulatory pathways. In this monograph several of the contributing pathways are reviewed, including light signaling (López-Juez and F. Devlin, Chapter 11), the classical hormones auxin (Zago et al., Chapter 8), ethylene (Dugardeyn and Van Der Straeten,

Chapter 10), and brassinosteroids (Clouse, Chapter 9), and signaling pathways including the TOR pathway (Anderson, Chapter 12), Armadillo repeat proteins (Coates, Chapter 15) and the MAPK cascades (Suzuki and Machida, Chapter 13), and protein dephosphorylation mechanisms (Schweigenhofer and Meskiene, Chapter 14). Devoto and Paccanaro (Chapter 17) describe the use of profiling and modeling to analyze signaling pathways on a genome-wide level. Downstream of those signaling pathways,, several key aspects of growth regulation itself are discussed, starting from the unicellular perspective of algae (Bišová, Chapter 18) to the regulation of cell growth, cell division (Doerner, Chapter 1), the switch between division and differentiation (Magyar, Chapter 5), the endoreduplication processes (Yoshizumi et al., Chapter 6) and interactions between cell size and cell numbers (Ferjani et al., Chapter 3) in higher plants. At the whole organ level the role of the epidermal layer in growth control is reviewed (Ingram, Chapter 7) and overall organ size control mechanisms are explored (Anatasiou and Lenhard, Chapter 2). Finally, emerging experimental approaches as proteomics (Schulze, Chapter 16) and kinematic analysis of growth (Walter, Chapter 4) are described.

We think it is timely to bring together this overview of the developments in various areas of plant-growth research in this monograph, firstly to give the reader a comprehensive insight into the current state of knowledge in the field. Reading through, it is possible to see common themes emerging from different fields of research and therefore we hope that this book will also stimulate an integrating perspective for future research aimed to better understand the fascinating process that plant growth represents.

March 2008

László Bögre and
Gerrit T.S. Beemster

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Signals and Mechanisms in the Control of Plant Growth

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Abstract Plant growth is mediated by three fundamental processes: cell growth, division, and expansion. The mechanistic analysis of their contributions are complicated by the observation that the balance of their contributions to organ growth are not hard-wired. Reduced cell proliferation, irrespective of whether this is caused by decreased cell growth or diminished cell division, can be, at least partially, compensated for by increased cell expansion. It is therefore argued that for a functional understanding of how gene regulatory networks control growth of the plant body, it is essential that all cellular parameters contributing to organ growth are quantified in concert. Plant growth behavior is exquisitely responsive to environmental change. Cell growth, division, and expansion, in aggregate, are promoted by nutrient availability and inhibited by abiotic stress. Recent studies that address how light intensity, CO_2 concentration, water activity, and temperature have complex effects on proliferation, cell expansion, and endoreplication that affect leaf organ growth are reviewed. Root growth rates and patterns are also very sensitive to mineral nutrient concentration and distribution. The mechanistic basis of plant organ growth still remains unknown; but such knowledge is critical for rational approaches to manipulate plant growth. Critical steps towards this goal are discussed.

1 Introduction and Background

Plants adapt exquisitely to their environment: physiology and metabolism change diurnally and in response to many environmental conditions, and reproductive development is generally sensitive to day length, temperature, or other proxies of seasonal change. The most fundamental adaptation to environmental change in plants is altered growth behavior, involving changes to root or shoot growth patterns, rates, or both.

Despite their fundamental importance for our understanding of plant growth, for rational approaches to sustainably enhance yields in agriculture and forestry and, ultimately, for human welfare, we still understand surprisingly little about the mechanisms that govern growth in plants. In this chapter, I will consider the signals and genetic mechanisms involved in controlling growth in aerial and underground organs.

1.1 Distinct Processes Contribute to Plant Growth

At the whole plant level, growth of the plant body proceeds by the linear extension of stems and branches, the production of leaf or floral organs, and the elongation and branching of roots, mediated by apical, axillary, and lateral meristems. Secondary growth, or radial thickening mediated by cambial cells, contributes to body size increase in many plants, but will not be considered further in this chapter. Primary stem or leaf and root organ growth, here defined simply as an increase in volume, proceeds in two stages, which I will call phase I, during which cells multiply in cycles of growth and division; and phase II, during which cells cease dividing but expand until differentiation is completed. High rates of proliferation are observed in meristems, in young leaf and floral primordia, but not in stem cells and the stem cell niche (Fig. 1).

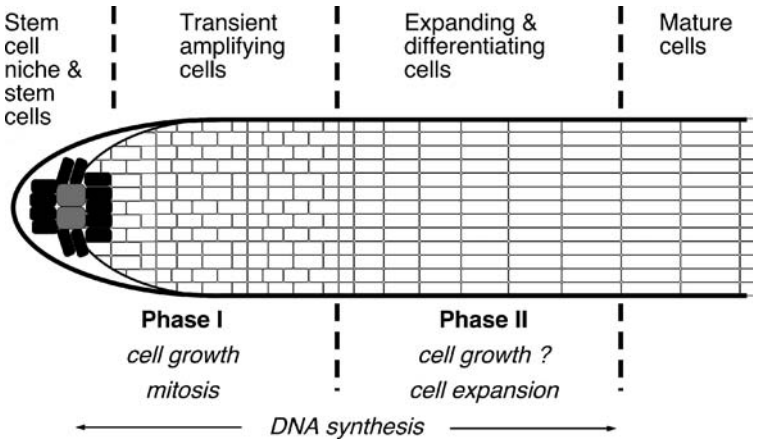

Fig. 1 Schematic representation of the root apical meristem illustrating the different zones of growth and the positional extent of various growth processes

