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Current Biology 22, 1–9, September 11, 2012 http://dx.doi.org/10.1016/j.cub.2012.06.050

Article

Mechanical Regulation of Auxin-Mediated Growth

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Summary

Background: The phytohormone auxin is a primary regulator of growth and developmental pattern formation in plants. Auxin accumulates at specific sites (e.g., organ primordia) and induces localized growth within a tissue. Auxin also mediates developmental responses to intrinsic and external physical stimuli; however, exactly how mechanics influences auxin distribution is unknown.

Results: Here we show that mechanical strain can regulate auxin transport and accumulation in the tomato shoot apex, where new leaves emerge and rapidly grow. Modification of turgor pressure, application of external force, and artificial growth induction collectively show that the amount and intracellular localization of the auxin efflux carrier PIN1 are sensitive to mechanical alterations. In general, the more strained the tissue was, the more PIN1 was present per cell and the higher the proportion localized to the plasma membrane. Modulation of the membrane properties alone was sufficient to explain most of the mechanical effects.

Conclusions: Our experiments support the hypothesis that the plasma membrane acts as a sensor of tissue mechanics that translates the cell wall strain into cellular responses, such as the intracellular localization of membrane-embedded proteins. One implication of this fundamental mechanism is the mechanical enhancement of auxin-mediated growth in young organ primordia. We propose that growth-induced mechanical strain upregulates PIN1 function and auxin accumulation, thereby promoting further growth, in a robust positive feedback loop.

Introduction

Morphogenesis is a mechanical phenomenon. Geometry and internal pressure determine the mechanical stress (force acting on the tissue), which induces mechanical strain (deformation) and reshapes the tissue depending on the material properties. Morphogenesis essentially is the accumulation of the strain over time [1, 2]. However, mechanics is not merely a read-out; it is becoming increasingly clear that mechanics instructs growth and developmental pattern formation in plants and animals [3–5]. Organogenesis at the shoot apex of flowering plants, in which new leaves emerge and rapidly expand (Figure 1A), has long been thought to be under mechanical control [6, 7]. Therefore, the shoot apex is an attractive system to study mechanical regulation of growth and development.

Plant growth is regulated at the level of cell wall extensibility—a material property of the tissue [8, 9]. Plant cells are surrounded by a rigid cell wall, which contains the turgor pressure within and restricts the cell volume. Localized loosening of the cell wall, via expansin activation or pectin methylesterification, in the periphery of the shoot apical meristem can induce the entire course of organ development [10–13]. Global activation of excess expansin expression or cell wall acidification loosens the cell wall and results in production of larger organs [14, 15]. The cells in the shoot apex also respond to mechanical cues and reorient the deposition of new structural polymers in the cell wall, so that they expand more in the less stressed direction [16]. Without this mechanical modulation of growth direction, cells tend toward spheres, and organs fail to realize their intended shapes.

Cell wall loosening is activated by the growth regulator auxin [8, 9]. Local accumulation of auxin is necessary and sufficient for organogenesis in the shoot apex [17, 18], and auxin maxima are maintained in developing primordia to regulate further growth and differentiation events [19]. The auxin efflux carrier PIN1, which is asymmetrically distributed within a cell, mediates the localized accumulation of auxin [18–21]. Auxin is transported against its concentration gradient and collects at specific locations [21–25]. Recently, polar PIN1 distribution was shown to correlate with the principal direction of mechanical stress in the *Arabidopsis* shoot apex, raising the possibility of mechanical regulation of auxin accumulation dynamics [26].

The intracellular redistribution of PIN family proteins also underlies developmental responses to mechanical stimuli, such as gravitropism and lateral root induction by bending of the primary root [27–29]. However, the mechanism by which PINs respond to physical cues is as yet unknown. Here we show that PIN1 responds to developmentally relevant degrees of mechanical strain and explore the mechanisms by which cells sense and respond to mechanical signals.

Results

Osmotic Changes Affect PIN1 Protein Level and Intracellular Localization

During morphogenesis, leaves typically grow at a rate of up to 5% per hour [30]. We thus imposed mechanical strain of similar degrees and time scales to living tomato shoot apices and observed PIN1 response. Changes in PIN1 protein level and intracellular localization were monitored quantitatively using ImageJ and the newly developed in-house software MorphoGraphX [31]. MorphoGraphX enables morphological characterization and quantification of fluorescent signals on the curved surface of 3D confocal data at cellular and subcellular resolutions (see Figure S1A available online).

We first modified the mechanical strain by osmotic manipulation. Upon exposure to 0–0.5 M mannitol solutions,

