Modulation of host nuclear ploidy: a common plant biotroph mechanism
Mary C Wildermuth

Plant biotrophs often establish highly specialized and localized interaction sites where sustained nutrient exchange occurs. Increased plant nuclear DNA ploidy at or adjacent to these sites has now been reported for a diverse set of interactions, including those with fungal and bacterial symbionts and parasitic fungi and nematodes. Also, novel regulators of induced endoreduplication have recently been identified. When localized host endoreduplication is reduced, so too is the growth and/or development of the biotroph, suggesting endoreduplication supports the enhanced metabolic demands imposed by these interactions. Transcriptome analyses support this function and further identify specific ploidy-impacted processes. Remarkably, notwithstanding differences in time scales, the ploidy-impacted processes are consistent with the Gene Balance Hypothesis, which can also be used to predict effector targets. As effector influence may diminish with enhanced ploidy, these interaction sites may be uniquely suited to identify effector-impacted processes as well as elucidate endocycle regulation and function.

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Introduction
During the classical mitotic cell cycle, DNA is duplicated during the S (synthesis) phase and equally divided during the M (mitotic) phase resulting in two daughter cells each with DNA content of 2C, identical to that of their parents. By contrast, during endoreduplication repeated cycles of chromosomal DNA replication occur without M and cell ploidy is doubled with each endoreduplication cycle (e.g. 4C, 8C, 16C, etc.). Endoreduplication occurs in a wide variety of cell types in insects, mammals, and plants [1]. In plants, it is most frequently observed in angiosperms with small genomes, including the model species Arabidopsis thaliana [2] and Medicago truncatula [3]. Though the cell and tissue-specific pattern of plant endoploidy is species-specific, it usually occurs in terminally differentiated, larger, single cells (e.g. Arabidopsis trichomes) and organs with enhanced metabolic capacity like endosperm and fruit. Therefore, it has been primarily studied in a developmental context using resource-rich model species such as A. thaliana (e.g. trichomes [4]) and agriculturally important systems including the maize endosperm [5] and tomato fruit [6]. These studies have identified a number of key mediators and components of endocycle initiation and progression. However, few transcription factors with roles in endoreduplication have been determined (recently reviewed in [7]) and specific functional roles of endoreduplication can be difficult to resolve in developmental systems.

Host endoreduplication at or adjacent to sites of sustained nutrient exchange has now been reported for a diverse set of biotroph–plant interactions. In these systems, endoreduplication is induced, highly localized, and not directly coupled to plant development, making them uniquely suited to investigations of endocycle regulation and function. In this review, I discuss (i) the biotroph/plant systems, (ii) functional impacts of ploidy, incorporating novel transcriptome analyses, and (iii) regulatory control of induced endoreduplication. The functional impacts of ploidy are discussed in the context of the Gene Balance Hypothesis that takes into account gene dosage and balance.

An emerging theme: host endoreduplication in biotroph–plant interactions
To date, host endoreduplication has been observed at or adjacent to the nutrient exchange sites of symbiotic interactions with arbuscular mycorrhizal fungi (AMF) and endosymbiotic rhizobia, and parasitic interactions with powdery mildews (PM) and nematodes (Figure 1). In parasitic interactions, the biotroph acquires all of its carbon (C) and nutrients from the living plant, whereas in symbioses, the microsymbiont supplies the plant with

1 C is defined as the haploid DNA content.

2 General term used to encompass both bi-directional and unidirectional transport.
limiting nutrients such as phosphorous (P) and nitrogen (N) in exchange for plant C.

