Supplementary Materials for

Coactivation of antagonistic genes stabilizes polarity patterning during shoot organogenesis

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Sci. Adv. 8, eabn0368 (2022)
DOI: 10.1126/sciadv.abn0368

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Figs. S1 to S12
Tables S1 and S2
**Fig. S1. PIN1 maxima formed within the REV domain before primordia protrusion.**

(A) The PIN1 signal (PIN1-CFP, green) combined with REV-2YPet (red) and KAN1-2GFP (blue) fluorescence signals in the epidermis of the inflorescence meristem. I3-P2, primordia from youngest to oldest. A heatmap of the fluorescence intensity of PIN1-CFP is shown below, and (m/n) indicates that m in n biological repeats show the displayed features. Scale bar, 20 μm.

(B-D) Enlargement of the I1 to I3 primordia in (A). Heatmaps of the fluorescence intensity of PIN1-CFP in I1 to I3 are shown below. Yellow stars indicate cells with PIN1 maxima. Scale bar, 20 μm.
Fig. S2. PIN1 convergence patterns in incipient primordia and the SAM.

(A) Schematic representation of PIN1 convergence patterns in incipient floral primordia I2 and I1. The blue arrows indicate PIN1 polarity. Note the observed PIN1 convergence pattern is generally consistent with previous reports (12).

(B) The PIN1 signal (PIN1-GFP, green) combined with propidium iodide (PI, red) fluorescence signals in the epidermis of the inflorescence meristem. Squares on the top panel marked with numbers are enlarged below. Magenta arrows indicated PIN1 polarities. Selected arrows use colors other than magenta, and the same color denotes the same walls in different enlarged squares. Scale bar, 20 μm.
Fig. S3. REV and KAN1 accumulation patterns in *pin1-1* and *arf5-1* mutants.

(A) REV-2YPet (green) and KAN1-2GFP (blue) fluorescence signals in a *pin1-1* inflorescence meristem (IM). The separated REV and KAN1 signals are shown in the middle and lowest panel, respectively. Scale bar, 20 μm.

(B) REV-Venus (green) fluorescence signal in an *arf5-1* IM. The REV signal alone is shown in the middle panel. Scale bar, 20 μm.

(C) KAN1-GFP (blue) fluorescence signal in an *arf5-1* IM. The KAN1 signal alone is shown in the middle panel. Scale bar, 20 μm. In A-C, the plasma membrane was visualized with the dye FM4-64 (red).

(m/n) indicates that m in n biological repeats show the displayed features.
Fig. S4. *PRS* expression pattern in an inflorescence meristem.

Pattern of *pPRS::3GFP* (green) abundance in the inflorescence meristem. A reconstructed view of the inflorescence meristem is shown. The GFP signal alone is shown below. Optical longitudinal sections through primordia P1 to P3 (along the yellow dashed lines) are shown on the right. M, meristem. P1-P3, primordia from youngest to oldest; (m/n) indicates that m in n biological repeats show the displayed features. Scale bars, 20 μm.
Fig. S5. Simulation results for MP temporal dynamics.
This plot shows the MP temporal dynamics obtained by simulating the model in the Materials and Methods with the kinetic parameters in Table S2.
**Fig. S6. Simulation results for PRS temporal dynamics.**

This plot was generated in the same way as that in fig. S5 except that the color indicates the PRS expression level.
In Simulations 5 and 6, we fixed other parameters but perturbed the strength of the link from MP to REV, the link from MP to KAN, the link from PRS to MP, or the production rate of the MP source. The headers of the subplots denote the strength of the link; for example, $0.2K_{MP \rightarrow REV}$ means that the half-saturation value $K_{MP \rightarrow REV}$ of REV caused by MP is reduced to 20% of the value used in Fig. 2 (also listed in Table S2); $0.5k_{MP}$ means that the basal production rate of MP ($k_{MP}$) in the third and fourth cell layers from the right-hand side is set to half of the value listed in Table S2. Each subplot shows the state at $t=1200$ h of the gene products to ensure that the steady state is reached. The red dashed boxes show that Simulation 6 is not as robust as Simulation 5 when REV is expressed at high levels in all cell layers. The black dashed boxes indicate that the KAN1 domain in Simulation 6 can encompass the last two cell layers, while it cannot in Simulation 5.

**Fig. S7. Comparisons of the robustness of the seesaw models used in Simulations 5 and 6.**
Fig. S8. Activation of KAN1 expression by MP is time-dependent.

