Arabidopsis JANUS Regulates Embryonic Pattern Formation through Pol II-Mediated Transcription of WOX2 and PIN7
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SUMMARY
Embryonic pattern formation relies on positional coordination of cell division and specification. Early axis formation during Arabidopsis embryogenesis requires WUSCHEL RELATED HOMEOBOX (WOX)-mediated transcription activation and PIN-FORMED7 (PIN7)-mediated auxin asymmetry. How these events are regulated is obscure. We report that Arabidopsis JANUS, a putative subunit of spliceosome, is essential for embryonic pattern formation. Significantly reduced transcription but not mRNA processing of WOX2 and PIN7 in janus suggested its role in transcriptional regulation. JANUS interacts with RNA polymerase II (Pol II) through a region outside of its spliceosome-association domain. We further show that Pol II mediates the transcription of WOX2 and PIN7 in a JANUS-dependent way and is essential for embryonic pattern formation. These findings reveal that JANUS recruits Pol II for the activation of two parallel pathways to ensure proper pattern formation during embryogenesis.

INTRODUCTION
Development of a specific body plan during embryogenesis requires precise cell fate determination based on the position of cells along the embryo axes. In Arabidopsis, the crucial cell types are established extremely early during embryogenesis as reflected by the stereotypic sequence of oriented cell divisions (Lau et al., 2012; ten Hove et al., 2015). Two pathways have been linked to the establishment of apical-basal axis and cell specification after zygotic division. One involves the transcription factors WUSCHEL RELATED HOMEOBOX2 (WOX2), WOX8, and WOX9, whereas the other depends on auxin, whose asymmetry is maintained by auxin efflux carrier PIN-FORMED7 (PIN7) (Lau et al., 2012). WOX2 and WOX8 are initially co-expressed in the zygote (Haecker et al., 2004). After zygotic division, WOX2 and WOX8 are restricted to the apical and basal cell lineage to control the following cell specification, respectively (Breuninger et al., 2008; Haecker et al., 2004). PIN7 is polarly localized to the apical plasma membrane (PM) of the basal cell, where it provides maternal auxin to the apical cell (Friml et al., 2003; Robert et al., 2018). The polar distribution of PIN7 ensures auxin maximum in the apical cell, which generates the proembryo and all apical structures of the plant. Functional loss of WOX2 or PIN7 compromised the formation of apical-basal axis during early embryogenesis. However, their defects at early embryonic pattern formation are later recovered (Friml et al., 2003; Robert et al., 2018). Whether these two pathways play redundant roles in embryogenesis and how their specific expression is controlled are unclear.

RNA polymerase II (Pol II) plays a pivotal role in regulating gene expression (Thomas and Chiang, 2006). Pol II in Arabidopsis consists of 12 core subunits (Ream et al., 2009), in which Nuclear RNA Polymerase B1 (NRPB1) and NRPB2 interact to form the catalytic center for RNA synthesis, whereas other subunits play structural and regulatory roles in transcription initiation, elongation, termination, or RNA processing (Cramer et al., 2008; Werner and Grohmann, 2011). Functional studies of genes encoding for Pol II subunits suggested its role in embryogenesis such that no homozygous mutants could be obtained for functional loss of NRPB2, as well as that of NRPB9 and NRPB11, genes encoding for the noncatalytic subunits of Pol II (Onodera et al., 2008; Ream et al., 2009; Tan et al., 2012). However, whether and how functional loss of Pol II affects embryogenesis is unclear.

Spliceosomes are large RNA-protein complexes mainly involved in pre-mRNA splicing. Nevertheless, subunits of spliceosomes also participate in many other processes, including mRNA export (Howard and Sanford, 2015; Muller-McNicoll et al., 2016), the maintenance of genome stability (Li and Manley, 2005; Xiao et al., 2007), and microRNA processing (Ben Chaabane et al., 2013; Wu et al., 2010). Interestingly, reports both in metazoans and in plants showed that a subunit of spliceosome, Serine/arginine-rich splicing

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factor 35 (SC35), interacts with a Pol II subunit and is required for the transcription of genes, in addition to its role in pre-mRNA splicing (Lin et al., 2008; Yan et al., 2017). A role of the spliceosome subunit in regulating Pol II-mediated transcription was proposed (Yan et al., 2017).