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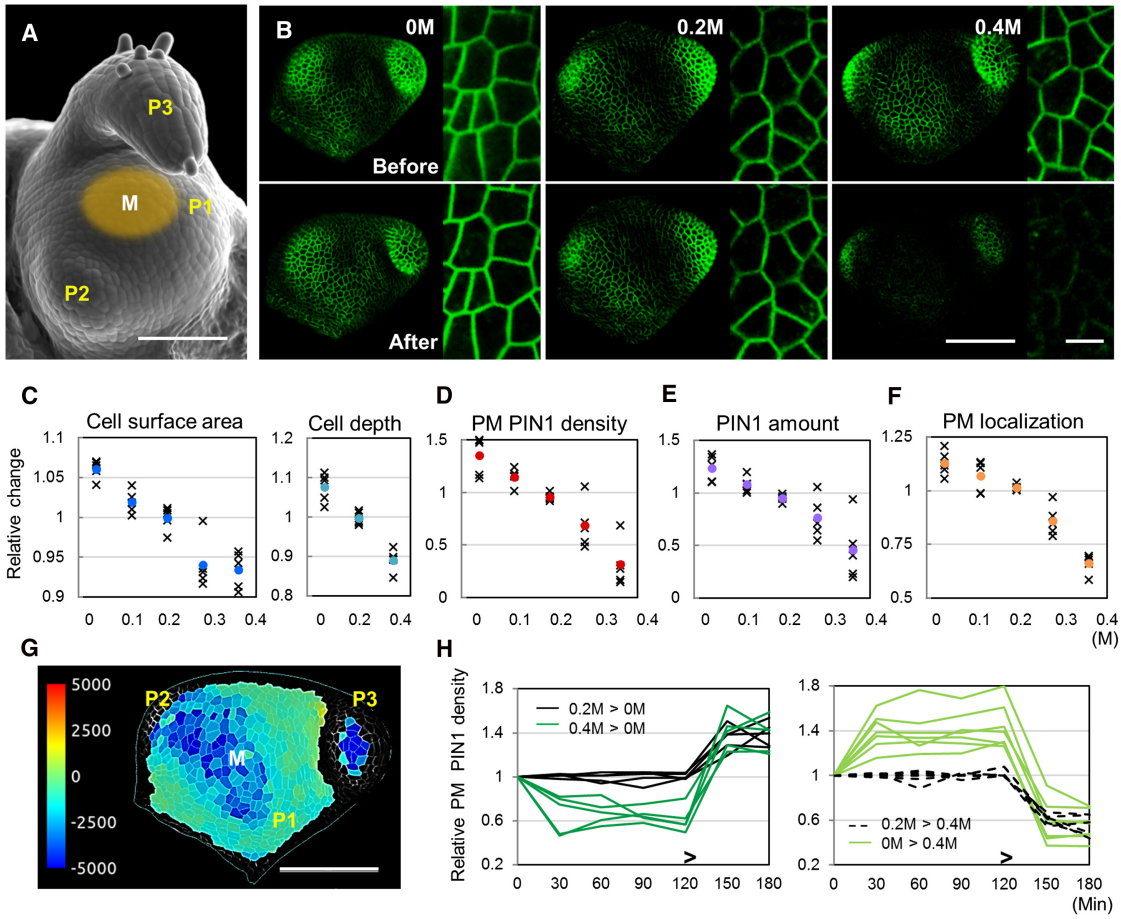

Figure 1. Osmotic Treatments Affect PIN1 Level and Intracellular Localization

(A) Tomato shoot apex showing the shoot apical meristem (M) and three youngest primordia (P1–P3). Scale bar represents 100 μm.

(B–F) Osmotic environment influences PIN1 level and intracellular localization.

(B) Snapshots of epidermal PIN1-GFP signal before and after 2 hr treatment in 0/0.2/0.4 M mannitol. Scale bars represent 100 μm (left panel) and 10 μm (right panel). MorphoGraphX was used to quantify the changes in (C) cell surface area and depth, (D) average PIN1 density on the plasma membrane (PM), (E) total PIN1 protein amount per cell, and (F) ratio of PM-localized to total PIN1 per cell. Changes are shown in after/before ratios (x, average per sample; n = 150 epidermal cells in the apex; ●, average of the averages; n = 5 samples).

(G) Heat map of absolute reduction in the PM PIN1 density after hyperosmotic (0.3 M) treatment showing the regional differences. P, primordia; M, meristem. Scale bar represents 100 μm.

(H) Time-course of the hyper- and hypoosmotic effects and reversion. Treatments were switched after 120 min (>). Each line shows the shifts in the average PM PIN1 density of epidermal cells in near median region of the apex (in after/before ratio; n = 7–12 per sample) from each sample (n = 5–7 samples). See also Figure S1.

plasmolysis was detected at 0.3 M and higher concentrations (Figure S1B). At 0.2 M, the cells maintained their original volume and thus the mechanical strain. In hypoosmotic conditions (below 0.2 M), tissues absorb water and inflate, increasing the mechanical stress and strain of the tissue, whereas in hyperosmotic conditions (above 0.2 M) they deflate and reduce the mechanical loads. The cells changed in size by roughly 6% in both directions (Figure 1C; Figure S1C.a).

Dissected shoot apices were immersed in 0–0.4 M mannitol for 2 hr, and PIN1 protein was monitored in vivo using a transgenic line containing GFP-tagged PIN1 [25]. In the hypoosmotic solutions, the density of GFP signal on the plasma membrane intensified, due to an increase in protein amount in the cell and enhanced plasma membrane localization (Figures 1B and 1D–1F; Figure S1C.b–d). In the hyperosmotic conditions, PIN1 signal in the cell was reduced and a higher fraction of PIN1 was cytosolic. There were regional specificities to the response; the effect was more pronounced in the meristem and primordia than the rest of the shoot apex (Figure 1G). PIN1 level and plasma membrane localization were unchanged in 0.2 M mannitol. Overall, the more strained the cells were, the more PIN1 was present per cell, and the higher fraction of PIN1 was localized to the plasma membrane.

The hyper- and hypoosmotic effects on PIN1 were fully reversible. Regardless of a prior treatment in 0.2 M or 0.4 M mannitol, plasma membrane PIN1 density increased to similar levels in 0 M (Figure 1H). Similarly, shifting from 0 M to 0.4 M decreased PIN1 on the membrane as effectively as shifting from 0.2 M. The osmotic regulation of membrane-localized PIN1 occurred primarily within the first 30 min, even in the reversion treatments, and stabilized by 2 hr.

