Supplementary Material for

Cyclic programmed cell death stimulates hormone signaling and root development in Arabidopsis

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This PDF file includes:
- Materials and Methods
- Supplementary Text
- Figs. S1 to S12
- Table S1
- Full Reference List

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencemag.org/content/351/6271/384/suppl/DC1)

Movies S1 to S9
Supplementary Information of Model

Model Description
To assess how programmed cell death (PCD) of the final lateral root cap cells affects auxin distribution, the auxin dynamics in the root apex were simulated. Taking a modelling approach, we tested whether PCD in lateral root cap creates a temporary increase in auxin levels in the oscillation zone, which was hypothesized to mediate lateral root initiation.

The model was based on the vertex-based simulations presented previously (21), which predicted dynamic auxin distributions within real multicellular root tip geometries. The multicellular geometries were obtained using confocal images of root tips with cell walls marked with propidium iodide. Using these images, the 2D cell geometries were extracted using the CellSeT and SurfaceProject image-analysis tools (21, 29). These geometries were read into a vertex-based modelling framework (based on the OpenAlea modelling framework), and rules (developed using experimental data) were applied to automatically populate the virtual root-tip geometries with distributions of the PIN, AUX1, LAX and ABCB auxin carriers (21). In the model, the supply of auxin from the shoot was represented by prescribing an auxin concentration in the vasculature cells at the shootward boundary of the modelled tissues. Auxin concentration was assumed to be spatially homogeneous within each cell cytoplasm and apoplast compartment. Thus, using this modelling framework, dynamic auxin distributions can be predicted by simulating a system of Ordinary Differential Equations (ODEs) that describe how the auxin concentration within each compartment evolves due to passive and carrier-mediated auxin transport across the cell membranes, auxin diffusion between adjacent apoplast compartments and auxin synthesis and degradation within each cytoplasm compartment. Using the model parameters given in Table S1, simulations were performed in the Python programming language (www.python.org) using the ODE solver LSODES from the ODEPACK suite to approximate numerically solutions of the system of differential equations (303). The solutions were then presented using the Python library matplotlib (31). Further details of the model assumptions and equations can be found in the main text and Supplementary Material of ref. 21, and model parameter values can be found in Table S1. Furthermore, a Graphical User Interface (GUI), SimuPlant, is available online (www.simuplant.org), which enables other researchers to interact with the previously published model.
The focus here is on auxin flows between the outer and inner layers of the root, which required a number of model developments:

(i) The 3D structure of the root tip was incorporated by rotating a multicellular geometry of half a root tip around the central root axis. By assuming auxin concentrations and dynamics to be axisymmetric (neglecting variations around the roots circumference), this approach, using real geometries in two dimensions with an idealized representation in the third dimension, enables us to capture the 3D structure of the root tip without significantly increasing simulation time.

(ii) Based on the localization of PINs proteins (21, 25, 32, 33), we introduced inward periclinal PINs on to the pericycle (i.e. PINs on the membrane faces towards the vasculature), and added low levels of periclinal PIN on to the most distal five lateral root cap cells (on faces towards the epidermis).

(iii) Considering the long-distance auxin transport from shoot, auxin from the shoot was supplied to only a single file of stele cells (in our half-root geometry), and AUX1 influx carriers were added to this file of cells (as shown using a AUX1-Ypet line (21)).

(iv) High auxin synthesis in the outer lateral root cap cells was introduced to capture the conversion of IBA to IAA (12). In light of the previous results (34), the high synthesis rate in the outer lateral root cap (representing IBA to IAA conversion) was set to be equal to that in the QC and columella initials where IAA is synthesized from tryptophan (35).

The model assumes that during PCD, the distal ring of lateral root cap cells release their auxin into the surrounding apoplast. Therefore, from the predicted steady-state auxin distribution, the most distal lateral root cap cells are removed, and the cells' auxin is distributed into the surrounding apoplast compartments. Simulating the model then predicts how the auxin distribution within the root apex evolves in response to the PCD and auxin release.

The simulations predicted that the auxin released from the lateral root cap undergoing PCD initially enters the underlying epidermal cells and some of this auxin is transported shootwards (due to the shootward epidermal PINs), with smaller amounts of auxin entering the inner layers. With the above model assumptions and parameter values, the model predicted that after PCD the auxin concentration increased by approximately 35% in the underlying epidermis and 6% in the stele cells within the oscillation zone.

Due to the periclinal endodermal and pericycle PINs, the model predicted that the auxin increase in the stele cells adjacent to the pericycle is larger than in the surrounding layers,
consistent with these cells initiating lateral root development. After auxin release and removal of the most distal lateral root cap cells, the auxin distribution equilibrates to a new steady state within 300 seconds.

