Cyclin-dependent protein kinases, mitogen-activated protein kinases and the plant cell cycle

Erwin Heberle-Bors

Vienna Biocenter, Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria

The basic cell cycle machinery is highly conserved in eucaryotes. Its central engine, the cyclin-dependent protein kinases (CDKs) and their associated cyclins, as well as a large number of turnover, upstream and downstream components also operate in plants. Despite their high evolutionary conservation, these components seem to assemble by a plant-specific combinatorial code, and the plant cell cycle machinery is steered by plant-specific forces. The mitogen-activated protein kinases (MAPKs), another class of highly conserved protein kinases, play a prominent role in turning on the central engine. In this review the roles of the two families of protein kinases in plant cell cycle regulation are linked to their role in growth, hormone action and the dynamics of the cytoskeleton.

SINCE Schleiden and Schwann's discovery that plants and other organisms consist of cells as units of life, cell division and its control has attracted the interest of plant biologists. The regular distribution of cells, seen in sections of plant organs, the files of cells emerging from the meristems or the directive changes in the orientation of the spindle in mitotic cells suggested a rigid mechanism of pattern formation, a strict lineage of cells, created by the orientation of the division plane. It was in a way ironic that in our days cell lineage has been discarded as a developmental mechanism in plants, while in animals with their less rigid and mobile cells, it turned out to be important. Nevertheless, cell division control in plants maintained centre stage in the scientific interest of plant biologists. Developmental processes do depend on the orientation of cell division, but in reaction to position, as has been demonstrated so beautifully in chimeras consisting of cells of different ploidy. Also the many instances in which developmental decisions are initiated by asymmetrical cell divisions give ample evidence for the importance of cell division control in plant development[1]. Cell division control also operates in situations when plants are attacked by predators or challenged by unfavourable weather and climate conditions (wind, rain, cold, drought, etc.). Plant cells retain a remarkable regeneration capacity, with many cells remaining totipotent, even after completion of differentiation programmes. Regeneration of a whole organism can occur from a single cell, both *in vivo* and in *in vitro* cultures of plant cells. Finally, in their quest to colonize the remotest places on our planet, plants evolved a variety of mechanisms to circumvent the relative stereotype cell division patterns which characterize sexual reproduction. The deviations in meiosis and embryo sac cell division patterns resulting in asexual or apomictic reproduction are legion.

All these features made plants to be seen very differently from animals, even to the eye of the scientist. Still, at a time when we start to value the diversity of life on our planet, we seem to neglect the similarities, the connectedness of life. Apart from the more obvious shared composition of cellular constituents or the common mechanisms of sex, molecular biology has shown us unforeseen and surprising similarities hidden to the eye. Many proteins of important functions are now known to be shared by all eukaryotes, encoded by genes of high sequence conservation. In contrast to prokaryotes, cellular compartmentalization, most notably the inclusion of DNA within the nucleus, required efficient and reliable, but at the same time diverse intracellular communication between sensor and effector mechanisms. Protein phosphorylation, catalysed by protein kinases, evolved as the most common mechanism for influencing protein conformation to make protein function dependent on signalling processes. Two classes of protein kinases, the cyclin-dependent protein kinases, or CDKs, and the mitogen-activated protein kinases, or MAPKs, are now known to play major roles in the regulation of how eukaryotic cells divide, grow, differentiate and communicate with other cells and the wider environment. CDKs have been central to understanding the control of cell division. However, other protein kinases, including MAPKs, placed in parallel, upstream or downstream of CDKs, also regulate cell divisions. In plants we are beginning to isolate the components, but little is known how they are connected which is crucial in understanding how different stimuli influence cell divisions and thereby meristem function and plant development. The focus in this review is on CDKs, but reference is given to MAPKs wherever mandated by evidence.

Basic eukaryotic cell cycle machinery

CDKs are now widely recognized as key players at various checkpoints in the eukaryotic cell cycle. More

than ten years ago, independent genetic approaches in yeasts and biochemical studies of mitotic controls in fertilized eggs of marine invertebrates revealed that the same serine–threonine protein kinase is involved both in G1/S and in G2/M transitions in yeast (the *cdc28* gene of *Saccharomyces cerevisiae* and its orthologue *cdc2* of *S. pombe*), and is the main component of the mitosis-promoting factor in fertilized eggs. The functional definition of a CDK is its requirement of cyclin binding for activity. In eucaryotes, CDK and cyclins form large superfamilies so that a very large number of CDK–cyclin complexes can occur with various substrate specificities at various locations within the cell and at various time points within the cell cycle. The CDK–cyclin complexes ensure a number of functions of which the direct regulation of the cell cycle is the most thoroughly characterized. The substrates of CDK–cyclin complexes include transcriptional regulators, cytoskeleton, nuclear matrix, nuclear membrane proteins as well as other cell cycle proteins[2,3].

The CDKs are, in the words of the early protagonists, the work horses of the cell cycle machinery which have to energize and dynamise all cellular components for their passage during the various phases of the cell cycle. In the yeast cell cycle CDK activity alternates between a high activity and a low activity state. A high CDK activity is required for G1/S transition and entry into mitosis, while a low CDK activity is required for chromosome segregation in anaphase and formation of DNA prereplication complexes[4].

