The RNA Polymerase-Associated Factor 1 Complex Is Required for Plant Touch Responses

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Abstract

Thigmomorphogenesis is a stereotypical developmental alteration in the plant body plan that can be induced by repeatedly touching plant organs. To unravel how plants sense and record multiple touch stimuli we performed a novel forward genetic screen based on the development of a shorter stem in response to repetitive touch. The touch insensitive (ths1) mutant identified in this screen is defective in some aspects of shoot and root thigmomorphogenesis. The ths1 mutant is an intermediate loss-of-function allele of VERNALIZATION INDEPENDENCE 3 (VIP3), a previously characterized gene whose product is part of the RNA polymerase II-associated factor 1 (Paf1) complex. The Paf1 complex is found in yeast, plants and animals, and has been implicated in histone modification and RNA processing. Several components of the Paf1 complex are required for reduced stem height in response to touch and normal root slanting and coiling responses. Global levels of histone H3K36 trimethylation are reduced in VIP3 mutants. In addition, THS1/VIP3 is required for wild type histone H3K36 trimethylation at the TOUCH3 (TCH3) and TOUCH4 (TCH4) loci and for rapid touch-induced upregulation of TCH3 and TCH4 transcripts. Thus, an evolutionarily conserved chromatin-modifying complex is required for both short- and long-term responses to mechanical stimulation, providing insight into how plants record mechanical signals for thigmomorphogenesis.

Key words: Histone methylation, Paf1 complex, TCH genes, thigmomorphogenesis, touch response, VIP3.

Introduction

It is firmly established that development in multicellular organisms relies on the local concentration of key biochemical signals such as hormones or growth factors (Wolpert, 1969). However, it is becoming increasingly clear that the temporal pattern of changes in the concentration of these biochemical signals is equally important for the final phenotypic output (Raj and van Oudenaarden, 2008; Lander, 2011; Oates, 2011; Webb et al., 2016). For instance, the intrinsic delays associated with transcription and translation have been proposed to play an instructive role in patterning (Monk, 2003; Gaffney and Monk, 2006). Understanding how the intensity as well as the frequency of a stimulus are measured and recorded is a key challenge for plant biologists, as post-embryonic development is continuously impacted by the environmental conditions in which the plant grows.

Abbreviations: Col-0, Columbia-0; FLC, Flowering Locus C; JA, jasmonic acid; LINC, linker of the nucleoskeleton and cytoskeleton; me3, trimethylation; Paf1, RNA polymerase II-associated factor 1; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; qRT-PCR, quantitative reverse-transcriptase PCR; SAM, S-ADENOSYLMETHIONINE SYNTHASE; TBST, Tris-buffered saline with Tween 20; TCH, TOUCHths1, touch insensitive 1; VIP3, VERNALIZATION INDEPENDENCE 3

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Mechanical stimuli are coded both in intensity and in frequency [for examples see (Chehab et al., 2009; Leblanc-Fournier et al., 2014)] but how this is accomplished at the molecular and genetic level is unknown. Here we begin to address the genetic pathways by which a repeated mechanical stimulus leads to a developmental change during plant thigmomorphogenesis, a stereotypical developmental alteration in the plant body plan that can be induced by shaking, rubbing, bending, or brushing leaves or stems (Chehab et al., 2009; Coutand, 2010). Thigmomorphogenesis is classically defined as producing a shorter and thicker plant due to decreased stem height and increased stem and/or petiole diameter relative to control plants (Jaffe, 1980; Biddington, 1986; Chehab et al., 2009; Coutand, 2010). Repeated mechanical stimulation can also result in a more flexible stem and an increase in root mass relative to the shoot. Taken together, these morphological changes are thought to produce a body plan that is more resistant to damage upon future mechanical challenges. This presents an intriguing conversion of energy from an elastic deformation (i.e. bending) into a developmental response (i.e. growth).

Three hallmarks of thigmomorphogenesis are: a dose responsiveness that prevents the plant from responding to isolated events, a systemic nature such that a stimulus applied to one organ leads to changes in overall plant morphology, and a characteristic delay between the initial stimulus and the developmental response (Chehab et al., 2009; Leblanc-Fournier et al., 2014; Moulia et al., 2015). The mechanical stimulus received by cells in the rosette leaves of plants that have been touched, sprayed, blown or bent must be transmitted to the cells of the shoot apical meristem in order to control elongation of the future shoot or stem (Moulia et al., 2015).

Indeed, there is evidence that plants are able to record the number and frequency of mechanical stimuli. The Venus flytrap uses action potentials to measure the number of times an insect touches the trigger hairs of its trap over the course of an hour (Böhme et al., 2016). Plants are also able to sense the number and frequency of stem bending events. When several bends are imposed on poplar stems, the impact on the transcriptome becomes less pronounced from the second bending event onwards. It also takes no less than a week without bending for a stem to recover full molecular sensitivity to such mechanical deformation (Martin et al., 2010). Thus, plants are indeed capable of recording successive mechanical deformations.

Plants also respond to touch in the short-term, though how these events are connected to long-term changes in body plan remains unclear. Following a single touch event, plants rapidly upregulate a large number of genes; in Arabidopsis as much as 2.5% of its genome (Braam and Davis, 1990; Lee et al., 2005). The best characterized of these in Arabidopsis are the TOUCH (TCH) genes, which encode calmodulins and calmodulin-like proteins, cell wall-modifying enzymes, and wound- and defense-inducible genes (Braam and Davis, 1990; Ling et al., 1991; Perera and Zielinski, 1992; Kagaya and Hattori, 2009; Lee et al., 2005). The timing of the induction of most TCH genes is between 5 and 30 minutes after the application of stimuli, suggesting a role in the early response to touch stimulation. The cml24 (tch2) mutation affects root morphology on hard agar and is implicated in microtubule structure and starvation-induced autophagy (Wang et al., 2011; Tsai et al., 2013) but a functional role for TCH genes in thigmomorphogenesis has yet to be established. While these results demonstrate that touch is perceived at the molecular level after a single event, no link between short-term and long-term responses to touch has been identified. In trees, the transcription factor gene ZFP2 is rapidly induced in response to bending (Leblanc-Fournier et al., 2008; Coutand et al., 2009). Interestingly, this induction can be attenuated after multiple bending events, suggesting that plants are able to record the number of mechanical perturbations, albeit through an unknown mechanism (Martin et al., 2010).

The growth response is likely to involve the phytohormone jasmonic acid (JA). JA accumulates in the stems of plants after touching of their rosette leaves, and JA biosynthesis and signaling are required for thigmomorphogenesis (Chehab et al., 2012). Conversely, application of the growth regulatory hormone gibberellin or a loss-of-function mutation in the gibberellin-catabolizing enzyme AtGA2ox7 can prevent thigmomorphogenesis (Lange and Lange, 2015). However, these effects are potentially far downstream in the pathway. No real known regulator of thigmomorphogenesis capable of sensing repetition has been identified yet.

