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Nematode-Induced Endoreduplication in Plant Host Cells: Why and How?

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Plant-parasitic root-knot and cyst nematodes have acquired the ability to induce remarkable changes in host cells during the formation of feeding sites. Root-knot nematodes induce several multinucleate giant cells inside a gall whereas cyst nematodes provoke the formation of a multinucleate syncytium. Both strategies impinge on the deregulation of the cell cycle, involving a major role for endoreduplication. This review will first describe the current knowledge on the control of normal and aberrant cell cycles. Thereafter, we will focus on the role of both cell-cycle routes in the transformation process of root cells into large and highly differentiated feeding sites as induced by the phytoparasitic root-knot and cyst nematodes.

Cytological studies indicate that syncytia contain many large nuclei through cell fusion and endoreduplication. In contrast, giant cells acquire many nuclei through acytokinetic mitosis. More detailed studies have revealed that endoreduplication is also important for giant cell maturation. Our recent studies have demonstrated a functional role for the *CCS52* gene family, *DEL1*, and *RHL1* genes in the endocycle of both giant cells and syncytia.

Furthermore, enhanced endoreduplication appears to stimulate more and faster initiation of both types of feeding cells. However, endoreduplication is not sufficient for syncytium or giant cell development and both feeding cells need the mitotic cell cycle for optimal maturation.

The mitotic and endoreduplication cell cycle in *Arabidopsis thaliana*.

A standard cell cycle is characterized by a round of DNA replication (S phase) followed by mitosis (chromosome and nuclear division, M phase) and cytokinesis (cell division) (Fig. 1B1). One gap phase (G1) precedes DNA synthesis and another (G2) separates DNA synthesis from mitosis. Two main forms of incomplete cell cycles leading to genome multiplication are known in plants: polyploidizing mitosis, in which the cell cycle is blocked after mitosis (such as acytokinetic mitosis) (Fig. 1B2), and endoreduplication (also called endocycle) (Fig.

1B3), which results from a block of the mitotic phase immediately after DNA synthesis. During polyploidizing mitosis, a higher number of chromosomes than normal can be observed whereas, during endoreduplication, chromatids do not separate after DNA duplication, leading to enlarged chromocenters. Endoreduplication is frequently observed in some but not all plants (D'Amato 1964; Galbraith et al. 1991; Nagl 1976; Sugimoto-Shirasu and Roberts 2003) and can occur reiteratively, resulting in substantial genome amplification. Nuclei with up to 32C are detected in *Arabidopsis* (Galbraith et al. 1991) but, for example, in maize endosperm, cell nuclei can reach a 96C DNA content and even higher (Kowles and Phillips 1985, Leiva-Neto et al. 2004).

Considerable progress has been made in recent years in understanding the molecular basis of endoreduplication in plants, focusing on the model system *Arabidopsis thaliana* (Boudolf et al. 2009; De Veylder et al. 2011; Lammens et al. 2008). Both the normal mitotic and the endoreduplication cycle mainly make use of the same general cell-cycle machinery (Bourdon et al. 2010). This consists of a cyclin-dependent kinase (CDK) activated by a cyclin. Eukaryotes have different types of CDK and cyclins involved in different cell-cycle phases. Because G and S phases are common to both the mitotic cycle and the endocycle, the same proteins are important. Retinoblastoma-related protein (RBR) controls the G1–S transition (Gutierrez et al. 2002). Transcription factors that regulate the expression of genes required for DNA replication are controlled by heterodimeric E2F-DP transcription factors. RBR negatively regulates E2F activity by blocking its activation domain in its hypophosphorylated form. When RBR is phosphorylated by the CDKA-Cyclin D complex, it promotes the activation of E2F. Changes in activity of specific CDK complexes will determine the cell-cycle mode. The CDK complex that is composed of CDKB1;1 and CYCA2;3 displays a maximum kinase activity at the G2–M transition and triggers the cell to enter into mitosis. Controlled degradation of CYCA2;3 downregulates CDKB1;1 activity, leading to the onset of endoreduplication (Boudolf et al. 2009). CYCA2;3 protein stability was found to be controlled by CCS52A1, a stimulator of the anaphase-promoting complex (APC) (Boudolf et al. 2009). Upon activation, the APC ubiquitinates specific substrates that are then targeted to the proteasome for degradation. However, the model in which the CDKB1;1/CYCA2;3 complex represents the core unit controlling mitosis versus endocycle is an oversimplification of the complex network.