1.2 Cell Growth

In phase I, cell growth alternates with division in mitotic cells. Cell growth is a prerequisite for division in meristems and organ primordia, and is driven by the increase of cell mass by synthesis of macromolecular cell constituents (Jorgensen and Tyers 2004). Ribosomes limit macromolecular synthesis and, therefore, their synthesis and its regulation is at the nexus of growth control. For example, yeast cells commit ~50% of their total transcription activity and a large fraction of their energy budget towards building ribosomes (Warner 1999) and quantitative studies reveal a strong positive correlation of ribosome synthesis with cell growth (Planta 1997; Warner 1999). There is good

evidence that impaired ribosome biosynthesis reduces plant growth (Van Lijsebettens et al. 1994; Weijers et al. 2001; Horvath and Bogre, this volume), but no detailed information is yet available on how well ribosome biosynthesis correlates with growth activity in plants. The expression of many components of the plant ribosome is regulated transcriptionally (McIntosh and Bonham-Smith 2006), but it is still poorly understood how ribosomal RNA and protein synthesis for ribosome production are coordinated mechanistically (for review, see McIntosh and Bonham-Smith 2006).

Cell growth is under control of the target of rapamycin (TOR) pathway, which couples nutritional cues to the regulation of ribosome biosynthesis, the rates of protein synthesis and proliferation. The TOR pathway interacts with the PI-3-kinase pathway, which mediates growth factor cues, and this interaction insures coordinate cellular growth responses (Arsham and Neufeld 2006; Jorgensen and Tyers 2004). The TOR pathway has been well characterized in animal and yeast systems, but much detail remains to be uncovered in plants: Orthologs of the TOR kinase, and of some additional components of the TOR signaling pathway have been identified in plants (Bogre et al. 2003; Menand et al. 2002; Wang et al. 2003), but their functional significance for plant cell growth control, specifically for coupling environmental change to growth responses, are only beginning to be examined in detail (Mahfouz et al. 2006). Likewise, plant homologs of PI-3-kinases and their effectors, the AGC kinases have been identified (Wang et al. 2003). At least one AGC kinase has been shown to be responsive to auxin and cytokinin growth regulator inputs (Anthony et al. 2004), and IRE (an AGC kinase) positively regulates root hair tip growth (Oyama et al. 2002). However, many gaps need to be filled until we understand the mechanisms of how growth regulator and nutrient inputs converge on cell growth control in plants.

1.3 Cell Division

In contrast, the mechanisms controlling cell division are much better understood than those regulating cell growth in plants (see Inze and De Veylder (2006) for an excellent recent review). Components of the plant cell cycle machinery (cyclins, cyclin-dependent kinases), orthologs of the retinoblastoma (Rb) gene, and E2F/DP-type transcription factors were identified based on their sequence homology (Vandepoele et al. 2002). Largely based on gain-of-function studies with transgenic plants over- or ectopically expressing cell cycle regulators and expression analysis, the following view is emerging: In association with CDKA (A-type cyclin-dependent kinase), D-type cyclins are involved in controlling the entry into the cell cycle (Menges et al. 2006, Riou-Khamlichi et al. 1999), whereas A- and B-type cyclins, in association with CDKA and CDKB play a major role in S-phase and entry into M-phase, respectively (Doerner et al. 1996; Weingartner et al. 2003). As in animal systems,

the E2F/DP and related genes, promote S-phase and DNA synthesis, but are also involved in controlling the switch between mitotic cell cycles and the endoreplication cycle. Likewise, CDK inhibitors function in post-translational control of cyclin–CDK complex activity. Anaphase promoting complex (APC) proteolytic activity at the metaphase-to-anaphase transition insures the irreversible directionality of cell cycle progression, as in other model systems.

Cell growth is coupled to cell division progression by mechanisms that monitor cell size. For example, in yeast, coupling of growth to cell cycle entry converges on the regulation of G1-type CLN3 cyclin abundance (Morgan 2007), although this view may be too simplified (Jorgensen and Tyers 2004). CLN3 abundance is regulated at the transcriptional, translational, and post-translational level (MacKay et al. 2001; Morgan 2007; Polymenis and Schmidt 1997). In aggregate, these mechanisms result in a steep stimulus–response coupling (ultrasensitive response) of CLN3 protein levels, and hence of CLN3–CDK complex activity, to the rate of mRNA translation by ribosomes, which reflects the activity of the TOR and other growth regulating pathways. Cell cycle entry in plants requires D-type cyclins. In Arabidopsis, cyclin D3;1 mediates the stimulatory effect of cytokinins on proliferation, while cyclin D2 abundance is responsive to sucrose levels (Riou-Khamlichi et al. 1999, 2000). Cyclin D3;1 is a labile protein (Planchais et al. 2004), as would be expected of a limiting regulator responsive to potentially rapidly changing environments. Moreover, cyclin D3;1 promotes the G1/S transition (Menges et al. 2006). Based on this limited information, it is therefore reasonable to predict that key aspects of the mechanisms that couple cell growth to cell division are conserved in all eukaryotes.