We therefore performed a novel forward genetic screen designed to identify the key molecular and genetic components that link successive elastic deformations to thigmomorphogenesis. Arabidopsis exhibits several stereotypical thigmomorphogenic responses that require multiple days of touch, including the inhibition of stem elongation, shortened petioles, reduced rosette diameter and delayed transition to flowering (Braam and Davis, 1990; Chehab et al., 2012; Cazzonelli et al., 2014; Lange and Lange, 2015). Here we concentrated on stem thigmomorphogenesis to identify new regulators of the touch response. We screened for plants that did not exhibit shortened stems after repeated touch. The first mutant isolated in this screen identified a known regulator of gene expression, the RNA Polymerase II-associated factor 1 (PaII) complex, as a key element of thigmomorphogenesis that serves to integrate touch stimuli over time.

**Materials and methods**

**Touch response assays**

For Fig. 1 and Fig. 2, plants were grown for one week in long day conditions of 16 hours of light and 8 hours of darkness at 23°C, then transferred to 24 hours of light at 25°C for one more week. These conditions were established during the screen and were replicated in later experiments to reduce variability due to growth conditions. Seedlings were allowed to germinate and grow in a large 16-hour chamber, then moved to a smaller 24-hour chamber that was set aside only for touch assay experiments. Two-week-old seedlings were then subjected to touch stimuli for 8–10 days, stopping when bolts were visible. For hand touching, a gloved hand was held parallel to the tray and moved across the pots, touching the tops of all plants within the tray with the palm once and then repeated for a total of ten passes each day. For paintbrushing, seedling leaves were brushed each day with ten passes of a 3-inch standard paintbrush held at a...
45 degree angle to the soil surface. Touched and untouched plants of the same genotype were grown side-by-side in the same tray and trays were rotated 180 degrees every day to reduce variability in temperature and airflow within the growth chambers. Stem height was measured 10–14 days later, whenever the stem height of wild type untouched plants reached over 15 cm high or approximately 30 days after germination. The number of days required for stems to reach 15 cm was dependent on the light and temperature conditions of the particular experiment and chamber used; similar results were obtained when wild type untouched stems were measured anytime beyond this point. In Fig. 3, plants were grown in 16 hours of light in order to allow the null vernalization independence (vip) mutants to grow as tall as possible. In Fig. 4, 12-day-old seedlings were grown in long day conditions of 16 hours light and 8 hours darkness at 21°C and were either untouched or touched with a large, soft paintbrush every 5–7 seconds for 2 minutes, with an average 18 brushes per treatment. Material was collected 30 minutes after the first touch treatment or 30 minutes after plants were subjected to a second touch treatment 2 hours following the first one.

Characterizing ths1 T-DNA
Templates in PCR reactions were either 1 μl genomic DNA or 1 μl cDNA made from 5 mg leaf tissue from wild type or ths1 mutant plants. PCR reactions were carried out using Hot Star Taq (Qiagen) and the following primer pairs: 29830-QPCR.F2/29830.F3, 29830-QPCR.F2/LBb1, 29830-QPCR.F2/Lba1, or ACTF2/ACTR2. The 29830-QPCR.F2/29830.F3 product includes an intron of approximately 450 bp from the VIP3 gene and the ACTF2/ACTR2 product includes an intron approximately 100 bp from the ACT genes. 5–10 μl of each PCR reaction was separated on an ethidium gel and photographed.

Mutant lines
The cfis1-1 (SAIL_367_F03), vip3-2 (SALK_083364), vip5-062223 (SALK_062223) and vip6-065364el/8-2 (SALK_065364) lines were obtained from the Arabidopsis Biological Resource Center. For genotyping, plant genomic DNA was isolated as described.
and wild type or mutant alleles amplified using the primer combinations listed in Supplementary Table S1 at JXB online.

VIP3 immunoblotting

50 mg of leaf tissue from 3–4 week old wild type, vip3-2, or ths1 mutant plants was ground in liquid nitrogen and immediately resuspended in 200 μl 2X SDS PAGE sample buffer. Equal volumes were run on an 8% SDS PAGE gel, blotted to polyvinylidene difluoride (PVDF), blocked in 5% milk/Tris-buffered saline with Tween 20 (TBST), and probed with a 1:1000 dilution of anti-VIP3 antibody overnight at 4°C. The blot was washed three times for 30 minutes each with TBST and incubated with anti-Rabbit HRP (Sigma) at 1:5000 for 60 minutes at room temperature, then washed again with TBST for 30 minutes. Peroxidase activity was detected with the SuperSignal West Femto kit (Thermo Scientific) and imaged on X-ray film. Equal loading was confirmed by Ponceau S staining and by comparison of non-specific bands.

Root slanting and coiling

Seeds were sown on square 100 x 15 mm plates (Falcon) containing 0.5X Murashige and Skoog medium, 1% sucrose and 1.6% phytagar, and in some cases with 3 μM propyzamide (Sigma). Plates were sealed with porous tape. After incubation at 4°C for 2 days in the dark, plates were transferred to a growth chamber and grown vertically for 2 days and then tilted 30° back from the vertical for 2–3 days. All plates were grown in 16 hours of light at 23°C. For curling assays, seedlings were grown on the same media for 2 days vertically and then 3 days horizontally.

Chromatin immunoprecipitation

All experiments were performed in triplicate with two technical repeats for each of the three biological repeats. For ChIP, 0.5 g of cauline leaf material was harvested from plants grown for three weeks under short day conditions of 8 hours light and 16 hours dark at 21°C followed by 2 weeks under continuous light at 16°C (as shown previously (Zhang et al., 2003). These conditions attenuate the phenotypic defects of the vip3 mutant, allowing us to collect sufficient tissue. Harvested tissue was placed on ice and crosslinked in 40 ml of infiltration buffer (13.69 g sucrose, 1 ml 100 mM PMSF, 1 ml 1M Tris/HCl pH 8, 200 μl 0.5M EDTA, 2.7 ml 37% formaldehyde in 100 ml distilled water). Vacuum was slowly introduced to the samples on ice, maintained for 10 minutes and slowly released. This was repeated three times. To stop the reaction 2 ml of 2M glycine was added to each sample. The samples were then infiltrated again for 5 minutes. Samples were washed with 500 ml of distilled water, dried on filter paper and frozen in liquid nitrogen.

