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Endoreplication as a potential driver of cell wall modifications

Rahul Bhosale[1,2], Steven Maere[3-5] and Lieven De Veylder[3,4]

Addresses

[1]Plant and Crop Sciences, School of Biosciences, University of Nottingham, Nottingham, LE12 5RD, United Kingdom
[2]Center for Plant Integrative Biology (CPIB), University of Nottingham, Sutton Bonington LE12 5RD, United Kingdom
[3]Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, B-9052, Belgium
[4]VIB Center for Plant Systems Biology, Ghent, B-9052, Belgium
[5]Bioinformatics Institute Ghent, Ghent University, Ghent, B-9052, Belgium

Corresponding author: De Veylder, Lieven (lieven.deveylder@psb.vib-ugent.be)

Mailing address: Lieven De Veylder
Center for Plant Systems Biology
VIB-Ghent University
Technologiepark 71
B-9052 Gent (Belgium)
Tel. +32 9 3313961;
Fax: +32 9 3313809;
E-mail: lieven.deveylder@psb.ugent.be

E-mail addresses: rahul.bhosale@nottingham.ac.uk
steven.maere@psb.vib-ugent.be

Highlights

- A DNA endoploidy map of the Arabidopsis root reveals a strict spatio-temporal regulation of endoreplication.
- Endoreplication is prominent in tissues that require a rapid increase in cell wall materials, such as root hairs and xylem.
- Endoreplication-dependent cell wall modifications might account for the pathogen sensitivity of ploidy mutants.
- Endocycle onset correlates with expression of cell wall-modifying genes that drive cell expansion.

Abstract

Endoreplication represents a variant of the mitotic cell cycle during which cells replicate their DNA without mitosis and/or cytokinesis, resulting in an increase in the cells' ploidy level. This process is especially prominent in higher plants, where it has been correlated with cell differentiation, metabolic output and rapid cell growth. However, different reports argue against a ploidy-dependent contribution to cell growth. Here, we review accumulating data suggesting that endocycle onset might exert an effect on cell growth through transcriptional control of cell wall-modifying genes to drive cell wall changes required to accommodate turgor-driven rapid cell expansion, consistent with the idea that vacuolar expansion rather than a ploidy-driven increase in cellular volume represents the major force driving cell growth.

Introduction

During a mitotic cell cycle, each round of DNA synthesis is followed by mitosis and cytokinesis to equally distribute the duplicated DNA among two daughter cells. In contrast, during an endocycle (also known as endoreplication), mitosis and/or cytokinesis are skipped, resulting in an increase of the cell's DNA content from 2C to 4C, 8C, 16C,…. While this process is widespread across different kingdoms, endoreplication is especially prominent in higher plants, where endocycle activity has been correlated with cell differentiation, increased metabolic output and rapid cell growth [1,2]. It is anticipated that the latter processes benefit from an increase in RNA transcription through an increase in gene templates. Accordingly, Bourdon, et al. [3] provided quantitative evidence in tomato fruits that endopolyploidy increases transcription of rRNA and mRNA on a per-nucleus basis.

The most frequently reported effect of endoploidy is its effect on cell size, illustrated in Arabidopsis by the observed correlation between the DNA ploidy level and the size of epidermal pavement cells and trichomes [4,5]. However, the relationship between cell size and DNA ploidy level is not that clear, as both pavement cells and trichomes grow as well when endoreplication is severely inhibited [6,7]. Correspondingly, dynamic mapping of endocycle onset in Arabidopsis epidermal sepal cells not only revealed that the differences in cell size between cells with a different ploidy level correlate with a variability in the timing of the exit from the mitotic cell cycle, but also that endocycling cells are mainly bigger because they grow without being halved by division [8], arguing against a ploidy-dependent control of cell growth rates. Moreover, the relationship between cell volume and ploidy appears to be tissue-specific, because a strong correlation between both was found for leaf epidermal pavement cells, but not for the palisade mesophyll cells [9•]. Likewise, based on analysis of the tomato pericarp, Bourdon, et al. [10] suggested that cell size is not only dependent on ploidy levels but also on the

position of the cell within the tissue. Therefore, to understand the role of the endocycle, and in particular its relationship to cell size, it might be important to have a clear view on the spatiotemporal distribution of endoploidy across a complete organ. Experimentally, this can be achieved through cytological techniques such as DNA densitometry or fluorescent in situ hybridization, combining microscopy with image analysis of quantitatively labeled nuclei [9-12].

