Supporting Online Material for

Generation of Leaf Shape Through Early Patterns of Growth and Tissue Polarity

Erika E. Kuchen, Samantha Fox, Pierre Barbier de Reuille, Richard Kennaway, Sandra Bensmihen, Jerome Avondo, Grant M. Calder, Paul Southam, Sarah Robinson, Andrew Bangham,* Enrico Coen*

*To whom correspondence should be addressed.
E-mail: enrico.coen@jic.ac.uk (E.C.); a.bangham@uea.ac.uk (A.B.)

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Materials and Methods

Plant Material

For tracking growth we used Arabidopsis lines pAR169 (ATML1::mCitrine-RCI2A) (22), or Lti6b (35S::EGFP-LTI6b) (23), which express fluorescently labelled plasma membrane markers and are in the Landsberg erecta background. For clonal analysis and calculating growth curves, we used lines carrying 35S::lox-uidA-loxGFP and hsp18.2::Cre (14) in the Landsberg erecta background. Some of the cutting experiments used wild-type Arabidopsis in the Columbia background to ensure comparability with Sena et al. (15).

Arabidopsis plants were grown in standard conditions (unless otherwise described): seeds were surface sterilised and sown on plates containing 25 ml of growth media [GM; 1x Murashige and Skoog salt mixture, 1 % (w/v) sucrose, 100 mg/ml inositol, 1 mg/ml thiamine, 0.5 mg/ml pyridoxin, 0.5 mg/ml nicotinic acid, 0.5 mg/ml MES, 0.8 % (w/v) agar, pH 5.7] then stratified at 4 °C for 3 days before being transferred to a controlled environment room at 20 °C in long day conditions (16 hours light/8 hours dark cycles). The unstable Antirrhinum line pal-2 was grown on soil under continuous light at 25 °C except for short periods when plants were transferred to 15 °C to induce transposon excision (24).

Growth curves and staging

To generate growth curves (fig. S1), widths and lengths of leaf 1 were measured for multiple seedlings at various days after stratification (DAS). For early stages (2 to 6 DAS), leaves were imaged under the confocal microscope after staining with propidium iodide (25), and measurements were taken in 3D using VolViewer (http://www.uea.ac.uk/cmp/research/cmpbio/VolViewer). At later stages leaves were flattened, photographed and measurements taken from the 2D images using Fiji (http://fiji.sc). Logistic curves were fitted to the leaf width and length data:

\[ y = -\frac{A}{1+\exp((-t-t_m)k))}+A \]

Where \(A\) is the upper asymptote, \(t\) is time (hours after stratification), \(k\) is the early exponential growth rate and \(t_m\) is the point of inflection, or time when \(y\) reaches \(A/2\). For \(y\) = leaf width, parameter values were estimated to be: \(A = 6\) mm, \(k = 0.020\) h\(^{-1}\) and \(t_m = 306\) h. For \(y\) = leaf length, \(A = 9.5\) mm, \(k = 0.021\) h\(^{-1}\) and \(t_m = 310\) h.

The logistic function was used to estimate when the primordium has a width of 0.02 mm (~ two cells wide), which was taken as the time of leaf initiation. This time was about 1 DAS. Thus, days after leaf initiation (DAI) were calculated by subtracting 1 day from DAS.

Time-lapse imaging and analysis

Seedlings were grown in standard conditions before being transferred to a Growth-Tracking chamber (26) at 4 or 6 DAI. The chamber was mounted onto the stage of a Zeiss LSM 5 EXCITER Laser Scanning Microscope and the growing abaxial epidermis of leaves imaged at regular intervals over several days. The GFP or YFP probe was excited using the 488-nm line of an argon ion laser, and emitted light filtered through a 500-550 nm band-pass filter and imaged with either a 10x dry, or 20x dry, objective. The chamber was illuminated by a cold light source set to a long-day photoperiod. Room temperature was maintained at 20 °C. Widths of tracked leaves showed good agreement with the widths of leaf 1 grown under standard conditions.