At the G1/S transition the Skp1-cullin-F-box protein complex (SCF) is the arbitrator in a tug of war between the trancriptionally-activated and growth-dependent G1-cyclins and the growth-arresting FAR1 protein by targeting CDK-phosphorylated FAR1 to proteolysis and turning on CDK activity. At the other side of the cell cycle, at mitosis, the eukaryotic cell is less concerned with growth control, but with reliable transmission of its constituents, primarily its chromosomes, to the daughter cells. So-called checkpoints have been defined at which cycling cells are transiently arrested in response to external and internal stresses and accidents which threaten fidelity in dividing up the cellular constituents, including the chromosomes, and thus genome integrity in the daughter cells. Elaborate signalling modules measure the end of DNA replication, cell size, proper alignment of the chromosomes in metaphase, proper attachment of the spindle microtubules to the kinetochore and separation of the sister chromatids. The anaphase-promoting complex (APC) controls several of these checkpoints by targeting cyclins and other proteins to proteolysis and brings down CDK activity to G1 levels.

Mitogen-activated protein kinases

MAPKs are encoded by another large family of serine-threonine protein kinases that are found in all eukaryotes and which phosphorylate a variety of substrates, including transcription factors[5]. Activation of MAPKs is brought about by upstream MAPK kinases (denoted as MAPKKs) through phosphorylation of conserved threonine and tyrosine. A given dual specificity MAPKK can only activate a specific MAPK and cannot functionally substitute for other MAPKKs. MAPKKs are themselves activated by phosphorylation through upstream kinases that belong to the class of MAPKK kinases (MAPKKKs), such as the Raf and Mos proteins. A specific set of three functionally interlinked protein kinases (MAPKKK–MAPKK–MAPK) forms the basic module of a MAPK pathway. Several modules may co-exist side by side in a cell and integrate a variety of upstream signals through interactions with other kinases denoted as MAPKKK kinase (MAPKKKK) or G proteins, such as Ras or heterotrimeric complexes. The latter factors often function as coupling agents between a plasma membrane located receptor protein that senses an extracellular stimulus and a MAPK module[6].

Animal paradigm of cell cycle control

The molecular events involved in G1-control in animal cells are well established[7,8]. Various types of growth factors activate tyrosine kinase receptors by inducing their oligomerization and phosphorylation. Receptor activation leads to the conversion of a small GTPase, Ras, from its inactive (Ras GDP) to its active (Ras GTP) form. The pathway bifurcates at Ras into two signalling cascades that co-operate in mitogenic stimulation. In one cascade, Ras binds and activates Raf which leads to the activation of the MAPK pathway through the phosphorylation of MEK1 or MEK2 and subsequently ERK1 or ERK2. The MAPKs, ERK1 and ERK2 translocate to the nucleus upon mitogenic stimulation and phosphorylate transcription factors, e.g. TCF, which leads to the expression of immediate early genes, such as *c-fos*, as well as the transcription of the G1 cyclin, cyclin D1.

The other pathway involves the activation of PI3-kinase by Ras and thereby the production of phospholipids (PIPs). PIPs activate two related protein kinases, PDK1 and PDK2, which phosphorylate and thereby activate PKB, another membrane-bound protein kinase. PKB has a dual role. It inactivates GSK3 kinase by phosphorylation, thus inhibiting apoptosis, an important step toward immortalized proliferation and, together with PDK1 and PDK2, it phosphorylates and in this case activates $p70^{Rsk}$, that also performs multiple functions. It phosphorylates the transcription factor SRF which together with TCF is required for the expression of immediate-early genes such as the proto-oncogene *c-fos* and for the expression of the G1-cyclin, cyclin D1. Another target of $p70^{Rsk}$ is the ribosomal protein S6, the phosphorylation of which enhances the translation of

mRNAs containing a 5′ tract of polypyrimidine, such as the cyclin D1 mRNA. $p70^{Rsk}$ is also known to phosphorylate the CDK inhibitor $p27^{Kip1}$ and in so doing targets it for degradation.

The increased transcription and translation of cyclin D1 and the proteolysis of $p27^{Kip1}$ leads to the subsequent activation of CDK4- or CDK6-cyclin D1 and CDK2-cyclin E complexes. All these events culminate in the phosphorylation of a master switch of G1 control, the retinoblastoma protein (Rb), first by the CDK4- or CDK6-cyclin D1 complexes and then by the CDK2–cyclin E complex. Hyperphosphorylated Rb does not bind any more to the transcription factor E2F, which induces the expression of genes required for DNA synthesis, such as thymidilate synthase, proliferating cell nuclear antigen (PCNA) and cyclin A.

The molecular events involved in G2-control in animal cells are not well established. The MAPKs are also activated during G2-phase[9]. MAPKs activate mitotic CDKs through the action of $p90^{Rsk}$ which phosphorylates and thereby inactivates the Myt1 kinase[10]. The Myt1 kinase is a partner enzyme of the Wee1 kinase for phosphorylating CDK1 on the Thr14 and Tyr15 inhibitory sites. Inactivation of Myt1 thereby activates CDK1.

In a wider sense, MAPKs have emerged recently as central regulator of growth[11]. Not only do they signal information to the basic cell cycle machinery, but they also have been shown to activate pathways to recruit ribosomes to nascent transcripts and thereby initiate protein synthesis. The Mnk1 kinase is activated by a MAPK and itself activates the elongation factor elF-4e. MAPKs also phosphorylate histones which, together with MAPK-phosphorylated transcription factors, control gene expression. Finally, nucleotide synthesis is also controlled by MAPKs. ERK MAP kinase regulates the activity of carbamyl phosphate synthetase II which catalyses the initial rate-limiting step in the *de novo* synthesis of pyrimidine nucleotides.

Plant cell cycle machinery

That all life forms are organized as cells and multimers thereof was first recognized in plants. Also the fact that cells divide by mitosis was first discovered in plants. In 1953, Howard and Pelc[12] observed that cells undergo DNA replication and that S phase and M phase are interrupted by two gap phases, G1 and G2. Cell division cycle times in higher plants can vary from five to hundreds of hours. M phase is usually of constant length, while differences in the duration of the G1, S and G2 phases account for major variations in total cell cycle lengths. When starved for nutrients or irradiated, cells typically stop in G1 or G2. These observations led van't Hof and Kovacs[13,14] to propose a hypothesis that involved two principal control points in the cell cycle at which cells may exit the cell cycle, one at the end of G1 and one during G2. Similar control points were later found in all eukaryotes.