A virtual root ploidy map

Although being highly informative, DNA densitometry and fluorescent in situ hybridization are rather laborious techniques and of too low throughput to be easily applied on a complete organ. Recently, a computational approach has been used to overcome these challenges and to map the DNA ploidy distribution across the complete Arabidopsis root tip [13••]. Within the root, all cells arise linearly from a group of stem cells surrounding the quiescent center (QC). Close to the QC, cells are dividing. As cells age, they gradually lose their division competence and gain the possibility to enter the endocycle, resulting in cells having a 4C, 8C, or 16C DNA content. To address how cells with a different ploidy level are integrated into the Arabidopsis root, first the transcriptome of cortex cells with different ploidy levels was determined. Subsequently, the obtained ploidy-dependent expression data of each Arabidopsis gene was used to pinpoint which genes in the dataset have a spatiotemporal expression profile that might be useful to predict the tissue- and development-dependent endoploidy state of every cell within the root. To this end, a mathematical model was built to predict the expression level of a gene in 12 different developmental stages and 17 different tissue marker lines (corresponding to 14 different tissues) as a function of its measured ploidy-specific expression levels in the cortex and the (unknown) ploidy transition boundaries in the different tissues along the longitudinal root axis (**Figure 1a**). The optimal ploidy boundaries were subsequently estimated by fitting modelled expression for a given gene to the spatiotemporal expression values previously obtained experimentally through transcriptome analysis [14]. Except for genes exhibiting trivial expression profiles with low variance across tissues and developmental stages, a good fit can only be expected for genes for which the ploidy-specific expression levels measured in the cortex cells are a good proxy for the ploidy-specific expression levels in other tissues, and for which the spatiotemporal expression levels are primarily determined by the endoploidy state of the cell, and thus not by tissue- or developmental stage-related factors acting independently of endoploidy. Eventually, 332 of the best-fitting genes, with 83 peaking at each of the endoploidy levels 2C, 4C, 8C and 16C, were selected as ploidy markers to construct a virtual endoploidy map for the complete root tip. The marker gene list was significantly enriched in genes functioning in biological processes related to DNA replication and epigenetic regulation of gene expression, which are arguably more likely to exhibit absolute expression

levels linked to DNA content. The predicted endoploidy map, which was subsequently experimentally validated, revealed that endoreplication is under strict spatiotemporal control, showing an overall concentric pattern with the ground tissues displaying higher endoreplication activity and the inner vascular tissues mostly being of lower ploidy (**Figure 1b**).

Causal links between cell ploidy and cell wall characteristics

The obtained ploidy map revealed that within the epidermis, hair cells undergo one extra round of endoreplication compared to non-hair cells, reaching a maximum ploidy level of 16C as opposed to 8C for non-hair cells (**Figure 1b)**. This observation suggests that endoreplication might support hair tip growth. However, up to date, it is unclear whether endoreplication is a prerequisite for root hair growth. For the *midget* and *rhl1/hyp7* mutants that are defective in the DNA topoisomerase VI complex, which is essential for decantation of replicated chromosomes, the inability to progress beyond an 8C ploidy level has been found to correlate with a root hair tip outgrowth phenotype [15,16] (**Figure 2**). In contrast, E Sliwinska et al. [17] reported that there is no clear correlation between the length of collet hair cells (hairs arising at the transition zone between the root and hypocotyl) and the level of endoreplication across a diverse set of mutants studied, with the short root hair *ethylene overproducer 1* (*eto1*) mutant accumulating even a higher ploidy level. A possible explanation for this apparent contradiction is that the replication factors MIDGET and RHL1/HYP7 likely directly affect the endoreplication process, whereas the hormone mutant *eto1* might indirectly exert a growth effect independent of the endocycle. Therefore, experiments aiming at a more direct manipulation of the endocycle might be required to draw any conclusions on the role of the endocycle in root hair tip growth.