Initial simulations predicted only a small increase in auxin levels within the oscillation zone because AUX1 in the epidermis and cortex holds auxin within these outer-cell files. However, assessing the model assumptions revealed that accounting for variations in AUX1 between the different cell types (see model parameter table) resulted in higher levels of auxin within the final lateral root cap cell and a more significant increase in auxin in the oscillation zone. The cell-type specific AUX1 distribution was prescribed based on AUX1-YPet images (Figures 6A and S15 of ref. 21), which presented lower AUX1 membrane concentrations in the epidermal, cortical and stele cells.
Materials and Methods

Plant growth conditions

Arabidopsis thaliana seeds were surface sterilized with 95% (v/v) ethanol for 5 minutes and 20% (v/v) bleach for 12 minutes. The seeds was subsequently rinsed 5 times with sterile water, and kept at 4°C in the refrigerator. After 3 days imbibition, seeds were sown on Petri dishes (12 cm X 12 cm) containing sterile half-strength Murashige and Skoog (½ MS) medium (0.5 x MS salts, 0.8% sucrose, 0.5 g/L 2-(N-morpholino)ethanesulfonic acid, pH 5.7, and 1% w/v agar), and grown under continuous light as described before (II). Root phenotypes were analyzed on plates or recorded by different imaging systems according to the objective of the experiment.

Treatment with chemicals

Indole-3-acetic acid (IAA, Product No. I5148), Dexamethasone (Dex, Product No. D4902), 5-Benzylxyindole-3-acetic acid (BZ-IAA, Product No. B0626), and propidium iodide (PI, Product No. P4864) were acquired from Sigma. Indole-3-butyric acid (IBA, Product No. I0902), 1-N-naphthylphthalamic acid (NPA, Product No. N0926) and D-luciferin (Product No. L1349) were acquired from Duchefa Biochemie. 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoic acid (BUM) was a kind gift from Prof. Dr. Markus Geisler. Dex, BZ-IAA, IBA, BUM and NPA were dissolved in 100% dimethylsulfoxide (DMSO) to make a 50 mM stock solution. IAA was dissolved in absolute ethanol to make a 5 mM stock solution. PI was dissolved in dH2O, and D-Luciferin was dissolved in 0.01% Tween 80.

For chemical treatment, the required amount of the stock solutions was added to (50°C) melted ½ MS agar-containing medium and mixed in 50-mL Falcon tubes before being poured into Petri dishes. Arabidopsis seeds were (1) germinated on the fresh ½ MS medium containing the compounds, or (2) germinated on ½ MS media under continuous light for 5 days (3-day-old), then transferred to fresh ½ MS medium with different compounds for indicated timings.

To induce gene expression by Dex treatment, seedlings were grown on ½ MS medium, and then transferred at day 5 after germination to fresh ½MS medium containing Dex at the indicated concentrations. The prebranch sites and lateral roots that developed in the newly formed primary root part of different seedlings after transfer were quantified by using a Lumazone imaging system equipped with a charge-coupled device (CCD) camera (Princeton Instruments, Trenton, NJ, USA) and a dissecting microscope respectively. Prebranch site
number was quantified at day 2 after transfer and lateral root number was quantified at day 5 after transfer.

For the Dex wash-out experiments, 2-day-old seedlings (day 2 after germination) were transferred to ½ MS medium containing 0.3 µM Dex for 2 days (day 4 after germination), and subsequently transferred to Dex-free medium for another 4 days (day 8 after germination). The positions of the root tips immediately after the transfer were marked on the plates by using a razor blade. The number of lateral roots formed in the different root regions was quantified at day 8 after germination.

**Plant Lines Used**

The *Arabidopsis* accessions used for this study were Columbia (Col-0) and C24. *Arabidopsis* transgenic lines DR5rev:VENUS-N7 (13), DR5:Luciferase (10), pAUX1:AUX1-YFP (36), pPASPA3->H2A-GFP (8), 35S:SMB-GR (17), UAS:HA-AUX1 aux1-22 (5), UAS:axr3-1 (5), R2D2 (28), WAVE131Y (37) and mutants aux1-21 (36), pin2 (38), pin2pgp1pgp19 (39), ibr1-2ibr3-1ibr10-1(40) and smb-3 (41), have been described previously. DR5rev:VENUS-N7 was backcrossed three times to Col-0 before being applied in all the experiments. The GAL4-GFP enhancer trap lines J3411, J1092, J0951 and J0121 were obtained from the Nottingham Arabidopsis Stock Centre (http://nasc.nott.ac.uk/). The GAL4 enhancer trap lines were crossed with transgenic plants containing the UAS:axr3-1 construct and the lateral root phenotype of the F1 generation was analyzed. Double and higher-order mutants harboring various marker lines were generated by crossing. F3 homozygous seedlings were analyzed in all experiments. For the aux1 complementation study, the enhancer trap lines J3411 and J0121 were first introduced into the aux1-21 mutant. Homozygous J3411 aux1-21 and J0121 aux1-21 seedlings were subsequently crossed with UAS:HA-AUX1 aux1-22 seedlings. F1 seedlings were used to quantify the root phenotype.

