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C2–CELL CYCLE CHECKPOINTS
Organised by Dennis Francis for the Cell Cycles Group

C.2.1 Connecting MEKs to their downstream MAP kinases in Arabidopsis

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Mitogen Activated Protein kinases (MAP kinases) are known to play key roles in signal transduction in all eukaryotes. The core of MAP kinase pathways is made of three protein kinases: the MAPKKK (MEKK), the MAPKK (MEK) and the MAP kinase that phosphorylates and activates each other in a linear way. Such MAPK signalling modules are prominently used in plants, as indicated by the expansion of the number of genes within these protein kinase families; there are 80 MEKKs, 10 MEKs and 20 MAPKs, in Arabidopsis, but their functions are largely unknown. The challenge in the field is to identify MEKK – MEK – MAPK connections and place these modules to signalling pathways with defined functions. Towards this goal we constructed loss of function and gain of function mutants of MEKs by *in vitro* mutagenesis of the ATP binding domain and the phosphorylation sites in the T-loop, respectively. We also created RNAi constructs based on gene-specific UTRs in order to silence the expression of MEKs. We will present our data on crosstalks among MAPKs pathways that play important roles to coordinate cell cycle with environmental conditions.

C2.2 Highlighting mitosis and cytokinesis with fluorescent proteins

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The position of the division plane in plant cells is established at the end of G2 before mitosis takes place by a process that depends on the formation of the preprophase band (PPB). The PPB disassembles upon entry into M-phase and leaves behind a hitherto unidentified positional cue that helps correct insertion of the cell plate into the mother wall. The phragmoplast emerges at the end of mitosis and serves as a scaffold, concentrating and keeping in place Golgi-derived vesicles containing cell plate synthesizing complexes at the equatorial plane. To identify components that are associated to these structures, we have explored the Arabidopsis genome for potential cytoskeleton and division related genes and localized the respective GFP-fusion proteins in dividing tobacco BY-2 cell suspension culture cells. A collection of ORF's were selected for analysis that included proteins with microtubule or actin binding properties, sequences with homology to *S. pombe*, *S. cerevisiae* and animal genes previously implicated in cytokinesis, and 25 Arabidopsis ORF's of unknown function that are the presumptive orthologues of tobacco cDNA tags specifically expressed during S-, G2-, or M-phase. The fluorescence profiles of BY-2 cultures transformed with 75 GFP-fusion constructs were determined. The analysis resulted in 56 specific intracellular localization patterns and allowed to discriminate different subcellular localizations of proteins belonging to a single, conserved protein family. Three fusion proteins associated with the PPB and 15 proteins distinctively labeled separate regions of the phragmoplast. Drug analysis distinguished the phragmoplast-associated proteins in vesicular and microtubule binding proteins. Furthermore, time-lapse confocal microscopy suggested that a protein of unknown function is implicated in the directional expansion of the cell plate and its insertion into the mother wall.

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C2.3 Regulation of *Arabidopsis* CycB1;1 expression

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The *Arabidopsis* B-type cyclin gene CycB1;1 is expressed late in the G2 and M phases of the cell cycle. We showed that abundance of CycB1;1 limits plant root growth without loss of developmental control. The CycB1;1 promoter can direct M phase-specific transcription of GUS reporter gene in synchronised dividing tobacco BY-2 cells. We functionally analyzed the CycB1;1 promoter to identify relevant control elements. In synchronised BY-2 cell cultures stably transformed with chimeric genes comprising CycB1;1 promoter deletions fused to GUS, CycB1;1 –351 (taking transcription start site as +1) is required for maximal M-phase expression. CycB1;1 -120 is sufficient to maintain the M phase specificity. Through DNA *in vivo* footprint analysis, we located the DNA binding sites of endogenous transcription factors involved in the regulation of CycB1;1 expression, and identified the GCCCR element. It is repeated four times in *AtCycB1;1* –351 promoter; possibly, the cognate transcription factor is a major determinant of *AtcycB1;1* transcript abundance. Functional analyses of GCCCR element in synchronised BY-2 cells and *Arabidopsis* plants, the GCCCR element indicate it is required for high-level CycB1;1 expression. Sequence analyses of CycB1;1 promoter indicated two MSA-like sequences in the *AtCycB1;1* promoter. Two MSA-likes sequences are sufficient for the M phase-specific expression of CycB1;1 by both loss-of-function and gain-of-function analyses.GCCCR elements are overrepresented in promoters of genes associated with cell growth. The crucial role for GCCCR elements in cyclin B1;1 expression provides the first evidence for a coordinated transcriptional programme of growth control in plants involving cell division regulators and genes required for fundamental growth processes such as protein synthesis.