**Symbiotic interactions**

Legumes establish a nitrogen-fixing symbiosis with rhizobia bacteria in which a *de novo* organ, the root nodule, is formed to supply the host plant with available N when it is limiting. The *Medicago truncatula*–*Sinorhizobium meliloti* interaction results in indeterminate nodules that develop longitudinally from the meristem (Figure 1a). Endoreduplication is primarily associated with the infection zone (Zone II) and continues into Zone III where fully differentiated bacteroids develop the capacity to fix nitrogen. DNA content increases from 2–4C up to 64C and is accompanied by nuclear and cell enlargement [8]. *M. truncatula* also serves as a model host for the study of the arbuscular mycorrhizal (AM) symbiosis in which Glomeromycota AMF, via their extensive extracellular hyphal networks, supply the plant with up to 70% of host P (Figure 1b). The AMF penetrate *Medicago* root cells harboring prepenetration apparatus (PPA), moving towards the inner cortex where highly branched arbuscules are formed [9,13*]. This process is not synchronous as there is continued movement into additional inner cortex cells. Furthermore, arbuscules tend to expand to a maximal size occupying much of the cytoplasmic space and then degrade. Enhanced chromatin decondensation and increased nuclear size have long been observed in arbuscule-containing cells of a variety of different species [11] and more recently linked with endoreduplication. For example, 90% of colonized tomato root cells exhibited endoreduplication (8C) compared with 18% of non-colonized cells from the same roots [12]. Also, nuclear enlargement was observed in PPA-containing inner cortical cells before fungal penetration, suggesting endoreduplication is part of pre-invasion cell preparation [13*].
Parasitic interactions

Recently, a role for endoreduplication in the growth and reproduction of a compatible PM on *A. thaliana* was established [14**]. Powdery mildews acquire their nutrients through the haustorial complex (HC), comprised of the fungal haustorium and surrounding extrahaustorial matrix and membrane (Figure 1c). This feeding structure develops at ~1 day post inoculation (dpi) in *Golovinomyces orontii*-infected leaf epidermal cells. By 5 dpi, extensive hyphae form at the fungal haustorium and surrounding extrahaustorial matrix through the haustorial complex (HC), comprised of the infected epidermal cell [14**]. These mesophyll cells exhibit a median DNA content of 32C compared to similar cells distal to the infection site or from leaves of uninfected plants (median: 8C). *Arabidopsis* is also among a wide variety of plant species that host parasitic nematodes. The sedentary endoparasitic cyst (*Globodera* spp. and *Heterodera* spp.) and root knot (*Meloidogyne* spp.) nematode larvae penetrate root tips and migrate towards the vascular cylinder where they induce nematode feeding sites (NFS) that serve as the exclusive nutrient source for the developing nematode [15**]. These NFS, respectively, consist of a large multinucleated cell called the synecium resulting from the incorporation of dividing neighboring cells (Figure 1d), or a gall containing several multinucleated giant cells (Figure 1e). Nuclei in both NFS are enlarged and amoeboid, and endoreduplication is associated with enlargement in both systems [15**].

For these systems, enhanced ploidy is accompanied by increased nuclear and cell size and decondensed chromatin. The biotrophs undergo considerable growth and development and thus impose a dramatic and sustained metabolic demand on the plant host, particularly at and adjacent to the site of nutrient exchange. This metabolic demand can be associated with the internal development of symbiotic cells housing arbuscules or bacteroids, that fill most of the cytoplasmic space, and/or the external growth and reproduction of the biotroph. AMF and PM form extensive surficial hyphal networks and reproductive structures, and parasitic nematodes increase dramatically in size. Though the number of endoreduplication cycles in cells at these interaction sites is fairly limited (2–4 cycles), it has a marked impact on the biotroph. Reduced host endoreduplication results in decreased biotroph growth and/or development [14**,15**,16,17**]. Therefore, endoreduplication may be a common mechanism required to support the enhanced metabolic demands associated with these interactions.

Functional impacts of enhanced ploidy on biotroph–plant interactions

Endoreduplication cycles are induced, repeated whole genome duplication events in which net gene dosage increases but gene balance is preserved. In general, both the expression of individual genes and total RNA (and mRNA) increase linearly with ploidy, as established using yeast [18], maize [19,20], and *Arabidopsis* [21] autopoloid series. Though net expression increases in each of these autopoloid series, the vast majority of genes exhibit no relative change in expression on a total RNA basis. However, 0.3–3% of assessed genes exhibit altered, ploidy-dependent expression [18,20*,21]. At the described biotroph–plant host interaction sites, increased ploidy is also likely to impact the relative expression of a similarly small subset of genes. To identify ploidy-impacted processes, transcriptome studies of the interaction sites [10*,14**,22,23,24*,25–27] were reanalyzed and compared with those from similar developmental plant systems. Such developmental systems are characterized by 2–4 endoreduplication cycles, decondensed chromatin, and enhanced metabolic capacity, and include trichomes of *Arabidopsis* [28] and peppermint [29] as well as the maize endosperm [30,31].