The discovery of a conserved set of genes controlling the eukaryotic cell cycle contributed largely to the unravelling of the plant cell cycle (for reviews see refs 15–17). Genes for CDK and mitotic cyclins were the first cell cycle genes characterized in plants.

Apart from the CDKs and cyclins, other constituents of the eucarytotic cell cycle are also conserved in plants. Much attention has focused recently on CDK inhibitors (CKIs). These proteins inhibit cell cycle progression through their association with CDK complexes[18]. The presence of CKIs has been inferred from differences in kinase activities in nucleus and cytoplasm[19] and a first plant CKI with limited sequence similarity to mammalian CKIs was isolated[20]. The ICK1 protein has been shown to bind to both, a CDKA and CycD3, *in vitro*[21]. High hopes are associated with the CKIs. As negative regulators and from what is known in animals they might be central to cell cycle arrest situations in the principal control points and checkpoints.

CDK phosphorylation, known to regulate the eukaryotic cell cycle on the conserved Tyr15, Thr14 and Thr160 residues, seems also to be conserved in plants since these residues are present in the most plant CDKs. Tyrosine 15 in CDK complexes of cytokinin-deprived *N. plumbaginifolia* cells seems to be the target of a yeast cdc25-like phosphatase[22], see also below. Tyr15 phosphorylation seems to play also a role in water stress responses in wheat[23]. However, transgenic *Arabidopsis* and tobacco plants expressing mutant versions of the *cdc2A*-gene in which the tyrosine 15 was mutated to phenylalanine and the threonine 14 to alanine, thought to be constitutively active, developed normally, except some tendency towards a reduced apical dominance. Unfortunately, these plants have not been analysed for cell number and cell size.

Finally there is little doubt that proteolytic degradation of cyclins exists in plants, given that the plant A- and B-type cyclins contain the destruction box, a conserved motif of ubiquitin-mediated degradation, and that the D-type cyclins contain the equally conserved PEST domains. However, very little is really known about this aspect of cell control in plants. Intriguingly, the plant homologues of a component of the yeast anaphase-promoting factor (APC), AXR1 and TIR1, seem to play a role in auxin response[24,25], see below).

Further downstream, the master switch of multicellular organisms in G1, the Rb-protein, has been isolated from plants[26,27], as have E2F-like proteins[28]. As in animals, Rb is hyperphosphorylated and can be phosphorylated by CDKs which are of the cdc2/cyclin D type, at least *in vitro*[29]. The upstream regulatory network is, however, not yet known. Nucleolin, a protein involved in rRNA biogenesis, and a target of CDKs in animals, has also been isolated from plants[30] and serves as a

valuable molecular marker for monitoring the entry of cells into a metabolically active state of proliferation.

In the past years, a rapid increase in the number of identified plant CDK and cyclin sequences has enabled the establishment of a structural classification and the proposal of a standardized nomenclature. The cyclins consist of two groups of B-type cyclins (*CYCB1* and *CYCB2* genes), three groups of A-type (*CYCA1*, *CYCA2* and *CYCA3*) and D-type cyclins (*CYCD1*, *CYCD2* and *CYCD3* genes)[31]. In animals, only one cyclin A, two cyclin B but several cyclin D genes have been identified. The higher complexity of the plant cell cycle is apparently matched by a larger number of variants of the basic cell cycle machinery. Information about their possible function was initially obtained from their cell cycle phase-specific expression (for a review see ref. 17). B-type cyclin genes have a clear G2-phase-specific expression pattern, while A-type cyclins are also expressed in S-phase. D-type cyclins are generally expressed at all phases of the cell cycle.

Although their number has increased at a lower rate than cyclins, 50 different sequences of plant CDKs in more than 20 species of the plant kingdom are now known. As cyclins, plant CDKs display both a high degree of homology and some structural and functional differences compared with animal and yeast CDKs[17,32]. A plant-wide nomenclature of CDKs has also recently been proposed[33]. Plant CDKs, from algae to angiosperms, are encountered in five evolutionarily conserved classes which, in comparison to other eucaryotes, represent a high level of complexity. The names *CDKA* to *CDKE* have been given to the five classes of plant *CDK* genes. The 31 plant CDKs present in the CDKA class are more closely related to yeast and human CDKs than to other plant CDKs of the same species. They contain the canonical PSTAIRE motif in the alpha-helical T-loop of the protein which is important for cyclin binding. Based on complementation of yeast mutants, expression pattern and activity profile, the CDKA kinases seem to play a dual function for plant CDKA during both S and M phase progression. The use of CDK-specific inhibitors such as the purine analogues olomoucine and roscovitine, also confirmed that the inhibited CDK(s) regulate(s) both transitions[34]. In addition the *CDKAs* seem to play a role in the maintenance of cell division competence in differentiated tissues during plant development[35,36].

The seven *CDKB* genes exist only in plants. They contain the PPTALRE or PPTTLRE motif. Plant *CDKBs* are unable to complement yeast *cdc2/cdc28* mutants, and their expression pattern and activity level is dependent on the cell cycle phase, unlike the *CDKAs*. The *CDKB* of *Medicago sativa* (formerly CDC2F), *f.e.*, is active only in mitosis[37]. Possibly CDC2B operates a checkpoint which is specific to plants.

