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*Invited review*

# The interface between the cell cycle and plant growth regulators: a mini review

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**Abstract**

The aim was to review knowledge about the interface between plant growth regulators and molecular checkpoints of the cell cycle. At what level of biochemical regulation of the cell cycle do plant growth regulators interface? Are there different levels of interfacing dependent on the plant growth regulator involved? As a preamble to tackling these questions, we overview the eukaryotic cell cycle with particular emphasis on checkpoints that regulate the transition from G0-G1-S-phase and G2-M. Cytokinins feature strongly as activators of cell division in plants both *in vivo* and *in vitro*. Recent research has shown that zeatin treatment led to the up-regulation of CycD3 in *Arabidopsis*. This is a D-type cyclin showing strong homology with vertebrate D cyclins which themselves are up-regulated by extracellular growth factors. Benzyladenine treatment can also shorten the duration of S-phase through recruitment of latent origins of DNA replication. Kinetin is involved in the phosphoregulation of the G2-M checkpoint; the major cyclin-dependent kinase (Cdk) at this checkpoint has recently been shown to be dephosphorylated as a result of cytokinin treatment, an effect which can also be mimicked by the fission yeast Cdc25 phosphatase. Hence, a picture emerges of a cytokinin-induced continuum of cell cycle activation through the up-regulation of a plant D-type cyclin at the G1 checkpoint and the phosphoregulation of the Cdk at the G2/M checkpoint. During S-phase, we argue for a link between cytokinins and the proteins associated with replication origins. Gibberellic acid (GA) treatment induces internode elongation. In deepwater rice, this response is mediated, at least partly, by a GA-induced up-regulation of a cyclin-Cdk at the G2-M checkpoint. Recent evidence has also linked abscisic acid to a cyclin-dependent kinase inhibitor. These, so-called CKIs are negative regulators of Cdks which fits with ABA's general role in growth inhibition; we await news of ethylene interactions. We highlight two instances of plant growth regulator-cell cycle interfacing during development, arguing for an involvement in microtubule orientation as a prerequisite to leaf initiation, and suggest a link between IAA and the activation of cell divisions in the pericycle required for lateral root initiation. A new D-type cyclin, recently discovered in *Arabidopsis*, may have a key role in this process. Finally, a model is presented which features a generalised cyclin-Cdk checkpoint exhibiting various interfaces with the plant growth regulators.

## 1. Introduction

There are numerous examples in the literature which show that cell division can be stimulated by plant growth regulators (pgrs) and by nutrient supply both *in vivo* and *in vitro* [e.g. 2, 40] In this review, we try to pin-point at which level of cell cycle regulation a pgr may be operating but, as a pre-amble, the major control points of the cell cycle are outlined.

The cell cycle is normally described in terms of four distinctive phases: post- mitotic interphase (G1), DNA synthetic phase (S), post-synthetic interphase (G2) and mitosis (M-phase). Additional subdivisions of mitosis into M and cytokinesis and the existence of a non-cycling G1-phase (G0) are also important descriptive inclusions (Figure 1). Arguably, the cell cycle represents a regulated running track stewarded by a series of checkpoints which determine whether

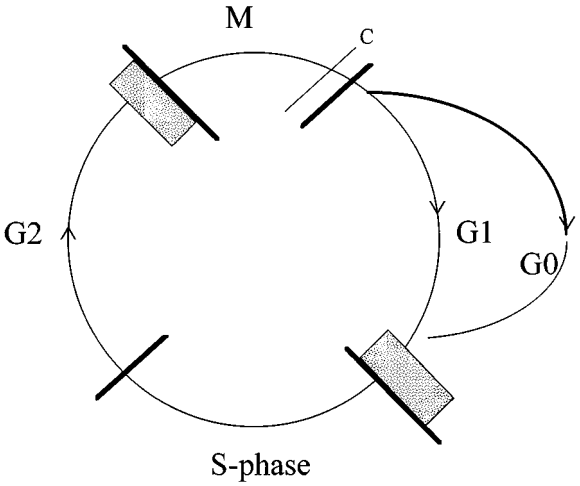


*Figure 1.* Schematic of the eukaryotic cell cycle with its traditional phases of mitosis (M-phase), cytokinesis (C), post-mitotic interphase (G1), and the side-branch for non cycling cells to arrest in G0, S-phase (period of DNA synthesis) and G2 (post synthetic interphase). The shaded boxes, adhering to the Principal Control Point Hypothesis [100], mark the positions of the key checkpoints of the cell cycle, G1-to-S, and G2-to-M.