Confocal z-stack images of time lapse data were processed using Merryproj (27) to create 2D projections of the leaf epidermis. Growth rates over 24 h intervals were extracted
using software called Point Tracker. The software is written in Python, uses NumPy and SciPy (28) for the data analysis and PyQt4 (http://www.riverbankcomputing.co.uk/software/pyqt) for the user interface. Cell vertices on the 2D projections were used as material points, which were linked to form regions (regions did not always correspond to biological cells). For each region, the growth tensor was estimated from the linear transformation that best approximated the deformation of the region (i.e. minimising the square error on the final vertex positions resulting from the transformation compared to the observed final positions). A polar decomposition allowed the rotation and scaling to be extracted from the transformation. From the scaling the strain tensor (II) was calculated. The scaling was further corrected to account for large time steps, with the assumption that the growth tensor is constant (i.e. the growth tensor is "attached" to the tissue and the tissue grows at a constant relative growth rate during the period covered, according to the Lagrangian formulation). The images shown in Fig. 1J and K, where growth is analysed over 5 days, are derived from two independent time lapse experiments

Growth in the direction of the midline (Fig. 1E to I) was calculated from the strain tensors of the cells in the midline region. The leaf midline axis was defined manually and the leaf rotated to align the midline axis with the y-axis. The position of each cell was recorded as the distance of its centroid from the petiole-lamina boundary along the y-axis. In cases where the midline was curved, it was divided into smaller segments, for each of which the midline axis was determined and aligned. Growth rate along the midline was calculated by projecting the midline unit vector onto the unit vectors defining the major and minor axis of growth for each cell.

Clonal analysis
Leaves were heat shocked at 3 or 6 DAI by submerging sealed plates containing seedlings into a water bath at 38 °C for 3 minutes. After 3 or 6 days of further growth in standard conditions, leaf 1 was removed, flattened and clones on the abaxial surface imaged on a Zeiss LSM 5 EXCITER Laser Scanning Confocal Microscope, or a Leica DM 6000 compound microscope. Leaves were imaged either at 6 DAI (leaf width ~ 0.34 mm), or 9 DAI (leaf width ~ 1.2 mm). For Antirrhinum, transposon excision was induced by moving plants from 25 °C to 15 °C for 24 hours, when metamer 4 (fourth node above the cotyledons) was ~ 50-100 µm wide. Mature metamer 4 leaves were flattened and photographed using a Kodak DCS Pro 14N camera. The Sector Analysis Toolbox (http://www.uea.ac.uk/cmp/research/cmpbio/SectorAnalysisToolbox) was used to analyse clonal patterns in Arabidopsis and Antirrhinum leaves. The program generates a virtual composite shape from the outline of individual leaves and the position and shape of their clones (Fig. 2, A, C and E, and Fig. 4J). Leaf outlines were captured by semi-automatic point placement and a mean leaf shape was calculated using Procrustes alignment (29), normalising for scale. Epidermal clones were segmented using a combination of automatic and manual segmentation. Each individual clone was warped onto the mean shape using a piecewise linear warp (30) in which the spatial transformations were maintained for each leaf. The Sector Analysis Toolbox was implemented in MATLAB. To simulate clones with the model, representative cell outlines were drawn on the canvas at the time of clone induction. The cell outlines were derived from confocal images of leaves at corresponding developmental stages. For the earliest stage (3 DAI) the curvature of the leaf surface precluded use of 2D projections for obtaining cell outlines. We therefore computed an approximation using a flattened curved section of the confocal image (fig. S3) using VolViewer (http://www.uea.ac.uk/cmp/research/cmpbio/VolViewer). The curved section was computed by first placing a 2D plane through the confocal image. The 2D plane was aligned to the leaf and discretised as a regular 2D (x, y) grid consisting of 64 x 64 vertices with connected
diagonals to form 8192 triangles. The 2D plane was made to curve independently along the $x$ and $y$ axis using two manually specified parameters. The parameters controlled the displacement along the $z$-axis at each vertex of the grid, using a sinusoid (sin) function. The parameters where adjusted until the curved section intersected the middle of the majority of the epidermal cells on the abaxial surface. To flatten the curved section image, the $x$, $y$-distances between the vertices were computed. The displacement along the $z$-axis was removed to flatten the curved section and the vertices where translated back to their computed $x$ and $y$-distances in order to obtain a flattened image of the cell outlines.

