RESEARCH ARTICLE

Ectopic KNOX Expression Affects Plant Development by Altering Tissue Cell Polarity and Identity

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Short title: KNOX Genes Control Polarity and Identity

One-sentence summary: KNOTTED1-like Homeobox (KNOX) gene expression reorients both tissue cell and regional polarity in Hooded barley, generating altered patterns of identity, anisotropic growth, and cell differentiation.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is Enrico Coen (enrico.coen@jic.ac.uk).

ABSTRACT

Plant development involves two polarity types: tissue cell (asymmetries within cells are coordinated across tissues) and regional (identities vary spatially across tissues) polarity. Both appear altered in the barley (Hordeum vulgare) Hooded mutant, in which ectopic expression of the KNOTTED1-like Homeobox (KNOX) gene, BKn3, causes inverted polarity of differentiated hairs and ectopic flowers, in addition to wing-shaped outgrowths. These lemma-specific effects allow the spatiotemporal analysis of events following ectopic BKn3 expression, determining the relationship between KNOXs, polarity, and shape. We show that tissue cell polarity, based on localization of the auxin transporter SISTER OF PINFORMED1 (SoPIN1), dynamically reorients as ectopic BKn3 expression increases. Concurrently, ectopic expression of the auxin importer LIKE AUX1 and boundary gene NO APICAL MERISTEM is activated. The polarity of hairs reflects SoPIN1 patterns, suggesting that tissue cell polarity underpins oriented cell differentiation. Wing cell files reveal an anisotropic growth pattern, and computational modeling shows how polarity guiding growth can account for this pattern and wing emergence. The inverted ectopic flower orientation does not correlate with SoPIN1, suggesting that this form of regional polarity is not controlled by tissue cell polarity. Overall, the results suggest that KNOXs trigger different morphogenetic effects through interplay between tissue cell polarity, identity, and growth.
INTRODUCTION

Class 1 KNOTTED1-like Homeobox (KNOX) genes are central to shoot meristem function (Barton and Poethig, 1993, Kerstetter et al., 1997). Loss-of-function mutants typically lack shoot meristems or have reduced organ complexity (Barton and Poethig, 1993, Long et al., 1996, Kerstetter et al., 1997, Vollbrecht et al., 2000), suggesting that class 1 KNOX genes contribute to meristem identity. When overexpressed, class 1 KNOX genes have a diverse range of developmental effects: enhanced leaf lobing, leaflet formation, ectopic meristems, knotted leaves, forked leaves and petal spurs (Vollbrecht et al., 1991, Smith et al., 1992, Sinha et al., 1993, Lincoln et al., 1994, Muller et al., 1995, Chuck et al., 1996, Williams-Carrier et al., 1997, Janssen et al., 1998, Golz et al., 2002, Hake et al., 2004, Ramirez et al., 2009, Shani et al., 2009). This raises the question of how a gene controlling meristem identity generates such a diverse range of morphological effects when ectopically expressed. One hypothesis is that KNOX genes have additional roles in organ outgrowth. This may be indicated by the localisation of KNOTTED1 protein in the base of developing maize (Zea mays) leaves (Jackson, 2002) and in the involvement of KNOX genes during evolution for leaf margin elaboration (Bharathan et al., 2002, Hay and Tsiantis, 2006, Shani et al., 2009, Piazza et al., 2010).

Previous studies have suggested that class 1 KNOX genes influence organ development by acting as regulators of cell fate determination (Smith et al., 1992, Sinha et al., 1993, Lincoln et al., 1994, Janssen et al., 1998, Shani et al., 2009), involving the modulation of the cytokinin to gibberellic acid ratio (Jasinski et al., 2005, Yanai et al., 2005). There is also evidence that class 1 KNOX genes influence cell division (Smith et al., 1992, Sinha et al., 1993, Schneeberger et al., 1995). For example the barley (Hordeum vulgare) Hooded (Hd) mutant, which ectopically expresses the class 1 KNOX gene BKn3 in the lemma/awn boundary (Muller et al., 1995), has altered cell division patterns (Stebbins and Yagil, 1966). The Hd mutant also develops wing-like outgrowths in the lemma margin, indicating that class 1 KNOX genes also influence growth patterns (Bonnett, 1938, Stebbins and Yagil, 1966, Williams-Carrier et al., 1997). Modulation of growth is also indicated in studies where leaf margins are modified (Hay and Tsiantis,
The effect of class 1 \textit{KNOX} genes on growth is further supported by their effect on the cytokinin: gibberellic acid ratio, influencing the distribution of cell division and cell elongation in a tissue (Sakamoto et al., 2001, Jasinski et al., 2005, Yanai et al., 2005, Bolduc and Hake, 2009, Bolduc et al., 2012).

Some of these developmental effects of class 1 \textit{KNOX} genes may also reflect their influence on polarity. We may distinguish between two types of polarity: regional polarity and tissue cell polarity. Regional polarity reflects spatial variation in regional identities. For example, the maize leaf has abaxial-adaxial, proximodistal and mediolateral polarities, which refer to the difference between the upper and lower leaf zones, the basal sheath and upper blade, and the midvein and lateral margins, respectively. Mathematically, regional polarity can be described as a field of values (e.g. gene expression levels) associated with positions in space (a scalar field (Lawrence et al., 2007)). Analysis of class 1 \textit{KNOX} gene overexpression mutants has led to the suggestion that morphological changes arise due to modulation of regional polarity (Golz et al., 2002, Ramirez et al., 2009). For example, the maize \textit{Knotted1} mutant produces proximal sheath-like outgrowths on the distal blade margins (Ramirez et al., 2009), suggesting a change in proximodistal polarity. The barley \textit{Hd} mutation also affects regional polarity. Wild-type (Wt) barley (Figure 1) has a protective bract-like floral organ, the lemma, which has a distal extension called the awn (Figure 1A-D). Instead of an awn, the \textit{Hd} mutant (Figure 1) develops ectopic flowers on the lemma (Figure 1E-H). These ectopic flowers have the same whorled structure of Wt flowers (Figure 1D vs Figure 1H), but inverted regional polarity, evidenced by the positions of the palea (Figure 1F-G) (Harlan, 1931, Bonnett, 1938, Stebbins and Yagil, 1966, Muller et al., 1995, Williams-Carrier et al., 1997). It has been proposed that the inversion of regional polarity in the \textit{Hd} lemma arises due to ectopic \textit{BKn3} expression in the lemma, inducing a new "polarising gradient centre" based on hormone concentrations (Stebbins and Yagil, 1966) or a new inflorescence meristem unit (Williams-Carrier et al., 1997), generating inverted ectopic flowers.

Tissue cell polarity refers to asymmetries across individual cells (cell polarity) and their coordination across a tissue. Mathematically, tissue cell polarity corresponds to a field
Figure 1. The Hd mutant exhibits polarity reversals. (A) Photograph of Wt barley (Bowman, 2-row) inflorescence, illustrating the arrangement of florets (Fl) along the rachis (Ra). Clusters of 3 spikelets are initiated in a distichous pattern along the rachis (only the central spikelet produces a mature floret). Each floret has an outer protective organ, the lemma, with a distal awn (Aw). Floral diagrams of Wt (B-D) and Hd (F-H). (B) Adaxial Wt morphology: the lemma and palea are visible, hairs on the lemma point distally (hair orientation, red arrows). (C) Longitudinal cross-section through a Wt spikelet: the floret has a dorsal lemma and ventral palea. (D) Transverse cross-section illustrating the organ whorls in the Wt floret. (E) The Hd inflorescence phenotype resembles Wt, although the lemma develops ectopic flowers (EF) instead of an awn. (F) One or more flowers can form on the Hd lemma’s adaxial surface. Analyses of hair and organ orientations (Harlan, 1931, Bonnett, 1938, Stebbins and Yagil, 1966, Muller et al., 1995, Williams-Carrier et al., 1997) have shown that the first ectopic flower (1) is inverted, pointing towards the lemma base, whereas the second ectopic flower (2) points towards the lemma tip. The Hd lemma develops wing-like (Wi) margin outgrowths below the first ectopic flower. (G) Longitudinal cross-section through an Hd spikelet. (H) Floral diagram of each Hd flower, showing that each ectopic flower retains the same whorled arrangement of organs. Each ectopic flower is thought to use the existing lemma as its own (dashed black line). Proximal-distal axis, Pr-Di. Dorsal-ventral axis, Do-Ve. Scale bars: 1 cm.
PIN-FORMED 1 (PIN1) can be used as a marker of tissue cell polarity and may also play a role in its establishment or coordination. Several models have been proposed for how PIN1 may be involved in the establishment of tissue cell polarity: (1) Up-the-gradient (Jönsson et al., 2006, Smith et al., 2006), (2) With-the-flux model (Sachs, 1969, Mitchison, 1980, Mitchison et al., 1981, Sachs, 1981, Stoma et al., 2008), and (3) Indirect cell-cell coupling (Abley et al., 2013). With all of these models, coordination of cell polarity across a tissue can be achieved through modulating auxin dynamics (Abley et al., 2016). Analysis of PIN1 localisation and class 1 KNOX gene expression in meristems and compound leaves has led to the suggestion that KNOX expression confers competency for PIN1 convergence point formation (Barkoulas et al., 2008, Hay and Tsiantis, 2010), indicating that class 1 KNOX genes and tissue cell polarity may interact. Tissue cell polarity has also been proposed to provide the axial information from which growth is oriented (Abley et al., 2013). In Drosophila melanogaster, epidermal wing hairs point distally on the wing and are used as a readout of planar cell polarity (Adler, 2002). Similarly, hairs on the adaxial surface of the Wt barley lemma point distally towards the lemma tip (Figure 1B). In the Hd mutant, this uniform orientation of hairs is disrupted, with hairs pointing proximally below the first ectopic flower (Figure 1F) (Bonnett, 1938, Stebbins and Yagil, 1966, Williams-Carrier et al., 1997). Taken together, these results suggest that some of the effects of ectopic expression of BKn3 in the developing lemma may be mediated through early changes in tissue cell polarity.