**Plant Constructs and Transformations**

The Gateway system® (Invitrogen, Carlsbad, CA, USA) was applied to generate most constructs. For transcriptional reporters, the SOMBRERO (SMB), and plant aspartic proteinase A3 (PAPSA3) (8), promoter fragments upstream of the coding sequence amplified from genomic DNA were cloned into pDONRP4P1R and subsequently introduced into different expression vectors (42). To generate the pSMB:axr3-GR construct, the gain-of-function axr3-1 cDNA fragment was amplified from UAS:axr3-1 seedling genomic DNA, and then fused between the SMB promoter and the GR tag in a destination vector (pB7m34GW).
Transgenic plants were created by *Agrobacterium tumefaciens* floral dipping with the construct described above into the appropriate genetic background (43). Primers used were as follows:

SMB_Promoter_attb4F,

GGGGACAACTTTGTATAGAAAAAGTTGGCTCGTTGAAGATGCCTGGATT TA;

SMB_Promoter_attb1R,

GGGGACTGCTTTTTTGTACAAACTTGCTATCCTTACTCT TTCTTTAAGC.

axr3-1_CDS_attb1F,

GGGGACAAGTTTGTACAAAAAAGCAGGCTATATGATGGGCAGTGTCGAGCT;

axr3-1_CDS_no stop_attb2R,

GGGGACCACTTTGTACAAAAAAGCAGGCTATATGATGGGCAGTGTCGAGCT;

Root phenotype analyses

To quantify the lateral root phenotype in wild-type plants and mutants, emerged lateral roots of the whole seedlings were counted under a dissecting microscope, 8 days after germination. Subsequently, whole seedlings were scanned for further analysis of the primary root length with NeuronJ (http://www.imagescience.org/meijering/software/neuronj/). For dexamethasone (Dex) and estradiol treatments, the length of the primary root grown during the treatment was measured and emerged lateral roots in this root region were counted. The vertical gravitropic index was obtained by calculating the ratio of vertical distance covered by the primary root and total primary root length (44). For microscopic analysis of primordium stages, root samples were cleared as described previously (45). All samples were analyzed by differential interference contrast microscopy (Olympus BX53).

Confocal microscopy and quantification

A Zeiss LSM710 and LSM780 laser-scanning microscope were used for fluorescence imaging of the *Arabidopsis* roots. For the propidium iodide (PI)-treated root images, seedlings were stained with 2 µg/mL PI for 3 minutes, washed with water, and mounted in water for confocal imaging. To generate 3D projection of z-stacks of root tip sections, stacks of ~70 optical z sections (1 ~ 1.5 μm step-size) were collected from the meristem zone.

To quantify the fluorescence signal in the lateral root cap cells, *Arabidopsis* seedlings were placed on the slide without PI staining, and the median plane of the root tip was immediately scanned with a Plan-Apochromat 20x/0.8 M27 lens. Arbitrary fluorescence units (a.u.) were determined by ImageJ. To obtain a better view of the fluorescence signal in the lateral root
cap cells, a C-Apochromat 40x/1.2 water-corrected lens was used. Excitation of YFP and PI was done with a 514nm laser line, and the emission between 519-564 nm was collected for YFP and between 614-735 nm for PI. For Fig.1B and C and fig. S1H, the model was based on z-stacks imaged with a iLCI Plan-Neofluar 25x/0.8 water-corrected lens. These files have been investigated using IMARIS. 3D reconstructions included smoothing of the PI/YFP signal and a threshold based surface construction.

The quantification of the lateral root cap cell number and DR5 signal intensity was performed on each individual root. Measurements were performed on a median confocal plane image along individual lateral root cap cell files, starting from the lateral root cap -columella boundary and ending with the most distal lateral root cap cell.

To investigate the auxin response during the PCD of the lateral root cap cells, 3-day-old seedling of R2D2 were placed on a on a PI (30µg/ml) containing ½ MS medium and carefully transferred to a cover slide. The time laps have been taken with a Zeiss780, equipped with a I LCI Plan-Neofluor 25X (0.8 imm Korr DIC M27) over a period of 3-5 hours. The images and movies have been investigated as mentioned above.

**Macroview stereo microscope setting up and imaging**

An Olympus MXV10 macroview stereo microscope ([http://www.olympusamerica.com/seg_section/product.asp?product=1013](http://www.olympusamerica.com/seg_section/product.asp?product=1013)) was tilted by 90 degrees and adapted to a holder, which enabled imaging the fluorescence signal from vertical growing Arabidopsis roots in square plates. A mobile microscope stage was installed to fix the plate close-up to the lens, and an automated ProScan™III system (Prior Scientific) was connected to the microscope to control the wheel containing three fluorescence filters. For the time lapse imaging, “Process Manage” function in Cellsens Dimension software (Olympus) was applied for all the settings. Pictures were taken every 2 min with 0.5s ~ 1.2s exposure times dependent on the expression level of the fluorescence signal. After finishing the time-lapse imaging, pictures were saved as video files and further analyzed by ImageJ.

To determine the time between the consecutive disappearances of the nuclear-localized fluorescence signals in the most distal lateral root cap cell files, Arabidopsis root tips were imaged over 20 hours, and the number of frames up to the frame with a complete absence of the fluorescence signal in the most distal lateral root cap, were counted. The time between two consecutive events was calculated based on the number of frames counted. 3-day-old seedlings were used for all the experiments unless stated otherwise.
Luciferase imaging and expression analysis

A Lumazone imaging system equipped with a charge-coupled device (CCD) camera (Princeton Instruments, Trenton, NJ, USA) or a Berthold NightSHADE LB985 imaging system was used for Luciferase imaging. The CCD camera is controlled by WinView/32 software. To perform time-lapse imaging of the DR5:Luciferase expression in the oscillation zone, square plates containing 1/2MS medium with or without chemicals were sprayed with 1mM D-Luciferin solution (0.01% Tween80) and left to dry in the dark, then 3-day-old DR5:Luciferase transgenic seedlings were transferred on the plates and imaged immediately with a macro lens every 10 minutes (exposure time) for indicated times. The picture series were saved as TIFF format by WinView/32 software for further analysis in ImageJ (http://imagej.nih.gov/ij/). The luciferase signals were quantified by measuring the analog-digital units per pixel, and the frequency of the DR5 oscillations during the primary root growth was calculated by recording the time interval between consecutive DR5 oscillations. More than 70 time points from at least 15 individual seedlings were collected to make a histogram.