In this study, we report that the Arabidopsis homolog of human SPliceosome-associated protein 49 (SAP49) and yeast Hsh49p, a subunit of spliceosome, is essential for pattern formation during early embryogenesis. We named it JANUS as it represents the god of new beginnings in ancient Rome and associates with the first steps of a journey. Functional loss of JANUS resulted in complete embryo lethality due to abnormal cell division immediately after the first zygotic division. The specific expression of PIN7 was disrupted in janus, resulting in defective auxin signaling. On the other hand, WOX2 was also transcriptionally downregulated in janus. Consistently, the disruption of both WOX2- and PIN7-dependent pathways resembled pattern formation defects of janus during early embryogenesis. We further showed that JANUS interacts with Pol II subunits independent of its role as a splicing factor and is required for Pol II-dependent transcription of WOX2 and PIN7. Indeed, functional loss of Pol II subunits mimicked embryonic defects of janus. Taken together, our findings demonstrate that JANUS recruits Pol II to transcriptionally activate WOX2- and PIN7-mediated pathways for pattern formation during early embryogenesis.

RESULTS

JANUS Is Essential for Pattern Formation during Embryogenesis

JANUS was isolated for characterization because of the complete embryo lethality of its mutant emb2444 (Meinke et al., 2008). JANUS contains two RNA recognition motifs (RRMs) and is homologous to a subunit of the spliceosome (Figures S1A, S1E, and S1F). Segregation ratio from reciprocal crosses between wild-type and janus/+ indicated that gametophytic transmission of the mutant was not affected (Table S1). The heterozygous mutant showed one-fourth of seed abortion (Figure 1A). Transcript abundance of JANUS was significantly reduced in janus/+ compared with that in the wild-type (Figure S1). In addition, the genomic fragment of JANUS with a GFP reporter gene in the control of its native promoter was introduced into janus/. Transgenic lines of JANUSg-GFP;janus were obtained, and all showed no seed abortion (Figures 1A and S1), indicating that JANUS is the causal gene for seed abortion of janus.
To determine at which stage developing seeds started to show defects in janus, we examined self-fertilized janus/+ by whole-mount clearing. Embryos developing within a single siliques are approximately at the same developmental stage (Breuninger et al., 2008), which enabled an estimate of janus embryos, which are much delayed compared with their wild-type siblings. After the first zygotic division, one-fourth of embryos from janus/+ plants displayed an asymmetric oblique division (Figures 1C and 1F) rather than symmetric vertical division as observed in three-fourth of the other embryos as well as in embryos of the wild-type (Figures 1B and 1F). The division pattern followed was impaired in these presumably janus embryos (Figures 1C and 1F), which showed severe morphological defects at the early globular stage and were eventually arrested at the late globular stage (Figures 1C and 1F). In the arrested embryos, the outer walls of protoderm cells were distended, producing an uneven surface on the embryo proper (Figures 1C and 1F). Abnormal divisions occurred both in the apical and the basal lineages (Figures 1C and 1F). Furthermore, the formation and specification of quiescent center (QC) was also compromised judged by the irregularly oblique divisions in hypophysis and by the absence of GFP signals in ProWOX2::GFP;janus (Figures 1D and 1E), which specifies the QC (Blilou et al., 2005). These results demonstrated that JANUS is an essential gene for early embryonic pattern formation and cell fate specification. Consistent with its role in embryogenesis, JANUS is highly expressed in developing embryos from the zygotic stage to the cotyledon stage (Figure S1).

