Figures and figure supplements

Two mechanisms regulate directional cell growth in Arabidopsis lateral roots

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Figure 1. Inhibition of RAB-A5c function caused increased anisotropy of the CMT array in meristematic cells. (A,B) Maximum intensity projections of confocal stacks of lateral roots co-expressing YFP:NPSN12 (top) and RFP:TUB6 (middle) either in wild-type (A) or in a RPS5a > Dex > RAB-A5c[N125I].
line (B) three days after seedlings were transferred to agar plates containing 20 μM Dex. Bottom image shows merge of both channels as well as manually drawn cell outlines (yellow) and vectors indicating mean orientation and degree of anisotropy of the CMT array in each cell as measured with FibrilTool (Boudaoud et al., 2014). (C) Plot showing anisotropy of CMTs in meristematic cells from lateral roots like those shown in (A) for wild-type (n = 114 cells) and RPS5a > Dex > RAB-A5c[N125I] line 1 (n = 43 cells) and line 2 (n = 41 cells). Note 0 corresponds to a fully isotropic array, one to a completely parallel (anisotropic) array. Mean CMT array anisotropy was significantly increased in the presence of RAB-A5c[N125I] (Welch’s t-test: p<0.001 (**)). Error bars are SD. (D) Rose diagrams showing mean orientation of the CMT array in cells used in (C) relative to the longitudinal and transverse axes of the lateral root. (E,F) CMT orientation at inner vs. outer periclinal cell walls in wild-type (E) and RP55a > Dex > RAB-A5c[N125I] (F) roots. Epidermal meristematic cells expressing YFP:NPSN12 were segmented in 3D using MorphoGraphX (Barbier de Reuille et al., 2015), co-expressed RFP:TUB6 was projected onto the 3D cell mesh, and mean CMT orientation at inner and outer periclinal faces was quantified using with FibrilTool (Boudaoud et al., 2014). Seedlings were imaged 24 hr after transfer to agar plates containing 20 μM Dex. (G,H) Average time projections of maximum intensity projections of confocal stacks of lateral roots expressing mCherry:CESA1 in a wild-type (G) or RPS5a > Dex > RAB-A5c[N125I] background (H). Seedlings were imaged 24 hr after transfer to agar plates containing 20 μM Dex. Stacks were acquired in 10 s intervals, each time average projection is based on 60 stacks (corresponding to 10 min total imaging time). (I,J) Maximum intensity projections of confocal stacks expressing CLASP:GFP in the RP55a > Dex > RAB-A5c[N125I] background showing lateral roots 3 days after transfer to agar plates containing 0.1% DMSO (I) or 20 μM Dex (J). Cell walls were stained with Propidium Iodide (PI). (K,L) Maximum intensity projections of confocal stacks expressing GCP2:3xGFP in a wild-type (K) and RP55a > Dex > RAB-A5c[N125I] (L) background showing lateral roots 3 days after transfer to agar plates containing 20 μM Dex. Scale bars 10 μm. Cell walls were stained with Propidium Iodide (PI). All scale bars 10 μm.

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Figure 1—figure supplement 1. CMTs in the absence and presence of RAB-A5cNI. (A,B) Confocal stacks of lateral roots co-expressing YFP:NPSN12 and RFP:TUB6 either in wild-type (A) or in a RPS5a > Dex > RAB-A5c[N125I] line (B) three days after seedlings were transferred to agar plates.

Figure 1—figure supplement 1 continued on next page
containing 20 μM Dex. Note the phragmoplasts in dividing cells (arrows). (C,D) Visualising microtubule orientation at inner versus outer periclinal cell walls using MorphoGraphX (as shown in Figure 1E,F). Epidermal meristematic cells expressing YFP:NPSN12 were segmented in 3D and co-expressed RFP:TUB6 was projected onto the 3D cell mesh to visualise CMT orientation at the outer (C) and inner (D) periclinal cell face. All scale bars 10 μm.

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Figure 1—figure supplement 2. CMT rearrangement occurs during early stages of RAB-A5c[N125I] induction. (A–F) Confocal stacks of seedlings expressing YFP:NPSN12 (red), RFP:TUB6 (green), and the Dex-inducible RAB-A5c[N125I]. Images were acquired 24 hr (A–C) and 44 hr (D–F) after induction. (G, H) Anisotropy index values of 24 and 44 hr DMSO, 300nM Dex, and 20μM Dex treatments. Asterisks indicate significant differences (***) compared to DMSO control. Images were acquired 24 hr (A–C) and 44 hr (D–F) after induction.
Figure 1—figure supplement 2 continued

