Historical perspectives on plant developmental biology

The pre-plant developmental biology era

Around 1950, B. McClintock’s and P. Peterson’s analyses in maize led to the description of mobile DNA in the genome. McClintock correlated chromosome breakpoints at specific positions with mobile DNA elements, called Dissociator (Ds) and Activator (Ac), that caused specific changes in phenotypes explained by the altered expression status of known gene loci such as the C locus (McClintock, 1950). This new vision of the genome being dynamic was confirmed in bacteria, animals and other plant species. The molecular basis of mobile DNA in several plant species was demonstrated later (Fedoroff et al., 1983; Döring et al., 1984; Pohlman et al., 1984; Pereira et al., 1985). The cloning of the bronze locus in maize with the Ac transposable element was one of the first examples of «gene tagging» in plants (Fedoroff et al., 1984). Moreover, the Ac/Ds and En/Spm elements, endogenous to the monocotyledon maize, were shown to be active in the dicotyledonous tobacco (Baker et al., 1986; Masson and Fedoroff, 1989; Pereira and Saedler, 1989). These studies pioneered the use of mobile DNA in large-scale mutagenization programs of the plant genome for gene discovery. Introduction of mobile DNA into heterologous plant genomes required a transformation step that was solved by the study of the tumor-inducing (Ti) principle of the plant-colonizing bacterium, Agrobacterium tumefaciens. In 1974, it was demonstrated that a plasmid was present in oncogenic Agrobacterium strains and absent from non-oncogenic strains (Zaenen et al., 1974). It resulted in the hypothesis that this plasmid was the Ti principle and it was indeed shown that a fragment of the plasmid, the so-called T-DNA was transferred to the plant genome (Chilton et al., 1977; De Beuckeleer et al., 1978). The T-DNA contained a number of genes, the so-called oncogenes, encoding plant hormone-synthesizing enzymes that were driven by eukaryotic promoters that became active in the plant cell upon infection (Joos et al., 1983; Zambrzyński et al., 1989). Only the T-DNA borders and the virulence genes on the Ti plasmid were essential for T-DNA transfer and integration into the plant genome. All the T-DNA genes could be replaced by chimeric selectable marker genes or other genes and stably integrated and expressed into the plant genome (Bevan et al., 1983; Fraley et al., 1983; Zambrzyński et al., 1983; De Block et al., 1984; Horsch et al., 1984). The T-DNA was further engineered as a versatile vector for plant transformation and such vector construction is still ongoing today (Karimi et al., 2002). The plant transformation procedures benefited from earlier research on in vitro propagation and regeneration of explants on sterile mineral salt solutions (Murashige and Skoog, 1962) that contained different ratios of phytohormones to promote either callus, root, or shoot growth from explants (Linsmaier and Skoog, 1965). Digestion of explants to single protoplasts and subsequent regeneration into fertile plants was a great advancement because these regenerated plants were clonal (Nagata and Takebe, 1970; Nagy and Maliga, 1976; Lörz et al., 1979). The integration of the

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protoplast regeneration procedure with *Agrobacterium* infection opened the way to produce clonal transgenic cell lines in tobacco at first (Márton et al., 1979). Some plants appeared to be recalcitrant to *in vitro* regeneration and Agrobacterium transformation. It took more than a decade to succeed in a wide variety of plant species. Efficient *Agrobacterium tumefaciens*-mediated transformation methods that were tissue culture-based and accessible to the entire academic community were established for the model plants *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays* (Valvekens et al., 1988; Hiei et al., 1994; Frame et al., 2002). In the meantime, a number of important technological breakthroughs were made in molecular biology, such as the cloning into plasmid vectors, the determination of the DNA sequence of the first viral organism (Fiers et al., 1978) and gene expression analysis (Kamalay and Goldberg, 1984). The subsequent automatization of the sequencing technology resulted in the whole genome sequence of the first flowering plant, namely that of *Arabidopsis* (Arabidopsis Genome Initiative, 2000). High density micro-arrays allowed for quantitative genome-wide expression analyses and contributed to the systems biology approach of biological questions (Lipshutz et al., 1999). The *in vitro* DNA amplification via the polymerase chain reaction (PCR) revolutionized plant biology because the large genomes were made accessible for experimentation (Mullis and Faloona, 1987). The β-glucuronidase gene of *Escherichia coli* was the first reporter gene adapted for use in plants (Jefferson et al., 1987) and was replaced ten years later by the green fluorescent protein (GFP) from jellyfish because of its

