

## MEETING REPORT

# Trends in Plant Cell Cycle Research

Plant growth can be considered as the sum of cell proliferation in the meristems and the subsequent elongation of cells. The continuous proliferative capacity of plant cells is crucial for the production of new organs and thus has a significant impact on plant architecture. The questions to be addressed are what controls the entry, maintenance, and exit of the cell cycle? What are the molecular components of the plant cell cycle machinery, and which roles do they play in differentiated cells and during development? How does the cell cycle machinery interact with the cytoskeleton? These areas of research have experienced considerable progress in recent years (Mironov et al., 1999), and the molecular tools required to approach them in a developmental context are gradually becoming available.

A workshop titled "Cell Cycle Regulation and Cytoskeleton" was recently sponsored by the Instituto Juan March de Estudios e Investigaciones in Madrid (Spain) on March 22–24, 1999. This meeting provided a timely platform for discussions of current topics in the regulation of the cell cycle and cytoskeleton in plants. The present meeting report reflects some of the meeting highlights.

### CYCLIN-DEPENDENT KINASES

As in other eukaryotes, cyclin-dependent kinases (CDKs) are central key regulators of the plant cell cycle (Doonan and Fobert, 1997). Extensive work in *Arabidopsis*, tobacco, and alfalfa demonstrates that plants possess at least four different classes of CDKs. Best characterized are the CDKs containing the hallmark PSTAIRE sequence, which play a role in both the

G<sub>1</sub>-to-S and G<sub>2</sub>-to-M transitions. An additional class of CDKs, unique to plants, is defined by a PPTALRE sequence and protein and activity levels that are highest at late G<sub>2</sub> and M phases. As postulated by several meeting participants, this group of CDKs might regulate plant-specific aspects of mitosis (Jim Murray, Institute of Biotechnology, University of Cambridge, Cambridge, UK; Dènes Dudits, Institute of Plant Biology, Szeged, Hungary; Dirk Inzé, Flanders Interuniversity for Biotechnology, Gent, Belgium). Dirk Inzé further showed that transgenic plants that overexpress a dominant negative mutant of the PPTALRE-containing CDK from *Arabidopsis* tend to develop cells with 4C content, indicating that the kinase plays a pivotal role in the G<sub>2</sub>-to-M transition.

### CYCLINS

The multiplicity of cyclins upon which plant CDKs depend generally exceeds that of animal cells (Renaudin et al., 1996). At least four different classes of plant cyclins (A, B, D, and H) can be found. Jim Murray and Dirk Inzé presented evidence that D-type cyclins control re-entry into the cell cycle and are important integrators of developmental signals into the cell cycle. As an example of such integration, two *Arabidopsis* D-type cyclins (CycD2 and CycD4) are induced by sucrose, whereas CycD3 is specifically induced by cytokinins. John Doonan (Department of Cell Biology, John Innes Institute, Norwich, UK) showed that the two D3-type cyclins of snapdragon are expressed in partly overlapping subsets of meristem cells. Most interestingly, Jim Murray showed that overexpression of CycD3

in *Arabidopsis* results in cytokinin-independent growth of callus tissue with dramatic effects on plant development (Riou-Khamlichi et al., 1999). However, the details are far from being fully understood, and considerable differences between various plant species are expected. For example, Eva Kondorosi (Institut des Sciences Végétales, Gif-sur-Yvette, France) reported that CycD3 of alfalfa is not induced by cytokinins and overproduction has no obvious effect.

Given the complexity of the biochemistry of the cyclins in plant cells, a particularly important goal is to identify which CDK is activated specifically by which cyclin(s). In this regard, Jim Murray presented evidence that D-type cyclins interact with the PSTAIRE-type kinases.

### CDK-ACTIVATING KINASE

CDK activity requires the phosphorylation of a specific Thr residue by a CDK-activating kinase (CAK). Maasaki Umeda (Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan) demonstrated that plants contain at least two CAK-related kinases, which differ in their substrate specificity toward human CDK2 and the C-terminal domain of the largest subunit of RNA polymerase II. Umeda furthermore suggested that cyclin H may be a physiological partner of CAK inasmuch as a cyclin-H homolog from poplar associates specifically *in vitro* with rice CAK.