The osmotic treatments interfered with PIN1 intracellular localization at another level: the maintenance of PIN1 polarity. Using MorphoGraphX, the degree of PIN1 polarity was deduced in each cell by calculating the ratio of the average PIN1 densities on the most enriched side to the least enriched

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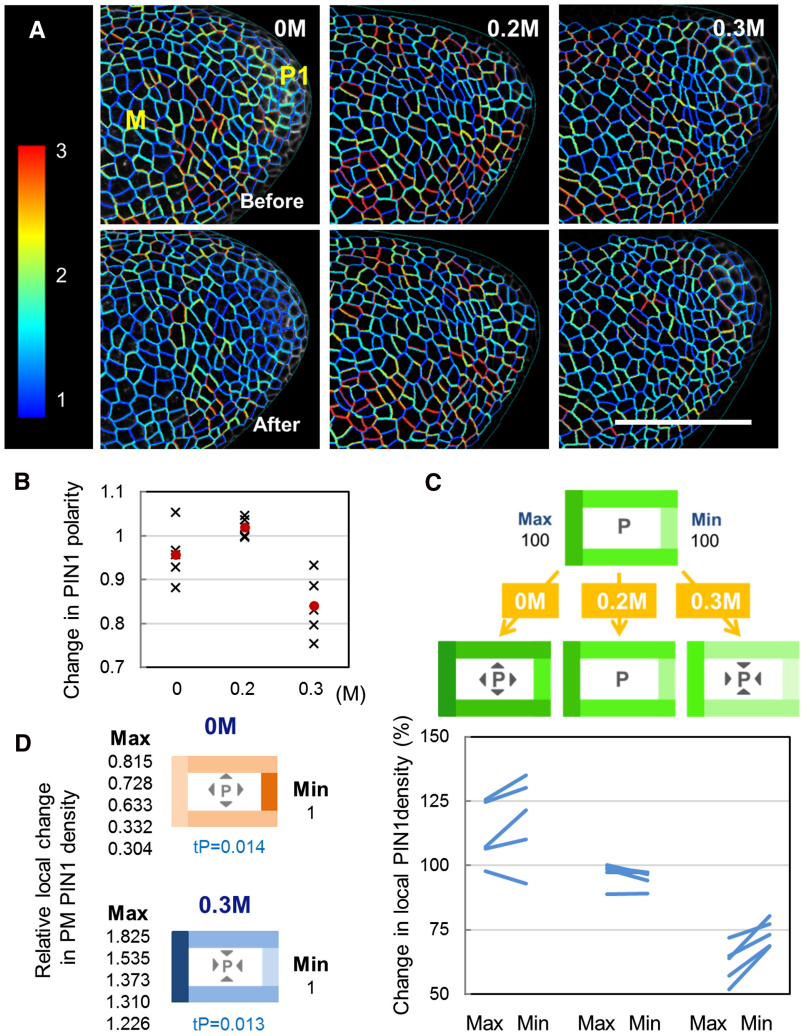

Figure 2. Osmotic Treatments Impair Maintenance of PIN1 Polarity

(A and B) Hypo- and hyperosmotic treatments reduce PIN1 polarity.

(A) Cellular heat maps of PIN1 polarity (i.e., the ratio of local PIN1 density to minimal PIN1 density, based on the average PIN1 density of each cell face), before and after 2 hr treatment in 0/0.2/0.3 M mannitol. The warmest color marks the polarized side. P, primordium; M, meristem. Scale bar represents 100 μm.

(B) Median change (after/before ratio) in average PIN1 polarity (x, median per sample; n = 300–400 cells all over the apex; red dot, average among the medians; n = 5 samples).

(C and D) Less plasma membrane (PM) deposition and more internalization occurred in the hypo- and hyperosmotic conditions, respectively, at the polarized side.

(C) Median change in the PM PIN1 density after 2 hr treatment in 0/0.2/0.3 M mannitol on the most (Max) or least (Min) enriched face of the cell in the pretreatment condition (shown in % to the pretreatment value). The rectangle represents a conceptual cell. The green color indicates PIN1 protein; the darker the green, the higher the PIN1 density.

(D) Median Max/Min ratio of changes (in %) in the PM PIN1 density. The redder shows the more increase in the PIN1 density; the bluer shows the more decrease. P, turgor pressure; tP, p value for Student's t test. n = 5.

side (Figure 2A). The polarity decreased in both hypo- and hyperosmotic treatments (Figures 2A and 2B), but it was more conspicuous in the primordia than the meristem in 0 M, whereas the polarity was reduced overall in 0.3 M. The PIN1 depolarization was due to differential effects depending on the cell faces; the hypoosmotic increase in the PIN1 density was less on the most enriched side than on the least side, whereas the opposite was the case for the hyperosmotic decrease (Figures 2C and 2D).

Osmotic Regulation of PIN1 Involves Shifts in Membrane Traffic and Protein Turnover

Next we examined the PIN1 specificity of the osmotic effects. We immunolocalized the endogenous PIN1 protein in the wild-type plants and confirmed that it responded similarly as the GFP-tagged PIN1 in the transgenic line (Figure 3A). Although immunolocalization is not suitable for fine quantitative assessment, reduction in PIN1 signal was apparent in 0.4 M and even in the milder 0.3 M mannitol treatments. Another plasma membrane-localized protein, H^+-ATPase, was also examined (Figure 3B); whereas the signal became lower in 0.4 M, it was unaffected in 0.3 M. Therefore, the hyperosmotic loss of plasma membrane-localized protein is a general phenomenon, although PIN1 responds particularly sensitively.