### TABLE 1

**TIMELINE OF PLANT DEVELOPMENTAL BIOLOGY MILESTONES INTEGRATED WITH RELEVANT TECHNOLOGICAL BREAKTHROUGHS**

<table>
<thead>
<tr>
<th>Year</th>
<th>Discovery</th>
<th>Scientist</th>
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<tbody>
<tr>
<td>1859</td>
<td>Speciation through genetic alteration and natural selection</td>
<td>C. Darwin and A.R. Wallace</td>
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<tr>
<td>1950</td>
<td>Mobile DNA in the maize genome and its mutagenicity</td>
<td>B. McClintock</td>
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<tr>
<td>1952</td>
<td>Regenerative capacity of the potato shoot apex shown by microsurgery</td>
<td>I. Sussex</td>
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<td>1955</td>
<td>Dependence of leaf dorsoventrality on communication with the shoot apex in potato</td>
<td>I. Sussex</td>
</tr>
<tr>
<td>1965</td>
<td>Organogenetic capacity of phytohormones in tobacco <em>in vitro</em> regeneration</td>
<td>E.M. Linsmaier and F. Skoog</td>
</tr>
<tr>
<td>1970</td>
<td>Evolution by gene duplication</td>
<td>S. Ohno</td>
</tr>
<tr>
<td>1974</td>
<td>Plasmid present in oncogenic agrobacteria</td>
<td>J. Schell and M. Van Montagu</td>
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<tr>
<td>1977</td>
<td>Tumor-inducing principle of <em>Agrobacterium tumefaciens</em> was a plasmid fragment that integrated into the plant genome</td>
<td>M.D. Chilton, M.P. Gordon and E.W. Nester</td>
</tr>
<tr>
<td>1978</td>
<td>Complete sequence of the viral SV40 genome</td>
<td>W. Fiers</td>
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<tr>
<td>1983-1984</td>
<td>Isolation of Ac/Ds elements and sequence determination</td>
<td>P. Starlinger and N. Fedoroff</td>
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<tr>
<td>1986</td>
<td>Maize transposons function in tobacco</td>
<td>B. Baker and N. Fedoroff</td>
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<tr>
<td>1987</td>
<td>DNA synthesis <em>in vitro</em> via polymerase chain reaction</td>
<td>K. Mullis and F. Faloona</td>
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<tr>
<td>1987</td>
<td><em>E. coli</em> β glucuronidase used as reporter gene in plants</td>
<td>R.A. Jefferson and M. Bevan</td>
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<tr>
<td>1989-1990</td>
<td>Floral homeotic mutants in <em>Arabidopsis</em> and <em>Antirrhinum</em> and the ABC model for flower formation</td>
<td>J. Bowman and E. Meyerowitz</td>
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<tr>
<td>1990</td>
<td>Cloning of the first floral homeotic gene in <em>Antirrhinum</em></td>
<td>R. Carpenter and E. Coen</td>
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<tr>
<td>1991</td>
<td>Cloning of the first floral homeotic gene in <em>Arabidopsis</em></td>
<td>M. Yanofsky and E. Meyerowitz</td>
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<tr>
<td>1991</td>
<td>Cloning of <em>KNOTTED</em> in maize important for indeterminacy in the SAM</td>
<td>E. Vollbrecht and S. Hake</td>
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<tr>
<td>1991</td>
<td>Genetic proof of embryonic axis formation</td>
<td>U. Mayer and G. Jürgens</td>
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<td>1993</td>
<td>Genetic control of meristem size: the <em>CLAVATA</em> signaling pathway</td>
<td>E. Meyerowitz and S. Clark</td>
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<td>1993-1994</td>
<td>Cell patterning in the root epidermis and meristem</td>
<td>L. Dolan and B. Scheres</td>
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<td>1995</td>
<td>Trafficking of <em>KNOTTED1</em> transfection factor through plasmodesmata</td>
<td>W. Lucas and S. Hake</td>
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<td>1995-1997</td>
<td>Cell identity in the root apical meristem dependent on short range signaling as shown by laser cell ablation</td>
<td>P. Weisbeek and B. Scheres</td>
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<td>1997</td>
<td>Gene silencing in plants</td>
<td>D. Baulcombe</td>
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<td>1997</td>
<td>Use of green fluorescent protein as reporter in plants</td>
<td>J. Haseloff</td>
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<tr>
<td>1999</td>
<td>High-density micro-array for monitoring genome-wide expression</td>
<td>R. Lipshutz and S. Fodor</td>
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<td>2001</td>
<td>Leaf polarity genes in <em>Arabidopsis</em></td>
<td>J. Bowman, R. Kerstetter and S. Poethig</td>
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<tr>
<td>2000</td>
<td>Sequence of the <em>Arabidopsis thaliana</em> genome</td>
<td>Arabidopsis Genome Initiative</td>
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<tr>
<td>2002</td>
<td>miniRNAs in plants</td>
<td>D.J. Bartel, B. Bartel and M.W. Rhoades</td>
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<tr>
<td>2003</td>
<td>Regulation of phytoxins by polar auxin transport</td>
<td>D. Reinhardt and C. Kuhlemeier</td>
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<tr>
<td>2003-2004</td>
<td>Sorting of cells by laser capture or flow cytometry</td>
<td>T. Nelson and P. Benfey</td>
</tr>
<tr>
<td>2003</td>
<td>Mathematical modeling and computer simulation of patterning</td>
<td>E. Coen and P. Prusinkiewicz</td>
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application in living explants using confocal microscopy (Haseloff et al., 1997). A timeline is presented in Table 1.