A putative scenario by which the endocycle might contribute to root hair tip growth is by providing the increased metabolic output needed for root hair tip outgrowth. Rapidly expanding root hairs express unique cell expansion and cell wall-modifying enzymes [18]. But root hair cells are not the only cells that demand a rapid increase in the production of cell wall materials, so do the xylem cells. Maturing xylem cells develop a thick secondary cell wall to provide the mechanical strength needed for transport of water and nutrients. The ploidy map revealed that, next to epidermal hair cells, xylem cells rapidly engage into the endocycle, in contrast to the phloem and phloem companion cells that mainly remain diploid. Strikingly, the endocycle-promoting *kaktus* mutation has been identified as a suppressor of the *esk1-5* mutation [19]. ESK1 encodes a xylan O-acetyltransferase and its mutation results in collapsed xylem vessels [20]. The observation that a ploidy-inducing mutation can rescue this phenotype again suggests a role of endocycle-driven gene expression in controlling cell wall biosynthesis.

A link between endoploidy and cell wall modification might also explain the observed altered pathogen response of ploidy mutant lines. Modification of the cell wall at sites of pathogen attack is a common response to infection [21,22] and the inability to do so, or the presence of a weakened cell wall, might explain in part the pathogen susceptibility phenotypes of endocycle onset mutants, such as observed for *smr1* and *sim smr1 [23,24]* (**Figure 2**). Likewise, ectopic overexpression of the endocycle-promoting *UVI4* and *OSD1* genes (see **Textbox** on molecular control of endocycle onset) results in enhanced disease resistance [25,26]. Strikingly, a transcriptome analysis revealed that 90% of the genes upregulated in the *CONSITUTIVE EXPRESSION OF PATHOGENESIS-RELATED GENES* (*cpr5*) mutant depend on SIM and SMR1 activity. Moreover, the pathogen resistance phenotype of the *cpr5* mutant is restored in the *cpr5 sim smr1* triple mutant [24], suggesting that SIM and SMR1 are directly responsible for endoploidy-dependent pathogen defense. Accordingly, transcriptome analysis of *SMR1* (also known as *LGO1*)-overexpressing lines revealed a constitutively activated defense response [27]. In agreement with a role for SIM and SMR proteins in pathogen defense, the rice *SIM/SMR* homolog *EL2* was originally identified as a gene being rapidly and strongly induced upon treatment with a biotic elicitor or flagellin [28,29].

More links between endoploidy and cell wall biosynthesis exist. For instance, the above-mentioned *midget* mutant not only displays a root hair phenotype but also an altered patterning of its seed coat, a phenotype speculated to be the result of a reduction in the secretion of mucilage, which is mainly composed of the cell wall components rhamnose and galacturonic acid [15]. Similarly, next to a constitutive pathogen response phenotype and reduced DNA content, trichomes of *cpr5* mutants display a thinner cell wall and reduced cellulose content [30,31]. Strikingly, *cpr5* mutant trichomes display an additional spontaneous cell death phenotype [31], similar to the phenotype observed for trichomes in which endocycle progression is specifically blocked through ectopic expression of the CDK inhibitor *KRP1* [6]. A speculative explanation might be that a reduction in ploidy results in insufficient cell wall components being produced to cope with turgor arising from the expanding vacuole, eventually resulting in bursting of the cells.

Interestingly, endoreplication onset precedes rapid cell expansion [13••,32]. Within the root, this rapid expansion at the cell division-to-cell expansion border depends on the antagonistic activity of cytokinin and auxin, resulting in an auxin minimum in the topmost meristematic cell [33]. This suggests that a low auxin level might act as a positional signal to trigger the switch from cell division to differentiation. More recently, it has been found that this drop in auxin content goes together with expression of cell wall-modifying genes, in particular encoding the cell wall-loosening expansins and plasma membrane-localised H^+-ATPases, together driving pH-dependent cell wall modifications,

further driving rapid cell elongation [34]. The observation that auxin represses endocycle onset [13••,35] suggests that endoreplication might contribute to such cell wall modifications. In support for this hypothesis, comparing the transcriptome of expanding wild-type versus *smr1* mutant cells revealed a correlation between endocycle onset, upregulation of specific cell wall-modifying genes, including xylan biosynthesis genes, and downregulation of *DEL3*, which encodes a transcriptional repressor of expansin genes and an UDP-glucose-glycosyl transferase [13••,36].