In order to gain further insights on the mechanism of the PIN1 regulation, we comonitored the dynamics of PIN1 and the membrane in sequential osmotic treatments (Figure 3C). In 0.2 M mannitol, most PIN1 (PIN1-GFP: green) is localized to the plasma membrane (FM4-64: red) on one side of the cell (panel 1). Within 5 min after the onset of hyperosmotic treatment, massive internalization of PIN1 and the membrane was observed (panel 2). After 30 min, most PIN1 signals had cleared away, whereas membrane signals were still abundant in the cytosol (panel 3). In the subsequent hypoosmotic treatment, PIN1 and membrane signals were restored on the cell surface within 30 min (panel 4). Thus, the osmotic treatments appear to influence plasma membrane localization of PIN1 via fast induction of endocytosis or exocytosis. PIN1 protein appears to undergo rapid turnover in response to osmotic alterations. Both in vivo imaging and immunolocalization showed that PIN1 did not persist for long once internalized (Figure 3C3; Figure S2), indicating that the protein is degraded in hyperosmotic environments.

Nonosmotic Mechanical Modulations Recapitulate the Osmotic Effects on PIN1

Osmotic treatments induce various cellular responses [32, 33], some of which are mechanics-independent. In order to determine whether PIN1 responds to the mechanical changes or to other effects of the osmotic treatment, we used nonosmotic, external force application methods to increase mechanical strain and observed whether they recapitulated the osmotic effects on the plasma membrane PIN1 density, within similar time frames (30 min).

We first applied external force to the shoot apex by gently pressing with a pulled glass rod (Figure 4A). Pressing increases mechanical stress and strain in the cells near the

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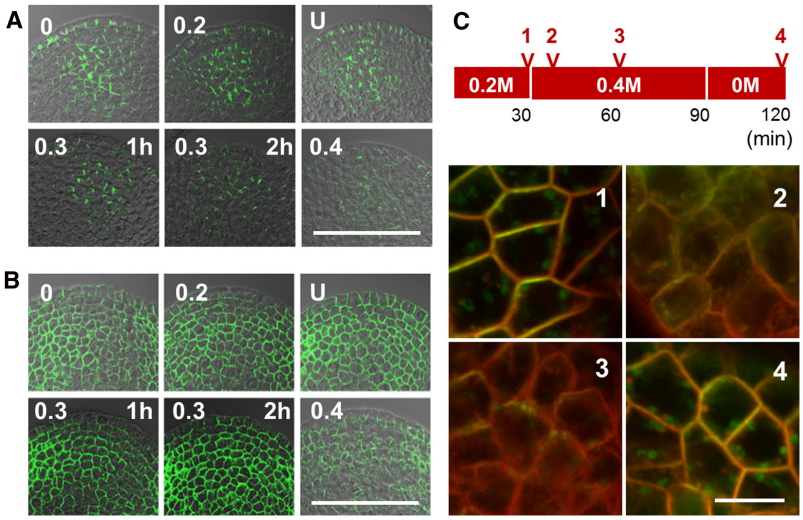

Figure 3. Mechanisms of Osmotic Effects on PIN1

(A and B) Immunolocalization of PIN1 (A) and a plasma membrane-localized H^+-ATPase on longitudinal sections of near median region of the shoot apex (B). Except for Untreated (U), samples were treated for 2 hr in 0/0.2/0.3/0.4 M or 1 hr in 0.3 M mannitol. The H^+-ATPase also responds to hyperosmotic stress, but PIN1 seems more sensitive. Scale bars represent 100 μm.

(C) FM4-64 stained cells on the flank of the shoot apex were monitored upon sequential osmotic treatments. The membrane (red) and PIN1-GFP (green) signals after 30 min in 0.2 M mannitol (1), 5 and 30 min in 0.4 M (2 and 3, respectively), and 30 min in 0 M following 1 hr in 0.4 M (4). The osmotic regulation of PIN1 involves rapid induction of endocytosis and exocytosis, as well as protein turnover. Scale bar represents 10 μm. Note: the green, spotty background signals were also seen in nontransgenic plants. See also Figure S2.

site of force application, where changes in the PIN1 density were monitored. Pressing increased the PIN1 density (Figure 4B). It also reversed the reduction in the density caused by the hyperosmotic treatments (Figure 4C). The restoration was specific to pressing, because the hyperosmotic effect returned if pressing was removed. The upregulation of PIN1 by external force application resembles the hypoosmotic effects, suggesting that the osmotic effect on PIN1 stems from mechanical alteration of the tissue.

Centrifugation can also increase mechanical stress of a tissue without physical contact. The shoot apices were mounted on solid media and subjected to centrifugal force of 1.2 g or 12 g sideways (Figure 4D). The PIN1 density was measured in the area of the tissue facing the center of the centrifuge, where mechanical strain is likely to be induced, and was found to be elevated in a dose-dependent manner. Centrifugation in hyperosmotic solutions partially rescued the hyperosmotic effect. These results further support the notion that osmotic effects on PIN1 are due to mechanical changes. The osmotic treatments and external force application both show a positive correlation between the PIN1 density and tissue mechanical strain.

Membrane Modulations Can Mimic the Osmotic Effects on PIN1

Next we tried to gain mechanistic insight into how cells sense mechanical strain and trigger the osmomechanical regulation of PIN1. Plant cells are thought to sense mechanical strain in the cell wall and/or on the plasma membrane [34]. In order to determine the site of mechanosensing, we modulated the plasma membrane without affecting the cell wall. Mechanical strain in the cell wall is directly transferred to the plasma membrane, because of the turgor pressure that presses the membrane against the cell wall [35]. The membrane strain in turn alters membrane properties (e.g., membrane tension—the in-plane force between membrane molecules), which can greatly influence cellular functions, such as vesicle fusion and fission, signal transduction, and the protein composition [34–42].

