Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured *Arabidopsis* cells

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Jasmonates (JAs) are plant-specific signaling molecules that steer a diverse set of physiological and developmental processes. Pathogen attack and wounding inflicted by herbivores induce the biosynthesis of these hormones, triggering defense responses both locally and systemically. We report on alterations in the transcriptome of a fast-dividing cell culture of the model plant *Arabidopsis thaliana* after exogenous application of methyl JA (MeJA). Early MeJA response genes encoded the JA biosynthesis pathway proteins and key regulators of MeJA responses, including most JA ZIM domain proteins and MYC2, together with transcriptional regulators with potential, but yet unknown, functions in MeJA signaling. In a second transcriptional wave, MeJA reprogrammed cellular metabolism and cell cycle progression. Up-regulation of the monolignol biosynthesis gene set resulted in an increased production of monolignols and oligolignols, the building blocks of lignin. Simultaneously, MeJA repressed activation of M-phase genes, arresting the cell cycle in G_2. MeJA-responsive transcription factors were screened for their involvement in early signaling events, in particular the regulation of JA biosynthesis. Parallel screens based on yeast one-hybrid and transient transactivation assays identified both positive (MYC2 and the AP2/ERF factor ORA47) and negative (the C2H2 Zn finger proteins STZ/ZAT10 and AZF2) regulators, revealing a complex control of the JA autoregulatory loop and possibly other MeJA-mediated downstream processes.

cell division | lignin | phenylpropanoid | secondary metabolism | stress

Jasmonic acid (JA) and its conjugates, such as methyl jasmonate (MeJA) and jasmonoyl-isoleucine (JA-Ile), collectively referred to as jasmonates (JAs), are small signaling molecules, specific for, but ubiquitous in the plant kingdom. Environmental stresses, such as wounding or pathogen attack, can trigger JA production *in planta*. Upon perception of JA signals, the plant cell activates several defense mechanisms, reflected in a massive reprogramming of gene expression. In addition, JAs are involved in the regulation of many developmental processes, including male fertility, fruit ripening, and root growth (1).

The F-box protein CORONATINE INSENSITIVE 1 (COI1) fulfills a key role in JA signaling, which is demonstrated by the JA insensitivity of the *coi1–1* loss-of-function mutant (2). In the presence of JA-Ile, COI1 binds to members of the JA ZIM domain (JAZ) protein family (3–5) and marks them for degradation by the 26S proteasome. The JAZ protein JASMONATE INSENSITIVE3 (JAZ3/TIFY6b) physically interacts with the basic helix–loop–helix (bHLH) transcription factor (TF) MYC2 (3), probably repressing its essential function for normal JA signaling (6).

The potency of JAs to elicit secondary metabolism in cell cultures has converted them into powerful tools to unravel this complex cellular process. Combined transcript and metabolite profiling approaches to tackle the MeJA-mediated regulation of secondary metabolism resulted in the establishment of gene-to-metabolite networks involved in the biosynthesis of the pharmaceutically valuable terpenoid indole alkaloids in periwinkle (*Catharanthus roseus*) cells (7), in the characterization of enzymatic steps in the isoflavone and triterpene biosynthetic pathways in barrel medic (*Medicago truncatula*) cells (8, 9), and in the isolation of novel regulators of nicotine and phenylpropanoid conjugate biosynthesis in tobacco (*Nicotiana tabacum*) cells (10–13) or of aliphatic glucosinolate biosynthesis in *Arabidopsis thaliana* cells (14).

We monitored the MeJA-triggered transcriptional cascade in a fast-dividing cell culture of the model plant *Arabidopsis*. In a first wave, MeJA induced expression of genes involved in transcriptional regulation or JA biosynthesis. In a second wave, transcriptional reprogramming resulted in a dual response: repression of cell cycle progression on the one hand and induction of phenylpropanoid metabolism on the other hand. Furthermore, a role was demonstrated for early MeJA-responsive transcriptional regulators of the bHLH, APETALA2/ETHYLENE-RESPONSIVE FACTOR (AP2/ERF), and C2H2 Zn finger families in the control of the JA autoregulatory loop.

