MOLECULAR-GENETIC APPROACH TO STUDY PLANT GROWTH AND DEVELOPMENT

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Due to their relative immobility, plants have evolved into organisms with a distinct pattern of growth and morphogenesis. Intrinsic to these are autotrophic growth, converting sun light and atmospheric carbon dioxide into chemical energy and chemical components necessary to sustain maintenance and growth of the organism, totipotency of cells and development through patterns of cell division at the meristematic zones that remain active over the entire life cycle. Processes of polar and non-polar cell expansion and differentiation lead to patterning and the formation of organs with inner and outer architecture adapted to their specific functions. Insight into the molecular mechanisms that direct and control the different steps in the ontogeny of organs, comes from the identification and study of the respective genes and their genetic manipulation in transgenic plants that nowadays can be obtained in many species.

The weed *Arabidopsis thaliana* has been widely accepted as a model system to study the molecular basis of plant growth and development and to address questions in plant physiology, biochemistry, cell biology, and pathology. The plant is ideally suited for molecular-genetic studies due to its minimal genomic DNA content of 100 Megabase, only five times the yeast genome, its small size, short life cycle, massive production of seed upon self-fertilization and the production of transgenic lines without limitation. In analogy to other models for other phyla, an *Arabidopsis* genome sequencing project has been initiated, with the aim of finishing the whole genome in the year 2004. It is clear that concerted research efforts on one particular species will accelerate the accumulation of biological knowledge in an exponential way.

One of our interests is to study the genetic control of leaf lamina formation. Leaf primordia originate from the shoot apical meristem by a difference in rate and polarity of cell division in the cell layers. Within the primordia cell division follows a gradient from base to tip and from the middle towards the margins, so that the oldest parts of the leaf are at the tip and the margins. Cell expansion occurs through an opposite gradient and from the lower side of the leaf to the upper side so that the oldest parts contain the expanded tissues. Patterns of differentiation lead to the formation of a venation network typical for each species and to spatial distribution of stomata and trichomes within the epidermal layer. We identify mutants in *Arabidopsis* populations mutagenized either with the *Agrobacterium tumefaciens* T-DNA, the maize transposons *Ac/Ds* or the chemical ethyl methanesulfonate. Some of them are tagged by an insertion element, others are non-tagged and either tissue-culture induced mutations or abortive integration events of the elements used. Inverse polymerase chain reaction strategies were designed to clone plant DNA sequences flanking the insertion elements in case of tagged alleles.

One of the mutant classes we study identifies genes that act on lamina formation in a quantitative way, i.e. all cell layers are present but the lamina width is reduced. With this class of mutants we would like to solve the question whether the polarity of cell division within the shoot apical meristem is responsible for lamina formation or whether lamina formation merely depends on the existence and activity of a marginal meristem within the leaf primordium. Identification of genes with an effect on mass production is of potential interest in the study of quantitative traits. Two tagged mutations of this type have been studied so far. The *pfl* mutation has a moderately reduced width of the leaf lamina resulting in a 20 % reduction in the fresh weight of full-grown rosettes. The respective gene, *RPS18A*, was cloned by T-DNA tagging and codes for the ribosomal protein gene S18, important in the formation of the translation initiation complex. The gene is predominantly expressed in meristematic tissues. Null mutation at this gene is not lethal because of the overlapping expressing of two additional copies of the gene family (Van Lijsebettens *et al.*, 1994). The complex regulation of this gene family is analyzed in more detail. The *nrl1* mutation has an extremely narrow leaf blade, with normal length. This phenotype is already apparent early, at the primordium stage and is due to a reduction in cell number and size in the width direction. The *NRL1* gene was cloned by *Ds* tagging, it encodes a protein homologous to the yeast KTI12 protein with a putative role in the cell cycle control. However, its exact role in lamina formation remains to be resolved.

A second class of mutants, called *tornado*, has a completely deteriorated leaf lamina with parts of the blade missing, and a defective vascular differentiation. They all have severely reduced apical dominance resulting in dwarf growth with twisting of all organs. Auxins are plant hormones known to play an important role in a variety of developmental processes such as cell expansion, apical dominance, vascular differentiation, lateral root formation and tropisms. The genetic control of auxin biosynthesis, transport and sensing is poorly understood, even few of the enzymes directing these processes in plants are known. Our mutant screenings have revealed loci with phenotypic alterations suggestive for involvement at the level of auxin metabolism, perception or transport. Several phenotypic aspects of the *trn* mutant phenotype suggest that auxin transport might be affected. Another mutant called *superroot*, *sur*, produces an excessive root system with a very poorly developed vegetative part due to increased levels of the auxin indole-3-acetic acid (Boerjan *et al.*, 1995). Map-based cloning techniques have been developed to clone these non-tagged *trn* and *sur* alleles. Recessive visible, dominant selectable (Van Lijsebettens *et al.*, 1996) and (co)dominant molecular markers such as CAPS and microsatellites were used to map the loci. We adopted the recently developed amplified fragment length polymorphism (AFLP) technique to generate

seventeen new molecular markers in a three centiMorgan genetic interval around the *TRN1* locus (Van Gysel *et al.*, 1996). Four of these markers cosegregate with the *trn1* mutation and allowed to isolate a yeast artificial chromosome (YAC) clone spanning the *TRN1* region (Cnops *et al.*, 1996). Corresponding and overlapping bacterial artificial chromosome (BAC) clones have been isolated and subclones are being introduced into the mutant for complementation analysis and isolation of the *TRN1* gene.

