

MYB36 regulates the transition from proliferation to differentiation in the *Arabidopsis* root

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Stem cells are defined by their ability to self-renew and produce daughter cells that proliferate and mature. These maturing cells transition from a proliferative state to a terminal state through the process of differentiation. In the *Arabidopsis thaliana* root the transcription factors SCARECROW and SHORTROOT regulate specification of the bipotent stem cell that gives rise to cortical and endodermal progenitors. Subsequent progenitor proliferation and differentiation generate mature endodermis, marked by the Casparian strip, a cell-wall modification that prevents ion diffusion into and out of the vasculature. We identified a transcription factor, MYB DOMAIN PROTEIN 36 (MYB36), that regulates the transition from proliferation to differentiation in the endodermis. We show that SCARECROW directly activates MYB36 expression, and that MYB36 likely acts in a feed-forward loop to regulate essential Casparian strip formation genes. We show that *myb36* mutants have delayed and defective barrier formation as well as extra divisions in the meristem. Our results demonstrate that MYB36 is a critical positive regulator of differentiation and negative regulator of cell proliferation.

differentiation | Casparian strip | MYB36 | endodermis | proliferation

Progression from stem cell through specification to differentiation is central to multicellular development. This process involves both the loss of proliferative potential and the acquisition of characteristic features that enable cells to perform specific functions. In plant roots, differentiated endodermis surrounds the central vasculature, which transports water and nutrients throughout the plant. The endodermis protects the root vasculature from the entry of harmful toxins and pathogens by means of a cell-wall modification called the Casparian strip (1). This lignin-composed extracellular barrier prevents the passive flow of water and solutes into and out of the vasculature (2). To enter the vasculature, ions must pass through the endodermis, which selectively filters nutrients.

Given this crucial cellular function, endodermal specification has been extensively studied. The cortex/endodermal initial cell divides asymmetrically to regenerate itself and produce a daughter cell. This daughter cell divides to produce the first cells of the cortex and endodermal lineages, which we will refer to as progenitors. Several transcription factors have been identified as essential regulators of the daughter cell division and of endodermal cell-fate specification. SHORTROOT (SHR) is expressed in the vasculature and moves into the daughter cell and endodermis, where it induces the expression of SCARECROW (SCR) (3) and regulates daughter cell division (4). Together, SHR and SCR regulate the expression of JACKDAW (JKD), MAGPIE (MGP), and NUTCRACKER (NUC), several members of the C2H2 transcription factor family (5–8). Collectively, these proteins pattern the cortical and endodermal progenitors. SCR, JKD, MGP, and NUC regulate SHR movement and nuclear localization in the daughter cell, thus regulating its asymmetric division (5, 6, 8). The molecular pathways that subsequently induce differentiation of these progenitors are not known.

Differentiated endodermis is marked by Casparian strip formation. Although the molecular mechanisms driving differentiation are not known, it has been shown that ectopic SHR expression results in ectopic Casparian strip formation (3, 9). Five functionally redundant Casparian strip proteins (CASP1–5) are

required for Casparian strip formation (10). CASPs are transmembrane proteins that are localized to a narrow zone within endodermal cells, where they anchor peroxidase and oxidase enzymes that polymerize lignin, the primary component of the Casparian strip (10, 11). Due to the irreversibility of lignin polymerization, Casparian strip formation can be used as a proxy for endodermal differentiation. Thus, understanding how CASP genes are regulated should provide insights into the mechanistic basis of differentiation.

We performed a genetic screen for mutants with altered *pCASP2::GFP* expression and identified a gene that codes for the transcription factor MYB DOMAIN PROTEIN 36 (MYB36). *myb36* mutant seedlings have no visible *pCASP2::GFP* expression and Casparian strip barrier formation is dramatically delayed. Both SHR and SCR have been shown to activate MYB36 expression, and we demonstrate that SCR activation is direct. Additionally, we show that MYB36 activates genes involved in Casparian strip formation, but this activation, although rapid, requires protein synthesis. RNA-sequencing (RNA-seq) transcriptional profiling from the endodermis of *myb36* mutants reveals that genes involved in daughter cell division and specification, including *SCR*, *JKD*, and *MGP*, are repressed by MYB36. These results demonstrate that MYB36 regulates essential genes for endodermal differentiation and represses transcription factors that regulate cell division and specification. We propose that MYB36 regulates the developmental switch from a proliferative state toward differentiation.

Significance

The process by which cells differentiate is central to multicellular development and cancer. Dramatic gene expression changes mediate this complex process, which involves the termination of proliferation and the acquisition of distinct cell-specific features. We identified a transcription factor, MYB DOMAIN PROTEIN 36 (MYB36), that regulates this developmental transition in the *Arabidopsis thaliana* root endodermis. Differentiated endodermis forms a protective waxy barrier called the Casparian strip. We found that MYB36 activates genes involved in Casparian strip formation and represses genes involved in proliferation. Our results suggest that MYB36 is a critical regulator of developmental timing in the root endodermis.

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The authors declare no conflict of interest.

