Meeting Review

Plant Development Meets Cell Proliferation in Madrid

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Cell division is intimately intertwined with plant development, and the mechanisms that link the control of cell proliferation and differentiation with the processes of organogenesis, morphogenesis, and growth are starting to be understood. A recent Juan March meeting explored this interface, and revealed a rich seam of exciting work that is leading toward an integrated view of the role of cell proliferation in the unfolding of developmental programs.

The excellent Juan March Foundation meetings are currently among the most exciting and enjoyable in biology, and the recent offering (Crosstalk between Cell Division and Development in Plants, November 12–14, 2001) was no exception. Hosted by the Juan March Foundation in Madrid under the strict but amiable guidance of Dr. Andres Gonzalez, director of the center, the meeting was structured to allow maximum opportunity for interaction and both formal and informal discussions, and led to an exciting exploration of the links between the cell cycle and plant developmental processes.

Plants are uniquely suited for analysis of the interaction between cell proliferation, organogenesis, and development because of the nature of plant ontogeny, in which embryogenesis produces not the organs of the adult but simply the necessary axes and stem cell populations that will later form them. Thus, not only is almost all organogenesis postembryonic in plants, but it is also highly responsive to the environment. Since cells are required to make organs, plasticity also implies appropriately responsive controls of the cell cycle integrated into the developmental pattern. A further unique feature of plants also has an influence: the rigid cell wall of plant cells precludes cell migration and severely restricts cell-cell flexibility, but does allow permanent cytoplasmic connections between cells to form symplastic domains. Consequently, the axis of cell growth and polarity of division are important aspects of the patterning process.

Several major questions underlying the interaction of cell proliferation and development were addressed at the meeting, such as: what is the relationship between pattern specification, the elaboration of pattern (which may require the differentiation of multiple cell types in complex organs), and cell cycle control (see Figure)? Where and how do developmental signals impinge on the cell cycle, and how does perturbation of the cell cycle affect development? How is the size of organs controlled and how does such control relate to cell number, cell size, and cell differentiation? How do plant hormones coordinate these processes? And what is the relationship between overall growth rate of the plant and cell division?

Cell Identity, Meristems, and Organogenesis

The outline development of many plant organs can be divided into three main phases: specification, proliferation, and differentiation. The initial specification and recruitment of cells to form a primordium, the proliferation of these cells to provide the required number to form the complete organ, and the subsequent differentiation and expansion of these cells that produces the vast majority of the visible increase in organ size are at least partly temporally separate processes. The spatial organization of organogenesis is different in the shoot, where primordia form in regions of active cell division on the flanks of the shoot apical meristem (SAM) and in the root, where laterals emerge after reactivation of cell division from a specialized layer of cells known as the pericycle.

It is well established that position, not lineage, determines cell identity in plants (van den Berg et al., 1997). Disorganized division does not destroy the fundamentals of pattern establishment (Torres-Ruiz and Jürgens, 1994). However, it can alter or prevent its proper elaboration, as seen in the tormoz (toz) mutant described by Venkatesan Sundaresan (Davis) which causes longitudinal divisions in the Arabidopsis embryo to be randomized. The apical parts form a callus-like growth, but nevertheless an apical-basal distinction remains.

Overexpression of cell cycle genes has so far generally been found to cause little effect on overall architecture, although growth rate or final organ size can be altered (Meijer and Murray, 2001; see Mironov et al., 1999 for a full review of cell cycle control in plants). Taken together, the previous results have suggested that cell division does not play a significant role in determining either pattern or organogenesis. However, work presented at this meeting shows that there is in fact a close and subtle interplay between patterning, organogenesis, and cell division, whose details differ depending on the developmental context. Jan Traas (Versailles) presented elegant experiments using optical sections of the SAM that show that there are important differences in cell cycle rates in different regions of the meristem. To address the significance of the coordination of cell division, meristems were treated with cell cycle inhibitors. The antimicrotubule drug oryzalin resulted in the blocking of cell division but the continuation of growth, although cycles of DNA replication still occurred, resulting in increased nuclear ploidy levels. When all cell cycle progression was blocked with the DNA polymerase inhibitor aphidicolin, both cell proliferation and growth were stopped, suggesting that growth

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The Role of Cell Division in Plant Development