The Hd mutant is a good model for exploring the relationship between class 1 KNOX genes, polarity, and shape, as all three aspects are affected. Ectopic expression of BKn3 in Hd is specific to the lemma/awn boundary (Muller et al., 1995, Williams-Carrier et al., 1997), so the subsequent effect on lemma development can be analysed in a specific spatiotemporal manner. Such spatiotemporal analysis is more difficult with other KNOX overexpression systems, like maize Kn1 (Ramirez et al., 2009), where ectopic expression patterns are more variable. The morphology of the Hd mutant is not unique to ectopic expression of BKn3, as the Hd phenotype can be replicated by overexpressing maize KN1 in barley (Williams-Carrier et al., 1997). This suggests that understanding the role of BKn3 in Hd development could shed light on class 1 KNOX
gene functions in general. Although the study of over-expression mutants generally identifies roles of genes outside of their wild-type context, they can also highlight wild-type functions not previously observed in the corresponding null mutants. This can be particularly important for a gene, like *KNOTTED1*, for which the null mutant exhibits complete loss of an organ or meristem, masking gene functions that are additional to those required for organ or meristem formation.

Here we characterise changes in *Hd* lemma morphology using 3D imaging and analyse the spatial and temporal effects of the *Hd* mutation on growth, tissue cell polarity, regional polarity, identity and differentiation. We show that at the time that ectopic *BKn3* expression is activated, there is a change in tissue cell polarity, as marked by SISTER OF PIN-FORMED1 (SoPIN1) cellular localisation. Concurrent with these changes in tissue cell polarity, changes in identity occur, illustrated by the upregulation of *BKn3* and ectopic activation of barley homologues of *LIKE AUX1* (*HvLAX1*) and *NO APICAL MERISTEM* (*HvNAM*) (*Hv*: *Hordeum vulgare*). These changes in tissue cell polarity and identity precede the later changes in regional polarity (inverted ectopic flowers), cell differentiation (inverted hair orientation) and growth (wing development). Experimental analysis and modelling of these developmental events suggest that interactions between identity and tissue cell polarity play a key role in regulating cell differentiation and growth. Ectopic flower polarity (regional polarity) does not directly reflect tissue cell polarity patterns, suggesting that this aspect of regional polarity is regulated via a separable mechanism. These studies on ectopic *BKn3* expression thus show how class 1 *KNOX* genes may regulate development through interactions between identity, tissue cell polarity and regional polarity.
RESULTS

Two separable morphological changes are observed for the *Hd* lemma

To determine the downstream effects of the *Hd* mutation, we first established the timing of both cellular and tissue level events in Wt and *Hd* inflorescence development (Figure 2). We used Optical Projection Tomography (OPT) (Sharpe et al., 2002, Lee et al., 2006) to image in 3D fixed inflorescences harvested at intervals over a 380 hour (h) period of development (Figure 2A and 2B). Using digital slices through the images, the width of floret 5 was measured for each timecourse sample and used to generate a growth curve (Figure 2C). This showed that the natural logarithm of floret width increased linearly at a similar rate in both Wt and *Hd*, corresponding to a growth rate of 0.46 % h⁻¹. Using the equation of this graph, we could assign a standard developmental time, in hours, based on floret width. 0 h (illustrated in Figure 2A, 2B, 2D, and 2J) corresponded to a floret width of 140 µm when the lemma primordium was just visible (Figure 2D-E and Figure 2J-K). At this stage, Wt and *Hd* were morphologically indistinguishable. Morphological divergence between Wt and *Hd* was first clearly detected at 170 h, when the Wt lemma had a smooth adaxial surface (Figure 2F), while an ectopic meristem was just visible as cushion on the adaxial surface of the *Hd* lemma (Figure 2L). By 240 h, an extended awn was visible at the distal end of the Wt lemma (Figure 2H-I) but absent from the *Hd* lemma (Figure 2N-O). At this stage the margins of the Wt lemma were curving (Figure 2I), while those of the *Hd* lemma showed small bulges that eventually developed into the wings (Figure 2O). Thus, two separable morphological changes are observed during *Hd* lemma development: formation of the ectopic meristem on the adaxial surface at 170 h, and outgrowth of the wings from the lemma margins at 240 h.

To visualise these changes at higher resolution, we carried out confocal imaging of the adaxial surface of fixed *Hd* lemmas at different developmental stages (Figure 3). At 130 h, when the Wt and *Hd* lemmas were morphologically indistinguishable, the *Hd* lemma had a smooth adaxial surface (Figure 3A). By 170 h, the *Hd* lemma had a distinct adaxial cushion (Figure 3B). This cushion corresponds to the ectopic meristem
identified in the *Hd* OPT images. The ectopic meristem continued to develop, forming distinct organ primordia. By 190 h, a distal palea primordium was visible (Figure 3C) and by 250 h other floral organ primordia started to emerge (Figure 3D,1). A second
ectopic meristem can also form on the *Hd* lemma distal to the first and was clearly visible by 250 h (Figure 3D, 2). At 250 h, small bulges were also visible in the margin of...
the *Hd* lemma (Figure 3D, Wi), which developed into more pronounced wing outgrowths by 350 h (Figure 3E, Wi).

**Ectopic BKn3 expression is activated by 110 h, and is strongly expressed by 170 h**

To establish when and where ectopic expression of BKn3 is activated during *Hd* lemma development, RNA *in situ* hybridisation of BKn3 was carried out on barley inflorescences (Figure 4). Throughout Wt development, BKn3 mRNA was completely excluded from the developing lemma and was restricted to the meristematic region at the base of the flower (Figure 4A-D, H, J). Similarly, in the *Hd* lemma at 90 h, BKn3 mRNA was localised to the meristematic region in the base of the flower (Figure 4E, white arrowhead) and was excluded from the developing organs, including the lemma. However, in contrast to Wt, by 110 h, faint BKn3 mRNA expression was detected in the distal adaxial region of the developing *Hd* lemma (Figure 4F). By 170 h (Figure 4G) when the ectopic meristem had started to form, BKn3 mRNA expression was strong within this meristem. This distribution of BKn3 mRNA localisation in Wt and *Hd* lemmas contrasts with previous observations of mRNA localisation by (Muller et al., 1995), who reported BKn3 mRNA throughout the lemma at early stages of development. Our observations are consistent with the protein localisation reported in (Williams-Carrier et al., 1997). However, both of these earlier studies did not establish timings, so direct comparisons cannot be made.

At later stages in *Hd* development, around 250 h, when the wings started to form, transverse cross-sections through the lemma indicated that BKn3 expression extended below the ectopic meristem region (Figure 4I, K-M). BKn3 was expressed in two short stripes (approximately 40-50 µm long) either side of the lemma midline, specific to the adaxial cell layers (Figure 4L, white arrowheads). This region was just above where the tissue starts to deform towards the wing outgrowths (Figure 4I, K-M) and may suggest a role of BKn3 in wing development. (No BKn3 expression was seen in transverse sections of Wt (Figure 4H, J).) The timing of the initial expression of the BKn3 in the *Hd* lemma, combined with the staging of ectopic flower and wing formation, is consistent with BKn3 modulating the development of the adaxial surface of the *Hd* lemma after 110 h.
Figure 4. In Hooded, ectopic BKn3 expression is activated around 110 h, is strong by 170 h, and extends beyond the ectopic meristem region by 250 h. BKn3 mRNA in situ hybridisation of longitudinal midsections through Wt florets (A-D) and Hd florets (E-G) (zoomed-in lemma images, black boxes) at 90 h (A, E), 110 h (B, F), 170 h (C, G), and 200 h (D, Wt only). At 90 h (A, E), BKn3 mRNA (dark precipitate) was localised to the base of the flower (white arrowhead) and excluded from developing organs, including the lemma, in both Wt and Hd. Throughout Wt development, this pattern is maintained, with BKn3 mRNA excluded from the lemma (B-D). In Hd at 110 h (F) there was faint BKn3 mRNA localisation in the middle of the lemma on the adaxial side (zoomed in image, white arrowhead). By 170 h in Hd (G), there was strong BKn3 mRNA localisation in the adaxial half of the lemma (zoomed in image, white arrowhead), where the ectopic meristem formed. (H) Diagram illustrating mature Wt morphology; the yellow box illustrates the approximate position of the sections in (J). In Hd, wings start to develop by 250 h (I). Confocal image of a calcofluor stained Hd lemma (I) at a similar developmental time as the sectioned lemma in (K-L); the yellow box indicates the approximate position of the sections. (J-L) Transverse sections through a 250 h lemma for Wt (J) and Hd (K-L). (J) In Wt, BKn3 was not expressed in the lemma. (K-L) Transverse sections through the Hd lemma. (K) 3D reconstruction of calcofluor stained slices, viewed from the top, showing the curve of the wing. (L) In situ hybridisation of transverse slices throughout the wing region indicated that BKn3 was expressed adaxially in two proximodistal stripes on either side of the lemma midline (the position of each slice within the 3D reconstructed region is indicated in μm). (M) Diagram depicting the predicted BKn3 expression pattern on the adaxial surface of the lemma (BKn3 expression, purple, lemma outline, black) in relation to the position of the wings (black arrows). Stamen (St), carpel (Ca), palea (Pa), abaxial (Ab), adaxial (Ad). Dotted white lines: lemma outline. n>4 for each timepoint in A-G, n= 3 J-L. Scale bars: 100 μm.
Ectopic *BKn3* expression leads to reorientation of tissue cell polarity

