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ARTICLES

Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal

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Re-orientation of *Arabidopsis* seedlings induces a rapid, asymmetric release of the growth regulator auxin from gravity-sensing columella cells at the root apex. The resulting lateral auxin gradient is hypothesized to drive differential cell expansion in elongation-zone tissues. We mapped those root tissues that function to transport or respond to auxin during a gravitropic response. Targeted expression of the auxin influx facilitator AUX1 demonstrated that root gravitropism requires auxin to be transported via the lateral root cap to all elongating epidermal cells. A three-dimensional model of the root elongation zone predicted that AUX1 causes the majority of auxin to accumulate in the epidermis. Selectively disrupting the auxin responsiveness of expanding epidermal cells by expressing a mutant form of the AUX/IAA17 protein, axr3-1, abolished root gravitropism. We conclude that gravitropic curvature in *Arabidopsis* roots is primarily driven by the differential expansion of epidermal cells in response to an influx-carrier-dependent auxin gradient.

Gravity represents an important environmental signal for plants that profoundly influences their growth and development[1–5]. Roots sense changes in their orientation using specialized gravity-sensing cells that are located within the columella root cap[6–9] (Fig. 1a). They correct changes in their orientation relative to gravity by means of a differential growth response that is termed root gravitropism; cell expansion on the lower side of the elongation zone is reduced relative to the upper side, which causes the root to bend downwards[10]. The root columella and elongation-zone tissues, that respectively sense[6–9] and respond[10] to the gravity stimulus, are spatially distinct (Fig. 1a), necessitating the transmission of a gravitropic signal(s). The plant growth regulator auxin represents a strong candidate for providing such a gravitropic signal, as a large number of auxin transport and response mutants exhibit root gravitropic defects[11–20]. Auxin transport components that are functionally required for root gravitropism include the auxin efflux facilitator PIN3; the gravity-induced retargeting of PIN3 to the lower face of columella cells helps create the initial lateral auxin gradient[16]. Similarly, the expression pattern of the auxin influx and efflux facilitators AUX1 (ref. 11) and PIN2 (AGR1/EIR1/WAV6)[12–15] seem to channel auxin from the root cap to the elongation zone. Nevertheless, it remains unclear whether auxin represents the gravitropic signal that acts directly on the elongation-zone cells or whether this involves other signalling intermediates[21–24]. Indeed, signalling molecules, such as cytokinin and nitric oxide, have recently been reported to be asymmetrically redistributed[25] or functionally required[26] during a root gravitropic response.

Auxin-responsive reporters have been used successfully to visualize dynamic asymmetric changes in gravity-sensing tissues[13,27–29], which is consistent with the creation of a lateral auxin gradient. However, determining whether auxin acts as the gravitropic signal has, so far, been hampered by the insensitivity of auxin-responsive reporters to visualize dynamic expression changes in gravity-responsive elongation-zone tissues. We have adopted an alternative approach to address this important issue by directly manipulating the ability of selected root tissues to transport and/or respond to the auxin signal using a targeted expression approach[30], followed by monitoring the phenotypic effects on root gravitropism. Tissues were selected for manipulation based, in part, on computer simulations of auxin flux in a three-dimensional model of the root. In this study, we provide definitive evidence that auxin acts as the intercellular gravitropic signal by functionally mapping the root tissues that transport and respond to auxin during a gravitropic response. Our results highlight the effectiveness of adopting a predictive biology-based approach that integrates modelling and experimental studies.

RESULTS

Root gravitropism requires AUX1 expression in the lateral root cap and epidermis

We initially addressed the question of whether root gravitropism requires auxin to be transported from the root cap to the elongation zone using the auxin influx facilitator AUX1 (ref. 31). Mutational studies had

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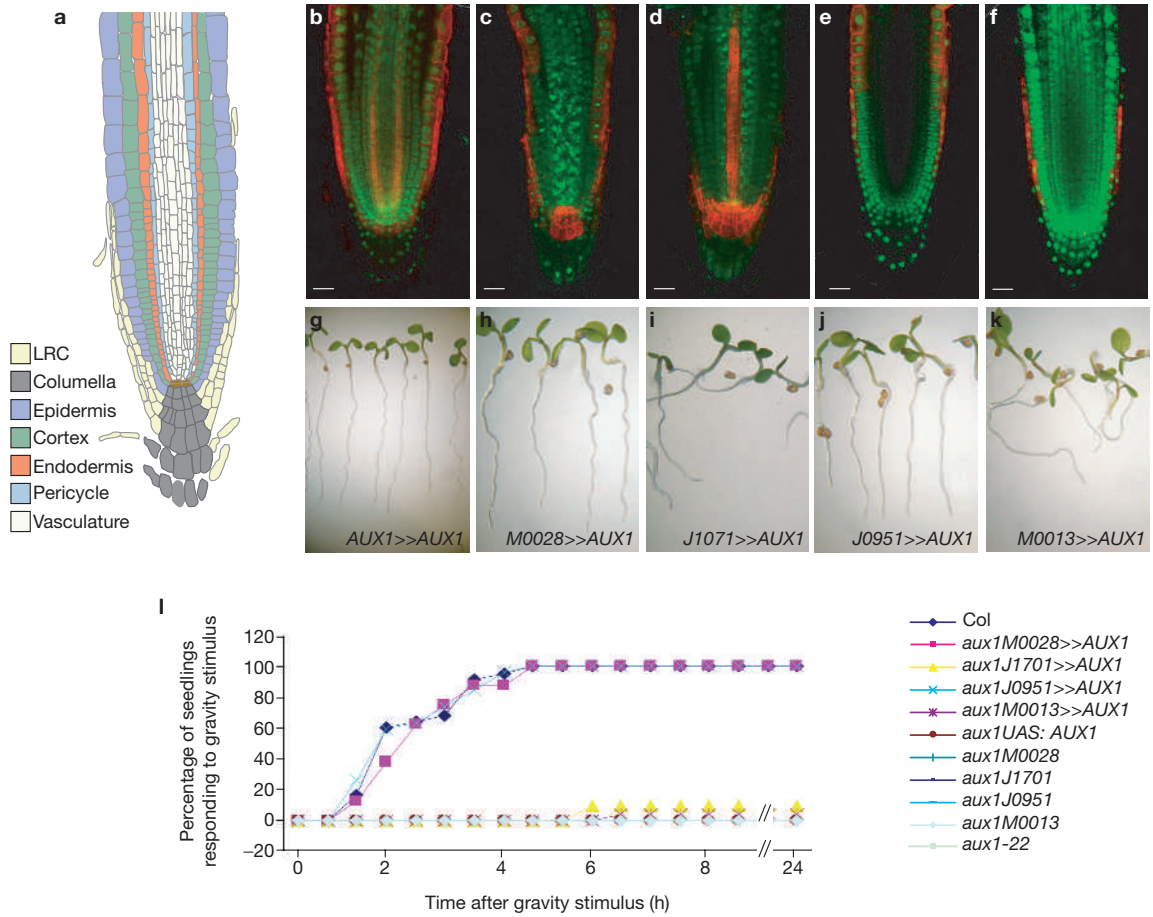

