Tissue folding at the organ–meristem boundary results in nuclear compression and chromatin compaction

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Artificial mechanical perturbations affect chromatin in animal cells in culture. Whether this is also relevant to growing tissues in living organisms remains debated. In plants, aerial organ emergence occurs through localized outgrowth at the periphery of the shoot apical meristem, which also contains a stem cell niche. Interestingly, organ outgrowth has been proposed to generate compression in the saddle-shaped organ–meristem boundary domain. Yet whether such growth-induced mechanical stress affects chromatin in plant tissues is unknown. Here, by imaging the nuclear envelope in vivo over time and quantifying nucleus deformation, we demonstrate the presence of active nuclear compression in that domain. We developed a quantitative pipeline amenable to identifying a subset of very deformed nuclei deep in the boundary and in which nuclei become gradually narrower and more elongated as the cell contracts transversely. In this domain, we find that the number of chromocenters is reduced, as shown by chromatin staining and labeling, and that the expression of linker histone H1.3 is induced. As further evidence of the role of forces on chromatin changes, artificial compression with a MicroVice could induce the ectopic expression of H1.3 in the rest of the meristem. Furthermore, while the methylation status of chromatin was correlated with nucleus deformation at the meristem boundary, such correlation was lost in the mutant. Altogether, we reveal that organogenesis in plants generates compression that is able to have global effects on chromatin in individual cells.

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forces act as instructive mechanical signals that affect all aspects of cell and developmental biology (1, 2). Although much attention has been paid to the role of forces at the cell cortex, forces can also propagate to the nucleus and affect chromatin and gene expression (3–6). In turn, chromatin changes can also affect nucleus stiffness and shape (7). However, the impact of forces on nuclei has mainly been studied in single cell systems using artificial micromechanical manipulations, so far. Patterns of forces are naturally present in multicellular tissues. In particular, mechanical conflicts result from differential growth and tissue morphogenesis (e.g., refs. 8 and 9). In plants, because cells are encaged in a wall, cell movement is hindered; when tissues grow and change their shape, cells have to cope with the resulting mechanical stress without resolving to cell intercalation (nor cell death in young tissues). In Arabidopsis shoot apical meristems, tissue folding occurs at the boundary between organs and meristem. Finite element model simulations suggest that this domain experiences highly anisotropic tension in the circumferential direction or radial compression (10). Radial compression of the outer cell wall has been reported in that domain in previous studies and would be consistent with compression at the boundary (11). Here, we characterized the nuclear compression at the organ boundary correlating with changes of chromatin organization and linker histone H1.3 activity.

Results

Nuclei Become Elongated as the Organ–Meristem Boundary Folds. Using the pdf1::CFP-N7 marker line, which marks nuclei (N7 nuclear localization signal) in the epidermis (PDF1 promoter), we observed that some nuclei at the boundary often display an elongated shape (Fig. 1A). These elongated nuclei appeared late in boundary formation, that is, when tissue folding is pronounced [i.e., beyond flower stage 2 (12)] and when major supracellular alignment of cortical microtubules is observed (Fig. 1A). When staining with FM4-64, we observed that these nuclei might be physically constrained by cell geometry (as the nuclear–cytoplasmic ratio is high in meristematic cells) (Fig. 1A), consistent with predicted compression in that domain (10, 13).

Because cell division follows the direction of maximal tension, cells at the boundary divide along one of their long axes (14). Therefore, cells appear more elongated in that domain. In principle, this may be sufficient to constrain nucleus shape. However, in that scenario, the presence of elongated nuclei