Extraction of nuclei was performed as described (Jaskiewicz et al., 2011) in extraction buffer containing 0.44 M sucrose, 1.25% Ficoll, 2.25% Dextran T40, 20 mM Hepes KOH pH 7.4, 10 mM MgCl2, 0.5% Triton X-100, 5 mM DTT, 1 mM PMSF and 1X complete protease inhibitor cocktail (Roche, Product No 04693116001). After spinning down, the pellet was diluted in 500 μl of lysis buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, 1% SDS and 1X complete protease inhibitor cocktail) and sonicated with a Bioruptor UCD-200 to fragments of 0.2–0.5 kilobases. 8 μg of chromatin diluted to a total volume of 500 μl with dilution buffer [1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCL pH 8, 167 mM NaCl, 0.2% complete protease inhibitor cocktail (Roche, EDTA-Free, Product No 04693116001)] was used for immunoprecipitation with specific antibodies on Protein G Plus-Agarose beads (Santa Cruz Technology, sc2002L) overnight on a spinning wheel at 4°C. Two independent immunoprecipitations with the H3K36me3 antibody (Abcam: ab9050, 3 μl per sample) and a no-antibody control were performed using each chromatin sample. Three independent chromatin samples were prepared for each genotype. Following the incubation, all samples were washed with wash buffer (150 mM NaCl, 0.1% Triton X-HCl pH 8, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 1 mM PMSF and 1X complete protease inhibitor cocktail). Precipitated DNA was extracted with the NucleoSpin PCR purification kit (Machery-Nagel REF-740809.250).
The quantity of DNA in each sample was determined using quantitative reverse-transcriptase PCR with the LightCycler 480 SYBR Green I Master Mix (Roche, Product No 04887352001) and the primers listed in Supplementary Table S1 to amplify a portion of exon 2 of TCH3, or the junction between exon 1 and exon 2 of TCH4. Enrichment for H3K36me3 at each region of interest was initially calculated as a percentage of the DNA recovered in the corresponding input samples. All samples were normalized against the enrichment for H3K36me3 at the SAM (At4g01850) locus (Cazzonelli et al., 2014). The signal intensity after immunoprecipitation with specific antibodies was at least 10 times higher than with the no-antibody control for the corresponding samples. The final result is represented as a fold difference over the wild type sample.

Chromatin immunoblotting
2.5 µg of chromatin extract from 0.5 g of cauline leaf tissue, prepared as described above, from each sample were run on a 10% SDS PAGE gel, blotted to PVDF, blocked in 3% BSA/phosphate-buffered saline (PBS) overnight at 4°C and probed with a 1:5000 dilution of each primary antibody. The antibodies Abcam ab9050 and mAbcam ab6002 were used to detect H3K36me3 and H3K27me3, respectively. Anti-Histone H3 antibody (Agrisera, AS10710) was applied as a loading control. After washing the membranes with 0.5% BSA, 0.1% Tween-20 in PBS, they were probed with a secondary anti-Rabbit HRP (Sigma) antibody at 1:10 000 for 90 minutes at room temperature. The signal was visualized with the Pierce™ ECL Western Blotting Substrate (ThermoFisher Scientific, No32106).

Quantitative RT-PCR (qPCR)
All experiments were performed in triplicate, with three technical repeats for each of the three biological repeats. For Fig. 4, RNA extraction from the aerial portion of 12-day-old plants grown under long day conditions of 16 hours of light and 8 hours of dark at 21°C was performed with the Spectrum™ Plant Total RNA Kit (STRN250 SIGMA). 800 ng of total RNA was used for cDNA synthesis with the oligo(dT)20 primer and RevertAid Reverse Transcriptase (Thermo Scientific #EPO441). Expression of target genes was measured using
LightCycler 480 SYBR Green I Master Mix (Roche, Product No 04887352001) in a StepOne Cycler (Applied Biosciences) in a reaction volume of 15 µl. The sequences of primers designed to amplify the coding regions of *TUB4* (AT5G44340), *TCTP* (AT3G16640), *TCH3* (AT2G41100), and *TCH4* (AT5G57560) are listed in Supplementary Table S1. *TUB4* and *TCTP* are classically used as reference genes for normalization because of their relatively invariant expression (Lee et al., 2005; Szecsi et al., 2006). Data were analyzed by the ΔΔCₜ approach with *TCTP* or *TUB4* as reference genes.

For Supplementary Fig. S1, cDNAs were generated using an oligo(dT)₂₀ primer and M-MLV Reverse Transcriptase (Promega) from template RNA extracted from rosette leaves of 4-week-old plants with TRIzol Reagent (Invitrogen). Primer mixes designed to amplify *CFIS1* (29820-QPCR.F/29820-QPCR.R), exon 1 of *VIP3* (29830-QPCR.F/29830-QPCR.R), exon 2 of *VIP3* (29830-QPCR.F/29830-QPCR.R3), or *ACTIN2/7/8* (ACTF-QPCR/equal volumes of Actin2.R-QPCR, Actin7.R-QPCR and Actin8.R-QPCR) were added to a cocktail containing 1X SYBR Green PCR Master
Mix (Applied Biosciences) and 0.5 µl cDNA to make a final 25 µl reaction. After amplification, the data were analyzed using StepOne software (Applied Biosciences).

Statistical analyses

ANOVAs and regressions were performed in R with the agricolae and car packages. Type II sum of squares was used for two-way ANOVAs and Tukey’s or Scheffe’s methods used as post-hoc means separation tests for balanced and unbalanced datasets, respectively. Student’s t-tests were performed in Excel.

Results

A forward genetic screen identifies a mutant defective in stem thigmomorphogenesis

The leaves of two-week-old soil-grown Arabidopsis thaliana seedlings, ecotype Columbia-0 (Col-0), were mechanically stimulated either by touching or brushing with a paintbrush. Stimulus was only administered to rosette leaves prior to bolting. We found that paintbrushing was almost as effective as hand touching at reducing subsequent stem height (Fig. 1A and 1B) and had the advantage of avoiding contact between experimenter and plant. The difference in stem heights between touched or paintbrushed and control plants was maintained until senescence. Repeated touch can lead to a delay in flowering (Chehab et al., 2012; Lange and Lange, 2015) but in our hands flowering time was not significantly different between hand-touched or paintbrushed plants and untouched plants (Fig. 1C). The reason for this discrepancy is not clear, though it is possible that it can be attributed to differences in growth conditions or in the severity of the mechanical stimulus. As expected, the decrease in stem height was proportional to the number of days of stimulus (Fig. 1D).

Twenty-four pools of 100 pROK2 T-DNA insertion lines (Alonso et al., 2003) were sown in flats and all seedlings subjected to 10 days of paintbrushing prior to bolting as described above. Thirty-three plants with stems taller than the wild type after touch were selected for further analysis. A representative example of a secondary screen in the next generation is shown in Fig. 1E. Line 6-4-2 showed insensitivity to touch as well as stem height similar to Col-0 and was named touch insensitive 1 (ths1). Twenty ths1 siblings were tested in the next generation and heritable insensitivity to touch was confirmed (Fig. 1F).

Touch insensitivity is caused by a T-DNA insertion located in the 5’UTR of the VERNALIZATION INDEPENDENCE 3 (VIP3) gene.

