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Cyclin/Cdk complexes: their involvement in cell cycle progression and mitotic division

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Dedicated to Professor Brian E. S. Gunning on the occasion of his 65th birthday

Summary. DNA replication and mitosis are dependent on the activity of cyclin-dependent protein kinase (CDK) enzymes, which are heterodimers of a catalytic subunit with a cyclin subunit. Cyclin binding to specific individual proteins is thought to provide potential substrates to Cdk. Protein binding by cyclins is assessed in terms of its mechanisms and biological significance, using evidence from diverse organisms including substrate specificity in animal Cdk enzymes containing D-, A-, and B-type cyclins and extensive cyclin gene manipulations in yeasts. Assembly of protein complexes with cyclin/Cdk is noted and the capacity of the cyclin-dependent kinase subunit Cks, in such complex, to extend the range of Cdk substrates is documented and discussed in terms of cell cycle regulation. Cell cycle progression involves changing abundance of individual cyclins, due to changing rates of their transcription or proteolysis, with consequent changes in the substrates of CDK through the cell cycle. Some overlap of the functions of individual cyclins in vivo has been identified by cyclin deletions and is suggested to follow a pattern in which cyclins can commonly complete functions initiated by the preceding cyclins well enough to preserve viability as groups of cyclins are removed by proteolysis. Cyclin accumulation is particularly important in terminating the G_1 phase, when it raises CDK activity and starts events leading to DNA replication. It is suggested that plants share this mechanism. The distribution of cyclins and Cdk in maize root tip cells during mitosis and cytokinesis indicates the presence of Cdk1 (Cdc2a) and cyclin CycB1zm;2 at the mature and disassembling preprophase band and the presence of CycB1zm;2 at condensing and condensed chromosomes. Both observations correlate with the earlier-reported capacity of injected metaphase cyclin/CDK to accelerate preprophase band disassembly and chromosome condensation and with observations of the location of Cdk and cyclins in other laboratories. Additionally CycB1zm;2 is seen at the nuclear envelope during its breakdown, which correlates with an acceleration of the process by injected metaphase cyclin B/CDK. A phenomenon possibly unique to the plant kingdom is the persistence of mitotic cyclins after anaphase. Participation of cyclins in cytokinesis is indicated by the concentration of the mitotic cyclin CycA1;zm;1 at the phragmoplast. It is suggested that cyclins have a general function of spatially focusing Cdk activity and that in the plant cell the concentrations of cyclins are important mediators of CDK activity at the cytoskeleton, chromosomes, spindle, nuclear envelope, and phragmoplast.

Keywords: Cyclin; Preprophase band; Cyclin-dependent protein kinase; Cytoplasmic retention signal; Maize; Phragmoplast.

1 Introduction

Common mechanisms for the control of the cell division cycle in plants and other eukaryotes were indicated by the detection in the plant kingdom of the cell division cycle (cdc) catalyst Cdc2 and its correlation with division activity (John et al. 1989, 1990). This protein kinase enzyme had previously been shown to operate at the core of the cell cycle, being necessary for both S phase and M phase in the fission yeast *Schizosaccharomyces pombe* (Nurse and Bissett 1981) and to be conserved in the evolutionarily remote budding yeast *Saccharomyces cerevisiae* (Beach et al. 1982) and in metazoa (for review, see Nurse 1990). The functional homology of the plant *cdc2* gene was unequivocally confirmed by its capacity, when cloned from *Arabidopsis thaliana*, alfalfa, and maize, to complement CDK1 mutants in yeasts (Ferreira et al. 1991, Hirt et al. 1991, Colasanti et al. 1991).

The hypothesis is widely held that cyclins direct CDK enzyme activities to particular protein substrates by affinity of the cyclin for particular proteins. To the extent that different cyclins bind different potential

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substrates, the pairing of a CDK with different cyclins allows a wide range of substrates to be recognised, phosphorylated, and thus regulated by a single CDK. The binding of different cyclins by one CDK is most strikingly evident in the unicellular yeasts, where only a single CDK is directly involved in the cell cycle and is encoded by *cdc2*$^+$ in fission yeast and by *CDC28* in budding yeast; these homologous genes are both here termed CDK1. Yeast Cdk1 can bind with four cyclins of the mitotic cell cycle in fission yeast, and with nine cyclins in budding yeast. Yeast Cdk1 thus is able to react with substrates that are involved in the biosynthetic events of gene transcription and DNA replication, then later, when in complex with different cyclins, Cdk1 regulates proteins that are involved in the structural changes of mitosis and cytokinesis.

Until quite recently it was wisely cautioned that the role of cyclins, as determinants of Cdk substrates and hence function, had not often been demonstrated (e.g., Nasmyth 1996). More recent evidence has underlined the importance of cyclins and we shall show first that selection of Cdk substrates by cyclin has been demonstrated in vitro and is dependent upon precisely defined regions in cyclin and substrate that can be termed respectively binding and docking regions. Interaction of these regions in D-type and A-type cyclins control commitment and execution of DNA replication. We next present in vivo evidence for the importance of individual cyclins by considering cyclin deletions and substitutions in yeast. These reveal specific contributions by cyclins to DNA replication, mitotic progress, cytoskeleton deployment and cell development. Finally we consider cyclin participation in the uniquely complex processes of plant mitosis and suggest that spatial concentrations of cyclins at sites of mitotic events are important in focusing CDK activity on cytoskeleton, chromosomes, spindle, nuclear envelope, and phragmoplast.

2 Cyclins: essential for Cdk activity but not conferring absolute specificity

Cyclins and Cdks must combine to perform their biological function. CDK enzymes consistently contain a region important for attachment of cyclins, containing the sequence EGVPSTAIRESLLKE (single-letter amino acid code) or variants of it. This region is often termed the PSTAIR helix and is adjacent to the catalytic loop (Nigg 1995, Morgan 1995). In cyclins the structural region for binding to CDK is equally strongly conserved. It contains 4–5 alpha helices, together referred to as a cyclin box that comprises about 250 amino acids and contributes about half of the cyclin molecule (Jeffrey et al. 1995, Bazan 1996, Kim and Cho 1997). The cyclin box is universal since it is present in the CLN yeast cyclins that are the most structurally remote from other cyclins (Huang et al. 1997).

Cyclins have a dual effect on CDK, one being activation of catalytic potential and the other concerned with holding substrate in proximity. Activation of the catalytic subunit occurs by two means; firstly the induction of a structural shift in the CDK due to binding cyclin, which aligns the PSTAIR helix, catalytic loop and β regions; and second an activating phosphorylation at Thr-161 catalysed by a separate specific kinase (Russo et al. 1996). Monomeric Cdk that is phosphorylated at Thr-161 has a low catalytic activity, but this is increased manyfold if cyclin is bound (N. Brown et al. 1999) and additionally cyclins hold potential substrates accessible to the CDK (Schulman et al. 1998), as will be assessed in more detail later. Therefore the cyclins potentially determine the substrates that are phosphorylated and hence the mitotic-division events that can be driven by Cdk activity.

The role of cyclins must be evaluated with care because of some indications that their specificity for performing particular cell cycle events is limited. Cell division can continue after deletion of any two of the three cyclins known to be present during the G_1 phase (the CLN cyclins) of budding yeast (Richardson et al. 1989, Hadwiger et al. 1989, Cross and Blake 1993), which argues against the existence of specific functions in an individual Cln protein. Furthermore when all three Cln are eliminated they can be substituted by constitutive expression of any of the known classes of mammalian cyclin, including mitotic cyclins that do not function in G_1 phase (Lew et al. 1991, Sherr 1993) and including plant mitotic cyclins (Day et al. 1996). Similarly in fission yeast the cell cycle can be traversed (albeit with abnormal timing) when all other known cyclins are eliminated except for the mitotic B-type cyclin named Cdc13 (Fisher and Nurse 1996). Thus specificity of cyclin function is not always so absolute as to be able to block the cell cycle if a particular cyclin is absent, although a block due to loss of a single cyclin can occur, as in fission yeast lacking Cdc13. Neither is absolute cyclin specificity necessary to confer some catalytic activity since cyclin can support Cdk activity

while truncated by up to 160 amino acids at the N terminus (Kobayashi et al. 1992).

3 Cyclin succession through the cell cycle

Cyclins are successively replaced by other cyclins during progression through the cell cycle (Evans et al. 1983). The most significant change in cyclins occurs at the time of commitment to DNA replication in late G_1 phase. Prior to this event only G_1 cyclins are present and after commitment these are replaced by mitotic cyclins. G_1 cyclins are distinguished by their continuing protein turnover and by their ability to commit the cell to DNA replication while not themselves being able to sustain replication. After commitment to DNA replication there is accumulation of mitotic cyclins that can drive DNA replication and mitosis (Nasmyth 1996).

Major factors in cyclin replacement at late G_1 phase are repression of G_1 cyclin transcription, initiation of mitotic-cyclin transcription and the cessation of proteolysis of the mitotic cyclins (Amon et al. 1994). It has now emerged that all organisms have G_1 cyclins, which in budding yeast are the CLN cyclins (Richardson et al. 1989); in fission yeast, cyclin Puc1 (Martín-Castellanos et al. 2000); and in multicellular plants and animals, D-type cyclins (Sherr 1993). Commitment to DNA replication occurs when G_1 cyclin/Cdk activity reaches a high enough level to activate transcription of the mitotic cyclins that are able to support DNA replication and mitosis. The shift to mitotic cyclins is enforced by cessation of their rapid ubiquitin-dependent proteolysis, which diminishes in late-G_1 phase and recommences at anaphase (Glotzer et al. 1991, Amon et al. 1994). Proteolysis is the key determinant of mitotic-cyclin presence since, even when transcribed from constitutive promoters, mitotic-cyclin proteins can only accumulate after the late-G_1 commitment to DNA replication when their proteolysis ceases (Amon et al. 1994).

The structural basis of differential proteolysis of cyclins is their possession of different recognition signals for ubiquitinating enzymes that are active at different times in the cell cycle and mark proteins for proteolysis by covalent attachment of multiple ubiquitin molecules (for reviews, see Deshaies 1995, Hoyt 1997). In mitotic cyclins, near their N terminus, is a sequence RTxLGxIG (x being variable residue) referred to as the "destruction box" by which they are recognised for ubiquitination and proteolysis (Glotzer et al. 1991, King et al. 1995) continuing from late mitosis through G_1 phase (Amon et al. 1994). In the animal B1 cyclins the box is perfectly conserved as RTALGDIGN and a similar sequence occurs in plant B1-type mitotic cyclins as RxxLxDIGN (Renaudin et al. 1998). Whereas G_1 cyclins have towards their C terminus an alternative ubiquitinating enzyme recognition signal that is rich in Pro, Glu, Ser, and Thr residues, hence termed a PEST region (Rogers et al. 1986), which is also found in many other unstable proteins. G_1 cyclins are subject to continuous proteolysis in metazoa and yeasts (Matsushime et al. 1991, Cross and Blake 1993; reviewed by Sherr 1993). Plant G_1 cyclins (cyclins D) also lack the destruction box and have a PEST region (Soni et al. 1995). After commitment, rising activity of mitotic cyclin/Cdk terminates transcription of G_1 cyclins that are under cell cycle control (Amon et al. 1993) and prevents reinitiation of DNA synthesis until mitosis has been completed (Amon et al. 1994; reviewed by Stern and Nurse 1996, Stillman 1996).

The mitotic cyclins include some that are involved in DNA replication, others that accumulate later and are involved in nuclear division, and some, like animal cyclin A, that are involved in both events. The S-phase cyclins in budding yeast are Clb5 and Clb6 then Clb3 and Clb4, in fission yeast Cig1 and Cig2, and in animal cells cyclin E then cyclin A. In plants, cyclins with some sequence similarities to animal cyclin A can be recognised (as discussed in Sect. 12) and some, like animal cyclin A, begin to be expressed in S phase (Setiady et al. 1995, Reichheld et al. 1996), but their function remains to be tested. The participation of some plant cyclins that are classified as A-type in events other than DNA replication is likely since some are expressed (Reichheld et al. 1996) or persist long after S phase (Sect. 11). Cyclins that are involved in nuclear division in budding yeast are Clb1 Clb2 and Clb3, in fission yeast Cdc13, and in metazoa cyclin A and cyclin B. In plants B1-type cyclins appear to follow this pattern since those that have been studied have predominantly mitotic expression, and some have locations at sites of mitotic events, as will be described and illustrated in Sect. 11.

Although the tasks performed by individual cyclins may be different in plants, it is reasonable to consider the core events of the eukaryote cell cycle and ask whether plant cyclins are expressed at times that are appropriate for performing these tasks. Different times of cyclin gene expression have been detected by

selecting nuclei from cells in specific phases of the cell cycle, which has shown Arabidopsis CycB1;at;1 transcripts to be more abundant in G_2 nuclei (Hemerly et al. 1992). Further indications have come from tissue probing in situ, which has shown in *Antirrhinum majus* that *CycB1;am;1* and *CycB1;am;1* cyclin genes are not expressed until S phase is finished (Fobert et al. 1994).

More precise temporal information has been obtained from synchronous cultures. During G_1 phase, expression of cyclin *cycD3;ms* in cultured alfalfa cells is consistent with function of this cyclin as a growth factor-signalling molecule (Dahl et al. 1995). In the S phase of synchronous tobacco cells, the expression of *cycA3;nt;1* and *cycA3;nt;2* is coincident with histone expression (Reichheld et al. 1996) and therefore suggestive of a cyclin involved in DNA replication, as will be discussed in Sect. 7 in connection with commitment to DNA replication. In G_2 and M phases both synchronous tobacco and Arabidopsis cells have distinct periods of preferential abundance for transcripts of individual cyclins. In the readily synchronised *Nicotiana tabacum* BY2 cells, cyclin *cycB1;nt;1* was expressed in G_2 and early-M phase (Qin et al. 1996) and precise differential timing of expression in these phases was revealed in this cell line by introduction of Arabidopsis cyclin promoters for the *CycA2;at;1* and *CycB1;at;1* genes. The former was consistently expressed 1 h earlier than the latter in the 5 h period between S phase and mid mitosis (Shaul et al. 1996). This differential timing depends upon repeats of a 9-base-pair motif T/C C T/C AACGG T/C T/C A (slash indicates alternative). This mitosis-specific activator (MSA) motif is found in the promoter regions of tobacco and other B-type cyclins, including soybean *cycB1;gm;3* and Arabidopsis *cycB1;at;2* and *cycB2;at;1* (Ito et al. 1998). The MSA motif is sufficient to confer M-phase expression to a reporter gene and is absent from plant A-type cyclins. It may confer expression on a number of genes during mitosis and interestingly has been found in the promoters of plant kinesin-like proteins that are expected to contribute to intracellular motility of such structures as microtubules, chromosomes, and vacuoles during mitosis.

The foregoing compendium is derived from observations on several cell types, but the pattern of cyclin expression nevertheless may well occur in individual cells. Thus cells of *N. tabacum* can express in succession D-type, A-type, and then B-type cyclins (Sorrell et al. 1999, Reichheld et al. 1996, Qin et al. 1996). While transcription times need not indicate the times when a cyclin protein is abundant, there is some evidence that plant cyclin proteins are indeed under temporal control. For example, levels of B1 cyclin protein of maize (CycB1;zm;2) change sharply with mitotic phase in the manner of animal cyclin B1 (King et al. 1995), accumulating only as cells approach mitosis and then being proteolysed in mid mitosis (Fig. 1) (Mews et al. 1997).