Using this approach, I identified specific ploidy-impacted metabolic processes of known or predicted functional importance to the biotroph–plant interactions (Table 1). Remarkably, despite dramatic differences in time scales, shared preferentially up-regulated metabolic processes associated with induced endoreduplication at interaction sites also tend to be over-retained following ancient whole genome duplication (WGD) events (e.g. in yeast [32,33**], *Paramecium* [34**], and *Arabidopsis* [35,36]). This accords with the Gene Balance Hypothesis [36–38], which states that more ‘connected’ genes encoding components of regulatory or metabolic macromolecular complexes with stoichiometric-sensitive components tend to be over-retained following WGD, and under-retained following partial duplication events. This finding also agrees with Metabolic Control Theory [39], which describes how increased expression of all genes in a metabolic pathway can effectively increase pathway flux, whereas metabolic fluxes are typically insensitive to dosage of an individual enzyme. Indeed, increased net gene dosage of metabolic pathway enzymes (e.g. in glycolysis) could confer an immediate selective advantage, resulting in their retention following WGD [32,33**].

Though nuclear DNA is replicated in endoreduplicated cells at the interaction sites, organelle ploidy does not change. This could disrupt gene balance for processes with both nuclear-encoded and organelle-encoded components. Organelle proliferation could presumably correct this imbalance if it accompanies endoreduplication. Though organelle proliferation occurs in specific cases (e.g. plastid proliferation in the arbuscule-containing...
cell), it is not uniformly observed. Therefore, one might expect processes such as respiration and photosynthesis that require both nuclear-encoded and organelle-encoded components to be negatively impacted by endoreduplication. However, both nuclear and mitochondrial genes involved in respiration are preferentially up-regulated, and alterations in photosynthetic gene expression are system-dependent and not associated with endoreduplication (Table 1). This is consistent with the evolution of compensatory mechanisms for dealing with nuclear–organelle gene dosage imbalance relating to fundamental processes [40,41]. As described in more detail below, the impact of biotroph effectors can also be considered in the context of gene dosage and balance with effector influence diminished as host ploidy increases.

### Metabolic pathways sensitive to net gene dosage

A net increase in metabolic activity in cells at and/or adjacent to the biotroph–plant interaction sites is considered critical to these interactions. However, processes required to generally enhance metabolic capacity are also specifically up-induced (that is, up-regulated on a total RNA basis) at interaction sites. Preferentially up-regulated processes include chromatin remodeling, ribosome biogenesis and protein synthesis/modification, carbohydrate metabolism, and energy generation (Table 1). Genes for such processes are also preferentially up-regulated in comparable plant developmental systems (Table 1) and tend to be over-retained following WGD, with a net increase in their dosage associated with generally enhanced metabolic rates [32,34,35,36]. In addition, my analysis identified specific metabolic subcategories of known or predicted functional importance with a subset discussed below.

### Fermentative capacity

Genes involved in glycolysis, respiration (TCA cycle and mitochondrial electron transport chain), and fermentation

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**Table 1**

<table>
<thead>
<tr>
<th>Shared ploidy-impacted metabolic processes</th>
<th>Chromatin</th>
<th>Protein/Ribosome</th>
<th>CH</th>
<th>Energy</th>
<th>Glycolysis</th>
<th>Respiration (M)</th>
<th>Fermentation</th>
<th>Transport</th>
<th>Photo-synthesis (P)</th>
<th>SM (P)</th>
</tr>
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**Parasitic biotroph – plant nutrient exchange sites**

- At-PM infection site [14••] UP UP/UP UP\(^1\) UP UP UP UP DOWN IG, Ph
- At-RKN induced galls [22] UP UP/UP UP UP UP UP UP ALT UP\(^2\) F
- At-RCN induced syncytia [24•] UP UP/UP UP UP UP UP ALT UP\(^2\) T, F

**Symbiotic biotroph – plant nutrient exchange sites**

- Mt-rhizobia nodules [17••,25-26] UP UP/UP UP UP UP UP UP ALT
- Mt-AM colonized roots\(^4\) [10•] UP UP/UP UP UP UP UP N.S. UP N.S. T, F, Ph