The major question addressed at this meeting was the role of cell proliferation in developmental processes, illustrated here by the coordinated control of cell division, organogenesis, and organ development needed to progress from the embryonic shoot meristem to the adult plant. What is the role of cell division in plant development? Postembryonic plant development occurs from root and shoot meristems specified during formation of the embryo. The shoot apical meristem (SAM) of the mature Arabidopsis embryo consists of about 100 cells (circled in top left panel, which shows an optical section through a mature embryo stained with propidium iodide). The SAM will give rise to all of the above ground organs of the adult plant, except the cotyledons which are already formed in the embryo, and are seen here pointing downward on either side of the SAM (Medford et al., 1994). The hypocotyl lies to the left. After the germination of the seed, cell division initiates within the SAM. The formation of the structures of the adult plant ([A], center and right) involves cell proliferation together with the initiation of organ primordia on the flanks of the SAM. Center, vertical section through vegetative meristem; right, mature Arabidopsis plant at transition to flowering.

Within the meristem, different zones are defined both by morphological characteristics and by characteristic gene expression (B). Left: a longitudinal section through a SAM (top), and a scanning SEM viewed from above (bottom). The zones of the meristem and associated areas are colored on the equivalent panels on the right. The central zone (blue) consists of a self-renewing population of stem cells with a relatively slow division rate, and is surrounded by a peripheral region of faster dividing cells (green). Primordia (magenta) initiate in an initiation zone on the flanks of the PZ. A single primordium is observed on the section ([B], top right), whereas the SEM view shows eight primordia numbered in increasing age. P0 indicates the position at which the next primordium will appear. Leaf primordia enlarge initially by “recruitment” of cells from the meristem, and then undergo a period of cell proliferation lasting until the forming leaf (colored reddish brown) is less than 1 mm² in size (De Veylder et al., 2001a). After this point, the majority of increase in the size of the leaf is due to cell expansion associated with the adoption of mature differentiated cell characteristics. The rib meristem (yellow) gives rise to the stem. Cell identity and cell division rates change in the different regions, raising the question not only of the regulation of division but how this is coordinated with changing cell identity and differentiation.

Can be uncoupled from cytokinesis but not from cell cycle progression. Alternatively, perhaps cell growth cannot occur during S phase.

Is cell cycle control involved in organogenesis? Use of GFP expression from promoters specific for different domains of the meristem allows the recruitment of cells to form a new leaf primordium to be visualized (Jan Traas, Versailles). Recruitment progresses until approximately 25 cells express the primordium-specific marker, and then outward growth of the primordium occurs through cell proliferation. Interestingly, the process of recruitment (and hence specification of the organ) can occur even in the presence of cell cycle inhibitors, except on “bare” (pin-like) meristems with no preformed primordia, produced using inhibitors of auxin transport. This leads to the conclusion that patterning does not require cell division in an organized apex with existing primordia, but importantly, that cell division may be necessary in meristems without prepatterning.

Further evidence for close links between patterning and cell cycle control was presented by John Doonan (Norwich). The mutation phantastica, which affects leaf development in Antirrhinum (snapdragon), causes partial loss of dorsoventrality (Waites et al., 1998). It also...
results in considerably larger early leaves, and since there is little difference in cell size, this suggests an increase in cell number and an effect on the mechanism specifying organ size. Phan mutants show an increase in the expression of the D-type cyclin CycD3a, but the related CycD3b and other “core” cell cycle genes such as histone and cyclin B were unaffected. As CycD3a is expressed specifically in organ primordia (Gaudin et al., 2000), this suggests it could play a role in coordinating cell proliferation in developing leaves, potentially acting downstream of phan.

D cyclins of the CycD3 group also appear to have important roles in leaf development in Arabidopsis. Overexpression of Arabidopsis CycD3:1 profoundly affects leaf morphology, causing hyperproliferation of leaf cells and affecting cell differentiation and leaf structure. This contrasts with the lack of morphogenetic effect of overexpression of most cell cycle genes, and suggests that D-type cyclins may play particular roles in the developmental control of cell division patterns (Jim Murray, Cambridge).