### CELL CYCLE KINASE INHIBITORS

CDK inhibitors (CKIs) play a pivotal role in arresting cell division at the end of developmental programs and/or in

## MEETING REPORT

response to environmental cues. Dirk Inzé demonstrated that *Arabidopsis* contains at least four CKIs. In situ hybridizations are consistent with a role in arresting or preventing cell division during development.

### RETINOBLASTOMA PATHWAY

A few years ago, it was demonstrated that plant cells use retinoblastoma-related proteins (hereafter designated RBRs) to control entry into S phase (Gutiérrez, 1998). Wilhelm Grissem (Department of Plant and Microbial Biology, University of California, Berkeley, CA) reported that an RBR exists in *Arabidopsis*, and both its overproduction and antisense inhibition cause the formation of fasciated inflorescences and distorted meristems, suggesting that RBRs execute important functions in the control of plant cell division and differentiation.

By analogy to animal systems, the activity of plant RBRs is most probably dependent on their phosphorylation and specific association with other proteins. Specifically, one or more as yet unidentified CDKs would likely phosphorylate plant RBRs, thereby modifying their binding to E2F-like transcription factors. Crisanto Gutiérrez (Centro de Biología Molecular, CSIC-UAM, Madrid, Spain) presented the characterization of a wheat E2F with domain organization similar to that of the human E2F1/2/3 subset of proteins. Interestingly, the maize RBR interacts strongly with the wheat E2F but only poorly with the human E2Fs. Eva Kondorosi further reported that alfalfa contains at least three E2F-like proteins, the expression of which appears to be differentially regulated. Currently, other interactions with RBRs are being characterized, such as that of the tomato *MSI1* gene product, a 48-kD WD40 protein. Wilhelm Grissem showed that antisense inhibition of *MSI1* expression

in *Arabidopsis* causes epinasty of cotyledons and a late flowering phenotype when grown under short days; however, overproduction produces no phenotypic consequences.

There appears to be at least two mechanisms by which RBRs interact with other proteins. One class of proteins, including the D-type cyclins and the RepA protein encoded by the Wheat Dwarf Geminivirus and other members of the *Mastrevirus* genus, contain an LXCXE motif (Gutiérrez, 1998) that mediates interaction with RBRs. Linda Hanley-Bowdoin (Department of Biochemistry, North Carolina State University, Raleigh, NC) provided evidence that the Rep protein (also named AL1) from the Tomato Golden Mosaic Virus, a geminivirus from the *Begomovirus* genus, interacts with RBR via a non-LXCXE sequence that overlaps the Rep dimerization domain.

Nicole Chaubet-Gigot (Institut de Biologie Moléculaire des Plantes, Strasbourg, France) demonstrated that S-phase-specific gene expression is under control of at least two different mechanisms. The gene for ribonucleotide reductase contains E2F-binding sites in its promoter, whereas histone gene expression depends instead on the presence of so-called OCT and NON sequences in the promoter. A protein complex interacts with the NON sequence throughout the entire cell cycle but appears to be altered in a phase-specific manner by phosphorylation.

### PROTEOLYSIS AS A CELL CYCLE REGULATORY MECHANISM

Many cell cycle proteins are destroyed at specific phases of the cell cycle. Pascal Genschik (Institut de Biologie Moléculaire des Plantes) discussed results that demonstrate that a specific sequence, or destruction box, present in both A- and B-type cyclins is both necessary and sufficient to drive their

specific proteolysis at anaphase (Genschik et al., 1998). In addition, several components of a putative anaphase-promoting complex (APC) from *Arabidopsis* have now been cloned. APC is one of two E3 ubiquitin ligase complexes essential to the degradation of a number of cell cycle proteins, including cyclins and CKIs in animals and yeast. Pascal Genschik also identified an *Arabidopsis* ubiquitin-conjugating enzyme (E2C). Interestingly, the gene that encodes E2C is expressed exclusively in M phase, suggesting that cell cycle phase-specific transcription might be an important regulatory mechanism to control the activity of APC.

In addition, Venkatesan Sundaresan (Institute of Molecular Agrobiolgy, National University of Singapore, Singapore) reported on the isolation of an *Arabidopsis* mutant of a CDC16 homolog. CDC16 is a component of the APC complex and is required for development of the embryo sac. Much progress was also reported by Eva Kondorosi in the characterization of *ccs52*, a *fizzy*-related gene of alfalfa. The CCS52 protein contains seven WD40 repeats and, in analogy to yeast, plays an important role in the regulation of the APC complex.

