

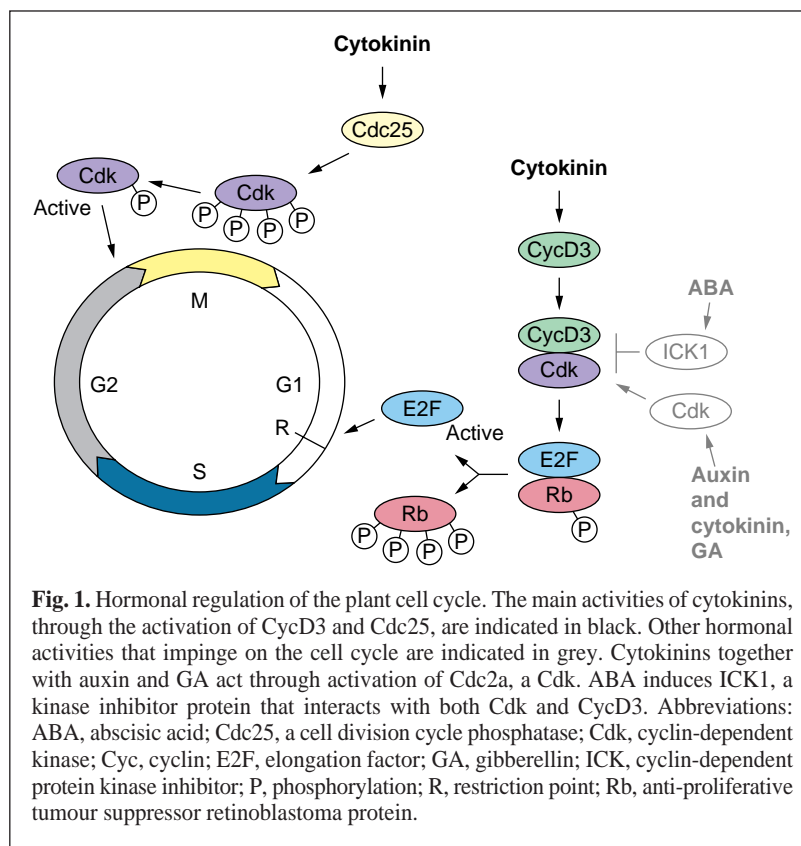
# Cytokinin cycles cells

Cytokinins were discovered during the 1950s because of their ability to induce cell division, in concert with auxin, in non-dividing plant cells. Until recently, the molecular targets of this hormone in the cell cycle regulatory machinery have remained elusive. Cloning genes coding for plant cyclin-dependent kinases (Cdks; e.g. Cdc2), cyclins (CycA, CycB and CycD families) and cyclin-dependent protein kinase inhibitors (ICKs) has made the cell cycle accessible to molecular studies. The cytokinin-dependent cell division activity in *Arabidopsis* is mediated by the transcriptional regulation of the *cyclin D3* (*CycD3*) gene at the G1/S transition<sup>1</sup>.

## Cytokinins regulate the G1/S transition through CycD3

The first three plant cyclin D gene homologues – *CycD1*, *CycD2*, *CycD3* – were identified by complementation of yeast G1 cyclin mutants<sup>2</sup>. They are similar to mammalian D-type cyclins, whose expression is strongly dependent on mitogenic stimuli, such as serum growth factor. Mammalian D-type cyclins are required during middle-to-late G1 phase and are considered to be the link between proliferative signals and emergence from the quiescent state<sup>3</sup>. In *Arabidopsis* cell culture it has been found that *CycD3* is induced in the G1 phase, shortly before the S phase, by cytokinins and sucrose<sup>1,2</sup>. *In situ* hybridizations show *CycD3* expression in proliferating tissues of the shoot meristem, young leaf primordia, axillary buds, procambium and vascular tissues of the maturing leaves. Cytokinin treatment enhances the expression in these responsive tissues. The cytokinin-overproducing *amp1* (which is allelic to *pt*, *hpt* and *cop2*) mutant has steady-state *CycD3* mRNA levels that are enhanced two- to three-fold<sup>1</sup>, further supporting the evidence for an *in vivo* link between cytokinins and *CycD3* expression.

How could CycD3 act to push the cell into the S-phase? In mammals, the CycD3–Cdk complex acts through hyperphosphorylation of the anti-proliferative tumour suppressor



**Fig. 1.** Hormonal regulation of the plant cell cycle. The main activities of cytokinins, through the activation of CycD3 and Cdc25, are indicated in black. Other hormonal activities that impinge on the cell cycle are indicated in grey. Cytokinins together with auxin and GA act through activation of Cdc2a, a Cdk. ABA induces ICK1, a kinase inhibitor protein that interacts with both Cdk and CycD3. Abbreviations: ABA, abscisic acid; Cdc25, a cell division cycle phosphatase; Cdk, cyclin-dependent kinase; Cyc, cyclin; E2F, elongation factor; GA, gibberellin; ICK, cyclin-dependent protein kinase inhibitor; P, phosphorylation; R, restriction point; Rb, anti-proliferative tumour suppressor retinoblastoma protein.

retinoblastoma (Rb) protein. Hyperphosphorylated Rb is unable to complex with elongation factor E2F, thus enabling the transcription of E2F-regulated genes, which are required for entry into S-phase<sup>3</sup>. Interestingly, the characteristic Leu-x-Cys-x-Glx Rb-binding motif (where x can be any amino acid) of D-type cyclins is conserved in plant CycD proteins at the equivalent position and plant CycD3 proteins are able to interact with proteins of the Rb family<sup>4</sup>. These data are consistent with a function for CycD3 at the G1/S-transition (Fig. 1). In contrast to emerging knowledge on interacting downstream partners, the links between the cytokinin signal and *CycD3* expression are unknown. The proteins involved in signalling are all present in the responding cell and protein phosphorylation plays a crucial role<sup>1</sup>, but none of the upstream elements is known.

