Sugar Signaling Induces Dynamic Changes during Meristem Development in Arabidopsis

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One Sentence Summary:

The increase in meristem size during the floral transition is regulated via a feedback regulation
involving sugar signaling.

15 Aerial parts of plants originate from pluripotent cells in the shoot apical meristem
16 (SAM). This population of stem cells is maintained via a negative feedback loop involving stable
17 expression of *WUSCHEL* (*WUS*) and *CLAVATA3*. SAM size is dynamic and undergoes a more than
18 2-fold expansion upon the transition to reproductive growth. The underlying mechanism
19 controlling this process is largely unknown, but coinciding increased levels of trehalose 6-
20 phosphate (T6P) suggest a participation of sugar signaling. Here we show that *TREHALOSE*
21 *PHOSPHATE PHOSPHATASE J*, a component of the T6P pathway, is directly regulated by *WUS*,
22 and controls SAM expansion at floral transition through *WUS*. Our findings demonstrate a
23 dynamic feedback-regulation between central meristem maintenance and flowering time
24 regulators with sugar signaling.

During the floral transition the *Arabidopsis thaliana* SAM undergoes a dramatic increase in size¹ (Fig. 1a). Since the transition between developmental stages requires a massive reorganization of organ development and sufficient energy, it is tightly controlled by environmental conditions and availability of nutrients²⁻⁵. Hence, plant development as a whole and SAM maintenance in particular demands continuous cross talk between its regulatory processes and the available resources. The sugar phosphate, trehalose 6-phosphate (T6P), serves as a signal for sucrose availability, which is conveyed to downstream metabolic and growth responses through a still largely unknown mechanism⁶. Also knowledge on the underlying mechanism controlling the morphological processes at the SAM at floral transition is scarce. However, increased levels of T6P coincide with the floral transition and the T6P pathway induces flowering by regulating key flowering genes expressed in leaves and the SAM⁴, which suggests a participation of sugar signaling in regulating the SAM size changes throughout the floral transition.

To assess whether the morphological changes at the SAM involve the T6P pathway, we investigated the effect on SAM architecture by decreasing *TPS1*, coding for the T6P synthesizing enzyme, by the means of an artificial microRNA also in the SAM proper (*35S:amiRTPS1*, Fig. S1). We compared these lines with plants with increased *TPS1* in the central zone (*CLV3:TPS1*, Fig. S2). These have smaller and bigger vegetative and reproductive meristems (Fig. 1b, 1c), resulting in smaller and bigger plants, respectively⁴. The homeodomain transcription factor *WUS* and the signaling peptide *CLV3* non-cell autonomously contribute to communication of position-dependent properties among SAM cells in a negative feedback loop and maintain the SAM cell population⁷. We therefore analyzed their expression in the SAM with altered *TPS1*. While the expression of *WUS* in the organizing centre (OC) and *CLV3* in the central zone (CZ) harboring

the stem cells are considered mutually exclusive⁷, we found reduced *TPSI* levels (35S:*amiRTPSI*) lead to an enlarged OC domain marked by *WUS* (Fig. 1d) that overlaps with the CZ, marked by *CLV3* expression (Fig. 1D). Plants overexpressing *TPSI* (35S:*TPSI*, Fig. S2) display an increased stem cell pool with otherwise little effect on *WUS* expression (Fig. 1d). However, the size of the CZ decreases in plants expressing *TPSI* under the *CLV3* promoter (Fig. 1d, Fig. S2), indicating a smaller stem cell pool in support of a much smaller SAM size phenotype (Fig. 1d) of the very early flowering *CLV3:TPSI* plants⁴. The expression of *HISTONE4*, *CYCLIN D3;1* and *CYCLIN-DEPENDENT KINASE2;1*, three cell cycle marker genes (Fig. S3), suggests a higher proliferation rate and an increased number of cells displaced from the CZ to adopt organ-specific cell fates at the periphery as a plausible reason for the decreased SAM size of *CLV3:TPSI* and *vice versa* increased SAM size of 35S:*amiRTPSI* plants (Fig. 1b, 1c).