RT-qPCR analysis of KAN1 expression in pMP:MPΔ-GR inflorescence meristems after 4 h (left) or 16 h (right) of treatment with 50 μM CHX in the absence or presence of 10 μM Dex. Error bars indicate the SD from three biological replicates.
Fig. S9. Phenotypes of *arf5-1* and *arf5-1 rev-10D* mutants.

Scanning electron microscopy images of 40-day-old *arf5-1* and *arf5-1 rev-10D* inflorescence meristems are shown in (A) to (C), respectively. (B) and (C) show two different *arf5-1 rev-10D* inflorescence meristems. Scale bar, 100 μm.
Fig. S10. Sensitivity of the seesaw model in Simulation 6 and the effect of varying strengths of MP→REV or PRS→REV regulation.

A. The circuit in Simulation 6 with the numbers labeling different regulatory relationships. B. Sensitivity analysis for the seesaw model in Simulation 6. The number in the x-axis denotes one regulatory link in A; for each regulatory link, the $K$ for this regulatory link is changed while other kinetic parameters are fixed. The y-axis of the bar denotes the range of the fold-change at which the system can maintain the pattern where $KAN1$ occupies the last three cell layers (in blue) or the last two cell layers (in green). The pattern at $t=1200$ h is considered to ensure that the steady state is reached. C-D. The REV-KAN1 patterns at $t=1200$ h with varying $K_{MP→REV}$ (C) or $K_{PRS→REV}$ (D).
**Fig. S11. Fluorescence intensity quantification for Figure 6J and 6K.**
Relative fluorescence intensity of GPF-positive cells within the primordia marked with P in (A) pPRS:SV40-GFP (Fig. 6J) and (B) KAN1-GFP (Fig. 6K) were quantified. *P < 0.05, **P < 0.01.
**Fig. S12. Model predictions based on the seesaw model in Simulation 6**

A-C. *PRS* expression levels for the circuit in Simulation 6 (A), the circuit without REV (B), and the circuit with high REV expression. In panel B, REV is set to zero in each column. In panel C, the basal production rate of REV, $k_{MP}$, is set to be 70, while the value used in Fig. 2 is 60 as shown in Table S2. D-F. *REV-KAN1* patterns for the circuit in Simulation 6 (D), the circuit without PRS (E), and the circuit without PRS and REV (F). In panel E, PRS is set to zero in each column. In panel F, PRS and REV are both set to zero in each column.
| Primer     | Primer sequence (5′-3′)                      |
|------------|---------------------------------------------|
| KAN1-F     | CTGCAGACGCCTCCGAGGATAGCTCGAATTTCTGCTAT      |
| KAN1-R     | CTTATCGATTTGCAACCCTTTCGCTGCAATCTGGTC       |
| WOX1-F     | GGTACCAATGTCGAGATGCGGTTCAAA                |
| WOX1-R     | TCGGAAATCTCCATAGGCAAGAAGACT                |
| PRS-F      | GGTACCAGTACCTGCTGGAATCTGGTCAC              |
| PRS-R      | TCGGAAATTTGGTTAAGTCTGCTTGT                |
| WOX1-RT-F  | CTGGATATGTCGCTGCAGATG                     |
| WOX1-RT-R  | CTCCACCCGTATATTCTGGT                      |
| PRS-RT-F   | TGTCTTTGAGCTGCTGCTC                      |
| PRS-RT-R   | TCTTCAGCTCCACTTTTGGTGCGA                  |
| KAN1-RT-F  | ATGTCTATGGAAGGTGTATTCTGAG                 |
| KAN1-RT-R  | AGAAGATCATTGTGATGG                     |
| REV-RT-F   | TCAACACTCTCAGTACATG                      |
| REV-RT-R   | ACCCAACTCAACAGCTGTC                       |
| MP-RT-F    | GTGGAAAGACAGCTGACT                       |
| MP-RT-R    | ATGTCTTTGAGCTGCTC                       |
| ACTIN2F    | GAGAGGATCATGTGCTCAACC                    |
| ACTIN2R    | GTGAACGATTTCCGACTGCTGCC                   |
| pREV-aF    | GTCGTGTTGTTACACATACA                    |
| pREV-aR    | TTTTCTAGGGTTATCACC                      |
| pREV-bF    | GAAACATACACTTCACAGAT                    |
| pREV-bR    | GATGATATTCAACTGATT                      |
| pREV-cF    | GAGCGGCCATAAAAGAGAC                    |
| pREV-cR    | CCACTGATGTAGTCCACA                      |
| pREV-dF    | GTACGTAATAAGTTGGA                    |
| pREV-dR    | GTGTGAGACTCAAATTTTGA                |
| pREV-eF    | GTCCTAATAATGAAGACAAA               |
| pREV-eR    | CAACAAAGTTTTTGCCACA                 |
| pREV-fF    | TATCAGGTAACTAAAACTC                  |
| pREV-fR    | GATGATGTGGTTGGCTCTGT                  |
| pREV-gF    | CTCTGTAATCTCTCTCTCTT                  |
| pREV-gR    | TCGTTCTCTCAAGGTTGTA                   |
| pKAN1-aF   | GAAAGTTAAACTAAGCCATT                 |
| pKAN1-aR   | GTCTCTCTCTCTCTTCTATT                |
| pKAN1-bF   | GCATACGTGACTCTCTTTACT                |
| pKAN1-bR   | TAATCCCTCTTCTCTTCTCC              |
| pKAN1-cF   | CACAGTAGATTAAGCTAAGCT                |
| pKAN1-cR   | GCTGATAGCAATTAGTCTT                 |
| pKAN1-dF   | GATAGGACAGAGAGAAGACT                |
| pKAN1-dR   | CATAGATAAAGTTGAGA                   |
| pKAN1-eF   | GATCATCTCACTCTCTCTCTC                       |
| pKAN1-eR   | CACCTTCCATAGTACATATT               |
| pKAN1-fF   | AATGGTGTTCTCATCAAGGCT           |
| pKAN1-fR   | GGAGCTCTCTAGCTGCTGCTT           |
| pKAN1-gF   | GACAAACCGCTAAGCCACA          |
| pKAN1-gR   | TGTTGTTGAAAAGGAAATCAC            |
| pKAN1-hF   | GAGACATTATAGGTGGT                   |
| pKAN1-hR   | ACTCTGGTTCTCTATGCT                  |
| pKAN1-iF   | GTAATAACTGCTCAAGCGAC            |
| pKAN1-iR   | CCAATCTCGTGCTGCTAATG            |
Table S2. Parameters used in Simulations 1 to 6.