Data deposition: All whole-genome sequencing data and RNA-seq data reported in this paper have been deposited in the Sequence Read Archive (SRA) and Gene Expression Omnibus (GEO) database under the umbrella BioProject, www.ncbi.nlm.nih.gov/bioproject (accession no. PRJNA292308). Seed lines generated for this paper have been sent to the Arabidopsis Biological Resource Center, abrc.osu.edu/order-stocks (ABRC).

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Results

MYB36 Is a Key Regulator of Endodermal Differentiation. To identify regulators of endodermal differentiation, we conducted a genetic screen for mutations that alter the expression of a *CASP2* transcriptional reporter (Fig. 1 *A* and *C*). Among mutagenized seedlings with a *pCASP2::GFP* reporter, we identified one recessive mutant that lacked any detectable GFP expression (Fig. 1 *B* and *D*). Using bulk segregant analysis and whole-genome resequencing, we identified a point mutation in the *MYB36* locus (At5g57620) resulting in a premature STOP codon in the predicted DNA-binding domain (Fig. 1*E*). Due to the nature and location of the mutation and its recessivity, we presume our allele is a genetic null. *MYB36* encodes an R2R3-MYB class transcription factor (12), which had previously been identified as a core expression marker for endodermal cell identity (13). We confirmed that our mutant is allelic to two other *myb36* alleles, FLAG_412E06 and WiscD-sLox442H5, which both fail to complement *pCASP2::GFP* expression when crossed to *myb36-1* mutants [$n = 20$; F1 (first generation progeny) seedlings examined from each cross]. These experiments collectively suggest that *MYB36* is the causal gene.

It has been shown that the intercalating agent propidium iodide (PI) is blocked from entering the vasculature by the Casparian strip (Fig. 2*A*) (14). We used this assay to determine Casparian strip function in *myb36-1* (Methods). In wild-type roots, the Casparian strip restricts PI penetration into the vasculature ~14 cells after the onset of elongation (Fig. 2*C*), coincident with the onset of differentiation. In *myb36-1* mutants, despite evidence of differentiation, such as root hairs (Fig. 1*B*), PI was able to penetrate into the vasculature (Fig. 2*B*). Restriction of PI occurred ~30 cells after the onset of elongation, indicating delayed barrier formation (Fig. 2*C*). Similar delays are observed in the *casp1/casp3* double

mutant (10) (Fig. 2*C*). These results indicate that *MYB36* regulates genes involved in Casparian strip formation, a proxy for endodermal differentiation.

We found that *MYB36* expression is enriched in the endodermis compared with both whole root and cortex in cell type-specific RNA expression analysis (15) as well as RNA-seq data (16). To examine the expression pattern of *MYB36* protein, we generated a translational fusion to GFP using recombineering to maintain the flanking DNA 5' and 3' of the *MYB36* genomic locus (*rMYB36::GFP*). After crossing *myb36-1* with plants expressing this construct, we found that *rMYB36::GFP* complements barrier function (Fig. 2*C*) and *pCASP2::GFP* expression (50/50 seedlings), providing further evidence that *MYB36* is the causal gene for both phenotypes. *rMYB36::GFP* is most highly expressed before the onset of differentiation (Fig. 2*D*) and is expressed exclusively in endodermal cells (Fig. 2*E*).

MYB36 Activates Genes Required for Endodermal Differentiation. To identify genes that are regulated by *MYB36*, we used RNA-seq to assess gene expression in *myb36-1* and wild-type roots. From RNA-seq libraries of whole roots, we found 178 genes down-regulated and 324 genes up-regulated in *myb36-1* (Dataset S1). Down-regulated genes are enriched for Gene Ontology (GO) terms associated with cell-wall organization, including all five *CASP* genes. Up-regulated genes are enriched for GO terms associated with extracellular region and peroxidase activity (Dataset S2).

Considering the cell-type specificity of *MYB36* expression, we wanted to investigate changes in gene expression specific to the endodermis. To this end, we used the *pSCR::ER::GFP* transcriptional reporter and fluorescence-activated cell sorting to enrich for endodermal cells (17) from wild-type (*pSCR::ER::GFP*) and *myb36-1* (*pSCR::ER::GFP*) seedlings. The endodermis-enriched RNA-seq data revealed 1,159 down-regulated genes and 949 up-regulated genes (Dataset S3). Down-regulated genes are associated with GO terms related to differentiation, including “peroxidase activity” and “Casparian strip.” Consistent with the whole-root data, all five *CASP* genes are significantly down-regulated in the mutant endodermis (Fig. 2*F*). Up-regulated genes are associated with GO terms related to response to stimulus, oxidoreductase activity, and binding, including “innate immune response,” “peroxidase activity,” and “RNA binding” (Dataset S4). Because peroxidase activity-related genes are enriched in both groups, *MYB36* may regulate reactive oxygen species (ROS) homeostasis, which has been shown to play a critical role in the transition from proliferation to differentiation (18). Expression of a key regulator of this transition, *UPBEAT1*, is down-regulated in *myb36-1* mutant plants (Fig. 2*F*).