**Milestones in plant developmental biology**

Milestones during the last 25 years were the cloning of genes corresponding to mutations affecting key steps in developmental processes (forward genetics), their molecular analysis and the study of their genetic interactions in order to build genetic models for a given process (reviewed for Arabidopsis by Somerville and Koornneef, 2002; Van Lijsebettens et al., 2002). This molecular-genetic approach is now replaced by a large-scale functional genomics approach in which the function of all members of a gene family is analyzed by reverse genetics. In the following sections, we will exemplify a number of specific cases, with milestones listed in Table 1.

**Embryogenesis**

Embryogenesis in plants starts with the asymmetric division of the zygote resulting in an upper cell that will develop into an embryo and in a lower cell that generates the suspensor, which is the connection to the maternal tissue. For ease of conceptualization approximately 20 stages have been distinguished in embryo formation of which the early ones are important for axis formation and patterning and the later ones for growth and maturation. Mature plant embryos have a very simple body plan, in which the apical-basal and radial axes are specified. At the end of the apical-basal axis the root and shoot apical meristems (SAM) are situated that become active upon germination and generate the primary root and shoot, respectively. Along the radial axis, patterning in progenitor tissue layers occurs during early embryogenesis. One of the key questions has been the identification of regulators that control the switches from globular to heart stage or from heart to torpedo stage. However, such master switches have not been detected despite extensive mutagenization programs for embryo-lethal (emb) mutants in Arabidopsis (Meinke and Sussex, 1979a, 1979b; Franzmann et al., 1995; McElver et al., 2001) and in maize and extensive studies of the Daucus carota embryogenic cell suspension (Giuliano et al., 1984; De Jong et al., 1992). Currently these structures are assumed to arise progressively. Approximately 750 EMB loci have been described to date that are essential for embryogenesis (Franzmann et al., 1995; McElver et al., 2001). Some of these genes are important in the communication between suspensor and embryo, in the control of cell number in the embryos and in the control of embryo maturation. A number of them will reveal essential enzymes for primary metabolism and numerous loci correspond to unknown proteins (Berg et al., 2004). A comprehensive database has been developed containing information on genes that give a seed phenotype upon mutation in Arabidopsis (Tzafrir et al., 2003). The conclusion is that the development of the zygote into the embryo is a progressive process in which the action of many genes together is required.

Key regulatory genes that control axis formation have been identified: phenotypes predicted from defects along the apical-basal or the radial axis were obtained upon mutagenization and screening for seedling lethals (Mayer et al., 1991); their corresponding genes are involved in cytokinesis and auxin transport (Shevell et al., 1994; Lukowitz et al., 1996; Hardtke and Berleth, 1998; Assaad et al., 2001). These studies confirmed the model for embryo formation obtained through cell biology and clonal analyses describing the different domains and boundaries within a developing embryo and the embryonal origin of the different parts of the germinating seedling (Dolan et al., 1993; Scheres et al., 1994).

**The self-regulatory shoot apical meristem**

The SAM has an embryonic origin based on the expression of the SHOOTMERISTEMLESS (STM) SAM marker gene and the stm knockout phenotype that produces seedlings without SAM

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**Fig. 1. Shoot apical meristem organization and leaf development.** (A) Drawing of an Arabidopsis thaliana median longitudinal histological section of the SAM with the different zones (FLM, flank meristem; CIZ, central initiation zone; CLZ, cambium-like zone; FLM, file meristem) (according to Vaughan, 1952). (B) Microsurgical incisions, represented by the white lines, in the potato SAM (top view) have an effect on the symmetry of the next leaf primordium (I1) to be formed (according to Sussex, 1955). (C) Paradermal section through an expanding Arabidopsis thaliana leaf lamina showing gradients of cell division at the basal zone and cell expansion at the tip (according to Pyke et al., 1991).
The STM gene is the orthologue of the maize KNOTTED1 (KN1) homeobox gene that upon ectopic expression in maize leaves reverts the determinate to the indeterminate state, resulting in the production of knots (Vollbrecht et al., 1991). KN1 was the first plant protein for which plasmodesmal trafficking has been shown to occur (Lucas et al., 1995); this report was one of the first to emphasize the importance of plasmodesmal cell-to-cell communication in developmental processes. After germination, the SAM starts to produce the lateral organs and stem tissue that are organized in the so-called phytomers. The SAM consists of zones that are distinct with respect to their cell division activity and developmental destination and was subject of early developmental research in plants and is still today (Vaughan, 1952; Steeves and Sussex, 1989; Potten and Loeffler, 1990; Laufs et al., 1998) (Figure 1A). In the central zone, stem cells stay in an indeterminate state. Upon division, stem cells replenish themselves but also produce daughter cells that are displaced into the peripheral zone where they are recruited to initiate leaf primordia or into the rib zone where they contribute to the formation of stem tissue. The SAM is also layered: L1, L2 and L3 layers that are the progenitor of epidermal tissue, of palisade and spongy parenchyma (and the sporogenic cells) and of vascular tissue, respectively. A genetic model has been proposed for the self-regulation of the SAM. The CLAVATA (CLV) genes are responsible for the repression of the growth in the central zone (Clark et al., 1993, 1997; Fletcher et al., 1999; Brand et al., 2000) and encode components of a signaling cascade that regulates WUSCHEL (WUS) activity (Laux et al., 1996; Trotta et al., 1999; Schoof et al., 2000). WUS is a homeodomain protein that keeps stem cells in their indeterminate state through a negative feedback loop with CLV3 (Mayer et al., 1998). The site of expression of the WUS domain, just beneath the stem cell zone is called the «organizing center» and is comparable to the quiescent center in the root apical meristem (van den Berg et al., 1997; for review, see Weigel and Jürgens, 2002).

