Understanding Regeneration: investigating the apical notch and meristem re-emergence in *Marchantia polymorpha*

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Abstract

Meristems are the growth centres of plants. The phytohormone auxin controls meristem maintenance and mediates regeneration of new meristems if existing ones are damaged. The Marchantia gemma is an experimentally tractable system featuring two meristematic regions (apical notches). We used laser ablation to precisely manipulate the apical notches, finding that the first apical cell layer is indispensable for meristem maintenance. Within this, a contiguous quorum of cells is required for meristem maintenance, depending on the other apical notch's integrity. Apical notches remodel in response to damage, demonstrating that the apical cell layer acts as a communicating population rather than containing one master-controller Apical Cell and subordinate subapical cells. We show communication between notches and regenerating meristems, with heterogeneity across the gemma tissue for carrying this communication signal. The signal represses cell division and requires both sources and sinks, features of auxin-mediated communication. We investigated expression of auxin-related genes (MpYUC2, MpPIN1, MpARF1 and MpARF2) during regeneration. Auxin biosynthesis becomes restricted to specific patches during meristem re-emergence, mediated by reorganization of auxin transport rather than auxin signalling. This is supported by pharmacological experiments: exogenous auxin prevents regeneration, while inhibiting auxin biosynthesis or transport stalls regeneration at a later stage. Another auxin-related gene, MpERF20/LAXR, appears to "prime" cells for regeneration. We present a model of Marchantia meristem organization, involving intra- and inter-meristem communication, and for meristem regeneration, which requires balance between auxin sources and sinks. This provides a framework for further study of meristem communication, formation and maintenance in Marchantia and plants generally.

Introduction

Meristems are the growth centres of plants, niches where pluripotent stem cells continually divide asymmetrically to produce a daughter and a meristematic cell. The cells at the centre divide slowly, while those at the periphery divide faster. This moves the daughter cells outwards as they mature, where they become differentiated into various tissues and cell types. The meristematic cells remain in the centre to replenish the stem cell pool. This spatial distinction between division and differentiation allows plant meristems to simultaneously sustain both growth and new organ formation (1). In angiosperms the two main meristems are found at either end of the plant: the Shoot Apical Meristem (SAM) and Root Apical Meristem (RAM) (1, 2). The SAM model is thought to be how meristems operated in the early land plants (3). The phytohormone auxin has a central role in plant meristem function and organization. Auxin is produced in the SAM (4) and flows outwards by polar transport, where it is involved in controlling cell expansion and differentiation (5, 6). Secondary meristem formation is induced by the formation of local auxin maxima and minima arising out of auxin gradients extending from the apical meristems (6). Apically derived auxin promotes shoot growth and inhibits shoot branching by preventing growth of secondary (axillary) meristems in the buds: this is apical dominance. If the SAM is removed or damaged, the flow of auxin is disrupted, apical dominance is removed and the axillary meristems are released from dormancy and begin growing (7). Plants can also regenerate new meristems in response to wounding of existing meristems. This is due to their inherent cellular plasticity, which allows cells from certain tissue types (e.g. embryonic tissue, pericycle) to reprogramme into meristematic tissue in response to signals caused by a wounding-induced changes in auxin and cytokinin ratios (6, 8).

Developing a better insight of the how meristems function is key to understanding plant development. Meristems are vital in agriculture for developing improved crop plant breeds with beneficial apical dominance traits (2). Inducing meristem regeneration is important in vegetative propagation of agricultural crops (9). Controlling the molecular mechanisms for meristem specification and reprogramming therefore offers an avenue for developing new transgenic methods (10). Considerable progress on understanding apical meristems has been made from study of angiosperms, particularly *Arabidopsis* (11), however these systems have limitations, such as difficult access for imaging (particularly live imaging) (12, 13) and the complexity of the gene networks involved (2, 5).

The liverwort *Marchantia polymorpha* has emerged as a new model system in plant biology with numerous advantages that compliment other plant systems. As a bryophyte,

Marchantia occupies an important position in the land plant phylogeny (14–17). The Marchantia genome is streamlined, with fewer gene copies for many gene families (18, 19), notably the minimal set of components related to auxin biosynthesis, transport and signalling (16, 20–22). It is highly experimentally tractable, with fast growth rates and well-established transgenic protocols (23, 24). Marchantia produces haploid spores as well as vegetative propagules called gemma (25, 26). Gemmae develop in gemma cups and remain dormant until they are removed from these cups, at which point they germinate and begin growing in a stereotypical pattern of development. Crucially, gemmae have open development where almost all of the cells and tissues are easily accessible for microscopy without the need for dissection, sectioning or clearing (23, 27).

Gemmae have two meristematic growth centres, each located in an apical notch at opposite ends (25) (see Fig. 1A, B). Cell division is focused around the apical notches with less cell division towards the middle of the gemma. As cells move outwards away from the meristem, division rates reduce, and cell expansion and differentiation increase. The gemma eventually grows into a thallus and branching produces additional meristematic apical notches, however the early stages of gemma development are characterized by growth centred around the two original notches (28). Marchantia shows massive capacity for meristem regeneration, with even single cells capable of reforming full thalli (29–32). In the case of gemma, the basic rules are that if one or other apical notch is removed then the remaining notch maintains apical dominance and prevents regeneration; however, if both apical notches are removed then new cell divisions and eventually meristems emerge in the remaining gemma fragment (29, 32). These new meristems produce apical notches as in normal thallus development from a gemma. Auxin has long been known to be involved in meristem regeneration in Marchantia (33, 34). Auxin is produced in the apical notch and exported to the rest of the thallus, where it inhibits the formation of new meristems (i.e., apical dominance). Removal of the apical notches removes these auxin sources. The levels of auxin initially drop in the remaining thallus, and then rise as new meristems appear (29). Although the Marchantia apical notch has received relatively little attention compared to angiosperm meristems (19) it offers a promising opportunity to gain insights into plant meristems generally.

Previous efforts at studying Marchantia meristem maintenance and regeneration have employed surgical methods such as cutting with scalpel blades (29, 32). Here we use laser ablation and transgenic marker lines to achieve a more precise disruption of the meristems in gemmae and to get a more sensitive measure of cell division and meristem-related processes

(35). Our results point towards a model with a dynamic population of apical cells communicating within the notch and to the rest of the gemma tissue, and with a balance of auxin sources and sinks maintaining normal meristem activity. Under this model, disruption of this balance induces new meristems to emerge. Auxin-based communication is central to this process of meristem regeneration, which occurs via reorganization of auxin biosynthesis and transport. Given the conserved nature of many developmental pathways in land plants (19), these results are relevant to auxin- and meristem-related processes in land plants as a whole.