Results

Dissection of the MeJA Transcriptional Cascade. For transcript profiling of early MeJA responses, an *Arabidopsis* cell suspension culture was subcultured and grown for 16 h before elicitation with 50 μM MeJA, or an equal volume of DMSO for mock treatment. The cultures were sampled at multiple time points ranging up to 12 h after treatments. Based on a pilot cDNA-amplified fragment length polymorphism assay in which a bird's eye view on MeJA-mediated transcriptional reprogramming was generated (data not shown), we designed a transcriptome-wide expression profiling experiment with the ATH1 GeneChip. In addition to the zero time point, three other time points were selected, 0.5 h, 2 h, and 6 h, for both mock- and MeJA-treated cultures. RNA from two independent experiments was used for microarray-based transcript profiling, and RNA of a third biological repeat experiment was used for real-time quantitative PCR analysis to validate the data. We considered 15,426 of 22,746 probe sets (68%) as reliably detected and genes corresponding to other probe sets as unexpressed. The MeJA-treated and control cultures were compared pairwise at the three time points of interest. In total, 598 different genes were judged as differentially regulated by MeJA at one or more time points (with a *P* value cutoff corrected for the false discovery rate

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Data deposition: The microarray data have been submitted to the ArrayExpress database, www.ebi.ac.uk/arrayexpress (accession no. E-ATMX-13).

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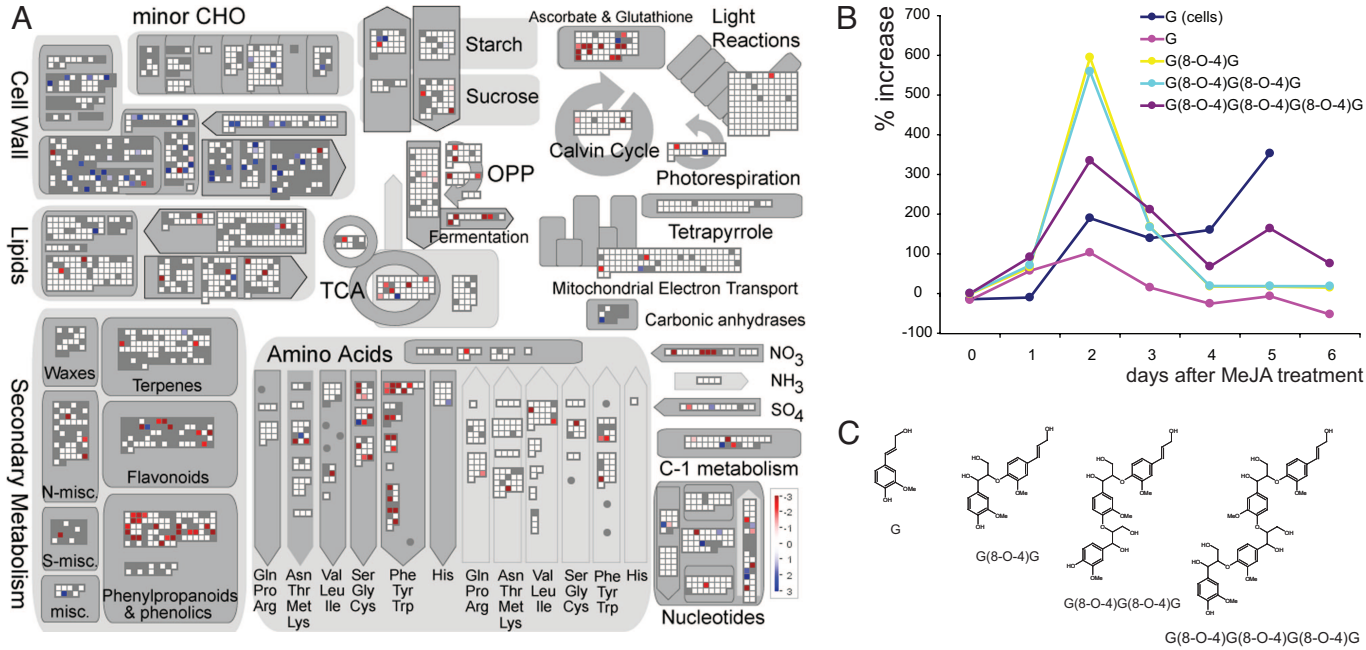

Fig. 1. Stimulation of monolignol biosynthesis in *Arabidopsis* cells by MeJA. (*A*) MAPMAN visualization of metabolism-related gene expression in MeJA-treated *Arabidopsis* cells. Genes were assigned to their associated metabolic pathway. Blue, red, and gray boxes represent repressed, induced, and unexpressed genes, respectively. *Limma P* values were adapted to MAPMAN for the MeJA vs. mock contrast at the 6-h time point. A value of 3 corresponds with a *P* value of 0.001. (*B*) Lignol level changes after MeJA treatment. Numerical values indicate the increase (%) in absolute lignol levels (see also SI Fig. 8) in MeJA-treated over mock-treated cultures for each day. The value for intracelllular coniferyl alcohol levels at day 6 was not included because it was out of range (SI Fig. 8). Oligolignols were reliably detected up to the level of tetramers (*SI Methods*). Coniferyl alcohol and β-aryl ether linkages are represented by G and (8-O-4), respectively. (*C*) Structures of the identified coniferyl alcohol monomer and oligomers.