The prebranch site numbers of 8-day-old transgenic DR5:Luciferase-harboring Col-0 or indicated mutants seedlings were quantified the by counting static DR5 expression sites that were visible along the primary root outside the oscillation zone. Seedlings were sprayed with D-Luciferin and immediately imaged in the Lumazone system with a 15-minute exposure time.

Time measurements

We determined the correlation between the timing of the disappearance of DR5rev:VENUS-N7 stripes in the distal lateral root cap cell files and DR5:Luciferase oscillation in the oscillation zone in 3-day-old Arabidopsis seedlings. All the regions (0.06 cm x 0.06 cm) used for quantifications were selected in the time-lapse movies based on the appearance of the formation of lateral root primordia and prebranch sites, respectively indicated by DR5rev:VENUS-N7 and DR5:Luciferase (Fig. 1C, and fig. S3, A, C, D and E).

To calculate the time required by a root tip to perceive a DR5:Luciferase oscillation signal and to produce a prebranch site, a square region (0.06 cm x 0.06 cm) where a prebranch site developed in the center was selected in the last frame of the movie. This region was subsequently used as the region of interest to integrate luciferase signal intensities starting upon the entering of the root apex (T1 = 10 min, 10 min interval between constitutive frames).
The changes of average luciferase signal in this region were quantified by using “Virtual Stack” in ImageJ. In all the measurements, luciferase signals from regions outside the root were measured as background and subtracted. An example is shown in fig. S3, D and E.

The same strategy was used to analyze the time required to lose the most distal DR5 stripes for which time-lapse movies of DR5rev:VENUS-N7 were used. A square region of the same size (0.06 cm x 0.06 cm) with a lateral root primordium located in the center was selected in the final frame of the movie. The time when the root tips entered the region was recorded as T1 (2 min interval between constitutive frames), and the time points when the most distal DR5 stripes disappeared were recorded and represented in a Histogram (Fig. 1C and fig. S3C).

**Distance measurements**

The distance from the oscillation zone to the root apex was measured when the expression level of DR5:Luciferase reached the highest value (fig. S3, F and G). The distance from the distal end of lateral root cap cells to the distal end of the root apex was measured on confocal images of root tips expressing DR5rev:VENUS-N7 in 3-day-old seedlings.

**Kymograph**

Kymographs ([http://www.embl.de/eamnet/html/body_kymograph.html](http://www.embl.de/eamnet/html/body_kymograph.html)) were generated by ImageJ to visualize the spatiotemporal changes of DR5:Luciferase signal in the root tips during primary root growth. For this purpose, a time-lapse movie (TIFF series) was loaded into ImageJ, and a “Z-projection” was performed to have an overview of the luciferase signal changes following primary root growth over time. Subsequently, a segmented line was drawn on the newly formed primary root and marked by the “ROI manage” function. This line was restored in the original TIFF series to generate “MultipleKymograph”. In our experiments, 3-day-old seedlings were used.

**Accession numbers**

Sequence data can be found in the Arabidopsis information resource (TAIR) databases under the following accession numbers: PASPA3 (AT4G04460), SOMBRERO (AT1G79580), AUX1 (AT2G38120), IBR1 (AT4G05530), IBR3 (AT3G06810), IBR10 (AT4G14430), IAA17/AXR3 (AT1G04250), PIN2 (AT5G57090), ABCB1 (AT2G36910), and ABCB19 (AT3G28860).
**Fig. S1. DR5rev:VENUS-N7 forms a stripe-like expression pattern in lateral root cap cells.**

(A) A scheme of the *Arabidopsis* root tip showing the three correlated developmental processes: PCD of lateral root cap cells, oscillation of gene expression and lateral root initiation. (B and C) Confocal images of DR5rev:VENUS-N7 (nuclear) and WAVE131Y (plasma membrane) expression (B) and pPASPA3>>H2A-GFP expression (PI counterstained) (C) in a wild-type root tip. Red arrow in (B) indicates the DR5 signal in an epidermal cell. White arrows in (C) indicate the PASPA3 signal in the lateral root cap cells. PI, propidium iodide. (D to G) Confocal images and quantification of DR5rev:VENUS-N7 and pSMB:NLS-GFP signals in lateral root cap. (H) Quantification of cell length of individual lateral root cap cells starting from columella until the most distal end of the lateral root cap. Data are means ± s.d. LRC, lateral root cap. All scale bars, 100 µm.
Fig. S2. Quantification of different periodic signals in the root tip.