Based on physical map data, ten YAC clones covering the genetically defined interval surrounding the *SUR* locus were isolated and subjected to an AFLP-based analysis to narrow down the genomic region around *SUR* to only two YACs. The YAC clones spanning the *TRN1* and *SUR* loci are used in a shot gun complementation approach in order to identify the corresponding genes. BAC clones covering the YACs are used to create sublibraries in a new plant transformation vector, especially designed for cloning large inserts directly in *Agrobacterium*. In that way, clones with inserts ranging from 20 to 50 kb can be readily obtained and stably maintained in *Agrobacterium*. To detect a subclone carrying the gene of interest, independent bacteria are randomly pooled and used to transform mutant plants in order to complement the mutation.

Over the last years, the tools and resources have been created in *Arabidopsis* to clone a gene based on mutation. The progress in understanding the molecular mechanisms underlaying developmental processes will come from focus on the most informative mutations.

Cell division is an integrated part of plant development and, similar to animals and yeast, cell cycle progression in plants depends on the activity of CDK/CYCLIN protein kinase. *Arabidopsis* contains two genes encoding cyclin-dependent kinases (*cdc2a* and *cdc2b*) and at least twelve different cyclins (Segers *et al.*, 1996). Based on expression analysis and sequence comparison cyclins can be subdivided into A-, B-, and D-types, reflecting a specific role during the different cell cycle phases (Shaul *et al.*, 1996). The structure and role of the CDK/CYCLIN kinases have been studied in detail by protein purification, two-hybrid interactions, expression analysis and overproduction of dominant negative CDK variants in transgenic cells (Hemerly *et al.*, 1995). A progress report will be presented.

Cyclins are excellent markers to follow the activation/inactivation of the cell cycle machinery by both intrinsic developmental signals and environmental factors. For example, transgenic *Arabidopsis* lines with cyclin promoter-β-glucuronidase fusions allowed us to describe the early events leading to the formation of lateral roots and to isolate novel *Arabidopsis* mutants with an altered cell division.

References

Boerjan, W., Cervera, M.-T., Delarue, M., Beeckman, T., Dewitte, W., Bellini, C., Caboche, M., Van Onckelen, H., Van Montagu, M. and Inzé, D. (1995). *superroot*, a recessive mutation in Arabidopsis, confers auxin overproduction. Plant Cell 7: 1405-1419.

Cnops, G., den Boer, B., Gerats, T., Van Montagu, M. and Van Lijsebettens, M. (1996). Chromosome landing at the Arabidopsis *TORNADO1* locus using an AFLP-based strategy. Mol. Gen. Genet.: in press.

Hemerly, A., de Almeida Engler, J., Bergounioux, C., Van Montagu, M., Engler, G., Inzé, D. and Ferreira, P. (1995). Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. EMBO J. 14: 3925-3936.

Segers, G., Gadisseur, I., Bergounioux, C., de Almeida Engler, J., Jacqmard, A., Van Montagu, M. and Inzé, D. (1995). The Arabidopsis cyclin-dependent kinase gene *cdc2bAt* is preferentially expressed turing S and G_2 phases of the cell cycle. Plant J. 10: in press.

Shaul, O., Mironov, V., Burssens, S., Van Montagu, M. and Inzé, D. (1996). Two *Arabidopsis* cyclin promoters mediate distinctive transcriptional oscillation in synchronized tobacco BY-2 cells. Proc. Natl. Acad. Sci. USA 93: 4868-4872.

Van Gysel, A., Cnops, G., Breyne, P., Van Montagu, M. and Cervera, M.T. (1996). An AFLP™-based strategy for chromosome landing at a genetic locus. In *Arabidopsis Protocols*, (Methods in Molecular Biology Series), J.M. Martinez-Zapater and J. Salinas (Eds.). Totowa, The Humana Press, in press.

Van Lijsebettens, M., Vanderhaeghen, R., De Block, M., Bauw, G., Villarroel, R. and Van Montagu, M. (1994). An S18 ribosomal protein gene copy, encoded at the *Arabidopsis PFL* locus, affects plant development by its specific expression in meristems. EMBO J. 13: 3378-3388.

Van Lijsebettens, M., Wang, X., Cnops, G., Boerjan, W., Desnos, T., Höfte, H. and Van Montagu, M. (1996). Transgenic *Arabidopsis* tester lines with dominant marker genes. Mol. Gen. Genet. 251: 365-372.