Supplemental Data

Local Cues and Asymmetric Cell Divisions Underpin Body Plan Transitions in the Moss *Physcomitrella patens*

C. Jill Harrison, Adrienne H.K. Roeder, Elliot M. Meyerowitz, and Jane A. Langdale

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell counts

The number of cells in transverse leaf sections was counted from adaxial and abaxial surfaces (Supplementary Figure 1A) in three sections close to the widest point of the leaf. Cells were counted in leaves half way up the bushy shoot in three median longitudinal sections for each leaf. Median sections were identified by the presence of multiple cell layers associated with the conducting hydroids (Supplementary Figure 1B). Counts from 25 leaves were obtained to evaluate the cell number in each dimension, and an ANOVA was used to test for differences between transverse counts on each face of the leaf and longitudinal counts.

X-ray irradiation and sector measurements.

Plants were irradiated with single doses of 250 kV X-rays at a dose rate of 2.2 Gy per minute. The X-ray generator was operated at 14 mA, with 0.5 mm Cu and 1 mm Al added filtration (half value layer = 1.32 mm Cu), with an open ended Perspex applicator. To determine the X-ray dose at which the maximum number of sectors could be induced without affecting plant growth, 12-14 day old spot cultures were first irradiated with 0 to 200 Gy at 50 Gy intervals. To verify that growth conditions did not affect the response to radiation, plants grown on medium with and without the growth supplement ammonium tartrate were irradiated. No differences in response were detected between plants grown in either regime, and all subsequent experiments were carried out using plants grown with ammonium tartrate (Figure1D). Irradiation appeared to have little effect on gross

colony and shoot morphology even at very high doses (Figure 1D). At doses above 50 Gy a subtle distinction in the number of shoots initiating from each colony was observed, with fewer shoots apparent on colonies irradiated at higher doses. Slight stunting of shoot growth was seen on leafy shoots exposed to higher doses. In contrast to the apparent minor effects on gross morphology, irradiation caused severe growth defects in leaves, including aberrations in the timing and plane of cell division, stunting, and the appearance of forked leaves (Figure 1D). These severe defects were initially overlooked due to an impressive capacity for shoot recovery that led to damaged leaves being camouflaged by normal leaves initiated following irradiation. However, once such defects became apparent, plants were irradiated at 0 to 100 Gy in 20 Gy increments, 0 to 20 Gy in 5 Gy increments, and 0 to 10 Gy in 2 Gy increments. Minor defects in growth were detected in some leaves at doses over 5 Gy, but few sectors were detected in plants irradiated at less than 5 Gy. Although no sectors were seen in non-irradiated plants, and few sectors were seen in irradiated WT plants, three cell autonomous sector types were seen in irradiated *glk* mutant plants, having a 'pale' 'beaded' or 'empty' appearance (Figure 1C). These sectors were examined in normal shaped leaves from plants irradiated at 2 Gy, 5 Gy and 10 Gy.

Calculation of apparent cell number (ACN).

The proportion of leaves occupied by each sector was calculated by counting the number of cells in each dimension of the leaf and sector. Estimates of the ACN at the time of irradiation were gauged using the reciprocal of the proportion occupied by each sector. Thus a sector that occupied both a half of the length and a half of the width of a leaf resulted from either one or two cells in each dimension, depending on the phase of the cell cycle and the plane of cell division [30]. By irradiating many plants with leaves at different stages of development, multiple representations of different sector types were obtained, allowing reconstruction of cell division patterns within the leaf.

Validation of live-imaging microscopy technique.

Studies of dynamic meristem activity have shown that normal cell division and growth patterns in *A. thaliana* can withstand repeated scanning using a confocal laser [1-3]. Previous studies of dynamic cell divisions in *P. patens* are not documented in the literature, so it was impossible to verify the consistency of dynamics observed in experiments presented here with previous results. However, results obtained from live-imaging experiments were completely consistent with cell division patterns inferred from clonal sector analysis, and with anatomical descriptions of development in other mosses. As growing plants were maintained in a solution of PI throughout experiments, cell death was also immediately apparent due to influx of PI and strong nuclear staining. Furthermore, in experiments where harsh dissection led to cessation of growth, this was immediately apparent. Where more gradual cessation of growth over the course of an experiment was detected, plants were excluded from further analysis. All reported analyses were carried out on plants that were still growing well at the end of experiments. Perturbations in growth detected following exposure of plants to X-irradiation were conspicuous, and because the leaf is mainly comprised of a single cell layer, even small disruptions in cell shape and size deformed overall leaf shape.