**Leaf phyllotaxis, initiation and polarity**

Leaf primordia initiate at the SAM peripheral zone and have a multicellular origin because cells are recruited from the different SAM layers. The leaf initiation site or phyllotaxis is delineated by molecular markers, such as ASYMMETRIC1, whose position at the periphery of the SAM depends on the position of previously formed primordia. Phyllotaxis is species specific, can be opposite, decussate, or spiral according to the mathematical Fibonacci series (Mitchison, 1977). «Biophysical forces» regulating local epidermal cell wall extensibility was one of the mechanisms proposed to explain phyllotaxis (Green, 1996; Fleming et al., 1997). Recently mutational analysis and pharmacological tests combined with micro-manipulation have demonstrated that the hormone auxin is crucial in determining the leaf initiation site (Reinhardt et al., 2000, 2003).

At the leaf inception site, no STM expression fits the exit from the proliferative state into a differentiation state of the primordium founder cells with a de-repressed AS1 gene activity. AS1 in Arabidopsis (Byrne et al., 2000), its orthologue PHANTASTICA (PHAN) in Antirrhinum (Waite and Hudson 1995; Waite et al., 1998) and ROUGH SHEATH2 (RS2) in maize (Timmermans et al., 1999; Tsiantis et al., 1999) are important for promoting adaxial fate in leaf primordia, their function being conserved in monocots and dicots. Upon recessive mutation of the AS1/PHAN/RS2 genes, some of the KNOX genes are ectopically expressed in the leaves where they are normally inactive (Schneeberger et al., 1998; Byrne et al., 2000; Ori et al., 2000). Microsurgical experiments on the potato shoot apex have shown that the SAM communicates with leaf primordia and that a signal is required to induce polarity in the leaf primordium. Incisions between the SAM and the primordium resulted in radial symmetrical rather than dorsoventral asymmetrical leaves (Figure 1B) (Sussex, 1951, 1955). Although the signal is still not known today, the genetic factors for polarity have been identified: these are AS1 and transcription factors of the HD-ZIPIII and GARP class (Sawa et al., 1999; Siegfried et al., 1999; Kerstetter et al., 2001; McConnell et al., 2001). Analysis of their genetic interactions resulted in a model for dorsoventrality in leaves (for review, see Bowman, 2004).

**Organ size and shape**

A very intriguing question in organ formation is how size and shape are determined. Cell expansion and its direction have been considered for a long time to be the major determinants. However, recent work has demonstrated that cell division activity, rate of cell division and termination of division activity are also important determinants for organ morphology as shown by mutational analysis and manipulation of the cell cycle. Two theories have been postulated: the Cell Theory which states organ size and shape are merely determined by their building blocks, the cells; in the Organismal Theory, cells just fill up the organ form that is determined by higher order control (for review, see Tsukaya, 2002; Beemster et al., 2003).

The leaf has been exploited as a model to study the genetic and environmental factors that control size and shape. Early leaf growth is mainly due to cell division processes that cease gradually from the tip to the base of the organ, from its margin to the midvein and from the ventral to the dorsal side of the lamina (Figure 1C) (Pyke et al., 1991; Van Lijsebettens and Clarke 1998; Donnelly et al., 1999). Interference with early growth by modulation of cell cycle regulatory genes has resulted in changes in leaf size and shape (De Veylder et al., 2001; Fleming, 2002; Wyrzykowska et al., 2002; Dewitte et al., 2003). The AINTEGUMENTA transcription factor controls organ size by regulating the number and the extent of cell divisions during organogenesis (Mizukami and Fischer, 2000). Later growth is assumed to be due to polar and non-polar cell expansion processes. Expansion growth is perturbed by modifying the expression of genes coding for enzymes involved in hormone biosynthesis or cell wall composition and results in altered leaf size and shape (Fleming et al., 1997; Kim et al., 1999; Cho and Cosgrove, 2000; Pien et al., 2001; Fleming, 2002). ANUGSTIFOLIA, a transcriptional co-repressor, is required for polar cell expansion (Folkers et al., 2002; Kim et al., 2002). Over 100 gene loci have been identified to date with a function in the making of the leaf, of which 94 originate from an ethyl methane sulfonate mutagenization program (Berná et al., 1999; Tsukaya, 2002). The systematic cloning and molecular-genetic analyses of these genes will further our knowledge on the molecular mechanisms directing organ formation.