(A) Illustration of how the time-interval between consecutive disappearances of DR5 stripes at the most-distal end of the lateral root cap (indicated in stereomicroscopic picture) was quantified. Black arrows in the graphs mark onset of DR5 stripe fluorescence decrease, red arrows mark the timing when the DR5 stripes were no longer visible. (B) Histograms showing the distribution of the time interval(s) between the consecutive disappearance of DR5rev:VENUS-N7, pPASPA3:NLS-tdTomato, pSMB:NLS-GFP stripes in the most-distal lateral root cap and DR5:Luciferase oscillations in the oscillation zone, determined as in (A). (C) Real-time imaging of DR5 stripes in the lateral root cap under during 135° gravistimulation-induced bending (Also see Movie S2). Arrows indicate the disappearing DR5 signal in the lateral root cap and appearance of lateral root primordia. LRP, lateral root primordium. (D) Quantification of the average time interval between consecutive disappearances of DR5rev:VENUS-N7 signals, pPASPA3:NLS-tdTOMATO signals in lateral root cap, and the period of DR5:Luciferase oscillation in the oscillation zone under normal conditions or during 135° gravistimulation-induced bending (n > 20). Different letters indicate significant differences among means (P < 0.05 by one way ANOVA and Tukey’s test as post hoc analysis). Data are means ± s.d. Scale bars, 200 µm.
Fig. S3. Disappearance of DR5 stripe precedes the DR5 oscillation in the oscillation zone. (A) Signal changes in the root tips over time. (B) Positioning along the root axis of the microscopic landmarks based on reporter gene analyses, expressed as distance from the root apex. LRC, lateral root cap. MZ, Meristem zone. OZ, oscillation zone. DZ, Differentiation zone. (C to G) Examples illustrating the procedures followed to determine the time points when the most distal DR5 stripes disappeared and to measure the amplitude of the DR5:Luciferase oscillations. (C) Histogram showing the measured distribution of the time span for the disappearance of the most distal DR5 stripes since it entered the yellow-boxed area (n > 60). An example was shown in Fig. 1C. Yellow-boxed area (0.06 cm x 0.06 cm) represents the region of interest to follow DR5 stripe over time upon the entering of the root apex. The time point when the root tip enter the yellow-boxed area was recorded as T0 (0'). (D and E) Determination of the time span needed for the root tips to perceive the DR5:Luciferase oscillation signal and to subsequently form a prebranch site. An example of a series of recordings is shown in (D). In this type of analysis, the average DR5:Luciferase signal is measured inside the selected region (0.06 cm x 0.06 cm, yellow boxed area in (D)) at different time-points. The values were obtained using the Visual Stack option of the software package ImageJ and are presented in the graph shown in (E). The red arrows in (E) correspond to the time points of the images shown in (D). (F and G) Analysis of the distance
between oscillation signals and the root apex in the root tips. An example is shown in (F): The DR5:Luciferase signal along the primary root was measured when the oscillation signal reached the maximum value in oscillation zone and the segmented yellow line on the panel representing the time point with maximal DR5:Luciferase expression indicates how the measurements were performed. The obtained DR5:Luciferase profiles are shown in (G) (n > 40). The lowest DR5:Luciferase signal detected at the beginning and the end of the oscillation zone are considered as the start and the end of the oscillation zone respectively; the distances from these signals to the root apex were collected and plotted in (B). Data are means ± s.d. All scale bars, 100 µm.
Fig. S4. Periodic lateral root cap cell death correlates with lateral root formation.

(A) Stereo microscope recordings using the YFP filter (upper panels) and RFP filters (lower panels) to analyze fluorescence signals in DR5rev:VENUS-N7 x pPASPA3:NLS-tdTomato (left), DR5rev:VENUS-N7 (right) and pPASPA3:NLS-tdTomato (middle) showing a weak DR5rev:VENUS-N7 bleed-through signal in the very root tip, but not at DR5 stripes in the lateral root cap when using the RFP-filter, demonstrating that the observed stripes in the RFP channel reflect pPASPA3:NLS-tdTomato expression. (B and C) Time series of real-time imaging of co-expression of DR5 stripes with PASPA3 stripes, and SMB stripes with PASPA3 stripes in the most-distal lateral root cap cells over time. White arrows indicate the most-distal SMB, DR5 and PASPA3 stripes that gradually disappear during the time course. All scale bars, 100 µm.
Fig. S5. Delay of PCD in the lateral root cap impairs lateral root spacing.

(A) Median plane image of DR5rev:Venus-N7 in root tips of 3-day-old Col-0 and smb-3 seedlings. Cell walls were stained with PI. LRC is lateral root cap, EZ is elongation zone, MZ is meristematic zone. Scale bars, 100 µm. (B and C) Quantification of lateral root cap cell number (B) and DR5 signal intensity (C) in 3-day-old Col-0 and smb-3 seedlings. (D) Quantification of DR5:Luciferase oscillation frequency in the oscillation zone in 3-day-old Col-0 and smb-3 seedlings. (E to G) Overview root phenotype (E) and quantification of prebranch site number (F) and lateral root number (G) of 8-day-old Col-0 and smb-3 seedling roots (n > 10). Scale bars, 1 cm. (H) Quantification of primary root growth of Col-0 and smb-3 over 6 days since germination (n > 14). Data are means ± s.d. **P < 0.01 compared to Col-0 by Student’s t test.
Fig. S6. Genetic ablation of the root cap abolishes lateral root formation capacity.