Ploidy-driven cell wall changes might not be restricted to endoploidy changes but might be generally related to polyploidization. When examining Arabidopsis plants with different somatic ploidy levels (2N, 4N, 6N and 8N), the ploidy level was found to be negatively correlated with lignin and cellulose abundance and positively with matrix polysaccharide content (hemicellulose and pectin) [37]. Interestingly, cross sections revealed that the shape of stem cortical cells was distorted in the high-ploidy plants, with cell walls being highly distorted and reduced in thickness. More specifically, stems of hexa- and octaploid plants appeared to be completely crushed, indicating that the strength of the cell walls was affected in the polyploid lines and again hinting to a link between ploidy and the cell wall characteristics. Correspondingly, transcriptome comparison of diploid versus tetraploid Col-0 plants revealed a limited set of differentially expressed genes, enriched for cell wall biosynthesis genes [38], whereas diploid and tetraploid etiolated hypocotyls were found to differ in permeability of the cuticle layer, an epidermal protective, hydrophobic waxy covering against pathogen attack and water loss [39]. Similarly, when comparing the mRNA transcriptome of diploid, tetraploid and octoploid plants, it was found that the absolute transcript levels of most genes scale with ploidy. Nevertheless, one cluster of genes showed a linear increase in expression per gene copy with increasing ploidy, and this cluster was found to be enriched for cell wall functions [40]. Therefore, it appears that similar to endoploidy, somatic ploidy might translate into cell wall changes, although currently it cannot be excluded that these changes represent indirect transcriptional responses that occur in reaction to changes in cytoplasmic versus cellular volume [40].

Conclusions

Although currently mainly supported by circumstantial data, recent reports suggest that endocycle onset may regulate the expression of cell wall-modifying genes to drive cell wall changes, presumably to prepare cells for the often massive cell enlargement following cell cycle exit. This is in line with the idea that vacuolar expansion likely represents the major force driving cell growth, rather than a ploidy-driven increase in cellular volume [41]. Likewise, recently a second mode of rapid root cell elongation was described as being dependent on actin dynamics but independent of endoreplication [42]. Indeed, plant cells in which endoreplication is impaired are still able to expand [6,7], indicating only a minor

contribution of the endocycle to growth compared to vacuolar expansion and actin reorganization. In the case of vacuole-driven cell expansion, the balance between the turgor generated by the vacuole and the strength of the cell wall determines the rate of cell growth. In this scenario, the endocycle could serve to modulate cell expansion by regulating the strength of the cell wall by stimulating the expression of both cell wall loosening and fortifying genes (**Figure 3**). Therefore, the endocycle might be of particular importance for tissues containing extremely rapidly expanding cells, where an increase in the gene copy number through endoreplication might be a way to cope with the high demand for new cell wall materials.

Seen the role of the cell wall in many biotic and abiotic responses, endoreplication-induced cell wall changes might also play an important role in adaptive stress responses. This may explain why the endocycle is mainly observed in species growing in variable environments [43,44]. How the endocycle specifically controls expression of cell wall biosynthesis and cell wall-modifying genes remains to be further elucidated. Results from the field of rhizobiology suggest a role for epigenetic reprogramming. Using nodule nuclei sorted according to their DNA content, a correlation between ploidy level, gene expression, and chromatin structure has been found [45]. In accordance, a high number of chromatin-related genes were found to be differentially expressed in wild-type versus *smr1* mutant plants [13], correlated with altered histone dynamics upon exit of the mitotic cell cycle [46]. Revealing the genetic network that accounts for reprogramming of the transcriptome in response to altered ploidy levels therefore represents a major future challenge.