Previous studies based on floral organ orientation have shown that regional polarity is inverted in the *Hd* mutant at late stages of development (Bonnett, 1938, Harlan, 1931, Muller et al., 1995, Williams-Carrier et al., 1997, Stebbins and Yagil, 1966). In addition to this, lemma hair orientation suggests that tissue cell polarity may also be altered in this mutant. To assess when and how *BKn3* first influences tissue cell polarity, we determined the distribution of the cell polarity marker, SoPIN1 (O'Connor et al., 2014), in the adaxial surface of the lemma using whole-mount immunolocalisation (Figure 5). At early stages in Wt and *Hd* lemma development, SoPIN1 (green signal, Wt 170 h Figure 5A-D, *Hd* 90 h Figure 5E-H) was localised at the distal end of epidermal cells (Figure 5C, and G white arrows), indicating that tissue cell polarity was coordinately oriented towards the lemma tip (Figure 5D,H, red arrows). As WT development progressed, SoPIN1 signal was lost.

In contrast to Wt, in *Hd*, epidermal SoPIN1 signal was not lost at later stages of development. At 120 h, after *BKn3* expression was activated in the adaxial half of the *Hd* lemma, SoPIN1 localisation became oriented towards the centre of the region of ectopic *BKn3* expression (Figure 5I-L, Figure 5J-K, white arrows, and L, red arrows). At 180 h, after the ectopic meristem had clearly formed and *BKn3* expression was strong in the *Hd* lemma, the reorientation of SoPIN1 in the adaxial surface, relative to its orientation at earlier stages, was more clearly seen (Figure 5M-R). At the distal end of the lemma, SoPIN1 pointed towards the lemma tip (Figure 5N-O, white arrows). Near the ectopic meristem, SoPIN1 was oriented towards the lemma midline (Figure 5N and P, white arrows). This midline-oriented SoPIN1 may have been focussed towards the centre of the *BKn3* expression region and the ectopic meristem (Figure 5R). Below the ectopic meristem, SoPIN1 pointed proximally (Figure 5N and Q, white arrows), the opposite orientation to that observed at 90 h. This suggests that *BKn3* is able to modulate tissue cell polarity early in lemma development.

To test whether the higher levels of SoPIN1 in the *Hd* lemma were due to transcriptional activation of *HvSoPIN1 (Hv. Hordeum vulgare)* by *BKn3*, we used RNA *in situ*
hybridisation (Figure 6). At 170 h in Wt florets, *HvSoPIN1* expression was largely excluded from the developing lemma (Figure 6A). By contrast, in the *Hd* lemma at 170 h, there was high expression of *HvSoPIN1* in the region of the ectopic meristem (Figure 5). SoPIN1 localisation reorients when ectopic BKn3 expression is activated. 3D projections of whole-mount immunolocalisation of SoPIN1 (green) and calcofluor stained cell walls (magenta) in whole Wt lemmas at 170 h (A-D) and Hd lemmas at 90 h (E-H), 120 h (I-L), and 180 h (M-R). Orientation of SoPIN1 is based on cellular SoPIN1 localisation in the epidermal layer only. Each panel illustrates SoPIN1 localisation alone (A,E,I,M), both SoPIN1 and calcofluor combined (B,F,J,N), and a zoomed-in image of the boxed regions (C,G,K,O,P,Q), alongside a cartoon illustrating the inferred relationship between the ectopic meristem (orange) and SoPIN1 localisation (red arrows) (D,H,L,R). Overlapping SoPIN1 and calcofluor signals appear white. In Wt lemmas, SoPIN1 pointed distally towards the lemma tip throughout development (A-D), although SoPIN1 signal in the adaxial surface was lost over developmental time. In Hd lemmas, SoPIN1 signal was not lost over developmental time (E-R). Before BKn3 was ectopically expressed in the Hd lemma, SoPIN1 was localised distally, towards the tip of the developing lemma (E-G, white arrows, H, red arrows). Around the time that ectopic BKn3 expression was activated in the lemma (110 h), SoPIN1 localisation reoriented (with respect to earlier stages in development) in the adaxial surface. Initially, SoPIN1 oriented laterally towards the centre of the adaxial surface (I-K, white arrows, L, red arrows). After the ectopic meristem had formed (M-R, 180 h), near the distal tip, cellular SoPIN1 was oriented distally towards the tip of the lemma (O), below the ectopic meristem SoPIN1 pointed towards the centre of the adaxial surface (P), and below this SoPIN1 was oriented proximally (Q, white arrows, R, red arrows). n=4 for each timepoint. Scale bars: 100 μm.
Figure 6. Ectopic expression of BKn3 leads to the expression of HvSoPIN1, HvNAM, and HvLAX1. RNA in situ hybridisation of HvSoPIN1 (A,E), HvNAM (B-C,F-G), and HvLAX1 (D,H). In Wt, HvSoPIN1, HvNAM, and HvLAX1 were excluded from the lemma, except in some vascular regions (A-D). At 120 h, HvNAM was expressed flanking the region of BKn3 expression (F, white arrowheads). By 170 h, when BKn3 expression was high, all three genes (HvSoPIN1, HvNAM, HvLAX1) were strongly ectopically expressed in the adaxial half of the Hd lemma (E, G-H, white arrowheads). Black box: zoomed-in image of the lemma. White dotted line: lemma outline. White arrowheads: ectopic expression. n>4 for each. Scale bars: 100 μm. (I) Approximate times (h) of different events during Hd development.

6E, white arrowhead). This HvSoPIN1 expression pattern supports the immunolocalisation observation that SoPIN1 protein levels are not detected in the adaxial surface of Wt, in contrast to what is observed for Hd at later stages of
development. Combined, the mRNA localisation patterns and the protein localisation suggest that BKn3 induces expression of HvSoPIN1 in the Hd lemma.

**BKn3 activates candidate polarity organisers**

It has previously been proposed that tissue cell polarity may be anchored by regions called organisers, which could influence auxin dynamics (Abley et al., 2013). Candidate organisers include the boundary gene NAM (Souer et al., 1996) and the auxin importer LAX1 (Péret et al., 2012). To test whether BKn3 may influence tissue cell polarity through the modulation of organisers, we carried out RNA *in situ* hybridisation (Figure 6) of barley homologues of candidate organiser genes: HvNAM and HvLAX1. RNA *in situ* hybridisation of these components in Wt florets showed that HvNAM and HvLAX1 mRNA were largely excluded from developing organs, including the lemma (Figure 6B-D). By contrast, after 110 h (when BKn3 is expressed in the Hd lemma), ectopic expression of HvNAM and HvLAX1 was detected in the Hd lemma. At 120 h, ectopic HvNAM expression was seen in the adaxial half of the developing lemma, flanking the region where BKn3 mRNA would be expected to be localised (Figure 6F, white arrowheads). By 170 h, ectopic expression of HvNAM had spread throughout the meristem cushion region (Figure 6G, white arrowheads). At this stage, HvLAX1 mRNA was localised in the epidermis of the ectopic meristem (Figure 6H, white arrowheads). This modulation of the expression patterns of the potential organiser components by BKn3 may contribute to the observed reorientation of tissue cell polarity.

The sequence of changes in identity, growth, tissue cell polarity and morphology is summarised in Figure 6I.

**Formation of the wings involves a reorientation of growth in the lemma margin**

In addition to floral meristem activation, ectopic expression of BKn3 also leads to the initiation of wings at around 240 h. To determine how these wings form, we analysed the pattern of cell files in the margin during Hd lemma development (Figure 7). Confocal images of calcofluor stained Hd lemmas (Figure 7A and 7B) showed that at 170 h, when the ectopic meristem had formed but before the wings had emerged, cell files were
largely oriented proximodistally in the lemma margin (Figure 7, magenta cell files). At 350 h, when the wings had formed (Figure 7B), cell files in the centre of the lemma body retained the proximodistal orientation, whereas in the margins where the wings were...
developing, the cell files were oriented towards the tip of the wing (Figure 7, magenta). This suggests that there is a change in growth orientation in the margins of the developing lemma, which leads to the formation of wings.

To evaluate whether the change in growth orientation may reflect a change in tissue cell polarity in the lemma margin, we assessed the orientation of mature hairs on the adaxial surface of the \textit{Hd} lemma using scanning electron microscopy (SEM) (Figure 7C). Below the wing outgrowths in the body of the lemma, hairs pointed distally (Figure 7H). In the middle of the lemma body, below the ectopic flower and above the emergence point of the wings, hairs pointed proximally (Figure 7D, red arrows). Closer to the proximal axil of the wing (the bend point of the tissue, Figure 7F), hairs from the body of the lemma point distally, whereas those from below the ectopic meristem point proximally, and along the edge of the wing they point towards the wing tip, suggesting a flow of margin hair orientation towards the wing tip. Hairs along the wings pointed towards the wing tip (Figure 7E, G, red arrows), indicating that the two polarity fields converged at the tips of the wings. This pattern of hair orientation suggests that during wing development, tissue cell polarity in the lemma margin may become oriented towards the wing tip.

