HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2016 October 23.

Published in final edited form as:

Science. 2015 October 23; 350(6259): 426–430. doi:10.1126/science.aad1171.

Transcriptional control of tissue formation throughout plant root development

Miguel A. Moreno-Risueno[1], **Rosangela Sozzani**[2,*], **Galip Gürkan Yardımcı**[2,†], **Jalean J. Petricka**[2,‡], **Teva Vernoux**[3], **Ikram Blilou**[4], **Jose Alonso**[5], **Cara M. Winter**[2], **Uwe Ohler**[6], **Ben Scheres**[4], and **Philip N. Benfey**[2,§]

[1]Department of Biotechnology, Center for Plant Genomics and Biotechnology, Universidad Politecnica de Madrid, 28223 Pozuelo de Alarcón (Madrid), Spain. [2]Department of Biology and Howard Hughes Medical Institute, Duke University, Durham, NC 27708, USA. [3]Laboratoire de Reproduction et Développement des Plantes, CNRS, INRA, ENS Lyon, UCBL, Université de Lyon, 69364 Lyon, France. [4]Department of Plant Biology, Wageningen University Research, Wageningen, Netherlands. [5]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA. [6]Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, 13125 Berlin, Germany.

Abstract

Tissue patterns are dynamically maintained. Continuous formation of plant tissues during postembryonic growth requires asymmetric divisions and the specification of cell lineages. We show that the transcription factors, the BIRDs and SCARECROW, regulate lineage identity, positional signals, patterning, and formative divisions throughout *Arabidopsis* root growth. These transcription factors are postembryonic determinants of the ground tissue stem cells and their lineage. Upon further activation by the positional signal SHORT-ROOT (a mobile transcription factor), they direct asymmetric cell divisions and patterning of cell types. The BIRDs and SCARECROW with SHORT-ROOT organize tissue patterns at all formative steps during growth, ensuring developmental plasticity.

Organs are formed, patterned, and maintained during growth. In the root of *Arabidopsis*, tissues are organized as concentric cylinders around the internal vascular tissue. Much progress has been made in identifying factors responsible for patterning some of these tissues, such as the ground tissue. The ground tissue lineage is continuously generated by the

[§]Corresponding author. philip.benfey@duke.edu.
[*]Present address: Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA.
[†]Present address: Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA.
[‡]Present address: Carleton College, Northfield, MN 55057, USA.

The authors declare that they have no competing interests.

SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/vol/issue/page/suppl/DC1
Materials and Methods
Figs. S1 to S7
Table S1 to S6
References (*16*–*30*)

cortex endodermis initial stem cell (CEI), which divides in the transverse orientation (anticlinal division) to produce a daughter cell (CEID) and regenerate itself. The ground tissue is patterned when the CEID divides asymmetrically in the longitudinal (periclinal) orientation, generating two cell types: endodermis and cortex (1). The mobile transcription factor SHORT-ROOT (SHR) moves from the stele to the ground tissue, where SCARECROW (SCR) and the C_2H_2 transcription factor JACKDAW (JKD) sequester it in the nucleus. Nuclear SHR is required for the periclinal asymmetric divisions of the CEID that pattern the ground tissue (2, 3). These divisions are activated through a bistable switch involving SHR, SCR, and other components and correlate with the temporal activation of transcriptional programs (4, 5). Absence of SHR results in abnormal ground tissue patterning, with loss of the endodermis and a remaining single layer of ground tissue due to absence of asymmetric cell divisions (5, 6). Because the ground tissue lineage remains, this indicates that other factors participate in its specification.

Specific roles for JKD and several close relatives have been recently identified (7). JKD and BALD-IBIS regulate SHR movement by promoting its nuclear retention, and cooperatively with MAGPIE (MGP) and NUTCRACKER (NUC) are required for the formative divisions that pattern the ground tissue into cortex and endodermis. Here we show that JKD, MGP, and NUC, along with two new members of this family (collectively known as the BIRDs; table S1) named BLUEJAY (BLJ) and IMPERIAL EAGLE (IME), organize the ground tissue after embryogenesis. They function as identity determinants of the CEI, which maintains and gives rise to the lineage, and act as effectors of asymmetric cell divisions of the CEID upon SHR activation. Furthermore, the BIRDs regulate the transcriptional identity of the two ground tissue cell types and form a regulatory network associated with lineage determination, asymmetric division, cell-type specification, and differentiation.