To assess whether the *WUS/CLV* feedback loop can uncouple in a wild-type SAM during normal growth, we analyzed *WUS* and *CLV3* expression in wild-type plants in a time series spanning the floral transition (Fig. 1e). Throughout the vegetative phase SAM size gradually increases due to rising cell numbers (Fig. 1a; Fig. S4)⁸, reaching its maximum at the floral transition, independent of whether the plants are grown in continuous long days (LD) (Fig. 1a) or in short days (SD) followed by a transfer to LD (Fig. S5). An enlarging SAM at the floral transition correlates with a larger *WUS* expression domain expanding into the CZ and the outer cell layer (L1) (Fig. 1e-g). This is supported by larger meristems of the 35S:*amiRTPSI* line, which express *WUS* in the central zone (Fig. 1d). This observation is also consistent with massive proliferation of meristematic cells detected when *WUS* is expressed from a *CLV3* promoter in the CZ that resulted in meristems, no longer producing organs⁹. The presence of

WUS transcript in the outer meristem layers might be explained by a transient cytokinin signal in L1, which induces *WUS* and was reported to respond to carbon in seedlings¹⁰⁻¹². However, cytokinin levels seem not to be altered in the L1 layer of cells as indicated by the synthetic cytokinin reporter *TCSn::GFP*¹³ (Fig. S6). Further, while *WUS* expands to L1 for a short period (8-10 DAG), the *CLV3* expression domain remains expanded post floral transition (Fig. 1e, 1h). This suggests a transient uncoupling, which is re-established at the reproductive SAM (16 DAG), resembling earlier vegetative SAM expression patterns (6 DAG) (Fig. 1e), allowing organ production to resume.

To understand how the T6P pathway might control this process, we first analyzed expression of the ten genes encoding TREHALOSE PHOSPHATE PHOSPHATASEs (TPP)⁶. Except for *TPPC* and *TPPD*, which are expressed below detection limit, all were expressed in distinct SAM domains (Fig. S7). *TPPJ* strongly increases in the enlarged SAM of *clv3-7* due to an ectopic expression in L1 and L2 (Fig. 2a-c, Fig. S8, S9). Notably, in *clv3-7* and *clv3-10* mutants high levels of *WUS* in the outer SAM layers (Fig S9)¹⁴ coincides with ectopic expression of *TPPJ* (Fig. S9). To assess whether ectopic expression of *TPPJ* contributes to the enlarged SAM of the *clv3* mutant, we next overexpressed *TPPJ* (*35S::TPPJ*, Fig. 2d). This results in significantly enlarged SAMs (Fig. 2e), and importantly, expands the *WUS* expression domain into L1 (Fig. 2f), consistent with our finding in wild-type plants at the floral transition (Fig. 1e).

Overlapping expression domains of ectopic *TPPJ* (Fig. 2b) and *WUS* in the *clv3* mutant SAM implies a direct influence of *WUS* on *TPPJ* expression. This is supported by an *in silico* analysis which predicts multiple, canonical *WUS* binding sites in the sequence upstream of the *TPPJ* coding sequence (*TPPJ*^{*WUS*}, Fig. 3a)^{10,15-17}. *WUS* is a bifunctional transcription factor, which was found to both activate and repress gene expression⁷. To understand if *WUS* directly

controls *TPPJ*, we performed *in vivo* transactivation assays. These show that WUS activates reporter gene expression when using a 2865 bp *TPPJ* 5' sequence, containing 17 putative *TPPJ*^{WUS} sites (Fig. 3a). Progressive deletion of the *TPPJ* 5' sequence results in a reduction of reporter gene activation, suggesting an additive effect of the individual *TPPJ*^{WUS} sites (#1-6; Fig. 3a, 3b). We confirmed the direct binding of WUS to three distinct regions of the *TPPJ* promoter (I, II, and III, Fig. 3c-e, Fig. S10) by Chromatin immunoprecipitation (ChIP) coupled to PCR using a specific antibody against WUS (Fig. S11), while all other regions did not indicate binding (Fig. S10, Fig. S12). We observed enrichment of WUS binding to *TPPJ*^{WUS} elements up to 0.67% of the input DNA in *clv3-7* and up to 0.75% of the input DNA in *clv3-10* apices, both of which express *WUS* at high levels in comparison to input DNA from wild-type apices, where *WUS* is expressed in only a few cells and input DNA from leaves, with no WUS (Fig. 3c-e; Fig. S10). Lastly, electrophoretic mobility shift assays (EMSA) confirm *in vitro* binding of WUS to the investigated sequences (I, II 1-3, III 1-3; Fig. 3f). In summary, we demonstrate that seven out of 17 putative *TPPJ*^{WUS} sites are directly targeted by WUS *in vivo*.