A small group of four plant CDKs from pea, alfalfa and *Arabidopsis* was characterized by the presence of the PITAIRE motif, also present in animal CDK-related kinases. The expression profile of these *CDKC* genes was constitutive in a synchronized cell suspension, while no *in situ* hybridization signal for an *Arabidopsis* member of the *CDKC* family could be obtained in actively dividing cells; evidence against the involvement of *CDKC* in cell cycle control.

Two plant sequences fall in the *CDCD* group which also includes a number of animal (CDK7) and yeast CDKs considered to be bifunctional proteins involved in phosphorylation-dependent activation of other CDKs during the cell cycle (i.e. CAK function) and in phosphorylation-dependent regulation of the activity of RNA polymerase II. These kinases have a conserved N(I/F)TALRE motif. In partially synchronized suspension cells[38] and during adventitious root growth[39], a preferential expression of a rice *CDKD* gene was recorded in the G1 and S phases. At the cellular level, *CDKD* was shown to be uniformly associated with the dividing region of the rice root apex[32], but a basal level of expression was detected in the differentiated zone of the internode, i.e. in cells in the G0 state[38].

A unique plant sequence, the alfalfa cdc2MsE, appears to be unrelated to any other plant sequence. It harbours a SPTAIRE motif. The most similar although distantly related protein is human CDK8 which contains the sequence SMSACRE. CDK8 is involved in the regulation of RNA polymerase II in association with cyclin C. The involvement of *CDKE* genes in the plant cell cycle has yet to be proven. At the mRNA level a weak constitutive signal has been detected during a synchronized cell cycle[37].

These sequence comparisons demonstrate on the one hand the high conservation of molecular mechanisms in the eukaryotic cell cycle and have allowed the identification of likely orthologues in the different eukaryotic and plant species – this is, for example, evidenced by the *CDKA* genes which display structural and functional properties very similar to those of their human and yeast counterparts. On the other hand, there is evidence that a CDK combines with one cyclin in the one species, but with another one in another species. In animals, binding to cyclin D is a property of the CDK4 and CDK6 kinases which are absent from the plant kingdom and plant CDKA kinases form complexes with plant homologues of D-type cyclins. Thus, in plants the precise combinatorial code, the interaction, co-ordination and activation of the different CDK complexes, as well as their effects on downstream components of the cell cycle machinery is different from other eucaryotes and has still to be described precisely at the molecular level. In fact, in only a few instances has CDK activity been traced down to a specific CDK[37] or cyclin[19].

Growth control in G1

Plant development is, among other signals, co-ordinated by metabolic signals providing information about the physiological state of its constituent organs. A large body of evidence suggests a role for soluble carbohydrates in controlling gene expression, growth, metabolism and differentiation in plants[40]. How the carbohydrate signals are transduced within the cell is still largely unknown. Eventually the signals arrive at the basic cell cycle machinery. At least this is what can be drawn as a conclusion from the fact that *Arabidopsis* cyclin D2/cdc2 and cyclin D3/cdc2 complexes are activated in G1 in response to sucrose[41]. This control is not growth phase-dependent, but is regulated by carbon source availability. The two D-type cyclins react differently to sucrose. While CycD2 responds very quickly in a hexokinase-dependent manner CycD3 is activated more slowly, in a hexokinase-independent manner and also requires cytokinin for activation. Similar to the yeast G1 cyclin CLN3, CycD2 and CycD3 induction in plants is not controlled directly by growth, but is induced by nutrient availability. This suggests that CycD2 and CycD3 form part of a cell cycle control in response to cellular carbohydrate status.

Plants can modulate their growth in response to environmental and developmental conditions. They involve specialized regions, the meristems, where cell division is concentrated and which consists of faster and slower growing cells. When expression of an *Arabidopsis* cyclin D2 was increased ectopically in transgenic tobacco plants, G1 phase was shortened resulting in an increase of the population of fast-cycling cells[42]. This suggested that nutrients may affect cell division by directly regulating the expression of the genes which code for the basic cell cycle machinery. In yeast and animals, increased expression of G1 cyclins has the direct effect of reducing the length of G1, leading to a reduction in cell size or to compensatory increases in the length of other cell-cycle phases. In the cyclin D2 overexpressing tobacco plants, however, neither effect was observed. This suggests that new relationships between cell growth and cell division controls exist in plant meristems.

Genetic dissection of plant meristem function has led to the view that cell division activity is subordinate to overall meristem controls by serving merely to subdivide cellular space[43]. Measurements of meristem size in the cyclin D2-overexpressing tobacco plants did not show an increase in the size or structure of the meristem, but resulted in the more rapid formation of new primordia[44]. This confirms on the one hand, the presence of higher-order regulatory circuits on the size and structure of the meristem, but shows that an increase in the turnover of meristem cell number has decisive effects on the rate of forming new organs at the shoot tip. This latter feature, the plastochron, indeed appears to be determined by the rate of cell division within the meristem.

Growth control in G1 is also affected by mineral salt availability. Tobacco BY2 cells are arrested in the G1 phase of the cell cycle when phosphate is omitted from the medium, while after refeeding with phosphate the cells resume proliferation. Entry into the cell cycle correlated with a rapid and transient activation of a tobacco MAPK related to the alfalfa MMK1, several hours before cells entered S phase[45]. In alfalfa cells, nucleolin was found to be expressed 4 h after re-feeding with phosphate, which is shortly before S-phase[30], while G1 cyclins were expressed 6 h after refeeding[46].

Cell cycle control in G2 and mitosis

Also in plants we learn that a number of checkpoints, before and during mitosis, operate to ensure the reliable transmission of the cell's constituents to the daughter cells. They are, however, much less well-defined and very little is known about the involvement of CDKs in the execution of these checkpoints.

