

## C2–PLANT AND FUNGAL CYTOSKELETONS

Organised by P.J. Hussey for the Cytoskeleton Group

### C2.1–The new tubulins: function and evolution

K. Gull, University of Oxford

The last few years has seen the discovery of a set of new tubulins -epsilon, zeta and eta. Examination of genome organization data (from completed or partial genome projects) suggests that these tubulins have a restricted evolutionary distribution. Such surveys and sequence comparisons provide clues to the roles of these tubulins and suggest that they are associated with the construction and function of the centriole/basal body and flagellum. Immunolocalisation of particular tubulins suggests that this is indeed their major site of their occurrence in the cell. We have also used RNAi ablation of gene expression in the African trypanosome *T.brucei* to down regulate expression of the new tubulins as well as gamma and delta tubulins to reveal specific effects on the microtubule organization of the basal body and flagellum. These primary effects are translated through to influence events such as organelle positioning and cytokinesis. Analysis of the phenotypes produced provides insights to the dependency relationships operating in the construction of the cytoskeleton.

### C2.2–Genetic analysis of basal body positioning in *Chlamydomonas* using VFL genes

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The direction of the effective stroke during the beat cycle of eukaryotic cilia is dependent on the rotational orientation of the basal body (centriole) from which the axoneme assembles. Genetic analysis of the biflagellate alga *Chlamydomonas* has provided valuable information on the function of the fibrous basal body apparatus that maintains basal bodies in the correct rotational orientation throughout the cell cycle. Mutations in each of several *vfl* loci result in cells with variable numbers of flagella, in flagella with variable beat direction, and in mispositioned and missing basal bodies. Previous studies have shown that the *vfl2* mutation identifies the gene encoding centrin, a component of several fibers of the basal body apparatus (Taillon et al., J. Cell Biol. 119:1613). The *VFL1* gene encodes a novel protein that localizes in a rotationally asymmetric pattern within basal bodies (Silflow et al., J. Cell Biol. 153:63). We will report recent results on the structure and function of the *VFL3* gene. A *vfl3* allele obtained by insertional mutagenesis was used to clone the *VFL3* gene which encodes

a basic protein of 603 amino acids with a central domain of predicted coiled-coil structure. A *VFL3* gene encoding a triple-HA epitope tag was shown to rescue the mutant phenotype when transformed into mutant cells. Results from immunofluorescence and immunogold localization experiments indicate that the Vfl3 protein is associated with a striated fiber that lies between each parental basal body and its adjacent nascent basal body. These results suggest that the *VFL3* gene plays a role in the pathway for assembly of a nascent basal body near a parental basal body, cosegregation of the pair at cytokinesis, and rotational positioning of the paired basal bodies. Sequences similar to the *VFL3* gene were found in several vertebrate genomes.

### C2.3–Microtubules, MAPs and plant development

P.J. Hussey, University of Durham

One of the most unique features of a plant is that the cells do not move. Embryogenesis is not dependent on cell migrations. The simple body plan of the seedling is established in the embryo, the cotyledons and the root primordium but most organ formation takes place post-embryogenesis and after seed germination. As plant cells do not move the development of tissues and organs is dependent on the balance of cell division and cell expansion, and in particular on the positioning of the new cross walls. The plant microtubule networks mainly govern this plant cell morphogenesis. Plant microtubules are structurally the same as their animal and fungal counterparts but they are more dynamic and have different biochemical properties. Moreover, microtubule associated proteins that regulate microtubule dynamics are not all conserved between the different kingdoms. For example there is no tau, MAP1, 2 or 4 but there are katanins, a homologue of MAP215, and many kinesins some of which have been identified genetically. Biochemical purifications have identified new plant MAPs, MAP65 and MAP190. Here, recent advances in the studies of MAP65 (1), MAP190 (2) and MAP215/MOR1/GEM1 (3) will be discussed.