CASP proteins are required to localize the lignin polymerization machinery enabling localized Casparian strip deposition (11). The NADPH oxidase respiratory burst oxidase homolog F (*RBOHF*) enables the production of H_2O_2 , which is used by peroxidase 64 (*PER64*) to polymerize monolignol subunits into lignin (11). Although *PER64* is significantly down-regulated in *myb36-1* (Fig. 2*F*), *RBOHF* did not meet our significance threshold. However, other genes involved in ROS homeostasis are significantly differentially expressed (gray bars, Fig. 2*F*), as are numerous other peroxidases and oxidases (Dataset S3). Additionally, *SCHENGEN3* (*SGN3*), a receptor-like kinase involved in localizing the *CASP* proteins (19), is significantly up-regulated in *myb36-1* (Fig. 2*F*). Considering that expression of ROS-related genes both increases and decreases in *myb36-1* mutants, *MYB36* may play a general role in maintaining ROS homeostasis during differentiation. Alternatively, disruption of Casparian strip formation may cause ROS imbalance.

To assess whether regulation of *CASP* genes or ROS-related genes is direct or indirect, we performed an induction experiment using a *MYB36* construct under its native promoter fused to the glucocorticoid receptor (GR) hormone-binding domain (*pMYB36::MYB36:GR*). GR fusions are sequestered in the cytoplasm and

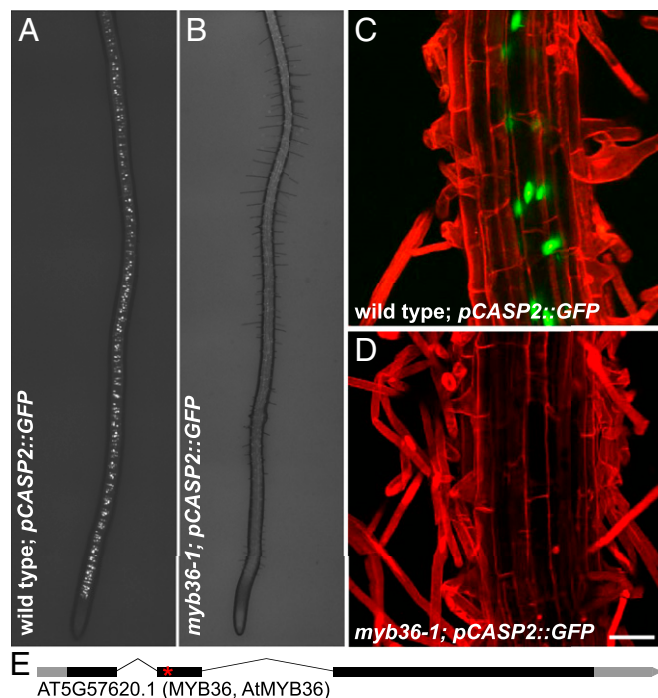


Fig. 1. Identification of a mutant lacking *pCASP2::GFP* expression. (*A* and *B*) Stitched dissecting scope image of (*A*) wild-type plants expressing the *pCASP2::GFP* transcriptional reporter and (*B*) *myb36-1* mutants that lack *pCASP2::GFP* expression. (*C* and *D*) Maximum-projection confocal images of (*C*) wild-type plants expressing the *pCASP2::GFP* transcriptional reporter and (*D*) *myb36-1* mutants that lack *pCASP2::GFP* expression. (*E*) The mutation was mapped by sequencing to reveal a premature STOP (asterisk) at W58 in the coding region of *MYB36*, a putative transcription factor. (Scale bar, 50 μ m.)