**Interaction between tissue cell polarity and growth could account for wing formation**

The observed cell file and hair orientations suggest that wings may form due to anisotropic growth, possibly oriented by the changes in tissue cell polarity in the lemma margin after the formation of the ectopic meristem. To evaluate this hypothesis, we used the Growing Polarised Tissue (GPT) framework to model the growth of the wings (Kennaway et al., 2011) (Figure 8, Supplemental Figure 1). This framework approximates plant tissues as a connected continuous sheet of material with two surfaces, termed the canvas. Anisotropic growth is oriented by a polarity field that propagates through the canvas, which may correspond to the tissue cell polarity field at the cellular level. Regional factors determine local specified growth rates parallel ($K_{par}$) and perpendicular ($K_{per}$) to the polarity field. The growth pattern is determined by three interacting networks: (1) the gene regulatory network (GRN) which defines the regional
factors in the model, (2) the growth regulatory network (KRN) which defines the specified growth rates, and (3) the polarity regulatory network (PRN) which defines the distribution of a diffusible factor called POLARISER (POL), the gradient of which
determines the polarity field. POL distribution is determined by the location of organiser regions that generate POL (plus organisers) and remove POL (minus organisers).

The model has two phases: (1) set-up, and (2) wing. The set-up phase is the same for all models and initially grows a small semicircular canvas (approximately the size of a lemma primordium at 0 h in our timecourse) to the shape of a lemma at 120 h (Supplemental Figure 1A-C). During this initial stage, the canvas has a proximodistal gradient in specified growth rates and has higher $K_{\text{par}}$ than $K_{\text{per}}$ (determined by the identity factor PGRAD, pink, which declines distally, Supplemental Figure 1A), consistent with higher growth rates in the base of monocot organs (Poethig, 1984, Sylvester et al., 1990, Fiorani and Beemster, 2006, Nelissen et al., 2012) and a proximodistal polarity field (determined by the gradient of POL, green, which is produced at PLUSORG, yellow, and degraded at MINUSORG, red, Supplemental Figure 1B), consistent with SoPIN1 localisation in early $Hd$ and Wt lemmas. If this simulation is run past 120 h, the canvas develops an elongated triangle shape with smooth margins (Supplemental Figure 1D); this shape is similar to that of a wild-type lemma. In this model, polarity is assumed to be proximodistal throughout the lemma, even though the marker of tissue cell polarity we use, SoPIN1 signal, is absent from much of the Wt lemma in later stages. The assumed proximodistal polarity pattern correlates with hair orientations in the mature Wt lemma.

After 120 h, distal BKN3 is added to the canvas. BKN3 promotes specified growth rates (both $K_{\text{par}}$ and $K_{\text{per}}$) simulating the formation of a new meristematic cushion. BKN3 also acts as a new minus organiser, and polarity is reset to orient towards the centre of the canvas surface and the tip, as we observe a reorientation of SoPIN1 localisation towards the centre of the lemma's adaxial surface soon after ectopic $BKn3$ expression. This generates the shape of a $Hd$ lemma at 170 h (Figure 8A-F). After 170 h is reached, the specified growth effect of BKN3 is removed but the polarity effect remains (Figure 8C). This is the starting canvas shape for all of the wing simulations (wing phase).

During the wing phase, hypotheses for wing development in the lemma margins are tested. At the start of the wing phase (170 h), the canvas has a distal region of BKN3
identity (Figure 8A, dark blue). The polarity field is proximodistal with a reorientation
towards the BKN3 region (Figure 8C,I). PGRAD (Figure 8B) promotes both $K_{par}$ and
$K_{per}$, promoting $K_{par}$ more than $K_{per}$ (Figure 8E, H). Clonal sectors in the margin of
canvas at 170 h are elongated proximodistally (Figure 8F-G), similar to the marginal cell
files in Figure 7A.

The wings could form as a result of the inversion of tissue cell polarity induced by BKn3.
To test this hypothesis, inversion of the model polarity field (consistent with the SoPIN1
patterns in later stage $Hd$ lemmas) is introduced by adding a PLUSORG region in the
boundary of BKN3 (Figure 8D, yellow), perhaps corresponding to the activation of
$HvNAM$, and then resetting the polarity. This simulation (Figure 8E) results in small
outgrowths forming in the margins at 240 h, where the polarity field converges (Figure
8J-K). However, the outgrowths remain much smaller than those observed at 350 h in
the confocal lemma images (compare to Figure 3E). These results suggest that a
convergent polarity field may be involved in generating the wing outgrowths, but
additional factors are also needed to account for their size.

One possible explanation for the size of the wings is that specified growth rates are
enhanced in the margin where the polarity field converges, possibly due to the
accumulation of auxin. To evaluate this hypothesis, we produce WING at the same
place as the polarity convergence points at 240 h (this could be a proxy for the
convergence points activating WING, Figure 8L, purple). WING promotes $K_{par}$ but also
inhibits $K_{par}$ where the concentration of WING is more than 65% of its maximum value
(Figure 8M-N; Without this inhibition of growth at the wing tip, the wings become sharp
and triangular; Supplemental Figure 1E). The inhibition of growth at the wing tip could
result from a reduction in the concentration of growth promoting factor. For example, if
WING corresponds to auxin, epidermal auxin at convergence points can be internalised
through the formation of vascular traces. Or, it could be achieved through threshold
responses; for example, different AUX-IAA (AUXIN/INDOLE-3-ACETIC ACID
protein)/ARFs (AUXIN RESPONSE FACTORS) combinations are proposed to respond
to different concentrations of auxin (Villalobos et al., 2012). By 350 h, the shape of the
outgrowths (Figure 8O-P) broadly matches the shape of the $Hd$ lemma wings at 350 h
(Figure 3E). The clonal sectors in the wing region of the canvas also become deformed towards the wing tip (Figure 8Q-R), broadly matching the flow pattern of the cell files observed in the confocal data (Figure 7B). The position and shape of the new PLUSORG in the BKN3 boundary can influence the position and shape of the outgrowths (Supplemental Figure 1F-I). Robustness in wing shape formation may result from activation of an additional factor, such as new MINUSORG at the wing tips possibly due to an accumulation of auxin.

It may be that the promotion of growth in the lemma margin alone can generate wing outgrowths. To evaluate this hypothesis, we do not invert the polarity field (same polarity field as Figure 8C), and we specify the production of WING in the margin (Figure 8S, purple) at 240 h, which is the approximate time when the wing outgrowths are first observed. WING promotes both $K_{\text{par}}$ and $K_{\text{per}}$, but it promotes $K_{\text{per}}$ more than $K_{\text{par}}$ (Figure 8T). This simulation results in the formation of outgrowths (Figure 8V-W) that have clonal sectors that do not seem to flow towards the tip of the outgrowth (compare Figure 8X-Y with Figure 3E and Figure 7B). This suggests that an increase in growth rate in the margin can generate outgrowths but is insufficient alone to account for the orientations of growth observed in wing formation.

The computational modelling suggests that the wing outgrowth in the margin may arise through the convergence of tissue cell polarity fields, combined with enhanced growth in the lemma margin.
DISCUSSION

Class 1 KNOX genes are central to meristem function, and when over-expressed, they have profound effects on morphology. Understanding how these effects are mediated may shed light on KNOX gene functions. We show that ectopic expression of the class 1 KNOX gene, BKn3, in the barley lemma leads to a defined series of temporal and spatial changes in tissue cell polarity (SoPIN1 localisation) and identity (ectopic expression of HvSoPIN1, HvNAM and HvLAX1). These events precede changes in growth (wing formation), regional polarity (inverted ectopic flowers) and oriented cell differentiation (hair development).

Ectopic BKn3 expression is activated between 90 h and 110 h in the distal region of the lemma. By 120 h, SoPIN1 in the lemma is upregulated and relocalises to point centrally, indicating a change in tissue cell polarity. Several hypotheses might account for how BKn3 alters SoPIN1 localisation. BKn3 could initially stimulate the formation of a minus organiser, causing the reorientation of polarity towards it. The possible molecular basis of the organiser would depend on the mechanism for coordinating tissue cell polarity (Abley et al., 2016). For example, if auxin is a component of tissue cell polarity, BKn3 could generate a localised increase in intracellular auxin, for example through enhancing auxin biosynthesis, as suggested by (Bolduc et al., 2012). SoPIN1 could perhaps then relocalise to point centrally if an up-the-gradient model applies (Jönsson et al., 2006, Smith et al., 2006). Alternatively, BKn3 could trigger a localised reduction in extracellular auxin, for example by promoting auxin import or vein formation. The observed upregulation of HvLAX1 expression in the epidermis of the ectopic meristem (Figure 6H) supports the hypothesis that BKn3 can promote local auxin import. SoPIN1 could perhaps then reorient towards the region of low extracellular auxin if the with-the-flux or indirect coupling models apply (Sachs, 1969, Mitchison, 1980, Mitchison et al., 1981, Sachs, 1981, Stoma et al., 2008, Abley et al., 2013). Whatever the mechanism, our results show that KNOX gene activity is intimately connected with the orientation of tissue cell polarity.
After strong BKn3 expression has been established and SoPIN1 has reoriented towards the BKn3 expression region (170 h), SoPIN1 below the ectopic meristem reorients to point proximally. This may be a result of the activation a new plus organiser in the boundary of the BKn3 expression region. For example, the HvNAM boundary gene is initially expressed in the margin of the BKn3 domain (120 h) and later throughout the BKn3 region. This activation of HvNAM could be a direct effect of BKn3, as KNOXs are able to bind upstream of NAM genes (Bolduc et al., 2012). Boundary identity regions themselves may act as plus organisers of SoPIN1 localisation (Abley et al., 2013). Thus, BKn3 is able to modulate tissue cell polarity during organ development, possibly through altering auxin dynamics and/or boundary identities. This may be a common class 1 KNOX function, as other overexpression phenotypes, like the altered vein patterns in maize Kn1 (Ramirez et al., 2009), could suggest changes to underlying tissue cell polarity.