1.4 Cell Expansion

After cells pass through the domain with high rates of cell growth and division, they cease dividing and cell size rapidly becomes larger. This transition from phase I to II is visually distinct in root meristems, whereas in leaf organs this transition is morphologically less conspicuous. Cell expansion in phase II is not driven by macromolecular synthesis but is the result of turgor-driven water uptake and concomitant cell wall loosening. The generation of increased osmotic pressure requires the activities of three major proteins or protein complexes in the tonoplast membrane: The V-type H^+ATPase, H^+pyrophosphatase and aquaporins (see Maeshima 2001 for review). This is balanced by cell wall loosening that permits the cell to expand mostly in one direction, and which involves several activities including expansins, xyloglucan endotransglycolase/hydrolase (XET), endo-(1,4)-β-D-glucanase, and hydroxyl radicals (see Cosgrove 2005 for review). In quantitative terms, cell expansion contributes most to organ growth: during cell expansion, volume increases from 20- to 1000-fold. Thus, the extent of cell growth and division

during phase I define the potential for organ growth by producing the cellular building blocks; during phase II, this latent ability is fulfilled during cell expansion.

The phase I/II boundary marks a transition of the cellular mechanism that mediates organ growth: from growth by cell production to organ growth by cell expansion. However, not all processes associated with organ and plant growth change at this transition. DNA synthesis persists during this transition, but in the absence of division, it leads to endoreplication. Therefore, DNA replication can be considered as the process that frames the entire organ growth process. In Arabidopsis, endoreplication can result in ploidy levels of up to 64C (with 1C being a haploid genome equivalent), indicating that cells undertake up to five additional rounds of DNA synthesis without dividing. In Arabidopsis leaves, cellular DNA content is positively correlated with mature, fully expanded cell size (Melaragno et al. 1993), however, in roots no such correlation was found (Beemster et al. 2002). DNA synthesis, and with it endoreplication and cell expansion, is thought to cease when cells become fully differentiated and primary organ growth is completed.

Although expanding cells increase their size by a different mechanism than cells growing in the proliferative zone, they continue entering the DNA replication cycle as long as they undertake endoreplication cycles. The bulk of the volume increase in expanding cells is mediated by inflation of the vacuole, but it is likely that the cytoplasm must also increase in mass to insure that the necessary concentration of reactants is thermodynamically favorable. This raises the interesting, and as yet unresolved, question whether the onset of S-phase in endoreplicating cells is also coupled to proxies of cell growth such as the rate of mRNA translation.

2 Regulation of Growth

Much progress has been made in identifying and functionally characterizing components of the plant cell division apparatus (Inze and Veylder 2006), and the mechanisms involved in cell expansion are also beginning to be quite well understood (Carol and Dolan 2006; Cosgrove 2005; Tsukaya 2006). In contrast, cell growth control is mechanistically still less well understood. Based on the preceding analysis of plant growth processes, I propose the existence of two major growth control points in plants likely to be sensitive to developmental or environmental inputs. The first is suggested to co-regulate cell growth and the onset of the cell cycle; the second is the switch of growth mechanisms at the phase I/II boundary to suppress mitosis and activate cell expansion. The identification of components involved in these control points, the mechanisms by which they operate and how they are coupled to cues will be major milestones to improve our understanding of plant growth control.

Recently, a few candidates for such components were identified. They are considered in detail below, because each one is a possible target or component of growth control pathways responsive to environmental or developmental cues. However, it will require more extensive analysis to unambiguously establish their specific function in growth control networks.

2.1 Coupling of Cell Growth and Division

There is increasing evidence that cell growth and division are co-regulated: rapidly dividing cells in young leaf primordia and in roots are remarkably uniform in size and recently, possible effector pathways for co-regulation of cell growth and division were identified. The best mechanistic evidence for co-regulation of cell division and cell growth currently comes from the analysis of Arabidopsis TCP20. TCP20, which belongs to a plant-specific class of transcription factors and is thought to promote gene expression, binds in vivo to the promoters of ribosomal protein genes as well as to the promoter of the mitotic cyclin B1;1 (Li et al. 2005). Elevated expression of cyclin B1;1 has been shown to promote organ growth (Doerner et al. 1996). However, the biological function of class I TCP genes in control of organ growth has not been reported yet.

EBP1 genes, identified in potato and Arabidopsis, are a further type of effector gene that affect phase I growth (Horvath et al. 2006). Putative orthologs have been identified in other eukaryotes, where they are thought to regulate ribosome biogenesis (Squatrito et al. 2004), modulate translational activity (Squatrito et al. 2006), as well as DNA replication by binding to the Rb protein (Zhang et al. 2003). This wide range of activities raises the interesting possibility that plant EBP1 genes are involved in promoting phase I growth (by promoting cell growth), as well as phase II growth (by regulating E2F activity). Over-expression of plant EBP1 leads to larger leaves with more cells, while reduced expression results in the opposite (Horvath et al. 2006). In this work, cell size at birth and ploidy were not analyzed and so the direct effects of EBP1 on cell growth and the phase I/II switch are not yet known.

Altered expression of many additional genes has been reported to enhance organ growth, including: ARGOS (Hu et al. 2003), AINTEGUMENTA (Mizukami and Fischer 2000), PEAPOD (White 2006), and BIG BROTHER (Disch et al. 2006). All these genes have opposing effects on organ size when either over- or under-expressed. Elevated expression (ARGOS, AINTEGUMENTA) or reduced expression (PEAPOD, BIG BROTHER) leads to extended phase I growth, with little or no effect on final cell size. However, cell size at birth in these plants (i.e., during phase I growth) was not reported, and therefore it is presently not clear whether these genes specifically control the timing of the phase I/II transition, or also affect the rate of cell growth.