In isolated tobacco pith parenchyma cells and *N. plumbaginifolia* tissue culture cells which are deprived of cytokinin arrest in G2 and have a reduced CDK activity and increased phosphotyrosine content[22]. Transition to mitosis upon cytokinin addition results in tyrosine dephosphorylation and kinase reactivation. The requirement for cytokinin can be completely alleviated by ectopic expression of the fission yeast *cdc25* gene which encodes the phosphatase for Tyr15 dephosphorylation[47]. In support, cells in transgenic tobacco plants expressing the fission yeast *cdc25* gene divided at reduced size[48,49], suggesting that the shorter cell cycle is caused by a shortened G2-phase.

As in animals, there is evidence for the involvement of MAPKs in plant G2 control. The first evidence for a role of MAP kinases in G2/M transition came from experiments in which the pea MAPK PsD5 was isolated from a growing bud library. PsD5 transcripts accumulated in proliferating cells[50], but transcript abundance was not correlated with any cell cycle phase. Its close relative in alfalfa, SIMK however, is expressed in a cell cycle-specific manner with a peak in transcript abundance in G2 (ref. 51). Furthermore, two MAPKKKs, BnMAP4Kalpha1 and 2 are also expressed in a cell cycle phase-specific manner with a peak in G2 (ref. 52).

More solid evidence came from the analysis of the two structurally similar plant MAPKs, the *Medicago* MAP kinase 3 (MMK3) and the *Nicotiana tabacum* FUS3-related kinase (NTF6). The two kinases which belong to the PERK4 class of plant MAP kinases[53] seem to play a role late in mitosis, i.e. in cytokinesis[54,55]. Although the PERK6 proteins are present throughout the cell cycle, their kinase activity is transiently induced during mitosis. Entry into mitosis is required for the

activation of these MAPKs, as seen in relation to the activity of mitosis-related CDK-complexes. Both the alfalfa and tobacco kinases, similar to the animal ERKs, were still inactive in metaphase cells with propyzamide-depolymerized microtubules and became active only after removing the drug as cells passed through anaphase and telophase. Later on, in G1, they were again inactive.

In *Xenopus*, a MAPK was found to control the spindle assembly checkpoint and arrest cells in metaphase until chromosomes are aligned at the cell equator. PERK4 however, is not active in metaphase-arrested cells. These data make it unlikely that PERK4 is involved in a checkpoint controlling microtubule integrity.

MMK3 and NTF6 localization in prophase is reminiscent of that of ERK1 and ERK2. Both ERKs, as well as both PERK4, are found in the cytoplasm in interphase cells and invade the nucleus at the end of G2. Though PERK4 is most active during anaphase and telophase, some activation could already be found at prophase. PERK4 and animal ERKs were found at comparable locations in late mitosis. PERK4 was associated with the phragmoplast in late anaphase and at the midplane of cell division in telophase cells, while active ERK1 and ERK2 were detected at the midzone region in late anaphase and at the midbody during telophase and cytokinesis in animal cells. The fact that both ERKs as well as PERK4 behave similarly throughout mitosis with respect to activity and location suggests that these MAPKs could play similar roles in animal and plant mitosis, respectively.

The tobacco MAPKKK, NPK1, has also been implicated in mitosis[56]. NPK1 protein levels increase as cells progress to mitosis[57]. A search for activators of NPK1 in a yeast screen led to the identification of kinesin-like proteins[57]. The animal ERK1 and ERK2 are also complexed with a microtubule motor protein, CENP-E. The kinesin-like tobacco proteins as well as CENP-E, specifically accumulate in mitosis. The microtubule association and mitosis-specific accumulation suggest that NPK1 might be an upstream activator of PERK4.

A dynamin-like protein, phragmoplastin, has been isolated from soybean and shown to be associated with cell plate formation[58,59]. Phosphorylation of animal dynamin by ERK2 inhibits the dynamin–microtubule interaction[60]. Phragmoplastin was found to appear first in the centre of the forming cell plate and, as the cell plate grew outwards, it redistributed to the growing margins of the cell plate. Contrary to this, PERK4 did not redistribute from the centre to the periphery, indicating that it is not associated with phragmoplastin during this process.

Recently, embryo patterning genes in *Arabidopsis thaliana* such as *knolle*[61] and *keule*[62] have been identified as being involved in cytokinesis, and the syntaxin-related KNOLLE protein was localized at the cell plate[63]. Similarly to PERK4 in late telophase, when the phragmoplast reached the lateral cortex of the cell, KNOLLE appeared to be present across the entire plane of division. Phragmoplast microtubules might be required to bring these proteins to the cell plate, but apparently the microtubules are not required to keep KNOLLE and PERK4 at this location. The link between cytokinesis and pattern formation in the embryo is exciting and warrants more detailed studies on the function of this MAP kinase[64].

Which events of cytokinesis might be regulated by PERK4 is not known at present. These may include the construction of the phragmoplast by regulating microtubule stability or microtubule-based motor proteins, vesicle transport along the phragmoplast by plus-end-directed motor proteins, or the fusion of these vesicles at the cell plate. Similarly, the signal which is transduced by these MAPKs is not yet known.

Plant hormones and cell cycle control

Plant hormones have a global effect on promoting (cytokinin, gibberellic acid, auxin) or inhibiting (ethylene, abscisic acid) cell divisions. Similar to animals, there is now evidence that MAP kinases are involved in the signalling of plant hormones to the basic cell machinery.

