Review

A kaleidoscopic view of the *Arabidopsis* core cell cycle interactome

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Although protein–protein interaction (PPI) networks have been shown to offer a systems-wide view of cellular processes, only a few plant PPI maps are available. Recently, the core cell cycle of *Arabidopsis thaliana* has been analyzed by three independent PPI technologies, including yeast two-hybrid systems, bimolecular fluorescence complementation and tandem affinity purification. Here, we merge the three interactomes with literature-curated and computationally predicted interactions, paving the way for a comprehensive picture of the plant core cell cycle machinery. Platform-specific interactions unveil the strengths and weaknesses of each detection method and give insights into the nature of the interactions among cell cycle proteins. Moreover, comparison of the obtained data reveals that a complete interactome can only be obtained when multiple techniques are applied in parallel.

The unique plant cell cycle interface

All eukaryotic organisms develop and reproduce as a result of cell growth and cell proliferation. Although the basic principles of cell division control appear to be conserved among eukaryotes, plants have unique features, including post-embryonic growth from the meristems, rigid cell walls preventing cell migration and the constant need to adapt to an ever-changing environment.

Over the past two decades of plant cell cycle research, multiple core components of the plant division machinery have been identified (Table 1) [1–5]. Cell division is driven by the timely activation of cyclin-dependent kinases (CDKs), the importance of which is revealed through the existence of multiple transcriptional and post-translational mechanisms that control their activity, including association with cyclins, CDK inhibitors, kinases and phosphatases, ubiquitin-dependent proteolysis and intracellular trafficking [6]. Intriguingly, *Arabidopsis thaliana*, and plants in general, encode more core cell cycle proteins than most other eukaryotes [7], possibly reflecting the developmental plasticity required to deal with a sessile lifestyle. The cell cycle, however, is an elaborate process controlled not by individual units, but by the concerted action of different protein complexes and their regulatory subunits, making the functional description of the individual components insufficient for its understanding. To comprehend how the cell cycle operates and gives rise to plant-specific cell division features, the biochemical and genetic interactions between all of its components have to be studied, and the behavior of the system emerging from these interactions has to be investigated [8]. This new paradigm is known as systems biology, which brings together high-throughput biochemical, genetic and molecular approaches (e.g. transcriptomics, proteomics, interactomics and metabolomics), and generates systems-wide data and computational tools that turn these data into models to create testable hypotheses concerning the biological system [9].

In a systems biology approach, the field of interactomics is a valuable component that studies networks of protein interactions [10]. To analyze protein–protein interactions (PPIs), multiple complementary technologies have been developed, some of which can be adapted easily for a high-throughput strategy [11], including the yeast two-hybrid (Y2H) assay [12], the bimolecular fluorescence complementation (BiFC) assay [13] and affinity purification coupled to mass spectrometry (AP–MS) [14] (Table 2). Although PPI data are still considered noisy and incomplete, numerous reports highlight their use in analyzing and understanding complex molecular processes [15–17].

Recently, the core cell cycle proteins of *Arabidopsis* have been analyzed by means of three independent PPI screens [7,18]. Here, to draw a more comprehensive picture of the plant core cell cycle machinery, we combined the obtained interactomes with available literature-curated data and computational predictions. In addition, in the light of their strengths and weaknesses, we discuss each detection method and review the nature of the interactions among the different cell cycle proteins.

Collecting the pieces of the cell cycle puzzle

To map interactions between 58 core cell cycle proteins, a binary *Arabidopsis* PPI network has been generated by means of two complementary interaction assays, BiFC and Y2H, [18]. Of the total 917 pairwise interactions tested by BiFC, 341 (37%) interacting pairs scored positively (Figure 1a). Of the 1339 pairs tested in the Y2H screen, 130 (9.7%) protein pairs scored positively. In a parallel study [7], interactions were mapped based on protein complex isolation through tandem affinity purification

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Table 1. Brief description of the core cell cycle proteins of *Arabidopsis thaliana*