**Distal leaf excision**

We repeated the leaf excision experiments in Sena *et al*. (15) as closely as possible by cutting leaf 3 of *Arabidopsis* accession Columbia-0 in half approximately perpendicular to the midvein (fig. S7) using Vannas micro-scissors (World Precision Instruments, 15 μm straight blades), as described by these authors. The excisions were carried out at 10 DAS, when leaf 3 width is ~ 0.2 mm. We found this to be the earliest stage that it was practical to cut the leaf using micro-scissors. We also repeated the excision experiments using leaf 1 of *Arabidopsis* Landsberg erecta at 6 DAI (leaf 1 width ~ 0.3 mm) and obtained the same results as for leaf 3 (no regeneration).

As the study by Sena *et al*. (15) shows that regeneration does not occur when leaves are cut at later stages (22 days after sowing), we wanted to ensure that our leaf cutting experiments had been done at a sufficiently early time point. We therefore compared the size of leaves 2 days and 5 days post cut to the published images by Sena *et al*. (15). In our experiments, leaf 3 was about 450 μm wide 2 days post cut, and 1.6 mm wide 5 days post cut. By contrast, in the experiments of Sena *et al*. (15), the leaf is 165 μm wide 2 days post cut, and 185 μm wide 5 days post cut, according to the scale bars in Fig. 4b and c of their paper. Thus it would seem that the leaves in the Sena *et al*. study are much smaller than ours and hardly grew during the 3 day period (the growth rate in width would be 0.16 %/h). However, closer inspection of their figure shows that the scale bar is most likely incorrect. The branches of mature Arabidopsis trichomes are about 250 μm long (31), yet according to the scale bars the trichome branches visible in Fig. 4b and c of Sena *et al*. (15) are about 20-25 μm long. Thus the scale bars seem to be out by a factor of about 10. Using the trichome branches for scaling, and assuming they are 200 μm long, we obtained estimated leaf widths of 1.25 mm for 2 days post cut, and 1.87 mm for 5 days post cut. These values are larger than those for leaves in our experiments, suggesting that our cuts had been carried out as early if not earlier than those of Sena *et al*. (15).

To improve the precision of leaf excision experiments, we used a Zeiss PALM laser dissecting microscope to cut the leaf. For these experiments we used leaf 1 as it is more accessible than leaf 3. Individual seedlings were placed on a drop of water on a glass slide and viewed using a 10x objective. The PALM software was then used to direct the laser beam to cut the lamina in half, perpendicular to the midvein. The laser was set to 100 % power and travelled at a speed of 5 mm/sec. The leaf tissue was severed completely (Fig. 3A) and the excised piece of leaf tissue removed using fine forceps. Seedlings were then returned to standard growing conditions for a further 3 or 6 days, and then imaged using a Leica MZ16 stereo microscope (Fig. 3, B and C). Alternatively, to track growth following excision, seedlings were mounted into a Growth-Tracking chamber immediately following laser cutting. The cut leaves were then imaged at regular intervals using a Zeiss LSM 5 EXCITER Laser Scanning Microscope (as described above). Cut edges grew in length by an average of
0.55 %/h in the tracking chamber, compared to 2.2 %/h for a corresponding region of an uncut leaf.

**SOM Text**

**Supporting model description**

1 **Basic factors and functions**

Growth patterns are determined by the pattern of factors distributed over the tissue, termed the canvas (8). Factors have one value for each segment or vertex of the canvas and are denoted by capital letters in the text. In the equations, factors that propagate through the canvas are denoted by the bold letter $s$ subscripted with the factor name, while those that are fixed to the canvas are denoted by $i$ subscripted with the factor name. For instance, the immobile factor PGRAD is described by $i_{pgrad}$ in the equations.