Figure 1 Mapping *Arabidopsis* root tissues that transport auxin during a gravitropic response. (**a**) Schematic diagram of tissues in the *Arabidopsis* root apex with colour-coded key. (**b**–**f**) Localization of HA-AUX1 (ref. 31) expression (red) in root tissues, counter stained with cytox green (green) of (**b**) *aux1,* AUX1>>HA-AUX1; (**c**) *aux1,* M0028>>HA-AUX1; (**d**) *aux1,* J1701>>HA-AUX1; (**e**) *aux1,* J0951>>HA-AUX1; and (**f**) *aux1,* M0013>>HA-AUX1. (**g**–**k**) Root gravitropic phenotypes of (**g**) *aux1,* AUX1>>HA-AUX1; (**h**) *aux1,* M0028>>HA-AUX1; (**i**) *aux1,* J1701>>HA-AUX1; (**j**) *aux1,* J0951>>HA-AUX1; and (**k**) *aux1,* M0013>>HA-AUX1. Note: Due to the requirement for limited proteolysis in the whole-mount immunolocalization procedure that was used[31], the signal for AUX1 (red) in columella and lower lateral root cap (LRC) cells seems to be less than expected compared with an AUX1–YFP fusion protein[48]. (**l**) Kinetics of root bending for selected wild-type Col., *aux1* and *aux1* GAL4>>AUX1 lines.

previously revealed that *Arabidopsis* root gravitropism required AUX1 (ref. 11). Given the broad pattern of AUX1 expression in stele, columella, lateral root cap (LRC) and epidermal tissues[31] (Fig. 1b), we attempted to determine which of these root tissues requires the auxin influx facilitator to mediate gravitropic signalling. This was performed by targeting the expression of AUX1 to specific tissue domains in *aux1* roots and examining the rescue of the mutant's agravitropic root phenotype (Fig. 1 and see Supplementary Information, Fig. S1). The targeted expression of a functional HA-epitope-tagged *AUX1* sequence (*HA-AUX1*)[31] was achieved using a GAL4-based transactivation expression approach[30]. The HA-AUX1 coding sequence was fused to the GAL4 recognition motif (termed the upstream activating sequence [UAS]), and then transformed into a null *aux1* background (see Methods). The *aux1, UAS:HA-AUX1* line was initially crossed with an $AUX1_{pro}$*:GAL4* line (also in an *aux1* mutant background) to demonstrate that transactivation did not interfere with AUX1-dependent gravitropic bending. Localization and gravitropic assays revealed that F1 progeny (termed *aux1*, AUX1>>AUX1) correctly expressed HA-AUX1 in stele, columella, LRC and epidermal tissues (Fig. 1b) and fully restored the *aux1* root gravitropic defect (Fig. 1g).

The *aux1, UAS:HA-AUX1* transgenic line was crossed with a selection of tissue-specific GAL4 driver lines (see http://www.plantsci.cam.ac.uk/Haseloff/geneControl/GAL4Frame.html; these driver lines had also been introgressed (a backcrossing method) into an *aux1* mutant background) in an attempt to rescue the mutant phenotype (see Fig. 1). Expressing HA-AUX1 in columella, LRC and epidermal tissues (Fig. 1c; M0028>>AUX1) successfully rescued *aux1* root gravitropism (Fig. 1h). By contrast, expressing HA-AUX1 in stele and columella tissues (J1701>>AUX1; Fig. 1d) did not rescue *aux1* gravitropism (Fig. 1i). Our M0028>>AUX1 results highlight the functional importance of basipetal (that is, root apex to base) auxin transport during a root gravitropic response. We next defined the domains of root tissues that are required to express AUX1 to rescue the *aux1* phenotype. Expressing AUX1 in LRC and expanding epidermal tissues (Fig. 1e; J0951>>AUX1) rescued *aux1* root gravitropism (Fig. 1j). By contrast, expressing AUX1 in just the LRC (M0013>>AUX1; Fig. 1f) was not able to rescue *aux1* root gravitropism (Fig. 1k). Our targeted expression results are consistent with auxin acting as the intercellular signal that requires AUX1 to facilitate its transport via LRC and expanding epidermal cells during a gravitropic response.

