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Modelling cell division and endoreduplication in tomato fruit pericarp

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AUTHOR-HIGHLIGHTS

- We model cell division in tomato fruit pericarp and its transition to endoreduplication.
- The model combines a cell cycle genetic regulatory network and auxin action.
- We show that auxin changes can cause transition from cell division to endoreduplication.
- Furthermore, the combined action of auxin to the cell cycle regulators improves the robustness of this shift.

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ABSTRACT

In many developing plant tissues and organs, differentiating cells switch from the classical cell cycle to an alternative partial cycle. This partial cycle bypasses mitosis and allows for multiple rounds of genome duplication without cell division, giving rise to cells with high ploidy numbers. This partial cycle is referred to as endoreduplication. Cell division and endoreduplication are important processes for biomass allocation and yield in tomato. Quantitative trait loci for tomato fruit size or weight are frequently associated with variations in the pericarp cell number, and due to the tight connection between endoreduplication and cell expansion and the prevalence of polyploidy in storage tissues, a functional correlation between nuclear ploidy number and cell growth has also been implicated (karyoplasmic ratio theory). In this paper, we assess the applicability of putative mechanisms for the onset of endoreduplication in tomato pericarp cells via development of a mathematical model for the cell cycle gene regulatory network. We focus on targets for regulation of the transition to endoreduplication by the phytohormone auxin, which is known to play a vital role in the onset of cell expansion and differentiation in developing tomato fruit. We show that several putative mechanisms are capable of inducing the onset of endoreduplication. This redundancy in explanatory mechanisms is explained by analysing system behaviour as a function of their combined action. Namely, when all these routes to endoreduplication are used in a combined fashion, robustness of the regulation of the transition to endoreduplication is greatly improved.

1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most important vegetable crops worldwide and the recent publication of the tomato genomic sequence has enormously increased our knowledge at the genetic level (Consortium, 2012). To use this improved genetic understanding in explaining phenotypic behaviour, functional links need to be established. In this paper we aim to improve understanding of the processes and interactions that underlie the formation of tomato fruits, by looking at phytohormonal influences on cell cycle regulation. Using a modelling approach, we zoom in on a key control point during tomato fruit development: the transition from the canonical cell cycle (Fig. 1A) to the partial cycle of endoreduplication (Fig. 1B) in tomato pericarp cells.

Abbreviation: CDK, cyclin dependent kinase; CKI, cyclin dependent kinase inhibitor; APC/C, anaphase promoting complex; KRP/ICK, Kip-related protein/Interactor of CDKs, family of CKIs; E2F, family of transcription factors; DP, dimerization partner; DEL, dimerization partner – E2F – like protein, alternative name for a-typical E2F factors; CCS52A, cell cycle switch protein 52A; RBR1, rhetinoblastoma-related protein; SIM/SMR, SIAMESE/SIAMESE-related plant-specific family of CKIs; CYC, cyclin, controls the cell cycle progression; SKP, S-phase kinase-associated protein; SCFSKP2A, SKP, Cullin, F-box, SKP2; PROPORZ1, Putative Arabidopsis Transcriptional Adaptor Protein, an Arabidopsis gene, important for the switch from cell proliferation to differentiation in response to the changes of phyto-auxin and cytokinin concentrations

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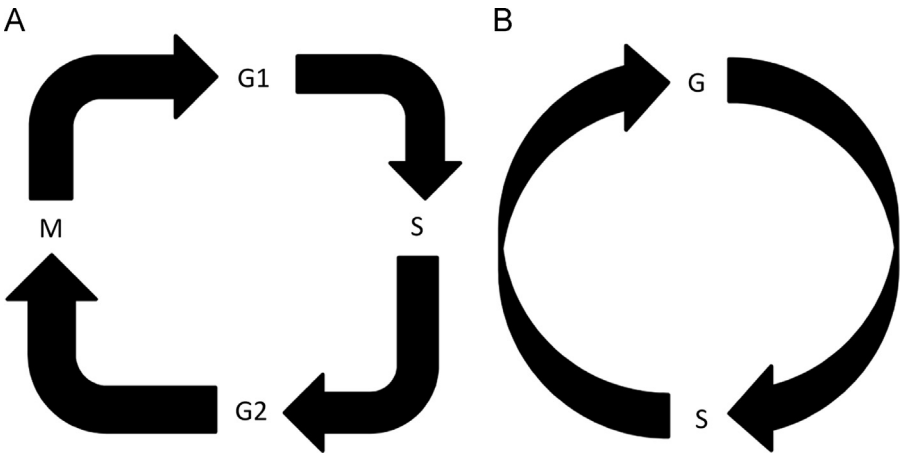

Fig. 1. *Cell cycle in plants*. (A) Canonical cell division consists of G1, S, G2, and M phases. In the S phase, the DNA is replicated whereas in the M phase, the nucleus and the cell divide. (B) Endoreduplication cycle. The phases are similar to that in the canonical cell cycle, except that the M phase is bypassed, effectively merging the G2 and G1 phases. Thus, the cell replicates its DNA, but it does not divide.