Factors may promote growth rates through the linear function $pro$, defined as:

$$pro(p_f, x_f) = 1 + p_f x_f,$$

where $x_f$ is a factor, $F$, and $x$ denotes either $i$ or $s$. $p_f$ is a promotion coefficient for that factor. Factors may inhibit growth through the function $inh$, defined as:

$$inh(h_f, x_f) = \frac{1}{1 + h_f x_f},$$

where $h_f$ is a inhibition coefficient for factor $F$. All multiplications and divisions are element-wise. Values of all coefficients are given in the tables below (section 2.2 for the 1D models and section 3.3 for the 2D models). All models run from $t = 87$ hours (end of day 3) to $t = 205$ hours (day 8 to 9).

2 **1D models**

Growth along the lamina midline (Fig. 1, E to G, black lines; Fig. 1, H and I, grey lines) was simulated using a one-dimensional model in MATLAB. An initial canvas comprises a line made up of multiple segments of equal length, $L_0$, joined at vertices (fig. S2A). Each line segment grows according to:

$$L(t) = L_0 e^{Kr},$$

where $K$ is the growth rate and $t$ is time in hours. The value of $K$ is promoted by PGRAD according to:

$$K = p_{pgrad} i_{pgrad},$$

where $p_{pgrad}$ is the promotion of growth by PGRAD. PGRAD has a linear gradient across the canvas with the highest level of 0.94 at the base and lowest level of $b_{pgrad}$ at the tip. Within each segment PGRAD levels are uniform (fig. S2B). The gradient in PGRAD forms
during setup (prior to the start of the growth simulation) and the value of PGRAD for each segment does not change as the line segments grow.

2.1 1D model with LATE

To decrease growth rates at later stages (Fig. 1, E to I, black lines), K is inhibited by LATE according to (Fig. 1P):

\[ K = p_{pgrad} \cdot pgrad \cdot \text{inh}(h_{late}, i_{late}), \]

where \( h_{late} \) is the inhibition coefficient of LATE. The value of LATE is the same for each segment and increases linearly with time after 148 hours (end day 6, fig. S2C):

\[ i_{late} \begin{cases} 0 & \text{if } t < 148 \text{ h} \\ g_{late}(t - 148 \text{ h}) & \text{if } t \geq 148 \text{ h} \end{cases} \]

where \( g_{late} \) defines the rate of increase of LATE with time.

2.2 1D models parameter list

<table>
<thead>
<tr>
<th>Growth parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( b_{pgrad} )</td>
<td>minimum levels of PGRAD</td>
<td>0.195</td>
</tr>
<tr>
<td>( p_{pgrad} )</td>
<td>growth promotion by PGRAD</td>
<td>0.04 h(^{-1})</td>
</tr>
<tr>
<td>( g_{late} )</td>
<td>increase in LATE over time</td>
<td>0.0048 h(^{-1})</td>
</tr>
<tr>
<td>( h_{late} )</td>
<td>growth inhibition by LATE</td>
<td>4.5</td>
</tr>
</tbody>
</table>

3 2D models

2D leaf models were specified using the growing polarised tissue framework as implemented in the MATLAB application GFtbox. Full details of both are given in Kennaway et al. (8) and models can be downloaded with the GFtbox software from (https://www.uea.ac.uk/cmp/research/cmpbio/Gftbox). In this method an initial finite element mesh, also termed the canvas, is deformed during growth. The pattern of deformation depends on growth-modulating factors, whose initial distribution is established during setup. Factors have one value for each vertex and values between vertices are linearly interpolated across each finite element. In the models described here, the initial canvas is oriented with regard to the external \( xy \)-coordinate system such that the canvas base is parallel to the \( x \)-axis and the midline is parallel to the \( y \)-axis. Growth at the baseline is constrained to be parallel to the \( x \)-axis, reflecting the anchoring of the leaf to the stem. The initial canvas consists of 3000 elements. Elements are not subdivided during the simulations.

Each model has two interconnected networks: the Polarity Regulatory Network (PRN) specifies tissue polarity and hence specified orientations of growth, the Growth rate Regulatory Network (KRN) determines how factors influence specified growth rates. In total, growth interactions are specified by three equations, one for the PRN and two for the KRN. These networks determine the specified polarity and growth fields across the canvas. Due to the connectedness of the canvas this specified growth differs from the resultant growth by
which the system is deformed. The time step of each model corresponds to one hour of developmental time. Models take about 20 min to run on a dual core desktop computer.