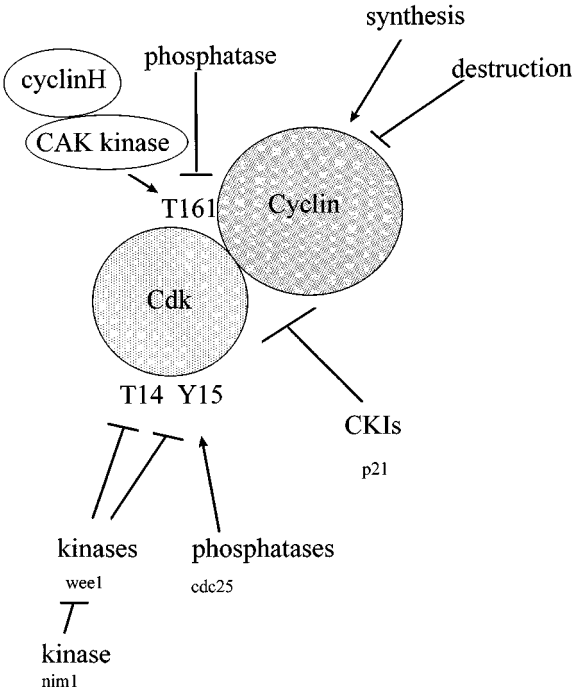


*Figure 2.* Schematic of a generalised checkpoint of the eukaryotic cell cycle featuring a cyclin-dependent protein kinase (Cdk) bound to a cyclin protein. Phosphorylation of threonine161 (T167 of Cdc2 of *S. pombe*, T160 of human Cdk2) of the Cdk by a cyclin-dependent activating kinase (CAK kinase) leads to binding of the Cdk with the cyclin, this binding can be repressed by phosphatase activity. Most cyclin proteins rise in concentration through the cell cycle, e.g. cyclinB peaks in late G2 and M-phase before rapid ubiquitin-dependent proteolysis which in turn incapacitates the Cdk's catalytic activity. The Cdk is phosphoregulated on threonine14 and tyrosine15 residues; *cdc25* and *wee1* are given as fission yeast examples of the phosphatases and kinases that are involved. In turn in fission yeast, Wee1 kinase can be repressed by a protein kinase encoded by Nim1 kinase. The activity of the cyclin-dependent kinase is also repressed by cyclin-dependent protein kinase inhibitors (CKIs e.g. p21) (adapted from [22]).

or not the chromosomes are to be duplicated during S-phase and whether or not chromatids are ready to be partitioned during M-phase. Hartwell and Weinert [32] defined a checkpoint empirically: when step 'B' is dependent on the completion of step 'A', that dependence is governed by a checkpoint unless a loss-of-function mutation exists that relieves the dependence. In other words the checkpoint is a fail-safe. Paulovich et al. [63] used, as an example, the DNA damage checkpoint of budding yeast. The transition from G2 to M is dependent on intact DNA but the dependence is eliminated by deletion of the RAD9 gene making the latter a functional part of the checkpoint that monitors DNA integrity. If the checkpoint hurdle proves insurmountable, perhaps because the cell is too small or its DNA exhibits a catastrophic number of lesions, it arrests more or less permanently, or it enters programmed cell death [49] or it rests temporarily making necessary repairs before rejoining the race. While this analogy may have some descriptive appeal it also has flaws because some cells divide without growing (e.g. the partitioning of mother cells to form sibling progenitors of adventitious roots) whilst others grow but do not divide, (e.g. descendants of the procambium and cambium that differentiate as xylem). Thus size *per se* is not everything. Note that Jacobs [38], in his tussle with cellular and organismal concepts, concluded that in different situations, cell division can drive expansion, or expansion can drive division.

Whatever the developmental outcome, proliferative cells in a meristem must pass a number of checkpoints in order to be competent to divide. Why some cells apparently do not need to satisfy all checkpoints while yet others grow beyond a particular checkpoint is unclear. What is clear is that in order to understand how the cell cycle is controlled in meristems we need to know more about these checkpoints and how they respond to extracellular growth factors.

We know of a number of checkpoints in the animal and yeast cell cycle and there may be a similar number, may be more, in the plant cell cycle. Certainly, the

G1/S-phase transition is best understood in budding yeasts and vertebrate cells and the G2/M transition in fission yeast. Each checkpoint comprises a cyclin-dependent protein kinase (Cdk) which can only exhibit catalytic activity when bound to a partner protein, a cyclin (Figure 2). The latter peaks in concentration at a particular point during the cell cycle coinciding with maximal Cdk activity; at least, most cyclins do [e.g. 78, 79]. In vertebrate cells, a major checkpoint operates at G1/S-phase and another at G2/M (Figure 1). The former checkpoint features Cdk4/6-cyclinD and Cdk2-cyclinE while the latter consists of Cdk1 kinase-cyclinB and Cdk1-cyclinA [see 60]. In fission yeast, in late G2, the Cdc2 kinase is also regulated through phosphorylation of *tyr*15 by Wee1 kinase. This is a negative regulation which effectively switches off the Cdc2 kinase. A positive regulation is effected by Cdc25 phosphatase on the same tyrosine residue while an indirect positive regulation is catalysed by Nim1 kinase which phosphorylates the Wee1 kinase [62, 70, 71, 72]. In fission yeast, the latter protein kinase can be substituted by Mik1 kinase [50]. Cdc25 can also be down-regulated by phosphorylation through association with so-called 14-3-3 proteins [9, 46, 64]. Hence, various "off signals" are mediated through phosphorylation which begs the question as to how pgr-mediated signal transduction chains could influence the plant equivalents to these protein kinases.