There are three *CCS52* genes in *Arabidopsis* divided into two classes, which most likely have a nonredundant function:

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CCS52B expression is detected from G2/M to M, while *CCS52A1* and *CCS52A2* are expressed from late M until early G1 (Fülöp et al. 2005). *CCS52A* is expressed in endoreduplicating cells targeting mitotic cyclins for degradation, and down-regulation results in reduced endoreduplication. Vanstraelen and associates (2009) showed that *CCS52A1* and *CCS52A2* are functional homologues in *A. thaliana* with a different expression pattern. The *CCS52B* gene is expressed in dividing cells, where it promotes the metaphase–anaphase transition (Fülöp et al. 2005). Therefore, *CCS52A* proteins might be crucial for APC activity in all cell-cycle phases, whereas the *CCS52B* proteins might play particular roles in M-phase progression (Fülöp et al. 2005). *CCS52A2* is a target gene of *DEL1* (Lammens et al. 2008). *DEL1*, an atypical E2F, acts as a transcriptional repressor and as a negative regulator of the endocycle (Vlieghe et al. 2005). *DEL1* transcripts are typically detected in mitotically dividing cells. Loss of *DEL1* function results in augmented ploidy levels, while ectopic expression diminishes endoreduplication (Vlieghe et al. 2005).

In addition to *DEL1*, several other transcription factors have been found to influence endoreduplication (Berckmans and De Veylder 2009). As an example, the myb-three-repeat transcrip-

tion factor *AtMYB3R4* can act as an activator or repressor of endoreduplication, depending on its phosphorylation state (Wildermuth 2010).

Furthermore, additional layers of regulation at the posttranscriptional level influence endoreduplication; for example, CDK inhibitors like *KRP* and *SIAMESE* prevent mitosis and promote endoreduplication in *A. thaliana* (Kasili et al. 2010; Verkest et al. 2005; Walker et al. 2000).

Rearrangements of microtubular arrays orchestrate cytokinesis.

At the onset of mitosis, the interphase cortical microtubular array transforms to a densely packed ring of microtubules encircling the nucleus. This structure is called the preprophase band and determines the cortical zone that corresponds to the future division plane (Van Damme 2009). As the cell progresses through mitosis, a bipolar mitotic spindle is formed that will ensure the precise segregation of the chromosomes during anaphase. Toward the end of mitosis, a cytokinesis-specific microtubular array named the phragmoplast assembles and this structure supports the formation and expansion of the cell plate (Jürgens et al. 2005). In recent years, insights have been gath-

Fig. 1. Sketches of a *Meloidogyne incognita*-induced gall and a *Heterodera schachtii*-induced syncytium showing the cell cycle and endocycle occurring within their feeding and neighboring cells. Green color is the epidermis, yellow the cortex, blue are neighboring cells, and white the feeding site. **A**, Sketches of a gall and a syncytium. **Ai**, A gall containing giant cells and its neighboring cells that undertake a normal cytokinetic cell cycle (1). Giant cells undergo (2) acytokinetic mitotic cycles, yielding several nuclei (violet spheres). Mitosis generates multiple giant cell nuclei that will subsequently undergo several rounds of endoreduplication (3) to generate nuclei with variable sizes and ploidy levels. At this stage, CDK/CYC activity decreases and mitotic cyclin expression is blocked. **Aii**, A syncytium and its neighboring cells that undertake a cytokinetic cell cycle (1). This mitotic activity results in additional neighboring cells that will fuse to the feeding site, resulting in a polynucleated syncytium. The syncytium will further undergo comparable endocycles (3) as occurring in maturing giant cells. **B1**, The mitotic cell cycle includes DNA synthesis and mitotic phases containing two resting Gap phases (G1 and G2), resulting in two daughter cells. This typically occurs in cells neighboring giant cells and the syncytium. **B2**, The acytokinetic mitotic cycle occurring in giant cells, and **B3**, the endocycle occurring in both galls and syncytia.

ered in the molecular basis of cytokinesis (Reichardt et al. 2007; Thiele et al. 2009) and several mutants are known that contain multinucleate cells with incomplete cell walls (Assaad 2001). However, an active mechanism that specifically prevents cytokinesis is unknown, although it can be mimicked by caffeine treatment (Samuels and Staehelin 1996; Yasuhara 2005).