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Textbox 1: Simplified molecular toolbox controlling endocycle onset

The molecular machinery driving endoreplication has been mainly studied in Arabidopsis, revealing a number of pathways seemingly operating in parallel and triggering endocycle onset by suppressing the abrupt rise in cyclin-dependent kinase (CDK) activity that is needed to trigger mitosis and cytokinesis. CDK inactivation is either achieved through association of the CDK subunit with inhibitory proteins or through preventing the accumulation of the cyclins that are rate-limiting for the G2-to-M transition, controlled by the SIM/SMR and CCS52A proteins, respectively. Biochemical and genetic data suggest that the SIM and SMR (mainly SMR1, also known as LGO1) proteins controlling endocycle onset target the G2/M phase-specific B1-type CDKs (CDKB1;1 and CDKB1;2) [47,48]. Although these SIM and SMR proteins bind as well to the more generic CDKA;1 kinase [47], recent data suggest that CDKA;1 might control SIM/SMR abundance post-transcriptionally by targeting phosphorylated SIM and SMR proteins for proteolytic turnover [49••].

Two other pathways operate through the Anaphase Promoting Complex/Cyclosome (APC/C), a multi-subunit E3 ubiquitin ligase complex targeting cell cycle proteins for destruction. Among these targets, the CDKB1;1-interacting cyclin CYCA2;3 has been best characterised in the context of endocycle onset [50]. Both pathways rely on a rate-limiting factor for APC/C activity, known as CCS52A1 and CCS52A2. These factors appear to control endocycle onset in a tissue-specific manner, with CCS52A1 being the main controller in the root elongation zone and trichomes, whereas both CCS52A1 and CCS52A2 contribute to endocycle onset in the leaf [51•]. CCS52A levels are controlled at multiple levels, including transcriptional repression of *CCS52A2* by the DEL1 transcription factor [52] and post-transcriptional control of CCS52A1 through the APC/C inhibitor UVI4 [53] and the UBIQUITIN-SPECIFIC PROTEASE 14 [54]. DEL1 itself is controlled by two counteracting E2F transcription factors (E2FB and E2FC) operating as transcriptional activator and repressor, respectively [55]. Both *CCS52A1* and *CCS52A2* transcription is further restrained in dividing cells through the repressive RBR1-E2FA complex [56]. Additionally, a rise in CCS52A1 activity appears to control an increase in CCS52A2 transcription through a yet unknown mechanism [51•].

More recently, the LATE MERISTEM IDENTITY (LMI) homeobox domain protein has been demonstrated to prevent the development of a stipule (a leaf-like outgrowth) into a leaf through stimulating endocycle onset. This process was speculated to be controlled through transcriptional activation of the WEE1 kinase that inhibits CDK activity through phosphorylation [57••], a mechanism described before to control endocycle onset in tomato [58]. This might represent yet another pathway

controlling endocycle onset, however probably operating in a tissue-specific manner, as WEE1 was demonstrated before not to control endocycle onset in the Arabidopsis leaf [59] (Figure I).