Significance

During development, growth deforms tissues and organs. This is notably the case during the formation of new flowers in plants, as the tissue folds during young floral bud emergence. Here, we provide further evidence that organogenesis compresses the cells at the boundary, separating the organ from the stem cell niche, and we show that this leads to nucleus compression and chromatin changes. While mechanical forces are well known to affect nucleus shape and chromatin in mammalian cells in culture, this demonstrates that such an effect also occurs in a developing organism and suggests that forces may help to define boundary domains through large-scale chromatin effects.
Fig. 1. Nucleus compression at the organ–meristem boundary in Arabidopsis. (A) (Upper) The maximal projections of confocal stack images with representative dissected shoot apex containing the fluorescent reporters for nuclei (pPDF1::CFP-N7, shown in gray), microtubules (pPDF1::mCitrine-MBD, shown in yellow), and membranes stained with the FM4-64 (magenta). (Lower) The boundary domain of representative shoot apex, showing cropped organ and boundary region of the respective sample. (Scale bars, 20 μm.) (B) Diagram representing the top and orthogonal views of a meristem (M) with a growing organ (O). Organ growth laterally pulls (blue arrows) and radially compresses (red arrows) cells at the boundary. (C) Representative images of a growing organ at the shoot apex of p35S::SUN1-YFP reporter line, acquired at four time points within 36 h. (Upper) The top view projections and the orthogonal sections of the respective stacks the growing organ and the boundary domain. (Bottom) Close-ups in the boundary region where the nuclei (marked with an asterisk) become more elongated following the 36 h. Green: SUN1-YFP; gray: cell membranes stained with the FM4-64. (Scale bars, 10 μm; for nuclei at the boundary, 5 μm.)

would not be the result of progressive compression emerging from neighboring tissue growth. To check whether organ growth can indeed actively compress nuclei at the boundary, we used the p35S::SUN1-YFP line (with SUN1 for SAD1/UNC-84 DOMAIN PROTEIN 1) that marks the nuclear envelope (15) and followed nuclear behavior in nondividing cells every 12 h. We could identify a population of nondividing cells that exhibit progressive nucleus deformation (Fig. 1 B and C). The magnitude of nucleus deformation increased with tissue curvature, consistent with organ growth being the main cause for nuclear compression in these cells (Fig. 1 B and C). In fact, we could mimic such nucleus deformation by mechanically compressing shoot apices laterally in a MicroVice with a force of ca. 0.1N (SI Appendix, Fig. S1). Altogether, this suggests that nuclear deformation in the boundary region is the result of compressive stresses caused by organ outgrowth.

To quantify these nuclear phenotypes, we used images obtained with the p35S::SUN1-YFP line and developed a computational pipeline to track cells and nuclei and quantify nuclear shapes (SI Appendix, Fig. S2). In brief, MorphoGraphX (16) was used to extract the meristem surface, and segment [two-dimensional (2D)] cells on which the curvature was mapped. In addition, three-dimensional (3D) cell segmentation of the epidermal layer followed by cell tracking was performed using multiangle image acquisition, three-dimensional reconstruction, and cell segmentation–automated lineage tracking (MARS/ALT, ref. 17). The nuclei were segmented in 3D using the MARS watershed algorithm. Finally, meristem regions and nuclear shapes were extracted and quantified using MATLAB (The MathWorks, Inc.) scripts after projecting the nuclear signal in 2D.

A subpopulation of nuclei at the boundary region exhibited a significant increase in their aspect ratio. In each boundary we could find a group of at least 10 nuclei with an aspect ratio superior to 1.55, whereas meristematic nuclei typically have an aspect ratio of 1.2 ± 0.15 (n = 132) (Fig. 2A and SI Appendix, Figs. S3 and S4). When backtracking these most affected nuclei via their cell lineages, we also found that they increased their aspect ratio following the increased growth of the primordia, correlating with the increased (negative) curvature of the boundary domain (Fig. 2B and C and SI Appendix, Figs. S3 and S4). Altogether, these quantities demonstrate the existence of active nucleus compression operating at the meristem boundary during organogenesis and highlight the presence of a subset of boundary cells with increasing negative curvature in time, indicative of cellular compression.