A genetic interaction has been demonstrated between the Arabidopsis mutant asymmetric leaf-1 (asl), which encodes the ortholog of phan (Byrne et al., 2000), the SHOOTMERISTEMLESS (STM) gene, and the STM relative KNAT1. Overexpression of STM can lead to degeneration of tissues into proliferating callus (Jim Murray, Cambridge), suggesting a link to cell proliferation. Venkatesan Sundaresan (Davis) showed that mutants in the yabby genes may also play a role, perhaps affecting STM/KNAT1 expression. Single mutants in the yabby1 (yll) gene have normal leaves and weakly radialized flowers (Sawa et al., 1999), and mutants in yabby3 have normal flowers and leaves. However, double yabby1/ yabby3 mutants have increased flower radialization and partially radialized leaves that themselves produce ectopic meristems. This is associated with derepression of the STM and KNAT1 genes. YABBY gene expression starts early in primordium development and persists at the edges of the leaf where marginal meristem activity ensures completion of the leaf blade, suggesting that YABBY genes may act to regulate or restrict activity of genes promoting cell division.

Andrew Fleming (Zurich) presented a fascinating approach to directly look at the role of cell proliferation in organogenesis and organ development. By placing microbeads containing inducer directly onto tobacco meristems, he saw localized gene induction. Expansins, which modulate cell wall extensibility, result in outgrowth of a primordium, suggesting a role in initiation processes. Induction of expression of an A-type cyclin (CycA3:2) or the yeast cmd25 phosphatase that promotes mitotic entry causes a local and transient increase in cell division. In the meristem, this does not lead to organogenesis, and morphology is unaffected. When induced on developing primordia, these genes again promote a transient increase in cell division rates. However, the effect on the overall lamina development seen after the leaf matures is an induration or reduced growth of the affected region. In other words, a transient increase in cell division results in decreased overall growth. Conversely, the application of a CDK-inhibitory compound results in inhibition of division and an outgrowth of the affected part of the lamina. These results show that leaf morphology can be modified by manipulating cell division and cell wall extensibility, and that the influence of cell division is context dependent. Whether these apparently counterintuitive results reflect simply a local alteration in growth rate or also a disturbance of differentiation resulting from altered proliferation is unclear. It is worth noting, however, that localized induction of cell division appears to be involved, as constitutive expression of the CycA3:2 gene has not been observed to have clear effects, suggesting that the relative timing of the proliferation phase of cells contributing to an organ may be important.

Organogenesis in the root may also be closely linked with regulation of cell cycle genes. Cells exit from the root meristem in G1 and respond to auxin cues both in their differentiation and in their activation to form lateral roots (Beeckman et al., 2001). A synchronous induction system for lateral roots (Dirk Inze, Gent) has allowed the sequential activation of cell cycle genes to be followed during this process. In particular, Inze and colleagues have found that CDK inhibitor genes (known as ICKs or KRP [Kip-related proteins]) are expressed with differential timing, suggesting roles for specific KRPs in either cell cycle reentry or G2/M control. Interestingly, expression of KRP2 is high before induction of lateral roots, and correlating with this, KRP2 overexpression severely reduces lateral root initiation although primary root growth was unaffected. Control of cell cycle reentry may therefore be rate limiting for organogenesis in the root. This forms an interesting comparison with the results discussed above for pin-like shoot meristems, as it could be argued that lateral root initiation occurs without the clear prepatternning normally present in the SAM (Figure, panel B).

Analysis of the stem cell system represented by the Arabidopsis root has shown particularly clearly that it is position that determines cell fate (van den Berg et al., 1997). There is strict control of division, with auxin as the cue. From this system comes a cautionary tale in the interpretation of phenotypes that appear to be cell cycle associated (Ben Scheres, Utrecht). The mutant plethora1 (plt1) has too many distal cell layers in the quiescent center and root cap. PLT1 encodes an AP2-type transcription factor, and double mutants of plt1 and the closely related gene plt2 have altered root/shoot axis patterning. It is likely that PLT1/2 regulate patterning genes rather than directly controlling genes regulating cell proliferation. The phenotype of plt1 is therefore not due to increased cell divisions but instead to an expansion of the domain of division competence. These results show that it is important to note that cell division consequences may follow from patterning changes.