In addition to the above-mentioned internal factors, leaf growth is also modulated by environmental factors, such as water, light and CO2 availability, that affect leaf size and shape. These parameters influence the number of cell cycles during leaf formation.
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(Tardieu and Granier, 2000) and the polar and non-polar cell expansion processes that contribute to leaf organogenesis as well (Granier and Tardieu, 1998).

In Arabidopsis, mutational analysis showed that leaf growth is controlled at the transcriptional level not only by transcription factors but presumably also by chromatin modification. Functional analysis of structural components and a putative regulator of the Elongator histone acetyltransferase complex, associated with the RNA polymerase II transcription elongation complex resulted in plants with a leaf phenotype (Nelissen et al., 2005), suggesting that the chromatin status is important during organogenesis (Figure 2).

Universal flower model

Flowers consist of four types of organs that are arranged in whorls. The genetic control of flower organ identity was a major discovery of plant developmental biology in the nineties and was based on the study of homeotic flower mutants with normal floral organs at ectopic positions, which replace the flower organs usually present. Such mutants were described in a number of species in ancient literature all over the world. Homeotic mutants and their genetic interactions have been studied extensively in snapdragon (Carpenter and Coen, 1990) and Arabidopsis (Komaki et al., 1988; Meyerowitz et al., 1989). This research resulted in the famous ABC flower model (Bowman et al., 1989; Schwarz-Sommer et al., 1990; Coen and Meyerowitz, 1991). In this model, floral organs are specified by the action of A, B and C genes, the so-called floral organ identity genes, that are active in two subsequent whorls. From 1990 on, the genes corresponding to the flower homeotic genes were cloned in Antirrhinum, Arabidopsis and Petunia by making use of the first mutant collections tagged either with endogenous transposable elements (Carpenter and Coen, 1990), with the Agrobacterium T-DNA (Azpiroz-Leehan and Feldmann, 1997), with the endogenous transposon dTph1 in petunia; or using reverse genetics strategies (Vandenbussche et al., 2003a, 2004). The first homeotic genes cloned were the C function gene AGAMOUS in Arabidopsis (Figure 3B) (Yanofsky et al., 1990) and the B function gene DEFICIENS in snapdragon (Sommers et al., 1990; Schwarz-Sommer et al., 1990). In the following years all the floral homeotic genes were cloned and appeared to be MADS-box transcription factors, except for APETALA2 that identified a plant-specific transcription factor class (Jofuku et al., 1994). The
ABC model has been verified by double and triple mutants, overexpression constructs and gene expression analyses; it still stands today even though it has been extended with D and E function genes (Colombo et al., 1995; Pelaz et al., 2000; Honma and Goto, 2001). The identity of the floral organs has been postulated to be specified by tetrameric complexes of floral organ identity gene products that bind to promoters of downstream targets thereby activating or repressing their activity and resulting in specific floral organ identities (Honma and Goto, 2001; Theißen and Saedler, 2001). ABCDE genes were identified in a lot of species of flowering plants, monocots and dicots, confirming the conservation of the overall molecular mechanism of flower organ formation in evolution (for recent review, see Ferrario et al., 2004).

All genes, except for A-type function, are present in gymnosperms indicating ancient mechanisms for reproductive organ formation (Tandré et al., 1995; Theißen et al., 2000).

In a b c triple mutants, every flower organ is converted into a leaf and overexpression of B and D function genes is sufficient to transform rosette leaves into petals, which is genetic proof of leaves being the ground state (Figure 3C) (Weigel and Meyerowitz, 1994; Honma and Goto, 2001). von Goethe (1790) had already pointed out that different types of organs, such as leaves, petals and stamens, were variations on a common underlying theme. His theory was also based on the study of abnormal flower morphologies in which one organ type was replaced by another one.

**Symmetry**

The famous botanist C. Linnaeus (1749) noticed that occasionally individuals of a plant species had altered flower morphologies. He described a naturally occurring mutant of Linaria vulgaris (toadflax) with radial flower symmetry instead of dorsoventral asymmetry; the mutant flower was called peloric (Greek for toad). Similar mutations have been obtained in Antirrhinum and Mohavea having been explained by ectopic expression patterns of the CYC and DICH genes (Hileman et al., 2003), which is one of the first examples that explains evolutionary morphological divergence in terms of variations in gene expression.