Cdc2 kinase activity is absolutely dependent on binding with its partner cyclin and this is achieved through another phosphorylation on thr167 residue of the kinase (thr161 in mammalian Cdk1) [45]. The kinase responsible for this phosphorylation in vertebrates, a cyclin activating kinase (CAK kinase) was originally called MO15 [25, 81] but is now known as Cdk7 [26]. This phosphorylation, by Cdk7, leads to binding of Cdc2 (Cdk1) with cyclin B. In turn, before Cdk7 exhibits catalytic activity it must bind to another partner, cyclinH [26]. This, in turn, could require another CAK kinase, and so on, which begs the question: where is the mother of all CAK kinases? This dilemma may be solved in a more overt way by Cdc2-cyclinB regulating the levels of other participating protein kinases at a given checkpoint. A safer assumption, is that each of the major checkpoints of the cell cycle is regulated by phosphorylation-dependent mechanism(s). Yet another regulation of the Cdk-cyclin kinase is by cyclin-dependent kinase inhibitors (CKIs). A CKI is capable of down-regulating a cdk-cyclin through binding to the Cdk [60] presumably denying it access to its substrate. However the exact details of all positive and negative regulation of each cell cycle checkpoint have yet to be resolved.

The scene set by animal and yeast cell cycle data clearly depicts each checkpoint as a multi-component fail-safe and a remarkably fastidious monitor of the cell's competence for division (Figure 2). In addition, elaborate feed-back controls form a complex mechanism sensing the integrity of DNA and ensuring a tight coupling between DNA replication and cell division [63]. As pointed out elegantly by Berridge [3], the decision to proliferate or not is *the* most critical decision in the life of a cell; small wonder, therefore, about the complexity of cell cycle checkpoints.

In animals (and yeasts), cell cycle checkpoints are sensitive to nutrient status and growth factors, which can induce calcium signals that impinge on the cell cycle [3]. Summarising various reports, it seems that extracellular signalling mechanisms operate at the level of G0-G1-S-phase in animals cells and yeasts whereas recent work suggests that in plants, pgrs affect both G0-G1-S-phase and the G2-M transition. Given this backdrop, it becomes increasingly important to discover the molecular nature of checkpoints in the plant cell cycle and how they might be regulated by pgrs. Hence, the aims of this review are to consider major transition points of the plant cell cycle: G0-to-G1, G1-to-S-phase and G2-to-M, and to try and integrate what is known about the effects of pgrs at these transitions with the function of particular checkpoints. As well as ascribing links between pgrs and cell cycle checkpoints, we shall also glimpse at this interface in a developmental context in specific examples and suggest a model which takes account of the various interactions that exist between the pgrs and the cell cycle. However, we do not promise an encyclopaedic account of all that is known about pgrs and the cell cycle but refer the reader to excellent reviews by Jacqmard et al. [40] and Mironov et al. [56]. Our aim is to provide some synthesis on the growing literature about the effects of plant growth regulators on molecular checkpoints in the plant cell cycle.

## 2. G0 to G1 – D-type cyclins and cytokinins

Cytokinins were first discovered in herring sperm DNA extract [56]. This class of pgrs promotes plant cell division either *in vivo*, when they are added exogenously to plant organs or *in vitro*, when added to tissue culture media. For example, substances such

as kinetin and benzyladenine, can stimulate cell division and shorten cell cycles if added exogenously to cultured cells. Many published examples of this exist [2]. In our view, the cytokinins represent the most enigmatic group of all plant growth regulators because whilst many papers show that cytokinins stimulate cell division, neither their biosynthetic pathways nor their sites of synthesis are resolved. Trewavas [95] pointed out that cytokinin levels *per se* are not reliable indicators of their sites of synthesis. He argued cogently that more cytokinin would be predicted in meristems simply on the basis of the number of cells there. To our knowledge, a convincing case for a given site of cytokinin synthesis is lacking. Hence our interpretations of the action of cytokinins are more to do with them "being there" as opposed to how they got there.

Cytokinin treatment can cause non-cycling cells to divide and can act at the G1/S-phase transition. For example, in dormant buds of *Fraxinus*, benzyladenine (20 $\mu$M), and indole butyric acid (0.1 $\mu$M) stimulated formerly quiescent G0 cells into S-phase [61]. Cytokinins may also increase the proportion of rapidly cycling cells in meristems by inducing G0 cells to enter the cell cycle. In *Sinapis alba*, the arrival of the floral stimulus at the shoot apical meristem results in an increase in the proportion of cycling cells [30]; an attractive hypothesis, therefore, is that cytokinins are part of the floral stimulus which brings about this change to the cell cycle. However, in a later paper [42] the same authors expressed some doubts about cytokinins in this process because the magnitude of increase in the proportion of rapidly cycling cells was not as great when the plants were treated with benzyladenine ($4.5 \times 10^{-5}$ M) compared with plants given an inductive (1 long day) treatment [42].