Cells and Plants

A major undercurrent of the meeting was the discussion of de Bary’s aphorism, an old chestnut of plant developmental biology, “the plant makes cells, not cells the plant.” Although attributed to de Bary in the form “Die Pflanzen bildet Zellen, nicht die Zellen bildet die Pflanzen,” there is in fact little evidence that de Bary actually expounded the dilemma in exactly this form (Barlow, 1982). Nevertheless, these extreme views have polarized much of the discussion and interpretation of the
relationship between cell division and plant development. What light was thrown on this question in Madrid? Clearly you cannot have a plant without cells, but you can have the proliferating cells of a callus without forming a plant. However, it is in the organization of callus to produce a functioning meristem and shoots, or in the formation of an adult plant from a germinating seedling that we see order imposed on cell division by patterning processes. Surely patterning must be dominant over, or upstream of, cell division controls, since otherwise the organization of cells into developing structures would not be possible. Or, are these processes independent, and cell division simply divides the internal space of the plant into conveniently managed units?

The discussion of organogenesis above already suggests that the question is more complex than the bald statement might imply. Although in many situations, patterning can generally be seen as dominant over cell division, specific examples are cited above where cell division may be important in driving developmental processes. Nevertheless, it has been suggested that evidence from various transgenic overexpressers of cell cycle proteins supports a largely organismal view, with cell division serving to subdivide the body of the plant. For example, overexpression of a dominant-negative cyclin-dependent kinase (CDK) that inhibited mitosis resulted in broadly normal tobacco plants whose leaves contain fewer, but larger, cells (Hemerly et al., 1995). Further evidence in this direction was presented by Dirk Inzé (Gent), who reported overexpression of the CDK inhibitor KRP2 results in dwarfism and small leaves composed of a reduced number of abnormally large cells (De Veylder et al., 2001a). Importantly, detailed analysis of the timing of the proliferation and differentiation/phases of leaf development in the KRP2 overexpresser revealed no differences in the timing of the transition between these phases (Gerrit Beemster, Gent). The important conclusion is that inhibition of cell division does not change developmental timing of when cell division stops or differentiation processes such as endoreduplication initiate. The increased cell size of KRP2 overexpressers can be interpreted as a “compensation” mechanism that attempts to achieve a normal leaf size independent of the number of cells available, fully consistent with an organismal view, or could simply be caused by a partial uncoupling of growth from cell division.

Inhibition of cell division throughout the leaf lamina can therefore result in smaller leaves (for complete confusion, compare this with the overly similar effect of the localized induction of cell division reported by Andrew Fleming [Zurich] discussed above!), but there are examples of manipulations that result in increased leaf size. These include overexpression of the AINTEGUMENTA gene (Mizukami and Fischer, 2000) and the increase of leaf size in the PHANTASTICA mutant of Antirrhinum. Nevertheless, the mechanism that determines leaf size, and whether this involves cell division control, remains an open question. However, promoting cell division by constitutive expression of cell cycle activators such as CdcD3 (J. Murray, Cambridge) or E2F/DP (Dirk Inzé, Gent) does not lead to larger leaves, but instead to a variety of ectopic cell divisions and effects on endoreduplication.

Most data concerning the role of cell proliferation during the initiation and elaboration of a determinate organ such as a leaf appear to point to controls operating at the organ level being predominant. Is there a difference in the mechanisms underlying the indeterminate growth of root and of the shoot apical meristem itself? Previous work in this area has suggested that some cell cycle regulators can increase plant growth rate by promoting more rapid cell cycling (Doerner et al., 1996; Cockcroft et al., 2000). An important contribution used modeling and measurement of growth parameters to address how the rate of growth is controlled (Gerrit Beemster, Gent). Soil stress caused by soil compaction results in reduced productivity and reduced elongation of wheat leaves. The imposition of soil stress results in a short-term change in the length of the cell cycle. However, this is replaced by a longer term response, in which the size of the meristem is reduced and cell cycle length is restored (Beemster et al., 1996). A smaller meristem results in reduced production of new cells and hence lower growth. Salt stress on Arabidopsis roots appears to lead to a similar long-term response, in that the root meristem becomes smaller but cell cycle duration is unchanged, although the short-term response is consistent with a transient block of cell cycle followed by recovery (Bursens et al., 2000). Overexpression of CKS1, the Arabidopsis homolog of yeast suc1, again reduces elongation rate, owing to a decrease in cell production and the absence of compensating changes in cell length (De Veylder et al., 2001b).