Seedlings were transferred to agar plates containing 0.1% DMSO (A,D), 300 nM Dex (B,E), or 20 μM Dex (C,F). Left: confocal stack as snap shots from MorphoGraphX, right: the same confocal stack with a heat map and vector (green) overlay displaying CMTanisotropy quantified using the fibril orientation plugin in MorphoGraphX (0 corresponds to a fully isotropic array, one to a completely parallel (anisotropic) array). Note the incomplete cell divisions (arrows). Scale bars 20 μm. (G,H) Mean anisotropy of cortical microtubules in meristematic cells from lateral roots shown in (A–C) (G) and (D–F) (H). n ≥ 88 cells (G) and n ≥ 53 cells (H). Anisotropy is significantly increased in the presence of RAB-A5c[N125I] (two-way ANOVA and post hoc Tukey’s test: p<0.001 (***)).
Figure 1—figure supplement 3. GCP2 and RAB-A5c localisation in lateral roots. (A, B) Average time projections of maximum intensity projections of confocal stacks of lateral roots expressing mCherry:CESA1 in a wild-type (A) or RPS5a > Dex > RAB-A5cn[N125I] background (B). Seedlings were imaged 48 hr after transfer to agar plates containing 20 μM Dex. Stacks were acquired in 10 s intervals, each time average projection is based on 60 stacks (corresponding to 10 min total imaging time). Scale bar 5 μm. (C) Maximum intensity projections of confocal stacks of a primary root (top and bottom left insert) and a lateral root (middle and bottom right insert) expressing GCP2:3xGFP (Nakamura et al., 2010). Scale bars 10 μm. (D) Maximum intensity projection of a confocal stack of a lateral root co-expressing GCP2:3xGFP (top) and YFP:RAB-A5c (middle). Scale bars 10 μm and 5 μm (insets). (E, F) Plots showing mean intensity and reciprocal colocalisation of GCP2:3xGFP and YFP:RAB-A5c at transverse and longitudinal edges (n ≥ 23, edge width 2 μm). Error bars are SD.

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Figure 2. A 3D FE model of a meristematic root cell. (A) Morphology of the uninflated model from the outside (left) and in section (right) of a cell with isotropic CMFs at all faces (left, 1), isotropic CMFs at transverse anticlinal and the outer periclinal face, and anisotropic CMFs at longitudinal anticlinal and the inner periclinal face (middle, 2), and isotropic CMFs at transverse anticlinal, and anisotropic CMFs at longitudinal anticlinal and periclinal faces (right, 3). Edges are colour-coded in red, isotropic faces in grey, and anisotropic faces in teal. R: radial, L: longitudinal, T: transverse. (B,C) Effect of selective increase or reduction by factor 10 of shear moduli shear moduli $\mu_{\text{edges}}$, $\mu_{\text{faces}}$, and $\mu_{\text{c}}$ at cell edges ($\mu_{\text{edges}}$) compared to shear moduli at faces ($\mu_{\text{faces}}$) on cell morphology for cells in which CMFs were oriented as described in 1, 2, and 3 in (A). Cells are colour coded for displacement. (B) idealised cases Figure 2 continued on next page
Figure 2 continued

with FA of 0 (fully isotropic) and 1 (fully anisotropic); (C) cases with FA corresponding to experimentally determined CMT orientation, with FA = 0.08 corresponding to the isotropic and FA = 0.2 corresponding to the anisotropic case. At faces, $K = 10$ GPa, $\mu_m = 18$ MPa, $\mu_i = 1.2$ GPa, $\mu_c = 18$ MPa and $\nu_i = 0.5$ in all cases. (D,E) Plots showing maximum displacement of cell models as those shown in (B,C).

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Figure 2—figure supplement 1. Stress distribution in *in silico* meristematic cells. (A,B) Effect of selective increase or reduction of the shear moduli at cell edges ($\mu_{\text{edges}}$) compared to shear moduli at faces ($\mu_{\text{faces}}$) on stress distribution for simulations shown in Figure 2B,C.

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Figure 2—figure supplement 2: Effect of varying model parameters. (A,B) Effect of variation in fibre fraction $\alpha_f$ on cell morphology when shear moduli at cell edges ($\mu_{\text{edges}}$) were selectively increased or decreased compared to shear moduli at faces ($\mu_{\text{faces}}$) for cells in which CMFs at all faces were anisotropic (FA=1).

C $\mu_c$ (Mpa): isotropic 0 9 18 36

D $\mu_c$ (Mpa): isotropic 0 9 18 36

E For faces isotropic: $\mu_m = 18$ MPa, $\mu_f = 1.2$ GPa, $K = 10$ GPa

For faces anisotropic: $\mu_m = 18$ MPa, $\mu_f = 1.2$ GPa, $K = 10$ GPa

5-fold smaller
5-fold larger

Figure 2—figure supplement 2 continued on next page
oriented isotropically (top), and cells in which CMFs at longitudinal anticlinal and periclinal faces were anisotropic in transverse orientation (bottom).