Although in plants, cells can arrest in G2, most data show G0 (non-cycling G1) as the resting-place for the majority of non-cycling cells both in plants and animals. As mentioned above, cytokinin treatment can cause cells to undergo the G0-to-G1 transition. In animals, serum growth factors induce passage from G0 into G1, a process requiring D-type cyclins; three have been isolated, cyclins D1, D2 and D3 [78, 79]. D-type cyclins are regarded as growth factor sensors due to their instability and because their transcription is absolutely dependent on the presence of serum growth factors rather than the cell's position in the cell cycle [78, 79]. They are strongly up-regulated by the addition of serum to serum-deprived or quiescent cells, whereas the removal of growth factors or the presence of anti-mitogens results in a rapid decline in transcript levels. Once induced by serum growth factors, D-cyclins form active complexes with Cdk4 and Cdk6, whose principal substrate is the Retinoblastoma (Rb) protein, a negative regulator of cell growth [79]. D-type cyclins interact with Rb via a characteristic LxCxE motif situated in the N-terminus of the cyclin [19]. During the early stages of G1, Rb is in an unphosphorylated active state and exerts its growth inhibitory effects by binding to, and inhibiting, E2F transcription factors which are required for the onset of S-phase [107]. The hyperphosphorylation of Rb during G1, initiated by cyclin D-dependent kinases in mid-to-late G1 results in the release of free E2F, which then promotes the transcription of genes necessary to propel cells into S-phase [79].

Plant D-type (CycD) cyclins were first isolated from *Arabidopsis* and alfalfa by their ability to functionally complement yeast strains defective in G1 cyclin activity [12, 85]. Subsequently, over 20 CycD genes have been isolated from a range of species [59, 67, 86]. Based on sequence criteria, these cyclins are classified into three distinct groups, CycD1, CycD2 and CycD3, originally defined in *Arabidopsis* [59, 67, 85]. Recently, a new *Arabidopsis* CycD (CycD4;1) has been isolated, which, based on a limited sequence analysis was classified as being the first member of a new CycD4 group [16]. However, a more detailed analysis indicates that it may in fact be a member of the CycD2 group [86]. Hence, the question of the existence of a CycD4 group remains open. Similar to animal D-type cyclins, plant CycDs contain the characteristic LxCxE Rb-binding motif.

The majority of CycDs show expression patterns reminiscent of animal D-type cyclins, including rapid transcript accumulation when quiescent cells in suspension culture are stimulated with growth-promoting substances, suggesting that they also regulate G1 progression in response to extracellular signals [12, 18, 69, 85, 87]. This opened up the exciting possibility that cytokinins may stimulate G1 progression by the induction of CycDs. Strong evidence to support this hypothesis has come recently, from a landmark study on *Arabidopsis* CycD3 [69]. In this study, CycD3 transcripts were found to be elevated in *Arabidopsis* mutants with high cytokinin levels and could be rapidly induced by the exogenous application of cytokinin in cell cultures (optimally with 1–10 $\mu$M zeatin or 1 $\mu$M benzylaminopurine) and in intact plants (1 $\mu$M zeatin). Moreover, in cultured leaf explants taken from transgenic plants

constitutively expressing CycD3, cell division could be induced and maintained, in the absence of exogenous cytokinin. Based on these and other results the authors concluded that cytokinin activates cell division by inducing CycD3 at the G1-S boundary [69]. In contrast, *Arabidopsis* CycD2 is not induced by pgrs, but is instead responsive to carbon source availability [59, 85]. Current data obtained using cell cultures suggest that sucrose may stimulate G0-to-G1 progression by rapidly inducing CycD2 [85, 88]. CycD4;1, the putative second *Arabidopsis* CycD2, may also be induced by sucrose [16], suggesting that sucrose induction may be a characteristic of the CycD2 group in general.

Plant CycD cyclins have been shown to interact with plant Rb proteins, via the LxCxE motif [1, 35], which, in turn, interact with recently identified plant E2F homologues [65, 77]. Therefore, it seems highly likely that CycD cyclins, like animal D-type cyclins, regulate G1 progression via an Rb-E2F based mechanism. However, the exact details of this mechanism remain to be determined.

Clearly much new data now exists regarding the G0-to-G1 transition and a strong case emerges for a role of cytokinins in up-regulating a plant CycD3 cyclin. It is of direct relevance, that various papers also show a role for cytokinins in DNA replication providing a cytokinin-induced continuum from G0 through to S-phase.

## 3. S-phase

DNA replication involves the activation of multitudes of replication origins spaced along the DNA molecule. From each origin, two replication forks diverge until they meet with forks from adjacent origins. The origin and its two diverging forks are known as a replicon [91] and groups that initiate replication simultaneously are said to comprise a replicon family [103]. S-phase can alter in length through changes in the rate of replication per single replication fork or through either activation or deactivation of replicons. For example, S-phase shortened due to the activation of latent origins of DNA replication during floral induction in three unrelated photoperiodically sensitive plants, *Sinapis alba* [39], *Silene coeli-rosa* and *Pharbitis nil* [20]. Clear evidence also exists for activation of latent origins of DNA replication by exogenous application of cytokinins. A single application of benzyl adenine ($4.5 \times 10^{-5}$ M) to vegetative shoot apices of *Sinapis alba* resulted in a 50% shortening in replicon size from 15 to 7.5 $\mu$m [34]. One other intriguing effect of applying cytokinins to shoot apices of *Sinapis alba* was the 100% synchronous activation of replication origins [34]. Hence, cytokinins must participate in, or trigger signal transduction chains that result in activation of latent replication origins.