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## C2.4—Helical growth mutants in *Arabidopsis*

Takashi Hashimoto, Nara Institute of Science and Technology, Japan

Handedness in plant growth may be most familiar to us as tendrils of twinning plants, which generally form consistent right- or left-handed helices as they climb. Also, the petals of several species are arranged like fan blades that twist in the same direction. To unravel molecular components and cellular determinants of handedness and anisotropic growth, we screened seedlings of *Arabidopsis thaliana* for mutants with either right- or left-handed helical growth. *spiral* mutants that consist of at least four genetic loci show right-handed helical growth in roots, hypocotyls, petioles, and petals; a series of semi-dominant *lefty* mutants show opposite left-handed growth in these organs. Cortical microtubule arrays are transverse to the elongation axis in elongating wild-type cells, whereas the arrays are arranged in left-handed helix in *spirall* and in right-handed helix in *lefty1* and *lefty2*. *lefty* mutations are epistatic to *spr* mutations, as far as several combinations tested. Both *SPR1* and *SPR2* genes are members of the distinct plant-specific novel gene families which encode proteins that, at least partially, associate with cortical microtubules, whereas *lefty* mutations are caused by dominant-negative amino acid exchanges of  $\alpha$  and tubulins at the intra- or inter-dimer interfaces. Proper control of microtubule functions is important for preventing elongating cells from potential twisting. SPIRAL proteins may define regulatory factors that control cortical microtubule organization, and include novel plant-specific microtubule-associated proteins (MAPs).

## C2.5—Microtubule-associated proteins

C.W. Lloyd and J. Chan, John Innes Centre, Norwich

Plant cells bonded together by their walls do not locomote into space but channel turgor pressure to produce directional cell expansion. The growth axis of the plant depends upon the ordered layering of cellulose microfibrils in the cell wall for only cellulose has the tensile strength to resist the large internal pressures generated by turgor. It is thought that the cytoplasmic microtubules, just inside the plasma membrane, form a temporary, dynamic scaffolding for the non-random deposition of the tough wall microfibrils; hence there is often a parallelism between the alignment of the cytoskeleton and the newest layer of wall fibrils. We are investigating how the patterns of microtubules are organised. One of the major difficulties of understanding microtubule organi-

zation in higher plants is in knowing how and where new microtubules are inserted into an array in the absence of fixed microtubule organizing centres, such as the centrosome found in other eukaryotic cells. It seems that microtubules, which wind around the cell, are severed from dispersed, amorphous nucleation sites and then organized into parallel sheets by various classes of microtubule-associated proteins (MAPs). We have been investigating the MAPs of *Arabidopsis* suspension cells. These have been biochemically isolated by affinity methods and then analysed by peptide mass fingerprinting. We will describe how MAPs, which are often conserved, form quite different microtubule arrays in higher plants.

## C2.6—Profilin function during pollen tube growth and signal transduction

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A remarkable example of cellular morphogenesis, pollen germination and tube growth, is essential for plant reproduction. In order to deliver non-motile sperm to the embryo sac, the vegetative cell of the pollen grain forms a tip-growing protuberance that extends at rates up to 1 cm/h. A dynamic network of filaments and associated proteins, the actin cytoskeleton, organizes the pollen cytoplasm, provides the molecular railroad tracks for cytoplasmic streaming, and orchestrates the delivery of secretory vesicles to the growing apex. To understand the molecular mechanism of tip growth, we have focused attention on a gene family for the actin monomer-binding protein, profilin, from the crop plant *Zea mays*. Our central hypothesis is that profilin is a fundamental regulator of actin polymerization and its cellular function is mediated through interactions with a diverse set of binding partners. We have characterized the interaction of recombinant and native maize profilins with three major ligands [G-actin, PtdIns(4,5)P<sub>2</sub> and polyproline] and discovered several important differences between plant and non-plant profilins and among plant profilin isoforms. Live cell and in vitro mutagenesis studies demonstrate that binding to both actin and proline-rich sequences is important for profilin's cellular function. Further, we show that the sequestering activity of profilin is regulated by calcium. Our results are consistent with two distinct populations of profilin playing different roles during pollen tube growth. We have also uncovered a role for profilin and other actin-binding proteins during the self-incompatibility reaction (SI) of poppy pollen. During SI, we have demonstrated a massive and sustained reduction of F-actin levels that occurs in response to a signal-mediated, cytosolic calcium flood. Molecular modeling and in vitro reconstitution

experiments suggest that pollen profilin can account for a small portion of the 50–80% reduction in filamentous actin that is induced. A search for other actin-binding proteins that could function as mediators of signal-induced actin rearrangements in pollen is underway.