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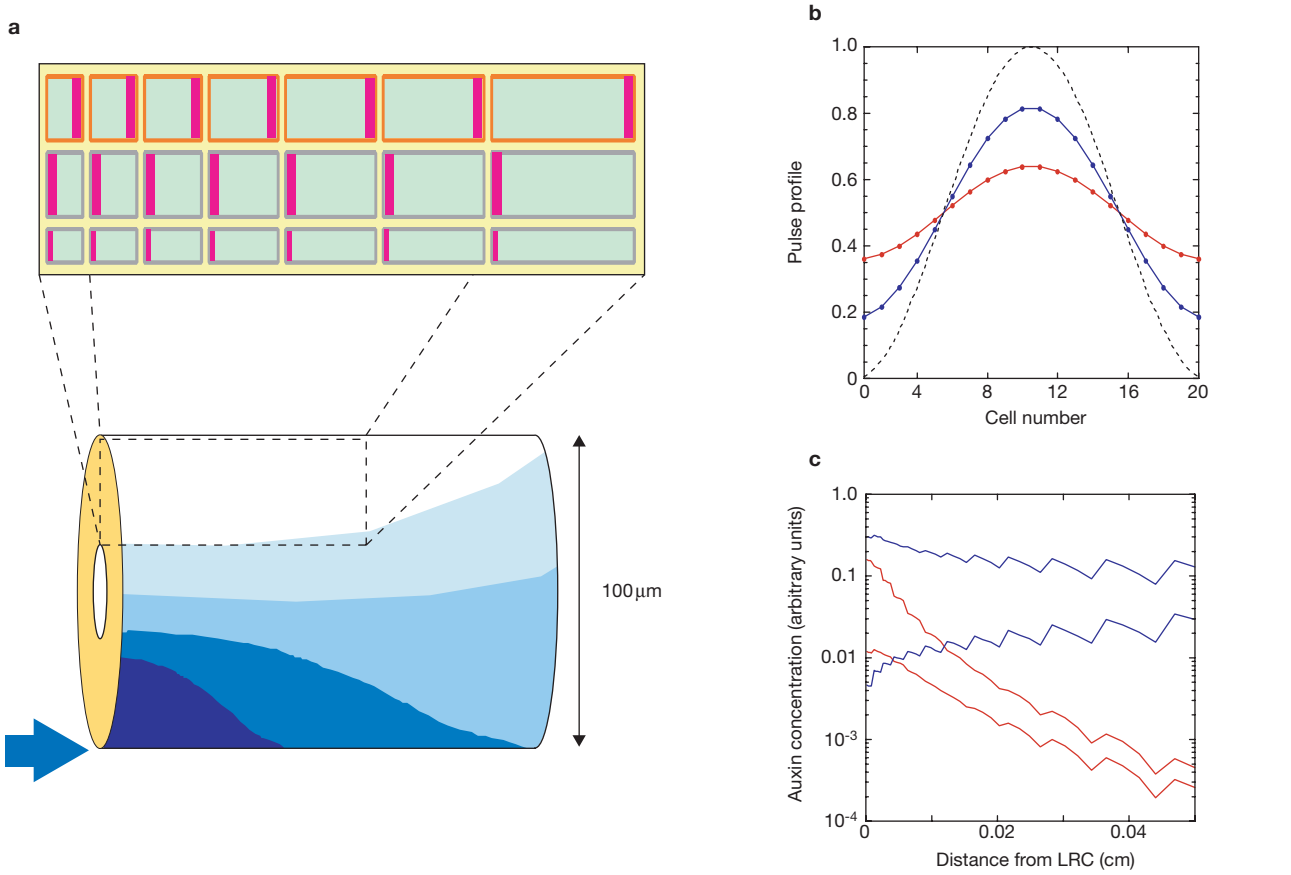

Figure 2 Modelling carrier-mediated transport of the lateral auxin gradient in the elongation zone. (**a**) Sketch showing a cylindrical section of the root elongation zone. During a gravistimulus, the lateral root cap (LRC; at left, not shown) deposits auxin into the lower side of the epidermis (blue arrow). Active transport then carries auxin basipetally. Shades of blue approximate the auxin concentration in the epidermis. Note that, from left to right, diffusion tends to smooth out the lateral gradient and also to reduce the overall auxin content. Inset: sketch of the model geometry, showing the three cell layers (top to bottom: epidermis, cortex and endodermis) and carrier localization. Green: cytoplasm; yellow: apoplast; grey: plasma membrane without carriers; pink: PIN2 in epidermis and cortex[14] plus weak PIN1 expression in epidermis, cortex and endodermis[32]; orange: AUX1 in epidermis[31]. (**b**) Cytoplasmic auxin concentration in all 20 epidermal cells around the circumference of the outer root. Dashed line is the profile of the auxin source applied at the LRC. Solid lines are profiles in the elongation zone, after 500 µm of basipetal transport, for the wild type (red) and *aux1* mutant (blue). Each profile is normalized to a mean of 0.5. (**c**) Cytoplasmic auxin concentration along the length of the model wild type root (blue) and *aux1* root (red). The upper red and blue lines denote auxin concentrations on the bottom side of the root, whereas the lower red and blue lines denote auxin concentrations along the top side.

Our targeted expression results indicate that the root gravitropic response is dependent on AUX1 LRC and epidermal (but not columella) expression domains. Nevertheless, it remains possible that AUX1 columella expression is required during the early root bending response, given that the lateral auxin gradient is generated in this tissue[13,27–29]. Root curvature was measured every 30 min following a 90° gravity stimulus in wild-type and *aux1* J0951>>AUX1 roots expressing AUX1 in LRC and epidermal tissues (Fig. 1l and see Supplementary Information, Fig. S1). Our measurements revealed that the kinetics of the root gravitropic bending response in *aux1*, J0951>>AUX1 roots was as fast as in the wild type (Fig. 1l). Hence, AUX1 columella expression was not required for root gravitropism. Instead, our targeted expression studies demonstrated that root gravitropism required AUX1 expression in just LRC and epidermal tissues, presumably to facilitate the delivery of the gravity-induced lateral auxin gradient to elongation-zone tissues.

Root gravitropism is dependent on AUX1 epidermal expression throughout the elongation zone

As auxin moves through the elongation zone, diffusion around the circumference will diminish the lateral gradient, and radial diffusion will recycle auxin from the epidermis back to the stele[32]. The auxin influx and efflux facilitators AUX1 (ref. 11) and PIN2 (refs 12–15) may function to minimize the effect of radial diffusion as well as facilitating basipetal auxin transport (that is, root apex to base; Fig. 2a). We probed the functional importance of AUX1 and PIN on the gravity-induced auxin pulse using a three-dimensional model of the *Arabidopsis* root elongation zone (see Methods; and see Supplementary Information text for further details). The model simulated the outer three elongation-zone tissues, encompassing ~1,200 cells and incorporating known auxin-carrier expression and localization patterns (Fig. 2a): PIN2 in the epidermis and cortex[14]; weak PIN1 expression in the epidermis, cortex and endodermis[32]; and AUX1 in the epidermis[11]. For the tissues under study, other members of the *Arabidopsis AUX1* and *PIN* gene families are not included in our gravitropic model based on their measured expression patterns and gravitropic phenotypes of knockout mutants (R.S. and M.J.B., unpublished observations)[32].

We simulated the gravitropic signal by supplying an auxin asymmetry (a higher concentration at the bottom) to the apical end of our virtual root model (Fig. 2a, b), then monitoring the movement of the lateral auxin gradient through the elongation-zone tissues (Fig. 2c and see

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Table 1a Characteristics of auxin transport in the epidermis of WT, *aux1*, *pin1* and *pin1pin2* mutants

	Lateral auxin gradient in CEZ (c_{bottom}/c_{top})	Fraction of all auxin in the apoplast	Fraction of auxin pulse that reaches the CEZ
WT	4.41	0.06	0.54
aux1	1.77	0.51	0.002
pin2	3.90	0.017	0.49
pin1pin2	NA	0.004	$< 10^{-8}$

The central elongation zone (CEZ) is here defined to be the portion of the root 500 μm behind the lateral root cap.