JANUS Mediates the Expression of WOX2 and PIN7

JANUS is nuclear localized (Figure S1), suggesting a potential role in gene expression. We thus tested whether the two major pathways in controlling early embryogenesis, i.e., WOX-mediated transcriptional pathway and PIN7-mediated auxin signaling pathway, were affected in janus. First, we analyzed the transcription activity of WOX2 and WOX8 by introducing ProWOX2::DsRed2/ProWOX8g::NLS-YFP3 (Yu et al., 2016) in janus/+ . WOX2 and WOX8 were transcriptionally activated in the apical and basal cells after the zygotic division in wild-type, respectively (Figure 2A), as reported (Breuninger et al., 2008; Haecker et al., 2004). By contrast, WOX2 showed a substantially reduced transcriptional activity in the apical cell lineage of one-fourth embryos in janus/+ , whereas WOX8 was not affected (Figures 2A and 2B). The reduced transcription of WOX2 but not WOX8 was confirmed by additional reporter line (Figure S2) or native transcript levels (Figure S3). These results suggested that the transcription activity of WOX2 but not WOX8 in early embryogenesis depended on JANUS.

Second, we examined whether PIN7 was transcriptionally affected by functional loss of JANUS. PIN7 (Blilou et al., 2005) was polarly localized to the apical PM of the basal cells in wild-type globular embryos (Figure 2C), as reported (Friml et al., 2003). In one-fourth embryos from PIN7::GFP;janus/+ , presumably of the janus genotype, GFP signals were significantly reduced or even undetectable (Figure 2C). Because JANUS is homologous to spliceosome subunits (Figure S1), we examined the transcription activity of PIN7 in ProPIN7::NLS-YFP3;janus/+ , which was reflected by fluorescence intensity, to exclude the possibility that mRNA splicing of PIN7 contributed to the expression difference. Consistent with the results obtained from PIN7::GFP, YFP signals were dramatically reduced or undetectable in the basal cells of one-fourth embryos in ProPIN7::NLS-YFP3;janus/+ (Figure 2D). These results implied that JANUS is crucial for the transcription of PIN7 during early embryogenesis. Indeed, no splicing defects of PIN7 as well as of WOX2 were detected in the siliques of janus/+ (Figure S2), confirming a role of JANUS in the transcription rather than RNA processing of the two genes. Because PIN7 is critical for auxin polar transport during early embryogenesis (Friml et al., 2003), the reduced PIN7 in janus would have severely compromised auxin signaling. It was indeed the case. By examining DR5::GFP, a synthetic auxin maximum reporter (Friml et al., 2003), we confirmed that GFP was accumulated in the apical cell lineage immediately after zygotic division and then restricted to the hypophysis of the globular embryos in wild-type plants (Figure 2E), as reported (Friml et al., 2003). By contrast, GFP signals were restricted to the basal cells and failed to establish an apical-basal gradient in one-fourth of embryos in DR5::GFP;janus/+ (Figure 2E). Similar defects were reported in embryos defective in PIN7 or treated with auxin efflux inhibitors (Friml et al., 2003).

Finally, we generated a double mutant of WOX2 and PIN7 under the rationale that if JANUS mediates the transcriptional activity of WOX2 and PIN7 during early embryogenesis, functional loss of both pathways would at least partially mimic the defects of janus. Compared with wild-type, the single mutant of PIN7 or WOX2, i.e., pin7-1 or wox2-4 (Friml et al., 2003; Zhang et al., 2017), showed a slight defect of apical-axis patterning in early stages during embryogenesis but finally recovered at the globular stage (Figures 2G and 2H), consistent with previous reports (Breuninger et al., 2008; Friml et al., 2003; Haecker et al., 2004). In comparison, the proportion of wox2-4/pin7-1 embryos with abnormal asymmetric division in early
stages substantially increased (Figure 2I). In the wild-type, the first division of the apical cell was vertical and symmetric (Figure 2F), as reported (Breuninger et al., 2008). By contrast, in *wox2-4;pin7-1*, the divisions were asymmetric, either oblique or horizontal in both embryo proper and hypophysis (Figure 2I), which largely resembled those of *janus* during early embryogenesis (Figure 2J). Instead of a full recovery at late stages as seen in each single mutant (Breuninger et al., 2008; Friml et al., 2003; Haecker et al., 2004), over 30% embryos of *wox2-4;pin7-1* arrested immediately after the first zygotic division (Figure 2I).