### **C2.7—Remodelling the cytoskeleton with ADF/cofilins, their role in cell locomotion**

S.K. Maciver, C. Yap, D. Ternent, and D. Price, University of Edinburgh

The ADF/cofilins (AC) are a family of actin binding and filament severing proteins that are essential for the viability of all eukaryotes so far tested. ACs bind both G-actin and F-actin. The interaction with the latter is complex, being co-operative, pH-dependent and so far, unique in that they alter the actin filaments helical twist. ACs increase the depolymerization rate of actin filaments by severing, and by increasing the rate at which subunits leave the pointed end. It is proposed that the main function of the ACs is to depolymerise filaments at the base of the leading lamella of locomoting cells in order that further polymerization of the recycled subunits can take place at the leading edge. We have investigated the role of AC in cell locomotion by increasing the concentration of these proteins in cells and measuring the effect on the locomotory rate of a glioma cell line. We have found that increasing the level of ACs by moderate amounts, in these cells increases the rate of locomotion, but that large increases of ACs decreases the locomotory rate. In addition to the signalling pathways that affect the ACs through their effect on actin, ACs are directly regulated by phosphorylation in cells principally by the LIM kinases. Our long-term goal is to unravel the pathways that work through the ACs to influence cell behaviour.

### **C2.8—Regulation of the pollen-specific actin-depolymerising factor, LIADF1**

E.G. Allwood and P.J. Hussey, University of Durham

Pollen tube growth is dependent on a dynamic actin cytoskeleton suggesting that actin regulating proteins are involved. Here we examine the regulation of the lily pollen-specific actin-depolymerising factor (ADF), LIADF1. Its actin binding and depolymerising activity is pH sensitive, is inhibited by certain phosphoinositides but is not controlled by phosphorylation. Compared to its F-actin binding properties its low activity in depolymerisation assays has been used to explain why pollen ADF decorates F-actin in pollen grains (Smertenko et

al., 2001). This low activity is incompatible with a role in increasing actin dynamics necessary to promote pollen tube growth. We have identified a plant homologue of actin interacting protein, AIP1 which we show enhances the depolymerisation of F-actin in the presence of LIADF1 by up to 60%. Both pollen ADF and pollen AIP1 bind F-actin in pollen grains but are mainly cytoplasmic in pollen tubes. We strongly suggest that together these proteins remodel actin filaments as pollen grains enter and exit dormancy (Allwood et al., 2002)

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### **C2.9—Regulation of cell polarity in budding yeast**

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Site-specific activation of the Rho-type GTPase Cdc24p by its guanine-nucleotide exchange factor (GEF) Cdc24p is critical for the establishment of cell polarity in yeast during the cell cycle and mating. We have investigated the molecular mechanisms of the spatial and temporal regulation of Cdc24p. We found that binding of Cdc24p to the small GTPase Rsr1p/Bud1p is required for its site-specific recruitment to the incipient bud site. Likewise, during mating, Cdc24p is recruited to the site of receptor activation by the adaptor protein Far1p. Both Rsr1p/Bud1p and Far1p bind to the CH-domain of Cdc24p, which is essential for its function in vivo. Our results suggest that Cdc24p is auto-inhibited by intra-molecular interaction with its carboxy-terminal PB1-domain. Rsr1p/Bud1p and Far1p also appear to activate the GEF-activity of Cdc24p, presumably by triggering a conformational change which dissociates the PB1-domain from its intra-molecular binding site. Finally, we identified the adaptor protein Bem1p as a positive regulator of Cdc24p in vivo. Bem1p interacts with the PB1-domain of Cdc24p thereby preventing its re-binding to the intra-molecular inhibitory site. Taken together, our results provide a two step molecular mechanism for the site-specific activation of Cdc24p, which involves Rsr1p/Bud1p and Far1p, respectively, and the adaptor protein Bem1p.

## C2.10—The role of ROPs in root hair development

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Root hair morphogenesis in *Arabidopsis* is one of the best-described examples of cell differentiation and morphogenesis in higher plants. Classical genetic analysis has identified a network of interactions controlling the process, but we are still identifying the proteins encoded by most of these genes. In other eukaryotes, Rho small GTPases are key regulators of morphogenesis. They act as 'molecular switches', responding to intra- and extracellular signals to orchestrate the actin and microtubule cytoskeletons, secretion, vacuole fusion, cell wall synthesis, calcium gradients, reactive oxygen species, and other cellular components and processes. We have used reverse genetics to test the roles of Rho-Of-Plants (ROP) genes in *Arabidopsis* root hair development. ROP2 is expressed in root hairs and can strongly regulate root hair growth and the actin cytoskeleton. ROP2-GFP fusion proteins localize to the site of root hair growth before morphogenesis begins and remain there throughout growth, disappearing once growth has ceased. ROP7 also affects root hair growth, but manipulating the expression or activity of ROPs from other phylogenetic groups has no effect (Jones et al., 2002). We have identified a negative regulator of ROP in root hairs. Loss-of-function mutations in this gene are unique in that they affect many steps of morphogenesis and interact strongly with mutations in almost every other root hair gene tested. The phenotypes of these mutants are similar to the phenotypes of gain-of-function ROP2 lines, supporting the hypothesis that this protein negatively regulates ROP2 in root hairs. Classical genetic analysis has identified many root hair genes that have strong genetic interactions with the ROP regulator, and we are identifying the sequences of these genes.