Cytokinins were discovered as substances promoting cell divisions in combination with auxin. Their signal transduction is just starting to be understood. Cytokinins were shown to induce the expression of the G1 cyclin, cycD3 in *Arabidopsis*[44]. Based on cytokinin feeding and mutant experiments, it was found that cytokinin modulates CycD3, but not CycD2 gene expression at physiological concentrations in *Arabidopsis* plants (see above). Cytokinins induced CycD3 expression in proliferating tissues of the shoot meristem, young leaf primordia, axillary buds, procambium and vascular tissues of the maturing leaves. Overexpression of CycD3 (via the FLP recombinase to allow regeneration of transgenic shoots in an off situation and CycD3 expression in offspring after induced recombination and activation of the 35S-CycD3 transgene) resulted in cytokinin-independent growth of *Arabidopsis* callus (and suppression of shoot formation). A detailed analysis established that CycD3 gene expression, and thus the likely point of cytokinin requirement, was in the G1-phase of the cell cycle. Similarly the alfalfa cycD3-ortholog is also activated shortly before entering S-phase in cells activated from a quiescent or resting state[47].

These, and other confirmatory results conflicted with the role of cytokinin in G2/M transition. Suspension cultures in a variety of species arrest in G2 or at various cell cycle stages when deprived of cytokinin. A peak in cytokinin levels is required for G2/M transition in tobacco BY-2 cells[65], and as mentioned above, *Nicotiana plumbaginifolia* cells arrest in G2/M after cytokinin removal, containing inactive CDK complexes which can be reactivated *in vitro* by the protein phosphatase cdc25 (ref. 21). The solution to this conflict lies probably in

the use of different suspension cultures. The G1-link in the former cases was found with cell cultures established from quiescent or resting cells, while the G2-link in the latter cases was found with established suspension cultures containing a majority of cycling cells.

Genes identified by the analysis of auxin-signalling mutants, such as aux1, show striking similarities to components of the protein degradation machinery, some of which are involved in G1 control in yeast[66]. AUXR1 and TIR1 are both involved in a common protein degradation pathway[25]. TIR1 is a member of the F-box family of proteins. Studies in yeast and animals indicate that F-box proteins are part of an E3 ubiquitin-ligase complex providing specificity in binding and targeting phosphorylated proteins for degradation. In yeast, the target proteins include regulators of the G1 phase of the cell cycle, such as the CDK inhibitor $p40^{sic1}$ or G1 cyclins[67]. Possible targets for the AXR1 and TIR1-mediated proteolysis might be the transcriptional regulators mediating auxin-induced gene expression, such as the auxin response factor (ARF1) or the AUX/IAA proteins. In this scenario, an auxin-dependent kinase would phosphorylate the transcriptional regulator protein(s), which is possibly a repressor, and target it for degradation.

The involvement of AUX1 in the degradation of a CDK inhibitor could provide a mechanism by which auxin stimulates cell division. Auxin is inducing the expression of Cdc2a, but in itself, it is not enough to stimulate cell division, probably due to missing cyclin partners or the inhibitory phosphorylation of cdc2a[35]. Changes in CDK inhibitor, G1 cyclin and CDK levels could operate the molecular switch of G1 control composed of the Rb and E2F, as has been found in animal cells[28].

MAPKs have also been implicated in auxin signalling. To connect auxin signalling to a MAPK pathway and early auxin gene transcription, components of signalling pathways together with an auxin-regulated promoter construct fused to a reporter gene were transfected into mesophyll leaf protoplast[68]. In these experiments it could be clearly shown that a constitutively active gain-of-function version of a MAPKKK, the tobacco NPK1, activated a MAPK-like protein and specifically inhibited auxin induced-gene expression. An involvement of MAPKs in auxin-regulated growth and cell division has, however, not yet been demonstrated convincingly.

Gibberellins (GAs) are growth-promoting plant hormones and act both on cell division and cell elongation. In deep-water rice, the submerged parts accumulate ethylene which in turn leads to an accumulation of GA and a decrease in abscisic acid levels. Internodal growth is induced by GA and leads first to cell cycle activation at the G1/S control point. An A-type cyclin gene and a *CDC2B*-like CDK gene have been shown to be transcriptionally induced after feeding GA to rice internodes[69], probably representing cell cycle events in G2, after cell cycle activation in G1. Further work will be required to identify the CDK and cyclins involved in cell cycle activation by GA. Abscisic acid, a growth-inhibitory plant hormone induces the expression of a CDK inhibitor, the *Arabidopsis* ICK1 (ref. 20).

On the MAPK side, ABA is able to induce a MAPK-like activity in barley aleurone protoplasts[70]. Besides inducing specific genes, abscisic acid (ABA) is known to inhibit gibberellic acid (GA)-induced effects in aleurone cells, and GA may function in this system in an antagonistic way to ABA, as indicated by the negative effect of GA on transcript accumulation of a MAPK gene in oat aleurone cells[71]. A role of MAPK in ABA- or GA-regulated cell division has not yet been shown.

Cytoskeleton and cell cycle control

Plants are multicellular organisms with cells in fixed positions. Thus, not only the regulation of the timing and the number of divisions, but also the orientation of these divisions and the orientation of cell enlargement are critical to elaborate the plant body shape. These aspects of cell division are regulated by the interchange of five major plant-specific cytoskeletal structures: the cortical array, the radial cytoplasmic array, the preprophase band (PPB) of cortical microtubules, the mitotic spindle and the phragmoplast. How these arrays interchange and how they are positioned are crucial during plant cell divisions and development.

PPB position depends upon a signal from the nucleus because misplacement of the nucleus by centrifugation induces the formation of the PPB at the new location, provided that it happens before or at early stages of PPB formation. CDK was found to co-localize with the PPB in maize root tip cells[72,73]. Microinjection of active CDK into plant cells led to the depolymerization of the preprophase band[74], while the inhibition of CDK activity with the drug roscovitine inhibited this process[34]. These results indicated that CDK might regulate the interchange of cortical microtubules with the mitotic spindle.