By contrast, enhanced expression of *PIN7* partially complemented the defects of *janus* such that early embryonic pattern formation was largely normal in *ProUBQ10:PIN7;janus/+* (Figure 2K). These results...
suggested that both WOX2- and PIN7-dependent pathways may be transcriptionally regulated by JANUS and play redundant roles in apical-basal patterning during early embryogenesis.

**JANUS Interacts with Pol II Whose Functional Loss Resulted in Embryo Lethality**

To determine how JANUS affected the transcription activity of WOX2 and PIN7 during embryonic pattern formation, we tested whether JANUS interacted with components of Pol II since Pol II is responsible for the transcription of most mRNAs in eukaryotes (Thomas and Chiang, 2006) and was shown to interact with other components of the spliceosome (Yan et al., 2017). Of 11 Pol II subunits tested, NRPB7, NRPB10, and NRPB11 showed interaction with JANUS by yeast two hybrid (Y2H) and bimolecular fluorescence complementation (BiFC) assays (Figures 3A–3C and S4). Interestingly, the interaction is evolutionarily conserved: JANUS homolog also interacts with Pol II components in mice (Figure S4).

The physical interaction between JANUS and Pol II suggested that they might function in the same pathway. Indeed, all three genes are highly expressed during embryogenesis based on reporter analysis (Figure S4). We thus hypothesized that functional loss of Pol II would also result in defective embryonic pattern formation. To test this hypothesis, we characterized mutants of NRPB10 and NRPB11, both of which interact with JANUS (Figure S4). Although NRPB7 also interacts with JANUS, the lack of its mutants in all stock centers prevented further analysis. Likely due to the redundancy with NRPB10-like genes (Ream et al., 2015), functional loss of NRPB10 did not show seed set reduction and embryo lethality (Figure S4). In comparison, functional loss of NRPB11, a single gene in Arabidopsis (Ream et al., 2015), resulted in embryo lethality (Figure S4), similar to nrpb2 (Onodera et al., 2008). Because both genes are required for Pol II activity (Kershnar et al., 1998;
Ream et al., 2009), these results hinted at an essential role of Pol II in embryogenesis. To identify the defects of embryogenesis due to Pol II loss of function, we examined developing embryos in nrpb2-1/+; nrpb2-2/+; and nrpb11-1/+; nrpb2-1/+; nrpb2-2/+; and nrpb11-1/+; nrpb2-1/+; nrpb2-2/+; and nrpb11-1/+ as well as in 29% of the nrpb2/+ mutants (Figures 3D and 3E). Similar to that in janus, the first division of the apical cell, i.e., symmetric and horizontal in wild-type, was asymmetric and oblique in nrpb11 and nrpb2 (Figures 3D and 3E). Irregular division patterns resulted in abnormal formation of embryo proper and hypophysis (Figures 3D and 3E). In most severe cases, embryos were arrested at as early as the two-cell stage (Figures 3D and 3E). Thus, these results demonstrated that Pol II is essential for early embryonic pattern formation and likely functions in the same genetic pathway as JANUS.

**Pol II-Mediated WOX2 and PIN7 Transcription Depends on JANUS**

That JANUS interacts with Pol II and affects the transcription activity of WOX2 and PIN7 suggested that Pol II might mediate WOX2 and PIN7 transcription during embryonic pattern formation in a JANUS-dependent way. We thus hypothesized that functional loss of Pol II would compromise WOX2 and PIN7 expression during early embryonic pattern formation. To test this hypothesis, we introduced ProWOX2:GFP and PIN7:GFP into nrpb2-1/+ and examined the fluorescence distribution during embryogenesis. Indeed, the expression of WOX2 and PIN7 was severely reduced or even undetectable in one-fourth embryos of the nrpb2-1/+ plants (Figures 4A and 4B), reminiscent of that in janus/+; Auxin signaling was also compromised during early embryogenesis by functional loss of NRPB2 such that ectopic GFP signals were detected in the basal cells of one-fourth DRS;GFP;nrpb2-1/+ embryos (Figure 4C), similar to that by functional loss of JANUS. These results suggested that Pol II mediates the transcription of WOX2 and PIN7 during early embryonic pattern formation.