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## C2.11—Triggering cell division in fission yeast: the spindle pole and protein kinases

I. Hagan, A. Bridge, F. H. MacIver, J. Petersen, A. Grallert, D. Mulvihill, A. Robertson, K. Tanaka, Paterson Institute for Cancer Research, Manchester

Commitment to mitosis is regulated by the protein kinase MPF. MPF is inhibited by phosphorylation on its catalytic sub-unit by protein kinases related to Wee1. Removal of this phosphate by Cdc25 promotes entry into mitosis. Full scale commitment to mitosis involves the promotion of Cdc25 activity and downregulation of

Wee1 in a positive feedback loop that is triggered by active MPF and involves the protein kinase polo. Fission yeast *cdc25.22* mutants can divide only if either *wee1*<sup>+</sup> or *cut12*<sup>+</sup> are also mutated. *cut12*<sup>+</sup> encodes an essential spindle pole body (SPB) component. Recessive *cut12* mutants block spindle formation while the dominant *cut12.s11* mutant permits division of cells which lack Cdc25. The polo kinase Plo1 usually only associates with the SPB of mitotic cells, however it associates with the SPB of *cut12.s11* mutants throughout the entire cell cycle. Cut12 binds to Plo1 in two hybrid and co-immunoprecipitation assays. Plo1 kinase activity is lost in recessive *cut12.1* mutants and boosted three fold in *cut12.s11* dominant mutants. The recruitment of Plo1 to the interphase SPB, and the suppression of *cdc25.22* by *cut12.s11* is dependent upon the function of both Plo1 kinase and the NIMA related kinase Fin1. Recessive temperature sensitive *fin1* mutants block spindle formation with the same defect as *cut12.1* and *plo1.ts* mutants while increasing *fin1*<sup>+</sup> transcription promotes the association of Plo1 with interphase SPBs of wild type cells and raises the restrictive temperature of both *cut12.1* and *cdc25.22* mutants. These data suggest that mutation of the SPB component Cut12 suppresses *cdc25.22* by promoting the activity of the feedback loop kinase, Plo1 independently of the normal controls that tie this activation to the prior activation of MPF. Thus, the association of key protein kinases with SPB proteins appears to play a critical role in driving commitment to mitosis.

## C2.12—Gene expression and functional analysis in the *Arabidopsis* cell-cycle

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Dispersed plant suspension cultures allow cell proliferation and growth to be analysed in the absence of developmental processes. We have recently established synchronisation procedures for *Arabidopsis* cell lines and used these to analyse cell cycle regulated gene expression by microarrays and Massively Parallel Signature Sequencing (MPSS) of cDNAs on immobilised microbead arrays. To extend the utility of the analysis, we have now also analysed gene expression in suspension cultures re-entering the cell cycle and during normal growth from sub-culture to stationary phase. Analysis of results shows that around 1100 genes show significant cell cycle regulation, and these are involved in a wide range of cellular processes. Identified genes include those already known to be cell cycle regulated, genes previously known to be cell cycle regulated in other eukaryotes and genes for novel plant-specific processes. We conclude that cell cycle regulation is an important mechanism in controlling cell division and

reflects the many interfaces by which cellular processes impinge on the cell cycle.

These data suggest that cell cultures can be used to model many cellular processes. Functional analysis of cell cycle genes is being carried out by overexpression in both cells and plants, and results with the D-type cyclin CYCD3;1 will be discussed to illustrate the utility and limitation of cell systems for such analysis.