Linker Histone H1.3 Is Induced by Compression. The boundary domain has a specific gene expression profile (18). This pattern relies on hormones, microRNA, and mechanical stress (19–21). Nuclear compression is likely to have a more global effect on chromatin than on specific gene expression, as shown in animal systems (5, 6, 22, 23). Furthermore, changes in global chromatin state have been associated with changes in chromatin compaction, which relies on linker histones (H1 isoforms). In plants, at least three isoforms of H1 have been studied. Their expression and competitive binding to DNA depend on cell cycle as well as on external stress factors. H1.3 has been associated with the cells response to abiotic stresses in past work (24). In particular, H1.3 is induced by drought stress (24) which, based on data from animals (e.g., refs. 25 and 26), might also drive nucleus compaction. When analyzing the expression pattern of three variants of histone H1 in the meristem, we found that H1.1 and H1.2 were expressed in the entire meristem, with a higher expression level in organs. Interestingly, H1.3 exhibited a patchy pattern, with the strongest signal deep in meristem boundaries (Fig. 3A). To go beyond this correlation, we next applied compression on meristem to check whether H1.3 expression would be induced. To do so, we used N-(1-naphthyl) phthalamic acid (NPA)-treated...
seedlings and placed the naked stems between two blades of the MicroVice (SI Appendix, Fig. S1A and Materials and Methods). When the blades were touching the meristem without deforming it, no clear H1.3 expression could be detected in the meristem (Fig. 3B and see SI Appendix, Fig. S5B) (n = 6/6 meristems with less than 3 nuclei exhibiting H1.3 induction at t = 8 h). However, when the meristem was deformed upon compression, most meristems expressed H1.3 ectopically (Fig. 3B and SI Appendix, Fig. S5B) (n = 10/11 meristems with more than 3 nuclei exhibiting H1.3 induction at t = 8 h). Our finding suggests that cells at the meristem boundary are experiencing high and long enough compressive stress to induce such expression.

Global Chromatin Changes Occur at the Organ–Meristem Boundary. Our results suggest that nuclear compression, naturally occurring during organogenesis, generates iterative chromatin changes each time a new organ emerges. To further test this hypothesis, we investigated whether nucleus compression is strong enough to affect chromatin architecture. To do so, we used whole-mount fixed meristems and stained them with DAPI, which displays a
brighter signal in heterochromatin regions corresponding to chromocenters (e.g., ref. 27). We analyzed their distribution in successive optical sections (SI Appendix, Fig. S6A). To check whether nucleus compression affects the distribution of these

**Discussion**

We show that organogenesis at the shoot apical meristem leads to nucleus compression in a specific set of cells, which is accompanied by major chromatin changes. This demonstrates that forces have a strong and direct impact on nuclei in a tissue context. The role of forces on chromatin is now well established, notably through experiments with artificial deformations of single cells in culture (4–6). Our results not only show that such effects also occur in plants but they also demonstrate that these effects occur in the natural context of a developing organ. In fact, several reports have proposed that the main role of forces in the nucleus is to affect the physics of chromatin, which in turn would affect gene expression (instead of affecting specific genes that would then affect chromatin). For instance, nuclei have been found to exhibit an auxetic behavior because of the decomposition of chromatin upon nucleus stretching (7). Our results are consistent with such conceptual framework and suggest that forces may primarily have global effects on chromatin, which would subsequently affect the expression of specific genes.