Table 1b Cytoplasmic auxin concentration ratios showing the partitioning of auxin between the three cell layers of the outer root

	Epidermis	Cortex	Endodermis
WT	21.4	1.73	1
aux1	1.55	0.78	1
pin2	75.7	4.16	1
pin1pin2	NA	NA	NA

Values normalized to the concentration in the endodermis.

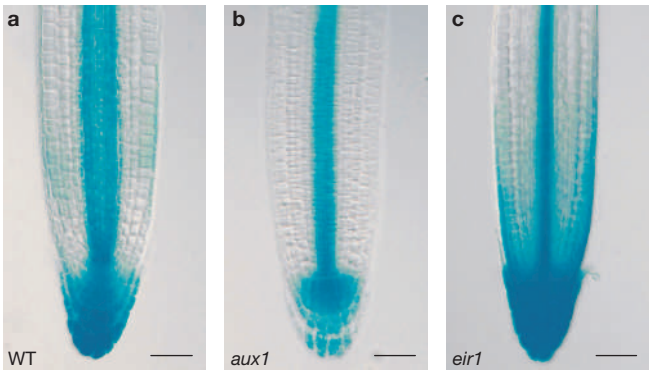

Figure 3 Auxin-responsive *IAA2:uidA* reporter expression. *IAA2:uidA* reporter expression in (**a**) wild-type (WT), (**b**) *aux1* and (**c**) *eir1/pin2* backgrounds. Note that the *IAA2:uidA* reporter was undetectable in lateral root cap cells of *aux1*, but was stained more intensely in *eir1/pin2* compared with the wild type. Scale bar, 20 μm.

Supplementary Information, Fig. S2). Our modelling studies indicate that the wild-type root epidermis is competent to transport the auxin pulse with only moderate diffusion (Fig. 2b, c). Wild-type root epidermal cells can retain more than 40% of the initial auxin asymmetry 500 μm into the elongation zone (Table 1a), and can maintain the lateral auxin gradient at least 2 mm from the root apex, which is consistent with auxin transport measurements[33]. Our simulations reveal that the *pin2* mutant root is still able to transport the auxin pulse in elongation-zone tissues (Table 1a) due to weak epidermal PIN1 expression[32], which is consistent with auxin transport measurements[27]. Instead, we predict that the *pin2* agravitropic phenotype results from a block in the auxin efflux activity of LRC cells (where PIN1 is not expressed)[34], which is consistent with elevated auxin-responsive reporter expression in the *pin2* LRC cells (Fig. 3c). Model roots lacking AUX1 are not competent to transport the auxin pulse (Table 1a), which is in agreement with the reduced root basipetal auxin transport[27], altered pattern of auxin-responsive reporter expression (Fig. 3b) and agravitropic root phenotype in the mutant[11]. In the absence of influx-carrier activity, the majority of auxin remains in the apoplast (Table 1a), where it is relatively free to diffuse back to the pericycle[32,35]. Apoplastic diffusion significantly diminishes the scale of the auxin pulse that is delivered to the *aux1* central elongation zone (Table 1a), resulting in a shallow auxin gradient that is insufficient to drive a differential growth response in the *aux1* root (Fig. 2b, c).

Simulations using the virtual root model predict that auxin levels drop rapidly (by at least two orders of magnitude) in the basal half of the *aux1* elongation zone (Fig. 2c). Such a reduction in auxin levels is likely to impact the rate of growth as expanding cells progress through the *aux1* elongation zone, prompting us to perform kinematic analysis (see Methods)[36] to provide detailed measurements of root growth at different positions within the *aux1* elongation zone (Fig. 4). These studies revealed that the velocity profile in the *aux1* distal elongation zone was equivalent to that in the wild type, but was curtailed from the central elongation zone onwards, corresponding with the region where the LRC is absent (Fig. 4a). Plotting the derivative of velocity values in Fig. 4a to provide relative expansion rates reveals that *aux1* cells do not expand to the same extent as does the wild type (Fig. 4b). However, a rate of growth almost the same as that in the wild type could be restored in the *aux1* elongation zone by expressing AUX1 in LRC/expanding epidermal cells using the GAL4 driver line J0951 (Fig. 4a, b). Our kinematic results highlight the importance of AUX1 epidermal expression to auxin-regulated root growth, as expanding cells migrate through the elongation zone.

Detailed growth measurements in *Arabidopsis* roots following a gravitropic stimulus have revealed that the initial curvature originated in the distal elongation zone[10]. The *Arabidopsis* primary root elongation zone contains ~16 rapidly expanding cells in each longitudinal file of cells[36]. We investigated how many of these expanding cells were required to be exposed to the lateral auxin gradient to result in a gravitropic bending response. This was determined by attempting to rescue *aux1* root gravitropism by expressing AUX1 in a variable number of expanding epidermal cells using the J1092 GAL4 driver line. Localization studies revealed that ~90% of J1092>>AUX1 F1 seedling roots expressed AUX1 in just columella and LRC tissues (Fig. 5a), but AUX1 was also expressed in up to five elongating epidermal cells in ~10% of roots (Fig. 5b). Nevertheless, gravitropic assays failed to observe any evidence of a root bending response in ~50 F1 seedlings (Fig. 5c, d). Gravitropic assays were subsequently performed on a larger number of F2 *aux1* J1092>>AUX1 seedlings ($n = 700$), which allowed identification of individuals that exhibited a wild-type gravitropic response (Fig. 5e, f). Localization studies revealed that gravitropic *aux1* J1092>>AUX1 seedlings strongly expressed AUX1 in the LRC plus expanding epidermal cells throughout the elongation zone (Fig. 5g and see Supplementary Information, Fig. S3a). By contrast, agravitropic *aux1* J1092>>AUX1 seedlings strongly expressed AUX1 in the columella, LRC and less than five expanding epidermal cells (Fig. 5h and see Supplementary Information, Fig. S3b). We therefore concluded from our studies on F1 and F2 *aux1* J1092>>AUX1 seedlings that the root gravitropic response requires AUX1 expression in every expanding epidermal cell, thereby ensuring that the lateral auxin gradient persists as it migrates through the elongation zone and drives asymmetric root growth.