Enhanced expression of some activating cyclin subunits of the CDK complexes that are rate-limiting regulators of cell cycle progression has led to

accelerated organ growth without affecting the final size of the plant (Cockcroft et al. 2000; Doerner et al. 1996; Li et al. 2005). These observations raise several intriguing possibilities: It is possible (although there is no experimental evidence yet) that CDK activity feeds back on cell growth control. This could be a parsimonious regulatory mechanism, in which for example, developmental pathways could directly regulate cell cycle activity. This would then suffice to entrain appropriate levels of cell growth activity. Alternatively, it is possible that cell division onset in meristems and organ primordia only occurs significantly later than the attainment of a minimal cell size in plants. In this scenario, CDK activity limits organ growth and the plant can cope with increased proliferation because cell mass is sufficient to sustain division at an earlier time. A third possibility is that a specific CDK activity could be required for mitosis and therefore become limiting at the phase I/II boundary. A delay of the phase I/II transition would enhance the growth capacity of the affected organ or meristem by increasing the size of the dividing cell population. In this scenario, CDK mitotic activity limits organ growth by controlling the switch in cellular growth mechanisms.

There is good evidence that cell division activity positively correlates with organ growth rates: High levels of CDK activity are associated with high proliferation (Granier et al. 2000). Enhanced expression of activating cyclin subunits of the cyclin-dependent kinase (CDK) complexes that are rate-limiting regulators of cell cycle progression has led to accelerated organ growth without affecting the final size of the plant (Cockcroft et al. 2000; Doerner et al. 1996; Li et al. 2005). Further, careful quantitative analysis of CDK kinase activity in relation to root organ growth rates support the notion that the level of CDK activity is a good predictor for the magnitude of organ growth rate (Beemster et al. 2002). Therefore, it appears possible that regulatory networks directly regulate CDK activity as a mechanism for plant growth control.

2.2 The Switch from Mitosis to Endoreplication

The switch from phase I to phase II growth mode involves two known mechanisms: (i) the suppression of mitosis and (ii) the stimulation of cell expansion, during which repeated rounds of DNA synthesis persist until cells are fully expanded. CDK–cyclin complexes control the commitment to S phase, but the execution of S-phase is enabled by a CDK-controlled hierarchy of enforcers that include the plant homolog of the retinoblastoma gene (Rb), and a family of related transcription factors that include E2F, DP, and DEL genes (Gutierrez et al. 2002; Inze and Veylder 2006). E2F and DP gene products heterodimerize to bind their canonical target sites, while DEL proteins can bind these as monomers and lack conspicuous activation domains. Rb keeps E2F proteins in check by binding them through a so-called A/B pocket, but upon hyperphosphorylation by CDKs, releases these so they can directly

activate expression of S phase genes. Over-expression of Arabidopsis E2F3 (also known as E2Fa) stimulates expression of S phase genes, and enhances proliferation and endoreplication. This phenotype is exacerbated when a DP gene is co-expressed (De Veylder et al. 2002). However, not all E2F genes promote S phase: E2F2 (also known as E2Fc) lacks an apparent activation domain and hence can suppress S-phase associated gene expression (e.g., CDC6) (del Pozo et al. 2002). Reduced E2F2/c expression results in increased expression of S-phase gene markers, enhanced cell production and plants with more, but smaller cells with reduced levels of endoreplication (del Pozo et al. 2006). Likewise, enhanced E2Fd (also known as DEL1) expression reduces ploidy levels, while reduced E2Fd/DEL1 activity increases endoreplication (Vlieghe et al. 2005). In contrast, altered levels of E2Ff/DEL3 had a modest impact on endoreplication, but elevated expression promoted precocious differentiation in roots, possibly because several expansins and xyloglucan endotransglycolase/hydrolase involved in cell wall extension during phase II growth are E2Ff/DEL3 targets (Ramirez-Parra et al. 2004).

Taken together, these results suggest that E2F/DEL genes are involved in regulation of S-phase-specific gene expression as well as promoting phase I/II transition. Therefore, they are likely targets of regulatory pathways that control the suppression of mitosis and the stimulation of cell expansion. However, how the activity of different E2F-like factors, in some cases possibly on common target genes, is regulated is still not well understood, but at least one of them, E2F2c, is unstable (del Pozo et al. 2002). Further evidence for a possibly pivotal role for regulated protein degradation in controlling the phase I/II transition comes from the observation that CCS52, a regulatory component of the anaphase promoting complex (APC) orthologous to CDH1 and fizzy-related (which functions as an inhibitor of mitosis), is required for endoreplication (Cebolla et al. 1999).

Although the experimental evidence clearly points to a complex involvement of Rb/E2F/DP/DEL proteins, as well as regulated proteolysis in controlling the phase I/II transition, the regulators that orchestrate the deployment of these enforcers, specifically their order of action, have not yet been identified.

3 Plant Growth Responses to Environmental Change

Plant growth patterns and rates adaptively respond to changes in the environment. Such adaptive changes confer competitive advantages and allow the plant to survive adverse conditions. Here, I will focus on adaptation to changes in nutrient availability.