To further assess the role of *TPPJ* at the SAM we used an artificial miRNA (*amiRTPPJ*) approach to downregulate *TPPJ* (*35S:amiRTPPJ*) (Fig. S13). Similar to a *tpj* T-DNA insertion mutant, plants overexpressing either of two versions of an *amiRTPPJ* (V1, V2) flower significantly earlier in both LD and SD (*35S:amiRTPPJ*, Fig. S14, Table S1). *MLI* expression specifically and stably localizes to the epidermis (L1) throughout the investigated developmental stages (Fig. S15). *MLI:amiRTPPJ* plants reduce *TPPJ* expression in L1 (arrowheads in Fig. S16), have smaller SAMs and flower significantly earlier in LD and SD (Fig. 4a-c, Fig. S17, S18, Table S1), while the level of *TPPJ* reduction is proportional to the acceleration of flowering (Fig. 4b and c, Fig. S13, Table S1). To date there are no reports that mutants in meristem

117 maintenance genes are affected in their flowering time. We found that plants mutant for *CLV3*
 118 are late flowering (Fig. 4c, Table S1). However, when *ML1:amiRTPPJ* is introgressed into either
 119 the *clv3-7* or *clv3-10* background, the late flowering phenotype of the mutant plants is restored to
 120 wild type (Fig. 4c, Fig. S18, Table S1), suggesting that *TPPJ* expression in the epidermis is also
 121 necessary for the late flowering phenotype of *clv3*. Hence, the early flowering of
 122 *ML1:amiRTPPJ* in a wild-type background is due to a reduction of *TPPJ* expression in L1 in its
 123 endogenous expression domain. In addition, other prominent morphological defects of *clv3-10*
 124 such as fasciated stems and enormous inflorescence SAMs are visibly reduced in the presence of
 125 *ML1:amiRTPPJ* (Fig. S18). *CLV3* expression increases to much higher levels in *ML1:amiRTPPJ*
 126 than in plants overexpressing *TPPJ* (Fig. 4d), suggesting an active role of the T6P pathway in the
 127 outer SAM layer with regard to stem cell maintenance. In addition, *WUS* is induced (Fig. 4d, Fig.
 128 S19), indicating uncoupling of the WUS/CLV feedback loop, consistent with our findings with
 129 altered T6P signaling at the SAM (Fig. 1d, Fig. 2f).

130 Since the T6P pathway influences the age pathway at the SAM⁴, we analyzed mature
 131 miR156 as well as the expression of *SPL3*, *SPL4*, *SPL5*, *SPL9* and *SPL15*, all associated with
 132 floral transition in general^{18,19} (Fig. S20, Table S1). We found a strong reduction of mature
 133 miR156 levels correlating with lower *TPPJ* expression in *ML1:amiRTPPJ*, and corresponding
 134 increased expression of *SPL3*, *SPL4*, *SPL5*, and *SPL9* (Fig. 4e). In line, miR156 abundance was
 135 highly increased in *TPPJ* overexpressing plants, while the corresponding *SPLs* were
 136 downregulated (Fig. 4e). However, expression of *SPL15*, previously described to be mainly
 137 responsible for integrating the aging stimulus into the regulation of the floral onset²⁰, was not
 138 differentially expressed in either of the transgenic lines (Fig. S21). In addition, we found miR156
 139 levels significantly increased in *clv3-10* mutant apices (Fig. 4f). In response, the levels of *SPL3*,