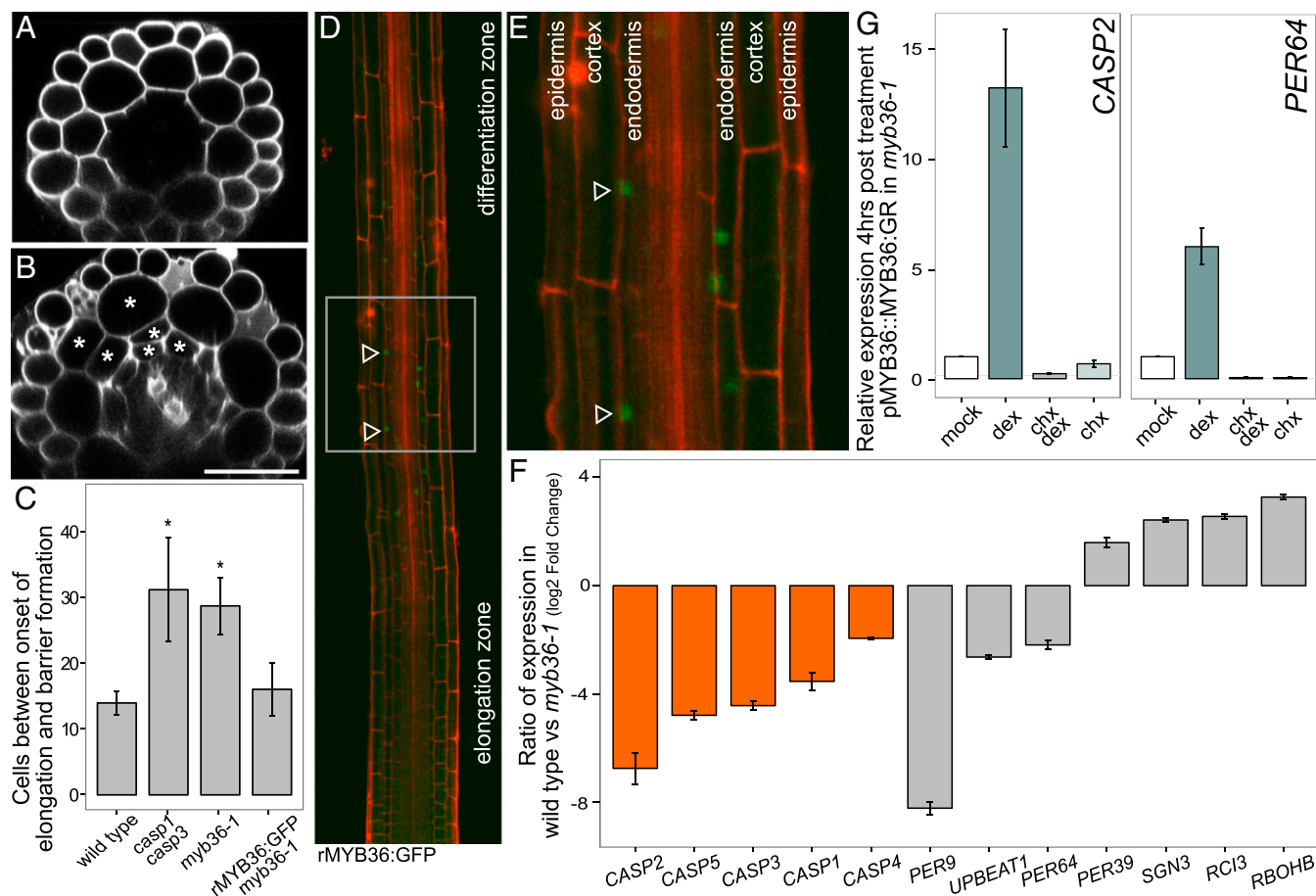


Fig. 2. *myb36-1* mutant seedlings exhibit delayed barrier formation and dramatic changes in gene expression. (A) Optical cross-section of wild-type root in the differentiation zone. (B) Optical cross-section of *myb36-1* mutant root displays Casparian strip defect and extra cell divisions (asterisks). (Scale bar, 50 μ m.) (C) Quantification of cells after onset of differentiation in wild type, *casp1 casp3*, and *myb36-1* and genetic complementation of *myb36-1* with rMYB36:GFP seedlings. Error bars are SD of the mean from three biological replicates. Significance was determined by Student's *t* test, **P* < 0.01 (for source data see Dataset S5). (D) Confocal images (25 \times) stitched together to reveal rMYB36:GFP expression. The fusion is most strongly detected in the elongation zone, before the onset of differentiation (arrowheads). (E) rMYB36:GFP is expressed specifically in the nuclei in the endodermis (arrowheads). Epidermis, cortex, and endodermis are labeled for orientation. (F) *myb36-1* mutant endodermis has reduced expression of *CASP* genes (orange) and altered expression of many genes involved in oxidoreductase activity (gray) compared with wild-type endodermis. Error bars are SEM from three biological replicates. Adjusted *P* value \leq 0.01 (Methods) (for source data see Dataset S6). (G) qRT-PCR of two putative MYB36 targets 4 h after induction of pMYB36::MYB36:GR. Mean expression values of three biological replicates are reported after normalization with PP2A expression. Error bars are SEM (for source data see Dataset S7).

translocated to the nucleus upon induction by dexamethasone (dex) (20). Following dex treatment, *myb36-1* mutants harboring this construct expressed *pCASP2::GFP* 4 h after induction (*n* = 100/100). Quantitative reverse transcription PCR (qRT-PCR) of *CASP2* and *PER64* confirmed that pMYB36::MYB36:GR induction resulted in increased expression of these genes (Fig. 2G). To assess whether this increased expression was the result of direct activation by MYB36, independent of protein synthesis, we induced with dex and added cycloheximide (chx), a potent protein synthesis inhibitor. Surprisingly, there was no significant increase in expression of either *CASP2* or *PER64*, suggesting that the activation is indirect (Fig. 2G). Alternatively, because protein synthesis is required for expression, MYB36 may induce a cofactor required for activation of *CASP* and *PER64* in a feed-forward loop.

MYB36 Represses Genes That Are Involved in Proliferative Divisions. In *myb36-1* mutants there is evidence of extra divisions in both the meristematic and differentiation zones of the endodermis (Fig. 3B and D). Extra divisions were previously observed for the WiscD-SLox442H5 allele of *myb36* at low penetrance (6/20 roots) (13), but we observed extra meristematic divisions at a higher penetrance (15/20 roots) in *myb36-1*, suggesting that our allele is more

severe. These results suggest that MYB36 suppresses meristematic cell divisions. Because MYB36 is most highly expressed in the elongation zone before the onset of differentiation, MYB36 regulation of meristematic cell divisions is likely indirect, either by feedback on meristematic regulators or possibly by misregulation of ROS homeostasis. There is a growing body of literature that implicates ROS in cell division and differentiation (21, 22).