Significant progress has also been made in the characterization of *Arabidopsis* SKP1-like (ASK) proteins, which are components of the SCF complex (for Skp1, Cdc53, F-box complex; the second of the two E3 ubiquitin-ligating activities in the ubiquitin-mediated protein degradation pathway). ASK1 was originally identified as a protein interacting with the UNUSUAL FLOWER ORGANS (UFO) protein. Already 11 members of the ASK family have been identified by sequence analysis (Eddy Risseuw, Plant Biotechnology Institute, Saskatoon, Canada). Furthermore, the cloning of a CDC53, or cullin, homolog will accelerate the understanding of the regulation of this important cell cycle proteolysis machinery.

## MEETING REPORT

## OTHER REGULATORS OF THE CELL CYCLE

Venkatesan Sundaresan described a genetic approach (to be presented in a forthcoming issue of THE PLANT CELL) to identify Arabidopsis mutants that are affected in the cell cycle. A screening for female gametophytic mutants yielded several novel loci that influence meiosis. In particular, the *SPL* gene encodes a novel protein, localized to the nucleus, which triggers the switch from a mitotic to a meiotic cell fate.

## CYTOSKELETON

Little is known about how the cell cycle machinery interacts with the cytoskeleton, although specific CDKs colocalize with the preprophase band, the spindle, or the phragmoplast. Richard Meagher (Department of Genetics, University of Georgia, Athens, GA) reported the characterization of eight actin genes from Arabidopsis. Most interesting is the finding that a knockout of the *actin2* (*act2*) gene results in swollen root hairs characteristic of the so-called schmoo phenotype when mutants are grown on a sucrose medium. A double knockout in both *act2* and *act7* results in a particularly severe schmoo phenotype when grown on a sucrose medium.

Christopher Staiger (Department of Biological Sciences, Purdue University, West Lafayette, IN) presented his characterization of the profilins, proteins that interact with G actins. Specifically, microinjection of profilins in *Tradescantia* stamen hair cells causes a reduction in cytoplasmic streaming. Anne-Marie Lambert (Institut de Biologie Moléculaire des Plantes) analyzed the dynamic behavior of  $\gamma$ -tubulins in tobacco BY-2 cells.

Polarized growth during cell morphogenesis is a fundamental issue in plant

development; however, the mechanisms underlying this process are largely unknown. Nam-Hai Chua (Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY) showed that the small GTP-binding protein Rac and phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar extension of pollen tubes (Kost et al., 1999). In the case of trichome morphogenesis, furthermore, the spatial pattern of the branches is established by microtubules, whereas microfilaments are involved in maintaining polarized growth.

## CYTOKINESIS

The rigid cell wall that surrounds the plant cell imposes mechanistic restraints on cytokinesis beyond those encountered by the yeast or animal cell (Verma and Gu, 1996; Heese et al., 1998). Although the cytokinetic mechanisms unique to plant cells are not fully elaborated, many ultrastructural features of dividing plant cells are well known. The preprophase band, for instance, is a microtubular array that determines the future division plane. At telophase, moreover, a cell plate, or phragmoplast, is formed in the center between incipient daughter cells. The temporal and spatial control mechanisms of phragmoplast formation are entirely unknown. Interesting candidate regulatory proteins are the MAP kinases, MMK3 and NTF6, reported by Erwin Heberle-Bors (Institute of Microbiology and Genetics, Vienna, Austria). Both kinases are located in phragmoplasts. Heberle-Bors furthermore showed that the formation of phragmoplast microtubules is required for MMK3 activity but not for retention of the kinase within the phragmoplast per se.

Andrew Staehelin (Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO) updated his earlier electron

microscopic investigations into the assembly pathway of the cell plate during cytokinesis. Specifically, recent experiments show that caffeine blocks the maturation of the tubulo-vesicular network and that dihydrocytochalasin B disrupts membrane trafficking to and from the cell plate.

A major step in cell plate formation is the fusion of vesicles into tubules. Natasha Raikhel (Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI) gave an outstanding overview of vesicle-mediated transport, a research area that highlights the continued importance of cell biology. Dash Pal Verma (Department of Molecular Genetics and Plant Biotechnology Center, Ohio State University, Columbus, OH) described the self-polymerization of phragmoplastin, a high-molecular weight GTPase, into a polymeric structure and suggested that this protein may be involved in the remodeling of vesicles during phragmoplast biogenesis.