It would be an oversimplification, however, to suggest that plant cyclin accumulation merely resembles that in metazoa since additional patterns are emerging in plants. To give just three examples, plant D-type cyclins include some that are expressed late in mitosis (Sorrell et al. 1999), some plant A-type cyclins are also expressed in mitosis or continuously (Reichheld et al. 1996, Meskeine et al. 1995), and conversely the B2 cyclin CycB2a-zm is expressed at the G_0-to-G_1 phase transition and accumulates in the nucleus when division is reactivated (Mews et al. 1996). The diversity of plant cyclins is discussed further in Sect. 5.

4 Multiple CDKs: mitosis-specific CDKs in plants

The range of CDK/cyclin complexes is extended in multicellular plants and animals by the presence of more than one CDK. In metazoa a succession of different Cdk proteins forms complexes with cyclins, including in G_1 phase, cyclin D/Cdk4; in S phase, cyclin E/Cdk2 and cyclin A/Cdk2; then in mitosis cyclin A/Cdk1 and cyclin B/Cdk1 (reviewed by Sherr 1993, 1996). The presence of multiple Cdks allows regulation by inhibition or activation that is specific to a particular Cdk or group of Cdks. For example the inhibitory protein $p27^{Kip1}$ preferentially affects Cdk4 and Cdk6 in mammals and its effect on them influences the major progression from G_1 phase to DNA replication (Resnitzky et al. 1995). A further example is the ability to use at two points in the cell cycle the control of Cdk activity by inhibitory phosphorylation of tyrosine at position 15 in the Cdk. This inhibition is supplemented by a small additional inhibition if threonine14 is also phosphorylated. In cells with a single Cdk (Cdk1), inhibitory phosphorylation regulates only the transition from G_2 phase to mitosis (Nurse 1990, Millar et al. 1991) and provides a means to restrain mitosis if DNA replication is incomplete (Nurse 1997). In metazoa this Cdk phosphorylation also controls S phase, but the dual occurrence does not lead to catastrophic simultaneous activation of S phase and mitosis because there are separate Cdks dedicated to

S phase, especially cyclin D/Cdk4, cyclin D/Cdk6 and cyclin A/Cdk2. These Cdks are substrates of the tyrosine/threonine phosphatase Cdc25A that can activate them but not Cdk1 (Hoffmann et al. 1994, Iavorone and Massaque 1997). DNA replication can therefore be sustained by Cdc25A or delayed by removal of Cdc25A, thus providing a mechanism for a checkpoint control that allows repair of any damage to the DNA template before it is copied (Mailand et al. 2000). Later, mitosis can be separately initiated, providing DNA replication has been completed (Lopez-Girona et al. 1999), by the different enzyme Cdc25C acting on the different Cdk in cyclin B/Cdk1.

Plant Cdks contain Tyr-15 in the same ATP-binding lobe of the enzyme as in CDKs from other taxa (for a review, see Dudits et al. 1998), but it is not yet established for most plant Cdks when in the cell cycle they may be regulated by phosphorylation. Clearly, regulation of some plant Cdks in S phase and of others in G_2 phase is possible by analogy with metazoa.

Assembly of the different cyclin-CDK complexes in metazoa, and presumably in plants, is facilitated by CDKs having different affinities for cyclins. The structural differences that underlie binding of different cyclins are often summarised by reference to modified sequence in the PSTAIR helix region that forms the major part of the cyclin docking site on CDK. In *A. thaliana* two CDKs have been characterised as Cdc2aAt and Cdc2bAt and their different structures can be summarised as PSTAIR and PTALRE (a fuller presentation of plant CDK structures is given by Dudits et al. 1998). The Cdc2aAt is active, like CDK1 in yeasts and animal cells, through S and M phases, while Cdc2b is active only in G_2 and M phases (Segers 1996; reviewed by Mironov et al. 1999). The functional homology of Cdc2aAt with CDK1 is underlined by the capacity of the plant gene, like other homologues from alfalfa and maize, to complement CDK1 mutants in yeasts (Ferriera et al. 1991, Hirt et al. 1991, Colasanti et al. 1991).

It is not general for CDKs additional to Cdk1 to be active in mitosis, since in mammalian cells Cdk4 and Cdk6 are active predominantly in G_1 phase and Cdk2 is active in S phase, but only Cdk1 is active in M phase (Sherr 1996). In plants Cdks expressed later in the cell cycle are common. In *Antirrhinum majus* there are two PSTAIR CDKs, Cdc2a and Cdc2b, that are expressed throughout the cell cycle and additionally Cdc2c (containing PPTALRE) and Cdc2d (containing PPTTLRE) that are expressed respectively through S, G_2, and M, or through G_2 and M phases (Fobert et al. 1994, Doonan and Fobert 1997). Furthermore alfalfa contains at least 6 CDKs, among which cdc2MsA, cdc2MsB, cdc2MsC, and cdc2MsE are expressed throughout the cell cycle, while cdc2MsD and cdc2MsF are expressed during G_2-to-M phases (Magyar et al. 1993, 1997, see also Huntley and Murray 1999). In plants that are known to have numerous CDKs it is not yet known when each one is enzymically active. However, it is known that their protein levels do not always determine their activities, thus measurement of Cdc2a activity, made in specific immunoprecipitates from *A. thaliana* and tobacco, shows greater activity during mitosis, although Cdc2a protein is relatively constant in amount through the cell cycle (Zhang et al. 1996, Mironov et al. 1999). Means by which CDK enzyme activity can be regulated by changing phosphorylation has been described, and regulation by cyclin availability and level of cyclin-dependent kinase inhibitor proteins will be described (Sect. 7).

The foregoing comparison shows that one special feature of plants is the common presence of CDKs that are preferentially expressed during mitosis. In all plant species in which two or more CDKs have been characterised, at least one Cdk has been found to be preferentially expressed during mitosis. Whereas in metazoa CDKs additional to CDK1 are expressed earlier, in G_1 and S phases, and Cdk1 alone is the significant Cdk during nuclear division. Consistent with this difference, plants express a greater variety of mitotic cyclins during nuclear division.

5 Diversity of plant mitotic cyclins

Individual plant cyclins have structural features similar to both A-type and B-type cyclins of animals and yeasts, and are therefore difficult to classify by their structure (Hata et al. 1991). The diversity of plant cyclins is at least as great as that of metazoa, and at least five major groupings, comprising cyclins A1, A2, and A3 with cyclins B1 and B2, have been recognised (Kouchi et al. 1995, Renaudin et al. 1998). Furthermore, within the A-type cyclins, Reichheld et al. (1996) have classified their isolates of tobacco cyclin A in three distinct groups additional to two groups isolated by Setiady et al. (1995), indicating possibly five A-type cyclins in tobacco. The presence of multiple A-type cyclins markedly distinguishes plant from animal cells, where there is only a single cyclin A (Sherr 1996). As

well as being diverse, plant A-type cyclins have different times of transcription (Reichheld et al. 1996); however, the full meaning of plant cyclin diversity will only become apparent as we gather information on their function. We shall later return briefly to the functional grouping of plant cyclins after presenting some information on locations of cyclin proteins. We suggest that the diversity of plant cyclins correlates with complexity of plant mitosis and cytokinesis, which requires progression through four separate microtubular arrays, rather than maintenance of the single centriole-organised array of animal cells (reviewed by Gunning 1982, Wick 1991).

6 Cyclin binding functions

Cyclin/CDK substrate specificity in vitro

In their patterns of changing abundance during the cell cycle, cyclins can bring a temporal order to cell cycle control. Cyclins could also bring spatial order by directing Cdk to particular compartments of the cell and cdk activity to specific substrates. The extent to which cyclins do specifically bind to potential substrates is a key question for evaluating cyclin function. Significant information is available for cyclins A, B, and D.

Discrimination between potential substrates has provided information about the specificities of cyclins A and B when in complexes with Cdks. Human cyclin A has been compared with cyclin B for the ability to direct phosphorylation to the "pocket protein" p107 when both cyclins are in complex with either human Cdk1 or Cdk2. Only cyclin A targeted p107 and either Cdk1 or Cdk2 was effective as partner (Peeper et al. 1993). Cdk activity depended upon cyclin binding to substrate since cyclin A/Cdk bound stably to p109, while Cyclin B/Cdk complexes did not bind. Similarly, cyclin A- but not cyclin B/CDK phosphorylated the related pocket protein p107 (Pan et al. 1993). Further selectivity is indicated by the ability of both cyclin A/Cdk2 and cyclin E/Cdk2 to phosphorylate another pocket protein, Rb, while only cyclin A/Cdk2 could phosphorylate the mammalian S phase transcription factor E2F (Dynlacht et al. 1994). Thus cyclin A has reproducible protein binding specificity in vitro.

The differential specificity of cyclins A and B is biologically significant since they catalyse the core sequence of events through S phase to mitosis. In essence, cyclin A targeting of CDK activity to the pocket proteins (Rb, p107, and p109) releases transcription factors that are otherwise held inactive in the "pocket" and allows transcription of genes required for DNA synthesis (described in Sect. 7). In animal cells the pocket protein Rb is initially phosphorylated by cyclin D/Cdk4 and this phosphorylation is then extended to further sites as S phase is imminent by activity of cyclin E/Cdk2 (Sherr 1993, 1996). As S phase progresses, the significance of changing cyclin associations with a single Cdk is seen as cyclin A replaces cyclin E in complex with Cdk2. The cyclin A/Cdk2 complex (Pines and Hunter 1991, 1994) has many key roles in DNA synthesis, which include phosphorylation of Rb and the transcription factor E2F that is released from Rb (Dynlacht et al. 1994). In turn the latter transcribes the key protein Cdc6, which initiates DNA synthesis in eukaryotes (Yan et al. 1998). Additionally, cyclin A in complex with p107 activates the thymidine kinase promoter in humans (Li et al. 1993) and at the origin-of-replication complex (ORC) cyclin A/Cdk1 is the sole cyclin/cdk pairing (Romanowski et al. 2000).

The changing pairings of cyclin and Cdk are again seen during progression through mammalian nuclear division since cyclin A enters an enzymically active pairing with Cdk1 (instead of the Cdk2 that it partners in S phase) and makes a significant contribution to early mitotic events (Pagano et al. 1992). Then Cdk1 remains the enzymically active Cdk, but as cyclin A declines through prophase cyclin B rises and becomes the predominant partner of Cdk1. Cyclin B/Cdk1 has high enzyme activity and CDK protein kinase activity peaks during mitosis in yeast (Stern and Nurse 1996), metazoan (Draetta and Beach 1988), and plant cells (Magyar et al. 1993, Zhang et al. 1996; reviewed by Mironov et al. 1999). Mitotic-cyclin B/Cdk1 activity is presumably targeted towards proteins that are involved in nuclear division. Few of these substrates are known, but they do include lamin proteins of the nuclear envelope which depolymerise when phosphorylated and contribute to the breakdown of the nuclear envelope (Enoch et al. 1991). Other substrates of cyclin B/Cdk1 are expected to include proteins that control chromosomes and spindle.

Cyclin-protein interaction: binding and docking sites

The mechanism by which cyclins select substrates of CDKs has been investigated for cyclins D and A and operates via specific binding regions in the cyclins and

docking regions in the substrates. Thus binding of cyclin D/Cdk4 to pocket proteins such as Rb which induces S phase, depends upon the sequence LxCxE (x being a nonspecific amino acid) at the amino terminus of the cyclin D. This site docks with the pocket region of Rb (Xiong et al. 1992, Dowdy et al. 1993). The importance of the LxCxE region and its relevance to plants is underlined by the presence of this motif in plant cyclin D (Soni et al. 1995) and the presence in plants of Rb protein (J. Murray et al. 1998, Huntley et al. 1998) and E2F-like transcription factors (Mariconti et al. 2000).

Cyclin A binding to pocket proteins depends upon the cyclin sequence MRAIL, which forms a hydrophobic patch at the N-proximal border of the cyclin box (Kobayashi et al. 1992, Jeffrey et al. 1995, Schulman et al. 1998). Additional factors must also operate since MRAIL is also present in cyclins B, D, and E, although (as noted above) cyclin B does not bind to Rb-like proteins. The MRAIL region in cyclin A is seen in X-ray crystallography to contact proteins that bind to cyclin A (Adams et al. 1996), and mutations that substitute less-hydrophobic residues prevent specific recognition of substrates in vitro. Furthermore the function of the MRAIL region in cyclin A is biologically significant since an accelerated entry to S phase, which can be induced by overexpression of cyclin A, depends upon an intact MRAIL region in the cyclin (Schulman et al. 1998). The docking region on substrates to which MRAIL binds is often RxL (e.g., Vlach et al. 1997, Schulman et al. 1998) and transfer of this region to other proteins can make them substrates of cyclin A/Cdk2 (Dynlacht et al. 1994). In Rb the docking region is KxL (Adams et al. 1996, 1999) and this motif is elaborated in the animal cell cycle control protein p53 to KKLMF (Luciani et al. 2000). The molecular basis of cyclin substrate specificity is therefore the precise interaction of binding and docking regions.

The manner in which cyclin presents substrates to the Cdk has also been tested. Flexible presentation of substrate was suspected when crystallography of the CDK/cyclin A complex (e.g., Jeffrey et al. 1995, Russo et al. 1996) showed that the MRAIL region is 3.5 nm distant from the active site in CDK, suggesting that the substrate may not be very precisely located (Schulman et al. 1998). Furthermore, numerous sites can be phosphorylated in a substrate, being recognised by their consensus sequence K/R *S*/*T* P x K/R (slash indicates alternative; italics indicate phosphorylation site) and adjacent basic residues (Nigg 1991, 1995), although these phosphorylation sites are at different distances from the docking region. Similarly, in peptides that are too small to be held by cyclin there is also accurate phosphorylation (Songyang et al. 1994). These considerations suggest that CDKs select regions for phosphorylation within the substrate and require only flexible presentation of substrate by cyclin.

An ingenious test by Schulman et al. (1998) used the binding affinity of the LxCxE sequence for pocket protein p107. LxCxE was introduced into cyclin A in which the MRAIL site was disabled and, although introduced at a point distant from MRAIL, activity was restored in phosphorylating p107 at all usual residues in spite of altered distances from the CDK active site. This restoration indicates that CDK does not require precise presentation of substrate relative to the active site, and rather cyclins can function as a scaffold on which the Cdk can operate (Schulman et al. 1998), deriving a high concentration of substrate tethered close to the Cdk by cyclin. This flexible mechanism depends upon the specificity of reaction between cyclin and substrate and implies that cyclins will have specific functions. The interesting possibility is raised that cyclins can bring together selected proteins and CDK in specific subcellular locations. The distribution of plant mitotic cyclins, which will be described and illustrated in Sect. 11, is consistent with cyclins focusing Cdk activity in this way.