To explore the role of the BIRDs in ground tissue specification, we used expression from the J0571 enhancer trap line as a robust marker for ground tissue identity (Fig. 1A). This is exemplified by its expression in the mutant layer of *shr* (Fig. 1B). However, its expression was lost in some cells of double- and triple-mutant combinations of BIRDs, and was almost undetectable in the quadruple mutant, *blj jkd mgp nuc* (Fig. 1, D to G), suggesting that these transcription factors play a role in ground tissue identity. We next introgressed combinations of the BIRD mutants into *scr*. In both *jkd scr* and *blj jkd scr*, ground tissue marker expression was reduced, and a number of *blj jkd scr* roots lacked the entire ground tissue (Fig. 1H). These results indicate that the BIRDs and SCR are required for maintenance of ground tissue identity in addition to their established role in patterning divisions and endodermis specification (1, 7).

The mRNAs of *JKD, MGP*, and *NUC* are primarily expressed in the ground tissue (6, 7). To accurately determine where the encoded proteins and BLJ and IME accumulate, we tagged the proteins with green fluorescent protein (GFP) (8). BLJ was specifically expressed in the ground tissue, more highly in the shootward part of the meristem, although it was occasionally detected in ground tissue stem cells. Similarly, IME was also expressed more strongly in the shootward part of the meristem. By contrast, JKD, MGP, and NUC expression was higher toward the root tip (fig. S1). Previously, expression of the *BIRDs* and *SCR* appeared to require SHR (5, 6). However, when we introgressed the expression

constructs into *shr*, we could detect all of the BIRD fusion proteins, although BLJ expression was reduced in this background (Fig 1, I to L). SCR is also detectable in the ground tissue of *shr* (9). Taken together, our results indicate that the BIRDs and SCR can be regulated in a manner that is both dependent and independent of SHR, which is consistent with the difference in the phenotypes of *blj jkd scr* and *shr*.

The ground tissue lineage is initially specified in the embryo (10). Inspection of *blj jkd scr* embryos revealed that they developed ground tissue during embryogenesis (Fig. 2A). In seedling roots, we found that the ground tissue was present in mature zones but frequently did not continue to the meristem (fig. S2, A to L). Loss of the ground tissue was detected after germination but became more severe over time (fig. S2M). When the ground tissue disappeared, the epidermis became directly juxtaposed with the stele, as shown by tissue-specific markers (Fig 2, B to E). These results indicate that the combined activity of the BIRDs and SCR is crucial to maintain the ground tissue lineage postembryonically.

The ground tissue marker J0571 was also lost in cells that were apparently formed by division of ground tissue cells (Fig. 1, D to G) and thus, the critical role of BLJ, JKD, and SCR in maintaining the ground tissue appears to be more than maintenance of the division potential of the CEI. Consistent with this hypothesis, other mutations affecting stem cell niche activity or the orientation plane of niche asymmetric cell divisions do not result in loss of cell lineages (11, 12). To determine if the loss of ground tissue was due to incorrect specification of the CEI, we regenerated roots from wild-type, *scr*, and *blj jkd scr* mutants after resection of the root tip (fig. S3, A to C). In *blj jkd scr*, there was a low regeneration frequency (fig. S3D), but in those meristems that did regenerate, we found severely impaired regeneration of ground tissue with, at most, one or two cells after 2 days (Fig. 2, F and G). These cells failed to establish a new ground tissue lineage and, although a small amount of division occurred, expression of the ground tissue marker was normally lost (Fig. 2, H to J). *scr* mutants also showed impaired regeneration of the ground tissue lineage and failed to complete an entire layer (fig. S4). Our results indicate that BLJ, JKD, and SCR maintain CEI stem cells and their progeny postembryonically through specification of CEI identity.