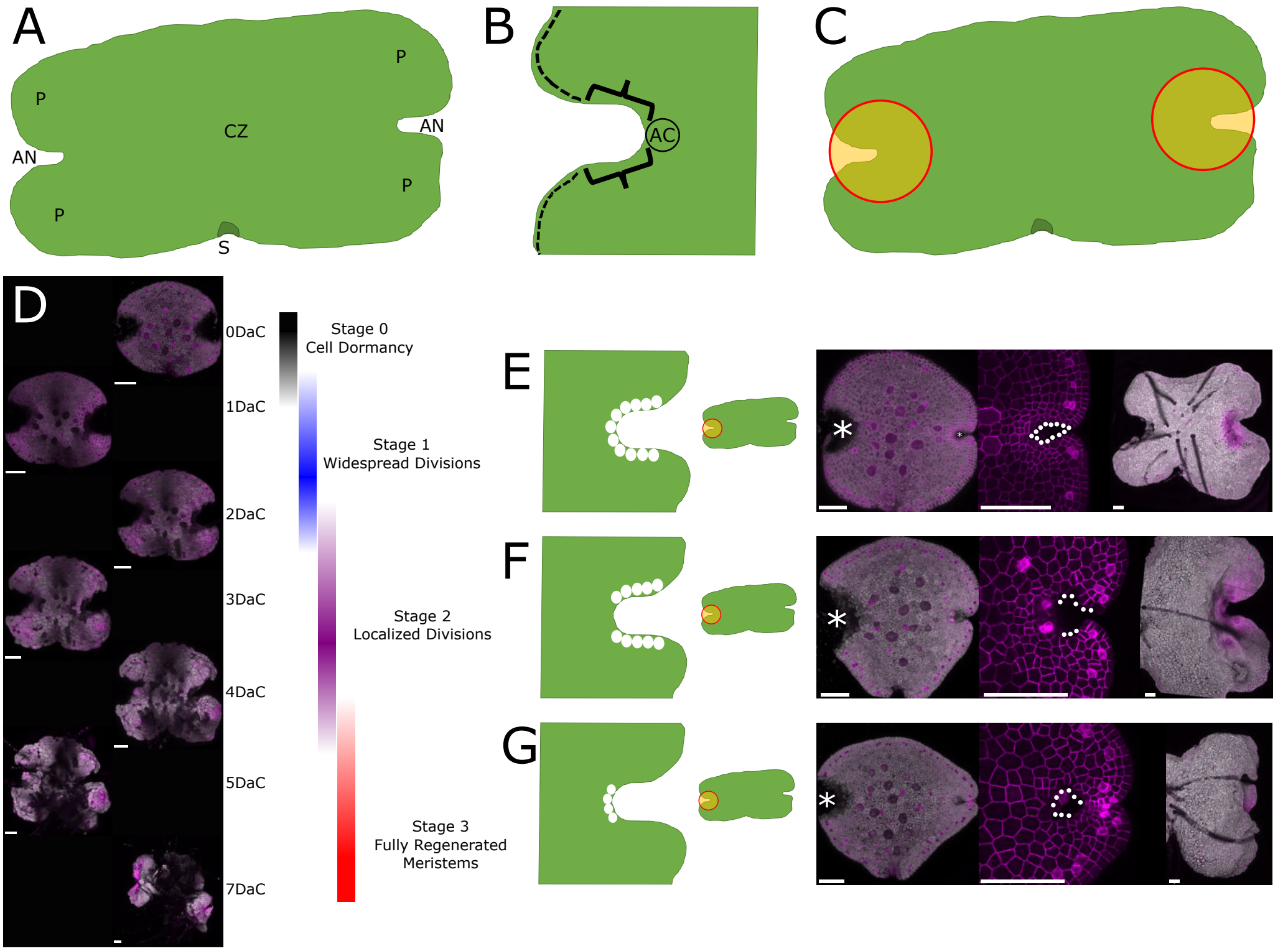

Fig. 1. Fine scale ablation shows that apical cells are indespensible for meristem function, but the cells within the apical notch are of equal competence. (A) Schematic outline of the Marchantia gemma showing the full gemma, with two apical notches (AN) at either end. To the sides of the notches are the peripheral regions (P) and behind the notches is the central zone (CZ). The stalk scar (S) is where the base of the gemma was attached to the parent plant in the gemma cup. (B) shows a close-up of an apical notch. AC marks the presumed "Apical Cell" at the apex of the notch, flanked by subapical cells (brackets) extending out the of "sides" of the notch. These cells are all in the first row of cells. Further out of the notch the first row of cells are comprised of margin tissue (dashed line) that forms from old notch cells and extends around the edge of the gemma and thallus. (C) is an outline schematic of the ablation pattern for complete excision of all notches (marked in orange with a red outline). (D) shows a time course of a pMpUBE2::mScarletI-lti6b gemma fragment following notch excision by laser ablation, with the four main stages of meristem regeneration marked to the right. The gemma images are a composite of membrane marker expression (magenta) and chlorophyll autofluorescence. The overlap and fading between the marked stages indicates the uncertainty and variability of the timing of the stage transitions. Images were acquired at the indicated time-points after meristem removal. (E), (F) and (G) show fine scale ablation experiments of the gemma, with a schematic outline of the ablation patterns used (orange bounded by red denotes excised tissue, white circles indicate ablated cells), and image of the 0DaA whole gemma (asterisk marking excised notch), a close up of the 0DaA notch (ablated cells marked by white circles) and an image of the 3DaA notch.(E) demonstrates that removal of only a small region around the apical cells is sufficient to induce meristem regeneration, just behind the precisely ablated notch. In both (F) and (G), the partially ablated notch continues to function as a meristem, with no new meristems emerging elsewhere in the gemma. This suggests that the apical cells are of approximately equal competence and function as a population as a whole. Gemma shown are from the pMpUBE2::mScarletI-lti6b membrane marker expression line. Confocal images show membrane marker expression in magenta and chlorophyll autofluorescence in grey. All scale bars 100μm.

Methods

Vector constructs were built according to the Loop Assembly protocol (23, 36) from L0 parts, L1 and L2 plasmids in the OpenPlant Loop Assembly toolkit (23). The lines used experimentally to produce each figure are given in SI Appendix Supplementary Information Text and full plasmid sequences for vector constructs are given in Supplementary Information Dataset 1. Plasmid vectors were transformed into Marchantia sporelings (Cam accession) by agrobacterium-mediated transformation according to the methods described previously (23, 24). T0 sporelings and subsequent G1 plants were grown at 21°C under continuous light (intensity= 150 µmol/m^2/s) on 1.2% w/v agar (Melford capsules A20021) plates of Gamborg B5 media with vitamins (Duchefa Biochemie G0210) prepared at half the manufacturer's recommended concentration and adjusted to pH 5.8. The media was supplemented with 100µg/ml Cefotaxime (BIC0111; Apollo Scientific, Bredbury, UK) to reduce bacterial or fungal contamination, and 20µg/ml Hygromycin (10687010; Invitrogen) for selection.

Inducible gene expression was carried out by growing gemmae on plates supplemented with 10µM dexamethasone (Sigma) and then heat shocking plants by incubating them at 37°C for 1 hour. For growing gemmae in elevated auxin treatments, 50mm ½ Gamborg +Hygromycin +Cefotaxime agar plates were prepared with addition of 10µM 1-Naphthaleneacetic acid (NAA) (Sigma) dissolved 1M NaOH and then diluted in water. Auxin biosynthesis inhibitor treatments were gone by growing gemmae on plates made with 100µM L-kynurenine (Sigma) dissolved in DMSO (37). Auxin transporter inhibitor treatments were done by growing gemmae on plates made with 100µM 2,3,5-triiodobenzoic acid (TIBA) (Aldrich) dissolved in water. Media plates were stored at 4°C wrapped in foil to protect from light and were used within 6 weeks of preparation. Auxin treatment time courses were all conducted with plants grown in the same growth cabinets as the control plants.

Imaging was carried out using a Leica Sp8 Confocal Microscope, equipped with a hybrid detector and a pulsed white-light laser, with a HC PL APO 10x/0.40 CS2 or a HC PL APO 20x/0.75 CS2 dry objective. Excitation and collection settings for each fluorophore are given in SI Appendix Supplementary Information Text. Images were taken in photon counting mode with sequential scanning. Time gating was active to supress autofluorescence. Maximum-intensity projections of the images were obtained from z-stack series, capturing z-slices at intervals between 1µm-12.5µm. The images were processed using the Leica LasX and Fiji/ImageJ software packages. Figures were produced using the QuickFigures (38) and Stitching (39) Fiji plugins.

Laser ablation experiments were performed using a Leica LMD6000 laser dissection microscope system equipped with a solid state 355nm cutting laser and controlled with the Leica LMD6 software. Ablation done under the 10x objective lens used settings 60 power, 45 aperture, 25 speed; ablation done under the 40x objective lens used 60 power, 35 aperture, 20 speed. All gemmae used for ablation were taken directly from the gemma cup of the parent thallus (i.e., 0 days post germination (dpg)). Intact gemmae were also taken from the same cups and used as controls to verify regular growth and marker expression in that plant line. Gemmae were planted on 50mm agar plates and all ablation and imaging was performed directly on the plates to minimize mechanical disruption to the plants. For wholescale notch removal a 90µm diameter circle was drawn with its centre point at the apical notch, and all tissue within this circle was excised. All other cells or regions ablated are given in a schematic accompanying the relevant figures. Gemma growth responses and marker gene expression were observed and consistent in at least three replicates, unless otherwise noted.

Propidium iodide (PI) staining was carried out to verify that the laser ablation settings did not cause widespread stress or uncontrolled damage to the gemma. WT and enhancer trap line gemmae were incubated in 10µl of 100µg/ml PI (Sigma) on a glass coverslip for 10 minutes at room temperature, ensuring that the gemma was not otherwise mechanically damaged or desiccated during incubation. Gemma staining and imaging was carried out before and/or after ablation in various combinations to ensure that any PI signal relating to cellular damage was solely connected to the laser ablation. All experiments showed that the laser ablation settings used did kill the targeted cells, that cellular damage was localized to the visible ablation zone and that the ablation process did not cause more widespread cell death or stress to the gemma (see Figure S1). This validates laser ablation as an approach to study the microarchitecture of the apical notch region.

Results and Discussion

Laser ablation of transgenic Marchantia lines reveals the minimum requirements for meristem regeneration

Marchantia plants expressing an mScarlet membrane marker enabled cell division events in the growing gemma to be observed by highlighting the formation of new cell membranes. Visual inspection of the density of new cell walls and tissue morphology allowed for recognition of developing meristematic regions in the apical notches. Where laser ablation microscopy was used to remove both notches of a 0dpg gemma (see Fig. 1C), these membrane marker lines were used to track the regeneration process (see Fig. 1D). This

confirmed previous findings (40), that there are four distinct stages of regeneration: Stage 0 (where the cells remain largely dormant), Stage 1 (rapid, widespread cell divisions across the gemma), Stage 2 (localized cell divisions restricted to specific clusters) and Stage 3 (complete meristem regeneration forming full apical notches with intact apical cells). Stage 0 lasts over the first 24 hours, Stage 1 begins within 24 hours, Stage 2 begins after ~48 hours and Stage 3 is complete approximately 4-5 days after ablation (DaA), although there is some variation in stage time and stages may overlap.