set at 0.05) [supporting information (SI) Table 1]. From a Venn diagram (SI Fig. 4) with the numbers of genes up-regulated or down-regulated at each time point, a few conclusions could already be drawn. First, MeJA clearly activated a transcriptional cascade: the number of MeJA-regulated genes increased from 75 after 0.5 h to 495 after 6 h. Hereafter, genes induced after 0.5 h, 2 h, or 6 h will be referred to as early, intermediate, and late MeJA response genes, respectively. Second, MeJA primarily triggered gene activation: all 75 early response genes had an enhanced expression, and, of the genes responding 2 h after treatment, only 17 were down-regulated. In contrast, 194 (60%) of the 328 genes differentially expressed only at the 6-h time point displayed repressed expression. Finally, approximately one-third of the early to intermediate MeJA-induced genes were transiently induced and had no significantly changed expression levels 6 h after MeJA elicitation. In conclusion, MeJA triggered a first, early, strictly inductive wave and a second wave, reflecting both positive and negative gene regulation and probably controlled by the first wave.

To study the expression kinetics in more detail, expression profiles of MeJA response genes were subjected to cluster analysis. Adaptive quality-based clustering (15) assigned 519 of the 598 MeJA-responsive expression profiles (87%) to eight clusters (SI Fig. 5). Subsequently, these clusters were analyzed for overrepresentation of gene ontology categories with BiNGO (16) (SI Table 2). Clusters 1 and 2 contained most early response genes, with genes belonging to cluster 2 more transiently induced than those of cluster 1. In these two clusters, genes involved in transcriptional regulation were most abundant. Clusters 3 and 4 grouped mostly intermediate to late MeJA-up-regulated genes with an overrepresentation of processes related to metabolism. Expression levels of genes assigned to cluster 5 were elevated over time in the mock-treated culture, but their induction was repressed in the presence of MeJA. BiNGO analysis indicated an overrepresentation of transcripts related to cell cycle and motor activity in this cluster. Cluster analysis also distinguished three clusters (clusters 6–8) without a clearly overrepresented functional category. The expression pattern of a representative set of genes of different clusters was confirmed by RT-PCR analysis (SI Fig. 6).

MAPMAN Analysis of MeJA-Regulated Processes. MAPMAN software (17) was used to gain insight into the biological processes affected by MeJA. MAPMAN allows the visualization in diagrams of gene expression changes of individual genes, with genes grouped in "BINs" by function or class. Because the MeJA response of entire BINs can be analyzed statistically with MAPMAN, it complements the BiNGO analysis. In the resulting MAPMAN diagram for *Arabidopsis* metabolism, displaying alterations in gene expression 6 h after elicitation, the BIN representing phenylpropanoid metabolism was strongly affected, in particular "lignin biosynthesis" (Fig. 1*A* and SI Table 3). Additionally, BINs corresponding to processes directly associated with the phenylpropanoid pathway, such as aromatic amino acid biosynthesis (the shikimic acid pathway) and ammonium metabolism, were also significantly responsive to MeJA (Fig. 1*A* and SI Fig. 7). MAPMAN analysis also indicated an induction of genes involved in ascorbate and glutathione metabolism. Similarly, Ser and Cys biosynthesis pathways, preceding the glutathione biosynthesis, did also respond to MeJA, as in seedlings (18). In all of the above-mentioned metabolic processes, the corresponding genes were up-regulated, matching clusters 3 and 4 (SI Fig. 5). In contrast, genes involved in cell wall modification, such as pectate lyases and cellulose synthases, appeared to be mainly down-regulated (Fig. 1*A*). In other categories, the BIN of JA metabolism was significantly responsive (SI Table 3).