SPL5, and *SPL9* were reduced in line with the late flowering phenotype of the mutant. In contrast to what would be expected, we found more *SPL4* in apices of the late flowering *clv3-10* (Fig. 4f). Higher order lines from *spl3*, *spl4* and *spl5* deletion mutant plants are significantly later flowering in all lines with *spl4* (Fig. S20, Table S1). This argues for an important role of *SPL4* in inducing flowering. *SPL4*, similar to *SPL3*, *SPL5* and *SPL9*, is induced at the wild-type SAM at floral transition²¹. However, it is expressed in the very center of the SAM, in a domain largely overlapping with the WUS protein domain^{5,17,20,22}, while *SPL3*, *SPL5*, and *SPL9* are expressed at the periphery of the SAM and in the vasculature of young leaves^{5,20}. This might denote a direct regulation of *SPL4* by WUS, which would explain increased *SPL4* in the *clv3* mutant background (Fig. 4f) and the previously identified partially miR156-dependent regulation of the *SPLs* by the T6P pathway⁴. Indeed, we identified a larger number of potential *SPL4*^{WUS} sites upstream of the *SPL4* coding sequence when compared to the 5' intergenic regions of the other *SPLs* (Fig. S22). However, this finding also suggests that additional players downstream of CLV3 are important for the onset of flowering, which cannot be bypassed by an otherwise inductive *SPL4* (Fig. 4f). Since carbohydrate status regulates timing of the floral transition⁴, it is interesting that T6P signaling also directly influences the re-organisation of the SAM during the floral transition (Fig. 4g). Given the ubiquitous nature of carbohydrate signaling and the large-scale change in sink-source relationships within the plants²³, it will be interesting to determine if this regulatory mechanism is widely present in the plant kingdom.

Methods

Materials and Methods

Plant material and growth conditions

All *Arabidopsis thaliana* plants used for this study are of the Columbia accession (Col-0). The lines *clv3-7*²⁴, *clv3-10*²⁵, *35S:amiRTPS1*, *CLV3:TPS1*⁴, and *WUS:WUS:GFP*²² have been described previously as indicated. Plants were grown in controlled growth chambers at 22°C in long day (LD, 16h light/8h dark) or short day (SD, 8h dark/16h light) conditions with a light intensity of approximately 160 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$ and a relative humidity of 60-65%. Controlled induction of flowering was performed using a previously described shift protocol²¹.

Phenotypic analysis

Flowering time was defined as days to flowering (DTF), recorded when shoots were 0.5 cm high (bolting), and by scoring the total leaf number (TLN). The TLN is the sum of rosette leaves (RLN, rosette leaf number) and cauline leaves (CLN, cauline leaf number) per plant (Table S1). On average 20 plants of each genotype were analyzed in one experiment. Plants were grown in the conditions described above, and randomized every second day to avoid position effects.

Generation of transgenic lines

Generally, Col-0 plants were transformed by the floral dip method²⁶. The presence of the transgene was confirmed by PCR and independent, single-insertion, homozygous T3 plants were selected and used for all further studies. Oligonucleotides used for cloning are provided in Table S2, those used for genotyping are given in Table S3. To generate the *35S:TPPJ* and