**The cellular organization in the primary root**

The development of the primary root has been neglected for a long time and it was only until Arabidopsis started to be a model that people got interested in root biology. Until then, the root was mainly studied for its role in gravitropism. The root is a good model for cell biology because it is transparent (no chlorophyll) and, hence amenable to confocal microscopy on living explants by using fluorescent dyes or reporter genes. Tissue patterning and cellular communication have been studied extensively and with great success in the root. Clonal analyses showed the embryonic origin of the root meristem initials and its radial organization in a constant cell number with root cell initials giving rise to one or two cell layers (Dolan et al., 1993; Scheres et al., 1994). The root meristem consists of quiescent center cells that keep the surrounding initials in an indeterminate state (van den Berg et al., 1997). The daughter cells of the initials differentiate into specific tissue, the identity of which is reinforced by signals from more mature cells (van den Berg et al., 1995). Patterning in the root epidermis was subject to cell biology and genetic analyses (Dolan et al., 1994) and a number of regulatory genes have been identified (Larkin et al., 2003). Mutagenization programs were initiated to look for regulatory genes of root cell specification (Benfey et al., 1993; Scheres et al., 1995). SCARECROW and SHORTROOT are essential for the asymmetric cell division in the generation of the cortex/endodermal tissue layers (Di Laurenzio et al., 1996). A huge number of marker lines exist with cell type-specific expression in the root, which have been obtained by promoter trapping with a modified GFP reporter gene (Haseloff et al., 1997). A major breakthrough technology was the use of these marker lines in cell sorting to purify specific cell types and study their transcript profiles (Birnbaum et al., 2003). The technology will become applicable to a large number of cell types or cell domains in planta that can be distinguished by marker genes. Cell differentiation studies were restricted in plant research because in vitro culture of a specific cell type has not been achieved so far.
Perspectives in plant developmental biology

Although the survey of milestones is far from complete it is obvious that in the past 15 years a lot of progress has been made in the identification of the genetic control of pattern formation during embryogenesis, organ (leaf and flower) formation and in tissue differentiation. Many of the transcription factors involved have been cloned and studied, however much less effort was investigated so far in the study of the upstream signaling cascades and intrinsic and external stimuli that direct these patterning processes through transcription factor activation or repression (Hay et al., 2004). It will be a future challenge to link the genetics to the physiology of the plant.

A lot of research needs to be done on the communication processes between the cells of the multicellular plant as well as between its different tissues and organs. Hormones have been shown to be important to direct developmental processes at the whole organism level, but the molecular mechanism of their circulation through the plant is still poorly understood. In addition other signaling molecules have been recognized as important communicators such as small peptides, oligosaccharides and metabolites such as salicylic acid. The peptides appear to have crucial functions in tissue domain interaction such as CLV3 in the regulatory loop for self-maintenance of the SAM and in cell-cell interaction such as SCR in the self-incompatibility response (Matsubayashi, 2003). A lot of small open reading frames are out there in the genome and their function remains to be solved. The role of volatiles such as jasmonic acid in plant development needs to be further explored. The regulation of plasmodesmata formation and closure between cells and tissue domains has been shown to be important in communication and needs further attention.

Another big question to be solved is how organ size and shape are determined. Over the last years it became clear that not only cell expansion but also cell division is important. At some point in development cells in meristems need to know when to leave the cell cycle and start the differentiation process. The signals and molecular mechanisms need to be determined that control the switch between cell cycle entry and exit during development and in response to environmental cues (Gutierrez, 2005).

It took a decade to functionally analyze 10% of the Arabidopsis genes using forward genetics. In the meantime large mutagenized seed collections have been generated that are exploited for reverse genetics of gene families. Within the next five years of Arabidopsis research the aim is to uncover the function of every gene; the National Science Foundation 2010 project is the leading initiative. This will be possible because there is a shift to large-scale experimentation in which not a single gene but rather its whole gene family is functionally analyzed. From the genome sequence, all the members of a given gene family can be retrieved; by reverse genetics, mutations can be looked for in the available collections and be analyzed for their phenotype. The function of large gene families such as the cellulose synthase-like genes (Bonetta et al., 2002) or myb-type transcription factors (Meissner et al., 1999) are analyzed by reverse genetics. In large gene families functions might be redundant because of recent gene duplication resulting in the lack of phenotypes by single gene knockout. In order to define functions, double or even triple mutant combinations of knockouts will have to be made in the respective paralogs. A few nice examples illustrate this approach, such as for the MADS-box SEPALLATA genes (Pelaz et al., 2000) and the B-function genes in petunia (Vandenbussche et al., 2004). Unknown proteins for which a mutant phenotype has been obtained are analyzed for their interactions with other proteins by means of yeast-two-hybrid analysis or TAP tagging to get a clue to their molecular function. A number of unknown proteins identified by embryo lethals are studied in this way (Berg et al., 2004). The wealth of information on gene function in model systems will serve to improve plant product quality and adaptation of plants to changing environments. Genes from model systems have been overexpressed in other species with success (Weigel and Nilsson, 1995); however, they mainly serve to isolate and study the orthologs in crops (Byzova et al., 2004). The synteny of large chromosomal domains between related species has been exploited to use gene knowledge obtained in model systems such as Arabidopsis for molecular breeding in related crops such as Brassica species (Lagercrantz et al., 1996). Synteny between cereal genomes is high and the rice genome sequence is used as reference to aid for instance in positional cloning of genes in maize (Devos, 2005). Quantitative trait loci analysis is an approach to identify and clone genes that contribute to complex phenotypes such as seed weight or leaf size and shape and has been successfully used in a number of plant species (Alonso-Blanco and Koornneef, 2000; Pérez-Pérez et al., 2002; Morgante and Salamini, 2003; Tanksley, 2004).