C2.4 The localisation of Arabidopsis CycB1;1 during the cell cycle

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Abstract not supplied

C2.5 The role of Arabidopsis E2Fb/DPa transcription factor in the control of cell division

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The plant E2F family of transcription factors play critical roles in the regulation of cell division and differentiation as well as endoreduplication. There are three E2F genes (E2Fa, E2Fb and E2Fc) and two DP genes (Dpa and DPb) in Arabidopsis with functions and activities that are similar to their animal counterparts. All the three E2Fs are able to dimerize with both DPs, but it is suggested that E2Fa and E2Fb are transcriptional activators while E2Fc is a repressor. The two E2F activator genes show different expression during the cell cycle: E2Fa is S phase specific, while the E2Fb is more ubiquitous. On the protein level however, the E2Fb is cell cycle regulated peaking both in S-phase and at G2-M transition. Therefore E2Fa and E2Fb could play not only overlaping but different roles.To further characterize their regulatory roles in the cell cycle we generated transgenic tobacco BY-2 cell cultures co-expressing E2Fa or E2Fb with their DPa dimerization partner. The growth and cell cycle characteristics of these transgenic tobacco lines revealed the functional differences of E2Fa and E2Fb.

C2.6 The Role of D-type Cyclins in Plant Growth and Development

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Progression through the cell cycle is mediated by the consecutive action of Cyclin Dependent Kinase complexes. In Arabidopsis, thirty genes encoding for cyclins are annotated and can be classified into four families. The D-type cyclins are postulated to play an important role in G1/S transition and are therefore a target for developmental pathways controlling cell proliferation. Currently, the D-type family in Arabidopsis has ten members, which may have redundant or complementary roles. Protein levels of ArathD2;1 and ArathD3;1 are differentially regulated during cell cycle re-entry. Furthermore, D-type cyclins display overlapping but distinct expression patterns and different responses to mitogens. The single knockout mutants already reported lack an obvious phenotype indicating a level of functional redundancy. Several lines of evidence support the model that CycD/CDK complexes hyperphosphorylate RB, preventing it from sequestering the E2F transcription factors which regulate genes involved in S-phase. Inter-

action with CDKA, which is present throughout the cell cycle, has been demonstrated for two D-type cyclins and the kinase activity of this complex towards RB has been confirmed. In addition, overexpression of ArathCycD3;1 results in upregulation of known E2F target genes. Strikingly, ectopic expression of ArathCycD3;1 and ArathCycD1;1 not only forces cell out of G1, but also programs the cell for a complete mitotic cycle. The capacity of D-type cyclins to trigger the mitotic cycle and their differential expression patterns suggest a role for D-type cyclins at the interface between developmental pathways and the cell cycle, a hypothesis supported by recent reports on patterning genes.

C2.7 Mutational Analysis of CycD3;1 in *Arabidopsis*

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Cyclin D3;1 regulates the G1-S phase transition of the cell division cycle through the formation of an active complex with CDKA. The complex is capable of hyperphosphorylating Rb, leading to the consequent release of Rb binding factors, such as the E2F/Dp transcription factors. Expression of CycD3;1 is induced by a number of exogenous signalling molecules, including sucrose and cytokinin, and thereby introduces a measure of cell cycle responsiveness to external conditions. The protein features a CDK-binding cyclin box, in common with all cyclins, and an Rb binding motif (LxCxE), but lacks the destruction box found in cyclins A and B, possessing polar PEST sequences which have been implicated in the degradation of the protein. Following related work in NictaCycD3;3, a number of ArathCycD3;1 mutants affecting these functional domains (S47, K145, E175, T190, S310, S343) have been generated and constitutively expressed in *Arabidopsis* in order to investigate the effects of these mutations with respect to the phenotype and stability of the native protein.