## Do cytokinins have additional targets in the cell cycle?

Cytokinins have also been reported to have a regulatory function at the mitotic control point of G2/M transition<sup>5</sup>. In excised stem pith parenchyma of *Nicotiana tabacum*, cytokinin activates a Cdc2-like kinase that accumulates in its inactive form after auxin treatment. Cytokinin stimulates a specific dephosphoryl-

ation of the kinase, a reaction that is carried out by the Cdc25 phosphatase. This indicates that cytokinins might control G2/M transition by Cdc25 mediated dephosphorylation of a Cdk. Consistent with a role for cytokinins in the G2/M transition is their distinct transient accumulation during this phase of the cell cycle<sup>6</sup>.

The different results with respect to the cytokinin cell cycle control points could be explained if different regulatory mechanisms are active in distinct cell types. Such distinct cell types might have been selected in different cell cultures, explaining differences reported in their cytokinin responses. An example of diverse modes of cell cycle regulation is found in *Drosophila*. Cell division during the start of

embryo development depends entirely on Cdc25, whereas it is regulated by CycD3 at later stages<sup>7</sup>. Consistent with different modes of cell cycle regulation in plants is the expression of the *Antirrhinum CycD3a* and *CycD3b* genes in distinct zones of the floral shoot meristem<sup>8</sup>. They might mediate specific signals in the cell cycle. Cell cycle control during floral transition might also be achieved by a different mechanism. This is indicated by a drastic decrease in cytokinin concentration during this period in spite of an increase in cell division activity<sup>9</sup>.

## Other hormonal players in the cell cycle

Cytokinin is apparently a specific trigger for cell cycle progression but other hormones with enhancing or inhibitory functions are involved in cell cycle regulation too. Auxin has multiple functions concerning Cdk activation and is involved in controlling ubiquitin-mediated destruction of cell cycle regulatory proteins<sup>10</sup>. Cdks also accumulate in response to gibberellins in specific tissues<sup>11</sup>. Recently, ICK1, which interacts *in vitro* with Cdc2a and CycD3, has been identified in *Arabidopsis*<sup>12</sup>. *ICK1* expression is induced by ABA and leads to a decrease in Cdc2a-like kinase activity. These results are compatible with the antagonistic functions of ABA and

cytokinins and suggest a molecular mechanism in which plant cell division is inhibited by ABA (Fig. 1). Taken together, a network of plant hormone activities, which are involved in the different cell cycle components, is emerging.

#### Plant proto-oncogene

Ectopic expression of *CycD3* has been found to alter the cytokinin requirement for growth of calli<sup>1</sup>. Callus formation from *CycD3*-overexpressing leaf tissue and growth of these calli is cytokinin autotrophic, which is characteristic of plant tumours. Deregulation of *CycD3* could perturb G1/S transition and contribute to tumour development. There is a parallel in animals, where deregulation of the cell cycle, also by means of the D-type cyclins, is important during tumourigenesis. The human *CycD1* gene and perhaps other D-type cyclins are putative proto-oncogenes that are possibly activated by deregulated transcription<sup>13</sup>. By analogy, plant *CycD3* can be regarded as a proto-oncogene.

#### Cell division and development

*CycD3* also provides a link between cytokinin and plant development. Plants overexpressing *CycD3* show extensive leaf curling, disorganized meristems, increased leaf number, late flowering and delayed senescence<sup>1</sup>. This indicates a role for *CycD3* in plant differentiation and contrasts with the results obtained with plants overexpressing other plant cell cycle genes, such as *Cdc2a* and *CycB1*. The ectopic expression of these genes leads to an altered cell number but fails to perturb plant growth or development<sup>14,15</sup>. This suggests that morphogenic control in plants is independent of the rate of cell division. Whether or not *CycD3* does play a privileged role at the interface of cell division and development needs further analysis. In *Drosophila* early development, a particular role is played by *Cdc25*. *Cdc25* is the limiting factor for entry into mitosis. Analysis of its promoter has shown that it is directly controlled by patterning information. Thus *Cdc25* is a single factor between patterning and cell division<sup>16</sup>. A detailed analysis of plant *Cdc25* has not yet been carried out.

It will be interesting to determine how cell cycle regulation and cell division activity are wired into the developmental programme of a plant and how hormonal, metabolic and environmental stimuli trigger these processes. As plant cells do not move and apoptosis is not a common means of altering morphogenesis, the right number and plane of divisions, as well as determining patterning, also determine plant shape. How regional patterns of cell division are established and maintained, such as during the formation of leaves from regions of the meristem, is unknown<sup>17</sup>.

- Do cytokinins play a role in regional organization?
- Where does the cytokinin that regulates *CycD3* originate?
- Is it an intrinsic signal?
- How is information from different clonally distinct cell layers coordinated and channelled to *CycD3*?

Homeodomain proteins, such as *KNAT1* and *STM*, which prevent differentiation and activate cell division, might play a role in determining cell fate through interaction with the cell cycle machinery. Interestingly, it was reported recently that the *KNAT1* and *STM* mRNA levels are increased in both the cytokinin-overproducing transgenic *Arabidopsis* and the *amp1* mutant<sup>18</sup>. This indicates that links between cytokinin, cell cycle genes and developmental genes exist. The challenge is to decipher the nature of these links.

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