Nematodes induce feeding cells via the activation of shortened cell cycles.

Sedentary endoparasitic nematodes can induce two different types of feeding sites, regardless of the plant host they infect (Perry and Moens 2011) but specific for the nematode genus. Root-knot nematodes induce 6 to 10 giant cells embedded in proliferating gall tissue, whereas cyst nematodes generate one large syncytium. Both types of feeding cells are induced within the vascular tissue due to the dedifferentiation of root cells near the xylem and phloem. Feeding cells are outsized and multinucleate, with a dense cytoplasm; they contain numerous organelles and display fragmentation of the large vacuole and cell wall ingrowths adjacent to the xylem.

Syncytium development. A syncytium is initiated when the nematode uses its stylet to puncture a single root cell and secrete a cocktail of effectors. Subsequently, cell wall dissolution leads to the fusion of adjacent cells as the syncytium expands. Neighboring cells clearly undergo mitosis before their incorporation into the syncytium (de Almeida Engler et al. 1999). Mitosis has been proposed to also occur inside the initial syncytium but this could not be unequivocally proven. The presence of transcripts of the mitotic marker *CYCBI;1* in young syncytia demonstrates that the cell-cycle progresses at least until the G2 phase (de Almeida Engler et al. 1999). Therefore, a shortened cell cycle (endoreduplication) is repeatedly stimulated, resulting in enlargement of nuclei in syncytia.

Giant cell development. The most obvious step in the formation of giant cells is the repeated acytokinetic mitosis of nematode-selected parenchymatic cells. Initially, cell wall stubs sometimes present in giant cells were thought to be a result of cell wall dissolution. It is now believed that stubs and fragments are the result of aborted cytokinesis (de Almeida Engler et al. 2004; Jones and Payne 1978). Occasionally, cell wall stubs are formed at the cell edge closest to the dividing nuclei (de Almeida Engler et al. 1999) or cell wall fragments are formed between the nuclei (Calliaud et al. 2008), both due to aborted cell plate formation. It will be interesting to find out how nematodes achieve this disruption in the cell cycle, and whether secreted effectors into the plant cell directly or indirectly could trigger incomplete cytokinesis.

Cytogenetic studies have shown that giant cell nuclei are polyploid or aneuploid. For example, chromosome counts of pea giant cell nuclei range from 15 ($2n = 14$) to over 100. This is most likely due to the high mitotic activity in giant cells that might result in fusion of adjacent metaphase configurations and unequal distribution of chromosomes to the daughter nuclei (Wiggers et al. 1990). However, the DNA content of giant cells can further increase for at least 1 week after mitosis has ceased, indicating that endoreduplication is not unique to syncytia. Confocal images of three-dimensional reconstruction of gall nuclei at different time points after nematode infection displayed no connected nuclei (Vieira et al. 2012). Thus, chromatin bridges previously observed (Bird 1961; Starr 1993; Wiggers et al. 1990) in giant cells might be rarely occurring or an artefactual observation due to sample preparation or examination of single sections.

Cell-cycle activation during syncytium and giant cell development.

Activation of the cell cycle has been demonstrated to be crucial for normal feeding site development and subsequent nema-

tode growth and reproduction (de Almeida-Engler et al. 1999; Van de Cappelle et al. 2008). What is the rationale behind this genome amplification?

One of the proposed consequences of polyploidy or endoreduplication is the increase in cell size and accelerated growth due to the shortened cell cycle. Less time and energy need to be spent for the production of mitosis- or cytokinesis-specific proteins and structures. Furthermore, the cell is proportionally longer in the metabolically active interphase than in mitosis. However, the concept that endoreduplication leads to larger cells has been challenged (Sugimoto-Shirasu and Roberts 2003) because overexpression of the cell-cycle inhibitor ICK/KRP genes causes a decrease in the ploidy level but, at the same time, an increase in cell size. Nevertheless, inhibition of endoreduplication in nematode-infected plants resulted in smaller feeding sites and affected nematode development (discussed below).