In both Wt and Hd lemmas, the orientation of epidermal hairs correlates with the pattern of SoPIN1 localisation. This suggests that differentiating cells have an inherent polarity based upon patterns of tissue cell polarity established earlier in development. Therefore, by modulating the local patterns of tissue cell polarity in the Hd lemma, BKn3 is able to influence oriented cell differentiation patterns at later stages.

Ectopic BKn3 expression also leads to changes in growth, as illustrated by the development of wings in the lemma margin below the ectopic meristem at 240 h. BKn3 expression extends a short distance below the ectopic flower into the wings but does not occur throughout the wings. Epidermal hair orientations and cell file patterns suggest that tissue cell polarity converges towards the tips of the developing wings. These observations suggest that the wings reflect effects of tissue cell polarity on orienting growth and may be a non-autonomous effect of BKn3 expression. Computational modelling results are consistent with this hypothesis. Models in which a polarity field (i.e. a vector field) orients specified growth, and in which growth rate is enhanced where polarity converges at the margin, lead to the formation of wing-shaped outgrowths. The position of the polarity convergence points may be reinforced by the formation of new minus organisers at the wing tips, possibly induced by the
accumulation of auxin. The increase in growth rate could be a consequence of an accumulation of a growth promoting factor in the margin; this factor would need to be activated outside of the $BKn3$ expression domain, as wing development is a non-cell autonomous effect. Auxin could be a strong candidate for this growth promoting factor, as it is known to promote growth (Teale et al., 2006), and it could accumulate in the margin due to the reversal of the SoPIN1 polarity field. The small stripe of $BKn3$ expression near the axil of the wing may also influence margin growth rates through influencing SoPIN1 localisation, auxin dynamics or other factors involved in growth (Sakamoto et al., 2001, Jasinski et al., 2005, Bolduc et al., 2012). Wing formation bears marked similarities with leaf margin outgrowths, such as serrations and lobes (Barkoulas et al., 2008, Bilsborough et al., 2011), suggesting similar underlying mechanisms may be involved. For example, both serrations and lobes involve PIN1 convergence points in the leaf margin and changes in margin growth rate and orientation. Overexpression of $KNOX$ genes can also influence lobe phenotype (Chuck et al., 1996), supporting a possible role for the stripe of $BKn3$ expression in modulating wing shape. Perhaps BKn3 near the distal edge of the wing outgrowth is involved in boundary formation, or is involved in modulating growth rate, reminiscent of the function of other homeodomain genes such as $RCO$ ($REDUCED\ COMPLEXITY$) (Vlad et al., 2014). Thus, through modulating tissue cell polarity, BKn3 is able to influence growth orientations and rates, leading to marginal outgrowths. This may explain the formation of marginal leaf flaps in other class 1 $KNOX$ overexpression mutants, such as maize $Kn1$ (Ramirez et al., 2009). In the $Kn1$ mutant, these leaf flaps exhibit a change in regional polarity, as they form in the margin of the blade and have sheath-like identity (Ramirez et al., 2009).

In addition to these effects on tissue cell polarity and growth, BKn3 induces a reversal in regional polarity in the $Hd$ lemma: the first ectopic flower has a distal palea while the second ectopic flower has a proximal palea. Tissue cell polarity does not match regional polarity, as both of these ectopic floral meristems form in the context of an initially proximodistal SoPIN1 polarity field. Thus, in this case, regional polarity may not be a consequence of tissue cell polarity but may instead be established through a separable mechanism. Several hypotheses might account for the inversion of regional polarity
Hypotheses for inverted regional polarity in *Hd*. Diagrams of the adaxial surface of the *Hd* lemma (black) outlining the hypotheses. **(A)** Induction of an inflorescence meristem. The expression of *BKn3* (A, dark blue hatch) results in the formation of an inflorescence meristem (pink), which then initiates floral meristems (orange) on its flanks, the palea (green) are initiated on the side of the floral meristem closest to the centre of the inflorescence meristem. **(B)** Underlying lemma properties. Ectopic *BKn3* expression (dark blue hatch) occurs in the lemma/awn boundary (light blue) and triggers the formation of ectopic floral meristems (orange). Palea (green) develop on the side of the ectopic floral meristem closest to the lemma/awn boundary.
This view is also consistent with the region between the ectopic flowers having a more rachis-like rather than lemma identity (Stebbins and Yagil, 1966). Another hypothesis is that identities within the lemma determine regional polarity (Figure 9B). For example, the ectopic floral meristems (Figure 9B, orange) may be initiated on the flanks of a lemma/awn boundary domain (Figure 9B, light blue), with the palea (Figure 9B, green) forming towards this boundary domain. This hypothesis is supported by the observation that the awnless mutation, lks2, is epistatic to Hd, suggesting that the awn identity region is required for the Hd mutant phenotype (Roig et al., 2004). In practice, it is hard to distinguish between this and the previous hypothesis, as the development of the second floral meristem is variable and it is difficult to assess the expression pattern of BKn3 across the lemma in 3D. According to either of these hypotheses, floral (regional) polarity results from an interaction between identities through signalling that does not depend on tissue cell polarity.

Interactions between identities may also explain the changes in regional polarity observed in the snapdragon (Antirrhinum majus) Hirzina mutant outgrowth, which exhibits a proximal-distal-proximal pattern of epidermal features (Golz et al., 2002). Similarly, alterations to the proximal-distal axis in Kn1 leaves (Ramirez et al., 2009) may reflect changes in identity, as KN1 protein at the base of the leaf is thought to confer proximal identity (Jackson, 2002).

Previous studies have shown that class 1 KNOX genes are essential for meristem identity (Barton and Poethig, 1993, Kerstetter et al., 1997) and have a diverse range of phenotypic effects when overexpressed. These studies indicate that class 1 KNOX genes regulate differentiation and growth (Stebbins and Yagil, 1966, Smith et al., 1992, Sinha et al., 1993, Lincoln et al., 1994, Janssen et al., 1998, Shani et al., 2009). KNOX genes may also have a role in leaf outgrowth, as indicated by the presence KNOX at the base of developing wild-type maize leaves and in the margin of compound leaves (Bharathan et al., 2002, Shani et al., 2009, Hay and Tsiantis, 2006) and by the loss of leaf formation in knox null mutants (Barton and Poethig, 1993, Long et al., 1996, Kerstetter et al., 1997, Vollbrecht et al., 2000). Our analysis of the Hd barley mutant suggests that KNOX genes may influence organ outgrowth through modulation of
regional polarity, tissue cell polarity and growth. Such modulations may also explain other KNOX over-expression phenotypes, such as the formation of knots and spurs. Our analysis of the Hd mutant suggests that these effects may involve changes in tissue cell polarity patterns, which influence oriented cell differentiation and growth patterns, as well as alterations in regional polarity. Thus, class 1 KNOX gene activity likely involves an interplay between identity, regional polarity and tissue cell polarity.
METHODS

Plants

Barley (*Hordeum vulgare* cv. Bowman) seeds (*Hd* BW341) were stratified at 4 °C for 48 h and germinated at room temperature. Five days after coleoptile emergence (dae), the seedlings were planted in P15 trays in John Innes Cereal Mix and grown in standard summer greenhouse conditions; timecourse samples were taken beginning at 17 dae.

Photography

Mature *Wt* and *Hd* inflorescences were photographed using an OLYMPUS XZ XZ-1.

OPT

Inflorescences collected in 100% EtOH were prepared for OPT as described in (Sharpe et al., 2002, Lee et al., 2006). Specimens larger than 1.5 cm were not embedded in agarose. Samples were imaged 400 times on an x/y rotation, either on the Prototype OPT scanner (Lee et al., 2006) (<1 cm), the Commercial Scanner Bioptons 3001 (SkyScan) (<1.5 cm) or the Macro OPT scanner (<4 cm) in BABB (1:2 benzyl alcohol: benzyl benzoate (Sigma-Aldrich). On the prototype scanner, we used white or UV light through the gfp1 filter, and UV light through the TXR filter. On the commercial scanner, we used white light through an infrared filter, and UV light through a GFP1, GFP+ or Cy3 filter. On the Macro scanner, we used white light through a GFP filter, or UV light through a GFP or TXR filter. Images were aligned using NRecon (NRecon Version 1.6.3.3 © SkyScan, 2010). Volviewer (http://cmpdartsvr3.cmp.uea.ac.uk/wiki/BanghamLab/index.php/VolViewer#Description) was used to reconstruct and edit the images.

Timecourse staging

Two timecourses were collected: the first covering 240 h and the second covering 380 h of *Wt* and *Hd* development. Initiation of flowering varied due to fluctuating growth conditions; therefore, to align both timecourses, a time zero morphology state (M0) was defined. To define M0, images in the first timepoint of timecourse 1 were measured
using FIJI (Schindelin et al., 2012) and the average dimensions of the fifth floret from the base of the inflorescence were calculated. The mean +/- 2 standard deviations defined a range of values that described the M0 reference. Comparing the average dimensions of the floret five in the second timecourse allowed both timecourses to be combined. Each timepoint was defined in hours since M0 (h) based on the time of harvest. The natural logarithm (ln) of floret 5 width was used to develop the growth graph, as ln(floret 5 width) did not vary significantly (p = 0.76) between Wt and Hd. The equation of the line of best fit was used to stage all experimental data in hours since M0 (h).