C2.8 Dissecting the role of cyclin D1 using conditional expression in BY-2 cells

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A putative G1 cyclin gene, *Antma;CycD1;1* (*CycD1*), from *Antirrhinum majus* is known to be expressed throughout the cell cycle in the meristem and other actively proliferating cells. To test its role in cell cycle progression, we examined the effect of *CycD1* expression in the tobacco cell suspension culture, BY-2. GFP: CycD1 is located in the nucleus throughout interphase, preferentially associated with the nucleoplasm, but disappears during mitosis. Using epitope-tagged *CycD1*, we show that it interacts *in vivo* with CDKA, a cyclin dependent protein kinase that acts at both the G1/S and the G2/M boundaries. A modified *AlcR/AlcA* two-component gene expression system was used to permit inducible gene expression in cell cultures in response to added dexamethasone instead of ethanol used for the original *AlcR/AlcA* system, to monitor the consequences of D1 cyclin gene expression. This experimental approach allowed us to induce D1 cyclin expression at specific times of the cell cycle and therefore temporally dissect the function of D1 cyclin during G1/S and S/G2 phases of the cell cycle. Expression in G0 cells accelerated entry into both S-phase and mitosis, whereas expression during S phase accelerated entry into mitosis. Continuous expression of *CycD1* led to moderate increases in growth rate. Therefore, in contrast to animal D cyclins, *CycD1* can promote both G1/S and S/G2 progression, indicating that D cyclin function may have diverged between plants and animals.

C2.9 Transcript profiling in synchronized rice cells

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Although the basic mechanism of cell cycle control and the key genes that mediate cell cycle progression are highly conserved in eukaryotes, certain pathways regulating cell proliferation in plants are different. Plant-specific features of the cell cycle have already been demonstrated e.g. the presence of G2/M phase-specific cyclin-dependent kinases. In order to identify plant genes involved in cell division and control of cell cycle progression, we initiated a genome-wide expression analysis of cell cycle genes in the rice Taipei T309 (japonica subspecies) cell line using cDNA-AFLP technique. This was successfully applied for transcriptome analysis using synchronized tobacco Bright-Yellow-2 cells. Our genome-wide expression analysis is far from being completed. So far more than 240 periodically expressed gene tags were analyzed. 53% of the tags were significantly homologous to genes of known functions. They belong to diverse functional classes: metabolism, signal transduction, kinases, phosphatases, proteolysis, DNA replication and modification, RNA processing, stress response, transcription, etc. This suggests that several biological processes are at least partially under temporal transcriptional control during the cell cycle. 21% of the tags had no homology with known sequences (neither *Arabidopsis thaliana* homologue was found) which shows that in addition to conserved genes different plant species possess unique sets of cell cycle genes. Further analysis on the rice system is under pro-

gress to identify proteins involved in cell cycle regulation, which may allow the control of agronomic traits involved in cell division, plant growth and development.

C2.10 A proteomic approach to study cell cycle progression in tobacco BY-2 cells

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Whereas the study of *Nicotiana tabacum* cv. Bright Yellow-2 (BY-2) delivered many important insights in the regulation of the plant cell cycle, the limited knowledge of the tobacco genome still impedes the analysis of its gene products. A strategy to analyze the proteome of the BY-2 cell would be a valuable tool for the further elucidation of the regulation of the plant cell division cycle. We have started to approach the BY-2 cell with contemporary proteomics techniques. We employ two-dimensional electrophoresis as the primary separation technique, with isoelectric focusing as the first and SDS PAGE as the second dimension. On a single gel approximately 1500 proteins (pI 3–10) are now separated in a reproducible way, and this number can be increased drastically by running multiple, slightly overlapping gels with more narrow pI ranges. After spot picking and digestion, proteins are identified by an in-house developed, automated LC-ESI-QTOF-MS-MS strategy, or with a MALDI-TOF-TOF setup. Cross-species identification is done by querying *in silico* fragmented gene-product databases with experimental spectra. The majority of the selected spots (a few hundred) are identified in a more or less automatic way, based on similarity to gene products of either closely related or more distant organisms. The obtained data are continuously integrated in a web-accessible database, which is federated by SWISS-PROT. Differentially fluorescently labeled protein samples (Cy2, Cy3, Cy5) are now separated on single 2D gels and the protein profiles are quantitatively compared. Furthermore, dyes specific for particular post-translational modifications, like phosphorylations, are introduced. The current status of these efforts will be discussed.