We first modulated the membrane properties with membrane-interactive chemicals. Ethanol and dimethyl sulfoxide (DMSO) are both known to expand the membrane, reduce the membrane rigidity and tension, and as a consequence enhance endocytosis [39, 43, 44]. Each chemical lowered the PIN1 density in a dose-dependent fashion, without causing cell wall strain (Figure 5A; Figure S3A). The effects could be reversed by a hypoosmotic treatment, indicating that the membrane retained healthy activity. Importantly, coincubation of these membrane-expanding reagents suppressed the hypoosmotic effect on PIN1.

Temperature also alters membrane properties. At higher temperatures (e.g., 37°C), the membrane is effectively softer and less tense, whereas it becomes more rigid and tense in the cold (e.g., 4°C) [45]. When incubated in 0.2 M mannitol at 37°C and 4°C, the cell size remained unchanged, yet the PIN1 density decreased and increased, respectively, compared to the control room-temperature treatment (Figure 5B; Figure S3B). These temperature effects on PIN1 were reversed by the second counteracting treatment in a hypo- or hyperosmotic solution at room temperature. Such osmotic reversions were suppressed, if the temperature was kept the same as the first treatment, either at 37°C or 4°C.

Exposure to ethanol, DMSO, or elevated temperature reduced the PIN1 density as much as 0.3 M mannitol did (by 20%–30%; Figures 4D and 5A and 5B), indicating that mimicking the changes in the membrane properties caused by tissue shrinkage could affect PIN1 as effectively as negative cell wall strain. Likewise, when the membrane was placed at 4°C and increased its rigidity and tension, the PIN1 density raised as much as in the 0 M treatment (by ~40%; Figure 5B), in which the cell wall strain was increased. Strikingly, 5% ethanol and 37°C treatment could suppress the hypoosmotic upregulation of PIN1 almost completely (Figures 5A and 5B). Taken together, these data suggest that the osmomechanical regulation of PIN1 occurs through changes in the plasma membrane.

Growth Upregulates PIN1

Next we investigated the effects of biologically relevant induction of mechanical strain. During organogenesis, auxin promotes growth via cell wall acidification (Figure 6A). According to the "acid growth" theory, auxin induces cell wall acidification (from pH ~5.5 to ~4.5) that activates cell wall loosening [8, 9]. PIN1 is highly expressed in young leaves (Figure 6B) and is polarized toward the tip of primordia, where auxin maxima are maintained to facilitate further growth (Figure 6C).

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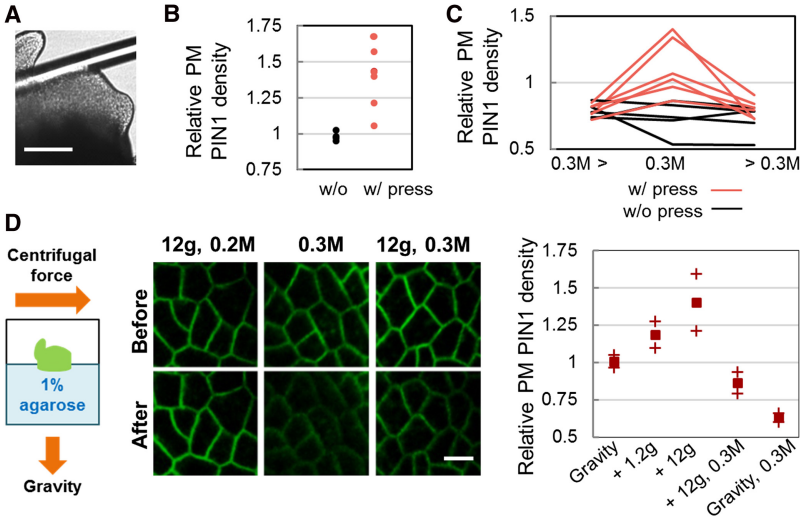

Figure 4. External Force Application Reverses the Hyperosmotic Effects on PIN1

(A–C) External force application by pressing.

(A and B) Gentle pressing with a pulled glass rod (A) increased the plasma membrane (PM) PIN1 density (B). Each dot denotes after/before ratio of the PIN1 density per sample (n = 8–12 epidermal cells in the area adjacent to the site of force application; n = 4–7 samples). Scale bar represents 100 μm.

(C) Pressing reversed hyperosmotic effects on PIN1. Samples were kept in 0.3 M mannitol throughout the experiment; after first 30 min, pressing was applied for 30 min to some of them, and then removed and incubated for another 30 min. The PM PIN1 density in 0.3 M mannitol, with or without pressing, is shown in ratios to the pretreatment density. Each line represents a sample.

(D) External force application by centrifugation. Centrifugation for 30 min increased the PIN1 density and lessened the hyperosmotic effect (■, average after/before ratio among samples; +, SD). n = 8–12 epidermal cells in the area facing the center of the centrifuge, per sample; n = 5–7 samples. Scale bar represents 10 μm.

When the cell wall loosens, the cells expand, increasing the cell wall strain. We therefore monitored PIN1 upon artificial growth induction. The cell wall was loosened with auxin or acid and then extended in a subsequent hypoosmotic treatment.

Application of exogenous auxin (IAA) increased the PIN1 density (Figure 6D). This probably reflects upregulation by auxin [46], which is mechanics-independent because the cells remained the same size. Upon the subsequent hypoosmotic treatment, the cells pretreated with 0.1 or 1 μ M IAA expanded more, and their PIN1 density increased further. Thus, IAA and the cell wall strain upregulated PIN1 additively. Although coincubation with 3% DMSO did not affect the growth induction, it did block the increase in PIN1 density by IAA or the cell wall strain. Auxin and mechanical strain seem to promote PIN1 abundance via plasma membrane strain.