The BIRDs and SCR are involved in regulation of transcription associated with formative divisions of the CEID and ground tissue cells (2, 6, 7). To further investigate the dual role of the BIRDs and SCR in generating and patterning the ground tissue, we reconstructed a gene regulatory network. Among the BIRDs, we focused on BLJ and JKD because they have a specific role in ground tissue establishment and identified their direct targets through chromatin immunoprecipitation (ChIP) followed by sequencing (table S2). To reconstruct the network, we used transcriptionally regulated targets that were identified by the intersection of ChIP-bound genes with previous genome-wide expression data (for SHR), as well as with new microarray data that we generated from *blj jkd scr* root meristems and from ground tissue sorted cells of *shr* mutants in which we amplified BIRDs and SCR expression using the J0571xUAS system [tables S3 and S4; a detailed description of network reconstruction is in (13)]. The resulting network (table S5) showed pronounced overlaps between the genes regulated by more than one transcription factor (Fig. 3A and fig. S5). A more detailed analysis of the network showed that in addition to directly regulating genes (~55%) in the SHR pathway (table S6), the BIRDs and SCR also directly regulate genes in

other pathways, suggesting that genes not in the SHR pathway are likely to be involved in maintaining ground tissue identity and may be activated through a transcriptional cascade (Fig. 3B).

To better understand how the BIRDs and SCR regulate ground tissue identity, we analyzed their ability to "rescue" ground tissue gene expression in the *shr* background. As these factors are down-regulated in *shr*, we amplified their expression using the J0571xUAS-driven lines in *shr* (see Methods). We then compared mRNA expression profiles of sorted ground tissue cells from these lines with the expression profile of wild-type ground tissue using principal component analysis (PCA) and hierarchical clustering. As an initial test, we asked if PCA could distinguish between the ground tissue and other root tissues as profiled in the RootMap (14) and found clear separation (fig. S6, A and B). This provided confidence to use PCA to infer the identity of the transcriptome when each of the BIRDs was expressed in the *shr* ground tissue. Consistently, PCA localized the BIRDs' ground tissue transcriptomes between *shr* and wild type (Fig. 3C). This was also true for SCR, indicating that a degree of rescue had occurred. In agreement with the PCA, hierarchical clustering separated the ground tissue transcriptome from other root tissues and showed different levels of rescue provided by the BIRDs and SCR (Fig 3D and fig. S6, C and D). JKD and SCR were able to rescue the ground tissue transcriptome more effectively than did BLJ. This suggests that BLJ's role may be to regulate a set of genes that are complementary to those regulated by JKD and SCR, leading to a combinatorial action responsible for the phenotype of *blj jkd scr*. The contrasting expression patterns of BLJ and JKD are consistent with complementary activities. In addition, there was greater overlap among genes regulated by SCR and the BIRDs that showed a higher level of rescue of the ground tissue transcriptome than with the BIRDs that showed a lower level of rescue (Fig. 3E).

Our network indicated that SCR, BLJ, JKD, MGP, and NUC are direct targets of SHR, whereas IME is regulated through an intermediate transcription factor. SCR, NUC, and MGP are activated upon SHR induction (5). To better understand the role of BLJ, JKD, and IME downstream of SHR, we performed a real-time polymerase chain reaction analysis of gene expression after SHR induction (Fig. 4A). We found that mRNA levels of all of them peaked at 6 hours, coincident with the time of onset of asymmetric divisions patterning the ground tissue. A large proportion of the genes down-regulated in *shr* are activated by the BIRDs and SCR (Fig 4B), and some of these had been previously identified to be activated at the time of the asymmetric divisions (fig. S7A). This indicates that SHR activates the BIRDs and SCR to induce gene expression associated with patterning. It is, therefore, possible that activation of downstream targets could be dependent on the level of SCR and the BIRDs, with SHR being the amplifying signal required for patterning. Inspection of the ground tissue in the J0571xUAS lines showed formative divisions (fig. S7, B to H) with high penetrance when BLJ was expressed in the *shr* background (80% of roots, $n = 20$), whereas other BIRDs and SCR could induce these divisions only with substantially lower penetrance (IME: 50%; others: 10%; $n = 20$).