Previously experiments investigating Marchantia regeneration were limited to relatively crude surgical manipulations using cutting by blades. Laser microscopy allows for the precise ablation of cells in the apical notch. This means that fine scale disruptions can be made in this region, to determine the minimum required amount of apical notch that needs to be removed to induce meristem regeneration. A gemma was manipulated so that one apical notch region was entirely excised, and in the other only the first row of apical cells at the centre of the meristematic apical notch were ablated. Removal of this first row was sufficient to induce meristem regeneration (see Fig. 1E-F). Meristem organisation was disrupted following apical cell ablation, with the notch expanding outwards. Cell divisions occurred in a region behind the row of ablated apical cells, and no signs of notch re-establishment were observed even 3DaA. This indicates that the apical cell population is indispensable for meristem maintenance.

We have previously generated a range of enhancer trap lines that mark the apical notch/meristem region of the Marchantia gemma (30). Removal of both of the entire apical notch regions of gemmae from these lines resulted in meristem regeneration, which could be observed by emergence of new nuclear mVenus signal in the enhancer trap lines (see Figure S2). The lines used (239-P21, 239-P33, 239-P125, 239-P153) marked the localized clusters of cell division of Stage 2 and only persisted in active meristematic regions that went on to produce full apical notches (*i.e.* Stage 3). This meant that the enhancer trap marker signal could be used as an easily observable indicator of meristem regeneration and to show which regions of the gemma had entered Stage 2 of regeneration. Persistence of localized marker signal also allowed simple verification of which regions were continuing as functioning meristems, whereas loss of marker signal would indicate loss of meristem identity and cell division activity.

Laser ablation was used to shave off rows of apical notch cells going progressively deeper into the gemma (with the other apical notch entirely removed). As before, shaving off just one row of cells was sufficient to induce meristem regeneration, in a region just behind

the ablated notch (see Figure S3A, B). Shaving off the first three rows of cells was required to induce regeneration elsewhere in the gemma, further away from the region immediately surrounding the shaved notch (see Figure S3C, D).

These results demonstrate that there is a substructure within the apical notch region, with the immediate first row of apical notch cells required to act as meristematic organizers and the second and third rows being in some way primed to be induced for meristem regeneration in preference to the rest of the gemma. This is in contrast to regenerations in gemma fragments with completely ablated meristems, where initial cell divisions of Stage 1 were widespread across the remaining gemma tissue (see Fig. 1D). We infer that the cells in the second and third rows of the apical notch may effectively already be in Stage 1, giving them a "head start" over other parts of the gemma with regards meristem regeneration. Outside of this, from examination of multiple gemmae with both notches removed there appears to be no clear predictability or bias as to which regions will proceed to form new meristem. This is in contrast to vascular plants, where in the post-embryonic plant only certain somatic cell types are competent for new meristem formation (8). The exception to this is the stalk scar region, which is the part of the gemma that forms first within the gemma cup (25, 26), and which was never observed to initiate cell divisions or to provide the basis for meristem regeneration.

The apical cells function collectively, and this involves intra-notch communication

The current Marchantia literature centres around a model where the key feature of each apical notch meristem is the Apical Cell, a pluripotent cell in the middle of the notch with four division planes (19, 25, 41). Under this hypothesis, the other "subapical" cells in the notch are products of and subordinate to the Apical Cell (19, 41). The Apical Cell has chiefly been defined by morphological characterization from light and electron microscopy images, rather than direct experimental evidence. Experiments on misexpression and knockout lines for the stem-cell promoting peptide factor MpCLE2/CLV3 were found to develop extra or reduced Apical and subapical cells respectively compared to wild-type plants (28), although no clear criteria was given as to how these were classified between the two cell types.

We therefore attempted to use laser ablation to interrogate the standard model of the apical notch, and to test its limitations by ablating portions of the cells in the first row of apical notch cells (*i.e.*, the Apical Cell or various subapical cells). Removing one side of the apical notch cells did not disrupt meristem function or apical dominance, irrespective of whether or not there was another apical notch in that gemma (Fig. S4A, B). However, if the

notch was ablated so that only a portion of apical notch cells were left intact on one side, then meristem function was disrupted. If the other apical notch in the gemma was intact (Fig. S4C) then this demonstrated apical dominance, and the partially ablated notch became inert (*i.e.* non-meristematic). If the other notch was removed (Fig. S4D, then new meristematic regions emerged elsewhere in the gemma, and the remaining apical notch cells were insufficient to retain apical dominance over these new meristems. If a few apical notch cells were left intact on each side (Fig. S4E, F) then the notch became inert irrespective of the status of the other notch- despite the total number of apical notch cells left intact being the same as notches with contiguous portions of apical notch cells that continued to function.

If both sides of the apical notch were removed, but a few cells at the base (including the presumed Apical Cell) were left intact, then the result was dependent on the status of the other notch in the gemma. If the other notch was removed (Fig. 2A), then the intact base continued to function as a meristematic apical notch, and retained apical dominance (that is, no new meristematic regions emerged elsewhere in the gemma). If the other notch in the gemma was intact (Fig. 2B), then the intact base alone could not sustain meristem functions and became inert, even if the remaining portion was larger than cases where notch function continued with the other notch excised (e.g., Fig. 2C). This shows that while the base of the apical notch has a greater functional capacity than the cells on the sides of the notch, this alone is insufficient for full meristematic capabilities and apical dominance if another, fully intact and functional notch is also present. These experiments also confirm that there is long-range communication between apical notches in a gemma.

We then proceeded to ablate portions spanning across the base and sides, including around the centre where, according to the traditional model of Marchantia notch architecture, the Apical Cell is located (Fig. 2D, E). If only a portion of the apical notch cells were removed, but a sufficient contiguous portion of apical notch cells remained, then not only did the notch continue to function as a meristem, but over the course of the next 48 hours it remodelled itself. The result was that by 3DaA, the centre of the apical notch had shifted to what was the centre of the contiguous portion of remaining apical notch cells on 0DaA. This was irrespective of where the centre of the original notch had been before ablation. The remodelling process did not appear to be influenced on whether the original location of the Apical Cell was ablated or not. The remodelled apical notches continued to be functional meristems with apical dominance regardless of whether there was another intact apical notch in that gemma. That is to say that during the first 48 hours after ablation, the remodelling process did not permit the initiation of new regions of cell division (if the other apical notch

had been removed), nor was it subject to apical dominance-related inhibition if the other notch was intact. This means that the apex remodelling is not simply meristem regeneration but is a continuation of the original meristem.

A flow chart of the outcomes of our ablation experiments is provided in Fig. S5. These experiments demonstrate that the key factor is not the presence or absence of a single, central and essential “Apical Cell” surrounded by dispensable “subapical cells”, but instead points towards there being a population of apical cells within the notch that are more-or-less equally competent. What is paramount for meristem integrity is that there is a sufficient number or quorum of these apical cells that are intact and in contiguous contact. The precise limits and requirements for this quorum remain to be determined. The characteristic notch shape of the Marchantia meristem emerges from self-organization of this quorum portion, as an outcome of the biophysics of differential rates of cell division and expansion from the centre of the quorum to the edges (42). The necessity for the portion to be contiguous could mean that intra-notch communication directly between the apical cells is vital to provide a mechanism to regulate faster rates of cell division at the centre of the notch. It may also be that non-contiguous portions compete and cancel each other out if they are in too close proximity. However, the evidence from the ablation experiments demonstrates that the single “Apical Cell” of previous models is not a unique, fixed or critical cell identity, and instead simply emerges from the architecture of the general meristematic apical cell population within the notch.