From the parasite's point of view, it is easier to control an interaction with few but large metabolically active cells rather than to feed on a group of many small cells. By puncturing one or a few cells with its stylet, the nematode will more efficiently retrieve all nutrients needed to complete its life cycle. Induction of the mitotic cycle coupled with the endocycle by root-knot nematodes during infection of plant roots might be decisive for the dedifferentiation of vascular parenchyma cells into giant feeding cells (Fig. 1Ai). On the other hand, for a syncytium (Fig. 1Aii), being multinucleated and expanding via cellular fusion, an increased DNA content might be sufficient to sustain cyst nematode development and reproduction.

Endoreduplication is important but not sufficient for giant cell and syncytium formation.

Although cytological studies support the idea that endoreduplication occurs in giant cells and syncytia (de Almeida Engler et al. 1999; Rohde and McClure 1975; Rubinstein and Owens 1964), only recently has a detailed functional analysis been reported of the genes involved (de Almeida Engler et al. 2012). Previously, observations of ^3H -thymidine incorporation into the nuclei of giant cells and syncytia have shown that additional DNA replication cycles take place in feeding sites (de Almeida Engler et al. 1999). Although giant cells show prominent DNA synthesis compared with syncytia, the latter show a longer ^3H -thymidine incorporation timing (de Almeida Engler et al. 1999). This shows that additional cycles continue to occur in syncytia until later stages of development (up to 20 days after inoculation [DAI]) whereas, in giant cells, the DNA replication phase is limited to approximately 14 DAI. To evaluate the importance of DNA synthesis for giant cells and syncytia, hydroxyurea has been used to block the G1-to-S transition. This drug inhibits the activity of the ribonucleotide diphosphate reductase enzyme and deprives the cells of newly synthesized deoxynucleotide triphosphates, hence preventing DNA replication (Young and Hodas 1964). These experiments have shown that nematodes can penetrate but are unable to induce feeding sites, demonstrating that DNA synthesis arrest in roots interferes with the ability of the plant to respond to the presence of the pathogen. Therefore, DNA synthesis is essential for the primary establishment of both giant cells and syncytia. Treatment after infection affected giant cells more than syncytia, possibly due to continuous cell fusion in the latter leading to cell-cycle-independent polynucleation. Finally, treatments on established feeding sites did not affect nematode maturation and reproduction.

Previous work has also demonstrated the early upregulation of key components of the core cell-cycle machinery in nematode feeding sites (*CDKA;1*, *CDKB1;1*, *CYCA2;1*, and *CYCBI;1*), as shown by promoter activity and transcript localization (de

Almeida Engler et al. 1999; Niebel et al. 1996). Expression of these genes increases in young galls and syncytia (Fig. 2B and C) and is downregulated as cell-cycle activity is diminished already at 14 DAI (Fig. 2D and E), as shown here (Fig. 2) for *CYCBI;1*, a gene typically controlled during mitosis. Different from other cell-cycle genes analyzed, induction of *CYCBI;1* is unique for galls and syncytia, considering that its expression is normally not observed in the root tissue where nematodes induce their feeding sites. Activity of this set of genes (*CDKA;1*, *CDKB1;1*, *CYCA2;1*, and *CYCBI;1*) persists in syncytia, possibly due to the long expansion phase accompanied by continued cell division of neighboring cells.

This study was followed by the functional analysis of genes reported to be involved in the plant endoreduplication process in feeding sites. Endocycles caused by overexpression of E2Fa and DPa in *Arabidopsis* plants induce key S-phase genes such as *ORC*, *CDC6*, *CDT1*, and *MCM* genes (Kondorosi and Kondorosi 2004). Most of these genes are somewhat expressed in galls and syncytia, suggesting the occurrence of a typical endocycle route in nematode feeding sites (J. de Almeida Engler, L. De Veylder, G. Gheysen, and G. Engler *unpublished data*).

The switch from mitotic to endoreduplication cycles is controlled by *CCS52A*. *CCS52A* is the plant ortholog of the yeast and animal Cdh1-type proteins while *CCS52B* is plant specific (Tarayre et al. 2004). As for its orthologous counterparts, *CCS52A* was observed to promote the onset and progression of endoreduplication (Cebolla et al. 1999; Vinardell et al. 2003). High promoter activity of *CCS52A1* has been shown to be induced by biotrophic phytopathogens, as in galls of root-knot nematodes (Favery et al. 2002), as well as in nodules of beneficial plant symbionts (Cebolla et al. 1999). Elevated *CCS52B* promoter activity has also been observed in young galls and syncytia (Fig. 3A and C). Decreased β -glucuronidase staining was observed in mature feeding sites (Fig. 3B and D), supporting the view that endoreduplication slows down during gall or syncytium maturation. High mRNA levels of both *CCS52A1* and *CCS52B* genes were detected at various developmental stages of galls induced by root-knot nematodes and syncytia induced by cyst nematodes, strongly suggesting their participation in the endocycle during nematode feeding site expansion (de Almeida Engler et al. 2012).