7 G_1 cyclins and commitment to G_1-to-S-phase progression: a universal mechanism

Recent evidence indicates that cyclin accumulation operates a common mechanism controlling progression from G_1 to S phase in yeasts, in metazoa, and probably in plants. The mechanism is dependent on the proteolysis of the cyclin-dependent kinase inhibitor (CKI). This protein inhibits G_1-cyclin/CDK activity prior to completion of G_1 phase. Universality of the mechanism that terminates G_1 phase could not be claimed while the fission yeast seemed not to have a G_1 cyclin equivalent to the CLN cyclins of budding yeast and cyclin D of animals and plants. However the cyclin Puc1 of fission yeast (Forsburg and Nurse 1994) has been found to share not only sequence similarity with Cln3 of budding yeast but also a function essential for completing a G_1 phase of normal duration (Martín-Castellanos et al. 2000).

It can now be seen that Puc1/Cdk1, like Cln1,2/Cdk1 and cyclin D/Cdk4, can phosphorylate and thus mark

for proteolysis (Hoyt 1997) a CKI protein that is able to bind and inhibit mitotic-cyclin/CDK complexes. Hence the cell division sequence of S to G_2 to M phases can only be started after G_1-cyclin/Cdk activity rises in late-G_1 phase and eliminates CKI. The CKI removed by phosphorylation and proteolysis in late-G_1 phase in fission yeast is Rum1 (Moreno and Nurse 1994, Martín-Castellanos et al. 2000), in budding yeast it is Sic1 (Schwob et al. 1994, Schneider et al. 1996, Tyers 1996) and in metazoa it is $p27^{Kip1}$ (Pagano et al. 1995, Sheaff et al. 1997). The Puc1/Cdk1 enzyme is resistant to inhibition by Rum1 and thus is well suited to trigger Rum1 proteolysis by the phosphorylation that leads to ubiquitination. Whereas the sensitivity of mitotic-cyclin/Cdk complexes to G_1-phase CKI inhibition (Moreno and Nurse 1994, Correa-Bordes and Nurse 1995) explains the long delay before S phase when only a mitotic cyclin remains (Fisher and Nurse 1996). Interestingly, several CKI proteins have been discovered in plants (Wang et al. 1997, 1998). But information is not yet available concerning when in the cell cycle plant CKIs are naturally abundant, and their precise role in regulating normal cell cycle progression has not yet been established, although an effect on cell cycle progression is implied since CKI expression is induced by abscisic acid, the plant hormone which commonly has the physiological function of slowing cell proliferation (Wang et al. 1998).

A model, drawn from these observations, of events at the termination of G_1 phase therefore includes the following; rising G_1-cyclin concentration, perhaps in proportion with increasing cell size, results in increasing cyclin/CDK activity which is resistant to inhibition by the CKI inhibitor protein that is abundant through G_1 phase (Sic1 or Rum1 in yeasts, $p27^{Kip1}$ in metazoa, and possibly an ICK in plants). Phosphorylation of the CKI targets it for ubiquitination and proteasome-mediated proteolysis. Removal of the CKI releases activity in mitotic-cyclin/cdk complexes that are more sensitive to inhibition by CKI than are G_1-cyclin/cdk. Mitotic CDK activity is then able to drive DNA replication, chromosome condensation, spindle formation, and nuclear division. Proteolysis of B-type cyclins and low CDK activity are necessary for completion of nuclear division and allow the re-accumulation of CKI. The stability of the genome is therefore protected by regular alternation of S phase and mitosis. Cdk activity must fall to low levels for completion of division, then, in the next cycle, rising G_1-cyclin/Cdk activity will lead to CKI proteolysis and chromosome duplication before Cdk activity has risen high enough to drive nuclear division.

Events at the G_1-to-S phase transition in multicellular animals, and probably in plants, include an additional mechanism that sets a threshold for adequate G_1-cyclin D/Cdk activity before progression to S phase. Cdk activity must be sufficient to multiply phosphorylate pocket proteins and thereby release the transcription factor E2F, which transcribes genes involved in DNA replication (the selective action of cyclins in this process was described in Sect. 6). The sequence of events in outline is that in animal cells Rb is initially phosphorylated by cyclin D/Cdk4 mediated by the LxCxE binding region of cyclin D and then by cyclin E/Cdk2. Released E2F can then transcribe cyclin E and cyclin A, which complex with Cdk2, so maintaining Rb phosphorylation as cyclin D transcription is turned off. DNA replication is promoted by cyclin A/Cdk2 phosphorylation of E2F and transcription by E2F of the replication-inducing protein Cdc6, and by direct cyclin A/Cdk2 participation at the ORC (Dynlacht et al. 1994, Yan et al. 1998, Romanowski et al. 2000). Interestingly, plant D cyclins may have additional functions outside the promotion of DNA replication since a novel cyclin D is transiently expressed in the establishment of new lateral-root primordia in *A. thaliana*, indicating a role in initiating rather than sustaining proliferation (De Veylder et al. 1999), and furthermore tobacco *cycD2;1* and *cycD3;1* are transcribed after S phase in synchronous suspension tobacco culture (Sorrell et al. 1999).

8 Regulatory interactions of proteins that complex with cyclin/Cdk

Cyclins can also juxtapose with Cdk molecules that are not substrates but regulators of the Cdk. The Cdc25C phosphatase that activates Cdk2 by removing inhibitory phosphate from Tyr-15 has been mentioned as allowing an important checkpoint during S phase (Sect. 4). Cdc25C activity affects cyclin E/Cdk2 but less so cyclin A/Cdk2 (Mailand et al. 2000), which suggests that cyclin E preferentially attracts to its cdk partner the regulator Cdc25C. Since cyclin E/Cdk2 acts in the initiation of S phase (Sect. 6) (Sherr 1996) this allows down-regulation of Cdc25C in response to DNA damage to prevent replication of corrupted genetic template.

Cyclins can also support larger groupings of proteins together with Cdk, which may be able to attract

proteins more effectively than cyclin/Cdk alone. In particular, B-type cyclins (Uetz et al. 2000) and Cdk (Brizuela et al. 1987) bind the small protein $p13^{suc1}$, which is thus termed cyclin-dependent kinase subunit (CKS). Cks in complex with cyclin/Cdk participates in a number of regulatory events during mitosis by its interactions with other proteins. Cks binds Cdc25 on entry into mitosis and participates in a positive feedback loop, in which Cdc25 activates Cdk1 by removal of phosphotyrosine (references in Sect. 4) and Cdc25 is in turn activated when it is multiply phosphorylated by activated Cdk1 (Patra and Dunphy 1998, Patra et al. 1999). The resulting feedback loop raises Cdk1 activity and protects the genome by decisive commitment of the cell to separation of the two copies of the genome. Cks also participates in a further protein interaction that protects the genome in case of incomplete DNA replication, when Cks competes for Cdc25 binding with another small protein, Pin1 (Shen et al. 1998, Lu et al. 1999). Pin1 is ubiquitous in eukaryotes including plants (Landrieu et al. 2000) and can interfere with Cdc25 activation and hence Cdk1 activation. In animal cells this is used in a checkpoint control that prevents attempted mitosis if duplication of the genome is not yet complete (Winkler et al. 2000). Since Cks and Pin1 compete for binding the same peptide sequence in Cdc25 (Landrieu et al. 2000), a possible mechanism of checkpoint arrest is the displacement of Cdc25 from contact with CKS and thus from proximity to Cdk1, with consequent blocking of Cdc25 activation by phosphorylation and failure of Cdk1 to become sufficiently enzymically active to drive mitosis.

The presence of Cks has been shown to extend the range of substrates of cyclin B/Cdk activity and this plays a part in an essential contribution of CKS to completion of mitosis. Cks is necessary in late mitosis for reducing cyclin B/Cdk1 activity to the low level that is essential for anaphase, which involves proteolysis of cyclin B (Moreno et al. 1989) (discussed in Sect. 10). The contribution of Cks to cyclin B proteolysis is explained at least in part by the ability of *Xenopus laevis* Suc1/Cks protein (known as p9) to direct activity of cyclin B/Cdk1 to proteins that are involved in ubiquitin-mediated proteolysis, in particular to Cdc27 and BIME, and thus to stimulate proteolysis of cyclin B (Patra and Dunphy 1998). Cks therefore extends the range of substrates that are recognised by the cyclin B/Cdk1 complex, such as to include Cdc27. Interestingly, plant Cdks also bind Suc1/Cks and the location of Cks injected into live plant cells closely correlates with that of Cdk1 (Cdc2a), as shown in Fig. 3 (Hepler et al. 1994).

9 Cyclin functions deduced from yeast cells

Evidence from genetically modified yeasts crucially supplements the biochemical and cell biological evidence from animal cells by providing an assessment of the importance of cyclins for efficient cell cycle progression. The yeasts have provided evidence of specific functions by some individual cyclins, but also evidence of some redundancy of cyclin function since small groups of cyclins can perform some functions of other cyclins. Extensive changes in the combinations of cyclins that are present have been made by making use of the homologous recombination that occurs between the genomic copy and an introduced (possibly disrupted, or modified) gene. Furthermore, speedy genetic crossing and Mendelian analysis allows the combination of multiple genetic changes in individual cells.

The most extensive cyclin redundancy has been found in budding yeast, where any one of the three CLN cyclins can complement the loss of the other two and allow progression to DNA synthesis (Richardson et al. 1989, Hadwiger et al. 1989, Cross and Blake 1993). Similarly, Clb5 or Clb6 are a functional pair in which either can complement the lack of the other and support DNA synthesis (Schwob and Nasmyth 1993). Furthermore, Clb3 and Clb4 readily complement each other (Richardson et al. 1992) and even deletion of both of these allows division if the four other mitotic cyclins, Clb5,6 and Clb1,2, remain. Clb1 and Clb2 can complement each other, but when Clb1 is the sole cyclin, there is slow progress through nuclear division (Fitch et al. 1992, Richardson et al. 1992).

An extreme demonstration of functional redundancy indicates similarity between all of the mitotic cyclins, since overexpression of Clb1p from a strong promoter can preserve the viability of cells in which all six endogenous Clb1–6 cyclin genes are inactivated (S. Haase and S. Reed unpubl. results reported by Stuart and Wittenberg 1998). Similarly fission yeast remains viable with cyclin B (cdc13) as sole cyclin (Fisher and Nurse 1996). However, closer consideration of the limitations of these tests, and more detailed examination of the effects of cyclin changes, supports the concept that cyclins have specific functions.

Cyclin deletions produce an artificial situation in which remaining cyclins, although they may have

lower affinities than the missing cyclin(s), can occupy sites left vacant by the missing cyclins and can bind heterologous substrates when there are no competing cyclins with higher affinity. Such abnormal low-affinity binding interactions may explain the ability of cyclins from taxonomically remote sources to replace deleted CLN cyclins in budding yeast (Lew et al. 1991). Truncated cyclins were more efficient in replacing Clns, presumably due to their impaired substrate recognition allowing them to more easily bind yeast proteins for which they have not evolved affinity. Furthermore, in cln complementation, very little discrimination between substrates is required since the sole essential G_1 substrate of Cln1–3/Cdk1 is the Sic1 inhibitor of Cdk1 activity. The Sic1 protein must be phosphorylated by Cdk1 and thus marked for proteolysis to allow entry into S phase (described in Sect. 7), but if Sic1 is deleted, cells become viable without having CLN genes (Schneider 1996, Tyers 1996), indicating that no other phosphorylation by Cln/Cdk1 is essential. The significance for cyclin function is that, in the test of complementing CLN cyclins, a heterologous cyclin need not discriminate subtly between proteins; if it can bind Sic1, even inefficiently, it can preserve cell viability.

A pattern in cyclin redundancy

We suggest that some overlap of function between cyclins can be of value for cell survival. The capacity to complete cell division may be threatened by proteolytic removal of cyclins (Evans et al. 1983, Sherr 1993, Nasmyth 1996) in cells that have progressed slowly through events that are dependent upon a particular cyclin. Thus a group of cyclins may disappear before their function is complete in a slowly progressing cell. There is not a reservoir of cyclin since extracted CDK is mostly monomeric (e.g., Wittenberg and Reed 1988), which indicates that cyclins are less abundant than CDK. This circumstance is advantageous in allowing newly synthesised cyclins to immediately find CDK partners and to alter the spectrum of proteins being phosphorylated. There is an associated risk to viability, from a process supported by a particular cyclin not being completed before it is proteolysed. The risk is alleviated if the next set of cyclins to be accumulated has some capacity to complete the preceding process. Such capacity of cyclins may be universal since in animal cells cyclin D expression normally precedes cyclin E, but cyclin D1 can be deleted and replaced by cyclin E that has been brought under the control of the cyclin D1 promoter and therefore is expressed with cyclin D1 timing (Geng et al. 1999). Furthermore, cyclin A, which normally accumulates after cyclins D and E have promoted initiation of DNA replication, can when overexpressed advance the time of S phase (Resnitzky et al. 1995, Rosenberg et al. 1995). This indicates that cyclin A can facilitate the initiation as well as execution of DNA synthesis and therefore that continued presence of cyclins D and E may be less critical than otherwise.

Complementation between yeast cyclins underlines the capacity of newly accumulating species of cyclin to continue cyclin-dependent events that are in progress and sometimes even to make good the lack of earlier progress. Deletion of all three Cln cyclins can be complemented by constitutive expression of cyclins Clb5,6 (Schwob and Nasmyth 1993, Epstein and Cross 1995). Cyclins Clb5,6 are expressed immediately after Clns (in S phase), but cyclins not expressed until after S phase are not effective in complementation of clns; thus, Clb1,2 are not effective unless overexpressed and rendered stable (Lew et al. 1991, Amon et al. 1994). Similarly the cyclins Clb5,6 can be partially replaced by Clb3,4 cyclins, which are expressed immediately after them, although division and growth are a little slowed in cells that are reliant on Clb3,4 for Clb5,6 function (Schwob et al. 1993, 1994). However, under the different conditions of sporulation Clb3,4 fail to replace Clb5,6 in supporting DNA replication (Stuart and Wittenberg 1998). This failure correlates with a later expression of Clb3,4 during sporulation that is too late to utilise the transient state of readiness for DNA replication established by Cln cyclins. Therefore overlap of cyclin functions can be exaggerated if a complementation test is performed under only one environmental condition. The cyclins Clb3,4, which are expressed in early mitosis, can be readily complemented by Clb1,2 with no apparent disadvantage (Fitch et al. 1992, Richardson et al. 1992). The Clb1,2 cyclins in budding yeast, which are expressed during mitosis, cannot however be replaced by other cyclins (Surana et al. 1991) and this correlates with their late expression in division and the absence of any later cyclins that might complete their function.

Distinct cyclin functions revealed in yeasts

Distinct cyclin functions can be revealed by assessing modifications to cell cycle events that are caused by

abnormal cyclin levels or cyclin combinations. Among the G_1 (CLN) cyclins, Cln3 activates transcription of genes (including *CLN1 CLN2*; Tyers et al. 1993) that are expressed in late G_1, due to Cln3/Cdk1 phosphorylation of the transcription factor SBF that binds to SCB regions in the promoters of these genes (Koch et al. 1993). Elevated activity of Cln3 (caused by hyperactive mutation, stabilisation of the protein against proteolysis, or expression of additional gene copies), results in earlier commitment to DNA replication and cell division and therefore causes smaller average cell sizes. Conversely *CLN3* deletion results in larger average cell size (Saul and Sudbery 1985, Nash et al. 1988).