180 35S:TPS1 lines, the coding sequences of *TPPJ* (At5g65140) and *TPS1* (At1g78580) were amplified
181 and introduced into the *pGEM®-Teasy* vector (Promega, Madison, Wisconsin, US), sub-cloned
182 into the Gateway® entry vector *pJLBlue reverse*²⁷ (pVW275 and pVW099), and recombined into
183 a *pGREEN-II*-based destination vector with 35S promoter (pVW279 – *TPPJ* and pVW161 – *TPS1*)
184 using the Gateway® LR clonase II Enzyme mix (Invitrogen, Carlsbad, CA). Generation of artificial
185 microRNAs targeting *TPPJ* (*ML1:amiRTPPJ* V1 and V2, *35S:amiRTPPJ* V1 and V2) was performed
186 according to a previously published protocol²⁸ using the WMD3–Web MicroRNA Designer
187 (<http://wmd3.weigelworld.org/cgi-bin/webapp.cgi>). The resulting *EcoRI/BamHI* fragment was
188 sub-cloned into the Gateway® entry vector *pJLBlue reverse*²⁷ (V1: pVW498, V2: pVW499) and
189 recombined using the Gateway® LR clonase II Enzyme mix (Invitrogen, Carlsbad, CA) into a
190 *pGREEN-II*-based destination vector with either the *ML1* or *35S* promoter (V1: pVW507, V2:
191 pVW508, V1: pVW504 V2: pVW505). Double mutant lines of *clv3* and *ML1:amiRTPPJ #1 and #2*
192 were generated through crosses. All lines were propagated to the F4 generation and tested for
193 homozygosity. To generate the *spl3*, *spl4*, *spl5*, knockout lines a modified version of the two
194 single guided RNAs (2sgRNA) CRISPR/*Cas9* technology was used^{29,30}. In brief, the NGG PAM
195 recognition sites in the 5'UTR and last exon of *SPL3*, first exon and 3'UTR of *SPL4* and 5'UTR and
196 3'UTR of *SPL5* were defined using the ATUM webtool (<https://www.atum.bio>). *pJF1033* vector,
197 containing 2sgRNA scaffold, was used as a template. Resulting *BsaI* products were restriction
198 cloned into the *pJF1031* binary vector³⁰. Plants with homozygous deletions for *SPL3*, *SPL4* or
199 *SPL5* were back-crossed to Col-0. Homozygous, *Cas9*-free lines were used for the experiments
200 and to generate all higher order mutations, i.e. the *spl34*, *spl35*, *spl45* double as well as the
201 *spl345* triple mutant lines.

RNA extraction and cDNA synthesis

Total RNA from homogenized rosette or dissected SAM samples was isolated by a phenol/chloroform extraction method using a modified TRIzol reagent with the following composition: phenol 38% (v/v), guanidine thiocyanate 0.8 M ammonium thiocyanate 0.4 M, sodium acetate 0.1 M pH 5.0, glycerol 5% (v/v), EDTA 5 mM pH 8.0, Na-lauroylsarcosine 0.5% (v/v). This was then followed by a sodium acetate precipitation to improve RNA purity. cDNA synthesis, preceded with removal of putative genomic DNA contamination with Dnase I Rnase-free (Ambion™/Thermo Fisher Scientific, Waltham, Massachusetts, US), was carried out using a SuperScript™IV Reverse Transcriptase Kit (Thermo Fisher Scientific, Waltham, Massachusetts, US) according to the manufacturer's instruction. For quantification of mature miR156 the respective stem-loop primers (Table S4) were added to the cDNA synthesis reaction (1:1 with oligo dT(18) primer) (Thermo Fisher Scientific, Waltham, Massachusetts, US) as previously described³¹.

RT-qPCR analysis

RT-qPCR analyses were performed with the ABI Prism 7900 HT fast real time PCR system (Applied Biosystems/Life Technologies, Darmstadt, Germany) using a Power SYBR® Green-PCR Master Mix (Applied Biosystems/Life Technologies, Darmstadt, Germany) in a 10 µl reaction volume for expression analyses and in a 5 µl volume for ChIP-PCRs. All oligos are listed in Table S4 and Table S5 (RT-qPCR and ChIP-PCR respectively). All data were analyzed using the SDS 2.4 software (Applied Biosystems/Life Technologies, Darmstadt, Germany) applying the criteria described by Czechowski and co-workers (2004). cDNA quality was determined with primers

binding to the 3' and 5' regions of *GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH, At1g13440)*, and samples with Ct 5'GAPDH/Ct 3'GAPDH values > 1 were excluded from further analyses. Expression values were calculated by the comparative Ct method using the reference gene index (RGI) as previously described⁴ and *POLYUBIQUITIN10 (UBQ10, At4g05320)* for figure 3C and S14³². The primer sequences for the reference and analyzed genes are given in the Table S4.

RNA *in situ* hybridization

Wax embedding was performed using an automated processor and embedding system (Leica EG1160, Solms, Germany). Sections of 8 µm thickness were prepared using a rotary microtome (Leica RM2265; Leica, Wetzlar, Germany).