Confocal microscopy of Hd lemmas

Hd inflorescences that had been fixed in 100% EtOH were imaged under brightfield light on a Leica M205C stereomicroscope with a DFC495 camera. Floret width was measured using FIJI (Schindelin et al., 2012), and each floret was allocated to a 20 h timeslot (80<t>100 h, 100<t>20 h, etc.) using the growth curve equation. Each lemma was fixed (adaxial surface up) to a slide, rehydrated and stained in 0.1% calcofluor (fluorescent brightener 28, F3543, Sigma-Aldrich) for 20 mins. Lemmas were imaged using a 25x multi-immersion lens on a Zeiss 780 confocal microscope, (violet laser diode (405 nm) excitation laser, PMT detectors 400-480 nm). Larger images were reconstructed in Adobe Photoshop from multiple tiles. Each figure image is a representative of the typical morphology in the 20 h timeslot, and the time stated is the median of the timeslot.

RNA in situ hybridisation probes

Probes targeted unique sequences in the coding sequence of each gene obtained from the published barley genome (Consortium, 2012): BKn3 (AK376780), HvLAX1 (AK369586), HvNAM (MLOC_65286.1), HvSoPIN1 (MLOC_293.1). Probe templates were amplified from Wt Bowman cDNA using the following primers: BKn3 (F:GCCATCAAGGCAAGATCTCC, R:GGTAGAAGACGAGCGTCTTCTTCATGCC, to give 600 bp), HvLAX1 (F:CTACCTCAGCGTCTCTACGTCG, R:CTAGCCGAACCTGTAGAGCCGCTG,
to give 530 bp), \textit{HvNAM} (F: AGATGGAGCGGTACGGTTCTCTG, R: GCAGGTAGATGTACTTGAACTTGGC, to give 399 bp), \textit{HvSoPIN1} (F: TTCCACTTCATCTCCTCCA, R: CCTCGCCTGCAGGTCCG, to give 413 bp). These products were cloned into pCR®4-TOPO® (Invitrogen Life Technologies TOPO®TA Cloning® kit and Maximum Efficiency One Shot® OmniMAX™2 T1 Phage-Resistant Chemically competent \textit{E. coli}) according to the manufacturer’s guidelines. Plasmids were sequenced using a BigDye® Terminator v3.1 cycle sequencing kit and Eurofins Genomics. RNA probes were made using the protocol from (Coen et al., 1990) with the following modifications: the probe template with the T7 or T3 transcription start site was amplified using PCR and purified using a QIAquick PCR purification kit (Qiagen) followed by phenol-chloroform extraction. 1 µg of purified PCR was used to make the digoxigenin-UTP labelled RNA using T7 or T3 RNA polymerase. All probes were hydrolysed in 200 mM carbonate buffer, pH 10.2 at 60 °C for 1 hour.

RNA \textit{in situ} hybridisation tissue fixation

Barley inflorescences were collected in ice-cold 4 % Paraformaldehyde (PBS pH7, 16 % Paraformaldehyde solution (Electron Microscopy Sciences), 4 % DMSO and 0.1 % Triton-X100), placed under vacuum 3x10 mins, and fixed overnight at 4 °C. Fixed samples were washed in 0.85 % Saline for 30 mins at 4 °C, 50 % EtOH/ 0.85 % Saline for 3 h at 4 °C, and 70 % EtOH/ 0.85 % saline for 3 h at 4 °C and stored at 4 °C in 70 % EtOH/ 0.85 % saline. Samples were transferred to Tissue-Tek® mesh biopsy cassettes and loaded into a Tissue-Tek® TEC VIP vacuum wax infiltrator (Sakura) with the following program: 4 h in 70 % EtOH at 35 °C, 4 h in 80 % EtOH at 35 °C, 4 h in 90 % EtOH at 35 °C, 3 cycles of 4 h in 100 % Xylene at 35 °C, 4 cycles of 4 h in 100 % paraffin wax at 60 °C; all steps were carried out with agitation and under vacuum. Samples were transferred to hot paraffin in a Tissue-Tek® TEC (Sakura) embedding machine and embedded, followed by storage at 4 °C. Blocks were sliced in 8 µm thick ribbons using a Reichet-Jung 2030 microtome at RT. The tissue slices were mounted on Polysine™ microscope slides (VWR, 631-0107) with water, dried on a 37 °C hotplate for 48 h, and stored at 4 °C.
RNA in situ hybridisation protocol

The RNA in situ hybridisation protocol was as described in (Coen et al., 1990) with the following modifications: 80 µl hybridisation buffer and 2-4 µl of RNA probe were used per slide and covered with HybriSlip™ Hybridization Covers (Grace Bio-Labs). Slides were washed with 0.2 % SSC (saline sodium citrate buffer) at 50 °C before washing in NTE (0.5 M NaCl, 10 mM Tris-HCl pH7.5, 1 mM EDTA) at 37 °C and RNase treatment. The samples were washed in NTE buffer, followed by Buffer 1 (100 mM of Tris-HCL, 150 mM of NaCl), at RT before incubating with blocking reagent (Roche) for 1 h. Anti-digoxigenin-AP (Roche) used at 1:3000 dilution in 1 % BSA, 0.3 % Triton-X100, Buffer 1 and incubated for 1.5 h. Subsequent washes were first performed with Buffer 1 with 0.3 % Triton-X100, then once with Buffer 1 only. Localisation of the anti-digoxigenin was visualised by incubating with 0.15 mg ml⁻¹ NBT (Nitro blue tetrazolium, Promega) and 0.075 mg ml⁻¹ BCIP (5-bromo-4-chloro-3-indolyl-phosphate, Promega) in 100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM MgCl₂ at RT overnight.

RNA in situ hybridisation imaging

Slides were imaged in water on a Leica DM600 microscope with a DFC420 digital camera under DIC light. Imaged slides were dried and mounted with VectaMount™ AQ (Aqueous mounting medium, H-5501), Vector Laboratories.

Antibodies

The SoPIN1 primary antibody was provided by Devin O’Connor (The Sainsbury Laboratory, Cambridge University (O’Connor et al., 2014)) and used at a 1:200 dilution. Standard anti-guinea-pig Alexa 488 secondary antibodies from Life Technologies (A11073, Lot: 1235789) were used to detect the SoPIN1 primary antibody at a 1:200 dilution.

Immunolocalisation tissue fixation

Barley inflorescences were fixed in FAA (50 % EtOH, 5% acetic acid, 3.7 % Formaldehyde (Sigma-Aldrich)) with 1 % DMSO and 0.5 % Triton-X100, placed under vacuum for 3 x 10 mins, then fixed overnight at 4 °C. Fixed samples were washed with
50 % EtOH for 3 h at 4 °C, followed by 70 % EtOH for 3 h at 4 °C and stored at 4 °C in 70 % EtOH.

**Whole-mount immunolocalisation**

The whole-mount immunolocalization protocol was adapted from (Conti and Bradley, 2007) with the following modifications: Digestion in 2 % Driselase ® (from Basidiomycetes sp, D9515, Sigma-Aldrich) and 1 % Pectolyase Y-23 (Yakult Pharmaceutical Ind., Co. LTD) for 30 mins at 37 °C and washing in PBS before the citrate boiling step. Permeabilisation was performed with a 2 h incubation in 1 % Triton-X100, 5 % DMSO in PBS before blocking for 1 h in 1 % BSA with 0.3 % Triton-X100. 1:200 dilutions for all antibodies were used. Samples were stained in 0.1 % calcofluor for 40 mins.

**Whole-mount Immunolocalisation Imaging**

Lemmas were imaged using a 25 x dip lens on an SP5 (II) Leica confocal microscope. Calcofluor: violet laser diode (405 nm) excitation laser, PMT detectors 400-480 nm. Alexa-488: argon ion (488 nm) excitation laser, PMT detectors 500-575 nm.

**SEM Imaging**

Cryo SEM images were taken using a Zeiss Supra 55 VP FEG with a Gatan Alto2500 cryo system and were generated by JIC Bio-Imaging Service (Elaine Barclay). Images were of the adaxial surface of mature *Hd* lemmas below the ectopic flower (which was removed).

**Image processing**

Figures were assembled in Adobe Photoshop 2015; the brightness and contrast were altered for better visualisation where appropriate. For large images, tiles were aligned in Adobe Photoshop and merged. The scale bars in every image were standardised to 100 µm, 200 µm, 250 µm or 1 cm where appropriate. Arrows and highlighted cells were added using Adobe Photoshop 2015.
Modelling was performed using the GPT framework (Growth toolbox rev. 4813) (Kennaway et al., 2011) run on MatLab R2013b. The canvas at the start of the wing simulations presented in this paper represents the morphology of the lemma at 170 h (0.66 mm high and 0.5 mm wide with 2786 finite elements; more elements are added to the wing region at 340 h to make 3352) with a fixed base, mimicking attachment to the base of the floret (m.fixedDFmap(id_base_p==1,2)=true, where 2 defines the y-axis). The model takes 20 mins to run from -50 to 380 h on an INTEL ® Core™ i7-2600 CPU processor. The model stops at 350 h when the canvas is 1.7 mm x 0.9 mm. Each step in the simulation represents 2 h.