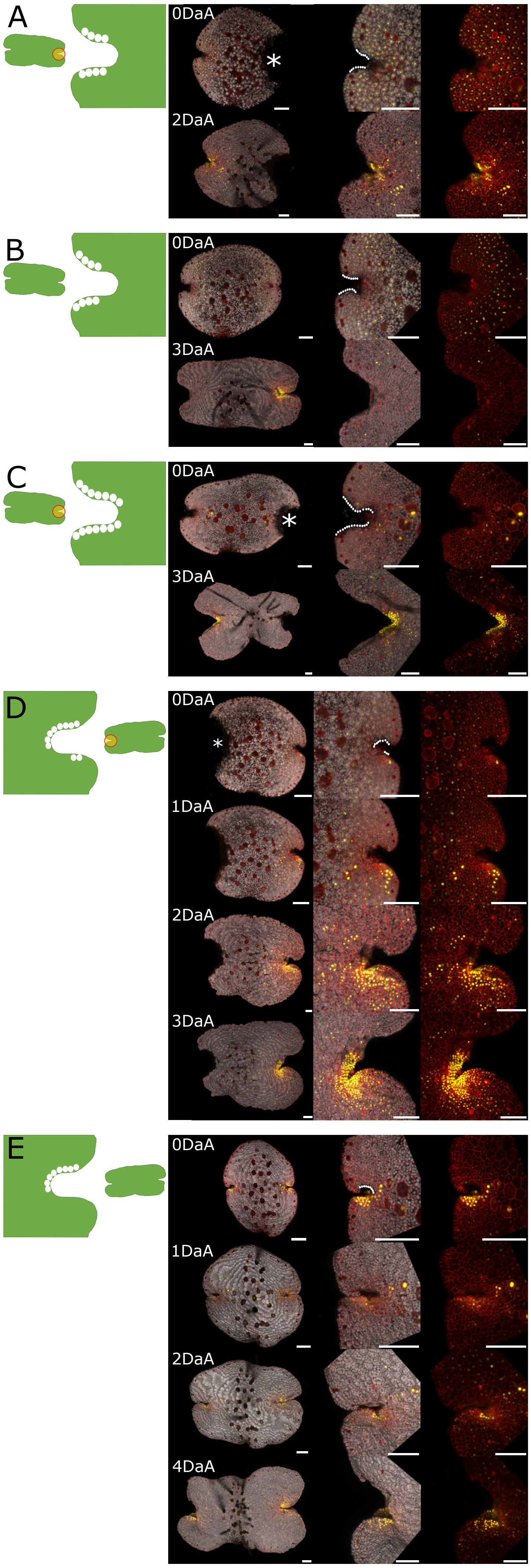

Fig. 2. The apical notch can remodel even after partial ablation across the notch side and base, independent of the status of the other gemma apical notch. (A) Ablation of the notch sides, leaving the notch base (including the "Apical Cell") intact, does not disrupt meristem status if the other apical notch in the gemma is excised. (B) if the other apical notch is intact then meristem function ceases because of apical dominance. (C) Meristematic activity continues even if more of the apical notch is ablated and fewer cells are left intact at the base, as long as the other notch is excised. (D) and (E) demonstrate that partially ablating the notch such that the intact portion spans across the base and one side of the notch, but excludes the "Apical Cell" location, results in remodelling of the apical notch and maintenance of meristematic function, as indicated by continuing mVenus expression. The apex of the notch changes so that it is found where the centre of the intact portion was. This is irrespective of whether the other notch in the gemma has been excised (D) or is intact (E). In each figure a schematic outline of the ablation pattern is shown with white circles marking the location of ablated cells and orange bounded by red denoting excised tissue. A time course is given in the format whole gemma, notch close up, notch close-up without the chlorophyll autofluorescence channel. Gemmae were imaged at the time points indicated. In the 0DaA images, white circles mark ablated cells, asterisks denote excised notch. The enhancer trap notch/meristem marker lines used were ET239-P21 (A, B, D, E) and ET239-P153 (C). Scale bars=100μm.

Meristem regeneration involves communication between regions of cell division

Partial ablation of the apical cells revealed further aspects of Marchantia apical dominance and meristem regeneration. Fig. S6 shows the case of where one apical notch region is removed entirely, and the other is partially ablated. The remaining apical cells of the partially ablated notch do not constitute a quorum initially, and therefore the rest of the gemma is released from apical dominance and proceeds to Stage 1 of regeneration (see Fig. S6C). Several patches of cell division can be observed in various regions across the gemma, and by 2DaA some of these have progressed to Stage 2, as indicated by their having dense patches of fluorescent marker signal (white arrows, Fig. S6 D, E). Simultaneously, the partially ablated original notch has also recommenced cell division and is well underway in Stage 2 of regeneration. By 3DaA this region is the most prominent patch of meristem, though emerging patches are visible and have marker signal. Five days after ablation (see Fig. S6 F), the notch that was originally partially ablated has once again exceed the quorum size of apical cells and is now a fully active, mature notch in Stage 3 of regeneration. This notch has established full apical dominance and the other patches have become inert (as indicated by loss of marker signal). However, it can be seen that these aborted meristems did progress far along the regeneration pathway, with significant amounts of cell division that have produced protrusions on the thallus (cyan arrows, Fig. S6 F).

This observation is especially noteworthy as it demonstrates that loss of meristem function and new meristem emergence is not irreversible. It also reveals that apical dominance in Marchantia is an active process that must be maintained by the meristematic apical notch, can be disrupted and can be re-established. Meristem “maturity” as well as size is a factor in this. This provides a possible checkpoint mechanism for progression from Stage 2 to Stage 3 of regeneration and may also prevent multiple meristems emerging in too close proximity. Our observations also show that Marchantia apical notches signal to the rest of the plant to inhibit cell division, thus generating the characteristic thalloid growth pattern.

There is a flow of signal from the apical cells

The signal produced at the apical notch must flow outwards in order to influence the rest of the gemma tissue. To investigate this, we used laser ablation to make incisions at a distance from the apical notch to see if the flow of this signal could be interrupted. Incisions made perpendicular to the notch had no obvious effect (see Fig. S7 A-E). Incisions made parallel to the notch (see Fig. S7 F-O) had the effect of increasing the rate of cell division proximal to the incision and increasing cell expansion distal to the incision. This effect was

not seen if the apical cells proximal to the parallel incision were also ablated. Instead, the cells on both sides of the incision ceased dividing and showed increased cell expansion. This shows that signals from the apical cells are responsible for controlling the balance between cell division and expansion in the region surrounding the apical notch. The cells in this region are the youngest in the gemma and will be acquiring new fates (25, 41, 43), so it is expected that they would be integrating multiple different positional and developmental cues from their surrounding tissue environment. It also reinforces the evidence that the apical cells exist as a population rather than being controlled by a central "Apical Cell", as the effect of removing the apical cells proximal to the incision was not dependent on the status of the cells at the very apex of the notch. Changes in cell division and expansion notwithstanding, it is important to note that there was no evidence for patches of cells distal to the parallel incision entering Stage 1 of meristem regeneration. This was the case whether the other apical notch in the gemma was present or not. Therefore, while some signals are dependent on direct flow from the notch, the signals involved in apical dominance and repression of new meristems are capable of being transported around the parallel incisions that were made in the gemma.

Further, more extensive incisions were made to test the limits of the transport of the repressive apical dominance signal (see Fig. 3). This found that the location of the tissue forming the connection to the rest of the gemma was crucial. Connections through central zone tissue (see Fig. 3 A-F) communicated the repressive signal and did not see any meristem regeneration elsewhere in the gemma. This was the case even when the connections were made from a small amount of tissue only a few cells wide. In contrast, connections formed from the thinner tissue around the gemma periphery (see Fig. 3 G-L) appeared not to communicate the repressive signal for apical dominance. Instead, multiple new meristematic regions emerged, even with there being a fully functioning meristematic apical notch present in the gemma. This demonstrates that there is a spatial heterogeneity across the gemma for transport of the repressive signal for apical dominance, suggesting that the signal is carried by a specific mechanism and that not all cell types possess this mechanism.

Signal export to a sink is required to maintain the apical notches as meristems

Making incisions in the central zone tissue from around the apical notch region, so that it is only linked to the rest of the gemma by peripheral connections, effectively isolates the apical notch. If the isolated region is large and includes tissue from outside the notch region, then it continues to grow and develop as normal, even as new meristematic regions emerge elsewhere in the gemma (see Fig. 3K, L). If the isolated region is small and only

contains the apical cells and cells immediately surrounding the notch, then the result is different (see Fig. S8). As expected, the rest of the gemma is released from apical dominance and proceeds through Stages 1 and 2 of meristem regeneration. The isolated notch sustains meristem identity for the first 24 hours after ablation, as shown by the persistence of marker signal. 48 hours after ablation the marker signal is fading and cell division has ceased, and by 3DaA the isolated notch has become totally inert. The cells do not divide and instead expand, and no apical notch/meristem marker signal is present whatsoever. This sequence of events is the same as cases where the apical notch and immediately surrounding region is totally disconnected from the rest of the gemma by laser ablation of the intermediary tissue (see Fig. S9).

This reinforces the conclusion that the repressive signal that is usually exported from the apical notch cannot be transported through these peripheral connections. It also appears that the meristematic tissue cannot degrade this signal. If it cannot be exported to a sufficient sink of non-meristematic tissue, then it accumulates and has a self-inhibitory effect. This repressive signal causes loss of meristematic identity: the former apical cells cease dividing, expand, and persist as regular thallus tissue.