Using thermal asymmetric interlaced PCR (Liu et al., 1995), we located a T-DNA insertion in the ths1 genome on chromosome 4, in the presumptive promoter of VERNALIZATION INDEPENDENCE 3 (VIP3) (At4g29830) (Fig. 2A). The first 23 bp of the VIP3 5’UTR were deleted, placing the left border of the T-DNA 57 bp upstream of the ATG of VIP3 and 332 bp upstream of the ATG of At4g29820 (CFIS1), which is expressed from the other strand of chromosome 4. We were unable to amplify the right border of the T-DNA. This T-DNA insertion upstream of VIP3 segregated with the observed touch insensitivity in ths1 mutants. When ths1 mutant plants were backcrossed to wild type Col-0 plants and the F2 progeny screened for inheritance of the T-DNA, the seven F2 lines homozygous for the T-DNA did not significantly respond to touch by reducing stem height, while F2 lines lacking the T-DNA did (see Supplementary Fig. S2). VIP3 encodes a WD-repeat protein that is implicated in chromatin remodeling, mRNA turnover, flowering time and vernalization (Zhang et al., 2003; Oh et al., 2004; Dorcey et al., 2012; Takagi and Ueguchi, 2012). CFIS1 encodes a protein proposed to function as a cleavage factor during mRNA polyadenylation (Hunt et al., 2008).

To determine which gene was responsible for the touch insensitive phenotype of the ths1 mutant, homozygous vip3-2 (Jolivet et al., 2006) and cfis1-1 (Fig. 2A and Supplementary Fig. S1) mutants were crossed to homozygous ths1 mutants and the F1 generation analyzed for stem touch responsiveness as in Fig. 1F. The offspring of two independent crosses between cfis1-1 and ths1 mutants were touch sensitive, ruling out CFIS1 as responsible for touch sensitivity in the simplest genetic scenario and establishing that ths1 is a recessive mutation (Fig. 2B). However, the offspring of two independent crosses between vip3-2 and ths1 mutants were touch insensitive, implicating VIP3 as responsible for touch insensitivity in ths1 mutants (Fig. 2B). The introduction of a transgene encoding a wild type copy of the VIP3 gene partially rescued touch sensitivity in the ths1 mutant background (Fig. 2C). Taken together, these data demonstrate that a defect in the VIP3 locus is responsible for the touch insensitive phenotype of the ths1 mutant. We therefore renamed ths1 as vip3-6, with other existing alleles named vip3-1, vip3-2, vip3-3, vip3<sup>zwh</sup> and bouquet-1 (Zhang et al., 2003; Jolivet et al., 2006; Dorcey et al., 2012; Takagi and Ueguchi, 2012).

Immuno blotting with an anti-VIP3 antibody (Zhang et al., 2003) was performed on whole cell extracts from the leaves of wild type, ths1 and vip3-2 plants. A VIP3-specific band of approximately 34 kDa present in extracts from wild type plants and absent from extracts from vip3-2 plants was only faintly detectable after long exposures in extracts from ths1 plants (Fig. 2D). We used quantitative RT-PCR to compare transcript levels from the first or second exon of VIP3 and exons 1–3 of CFIS1 in ths1, vip3-2, and wild type plants. The ths1 mutant showed a modest 1.5 to 3-fold increase in VIP3 and CFIS1 transcript levels compared to wild type (see Supplementary Fig. S3A). We note that the data shown in Fig. 2B show that the touch insensitive phenotype of ths1 is not due to a recessive defect in CFIS1 and that the ths1 phenotype is not dominant; thus this modest overexpression of CFIS1 due to the T-DNA insertion is unlikely to be responsible for the observed phenotype.

Semi-quantitative RT-PCR detected an abundant transcript stretching from the left border of the T-DNA to the second exon of VIP3, along with several larger, less abundant transcripts (see Supplementary Fig. S3B). These transcripts may be derived from the NOS or CaMV 35S promoters present
inside the pROK2 vector. Though present at levels similar to or higher than the wild type transcript, this transcript may lack proper start site or splicing information due to its aberrant start site. Thus, while thsl plants produce mRNA from the VIP3 locus, it is unstable and/or not properly translated.

vip3-2 mutants are small with narrow, crinkled leaves and short, misshapen floral organs, as shown previously (Jolivet et al., 2006). thsl mutants grown side-by-side were smaller and had narrower leaves than wild type plants and exhibited mild floral organ defects (Fig. 2E). We also assessed the flowering time of wild type and thsl homozygous mutant siblings derived from a thsl x Col-0 backcross, Col-0, and vip3-2 mutants. thsl mutant siblings showed slightly but significantly earlier flowering, while wild type siblings were indistinguishable from Col-0, and vip3-2 mutants had a clear early flowering phenotype (Fig. 2F). Taken together, the data presented in Fig. 2 show that an intermediate loss-of-function allele of VIP3 is responsible for touch insensitivity in thsl mutants. From these results we infer that the low amount of VIP3 protein produced in the thsl background (Fig. 2D) is sufficient for most developmental functions of VIP3.

Two members of the Paf1 complex are required for stem thigmomorphogenesis

The VIP3 protein has been detected both in the Paf1 complex and in the Superkiller (SKI) complex (Dorcey et al., 2012). Several other proteins (VIP4, VIP5, VIP6/ELF8 and VIP2/ELF7) are known to be part of the Paf1 complex but not the SKI complex (Zhang and van Nocker, 2002; Zhang et al., 2003; He et al., 2004; Oh et al., 2004; Oh et al., 2008; Shiraya et al., 2008; Park et al., 2010). To determine if the touch insensitivity of thsl can be attributed to the action of the Paf1 complex, we tested null vip5-062223 mutants (Oh et al., 2004) in the paintbrush assay. When grown in 16 hours of light to produce taller plants, vip3-2 and vip5-062223 mutants were similarly touch insensitive, while wild type plants still showed an approximately 40% reduction of stem height when touched (Fig. 3A). Early flowering is not sufficient to confer touch insensitivity as two early flowering lines showed a normal touch response (Supplementary Fig. S4).

Root skewing and coiling is abnormal in thsl and vip mutants

Assays for root touch response that involve growing seedlings on agar plates include waving (Okada and Shimura, 1990), skewing or slanting (Simmons et al., 1995; Rutherford and Masson, 1996), and curling or looping (Mirza, 1987; Simmons et al., 1995; Buer et al., 2000). These assays are thought to reveal the interplay between the response to touching of the agar surface and the response to gravity (Okada and Shimura, 1990; Thompson and Holbrook, 2004). Forward and reverse genetic approaches have revealed that slanting is affected by a diverse array of pathways, including the cortical microtubule array, hormones such as auxin and ethylene and environmental factors such as sucrose and salt stress (Buer et al., 2000; Shoji et al., 2006; Oliva and Dunand, 2007). In addition, lateral root initiation can be modulated by physical bending with forceps or by growth into barriers (Ditengou et al., 2008; Laskowski et al., 2008; Richter et al., 2009). This touch perception pathway appears to modulate the endogenous auxin-regulated pathway for lateral root initiation (Ditengou et al., 2008; Laskowski et al., 2008; Richter et al., 2009).