The epidermal auxin response is essential for differential root growth

Simulations using our root elongation zone model suggest that 10–20 times more of the lateral auxin gradient accumulates in the

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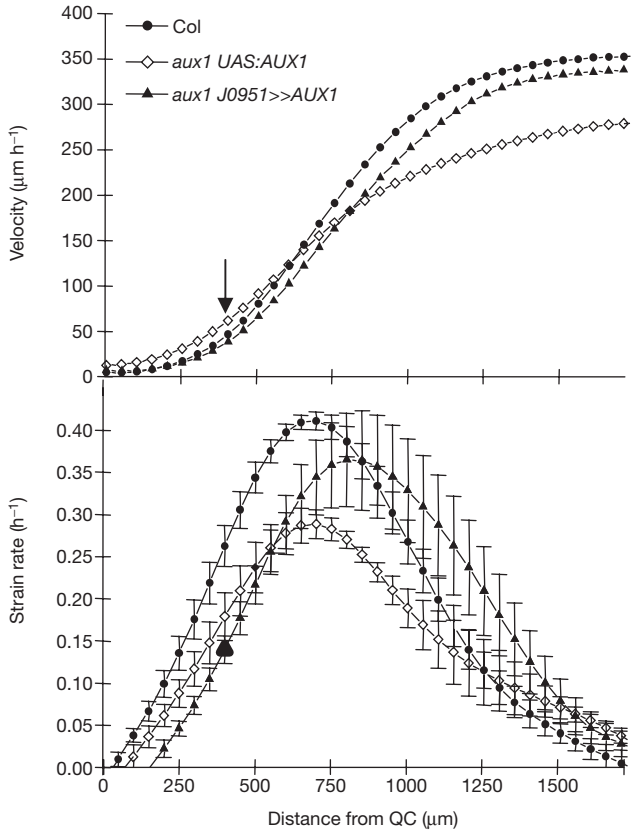

Figure 4 Kinematic analysis of AUX1-dependent root growth. Profiles of velocity (**a**) and relative rates of cell expansion (**b**) along the root elongation zone of the wild type (circle), *aux1* (diamond) and *aux1* J0951>>AUX1 (triangle) obtained from kinematic analysis[36] (see Methods). Note that the growth rates in *aux1* are similar in the apical part of the root, but decrease below that of the controls from the end of the lateral root cap (denoted by the arrow). QC denotes quiescent centre at the root apex.

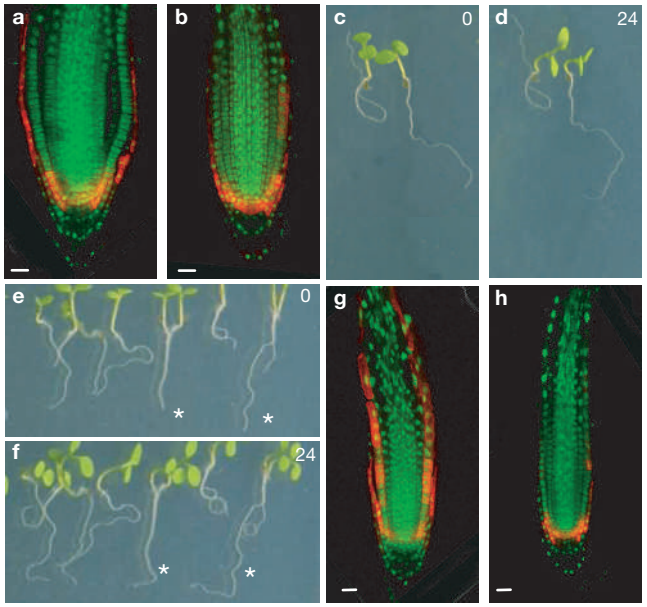

Figure 5 Root gravitropism requires AUX1 expression in all expanding epidermal cells. (**a**–**b**) Confocal images of F1 *aux1* J1092>>AUX1 seedlings expressing AUX1 (red) in (**a**) columella and lateral root cap and (**b**) up to five expanding epidermal cells in the elongation zone. Note that root tissues were counter-stained with cytox green (green). (**c**–**d**) All F1 *aux1* J1092>>AUX1 seedlings that were characterized were agravitropic (**c**) even 24 h after a gravity stimulus (**d**). (**e**–**f**) F2 *aux1* J1092>>AUX1 seedling roots were agravitropic (**e**) even 24 h after a gravity stimulus (**f**), with the exception of those seedlings denoted with an asterisk. (**g**–**h**) Localization studies revealed that (**g**) gravitropic F2 *aux1* J1092>>AUX1 seedling roots expressed AUX1 (red) in epidermal cells throughout the elongation zone, whereas (**h**) agravitropic F2 *aux1* J1092>>AUX1 seedling roots expressed AUX1 in only a few epidermal cells.

epidermis compared with the underlying cortical and endodermal tissues (Table 1b). The large difference in wild-type auxin distribution that was predicted to occur in inner versus outer root tissues primarily results from AUX1 epidermal expression, as the simulated ratio of cytoplasmic auxin concentrations in the root epidermis:cortex:endodermis of the *aux1* mutant was 1.55:0.78:1.00, respectively (Table 1b). Partitioning the lateral auxin gradient in wild-type roots in such a manner is predicted to result in root gravitropic bending being driven primarily by differential cell elongation in the epidermis.

We tested the predicted functional importance of the epidermis for root gravitropism by selectively disrupting the auxin response in this tissue using the axr3-1 protein[17,37]. The axr3-1 protein is able to disrupt auxin responses in a wide range of root tissues, including in the epidermis (Fig. 6c). For example, although external addition of the auxin indole-3-acetic acid (IAA) caused every root cell to express the auxin-responsive reporter *IAA2:GUS* (Fig. 6b), *axr3-1* significantly disrupted reporter expression in every root tissue (Fig. 6d). As an AUX/IAA protein[17], axr3-1 negatively regulates the activity of transcription factors termed auxin response factors[38] (ARFs), several of which have been demonstrated to regulate differential growth processes[39,40]. The ability of axr3-1 to disrupt ARF function was illustrated when an *UAS:axr3-1* line was expressed under the control of the GAL4 driver line J1701, resulting in a *root-less* phenotype (R.S. and M.J.B., unpublished observations), which mimicks the ARF5 mutant, *monopteros*[41]. The *UAS:axr3-1* line was next crossed with the epidermal- and/or LRC-expressed GAL4 driver lines J0951 and M0013 (Fig. 6e, f) in an attempt to disrupt auxin responses in these tissues. Consistent with this experimental goal, root bioassays revealed that J0951>>axr3-1 seedlings (but not *UAS:axr3-1*, J0951, M0013 or M0013>>axr3-1 lines) exhibited a growth-resistance phenotype to the auxins IAA and 2,4D (see Supplementary Information, Fig. S4).