As suggested by our network, we next asked if BLJ, JKD, and SCR are required for SHR function in the ground tissue. For this purpose, we fused SHR to a nuclear localization signal and expressed it directly in the ground tissue under a two-component system driven by the

En7 promoter, which is specific to the endodermis and CEI (fig. S7, I to L). When expressed in a wild-type background, SHR generated additional ground tissue layers, as previously described (7, 9). However, when expressed in combinations of mutants involving *blj jkd* and *scr*, SHR failed to rescue the formative cell divisions within the ground tissue. These results indicate that BLJ, JKD, and SCR are essential for SHR to carry out ground tissue patterning. Furthermore, analysis of the contribution of the BIRDs and SCR to generate specific gene expression patterns showed that these transcription factors were able to activate expression of endodermis and cortex genes (Fig. 4C). Staining for endodermis-specific attributes (the Casparian strip) in the *shr* J0571xUAS lines showed that BLJ (Fig. 4, D to F), along with the other BIRDs and SCR (fig. S7, M to Q), could induce Casparian strip formation subsequent to periclinal divisions of the ground tissue. Expression of cortex-specific markers required at least JKD, MGP, and NUC (Fig. 4, G and H). SCZ, which is required for expression of some cortex-specific markers (15), is also a target in the network. Our analysis suggests that cortex identity requires multiple inputs from the BIRDs. Therefore, the BIRDs and SCR, in addition to mediating SHR transcriptional competence (7), are endogenous effectors of ground tissue patterning and can provide all the necessary information for the asymmetric divisions that are activated by SHR to pattern the ground tissue.

Cell fate choices in all multicellular organisms are governed by transcription factors. Their combinatorial expression and interactions are key to tissue identity. The BIRDs and SCR play critical roles in maintaining ground tissue identity in postembryonic roots by specifying the CEI stem cells that generate the ground tissue lineage (Fig. 4I). In addition, they are effectors of asymmetric divisions that pattern the progeny of the CEIs (Fig. 4J). The continuous control of multiple steps of tissue formation by the same set of transcription factors, independently of and dependent on positional cues, is a sophisticated mechanism ensuring plasticity in the regulation of cell fate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by grants from the NIH (R01-GM043778) and the Gordon and Betty Moore Foundation (GBMF3405) to P.N.B.; from the NSF (IOS-1021619-002) to U.O. and P.N.B.; and from MINECO, FEDER/EFDR (BFU2013-41160-P), and FP7 (PCIG11-GA-2012-322082) to M.A.M-R. M.A.M-R is supported by the Ramon y Cajal program (MINECO), C.M.W. by a NRSA F32 GM106690-01 fellowship, I.B. by a NWO VIDI grant, and B.S. by an ERC Advanced Grant SysArc. J.J.P. was supported by a NIH Ruth L. Kirschstein NRSA F32 GM086976 fellowship. We thank D. R McClay, M. Pernas, and Y. Long for critical reading of the manuscript. Additional data are in the supplementary materials. The National Center for Biotechnology Information Gene Expression Omnibus accession numbers are GSE60157 (microarrays) and GSE60011 (ChIP-Seq).

REFERENCES AND NOTES

1. Petricka JJ, Van Norman JM, Benfey PN. Cold Spring Harb. Perspect. Biol. 2009; 1:a000497. [PubMed: 20066115]
2. Welch D, et al. Genes Dev. 2007; 21:2196–2204. [PubMed: 17785527]
3. Cui H, et al. Science. 2007; 316:421–425. [PubMed: 17446396]
4. Cruz-Ramírez A, et al. Cell. 2012; 150:1002–1015. [PubMed: 22921914]
5. Sozzani R, et al. Nature. 2010; 466:128–132. [PubMed: 20596025]