3.1 Non-deforming model

This model involves growth orientations being held parallel to the midvein (y-axis) throughout growth (Fig. 1, L and T).

Setup

The initial canvas shape and distribution of factors are shown in Fig. 1Q and R. The PGRAD gradient is specified in the same way as in the 1D model, where PGRAD levels are 1 at the base and \( b_{pgrad} \) at the tip. PGRAD levels are calculated according to the initial y-coordinate of each vertex and interpolated across each element. LAM is highest in the proximal regions of the lamina with lower levels in the distal regions and in the proximal region that will form the petiole. MID is expressed along the midline and declines distally with a linear gradient. The value of all these factors at each vertex does not change during growth (they are fixed to the canvas). The maximum level of these factors is 1.

PRN

A proximodistal polarity field is specified as being oriented parallel to the midline throughout growth by the gradient of a polarity factor, POLARISER (POL, Fig. 1T). Similar to PGRAD, POL has a linear gradient across the canvas with highest levels, determined by \( b_{pol} \), at the base and at level of 0 at the tip. POL levels are calculated according to the y-component of each vertex and interpolated across each element. To maintain polarity parallel to the y-axis throughout growth the POL gradient is re-established at every iteration and therefore does not deform with the canvas during growth (unlike PGRAD, LAM and MID).

KRN

Specified growth rate parallel to the polarity gradient, \( K_{par} \), is defined in the same way as for the 1D model (Eqn. 2.3 and Fig. 1S):

\[
K_{par} = p_{pgrad} i_{pgrad} \cdot inh(h_{late}, i_{late})
\]  

Specified growth perpendicular to the polarity is promoted by LAM and LATE and inhibited by MID:

\[
K_{per} = p_{lam} i_{lam} \cdot pro(p_{late}, i_{late}) \cdot inh(h_{mid}, i_{mid})
\]

3.2 Organiser-based model (Deforming model)

These models involve growth orientations being established by POL propagation from an organiser, which was expressed at the canvas base. The gradient of POL defines the local polarity and hence local orientations of specified growth, which reorient with changes in the POL gradient. The KRNs for all organiser model versions are the same as for the previous model (Eqn. 3.1 and Eqn. 3.2).

PRN

An identity factor, PROXORG, is expressed at a level of 1 along the base of the canvas and 0 elsewhere. The value of POL is fixed at a value of 0.1 (\( b_{pol} \)), where PROXORG is expressed (i.e. where PROXORG > 0). POL diffuses according to the equation:
\[
\frac{\partial s_{\text{pol}}}{\partial t} = D_{\text{pol}} \nabla^2 s_{\text{pol}} - \mu_{\text{pol}} s_{\text{pol}},
\]  

(3.3)

where \( D_{\text{pol}} \) is the diffusion rate and \( \mu_{\text{pol}} \) the decay rate of POL throughout the tissue. POL distribution is allowed to establish during the setup phase for 20 time steps before the commencement of growth.

The importance of POL propagation for the polarity field maintenance is explored using POL propagation rates at two extremes. At one extreme (fixed version), the local POL gradient is fixed to the initial canvas after setup and thus the gradient and polarity field deform with the canvas (Fig. 1, U and M). In the other version (dynamic), POL continues to propagate and the polarity field readjusts according to POL levels throughout growth (fig. S5, A and B; see 2D models parameter list). In the dynamic version, the polarity field deforms during growth because changes in tissue geometry affect the way POL becomes distributed. The patterns and orientations of resultant growth for the dynamic and fixed versions of the organiser-based model are very similar (Fig. 1O and fig. S5C) and match the data (Fig. 1, J and K). Thus, both versions of the organiser-based (deforming) model are compatible with our observations.

To confirm that the propagation rate in the dynamic model is high enough to polarise the tissue throughout growth and to explore the importance of canvas geometry on the polarity field, the polarity field is perturbed at 148 hours (fig. S5, D and E). POL levels are reduced 10-fold and spatially randomised everywhere, except at PROXORG, to give randomly orientated polarity. \textit{De novo} production of POL at PROXORG and POL diffusion (Eqn. 3.3) restores the polarity pattern within 1 hour, with orientations diverging at the base and converging towards the tip (fig. S5F). Model shape and the polarity field are indistinguishable from an undisturbed run of the dynamic model (fig. S5, B and G).