We also induced extra growth by cell wall acidification. The cell wall pH was calibrated with MES buffers to pH 4.5, 5.5, or 6.5, and then the tissue was strained with a hypoosmotic treatment (Figure 6E). The apices that were preadjusted to pH 4.5 expanded 4%–5% more than the other samples. The acid treatment induced growth as much as 0.1 or 1 μM auxin did (Figure 6D), consistent with the acid growth theory of auxin-mediated cell expansion. The pH 4.5 samples also raised the PIN1 density by ~30%, but this upregulation of PIN1 was blocked by coincubation with 3% DMSO. These findings also suggest that growth upregulates PIN1 through the membrane properties.

Mechanics Affects Auxin Accumulation and Organ Growth

In order to assess whether mechanical regulation of PIN1 level and intracellular localization also affect the protein function, we monitored in vivo auxin accumulation pattern using DR5::*YFP* transgenic plants. They contain a nuclear-targeted, VENUS-type *YFP* reporter gene, which is under the influence

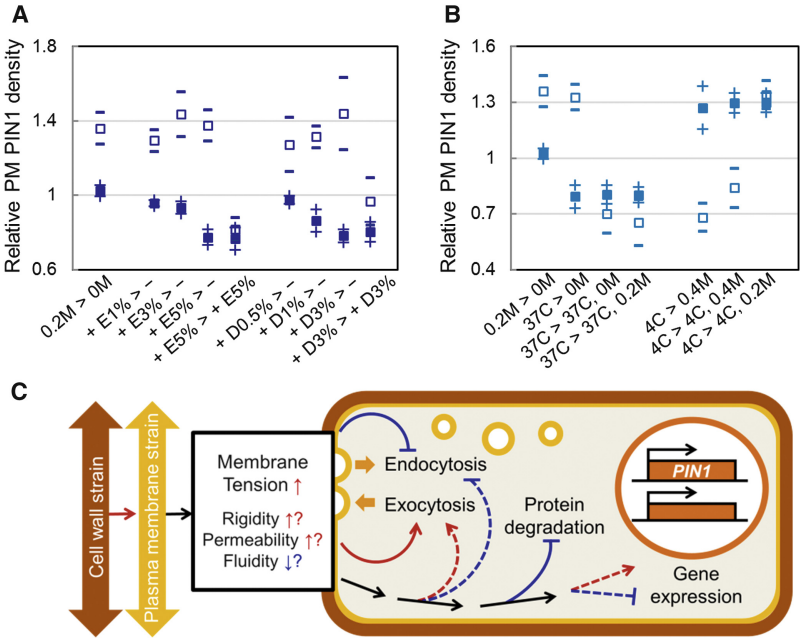

Figure 5. Membrane Modulation Is Sufficient for the Osmomechanical Effects on PIN1

(A) Membrane modulation by chemicals. Changes in the plasma membrane (PM) PIN1 density (shown in ratio to the pretreatment density) after 30 min incubation in 0.2 M mannitol with/without ethanol or DMSO (E or D; concentration in %) (■, average; +, SD) and after another 30 min in 0 M with/without E or D (□, average; –, SD). n = 8–12 near median epidermal cells per sample; n = 5–7 samples.

(B) Membrane modulation by temperature. Changes in the PM PIN1 density after 30 min treatment in 0.2 M mannitol at RT/37°C/4°C (■, average; +, SD) and after the second 30 min incubation in 0/0.2/0.4 M mannitol at RT/37°C/4°C (□, average; –, SD). n = 8–12 near median epidermal cells per sample; n = 5–8 samples.

(C) Schematic of how strain in the cell wall (brown) can alter the strain and mechanical parameters of the plasma membrane (yellow), triggering such cellular responses as shifts in membrane trafficking and signaling cascades. The mechanotransduction may further modulate endocytosis and exocytosis, as well as the chemical composition of the cell via protein synthesis and degradation, and transcriptional regulation. Red and blue arrows respectively indicate positive and negative influences. Dotted arrows represent hypothetical, as yet unverified effects that are likely to take place. See also Figure S3.

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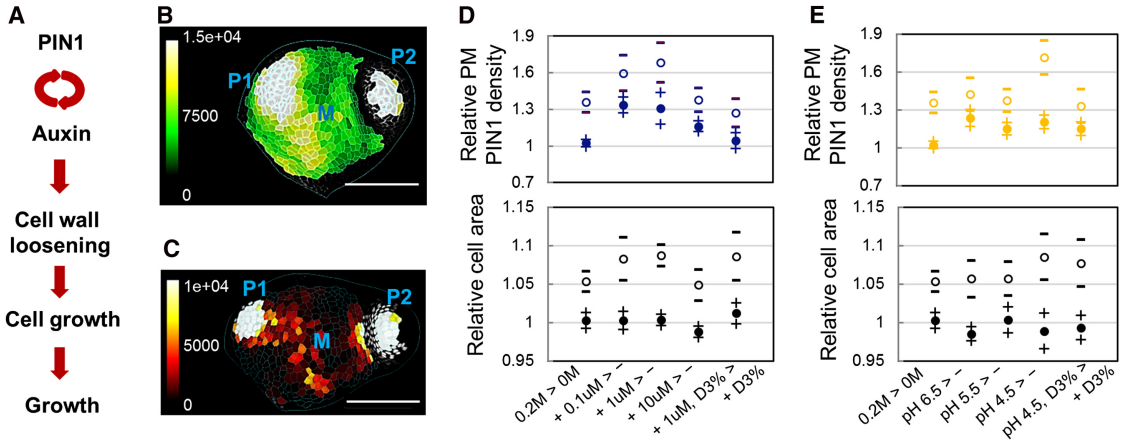

Figure 6. Growth Upregulates PIN1

(A) Previous view of auxin-mediated growth at the shoot apex.