Surprisingly, the second most significant MeJA-affected BIN was that of "cell organization." The expression profiles of genes identified in this BIN grouped mostly in cluster 5 with reduced gene activation over time in the presence of MeJA (SI Fig. 5). BiNGO analysis exposed an overrepresentation of the categories

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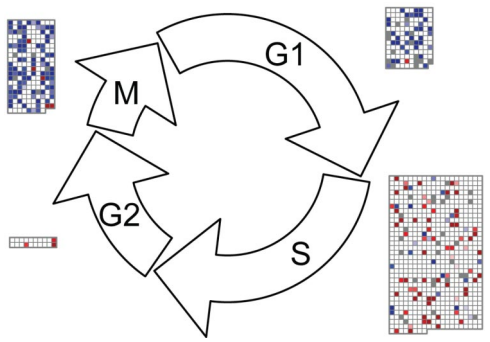

Fig. 2. Repression of *Arabidopsis* cell cycle progression by MeJA. Shown is MAPMAN visualization of cell cycle-related gene expression in MeJA-treated *Arabidopsis* cells. Genes were assigned to their associated cell cycle phase (M, G_1, S, or G_2). Color codes and values are as in Fig. 1.

"cell cycle" and "motor activity" in this cluster (SI Table 2), the latter because of the abundance of kinesins, proteins functioning during mitosis. Because the identity of these genes was particularly related to the M-phase of the cell cycle, the 40 genes of cluster 5 were compared with a data set of 198 M-phase-associated genes, identified in a transcriptome-wide transcript profiling experiment of synchronized *Arabidopsis* cell cultures (19). Most (33, or 83%) of the transcripts in cluster 5 turned out to be shared, indicating that it consisted mainly of mitosis-associated genes. Based on the annotation of cell cycle-regulated or associated genes (19), a custom cell cycle MAPMAN BIN was designed to visualize the overall influence of MeJA on cell cycle gene expression. The repressive effect of MeJA on cell cycle progression was clearly apparent from the M-phase on, in which it was widespread, and also extended to the G_1 phase (Fig. 2).

Together, these findings reveal that MeJA causes a concurrent and dual effect in *Arabidopsis* cells. MeJA activated defense responses by triggering, for instance, phenylpropanoid biosynthesis gene expression, whereas it repressed expression of M-phase-associated genes.

MeJA Increases the Flux Through the Monolignol Biosynthesis Pathway. Within the MeJA-responsive genes in the MAPMAN BIN representing phenylpropanoid metabolism, genes known to be involved in monolignol biosynthesis were strongly enriched (SI Tables 1 and 3) (20). Therefore, phenylpropanoid accumulation was evaluated by metabolite profiling of both mock- and MeJA-treated cells and the corresponding culture media, which were separated from the cells by vacuum filtration. The cellular phenolic profiles revealed UV/Vis absorption spectra characteristic of simple phenolic compounds and phenylpropanoids. The monolignol coniferyl alcohol was clearly detectable, and its abundance differed increasingly in the MeJA-elicited cultures compared with that in the mock-treated cells (Fig. 1*B* and SI Fig. 8). In addition, MeJA treatment yielded significantly higher levels for the major HPLC peaks corresponding to hydroxycinnamate derivatives (data not shown). No UV/Vis spectra reminiscent of flavonoids were observed. In the medium of both mock- and MeJA-treated cells, HPLC revealed the presence of increasing amounts of oligolignols over time (Fig. 1*B* and SI Fig. 8), in which the main units were β-aryl ethers, as in lignin (21, 22). In 3-week-old cultures, the culture medium could be stained with phloroglucinol and no more oligolignols could be detected, suggesting that these compounds were used in higher-order lignin (data not shown). Overall, the total amount of oligolignols, expressed as the sum of their individual peak heights, was consistently higher in the MeJA-treated than in the control cells throughout the time course, with a peak after 2 days, supporting a higher monolignol biosynthetic flux due to MeJA treatment.

MeJA Inhibits Growth and Halts Cell Cycle Progression in G_2. To verify whether the repression of cell cycle gene activation was also reflected at the cellular level, the DNA content of suspension cells grown in the absence or presence of MeJA was analyzed with flow cytometry (SI Fig. 9). At the moment of subculturing, most cells (70%) resided in cell cycle phase G_1, confirming previous observations (23). Subculturing was followed by a nearly synchronized reactivation of the cell cycle. At the time of MeJA elicitation, 16 h after subculture, most cells (62%) were in G_2 phase. In the mock-treated culture, the G_2 population gradually decreased over time leading to 20% G_2 cells 96 h after the mock treatment. Upon treatment with MeJA at the concentration used for transcript profiling (50 μM), only a minor shift (2%) toward G_2 cells was observed. However, when MeJA concentrations were increased, the applied MeJA dose and the number of cells residing in G_2 phase clearly positively correlated. At 200 μM MeJA, 40% of cells were obstructed in G_2 phase, doubling the amount of G_2 cells compared with those in the mock-treated culture. A similar MeJA dose–response, but with an inverse correlation, was obtained when *Arabidopsis* callus growth was scored on solid medium with increasing MeJA concentrations (SI Fig. 10), suggesting that MeJA-modulated growth inhibition is at least in part mediated by the imposed G_2 arrest of cell cycle progression.