C2.11 Genome-wide gene expression in an *Arabidopsis* cell suspension

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Plant cell suspension cultures are invaluable models for the study of cellular processes. We have developed the recently described highly synchronisable Arabidopsis suspension culture MM1 and MM2d as a powerful transcript profiling platform for further cell cycle studies using Affymetrix ATH1 microarrays and massively parallel signature sequencing (MPSS) as analytical tools. Analysis of gene expression profiles during normal culture growth, during synchronous cell cycle re-entry and during synchronous cell cycle progression provides a unique integrated view of gene expression responses in a higher plant system. Particularly striking is that expression of over 14,000 genes belonging to all defined categories can be reliably detected, suggesting that integrated and comparative analysis of datasets derived from transcript profiling of cultures is a powerful approach to identify candidate components involved in a wide range of biological processes. Combinatorial analysis of independent cell cycle synchrony methods allows the identification of genes apparently cell cycle regulated but are most likely responding to the induction of synchrony. We thus present a first integrated genome-wide view of the transcriptional profile of a plant suspension culture, and identify a refined set of 1,082 cell cycle regulated genes largely independent of the synchrony method.

C2.12 The number of retinoblastoma genes may reflect phylogenetic divergence of dicot and monocot plants

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Cell fate decision manifesting during division, differentiation or death can be defined by an intricate balance of many regulators, including retinoblastoma proteins (Rb). The phosphorylation status of Rb proteins plays important role in the cell cycle control. Rb genes are present in several copies in mammalians: human genome contains three Rb gene variants encoding pRb, p107 and p130. In our laboratory we have identified single *Medicago* and two rice cDNA clones encoding retinoblastoma-related proteins. This difference between these species raised the question whether other plants differ in Rb gene number according their phylogenetic history. Searches in the plant genome databases and already sequenced plant genomes generalized the previous observation, namely one Rb could be identified in dicotyledonous plants as *Arabidopsis thaliana*, *Nicotiana tabacum* and *Medicago sativa*, while two Rb related genes were found in monocotyledonous plants as *Oryza sativa*, *Triticum aestivum* and *Zea mays*. Gene structure, including intron length was also investigated. Since retinoblastoma proteins are integrated into multiple cellular pathways, a complex view has emerged how the individual members respond to various external and internal stimuli. To find the functional characteristics of the two

different Rbs in rice, we have investigated the transcriptional profile and interacting protein partners. The differences in the complexity of the Rb-centered cell cycle regulatory pathways between dicot and monocot species may have significant consequences as the growth and developmental characteristics are concerned.

C2.13 E2Fa-DPa and the mitotic—specific CDKB1;1 co-operate to drive the mitotic cell cycle

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Transgenic plants overproducing the E2Fa-DPa transcription factor display two distinct tissue-specific phenotypes: cells are stimulated to re-enter the mitotic cell cycle, or undergo endoreduplication (De Veylder et al., 2002). The decision of cells to undergo extra mitotic divisions was postulated to be depending on the presence of a Mitosis-Inducing Factor (MIF). Plants possess a unique class of CDKs (B-type CDKs) for which no counterpart can be detected in other species. The peak of kinase activity of CDKB1;1 at G2/M suggested that this CDK might be a good candidate to be part of the MIF. Plants overexpressing a dominant negative CDKB1;1 indeed show extra endoreduplication, demonstrating CDKB1;1 kinase activity is required to suppress endoreduplication. Moreover, when the wild type CDKB1;1 gene is expressed in a E2Fa-DPa overexpressing background, it partially suppresses the endoreduplication phenotype. Surprisingly, promoter and transcription analysis demonstrated that CDKB1;1 transcription is regulated by E2Fa-DPa, suggesting a cross talking mechanism between the G1/S and G2/M transition points. It implies that in endoreduplicating cells a mechanism must be operational suppressing the activation of CDKB1;1.