| Kinetic parameter | Value |
|-------------------|-------|
| $D$               | 0.2   |
| $k_{MP}$          | 60 in Simulations 2 to 6 |

**Simulation 1**

| Parameter          | Value |
|--------------------|-------|
| $(k_{b,REV}, v_{rev}, d_{REV})$ | (0, 60, 2) |
| $(k_{b,KAN1}, v_{KAN1}, d_{KAN1})$ | (0.8, 60, 2) |
| $K_{KAN1\rightarrow REV}$ | 8 |
| $K_{REV\rightarrow KAN1}$ | 20 |
| $K_{REV \rightarrow REV}$ | 10 |
| $K_{KAN1 \rightarrow KAN1}$ | 10 |

**Simulation 2 (including parameters in Simulation 1)**

| Parameter          | Value |
|--------------------|-------|
| $(k_{b,MP}, v_{MP}, d_{MP})$ | (0, 20, 2) |
| $(k_{b,PRS}, v_{PRS}, d_{PRS})$ | (0.4, 40, 2) |
| $K_{MP\rightarrow MP}$ | 1 |
| $K_{KAN1\rightarrow PRS}$ | 50 |
| $K_{MP \rightarrow PRS}$ | 100 |
| $K_{PRS\rightarrow MP}$ | 1 |

**Simulation 3 (including parameters in Simulation 2)**

| Parameter          | Value |
|--------------------|-------|
| $K_{MP \rightarrow REV}$ | 5 |

**Simulation 4 (including parameters in Simulation 3)**

| Parameter          | Value |
|--------------------|-------|
| $K_{MP\rightarrow KAN1}$ | 0.1 |

**Simulation 5 (including parameters in Simulation 3)**

| Parameter          | Value |
|--------------------|-------|
| $K_{MP\rightarrow KAN1}$ | 100 |

**Simulation 6 (including parameters in Simulation 5)**

| Parameter          | Value |
|--------------------|-------|
| $K_{PRS\rightarrow KAN1}$ | 7 |
| $K_{REV \rightarrow MP}$ | 400 |
| $K_{REV \rightarrow PRS}$ | 300 |
| $K_{PRS \rightarrow REV}$ | 100 |