According to publically available gene expression databases, components of the Paf1 complex are widely expressed in most plant tissues, including the root (Winter et al., 2007). We therefore analyzed root thigmomorphogenetic responses using root slanting and coiling assays (Bidzinski et al., 2014; Swanson et al., 2015). As previously observed, the roots of wild type plants slant to the left when grown on hard agar plates tilted 30 degrees from the vertical, and this phenomenon was enhanced in the presence of the microtubule-destabilizing drug propyzamide (Furutani et al., 2000; Nakamura et al., 2004), while thsl and vip3-2 mutant roots slanted strongly to the right under these conditions (Fig. 3B). Similar results were obtained for root coiling assays, where seedlings were grown horizontally on hard agar plates (Fig. 3C). Plotting the distribution of slanting angle within a population of seedlings grown in the absence of propyzamide revealed that wild type plants slanted on average 6 degrees to the left when viewed from above the agar surface (Fig. 3D). While the thsl mutant did not show a clear difference from wild type, vip3-2, vip5-062223 and null vip6-065364 (elf8-2) (He et al., 2004; Oh et al., 2004; Shiraya et al., 2008) mutants showed a dramatic difference in how their roots responded to growth on hard agar, with a wider range of slanting angles and no preference for slanting direction. In summary, thsl mutants have defects in root skewing and coiling assays when propyzamide is added, and vip null mutants have defects in root skewing and coiling assays both with and without propyzamide.

ths1 and vip3-2 mutants exhibit a histone H3 trimethylation pattern associated with inactive chromatin globally and at TCH3 and TCH4 loci

The Paf1 complex is known to promote histone methylation patterns associated with active chromatin. At Flowering Locus C (FLC), the Paf1 complex promotes lysine methylation of histone H3 associated with transcriptionally active regions, lysine 4 (H3K4) and lysine 36 (H3K36), while reducing lysine methylation associated with transcriptional repression at lysine 27 (H3K27) (Zhao et al., 2005; Ko et al., 2010). Global levels of trimethylation at H3K36 and H3K27 in cauline leaves were assessed by western blotting with specific antibodies. Under these conditions, a reduction in the level of H3K36 trimethylation (H3K36me3) was detected in thsl and vip3-2 mutants (Fig. 4A). As a positive control, we also observed reduced H3K36me3 levels in the sdg8 background under the same conditions (Fig. 4A). SDG8 encodes an H3K36-directed methyltransferase and is required for global and genic H3K36me3 (Zhao et al., 2005; Xu et al., 2008). H3K27me3 levels were higher than the wild type in vip3 mutants but indistinguishable from the wild type in thsl and sdg8 mutants.
H3K36me3 levels were also assessed by chromatin immunoprecipitation at TCH3 and TCH4 loci. TCH3 is one of the most studied TCH genes and encodes a calmodulin-like calcium-binding protein (Sistruk et al., 1994). TCH4 encodes a xyloglucan endotransglycosylase (Xu et al., 1995). We found that levels of H3K36me3 were not significantly changed at the TCH3 locus in vip3-6/ths1. However, in the null vip3-2 mutant they were reduced two-fold compared to the wild type (Fig. 4B). Levels of H3K36me3 were significantly reduced at the TCH4 locus in both the vip3-6/ths1 and vip3-2 mutants when compared to wild type.

To determine if these chromatin marks were related to TCH gene expression, we tested the rapid touch induction of TCH4. Thirty minutes after one touch treatment with a paintbrush, a significant induction of TCH4 expression was observed in the wild type (Fig. 4C). The same trend, albeit not statistically significant, was observed for TCH3. Two touch treatments also led to high levels of expression of both TCH genes in the wild type (Fig. 4C). These responses were reduced and/or delayed in both vip3 mutants (Fig. 4C). Note that the induction was not totally absent in both vip3 mutants and could even reach wild type levels after two subsequent touches for TCH4 expression in the vip3-6/ths1 mutant. Nonetheless, the induction was never detected after a single touch event in the vip3 mutants (Fig. 4C). VIP3 transcript levels were not significantly altered in response to touch (Supplementary Fig. S5). Thus, the Paf1 complex is not only required for long-term thigmomorphogenesis responses but is also involved in short-term touch-induced transcription.

**Discussion**

We show here that the Arabidopsis Paf1 complex is a critical factor for stem and root thigmomorphogenesis, stereotypical alterations in plant form that are made in response to repeated mechanical disturbance. We also show that THS1/VIP3 is required for both global and genic levels of H3K36 methylation and for upregulation of two canonical touch-inducible loci in response to touch stimulation. VIP3 was identified in a genetic screen with no a priori expectations and our findings are consistent with previous observations that sgld8 mutants exhibit defects in wind-induced alterations in leaf morphology and in H3K4 trimethylation at the TCH3 gene (Cazzonelli et al., 2014). It has been proposed that mitotically stable chromatin marks serve to establish and stabilize certain transcriptional states (Iwaski and Paszkowski, 2014; Alabert et al., 2015; Avramova, 2015; Crisp et al., 2016). The data presented here are consistent with this model, demonstrating that the state of chromatin is modulated by the Paf1 complex and facilitates both the spatial and temporal aspects of thigmomorphogenesis, namely its systemic nature and need for repetitive touch.

**Linking VIP3 and the Paf1 complex to stem and root thigmomorphogenesis.**

We present four lines of evidence to support the conclusion that a lesion in VIP3 is responsible for the thigmomorphic defects seen in thsl mutants: 1) The T-DNA inserted into the VIP3 3’ UTR in thsl mutants segregates with its touch insensitive phenotype, ruling out a causal mutation at a second site (Supplementary Fig. S2); 2) While the T-DNA insertion is associated with a modest increase in expression of both VIP3 and CFIS1 (Supplementary Fig. S3A), the complementation test shown in Fig. 2B, where the cfis1 mutant complements the thsl mutant but a vip3 null mutant does not, indicates that it is a recessive lesion in VIP3 that is responsible for the touch insensitivity of thsl mutants; 3) A VIP3g genomic construct partially restores touch responsiveness to the thsl mutant. This result is difficult to explain if a gene other than VIP3 were involved given that no CFIS1 sequences were included in the VIP3g construct; and 4) vip3-2 null mutants do not respond to touch.

These lines of evidence link VIP3 to stem thigmomorphogenesis. We also present evidence that other components of the Paf1 complex are required for thigmomorphic responses in both the stem and the root. The stems of vip3-2 and vip5 null mutants do not shorten in response to paintbrushing (Fig. 3A) and thsl, vip3-2, vip5, and vip6 mutants show defects in root coiling and slanting assays (Fig. 4). However, we cannot rule out the possibility that vip null mutants are simply too short, even when grown in 16 hours of light, to get any shorter. Furthermore, we acknowledge that the root coiling and slanting assays used here do not solely assess touch responses but also integrate the perception of gravity (Thompson and Holbrook, 2004). Nonetheless, these additional data help support our hypothesis that the plant Paf1 complex is required for normal thigmomorphogenesis in the stem and in the root.