The Early MeJA Response: Identification of Regulators of JA Biosynthesis. As mentioned, MAPMAN analysis pointed to a significant response in the JA metabolism BIN (SI Table 3). Five genes involved in the JA biosynthesis pathway, lipoxygenase 3 (*LOX3*), allene oxide cyclase 3 (*AOC3*), OPDA reductase 2 (*OPR2*), *OPR3*, and OPC-8:0 CoA ligase 1 (*OPCL1*), were rapidly up-regulated by MeJA, whereas two other known JA biosynthesis genes, allene oxide synthase (*AOS*) and acyl-CoA oxidase 1 (*ACX1*), were significantly induced at later time points (SI Fig. 11). This phenomenon of self-activation of JA biosynthesis has already been extensively reported in the literature (1), but the regulatory mechanism behind this positive feedback loop has not been elucidated yet. Besides the five early responsive JA biosynthesis genes mentioned above, the expression of 70 other genes, of which more than one-third corresponded to genes putatively involved in transcriptional regulation, was simultaneously enhanced 0.5 h after MeJA treatment (SI Fig. 4 and SI Table 1). Transcription factors from eight different TF families (http://attfdb.cbi.pku.edu.cn) were found within this set, including JAZ/TIFY, AP2/ERF, WRKY, bHLH, MYB, NAC, and C2H2 Zn finger family members.

To unravel the autoregulation of JA biosynthesis gene expression, TFs controlling the expression of the JA biosynthesis gene *LOX3* were identified in two parallel screens. A set of 21 early MeJA-responsive TFs, covering all relevant expression patterns and TF families (SI Fig. 12), was selected and tested (*i*) for interaction with the *LOX3* promoter (PLOX3) by a yeast one-hybrid (Y1H) screen and (*ii*) for PLOX3 transactivation in a protoplast-based transient expression assay (TEA). For the Y1H analysis, a reporter strain was constructed containing a 1,997-bp PLOX3 fragment fused to the *HIS3* reporter gene. The reporter strain was subsequently supertransformed with expression clones containing the ORFs of the 21 TFs fused to the yeast GAL4 activation domain (GAL4AD). Promoter binding was scored on media with increasing concentrations of 3-amino-1,2,4-triazole (3-AT). Growth of a control reporter strain, as well as of most other supertransformants, was completely arrested at 2.5 mM 3-AT, except for yeast cells producing the GAL4AD-STZ/ZAT10 and GAL4AD-AZF2 fusion proteins (Fig. 3*A*). These results indicate that the closely related C2H2 Zn finger proteins STZ/ZAT10 and AZF2, which contain an ERF-associated amphiphilic repression domain and act as transcriptional repressors, can bind PLOX3 (SI Fig. 13*A*) (24).