(A) Impact of mild SMB-GR activation on root growth and quantification of lateral root number and primary root length in 8-day-old Col-0 and 35S:SMB-GR transgenic seedlings after 5 days treatment with Dex since day 3 (n > 10). The lateral roots and primary root formed after transfer were measured. **P < 0.01 indicates significant difference by Student’s t test. (B) Detail of the root meristems of 35S:SMB-GR treated different concentrations of Dex for 1 day since day 3 after germination. Red arrows indicate lateral root cap, and black arrows indicate epidermis. (C) Prebranch site distribution in 5-day-old 35S:SMB-GR seedlings treated with Dex since day 3 after germination. Red arrow heads indicate the prebranch sites. Dashed line indicates the location of root tip when seedlings were transferred to Dex medium. (D) Two-day old Col-0 and 35S:SMB-GR were transferred to Dex-containing medium for 2 days and transferred back to normal medium for 4 days. Red and white dashed lines indicate the root tips’ position at the moment of the transfer. (E) Quantification of lateral roots formed in Col-0 and 35S:SMB-GR in the root segments formed during Dex treatment (upper panel) and during recovery growth (lower panel) (n > 10). *P < 0.05 and **P < 0.01 indicate
significant difference by Student’s $t$ test. (F) Lateral root cap formation of 35S:SMB-GR transgenic seedlings was recovered after transfer to Dex-free medium for 4 days, red arrows indicate the outer layer of lateral root cap. (G) Quantification of the lateral root number in 8-day-old Col-0 and 35S:SMB-GR seedlings treated with various concentrations of IBA, Dex or the combination of IBA and Dex since day 3 ($n > 10$). The lateral roots formed after transfer were measured. **$P < 0.01$ indicate significant different by Student’s $t$ test. Data are means ± s.d. White scale bars, 100 µm; red scale bars, 0.5 cm.
Fig. S7. Local auxin signaling in lateral root cap is not required for lateral root formation.

(A) $pSMB:axr3-GR$ seedlings germinated on ½ MS medium without Dex and transferred after 3 days of growth (indicated by dashed lines) to medium with 10 µM Dex. (B) Macroview stereomicroscope (left) and confocal microscope (right) images of $DR5rev:VENUS-N7$ expression in the root tips of 3-day-old Col-0 and $pSMB:axr3-GR$ seedlings grown on 10 µM Dex. White and red arrows indicate the nuclear $DR5$ signal in lateral root cap and epidermal cells, respectively. Scale bar, 50 µm. (C) Quantification of DR5 signal intensity in lateral root cap (upper panel) and epidermis (lower panel) of 3-day-old seedlings ($n > 30$). **$P < 0.01$ compared to Mock-treated by Student’s $t$ test. (D) Macroview stereomicroscopy images of $pPASPA3:NLS-tdTomato$ expression in the root tips of 3-day-old Col-0 and $pSMB:axr3-1-GR$ seedlings germinated on 10 µM Dex. Scale bar, 50 µm. (E) Quantification of lateral root number and primary root length in 8-day-old Col-0 and $pSMB:axr3-1-GR$ transgenic seedlings treated with 10 µM Dex since day 3 ($n > 10$). The lateral roots and primary roots formed after transfer were measured. **$P < 0.01$ indicates significant different by Student’s $t$ test. Bar = 1 cm. (F) Lateral root number of the F1 progeny of $UAS:axr3-1$ crossed with different enhancer trap lines: J3411 (lateral root cap domain), J1092 (lateral root cap initials domain), J0951 (outer layer of lateral root cap and pericycle in the different zone), and J0121
(xylem pole pericycle) \((P < 0.05\) by one way ANOVA and Tukey’s test as post hoc analysis, \(n > 10\)). Data are means ± s.d.

Fig. S8. Predicted auxin dynamics after PCD of most distal lateral root cap cells.
(A to C) Distribution of auxin influx and efflux carriers in the root tip. Colors show the permeability associated with the carriers on the marked membrane: 0.35 \(\mu\)m/s (green) 0.035 \(\mu\)m/s (blue) 0.85 \(\mu\)m/s (red) and 0.17 \(\mu\)m/s (purple). Scale bars, 50 \(\mu\)m. (D) Key highlighting 6 epidermal cells and 7 stele cells underlying the lateral root cap cells that undergo PCD. The colors correspond to the predictions shown in panels E to H. (E to H) Predicted evolution of auxin concentration in cells (shown in panel D) after auxin deposition following PCD of the most distal lateral root cap cells; (E) Wild type; (F) Defect in IBA-to-IAA conversion; (G) Defect in AUX1-mediated influx in lateral root cap; (H) Defect in polar carrier-mediated efflux.
Fig. S9. Lateral root spacing requires root cap-AUX1.