C2.14 Role of protein kinase CK2 in the plant cell cycle

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Protein kinase CK2 is an ubiquitous Ser/Thr kinase, with multiple substrates, and an important component of the regulatory signalling pathways relevant to the growth and division of cells. CK2 is a tetrameric holoenzyme, $\alpha_2\beta_2$, where α is the catalytic subunit and β is the regulatory subunit, that acts by modulating both substrate specificity and response to different effectors. We have demonstrated that CK2 is present in *Arabidopsis thaliana*, both as a monomeric enzyme, composed only by one α subunit, and as a tetrameric holoenzyme $\alpha_2\beta_2$. In BY2 synchronised cells, CK2 activity oscillates cyclically, peaking at G1/S or early S and at M-phase. *In vivo* inhibition of CK2 activity corroborates the requirement of a functional CK2 to progress through the cell cycle and suggests that CK2 might play an important role at the G2/M checkpoint. Moreover, CK2 activity increases from a resting to a proliferative state, and shows a complex pattern of regulation, including allosteric activation by polyamines and post-transcriptional regulation of β polypeptide levels. By *in situ* hybridization, a high expression of CK2 subunits correlates with the appearance of the meristems and with high mitotic activity during plant morphogenesis. At present, our work is focused on the study of the subcellular localization dynamics and stability of CK2 throughout the cell cycle, by following a GFP reporter protein approach in BY-2 stably transformed cells. We have also generated *Arabidopsis* transgenic plants that overexpress a dominant-negative mutant of the catalytic subunit, that has been cloned under the control of a glucocorticoid-inducible promoter.

C2.15 Abstract not supplied

C2.16 Abstract not supplied

C2.17 Targeting and functional analysis of DNA ligase I isoforms in *Arabidopsis*

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DNA ligases catalyse the formation of phosphodiester bonds at single or double-strand breaks in DNA and play a fundamental role in genomic DNA replication, repair and recombination. DNA ligase activity is also required to propagate and maintain the genomes of mitochondria and chloroplasts. However, mitochondrial and chloroplast genomes do not encode a DNA ligase, which suggests that ligase activity must be imported from the cytoplasm. Analysis of the *Arabidopsis* DNA ligase I (AtLig1) ORF has highlighted the presence of three consecutive in-frame initiation codons separated by putative mitochondrial, chloroplast and nuclear targeting sequences, suggesting AtLig1 may encode three distinct isoforms, each targeted to different subcellular locations. A single transcription initiation site of AtLig1 has been mapped upstream of the first initiation codon, showing that expression of AtLig1 isoforms is under translational control. *In vitro* expression of mutagenised AtLig1 constructs (that each contain a single initiation codon) demonstrates that translation is favoured from the first two

initiation codons. To investigate the subcellular location of AtLig1 isoforms*, in planta, Arabidopsis* plants have been transformed with an AtLig1-GFP fusion construct, under the control of the CaMV 35S promoter, and imaged using confocal laser scanning microscopy. AtLig1-GFP co-localised to the nucleus and mitochondria but was not detected in chloroplasts. Mutagenised AtLig1-GFP constructs that contain a single initiation codon have been used to determine both the efficacy of each initiation site to target specific AtLig1 isoforms to different subcellular compartments and the extent of any hierarchical relationship between the different targeting sequences.