Cln1,2 are instead concerned with bud emergence and with the initiation of DNA synthesis through the removal of the Cdk activity inhibitor Sic1. Proteolysis of Sic1 follows its phosphorylation by Cln1/Cdk1 and Cln2/Cdk1 (Schneider et al. 1996) and the removal of its inhibitory effect allows mitotic-cyclin/Cdk activity to reach levels that drive DNA replication and bud emergence (described in more detail in Sect. 10). Both replication and budding are delayed in the absence of Cln1,2, or hastened by their raised level (Lew et al. 1992, 1997). Therefore accurate timing of the start of DNA replication and maintenance of optimum cell size depend upon the distinctive functions of individual G_1 cyclins in *S. cerevisiae*, although they can perform other functions slowly and by the crude test of survival G_1 cyclins are interchangeable.

Later in the cell cycle a special contribution of Clb5 has been identified by deleting it from cells that carry a partially disabled CDK1 (*cdc28-4^{ts}*), revealing defective development of mitotic spindle and nuclear positioning in diploid cells (Segal et al. 2000). Only deletion of *CLB5* caused this defect and no rescue of *clb5* mutation was possible by overexpression of other cyclins, therefore a specific contribution of *CLB5* to spindle and nuclear positioning is revealed.

Furthermore, a key function of Clb5 cyclin/Cdk1 occurs at the completion of mitosis (Shirayama et al. 1999). Clb5 cyclin/Cdk1 is the most significant kinase phosphorylating and marking for proteolysis the CDK inhibitor protein Sic1 during mitosis. Largely due to its proteolysis Sic1 is of low abundance during S phase and early mitosis. This low level of Sic1 allows high CDK activity that is necessary for early events in nuclear division, but completion of anaphase requires the reaccumulation of Sic1 and consequent low Cdk activity. The recovery of Sic1 levels involves elimination of Clb5. The experimental deletion of this cyclin, and of no other cyclin, is effective in reducing the phosphorylation of Sic1, thereby increasing its stability and accumulation. This special function of Clb5 was seen in double-mutant cells that lacked the protein Cdc20, which normally leads to proteolysis of Clb5 at anaphase, and the mutant also lacked the protein Pds1, which regulates chromatid separation and also releases the phosphatase cdc14 that removes phosphate from Sic1 (Clarke et al. 1999). In these double-mutant cells mitosis was stalled in anaphase because Sic1 protein remained phosphorylated and could not accumulate, therefore Cdk1 activity remained high. Only deletion of *CLB5* could rescue cell division because this cyclin is so specifically targeted to the Sic1 protein that *CLB5* deletion is able to sufficiently reduce Sic1 phosphorylation (Shirayama et al. 1999). Clb5/Cdk1 activity is therefore inversely related to Sic1 stability, and since Sic1 can inhibit all mitotic Cdk complexes, the Clb5 level acts as a switch controlling the two major cell cycle transitions: exit from G_1 phase and completion of mitosis.

Many distinct cyclin functions have therefore been verified by intensive testing in budding yeast. It can now be well understood that, while absence of an individual cyclin may not cause immediate death of the cell, the requirement for timing of division events and retention of optimum cell size ensures that wild cells retain all nine cyclins as essential for effective competition in the wild environment.

10 Cyclin specification of structural events in division

Manipulation of cyclin expression in yeasts has been more extensive than any yet achieved in plants and indicates cyclin control over the cytoskeleton and cell development. Cyclins that are expressed in G_1 phase and cyclins expressed during nuclear division have opposite effects on the cytoskeleton and growth distribution (Lew and Reed 1993). Accumulation of Cln1,2 cyclins in G_1 phase induces aggregation of the actin cytoskeleton and localised wall growth that initiates a bud. Then during G_2 phase, Clb1,2 cyclin accumulation disperses actin and there is an accompanying dispersion of wall growth to occur in both mother cell and bud. Significantly, overexpression of *CLN* genes in G_2 phase restores concentration of actin and growth in the bud. Conversely overexpression of the Clb1,2 cyclins (modified by deletion of destruction box to

evade proteolysis) during G_1 phase disperses the actin and prevents localised growth and bud formation (Lew and Reed 1993). Similarly in fission yeast overexpression of Cdk1 and the mitotic cyclin Cdc13 induces attempted mitosis during G_1 phase (Hayles et al. 1994). Thus the effect on cell development is consistently determined by the identity of the abundant cyclin.

Cyclins exert their effects, at least in part, by their specific subcellular locations (Pines and Hunter 1991, 1994). In mammalian cells B-type cyclins are retained in the cytoplasm through G_2 phase until their release into the nucleus in late-G_2 phase, when cyclin B associates with chromosomes during their condensation and with the spindle. In B-type cyclins a cytoplasmic retention signal (CRS) of about 50 amino acids has been detected upstream of the cyclin box and is found responsible for the initial retention of cyclin B in the cytoplasm. Whereas A-type cyclins lack the CRS and are predominantly nuclear through out the cell cycle. The CRS is crucial to location since if it is removed cyclin B becomes nuclear, and if the CRS is attached to cyclin A it is retained in the cytoplasm (Pines and Hunter 1994). Neither of these mislocations of cyclins expressed from introduced genes disrupts cell cycle progress, but this does not mean that cyclin location is unimportant since unmodified endogenous cyclins are still in their normal locations.

Entry of cyclin B into the nucleus is certainly important since activation of quiescent oocytes by injected cyclin B mRNA depends upon the expressed cyclin protein moving into the nucleus. In this system, cyclin that was unable to leave the cytoplasm because it contained mutated CRS that could not be phosphorylated, was inactive in inducing division. Activity was restored if the cyclin was further modified to restore nuclear location, by introduction of a functional CRS or a nuclear location signal (Li et al. 1997, Hagting et al. 1999).

It is appropriate to consider how plant cell division and development may be similarly influenced by the presence and location of cyclin/Cdks since many forms of evidence point to the importance of these catalysts. In cell division, which is induced by developmental progression, hormonal stimulus, or wounding, there is consistently induction of Cdk (e.g., Gorst et al. 1991, Zhang et al. 1996) and of cyclin gene expression (Ferreira et al. 1994, Riou-Khamlichi et al. 1999, Gaudin et al. 2000, Mews et al. 2000; for reviews, see Jacobs 1995, Huntley and Murray 1999). Rising activity of Cdk has been found to drive progression through the yeast cell cycle (for a review, see Stern and Nurse 1996), and in plants essential cyclin/Cdk involvement in division is indicated by the inhibitory effect of dominant negative CDK1 on cell proliferation (Hemerly et al. 1995) and the stimulatory effect of injected plant mitotic cyclin B/Cdk (Hush et al. 1996; for a review see, Mironov et al. 1999). It is timely therefore to begin to consider the mechanisms by which the uniquely complex structural events of plant cell division may be influenced by the location of cyclin/Cdk enzymes.

11 Location of cyclin/Cdk in dividing plant cells

Methodology: in vivo location of plant proteins

The accurate location of proteins in live cells remains problematical. A long-established method for locating proteins by indirect immunofluorescence microscopy (Osborn and Weber 1982) requires killing the cell with fixative to immobilise proteins and then permeabilising to allow penetration of antibody that will bind specifically to the protein under investigation. Usually a secondary antibody with a fluorescent tag is then applied to allow indirect detection of the target protein detection. Results from many laboratories, using this method with different tissues, fixation techniques, and antibodies, have revealed something of in vivo locations, particularly of structural proteins. However, the technique can give both false negative and false positive results. False negative results can arise, particularly with soluble proteins, due to failure of fixation to cross-link the protein to adjacent structures with sufficient stability to withstand the leaching effects of incubations with cell-wall-dissolving enzymes, permeabilising agents, and antibodies. False negative results can arise by masking of the antigenic site of the subject protein by other proteins. False positive results can arise from localised precipitation of the target protein by fixative and by differential loss of the target protein during the staining. These are real concerns since artefacts of immunolocation have been demonstrated by injecting into live cells heterologous proteins, such as serum protein, which disperse evenly because they have no affinity for plant cell structures yet upon fixation and immunostaining appear to indicate localised distributions (Melan and Sluder 1992). To underline the point, fluorescent spinach calmodulin is evenly distributed when microinjected into live *Tradescantia virginiana* stamen hair cells, but when the

cells are fixed, the calmodulin takes on false localised distributions (Vos and Hepler 1998) that others had considered meaningful.

An alternative approach is to make the subject protein under study fluorescent, so that when introduced by expression of a recombinant transgene or by microinjection it can be detected directly in the live cell without fixing and processing and also without requiring that an antigenic site be accessible. This method also brings problems since the fluorescent tag may alter the properties of the protein. In the case of small fluorescent chemical groups such as carboxyfluorescein the attachments are physically small but, in the creation of the tagged molecule, their binding to reactive elements such as free amino groups that are usually scattered throughout the protein, the tag may bind to several locations. This may interfere with binding to other proteins and may distort cellular locations.

More controlled placing of the fluorescent tag is possible with biologically synthesised fusion-proteins that contain a fluorescent-protein moiety such as green-fluorescent protein (GFP) as part of a composite protein expressed from a single recombinant open reading frame. Such protein fusion fixes the area on the subject protein that is influenced by the tag, but that influence may be great since the 28 kDa GFP is almost as big as an average protein. There is also the problem that folding of the GFP may hinder normal folding of the subject protein under study. This can be addressed by introducing a flexible linker peptide. But the juxtaposition of subject protein, possible linker, and fused tag can unintentionally create structural features in the composite molecule such as bipartite nuclear localisation signals (Smith and Raikhel 1999). These reservations may be partially addressed if the function(s) of the subject protein is known and can be assayed in vitro to test the effect of fluorescent tags, as for example the capacity of carboxyfluorescein-Cks1 to bind Cdk, or of GFP-calmodulin to show conformational shift with calcium (Hepler et al. 1994, Vos and Hepler 1998). However, it is rarely possible to know and to test all functions, or to test them in the milieu of competing proteins that occurs in the live cell. These problems may be countered by use of small chemical tags as well as fusion proteins, and by comparison of fusions made at each end of the subject protein, both with and without a flexible linker.

The in vivo location of proteins will be best indicated by a convergence of evidence, especially by correlation between microinjection of the unfixed cell and immunostaining of the unmodified protein. It is also useful if independent evidence that is not based on location can indicate whether the protein does have the function(s) that its location may imply. Some convergence of this sort is now emerging for a few cyclins and Cdks in plant cells as considered below.

Mitotic locations of Cdk1 (Cdc2Azm) and of cyclins CycB1zm;2 and CycA1;zm;1

Four mitotic cyclins (Renaudin et al. 1994) and the CDK Cdc2Azm (Colasanti et al. 1991) have been located during cell division in maize by antibody probing of sections cut from fixed and embedded roots. The antibodies were raised against the carboxy-terminal sequences of the cyclins and CDK, which are variable regions in these classes of protein, and are thus specific for the particular species. The CDK studied has been found able to complement mutation of the yeast *CDC28* gene (Colasanti et al. 1991) and is therefore a functional homologue of CDK1. Encouragingly there was no evidence of common distortions from fixation since the five antibodies indicated distinct distributions of their target proteins. At two locations cell cycle proteins have been detected by more than one technique, and other forms of evidence support their deduced function at these locations.

At the preprophase band (PPB), both Cdk1 and CycB1zm;2 are detected (illustrated in Fig. 1I–K) in serial sections that reveal its circumferential structure (Gunning 1982, Wick 1991) (illustrated diagramatically in Fig. 3). This location for Cdk1 correlates with observations made in onion with an antibody raised against the cyclin binding region (PSTAIR region), which is expected to find Cdk1 and closely related variants (Mineyuki et al. 1991, 1996), and it also correlates with observations in maize, made with a different antibody which was raised against the same carboxy-terminal region of Cdc2Azm (Colasanti et al. 1993). Finally a further different antibody locates Arabidopsis Cdc2a at the PPB (Stals et al. 1997). In maize the PPB location of CycB1zm;2 and Cdc2Azm is not shared with others of the four cyclins studied, but it cannot be concluded that CycB1zm;2 and Cdc2Azm are necessarily in a functional complex. A strong temporal element in location at the PPB was indicated by detection of CycB1zm;2 and Cdc2Azm only at the mature narrowed PPB, in which microtubules are about to be, or are being, disassembled at the

prophase–prometaphase transition. Significantly, in live tobacco cells there was a similar location of alfalfa Cdc2b (a PSTAIR-containing CDK1) in a fusion with GFP without linker peptide. When expressed in the BY2 cell line, preliminary observations have detected Cdc2-GFP at the PPB and more precisely only at the mature PPB (Weingartner et al. 2000), exactly as has been revealed by immunolocation in maize (Mews et al. 1997).

This timing and location of Cdk1 and cyclin CycB1zm;2 suggested a likely function in PPB disassembly that was supported by a direct functional test (Hush et al. 1996, John 1996, Wu et al. 1997). PPB disassembly was greatly accelerated in live stamen hair cells of *Tradescantia virginiana* by microinjection of cyclin B/Cdk enzyme taken from unicellular plant cells in metaphase of mitosis and purified by affinity for yeast Cks(Suc1) protein that binds Cdk (John et al. 1991) (see Sect. 8). The purified plant enzyme contained cyclin B, which reacted with antibody against fission yeast cyclin B, and contained Cdk, which reacted with antibody against the PSTAIR motif (Hush et al. 1996). The purified enzyme from metaphase cells had high protein kinase activity, but control enzyme from cells in telophase had low activity. Metaphase enzyme caused disassembly of the PPB so rapid that there was no detectable incorporation of fluorescently tagged tubulin monomer into microtubules of the PPB, although such incorporation into the PPB microtubules occurred in controls (Hush et al. 1996). We consider it significant that CycB1zm;2 and Cdc2Azm are only found at the mature PPB that is beginning to be disassembled and that this disassembly has been demonstrated to be limited by activity of cyclin B/Cdk since it is accelerated by injected active enzyme. We suggest that localisation of CycB1zm;2 and Cdc2Azm at the PPB is a mechanism by which CDK activity is focused on PPB breakdown at the transition to prometaphase.