One challenging aspect of the paintbrush assay used here is the variability in stem height; both in the average untouched thsl stem heights compared to average untouched wild type stem heights and in the extent to which stem height in the thsl mutant is insensitive to touch (compare Fig. 1F, 2B, 2C and 3A). We found that the average stem height of any genotype depends on a number of growth conditions including, but not limited to, light and temperature. We controlled for these variables as much as possible by using the same shelf and chamber, rotating trays and growing touched and untouched pots side-by-side. We were nonetheless unable to completely prevent stem height variability. However, we note that the key comparison in this study is between touched and untouched individuals of a single genotype, and that thsl mutants were consistently less sensitive to paintbrushing than the wild type under all conditions tested.

**The Paf1 complex as a conserved and multitasking regulator of gene expression.**

First identified in yeast as a transcriptional regulator, the Paf1 complex was later shown to be present and functionally conserved in all kingdoms (Koch et al., 1999; Penheiter et al., 2005; Sheldon et al., 2005; Nordick et al., 2008; Oh et al., 2008; Dermody and Buratowski, 2010; Chu et al., 2013; Sadeghi et al., 2015). The Paf1 complex plays a role in a number of transcription-related processes, such as facilitating
elongation, recruitment and regulation of chromatin-modifying factors like histone-methylation factors, transcription termination and polyadenylation (Krogan et al., 2003; Ng et al., 2003; Nordick et al., 2008; Penheiter et al., 2005; Rozenblatt-Rosen et al., 2009; Zhang et al., 2009; Tomson and Arndt, 2013; Sadeghi et al., 2015). In yeast, it was also shown to participate in small nuclear RNA formation (Sheldon et al., 2005; Tomson et al., 2013). Pafl complex activity has been reported to be necessary for the maintenance of the active chromatin marks histone H3K4me3 and H3K36me3 in yeast, plant, human and mouse cells (Jaehning, 2010; Zhang et al., 2013), providing further evidence for its functional conservation among kingdoms.

Impaired Pafl complex function leads to developmental defects in animals. The activity of the Pafl complex is required for heart and neural crest development in zebrafish (Nguyen et al., 2010), embryonic epidermal morphogenesis in C. elegans (Kubota et al., 2014), Notch and Wnt signaling in Drosophila (Bray et al., 2005; Mosimann et al., 2009; Bahrampour and Thor, 2016), key lineage specific factor expression in mice embryos (Zhang et al., 2013), oligodendrocyte differentiation (Kim et al., 2012) and cardiomyocyte specification (Langenbacher et al., 2011). It also affects cancer progression in human cell lines (Dey et al., 2011; Tomson and Arndt, 2013) and anti-viral immune responses (Liu et al., 2011). In plants, the Pafl complex functions in the regulation of flowering time through establishment of histone marks at the TCH locus (Zhao et al., 2005; Oh et al., 2008; Xu et al., 2008).

Chromatin, a new frontier in plant mechanotransduction?

Given that we find that TCH gene induction in response to touch is delayed in vip3 mutants, the function of the Pafl complex does not seem absolutely required for TCH induction, but instead may prime a quick response to mechanical perturbations through histone modifications at TCH loci. The role of the Pafl complex in thigmomorphogenesis might therefore reside in providing chromatin with the ability to respond to mechanical perturbations rapidly.

This scenario echoes a number of recent reports in animal systems suggesting that mechanotransduction incorporates a chromatin component. In fact, because chromatin modifications change the shape of the nucleus, notably through chromatin condensation and decondensation states, it has been proposed that mechanical forces may in turn be the primary modifiers of chromatin (Pagliara et al., 2014). Global chromatin modifications have consistently been associated with nuclear stiffness [for example (Schreiner et al., 2015)] and nuclear stiffness has been shown to increase with differentiation (Hampoezl and Lecuit, 2011). Given that chromatin is associated with the nuclear envelope through lamins, its modification may result from deformations originating within the extracellular matrix that are propagated through the cytoskeleton and the LINC (linker of the nucleoskeleton and cytoskeleton) complex (Isermann and Lammerding, 2013). Applying mechanical strains to mesenchymal stem cells in culture leads to a lamin-dependent decrease in histone deacetylase activity and an increase in histone acetylation (Li et al., 2011). The finding that the Pafl complex is involved in thigmomorphogenesis in plants may thus provide an opportunity to unravel potential connections between cytoskeletal and gene expression responses to mechanical perturbation.

Our results build a foundation for future investigations into the possible function of the Pafl complex and chromatin marks in priming TCH genes for rapid induction upon mechanical stimulation and in translating discrete touch events into a continuous growth response. Given that the Pafl complex is conserved across kingdoms and because animals also alter their body plan in response to repeated mechanical stresses (Kahn et al., 2009; Heckel et al., 2015; Steed et al., 2016), these findings also raise the possibility of a common mechanism for recording multiple mechanical deformations and translating them into developmental changes.

Supplementary Data

Supplementary data are available at JXB online.

Fig. S1. The cis1-1 allele produces little to no CFISI transcript.

Fig. S2. T-DNA in the VIP3 gene is linked to the touch insensitive phenotype.

Fig. S3. Transcript levels of VIP3 and CS1F in the thsl background.

Fig. S4. Two established early flowering lines are touch sensitive in the paintbrushing assay.

Fig. S5. VIP3 transcript levels are not significantly affected by touch.

Table S1. Oligos used in this study.

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References

Alabert C, Barth TK, Reverón-Gómez N, Sidoli S, Schmidt A, Jensen ON, Imhof A, Groth A. 2015. Two distinct modes for propagation of histone PTMs across the cell cycle. Genes & Development 29, 585–590.

Alonso JM, Stepanova AN, Leisse TJ, et al. 2003. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science (New York, N.Y.) 301, 656–657.

Avramova Z. 2015. Transcriptional ‘memory’ of a stress: transient chromatin and memory (epigenetic) marks at stress-response genes. The Plant Journal: for cell and molecular biology 83, 149–159.

Bahrampour S, Thor S. 2016. Ctr9, a Key Component of the Pafl Complex Affects Proliferation and Terminal Differentiation in the Developing Drosophila Nervous System. G3 (Bethesda). Epub ahead of print.

Biddington NL. 1986. The effects of mechanically-induced stress in plants—a review. Plant Growth Regulation 4, 103–123.

Bidzinski P, Noir S, Shahi S, Reinštäder A, Gratkowska DM, Panstruga R. 2014. Physiological characterization and genetic modifiers of aberrant root thigmomorphogenesis in mutants of Arabidopsis

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References

Alabert C, Barth TK, Reverón-Gómez N, Sidoli S, Schmidt A, Jensen ON, Imhof A, Groth A. 2015. Two distinct modes for propagation of histone PTMs across the cell cycle. Genes & Development 29, 585–590.