Gravitropic assays revealed that disrupting the auxin response in LRC and expanding epidermal cells in J0951>>axr3-1 seedlings completely blocked root gravitropism (Fig. 6j and see Supplementary Information, Fig. S5). By contrast, expressing axr3-1 in just LRC cells in M0013>>axr3-1 seedlings did not affect root gravitropism (Fig. 6l). Kinetic studies revealed that the gravitropic defect in J0951>>axr3-1 seedlings was comparable to the *axr3-1* mutant phenotype (Fig. 6m). However, J0951>>axr3-1 exhibited only a small reduction in basal root elongation compared with its equivalent Col × C24 control (Fig. 6n; Student's *t*-test, $P = 0.0129$; see Supplementary Information, Table S1). Note that the genetic background of the GAL4 driver lines (C24) versus the *UAS:axr3-1* line (Col) had a far more significant effect on root growth (Fig. 6n; Student's *t*-test, $P = 9.08, 10^{-10}$; see Supplementary Information, Table S1). Our results demonstrate that disrupting auxin response in the expanding epidermal cells is sufficient to block differential growth during root gravitropism, without greatly affecting basal-cell elongation.

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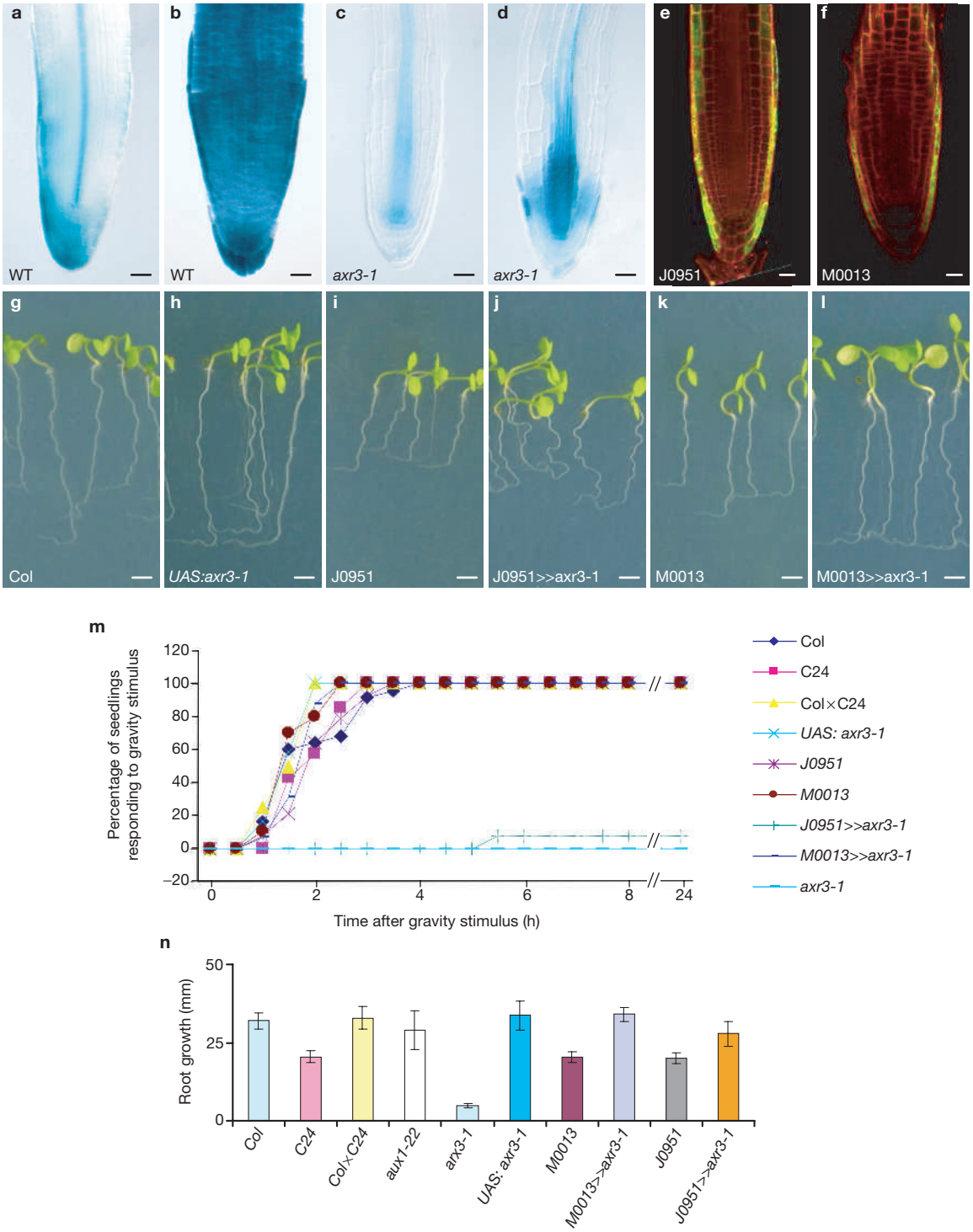