High *CCS52* expression, intense ^3H -labeling (de Almeida Engler et al. 1999), and high transcript levels of cell-cycle genes involved in DNA synthesis (J. de Almeida Engler, L. De Veylder, G. Gheysen, and G. Engler *unpublished data*) are also seen in cells neighboring the syncytia, suggesting that cell

division might be followed by cycles of endoreduplication in these cells prior to incorporation. Then, additional cycles might take place in the nuclei after incorporation of neighboring cells into the feeding site. This is supported by the fact that flow cytometrical analysis of syncytium nuclei shows peaks ranging from 2C to 32C (J. de Almeida Engler, L. De Veylder, G. Gheysen, and G. Engler *unpublished data*).

Considering the importance of the *CCS52* gene family and *DEL1* in the endocycle, we recently performed a functional analysis of these genes in nematode feeding sites (de Almeida Engler et al. 2012). Overexpression as well as knock down of *CCS52* genes and *DEL1* have an impact on giant cell and syncytium development. Analysis of propidium iodide-stained and cleared nuclei of galls overexpressing *CCS52B* revealed large nuclei with bulky chromocenters (Fig. 3E), compared with the smaller nuclei present in wild-type galls (Fig. 2F) and uninfected *Arabidopsis* roots (Fig. 2G). Interestingly, upregulation of the different *CCS52* genes (*CCS52A2* and *CCS52B*) reported to be activators of the endocycle also speeds up gall and syncytium initiation and induces higher numbers of both types of feeding sites (compare Fig. 4A with 4 for galls and C with D for syncytia). These observations suggest that cells competent to dedifferentiate and enter the endocycle are possibly preferentially used for induction of root-knot and cyst nematode feeding sites. Therefore, nematodes might favor the choice of cells in the root elongation zone, a transition zone dealing with a state of differentiation where the mitotic cycle is taken over by the endocycle. However, accelerated induction of the endocycle puts a stop to the mitotic phase, also crucial for gall and syncytium development, as illustrated by their hampered development in the *CCS52*-overexpressing plants (de Almeida Engler et al. 2012). These findings support the idea that the endocycle alone is not sufficient for nematode feeding-site maturation.

On the other hand, downregulation of *CCS52A* and *CCS52B* prevented the development of a large fraction of galls and syncytia compared with wild-type *Arabidopsis* (Fig. 4E and F). These analyses clearly show the active involvement of the *CCS52* genes in root-knot and cyst nematode feeding-site development, most likely by positively regulating the endocycle. *CCS52A* might promote endoreduplication in developing giant cells and syncytia through degradation of mitotic cyclins that would otherwise promote successive re-entry of giant cell nuclei into mitosis. During the symbiotic interaction of *Medicago truncatula* and rhizobial bacteria, cells deficient in *CCS52A* do not undergo endoreduplication and are refractory to bacterial infection (Kondorosi and Kondorosi 2004). As ex-

Fig. 2. Promoter activity of *CYCBI;1* in an *Arabidopsis* root apical meristem, in galls and syncytia. **A**, *CYCBI;1* expression in a root apex showing a patchy expression pattern. *CYCBI;1* expression in **B**, a gall and **C**, a syncytium 7 days after inoculation (DAI), showing high promoter activity in both galls and syncytia. *CYCBI;1* expression in **D**, a gall and **E**, a syncytium 14 DAI showing low promoter activity in both galls and syncytia. RAM, root apical meristem; EZ, elongation zone; G, gall; n, nematode; S, syncytium.