6. Levesque MP, et al. PLOS Biol. 2006; 4:e143. [PubMed: 16640459]
7. Long Y, et al. Plant Cell. 2015; 27:1185–1199. [PubMed: 25829440]
8. Zhou R, Benavente LM, Stepanova AN, Alonso JM. Plant J. 2011; 66:712–723. [PubMed: 21294796]
9. Helariutta Y, et al. Cell. 2000; 101:555–567. [PubMed: 10850497]
10. Pernas M, Ryan E, Dolan L. Curr. Biol. 2010; 20:818–823. [PubMed: 20417101]
11. Aida M, et al. Cell. 2004; 119:109–120. [PubMed: 15454085]
12. Willemsen V, et al. Dev. Cell. 2008; 15:913–922. [PubMed: 19081078]
13.
Materials and methods are available as supplementary materials on Science Online.
14. Brady SM, et al. Science. 2007; 318:801–806. [PubMed: 17975066]
15. ten Hove CA, et al. Curr. Biol. 2010; 20:452–457. [PubMed: 20171102]

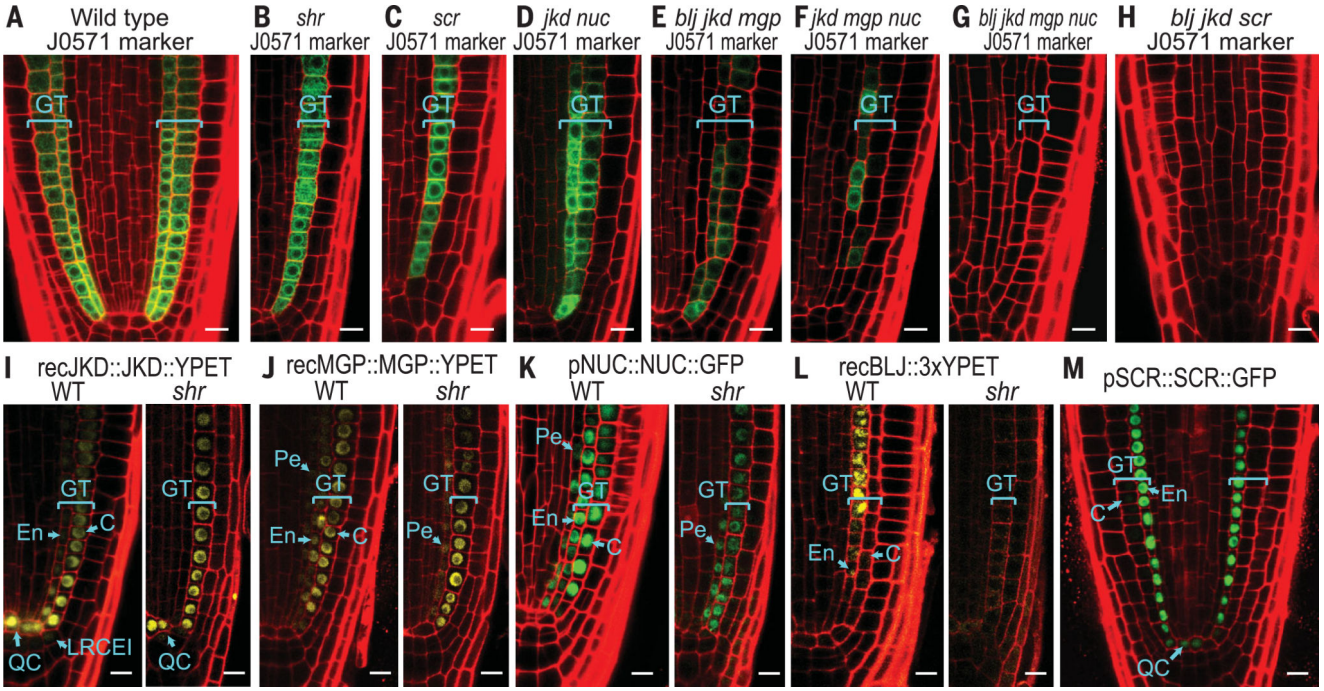

Fig. 1. BIRDs are required for ground tissue maintenance and are expressed in the ground tissue

(**A to H**) Confocal images of roots of wild-type (WT), *shr, scr, jkd, nuc, blj, and mgp*, at 6 days post-imbibition (dpi), showing the ground tissue (GT) marked by J0571. (**I** to **L**) Expression patterns of the BIRDs in WT or *shr* roots at 6 dpi; rec: regulatory regions using recombineering; p: promoter. (**M**) SCR expression. En: endodermis; C: cortex; QC: quiescent center; LRCEI; lateral root cap/epidermis initial; Pe: pericycle. Scale bars: 10 μm.