In previous work, we had already shown that STM and CUC3 are induced by mechanical stress at the organ–meristem boundary (20, 21). However, both of these genes are induced in shallow boundaries (i.e., before we can detect significant nucleus deformation). We thus believe that nucleus deformation may contribute to their expression, but it is unlikely that we would find a clear-cut contribution of nucleus deformation on the

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Fig. 4. Chromatin changes in compressed nuclei at the organ–meristem boundary. (A and B) Maximal projection of the confocal stacks showing the expression of the pHTR5::HTR5-GFP (A) and pHTR5::mCG-MBD-GFP (B) reporter lines with the global top view (Left) and close-up in the organ boundary area (Right). (Scale bar, 20 μm.) (C) Representative pHTR5::mCG-MBD-GFP and pPDF1::CFP-N7 patterns in organ and meristem domains. (Scale bar, 5 μm.) (D) Number of mCG regions in the nuclei correlates negatively with nuclear aspect ratio. (Left) Violin plots illustrating the distributions of mCG regions in the nuclei with the aspect ratio below 1.55 (n = 256) and above 1.55 (n = 51). The black line in the violin plot represents the median, and the dots are the values of individual samples; P value < 0.05 (P value = 7.3 × 10^{-5}, n = 5 meristems). (Right) A plot of the average number of mCG regions in the nuclei as a function of nuclear aspect ratio. The error bars represent the SEM, where 9 ≤ n ≤ 93. (E) (Left) The violin plot of average nuclear signal density in the nuclei with the aspect ratio below 1.55 (n = 331) and above 1.55 (n = 74). The black line in the violin plot represents the median, the dots are the values of individual samples; P value < 0.05 (P value = 4.5 × 10^{-6}, n = 6 meristems). (Right) A plot of average nuclear signal density in the nuclei in correlation with the nuclear aspect ratio. The error bars represent the SEM, where 19 ≤ n ≤ 133.
expression of these genes since they are already highly expressed when nuclei become more deformed. Instead, the analysis of chromatin status appears more convincing and also better fits with the idea that mechanical stress would have a more global role on the structural component of the nucleus (i.e., nucleoskeleton and chromatin, rather than an instructive role on specific genes). These data are important as they establish a link between growth-derived patterns of compression in tissue to nuclear shape and chromatin status.

Further studies would be required to elucidate the structural role of histones in response to growth-derived forces in plants. Here, we reveal the induction of linker histone H1.3 at the organ–meristem boundary. One may speculate whether H1.3 expression could scale with mechanical stress intensity in different tissues. Interestingly, H1.3 expression has never been detected in the female megaspore mother cell (i.e., in one of the most mechanically protected cells, arguably) (32). Yet, we could not detect major phenotypic defects in the H1.3 mutant meristem; at least in control growth conditions (SI Appendix, Fig. S8).

The situation might actually be more complex. Many cross-talks between linker histones have been identified (33) and, unsurprisingly, chromatin changes are associated with many pathways, from drought (26) to light (34). Integrating the contribution of mechanical forces in this network is thus likely going to be a long endeavor, but also an exciting one. In particular, this may compel us to see the structural side of chromatin and analyze the role of the relevant regulators with this lens, also echoing results obtained in animal cells in culture (e.g., ref. 35).

This work also opens the path to the investigation of the mechanisms leading to such nucleus compression and chromatin compaction. For instance, nuclei may be simply squeezed by pressure, meaning that nuclear density would increase, resulting in chromatin changes. Interestingly, hyperosmotic stress leads to nucleus deformation and chromatin changes in animal cells in culture as well as in isolated nuclei (36). The behavior of nuclei in the cells at the organ–meristem boundary may recapitulate such effects, albeit with mechanically driven, instead of osmotically driven, nucleus deformation and in a growing tissue context. The role of the nuclear envelope is also likely to be important in that pathway (e.g., refs. 37, 38). In particular, chromatin organization depends on proteins from the Linker of Nucleoskeleton to Cytoskeleton complex in plants (39).

