



## EDITORIAL: REFLECTIONS ON *THE PLANT CELL CLASSICS*

# The Discovery of Plant D-Type Cyclins<sup>[OPEN]</sup>

Across all eukaryotes, cell cycle progression is controlled by heterodimeric protein complexes composed of a Ser/Thr-specific cyclin-dependent kinase (CDK) and cyclin, a regulatory subunit that controls both the timing of CDK activation and its substrate specificity. All cyclins possess a homologous region of ~100 amino acids named the cyclin box, which is required for interaction with the CDK. Typically, every eukaryotic organism holds different types of cyclins that can be phylogenetically grouped into different classes based on the cyclin box. In mammals, A- and B-type cyclins show a transcriptional peak and predominant role during the S-G2 and G2-M phases, respectively. By contrast, the expression of the D-type cyclins is not necessarily linked to a particular cell cycle phase, but rather correlates with the presence of mitogens.

Before the availability of any plant genome sequence, PCR was one of the most successful strategies to isolate putative orthologous plant genes. The first partial plant cyclin genes were isolated using primer sequences deduced from the conserved mammalian cyclin box, followed by the identification of the corresponding full-length sequences through screening of genomic libraries by DNA hybridization experiments. Although this strategy resulted in the successful identification of the first plant A- and B-type cyclins, it failed to identify any plant D-type cyclin, which in retrospect can be explained by the relatively poor sequence conservation between the D-type cyclins of different species.

In the early 1990s, Jim Murray's team took a different approach (Soni et al., 1995), based on the observation that cyclins that are rather weakly conserved at the sequence level can be functionally conserved to the extent that they can take over the role of cyclins within a heterologous species. A yeast complementation experiment had previously demonstrated that mammalian D-type cyclins can take over the role of the budding yeast (*Saccharomyces cerevisiae*) G1/S-specific CLN cyclins (Xiong et al., 1991). Budding yeast holds three such CLN genes, and knocking them all out impairs cell division, resulting in a no-grow phenotype. The latter can be circumvented by deleting only two of these cyclins (CLN1 and CLN2) and putting the third one (CLN3) under the control of a Gal-inducible promoter. This allows the yeast to grow in the presence of Gal but not on Glc-containing medium. Using a nearly identical approach to the one that led to the identification of the first mammalian D-type cyclins, Murray and his team transformed the CLN mutant yeast strain with a library of Arabidopsis (*Arabidopsis thaliana*) cDNAs cloned under the control of a yeast promoter and screened the collection of transformed cells for growth on Gal-free medium. This strategy resulted in the identification of the first plant D-type cyclins, originally nominated  $\delta$ -type cyclins (later renamed as CYCD1;1, CYCD2;1, and CYCD3;1).

The isolated plant D-type cyclins showed the highest sequence similarity to mammalian G1 cyclins. Whereas both A- and B-type cyclins characteristically hold an N-terminally located destruction box (D-box) that marks them for degradation by ubiquitination, D-type cyclins do not have such a domain; rather, they hold regions rich in Pro, Glu, Ser, and Thr—sequences typically associated with proteins that have a short intracellular half-life. More strikingly, all three isolated D-type cyclins were found to also hold the LxCxE domain, a motif that was known to allow mammalian D-type cyclins to bind the RB tumor suppressor protein, hence hinting at the existence of plant RB-related proteins well before their final discovery one year later (Grafi et al., 1996; Xie et al., 1996). Additionally, through DNA gel blotting, it was concluded that probably many more D-type cyclins existed in Arabidopsis, later confirmed to be 10 in total (Vandepoele et al., 2002).

Mammalian D-type cyclins are unique among the cyclins in the way that their transcription does not show a strong periodicity during the cell cycle. Rather, their expression levels depend on the presence of mitogens, which suggested that they act as primary growth factor sensors feeding information about internal and environmental signals to the cell cycle control system. In their work, Soni et al. (1995) were the first to hint at a similar role for the plant D-type cyclins. Cell cultures were depleted for their most important growth factors (auxin, cytokinin, and Suc as carbon source), forcing the cells into a “quiescent state.” Strikingly, replenishment of one or more of the growth factors was followed by an instant transcriptional induction of the D-type cyclins, with *CYCD3;1* responding to the addition of cytokinin or Suc. These data gave the early suggestion that, similar to other eukaryotes, plant D-type cyclins also communicate cellular and environmental signals to the cell cycle machinery, data that were later substantiated by the phenotypic analysis of knockout and overexpressor lines.

Being essential proteins linking environmental factors to cell proliferation makes the D-type cyclins ultimate targets to arrest the cell cycle in response to stresses, both from biotic and abiotic origin. Similarly, D-type cyclins may be part of the key CDK/cyclin complexes to be inhibited upon the execution of important developmental decisions, such as cell cycle exit or the shift from a mitotic to a meiotic cell cycle. Indeed, through my own work and that of many others, later two distinct groups of plant-specific proteins were identified as being responsive to stress and developmental cues and that interact with D-type cyclins to inhibit the activity of the bound CDK. These CDK activity inhibitory proteins are now known in the field as KIP-RELATED PROTEINS (KRPs) and SIAMESE/SIAMESE-RELATED (SIM/SMR) CDK inhibitors (De Veylder et al., 2001; Yi et al., 2014) and clearly demonstrate that the D-type cyclins are the key factors that are inhibited to arrest cell cycle progression in response to stress and developmental signals. In retrospect, the high number of publications reporting on the involvement of either D-type cyclins,

KRP, or SIM/SMR proteins in the context of development or stress underscores the importance of the first report by Soni et al. (1995) on the discovery of the D-type cyclins.

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\*Reference highlighted for the 30<sup>th</sup> Anniversary of *The Plant Cell*.

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