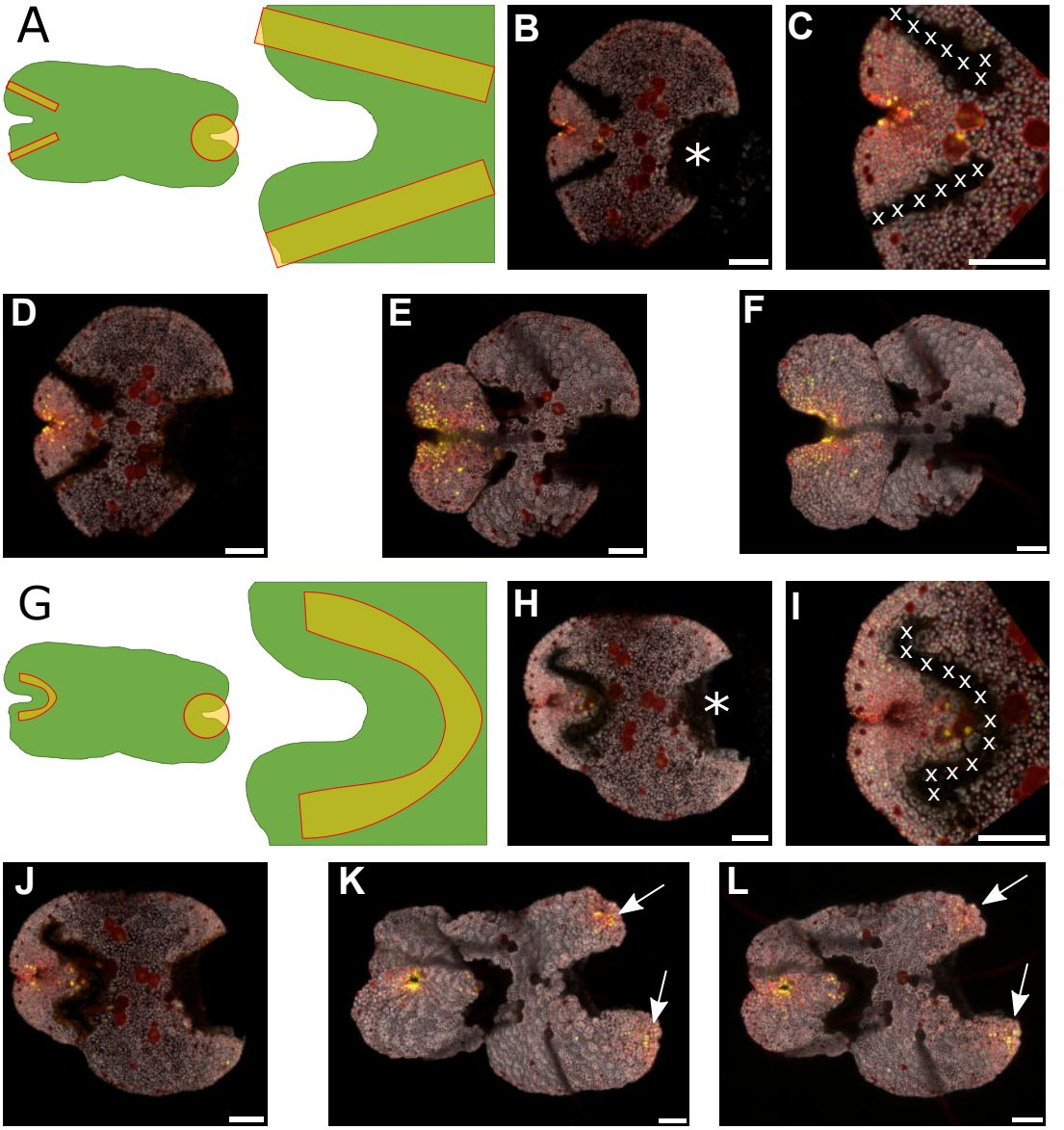

Fig. 3. Apical Notch Isolation. (A) shows an outline schematic of the ablation pattern used on the 0dpg gemma shown in (B) (whole gemma) and (C) (close-up of notch). (D)-(F) shows a time course from 1DaA (D), through 2DaA (E) until 3DaA (F). The isolated notch continues to be mertistatically active. The gemma fragment attached to this notch by a central connection does not show any evidence for the emergence of new meristems. (G) shows an outline schematic of the ablation pattern used on the 0dpg gemma shown in (H) (whole gemma) and (I) (close-up of notch). (J)-(L) shows a time course from 1DaA (J), through 2DaA (K) until 3DaA (L). The isolated notch continues to be meristematically active. However, the gemma fragment attached to this notch by the peripheral connections does have new meristems emerging (white arrows). Asterisk marks excised notch, X marks removed tissue, orange bounded in red denotes excised tissue in the outline schematics. Gemmae were from the enhancer trap notch/meristem marker line ET239-P153. Scale bars= 100μm.

Auxin-related gene expression and meristem regeneration in Marchantia

The observed self-inhibitory feedback of these isolated apical notches is characteristic of auxin (21, 44). Auxin controls and regulates processes such as apical dominance (7), meristem regulation (5, 6) and meristem regeneration (8) in plants, including Marchantia (33, 34). Auxin is produced in the apical notch and exported to the rest of the thallus. Surgically removal of the apical notch removes this auxin source, causing auxin levels in the remaining thallus fragment to drop initially before rising again as new meristems regenerate (29).

We therefore produced transgenic reporter lines for several auxin-related genes (18) to study auxin dynamics in the 0dpg gemma and how the spatial distribution of expression changes in response to excision of the apical notch regions by laser ablation. One reporter line marked nuclei expressing the auxin biosynthesis gene MpYUC2 (45). In intact 0dpg plants, MpYUC2 is expressed throughout the gemma with the apical cells having particularly high expression levels (see Fig. S10). A second line marked expression of the polar auxin transporter MpPIN1 (17, 22). MpPIN1 expression is focused around the apical notch regions (see Fig. S11), as well as being present around the gemma edge and in occasional cells across the central zone. A third line marked expression of the auxin responsive transcription factors MpARF1 (which upregulates expression of downstream genes in response to auxin) and MpARF2 (which downregulates expression of downstream genes in response to auxin) (16, 20). MpARF1 and MpARF2 are both expressed throughout the gemma (see Fig. S12), with no particular expression patterns (higher or lower expression of one or other or both genes) around the apical notch. Some cell types did display variation in expression, for example higher ARF2 and lower ARF1 expression in rhizoid precursors, or in some cells in the margin tissue (20, 30). In older gemmae (4pdg), MpYUC2 and MpPIN1 expression can be seen to become more focused around the apical cells (see Figs. S10 and S11). In older gemmae MpARF1 expression changes to be relatively lower around the meristem compared to MpARF2 expression and compared to the 0dpg expression patterns (see Fig. S12). Collectively, these results show that the apical notch meristem acts as an auxin source in intact gemmae, but that auxin signalling is more widespread.

When the apical notch regions are removed, there is widespread up-regulation of MpYUC2 across the gemma (see Fig. 4A, B, Fig S13A). As meristem regeneration proceeds the expression of MpYUC2 becomes restricted to the patches of dividing cells, and eventually the new apical notches. This reinforces the view of meristems as auxin sources in the Marchantia gemma, with auxin production becoming increasingly restricted to the area(s) of meristem regeneration. It is well established that MpYUC2 expression is inversely

correlated with auxin levels (21). As auxin levels fall in the gemma following removal of the apical notches, the downregulation of MpYUC2 is lifted. This is seen in gemmae treated with 10µM NAA, where scarce nuclear fluorescence is visible two days after notch removal (2DaA, see Fig. S13 B), and meristem regeneration does not occur (29, 34).

MpPIN1 expression is low all across the gemma following notch removal (see Fig. 4C, D). Two days after ablation MpPIN1 expression levels are high and widespread all across the gemma, in a fashion similar to MpYUC2 expression. As meristem regeneration proceeds, MpPIN1 expression then becomes restricted to the cells at the edge of the regenerating areas in Stage 2. This is different from MpYUC2 expression (which is present in cells throughout the regenerating areas at Stage 2), implying that focusing of auxin transporter expression somehow precedes the focusing of auxin biosynthesis genes during meristem regeneration. It also demonstrates that the reconfiguration of auxin transport, by initial widespread upregulation and then becoming restricted to emerging meristems, is a key feature of regeneration.

PIN1 distribution does not explain why the repressive signal is carried through central connections but not peripheral connections between the apical notch and the rest of the gemma. As mentioned previously, MpPIN1 expression is high at the gemma edge. The gemma in Fig. S14 A-D has no MpPIN1 expression in the cells of the central tissue connection, yet the intact notch retains apical dominance over the rest of the gemma and no new meristems emerge. Conversely, Fig. S14 E-H shows a gemma ablated such that the one remaining notch is only connected to the rest of the gemma by peripheral tissue. The cells of the peripheral connection clearly display MpPIN1 expression (e.g., Fig, S11D, Fig. S14E-H), yet meristem regeneration occurs elsewhere in the gemma. It may be that other auxin transporters are involved, for example the Marchantia non-canonical PIN, ABCB or AUX/LAX transporters (18, 22, 46). Alternatively, there could be another mechanism of apical dominance at work independent of directional auxin transport.

There is no clear upregulation of MpARF1 or MpARF2 immediately following notch removal, however MpARF1 appears to be expressed more in dividing cells at 2DaA (see Fig. 4E-G). Four days after ablation (approximately the transition from Stage 2 to Stage 3) we saw a clear excess of MpARF1 expression compared to MpARF2 in clusters of dividing cells. This is the opposite pattern seen in the division zone of intact meristems (see Fig. S12). MpARF2 expression was elevated along the newly forming thallus edge, similar to the situation in intact meristems and consistent with previously reported higher expression of MpARF2 along the gemma edge (20). This high relative abundance of MpARF1 compared to

MpARF2 suggested that auxin producing, dividing cells may actually be hyper-sensitive to auxin signalling, during Stage 2 and 3 of meristem regeneration.

These results show progressive restriction of auxin biosynthesis to ever more restricted patches of the regenerating gemma, and eventually to the apical cells of the newly emerged notch, is central to meristem regeneration in Marchantia. Furthermore, it argues against a role of auxin response (as measured by ARF stoichiometry) in stabilising localised auxin biosynthesis, and instead points to reorganization of auxin transport (as measured by localization of PIN expression) being responsible.