More model species for developmental studies are emerging such as Medicago truncatula to study nodule formation upon symbiosis with Sinorhizobium (Cook, 1999; Young et al., 2003) and the tree model Populus to study wood formation and, more recently, cambium activity (Bhalerao et al., 2003; Brunner et al., 2004). These model systems fulfill a number of criteria such as diploidy, easy transformation, small genome, ongoing genome sequencing, big consortia for coordinated international research and maintenance and availability of genetic resources. In this new research tendency the diversity in plant developmental processes is recognized to exceed the potential of just a few model systems.

Soon there will be a shift from the model species to a wide range of species to study species-specific development or morphologies as for instance the «cluster» root (Shane et al., 2004) and to study processes for which Arabidopsis is not a good model such as for domestication, mycorrhizae interaction or nodule formation. With the increasing functional analyses of genes from model species, comparative analysis will become more important and powerful. DNA sequencing technology is automated and its efficiency has improved tremendously over the last five years, so that not the amount of work but rather the cost and bioinformatics tools will be the limiting factors for sequence analysis of a specific species in the near future (Venter, 2004). New areas of research, such as comparative genetics, will exploit this sequence information and couple it to questions related to gene function conservation or divergence. A well-studied case is the homeobox gene function divergence between plants and animals (Meyerowitz, 2002). The conclusion is that similar processes of pattern formation are used in plant and animal developmental programs; however different classes of regulatory genes have been re-
recruited for it during evolution. Comparative genetics relies on DNA sequence information and aims at studying a genetic trait within a plant family or even between incompatible species and overcomes the genetic barrier of crossing inhibition.

Another emerging field is evolutionary developmental biology the so-called “Evo-Devo” that also exploits DNA sequence information to explain morphological diversity. Function conservation of key regulators in development, such as the MADS-box transcription factors with a role in flower organ specification, begins to explain the main aspects of flower morphology in different species, such as the different floral organ types and the floral whorls. Gene duplication and function divergence by coding sequence changes in addition to ectopic expression patterns clarify the diversity in flower morphology in a number of cases (Kramer and Irish, 1999; Vandenbussche et al., 2003b; Ferrario et al., 2004).

Evolutionary developmental biology studies have investigated some aspects of diversity in leaf morphology as well (Cubas et al., 1999b; Bharathan et al., 2002; Kanno et al., 2003; Hileman et al., 2003; Tsiantis and Hay, 2003). Bioinformatics research showed that diploid genomes, such as that of Arabidopsis and other model systems contain large genome duplications (Arabidopsis Genome Initiative, 2000; Simillion et al., 2002; Blanc and Wolfe, 2004). Genome duplications have been postulated to allow for diversification in gene function and to be the major mechanism to achieve morphological diversity in the flowering plants and also in the animal kingdom in combination with natural selection as postulated by C. Darwin in the late 19th century (Darwin, 1859; Ohno, 1970). Computational approaches to unveil ancient genome duplications are under development and may contribute to new insights into evolutionary genetics (Van de Peer, 2004).

Significant progress in the unraveling of molecular networks is to be expected from the systems biology approach in which the entire transcriptome, proteome, or metabolome is analyzed upon perturbation rather than single genes. The aim is to identify the complex networks responsible for biological processes and their mutual interactions (Gutiérrez et al., 2005). Integration with computational science and mathematics will be indispensable to interpret the large data sets, generate network visualization and build models. The number of computer programs for visualization and integration of different data sets, such as MAPMAN (Thimm et al., 2004) is increasing and is a prerequisite to understand the biology. The integration of biological data into regulatory networks will allow further testing and predictions (Ideker et al., 2001; Davidson et al., 2002). Models on plant growth and development are being generated for plant architecture, organs and tissues and incorporating genetic regulatory networks. These models are an integration of mathematical modeling and computer simulations with biological components such as modules for architecture, growth parameters for organs and tissues, or genes and their domains of action and genetic interaction for regulatory networks (Rolland-Lagan et al., 2003; Kwiatkowska and Dumas, 2003; Gielis, 2003; Prusinkiewicz, 2004). Future goals are the integration of models for architecture with those for organs and tissues and for genetic regulatory networks in order to obtain in-depth understanding of the mechanisms of plant development from genes to phenotypes (Prusinkiewicz, 2004).