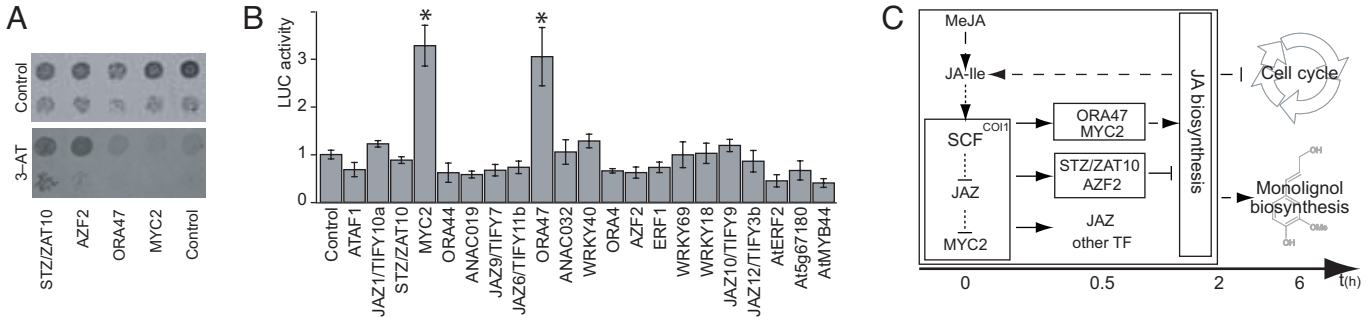

Fig. 3. Mapping of the transcriptional network regulating the MeJA response in *Arabidopsis* cells. (*A*) Y1H interaction with the *LOX3* promoter. Full-length ORFs fused to GAL4AD were expressed in a PLOX3:HIS3 reporter strain. The empty Gateway destination vector was used as a negative control. Yeast was grown for 2 and 6 days on control and selective medium (3-AT), respectively. (*B*) Regulation of *LOX3* expression by transient expression of early MeJA-responsive TFs. Tobacco protoplasts were transfected with a PLOX3:fLUC reporter construct, a P35S:ORF effector construct, and a P35S:rLUC normalization construct. Averaged ($n \geq 4$) normalized fLUC activities are plotted relative to the P35S:GUS control. Error bars represent standard error. Asterisks indicate significant effects (*t* test, $P < 0.01$). (*C*) Model of MeJA signal transduction in *Arabidopsis* cells. MeJA is converted to JA-Ile, which promotes interaction between COI1 and JAZ proteins, leading to degradation of the latter proteins and release of the positive regulator MYC2. In a first wave, this process, in turn, induces expression of a set of early responsive TFs that exert a positive and negative control on the JA biosynthesis amplification loop. The MeJA-initiated signaling cascade extends to the second wave, leading to transcriptional reprogramming of cellular metabolism and cell cycle progression, toward increased monolignol biosynthesis and a G_2 arrest, respectively. The bar at the bottom gives the time (in hours) at which the signal transduction takes place. Dotted, full, and dashed lines mark physical interactions, direct transcriptional regulation, and incompletely characterized metabolic or signaling pathways, respectively.

In parallel, regulation of *LOX3* expression was investigated in tobacco protoplasts by an automated TEA (11). To this end, the PLOX3 fragment was fused with the firefly luciferase (*fLUC*) reporter gene, and the resulting construct (PLOX3:fLUC) cotransfected with overexpression cassettes of the 21 TFs. No significant effect on *PLOX3:fLUC* expression was observed when cotransfecting with the *STZ/ZAT10* or *AZF2* overexpression constructs (Fig. 3*B*). In contrast, *PLOX3:fLUC* expression increased >3-fold when the transcriptional activators ORA47, an AP2/ERF protein, and MYC2, a bHLH protein (Fig. 3*B* and SI Fig. 13*B*), were overproduced. Both proteins are known to be positive actors in the MeJA signaling cascade (1), but a direct involvement in the regulation of JA biosynthesis had not been established yet.

Discussion

MeJA Has a Dual and Direct Effect on Growth and Defense of *Arabidopsis* Cells. JAs are signaling molecules implicated in the regulation of various biological processes. The transcriptional cascade after MeJA perception in fast-dividing suspension-cultured cells of the model species *Arabidopsis* was studied. This approach revealed a surprisingly swift- and concurrent-acting dual effect of MeJA on the transcriptome of *Arabidopsis* cells. On the one hand, MeJA impaired the G_2-to-M transition by repressing M-phase gene activation, and, on the other hand, MeJA coordinately induced genes from phenylpropanoid-associated pathways. That both gene expression trends, which might broadly be redefined as repression of multiplication or growth and activation of the defense response, respectively, were set concomitantly within a few hours after MeJA elicitation clearly indicates that both processes occur independently of each other and can consequently be considered as direct effects of MeJA treatment (Fig. 3*C*).

The Monolignol Biosynthesis Pathway Is Subject to MeJA Elicitation at Both Transcript and Metabolite Levels. MeJA elicitation of *Arabidopsis* cell cultures significantly induced expression of genes involved in general phenylpropanoid and monolignol biosynthesis and associated processes—more specifically, the shikimate pathway that supplies the phenylpropanoid pathway with phenylalanine and the nitrogen-recycling mechanism that salvages the ammonium released by the action of phenylalanine ammonia lyase, the enzyme catalyzing the first committed step of phenylpropanoid metabolism. In agreement with the transcriptome data, targeted metabolite profiling showed that gene induction was accompanied by an increased flux in the monolignol pathway as manifested by elevated levels of extracellular coniferyl alcohol, as well as oligomers thereof. Indeed, once produced, monolignols, i.e., coniferyl and sinapyl alcohols in angiosperms, are mainly exported outside the cell where they can be oxidized by peroxidases and/or laccases and polymerized to lignin by radical–radical combinatorial coupling reactions (20). Therefore, this inducible cell suspension culture might be a suitable model system for monolignol biosynthesis studies.