(A) Confocal microscopy images of 3-day-old pAUX1:AUX1-YFP root tip. LRC, lateral root cap. Ep, epidermis. Scale bar, 200 µm. (B) Quantification of the number of the lateral root cap cells that are adjacent to pAUX1:AUX1-YFP expressing epidermal cells. (C) Prescribed AUX1 distribution reflecting J3411>>AUX1 aux1 pattern. LRC, lateral root cap. Scale bar, 50 µm. (D) Predicted auxin distribution mediated by AUX1 in lateral root cap. Scale bar, 50 µm. (E) Predicted lateral root cap-localized AUX1-mediated auxin dynamics in the epidermal cells and the stele cells underlying the most distal lateral root cap cells after PCD. (F and G) Root phenotype and quantification of lateral root number, primary root length and gravity index in 8-day-old Col-0, aux1 single mutant and F1 progeny of J3411>>AUX1 aux1-22 and J0121>>AUX1 aux1-22 seedlings. *P < 0.05 and **P < 0.01 compared to Col-0 by Student’s t test. Data are means ± s.d. Scale bar, 1 cm.
Fig. S10. Predicted auxin distributions and dynamics in the root tip with different rates of apoplastic diffusion.

(A) Predicted steady-state auxin distribution for wild-type, with an apoplastic diffusion rate of 32 μm²s⁻¹, 160 μm²s⁻¹ or 0 μm²s⁻¹. (B) Predicted steady-state auxin distribution with defects in carrier-mediated auxin efflux and an apoplastic diffusion rate of 32 μm²s⁻¹, 160 μm²s⁻¹ or 0 μm²s⁻¹. (C and D) Predicted auxin dynamics in the epidermal cells and the stele cells underlying the most shootward LRC cells after PCD for wild type and different rates of apoplastic diffusion. (E and F) Predicted auxin dynamics in the epidermal cells and the stele cells underlying the most distal lateral root cap cells after PCD with defects in carrier-mediated auxin efflux and different rates of apoplastic diffusion. Results in C to F show the auxin concentrations relative to that at t = 0. The model predicts that the apoplastic diffusion rate affects the magnitude of the auxin accumulation in wild type (panel D), suggesting that the auxin flux from the lateral root cap to the stele is due to both apoplastic and cell-to-cell transport. In contrast, no auxin accumulation is predicted if carrier-mediated efflux is defective and the apoplastic diffusion rate does not affect this prediction (panel F). Scale bars, 50 μm.
Fig. S11. Lateral root spacing requires polar auxin transport.

(A) Localization of PIN2, ABCB1 and ABCB19 proteins in 3-day-old indicated transgenic seedlings. Arrows indicate the periclinal localization of PIN2 in the lateral root cap. (B and C) Quantification of (B) number of primordia, lateral roots, and (C) primary root length in 8-day-old Ws, pin2 abcb1 abcb19, Col-0 and pin2 (n > 10). *P < 0.05 and **P < 0.01 compared to
ecotype controls Col-0 or Ws by Student’s t test. (D) Quantification of lateral root number in 8-day-old indicated genotypes treated with or without IBA for 5 days since day 3 (n > 10). *P < 0.05 and **P < 0.01 indicate significant differences by Student’s t test. (E) Expression pattern of pPASPA3:NLS-tdTomato in 4-day-old Col-0 seedlings treated with or without indicated chemicals for 24 h since day 3 after germination. White arrows indicate the PASPA3 stripes in the lateral root cap. (F and G) Confocal microscopy (F) and quantification (G) of DR5rev:VENUS-N7 expression in 4-day-old Col-0 seedlings treated with or without NPA, BUM and BZ-IAA for 24 h since day 3 (n > 20). *P < 0.05 and **P < 0.01 compared to DR5 signal intensity in lateral root cap, epidermis, and vascular tissue under Mock treatment by Student’s t test. LRC, lateral root cap; EP, epidermis. (H and I) Root growth (H) and lateral root formation (I) in 8-day-old Col-0 seedlings treated with or without BUM and BZ-IAA for 5 days since day 3 after germination. The lateral roots formed in the newly grown root segment were measured. Black dashed lines indicate the location of root tip when seedlings were transferred to medium containing different chemicals (n > 10). *P < 0.05 and **P < 0.01 compared to Mock-treatment by Student’s t test. In all experiments, NPA, BUM, and BZ-IAA was used at 10 µM, 0.3 µM, and 3 µM respectively, unless mentioned differently. Data are means ± s.d. White scale bars, 100 µm; black scale bar, 0.5 cm.
Fig. S12. Scheme indicating the order of developmental events upstream of lateral root initiation.
In the Arabidopsis root tip, the most distal lateral root cap cells undergo PCD which facilitates the redistribution of auxin from lateral root cap into the elongation zone through auxin transport. In the elongation zone, xylem pole pericycle cells will become primed and recruited into the developmental program of lateral root development. As the root grows this cascade of events is repeated when the next lateral root cap cells reach maturity and undergo PCD.
Table S1. Model parameter values.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Parameter value</th>
<th>Reference</th>
</tr>
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<tr>
<td>Passive membrane permeability</td>
<td>0.35 \text{ mu m/s}</td>
<td>ref. 46</td>
</tr>
<tr>
<td>Membrane permeability due to AUX1 influx carriers in columella</td>
<td>0.35 \text{ mu m/s}</td>
<td>ref. 46</td>
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<tr>
<td>Membrane permeability due to AUX1 influx carriers in lateral root cap, epidermis, cortex and stele</td>
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<td>Assumed to be 10% of that in the columella</td>
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<td>0.35 \text{ mu m/s}</td>
<td>ref. 46</td>
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<tr>
<td>Membrane permeability due to PIN efflux carriers</td>
<td>0.85 \text{ mu m/s}</td>
<td>ref. 46</td>
</tr>
<tr>
<td>Membrane permeability due to non-polar efflux carriers</td>
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<td>Assumed to be 30% of polar PIN permeability</td>
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<td>Cytoplasmic pH</td>
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<td>ref. 47 and ref. 48</td>
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<td>Apoplastic pH</td>
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<td>ref. 47</td>
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<td>Dissociation constant for auxin</td>
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<tr>
<td>Cell membrane potential</td>
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<tr>
<td>Temperature</td>
<td>300 \text{ K}</td>
<td>ref. 5</td>
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<td>Apoplast thickness</td>
<td>0.14 \text{ mu M}</td>
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<td>Diffusion constant within the apoplast</td>
<td>3.2 \times 10^{-11} \text{ m}^2/\text{s}</td>
<td>ref. 51</td>
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<td>High auxin synthesis rate (QC, columella initials and outer two layers of lateral root cap)</td>
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<td>Assumed to be a small value</td>
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<td>Background auxin synthesis rate</td>
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<td>Assumed to be 10% of the high synthesis rate</td>
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<tr>
<td>Auxin degradation rate</td>
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<td>Assumed to be a small value</td>
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**Supporting Movies**