To test whether Pol II-mediated transcription of WOX2 and PIN7 depended on JANUS, we examined Pol II occupancy at the promoter regions of WOX2 and PIN7 by chromatin immunoprecipitation (ChIP) using an antibody against NRPB2 (Yan et al., 2017). Because no homozygous janus plants could be obtained, we generated Pro35S::JANUS-RNAi transgenic plants. Transcript analysis as well as fluorescence quantification supported a significant reduction of JANUS by RNAi in Pro35S::JANUS-RNAi transgenic plants (Figure S5). ChIP assays indicated that Pol II occupancy was significantly reduced at the promoter regions of PIN7 in Pro35S::JANUS-RNAi lines (Figures 4F and 4G) as compared with that in the wild-type (Figures 4F and 4G). Consistent with the reduced Pol II occupancy, PIN7 was transcriptionally downregulated in Pro35S::JANUS-RNAi lines, either by quantitative PCRs for the endogenous genes (Figures 4J and 4K) or by fluorescence quantification of the PIN7-GFP transgenic plants (Figures 4D, 4E, 4H, and 4I). Except for embryos, WOX2 was weakly expressed in a few cells at root maturation zone (Figure 4D), which hindered the application of ChIP assays on its promoter regions. However, by analyzing the fluorescence in the Pro35S::JANUS-RNAi; ProWOX2;H2B-GFP or ProWOX2;DsRed2;janus/+ plants, we confirmed that JANUS-RNAi or janus significantly reduced the transcription activity of ProWOX2 (Figures 2A and 4D). These results suggested that Pol II-mediated transcription of WOX2 and PIN7 depends on JANUS. By contrast, the transcription of WOX8 and SCARECROW (SCR), a critical embryonic gene (Wysocka-Diller et al., 2000), depends on Pol II but not JANUS (Figure S3), suggesting that JANUS did not affect the transcriptional activity of Pol II in general.

**DISCUSSION**

Early embryogenesis is the critical developmental phase during which the basic features of the plant body are established. Although distinct expression domains of WOX family transcription factors as well as directional auxin transport are known to be involved in early apical-basal patterning, their upstream regulators and potential interactions were obscure. Our results demonstrated that the two pathways control early embryonic pattern formation in a parallel way and both mediated by JANUS. First, functional loss of WOX2 and PIN7 caused a severe defect immediately after the zygotic division, which cannot be restored at late stages, unlike the disruption of each pathway. Second, both WOX2 and PIN7 were transcriptionally downregulated by JANUS loss of function. The effect of JANUS on PIN7 and WOX2 is specific because WOX8, another embryonic gene, requires RNA Pol II but not JANUS for its expression during early embryogenesis. Third, woxx2-4;pin7-1 resemble janus in early embryogenesis. Finally, the accumulation of Pol II at PIN7 was significantly reduced by JANUS-RNAi, suggesting JANUS-dependent recruitment of the transcriptional machinery at PIN7.

Although transcription and RNA processing are coupled in vivo (Bentley, 2002; Hirose and Manley, 2000; Lee and Tarn, 2013; Proudfoot et al., 2002; Yan et al., 2017), studies have proven that splicing factors may interact
with transcription machinery to directly influence gene expression in metazoans (Braunschweig et al., 2013; Das et al., 2007). Although we cannot exclude a role of JANUS in RNA splicing owing to its homology to the component of the spliceosome during embryogenesis, results presented here strongly suggested its role through transcriptional regulation. First, JANUS is critical for the transcription but not RNA splicing of
WOX2 and PIN7, two genes critical for early embryonic pattern formation. Second, the RRM1 domain of yeast SAP49 or metazoan Hsh49p, homolog of Arabidopsis JANUS, is responsible for RNA binding and spliceosome association and is essential for spliceosome-mediated RNA splicing (Igel et al., 1998; Kuwasako et al., 2017; Pauling et al., 2000; van Roon et al., 2017). However, we found that JANUS directly interacts with Pol II through its RRM2 but not RRM1 domain, suggesting that its interaction with Pol II does not require its association with the spliceosome. Third, yeast and metazoan homologs of JANUS are part of an SF3b complex within the spliceosome (Kuwasako et al., 2017; van Roon et al., 2017). However, although other components of the SF3b complex in Arabidopsis have been functionally characterized (Aki et al., 2011; Wang and Brendel, 2006), none of the related mutants showed embryo lethality.