### **C2.13–Spindle-Kinetochores Interaction in Fission Yeast**

T. Toda, Cancer Research UK, London Research Institute, London

Accurate chromosome segregation is vital for cell proliferation and genome stability in all organisms. In eukaryotic cells, spindles, mitosis-specific structures of polarised microtubules, play a fundamental role in capturing, pulling and segregating sister chromatids. Central to the spindle function is the establishment of bipolarity. Spindles must attach each sister chromatid at a specific site, called the kinetochore. The bivalent attachment of the kinetochores to the spindles emanated from both poles results in chromosome alignment at the equatorial plane (metaphase plate), which is a prerequisite for subsequent sister chromatid segregation. Using fission yeast as a model system, we have demonstrated, for the first time, that evolutionarily conserved microtubule-destabilising Kin I family proteins, Klp5 and Klp6, play a crucial role in chromosome alignment.

Further analysis shows that Klp5 and Klp6 are not sole molecules that are required for chromosome alignment. Conserved microtubule-associated proteins Alp14 and Dis1, whose vertebrate homologues (ch-TOG and Mor1 in plant) are overproduced in hepatic tumour cells, play a collaborative role with Klp5 and Klp6 in chromosome alignment. Intriguingly whilst Klp5 and Klp6 function as microtubule-destabilising activities, Alp14 and Dis1 stabilise microtubules. Therefore factors that apparently play an antagonising function in microtubule stability are in fact both needed to provide spindle dynamics in the process of spindle-kinetochore interaction and subsequent chromosome segregation. Molecular network that is necessary for regulating Klp5/6 and Dis1/Alp14 will be discussed.

### **C2.14–Role of astral microtubulus in spindle orientation and sister chromatid separation in fission yeast**

J. Hyams, University College, London

We recently described a new spindle orientation checkpoint (SOC) that delays entry into anaphase in fission

yeast when the actin cytoskeleton is disturbed [Gachet et al., *Nature* 412, 352–355, 2001]. We postulated that the cytotkinetic actin ring (CAR) is necessary for the positioning of proteins or protein complexes that mediate the dynamic interaction of the growing ends of astral microtubules with the medial cell cortex and that disruption of this structure activates the SOC. In the present study, we have addressed this hypothesis directly by comparing the behaviour of astral microtubules and spindle orientation in living cells in the presence or absence of Lat B and in mutants in which either the actomyosin ring is disorganised (*cps8*, *myo52*) or the dynamic association of astral microtubules with the spindle pole body is disturbed (*cdc11*). In all cases, cells entered mitosis normally but the spindle failed to orient and sister chromosome segregation was correspondingly delayed. Defective spindle orientation equated to changes in astral microtubule dynamics. In control cells, the balanced interaction of astral microtubules from both poles with a region at the medial cell cortex served to orient the spindle. Spindle elongation was delayed until the spindle achieved an angle of  $\sim 300^\circ$  to the cell axis. In Lat B or in the above mutants, astral microtubules showed exaggerated phases of growth and shrinkage although these were ineffective in orienting the spindle. This is the first demonstration of an active spindle orientation mechanism in fission yeast. How defective spindle orientation translates into a delay in sister chromosome segregation is the subject of active investigation.

### **C2.15–The role of cell polarity proteins in mitotic exit**

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The transition from mitosis to G1 in budding yeast is regulated by a signal transduction network called the mitotic exit network (MEN). One of the most upstream component of this network is the GTPase Tem1, which is activated by its guanine nucleotide exchange factor (GEF) Lte1. Here we describe how cell polarity proteins regulate mitotic exit. First, the Rho-like GTPase Cdc42, its GEF Cdc24 and its effector Cla4 (a PAK kinase) control the initial binding and activation of Lte1 to the bud cortex. Cla4-mediated phosphorylation of Lte1 probably targets Lte1 to the plasma membrane. Furthermore, Cla4 activates the mitotic exit network and consequently cells lacking *cla4* arrest at the end of anaphase. Secondly, Ste20 another PAK kinase that is regulated by Cdc42 facilitates mitotic exit independently of Lte1. Furthermore, we present data how other cell polarity proteins regulate mitotic exit.

### **C2.16—Characterization of plant proteins involved in microtubule dynamics during the cell cycle**

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Plant microtubules undergo dramatic architectural changes in the course of cell cycle, specifically at the entry into and exit from mitosis. These changes underlie the acquisition of specialized properties and functions. In living cells, they are stable or dynamic depending on the cellular process in which they are involved and on the interactions they develop with cellular effectors. These effectors include several families of proteins that interact with microtubule polymers or tubulin dimers. They are mainly classified in two major groups, those that favor the polymerized state of microtubules (MAPs), and those that promote microtubule depolymerisation. Up to now, our knowledge of such proteins in plant cells is still very limited.