In mammalian cells, stretching was recently proposed to soften chromatin through a reduction in heterochromatin formation, thereby insulating the genetic material from cellular and supracellular mechanical stress (40). Interestingly, nuclei become stiffer in plant cells through heterochromatin clustering when exposed to hyperosmotic stress (i.e., when tensile stress level at the cell cortex decreases) (41). This could suggest that cortical tension might positively correlate with softer nuclei across kingdoms. Whether this applies to our system remains an open question. The observed reduction in the number of chromocenters at the meristem boundary, both via DAPI staining and mCG-MBD-GFP signal quantification, echoes the behavior of human stem cells placed on stiff environment (42). In our system, such reorganization of chromatin may involve a decrease in individual heterochromatin clusters as well as the spreading of

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Fig. 5. Chromatin changes in compressed nuclei at the organ–meristem boundary in h1.3 mutant. (A) Cartoon drawing illustrating the approach, used for measuring the average nuclear signal density and aspect ratio at the boundary region of a dissected meristem. (Left) Shows the top and side views of the region between the shoot apical meristem and a young organ; the L1 layer at the boundary region is shaded in pink with the contours of the nuclei traced. The Right represents a single nucleus, selected within the target region. The average fluorescence signal is projected from the portion of the z-stack corresponding to the thickness of the cell layer. This data was subsequently used to extract the values of nuclear aspect ratio and average nuclear signal density. (B) Plots illustrating the areas (Left) and aspect ratios (Right) of the nuclei at the organ boundary regions in WT and h1.3 mutant plants containing the p35S::SUN1-YFP reporter. Sample size for WT: \( n_{WT} = 201 \) nuclei from 5 meristems; \( n_{h1.3} = 223 \) nuclei from 5 meristems. \( P \) value for area \( = 0.07 \) and \( P \) value for aspect ratio \( = 0.05 \). The black line in the violin plots represents the median; the dots are the values of individual samples. (C) Violin plot illustrating the average nuclear signal density in the WT and h1.3 nuclei with the aspect ratio below 1.55 (\( n_{WT} = 363 \) and \( n_{h1.3} = 473 \) in 10 meristems for both genotypes) and above 1.55 (\( n_{WT} = 106 \) and \( n_{h1.3} = 106 \) in 10 meristems for both genotypes). The black line in the violin plots represents the median; the dots are the values of individual samples. Higher average signal density is detected in the nuclei of the WT with the aspect ratio above 1.55 (\( P \) value \( = 1.35 \times 10^{-5} \)), compared to below 1.55, a trend not seen in the h1.3 mutant (\( P \) value \( = 0.1 \)).

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more transient CHH methylation. The accumulation of H1.3 histones would compete with other linker histones (24), but it remains to be shown whether this would also affect nuclear stiffness ultimately.

From a developmental point of view, our work adds another layer of complexity to the definition of the boundary domain in plant shoot apices (19). Beyond morphology (13), hormone signatures (43), gene expression (18), microRNA regulation (44), and stress pattern (10), boundary domains may indeed be defined by nucleus compression and associated chromatin changes. This likely contributes to its cadaster role and may be conserved beyond the plant kingdom.

Materials and Methods

Plant Materials and Growth Conditions. All procedures were performed on plants from the Col-0 ecotype, grown in “long day” conditions, with 16 h/day light/dark period at 21 °C. The pPDF1::CFP-N7 (20), pPDF1::mCitrine-MBD (45), pPDF5::SUN1-YFP (15), pH1.1::H1.2-EGFP; pH1.2::H1.2-EGFP (32), pH1.3::H1.3-EGFP (24), pHTR5::HTRS-GFP (46), pPHTR5::mCG-MBD-GFP, and pPHTRS::mCHH-GFP (30) reporter lines were used for this study.

Meristem Dissection. Stems were cut when the shoot apical meristem switched to an inflorescence meristem identity, that is, between the appearance of the first flower to the appearance of the first silique [stages 13 through 17 (12)] and transferred to medium without inhibitor. Within 48 h, plants were placed in a petri dish (SI Appendix, Fig. S1A). The two blades of the Microvace were brought closer so as to induce a strong deformation of the shoot apex. Images were acquired with an upright Leica SP8 or Zeiss LSM700 laser scanning confocal microscope, as described below (n > 5).