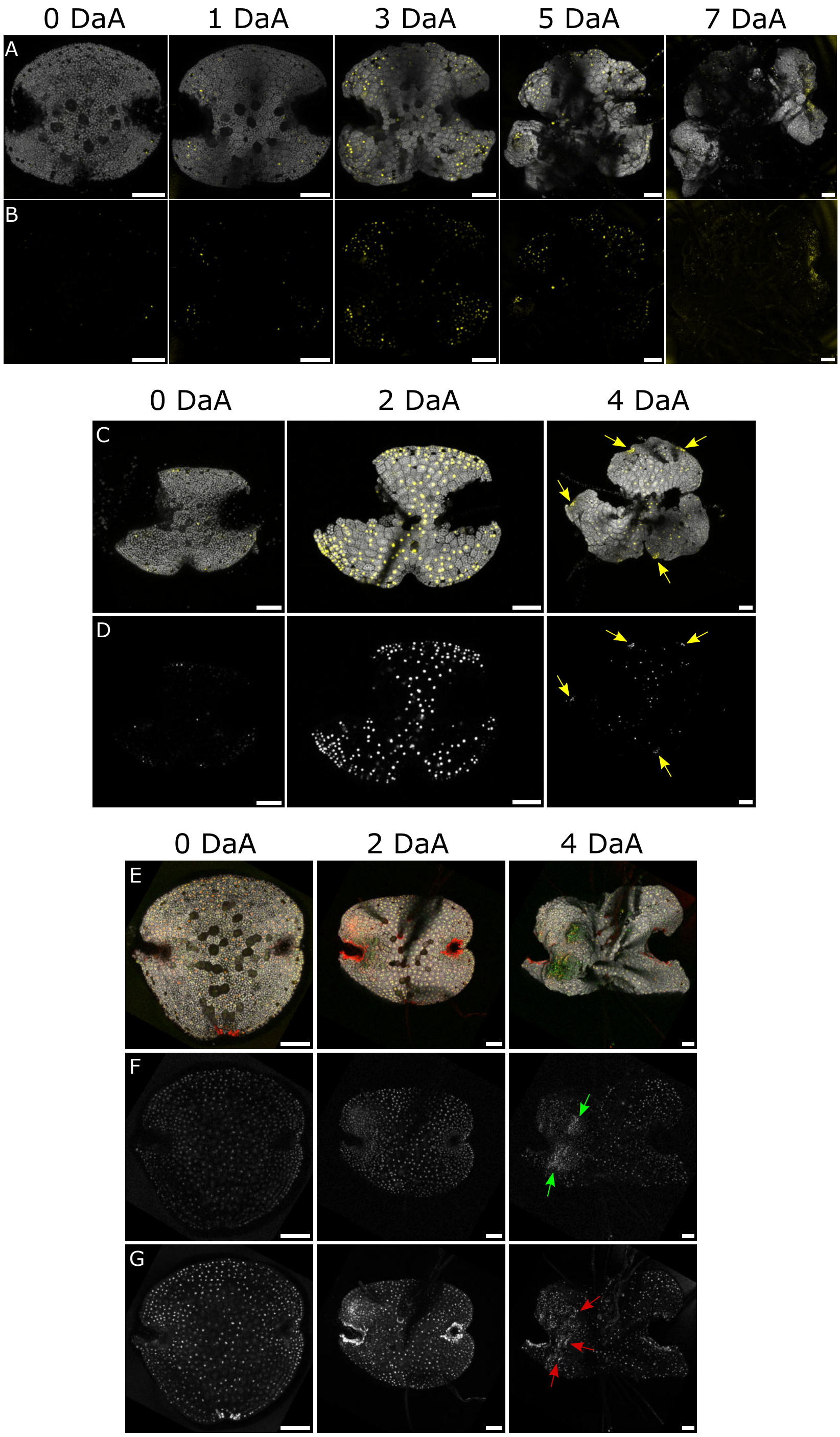

Fig. 4. Expression of YUC, PIN and ARF reporters during meristem regeneration in gemma fragments.
(A) shows a regenerating gemma fragment of pMpYUC2(5.5kb)::mTurqouise2-N7 gemma, with MpYUC2 reporter expression is shown in yellow and chlorophyll autofluroescence is shown in grey. (B) is the same gemma with only MpYUC2 reporter expression shown. There is widespread up-regulation of MpYUC2 expression across the gemma following meristem excision, which then becomes restricted to the patches of dividing cells, and eventually to the new meristems.
(C) shows a regenerating gemma fragment of the MpPIN1 transactivation reporter (pMpPIN1::GAL4-VP16, pUAS::mVenus-N7) with reporter expression in yellow. (D) is the same gemma with only MpPIN1 reporter expression shown in greyscale. MpPIN1 expression is initially low after meristem excision, but is then upregulated across the gemma fragment. As meristem regeneration proceeds, expression becomes restricted to the edge of regenerating areas (yellow arrows).
(E) shows a time course series of a regenerating pMpARF1::mVenus-N7, pMpARF2::mScarletI-N7 gemma fragment. The composite image has pMpARF1::mVenus-N7 in green, pMpARF2::mScarletI-N7 signal in red and chloroplast autofluorescence in grey. (F) shows the fluorescence of the pMpARF1::mVenus-N7 reporter in greyscale. (G) shows the fluorescence of the pMpARF2::mScarletI-N7 reporter in greyscale.There is no clear up-regulation of MpARF1 or MpARF2 immediately following meristem excision. MpARF1 appears to be more expressed compared to MpARF2 in patches of dividing cells (green arrows), with MpARF2 expression being elevated along the newly forming thallus edge (red arrows).
The ablation patterns used were the same as given in the outline schematic in Fig. 1C. Gemmae were imaged at the time point indicated, with the 0DaA images acquired within 1 hour of meristem excision. Scale bars= 100μm.

Auxin transport and auxin source-sink dynamics in meristem regeneration

It is not only re-establishment of auxin biosynthesis that is important in meristem regeneration in Marchantia: auxin mediated signalling is also vital. Communication must take place during regeneration between auxin sources (patches of dividing cells and eventually apical notches) and sink tissues (the central zone or non-dividing cells) to preserve the balance between auxin production, transport and degradation. This can be demonstrated by manipulation of auxin-related processes by pharmacological treatments. It is well established that applying exogenous auxin to gemmae from which the notches have been removed inhibits regeneration (29, 34). This affects Stage 1 of regeneration: gemma tissues continue to act as an auxin sink, there is no burst of MpYUC2 expression (see Fig. S13B), no new cell divisions occur, and no new meristems emerge.

Auxin biosynthesis inhibitors also prevent meristem regeneration, but at a later stage. Growing intact gemmae on media supplemented with auxin biosynthesis inhibitors causes widespread and elevated MpYUC2 expression compared to controls (30). This effect is also observed in gemma which have had the apical notches removed and grown on 100µM kynurenine (see Fig. S13C). Kynurenine treated plants were still able to progress to Stage 2 of regeneration and to establish zones of localized cell division. This may be due to incomplete inhibition of auxin synthesis, which is probable given the competitive nature of kynurenine inhibition (47). However, kynurenine treatment prevented the transition to Stage 3 of regeneration (the appearance of full apical notches), even 7DaA. There was no evidence of MpYUC2 expression becoming more localized or specific (see Fig. S13C). While kynurenine treatment at this concentration may not abolish the establishment of patches of dividing cells, the fact that it does block the transition to Stage 3 suggests that auxin is required for the re-establishment of mature meristems containing apical cells. This is consistent with the phenotypes of strong genetic perturbations to auxin signalling (48) and treatment of gemmae with very high concentrations of auxin biosynthesis inhibitors (49), which both result in undifferentiated callus-like thalli that resemble the early stages of meristem regeneration. This reinforces the findings that progressive restriction of auxin biosynthesis is vital for meristem regeneration in Marchantia.

Crucially, inhibiting auxin transport also disrupts meristem regeneration, in a manner similar to inhibiting auxin biosynthesis. Growing regenerating gemma fragments on media supplemented with TIBA results in the meristem regeneration process becoming stalled at Stage 2 (see Fig. S13D). The corroborates the observation that changes in the localization of MpPIN1 expression precede the restricting of cell divisions to regenerating meristems.

Instead, cell divisions continue in larger dividing zones, eventually exhausting the reservoir of potential stem cells and ceasing meristem regeneration, just as in regenerating gemma fragments treated with kynurenine (see Fig. S13C).

MpERF20/MpLAXR primes cells to acquire apical cell identity

Another Marchantia auxin-related gene involved in meristem regeneration is the transcription factor MpERF20, also recently called Low Auxin Responsive (MpLAXR) (29). MpERF20/LAXR has increased expression in plants with inhibited auxin biosynthesis or signalling and shows strong and significant upregulation after notch removal, similar to the response of MpYUC2 (18, 21, 29, 49). Ishida and colleagues (29) performed experiments to demonstrate that MpERF20/LAXR is pivotal in meristem regeneration in excised thalli, with knockout mutants having severe retardation of the early stages of regeneration, and overexpression lines forming exogenous Stage 2 division zones and even being able to regenerate meristems in spite of elevated auxin treatment.

We performed experiments with young gemmae from MpERF20/LAXR reporter lines with nuclear fluorescence marking expression of MpERF20/LAXR (see Fig. S15A-D), complementing the work done by Ishida et al. (29) on older thalli. Expression in 0dpg and 1dpg gemmae was heavily localized to the meristems of the apical notch (albeit with occasional expression in oil cells). In 5dpg gemmae expression was observed in clusters of cells across the meristem but was absent from developing air pores. This pattern is broadly similar to MpYUC2 localization.