REFERENCES

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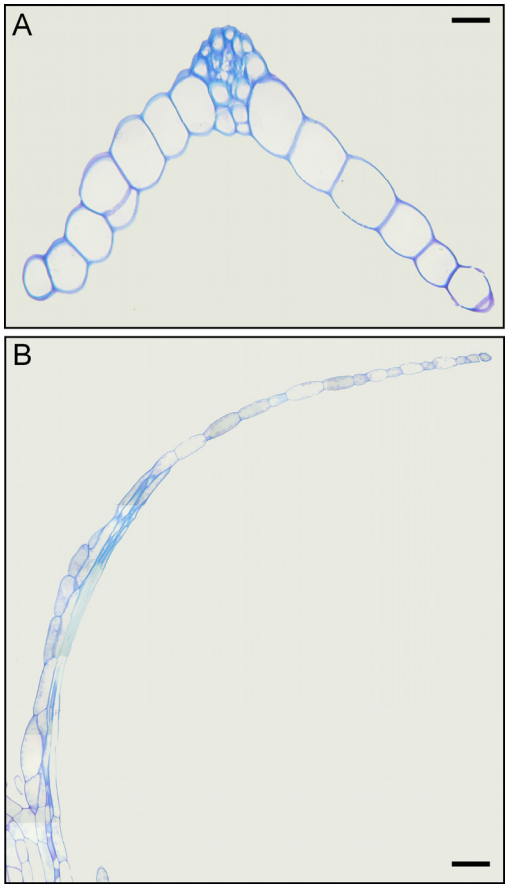

FIGURE S1.

A) Transverse section through widest point of a leaf on a 3 week old bushy shoot showing cell layers in conducting tissue. B) Median longitudinal section through a leaf on a 3 week old bushy shoot showing cell layers in conducting tissue at base. Scale bars: (A) 30 µm; (B) 100 µm.

Table S1. Balanced sectors spanning the leaf tip.

Sector length		Deduced p-d cell	Number of sectors
Edge 1	Edge 2	number	
Full	Full	1	1
Full	Half	1-2	1
Half	Half	2	0
Half	Third	2-3	2
Third	Third	3	2
Third	Quarter	3-4	7
Quarter	Quarter	4	0
Quarter	Fifth	4-5	3
Fifth	Fifth	5	3
Fifth	Sixth	5-6	3
Sixth	Sixth	6	4
Sixth	Seventh	6-7	0
Seventh	Seventh	7	0
Seventh	Eighth	7-8	0
Eighth	Eighth	8	2 (+2)
Eighth	Ninth	8-9	0
Ninth	Ninth	9	2 (+2)
Ninth	Tenth	9-10	0
Tenth	Tenth	10	4
Tenth	Eleventh	10-11	0
Eleventh	Eleventh	11	1
Eleventh	Twelfth	11-12	0
Twelfth	Twelfth	12	3
Twelfth	Thirteenth	12-13	0
Thirteenth	Thirteenth	13	6
Thirteenth	Fourteenth	13-14	0
Fourteenth	Fourteenth	14	1
Fourteenth	Fifteenth	14-15	0
Fifteenth	Fifteenth	15	2
Fifteenth	Sixteenth	15-16	0
Sixteenth	Sixteenth	16	0
Sixteenth	Seventeenth	16-17	0
Seventeenth	Seventeenth	17	1

Length of tip sectors at each edge of the leaf, cell numbers deduced to be present in the leaf at the time of irradiation and the number of sectors representing each class.

Table S2. Imbalanced sectors spanning the leaf tip.