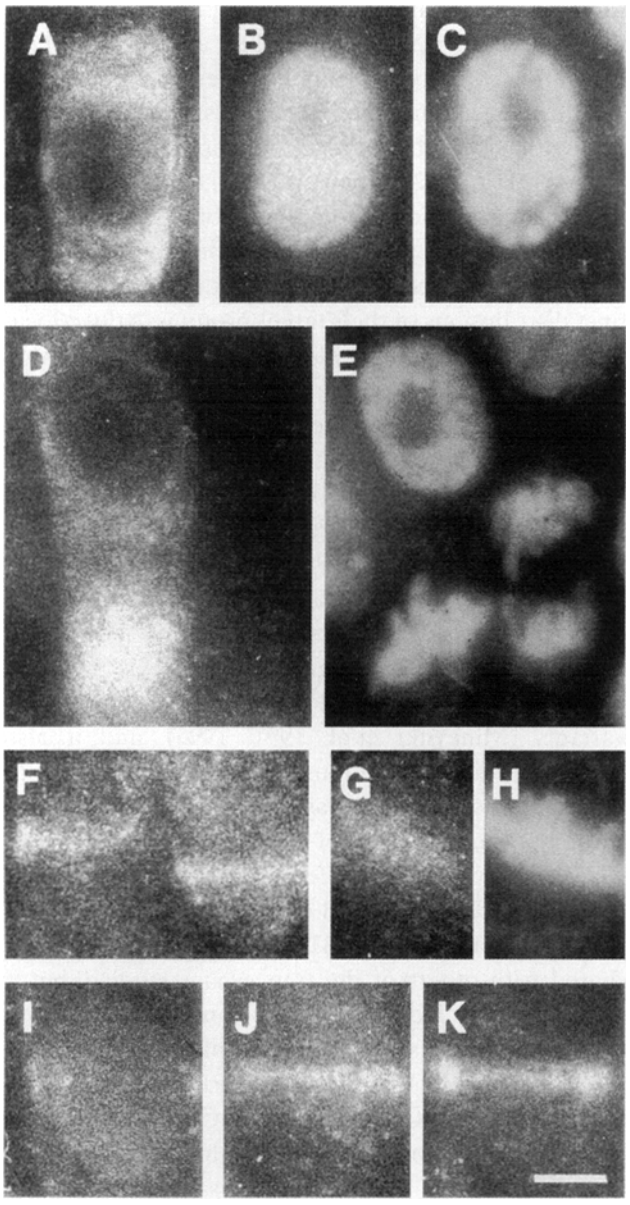

Fig. 1 A–F. Cyclin CycB1zm;2 localisations during mitosis. Sections were labelled with anti-CycB1zm;2 antibody (**A**, **B**, **D**, **F**, **G**, **I**, and **J**), with anti-α-tubulin (**K**), and DNA was stained with 4′,6-diamidino-2-phenylindole (**C**, **E**, and **H**). **A** Cyclin CycB1zm;2 accumulates first in the cytoplasm during late-G_2 phase when it impinges upon, and begins to concentrate at, the nuclear envelope. **B** and **C** During prophase the cyclin is released from cytoplasmic retention and accumulates within the nucleus in association with the chromosomes. **D** and **E** The cycle of CycB1zm;2 abundance and location through mitosis is encapsulated in four contiguous cells, where initial cytoplasmic retention during early prophase (top left) is followed by penetration of the nucleus and persistent association with the chromosomes until metaphase (bottom left, and also **G** and **H**), after which there is abrupt proteolysis of this cyclin, which reduces levels to a minimum by anaphase (bottom right), and minimal level persists through interphase (top right). **F** During late G_2 and early prophase, as CycB1zm;2 is moving into the nucleus (cells left and right), the cyclin associates with the PPB microtubules that is tightly focused and about to be disassembled. **I** and **J** The circumferential nature of the PPB is seen in serial sections through the same cell, cutting the PPB transversely (**I**) and in a glancing plane (**J**). **K** The coincidence of the structure labelled with anti-CycB1zm;2 with the microtubular PPB is seen by double labelling the section in **J** with anti-α-tubulin. These results, and those in Fig. 2, were obtained in parallel with those reported by Mews et al. (1997), where the methods are fully described and the specificity of antibodies for proteins of appropriate size and recognition by epitope-specific binding are demonstrated. Bar: 5 μm

We cannot eliminate the possibility suggested by Sundaresan and Colasanti (1998) that Cdc2A plays a part in the maturation of the PPB and in marking of the future cross wall attachment site. But we note that CDK driving PPB maturation is not consistent with the late time of arrival of the Cdk and cyclin at the PPB, nor the inhibition of PPB breakdown caused by inhibition of CDK activity (Katsuta and Shibaoka 1992), while conversely accumulation of phosphate on proteins by inhibition of protein phosphatase does not block PPB breakdown (Hasezawa and Nagata 1992, Zhang et al. 1992). Concerning the function of marking the future cross wall attachment site, if this occurs by activity of cyclin B/Cdk the most precise marking will occur if the cyclin B/Cdk arrives at the tightly focused mature PPB as we suggest, rather than at the dispersed early PPB. A possible substrate of Cdk1 at the PPB is the product of the *Tangled* gene that is necessary for proper cross wall location and orientation of division planes (Cleary and Smith 1998).

At the chromosomes, concentration of CycB1zm;2 was detected in the immediate chromosomal area throughout the period in which they were condensed; from prophase after this cyclin entered the nucleus to the metaphase–anaphase transition when CycB1zm;2 was proteolysed (Fig. 1 B, D, G). There was also an association of this cyclin with the spindle region (Mews et al. 1997). This chromosomal location is in contrast to CycB2a-zm, which was intranuclear but sharply excluded from chromatin (Mews et al. 1997), and this differential location is consistent with absence of fixation artefacts concerning cyclins within the nucleus. Significantly a chromosomal location similar to CycB1zm;2 was detected for the structurally similar tobacco cyclinB1;nt;2 (Genschik et al. 1998) by expression of a GFP fusion in the BY2 cell line of tobacco (Parmentier et al. 2000). The fusion protein had no peptide linker but retained biological function indicated by its Cdk protein kinase activity in an anti-GFP immunoprecipitate. Again there is independent evidence not only of location but also of likely function of the cyclin B/Cdk at the chromosomes because injected cyclin B/Cdk dramatically accelerated chromosome condensation (Hush et al. 1996). We therefore suggest that chromosome condensation is an in vivo function of plant cyclin B1. The Cdk partner at this location might be Cdc2a (Stals et al. 1997), but Cdks expressed late in the cell cycle (Magyar et al. 1997) cannot be discounted and Meszaros et al. (2000) recently reported Cdc2MsF at this location.

Taking into account this convergence of evidence from ourselves and others, together with the genetic evidence for the essential contribution of cyclin/Cdk in mitosis (Nurse 1990, Mironov et al. 1999) and the evidence for cyclin/Cdk association with microtubule-associated proteins (Ookata et al. 1993, 1995), we therefore deduce a direct involvement of cyclin/Cdk enzymes in the cytoskeletal machinery of plant mitosis.

Further evidence, that is less extensively corroborated, indicates location of CycB1zm;2 also at the nuclear envelope (Mews et al. 1997) (Fig. 1 A). This is indirectly supported by the effects of microinjection but awaits confirmation of location in other plants. CycB1zm;2 behaves in several ways like animal cyclin B1 (Pines and Hunter 1991) since it accumulates first in the cytoplasm through G_2 phase (Fig. 1 A, D) and then during prophase relocates into the nucleus (where it becomes associated with the chromosomes and spindle as noted). Cytoplasmic retention of human cyclin B1 derives from the presence of a CRS that can act as a cytoplasmic anchor if artificially transferred to other cyclins (Pines and Hunter 1994).

A putative plant CRS has been recognised in CycB1zm;2 and in others of the CycB1-group, including CycB1gm;1, -am;1, -am;2, -at;2, -nt;1, and -nt;2 (Mews et al. 1997), but the plant CRS has not yet been tested. It is an indication of the robustness of the present classification that, although it was not based upon the putative CRS region since cytoplasmic retention was not yet reported in plant cyclin proteins, the cyclins that are now identified as having putative CRS region (Mews et al. 1997) were classified as B1-type by Renaudin et al. (1998). An anomaly may be CycB1zm;1, which is not cytoplasmically retained, although it is structurally similar to the B1-type plant cyclins, and especially similar to CycB1zm;1, in the cyclin box. We consider it significant that a major difference between CycB1zm;1 and CycB1zm;2 is the absence of a CRS in the former and that cytoplasmic retention correlates with the CRS.

The importance of transfer into the nucleus for animal cyclin B function (Li et al. 1997, Hagting et al. 1999) has been mentioned in Sect. 10. During the transfer of CycB1zm;2 the cyclin is transiently visible concentrated at the nuclear envelope (Fig. 1 A, D) (Mews et al. 1997). The association probably then persists during the brief period while cyclin B1 has entered the nucleus and envelope breakdown is not yet complete, but the cyclin is not then clearly visible

at the envelope against the background of cyclin that accumulates in the nucleus. Location at the nuclear envelope may be functionally significant since the cyclin arrives immediately prior to envelope breakdown, which is greatly accelerated by injection of cyclin B/Cdk and begins nearest the point of injection where cyclin B/Cdk concentration is highest (Hush et al. 1996, Wu et al. 1997). It is known that Cdk1 phosphorylation of lamin proteins in the nuclear envelope is necessary for nuclear-envelope breakdown (Enoch et al. 1991). The weight of evidence therefore suggests that a further likely function of CycB1zm;2/Cdk1 is in nuclear-envelope breakdown. The several locations and probable functions of CycB1zm;2 just indicated are not unprecedented, and indeed they resemble those of metazoan cyclin B1 (Pines and Hunter 1991, 1994; Jackman et al. 1995).

Phragmoplast location of CycA1;zm;1 was an unexpected observation (Mews et al. 1997) (Fig. 2) since mitotic cyclins in other eukaryote kingdoms are proteolysed during anaphase and therefore are at low levels in cytokinesis. The proteolysis of B-type cyclins is part of a wave of proteolysis at anaphase that reduces mitotic Cdk activity by cyclin degradation (Nigg 1995, Nasmyth 1996, A. Murray et al. 1989) and eliminates proteins that are inhibitory to chromosome separation, especially Pds1, cohesins, and chromokinesins (Shirayama et al. 1999, Michealis et al. 1997, Funabiki and Murray 2000). If Cdk activity is kept high by overexpression of cyclins and Cdk, or by making cyclin more stable through deletion of the destruction box, then anaphase is inhibited (King et al. 1995). A similar failure to progress past metaphase is seen in a unicellular plant with a genetic block to cyclin proteolysis (Wu et al. 1997) and in higher-plant cells with an inhibitor block to proteolysis (Genschik et al. 1998). The cyclin behaviour seen in maize is not in contradiction of this since the B-type cyclin CycB1zm;2, which is seen to be most directly involved in nuclear division, is indeed eliminated at anaphase (Fig. 1 D, E).

The persistence of the CycA1;zm;1 cyclin and its concentration at the phragmoplast suggest that the microtubular dynamics of this structure which is unique to plants, are regulated by cyclin/Cdk much like the mitotic spindle that precedes it. Observations that cyclin/Cdk interacts with the microtubule-associated protein MAP4 at the spindle (Ookata et al. 1993, 1995) raises the possibility that CycA1/Cdk substrates at the phragmoplast may include MAPs and motor proteins

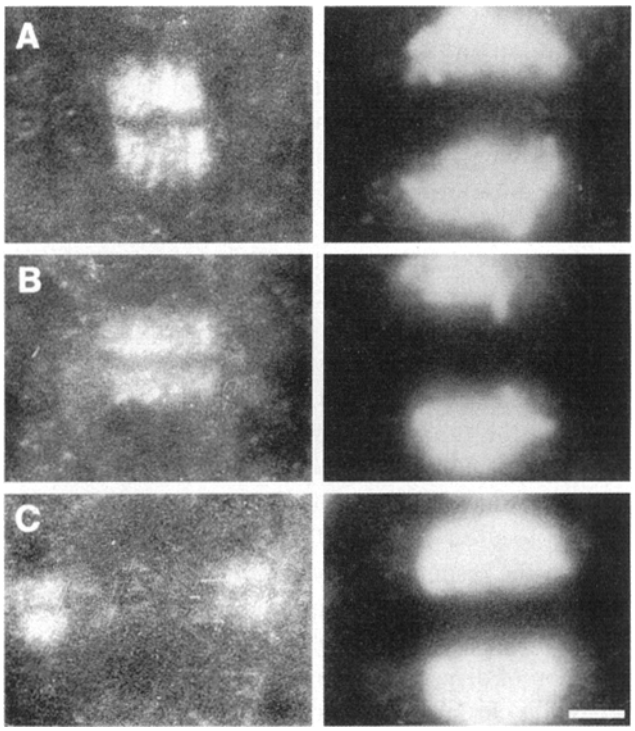

Fig. 2 A–C. Cyclin CycA1;zm;1 localisation at the phragmoplast. Sections were labelled with anti-CycA1;zm;1 (left) and DNA was stained with 4′,6-diamidino-2-phenylindole (right). Cyclin CycA1;zm;1 concentrates at the phragmoplast from the time of initial phragmoplast formation in late anaphase (**A**), continuing through the centrifugal extension of the phragmoplast to the margins of the cytoplasm by telophase (**B**), and remaining during the completion of cytokinesis as the phragmoplast is eroded beginning centrally (**C**). The concentration of CycA1;zm;1 at the phragmoplast relative to that in the surrounding cytoplasm far exceeds that which occurs nonspecifically when soluble proteins penetrate the greater available space within the pallisade of phragmoplast microtubules (Hepler et al. 1994). Cyclin CycA1;zm;1 also associates with other microtubular structures (illustrated in Mews et al. 1997) but is most concentrated at the phragmoplast. Bar: 5 μm

such as phragmoplast-located kinesin-like proteins (Liu et al. 1996, Asada et al. 1996). The MAP kinase MMK3 is concentrated in the phragmoplast of alfalfa and depends upon integrity of microtubules for its accumulation at that location and for its retention of enzyme activity (Bögre et al. 1999). Interaction of the CycA1/Cdk and MMK3 kinases is an evident possibility. The function of plant cytokinesis is finally yielding to genetic analysis (for a review, see Heese et al. 1998) and may reveal the phragmoplast proteins that interact with cyclin/Cdk.

Successional changes of cyclins through nuclear division are not unique to plants. In *Drosophila melanogaster* cyclins A, B, and B3 are sequentially degraded during nuclear division and introduction of modified nondegradable (stable) cyclin A results in

metaphase arrest, while stable cyclin B causes anaphase arrest and stable cyclin B3 causes late-anaphase arrest, suggesting that the sequential disappearance of cyclins orders progression from metaphase to telophase (Sigrist et al. 1995). In budding yeast cyclin-like proteins Pcl2 and Pcl9 are expressed in late mitosis, but both genes can be deleted without causing any phenotype and their expression is not linked to cytokinesis, which begins earlier with formation of the bud (Aerne et al. 1998).

Plants are not unique in having protein kinase activity during cytokinesis since protein kinases of the polo family, which are not cyclin dependent, are located in the persistent midzone of the *Caenorhabditis elegans* spindle and are required for the location there of microtubule-bundling proteins (Schumacher et al. 1998) and the *S. pombe* polo kinase phosphorylates and promotes movement from nucleus to the cortex of the protein Mid1 that marks the site of the cytokinesis cleavage furrow (Bähler et al. 1998). These sorts of cytoskeletal and positioning functions may be carried out by cyclin-dependent kinases in plants. Interestingly, in *Aspergillus nidulans* Cdk activity is necessary and rate limiting for septation (Harris and Krauss 1998), although it is not in fission yeast, but it is not known if cyclin/cdk is present at the Aspergillus septum, and since this is very different from the plant cell plate, the fungus is not likely to be a useful model.

Plants appear to be unique among higher eukaryotes in extending cyclin participation into cytokinesis. It has been suggested (Mews et al. 1997) that this could have evolved in the plant kingdom from situations in which cytokinetic microtubular structures are present concurrently with the mitotic spindle when cyclin/Cdks are generally associated with microtubular structures (Jackman et al. 1995, Ookata et al. 1995). Concurrent presence of spindle and cytokinetic apparatus is seen, for example, in some members of the charophycean algae, from which higher plants are thought to have evolved (Lokhorst et al. 1988), and in the spore mother cells of bryophytes (reviewed by R. Brown and Lemmon 1997). It may also be functionally important that the phragmoplast is attended by cyclins that do not undergo the anaphase proteolysis seen with the B1-type cyclin CycB1zm;2. This may be particularly advantageous in developmental programs where cytokinesis does not follow on from mitosis, as when cytokinesis is suspended in the development of coenocytic tissues such as the endosperm (reviewed by Gunning 1982, Heese et al. 1998). Thus some separation of cyclin functions in phragmoplast and spindle may facilitate independent regulation of nuclear division and mitosis, as in endosperm development and subsequent cellularisation.