After polymerization of microtubules around mitotic chromatin, the randomly oriented microtubule fibres are organized into a bipolar mitotic spindle. Self-organization requires neither centrosomes nor centromeres, but is organized by plus- and minus-end-directed microtubule motors. Recently, a molecular motor similar to the animal Eg5 motor has also been isolated from tobacco and possesses a consensus phosphorylation site for CDKs and MAPKs[75]. Similar to microtubule stability, microtubule movements are also regulated by phosphorylation during mitosis[76]. Eg5 is phosphorylated by CDK1 on a site conserved in yeasts, animals and higher plants, and this phosphorylation is required for the association of Eg5 with the mitotic microtubules.

1. Sundaresan, V. and Colasanti, J., in *Plant Cell Division* (eds Francis, D., Dudits, D. and Inzé, D.), Portland Press, London, 1998, pp. 47–65.
2. Morgan, D. O., *Annu. Rev. Cell. Dev. Biol.*, 1997, **13**, 261–291.
3. Nigg, E. A., *Bioessays*, 1995, **17**, 471–480.
4. Peters, J.-M., *Curr. Opin. Cell Biol.*, 1998, **10**, 759–768.
5. Kültz, D., *J. Mol. Evol.*, 1998, **46**, 571–588.
6. Marshall, C. J., *Curr. Opin. Cell Biol.*, 1996, **8**, 197–204.
7. Treisman, R., *Curr. Opin. Cell Biol.*, 1996, **8**, 205–215.
8. Bottazzi, M. E. and Assoian, R. K., *Trends Cell Biol.*, 1997, **7**, 348–352.
9. Tamemoto, H. *et al.*, *J. Biol. Chem.*, 1992, **267**, 20293–20297.
10. Palmer, A., Gavin, A. C. and Nebrada, R. A., *EMBO J.*, 1998, **17**, 5037–5047.
11. Whitmarsh, A. J. and Davis, R. J., *Nature*, 2000, **403**, 255–256.
12. Howard, A. and Pelc, S. R., *Heredity Suppl.*, 1953, **6**, 261–273.
13. van't Hof, J. and Kovacs, C. J., *Adv. Exp. Med. Biol.*, 1972, **18**, 15–30.
14. van't Hof, in *Cell Cycle Controls* (eds Pallida, G. M., Cameron, I. L. and Zimmerman, A.), Academic Press, New York, 1974, pp. 77–85.
15. Hirt, H. and Heberle-Bors, E., *Sem. Dev. Biol.*, 1994, **5**, 147–154.
16. Sopory, S. K. and Munshi, M., *Crit. Rev. Plant Sci.*, 1998, **17**, 245–318.
17. Mironov, V., Deveylder, L., van Montagu, M. and Inze, D., *Plant Cell*, 1999, **11**, 509–521.
18. Nakayama, K.-I. and Nakayama, K., *Bioessays*, 1998, **20**, 1020–1029.
19. Bögre, L. *et al.*, *Plant Physiol.*, 1997, **113**, 841–852.
20. Wang, H., Fowke, L. C. and Crosby, W. L., *Nature*, 1997, **386**, 451–452.
21. Wang, H., Qi, Q. G., Schorr, P., Cutler, A. J., Crosby, W. L. and Fowke, L. C., *Plant J.*, 1998, **15**, 501–510.
22. Zhang, K., Letham, D. S. and John, P. C. L., *Planta*, 1996, **200**, 2–12.
23. Schuppler, U., He, P.-H., John, P. C. L. and Munns, R., *Plant Physiol.*, 1998, **117**, 667–678.
24. Rouse, D., Mackay, P., Stirnberg. P., Estelle, M. and Leyser, O., *Science*, 1998, **279**, 1371–1373.
25. Del Pozo, J. K. and Estrelle, M., *Trends Plant Sci.*, 1999, **4**, 107–112.
26. Grafi, G., Burnett, R. J., Helentjaris, T., Larkins, B. A., Decaprio, J. A., Sellers, W. R. and Kaelin, W. G., *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 8962–8967.
27. Ach, R. A., Durfee, T., Miller, A. B., Taranto, P., Hanley-Bowdoin, L., Zambryski, P. C. and Gruissem, W., *Mol. Cell Biol.*, 1997, **17**, 5077–5086.
28. Gutierrez, C., *Curr. Opin. Plant Biol.*, 1998, **1**, 492–497.
29. Nakagami, H., Sekine, M., Murakami, H. and Shinmyo, A., *Plant J.*, 1999, **18**, 243–252.
30. Bögre, L. *et al.*, *Plant Cell*, 1996, **8**, 417–428.
31. Renaudin, J. P. *et al.*, *Plant Mol. Biol.*, 1996, **32**, 1003–1018.
32. Umeda, M., Bhalerao, R. P., Schell, J., Uchimiya, H. and Koncz, C., *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 5021–5026.
33. Joubès, J., Chevalier, J., Dudits, D., Heberle-Bors, E., Inzé, D., Umeda, M. and Renaudin, J.-P., *Plant Mol. Biol.*, 2000, in press.
34. Binarova, P., Dolezel, J., Draber, P., Heberle-Bors, E., Strnad, M. and Bögre, L., *Plant J.*, 1998, **16**, 697–707.
35. Hemerly, A. S., Ferreira, P. J. E., van Montagu, M., Engler, G. and Inzé, D., *Plant Cell*, 1993, **5**, 1711–1723.