Sector length		Deduced p-d cell	Number of sectors
Edge 1	Edge 2	number	
Third	Sixth	3-6	1
Fifth	Tenth	5-10	1
Sixth	Eighth	6-8	1
Sixth	Ninth	6-9	1
Sixth	Twelfth	6-12	4
Seventh	Fourteenth	7-14	6
Eighth	Eleventh	8-11	1
Eighth	Sixteenth	8-16	2
Eighth-Ninth	Seventeenth	8/9-17	3
Ninth	Eighteenth	9-18	2
Ninth	Fourteenth	9-14	4
Ninth	Thirteenth	9-13	1
Ninth-Tenth	Nineteenth	9/10-19	1
Tenth	Fifteenth	10-15	2
Tenth	Twentieth	10-20	2
Twelfh	Twenty-fourth	12-24	4
Twelfth	Twenty-third	12-23	1
Twelfth	Twenty-fifth	12-25	2
Fourteenth	Twenty-seventh	14-27	3
Fifteenth	Thirtieth	15-13	3
Sixteenth	Thirty-Third	16-33	1

Length of tip sectors at each edge of the leaf, cell numbers deduced to be present in the leaf at the time of irradiation and the number of sectors representing each class.

Table S3. Segment sectors occupying between a half and third of the medio-lateral extent of the Leaf.

Length	Deduced p-d cell number	Number of sectors
Half	2	8
Third	3	4
Quarter	4	3
Quarter-fifth	4-5	1
Fifth	5	2
Sixth	6	10
Sixth-seventh	6-7	2
Seventh	7	3
Eighth-ninth	8-9	2
Ninth	9	3
Ninth-tenth	9-10	1
Tenth	10	8
Tenth-eleventh	10-11	1
Eleventh	11	5
Eleventh-twelfth	11-12	3
Twelfth	12	6
Thirteenth	13	3
Thirteenth-fourteenth	13-14	3
Fourteenth	14	1
Fifteenth	15	1
Fifteenth-sixteenth	15-16	1
Sixteenth	16	1
Seventeenth	17	1
Eighteenth	18	2
Nineteenth	19	1
Twentieth	20	1
Twenty-third	23	1
Twenty-fifth	25	1
Twenty-sixth	26	1
Twenty-eighth	28	1

Length of lateral segment sectors at the edge of the leaf, cell numbers deduced to be present in the leaf at the time of irradiation and the number of sectors in each class.

Table S4. Sectors consistent with early divisions within lateral segments.

Sector length	Deduced p-d number of cells	Sector width	Deduced m-l number of cells	Number of sectors	Most frequent width
Half	2	Third to sixth	3-6	13	Sixth (10/13)
Half to third	2-3	Tenth	10	1	n.a.
Third	3	Third to thirteenth	3-13	9	Quarter and Sixth (3/9)
Third to quarter	3-4	Third to ninth	3-9	4	Ninth (2/4)
Quarter	4	Quarter to twentyeigth	4-28	21	Sixth (6/21)
Quarter to fifth	4-5	Third to fourteenth	3-14	3	N.a.
Fifth	5	Third to twelfth	3-12	12	Quarter and eighth (3/12)
Fifth to sixth	5-6	Sixth to sixteenth	6-16	6	Tenth (2/6)
Sixth	6	Quarter to fifteenth	4-15	27	Sixth (10/27)
Sixth to seventh	6-7	Quarter to thirteenth	4-13	8	Sixth (3/8)
Seventh	7	Third to sixteenth	3-16	13	Quarter (4/13)
Seventh to eighth	7-8	Eighth to fifteenth	8-15	2	n.a.
Eighth	8	Quarter to sixteenth	4-16	11	Fifth to Sixth (2/11) and Fourteenth (2/11)
Eighth to ninth	8-9	Quarter to tenth	4-10	5	Tenth (2/5)
Ninth	9	Sixth to twelfth	6-12	4	Sixth (2/4)
Ninth to tenth	9-10	Quarter	4	1	N.a.
Tenth	10	Quarter to sixteenth	4-16	6	N.a.
Tenth to eleventh	10-11	Third to eighth	3-8	2	N.a.
Eleventh	11	Sixth	6	1	N.a.
Twelfth	12	Quarter to tenth	4-10	5	Quarter (2/5)
Twelfth to thirteenth	12-13	Sixth	6	1	N.a.
Fourteenth	14	Third	3	1	N.a.
Twentieth	20	Third	3	1	N.a.
Twenty-second	22	Third	3	1	N.a.
Twenty-fourth	24	Third	3	2	N.a.
Twenty-eighth	28	Third	3	1	N.a.

Length of sectors, breadth of sectors and cell numbers deduced to be present in the leaf at the time of irradiation. The number of sectors in each class and the median sector width for each length class are also shown.