Probes for RNA *in situ* hybridization were synthesized using the DIG RNA Labeling Kit (Roche, Mannheim, Germany). For this the ORFs of the target genes were cloned into the pGEM®-T Easy vector (Promega, Madison, Wisconsin, US) as a template according to manufactures instructions (oligonucleotides and construct IDs listed in the Table S6) and the sense and antisense RNA probes were synthesized using T7 and SP6 polymerases, respectively. RNA *in situ* hybridizations were carried out as previously described⁴. The final sections were imaged with an Olympus BX-61 microscope equipped with a DC View II digital camera (Olympus Europa SE & Co, Hamburg, Germany).

Chromatin immunoprecipitation (ChIP)

For ChIP-PCR, 100 apices per biological replicate (Col-0, *clv3-7* and *clv3-10*) were collected and snap frozen in liquid N₂. Samples were fixed in 1% (v/v) formaldehyde buffer (10 mM

sodium phosphate buffer, pH 7; 50 mM NaCl; 100 mM sucrose) under vacuum. ChIP was performed as previously described³³ with modifications: Antibody incubation (anti-WUS; AS11 1759; Agrisera) was extended to o/n at 4°C and incubation with Agarose beads (Protein A-Agarose; sc-2001; Santa Cruz Biotechnology) to 6 h at 4°C. Immunoprecipitated DNA was analyzed by RT-qPCR assay. Data were analyzed using the SDS 2.4 software (Applied Biosystems/Life Technologies, Darmstadt, Germany). Ct values of *TPPJ* promoter regions were normalized to the Ct value of a region within the *UBQ10* promoter. The % of enrichment was calculated as relative to the input expression of the individual *TPPJ* promoter regions. The following controls were performed: ChIP on 100 apices of Col-0, *clv3-7* and *clv3-10* omitting addition of AB and ChIP on 1.5 g of Col-0, *clv3-7* and *clv3-10* leaves (Fig. S. 10). Please note that in contrast to the input samples there was no amplification in any of the other ChIP control samples. Oligonucleotides are listed in the Table S5.

Transactivation assay

For the effector construct line, the *WUS* (At2g17950) coding sequence was PCR amplified using the Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, US). The resulting fragment was cloned into the pGEM®-Teasy vector (Promega, Madison, Wisconsin, US) (pVW310), sub-cloned into the Gateway® entry vector pJLBlue reverse²⁷ (pMML056) and recombined using the Gateway® LR clonase II Enzyme mix (Invitrogen, Carlsbad, CA) into a Gateway® destination vector with 35S promoter (pMDC32) (pMML058). For the reporter gene constructs, designated regions of the 5' *TPPJ* region were amplified with specific primers (Table S2) using the Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, US). The resulting *KpnI*/*Acyl* fragment was

sub-cloned into the Gateway® entry vector pMDC162 to obtain GUS gene fusions (Table S2). The 35Somega:LUC-NOS plasmid containing the LUC gene driven by the 35S promoter was used as an internal control. Col-0 protoplasts were isolated from leaves of 4-week-old plants and transfected by a modified polyethylene glycol method³⁴. The transfected cells were incubated for 20 h at 22°C in the light (100 µmol/m²s⁻¹), harvested by centrifugation at 100 g for 2 min, and then lysed³⁵. Luciferase activity was measured with a luciferase assay kit (Promega, Madison, Wisconsin, US) according to the manufacturer's instruction, and GUS activity was determined according to a previously described protocol³⁵.