Compared to uninduced gemmae (see Fig 5A), gemmae from MpERF20/LAXR overexpression lines showed severe developmental defects after induction (see Fig. 5B-D). Control experiments demonstrated that these defects did not result from the activity of the inducible transactivation system itself (see Fig. S16) but from MpERF20/LAXR overexpression. Numerous ectopic notches formed along the gemma edge in addition to the normal pair of apical notches (see Fig. 5B-D), similar to the findings of Ishida et al. (29). The notch shape itself is thought to form as a result of low rates of cell division and tissue expansion in the apex of the notch, which comprises the apical cell population, compared to surrounding cells (42). Most ectopic notches were already visible in gemmae at 0dpg, suggesting that they may have emerged during gemma development, but we did observe examples of notch formation and loss during the four-day time course. This suggests that ectopic notch formation is not restricted to gemma development in the gemma cup but can also occur after gemma germination. Gemma growth was severely retarded in our

MpERF20/LAXR overexpression lines, particularly where overexpression was induced by a combination of heat shock and dexamethasone treatment. Most of the ectopic notches appeared to be dormant, based on a lack of tissue expansion or cell divisions in the surrounding cells. We typically observed only a single actively dividing notch in 4dpg plants, compared to wild type plants where both notches always mark active meristems. Qualitatively, we observed cases where one of the original notches showed meristematic activity as well as cases where an ectopic notch became active while the original notch remained dormant.

These results suggest that MpERF20/LAXR overexpression alone is sufficient to induce ectopic notch formation and that ectopic notches are functionally equivalent to the pair of notches that form during gemma development. The fact that the original pair of notches is still clearly visible and correctly positioned in our over-expression lines suggests that tissue patterning during gemma development in gemma cups is not disrupted by MpERF20/LAXR activity, and that MpERF20/LAXR expression induces apical cell fate, resulting in notch formation. The slow growth of plants and small number of notches that become mitotically active could suggest competition between notches. We hypothesize that ectopic apical cells may represent new centres of auxin production and export. Since there is very little space between notches, neighbouring notches may inhibit each other via auxin accumulation, while the strongest source of auxin production may eventually repress all other notches, permitting outgrowth of an active meristem. This is reminiscent of the situation where a partially ablated apical notch can re-establish apical dominance over other emerging meristems even at a relatively advanced stage (see Fig. S6).

In gemmae whose notches have been removed, MpERF20/LAXR expression is rapidly induced after less than 24 hours all across the gemma (see Fig. S15 E, F), again similar to the expression pattern of MpYUC2 (see Fig. 4A, B). However, while 3DaA MpYUC2 expression was largely restricted to dividing cell clusters, MpERF20/LAXR expression remained widespread. Seven days after ablation expression of both genes was specifically localised to the centre of fully regenerated meristems, just like in intact gemmae. Our localization findings are in contrast to Ishida et al. (2022), who observed a focus of expression at the part of the midrib closest to where the apical notch was previously located. In regenerating fragments from older thalli by surgical cutting, meristem always emerges at the midrib closest to the location of the former notch (29). Gemmae with notches excised immediately after removal from the gemma cup do not display such a pattern, with patches of dividing cells and meristem emerging apparently stochastically across the gemma (which at

that stage lacks a midrib). Collectively we interpret these results as pointing towards MpERF20/LAXR having a role in “priming” cells for regeneration, and in promoting apical cell fate once auxin signalling pathways have re-emerged and stabilized. If certain cells have higher MpERF20/LAXR expression (for example younger cells newly generated from meristem) then they would be predisposed to regenerate meristem faster than elsewhere in the regenerating fragment. This could explain why the apical midrib is the focus of meristem regeneration in older thalli fragments (32), and the bias towards regeneration nearer the former apical notch in young gemmae, particularly in the second and third rows of apical notch cells (see Fig. 1E-G and Fig. S3). Conversely, it may explain why meristem regeneration rarely (if ever) occurs in the oldest cells of the gemma, that is at the stalk scar (25, 26).

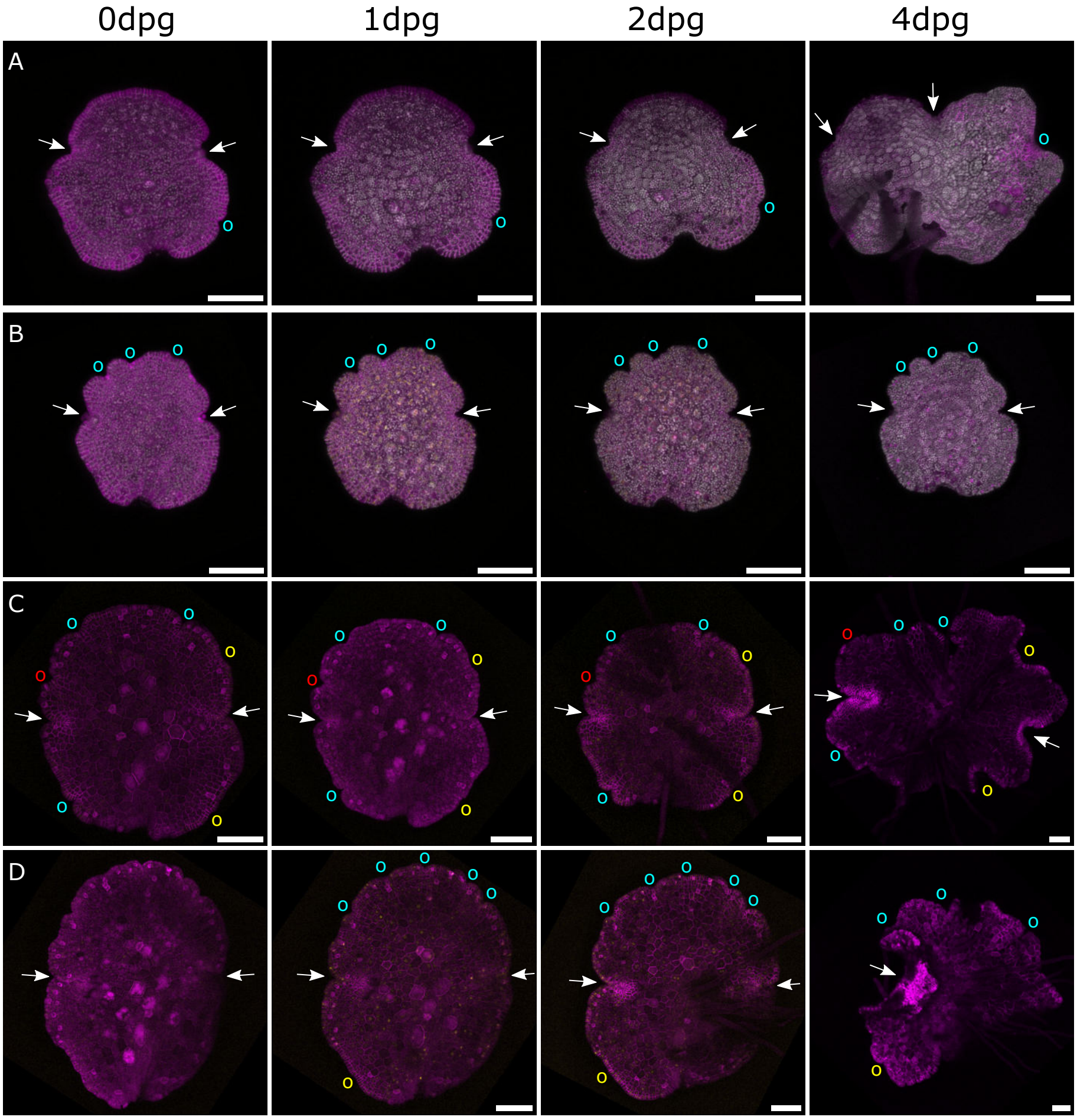

Fig 5. MpERF20/LAXR over-expression in gemmae. (A) and (B) are composite images with pMpHSP18.7A1::GAL4-VP16-DEX, pUAS::MpERF20/LAXR-mVenus expression in yellow, pMpUBE2::mScarletI-lti6b membrane marker expression in magenta and chloroplast autofluorescence in grey. (A) was grown on standard 1/2 Gamborg media agar. (B) was grown on agar supplemented with 10μM dexamethasone and heat-shocked in a 37°C incubator for 1h at 1dpg, before imaged and being returned to normal growth conditions. (C) and (D) composite images show pMpYUC2::GAL4-VP16-DEX, pUAS::MpERF20/LAXR-mVenus expression in yellow and pMpUBE2::mScarletI-lti6b membrane marker expression in magenta. (C) was grown on standard 1/2 Gamborg media agar. (D) was grown on agar supplemented with 10μM dexamethasone. Induction of MpERF20/LAXR over-expression causes numerous developmental abberations, with numerous ectopic notches formed. Ectopic notches are functionally equivalent to apical notches and are competent to form fully-functional meristems. White arrows indicate the normal pair of gemma meristems, cyan circles denote ectopic meristems visible throughout the time course, yellow circles indicate ectopic meristems that form during the time course and red circles indicates ectopic meristems that disappear during the time course. All scale bars= 100μm.