In the goal to study plant microtubule destabilisation, we cloned the cDNA encoding for the catalytic subunit of the *Arabidopsis* katanin (Atkat60), a microtubule-severing protein. We report the first functional evidence that Atkat60 binds to microtubules and severs them in an ATP-dependent manner *in vitro*. The search for its molecular mechanisms and its functions in plants are further developing.

In an attempt to identify new microtubule-interacting proteins which regulate plant microtubule dynamics in a cell cycle dependant manner, we screened plant expression libraries with antibodies directed against animal microtubule-interacting proteins which shares common epitope(s) with plant proteins. We identified novel proteins which are putatively involved in microtubule assembly in a cell cycle dependant manner. Their properties and putative functions will be discussed.

### **C2.17—Developmental control of the cell cycle in plants**

J.H. Doonan, John Innes Centre, Norwich

Developmental patterning and cell cycle control are coordinated throughout plant growth. Developmental mutants alter both the timing and rate of cell division, suggesting that cell cycle progression is controlled, directly or indirectly, by developmental master control genes. Cyclins are positive rate-limiting activators of cyclin-dependent protein kinases that are implicated in driving cell cycle progression. Cyclins are therefore good candidates for coordinating cell cycle control with growth and development. In both animals and plants, the D-cyclins are implicated in the cell's response to extracellular growth factors and are believed to determine the balance between cell proliferation and

differentiation. On the other hand, A- and B-type cyclins are believed to regulate cell cycle progression.

Using RNA *in situ* hybridisation, we have screened for cyclin genes whose expression pattern is related to either cell cycle progression or development. B cyclins display cell cycle-specific gene expression in both *Arabidopsis* and Antirrhinum and this is regulated by a small family of Myb transcription factors related to cMyb from mammalian cells. An unusual A-type cyclin is expressed in growing cells but is not directly related to either cell cycle phase or developmental stage. Three D3 cyclins display differential expression within the meristem that is related to meristem development and is modified in certain developmental mutants, suggesting that the D3 class of cyclins to play a role in co-ordinating the interface between development and cell cycle control. To define the role of these cyclins, we are taking a combined genetic and transgene approach to examine the effect of experimentally altering the levels of particular cyclins in plants and cell cultures

### **C2.18—Entry and exit from mitosis in plants**

Anne Helfer, Tamas Meszaros and Laszlo Bogre, Royal Holloway, University of London; Pavla Binarova, Academy of Sciences of the Czech Republic, Prague

Mitotic progression is timely regulated by the accumulation and degradation of two classes of B-type cyclins in plants, B1 and B2 and thus by the activation and localisation of cyclin-dependent kinases (CDK-A and CDK-B). We found that cyclin B2 is a critical regulator for the entry into mitosis, and targeted by hormonal as well as by the DNA condensation and DNA replication checkpoint pathways. Cyclin B2 degrades early in mitosis, during prometaphase, which surprisingly does not depend on its destruction box motif, or the proteasome pathway. Contrary to this, cyclin B1 in plants is degraded during meta- to anaphase transition through the anaphase-promoting complex, and its degradation is critical for cytokinesis and for phragmoplast formation. During cytokinesis a specific MAPK pathway is activated. We will describe our findings how this CDK/MAPK switch during meta/anaphase to cytokinesis might regulate plant-specific events of microtubule organisation and membrane trafficking.

### **C2.19—Cytoskeleton in *Arabidopsis*: a genetic approach**

D. Bouchez and M. Pastuglia, SGAP, INRA-Versailles, France

The *Arabidopsis ton* mutations drastically change plant morphology and cell organization, and mutant cells are unable to form a preprophase band of microtubules (Traas et al. 1995).

The *ton1* mutation affects two similar genes in tandem which encode small polypeptides with no similarity to known proteins. TON1 proteins are associated with the cortical cytoskeleton. Putative TON1b interacting proteins have been identified using the 2-hybrid system, including Centrin. Eleven other interactants define a new, plant-specific family of large proteins containing five conserved motifs.