**Plant developmental systems**

- At trichome [28] UP UP/UP UP UP UP UP UP UP DOWN\(^5\) T, F
- Peppermint trichome secretory cells\(^6\) [29] UP/UP UP UP UP UP UP UP UP T, F
- Maize endosperm [30,31] UP/UP UP UP UP UP UP UP UP IA

Transcriptome data at the biotroph–plant interaction sites and for comparable plant developmental systems was reanalyzed. Endoreduplication-associated data points were utilized with greater coverage arrays prioritized. Transcriptome data is normalized on a total RNA/mRNA basis. Bold: statistically significant (\(p\)-value \(< 0.05\)) determined using MapMan or BioMaps in Virtual Plant for *Arabidopsis* arrays as in [14•] or GeneBins [57] for the *Medicago* 61K Affymetrix array. Abbreviations: ALT, altered (not predominantly UP or DOWN); AM, arbuscular mycorrhiza; At, *Arabidopsis thaliana*; CH, carbohydrate; F, flavonoids; IA, indole alkaloids; IG, indole glucosinolates; M, mitochondria; Mt, *Medicago truncatula*; N.S., not significant; P, plastid; Ph, phenolics; PM, powdery mildew; RCN, root cyst nematode; RKN, root knot nematode; SM, specialized metabolites (largely) produced in the plastid, nuclear-encoded; T, terpenoids.

\(^1\)Minor CH; \(^2\)Artifact of experimental protocol; \(^3\)Red (up-regulated), green (down-regulated), a significant component (if not all) of the biosynthetic pathway is plastid-localized; \(^4\)Similar, less comprehensive results were obtained using lower coverage arrays [23,27]; \(^5\)DOWN when compared with processed shoots [28], N.S. when mature trichomes compared with trichome initial cells [58], for other categories, results from [58] support those of [28]; and \(^6\)EST data.

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Localized endoreduplication may function at and adjacent to biotroph–plant interaction sites to enhance metabolic capacity and reduce the impact of biotroph effectors. Shared ploidy-impacted processes are presented for the powdery mildew interaction. Shown are mesophyll cells underlying the infected epidermal cell before and after endoreduplication (at 5 dpi). Plant cell wall is not shown. Nucleus is in blue with ploidy indicated, and chloroplasts are green. (a) Enhanced metabolic capacity can be attributed to enhanced (i) primary metabolism (glycolysis, respiration, and fermentation), (ii) transport, and (iii) plastid-localized production of specialized metabolites (S). These processes are sensitive to net gene dosage. At the PM interaction site, glycolysis, respiration, and fermentation are all up-regulated with flux through pyruvate decarboxylase (PDC) favored over pyruvate dehydrogenase (PDH) when pyruvate levels are high. Enhanced transporter expression (red) coupled with increased cell surface area can result in dramatically enhanced transport rates. Specialized metabolites encoded in the nucleus and (largely) produced in the plastid exhibit dramatic alterations in expression at the site of these interactions and play important roles in these interactions. (b) The impact of a biotroph effector on a plant host regulatory or metabolic pathway may be diminished as a result of endoreduplication. Here, a simple example is provided in which defense genes that are typically expressed early in non-host or incompatible PM interactions are only expressed much later (e.g., at 5 dpi) in a compatible PM interaction. A PM effector could target a regulatory protein (yellow) that must form a dimer with a distinct regulatory protein (purple) to induce defense gene expression. Initially, the effector would suppress defense gene expression. However, as ploidy increases, heterodimers can form, resulting in defense gene expression. As evident from this example, biotroph effectors could effectively target genes in macromolecular complexes that are sensitive to stoichiometry (gene balance) and are low copy number.

Transporters tend to respond favorably to net increases in gene dosage and are over-retained following WGD (e.g., yeast [33**]). From a metabolic perspective, transporters can be viewed as important components of a metabolic pathway that are preferentially retained in concert with genes encoding the metabolic enzymes. In addition, specific transporters exhibit particularly high flux control coefficients (extent pathway flux increases with increasing concentration of an individual enzyme). In these cases, increased transporter expression can dramatically impact pathway flux (e.g., glycolysis is limited by hexose import [32]). Therefore, when beneficial, these transporters can also be selectively retained following partial duplication events (e.g., [32]).