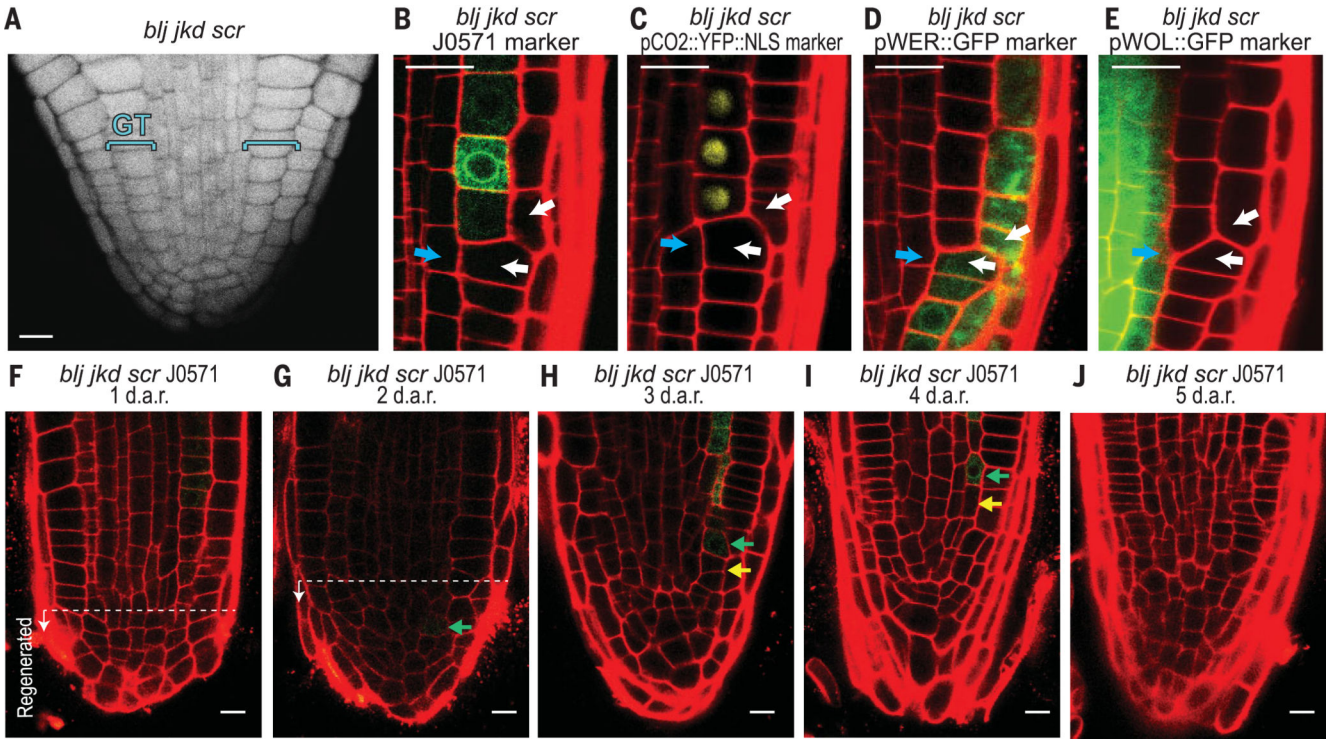

Fig. 2. BLJ, JKD, and SCR specify identity of ground tissue initial cells

(**A**) Mature embryos of *blj jkd scr*. (**B to E**) Lineage analyses in *blj jkd scr* using the J0571, cortex (CO2), epidermis (WER), and stele (WOL) markers. Blue arrows: stele; white arrows: epidermis. (**F** to **J**) *blj jkd scr* roots from 1 to 5 days after resection (d.a.r.). Regenerated ground tissue (green arrows) is visualized with J0571 marker. Yellow arrows: cells missing J0571 expression. Scale bars: 10 μm.