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Figures

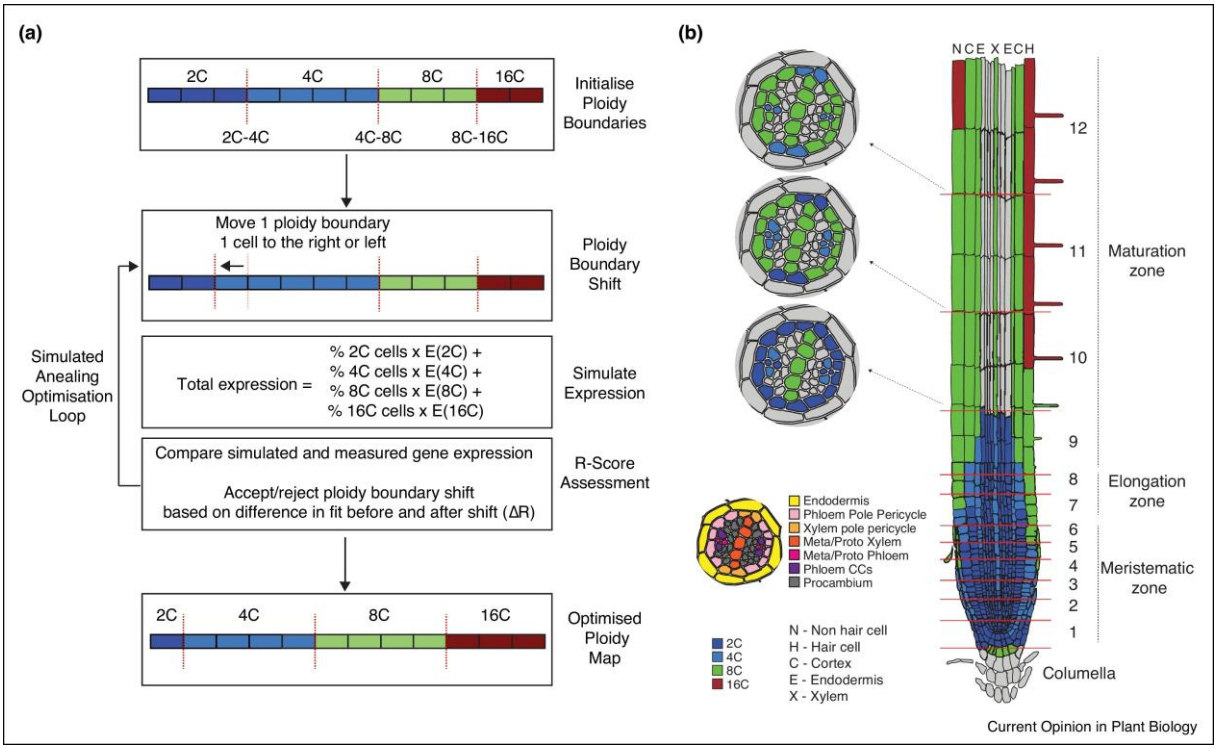


Figure 1. Virtual DNA ploidy map of the Arabidopsis root tip. **(a)** Simplified schematic representation of the mathematical modelling approach used in Bhosale, et al. [13••]. For simplicity, only one tissue layer is shown here, subdivided into 12 sections. Ploidy boundaries (2C-4C, 4C-8C and 8C-16C) are initially randomly assigned. Subsequently, one of the boundaries is shifted one cell up or down the cell file, and the obtained ploidy pattern is used to calculate a gene's total expression value in the tissue file (simulated expression), using the ploidy-dependent expression values of that particular gene in the root cortex cells. The simulated expression profile is then compared to the expression profile experimentally measured by Brady et al. [14] (R-score assessment) and the proposed boundary shift is accepted or rejected, after which the process is repeated until the best possible fit (low R-score) between the simulated and measured expression levels is obtained, resulting in an optimised ploidy map. **(b)** Root ploidy map obtained upon simultaneous model optimization for 332 ploidy reporter genes across 14 tissues and 12 root sections. Radial cross sections of slices 10-12 are shown to detail the vascular tissue. Procambium tissue is left unassigned because there is less confidence in the predicted ploidy levels as a result of the unavailability of expression profiles obtained using a specific procambium tissue reporter.