The *Arabidopsis* mutant *constitutive expression of VSP1* (*cev1*) is defective in the cellulose synthase gene *CESA3* and shows constitutive JA responses together with an increased production of JA and ethylene (25). The *ectopic lignin 1* (*eli1*) mutant is allelic to *cev1* and has been identified in a screen for mutants with ectopic lignification patterns. Additionally, isoxaben, an inhibitor of cellulose biosynthesis, phenocopies *eli1* lignification in wild-type seedlings. In JA-insensitive *coi1* plants, ectopic lignification upon isoxaben treatment is reduced, indicating a partial requirement of JA signaling (26). Our transcriptome analysis clearly supports an active role of JAs in the regulation of *Arabidopsis* monolignol biosynthesis.

M-Phase Gene Repression Correlates with a G_2-Phase Blockade. JA treatment has recently been demonstrated to arrest synchronized tobacco cells in both G_1 and G_2 phases (27), and the G_2 arrest has been found to be accompanied by reduced accumulation of B-type cyclin-dependent kinases and cyclin B1;1 (28), hinting at a G_2 checkpoint possibly controlled by JAs. Our data indicated that the G_2 arrest could occur together with an unexpectedly rapid blockade at the transcript level, as reflected by the repressive effect of MeJA on M-phase gene expression. Correspondingly, a dose-dependent effect of MeJA on cell cycle progression and growth of *Arabidopsis* cells was observed. The concentration of MeJA used for elicitation correlated positively with the amount of cells residing in G_2 phase 96 h after elicitation and negatively with callus growth. These observations support previous research in which inhibition of cell cycle progression and consequential growth reduction had been linked to altered transcriptional regulation of cell cycle genes (29). Furthermore, these observations might enlighten the long-reported negative impact of JAs on plant growth (1) that is, for instance, also linked to the dwarfed phenotype of *cev1*/*eli1* mutants (25, 26).

MeJA application repressed activation of a large group of M-phase-associated genes rather than a specific subset. M-phase-specific gene activation has been attributed to M-phase-specific

activator (MSA) promoter elements that are overrepresented in promoters of M-phase-associated genes and are both essential and sufficient for M-phase-specific promoter activity (19, 30). Whether the MSA element plays a role in this MeJA-induced repression and whether it involves *de novo* synthesis or posttranslational modification of a transcriptional regulator remains to be determined. Alternatively, JA signaling may activate secondary messenger molecules that, in turn, affect cell cycle progression. For instance, hydrogen peroxide is believed to act as a secondary messenger in JA-mediated induction of late-responsive defense genes (31). Oxidative stress can impair cell cycle progression and repress cell cycle gene activity in tobacco cells (32). Genes involved in the ascorbate recycling and glutathione biosynthesis pathways are induced by MeJA treatment in cultured cells and seedlings of *Arabidopsis* (ref. 18 and this study), which could be indicative of an oxidative burst after JA perception.

Early MeJA-Responsive TFs Encode Both Activators and Repressors, Acting at the First Level of the MeJA Signaling Cascade. MeJA perception in *Arabidopsis* cells was rapidly followed by gene activation. Within 0.5 h, expression of a distinct set of early response genes was significantly enhanced. Besides known regulators of MeJA responses, such as MYC2 (6) and several members of the JAZ repressor family (3–5), this set included several other early MeJA-responsive TFs. These TFs mediate the altered expression of other early MeJA-responsive genes, encoding enzymes involved in JA biosynthesis, corresponding to the autoregulatory JA biosynthesis loop, or later-response genes involved in monolignol biosynthesis and cell cycle progression (Fig. 3*C*).

Two parallel screens for transcriptional regulators of JA biosynthesis identified both potential activators and repressors. The activators were encoded by MYC2, the bHLH-type TF that directly interacts with the JAZ proteins and seems to act at the first level of the JA cascade (3, 6), and ORA47, the AP2/ERF protein recently postulated to be a positive regulator of JA biosynthesis (33). The repressors were encoded by STZ/ZAT10 and AZF2. These closely related C2H2 Zn finger proteins contain an ERF-associated amphiphilic repression domain and might act as both positive and negative regulators of plant defenses (24, 34). These findings are summarized in a model (Fig. 3*C*).