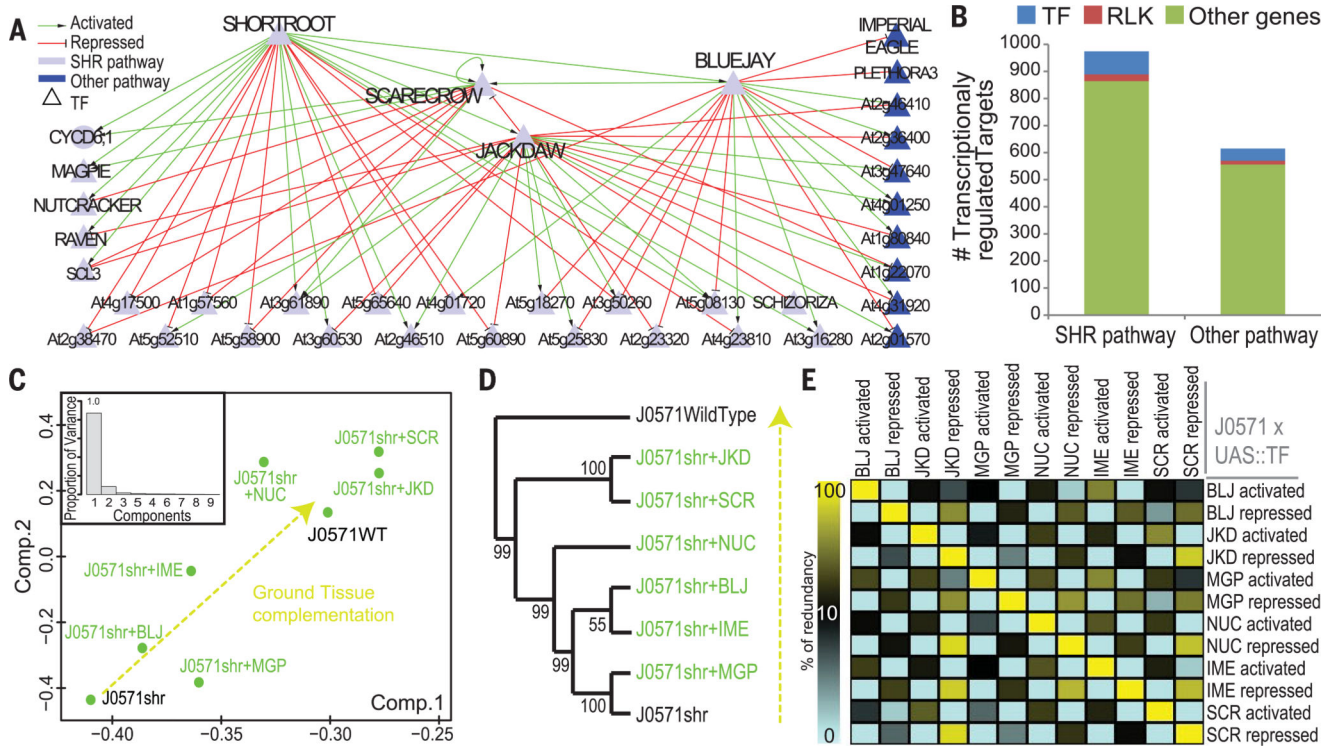

Fig. 3. BIRDs are determinants of ground tissue gene expression and form a network with SHR and SCR

(**A**) Network of SHR, BIRDs, SCR, and downstream transcription factor hubs. (**B**) Comparison of transcriptionally regulated targets. (**C**) Principal component analysis of the transcriptional profiles of ground tissue–expressed genes in different mutants; inset: component weights. (**D**) Same profiles hierarchically clustered. (**E**) Redundancy among regulated genes.