Mutants defective in mitosis and in cytokinesis are expected to be embryo lethal. In an exhaustive screen, Gerd Jürgens (Department of Developmental Genetics, Universität Tübingen, Germany) recovered both classes of mutants. *Pilz* mutant embryos are defective in cell division and consequently consist of only one or a few large cells. Moreover, each cell contains one or more enlarged nuclei and cell wall stubs. The genetic screen also identified at least six genes involved in cell plate formation, of which the best studied is *KNOLLE*. This gene encodes a syntaxin that is probably involved in vesicle fusion during cell plate formation.

## PERSPECTIVES

In the light of data presented at this workshop, it is clear that future plant cell cycle research will concentrate on

## MEETING REPORT

three major areas. The first will be the continued identification of novel components of the cell cycle machinery. The detailed molecular characterization of these components is crucial to an understanding of their mechanism of action, which is frequently dependent on the dynamic associations of multiple proteins. Second, more emphasis will be given to the study of the interaction between the cell cycle machinery and the cytoskeleton. Third, the accumulating evidence strongly indicates that certain cell cycle regulatory proteins also play roles in differentiated cells. The identification of these proteins and the developmental consequences of their activities should contribute to an understanding of their impact on plant growth and architecture.

**Dirk Inzé**  
**Laboratorium voor Genetica**  
**Departement Plantengenetica**  
**Vlaams Interuniversitair Instituut**  
**voor Biotechnologie (VIB)**  
**Universiteit Gent**  
**K.L. Ledeganckstraat 35, B-9000**  
**Gent, Belgium**  
**diinz@gengenp.rug.ac.be**

**Crisanto Gutiérrez**  
**Centro de Biología Molecular**  
**"Severo Ochoa"**  
**CSIC-UAM, Universidad Autónoma**  
**de Madrid**  
**Cantoblanco**  
**E-28049 Madrid**  
**Spain**  
**cgutierrez@cbm.uam.es**

**Nam-Hai Chua**  
**Laboratory of Plant Molecular**  
**Biology**  
**The Rockefeller University**  
**1230 York Avenue**  
**New York, NY 10021-6399**  
**chua@rockvax.rockefeller.edu**

## REFERENCES

- Doonan, J., and Fobert, P.** (1997). Conserved and novel regulators of the plant cell cycle. *Curr. Opin. Cell Biol.* **9**, 824–830.
- Genschik, P., Criqui, M.C., Parmentier, Y., Derevier, A., and Fleck, J.** (1998). Cell cycle-dependent proteolysis in plants: Identification of the destruction box pathway and metaphase arrest produced by the protease inhibitor MG132. *Plant Cell* **10**, 2063–2075.
- Gutiérrez, C.** (1998). The retinoblastoma pathway in plant cell cycle and development. *Curr. Opin. Plant Biol.* **1**, 492–497.
- Heese, M., Mayer, U., and Jürgens, G.** (1998). Cytokinesis in flowering plants: Cellular process and developmental integration. *Curr. Opin. Plant Biol.* **1**, 486–491.
- Kost, B., Lemichez, E., Spielhofer, P., Hong, Y., Tolia, K., Carpenter, C., and Chua, N.-H.** (1999). Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *J. Cell Biol.* **145**, 317–330.
- Mironov, V., De Veylder, L., Van Montagu, M., and Inzé, D.** (1999). Cyclin-dependent kinases and cell division in higher plants—The nexus. *Plant Cell* **11**, 509–521.
- Renaudin, J.-P., Doonan, J.H., Freeman, D., Hashimoto, J., Hirt, H., Inzé, D., Jacobs, T., Kouchi, H., Rouzé, P., Sauter, M., Saviouré, A., Sorrell, D.A., Sundaresan, V., and Murray, J.A.H.** (1996). Plant cyclins: A unified nomenclature for plant A-, B-, and D-type cyclins based on sequence organisation. *Plant Mol. Biol.* **32**, 1003–1018.
- Riou-Khamlichi, C., Huntley, R., Jacquard, A., and Murray, J.A.H.** (1999). Cytokinin activation of Arabidopsis cell division through a D-type cyclin. *Science* **283**, 1541–1544.
- Verma, D.P.S., and Gu, X.** (1996). Vesicle dynamics during cell-plate formation in plants. *Trends Plant Sci.* **1**, 145–149.