Altered nutrient availability can impact plant growth at the cellular, organ, and whole plant level: Local (in the order of 50–100 μm) differences in soil

phosphate availability suffice to alter root hair growth, which in low phosphate, is stimulated in a cell-autonomous manner (Bates and Lynch 1996). Likewise, cell expansion in leaves subjected to low water activity is reduced, but can recover when water is available again (Granier and Tardieu 1999). When mature leaves are exposed to high light or CO_2, leaf growth is stimulated and stomatal density increases. Developing leaf primordia exposed to low light or low CO_2 will develop with the characteristics of the mature leaves, indicating that at least some aspects of leaf growth are controlled by systemic signals (Lake et al. 2001; Yano and Terashima 2001; Ferjani et al., this volume). Unfortunately, the precise mechanisms by which any nutritional cue elicits one or more signals controlling cell growth, division, or expansion are not yet known.

3.1 Shoot Growth

Shoot meristems and developing leaf organs adapt exquisitely to the abundance of light, CO_2, and water by modulating leaf production rate, leaf size and shape, anatomy, and physiology to confer competitive advantages in an environment where competition for light is fierce. Many genes and growth factor signaling pathways have been identified that contribute to specifying final leaf size and shape, but it is not yet known whether or how these mediate specific environmental cues as well.

Most leaves are determinate organs, but monocot and dicot leaves grow differently. In monocots, meristematic cells across the leaf base produce cells until the blade has reached its full longitudinal extent, and lateral growth of the blade does not occur. By contrast, growth is more complex in dicot leaves: all cells initially grow and divide, but quiescence sets in in a basipetal direction from the leaf tip to the base, and cell divisions cease early in leaf development. However, proliferation persists at a low rate in vascular tissues and in isolated cells (e.g., cells of the stomatal lineage), and endoreplication continues. Analysis of dicot leaf organ growth in mutants and transgenic plants has also revealed a compensatory mechanism: reduced proliferation can be mitigated by enhanced cell expansion, thereby maintaining a similar leaf area (Hemerly et al. 1995; Horiguchi et al. 2006). Such compensatory control of final leaf organ area has been suggested to result from an organ size control mechanism (Hemerly et al. 1995), but components of such a regulatory mechanism have remained elusive. Since the extent of cell expansion in leaf epidermal cells positively correlates with ploidy level (Melaragno et al. 1993), it has been proposed that the observed compensatory increase in cell expansion could depend on modulation of ploidy. In a recent study, expansion at the cellular and leaf organ level was analyzed in response to low light and water deficit (Cookson et al. 2006). Plants growing in low light produced smaller leaves comprised of fewer, but larger cells; while those growing in water deficit

conditions produced smaller leaves comprised of smaller cells. However, the mean number of endoreplication cycles was reduced under both experimental conditions (Cookson et al. 2006). Taken together, these observations are not consistent with a role for endoreplication in governing final cell size in response to a nutrient and environmental cue such as light.

3.2 Light and CO_2

Light quality and intensity has a profound effect on plant growth. Changes to the red–far red ratio trigger the photoreceptor-dependent shade avoidance response, which involves increased cell expansion and, in extreme cases, an acceleration of plant development (Franklin and Whitelam 2005). Here, I will focus on the effects of light quantity on plant growth, specifically leaf organ growth, and the emphasis will be on Arabidopsis. It should be noted that most Arabidopsis experiments are performed in laboratory growth chamber conditions, where "high light" corresponds to 150–250 $\mu mol\ m^{-2}\ s^{-1}$, and "low light" corresponds to 15–75 $\mu mol\ m^{-2}\ s^{-1}$. However, in nature, exposure to sunlight corresponds to 150–2000 $\mu mol\ m^{-2}\ s^{-1}$, and "shade" in nature can span the whole high-light/low-light range examined in the laboratory. Therefore, the relevance of the observations described below remains to be validated for natural conditions.

Leaf organ growth responds to light intensity in several ways: In constant conditions, leaf initiation rate is reduced by low light (Cookson et al. 2005); blade anatomy is altered such that in "sun" leaves, two layers of palisade cells are produced (Kim et al. 2005); and the density of stomata is increased (Lake et al. 2001) in high light. In low light, leaf blade area is decreased, mediated by a reduction in cell number, but it is not yet known whether this is caused by reduced cell division, or whether cell growth (and as a consequence, cell division) is reduced (Cookson et al. 2005; Granier and Tardieu 1999). However, reduced proliferation is compensated for in part by increased cell expansion (Cookson et al. 2005; Cookson and Granier 2006) Moreover, the growth characteristics of the leaf organs are altered in low light so that maximal organ expansion rates are reduced and delayed (Cookson et al. 2005). Interestingly, a strong correlation was observed between leaf initiation rates and leaf epidermal cell number (Cookson et al. 2005). This raises the intriguing possibility that light intensity generates a signal that acts directly on the meristem to control the rate of primordium formation *and* the number of cells committed to a primordium. Such a possibility is consistent with the observation that all early processes in leaf organ development are correlated with each other (Cookson et al. 2005), implying that they are co-regulated.

Non-stressing levels of high light also increase photosynthesis and carbon assimilation and are therefore likely to also affect whole plant growth. Increased root growth (and an associated improved ability for mineral nu-

trient assimilation), would positively affect leaf growth. Such indirect effects on Arabidopsis leaf growth parameters in different light intensities have not been reported.