Eukaryotes decode their genomes using three essential nuclear DNA-dependent RNA polymerases (Cramer et al., 2008; Werner and Grohmann, 2011). In Arabidopsis, several subunits of Pol II, including NRPB2, NRPB5, NRPB9, and NRPB11, have been reported to affect plant viability (Onodera et al., 2008; Ream et al., 2009; Tan et al., 2012). However, whether and how Pol II regulates embryogenesis was unclear. We showed here that mutations at a few Pol II components resulted in complete embryo lethality (Figures 3A and S3I). A few components of Pol II are shared by two plant-specific RNA polymerase complexes, Pol IV and Pol V (Ream et al., 2009). However, we believe that Pol II is key for JANUS-mediated transcription of WOX2 and PIN7 because the JANUS-interacting component NRPB7 is specific for Pol II (Figures 3A and S3B) and only Pol II was reported to be essential for viability (Herr et al., 2005; Kanno et al., 2005; Onodera et al., 2005; Pontier et al., 2005; Ream et al., 2009).

Data presented here suggested that JANUS is important for the Pol II-mediated transcription of WOX2 and PIN7 during early embryogenesis. However, JANUS has no recognizable DNA-binding domains. It is unclear how JANUS determines the selectivity of Pol II on target genes such as WOX2 and PIN7. In eukaryotes, mediators, multi-subunit complexes, bridge transcription activators with Pol II at specific cis-elements for transcription initiation (Dolan and Chapple, 2017). Whether JANUS fulfills a role of mediator, recruiting DNA-binding transcription factors together with Pol II, to the promoter regions of WOX2 and PIN7, is an interesting scenario worthy of further investigation.

Limitations of the Study
We would like to note that, because both janus and Pol II mutants are embryo lethal, some molecular and biochemical experiments can only be carried out in Pro35S:JANUS-RNAi plants. Future efforts will be dedicated to generate weak mutant alleles of JANUS, for which homozygous plants can be used for further mechanistic analysis. We also would like to point out that, although JANUS is important for Pol II-mediated transcription of WOX2 and PIN7 during early embryogenesis, it is unclear how JANUS, a protein containing no DNA-binding domains, determines target selectivity for Pol II. Whether JANUS contains untraditional DNA-binding domains or interacts with transcription factors for its function will be an interesting scenario for future studies.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2019.09.004.

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AUTHOR CONTRIBUTIONS
Conceptualization, F.X.; Methodology, F.X.; Investigation, F.X., H.-H.L., C.-Y.D., B.-K.Z., and G.W.; Writing – original draft, F.X.; Writing – review and editing, Y.Z. and S.L.; Supervision, Y.Z. and S.L.; Funding acquisition, Y.Z. and S.L.
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Supplemental Information

Arabidopsis JANUS Regulates Embryonic Pattern Formation through Pol II-Mediated Transcription of WOX2 and PIN7

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Figure S1. JANUS is homologous to a component of the spliceosome and is present in the nucleus.

(A) Domain organization of Arabidopsis JANUS and its yeast and human homologs. RRM, RNA recognition motif. Number of amino acid is on top.

(B) Schematic illustration of the JANUS genomic locus. LB, left border primer for T-DNA; LP, left primer; RP, right primer.

(C) Genotyping PCRs verify the genetic background of wild type (WT), janus/+; and three JANUSg-GFP;janus lines (Comp).

(D) Transcript analysis showing JANUS abundance. Results are means ± standard deviation (sd, n=3). Results are representative of three biological replicates. Different letters indicate significant difference (Tukey's multiple comparison test, P<0.05).

(E) Phylogenetic tree of JANUS and its homologs from Arabidopsis lyrata (XP_002886178.1), Brachypodium distachyon (XP_003574000.1), Brassica napus (CDY59539.1), Dictyostelium purpureum (XP_003287607.1), Drosophila sechellia (XP_002036808.1), Glycin max (XP_003540544.1), Homo sapiens (NP_005841.1), Macaca mulatta (NP_001248161.1), Medicago truncatula (XP_003600696.1), Nicotiana tabacum (XP_016437621.1), Oryza sativa (XP_015614791.1), Physcomitrella patens (XP_001764808.1), Rattus norvegicus (NP_001011951.1), Saccharomyces cerevisiae (AJT72224.1), and Xenopus laevis (NP_001080100.1).