(B and C) Heat maps of epidermal (B) plasma membrane (PM) PIN1 density and (C) auxin signaling output (visualized with DR5::YFP). Scale bars represent 100 μm.

(D and E) Growth induction assay by (D) exogenous auxin treatment or (E) cell wall acidification. Changes (shown in the ratio to the pretreatment value) in the PIN1 density (upper panel) and cell area (lower panel) after 30 min treatment in 0.2 M mannitol with/without (D) 0.1/1/10 μM active auxin IAA or (E) pH 4.5/5.5/6.5 buffer (●, average; +, SD), followed by 30 min treatment in 0 M with/without 3% DMSO (○, average; –, SD). For each treatment, n = 8–12 near median epidermal cells per sample; n = 5–7 samples.

of the auxin-responsive element DR5 [20, 47]. Changes in the YFP signal were quantified in the two youngest primordia (P1 and P2), as well as in the meristem (M) where the next leaf will emerge, before and after osmotic treatments with or without 3% DMSO.

Both 0 M and 0.4 M mannitol treatments interfered with the normal auxin accumulation pattern in the shoot apex. In the hypoosmotic condition, the auxin signal output was reduced by ~20% both in the primordia and the meristem (Figure 7A), which probably resulted from the less polar distribution of PIN1 (Figures 2A and 2B). On the other hand, the hyperosmotic treatment did not affect the auxin maxima in the primordia, where auxin maxima were already present, but reduced auxin accumulation in the meristem, where a new auxin maximum was forming, by ~60%, suggesting that reduced functional level and polarity of PIN1 interfered with auxin movement. Incubation with 3% DMSO in 0.2 M mannitol had similar effects as the hyperosmotic treatment, but the effect was recovered if it was in 0 M mannitol. Remarkably, the combination of membrane straining hypoosmotic condition and membrane softening DMSO led to nearly normal auxin distribution, unlike the ones observed in either single treatment.

Such effects on auxin accumulation were reflected in primordia growth (Figure 7B; Figure S4). The primordia treated with the hypo- or hyperosmotic solutions grew less. Incubation with 3% DMSO also reduced growth in 0.2 M mannitol. DMSO application in the hypoosmotic solution instead rescued the hypoosmotic or DMSO-dependent growth reduction, at least partially, similarly to the DR5 signal. These results together suggest that a fine-tuned mechanical balance is critical for proper auxin accumulation dynamics and growth control in the tomato shoot apex.

Discussion

In order to investigate whether mechanics regulates auxin dynamics, we modified tissue mechanics in living tomato shoot apex by osmotic treatments, external force applications, membrane modulations, and growth inductions. Because mechanical manipulations are intrinsically pleiotropic, we took the strategy to employ multiple independent assays. It is the combination of different approaches and especially the counteracting effects of different treatments that lead us to conclude that tissue mechanics affects PIN1 abundance and intracellular localization.

How could this work mechanistically? The mechanical load of plant tissues is borne by the cell wall, and cells are thought to perceive cell wall mechanics through the interaction between the cell wall and the plasma membrane. The mechanical status of the cell wall is transmitted to the plasma membrane, affecting the proteins embedded in it. A simple scenario by which tissue mechanical strain could regulate the abundance and subcellular distribution of PIN1 is as follows (Figure 5C). Local cell wall strain is directly transferred to the plasma membrane. Accommodating this strain, the membrane increases its tension. This increase in the in-plane tension of the membrane facilitates exocytosis and inhibits endocytosis [36–42], shifting the ratio between plasma membrane-localized and cytosolic PIN1, the latter of which is degraded. This biophysical principle is likely to apply to all membrane proteins; however, because PIN proteins rapidly cycle between the membrane and intracellular compartments, they would be exquisitely sensitive to small changes in the balance between the endo- and exocytosis [48].

The above hypothesis where the plasma membrane itself acts as a mechanosensor does not exclude more complex models. Plasma membrane strain could modify membrane rigidity and/or permeability, depending on the material properties of the membrane. Mechanical stimuli are also known to rearrange the cytoskeleton [16, 49]. Furthermore, plasma membrane-localized stretch-activated channels and receptor-like kinases are thought to be involved in the perception of mechanical signals [34, 35]. Activation of such factors triggers calcium- or phosphorylation-dependent signaling cascades [50–52]. Although the molecular architecture of such pathways and their roles in developmentally relevant