Electrophoretic mobility shift assay (EMSA)

WUS coding sequence without STOP codon was PCR amplified using the Phusion DNA polymerase (New England Biolabs). The resulting fragment was sub-cloned into the Gateway® entry vector pDONR207 (pMML059) and recombined into a Gateway® destination vector with 35S promoter (pDEST24) using the Gateway® LR clonase II Enzyme mix (Invitrogen, Carlsbad, CA) resulting in pMML063. The plasmid was transformed into the *Escherichia coli* strain Rosetta plysS and the protein production was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside at 30°C over-night. Bacterial cell lysis was performed by sonication (1x, 5 sec, 20% power, 4 cycles, Sonoplus Hd 2070 Sonicator, Badelin, Berlin, Germany) preceded by incubation on ice for 20 min in the freshly prepared protein extraction buffer (20 mM Na-phosphate buffer, pH 7.4; 0.5 M NaCl; 1 mM phenylmethylsulfonyl fluoride; 1 mM Ethylenediaminetetraacetic acid; 1 tablet of cOmplete™ Protease Inhibitor Cocktail (Merck, Darmstadt, Germany) per 10 ml of the buffer). For the EMSA 2 µg of protein (crude extract, determined by Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham,

Massachusetts, US) was used. The EMSA was performed using 5'-IRDeY-682-labeled, double-stranded oligos of 50 bp spanning the putative WUS binding sites in the 5' regulatory region of *TPPJ* (Table S7). Binding reactions were carried out using the Odyssey® EMSA Kit (LI-COR®) according to the manufacturer's instruction with a competitor to probe ratio of 1:200. Results were visualized using an Oddysey Infrared Imaging System (Li-Cor, Lincoln, NE).

Confocal microscopy

Apices from developmental series of Col-0 TCSn:GFP cytokinin reporter line lines were excised using a diamond knife leaving a stalk of stem tissue. This stalk was used to fix the sample for imaging in a droplet of 0.1% agar covered with Perfluorodecalin (F2 Chemicals, Lancashire, UK) on a glass slide. A Leica SP8 confocal laser scanning system equipped with a M6000B-CS microscopy stage, an Argon laser (65 mV), and a 40x water immersion HCX APO objective was used to image SAMs with following settings: Laser output power 20%; GFP Excitation (green): wavelength 488 nm / emission detection channel 3: 495 - 520 nm, gain PMT 800 V; plastid auto-fluorescence (blue) emission detection channel 4: 700 - 800 nm, gain PMT ~500 V; scan speed 600 Hz in xyz bi-directional scanning mode with a z-stack distance of approx. 10 µm. Offset = 0; pixel dimension: 1024 × 1024. To visualize the differences in GFP presence middle sections of representative SAMs were extracted from z-stacks using the Fiji software package³⁶, version 2.0.0-rc-69/1.52 max-intensity Z-Projection function.

Statistical consideration

Statistical significance of flowering time and RT-qPCR data was analyzed both, by one-way ANOVA (Analysis of Variance) with Tukey' Post Hoc HSD (Honestly Significant Difference) based

on Tukey-Kramer correction (P value <0.05) and two-tailed Student's *t*-test. Significance of the data was marked with asterisks (*) based on the following criteria: $P \leq 0,05$: *, $P \leq 0,01$: **, $P \leq 0,001$: ***. The presented statistical data are based on: flowering time experiments – min. 20 plants of each genotype; SAM area measurements – longitudinal middle section through min. 7 individual SAMs; *WUS* and *CLV3* expression domain size and distance to the summit – longitudinal middle section through min. 6 individual SAMs RT-qPCR – min. three biological replicates; Transactivation assay – 6 biological replications; ChIP – 3 biological replications.

Data availability

All data supporting the findings of this study are available in the main text or the Supplementary Information. All biological materials used in this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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Authors contribution

VW conceived and designed the experiments and prepared the figures with contributions from MML. VW and MML analyzed the data and wrote the manuscript. MML, KF, AK, and VW performed all essential experiments, i.e. VW generated most of the lines used, except for *ML1:amiRTPPJ* (KF), the *spl* CRISPR/*Cas9* deletion lines (MML), and all crosses thereof (MML). MML performed all transactivation assays, ChIP-PCRs, EMSAs and qPCR analyses. MML, AK, KF, CA, and VW analyzed SAM sizes and scored flowering time. RNA *in situ* hybridizations were performed by VW, AK, KF and MML. FK and VW took the confocal images. All authors have read and commented on the text and figures within this manuscript.

Competing interests

Authors declare no competing interests.