Class	Name[a]	Function	Refs
CDK	CDKA;1	Ortholog of yeast Cdc28 and human CDK1/2, characterized by PSTAIRE hallmark in the cyclin-binding domain; key regulator of the G1–S and G2–M transition points; essential for cell division during male gametogenesis; must be phosphorylated on so-called 'T-loop' by CDK-activating kinases (CAKs) to be active; negatively regulated through binding with inhibitory proteins (KRPs/SMRs) and phosphorylation (WEE1)	[57–62]
CDK	CDKB (4)	Plant-specific CDK, divided into two major classes (B1 and B2); expressed during S/G2 (B1) or G2/M (B2); involved in skotomorphogenesis, stomatal development and maintenance of shoot apical meristem identity; essential for mitosis; negative regulators of cell cycle exit and endoreduplication	[35,63–65]
CDK	CDKC (2)	Acts together with CYCT in transcript elongation and splicing; mainly active in differentiated tissues	[66–69]
CDK	CDKE;1	Identical to HUA ENHANCER3; has a role in leaf cell expansion and floral cell-fate specification; phosphorylates the C-terminal domain of the largest subunit of the RNA polymerase II (CTD)	[70]
CDK	CDKG (2)	Acts together with CYCL;1; possibly involved in coupling cell cycle progression with transcript splicing	[2,7]
CAK	CDKD (3)	Phosphorylate CDKs and CTD; acts together with CYCH;1 and MAT1 to determine the growth rate and the differentiation status of cells	[59,71–73]
CAK	CDKF;1	Plant-specific CAK and CAK-activating kinase (CAKAK); phosphorylates CDKA;1 and CDKDs without need to bind a cyclin partner; no activity towards CTD	[59,71,74]
CDK-like	CKL (15)	CDK members discovered through genome sequence analysis	[2]
CKS	CKS1-2	Docking factors for positive and negative regulators of CDK activity; CKS1 transcripts are present in both mitotic and endoreduplicating cells; CKS2 is mitosis specific	[2,75,76]
Cyclin	CYCA1 (2)	Mitotic cyclins; possess a destruction box recognized by the anaphase-promoting complex (APC) ubiquitin ligase; CYCA1;1 is upregulated in G2 phase. CYCA1;2 is required for meiosis during male gametogenesis.	[2,77,78]
Cyclin	CYCA2 (4)	Mitotic cyclins; has a destruction box recognized by the APC ubiquitin ligase; CYCA2;3 negatively regulates endoreduplication through its interaction with CDKB1;1; CYCA2;4 is induced during lateral root initiation; expression is repressed by the transcriptional regulator ILP1	[79–82]
Cyclin	CYCA3 (4)	Upregulated at the G1–S transition before DNA replication; involved in the control of cell division and differentiation	[2,83,84]
Cyclin	CYCB (11)	M phase-specific cyclins divided into three subclasses: B1 (5), B2 (5) and B3 (1); transcriptionally controlled by M-specific activator promoter elements; CYCB1;1 expression is controlled by TCP20, coupling cell growth and division; proteins are destroyed by the APC ubiquitin ligase; ectopic CYCB1;1 expression promotes root growth; CYCB1;2 induces mitotic divisions in trichomes	[2,85–87]
Cyclin	CYCC (2)	Not functionally characterized yet	
Cyclin	CYCD (10)	D-type cyclins subdivided into seven classes; probably regulate the G1–S transition through phosphorylation of RBR; expression of D-type cyclins is modulated by phytohormones and sucrose; CYCD6;1 is involved in formative divisions generating the root ground tissue; CYCD4;1 controls lateral root initiation	[33,88–94]
Cyclin	CYCH;1	Regulatory subunit of D-type CDKs (see above)	
Cyclin	CYCL1	Regulatory subunit of G-type CDKs (see above)	[7,95]
Cyclin	CYCP (7)	Poorly characterized CDKA;1-interacting cyclins possibly linking cell division to the nutritional status of a cell	[96]
Cyclin	CYCT (5)	Regulatory subunit of C-type CDKs (see above)	
Cyclin	SDS	'SOLO DANCERS' encodes a cyclin-like protein interacting with CDKA;1 and CDKB1;1; involved in meiosis	[97]
E2F/DP	E2Fa, E2Fb, E2Fc	Control the transcription of G1/S-phase genes (e.g. *MCM3*, *CDC6*, *CDT1a*, *PCNA* and *RBR*); E2Fa and E2Fb overexpression induce ectopic divisions and endoreduplication; E2Fc lacks a transactivation domain, and is degraded after CDK phosphorylation in a SCF ubiquitin ligase-dependent manner, controlling the transition from skoto- to photomorphogenesis; E2Fc negatively regulates the entry into G1/S and positively regulates the switch to the endocycle	[35,98–101]
E2F/DP	DPa, DPb	Dimerization partners of E2Fs (see above)	
E2F/DP	DEL1, DEL2, DEL3	DP/E2F-like proteins; similarity with E2F and DP transcription factors is restricted to the DNA-binding domain, which is present in tandem, enabling binding to E2F-responsive sites as monomers; DEL1 inhibits the endocycle; DEL2 promotes cell proliferation and represses cell elongation; DEL3 controls cell expansion by repressing cell wall biosynthesis genes	[102–106]
E2F/DP	RBR	Cell cycle phase-dependent phosphorylation of RBR by CDKs releases the repression of E2F/DP transcription factors; RBR recruits DNA-modifying enzymes, such as histone deacetylases, and is involved in stem cell maintenance, cell differentiation and imprinting; controls the arrest of unfertilized gametophytes	[90,107–113]
Inhibitor	KRP1-KRP7	CDK inhibitors induced in response to extracellular or intracellular signals; interact with CDKA;1 and D-type cyclins; KRP1/2 are phosphorylated by CDKA;1 and CDKB1;1 preceding their destruction; KRP6/7 are targeted by the SCF^{FBL17} ubiquitin ligase for degradation during male gametogenesis; KRP2 regulates endoreduplication onset in a dosage-dependent manner and KRP1 influences cell size through balancing DNA ploidy levels; activity of some KRPs is regulated by PROPORZ1 in response to auxin	[114–119]
Inhibitor	SIM/SMR (13)	CDK inhibitors, comprising SIAMESE (SIM) and SIAMESE-related (SMR) proteins; SIM and SMR1 represent trichome- and sepal-specific activators of endoreduplication, respectively	[42–44]
Inhibitor	WEE1	Putatively involved in the inhibitory phosphorylation of Tyr residues in the ATP-binding site of CDKA;1 and D-type CDKs; controls cell cycle arrest in response to activation of the DNA integrity checkpoint	[71,120]

[a]Numbers between parentheses indicate members in the family.