C2.18 DNA double strand break repair and gene targeting in Arabidopsis

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Double strand breaks (DSBs) are one of the most serious forms of DNA damage. The major DSB repair pathway in yeast and prokaryotes is homologous recombination (HR) which employs a gene conversion mechanism. In higher eukaryotes DSBs are usually repaired by non-homologous end-joining (NHEJ) whereby broken DNA ends are simply rejoined, end to end, in what appears to be a random fashion. DSB repair pathways are also involved in the integration of transgenes. The natural tendency of higher plants to incorporate exogenous DNAs (transgenes) via NHEJ into random rather than homologous sites in the genome is problematic for the production of transgenic plants where either true gene replacement or site specific insertion of the transgene into the plant is preferred. We are attempting to influence the relative rates of gene replacement versus random integration events through manipulation of the expression and activities of key components of HR and NHEJ pathways, thereby enhancing the precision of transgene insertion via HR. To achieve this objective we have employed a combined biochemical and molecular genetic approach to enhance our fundamental knowledge of the mechanism of NHEJ in plants. We have identified several components involved in Arabidopsis NHEJ pathway(s) including DNA ligase IV, which forms a strong association with XRCC4, and the Ku70/ Ku80 complex which displays binding activity specific for dsDNA ends. Furthermore we have identified a putative Arabidopsis NBS1 homologue and we are investigating the role of this gene and the other NEHJ components in DSB repair *in planta*.

C2.18a Medicago CDK inhibitor

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Cyclin-dependent protein kinases (CDKs) have a central role in cell cycle regulation and can be inhibited by the binding of small protein CDK inhibitors (CKIs). Seven genes that encode proteins with distant sequence homology with p27Kip1 have been isolated from Arabidopsis. Using the yeast two-hybrid system we have identified a CDK inhibitor gene in *Medicago truncatula*, named CKIMt. Searches of the GenBank databases revealed that CKI shares limited amino acid identity to Arabidopsis KRPs. In all cases the highest degree of amino acid similarity was detected near the carboxy terminal of these proteins. This region of similarity is also related to the CDK2 binding site of the mammalian p27kip1, p21kip1 and p57Kip2. The mRNA of CKIMt was present in all cell cycle phases. However the transcript level increased moderately in G2/M phase. The CKIMt expression was induced by ABA. In a yeast two-hybrid matrix analysis revealed that the inhibitor protein can interact with the cdc2MsA and three *Medicago* D-type cyclins. The recombinant CKI was effective in inhibition of the histone H1 kinase activity of kinase complexes such as the $p13^{SUC1}$-associated kinases or immuneprecipitated cdc2A/B and cdc2F from *Medicago* A2 suspension. The inhibitory function of the recombinant CKIMt varied in the case of different kinase complexes and significant increase in its activity was observed after phosphorylation with the stress related MsCPK3 (calmodulin like domain protein kinase). Based on *in vitro* phosphorylation studies we propose CKI proteins as a molecular links between Ca^{2+} signaling and cell cycle control.

C2.19 Signal molecules in the control of cell cycle progression in tobacco BY-2 cells

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Mass spectrometrical analysis of plant hormones and other signal molecules throughout the cell cycle of synchronised tobacco BY-2 cells reveals a stringent control of the synthesis of zeatin type cytokinins and cyclic AMP during cell division. Work with inhibitors of cytokinin synthesis and subsequent feeding with various types of cytokinins points to a specific regulation of G2/ M transition in BY-2 by zeatin. Work with inhibitors of

the synthesis of cyclic nucleotides or with compounds that interfere with their downstream function equally points to a role for these molecules in the plant cell cycle. The levels of jasmonic acid and abscisic acid do not seem to change during cell cycle progression, but their application to cycling cells evokes dramatic effects in a fashion that is very dependent on the time of addition. This report will elaborate on our efforts to elucidate the function of these signal molecules in the plant cell cycle and our efforts to identify their downstream targets.

C2.20 Regulation of plant ethylene responses by ubiquitylation

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The plant hormone ethylene regulates a wide range of developmental processes and the response of plants to stress and pathogens. Several components involved in the ethylene signal transduction cascade have already been identified in Arabidopsis and led to a partial elucidation of the mechanisms of ethylene action. Ethylene signal transduction initiates with ethylene binding to a family of ethylene receptors and terminates in a transcription cascade involving the EIN3/EIL and ERF families of plant-specific transcription factors. Here, we identified two novel Arabidopsis F-Box proteins,EBF1 and EBF2, that interact physically with EIN3/EIL transcription factors. EBF1 overexpression results in plants insensitive to ethylene. In contrast, plants carrying the ebf1 and ebf2 mutations display a constitutive ethylene response and accumulate the EIN3 protein in the absence of the hormone. Our work places EBF1 and EBF2 within the genetic framework of the ethylene response pathway and supports a model in which ethylene action depends on EIN3 protein stabilisation.