Alonso JM, Stepanova AN, Leisse TJ, et al. 2003. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science (New York, N.Y.) 301, 656–657.

Avramova Z. 2015. Transcriptional ‘memory’ of a stress: transient chromatin and memory (epigenetic) marks at stress-response genes. The Plant Journal: for cell and molecular biology 83, 149–159.

Bahrampour S, Thor S. 2016. Ctr9, a Key Component of the Pafl Complex Affects Proliferation and Terminal Differentiation in the Developing Drosophila Nervous System. G3 (Bethesda). Epub ahead of print.

Biddington NL. 1986. The effects of mechanically-induced stress in plants—a review. Plant Growth Regulation 4, 103–123.

Bidzinski P, Noir S, Shahi S, Reinštäder A, Gratkowska DM, Panstruga R. 2014. Physiological characterization and genetic modifiers of aberrant root thigmomorphogenesis in mutants of Arabidopsis
thaliana MILDEW LOCUS O genes. Plant, cell & environment 37, 2738–2739.
Böhm J, Scherzer S, Krolof E, et al. 2016. The Venus Flytrap Dionaea muscipula Counts Prey-Induced Action Potentials to Induce Sodium Uptake. Current Biology: CB 26, 286–295.
Braam J, Davis RW. 1990. Rain-, wind-, and touch-induced expression of calmodulin and calmodulin-related genes in Arabidopsis. Cell 60, 357–364.
Bray S, Musisi H, Bienz M. 2005. Bre1 is required for Notch signaling and histone modification. Developmental cell 6, 279–286.
Buér CS, Masle J, Wasteneys GO. 2000. Growth conditions modulate root- wave phenotypes in Arabidopsis. Plant & cell physiology 41, 1164–1170.
Cazzonelli CI, Nisar N, Roberts AC, Murray KD, Borevitz JO, Pogson BJ. 2014. A chromatin modifying enzyme, SDG8, is involved in morphological, gene expression, and epigenetic responses to mechanical stimulation. Frontiers in Plant Science 5, 533.
Chehab EW, Eich E, Braam J. 2009. Thigmomorphogenesis: a complex plant response to mechano-stimulation. Journal of Experimental Botany 60, 43–56.
Chehab EW, Yao C, Henderson Z, Kim S, Braam J. 2012. Arabidopsis touch-induced morphogenesis is jasmonate mediated and protects against pests. Current Biology: CB 22, 701–706.
Chu X, Qin X, Xu H, Li L, Wang Z, Li F, Xie X, Zhou H, Shen Y, Long J. 2013. Structural insights into Pafl complex assembly and histone binding. Nucleic Acids Research 41, 10619–10629.
Coutand C. 2010. Mechanosensing and thigmomorphogenesis, a physiological and biomechanical point of view. Plant Science 179, 168–182.
Coutand C, Martin L, Leblanc-Fournier N, Decourteix M, Julien JL, Moulia B. 2009. Strain mechanosensing quantitatively controls diameter growth and PtAZFP2 gene expression in poplar. Plant Physiology 151, 223–232.
Criss PA, Ganguly D, Eichten SR, Borevitz JO, Pogson BJ. 2016. Reconsidering plant memory: Intersections between stress recovery, RNA turnover, and epigenetics. Science Advances 2, e1501340.
Dermody JL, Buratowski S. 2010. Leot1 subunit of the yeast Pafl complex binds RNA and contributes to complex recruitment. The Journal of biological chemistry 285, 33671–33679.
Dey P, Ponnusamy MP, Deb S, Batra SK. 2011. Human RNA polymerase II-association factor 1 (hPaf1/PCD2) regulates histone methylation and chromatin remodeling in pancreatic cancer. PLoS One 6, e26926.
Ditengou FA, Teale WD, Koschersperger P, et al. 2008. Mechanical induction of lateral root initiation in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America 105, 18818–18823.
Dorcey E, Rodríguez-Villalon A, Salinas P, Santuari L, Pradrervand S, Harshman K, Hardtke CS. 2012. Context-dependent dual role of SKL homologs in mRNA synthesis and turnover. PLoS Genetics 8, e1002652.
Edwards K, Johnstone C, Thompson C. 1991. Nucleic Acids Research 19, 1349.
Furutani I, Watanabe Y, Prieto R, Masukawa M, Suzuki K, Naoi K, Thitamadee S, Shikanai T, Hashimoto T. 2010. Mechanosensing and thigmomorphogenesis, a complex plant response to mechano-stimulation. Journal of Experimental Botany 61, 95–99.
Kagaya Y, Hattori T. 2009. Arabidopsis transcription factors, RAV1 and RAV2, are regulated by touch-related stimuli in a dose-dependent and biphasic manner. Genes & genetic systems 84, 95–99.
Kahn J, Shwartz Y, Blitz E, et al. 2009. Muscle contraction is necessary to maintain joint progenitor cell fate. Developmental Cell 16, 734–743.
Kim S, Kim JD, Chung AY, et al. 2012. Antagonistic regulation of PAFlC and p-TEFb is required for oligodendrocyte differentiation. The Journal of Neuroscience: the official journal of the Society for Neuroscience 32, 8201–8207.
Ko JS, Mitina I, Tamada Y, Hyun Y, Choi Y, Amasino RM, Noh B, Noh YS. 2010. Growth habit determination by the balance of histone methylation activities in Arabidopsis. The EMBO Journal 29, 3208–3215.
Koch C, Wollmann P, Dahl M, Lottspeich F. 1999. A role for Ctr3p and Paflp in the regulation G1 cyclin expression in yeast. Nucleic Acids Research 27, 2126–2134.
Krogan NJ, Dover J, Wood A, et al. 2003. The Pafl complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Molecular Cell 11, 721–729.
Kubota Y, Tsuyama T, Takabayashi Y, Haruta N, Maruyama R, Iida N, Sugimoto A. 2014. The Pafl complex is involved in embryonic epidermal morphogenesis in Caenorhabditis elegans. Developmental Biology 391, 43–53.
Lander AD. 2011. Pattern, growth, and control. Cell 144, 955–969.
Lange MJ, Lange T. 2015. Touch-induced changes in Arabidopsis morphology dependent on gibberellin breakdown. Nature Plants 1, 14025.
Langenbacher AD, Nguyen CT, Cavanaugh AM, Huang J, Lu F, Chen JN. 2010. The Pafl complex differentially regulates cardiomyocyte specification. Developmental Biology 353, 19–28.
Laskowski M, Grienisen VA, Hofhuis H, Hove CA, Hogeweg P, Marte AF, Scheres B. 2008. Root system architecture from coupling cell shape to auxin transport. PLoS Biology 6, e3096.
Leblanc-Fournier N, Coutand C, Crouzet J, Brunel N, Lenne C, Moulia B, Julien JL. 2008. JR-ZFP2, encoding a Cys2/His2-type transcription factor, is involved in the early stages of the mechanosensation pathway and specifically expressed in mechanically stimulated tissues in woody plants. Plant, cell & environment 31, 715–726.
Leblanc-Fournier N, Martin L, Lenne C, Decourteix M. 2014. To respond or not to respond, the recurring question in plant mechanosensitivity. Frontiers in Plant Science 5, 401.
Lee D, Polisensky DH, Braam J. 2005. Genome-wide identification of touch- and darkness-regulated Arabidopsis genes: a focus on calmodulin-like and XTH genes. The New phytologist 165, 429–444.
Li Y, Chu JS, Kurupski K, Li X, Bautista DM, Yang L, Sung KL, Li S. 2011. Biophysical regulation of histone acetylation in mesenchymal stem cells. Biophysical Journal 100, 1902–1909.
Ling V, Perera I, Zielinski RE. 1991. Primary structures of Arabidopsis calmodulin isoforms deduced from the sequences of cDNA clones. Plant Physiology 96, 1196–1202.
Liu L, Oliveira NM, Chenev KM, et al. 2011. A whole genome screen for HIV restriction factors. Retrovirology 8, 94.
Hughes CM, LaFramboise T, Manley JL, Meyerson M. 1855–1866.
Richter GL, Monshausen GB, Krol A, Gilroy S. gene expression and its consequences. Cell
Raj A, van Oudenaarden A. Arabidopsis CaM-3 calmodulin gene. Plant Molecular Biology
Perera IY, Zielinski RE. Penheiter KL, Washburn TM, Porter SE, Hoffman MG, Jaehning
Park S, Oh S, Ek-Ramos J, van Nocker S. stem cells exiting pluripotency. Nature Materials
Ojima J, Kato T, Tabata S, Iwasaki T. 2008. Arabidopsis VIP6/ ELFB, the homolog of CTR9 component of the transcriptional complex PAFT1, is essential for plant development. Plant Biotechnology 25, 447–455.
Shoji T, Suzuki K, Abe T, Kaneko Y, Shi H, Zhu JK, Rasa A, Hasegawa PM, Hashimoto T. 2006. Salt stress affects cortical microtubule organization and helical growth in Arabidopsis. Plant & cell physiology 47, 1158–1168.
Simmons C, Soll D, Migliaccio F. 1995. Circumnutation and gravitropism cause root waving in Arabidopsis thaliana. Journal of Experimental Botany 46, 143–150.
Sistrunk ML, Antosiewicz DM, Purugganan MM, Braam J. 1994. Arabidopsis TCH3 encodes a novel Ca2+ binding protein and shows environmentally induced and tissue-specific regulation. The Plant cell 6, 1553–1565.
Steed E, Boselli F, Vermot J. 2016. Hemodynamics driven cardiac valve morphogenesis. Biochimica et Biophysica Acta 1863, 1760–1766.
Swanson SJ, Barker R, Ye Y, Gilroy S. 2015. Evaluating mechanotransduction and touch responses in plant roots. Methods in molecular biology (Clifton, N.J.) 1309, 143–150.
Szács J, Holcik M, Varadi E, Cock JM, Dumas C, Bendahmane M. 2006. BIGPETAlp, a bHLH transcription factor is involved in the control of Arabidopsis petal size. The EMBO Journal 25, 3912–3920.
Takagi N, Ueguchi C. 2012. Enhancement of meristem formation by bouquet-1, a mis-sense allele of the vernalization independence 3 gene encoding a WD40 repeat protein in Arabidopsis thaliana. Genes to Cells: devoted to molecular & cellular mechanisms 17, 982–993.
Thompson MV, Holbrook NM. 2004. Root-gel interactions and the root waving behavior of Arabidopsis. Plant Physiology 135, 1822–1837.
Tomson BN, Arndt KM. 2013. The many roles of the conserved eukaryotic Pafl complex in regulating transcription, histone modifications, and disease states. Biochimica et Biophysica Acta 1829, 116–126.
Tomson BN, Crisucci EM, Heisler LE, Gebbia M, Nislow C, Arndt KM. 2013. Effects of the Pafl complex and histone modifications on snoRNA 3’-end formation reveal broad and locus-specific regulation. Molecular and Cellular Biology 33, 170–182.
Tsai YC, Koo Y, Delk NA, Gehl B, Braam J. 2013. Calmodulin-related CML24 interacts with ATG4b and affects autophagy progression in Arabidopsis. The Plant Journal: for cell and molecular biology 73, 325–335.
Wang Y, Wang B, Gilroy S, Chehab EW, Braam J. 2011. CML24 is involved in Root Mechanosensors and Cortical Microtubule Orientation in Arabidopsis. Journal of Plant Growth Regulation, 467–479.
Webb AB, Lengyel IM, Jorg DJ, Valentin G, Julicher F, Morelli LG, Oates AC. 2016. Persistence, period and precision of autonomous cellular oscillators from the zebrafish segmentation clock. Elife 5.
Winter D, Vinegar B, Nahal A, Ammar R, Wilson GV, Provart NJ. 2007. An “Electronic Fluorescent Pictograph” browser for exploring and analyzing large-scale biological data sets. PLoS One 2, e718.
Wolpert L. 1969. Positional information and the spatial pattern of cellular differentiation. Journal of Theoretical Biology 25, 1–47.
Xu L, Zhao Z, Dong A, Soubigou-Taconnat L, Renou JP, Steinmetz A, Shen WH. 2008. Di- and tri- but not monomethyllysine on histone H3 lysine 36 marks active transcription of genes involved in flowering time regulation and other processes in Arabidopsis thaliana. Molecular and Cellular Biology 28, 1348–1360.
A Role for the Paf1 Complex in Touch Responses