Overall growth of most plants, including Arabidopsis, is promoted in elevated CO_2 concentrations (Pritchard et al. 1999; Tocquin et al. 2006). Leaf organ growth in Arabidopsis is stimulated, and this effect is more pronounced when nitrogen is not limiting (Tocquin et al. 2006). Kinematic analysis of Arabidopsis leaf growth under these conditions has not yet been reported. However, in monocot leaves such analysis is more straightforward. In a detailed analysis of leaf growth kinetics in two wheat cultivars, elevated CO_2 concentration led to enhanced cell production and increased meristem size, but no change of cell size at cytokinesis or of final expanded cell size was observed (Masle 2000). Together, this suggests that cell cycle entry is directly stimulated by CO_2 and that control of this parameter mediates CO_2 concentration-dependent organ growth changes. Interestingly, growth in elevated CO_2 concentration leads to significantly increased foliar concentrations of cytokinins, gibberellins, and auxin, while concentrations of growth-inhibitory ABA are reduced in Arabidopsis (Teng et al. 2006). If leaf growth control in dicotyledonous leaves mirrors that in monocots, then Arabidopsis CYCD3;1, which is one of the D3-type cyclins that limits cell cycle entry (Menges et al. 2006) and is also involved in mediating cytokinin-dependent stimulation of cell division (Riou-Khamlichi et al. 1999), may be a direct target of CO_2 concentration-dependent organ growth control.

3.3 Water Activity and Temperature

Water deficits and elevated temperatures are stressful conditions that negatively affect plant and leaf growth. Detailed kinematic analyses of leaf growth at non-stressful temperatures or a range of water activities have not been reported for Arabidopsis. However, in maize, a good correlation between leaf elongation and cell production rates was observed in a range of temperatures and when comparing watered plants with plants experiencing water stress (Granier et al. 2000). Moreover, CDK activity and cell division rate were strongly correlated, but not $p34^{cdk}$ abundance. This observation suggests that post-translational modification of $p34^{cdk}$ or transcriptional control of cyclin expression are potential targets for these signals. In a recent study (Rymen et al. 2007), the effect of cold nights on leaf growth in maize was examined. These conditions did not affect mature cell size, or the size of the meristem, but major changes were observed in the dividing cells of the meristem: cell size of dividing cells was reduced (hence the meristem had more, but smaller cells), and cell cycle duration was extended. This indicated that cell growth was strongly affected, and furthermore, that low

temperature resets the size threshold for division. Interestingly, the latter is reminiscent of the response of budding yeast to growth at different levels of nutrient availability: in low nutrients, the size threshold for division is reduced, but when nutrients are abundant, cells divide with a larger mass (Tyson et al. 1979). Ploidy levels were not significantly changed, suggesting that all cell cycle phases were equally affected by the low temperatures, but the expression of some cell cycle regulators that function in the G1/S transition and in S-phase (e.g., cyclin CYCA3;1, CDKA1;1, E2F) was strongly down-regulated.

4 Root Growth

Roots are indeterminate organ systems that grow apically, potentially indefinitely, and that form lateral roots at a distance from the growing apex. The patterns and rates of root system growth are influenced by the distribution and concentration of mineral nutrients in the soil, the availability of water and the degree of soil compaction. The distribution of some mineral nutrients such as phosphate and iron is very heterogeneous, due to their strong ionic interactions with the soil matrix and the strong pH-dependency of their solubility. The abundance of such immobile minerals can vary by an order of magnitude at scales of a 100 μm (Strawn et al. 2002). In contrast, other nutrients such as nitrate and potassium are at least tenfold more mobile in the soil (Marschner 1995), and therefore tend to accumulate as solutes above water-impermeable clay layers. The distinct physicochemical properties of plant mineral macronutrients implies that there should be at least two distinct growth or foraging strategies in response to limitation of soil minerals: for immobile minerals, the most efficient response to enhance uptake is to increase the surface area of the root to directly contact soil particles in previously unexploited domains of the soil. Increased branching, radial thickening, and growth of root hairs, while suppressing primary root growth, best accomplish this objective. In contrast, for mobile elements, the optimal strategy is to enhance root apical growth to reach deeper layers where such solutes accumulate.

Both syndromes are observed: under conditions of phosphate starvation, reduced primary root growth, enhanced lateral root formation, and stimulation of root hair growth is observed (Lopez-Bucio et al. 2002), while during iron starvation, mostly root hair growth is stimulated (Muller and Schmidt 2004). Both iron and phosphate have low mobility in the soil column. In contrast, root apical growth is stimulated in low nitrate (0.1–2.5 mM), when compared to higher concentrations, and this is due to a larger population of dividing cells and a delayed phase I/II transition (Dubrovsky and Doerner, unpublished). At lower concentrations (< 50 μM), primary root growth per-

sists for a while without stimulation of lateral root growth (López-Bucio et al. 2003). Nitrate is relatively mobile in the soil column and accumulates above water-impermeable layers in the ground.

The analysis of root growth parameters is in many ways more straightforward than in shoots: organ growth is essentially anisotropic, and because the different processes contributing to organ growth occur in spatially distinct domains, they can be more readily analyzed. Kinematic analysis is very powerful in this respect, but has surprisingly only been used in a few cases for root growth analysis in Arabidopsis (Beemster and Baskin 1998, 2000; Beemster et al. 2002). In Sect. 4.1, I will focus on growth responses to phosphate starvation as these have been analyzed in greater detail than for other mineral nutrients.