### 3.2.1 Distal leaf excision

The above models assume that a basic pattern of growth rates is established early. This is verified by excising the distal half of the canvas (Fig. 3, H and I). During setup the distal part of the canvas is marked by DISTAL (fig. S2D). Regions expressing DISTAL are removed from the canvas at 148 hours (day 6), corresponding to the cuts performed experimentally. After deletion of the distal half, the remaining canvas grows to a time of 205 hours, but is inhibited at the cut edge by inhibitor induced by cut (INC), which is expressed after cutting along the cut edge (fig. S2E). INC is expressed at a level of 1 and inhibits \( K_{\text{par}} \) and \( K_{\text{per}} \) through an additional term to equations 3.1 and 3.2, giving the overall KRN equations:

\[
K_{\text{par}} = p_{\text{grad}} i_{\text{grad}} \cdot \text{inh}(h_{\text{late}}, i_{\text{late}}) \cdot \text{inh}(h_{\text{inc}}, i_{\text{inc}}) 
\]  

(3.4)

\[
K_{\text{per}} = p_{\text{lam}} i_{\text{lam}} \cdot \text{pro}(p_{\text{late}}, i_{\text{late}}) \cdot \text{inh}(h_{\text{mid}}, i_{\text{mid}}) \cdot \text{inh}(h_{\text{inc}}, i_{\text{inc}}) 
\]  

(3.5)

### 3.3 2D models parameter list

For organiser-based models, the POL diffusion constant \( D_{\text{pol}} = 0.0001 \text{ mm}^2\text{h}^{-1} \) in the fixed polarity version and 0.01 mm\(^2\)h\(^{-1}\) in the dynamic polarity version. The other parameter values in all 2D models are:
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
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<tr>
<td>$b_{pol}$</td>
<td>maximum POL levels</td>
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<tr>
<td>$\mu_{pol}$</td>
<td>POL decay rate</td>
<td>0.1 h$^{-1}$</td>
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<tr>
<td><strong>Polarity parameters</strong></td>
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<tr>
<td>$b_{grad}$</td>
<td>minimum levels of PGRAD</td>
<td>0.195</td>
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<tr>
<td>$g_{late}$</td>
<td>increase in LATE over time</td>
<td>0.0048 h$^{-1}$</td>
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<tr>
<td>$p_{grad}$</td>
<td>$K_{par}$ promotion by PGRAD</td>
<td>0.041 h$^{-1}$</td>
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<td>$p_{lam}$</td>
<td>$K_{per}$ promotion by LAM</td>
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<tr>
<td>$h_{late}$</td>
<td>$K_{par}$ inhibition by LATE</td>
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<tr>
<td>$h_{mid}$</td>
<td>$K_{per}$ inhibition by MID</td>
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<tr>
<td><strong>Growth parameters</strong></td>
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<tr>
<td>$h_{inc}$</td>
<td>growth inhibition by INC</td>
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<tr>
<td><strong>Excision parameters</strong></td>
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</table>

Fig. S1. Growth analysis of leaf 1.
Growth curves for leaf width (black) and length (red), with outlines of representative leaves. Several small cuts were made in the oldest leaf, allowing it to be flattened. Inset shows measurements on a logarithmic scale.
Fig. S2. Model methods.