(F) Structure model of JANUS N-terminus including both RRM motifs.

(G) Confocal laser scanning microscopy (CLSM) of JANUSg-GFP embryos at various developmental stages and root epidermal cells. Images shown are merges of the GFP channel and the transmission channel for embryos, and of the GFP channel and the RFP channel (FM4-64) for root cells. Scale bars, 20 µM.

Related to Figure 1.
Figure S2. JANUS mediates the expression but not RNA processing of WOX2 and PIN7.
(A) CLSM of a ProWOX2:H2B-GFP embryo at early developmental stages in wild type or in janus. Scale bars, 50 µm.
(B) CLSM of embryos from a different PropPIN7:NLS-YFP or PropPIN7:NLS-YFP;janus/+ plant. Scale bars, 50 µm.
(C) Transcript abundance of WOX2 in WT, wox2-4;pin7-1, and janus/+ by qPCR.
(D) Transcript abundance of PIN7 in WT, wox2-4;pin7-1, janus/+ and two lines of PropUBQ10:PIN7;janus/+ by qPCR. Results shown in (C) and (D) are means ± standard error (SEM) from three technical repeats. qPCRs were repeated three times with similar results. Different letters indicate significantly different groups (Tukey’s multiple comparison test, P<0.05).
(E) RT-PCRs showing that both PIN7 and WOX2 have no alternative spliced forms in janus/+ or in Prop35S:JANUS-RNAi. The above numbers indicate relative abundance. Experiments were repeated in three biological replicates with similar results.
(F) Schematic diagram of the genomic regions of PIN7 and WOX2. Primers used for detecting intron retention and splicing efficiency are shown in the regions of interest. F, forward primer; R, reverse primer; Black box, exon; Gray box, UTR; Black line, intron.
(G) Intron retention that was detected via RT-PCRs shows no significant difference between WT, janus/+ and RNAi-1 siliques.
(H) Splicing efficiency of PIN7 and WOX2 was unaffected in janus/+ and RNAl-1 siliques. Splicing efficiency was calculated as the ratio of spliced to total (spliced + unspliced) transcripts for two representative PIN7 introns and WOX2 intron. Results are means ± SE from three experiments. ns indicates no significant difference (t-test, P>0.05).
Related to Figure 2.
Figure S3. The expression of SCR is reduced by functional loss of Pol II but not that of JANUS.

(A) CLSM of ProSCR:H2B-YFP or ProSCR:H2B-YFP:janus embryos at various developmental stages. Images shown are merges of the GFP channel and the transmission channel. Scale bars, 50 µM.

(B) CLSM of a root from 4 DAG seedlings of ProSCR:H2B-YFP or ProSCR:H2B-YFP;Pro35S:JANUS-RNAi. Images shown are merges of the GFP channel and the RFP channel (FM4-64). Scale bars, 50 µM.

(C-D) Relative transcript abundance of SCR (C) and WOX8 (D) in the siliques of wild type, janus/+, or nrpb2-1/+ by qPCRs. Results are means ± SEM from three technical repeats. Different letters indicate significantly different groups (Tukey’s multiple comparison test, P<0.05).