The *ton2* gene encodes a 55 kDa protein with high similarity with a human protein of unknown function (Camilleri et al, 2002). The C-terminal part of the TON2 protein is similar to B'' regulatory subunits of type 2A phosphatases (PP2A). The TON2 protein interacts with an *Arabidopsis* type A subunit in yeast and likely defines a novel subclass of PP2A subunits possibly involved in the control of cytoskeletal structures. The target of such phosphatase activity is not known at present.

We used reverse genetics to isolate  $\gamma$ -tubulin knockout mutants in *Arabidopsis*. Simultaneous disruption of the two  $\gamma$ -tubulin genes of *Arabidopsis* is lethal at the plantlet level, but neither affects embryogenesis or early seedling development, where normal cortical microtubule arrays are assembled. At later stages of development, disruption of microtubule organisation is observed.

#### References

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### C2.20–Membrane trafficking and cytoskeleton in plant cytokinesis

G. Jürgens, ZMBP, Entwicklungsgenetik, Universität Tübingen

Higher plants have evolved a unique mode of cytokinesis. Targeted delivery of membrane vesicles to the centre of the division plane initiates the formation of a transient membrane compartment, the cell plate, which then expands towards the cell periphery to fuse with the parental plasma membrane. Cytokinesis is supported by a dynamic cytoskeletal array, the phragmoplast, consisting of two opposing sets each of microtubules and actin filaments. The phragmoplast undergoes lateral translocation during cell-plate expansion such that additional vesicles are delivered to the growing margin of the cell plate. We are using the *Arabidopsis* embryo as an assay system for identifying genes involved in cell division. Two classes of mutants have been obtained. Cytokinesis mutants are defective in the actual process of division whereas cell-division mutants stop dividing altogether at a very early stage of embryogenesis. The genes identified encode components of the cytokinetic vesicle fusion

machinery or proteins required for microtubule formation or reorganisation. Our results suggest that microtubules play a prominent role in the targeted delivery of membrane vesicles during cytokinesis.

### C2.21–Cytoskeleton and Endomembrane Interactions

C. Hawes, C. Saint-Jore and F. Brandizzi, Biology, Oxford Brookes; Ian Moore, Plant Sciences, Oxford

By applying a fluorescent protein strategy we have shown in tobacco leaf epidermal cells and in BY2 cells that movement of the individual stacks of the Golgi apparatus over the polygonal network of cortical ER is actin based. Similarly, the dynamics and growth of the cortical ER tubules are actin-dependent. In the absence of actin, Golgi bodies aggregate and their movement ceases. Likewise, the growth and movement of ER tubules is arrested. However, the basic geometrical integrity of the ER network does not appear to rely on any cytoskeletal elements. The role of the cytoskeleton in transport of membrane protein between the ER and Golgi was investigated through the use of the secretory inhibitor brefeldin A and by fluorescence recovery after photobleaching (FRAP) experiments. Brefeldin A induced a redistribution of fluorescent protein from Golgi membranes back into the ER, which was independent of the cytoskeleton. Likewise reformation of fluorescent Golgi bodies on removal of the drug was also independent of actin and microtubules. By photobleaching individual Golgi and measuring the rate of fluorescence recovery, transport of new membrane protein from the ER to Golgi could be assayed. Our results showed that all the Golgi markers used in the study cycle to and from the Golgi in an energy-dependent and NEM sensitive manner and do not require the involvement of the cytoskeleton.

### C2.22–Class I myosin in hyphal growth and morphogenesis

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The genetically tractable fungus *Aspergillus nidulans* has a single class I myosin gene, *myoA*, that is essential. We have used molecular genetics in *A. nidulans* to investigate the functions of this class I myosin in hyphal growth and morphogenesis. Myosin I mutants have demonstrated that MYOA functions in endocytosis, establishing polar hyphal growth and septation. Like all amoeboid class I myosins, MYOA contains a proline-rich domain and an SH3 domain in the carboxyl terminal region of the polypeptide. Mutational studies have shown that the SH3 domain is not required for MYOA function. They have identified an approximately 30 amino acid proline-rich domain adjacent to the SH3 domain

as essential for function. Interestingly, mutations that affect the ability of MYOA to hydrolyze ATP to generate mechanochemical force to move along F-actin are viable. These mutational studies suggest that the primary function of MYOA is to establish polar hyphal growth and actin assembly, not as a molecular motor. We have identified proteins that bind to the SH3 and proline-rich domains of MYOA using a fragment of MYOA made in *E. coli* as an affinity matrix. Two of the proteins identified are the actin related proteins Arp2 and Arp3.