(A) 1D example canvas with spatial discretisation. $L_0$ is the initial size of each segment. The canvas used in the 1D models consists of 1000 segments. (B) PGRAD levels along the 1D canvas. Levels are uniform in each segment. (C) LATE levels over time. (D) Canvas region to be excised at later stages was marked by DISTAL on the starting canvas (end of day 3). (E) Canvas shape just after cutting (day 6). Growth at the cut edge was inhibited by INC after excision of the DISTAL region. Scale bars for D and E, 100 µm.
Fig. S3. Flattening the leaf using VolViewer.
(A) Volume rendering of leaf 1 at 3 DAI. (B) User-placed curved section through the abaxial epidermal layer. (C) Resulting flattened section image, which was used as the basis for the initial canvas shape for 2D models. Representative cells are highlighted. For clarity (in this figure) the grid resolution was reduced. Scale bar, 50 μm.
Fig. S4. Observed growth directions for additional images.
Principle directions of growth (black lines, calculated over 24 hour intervals and shown where anisotropy > 10%), from four different tracking experiments Comparisons to the model were quantified for the tracked regions covered by ellipses. Scale bars, 100 μm.
Fig. S5. Dynamic version of organiser-based model.

(A and B) Distribution of PROXORG (green) and polarity factor POL in the canvas for dynamic version of the organiser-based model (see section 3.2 of SOM text) at 3 days (A) and 8 days (B). (C) Resultant areal growth rates (heat map) and orientations of growth (black lines, shown where anisotropy > 5%). (D to G) Transient POL reduction and redistribution. (D) Before POL reduction. (E) Immediately after POL reduction. (F) POL regains its initial distribution within one hour (one time step). (G) POL distribution and polarity field on day 8. Scale bars, 100 μm. POL (blue), polarity field (arrows).
Fig. S6. Validation of direct tracking using clonal analysis.

(A and C) Cell fates from time-lapse imaging: (A) Cells tracked from 4 DAI (small leaf on left) to 6 DAI (right leaf) and (C) from 6 DAI (left leaf) to 8 DAI (right leaf). Examples of tracked clones are shown enlarged. (B and D) Clones induced at 3 DAI (B) or 6 DAI (D) and imaged at comparable developmental stages to (A) and (B) respectively (6 DAI and 9 DAI). Clones from several leaves have been superimposed. Scale bars, 100 μm.
Fig. S7. Excision of leaf 3.
Excision of the distal half of the leaf 3 lamina at 10 DAS using micro-scissors. (A) Leaf shape after 2 days of growth, arrow points to cut. (B) Leaf viewed from the top after 5 days of growth, showing apparent regeneration of tip. (C) Same leaf as (B) viewed from lower (abaxial) side, showing curved indentation at tip (arrow). Scale bar, 100 μm.
Fig. S8. Model sensitivity analysis.
Resultant canvas shapes and clones (circles on the initial canvas) with parameter values varied by +/- 20%. **Box**, Growth regulatory network of the 2D models with growth factors (black) and growth parameters (red). For explanation of growth parameters see section 3 of SOM text. Scale bar, 500 μm.
Table S1. Quantitative comparisons between output from models and observed principal orientations of growth.

The results in Fig. 1K and fig. S4 provide 6 data sets for comparing observed orientations with those generated by the models. Comparisons were quantified by fitting the best matching growth tensor for the tracked regions covered by ellipses. The principal directions of growth were then determined, expressed as an angle in degrees clockwise from the midline. For the models, we performed the same computation, using the vertices of the finite element mesh instead of experimentally tracked points, at the times corresponding to the leaf observations. Model output and experimental data were scaled and aligned using reference points at the tip of the leaf and the base of the lamina. In all 6 cases the angles based on experimental data were positive (splaying outwards from the midline for green ellipses, and inwards towards the tip for the brown ellipses) as were those from the deforming (organiser-based) model. By contrast, the angles predicted by the non-deforming model were near zero or negative.

<table>
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<tr>
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<th>Observed angle</th>
<th>Non-deforming model output</th>
<th>Deforming model output</th>
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<tbody>
<tr>
<td>Brown ellipse</td>
<td>46 °</td>
<td>3 °</td>
<td>15 °</td>
</tr>
<tr>
<td>(Fig. 1K)</td>
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<tr>
<td>Brown ellipse</td>
<td>52 °</td>
<td>3 °</td>
<td>15 °</td>
</tr>
<tr>
<td>(fig. S4A)</td>
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<tr>
<td>Brown ellipse</td>
<td>22 °</td>
<td>3 °</td>
<td>15 °</td>
</tr>
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<td>(fig. S4B)</td>
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<td>(fig. S4D)</td>
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References