Xu W, Purugganan MM, Polisensky DH, Antosiewicz DM, Fry SC, Braam J. 1995. Arabidopsis TCH4, regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. The Plant cell 7, 1555–1567.

Zhang H, Ransom C, Ludwig P, van Nocker S. 2003. Genetic analysis of early flowering mutants in Arabidopsis defines a class of pleiotropic developmental regulator required for expression of the flowering-time switch flowering locus C. Genetics 164, 347–358.

Zhang H, van Nocker S. 2002. The VERNALIZATION INDEPENDENCE 4 gene encodes a novel regulator of FLOWERING LOCUS C. The Plant journal: for cell and molecular biology 31, 663–673.

Zhang K, Haversat JM, Mager J. 2013. CTR9/PAF1c regulates molecular lineage identity, histone H3K36 trimethylation and genomic imprinting during preimplantation development. Developmental Biology 383, 15–27.

Zhang Y, Sikes ML, Beyer AL, Schneider DA. 2009. The Paf1 complex is required for efficient transcription elongation by RNA polymerase I. Proceedings of the National Academy of Sciences of the United States of America 106, 2153–2158.

Zhao Z, Yu Y, Meyer D, Wu C, Shen WH. 2005. Prevention of early flowering by expression of FLOWERING LOCUS C requires methylation of histone H3 K36. Nature Cell Biology 7, 1256–1260.