To investigate whether MYB36 regulates transcription factors involved in proliferative divisions, we reexamined the endodermis-enriched RNA-seq data. We found that *SCR* as well as *MGP* and *JKD* genes encoding the C2H2 transcription factors are significantly up-regulated in the endodermis of *myb36-1* mutants (Fig. 3E). The change in expression from the closely related gene *NUC* did not satisfy our significance threshold. These BIRD transcription factors have been shown to play a role in early endodermal specification and proliferation (5, 6, 8). Thus, we speculate that MYB36 represses genes regulating proliferation as cells transition to a differentiated state.

SCARECROW Directly Activates MYB36 Expression in the Endodermis. Based on the endodermal specificity of MYB36 expression, we hypothesized that SHR and/or SCR could be involved in its

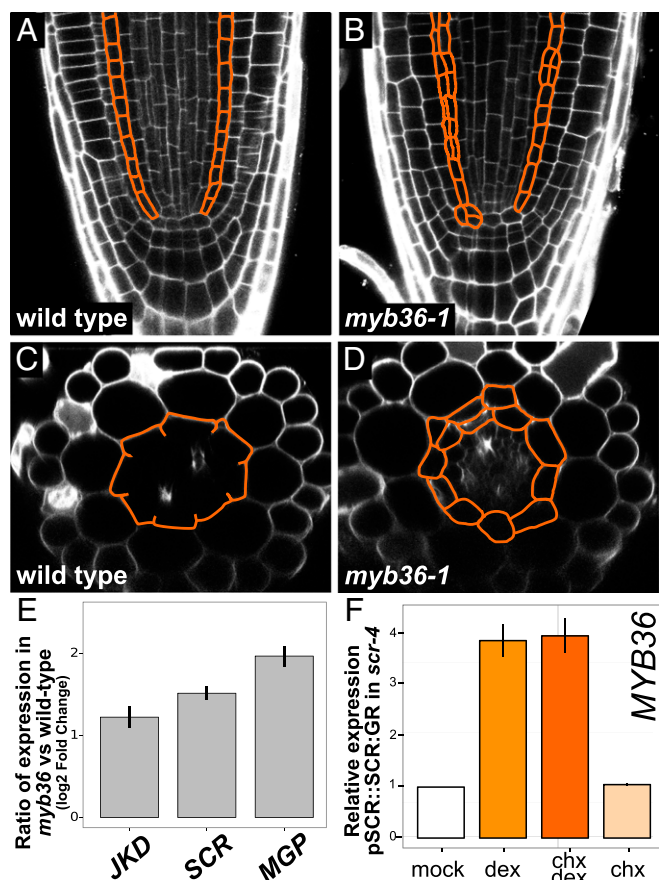


Fig. 3. *myb36-1* has more divisions in the meristem and in the differentiation zone than wild type. (A) Wild-type root meristem; the endodermis is outlined in orange ($n = 20$). (B) *myb36-1* mutant root meristem displays additional divisions (outlined in orange) with 75% penetrance ($n = 15/20$). (C) Optical cross-section of wild-type seedling at the onset of the differentiation zone. Note the stereotypical eight cells present in both the endodermis and cortex. (D) Optical cross-section of *myb36-1* at the onset of the differentiation zone. Additional divisions are outlined in orange. (E) *myb36-1* has increased endodermal expression of *JKD*, *SCR*, and *MGP* compared with wild type (for source data see [Dataset S8](#)). (F) qRT-PCR of *MYB36* expression 3 h after induction of pSCR::SCR:GR. Mean expression values of three biological replicates are reported after normalization with PP2A expression. Error bars are SEM (for source data see [Dataset S9](#)).

regulation. Time course gene expression data from inducible *SHR* and *SCR* proteins in their respective mutant backgrounds revealed that *MYB36* is significantly up-regulated after induction of either *SCR* or *SHR* (4). To verify that *SCR* activates *MYB36* expression, we performed an induction experiment using an inducible *SCR* construct under its native promoter fused to the GR hormone-binding domain (pSCR::SCR:GR) in the *scr-4* mutant (4, 5, 7). We found that *MYB36* expression is induced 3 h after induction, consistent with previous results (4). Treatment with dex and chx induced *MYB36* expression as much as dex alone, providing strong evidence that *SCR* directly activates *MYB36* expression (Fig. 3F).

A Key Link Between Proliferation and Differentiation. The progression from stem cell to differentiated tissue involves transitions through different gene expression states. We have identified and characterized *MYB36* as a key mediator of the transition from proliferation to end-stage differentiation. When mutated, critical genes for Casparian strip formation are not expressed, indicating that *MYB36* controls this hallmark of differentiation. We find it interesting that *MYB36* activates *CASPs* but represses the gene

encoding the receptor-like kinase *SGN3*. This provides evidence for a more complex role of *SGN3* in Casparian strip deposition. Although a barrier to small molecules forms eventually, future work will reveal whether this barrier is a fully functional Casparian strip.