12 Towards functional groupings of plant cyclins

There are implications for the functional groupings of plant cyclins from the involvement of cyclins and Cdks in many processes that are not found in animal mitosis (Fig. 3). Plants are not unique in having cyclins that accommodate special features of cell division since in animal cells the large single Golgi body, which is disassembled during mitosis and then reassembled in daughters, is attended by cyclin B2, while cyclin B1 attends chromosomes and spindle (Jackman et al. 1995). By contrast the numerous small persistent Golgi stacks of plants are inherited by segregation to a "Golgi belt" that is bisected by the new cell wall (Nebenführ et al. 2000). We need not expect plant equivalents of animal cyclin B2. Nor, conversely, need there be precise animal equivalents of plant cyclins that are dedicated to the formation and operation of a mitotic spindle that functions without a polar organiser. Similarly, control of the plant phragmoplast will have evolved distinctively because the phragmoplast outlasts the spindle in the cell cycle, has more numerous shorter tubules, and makes the new cell wall by transporting wall material centripetally towards a cell plate, which expands towards the cell margins.

In spite of these differences between plant and animal cell division, and although plant cyclin classification was not undertaken to predict function (Renaudin et al. 1998), the plant cyclin B1 group is now seen to contain some cyclins with significant functional similarities with animal cyclin B1. The cyclin CycB1zm;2 (described earlier as located at the PPB) is initially cytoplasmic, relocates to the nucleus during prophase and associates with chromosomes and spindle, and then is abruptly proteolysed during entry to anaphase (Fig. 1 D). All of these general properties are shared with animal cyclin B1 (Pines and Hunter 1991, 1994; Jackman et al. 1995); however, a simple conservation of cyclin B between animals and plants is not likely since there is only 30% amino acid sequence similarity and the 9 bp MAS motif (Ito et al. 1998) is present only in the plant promoter (Sect. 3).

Plant A-type cyclins are likely to be more plant-specific in structure and function. Plant A-type cyclins with the functions of animal cyclin A would have a

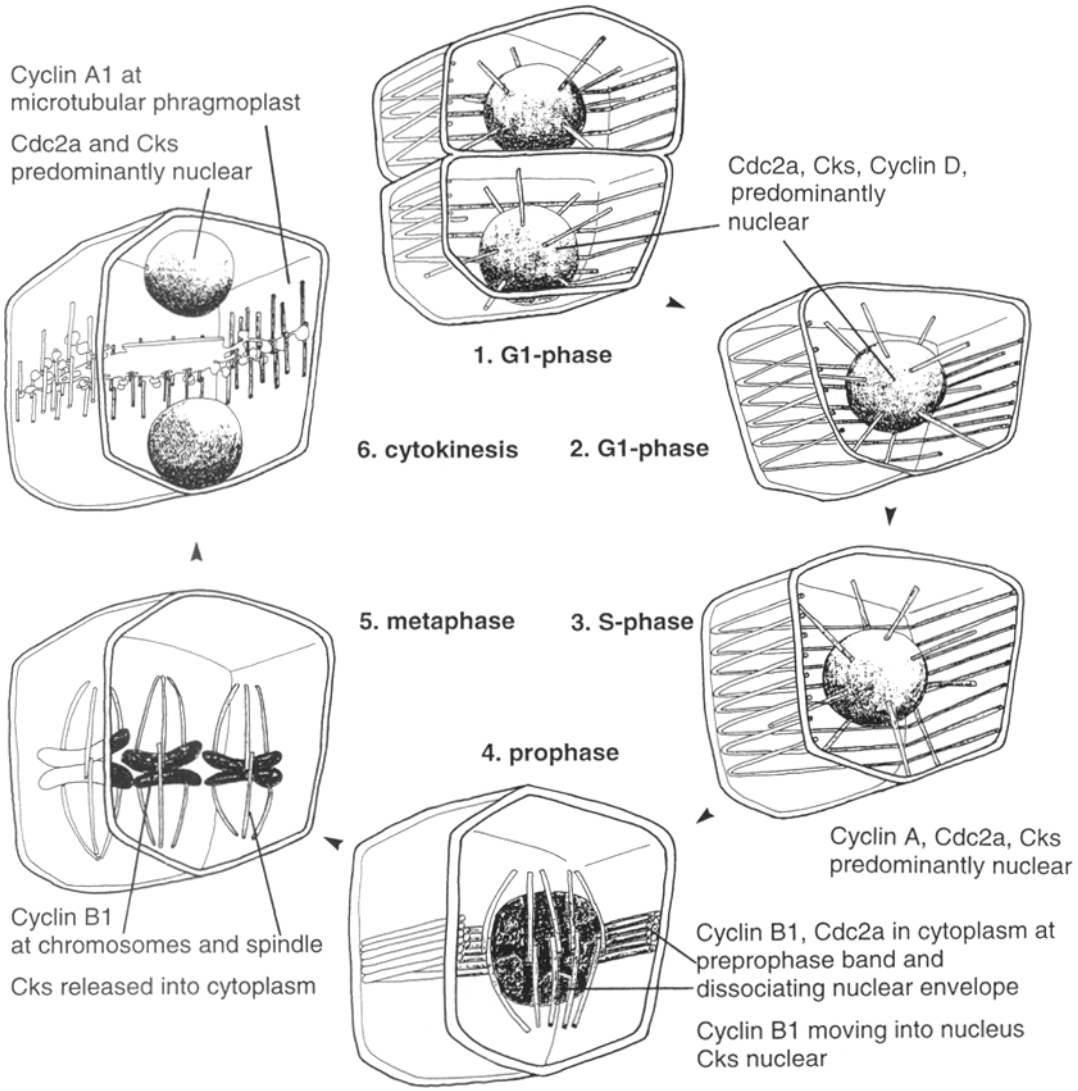

Fig. 3. Summary of locations of some cyclins and associated proteins through the cell cycle. Only selected stages of division are illustrated and for clarity microtubules are increased in diameter and greatly reduced in number. In reality microtubules are so numerous that they form a haze in which individual tubules cannot be distinguished by light microscopy; for example, cell 4 corresponds with Fig. 1 A, cell 5 corresponds with Fig. 1 D bottom left, and cell 6 corresponds with Fig. 2 C. Although Cdc2a is described as predominantly nuclear at times when the nucleus is enclosed by membrane, its cytoplasmic presence is quantitatively significant because of the greater volume and it allows an association with PPB microtubules at prophase (*4*). Functions of cyclins, Cdc2a, and Cks are described in the text together with references. It is expected that future study of the many plant cyclins and interacting proteins will reveal many more locations and functions

nuclear location and function in the transcription of genes for DNA replication and in the activity of DNA replication origins (Sects. 6 and 7) but would not be concerned with the later events of mitosis. However, plant cyclins that are structurally similar to animal A-type cyclins are difficult to recognise. Sequence comparison focuses on small regions including the 9-amino-acid destruction box motif (Sect. 2) and the cyclin box region of about 250 amino acids. Outside the cyclin box there is little sequence conservation for comparison, yet the cyclin box is mainly concerned with binding Cdk and it is the remaining half of the molecule that has the prime function of binding substrate proteins. In the cyclin box, plant cyclins designated as A-type are approximately 37% similar with animal and yeast cyclin A, but also 26% similar with the B-cyclins. Clearly overall similarity in the cyclin box is not striking, therefore small regions whose biological functions are not known have been used in attempted discrimination. Plant A-type cyclins have

L_{59} VEV-EEY (which is close to the MRAIL region) and plant B-type cyclins have the small motif H_{63}-KF found in yeast B-cyclins (Renaudin et al. 1998).

It has therefore been cautioned that designations of plant cyclins as A-type or B-type does not imply homology of function with animal cyclins. This is underlined by the behaviour of maize CycA1;zm;1, which has properties typical of both animal A-type and B-type cyclins. Persistence of CycA1;zm;1 through telophase is unlike the anaphase proteolysis of animal B-type cyclins, but cytoplasmic retention of CycA1;zm;1 and presence of a putative CRS (Mews et al. 1997) is like animal B-type cyclins, and it is significant in allowing retention of this cyclin in the cytoplasm at the phragmoplast, after nuclei have re-formed at telophase.

Difficulty of predicting function is also seen in the pair of cyclins CycB1zm;1 and CycB1zm;2 that are classified together from structural similarity in most of the molecule (Renaudin et al. 1998) but behave quite differently. CycB1zm;1 behaves like animal cyclin A (Mews et al. 1997) in being essentially nuclear and dispersing evenly at nuclear-envelope breakdown, exactly like CKS (Hepler et al. 1994), whereas CycB1zm;2 has cytoplasmic retention and the range of mitotic locations described (Fig. 1). Further functional difference is seen in the marked anaphase proteolysis of CycB1zm;2 but not CycB1zm;1, although both are highly conserved in the destruction box itself. Functional difference is also implied in the different times of accumulation, which for cyclin CycB1zm;1 occurs in G_1 phase but for CycB1zm;2 in G_2 phase. This difference in timing is consistently seen during ongoing cell proliferation and also when cell division is reinitiated (Mews et al. 1997, 2000). One structural clue to the different in vivo properties of these cyclins is the presence of a putative CRS sequence in CycB1zm;2 upstream of the cyclin box. This retention sequence, together with other regions outside the cyclin box that are not yet understood in terms of effect on function, may explain the difference in behaviour and function.

Plant cyclins therefore have mixed properties and functions compared with those of animals, as well as some structural and functional elements that have no close animal counterpart. Such mixed properties are indeed not unknown in animals, where cyclin B3 shares properties with both A- and B-type cyclins (Gallant and Nigg 1994).

13 Conclusion

In the plant kingdom it is becoming clear that cyclins make multiple contributions, being involved at sites of change to the cytoskeleton, nucleus, chromosomes, spindle, and phragmoplast. Cyclins bind diverse proteins, including some like Cks that are not themselves Cdk substrates but have the capacity to bind other proteins that are substrates. The binding activities of cyclins, and their diverse locations in the plant cell, suggest that cyclins function as scaffolds in appropriate locations, on which different groups of proteins including substrates of Cdk or modifiers of Cdk activity can assemble and where Cdk enzyme can select multiple phosphorylation sites in the assembled proteins.

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References

Adams PD, Sellers WR, Sharma SK, Wu AD, Nalin CM, Kaelin WG Jr (1996) Identification of a cyclin-cdk2 recognition motif present in substrates and p21-like cyclin-dependent kinase inhibitors. Mol Cell Biol 16: 6623–6633

– Li X, Sellers WR, Baker KB, Leng X, Harper JW, Taya Y, Kaelin WG Jr (1999) Retinoblastoma protein contains a C-terminal motif that targets it for phosphorylation by cyclin-cdk complexes. Mol Cell Biol 19: 1068–1080

Aerne BL, Johnson AL, Toyn JH, Johnston LH (1998) Swi5 controls a novel wave of cyclin synthesis in late mitosis. Mol Biol Cell 9: 945–956

Amon A, Tyers M, Futcher B, Nasmyth K (1993) Mechanisms that help the yeast cell cycle clock tick: G2 cyclins transriptionally activate G2 cyclins and repress G1 cyclins. Cell 74: 993–1007

– Irniger S, Nasmyth K (1994) Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle. Cell 77: 1037–1050

Asada T, Kuriyama R, Shibaoka H (1996) TKRP125, a kinesin-related protein involved in the centrosome-independent organization of the cytokinetic apparatus in tobacco BY-2 cells. J Cell Sci 110: 179–190

Bähler J, Steever AB, Wheatley S, Wang Yl, Pringle JR, Gould KL, McCollum D (1998) Role of polo kinase and Mid1p in determining the site of cell division in fission yeast. J Cell Biol 143: 1603–1616

Bazan JF (1996) Helical fold prediction for the cyclin box. Proteins Struct Funct Genet 24: 1–17

Beach D, Durkacz B, Nurse P (1982) Functionally homologous cell cycle control genes in budding and fission yeast. Nature 300: 706–709

Bögre L, Calderini O, Binarova P, Mattauch M, Till S, Kiegerl S, Jonak C, Pollaschek C, Barker P, Huskisson NS, Hirt H, Heberle-

Bors E (1999) A MAP kinase is activated late in plant mitosis and becomes localized to the plane of cell division. Plant Cell 11: 101–113

Brizuela L, Draetta G, Beach D (1987) $p13^{suc1}$ acts in the fission yeast cell cycle as a component of the $p34^{cdc2}$ protein kinase. EMBO J 6: 3507–3514

Brown NR, Noble MEM, Lawrie A, Morris M, Tunnah P, Divita G, Johnson LN, Endicott JA (1999) Effects of phosphorylation of threonine 160 on cyclin-dependent kinase-2 structure and activity. J Biol Chem 274: 8746–8756

Brown RC, Lemmon BE (1997) The quadripolar microtubule system in lower land plants. J Plant Res 110: 93–106

Clarke DJ, Segal M, Mondesert G, Reed SI (1999) The Pds1 anaphase inhibitor and Mec1 kinase define distinct checkpoints coupling S phase with mitosis in budding yeast. Curr Biol 9: 365–368

Cleary AL, Smith LG (1998) The Tangled1 gene is required for spatial control of cytoskeletal arrays associated with cell division during maize leaf development. Plant Cell 10: 1875–1888

Colasanti J, Tyers M, Sundaresan V (1991) Isolation and characterisation of cDNA clones encoding a functional $p34^{cdc2}$ homologue from *Zea mays*. Proc Natl Acad Sci USA 88: 3377–3381

– Cho S-O, Wick S, Sundaresan V (1993) Localisation of the functional $p34^{cdc2}$ homologue of maize in root tip and stomatal complex cells: association with predicted division sites. Plant Cell 5: 1101–1111

Correa-Bordes J, Nurse P (1995) $p25^{rum1}$ orders S-phase and mitosis by acting as an inhibitor of the $p34^{cdc2}$ mitotic kinase. Cell 83: 1001–1009

Cross FR, Blake CM (1993) The yeast Cln3 protein is an unstable activator of Cdc28. Mol Cell Biol 13: 3266–3271

Dahl M, Meskiene I, Bögre L, Ha DTC, Swoboda I, Hubman R, Hirt H, Heberle-Bors E (1995) The D-type alfalfa gene cycMs4 complements G1cyclin-deficient yeast and is induced in the G1 phase of the cell cycle. Plant Cell 7: 1847–1857

Day IS, Reddy AS, Golovkin M (1996) Isolation of a new mitotic-like cyclin from Arabidopsis: complementation of a yeast cyclin mutant with a plant cyclin. Plant Mol Biol 30: 565–575

Deshaies RJ (1995) The self destructive personality of a cell cycle in transition. Curr Opin Cell Biol 7: 781–789

De Veylder L, Engler JD, Burssens S, Manevski A, Lescure B, Van Montagu M, Engler G, Inzé D (1999) A new D-type cyclin of *Arabidopsis thaliana* expressed during lateral root primordia formation. Planta 208: 453–462

Doonan J, Fobert P (1997) Conserved and novel regulators of the plant cell cycle. Curr Opin Cell Biol 9: 824–830