Video files S1-S9

**Movie S1.** Movie of *DR5rev:VENUS-N7* expression in a 3-day-old seedling for 14 hours. Red arrows indicate the disappearance of *DR5* expression in the root cap; yellow arrows indicate the formation of a lateral root primordium. Movie is at 30 frames per second. Scale bar, 0.2 mm.

**Movie S2.** Movie of *DR5rev:VENUS-N7* expression in a 3-day-old seedling after a gravistimulation (135° turn) for 20 hours. Red arrows indicate the disappearance of *DR5* expression in the root cap during root bending; yellow arrows indicate a lateral root primordium. Movie is at 30 frames per second. Scale bar, 0.2 mm.

**Movie S3.** Movie of *pPASPA3:NLS-tdTOMATO* expression in a 3-day-old *pPASPA3::NLS-tdTOMATO x DR5rev:VENUS-N7* seedling under normal condition over 18 hours. At the end of the movie, the corresponding *DR5* expression (green) at 19 hours was included to visualize the position of lateral root primordia. Red arrows indicate the sites where *PASPA3* stripes disappeared from the most distal root cap cell and green arrows indicated the positions of *DR5* expressing lateral root primordia. Movie is at 30 frames per second. Scale bar, 0.2 mm.

**Movie S4.** Movie of *pPASPA3:NLS-tdTOMATO* expression in a 3-day-old *pPASPA3::NLS-tdTOMATO x DR5rev:VENUS-N7* seedling after gravistimulation (135° turn) for 18 hours. At the end of the movie, the corresponding *DR5* expression (green) at 19 hours was included to visualize the position of lateral root primordia. Red arrows indicate the sites where *PASPA3* stripes disappeared from the most distal root cap cell and green arrows indicated the positions of *DR5* expressing lateral root primordia. Movie is at 30 frames per second. Scale bar, 0.2 mm.

**Movie S5.** Movie of *DR5:Luciferase* expression in 3-day-old seedlings Col-0 and *35S:SMB-GR* Dex-treated seedling over 24 hours. One root from the Col-0 seedlings (on the left) and five roots from the *35S:SMB-GR* seedlings were imaged over time. Movie is at 24 frames per second. Scale bar, 0.5 cm.
**Movie S6.** Movie of D2R2 expression in a 3-day-old seedling root tip over 170 minutes. Seedling was placed in the growth chamber covered with 1/2 MS agar, and horizontally scanned by confocal every 10 min. 3D-projection of the images were performed in ImageJ and stacked as video files. Yellow box indicates the region where the disappearance of YFP signals in epidermal cells and the PCD of lateral root cap cells were observed. Movie is at 6 frames per second. Scale bar, 50 μm.

**Movie S7.** Movie of predicted dynamics of deposited auxin over 300 seconds after programmed cell death of the final lateral root cap cells in wild type. Panels show dynamics for wild type, defect in IBA-IAA conversion, defect in AUX1-mediated influx and defect in carrier-mediated efflux. Movie represents 20 frames per second where each frame represents 1 second. Scale bar, 50 μm.

**Movie S8.** Movie of *DR5rev:VENUS-N7* expression in 3-day-old Col-0 seedlings treated with Mock, 10μM NPA, 0.3μM BUM, and 3 μM BZ-IAA for 18 hours. Movie is at 30 frames per second. Scale bar, 0.5 mm.

**Movie S9.** Movie of *DR5:Luciferase* expression in a 3-day-old Col-0 seedling treated with Mock, 10μM NPA, 0.3μM BUM, and 3 μM BZ-IAA for 20 hours. Movie is at 24 frames per second. Scale bar, 0.1 cm.
References and Notes


12. W. Xuan, D. Audenaert, B. Parizot, B. K. Möller, M. F. Njo, B. De Rybel, G. De Rop, G. Van Isterdael, A. P. Mähonen, S. Vanneste, T. Beeckman, Root cap-derived auxin pre-