pected, overexpression of DEL1, a repressor of the endocycle (Vlieghe et al. 2005), inhibits giant cell and syncytium development (de Almeida Engler et al. 2012). In DEL1-overexpressing plants, mitosis is stimulated in giant cells as well as in surrounding cells, causing total gall disarrangement (Fig. 4G). Giant cells which succeeded in developing displayed multiple small nuclei, and others accomplished cytokinesis (Fig. 4G) (de Almeida Engler et al. 2012). In agreement with the idea that mitosis is not normally occurring inside a syncytium, no nuclear division is observed in this type of feeding site except for neighboring cells (Fig. 4H). In any case, syncytia in the DEL1^{OE} line were smaller possibly due to the inhibition of the endocycle. Finally, the implication of another endocycle regulator, ROOT HAIRLESS 1 (RHL1), was investigated upon nematode infection. The RHL1 forms a multiprotein complex with plant topo VI, participating in decatenation of duplicated chromosomes, and is linked with defects in the third round of the endocycle (Sugimoto-Shirasu et al. 2002). The mutant *rhl1* allowed root-nematode infection but only allowed the development of minute giant cells while cyst nematodes did not even succeed in infecting *rhl1* roots (de Almeida Engler et al. 2012).

Our data demonstrated that recurrent endocycles during feeding site ontogeny could assure the enlargement of nuclei and

giant cell size and syncytium expansion related to the high metabolic activity of the outsized feeding cells which would, in consequence, provide sufficient food for nematode growth and reproduction.

The mitotic cycle is critical for giant cell and syncytium development.

Although the endocycle seems essential for both giant cell and syncytium development, it is not sufficient to drive feeding site formation (de Almeida Engler et al. 1999, 2012). Nuclei in giant cells are not only enlarged but also abundant as a result of successive mitotic events without cytokinesis (Vieira et al. 2012). Although mitosis seems to be a common event in giant cells, mitotic figures have not been observed in syncytia (de Almeida Engler et al. 2004; Huang and Maggenti 1969; Wiggers et al. 1999). Conversely, syncytia contain multiple nuclei as a result of fusion of surrounding cells which undergo division within the vascular tissue. There, mitosis is essential to occur in neighboring cells prior to their incorporation to allow lateral and longitudinal syncytium expansion. Markers of the G2/M phase such as *CDKB1;1* and *CYCBI;1* are highly expressed in mitotic giant cells as well as in dividing neighboring cells. In the case of syncytia, promoter activity of these genes is often higher in neighboring cells than in the syncytia

Fig. 3. Expression of *CCS52B* in galls and syncytia, and nuclei in giant cells of the *CCS52B*-overexpressing line. Activity of the *CCS52B* promoter in galls **A**, **7** and **B**, 21 days after inoculation (DAI). Activity of the *CCS52B* promoter in syncytia **C**, **7** and **D**, 21 DAI. Fluorescence image of propidium iodide-stained cleared nuclei in **E**, a gall of *CCS52B* overexpressing line 21 DAI; **F**, a wild-type gall 21 DAI; and **G**, an uninfected *Arabidopsis* root. **E** to **G**, Fluorescence images are projections of serial confocal optical sections revealing propidium iodide-stained nuclei (red). Note the increased nuclear and chromocenter size in giant cells of the *CCS52B*-overexpressing line. G, gall; n, nematode; S, syncytium; UR, uninfected root. Bars = 100 μ m (**A** to **C**) and 20 μ m (**E** to **G**).

themselves (de Almeida Engler et al. 1999). In addition, analysis of a set of mitotic cyclins shows strong expression in these dividing cells before syncytial incorporation (J. de Almeida Engler, L. De Veylder, G. Gheysen, and G. Engler *unpublished data*).

Cell-cycle progression in galls and syncytia is obstructed (de Almeida Engler et al. 1999) by the inhibitor oryzalin, a dinitro-aniline herbicide with strong binding affinity for plant tubulins inhibiting microtubule polymerization in plant cells and, consequently, blocking mitotic events to occur (Morejohn et al. 1987). Prior observations of giant cell development in plants treated (1 to 7 DAI) with oryzalin revealed that the cell cycle was blocked at the G2 phase and their development was delayed. The cytoplasm was less dense compared with untreated giant cells and a large number of nematodes failed to mature. Similar treatments on syncytia showed that the feeding site expanded normally by incorporating neighboring cells but, as these ceased to divide, narrower syncytia resulted compared with untreated ones. Slightly fewer nematodes matured in this treatment compared with control roots. At later developmental stages, oryzalin treatment had no effect on feeding sites and allowed nematodes to complete their life cycle (de Almeida Engler et al. 1999). Further functional analysis using knockout or knockdown lines and overexpression of genes involved in the mitotic cell cycle in nematode feeding sites will endorse the data obtained from expression analyses and inhibitor treatments.