Dowdy SF, Hinds PW, Louie K, Reed SI, Arnold A, Weinberg RA (1993) Physical interaction of the retinoblastoma protein with human D cyclins. Cell 73: 499–511

Draetta G, Beach D (1988) Activation of cdc2 protein kinase during mitosis in human cells: cell cycle-dependent phosphorylation and subunit rearrangement. Cell 54: 17–26

Dudits D, Magyar Z, Deak M, Meszaros T, Miscolczi P, Feher A, Brown S, Kondorosi E, Athanasiadis A, Pongor S, Bako L, Koncz C, Gyorgyey J (1998) Cyclin dependent and calcium dependent kinase families: response of cell division cycle to hormone and stress signals. In: Francis D, Dudits D, Inzé D (eds) Plant cell division. Portland, Colchester, pp 21–45

Dynlacht BD, Flores O, Lees JA, Harlow E (1994) Differential regulation of E2F transactivation by cyclin/cdk2 complexes. Genes Dev 8: 1772–1786

Enoch T, Matthias P, Nurse P, Nigg EA (1991) $p34^{cdc2}$ acts as a lamin kinase in fission yeast. J Cell Biol 112: 797–807

Epstein CB, Cross FR (1995) CLB5 a novel B cyclin from buding yeast with a role in S phase. Genes Dev 6: 1695–1706

Evans T, Rosenthal ET, Youngblom J, Distel D, Hunt T (1983) Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell 33: 386–389

Ferreira PCG, Hemerly AS, Vilarroel R, Van Montagu M, Inzé D (1991) The Arabidopsis functional homolog of the $p34^{cdc2}$ protein kinase. Plant Cell 3: 531–540

– – Engler JD, van Montagu M, Engler G, Inze D (1994) Developmental expression of the Arabidopsis cyclin gene *cyc1At*. Plant Cell 6: 1763–1774

Fisher D, Nurse P (1996) A single fission yeast mitotic cyclin B $p34^{cdc2}$ kinase promotes both S-phase and mitosis in the absence of G1 cyclins. EMBO J 15: 850–860

Fitch I, Dahmann C, Surana U, Amon A, Nasmyth K, Goetsch L, Byers B, Futcher B (1992) Characterization of four B-type cyclin genes of the budding yeast *Saccharomyces cerevisiae*. Mol Biol Cell 1992 3: 805–818

Fobert PR, Coen ES, Murphy GJP, Doonan JH (1994) Patterns of cell division revealed by transcriptional regulation of genes during the cell cycle in plants. EMBO J 13: 616–624

Forsburg SL, Nurse P (1994) Analysis of *Schizosaccharomyces pombe* cyclin puc1: evidence for a role in cell cycle exit. J Cell Sci 107: 601–613

Funabiki H, Murray AW (2000) The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. Cell 102: 411–424

Gallant P, Nigg EA (1994) Identification of a novel vertebrate cyclin: cyclin B3 shares properties with both A- and B-type cyclins. EMBO J 13: 595–605

Gaudin V, Lunness PA, Fobert PR, Towers M, Riou-Khamlichi C, Murray JA, Coen E, Doonan JH (2000) The expression of D-cyclin genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the Cycloidea gene. Plant Physiol 122: 1137–1148

Geng Y, Whoriskey W, Park MY, Bronson RT, Medema RH, Li T, Weinberg RA, Sicinski P (1999) Rescue of cyclin D1 deficiency by knockin cyclin E. Cell 97: 767–777

Genschik P, Criqui MC, Parmentier Y, Derevier A, Fleck J (1998) Cell cycle-dependent proteolysis in plants: identification of the destruction box pathway and metaphase arrest produced by the protease inhibitor MG132. Plant Cell 10: 2063–2075

Glotzer M, Murray AW, Kirschner MW (1991) Cyclin is degraded by the ubiquitin pathway. Nature 349: 132–138

Gorst JR, Sek FJ, John PCL (1991) Level of $p34^{cdc2}$-like protein in dividing, differentiating and dedifferentiating cells of carrot. Planta 185: 304–310

Gunning BES (1982) The cytoskeletal apparatus: its development and spatial regulation. In: Lloyd CW (ed) The cytoskeleton in plant growth and development. Academic Press, New York, pp 230–288

Hadwiger JA, Wittenberg C, Richardson HE, de Barros Lopes M, Reed SI (1989) A family of cyclin homologues that control the G1 phase in yeast. Proc Natl Acad Sci USA 86: 6255–6259

Hagting A, Jackman M, Simpson K, Pines J (1999) Translocation of cyclin B1 to the nucleus at prophase requires a phosphorylation-dependent nuclear import signal. Curr Biol 9: 680–689

Harris SD, Kraus PR (1998) Regulation of septum formation in *Aspergillus nidulans* by a DNA damage checkpoint pathway. Genetics 148: 1055–1067

Hasezawa S, Nagata T (1992) Okadaic acid as a probe to analyse the cell cycle progression in plant cells. Bot Acta 105: 63–69

Hata S, Kouchi H, Suzuka I, Ishii T (1991) Isolation and characterisation of cDNA clones for plant cyclins EMBO J 10: 2681–2688

Hayles J, Fisher D, Woolard A, Nurse P (1994) Temporal order of S phase and mitosis in fission yeast is determined by the state of the $p34^{cdc2}$-mitotic B cyclin complex. Cell 78: 813–822

Heese M, Mayer U, Jurgens G (1998) Cytokinesis in flowering plants: cellular processes and developmental integration. Curr Opin Plant Biol 1: 486–491

Hemerly A, Bergounioux C, Van Montagu M, Inze D, Ferreira P (1992) Genes regulating the plant cell cycle: isolation of a mitotic-like cyclin from *Arabidopsis thaliana*. Proc Natl Acad Sci USA 89: 3295–3299

– Engler Jde A, Bergounioux C, Van Montagu M, Engler G, Inzé D, Ferriera P (1995) Dominant negative mutants of Cdc2 kinase uncouple cell division from iterative plant development. EMBO J 14: 3925–3936

Hepler PK, Sek FJ, John PCL (1994) Nuclear concentration and mitotic dispersion of the essential cell cycle protein, $p13^{suc1}$, examined in living cells. Proc Natl Acad Sci USA 91: 2176–2180

Hirt H, Pay A, Gyorgyey J, Bako L, Newmeth K, Borge L, Schweyen R, Heberle-Bors E, Dudits D (1991) Complementation of a yeast cell cycle mutant by an alfalfa cDNA encoding a protein kinase homologous to $p34^{cdc2}$. Proc Natl Acad Sci USA 88: 1636–1640

Hoffmann I, Draetta G, Karsenti E (1994) Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. EMBO J 13: 4302–4310

Hoyt MA (1997) Eliminating all obstacles: regulated proteolysis in the eukaryotic cell cycle. Cell 91: 149–151

Huang KN, Odinsky SA, Cross FR (1997) Structure-function analysis of the *Saccharomyces cerevisiae* G1 cyclin Cln2. Mol Cell Biol 17: 4654–4666

Huntley RP, Murray JAH (1999) The plant cell cycle. Curr Opin Plant Biol 2: 440–446

– Healy S, Freeman D, Lavender P, de Jager S, Greenwood J, Makker J, Walker E, Jackman M, Xie Q, Bannister AJ, Kouzarides T, Gutierrez C, Doonan JH, Murray JAH (1998) The maize retinoblastoma protein homologue ZmRb-1 is regulated during leaf development and displays conserved interactions with G1/S regulators and plant cyclin D (CycD) proteins. Plant Mol Biol 37: 155–169

Hush JM, Wu L, John PCL, Hepler LH, Hepler PK (1996) Plant mitosis promoting factor disassembles the microtubule preprophase band and accelerates prophase progression in *Tradescantia*. Cell Biol Int 20: 275–287

Ito M, Iwase M, Kodama H, Lavisse P, Komamine A, Nishihama R, Machida Y, Watanabe A (1998) A novel cis-acting element in promoters of plant B-type cyclin genes activates M phase-specific transcription. Plant Cell 10: 331–341

Jackman M, Firth M, Pines J (1995) Human cyclins B1 and B2 are localised to strikingly different structures: B1 to microtubules, B2 primarily to Golgi apparatus. EMBO J 14: 1646–1654

Jacobs T (1995) Cell cycle control. Annu Rev Plant Physiol Plant Mol Biol 46: 317–339

Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massague J, Pavletich NP (1995) Mechanism of CDK activation revealed by the structure of a cyclin A-CDK2 complex. Nature 376: 313–320

John PCL (1996) The plant cell cycle: conserved and unique features in mitotic control. In: Meijer L, Guidet S, Vogel L (eds) Progress in cell cycle research, vol 2. Plenum, New York, pp 59–72

– Sek FJ, Lee MG (1989) A homologue of the cell cycle control protein $p34^{cdc2}$ participates in the cell division cycle of *Chlamydomonas* and a similar protein is detectable in higher plants and remote taxa. Plant Cell 1: 1185–1193

– – Carmichael JP, McCurdy DW (1990) $p34^{cdc2}$ homologue level, cell division, phytohormone responsiveness and cell differentiation in wheat leaves. J Cell Sci 97: 627–630

– – Hayles J (1991) Association of the plant $p34^{cdc2}$-like protein with $p13^{suc1}$: implications for control of cell division cycles in plants. Protoplasma 161: 70–74

Katsuta J, Shibaoka H (1992) Inhibition by kinase inhibitors of the development and disappearance of the preprophase band of microtubules in tobacco BY-2 cells. J Cell Sci 103: 397–405

Kim HY, Cho Y (1997) Structural similarity between the pocket region of retinoblastoma tumour suppressor and the cyclin-box. Nat Struct Biol 4: 390–3955

King RW, Peters JM, Tugendreich S, Rolfe M, Hieter P, Kirschner MW (1995) A 20s complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. Cell 81: 279–288

Kobayashi H, Stewart E, Poon R, Adamczewski JP, Gannon J, Hunt T (1992) Identification of the domains in cyclin A required for binding to, and activation of, $p34^{cdc2}$ and $p32^{cdk2}$ protein kinase subunits. Mol Biol Cell 3: 1279–1294

Koch C, Moll T, Neuberg M, Ahorn H, Nasmyth K (1993) A role for the transcription factors Mbp1 and Swi4 in progression from G1 to S phase. Science 261: 1551–1557

Kouchi H, Sekine M, Hata S (1995) Distinct classes of mitotic cyclins are differentially expressed in the soybean shoot apex during the cell cycle. Plant Cell 7: 1143–1155

Landrieu I, Odaert B, Wieruszeski JM, Drobecq H, Rousselot-Pailley P, Inze D, Lippens G (2000) $p13^{suc1}$ and the WW domain of PIN1 bind to the same phosphothreonine-proline epitope. J Biol Chem 276: 1434–1438

Lew DJ, Reed SI (1993) Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins. J Cell Biol 120: 1305–1320

– Dulic V, Reed SI (1991) Isolation of three novel human cyclins by rescue of G1 cyclin (Cln) function in yeast. Cell 66: 1197–1206

– Marini NJ, Reed SI (1992) Different G1 cyclins control the timing of cell cycle commitment in mother and daughter cells of the budding yeast *S. cerevisiae*. Cell 69: 317–327

– Weinert T, Pringle JR (1997) Cell cycle control in *Saccharomyces cerevisiae*. In: Pringle JR, Broach JR, Jones EW (eds) The molecular and cellular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Plainview, NY, pp 607–695

Li LJ, Naeve GS, Lee AS (1993) Temporal regulation of cyclin A-p107 and $p33^{cdk2}$ complexes binding to a human thymidine kinase promoter element important for G1-S phase transcriptional regulation. Proc Natl Acad Sci USA 90: 3554–3558

Li J, Meyer AN, Donoghue DJ (1997) Nuclear localization of cyclin B1 mediates its biological activity and is regulated by phosphorylation. Proc Natl Acad Sci USA 94: 502–507

Liu B, Cyr RJ, Palevitz BA (1996) A kinesin-like protein, KatAp, in the cells of *Arabidopsis* and other plants. Plant Cell 8: 119–132

Lokhorst GM, Sluiman HJ, Star W (1988) The ultrastructure of mitosis and cytokinesis in the sarcinoid *Chlorokybus atmosphyticus* (Chlorophyta Charophyceae) revealed by rapid freeze fixation and freeze substitution. J Phycol 24: 237–248

Lopez-Girona A, Furnari B, Mondesert O, Russell P (1999) Nuclear localisation of Cdc25 regulated by DNA damage and 14-3-3 protein. Nature 397: 172–175

Lu PJ, Zhou XZ, Shen M, Lu KP (1999) Function of WW domains as phosphoserine- or phosphothreonine-binding modules. Science 283: 1325–1328

Luciani MG, Hutchins JRA, Zheleva D, Hupp TR (2000) The C-terminal regulatory domain of p53 contains a functional docking site for cyclin A. J Mol Biol 300: 503–518

Magyar Z, Bako L, Bögre L, Dedeoglu D, Dudits D (1993) Active *cdc2* genes and cell cycle phase-specific cdc2-related kinase complexes in hormone-stimulated alfalfa cells. Plant J 4: 151–161

– Mészáros T, Miskolczi P, Deák M, Fehér A, Brown S, Kondorosi E, Athanasiadis A, Pongor S, Bilgin M, Bakó S, Koncz C, Dudits