Growth rates of whole plants and consequently of indeterminate meristems such as the SAM and primary root meristem may therefore be primarily driven by the cell cycle. A substantial body of data supports the idea that control of the cell cycle is a primary response of plant growth rates to temperature (Francis and Barlow, 1988) and CO2 levels (Kinsman et al., 1997), but the direct links between environmental signals and cell division rates are as yet unknown. Cell division can also determine organ growth, but apparent compensation suggests a role for organ or plant level controls, possibly involving coordination of division and cell expansion.

Core Cell Cycle Processes

Further progress in understanding a number of core cell cycle processes was presented, particularly involving G2 and mitotic processes (see Mironov et al., 1999 for a review). John Doonan (Norwich) reported on work with Masaki Ito (Tokyo) showing that the G2/M-specific expression of genes such as cyclin B1 is controlled by constitutive inhibitory B-myb proteins and activating A-mybs. The A-myb is cell cycle regulated and expressed in G2/M when it may displace the B-myb from the promoter of regulated genes. Denes Dudits (Szeged) showed that the mitotic-specific CDK of the CDKB2 class (Cdc2MsF; Magyar et al., 1997) interacts with myosin. He also presented data that the phosphatase inhibitor endothall inhibits serine/threonine phosphatases, resulting in a prophase ring forming in the absence of chromosome condensation. This correlated with premature activation of CDKB2 (Cdc2MsF) in endothall-treated cells, suggesting this CDK could coordinate chromosome condensation with microtubule organization.
Retinoblastoma Protein and Other
transitions, with a specific defect in the autonomous
cyclin D-containing kinases. KEULE encodes a protein related to yeast Sec1 that binds KEULE protein in vitro. Double mutants produce a cellular bag containing nuclei, as a result of synchronous cell cycles without intervening cytokinesis. A KEULE-interacting SNAP25 homolog (SNP33) was shown to colocalize with KNOLLE (Heese et al., 2001). Using a three-component interaction assay, a candidate synaptic vesicle component that may represent the third member of the cytokinesis-specific SNARE complex, potentially targeting vesicles to membranes containing KNOLLE and SNP33.

The control of cytokinesis is mediated by a MAP kinase cascade described by Yasunori Machida (Nagoya), involving NPK1 (Nishihama et al., 2001). This is present from G1 to M, localizes to the spindle midzone and equator at mitosis, and is activated in late M phase. Expression of kinase-negative NPK1 results in multinucleate cells with stubs of cell wall. NACK1, a motor domain protein, was identified in a functional assay in yeast as an activator of NPK, and functional analysis suggests that it has a direct role in cell division control.

Lethal embryos consisting of one or a few cells are represented by the pilz (Gerdy Jürgens, Tübingen) and some titan (David Meinke, Oklahoma) mutants. PILZ genes turn out to encode proteins required for the folding of tubulin to form heterodimers. Cell division but not cell growth is blocked in the mutants, suggesting that microtubules deliver vesicles for formation of the cell plate and hence cytokinesis, but actin filaments are responsible for the delivery required for overall growth of the cell wall. These results also serve as a reminder that cell division defects can result from disruption of cellular processes, and not necessarily from loss of control mechanisms.

Retinoblastoma Protein and Other Chromatin-Linked Processes
In mammalian cells, the retinoblastoma tumor suppressor protein Rb plays a key role in preventing uncontrolled cell proliferation and is also involved in differentiation (Lee et al., 1994; Zacksenhaus et al., 1996). Rb normally blocks activation of E2F-regulated genes by binding to E2F and recruiting histone deacetylases to promoters. Cell cycle progression requires inactivation of Rb by cyclin D-containing kinases.

Crisanto Gutierrez (Madrid) presented evidence that the phosphorylation of Rb protein is cell cycle dependent and, in Arabidopsis, that an Rb-associated kinase contains cyclin CycD2. He also showed that E2F regulates the CDC6 gene (encoding a protein essential for origin firing in yeast), which is expressed during S phase in both normal and endoreduplication cycles. Ectopic expression of AAtCDC6 induces extra endoreduplication cycles (Castellano et al., 2001). Masami Sekine (Nara) showed that in tobacco BY-2 cells, CycD3;3-containing kinases phosphorylated tobacco Rb only at the G1/S transition, although interestingly, they phosphorylate histone H1 at both the G1/S and G2/M transitions.