To attempt to summarise literature on the regulation of DNA replication would be a review in itself. Hence, what follows can only be a brief, arguably superficial, stab at how cytokinins may be involved in such activation of initiation points. There is a group of six polypeptides, the origin recognition complex (ORC) that remains bound to replication origins throughout the cell cycle [21]. DNA replication is thought to be initiated when Cdc6p (in budding yeast) or Cdc18p (in fission yeast) facilitates the loading of licensing factors, recently referred to as the MCM (minichromosome maintenance) complex, to the ORC [17, 90]; the MCM complex may function as a helicase catalyzing the unwinding of DNA at an origin [48]. In budding yeast, this cascade of proteins acts downstream of CDC28-G1 cyclins. Seemingly, MCM is phosphorylated by Cdc7p, a widely conserved protein kinase [43] with an activating subunit, Dbf4p [37]; this phosphorylation is required for origin activation. Plant homologues to MCMs have been discovered [89] (reviewed by Bryant [7]), raising the expectation that there are plant homologues to all of these co-factors. As elegantly shown by Jacqmard and her colleagues [34, 41] pgrs also activate or, indeed inactivate, initiation points for DNA replication. For example, in *Sinapis alba*, abscisic acid treatment resulted in an increased distance between initiation points [41]. This would suggest a regulatory role for pgrs at the level of the ORCs, or Cdc7p or, through alterations of the DNA loops at the nuclear matrix, a conclusion not dissimilar to that reached by Ivanova and Rost [36].

## 4. G2 to M

Classically, Van't Hof [99] demonstrated that in pea root tips, provision of sucrose to the culture medium enabled cells that were arrested in G1 to enter S-phase, or those arrested in G2 to enter M.

There are many examples of cell cycle activation by exogenous application of pgrs *in vitro* [2, 10, 27, 40, 44]. In many of these cases, formerly quiescent cells in G1 or G2 were stimulated to enter S-phase and M-phase respectively [13, 19, 108].

Hence the activation of these cells must be dependent on checkpoints. A hitherto unexplored possibility could be that these cells were quiescent because they accumulated DNA lesions faster than they repaired them. Perhaps cytokinins stimulate signal transduction chains which up-regulate enzymes which sense the damage, leading to the engagement of enzymes for the repair process. DNA repair-like enzymes, would be obvious candidates for up-regulation if this were the case. In fission yeast, DNA-dependent protein kinases (reviewed by Russell and Rhind [68]) have been identified as important players in the repair process at the G1-to-S and G2-to-M checkpoints. DNA repair enzymes and associated proteins would be expected to remove, or reverse lesions thereby restoring DNA integrity for successful entry into S- or M-phase [63].

As mentioned in the Introduction, the G2 to M-phase checkpoint is one of the best understood of all cell cycle checkpoints. In plants, Van't Hof [100] showed that the G2-to-M, and G1-to-S transitions could be blocked by denying cells an energy source through removal of carbohydrate from the medium, by exposing cells to inhibitors of protein synthesis, or, by perturbing substrate phosphorylation with the uncoupler, dinitrophenol [106]. Hence, it is important to note that nutrient sensing and metabolic status have a critical bearing on pgr interfacing with the cell cycle.

Fosket et al. [27] observed that in soybean, zeatin (0.5 $\mu$M) caused qualitative changes in protein synthesis and concluded that these changes were necessary to permit the entry of cells into division. More recently, in tobacco TBY-2 cells, peaks of zeatin, zeatin riboside and zeatin-5′ monophosphate levels were observed to coincide with the end of S-phase, mitosis and G1-phase [47, 66]. These authors concluded that fluctuations in the levels of specific cytokinins at specific stages of the cell cycle reflect their essential role during these phases [47, 66]. The lingering question is at what level in the cell cycle would they operate? A clue comes from experiments where olomoucine, a cytokinin analogue, blocked the cell cycle at G1/S and G2/M; this molecule locates in a pocket where ATP binds to, and inhibits Cdk2 [75]. Conversely, Crowell and Salaz [11] showed that the growth of TBY-2 cells was inhibited by low concentrations of lovostatin, which inhibits the isoprenoid pathway that leads to biosynthesis of the cytokinin side-chain; this was reversed by cytokinin. What emerges is a somewhat complex picture of inhibition of Cdk activity by cytokinins at one level, and promotion of growth by zeatin at another. Redig et al. [66] concluded that this type of "stop-go" could be explained in that these regulatory kinases of the cell cycle need to be active only at restrictive phases of the cell cycle and then need to be inactivated immediately after. Note that this was described by Elledge [22] as a molecular logic whereby a transition turns off the previous state while promoting the future state (see Introduction).