D (1997) Cell cycle phase specificity of putative cyclin-dependent kinase variants in synchronized alfalfa cells. Plant Cell 9: 223–235
Mailand N, Falck J, Lukas C, Syljuasen RG, Welcker M, Bartek J, Lukas J (2000) Rapid destruction of human Cdc25A in response to DNA damage. Science 288: 1425–1429
Mariconti L, Albani D, Pitto L, Moroni C, Helin K, Cella R (2000) Molecular characterisation of a plant E2F-like transcription factor. Plant Mol Biol Rep 18: 20
Martín-Castellanos C, Blanco MA, de Prada JM, Moreno S (2000) The puc1 cyclin regulates the G1 phase of the fission yeast cell cycle in response to cell size. Mol Biol Cell 11: 543–554
Matsushime H, Roussel MF, Ashmun RA, Sherr CJ (1991) Colony-stimulating factor 1 regulates novel cyclins during the G1 phase of the cell cycle. Cell 65: 701–713
Melan MA, Sluder G (1992) Redistribution and differential extraction of soluble proteins in permeabilized cultured cells. J Cell Sci 101: 731–734
Meskiene I, Bögre L, Dahl M, Pirck M, Ha DTC, Swoboda I, Heberle-Bors E, Ammerer G, Hirt H (1995) cycMs3, a novel B-type cyclin gene, is induced in the G0-to-G1 transition of the cell cycle. Plant Cell 7: 759–771
Mews M, Sek FJ, Moore R, Volkmann D, Gunning BES, John PCL (1997) Mitotic cyclin distribution during maize cell division: implications for the sequence diversity and function of cyclins in plants. Protoplasma 200: 128–145
– – Volkmann D, John PCL (2000) Immunodetection of four mitotic cyclins and the Cdc2a protein kinase in the maize root: their distribution in cell development and dedifferentiation. Protoplasma 212: 236–249
Michealis C, Ciosk R, Nasmyth K (1997) Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91: 35–45
Millar JBA, McGowan CH, Lenaers G, Jones R, Russell P (1991) $p80^{cdc25}$ mitotic inducer is the tyrosine phosphatase that activates $p34^{cdc2}$ kinase in fission yeast. EMBO J 10: 4301–4309
Mineyuki Y, Yamashita M, Nagahama Y (1991) $p34^{cdc2}$ kinase homologue in the preprophase band. Protoplasma 162: 182–186
– Aioi H, Yamashita M, Nagahama Y (1996) A comparative study on stainability of preprophase bands by the PSTAIR antibody. J Plant Res 109: 185–192
Mironov V, De Veylder L, Van Montagu M, Inzé D (1999) Cyclin-dependent kinases and cell division in plants: the nexus. Plant Cell 11: 509–522
Moreno S, Hayles J, Nurse P (1989) Regulation of $p34^{cdc2}$ protein kinase during mitosis. Cell 58: 361–372
– Nurse P (1994) Regulation of progression through the G1 phase of the cell cycle by the *rum1* gene. Nature 367: 236–242
Morgan DO (1995) Principles of CDK regulation. Nature 374: 131–134
Murray AW, Solomon MJ, Kirschner MW (1989) The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. Nature 339: 275–280
Murray JAH, Freeman D, Greenwood J, Huntley R, Makkerh J, Riou-Khamlichi C, Sorrell DA, Cockroft C, Carmichael JP, Soni R, Shah ZH (1998) Plant D cyclins and retinoblastoma protein homologues. In: Francis D, Dudits D, Inzé D (eds) Plant cell division. Portland, Colchester, pp 99–127
Nash R, Tokiwa G, Anand S, Erickson K, Futcher AB (1988) The WHI1+ gene of *Saccharomyces cerevisiae* tethers cell division to cell size and is a cyclin homolog. EMBO J 7: 4335–4346
Nasmyth K (1996) At the heart of the budding yeast cell cycle. Trends Genet 12: 405–412
Nebenführ A, Frohlick JA, Staehelin A (2000) Redistribution of Golgi stacks and other organelles during mitosis and cytokinesis in plant cells. Plant Physiol 124: 135–151
Nigg EA (1991) The substrates of the cdc2 kinase. Semin Cell Biol 2: 261–270
– (1995) Cyclin-dependent protein kinases; key regulators of the eukaryotic cell cycle. Bioessays 17: 471–480
Nurse P (1990) Universal control mechanism regulating onset of M-phase. Nature 344: 503–508
– (1997) Checkpoint pathways come of age. Cell 91: 865–867
– Bissett Y (1981) Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. Nature 292: 558–560
Ookata K, Hisanaga S-I, Okumura E, Kishimoto T (1993) Association of $p34^{cdc2}$/cyclin B complex with microtubules in starfish. J Cell Sci 105: 873–881
– – Bulinski JC, Murofushi H, Aizawa H, Itoh TJ, Hotani H, Okumura E, Tachibana K, Kishimoto T (1995) Cyclin B interaction with microtubule-associated protein 4 (MAP4) targets p34cdc2 kinase to microtubules and is a potential regulator of M-phase microtubule dynamics. J Cell Biol 128: 849–862
Osborn M, Weber K (1982) Immunofluorescence and immunocytochemical procedures with affinity purified antibodies: tubulin containing structures. Methods Cell Biol 24: 98–132
Pagano M, Pepperkok R, Verde F, Ansgorge W, Draetta G (1992) Cyclin A is required at two points in the human cell cycle. EMBO J 11: 961–972
– Tam SW, Theodoras AM, Beer-Romero P, Del Sal G, Chau V, Yew PR, Dreatta GF, Rolfe M (1995) Role of the ubiquitin-proteosome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. Science 269: 682–685
Pan ZQ, Amin A, Hurwitz J (1993) Characterization of the in vitro reconstituted cyclin A or B1-dependent cdk2 and cdc2 kinase activities. J Biol Chem 268: 20443–20451
Parmentier Y, Criqui MC, Derevier A, Shen W-H, Genschik P (2000) Cell cycle-dependent proteolysis and ectopic expression of cyclin B1 in tobacco BY2 cells. Plant Mol Biol Rep Suppl 18: S01–S64
Patra D, Dunphy WG (1998) Xe-p9, a Xenopus Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase-promoting complex at mitosis. Genes Dev 12: 2549–2559
– Wang SX, Kumagai A, Dunphy WG (1999) The Xenopus Suc1/Cks protein promotes the phosphorylation of G_2/M regulators. J Biol Chem 274: 36839–36842
Peeper DS, Parker LL, Ewen ME, Toebes M, Hall FL, Xu M, Zantema A, van der Eb AJ, Piwnica-Worms H (1993) A- and B-type cyclins differentially modulate substrate specificity of cyclin-cdk complexes. EMBO J 12: 1947–1954
Pines J, Hunter T (1991) Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. J Cell Biol 115: 1–17
– – (1994) The differential localisation of human cyclins A and B is due to a cytoplasmic retention signal in cyclin B. EMBO J 13: 3772–3781
Qin LX, Perennes C, Richard L, Bouvier-Durand M, Tréhin C, Inze D, Bergounioux C (1996) G2- and early-M-specific expression of the NTCYC1 cyclin gene in *Nicotiana tabacum* cells. Plant Mol Biol 32: 1093–1101
Reichheld J-P, Chaubet N, Shen WH, Renaudin J-P, Gigot C (1996) Multiple A-type cyclins express sequentially during the cell cycle in *Nicotiana tabacum* BY2 cells. Proc Natl Acad Sci USA 93: 13819–13824
Renaudin JP, Colasanti J, Rime H, Yuan ZA, Sundaresan V (1994) Cloning of four cyclins from maize indicates that higher plants have three structurally distinct groups of mitotic cyclins. Proc Natl Acad Sci USA 91: 7375–7379
– Savouré A, Philippe H, Van Montagu M, Inzé D, Rouzé P (1998) Characterization and classification of plant cyclin sequences

related to A- and B-type cyclins. In: Francis D, Dudits D, Inzé D (eds) Plant cell division. Portland, Colchester, pp 67–98

Resnitzky D, Hengst L, Reed SI (1995) Cyclin A-associated kinase activity is rate limiting for entrance into S phase and is negatively regulated in G1 by p27Kip1. Mol Cell Biol 15: 4347–4352

Richardson H, Lew DJ, Henze M, Sugimoto K, Reed SI (1992) Cyclin-B homologues in *Saccharomyces cerevisiae* function in S phase and in G2. Genes Dev 6: 2021–2034

– Wittenberg C, Cross FR, Reed SI (1989) An essential G1 function for cyclin-like proteins in yeast. Cell 59: 1127–1133

Riou-Khamlichi C, Huntley R, Jacqmard A, Murray JA (1999) Cytokinin activation of *Arabidopsis* cell division through a D-type cyclin. Science 283: 1541–1544

Rogers S, Wells R, Rechsteiner M (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 234: 364–368

Romanowski P, Marr J, Madine MA, Rowles A, Blow JJ, Gautier J, Laskey RA (2000) Interaction of Xenopus Cdc2 × cyclin A1 with the origin recognition complex. J Biol Chem 275: 4239–4424

Rosenberg AR, Zindy F, Le Deist F, Mouly H, Metezeau P, Brechot C, Lamas E (1995) Overexpression of human cyclin A advances entry into S phase. Oncogene 10: 1501–1509

Russo AA, Jeffrey PD, Pavletich NP (1996) Structural basis of cyclin-dependent kinase activation by phosphorylation. Nat Struct Biol 3: 696–700

Saul DJ, Sudbery PE (1985) Molecular cloning of WHI2, a gene involved in the regulation of cell proliferation in *Saccharomyces cerevisiae*. J Gen Microbiol 131: 1797–1806

Schneider BL, Yang Q-H, Futcher AB (1996) Linkage of replication to Start by the Cdk inhibitor Sic1. Science 272: 560–562

Schulman BA, Lindstrom DL, Harlow E (1998) Substrate recruitment to cyclin-dependent kinase 2 by a multipurpose docking site on cyclin A. Proc Natl Acad Sci USA 95: 10453–10458

Schumacher JM, Golden A, Donovan PJ (1998) AIR-2: an Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos. J Cell Biol 143: 1635–1646

Schwob E, Nasmyth K (1993) *CLB5* and *CLB6*, a new pair of B cyclins involved in DNA replication in *Saccharomyces cerevisiae*. Genes Dev 7: 1160–1175

– Böhm T, Mendenhall MD, Nasmyth K (1994) The B-type cyclin kinase inhibitor p40 *SIC1* controls the G1 to S transition in *S. cerevisiae*. Cell 79: 233–244

Segal M, Clarke DJ, Maddox P, Salmon ED, Bloom K, Reed SI (2000) Coordinated spindle assembly and orientation requires Clb5p-dependent kinase in budding yeast. J Cell Biol 148: 441–452

Segers G, Gadisseur I, Bergounioux C, Engler JdeA, Jacqmard A, Van Montagu M, Inzé D (1996) The *Arabidopsis* cyclin-dependent kinase gene *cdc2bAt* is preferentially expressed during S and G2 phases of the cell cycle. Plant J 10: 601–612

Setiady YY, Sekine M, Hariguchi N, Yamamoto T, Kouchi H, Shinmyo A (1995) Tobacco mitotic cyclins: cloning, characterization, gene expression and functional assay. Plant J 8: 949–957

Shaul O, Mironov V, Burssens S, Van Montagu M, Inzé D (1996) Two Arabidopsis cyclin promoters mediate distinctive transcriptional oscillation in synchronized tobacco BY-2 cells. Proc Natl Acad Sci USA 93: 4868–4872

Sheaff RJ, Groudine M, Gordon M, Roberts JM, Clurman BE (1997) Cyclin E-CDK2 is a regulator of p27Kip1. Genes Dev 11: 1464–1478

Shen M, Stukenberg PT, Kirschner MW, Lu KP (1998) The essential mitotic peptidyl-prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins. Genes Dev 12: 706–720

Sherr CJ (1993) Mammalian G1 cyclins. Cell 73: 1059–1065

– (1996) Cancer cell cycles. Science 274: 1672–1677

Shirayama M, Tóth A, Gálová M, Nasmyth K (1999) APC^{Cdc20} promotes exit from mitosis by destroying the anaphase inhibitor Pds1 and cyclin Clb5. Nature 402: 203–207

Sigrist S, Jacobs H, Stratmann R, Lehner C (1995) Exit from mitosis is regulated by *Drosophila* fizzy and the sequential destruction of cyclins A, B and B3. EMBO J 14: 4827–4838

Smith HM, Raikhel NV (1999) Protein targeting to the nuclear pore: what can we learn from plants? Plant Physiol 119: 1157–1164

Songyang Z, Blechner S, Hoagland N, Hoekstra MF, Piwnica-Worms H, Cantley LC (1994) Use of an oriented peptide library to determine the optimal substrates of protein kinases. Curr Biol 4: 973–982

Soni R, Carmichael JP, Shah ZH, Murray JAH (1995) A family of cyclin D homologs from plants differentially controlled by growth regulators and containing the conserved retinoblastoma protein interaction motif. Plant Cell 7: 85–103

Sorrell DA, Combettes B, Chaubet-Gigot N, Gigot C, Murray JAH (1999) Distinct cyclin D genes show mitotic accumulation or constant levels of transcripts in tobacco bright yellow-2 cells. Plant Physiol 119: 343–351

Stals H, Bauwens S, Traas J, Van Montagu M, Engler G, Inzé D (1997) Plant CDC2 is not only targeted to the preprophase band, but also co-localises with the spindle, phragmoplast and chromosomes. FEBS Lett 418: 229–234

Stern B, Nurse P (1996) A quantitative model for the cdc2 control of S phase and mitosis in fission yeast. Trends Genet 12: 345–350

Stillman B (1996) Cell cycle control of DNA replication. Science 274: 1659–1664

Stuart D, Wittenberg C (1998) Clb5 and Clb6 are required for premeiotic DNA replicatiion and activation of the meiotic S/M checkpoint. Genes Dev 12: 2698–2710

Sundaresan V, Colasanti J (1998) Cyclin dependent kinases in higher plants: spatial and temporal control of cell division. In: Francis D, Dudits D, Inzé D (eds) Plant cell division. Portland, Colchester, pp 47–65

Surana U, Robitsch H, Price C, Schuster T, Fitch I, Futcher AB, Nasmyth K (1991) The role of CDC28 and cyclins during mitosis in the budding yeast *S. cerevisiae*. Cell 65: 145–161

Tyers M (1996) The cyclin dependent kinase inhibitor p40 SIC1 imposes the requirement for Cln G1 cyclin function at Start. Proc Natl Acad Sci USA 93: 7772–7776

– Tokiwa G, Futcher B (1993) Comparison of the *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. EMBO J 12: 1955–1968

Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, Qureshi-Emili A, Li Y, Godwin B, Conover D, Kalbfleisch T, Vijayadamodar G, Yang M, Johnston M, Fields S, Rothberg JM (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. Nature 403: 623–627

Vlach J, Hennecke S, Amati B (1997) Phosphorylation-dependent degradation of the cyclin-dependent kinase inhibitor p27. EMBO J 16: 5334–5544

Vos JW, Hepler PK (1998) Calmodulin is uniformly distributed during cell division in living stamen hair cells of *Tradescantia virginiana*. Protoplasma 201: 158–171

Wang H, Fowke LC, Crosby WL (1997) A plant cyclin-dependent kinase inhibitor gene. Nature 386: 451–452

– Qi Q, Schorr P, Cutler A, Crosby WL, Fowke LC (1998) ICK1, a cyclin dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3,

and its expression is induced by abscisic acid. Plant J 5: 501–510

Weingartner M, Schweighofer A, Heberle-Bors E, Doonan J, Bögre L (2000) Visualisation of microtubule movements in living mitotic cells. Plant Mol Biol Rep Suppl 18: 2

Wick SM (1991) Spatial aspects of cytokinesis in plant cells. Curr Opin Cell Biol 3: 253–260

Winkler KE, Swenson KI, Kornbluth S, Means AR (2000) Requirement of the prolyl isomerase Pin1 for the replication checkpoint. Science 287: 1644–1647

Wittenberg C, Reed SI (1988) Control of the yeast cell cycle is associated with assembly/disassembly of the Cdc28 protein kinase complex. Cell 54: 1061–1072

Wu L, Hepler PK, John PCL (1997) The *met1* mutation in *Chlamydomonas reinhardtii* causes arrest at mitotic metaphase with persisting $p34^{cdc2}$-like H1 histone kinase activity that can promote mitosis when injected into higher-plant cells. Protoplasma 199: 135–150

Xiong Y, Zhang H, Beach D (1992) D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. Cell 71: 505–514

Yan Z, DeGregori J, Shohet R, Leone G, Stillman B, Nevins JR, Williams RS (1998) Cdc6 is regulated by E2F and is essential for DNA replication in mammalian cells. Proc Natl Acad Sci USA 95: 3603–3608

Zhang K, Tsukitani Y, John PCL (1992) Mitotic arrest in tobacco caused by the phosphoprotein phosphatase inhibitor okadaic acid. Plant Cell Physiol 33: 677–688

Letham DS, John PCL (1996) Cytokinin controls the cell cycle at mitosis by stimulating the tyrosine dephosphorylation and activation of $p34^{cdc2}$-like H1 histone kinase. Planta 200: 2–12