Dirk Inzé (Gent) showed that overexpression of an Arabidopsis E2F together with its partner protein DPa resulted in strong curling of leaves and ectopic cell division in cells with the potential to divide, and in other cells appeared to trigger endoreduplication. In these plants, genes predicted to be under E2F control such as those involved in DNA replication and its initiation were strongly upregulated.

Rb also appears to link cell cycle control to a number of developmental processes, possibly through effects on chromatin (Harbour and Dean, 2000). MSII (a homolog of RbAp48; Ach et al., 1997) is an Rb binding protein that is part of several complexes including chromatin assembly factor 1 (CAF-1) and the chromatin remodeling complex NURF (Wilhelm Gruissem, Zurich). CAF-1 is a trimeric complex of MSI1 with p93 (FAS1) and p74 (FAS2; Kaya et al., 2001). CAF-1 is associated with histone H3 and H4 acetylated at the K5/K12 positions, and in vitro facilitates deposition of nucleosomes on newly replicated DNA. Arabidopsis lines in which expression of MSI1 is subject to cosuppression (resulting in reduced gene expression) show altered phyllotaxy with organs that initiate but can arrest in development. Examining global changes in gene expression between MSI1 cosuppressed, wild-type, and MSI1 overexpression lines shows differences in expression of genes involved in (among others) chromatin organization and the cell cycle (such as CDKs, cyclins, and CKS1). These results, coupled with the mutant phenotypes of the CAF-1 components fas1 and fas2, suggest that Rb may be involved in changes in cell proliferation and identity that occur within the shoot meristem (Figure, panel B). Both the transition from the central zone (stem cell identity) to the peripheral zone and the subsequent initiation of primordia may involve Rb function, although only the second step appears to require the CAF-1 complex.

Interestingly, there are additional MSI genes in Arabidopsis. José Miguel Martinez-Zapater (Madrid) showed that one of these (MSI4) is mutated in the fve mutant. fve is heterochronic and delayed in all developmental transitions, with a specific defect in the autonomous flowering promotion pathway. This suggests that chromatin modulation may be important in driving a number of developmental transitions in plants.

Genomic imprinting in plants has been described for the medea mutation, which confers a maternally controlled seed abortion phenotype depending on the genotype of the female gametophyte, because only the maternally inherited allele is active due to imprinting. In yeast and in vitro, MEDEA was shown to interact with FIE, a protein identified as conferring fertilization-independent endosperm development. mea and fie mutations share both these aspects of the phenotype, and the proteins are related to the Polycomb group of proteins homologous to Enhancer of Zeste and Extra sex combs of Drosophila, which are involved in regulation of Hox genes as part of a 600 kDa complex that is thought to modulate higher order chromatin structure. The MEA and FIE proteins coelute at high molecular weight after
gel filtration using plant nuclear extracts, suggesting they are part of a similar multimeric protein complex that either directly or indirectly controls cell proliferation in the female gametophyte and developing seed.

The End of the Line
The key role of protein destruction by both the anaphase-promoting complex (APC) and SCF complexes (see below) in controlling and integrating a variety of plant cellular processes was underlined in a number of presentations. Pascal Genschik (Strasbourg) showed that the APC, responsible for destroying mitotic cyclins through their destruction box, is conserved between plants and animals. Surprisingly, the APC machinery remains active in differentiated nondividing cells, since a CycB1–GUS fusion protein is unstable even in mature leaves. He also showed that the APC is essential for division, as a knockout mutant in APC2 blocks after meiosis, managing only a single division before arresting. Similarly, a mutant in the APC component CDC16 causes the female gametophyte to arrest at two cells, with abortion of the mature ovule (Venkatesan Sundaresan, Davis). By contrast, mutants in HOBBIT, which encodes the APC component CDC27, show specific developmental defects such as loss of both cell division and identity markers in the quiescent center of the root meristem and lateral root cap. This suggests that some APC components may serve specialized roles in plant development (Ben Scheres, Utrecht).