Nematode effectors that trigger cell-cycle changes in the plant are yet to be verified.

Recent years have witnessed the identification of several nematode-secreted effectors that interact with plant proteins and, in this way, manipulate plant developmental pathways. For example, a cellulose-binding protein from the cyst nematode *Heterodera schachtii* has been shown to interact with and enhance activity of an *A. thaliana* pectin methylesterase, to facilitate syncytium development (Hewezi et al. 2008). Pectin methylesterases contribute to cell growth by regulating the properties of plant cell walls via demethylesterification of pectin.

Another appealing example is the novel effector protein Hs19C07 from *H. schachtii* that was proven to interact with the auxin influx protein LAX3 of *A. thaliana*, and overexpression of this effector increased the rate of lateral root emergence, indicating an enhanced auxin influx (Lee et al. 2011). Auxin accumulation has been shown to be important for syncytium development (Goverse et al. 2000).

It will be interesting to find out which nematode effectors might directly or indirectly affect the endoreduplication cycles occurring in nematode feeding sites or the acytokinetic mitosis in giant cells. Several potentially secreted nematode proteins with a possible role in cell-cycle regulation, including a CDC48-like protein, a ubiquitin, and a SKP1-like protein, have been identified; however, a role in cell-cycle regulation during feeding site formation remains to be shown (Bellaafiore et al. 2008).

Fig. 4. Effect of *CCS52A2*, *CCS52B*, and *DEL1* overexpression and *CCS52AB* knockdown in galls. Bright-field images of longitudinal tissue sections stained with toluidine blue. **A**, Section through a gall 7 days after inoculation (DAI) showing giant cells formed in a wild-type root. **B**, Section through a gall 7 DAI formed on a plant overexpressing *CCS52A2* showing larger giant cells containing enlarged nuclei (white arrowheads) compared with the wild-type. **C**, Section through a syncytium formed in a wild-type root 7 DAI. **D**, Section through a syncytium 7 DAI overexpressing *CCS52B* showing larger nuclei (white arrowhead) than in the wild type, and accelerated feeding site differentiation. **E**, Section through a gall 14 DAI in *CCS52AB* knockdown line shows blocked giant cell development and delayed nematode maturation. **F**, Section through a syncytium 14 DAI in *CCS52AB* knockdown line showing blocked syncytium development. **G**, Section through galls overexpressing *DEL1* showing disturbed ectopic mitoses in giant cells and neighboring cells. **H**, Section through a syncytium overexpressing *DEL1* 14 DAI do not show an increased number of nuclei compared with the wild type. Abbreviations: Asterisk, giant cell; n, nematode; S, syncytium. Bar = 50 μ m.

Conclusions.

This review assembles data on cyst and root-knot nematodes with an attempt to better identify the relevance and essential components of the endoreduplication cycle in nematode feeding sites. Nematodes seem to employ the classical cell-cycle machinery composed of the mitotic cycle and the endocycle, using them for their benefit. Initially, it was thought that giant cells mainly deployed acytokinetic mitoses and that endoreduplication was only marginally involved in the maturation of giant cells. In contrast, a syncytium would mainly depend on endoreduplication. Our current knowledge, however, indicates that galls and syncytia both need mitosis for their efficient maturation whereas, in both cases, endoreduplication enhances feeding site expansion. Surprisingly, premature endocycle activation can accelerate initiation of both types of feeding sites. Although, currently, the components involved in the endocycle are being deciphered by functional analysis in giant cells and syncytia, many questions remain unresolved. Are nematodes able to induce both the mitotic cell cycle and the endocycle simultaneously in one giant cell but involving different nuclei? Is the mitotic cell cycle switched off after forming a polynucleated cell, so that the endocycle can take over and act on all nuclei simultaneously in a feeding cell? Do some large nuclei result from incomplete mitosis or metaphase fusion or can nuclei undergo fusion after completing mitosis? Data based on whole-mount confocal imaging suggest that nuclei in giant cells first go through a highly synchronized mitotic stage which then switches to an endoreduplication stage to allow giant cell expansion (Vieira et al. 2012). However, mitotic events occurring in distinct giant cells building up a gall are not always synchronized. A more profound analysis of nuclear ploidy levels and time-lapse in vivo imaging of nuclei in galls might help to better address these questions.

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