36. Martinez, M. C., Jorgensen, J. E., Lawton, M. A., Lamb, C. J. and Doerner, P. W., *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 7360–7364.
37. Magyar, Z. *et al.*, *Plant Cell*, 1997, **9**, 223–235.
38. Sauter, M., *Plant J.*, 1997, **11**, 181–190.
39. Lorbiecke, R. and Sauter, M., *Plant Physiol.*, 1999, **119**, 21–29.
40. Jang, J. C. and Sheen, J., *Trends Plant Sci.*, 1997, **2**, 208–214.
41. Riou-Khamlichi, C., Menges, M., Healy, J. M. S. and Murray, J. A. H., *Mol. Cell Biol.*, 2000, **20**, 4513–4521.
42. Cockcroft, C. E., den Boer, B. G. W., Healy, J. M. S. and Murray, J. A. H., *Nature*, 2000, **405**, 575–579.
43. Clark, S. E. and Schiefelbein, J. W., *Trends Cell Biol.*, 1997, **7**, 454–458.
44. Riou-Khamlichi, C., Huntley, R., Jacqmard, A. and Murray, J. A. H., *Science*, 1999, **283**, 1541–1544.
45. Wilson, C., Pfosser, M., Jonak, C., Hirt, H., Heberle-Bors, E. and Vicente, O., *Physiol. Plant.*, 1998, **102**, 532–538.
46. Dahl, M., Meskiene, I., Bögre, L., Ha, D. T. C., Swoboda, I., Hubmann, R., Hirt, H. and Heberle-Bors, E., *Plant Cell*, 1995, **7**, 1847–1857.
47. John, P. C. L., *J. Exp. Bot.*, 1998, **49** (Suppl), 91.
48. Bell, M. H., Halford, N. G., Ormrod, J. C. and Francis, D., *Plant Mol. Biol.*, 1993, **23**, 445–451.
49. McKibbin, R. S., Halford, N. G. and Francis, D., *Plant Mol. Biol.*, 1998, **36**, 601–612.
50. Stafstrom, J. P., Altschuler, M. and Anderson, D. H., *Plant Mol. Biol.*, 1993, **22**, 83–90.
51. Jonak, C., Páy, A., Bögre, L., Hirt, H. and Heberle-Bors, E., *Plant J.*, 1993, **3**, 611–617.
52. Leprince, A.-S., Jouannic, S., Hamal, A., Kreis, M. and Henry, Y., *Biochem. Biophys. Acta*, 1999, **1444**, 1–13.
53. Ligterink, W., in *MAP Kinases in Plant Signal Transduction* (ed. Hirt, H.), Springer-Verlag, 2000, pp. 11–28.
54. Calderini, O., Bögre, L., Vicente, O., Binarova, P., Heberle-Bors, E. and Wilson, C., *J. Cell Sci.*, 1998, **111**, 3091–3100.
55. Bögre, L. *et al.*, *Plant Cell*, 1999, **11**, 101–113.
56. Banno, H., Hirano, K., Nakamura, T., Irie, K., Nomoto, S., Matsumoto, K. and Machida, Y., *Mol. Cell Biol.*, 1993, **13**, 4745–4752.
57. Machida, Y., Nakashima, M., Morikiyo, K., Banno, H., Ishikawa, M., Soyano, T. and Nishihama, R., *J. Plant Res.*, 1998, **111**, 243–246.
58. Gu, X. and Verma, D. P. S., *EMBO J.*, 1996, **15**, 695–704.
59. Gu, X. and Verma, D. P. S., *Plant Cell*, 1997, **9**, 157–169.
60. Earnest, S., Khokhlatchev, A., Albanesi, J. P. and Barylko, B., *FEBS Lett.*, 1996, **396**, 62–66.
61. Lukowitz, W., Mayer, U. and Jürgens, G., *Cell*, 1996, **84**, 61–71.
62. Assaad, F. F., Mayer, U., Wanner, G. and Jürgens, G., *Mol. Gen. Genet.*, 1996, **253**, 267–277.
63. Lauber, M. H. *et al.*, *J. Cell Biol.*, 1997, **139**, 1485–1493.
64. Heese, M., Mayer, U. and Jürgens, G., *Curr. Opin. Plant Biol.*, 1998, **1**, 486–491.
65. Redig, P., Shaul, O., Inzé, D., van Montagu, M. and van Onckelen, H., *FEBS Lett.*, 1996, **391**, 175.
66. Walker, L. and Estelle, M., *Curr. Opin. Plant Biol.*, 1998, **1**, 434–439.
67. Patton, E. E., Willems, A. R. and Tyers, M., *Trends Genet.*, 1998, **14**, 236–243.
68. Kovtun, Y., Chiu, W.-L., Zeng, W. and Sheen, J., *Nature*, 1998, **395**, 716–720.
69. Fabian, T., Lorbiecke, R., Umeda, M. and Sauter, M., *Planta*, 2000, **211**, 211–376.
70. Knetsch, M. L. W., Wang, M., Snaar-Jagalska, B. E. and Heimovaara-Dijkstra, S., *Plant Cell*, 1996, **8**, 1061–1067.
71. Huttley, A. K. and Phillips, A. L., *Plant Mol. Biol.*, 1995, **27**, 1043–1052.
72. Colasanti, J., Cho, S. O., Wick, S. and Sundaresan, V., *Plant Cell*, 1993, **5**, 1101–1111.
73. Mews, M., Sek, F. J., Moore, R., Volkmann, D., Gunning, B. E. S. and John, P. C. L., *Protoplasma*, 1997, **200**, 128–145.
74. Hush, J., Wu, L., John, P. C., Hepler, L. H. and Hepler, P. K., *Cell Biol. Int.*, 1996, **20**, 275–287.
75. Asada, T. and Collings, D., *Trends Plant Sci.*, 1997, **2**, 29–37.
76. Verde, F., Berrez, J.-M., Antony, C. and Karsenti, E., *J. Cell Biol.*, 1991, **112**, 1177–1187.