The involvement of cytokinins at the G2-to-M phase transition was also shown in *Nicotiana plumbaginofolia* cells in culture [109]. Cells that arrested in G2, because of a lack of cytokinin, exhibited high levels of inactive Cdc2 which was phosphorylated at tyr15 [109]. One immediate consequence of cytokinin (0.23 $\mu$M kinetin) induced entry into M-phase was dephosphorylation of Cdc2. Subsequently, the same workers observed that this dephosphorylation could also be mediated by fission yeast Cdc25 phosphatase [109]. Moreover, a plant protein phosphatase activity was observed that peaked during prophase.

Each protein that influences Cdk activity is a potential interface for signal transduction pathways [22]. This is particularly germane for Zhang et al.'s [109] work which provides a clear link between kinetin, dephosphorylation and Cdc2 activation thereby representing the core of a cytokinin-dependent activation of cells into mitosis. Can we marry these observations of cytokinin activation of the cell cycle [109], with the suggestion of Redig et al. [66], that cytokinins may interfere with Cdk-cyclin complexes? Actually there should be no need to because the effects are mediated by different cytokinins and at different concentrations. Often, published papers indicate that one class of pgr can result in different effects at different concentrations; such concentration-dependent effects will be discussed towards the end of this article.

The transition from G2-to-M in the plant cell cycle is made more complicated because, in addition to stopping in G0, plant cells can arrest in G2. For example, meristematic cell arrest in G2 occurs in *Vicia faba* [104], *P. sativum* [23, 24] and *Lactuca sativa* [54]. Cell arrest in G2 was pronounced in response to trigonelline (n-methyl nicotinic acid), a substance that is synthesised in the cotyledons and transported to the root system [23, 24]. The latter perturbed replicon ligation at the S/G2 boundary [101]. The consequent arrest in G2 may stem, in part, from DNA damage that is too extensive to be overcome at the DNA damage checkpoint. However, trigonelline did not arrest all cells in G2 [101; S.Mazzuca, M.B. Bitonti

and D. Francis unpublished data] so that meristematic cells are differentially sensitive to cell cycle regulators in G2 perhaps because of a flip mechanism which regulates competence / non-competence for cell cycle progression.

## 5. Gibberellins and the cell cycle

Jacobs [38] raised the issue as to whether gibberellins are mitogens. In reviewing the effects of $GA_3$ on elongation of deep-water rice, he plumped for cell division as a secondary consequence of cell enlargement. An inherent difficulty in this interpretation, not overlooked by Jacobs [38], is that $GA_3$ has pleiotropic effects, on the cell wall at one concentration and in the cytoplasm at another.

In deep-water rice, the major growth response to $GA_3$ resides in the youngest internodes. In this system, GA-induced growth depends on an increase in both the rate of cell elongation and the rate of cell production in the intercalary meristem [6, 55, 73]. The initial response was an increase in cell size followed by an activation of G2 cells into mitosis and then an increase in the rate of DNA synthesis. The data were clearly consistent with an induction of cell cycle activation at the G2/M transition and subsequent entry of these cells into S-phase [73]. Indeed, the data suggested an interaction of GA with the G2-M checkpoint. This was confirmed when it was subsequently shown that the mRNA level of one *cdc2* homologue (*cdc2*Os-2) increased rapidly, one hour following GA treatment (50 $\mu$M) [74]. Two rice cyclin homologues were also up-regulated which led to the conclusion that GA treatment resulted in transcriptional activation of G2-M checkpoint proteins [74].

We investigated the role of GA in the plant cell cycle but could not find evidence of GA stimulation of cell division in shoot apical meristems of dwarf peas. Indeed, the cell cycle was longer in meristems treated with exogenous $GA_3$ (290 $\mu$M) compared with the untreated controls [14]. The major effect of applied $GA_3$ on cell division was in the developing internode. We measured rates of division in the 7th internode, from its inception to maturity, in the same dwarf plants $\pm$ $GA_3$ [15]. In the $GA_3$ treatment (290 $\mu$M), cell doubling times in the epidermis, cortex and pith were about half that of the controls (17 h cf. 39 h in the controls). This rapid rate of cell division was reflected in specific cell reproduction rates which for the pith and epidermis, declined rapidly and steadily with time, but for the cortex, declined slowly at first, but rapidly thereafter. In the controls, the slower rates of cell production reflected a cell doubling time which remained constant until the 18th day of the experiment, before declining to zero within a few hours [15]. In other words, whilst GA stimulated higher rates of cell production over a relatively short interval, in the controls, rates of cell division were slower but the division phase lasted longer. One clear feature of the response was the similarity of cell lengths in the $+GA_3$ and $-GA_3$ treatments. We argued that the $GA_3$ effect was regulated through cytoplasmic parameters particularly during the early period of internode growth and that the GA effect was mediated by signalling-through-cytoplasm-monitoring [15]. However, this is not to deny that $GA_3$ affects cell wall properties but it reminds us of the pleiotropic effects $GA_3$ can have.

Hence, it seems that internodal growth in both a monocot and dicot is regulated, at least in part, by a GA induced increase on the rate of cell production and that this pgr impinges on the G2-M checkpoint at the level of the Cdc2 kinase [74].