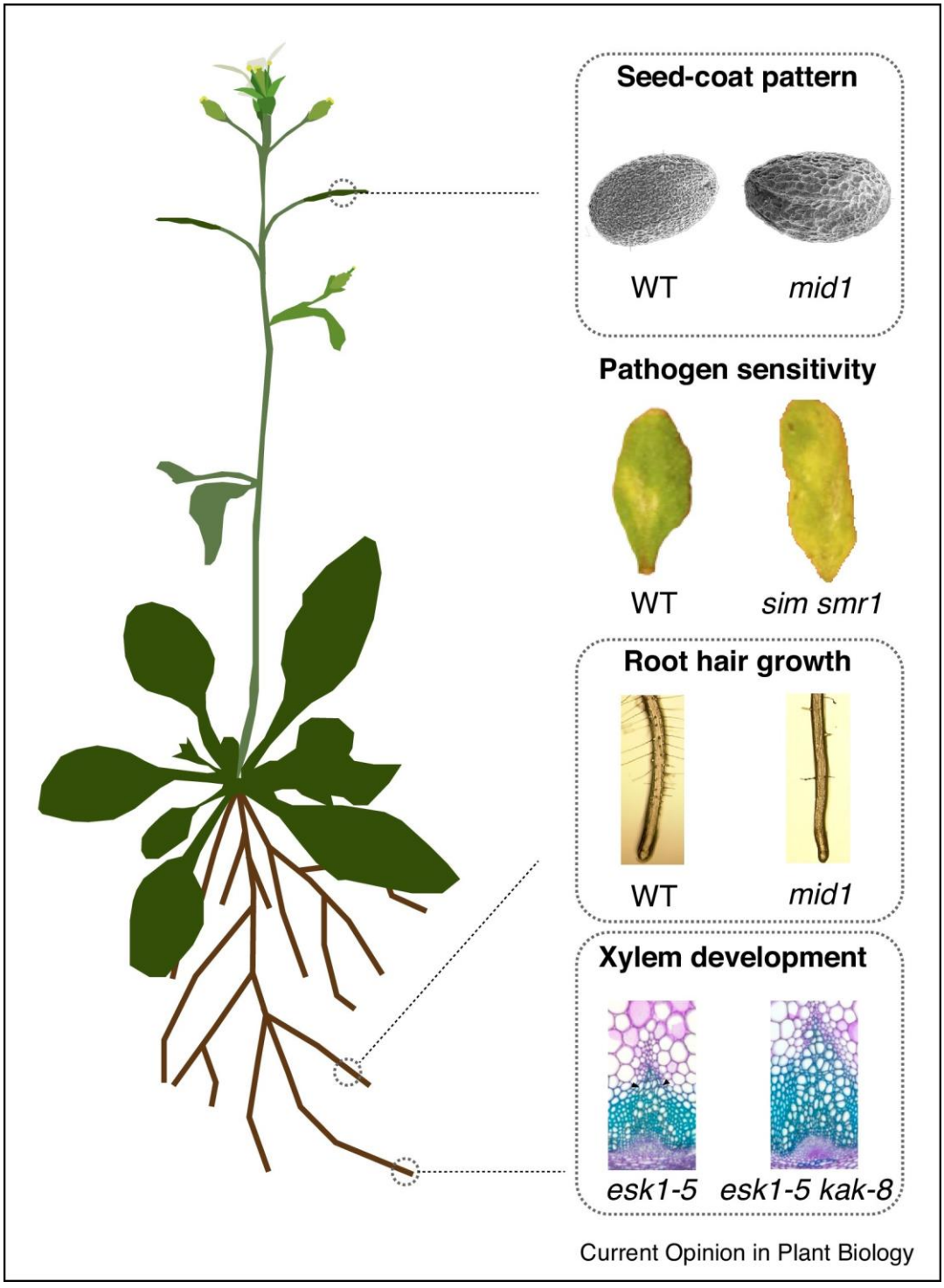


Figure 2. Causal links between endoreplication and cell wall biosynthesis or fortification. A reduced endoploidy level in the *mid1* mutant has been found to correlate with altered seed-coat composition and inhibition of root cell tip growth [15], whereas the *sim smr1* mutations have been correlated with altered pathogen sensitivity [23]. Reversely, the endocycle promoting *kak-8* mutation was found as a suppressor mutation rescuing the xylem vessel *esk1-5* mutant phenotype [19]. Part of the figures are reused from [15, 19, 23] (www.plantphysiol.org / www.plantcell.org). Copyright American Society of Plant Biologists.