C2.21 Restarting the cell cycle during lateral root initiation

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Root systems explore the soil mainly by the generation of lateral roots. Branching of roots takes off by a process called 'lateral root initiation'. The first visible feature of this process is the appearance of a set of isodiametric cells resulting from a series of anticlinal divisions within the monolayered pericycle. These divisions only seem to happen in a restricted number of pericycle cells at very specific positions in the root. The phytohormone auxin plays a crucial role in the timing and spacing of lateral root initiation by inducing cell divisions in these pericycle cells. We intend to unravel the signal cascade that links the auxin signal with the induction of cell division in the context of lateral root initiation by following two inverse approaches. In one approach, we start from the cell cycle regulation itself by studying cell cycle gene expression in the pericycle and/or studying the root phenotype after having perturbated the cell cycle protein levels in transgenics. The second approach departs from the auxin signal. By interfering with auxin distribution in young seedling roots, we were able to synchronize lateral root initiation allowing extensive gene expression studies through microarrays. We could visualize the transcriptional changes from the very early perception of auxin by the pericycle cells until the first cell divisions. The results obtained via both approaches will be discussed.

C2.22 Is cell division involved in plant growth responses to environmental constraints? Carbon dependent early cessation of elongation in lateral roots is due to early cessation of cell division

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The role of cell division in controlling growth or morphogenesis is for long a matter of debate. In lateral roots of maize as in most species, the duration of elongation (ie the time when elongation rate reaches zero) is strongly variable from 1-2 days to several weeks. The proportion of roots in each category of duration of elongation depends on the C status of the plant with situations of C limitation leading to increased proportion of roots rapidly ceasing to elongate. At the tip of roots which show decelerating elongation, cell length profiles are progressively shifted up. This strongly resembles what can be observed in leaves of both dicots and monocots during the days following cessation of expansion suggesting cell division is more (or earlier) affected than tissue expansion. This suggestion was confirmed by both kinematic studies and spatial distribution of mitosis in roots with decelerating elongation which showed a progressive shortening of the meristematic zone. The precocity of the decrease of cell division as compared to tissue expansion was further confirmed by the lack of mitosis in roots with little (though measurable) elongation. These results thus suggest that the carbon dependent early cessation of elongation in lateral roots is due to an early cessation of cell division.

C2.23 The relationship between *WEE1,* cell size and the plant cell cycle

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In eukaryotes the regulatory cell cycle gene, *WEE1*, encodes a protein kinase. In late G2, it inactivates cyclin-dependent kinases (CDKs) in the CDK-cyclinA/B complexes, by phosphorylating the CDK on tyrosine15. This can result in a delay in mitosis. Over expression of the *Arabidopsis thaliana* homologue of *WEE1* in fission yeast results in an elongated cell length phenotype in the same way as over expression of fission yeast *wee1*. We have tested whether *AtWEE1* could also induce this effect in plant cells. The tobacco TBY-2 cell line has been transformed with *AtWEE1*, both under constitutive and inducible promoters. Phenotypic characteristics observed compared with the control are premature entry to mitosis and a reduced mitotic cell size through a shortening of the G2 phase with a compensatory increase in the duration of G1. However, cell cycle length was two hours shorter in the *AtWEE1* expressing lines compared with empty vector lines. Hence, the phenotype and cell cycle response is the exact opposite of the known effects of over expression of this gene in fission yeast. CDKa and CDKb activity is not inhibited in the transgenic lines compared with wild type suggesting that the AtWEE1 expression may be perturbing the native NtWEE1 kinase. We hypothesise that the down regulation of NtWEE1 activity by ATWEE1 results at in premature entry of cells into division at reduced mitotic cell size.