The CCS52 protein of alfalfa has homology to FZR (fizzy-related) of Drosophila (Eva Kondorosi, Gi-sur-Yvette). These WD40 repeat proteins are substrate-specific activators of the APC. CCS52A is expressed in nodules, and when overexpressed in fission yeast, causes growth arrest, cell elongation, and endoreduplication. CCS52B, however, which is only expressed in roots, has no effect in fission yeast. The two genes appear to have specific functions. CCS52A is repressed during initial cell divisions in nodule formation but turned on in the central region before differentiation at the time when cells enter endoreduplication. Plants expressing antisense CCS52A show aborted nodule formation and decrease in ploidy level, and Rhizobium infection is followed by lysis and early death of plant cells. These results show not only that CCS52A plays an essential role in the switch to endoreduplication, but also suggests that endoreduplication is itself necessary for the developmental process to occur. By contrast, CCSB is only present in G2/M phase and is likely to have a primary role in cell cycle progression.

The hormone auxin plays central roles in plant cell proliferation and differentiation. Mark Estelle (Austin) showed that auxin produces its molecular responses by promoting the degradation of transcriptional repressors through targeted destruction by the SCF complex and the ubiquitin pathway, present in all eukaryotes. SCF is named after its composition: it contains a Skp1 homolog, a cullin homolog, and an F box protein that confers specificity. The Arabidopsis genome encodes over 600 F box proteins, one of which is TIR1, which is involved in the auxin response along with ASK1 (Skp1 homolog), AtCul1, and RBX1 (a RING finger protein). The AXR2 (isolated as an auxin-resistant mutant) gene encodes a repressor of auxin action—is it a substrate of SCF TIR1? Genetic interaction evidence and the binding of AXR2 and the related AXR3 to TIR1 in pull-down assays indicate that in fact, both of these proteins are indeed substrates. A further auxin response gene, AXR6, encodes a cullin, and the axr6 mutant is auxin resistant. Interestingly, a reduction in Cul1 levels inhibits organogenesis, and the resulting pin-like meristems do not respond to auxin, suggesting that the SCF complex containing TIR1 and AXR6 may be a key element in the auxin response.

Recent evidence suggests that responses to the plant defense hormone jasmonic acid involve analogous pathways, and Dao-Xin Xie (Singapore) showed that COII1, an Arabidopsis gene required for jasmonate responses, encodes an F box protein related to TIR1 that is likely involved in forming an SCF complex. SCF complexes are almost certainly also involved in the timely destruction of cell cycle components, and Crisanto Gutierrez (Madrid) showed that Arabidopsis E2F2 (which has sequence similarity to inhibitory E2Fs such as Drosophila E2F2 and human E2F6; de Jager et al., 2001) is stabilized in proliferating and differentiated cells by the proteasome inhibitor MG132, although the F box targeting protein involved has yet to be identified.

Nam-Hai Chua (Rockefeller) described NAC1, a new member of the NAC protein family (named after Nam/Ataf1/2/CUC2, proteins involved in meristem organization and transcriptional regulation). This is a positive regulator of the auxin signaling pathway, and can rescue tir1 when overexpressed. NAC1 binds the E2 ubiquitin-conjugating enzyme AtUBC9 and the E3 enzyme SINAT5, a homolog of seven in absentia of Drosophila, where it is involved in determination of eye fate. Both SINAT5 and NAC1 are induced by auxin. As SINAT5 ubiquitiniates NAC1 in vitro, Chua proposed that NAC1 acts as a positive regulator of the auxin response and SINAT5 destroys NAC1 to reset the pathway. Taken together with the data of Mark Estelle (Austin) discussed above, these results show that the biochemical pathways mediating auxin control in plants are becoming increasingly well understood.

Afterword
The overall impression of this meeting was that the field is poised for a revolutionary convergence of cell cycle, the function and control of the meristem, and developmental processes. The common themes emerging from the presentations of many speakers and the general embracing of inclusive rather than overly reductionist approaches auger well for the future of this area.

Those who have attended Juan March Meetings will know of Dr. Gonzalez’s strict interdiction on thanking organizers or funders of the meeting to preserve more time for discussion. I will risk his belated wrath here, and thank both for a highly successful and stimulating congress of ideas. See you in Madrid!

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References