Loss of *MYB36* activity also results in increased cell proliferation and expression of *SCR* and certain genes encoding C2H2 transcription factors, suggesting a dual role of damping down cell division while promoting differentiation. This is analogous to the retinoblastoma (*Rb*) tumor suppressor protein in animals. *Rb* normally regulates progression through the cell cycle; however, when mutated or hyperphosphorylated, errant cell divisions result (23). Why ectopic cell divisions in plants appear to be more circumscribed than in animals is an interesting question for future research.

Expression of rMYB36:GFP is highest in the region just below the differentiation zone where the *CASP* proteins are expressed. The protein localization pattern mimics this expression (Fig. 2D). Considering that *SCR* and *SHR* are expressed throughout the endodermis, the expression pattern of *MYB36* suggests that either there is an additional activator, which is also spatially restricted, or a repressor localized to the meristematic zone. Further studies will reveal additional upstream regulators of *MYB36* and how network perturbation alters differentiation.

Our expression and direct activation data combined with results from the literature allowed us to construct a model describing the role of *MYB36* in regulating the transition from proliferation to differentiation in the endodermis (Fig. 4). *SCR* and *SHR* activate *MYB36* expression, which promotes expression of genes essential for localized Casparian strip formation. Additionally, *MYB36* represses the genes encoding *JKD* and *MGP*, which regulate periclinal divisions in the endodermis and cortex (5, 6, 8). This repression by *MYB36* results in restriction of proliferative divisions once the endodermis starts to terminally differentiate. These interactions generate an incoherent feed-forward loop (Fig. 4D and E), which could provide a mechanism for the transitions between proliferation and differentiation (Fig. 4F).

Methods

Plant Materials, Growth Conditions, and Steroid Treatment. *Arabidopsis* accession Columbia-0 (Col-0) or Col-0 with the pCASP2::NLS:GFP::GUS (pCASP2::GFP) transgene was used as wild type for all experiments. The *myb36-1* ethylmethane sulfonate (EMS)-generated line was used for all mutant experiments unless otherwise noted. For all experiments, seeds were sterilized, stratified, and imbibed 48 h at 4 °C. For the barrier assay, seedlings were plated on 0.5× Murashige and Skoog (MS) media, 1% agar, with no sucrose, and sealed with Micropore tape. Plates were grown vertically on square plates under long-day conditions and assayed at 6 d post exposure to light.

Induction and cyclohexamide experiments were performed as described (24) with modifications for younger plants. Briefly, pMYB36::MYB36:GR *myb36-1* seeds were plated on sterile mesh, 0.5× MS media, with no sucrose. Seedlings were transferred 6 d post exposure to light to fresh 0.5× MS media (mock), 10 mM dexamethasone-containing media (dex), 10 mM cyclohexamide/10 mM dexamethasone-containing media (chx dex), or 10 mM cyclohexamide-containing media (chx). After 4 h of treatment, root tissue was harvested with a scalpel and frozen in liquid nitrogen. pSCR::SCR:GR *scr-4* seeds were plated on sterile mesh, 1× MS, 1% sucrose media. Seedlings were transferred 6 d post exposure to light to fresh 1× MS, 1% sucrose media (mock), 10 mM dexamethasone-containing media, 10 mM dexamethasone/10 mM cyclohexamide-containing media, or 10 mM cyclohexamide-containing media. After 3 h of treatment, root tissue was harvested with a scalpel and frozen in liquid nitrogen.

Microscopy. Barrier assays assessing Casparian strip function were performed as previously described (14). Briefly, seedlings were incubated in the dark for 10 min in fresh 10 µg/mL propidium iodide (Invitrogen) and rinsed two times in water. Stock solution (100×) was stored at 4 °C in water. The onset of elongation was defined as the first endodermal cell. Cortical cells were counted from the onset of elongation in a median optical section that was twice the size of its younger neighbor until the neighboring endodermal cell restricted PI penetration. The barrier assay and aberrant meristematic cell divisions were imaged and quantified using a Zeiss 510 upright confocal microscope.