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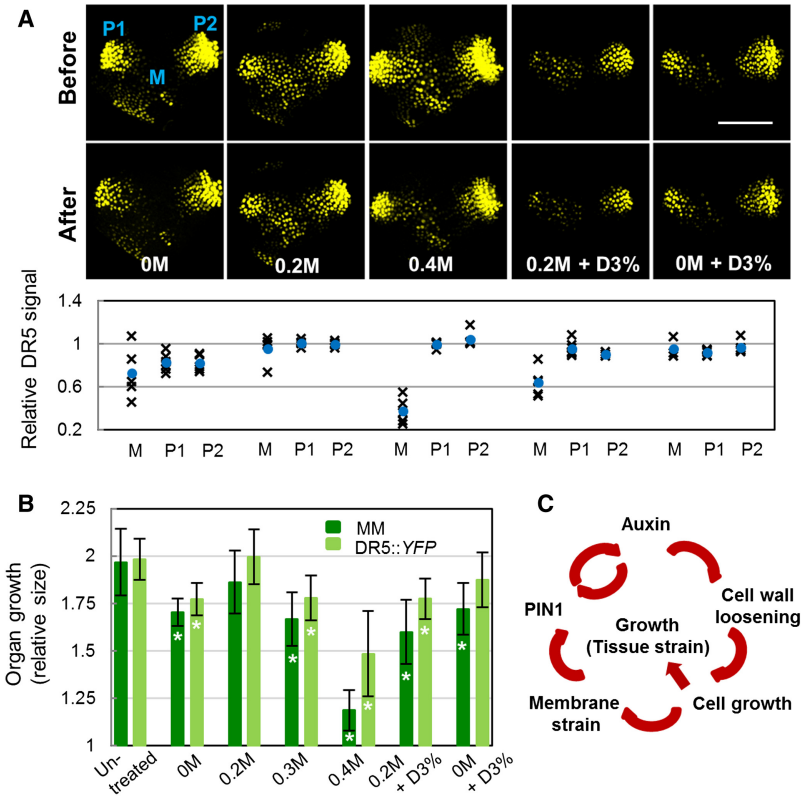

Figure 7. Mechanical Modulation Affects Auxin Accumulation and Organ Growth

(A) Osmomechanical effects on auxin accumulation pattern. Change (in after/before ratio) of DR5::YFP signal intensity in the meristem (M) or two youngest primordia (P1 and P2) after 2 hr treatment in 0/0.2/0.4 M mannitol or 30 min 3% DMSO treatment in 0/0.2 M mannitol (x, average per sample; n = 10 epidermal cells; blue dot, average among the averages; n = 5–6 samples). Scale bar represents 100 μm.

(B) Osmomechanical effects on organ growth. Primordia size change (in after/before ratio; average ± SD) after 20 hr growth following 2 hr osmotic or 30 min 3% DMSO treatment. The samples consisted of two genotypes (wild-type "MM" and DR5::YFP). n = 5–8. *, different from Untreated samples at 95% confidence.

(C) New model of auxin-mediated growth via mechanical regulation of PIN1: the mechanical strain due to growth upregulates PIN1 and facilitates further auxin accumulation and growth in young primordia. See also Figure S4.

mechanical transduction are not well established, yet they are plausible candidates mediating the regulation of PIN1 abundance and subcellular localization.

Because PIN-directed auxin accumulation guides numerous developmental patterning events, from embryogenesis and organogenesis to tropic growth in response to environmental stimuli [53, 54], mechanical sensitivity of PIN proteins has many implications. One example is promotion of growth in young primordia. PIN1 accumulates auxin, and auxin induces growth. Auxin maxima are reinforced by activation of *PIN1* gene expression by auxin [46] (Figure 6A). Our findings add another layer to this feedback loop: growth, via increasing cell wall strain and membrane modulation, upregulates PIN1 and ensures its localization to the plasma membrane and the polarity toward auxin maxima (Figure 7C). Auxin and mechanics together potentiate organ growth in a robust, multi-layered positive feedback loop.

PIN polarity is thought to be maintained through more exocytosis and less endocytosis on one cell face [22, 48, 55–57]. Higher membrane tension (generally corresponding to higher cell wall stress and strain) at one side of the cell can explain the asymmetric membrane trafficking in normal conditions, as well as the cell face-dependent effects of the osmotic treatments (Figure 2). Plasmolysis of *Arabidopsis* root cells also resulted in internalization and less polar distribution of PIN1 and PIN2 proteins [58], suggesting that similar mechanisms regulate PIN proteins in general. The seemingly unrelated factors that have been implicated in polar targeting of PINs, such as auxin [22], mechanical stress [25], plasma membrane-cell wall connections [58], steroid [59], and cell curvature [60], share the common theme that they can locally increase membrane tension. Polar targeting of PIN proteins may be specified through intracellular variations in plasma membrane properties.

Experimental Procedures

Plant Materials

The vegetative shoot apices of 14- to 21-day-old tomato plants of the transgenic lines transformed with the At-PIN1p::*PIN1-GFP* or DR5::*VENUS*X6 construct [25, 51] or the wild-type cultivar Moneymaker (Wyss, Zuchwil, Switzerland) were used after dissection down to two to three primordia. For more details of the growth condition and sample preparation, see Supplemental Experimental Procedures.

Image Collection and Analysis

GFP/YFP signal in the epidermal cells was quantified using ImageJ (rsbweb.nih.gov/ij) and/or the newly developed software MorphoGraphX (www.morphographx.org). 3D stacks of optical sections were collected using a TCS SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany). See Supplemental Experimental Procedures for more details on confocal imaging and data analysis, including PIN1 polarity calculation, and for the protocols for individual experiments.

Supplemental Information

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.06.050.

Acknowledgments

We thank Marc Boutry for the H^+-ATPase antibody, Naomi Ori for the DR5::*YFP* seeds, and Jacques Dumais, Bruno Moulia, and the Kuhlemeier group for helpful discussion. This work was supported by EMBO LT and Roche Research fellowships to N.N., EMBO LT fellowship to S.R., MEXT Japan Grant-in-Aid for Young Scientist (#22870031 and 24770047) to S.K., EU New and Emerging Science and Technology grant "MechPlant" to A.B. and C.K., and SystemsX.ch grant "Plant Growth in a Changing Environment" to R.S.S. and C.K.

Received: January 17, 2012
Revised: April 26, 2012
Accepted: June 19, 2012
Published online: July 19, 2012

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Please cite this article in press as: Nakayama et al., Mechanical Regulation of Auxin-Mediated Growth, Current Biology (2012), http://dx.doi.org/10.1016/j.cub.2012.06.050

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