There are two model phases: set-up (-50-170 h) and wing (170-380 h). The set-up phase generates the starting shape of the canvas for the wing simulations. (The wing simulation phase is focussed on in this paper.) In the set-up phase, the canvas deforms from a semi-circular canvas (0.15 mm x 0.1 mm) with a layer of dividing virtual cells to a shape similar to that of a lemma at 170 h of development. If a random selection of virtual cells is labelled early in the model, the resulting clonal sectors are proximodistally elongated by 170 h. In the wing phase, hypotheses for how wing outgrowths could form in the margin of the canvas based on changes in polarity and growth rates are tested. Virtual clonal sectors in this phase of the model are activated at 170 h, and the resulting sector shapes are observed at 350 h.

The set-up phase defines factors (Table 1) that remain the same throughout the model. There are signal factors, denoted by “s”, which have production (P), diffusion (D) and decay (De) parameters (Table 2), and identity factors denoted by “i”, which have a value of 1, and if defined by a signal factor, they have the same gradient as the signal factor at that point in time. Signal factors diffuse according to the following equation:

$$\frac{\partial S_x}{\partial t} = D_x \nabla^2 S_x - De_x S_x$$

, where x denotes the specific signal factor (S). The gradient of diffusible factors is frozen using the following function:

$$m.morphogenclamp (((x ==1)| (y ==1)), s ) = 1,$$
where $x$ and $y$ define regions of the canvas and $s$ is the signal factor. The diffusion and
decay parameters of signal factors are set to 0 after a period of time to maintain the
gradient pattern.

In this framework, a polarity field is used to define the orientations of growth, $K$ (growth
can be defined parallel, $K_{par}$, perpendicular, $K_{per}$, or normal, $K_{nor}$ [thickness], to the
polarity field). This polarity field is based upon the local concentration of a signal factor,
POLARISER (POL). POL is produced at sources, defined by iPLUSORG, and degraded
at sinks, defined by iMINUSORG. This forms the basis of the polarity regulatory network
(PRN). During the set-up phase, iPLUSORG is at the base of the canvas and
iMINUSORG is at the distal tip of the canvas. During the wing phase, the POL gradient
is reset when $sBKN3$ is added as a sink and when iBOUNDARY is introduced as an
additional iPLUSORG region to the PRN.

Growth rates are influenced by the factors in model. Promotion ($pro$) and inhibition ($inh$)
of growth are defined using the following functions:

$$pro(n, x) = 1 + nx \quad \text{and} \quad inh(n, x) = \frac{1}{1 + nx}$$

where $x$ is the factor influencing growth and $n$ is the constant that defines how much
the factor promotes or inhibits growth rates.

During the set-up phase growth is defined with the following equations:

$$K_{par} = b \cdot pro(n, S_{GRAD}) \cdot inh(n, i_{MINUSORG}) \cdot pro(n, S_{BKN3} > 0.2)$$

$$K_{per} = b \cdot pro(n, S_{BKN3} > 0.2) \cdot pro(n, S_{GRAD})$$

$$K_{nor} = b$$

During the wing phase of the most complex model (outlined in Figure 8 N-R), growth is
defined with the following equations:

$$K_{par} = b \cdot pro(n, S_{GRAD}) \cdot inh(n, i_{MINUSORG}) \cdot pro(n, S_{WING}) \cdot inh(n, S_{WING} > 0.65)$$

$$K_{per} = b \cdot pro(n, S_{GRAD})$$

$$K_{nor} = b$$

The values of all of the parameters at different times in the model are outlined in Table
3.
Accession Numbers

Sequences used to design the RNA *in situ* hybridisation probes can be obtained from the published barley genome sequence (Consortium, 2012): *BKn3* (AK376780), *HvLAX1* (AK369586), *HvNAM* (MLOC_65286.1), *HvSoPIN1* (MLOC_293.1).

The seeds used in this work were Bowman barley wild-type seeds and Bowman *Hooded* mutant (*Hd BW341*) obtained from Arnis Druka, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, Scotland UK.

SUPPLEMENTAL DATA

Supplemental Figure 1. Set-up phase of the model and the effect of modulating different features.

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We thank Catherine Taylor for plant care, Karen Lee for help with OPT imaging and processing, Grant Calder for help with confocal microscopy, Elaine Barclay for Cryo SEM imaging, Richard Kennaway for help with computational modelling, Desmond Bradley for extensive help with methods and useful discussion, Chris Whitewoods, Catherine Mansfield for useful discussions on the manuscript. We also thank Sarah Hake and Devin O’Connor for the maize SoPIN1 antibody and for useful discussions. We also thank Arnis Druka at the James Hutton Institute for the Bowman barley seeds. This work was supported by the John Innes Centre Rotation PhD program funded by BBSRC and the John Innes Society (A.R.). This work was also supported by BBSRC grants BB/M023117/1 and BB/F005997/1.

AUTHOR CONTRIBUTIONS

E.C., A.B.R. and A.R. designed the research. A.R. generated and analysed the data and built the computational models. A.B.R. and A.R. worked on method development
and cloning. E.C. and A.R. wrote the article, A.B.R. edited the article. E.C. provided funding.

**TABLES**

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**Table 1: Identity (i) and signalling (s) factors used in the model.** Outlines of the name and category of each factor, when it is activated, the canvas position, and its role in the model are provided.

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Table 2. Parameters for signalling factors. This table outlines all of the parameters relevant for the signal factors in the model, including diffusion rate (D), decay rate (De), and production (P, maximum value).

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Table 3. Parameters for the growth regulatory network (KRN) at different times in the model.

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FIGURE LEGENDS

Figure 1. The Hd mutant exhibits polarity reversals. (A) Photograph of Wt barley (Bowman, 2-row) inflorescence, illustrating the arrangement of florets (Fl) along the rachis (Ra). Clusters of 3 spikelets are initiated in a distichous pattern along the rachis.
(only the central spikelet produces a mature floret). Each floret has an outer protective organ, the lemma, with a distal awn (Aw). Floral diagrams of Wt (B-D) and Hd (F-H). (B) Adaxial Wt morphology: the lemma and palea are visible, hairs on the lemma point distally (hair orientation, red arrows). (C) Longitudinal cross-section through a Wt spikelet: the floret has a dorsal lemma and ventral palea. (D) Transverse cross-section illustrating the organ whorls in the Wt floret. (E) The Hd inflorescence phenotype resembles Wt, although the lemma develops ectopic flowers (EF) instead of an awn. (F) One or more flowers can form on the Hd lemma’s adaxial surface. Analyses of hair and organ orientations (Harlan, 1931, Bonnett, 1938, Stebbins and Yagil, 1966, Muller et al., 1995, Williams-Carrier et al., 1997) have shown that the first ectopic flower (1) is inverted, pointing towards the lemma base, whereas the second ectopic flower (2) points towards the lemma tip. The Hd lemma develops wing-like (Wi) margin outgrowths below the first ectopic flower. (G) Longitudinal cross-section through an Hd spikelet. (H) Floral diagram of each Hd flower, showing that each ectopic flower retains the same whorled arrangement of organs. Each ectopic flower is thought to use the existing lemma as its own (dashed black line). Proximal-distal axis, Pr-Di. Dorsal-ventral axis, Do-Ve. Scale bars: 1 cm.

Figure 2. The Hd phenotype emerges early in development. Optical Projection Tomography (OPT) of fixed timecourse samples of Wt (A, D-I) and Hd (B, J-O) Bowman barley inflorescences. (A-B) Examples of inflorescence morphology at 0 h and 340 h: yellow boxes indicate the position of floret 5. The natural logarithm (ln) of floret 5 width (yellow line in the inset in C) was used to generate a combined log-linear growth curve (C) for Wt (dark blue) and Hd (red) (n = 2-4 for each timepoint). The equation of the line of best fit (y = 0.0046x + 4.9305) ($R^2$ value of 0.8482) was used to stage all further experimental data. All times are hours since the initial morphology stage (0 h, illustrated in A and B). (D-O) Lateral cross-sections through the centre of floret 5 (D,F,H, J, L,N) and images of the abaxial surface of the lemma (E,G,I,K,M,O) for different timepoints identified key morphology changes in the Hd lemma (J-O), versus Wt (D-I). At 0 h (D-E and J-K) Hd and Wt shared the same morphology: by 170 h (F-G and L-M), the ectopic meristem started to form on the adaxial surface of the Hd lemma (yellow box, white
arrowhead), whilst the Wt lemma elongated to form the awn (F-I). By 240 h, the wing outgrowths started to form in the margins below the ectopic flower in the *Hd* lemma (N-O). Zoomed-in images (yellow boxes) of the ectopic meristem and wings in *Hd* and the corresponding positions in Wt are shown. Floral meristem (FM), lemma (Le), stamen (St), carpel (Ca), palea (Pa), wings (Wi) and awns (Aw). White dotted lines: shape of the lemma. White arrowheads: position of the ectopic meristem and wings. Scale bars: 500 μm A-B, 200 μm D-O.

**Figure 3. Ectopic flowers emerge on the adaxial surface of the *Hd* lemma.**
Confocal microscope images of the adaxial surface of *Hd* lemmas (cell walls highlighted by calcofluor staining) illustrating ectopic meristem and wing development. The lemma initially had a smooth surface and triangular shape (A, 130 h). At 170 h (B), the first ectopic meristem (EM) had started to develop. By 190 h (C), organ primordia were initiated, first forming a distal palea (Pa). If a second EM formed (D,2), the palea was proximally positioned (D, 2, Pa). By 250 h (D), the wings (Wi) started to develop from the margins below the first EM. The wings formed distinct rounded outgrowths by 350 h (E). n>4 for each timepoint. Scale bars: 100 μm.