Cells are colour coded for displacement, maximum displacement for each simulation is displayed in white for each cell. \( \nu_f \) was either 0.25 (A) or 0.5 (B); at faces, \( K = 10 \text{ GPa} \), \( \mu_m = 18 \text{ MPa} \), \( \mu_c = 1.2 \text{ GPa} \), \( \mu_c = 18 \text{ MPa} \) in all cases. (C,D) Effect of variations in the cross-linking shear modulus \( \mu_c \). (C) idealised cases with FA of 0 (fully isotropic) and 1 (fully anisotropic) as shown in (B); (D) cases with FA corresponding to experimentally determined CMT orientation, with FA = 0.08 corresponding to the isotropic and FA = 0.2 corresponding to the anisotropic case. At faces, \( K = 10 \text{ GPa} \), \( \mu_m = 18 \text{ MPa} \), \( \mu_c = 1.2 \text{ GPa} \), and \( \nu_f = 0.5 \) in all cases. (E) Effect of variations in \( \mu_m \), \( \mu_c \), and \( K \) for idealised cases with FA of 0 (fully isotropic) and 1 (fully anisotropic) as shown in (B).

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Figure 3. Phenotypic interactions between microtubule mutants and RAB-A5c[N125I]. (A–H) Confocal stacks of lateral roots expressing RPS5a > Dex > RAB-A5c[N125I] in wild-type (A,C,D,E,G), erh3-3 (B), pom2-4 (F), or clasp-1 (H) backgrounds; 3 days after transfer to 0.1% DMSO (left) or 1 μM Dex (right). Figure 3 continued on next page.
Figure 3 continued

(right), in (D), plates additionally contained 250 nM Ory. Cell walls were stained with Propidium Iodide (A,B,E–H), or cell outlines were visualised using the plasma membrane marker YFP NPSN12 (C,D). Images are snapshots from MorphoGraphX or maximum intensity projections of confocal stacks. Scale bars 50 μm. (I–L) Mean diameter of lateral roots such as those shown in (A–H) (n ≥ 21). Difference in diameter (%) between DMSO and 1 μM Dex treatments for each genotype noted above respective columns. Two-way ANOVA and post hoc Tukey’s test: same letter indicates no significant difference in relative diameter increase (p≥0.05), different letters indicate significant difference (p<0.05).

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Figure 3—figure supplement 1. Synergistic effect of microtubule inhibitors and RAB-A5c[N125I] expression on lateral root morphology. (A,B) Maximum intensity projections of confocal stacks of lateral root meristematic cells expressing YFP:NPSN12 (green) and RFP:TUB6 (red) in a wild-type (A, Figure 3—figure supplement 1 continued on next page
C,E) and RPS5a > Dex > RAB-A5c[N125I] (B,D,F) background after 24 hr induction with Dex in the absence (A,B) or presence of taxol (C,D) or oryzalin (E,F). Scale bars 5 μm. (C,H,I) Maximum intensity projections of confocal stacks from lateral roots expressing YFP:NPS12 in a RPS5a > Dex > RAB-A5c [N125I] background grown for 3d in the absence (G) or presence of taxol (H) or oryzalin (I) and the presence of subsaturating (middle) or saturating (bottom) concentrations of Dex. Scale bars 50 μm. (J–L) Mean diameter of lateral roots as those shown in (G–I) (n ≥ 21). Differences in diameter (%) between treatments noted above respective columns. Two-way ANOVA and post hoc Tukey’s test: same letter indicates no significant difference in relative diameter increase (p>0.05), different letters indicate significant difference (p<0.01).

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Figure 4. YFP:RAB-A5c localisation and CMT organisation. (A) Maximum intensity projections of 3D confocal stacks from lateral roots expressing YFP: RAB-A5c (left) and RFP:TUB6 (middle). Scale bar 20 μm. (B,C) Maximum intensity projections of 3D confocal stacks from lateral roots expressing YFP: Kirchhelle et al. eLife 2019;8:e47988. DOI: https://doi.org/10.7554/eLife.47988