Relationship between regeneration speed and size of the regenerating gemma fragment

An extension of the hypotheses we present is that if there are a greater number of cells that can be "primed" by MpERF20/LAXR and act as auxin sources, combined with a greater number of cells available to act auxin sinks, then regeneration will proceed faster compared to fragments with fewer cells. We tested this by using laser ablation microscopy to remove a certain amount of tissue (expressed as a percentage of the original gemma diameter) from a gemma that had a set amount of the apical notch region removed. The gemmae used were taken from the enhancer trap line 239-P125, a line marking the apical notch/meristem and in which clusters of fluorescent signal re-emerges at Stage 2 of regeneration (30). We used the timing reappearance of this signal as a proxy for measuring the speed of the meristem regeneration process. In a 108-hour time course (imaging every 12 hours) it was seen that larger fragments consistently had marker signal reappear faster after ablation compared to smaller fragments (see Fig. S17). This supports our hypothesis that having a greater pool of cells to provide a balance between auxin sources and sinks is important for promoting meristem regeneration. An extreme version of this is the case of regeneration from a very small number of isolated thallus cells (30, 31, 50). In contrast to meristem regeneration from full gemmae, which is complete after ~7 days, isolated single cells or small clusters can take at least 5-7 days just for cell divisions to occur (*i.e.,* to enter Stage 1), another 4+ days for localized patches of cell division to stabilize (*i.e.,* Stage 2), with recognizable notches only appearing 14 days after cell isolation (even longer for protoplasts).

Conclusions

Our outline model for Marchantia meristem maintenance and regeneration in the gemma is given in Fig. 6. A population of apical cells are found in the first row of the notch, behind which are 2 rows of cells "primed" to acquire apical cell fate. The centre of the apical cell population forms the apex of the notch by having lower rates of cell division and expansion compared to the edges of the apical cell quorum. The apical cells produce signals controlling cell division and expansion, and signals maintaining apical dominance across the gemma. The apical dominance signal, likely auxin, is carried through the centre of the gemma and not the peripheral regions. If neither meristematic apical notch has a sufficient quorum of apical cells in contiguous contact, or if the apical dominance signal cannot flow to the rest of the gemma, then meristem regeneration occurs elsewhere.

Removing the apical notches causes a drop in auxin levels across the gemma, initiating meristem regeneration. Regeneration can be inhibited by application of exogenous

auxin. Falling auxin concentrations cause auxin biosynthesis and transport genes to be widely upregulated, as well as genes associated with apical cell fate. This results in cell division across the gemma fragment. As regeneration proceeds cell division and auxin biosynthesis become localized to certain patches of the gemma fragment. What determines which cells sustain division and which dividing regions go on to become full meristems remains unknown: it may be entirely stochastic, or there could be underlying governing factors such as tissue type, patch size or distance between patches. Genes related to auxin transporter expression become reconfigured to the edges of these patches. This mediates auxin-related communication between patches of regenerating cells, which is vital for establishing a gradient between auxin sources and sinks. If auxin biosynthesis or transport is inhibited, regeneration stalls at this stage until the pool of potential meristematic stem cells is exhausted. This communication process also produces the stochastic pattern of meristem emergence in the gemma, both in number and location, and may also result in the most advanced meristems (including previously damaged, but now repaired, apical notches) asserting apical dominance over other incipient meristems. Eventually a balance is reached where one or more new meristems culminate as mature apical notches. These act as auxin sources exporting to the rest of the gemma and are the focus of expression of genes for auxin biosynthesis, transport and apical cell fate.

The experimental results we present here refine our understanding of the apical notch substructure, and of the requirements to sustain notch meristematic activity or to induce meristem regeneration. We also demonstrate that there is intra- and inter-notch communication in Marchantia, and the role of auxin biosynthesis and transport in meristem regeneration. As such developmental processes and pathways show widespread conservation across the land plants, this provides an avenue to provide new insights into the nature of meristem communication, maintenance and regeneration in more complex plant systems.

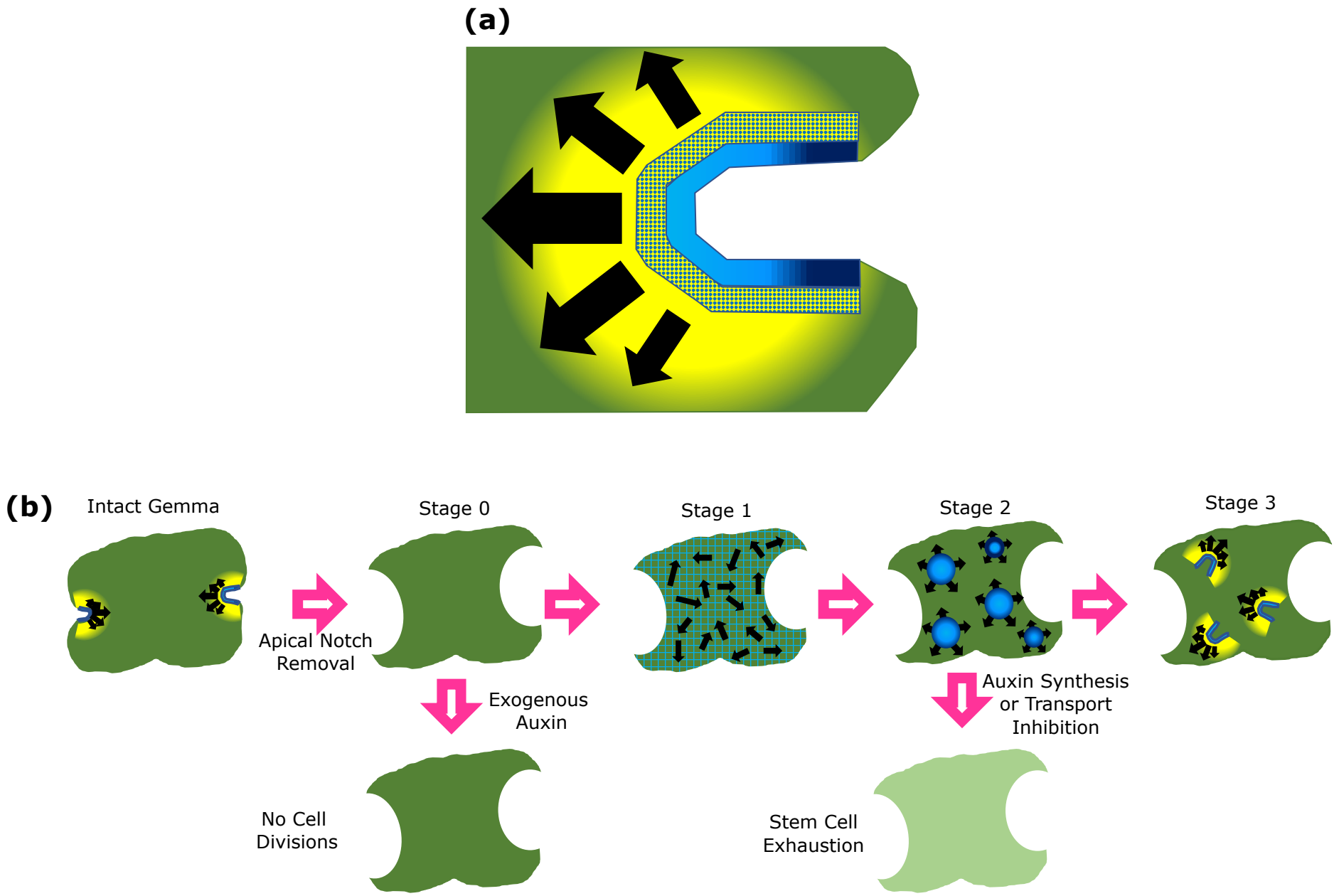

Fig. 6. Outline model of meristem organization and regeneration in *Marchantia*. (A) shows a schematic of the apical notch model. The first row of cells contains the critical meristem (in blue). The cells at the centre of this population (wherever that centre is) become distinct, from the cells towards the edge of the apical notch (blue gradient). The cells in the two rows behind this are primed to form new meristem (blue hatching). The apical cells emit a signal controlling the balance of cell division and expansion in the region surround the notch (yellow gradient). The meristem emits a repressive signal (auxin) that maintains apical dominance, transmitted through the central region (black arrows). (B) shows a schematic of the meristem regeneration model. Removal of both apical notches leads to Stage 0. If exogenous auxin is added, no cell divisions occur and the gemma fragment remains at Stage 0. Otherwise, without exogenous auxin cell divisions occur across the gemma fragment (blue hatching) during Stage 1. During Stage 1 auxin biosynthesis and transport is up-regulated (black arrows). With time, cell divisions (blue circles), auxin biosynthesis and export (black arrows) become restricted to multiple cell clusters (Stage 2). Cells in the remaining tissue stop dividing, indicating rising auxin signaling in the remaining tissue. At Stage 3, apical notches (blue gradient) are fully reformed and divisions occur predominantly in surrounding cells (yellow gradient), indicating complete reformation of meristem domains. Inhibition of auxin biosynthesis or transport prevents the transition into Stage 3. Eventually the pool of stem cells in the gemma fragment becomes exhausted and the regeneration process ceases. This indicates that auxin signaling gradients are essential for the re-establishment of the apical cell population.

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