Nutrient exchange is a central feature of biotroph–plant interactions, and thus transporters play a particularly important role. Endoreduplication results in both the enhanced expression of specific transporters and in increased cell surface area. Therefore, net transport rates could increase dramatically (Figure 2a). I found transporter expression was preferentially altered in all systems
examined (Table 1). Moreover, strongly up-regulated genes for each of the plant–biotroph interactions include nutrient transporters of known significance to that interaction. For example, the phosphate transporter MtPT4 is expressed specifically in arbuscule-containing cells (158-fold enhanced expression [10*]). Not only is this PAM-localized protein critical to phosphate transport out of the arbuscule, but also for proliferation of the AMF in the root and thus for the symbiosis [43]. In addition, specific sucrose transporters and water channel proteins (aquaporins) are expressed at the NPS of parasitic nematodes, reduced nematode development being associated with reduced function or expression of these proteins [44,45]. And, the plasma membrane hexose transporter AtSTP4 is expressed in mesophyll cells surrounding PM-infected epidermal cells [14**,46] with a 13-fold increase in expression at the PM infection site [14**].

**Plastid production of specialized metabolites**

Genes involved in the production of specialized metabolites (largely) produced in the plastid (e.g. flavonoids, terpenoids, indole glucosinolates) tend to be strongly impacted by increased ploidy, with the specific class of affected metabolite being dependent on the system (Table 1 and Figure 2a). These metabolites are also known or predicted to play important roles in the described biotroph–plant interactions. For example, RNAi-mediated reduction of apocarotenoid biosynthesis in arbuscule-associated plastids increases arbuscule degeneration [47*]. Enzymes in these plastid-localized pathways are exclusively nuclear-encoded and thus expression of pathway genes could be preferentially enhanced with increased ploidy. These pathways are often metabolically demanding (e.g. in their requirement for C and NADPH), and thus the overall enhanced metabolic capacity of endoreduplicated cells would allow for their heightened synthesis. Genes involved in specialized metabolism are preferentially retained following WGD in *Arabidopsis* [35] and increased plant ploidy is associated with enhanced specialized metabolism production (e.g. terpenoids, flavonoids, alkaloids) in a variety of medicinal plants [48] indicating these pathways respond to increased net gene dosage.

**Impact of increased ploidy on biotroph effectors**

Biotroph effectors are defined here as symbiont-produced or parasite-produced secreted proteins or small molecules that impact host cell structure or function. Using the Gene Balance Hypothesis [36–38], one can predict candidate effector targets and effector-impacted processes. Effector action can be viewed as altering/disrupting both gene dosage and balance. As effector concentrations are limited, I would expect them to most dramatically impact particularly dosage and/or balance sensitive proteins and processes. Dosage-sensitive proteins tend to be encoded by single or low copy number genes, which may also be uniquely sensitive to gene dosage imbalance [37,49**]. For example, genes encoding host regulatory proteins that can interact with multiple protein partners or participate in regulatory loops in which some components have opposing functions are sensitive to gene dosage imbalance. Indeed, the known effector targets RAR1 and RIN4 are (i) balance-sensitive genes that interact with multiple distinct partners to promote plant immune signaling and defense responses [50], and (ii) preferentially retained as single copy genes in multiple plant genomes, despite duplication events [49**].

As host ploidy (and gene copy number) increases, effector influence could be predicted to diminish. This could lead to incomplete suppression, for example of defense-associated genes, with enhanced ploidy allowing the now partially suppressed process to be identified (Figure 2b). For example, (a)biotic stress response and hormone categories often fall below significance thresholds in the transcriptome analyses. However, distinct and interaction-specific subsets of genes in these categories exhibit altered expression. Genes in these categories are impacted by known effectors [50] including biotroph effectors (e.g. *Sinorhizobium* NopL [51]). Therefore, the enhanced expression observed when ploidy increases may be owing to the reduced impact of an effector on its target and associated pathway. In the compatible PM-*Arabidopsis* interaction, host defense responses that are rapidly expressed in response to non-host PMs are induced much later concurrent with endoreduplication [14**]. Genetic studies indicate that the early suppression of these defenses may be essential for establishment of the PM infection, whereas they are tolerated at later stages (discussed in [14**]). Similarly, as ploidy increases, biotroph effectors that target low copy number metabolic proteins in a balanced metabolic complex/pathway would probably be unsuccessful in the complete redirection or inhibition of a given pathway. This could confer the benefit of allowing multiple metabolic pathways to operate simultaneously, perhaps to furnish specific compounds required by the biotroph while also allowing essential host metabolism to proceed to maintain cell viability. In summary, these biotroph–host interaction sites may be uniquely suited for the elucidation of effector-impacted genes and products, and thus, the parallel identification of the associated effector targets.