An interesting parallel can be drawn with the transcriptional regulation of the periwinkle gene encoding strictosidine synthase (STR), the enzyme that catalyzes the first step in terpenoid indole alkaloid biosynthesis. Various TFs were shown to bind the *STR* promoter, including CrMYC1, the putative ortholog of MYC2; CrZCT1 to CrZCT3, homologs of STZ/ZAT10 and AZF2; and CrORCA1 to CrORCA3, encoding AP2/ERF proteins homologous to ORA47 (35). It is tempting to speculate that proteins such as MYC2, ORA47, STZ/ZAT10, AZF2, and perhaps other early-responsive *Arabidopsis* TFs regulate not only early MeJA responses such as JA biosynthesis, but also later responses, such as activation of phenylpropanoid metabolism. The finding that the MeJA responsiveness of several genes involved in phenylpropanoid/flavonoid biosynthesis was reduced in the *jin1/myc2* mutant (36) corroborates this hypothesis. It will be exciting to view the outcome of future large-scale TF interaction screens with promoters of the phenylpropanoid and cell cycle genes as baits. Such screens will undoubtedly enrich our current understanding of the complex transcriptional cascade that is steered by JAs and in which both repressors and activators of the same cellular and metabolic pathways are induced to control the plant's defense response.

Materials and Methods

Maintenance, Treatments, and Sampling of the Cell Suspension Culture. Suspensions of cultured cells of *A. thaliana* (L.) Heynh. (ecotype Columbia-0) were maintained as described (37). For elicitation, a 7-day-old culture was diluted 10-fold in fresh medium and grown for 16 h. Subsequently, MeJA (Duchefa) at a final concentration of 50 μM or an equal volume of solvent (DMSO) was added to the cultures. For transcript profiling, cultures were sampled 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h after treatments. Samples for flow cytometry were taken before subculture and daily for 1–4 days after elicitation. For metabolite profiling, cultures treated with MeJA or DMSO were sampled every day for 1 week after treatments.

Transcript Profiling. Filtered cells were ground in liquid nitrogen, and total RNA was isolated with TRIzol Reagent (Invitrogen). The *Arabidopsis* ATH1 GeneChip (Affymetrix) was hybridized at the Flanders Institute for Biotechnology microarray facility (www.microarrays.be) according to the manufacturer's instructions. Microarray data processing and statistics as well as real-time quantitative PCR are described in *SI Methods*. The microarray data have been submitted to the ArrayExpress database (www.ebi.ac.uk/arrayexpress; accession no. E-ATMX-13).

Oligolignol Profiling. Cells and medium were separated by vacuum filtration. Approximately 300 mg of the filtered cells were ground in liquid nitrogen, extracted with 3 ml of methanol, and, after lyophilization of 1 ml of the supernatant, extracted and analyzed by reversed-phase HPLC. Additionally, 350 μl of the culture medium was analyzed (21, 22). Peaks with UV/VIS spectra similar to those of lignans and oligolignols were collected and identified through LC/MS (21, 22). Peak heights of oligolignols were measured with the maxplot option (200–450 nm) in Xcalibur version 1.2. The β-aryl ether tetramer was identified as described (*SI Methods*).

Flow Cytometry. Culture samples were vacuum filtered and frozen in liquid nitrogen. Nuclear DNA content distribution was analyzed (29) and quantified with the multicycle flow cytometry software (Phoenix Flow System).

TEA and Y1H Analysis. A 1,997-bp PLOX3 fragment was obtained from the *Arabidopsis* promoterome (www.psb.ugent.be/SAP) and Gateway recombined with pDONRP4P1R (Invitrogen) to yield pENTRY-PLOX3. For generation of TEA reporter constructs, pENTRY-PLOX3 or pENTRY-PUAS (6xUAS-P35S$_{min}$) were recombined by Gateway MultiSite LR cloning with pENTRY-fLUC and pm42GW7,3 (38) to yield PLOX3:fLUC or PUAS:fLUC, respectively. The PLOX3:HIS3 Y1H reporter plasmid was developed by MultiSite LR cloning (39). TF ORFs were amplified by PCR with Platinum *Taq* High Fidelity (Invitrogen) and cDNA synthesized from MeJA-treated *Arabidopsis* cells as template. All primers used are listed in SI Table 4. ORFs were introduced by Gateway BP and LR cloning for Y1H into pGAD (40) and for TEA into p2GW7 (38) and p2GAL4DBGW6. The latter destination vector was constructed by inserting a Gateway cassette into the P35S-GAL4DB vector (41). TEA and Y1H were performed as described (11, 39). For the growth test on 3-AT, 10- and 100-fold dilutions of a 2-day-old liquid culture were dropped on media containing increasing 3-AT concentrations and allowed to grow for several days at 30°C.

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