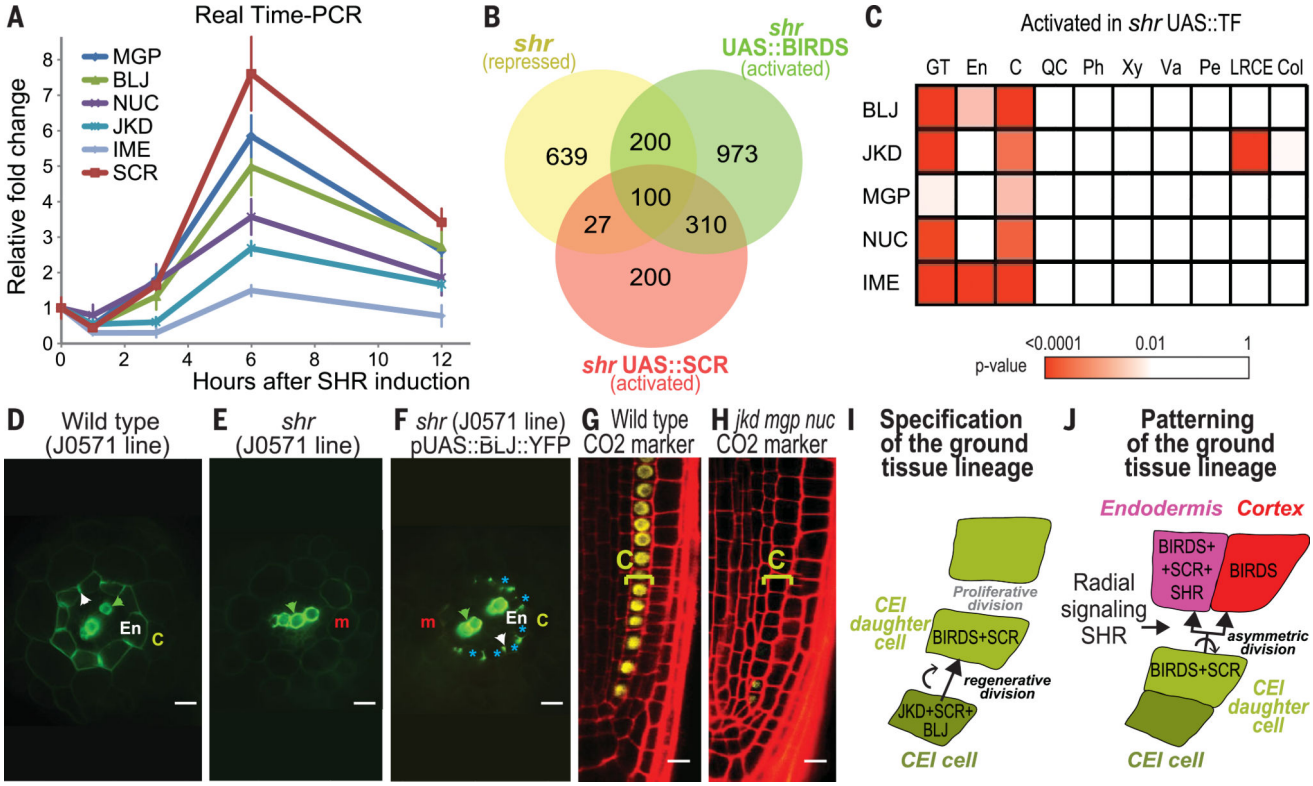

Fig. 4. BIRDs are activated by SHR and specify endodermis and cortex

(**A**) BIRD expression in ground tissue cells at different times after SHR induction. (**B**) Venn diagrams comparing genes activated by BIRDs and SHR. (**C**) Heatmap of BIRD contribution to tissue-enriched expression. Ph: phloem; Xy: xylem; Va: vasculature; Col: columella. (**D** to **F**) Optical root sections at 10 dpi. White arrows: Casparian strip; green arrows: lignin in xylem. (**G** to **H**) Cortex marker in 6 dpi roots. (**I** and **J**) Model of postembryonic formation and maintenance of ground tissue. Scale bars: 10 μm.