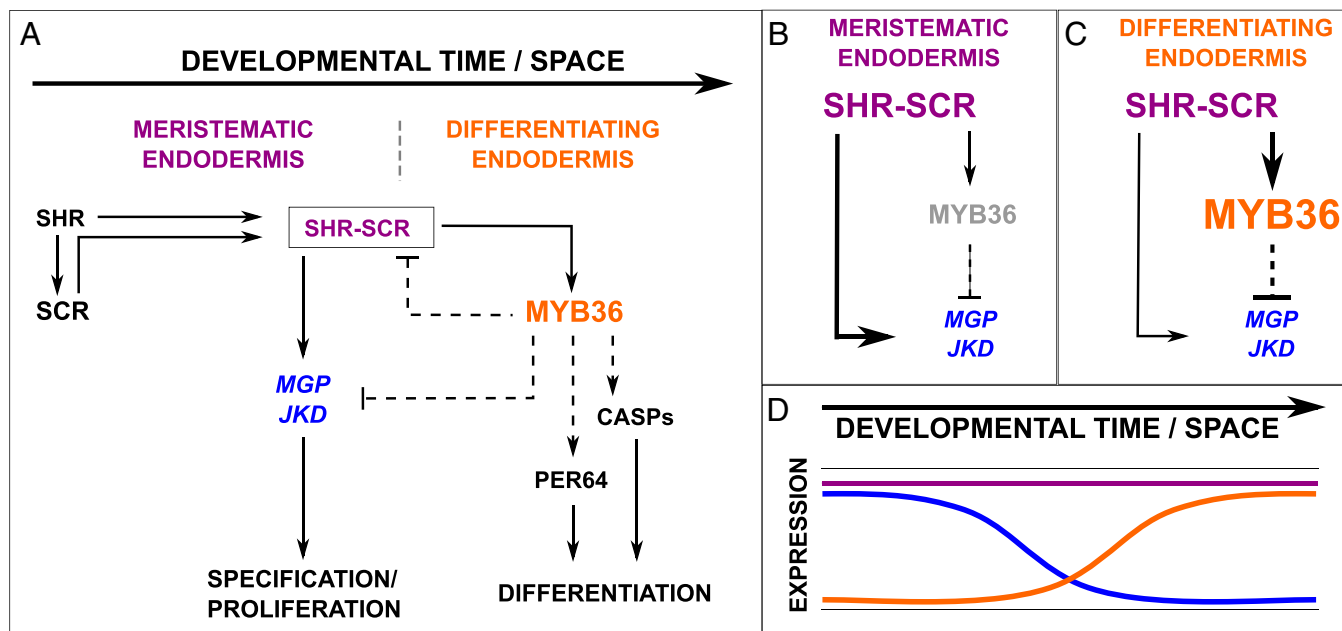


Fig. 4. Model of the transition from specification to differentiation. (A) Wiring diagram describing the transition from proliferation to differentiation based on this work and published data discussed in the text. SHR activates expression of SCR. SHR and SCR form a complex that regulates expression of MGP and JKD, which regulate proliferation. At the transition between specification and differentiation, SHR/SCR, MYB36, and MGP/JKD form an incoherent feed-forward loop predicting two behaviors, as follows. (B) MGP and JKD gene expression should be high in the meristem in the absence of MYB36 expression. (C) Increased expression of MYB36 in the differentiation zone rapidly reduces MGP and JKD expression. (D) Predicted expression of proliferation-inducing genes (MGP and JKD) (blue) and MYB36 (orange) throughout developmental time. Thickness of lines represents the strength of interaction.

Dissecting scope images were acquired with an Axio Zoom.V16 fluorescence dissecting scope (Zeiss) at 25× magnification. Images were stitched using Fiji (25) with the Pairwise Stitching plugin (26).

Mutagenesis Screen. Seeds were mutagenized as previously described (27). Briefly, ~7,500 seeds expressing homozygous *pCASP2::GFP* were imbibed overnight, mutagenized with 0.2% EMS for 15 h, washed with sterile water 10 times for a total of 4 h, and sown on soil. Approximately 5,000 mutagenized (M0) seeds germinated and allowed to self-cross to produce M1 seeds. Seeds were collected as bulks with 5–10 M1 plants per bulk. Nine hundred thirty bulks were collected and screened. In the next generation, ~60 M2 seedlings per bulk were screened for changes in *pCASP2::GFP* expression intensity using a fluorescence stereo dissecting scope (Leica).

Mapping. Bulk segregant analysis and whole-genome resequencing were used to map the EMS-generated *myb36-1* mutant as previously described (28). Briefly, mutant plants were back-crossed to the *pCASP2::GFP* parental line and map-crossed to *Landsberg erecta* (*Ler*) to generate F1 heterozygotes. These plants were self-crossed, producing two populations of segregating F2 plants. Approximately 50 mutant plants were bulked from each cross. Unmutagenized *pCASP2::GFP* and *Ler* parental plants were grown and 100 seedlings were sequenced. Four pools were used to make DNA libraries using a TruSeq Library Prep Kit (Illumina). SNPtrack (genetics.bwh.harvard.edu/snptrack/) was used to analyze the data and determine the causative mutation (28). dCAPS genotyping primers were designed (helix.wustl.edu/dcaps/dcaps.html) (29) to screen for a mutant allele: genotype forward (F)/reverse (R): 5'-AGATGTGGTAAGAGTTGCA-GACTGAGATA-3', 5'-caagagagatagatcacgcacCG-3'. Product size is 144 bp; wild type is cut into 114- and 31-bp fragments with AluI enzyme (New England Biolabs; R01375). Whole-genome resequencing data have been uploaded to NCBI BioProject (www.ncbi.nlm.nih.gov/bioproject) accession no. PRJNA289027.