Additional Information

Supplementary Information: Figures S1-S22, Tables S1-S7, References (1-3).

Figures captions

Fig. 1. The T6P pathway impacts SAM size during development. (a) SAM area throughout development. (b) Vegetative and (c) inflorescence SAM size of *CLV3:TPS1* and *35S:amiRTPS1* lines. (d) *WUS* and *CLV3* expression by RNA *in situ* hybridisation on longitudinal sections through Col-0, *35S:amiRTPS1*, *35S:TPS1* and *CLV3:TPS1* SAMs of LD-grown plants 8 days after germination. (e) *WUS* and *CLV3* expression by RNA *in situ* hybridisation in vegetative (6 and 8 DAG), transition (10 DAG, dark grey) and inflorescence SAMs (12 and 16 DAG) of LD-grown Col-0 plants. (f) *WUS* expression domain sizes, (g) *WUS* expression domain distance to SAM summit, and (h) *CLV3* expression domain area, in vegetative, transition (dark grey) and inflorescence SAMs of LD-grown wild-type plants. (i) Schematic picture of *WUS*, *CLV3* and *TPS1* expression dynamics with arrows indicating direction of increasing expression domain at transition. Error bars denote s.d.; significance was calculated based on one-way Anova and a Student's *t*-test, ****P*<0.001. ☆ indicates SAM summit. Scale bars 25μm.

Fig. 2. *WUS* and *CLV3* expression in response to changes of *TPPJ* in the SAM. (a, b) *TPPJ* expression by RNA *in situ* hybridisation on longitudinal sections through inflorescence SAMs of Col-0 (a), and *clv3-7* (b), and by qRT-PCR in apices collected from *clv3-7*, *clv3-10* (c), and *35S:TPPJ* (d) plants. (e, f) SAM size of plants overexpressing *TPPJ* (e), expression of *TPPJ*, *WUS*, *CLV3* by RNA *in situ* hybridization (f). Error bars denote s.d.; significance calculated by one-way Anova and Student's *t*-test, ****P*<0.001. ☆ indicates SAM summit. Scale bars are 50μm.

Fig. 3. WUS directly regulates *TPPJ* in the SAM. (a) Overview of *TPPJ* 5' regulatory region with putative WUS binding sites (●, ■), position of ChIP-PCR amplicons corresponding to the results shown in (c-e). Boxes marked with I, II, and III indicate 5' TPPJ regions with in total seven core WUS binding sites – I: -2795 – -2789 bp, II: -2073 – -1830 bp, and III: -652 – -564 bp. Sequence location and lengths used in (b) indicated with #1-6. (b) Protoplast transactivation assay showing activation of the GUS reporter when coupled to the regions indicated in (a), relative to LUC activity. c indicates untransformed control. (c-e) Enrichment of (c) region I, (d) region II, and (e) region III as indicated in (a) measured by ChIP-PCR relative to the input. PB – post binding fraction. (f) EMSA for WUS binding to the indicated regions (a, I-III). Error bars denote s.d.; significance based on one way Anova and Student's *t*-test, ****P*<0.001. Scale bars are 50µm.

Fig. 4. The role of TPPJ in the outer SAM layer. (a) Meristem area of Col-0, *ML1:amiRTPPJ* V1 and V2, (b-c) Flowering time of *ML1:amiRTPPJ* (b) and *clv3-10;ML1:amiRTPPJ* (c) shown as days to bolting (a, c) relative to the wild type. V1 and V2 indicate two independent versions of artificial microRNAs designed to target *TPPJ* transcript. (d, e) Relative expression of *SPL* genes, mature miR156 (d), and *TPPJ*, *CLV3*, and *WUS* (e) in SD-grown *ML1:amiRTPPJ* and *35S:TPPJ* at 40 days after germination. (f) Relative expression of mature miR156 and *SPL* genes in SD-grown *clv3-10* at 40 days after germination. (g) Schematic model of vegetative and transition SAM circuits. Error bars denote s.d.; significance calculated based on one way Anova and Student's *t* test; **P*<0.05, ***P*<0.01, ****P*<0.001. ☆ indicates SAM summit.