Figure 6 Mapping root tissues that respond to the lateral auxin gradient using axr3-1. (**a**–**d**) Auxin-responsive IAA2:GUS reporter[13,31] expression in (**a**, **b**) wild-type (WT) and (**c**, **d**) *axr3-1* backgrounds in response to endogenous (**a**, **c**) and external 50 μM IAA stimuli for 16 h (**b**, **d**). (**e**, **f**) Tissue-specific UAS–GFP expression (green) driven by GAL4 lines (**e**) J0951 in expanding epidermis plus lateral root cap (LRC); and (**f**) M0013 in LRC alone. Background root tissues are stained with propidium iodide. (**g**–**i**) Root growth behaviour of wild type (**g**), J0951 (**h**), UAS:axr3-1 (**i**) and J0951>>axr3-1 (**j**), M0013 (**k**) and M0013>>axr3-1 (**l**). (**m**) Kinetics of root bending for selected wild-type Col., *axr3-1* mutant, UAS:axr3-1 and M0013 and J0951 GAL4 driver lines, plus F1 seedlings expressing axr3-1, in epidermal and/or LRC tissues (J0951>>axr3-1 and M0013>>axr3-1), respectively. (**n**) Root growth measurements 7 d after germination for wild type (Col., C24 and F1 Col. × C24 control), *aux1* and *axr3-1* mutants, UAS:axr3-1 and M0013 and J0951 GAL4 driver lines, plus F1 seedlings expressing axr3-1, in epidermal and/or LRC tissues (J0951>>axr3-1 and M0013>>axr3-1), respectively. Error bars denote standard error. Statistical significance of differences observed are displayed in Supplementary Information, Table S1. Scale bars represent 20 μm for panels **a**–**f** and 200 μm for panels **g**–**l**.

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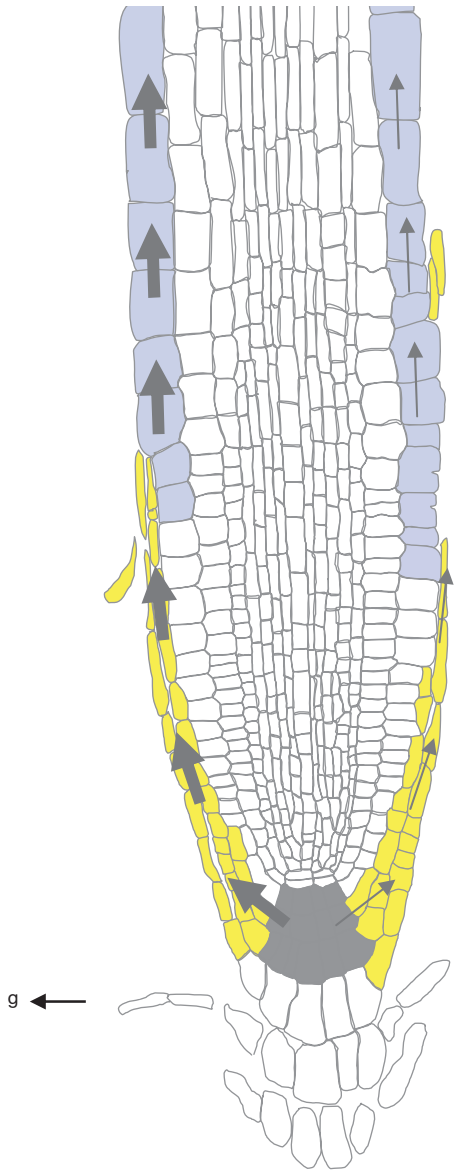

Figure 7 A schematic model for auxin-regulated root gravitropism. A gravity stimulus (denoted by **g**) causes auxin to be asymmetrically redistributed (denoted by arrow width) at the root apex due to the gravity-induced retargeting of PIN efflux facilitators, such as PIN3 (ref. 16), to the lower side of the gravity-sensing columella cells (coloured in grey). The resulting lateral auxin gradient is mobilized through the combined action of auxin influx and efflux facilitators AUX1 (ref. 31) and PIN2 (refs 14, 34) via lateral root cap cells (coloured in yellow) to expanding epidermal cells in the elongation zone (coloured in lilac). The lateral auxin gradient inhibits the expansion of epidermal cells on the lower side of the root relative to the upper side, causing a differential growth response that ultimately results in root curvature downwards.

DISCUSSION

For more than 80 years, auxin has been hypothesized to represent the signal that regulates gravitropic responses in plants[1–5]. Recent work has demonstrated gravity-induced changes in auxin distribution at the root apex[13,27–29]. This current study provides conclusive evidence that auxin functions as the primary gravitropic signal linking columella tissues that perceive a gravitropic stimulus and elongation-zone tissues that mediate the root bending response (Fig. 7). Our targeted expression experiments initially demonstrated that root gravitropism is dependent on the activity of the auxin-influx facilitator AUX1 in LRC and elongating epidermal cells (Fig. 1), which is consistent with the basipetal transmission of an auxin signal from the root tip to gravity-responsive tissues in the elongation zone[42] (Fig. 7). We subsequently demonstrated the functional importance of the auxin response in the elongation zone for root gravitropism through targeted expression of axr3-1 (Fig. 6). Auxin is therefore linked with the gravitropic functions of tissues that mediate the perception, transmission and response to a gravity stimulus, which is consistent with its role as the primary gravitropic signal.

The targeted expression approach has also enabled us to probe the gravitropic signalling function(s) of root apical tissues (Fig. 7). Our demonstration that root gravitropism requires expression of the auxin influx facilitator AUX1 by both the LRC and the epidermis (Fig. 1) implies that both tissues perform auxin transport functions. However, LRC cells do not seem to be required for the subsequent gravitropic response phase. Disrupting the auxin response in LRC cells had no effect on root gravitropism (Fig. 6m). Therefore, LRC cells seem to function primarily to facilitate the transmission of the auxin signal from columella to elongation-zone tissues. By contrast, disrupting the auxin response in expanding epidermal (plus LRC) cells was observed to abolish root gravitropism (Fig. 6m). Taken together, these results indicate that expanding epidermal cells are required to perform both auxin transport and response functions, whereas LRC is required for auxin transport following a gravitropic stimulus.

AUX1 (ref. 11) and PIN (refs 12–16) auxin influx and efflux facilitators are crucial for epidermal auxin transport function during a root gravitropic response. As only the epidermis expresses an auxin influx facilitator (AUX1) in the elongation zone[31], simulations indicate that this tissue accumulates significantly more auxin than the cortex and endodermis (Table 1b), which is in agreement with experimental observations[32,33,43]. Hence, the polar localization of PIN auxin efflux facilitators in the epidermis establishes the basipetal direction of flux through the outer root. Flux may be regarded as the product of two terms — a speed (set by the carrier permeabilities and the cell size) multiplied by a concentration. The relative depletion of auxin from the cortex and endodermis makes these tissues minor players in the auxin flux. Models that are run with different choices for the direction of efflux from cortical cells show only small changes in the auxin distribution (see Supplementary Information, Tables S2, S3). Thus, whereas PIN efflux facilitators establish the direction of transport, the AUX1 influx facilitator determines which cell types participate in the transport stream. There is a common misconception that, because protonated IAA is membrane permeable, influx carriers play only a supplementary role in auxin distribution. However, we estimate that cells expressing AUX1 can have a carrier-mediated IAA influx 15 times greater than the diffusive contribution (see Supplementary Information). In the absence of AUX1, the rate of IAA membrane diffusion is too slow for root gravitropism to occur. This biophysical constraint in the *aux1* mutant can be overcome through the addition of the membrane-permeable auxin 1-NAA (naphthalene-1-acetic acid), which rescues the gravitropic defect[44,45].