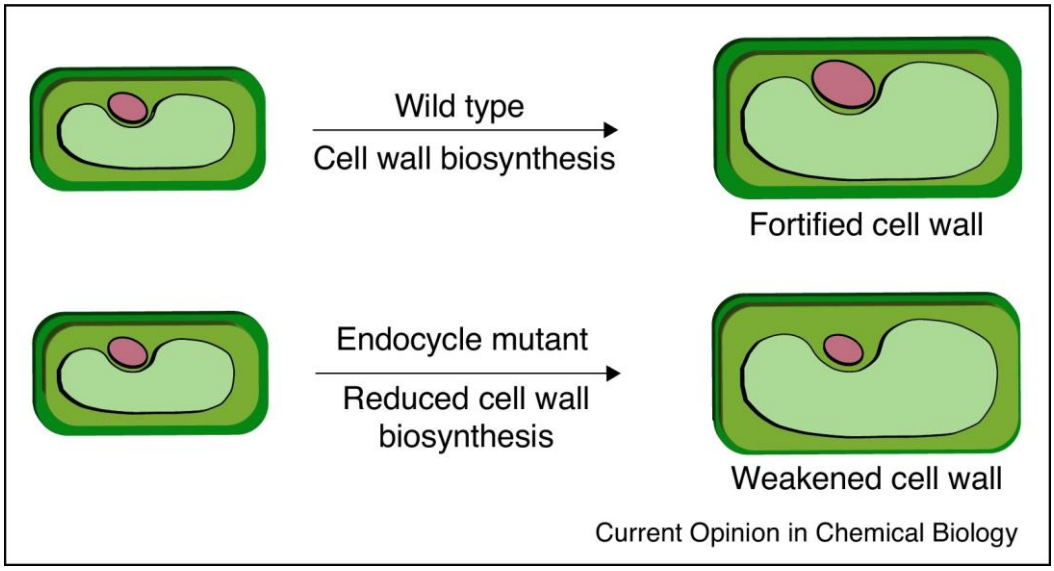
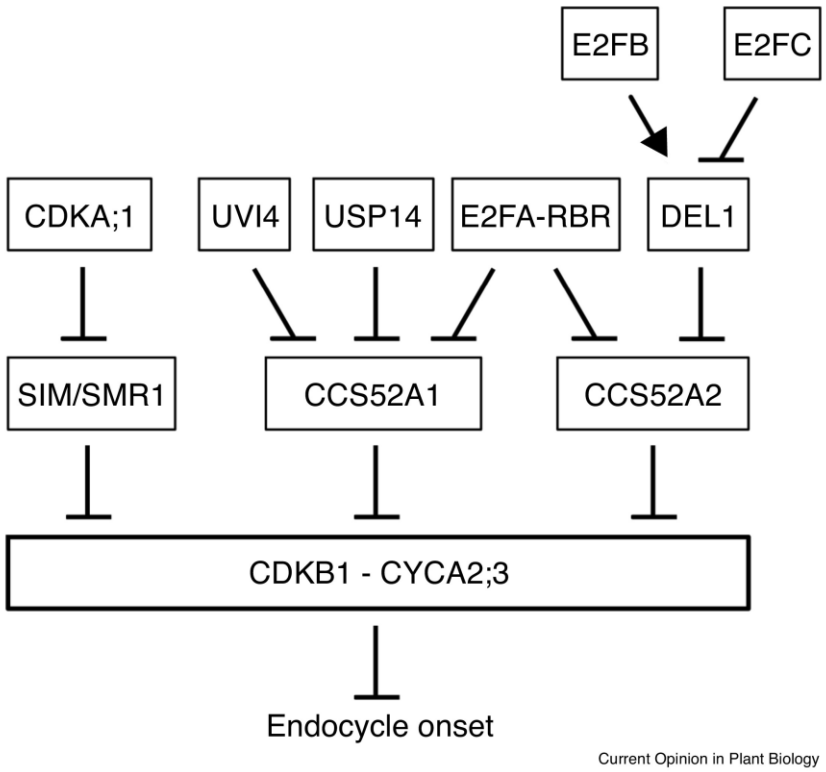


Figure 3. Model for the endocycle contributing to cell wall fortification. When cells stop to divide mitotically, the vacuole (light green) likely represents the major force for growth through exerting turgor pressure on the plasma membrane. We speculate that an increase in nuclear size (pink) through endoreplication helps in supporting such rapid growth with the expression of cell wall biosynthesis and -modifying genes, resulting in a fortified cell wall (dark green) matching the cell's rapid expansion. In endocycle mutants, such cell wall modification might lag behind, resulting in altered cell wall characteristics.



Text Box Figure I. Molecular toolbox of endocycle onset