**Regulation of induced host endoreduplication at nutrient exchange sites of biotroph–plant interactions**

The highly localized induced nature of endoreduplication in cells at and/or adjacent to sites of biotroph-plant nutrient exchange may make these systems uniquely suited to identify endoreduplication-associated transcriptional regulators and their targets. In fact, site-specific expression profiling led to the recent discovery that AtMYB3R4 regulates induced endoreduplication at PM
Arabidopsis acts as a competitive repressor of NtMYBA2; however, dependent kinase (CDK)/cyclin (CYC) complexes full activation of the M-promoting activity of AtMYB3R4. Similar to the orthologous tobacco protein, NtMYBA2, endoreduplication had not previously been described. Un-phosphorylated MYB3R4 could result from (i) a reduction in CDK/CYC complex components or activity or (ii) the action of a yet unknown phosphatase (Pase). CDK inhibitors and/or the anaphase promoting complex/cyclosome (APC/C) could reduce CDK/CYC complex function.

Fig. 3

Proposed model for induced endoreduplication at a biotroph–plant interaction site. Arabidopsis MYB3R4, a MYB three repeat transcription factor, mediates powdery mildew-induced endoreduplication in mesophyll cells underlying the infected epidermal cell. Phosphorylation of MYB3R4 by specific cyclin dependent kinase (CDK)/cyclin (CYC) complexes is required for its function as a transcriptional activator of M (mitotic phase of the cell cycle). Un-phosphorylated MYB3R4 is proposed to function as an M repressor that promotes the endocycle. Un-phosphorylated MYB3R4 could result from (i) a reduction in CDK/CYC complex components or activity or (ii) the action of a yet unknown phosphatase (Pase). CDK inhibitors and/or the anaphase promoting complex/cyclosome (APC/C) could reduce CDK/CYC complex function.

infection sites [14**]. Genes encoding similar MYB three repeat (MYB3R) transcription factors are also induced in syncytia [24*] and AM roots [23], suggesting MYB3R proteins may be a common control point for these systems (Figure 3).

Though MYB3R4 was known as a transcriptional activator of the mitotic phase (M) that binds the MSA (mitosis-specific activator) element [52], its functional impact on endoreduplication had not previously been described. Similar to the orthologous tobacco protein, NtMYBA2, full activation of the M-promoting activity of AtMYB3R4 requires C-terminal phosphorylation by specific cyclin-dependent kinase (CDK)/cyclin (CYC) complexes [52,53]. In tobacco, a distinct MYB3R protein, NtMYBB, acts as a competitive repressor of NtMYBA2; however, Arabidopsis [52], rice [52], and Medicago* do not contain MYB3R repressors. In the PM–Arabidopsis interaction, AtMYB3R4 may itself function as a transcriptional repressor of M and promoter of endoreduplication since (i) its expression was elevated while putative targets were exclusively down-regulated, and (ii) PM-induced endoreduplication was not observed in myb3r4 mutants [14**]. Therefore, AtMYB3R4 may function as a transcriptional repressor or activator of M, depending on its phosphorylation status. In this case, localized regulated proteolysis (e.g. via the anaphase promoting complex/cyclosome (APC/C)) and/or reduced expression of specific CDKs and/or CYCs (e.g. CYCB1) could modulate MYB3R4 function (Figure 3). Reduced expression of CDKB1;2, CYCB1;2, and CYCB1;5 at the PM infection site suggests their possible involvement [14**]. However, preliminary experiments using two homozygous T-DNA insertion lines in the promoter (SALK_110587c) and exon I (SALK_133560c) of CDKB1;2, respectively, found no difference in the growth or reproduction of the compatible PM (Chandran and Wildermuth, unpublished) suggesting additional CDKs may functionally compensate for loss of CDKB1;2. Induced, locally expressed CDK inhibitors or as yet undetermined phosphatase(s) could also limit MYB3R4 phosphorylation, thereby promoting MYB3R4 repressor function and endoreduplication. Expression profiling studies have shown specific CDK inhibitors of the SMR (SIAMESE-related) and KRP classes to be expressed at the PM infection site [14**] and in nematode-induced galls [22] and cysts [24*]. Though functional data regarding the role of these inhibitors in plant–biotroph interactions have not yet been described, the CDK inhibitor SIAMESE was recently shown to cooperate with CCS52A to establish endoreduplication in trichomes [54**]. The cell cycle switch protein CCS52A is an APC/C activator that promotes the destruction of mitotic cyclins (e.g. CYCB1) and entry into the endocycle. CCS52A transcript and protein are observed in endoreduplication-competent cells of nodules and required for endoreduplication, symbiosome development, and nitrogen fixation [16]. CCS52A is also expressed in RKN galls [55] and in cyst nematode-induced syncytia where down-regulation of its expression inhibits nematode development [15*]. Moreover, partial suppression of CDC16, a core APC component that is expressed in nodules, resulted in nodules with an enlarged infection zone that were less efficient at fixing nitrogen, a phenotype consistent with delayed endoreduplication [56*]. Thus, the APC/C could function (in part) through its impact on the phosphorylation status of a MYB3R protein.