Having explored some potential pgr / cell cycle interfaces, we turn our attention, albeit tentatively, to developmental controls. Once again, the subject could spark a substantial review of its own and, most certainly, there are others more qualified to do this. Instead, we examine two aspects of development, one in the shoot and the other in the root, in an effort to demonstrate a potential area of interfacing between the cell cycle and pgrs during morphogenesis.

## 6. Auxin, the shoot apical meristem and the cell cycle

The shoot apical meristem is a dome of cells which perpetually changes its shape. It gets bigger through cell division during a plastochron and drops back to a smaller size upon initiation of primordia [51]. This is most dramatically demonstrated during vegetative growth through an exponential increase in the volume of the apical dome and can be illustrated by saw-tooth plots of temporal changes in apical dome volume on a loge scale [51]. During the transition to floral growth the apex enlarges maximally and is due to a substantial increase in the rate of cell division (reviewed in [29]). The increase in volume accommodates the floral primordia usually in a different pattern to the vegetative primordia. In the floral apex, there are many more organs which are initiated rapidly

in short plastochrons e.g. in *Silene coeli-rosa* from 3.8 d in vegetative growth to 0.5 d for sepals [51]. As floral morphogenesis progresses, the dome gets used up until a flattened top is consumed by carpel primordia [51]. Hence it is reasonable to conclude that shoot apical meristem cells, through cell division, make leaves during vegetative growth and floral whorls during floral growth.

An involvement of pgrs in regulating shoot apical cells could be through altering the kinetics of cell division through checkpoints (see above). However, another level of control could be exercised through alterations in microtubule orientation. For example, in elegant studies of leaf morphogenesis, Green [31] identified the organisation of cell wall microfibrils as key elements in the control of organ initiation. Coupling this with changes in cell surface extensibility, he emphasised how changes in hoop reinforcement of microfibrils led to primordium initiation. As pointed out previously [28], such changes in surface microstructure ought to be coupled with changes in the plane of cell division for a cohort of peripheral zone cells which is destined to form the new primordium. Long standing theories state that auxin is involved in leaf initiation [82, 83, 84] and that IAA can change the mechanical properties of cell walls [53] and alter the orientation of cortical microtubules (reviewed by Shibaoka [80]). All of this leads us to suggest an interface for auxins and the cell cycle in terms of regulating planes of cell division, maybe at the level of microtubule associated proteins (MAPs) and mitogen associated protein kinases (also, confusingly known as "MAP" kinases). Certainly, any discussion of the dynamics of microtubule assembly / disassembly includes MAPs as potential substrates for cyclin dependent kinases [98]. A link between this perceived level of interaction, organ initiation and cell cycle control could perhaps emerge through studies of leaf morphogenesis in auxin *deficient mutants* of *Arabidopsis* where the possibilities of uncovering specific gene functions are maximised.

### 6.1 *Auxin, roots and the cell cycle*

Once again, the heading could prompt a complete review in its own right. Our chosen example of pgr interfacing is the lateral root which is initiated from the outermost layer of the stele, the pericycle, in regions immediately adjacent to protoxylem poles. Using radish roots, Blakely et al. [5] demonstrated how the number of laterals forming in culture could be regulated by exogenous IAA. Polar transport of auxin from shoot to root is also a well established phenomenon and, hence, the delivery of auxin to the pericycle by the xylem conduit, and subsequent triggering of cell division in the pericycle, became an attractive hypothesis [76]. However, the pathway of auxins through roots is more problematic. Summarizing various reports, the movement of this pgr is probably bidirectional in the root system moving up in one tissue while moving down in another [57, 93]. This does not weaken the case for a requirement for auxin in lateral root initiation but inevitably signalling might be more complicated. These elegant studies from the 1970's proved very useful for the genetic analyses of the 1990's which have employed *auxin deficient mutants* and *auxin over-producing mutants* of *Arabidopsis* [8].

A whole variety of mutants with abnormal levels of IAA have been characterised and of these, we highlight one that makes an excess number of lateral roots and adventitious roots (*alf1*), in which it was concluded that the phenotype results from an overproduction of IAA [8]. Another is defective in lateral root formation (*alf4-1*). Applications of IAA to ALF4 plants led to lateral root formation [8]. What clearly emerges, is the requirement of IAA to initiate cell division in the pericycle. Studies of other mutant phenotypes, which can initiate primordia that develop no further, led to the conclusion that the development of the primordium across the cortex is a separately controlled event [8].