In most fleshy fruits, growth starts with intense cell division, which after the first weeks gradually declines and is replaced by cell enlargement (Gillaspy et al., 1993). During this expansion phase, individual cells spectacularly increase in volume: more than 10,000-fold in tomato mesocarp cells! (Cheniclet et al., 2005). In many fleshy fruits, as well as in maize endosperm and Arabidopsis trichomes, this huge cell expansion is accompanied by an increase in ploidy through the process of endoreduplication, i.e., an incomplete cell cycle in which cells continue to replicate their DNA without subsequent mitosis (Bourdon et al., 2010). The endoreduplication cycle is a developmental, by default irreversible process, which in tomato pericarp tissue marks the onset of differentiation in parenchyma cells.

Endoreduplication (sometimes also referred to as endocycle or endoreplication) is widespread in nature. It can be observed, e.g., in mammals, drosophila (Sher et al., 2013), yeast (Labib et al., 1995), and higher plants (Cheniclet et al., 2005). Numerous studies after the role of endoreduplication in nature have been conducted (Lee et al., 2009). It is found that endoreplication is fundamental for early development, e.g., in *Drosophila melanogaster* females endoreplication is employed to provide nutrients and proteins required to support egg production. Endoreduplication is also utilized for tissue regeneration under stress conditions. E.g., the negative effect of water deficit on leaf size in Arabidopsis can be reduced by increasing the level of endoreduplication. In mammals and higher plants such as tomato endoreduplication is employed to enable growth.

In view of the importance of endoreduplication, much research has been devoted to find the mechanisms that regulate the transition between canonical cell division and endoreduplication. Here, we focus on targets for regulation of the transition to endoreduplication by the phytohormone auxin, which plays a vital role in the onset of cell expansion and differentiation in developing tomato fruit.

The state of the art of molecular control and functioning of endoreduplication has recently been reviewed by De Veylder et al. (2011) and more specifically for tomato by Chevalier et al. (2011). In plants, normal G2-M progression is supposed to require significant activity of the 'mitosis promoting factor' (MPF). In plants the MPF is composed of the plant M-phase specific cyclin-dependent kinase CDKB1;1, which is activated by the A-type cyclin CYCA2;3. The quantitative presence of MPF is a major control factor in determining whether the cell divides mitotically or undergoes repeated rounds of duplicating its DNA without subsequent mitosis (Boudolf et al., 2009). Consequently, mechanisms that reduce the activity of the functional complex CDKB1;1/CYCA2;3 should inhibit cytokinesis and could promote endoreduplication. Contrary to the M-phase specific CDKB1;1, A-type CDKs (referred to as CDKA) are needed both for G1-S and G2-M transitions. As a consequence, the transition from mitotic to endoreduplicating cycles could also be sensitive to factors influencing CDKA activity. In the following paragraphs, we summarise putative mechanisms involved in the onset of endoreduplication.

1.1. *Proteolytic degradation of M-phase specific cyclins*

To form an active complex, CDKs depend on the presence of activating cyclins. Specific degradation of M-phase specific cyclins, such as the A-type cyclin CYCA2;3, could therefore promote endoreduplication. In the ubiquitin-mediated proteolysis pathway, the E3 ubiquitin ligase anaphase promoting complex/cyclosome (APC/C) selectively labels proteins for destruction (for reviews see Capron et al., 2003; Peters, 2006), based on the binding of the APC to the activating proteins CDH1 or CDC20 (Vodermaier, 2001). It was shown in Boudolf et al. (2009) that CCS52A (the higher plant orthologue of CDH1) affects the stability of CYCA2;3 in Arabidopsis. A further analysis on the APC activating subunits in tomato in Mathieu-Rivet et al. (2010) showed that SlCCS52A overexpression in young developing fruits led to significant alterations in cell division and DNA ploidy levels after eight days post-anthesis (dpa), whereas in fruits younger than eight dpa, cyclin transcription rates were suggested to be high enough to render the cell cycle progression insensitive to CCS52A expression (Joubès and Chevalier, 2000).

CCS52A expression is regulated by E2F transcription factors (Vlieghe et al., 2005; Lammens et al., 2008). In *Arabidopsis thaliana*, the E2F family of transcription factors is composed of six transcription factors E2F A–F and two dimerization partners DP-A and DP-B (Mariconti et al., 2002). The interplay between E2F transcriptional factor with Retinoblastoma-related protein 1 (RBR1) forms an important regulator of the expression of many prominent cell cycle control genes. Typical E2F factors A, B, and C dimerize with a DP to gain high DNA-binding specificity and can manipulate transcription via a transactivation domain. In contrast, atypical E2F factors D, E, and F (also called [DP-E2F-LIKE] DEL1-3, see Vandepoele et al., 2002) have two DNA-binding domains, and as a result can bind DNA as monomers. Because E2FD-F/DEL1-3 lack the typical transactivation domain, they can inhibit (but not cause) transactivation of E2F responsive elements, by competing for DNA-binding with E2FA and E2FB (Mariconti et al., 2002). The a-typical E2FE/DEL1 is directly involved in regulation of CCS52A by repressing its expression (Vlieghe et al., 2005; Lammens et al., 2008). It was also shown in Berckmans et al. (2011) that E2FB and E2FC have an opposite regulatory effect on E2FE/DEL1, whereas