**Figure 4. In Hooded, ectopic *BKn3* expression is activated around 110 h, is strong by 170 h, and extends beyond the ectopic meristem region by 250 h.**
*BKn3* mRNA *in situ* hybridisation of longitudinal midsections through Wt florets (A-D) and *Hd* florets (E-G) (zoomed-in lemma images, black boxes) at 90 h (A, E), 110 h (B, F), 170 h (C,G), and 200 h (D, Wt only). At 90 h (A, E), *BKn3* mRNA (dark precipitate) was localised to the base of the flower (white arrowhead) and excluded from developing organs, including the lemma, in both Wt and *Hd*. Throughout Wt development, this pattern is maintained, with *BKn3* mRNA excluded from the lemma (B-D). In *Hd* at 110 h (F) there was faint *BKn3* mRNA localisation in the middle of the lemma on the adaxial side (zoomed in image, white arrowhead). By 170 h in *Hd* (G), there was strong *BKn3* mRNA localisation in the adaxial half of the lemma (zoomed in image, white arrowhead), where the ectopic meristem formed. (H) Diagram illustrating mature Wt morphology; the yellow box illustrates the approximate position of the sections in (J). In *Hd*, wings start to develop by 250 h (I). Confocal image of a calcofluor stained *Hd*
lemma (I) at a similar developmental time as the sectioned lemma in (K-L); the yellow box indicates the approximate position of the sections. (J-L) Transverse sections through a 250 h lemma for Wt (J) and Hd (K-L). (J) In Wt, BKn3 was not expressed in the lemma. (K-L) Transverse sections through the Hd lemma. (K) 3D reconstruction of calcofluor stained slices, viewed from the top, showing the curve of the wing. (L) In situ hybridisation of transverse slices throughout the wing region indicated that BKn3 was expressed adaxially in two proximodistal stripes on either side of the lemma midline (the position of each slice within the 3D reconstructed region is indicated in μm). (M) Diagram depicting the predicted BKn3 expression pattern on the adaxial surface of the lemma (BKn3 expression, purple, lemma outline, black) in relation to the position of the wings (black arrows). Stamen (St), carpel (Ca), palea (Pa), abaxial (Ab), adaxial (Ad). Dotted white lines: lemma outline. n>4 for each timepoint in A-G, n= 3 J-L. Scale bars: 100 μm.

**Figure 5. SoPIN1 localisation reorients when ectopic BKn3 expression is activated.** 3D projections of whole-mount immunolocalisation of SoPIN1 (green) and calcofluor stained cell walls (magenta) in whole Wt lemmas at 170 h (A-D) and Hd lemmas at 90 h (E-H), 120 h (I-L), and 180 h (M-R). Orientation of SoPIN1 is based on cellular SoPIN1 localisation in the epidermal layer only. Each panel illustrates SoPIN1 localisation alone (A,E,I,M), both SoPIN1 and calcofluor combined (B,F,J,N), and a zoomed-in image of the boxed regions (C,G,K,O,P,Q), alongside a cartoon illustrating the inferred relationship between the ectopic meristem (orange) and SoPIN1 localisation (red arrows) (D,H,L,R). Overlapping SoPIN1 and calcofluor signals appear white. In Wt lemmas, SoPIN1 pointed distally towards the lemma tip throughout development (A-D), although SoPIN1 signal in the adaxial surface was lost over developmental time. In Hd lemmas, SoPIN1 signal was not lost over developmental time (E-R). Before BKn3 was ectopically expressed in the Hd lemma, SoPIN1 was localised distally, towards the tip of the developing lemma (E-G, white arrows, H, red arrows). Around the time that ectopic BKn3 expression was activated in the lemma (110 h), SoPIN1 localisation reoriented (with respect to earlier stages in development) in the adaxial surface. Initially, SoPIN1 oriented laterally towards the centre of the adaxial surface (I-K, white arrows, L, red arrows). After the ectopic meristem had formed (M-R, 180 h), near the distal tip, cellular
SoPIN1 was oriented distally towards the tip of the lemma (O), below the ectopic meristem SoPIN1 pointed towards the centre of the adaxial surface (P), and below this SoPIN1 was oriented proximally (Q, white arrows, R, red arrows). n=4 for each timepoint. Scale bars: 100 μm.

**Figure 6. Ectopic expression of BKn3 leads to the expression of HvSoPIN1, HvNAM, and HvLAX1.** RNA in situ hybridisation of HvSoPIN1 (A,E), HvNAM (B-C,F-G), and HvLAX1 (D,H). In Wt, HvSoPIN1, HvNAM, and HvLAX1 were excluded from the lemma, except in some vascular regions (A-D). At 120 h, HvNAM was expressed flanking the region of BKn3 expression (F, white arrowheads). By 170 h, when BKn3 expression was high, all three genes (HvSoPIN1, HvNAM, HvLAX1) were strongly ectopically expressed in the adaxial half of the Hd lemma (E, G-H, white arrowheads). Black box: zoomed-in image of the lemma. White dotted line: lemma outline. White arrowheads: ectopic expression. n>4 for each. Scale bars: 100 μm. (I) Approximate times (h) of different events during Hd development.

**Figure 7. Reorientation of growth and tissue cell polarity in the margins of the Hd lemma.** Confocal images of the adaxial surface of the Hd lemma, stained with calcofluor (A-B). Zoomed-in images of the region where wings form (yellow boxes) are also shown. Before wing formation (A), cell files (magenta) in the margin of the Hd lemma were oriented proximodistally. After wing formation was initiated (B), the cell files in the margins became aligned with the outgrowing tip of the wing (n = 6). SEM images of mature Hd wings (zoomed-in images of the boxed regions in C shown in D-H) show the orientation of hairs on the adaxial surface of the Hd lemma. Below the ectopic flower (removed), the hairs pointed basally (D, red arrows). There is also a ridge where different cell patterns and hairs seem to collide; this may correspond to the convergence of two different polarity fields, as in the lemma body (H), the hairs pointed distally. In the region of the wing outgrowth, the hairs pointed towards the wing tip (E, G). In the proximal axil of the wing (F), the hairs in the main lemma body orient distally, and those above in the wing orient proximally; near the node, the hairs appear to flow towards the wing. (n = 2) Scale bars: 100 μm.
Figure 8. Changes in growth and polarity can account for the formation of wings. GPT framework models exploring wing development hypotheses. Initially at 170 h, there is a distal region of BKN3 identity (dark blue) (A) and a proximodistal gradient of the identity factor PGRAD (B, pink). To start with, there is a proximodistal polarity field, which converges towards BKN3 (C, small black arrows), defined by the local gradient of POL (green). POL is produced at PLUSORG (yellow) and degraded at MINUSORG (red) and BKN3 (PRN in I). A reversal in polarity is introduced at 170 h (by adding PLUSORG to the lower boundary of BKN3 (D)). Initially, growth is promoted by PGRAD (KRN in H, growth rate in E). If a layer of virtual dividing cells is added to the surface of the canvas at 0 h and a random selection of cells are shocked, clonal sectors by 170 h are proximodistally elongated (F-G). When this model is run to later stages, it forms small outgrowths in the margins (240 h in J) where the polarity fields converge (K, black arrows); however, these outgrowths remain small in size. To enhance the size of the outgrowths, both the inversion in polarity and a change in growth rate pattern can be combined (L-R). A diffusible growth factor, WING (L, purple) is produced at the wing tips at 240 h. WING promotes $K_{par}$ where the concentration of WING is less than 65% (M-N). This model generates larger outgrowths by 350 h (O-P). Clonal sectors induced at 170 h in the margin of the canvas become deformed towards the wing tip by 350 h (Q-R). Alternatively, enhanced growth rates in the margins could generate the outgrowths (S-Y). This model has the same set up (A-B), initial KRN (H), PRN (I) and proximodistal polarity field (C) as the previous model, but no inversion of polarity occurs (the polarity and growth rate patterns for this model at 170 h are summarized in U). WING is promoted in the margins at 240 h (S) and WING promotes $K_{per}$ more than $K_{par}$ (T). By 350 h, outgrowths form in the margins where WING is promoting growth (V-W). Clonal sectors induced at 170 h, are mostly proximodistal and do not deform towards the wing tip by 350 h (X-Y). Black arrows illustrate the orientation of the polarity field defined by the local concentration of POL. Scale bars: 100 μm.

Figure 9. Hypotheses for inverted regional polarity in Hd. Diagrams of the adaxial surface of the Hd lemma (black) outlining the hypotheses. (A) Induction of an inflorescence meristem. The expression of BKn3 (A, dark blue hatch) results in the
formation of an inflorescence meristem (pink), which then initiates floral meristems
(orange) on its flanks; the palea (green) are initiated on the side of the floral meristem
closest to the centre of the inflorescence meristem. (B) Underlying lemma properties.
Ectopic BKn3 expression (dark blue hatch) occurs in the lemma/awn boundary (light
blue) and triggers the formation of ectopic floral meristems (orange). Palea (green)
develop on the side of the ectopic floral meristem closest to the lemma/awn boundary.


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### Ectopic KNOX Expression Affects Plant Development by Altering Tissue Cell Polarity and Identity

Annis Elizabeth Richardson, Alexandra B Rebocho and Enrico S. Coen

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