### C2.23—Microtubule organization in *Ustilago maydis*

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Growth of most eukaryotic cells requires directed transport along microtubules (MTs) that are nucleated at nuclear associated microtubule organizing centers (MTOCs), such as the centrosome and the fungal spindle pole body (SPB). Here we show that the pathogenic fungus *Ustilago maydis* uses different MT nucleation sites to rearrange MTs during the cell cycle. In vivo observation of GFP-MTs and MT plus-ends, tagged by a fluorescent EB1 homologue, provided evidence for antipolar MT orientation and dispersed cytoplasmic MT nucleating centers in unbudded cells. On budding gamma-tubulin containing MTOCs appeared at the bud neck and MTs reorganized with >85% of all minus-ends being focused towards the growth region. Experimentally-induced lateral budding resulted in MTs that curved out of the bud, again supporting the notion that polar growth requires polar MT nucleation. Depletion or over-expression of Tub2, the gamma-tubulin from *U. maydis*, affected MT number in interphase cells. The SPB was inactive in G2 phase but continuously recruited gamma-tubulin until it started to nucleate mitotic MTs. Taken together, our data suggest that MT reorganization in *U. maydis* depends on cell cycle-specific nucleation at dispersed cytoplasmic sites, at a polar MTOC and the SPB.

### C2.24—Fission Yeast Myosins

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Fission yeast possesses two type II myosins, Myo2 and Myp2, two type V myosins, Myo51 and Myo52, and a single type I myosin, Myo1. Myo2, Myp2 and Myo51 are components of the cytokinetic actomyosin ring (CAR) whilst Myo1 and Myo52 are involved in the deposition of the cytokinetic septum (Win et al. 2002. Cell Motil Cytoskel 51: 53–56). We have developed new procedure to directly examine the spatial and temporal relationships between CAR formation and contraction, mitotic spindle formation and elongation and septum formation in living fission yeast cells. We have dem-

onstrated that CAR formation at anaphase onset requires a stable microtubule cytoskeleton (Mulvihill & Hyams. 2002. J Cell Sci 115: 3575–3586). However, microtubules are not required for the positioning and stability of the CAR. In wild type cells, CAR contraction is a continuous smooth process and the centripetal deposition of the septum precisely follows the constricting ring. At 22 °C the velocity of CAR contraction in wild type cells is 4.46 nm/sec  $\pm$  0.78 and occupies ~35 min. No significant difference is observed in cells lacking either Myp2 or Myo51. However, in the absence of Myp2 the CAR persists for longer in a contracted state and is often seen to split in two discrete rings, thus, demonstrating that Myp2 contributes to the stability of the CAR, particularly in the late stages of contraction (Mulvihill & Hyams. 2003. Cell Motil Cytoskel 54: In press). In contrast, in the absence of the type V myosin, Myo52, CAR contraction is discontinuous and occupies up to 55 min. Unlike Myo2, Myp2 and Myo51 which are CAR components, Myo52 colocalises with the newly deposited septal material. These findings demonstrate the interdependence of CAR contraction and septum deposition and show that Myo52 links the two processes. At cytokinesis, Myo52 is translocated to the division site from the cell poles. We have examined the dependence of Myo52 movement upon different cytoskeletal components and examined which regions of its structure are required for Myo52 function and localisation. Whilst localisation of Myo52 at the cell tips and equator is dependent upon both actin and microtubules, its movement over long distances within the cell is solely microtubule-dependent. The biological implications of these phenomena will be discussed.

### C2.25—Structure and Regulation of the Dynein Microtubule Motor

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Outer arm dynein from *Chlamydomonas* flagella is a 1.9 MDa complex consisting of three motor-containing heavy chains (HCs) associated with several intermediate and light chains that are responsible for cargo attachment and motor regulation. Furthermore, an additional trimeric structure (the docking complex) is required to specify the precise dynein attachment site within the flagellar axoneme. The light chain 1 protein (LC1) is associated with the ATP hydrolytic site of the gamma HC. NMR structural analysis revealed that LC1 consists of an N-terminal helix, a central barrel composed of six beta-beta-alpha repeats and a C-terminal helical domain that protrudes from the main protein axis and is predicted to insert within the motor domain. This C-terminal domain contains two Arg residues that potentially could affect ATPase activity in a manner similar to the GAPs that activate Ras and Rho. Expression of an