4.1 Phosphate

Arabidopsis responds to phosphate starvation with a complex adaptive growth response. Initially, this involves a rapid inhibition of cell expansion in roots (Lai et al. 2007; Sanchez-Calderon et al. 2005; Williamson et al. 2001) and stimulation of lateral root initiation and emergence (Lopez-Bucio et al. 2002; Williamson et al. 2001). Prolonged starvation involves progressively reduced cell division, quiescence, and differentiation of cells in the apical meristem (Lai et al. 2007; Sanchez-Calderon et al. 2005; Ticconi et al. 2004). While the sequence of these events appears invariant, their kinetics and severity are quite variable between experiments and laboratories, possibly because it is very difficult to completely remove traces of phosphate from the growth media. This sequence of events implies that signaling networks involved in controlling responses to phosphate starvation target more than one of the fundamental mechanisms regulating organ growth.

Recent work indicates that the timing of onset, rate of progression, and severity of growth responses to phosphate depletion depends on the overall growth activity of the plant. Under phosphate starvation conditions, root growth is promoted by sugars and inhibited by nitrate, osmotic stress, or treatments with plant growth regulators (Lai et al. 2007). The emerging concept is that the scale of organ growth activity determines the level of demand for phosphate, which in turn influences the rate at which the plant goes through the series of adaptive growth responses.

The targets of phosphate signaling pathways involved in controlling cell growth, division, or expansion have not yet been identified. However, mutational dissection of adaptive responses to phosphate starvation has resulted in the identification of two interesting classes of mutants: the *pdr* (phosphate deficiency response) and the *lpi* (low phosphate insensitive) mutants. The *pdr2* mutant is hypersensitive to low phosphate availability and shows a short root phenotype under these conditions that is caused by inhibition of cell expan-

sion and division (Ticconi et al. 2004). The onset of quiescence and terminal differentiation observed in wild-type plants only upon extended phosphate starvation (Sanchez-Calderon et al. 2005), occurs earlier and at higher external phosphate levels, and also leads to cell death. This suggests that *PDR2* might be involved in phosphate sensing or coupling perception to root growth responses.

The *lpi* mutants show the opposite phenotype: these mutants are hyposensitive to phosphate starvation. Four complementation groups have been identified, all of which continue root apical growth in the absence of phosphate (Sanchez-Calderon et al. 2006). However, this is not because these plants do not know that they are experiencing phosphate starvation: these mutants activate physiological and gene expression responses to phosphate starvation to a very similar degree as wild-type (Sanchez-Calderon et al. 2006). The *lpi* mutants have constitutively slightly reduced cell expansion, but dramatically increased cell division activity when compared to the wild-type in phosphate-starved conditions. These phenotypes suggest that *LPI* genes are involved in restraining cell division during phosphate limitation. This would serve two complementary purposes: (i) to insure the functional integrity of the root apical meristem for the longest possible time, and (ii) possibly to direct resources to incipient lateral roots to shift the patterns of root growth in favor of increasing root surface area. The cloning of *PDR* and *LPI* genes has not yet been reported, but their identification will facilitate the identification of their targets in the growth control machinery.

5 Integration of Growth Control

Shoots and roots are interdependent for nutrients, with overall shoot growth limited by nutrients assimilated by the root, and root growth limited by fixed carbon (C) translocated from the shoot. Nitrogen (N) limitation and uptake by the root plays a key role in controlling shoot growth and, taken together, this suggests that just as in heterotrophic multicellular organisms, N (amino acid) and C (sugar) availability provide crucial cues in overall plant growth control (Lorberg and Hall 2004). In limiting conditions, nutrients are re-allocated to meristems and developing organs to sustain growth for the longest period possible. Unfortunately, the kinetics of change in nutrient concentrations, transport, and translocation have not yet been examined in whole plants with cellular or high temporal resolution. Therefore, it is presently not clear whether the growth responses observed in response to altered nutrient abundance are due to direct sensing of nutrient levels in meristematic cells, or whether these cells respond to surrogate systemic or mitogenic signals such as plant growth regulators or miRNAs. Novel tools for such measurements are currently being developed (Deuschle

et al. 2006; Gu et al. 2006; Lager et al. 2006), and therefore it will be interesting to re-visit some of the experiments relating to plant-mobile nutrients to carefully re-assess plant growth responses when these nutrients are limiting.

A characteristic feature of plant adaptive growth responses is that different shoot or root apices, or leaf organs, grow at different rates. Growth of organs or meristems directly exposed to the nutrient is promoted. The spatially selective allocation of resources to meristems or organs experiencing conditions more conducive to growth than others in effect constitutes foraging behavior, in which the "winners are fed" and which may be cued by the physiology of the affected tissues. For example, if barley root systems are separated into different compartments, and the bulk of the root system is grown in nutrient-limiting conditions, then roots in a compartment that is provided with higher mineral nutrient levels grow faster and branch more, leading to a more effective exploitation of such localized resource "jackpots" (Drew and Saker 1975). Importantly, if the whole root system is uniformly exposed to optimal mineral nutrient levels, stimulated growth is not observed, indicating that the selective growth stimulation observed upon localized nutrient availability is an internally regulated process. Likewise, it was recently reported that the sun leaves, with their higher rates of photosynthesis and transpiration, import almost three times more cytokinins than shade leaves (Boonman et al. 2007). When cytokinins were applied to shaded or water-deficit leaves, these behaved like sun leaves. Taken together, these data are consistent with a model in which the rate of metabolism or physiology cues changes in plant growth regulator concentrations or flux to regulate growth activities.