Plant cell sorting has recently been achieved by several groups to purify living cells of the same type with cell-specific GFP markers, or alternatively small tissue domains with laser technology (Kerk et al., 2003; Birnbaum et al., 2003). Plant cell sorting is a breakthrough technology since research on cell differentiation in plants was limited to molecular-genetic analysis because of the inability to culture differentiated plant cells in vitro unlike in mammalian systems. The genome-wide profiling techniques are applied to this sorted plant material and the results will undoubtedly further our knowledge on the progressive process of cell specification to differentiation and cell function. Increasing the resolution of sorting and systems biology up to single cell level will open up new opportunities in the study of cell specification. Then, genetic programs would be analyzed that distinguish, for instance, between the different fate of the daughter cells after asymmetric cell division, such as in the case of the first zygotic division or upon lateral root induction or in stomatal development.

Another challenge for future research on plant development will be to understand other mechanisms besides the transcriptional control of genetic programs exerted by transcription factors. Recently, microRNAs have been discovered in plants and a number of them are complementary to transcription factors with a function in developmental processes (Reinhart et al., 2002; Rhoades et al., 2002; Bonnet et al., 2004). For instance in leaf development several transcription factors, such as PHASULOSA, PHAVOLUTA and CINCINNATA-like genes are targeted by miRNAs (Nath et al., 2003; Palatnik et al., 2003; Juarez et al., 2004; Kidner and Martienssen 2004). Temporal and spatial regulation of expression of microRNAs is of utmost importance for the proper destruction of transcription factor mRNAs during developmental processes and it is based on the silencing pathway (Baulcombe, 2004). However, the regulation of expression of the miRNAs is still unknown and needs to be explored because it adds another level to gene expression regulation and it may contribute to the delineation of boundaries and domains in developing organs.

Protein degradation through the ubiquitination pathway is an important control mechanism for developmental pathways. E3 ubiquitin ligases target specific substrates for degradation at the proteasome and more than 460 are represented in the Arabidopsis genome (Stone et al., 2005). A number of their targets will be important in developmental control and their nature will be revealed in the coming years by functional genomics.

Epigenetic control of developmental transitions and morphogenetic processes needs to be further explored (Reyes et al., 2002). The naturally occurring peloric mutant of Linaria vulgaris described by Linnaeus more than 250 years ago has an epigenetic mutation in a CYC ortholog (Linnaeus, 1749; Gustafsson, 1979; Cubas et al., 1999b). Other well-studied epimutations are at the P locus in maize and at the SUPERMAN locus in Arabidopsis (Das and Messing, 1994; Jacobsen and Meyerowitz, 1997). From these studies it became clear that the DNA methylation status has a great impact on gene expression and can be transferred to subsequent generations in plants. As mentioned, the DRL1 and ELO genes studied in our unit (Nelissen et al., 2003, 2005) identified a histone acetyltransferase complex, named Elongator that associates with the RNA polymerase II transcription elongation complex. The drl1-2 and elo mutants have a narrow leaf phenotype indicating that leaf form is also regulated by reversible chromatin modification. The drl1-2 and elo mutants have in addition reduced root growth, a stunted inflorescence and an altered phyllotaxis (Figure 2). Reversible histone modifications,
such as acetylation/deacetylation, are of critical importance to make DNA available for transcription or to repress transcription. A well-studied case is the vernalization-dependent deacetylation and, hence, inactivation of the FLOWERING LOCUS C gene that codes for a repressor of flowering (Sung and Amasino, 2004). A number of histone acetylases and deacetylases (HDAC) are present in the plant genome, amongst them the plant-specific HD2 subfamily of HDACs (Lusser et al., 1997; Pandey et al., 2002). It will be a challenge to find out about their upstream signaling, downstream targets and function in plant development.

On the longer term, more than 10 years from now, it is difficult to predict the future because a major input of technology and expertise from other disciplines in biological research is to be expected. The biology-driven research relies to a great extent on breakthrough technologies to take the research to the next level. No doubt, technology will have a huge impact on experimentation and thinking in biological research in the next decade.

**Summary**

The early studies of plant growth and development focused on embryogenesis. In the past twenty five years, it became possible to successfully analyze many more developmental processes, hence plant developmental biology became the generally accepted terminology. It refers to a multidisciplinary approach using expertise from other disciplines in biological research is to be expected. The biology-driven research relies to a great extent on breakthrough technologies to take the research to the next level. No doubt, technology will have a huge impact on experimentation and thinking in biological research in the next decade.

**KEY WORDS:** model plant, regulatory network, forward and reverse genetics, evo-devo, systems biology and modeling

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