Conclusions

Induced, highly localized endoploidy at and/or adjacent to sites of sustained nutrient exchange between biotrophs and plant hosts has emerged as a common theme for these interactions. While future research will uncover the extent of this phenomenon, the sustained nature of the nutrient exchange site is notable, since fewer endoreduplication cycles are associated with the more transient interaction sites of the AM symbiosis. As discussed, biotroph–plant interaction sites appear uniquely suited to elucidate regulatory control and function of the endocycle as well as effector-impacted processes. Transcriptome analyses support induced endoreduplication as a mechanism to enhance metabolic capacity and identify specific processes and genes of known or predicted importance to these systems. Despite dramatic differences in time scale, preferentially up-regulated ploidy-impacted processes associated with
induced endoreduplication are similar to those retained following ancient WGD events and follow the tenets of the Gene Balance Hypothesis. This suggests that these systems may also be used to explore fundamental mechanisms underlying ploidy and its impact on plants.

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References and recommended reading
Papers of particular interest, published within the last two years have been highlighted as:

- of special interest
- of outstanding interest


Used Medicago GeneChip (61K array) to profile AM-associated expression providing a rich dataset that identifies novel AM-induced genes. Developed methodology for laser microdissection of arbuscular mycorrhizal nodules. Laser microdissection and its application to analyze gene expression in arbuscular mycorrhizal symbiosis.


Groundbreaking paper established prepenetration apparatus formation is required for AMF colonization. Nuclear enlargement and chromatin condensation are observed in PPA-containing inner cortical cells before fungal infection suggesting endoreduplication plays a role in this process.


Host endoreduplication in mesophyll cells underlying PM-infected epidermal cells at a late stage of a compatible interaction was predicted using global site-specific expression profiling data (performed using laser microdissection) and observed microscopically. A novel transcriptional regulator (AtMYB3R4) of this induced endoreduplication was identified with myb3r4 mutant compromised in induced endoreduplication and supporting less growth and reproduction of the fungus. Thus, this paper established a role for endoreduplication in the sustained growth and reproduction of the compatible PM on its host plant. It further provides a rich, infection-site-specific dataset and sets the stage for additional temporally and spatially resolved PM-host analyses.


Preliminary experiments cited as unpublished data in this excellent review establish a role for CCS2A in cyst nematode growth and development.


Careful examination of global gene expression in 1N-4N maize autopolyploid series, as well as tetraploid inbred and hybrid lines. Found most genes do not show significant changes in expression on a total RNA basis with increasing ploidy, consistent with previous smaller scale study (Guo et al. [19]). Non-additive gene expression was common in hybrids and increased with ploidy. Very rich dataset for future functional analyses.


Microaspiration was used to harvest pure syncytium for Arabidopsis Affymetrix GeneChip expression profiling enabling statistical analysis of altered genes and processes in the syncytia.


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