When the promoter of *cdc2aAt* was used to drive GUS expression, *Arabidopsis* roots exhibited not only an intensely stained primary root meristem but also a prominently stained pericycle along its entire length [33], which is consistent in showing the mitotic competence of this tissue. However, branch roots are initiated from the pericycle in clusters separated by other clusters. This means that an IAA-mediated signal transduction pathway operates in such a way that all of the necessary components of a checkpoint are satisfied in some pericycle cells but not others. The recently discovered D-type cyclin in *Arabidopsis* (CycD4;1), which is preferentially expressed during lateral root formation, holds a likely key to acquisition of mitogenic competence in the pericycle [16]. As noted by these authors, this D-type cyclin might be one of the key limiting factors in lateral root formation and could be sensitive to IAA-mediated signal transduction. If so, a classic case emerges for an interface between a pgr, the cell cycle and development notwith-

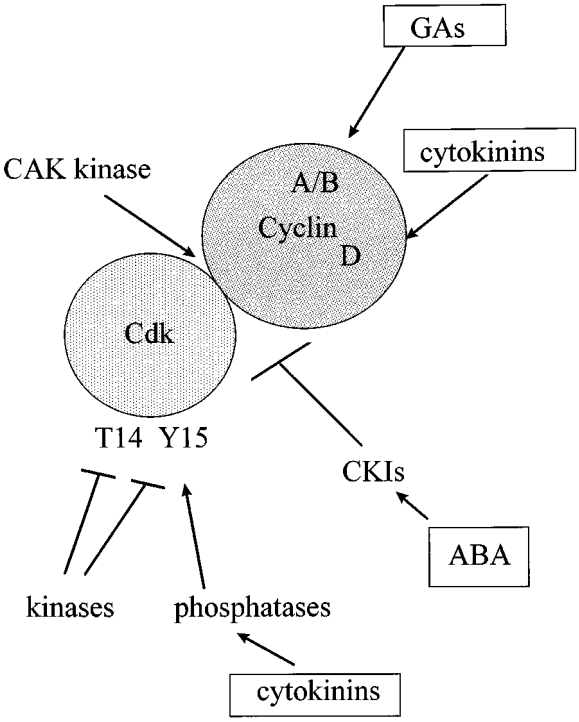


*Figure 3.* Schematic of a plant cell cycle checkpoint with known interfaces between the checkpoint and plant growth regulators. The schematic indicates up-regulation of cyclin A/B by gibberellins [74], of D-type cyclins by cytokinins [69, 85] of cyclin-dependent kinase inhibitors (CKIs) by abscisic acid [105], and of cytokinin-activated phosphatases that dephosphorylate tyrosine15 of the Cdk [109].

standing the likelihood of other contributing factors including nutrient status and other interacting pgrs.

## 7. Conclusions

From this fairly short tour of some pgr-cell cycle interactions it becomes clear that major checkpoints can be regulated by pgrs via specific moieties and at specific concentrations.

By revisiting the Elledge [22] cyclin-cdk model, it also now seems possible to fit pgr-mediated signal transduction pathways to some of the potential interfaces of a typical Cdk-cyclin complex (Figure 3). From our review of the literature, interfaces exists between cell cycle checkpoints and: cytokinins, gibberellic acid and abscisic acid. To our knowledge, there are no molecular data linking ethylene to a checkpoint but, clearly, this does not exclude the possibility of such an interface. In the same way, data are not yet available to link a pgr with a CAK kinase that activates a Cyclin-cdk complex. Clearly, the discovery of plant homologues to regulatory genes of the cell cycle will widen the possibility of testing the level at which pgrs are involved with the checkpoints.

Equally clear cases exist for pgr-mediated regulation of S-phase through the recruitment of latent origins of DNA replication or through inactivation of primary origins. Establishing a wider base of plant homologues to MCMs, Cdc6p and Cdc7p is now necessary in order to test the molecular circuitry and at which level DNA replication is most sensitive to pgr-mediated signal transduction chains.

Note that it has only been within the past 4–5 years that data have become available linking pgrs with plant cell cycle checkpoints. Hence these are early days in what will turn out to be an exciting story of interfacing between plant growth regulators and the cell cycle.

Amidst such optimism we end with a sense of caution on the interface between pgrs and the cell cycle, borne out of a sense of *déjà vu* about the effects of pgrs themselves. We are still left with a bewildering array of effects that pgrs have on development with one pgr at different concentrations apparently having diametrically opposed effects on cells in different tissues and at different stages of development. The same is true for pgrs effects on the cell cycle. We can invoke models to engage a specific pgr with a particular cell cycle checkpoint (Figure 3), but how much do we know about the interaction of each pgr with its receptor? How much do we know about the events down-stream of receptor binding that impinge on the cell cycle? Clear and complete answers are not immediately forth-coming. Hence mechanisms of pgr action on the cell cycle have to be couched carefully until more becomes known about pgr-receptors and their immediate down-stream events. Are the down-stream events the same regardless of developmental phase or regardless of cell cycle phase? We don't know. Trewavas [94, 95, 96, 97], in a series of seminal papers, outlined the risks of dogma that would govern how a pgr works. Indeed Trewavas [97] pointed out that whilst growth substances are part of the genetic potential for rapid cell division, that potential can only be fulfilled if other resources (e.g. nutrient, calcium signalling) are supplied adequately. Hence, research on nutrient sensing and its interaction with the cell cycle will have an increasingly important part to play. Discovering plant homologues to vertebrate and yeast cell cycle genes is important, but determining their function will require a clear understanding of cell

cycle interfaces. Clearly, understanding pgr-cell cycle interfacing will provide a unique insight into both cell cycle control and the development of plants. Also, such an understanding will help us appreciate the evolutionary divergence of cell cycle checkpoints in plants, yeasts and animals. Clearly, there are exciting times ahead.

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