RNA-Seq Library Preparation. Seeds were sterilized with 3% (vol/vol) sodium hypochlorite and 0.1% Tween for 7 min and rinsed five times in sterile water. Seeds were plated on sterile mesh, 1× MS, 1% agar, 1% sucrose media, and sealed with parafilm. Plates were grown under long-day conditions for 6 d. Whole-root samples from wild type (Col-0) and *myb36-1* were harvested and frozen in liquid nitrogen. Endodermal-enriched samples expressing the transcriptional reporter *pSCR::ER::GFP* (30) in the Col-0 and *myb36-1* backgrounds were obtained by fluorescence-activated cell sorting as described previously

(17). Total RNA from whole roots and sorted cells was extracted using a Plant RNeasy Kit (Qiagen). rRNA was depleted from total RNA using a RiboMinus Plant Kit (Invitrogen). RNA quantity and integrity were assessed by Qubit (ThermoFisher Scientific) and Agilent Bioanalyzer. A ScriptSeq v2 RNA-Seq Library Prep Kit (Epicentre) was used to generate strand-specific RNA-seq libraries. Paired-end reads (50-bp) were obtained using the Illumina HiSeq 2000 platform at the Duke University Genome Sequencing & Analysis Core.

RNA-Seq Data Analysis. Reads were quality-filtered and mapped to the Arabidopsis genome version TAIR10 using TopHat (31), and read counts were determined by HTSeq (32). Differentially expressed genes were determined using the R package DESeq (33). Significance was assessed using a threshold of log2 fold change ≥ 1 and *padj* ≤ 0.01 [*padj*: *P* value adjusted for multiple testing and controlled for false discovery rate with the Benjamini–Hochberg statistical procedure (33)]. Libraries were also run through Cufflinks (34) to determine fragments per kilobase of transcript per million mapped reads (FPKM) values for whole-root (Dataset S5) and sorted libraries (Dataset S6). All RNA-seq data have been uploaded to the GEO accession no. GSE70584. AGI numbers or locus identifiers for selected genes are as follows: AT2G36100 *CASP1*; AT3G11550 *CASP2*; AT2G27370 *CASP3*; AT5G06200 *CASP4*; AT5G15290 *CASP5*; AT5G03150 *JACKDAW*; AT1G03840 *MAGPIE*; AT5G44160 *NUTCRACKER*; AT4G11290 *PER39*; AT5G42180 *PER64*; AT1G09090 *RBOHB*; AT1G64060 *RBOHF*; AT1G05260 *RCI3*; AT4G20140 *SGN3/GSO1*; AT4G37650 *SHR*; AT3G54220 *SCR*; AT2G47270 *UPBEAT1*.

Functional categories were assigned to differentially expressed genes using the GOzilla online tool (35). A background set of detected genes was defined for whole roots and sorted endodermis independently. A gene was called detected if it was found in at least two biological replicates with an arbitrary cutoff of two normalized counts per replicate. A *P* value of 1E-04 was used for both conditions; only the most descendant Gene Ontology terms are reported for the sorted endodermis analysis.

Plasmid Construction and Transformation. The JaTy clone JaTy60A10 (64,154 bp) was used to generate the rMYB36::GFP recombinering line as described (36). The oligos MYB36_Rec_F/R were designed to remove the STOP codon upon recombination with the GFP cassette. Homology to the MYB36 locus is in capital letters: MYB36_Rec_F/R: 5'-TGGTTATGCTTCAAGATTACGCTCAGATG-AGCTACCACAGTGTGGAGTGGAGTGG-3', 5'-ATCCATCCCTATAGTTACGCATTAT-ATATATGATGATATAACTTActaagcgaatcaggaacatcgaagggttagcccgccgccc-3'. This construct was transformed into Col-0 and crossed to *myb36-1*.

Standard molecular biology procedures and the Gateway Cloning System (Invitrogen) were used for cloning. The 3.0-kb region upstream of the MYB36 transcription start site and the genomic region of the MYB36 gene were used to generate the pMYB36::MYB36:GR construct. pMYB36::MYB36:GR was transformed into the *myb36-1* mutant.

qRT-PCR. RNA was prepared from whole roots as described for RNA-seq libraries. Five hundred nanograms of RNA was used to generate cDNA with SuperScript III (Invitrogen) and diluted 1:10 for assays. Primers were designed and tested for amplification efficiency. qRT-PCR was performed using FastStart Universal SYBR Green Master Mix (Roche) on a StepOnePlus instrument (Applied Biosciences). Three biological replicates and three technical replicates were used for each experiment. Standard curves were run for each primer pair. Values reported are the means of three biological replicates after efficiency corrected quantification with PP2A as the reference. The following pairs were used: CASP2exon1F/R: 5'-TACAACAGAGGACTCGCATC-3', 5'-TGGAAAGTTGGTAGATCGTCG-3'; PER64exon2F/R: 5'-ACAAGCAGAGAAAGATGGACC-3', 5'-CTCCTGAGAGAGCGACAGCATC-3'; MYB36exon3F/R: 5'-GGTCCATAATTGCAGCTCAG-3', 5'-AATCGTTATGAGTCTTGACG-3'; PP2AF/R: 5'-TAACGTGGCCAAATGATGC-3', 5'-GTTCTCCACAACCGCTTGGT-3'.

Note. While our paper was in revision, another paper was published in PNAS that describes the cell biology of MYB36 (37). Our paper demonstrates how MYB36 regulates the transition from proliferation to differentiation. We find the two papers to be complementary.

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