The fact that the majority of the lateral auxin gradient accumulates in the epidermis indicates that this is the root tissue that is vital for a gravitropic response. Indeed, results from our axr3-1 targeted expression studies clearly demonstrate that the auxin response in expanding epidermal cells is essential for the differential root growth response, gravitropism (Fig. 6). By contrast, the basal rate of root growth is only slightly reduced

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when the auxin response is disrupted in this tissue (Fig. 6n). These contrasting growth effects could be explained if the epidermis primarily regulates differential root growth, whereas basal root growth was driven by the expansion of the inner tissues. The idea that the basal rate of organ elongation is established by the inner tissues, rather than the epidermis, has received support from experiments with sunflower hypocotyls[46]. In such a model, the epidermis would function to restrain basal root growth in response to a lateral auxin gradient. As the outermost root tissue, the epidermis is ideally positioned to regulate bending[47]. Generating sufficient force to modify the pattern of growth of the underlying tissues is likely to necessitate the involvement of multiple epidermal cells in the elongation zone. Indeed, our J1092>>AUX1 results (Fig. 5) highlight the importance of an integrated differential growth response that involves multiple expanding epidermal cells that are resident in both distal and central elongation zones. □

METHODS

DNA constructs and transgenic materials. The *GAL4* recognition sequence *UAS* was sub-cloned from pIC–UAS–tNOS[30] (a gift from Dolf Weijers) into pBC (Stratagene, La Jolla, CA) to create pBC UAS. The *UAS:HA-AUX1* construct contained *UAS:HA-Aux1* and *UAS:uidA* cassettes, which were originally created as two separate constructs in pBC UAS. Full-length *GUS* gene (*uidA*) containing the 5′ untranslated region (UTR), and the *Nos* terminator and HA-tagged *AUX1* genomic sequence[31] containing the 5′ and 3′ UTRs, were fused downstream of the *UAS*. The resulting *UAS:uidA* and *UAS:HA-AUX1* cassettes were then cloned into a BIN19-based kanamycin-resistant plant transformation vector to create the final construct — *UAS:HA-AUX1*. The *axr3-1* sequence[17] was subcloned downstream of the *UAS* to create *UAS:axr3-1*. The *AUX1pro:GAL4* construct was created by cloning the *GAL4-VP16* (ref. 30) sequence (a gift from Dolf Weijers) into a BIN19-based kanamycin-resistant plant transformation vector, followed by insertion of a 2-kb *AUX1* promoter sequence upstream of the *GAL4* coding sequence. The *UAS:axr3-1* was transformed into the Col background, whereas *UAS:HA-AUX1* and *AUX1pro:Gal4* constructs were transformed into an *aux1* mutant background[48]. In parallel, existing tissue-specific-expressing GAL4 driver lines (see Fig. 1) were introgressed into an *aux1* mutant background. GAL4-dependent transactivation of *UAS:HA-AUX1* was verified by GUS staining (as the *UAS:HA-AUX1* construct also encoded an *UAS:uidA* gene), as well as by immunolocalization of HA-AUX1.

Immunolocalization. Tissue-specific AUX1 expression was assayed by immunolocalization of HA-AUX1 (ref. 31) using anti-HA primary antibody (Roche, Lewes, UK) and Alexa-Fluor-555-coupled secondary antibody (Molecular Probes, Carlsbad, CA), then visualised using confocal microscopy. Background staining was performed with Sytox Green (Molecular Probes).

Root growth measurements. To assess basal root growth, seedlings were grown vertically for 5 d and the root length measured. Two-tail *t*-tests were performed using Microsoft Excel software. For 2,4-dichlorophenoxyacetic acid (2,4-D) and IAA root bioassays, seedlings were grown vertically on MS plates for 5 d and then transferred to fresh MS, MS + 2,4-D (10^{-7} M) or MS + IAA plates (2.5×10^{-7} M) for a further 2 d. The delta root growth in the presence or absence of auxin was measured and expressed as percentage root growth compared with the MS control. Kinematic analyses were performed 3 d after germination of seedlings on vertically placed seedlings. To this end, a series of overlapping time-lapse images were obtained that covered the entire growth zone using a vertically oriented microscope (Axiolab, Zeiss) with a charge-coupled device camera connected to a PC fitted with a Scion LC3 framegrabber board and ScionImage software. For each treatment, at least five replicate roots were imaged nine times at 10-s intervals. The obtained image stacks were used for calculation of velocity and strain rate profiles using RootflowRT software[49].

Gravitropic assays. Seedlings were grown vertically for 4 d on MS plates and the plates were then turned at an angle of 90°. Photographs were taken every 30 min up to 8 h after the gravity stimulus and then finally at 24 h (see Supplementary Information, Fig. S1, S4). The kinetics of the root gravitropic response was expressed as a percentage of seedlings responding to the gravity at a given time point. Seedlings were scored as positively responding to the gravity stimulus once they had reoriented by 45°.

Computer simulations. Our computer model of auxin flux in root tissue used the program AuxSim, written by E.M.K.[35] The new version modelled cells in three-dimensions, rather than in two-dimensions. The root tip was modelled as a rectangular array of cells that was 20 long × 20 wide × 3 deep, with periodic boundary conditions in *y* to mimic a cylindrical geometry. The three cell layers in *z* were the epidermis, cortex and endodermis. In transverse view, epidermal and cortical cells were $14 \times 14\ \mu m^2$ and endodermal cells were $14 \times 7\ \mu m^2$. Cell lengths increased exponentially from the apex back (doubling every third row), with the first row being 10 μm long (see Fig. 2a). Cell walls were 0.5 μm thick. An additional description of the model, including model membrane permeabilities, can be found in the Supplementary Information text and Supplemental Table S4.

Note: Supplementary Information is available on the Nature Cell Biology website.

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COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.

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