DAPI Staining of the Whole-Mount Meristems. Prior to staining, the dissected shoot apices were fixed 10 min on ice and then under vacuum (3 times for 20 min at room temperature) in a fixation solution containing phosphate-buffered saline (PBS) 1x, ethylene glycol-bis[-iminomethyl ether]-N,N,N’,N’-tetraacetic acid 0.5M, dimethyl sulfoxide 10% formaldehyde 4%. The samples were then dehydrated in ethanol-methanol series as follows: 1× ethanol 70% for 5 min, 1× ethanol 100% for 5 min, 2× methanol 100% for 5 min, 2× ethanol 100% for 5 min, and incubated overnight in ethanol 100% at 4 °C. Rehydration was performed as follows: 1× ethanol 100% for 5 min, 1× ethanol 70% for 5 min in PBS (1% Tween20 in PBS), 1× ethanol 50% for 5 min in PBS, 1× ethanol 30% for 5 min in PBS, and 3× PBS for 5 min. Then, the samples were incubated for 30 min at 37 °C with the mix of cell wall digesting enzymes (48) and RNase A (0.1 mg/mL) and refixed in 4% formaldehyde in PBS. After washing with PBS buffer, the samples were incubated with the DAPI solution (5 µg/mL). The mounting of the samples was performed as described in (48).

Confocal Laser Scanning Microscopy and Image Analysis. Dissected shoot apical meristems from greenhouse plants or dissected meristems from in vitro-grown NPA-treated seedlings were imaged with a water-dipping lens (×25, numerical aperture = 0.8) using an upright SP8 confocal microscope to generate a stack of optical sections with an interval of 0.15 µm between slices. The membranes were stained with FM4-64 (also called N-(3-triethylamino- niumpropyl)-4-(6-(4-dihexyloxyphenyl)hexatienyl)-pyridinium dibromide, Thermofisher). Images are presented using the Fire lookup table (ImageJ). Image analysis for chromosome and CG-methylated regions detection was performed using the 3D object counter option in Fiji software (https://fiji.sc/wiki/index.php/Fiji). The nuclear average signal density was calculated following along the line of the protocol, described in ref. 31. In the case of imaging whole meristems, the area of the corresponding nuclei and then correlated with their aspect ratio.

Confocal images of the membrane-marked channel using the Block-Subtractive protocol for executing the steps of the pipeline is provided. All scripts and computational workflows for executing the Lineage Tracking function.

Cell lineage tracking. To compute cell lineages, affine transformation and, subsequently, nonlinear transformation was applied to the corresponding segmented images and optimal cell pairings between registered segmented image and reference segmented image were computed by identifying maximally overlapping cell labels from the two images.

Cell and nucleon size and shape quantification. For each segmented object (cell or nucleus), the z-slice with the largest area was extracted for further analysis. The aspect ratio was calculated as the length of the main axis divided by the length of the minor axis of the ellipse representing the covariance matrix of the pixel positions for the region. All properties were extracted using the MATLAB function regionprops() and MARS/ALT algorithms.

Software/protocol. Scripts required to do the segmentation and quantitative analysis are provided via the Sainsbury Laboratory Gitlab repository (https://gitlab.com/slcu/teamHJ/publications/fal_etal_2020), where also a more detailed protocol for executing the steps of the pipeline is provided. All scripts have been developed in Python (https://python.org) and MATLAB (Math-Works Inc.), and the protocol is dependent on the MorphoGraphX [https://www.mpipz.mpg.de/MorphoGraphX] and MARS/VALT (50) software.

Data Availability. Original confocal data are available via the University of Cambridge Data Repository [https://doi.org/10.17863/CAM.64310]. Scripts for the analysis pipeline are available via the Sainsbury Laboratory Gitlab repository (https://gitlab.com/slcu/teamHJ/publications/fal_et_al_2020).

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Table 1

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