C2.24 Cell division and cell expansion in *Arabidopsis* mutants with altered leaf development

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Final leaf size differences caused by environmental changes are correlated to differences in cell number, as opposed to appreciable changes in cell size. There are two possible explanations: either, leaf expansion and cell division are dependent upon one another, or, they are regulated independently but similarly. We investigated the respective contributions of cell number and cell size in *Arabidopsis thaliana* leaves with different final area in 3 mutants with altered final leaf shapes and sizes (from the collection of J.L. Micol, Universidad Miguel Hernández, Alicante) and the Ler wild type plants. The 3 mutants were: *ang 4*-smaller leaves than the wild-type, *ron 2-1*-larger leaves than the wild-type and *elo 1*-leaves of similar size but with altered shape and morphology. Plants were grown in controlled environments in optimal light conditions (12 mol day^{-1}) and in two shade treatments (3.2 and 2.6 mol day^{-1}). Mean final area of the 6th leaf of the rosette varied from 11.83 mm^2 to 194.41 mm^2 depending on the genotype and the light conditions. Shade treatments caused a reduction in final leaf area and final cell number for Ler, *ang 4* and *ron 2-1*. However, this reduction was not proportional as cell size was increased by the shade treatments. Differences in final leaf area among the mutants were not only associated to differences in cell number but also in cell size. The results suggest that cell size is not so stable and can be affected by internal and external factors.

C2.25 Cell cycle regulation during leaf development in *Arabidopsis*

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Organ growth results from the progression of component cells through subsequent phases of proliferation and expansion before maturity. We developed a kinematic analysis of cell cycle and cell expansion parameters throughout the development of the first leaf pair of *Arabidopsis thaliana*. The data showed that the epidermis proliferates until day 12. Cells then expand until day 19 when leaves reach maturity. Endoreduplication occurs from the time cell division rates decline until the end of cell expansion. Using this model we established the effect of mis-expression of various cell cycle genes: KRP2 overexpression inhibited division and endoreduplication rates in the absence of an effect on timing of the transitions between subsequent stages. E2Fa/Dpa overexpression, in contrast, strongly prolonged the proliferative phase at the cost of differentiation. The experimental system also proved suitable for microarray analysis of gene-expression associated with transitions between subsequent developmental stages. Though the timing of mitotic exit differs between tissues and positions across the leaf, transcriptome data from whole leaf samples closely reflect kinematic data from the abaxial epidermis. The data demonstrate the suitability of the experimental system for exploring the growth regulatory network and the cell cycle circuitry in multicellular organ development.

C2.26 The role of Cdc23/Mcm10 in the cell cycle of *Schizosaccharomyces pombe*

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Faithful DNA replication is vital to maintain genome stability. Numerous genes have been demonstrated to be involved in eukaryotic DNA replication, although we are far from understanding the complexity of this process. The conserved Cdc23/Mcm10 protein was identified in the fission yeast *Schizosaccharomyces pombe* as crucial for normal cell cycle progression: mutants fail to initiate DNA replication or exhibit a slowed replication, resulting in death. The gene encodes a 593 amino acid protein, including two putative Cdc2 phosphorylation sites and a central region containing a zinc finger-like domain. *In vitro* studies have shown Cdc23 to promote Mcm2 and Mcm4 phosphorylation by stimulating the Cdc7 kinase homologue. Chromatin binding studies have shown that Cdc23/Mcm10 is needed to load the Cdc45 homologue (DNA polymerase-α loading factor) onto chromatin after pre-replicative complex formation. The inability of *cdc23* mutants to complete S-phase after release from the hydroxyurea block suggests an additional role in the elongation phase of DNA replication. Our experiments have revealed a constant level of Cdc23/Mcm10 mRNA and protein throughout the cell cycle. Genetic studies and yeast 2-hybrid screening have shown interaction with MCM and ORC subunits, and with the Rad4 protein which has also been shown to have a role in the intra-S checkpoint. Full length and deleted Cdc23/Mcm10 were assayed in the yeast 2-hybrid screen with MCM and ORC subunits, revealing binding to require a central region containing the zinc finger-like domain. Intriguingly, recent data seem to suggest that Rad4 binds to the same region of the Cdc23/Mcm10 protein.