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Figure 4 continued

RAB-A5c (left) and RFP:MBD (middle). Scale bars 5 μm. White arrows indicate YFP:RAB-A5c compartments at cell faces co-localising with RFP:MBD, blue arrows indicate CMTs at cell edges not labelled with YFP:RAB-A5c, white arrowheads indicate preprophase bands. (D) Schematic summary of RAB-A5c localisation in relation to microtubule localisation at different stages of the cell cycle based on images such as those shown in (A–C) and S6. (E–H) Maximum intensity projections of confocal stacks of lateral roots expressing YFP:RAB-A5c either in wild-type (E), clasp-1 (F), erh3-3 (G), and spr3 (H) backgrounds. Cell walls were stained with propidium iodide (PI). Scale bars 10 μm. (I,J) Plots showing relative enrichment of YFP:RAB-A5c intensity at an edge normalised against mean edge intensity in the respective cell (I) and mean transverse and longitudinal edge length in those cells (J) for lateral roots like those shown in (E–H). (n ≥ 103 cells for each genotype; two-way ANOVA and post hoc Tukey’s test: same letter indicates no significant difference (p>0.05), different letters indicate significant difference (p<0.01).

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Figure 4—figure supplement 1. YFP:RAB-A5c and RFP:TUB6 dynamics during lateral root development. (A) Maximum intensity projections of a 4D confocal stack from a lateral root expressing YFP:RAB-A5c (green) and RFP:TUB6 (red) at consecutive 40 min time intervals. (B) Close-up of the box shown in (A), as well as intermediate time-points (20 min, 60 min, 100 min, 140 min) following one cell’s progression through the cell cycle. Scale bars 20 μm (A) and 5 μm (B).

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Figure 4—figure supplement 2. YFP:RAB-A5c localisation in microtubule mutants. (A,B) Plots as those shown in Figure 4 showing relative enrichment of YFP:RAB-A5c intensity at an edge normalised against mean edge intensity in the respective cell (Figure 4I) and mean transverse and longitudinal edge length in those cells (Figure 4J). Plots differentiate between cells of different roots (n ≥ 3) and left and right longitudinal and apical and basal transverse walls, respectively.

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Figure 5. Two mechanisms drive growth anisotropy in meristematic lateral root cells. (A,B) Snapshots from time-lapse confocal series of two lateral roots expressing YFP:NPSN12 at 0 hr (top) and 6 hr (middle, bottom). Epidermal cells were segmented in 3D using MorphoGraphX (Barbier de Reuille et al., 2015) and are colour-coded for absolute volume (top, middle) or relative volume growth (bottom). (C) Boxplot showing relative volume (vol), longitudinal (long), radial (rad), and circumferential (circ) growth for cells shown in (A,B). Cells were split up in two populations based on volume growth to separate slow-growing meristematic cells (growth <1.5, n = 51) from more rapidly growing elongation zone cells (growth >1.5, n = 31). Two-way ANOVA and post hoc Tukey’s test: same letter indicates no significant difference (p >0.05), different letters indicate significant difference (p<0.01). Note that while cells grow substantially faster once they have entered the elongation zone, growth is predominantly longitudinal in both meristematic and elongation zone cells, and there is no significant difference in radial or circumferential growth between both cell populations. (D) Proposed model of growth anisotropy regulation in epidermal lateral root cells. Top: in wild-type meristematic cells, CMTs (red) and consequently CMFs (grey) are largely isotropic. Growth anisotropy is predominantly conferred through local modification of cell wall properties at edges (green) through a RAB-A5c-dependent trafficking pathway. In elongation zone cells, CMTs and CMFs are aligned transverse anisotropic, further contributing to anisotropic growth. Figure 5 continued on next page.
Middle/Bottom: inhibition of RAB-A5c function through RAB-A5c[N125I] abolishes local modification of cell wall properties at edges, leading to the lack of a mechanism promoting anisotropic growth in meristematic cells. This can be partially compensated through premature transverse alignment of CMTs, leading to moderate swelling of cells. If compensatory CMT alignment is prevented through genetic or pharmacological means, cells lack both mechanisms promoting growth anisotropy, and cells swell dramatically.

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Figure 5—figure supplement 1. Cell growth rates in lateral roots. (A) Plot showing relative volume growth for roots shown in Figure 5A along the longitudinal axis of the roots. The dotted line corresponds to 1.5-fold volume growth, which was identified manually as the point where cells entered into rapid elongation.

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Figure S—figure supplement 2. YFP:RAB-A5c localisation in young primary roots. (A–C) Maximum intensity projections of confocal stacks from 2d old seedlings expressing YFP:RAB-A5c (left) and mCherry:NPSN12 (middle). (B) Arrows indicate cell plates. Note that edge label is most prominent in epidermal cells not covered by the lateral root cap. (C) Boxed area in (B). Arrowheads, YFP:RAB-A5c labelled edges. Scale bars 50 μm (A,B) or 20 μm (C).

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