All classical plant growth regulators: auxins, cytokinins, gibberellins, brassinosteroids, ethylene, and abscisic acid have been shown to be involved directly or indirectly in controlling adaptive growth responses to environmental change. Auxins are required for the initial specification of lateral shoot organs (Reinhardt et al. 2000) and lateral root initiation (Torrey 1950), but it is less clear how it is mechanistically involved in adaptive growth responses to nutrients. Cytokinins are involved in controlling sink-source relationships and the balance of shoot and root growth (Werner et al. 2001, 2003), and at least partially mediate nitrogen nutrient cues (Miyawaki et al. 2004; Rahayu et al. 2005). They may also be involved in controlling root growth rates by affecting the phase I/II transition. Gibberellins (GA) are required for auxin stimulation of root growth (Fu and Harberd 2003), for organ expansion in shoots, and for maintenance of the indeterminate state in axillary meristems, and hence are possibly involved in determining the dividing cell population size in early leaf primordia (Keller et al. 2006). Biosynthesis of GAs is enhanced in low light (Potter et al. 1999), and in high concentrations of CO_2 (Teng et al. 2006), and therefore they likely play a role in stem and leaf organ growth. Brassinosteroids (BR) are required for cell expansion and cell

division in leaves and roots (Nakaya et al. 2002). Their biosynthesis is stimulated by light, but since BR concentration is subjected to complex feedback mechanisms (Nomura and Bishop 2006), it is not clear whether BRs mediate light-intensity signaling. Ethylene is involved in many growth responses, particularly involving cell expansion, but is also involved in adaptive changes to leaf blade growth in low light (Vandenbussche et al. 2003). Abscisic acid (ABA), which mediates water deficiency cues, plays a negative role in leaf and root growth.

With the exception of ABA, which has been shown to stimulate expression of CDK inhibitors (KRP genes) (Wang et al. 1998), the mechanisms by which growth processes are controlled by these regulators are not yet clear. However, it is expected that growth regulators that move between different plant organs, i.e., auxin, cytokinin, ABA, as well as novel and still poorly characterized molecules (Booker et al. 2005), will play a major role in integration of growth responses at the whole plant level.

At the whole plant level, it is presently not clear whether cues that appear to promote growth (e.g., mineral nutrients and high, but not stressful, levels of light) and those that inhibit growth (e.g., water deficit or low temperature) act by the same mechanisms to modulate the activity of common targets. In other words, it is unclear whether promoting growth is relieving growth inhibition. Based on first principles, it is simpler, faster, and more economical to arrest growth, because it would suffice to interfere with an essential step, than to promote growth, which would require coordinate regulation of disparate processes. The principles underpinning plant growth regulation will become clearer once the targets of growth signaling pathways are identified and can be subjected to experimental manipulation.

6 Conclusions and Perspectives

Two significant gaps in our understanding of plant growth control remain:

- How environmental, nutritional, and growth factor cues are perceived and processed by sensory networks
- Mechanistic detail on how such networks control and coordinate the activity of cell growth, division, and expansion

Although increasing numbers of genes involved in these mechanisms are uncovered, very little is still known about how these genes interact to form a regulatory network that couples exogenous and endogenous signals to orchestrate growth responses.

Rapid progress in our understanding of environmental (specifically nutrient) control of adaptive growth responses in plants would be very much facilitated if a minimal set of parameters necessary for analyzing how spe-

cific cues effect changes in growth processes were determined in future experiments. These include the establishment of size at cell birth, kinematic analysis of the spatio-temporal scale and pattern of growth, ploidy analysis, and final cell size. The analysis of several of these parameters is still very challenging, but novel technical approaches, for example FRET-based sensors (Looger et al. 2005), and approaches that could help determine ploidy levels with cellular resolution (Matzke et al. 2005) are being developed. Although comprehensive data sets reflecting genome-wide responses at the level of gene expression, the proteome, and various post-translational modifications are becoming available, I posit that as long as these are obtained from, for example, whole tissues, which correspond to mixed populations of cells undertaking different, often opposite responses, they will be confusing and potentially misleading. Fortunately, novel tools and techniques are becoming available that should soon allow the analysis of such genome-wide responses at the cellular level (Birnbaum et al. 2005; Casson et al. 2005; Lee et al. 2006; Mace et al. 2006; Schad et al. 2005).

Finally, a conceptual debate about the most efficient and comprehensive experimental approaches for characterization of growth signaling is also necessary. Recent analysis of large collections of systematically generated knock-out mutants in budding yeast have led to revised views of signaling pathways. Instead of essentially linear pathways with only few lateral inputs, it has been proposed that much larger numbers of genes and their products participate in signaling networks with many products, associated in complexes, contributing quantitatively to signaling in minor ways (Friedman and Perrimon 2007). These conclusions have been drawn on the basis of end-point results, for example the quantitative effect of loss-of-function mutations on a specific trait under investigation. Such approaches are useful for assembly of a collection of cellular components even peripherally involved in signaling. However, the defining feature of signaling networks are that they respond dynamically to constant changes of specific cues to orchestrate desired outcomes at the cellular, organ, or whole-plant level by processing cues and propagating resultant signals. Thus, signaling networks contain two types of components: (i) those that change their activity as they process and transduce signals, and (ii) those that are minor accomplices to assist signal flux. To understand how the environment controls adaptive growth responses, we must focus on those network components that change properties when signaling is active and on their targets by examining the behavior of such networks under conditions of dynamic change.

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