Related to Figure 2 and Figure 4.
Figure S4. Functional loss of Pol II components resulted in embryo lethality.
(A) Domain organization of JANUS, its variants, and its mice homolog SF3B4. Number of amino acid is on top.
(B-C) Y2H assays demonstrating RRM1-dependent interaction between JANUS and Pol II components. Results shown are representative of three biological replicates.
(D-G) CLSM of ProNRPB2:NLS-YFP (D), NRPB7g-GFP (E), ProNRPB11:NLS-YFP (F), or NRPB10g-GFP (G) embryos during development. Images shown are merges of the GFP and transmission channels. Scale bars, 20 µM.
(H) Schematic illustration of the NRPB2, NRPB10, and NRPB11 genomic loci. LB, left border primer for T-DNA; LP, left primer; RP, right primer.
(I) Seed set of different genotypes. Results are means ± sd (n=8). Scale bars, 1 mm.
(J) Embryogenesis of nrpb10 by ovule clearing. Scale bars, 50 µM.
(K) Relative transcript abundance of NRPB2, NRPB10, or NRPB11 in corresponding mutants versus in wild type by qPCRs. Results are means ±SEM from three technical repeats. Experiments were repeated three times with similar results. Asterisks indicate significant difference (t-test, P<0.05). Related to Figure 3.
Figure S5. JANUS-RNAi caused delayed embryogenesis.
(A-C) CLSM of JANUSg-GFP (A), and two lines of Pro35S::JANUS-RNAi::JANUSg-GFP (B-C). Left images are whole root tips; right images are merges of the GFP channel (green, JANUS-GFP) and the RFP channel (magenta, FM4-64). Dotted circles indicate ROI. 
(D) Quantification of fluorescence intensity. Results shown are average fluorescence intensities within a ROI, showing in (A-C). Scale bars, 20 µM.
(E) Relative transcript abundance of JANUS in WT or in Pro35S::JANUS-RNAi lines by qPCRs. Results are means ± SEM from three technical repeats. Experiments were repeated three times with similar results. Different letters indicate significantly different groups (Tukey’s multiple comparison test, P<0.05).
(F) Representative stages of embryogenesis in wild type versus in two Pro35S::JANUS-RNAi lines. Scale bars, 50 µM.
Related to Figure 4.
Table S1. JANUS loss-of-function does not compromise gametophytic transmission.

| Parents            | Genotype                    | Expected ratio | Observed ratio |
|--------------------|-----------------------------|----------------|----------------|
| janus/+ X WT       | WT : janus/+                | 1 : 1          | 66 : 67<sup>a</sup> |
| WT X janus/+       | WT : janus/+                | 1 : 1          | 83 : 79<sup>a</sup> |
| janus/+ X janus/+  | WT : janus/+ : janus        | 1 : 2 : 1      | 53 : 113<sup>b</sup> |

<sup>a</sup> Not significantly different from the segregation ratio 1:1 ($\chi^2$, P>0.05).

<sup>b</sup> Significantly different from the segregation ratio 1:2:1 ($\chi^2$, P>0.05).

Related to Figure 1.
Table S2. Segregation of self-fertilized heterozygous Pol II mutants.

| Parents            | Genotype              | Expected ratio | Observed ratio |
|--------------------|-----------------------|----------------|----------------|
| nrpb2-1/+ X nrpb2-1/+ | WT : nrpb2-1/+ : nrpb2-1 | 1 : 2 : 1      | 44 : 78 : 0<sup>a</sup> |
| nrpb2-2/+ X nrpb2-2/+ | WT : nrpb2-2/+ : nrpb2-2 | 1 : 2 : 1      | 36 : 65 : 0<sup>a</sup> |
| nrpb10/+ X nrpb10/+  | WT : nrpb10/+ : nrpb10  | 1 : 2 : 1      | 28 : 61 : 33<sup>b</sup> |
| nrpb11/+ X nrpb11/+  | WT : nrpb11/+ : nrpb11  | 1 : 2 : 1      | 15 : 33 : 0<sup>a</sup> |

<sup>a</sup> Significantly different from the segregation ratio 1:2:1 ($\chi^2$, P>0.05).
<sup>b</sup> Not significantly different from the segregation ratio 1:2:1 ($\chi^2$, P<0.05).

Related to Figure 3.
| Application | No.   | 5'-3' sequences                                      |
|------------|-------|-----------------------------------------------------|
| Genotyping |       |                                                     |
| SALK LB    | ZY1   | ATTTTGGCGATTTCGGAAC                                 |
| SAIL LB    | ZY4   | GCTTCCTATTATATCTTCACAAAAATTACCAATACA               |
| layus/+    | ZY7860| AGAAGCAATCACACACCAATGC                              |
|           | ZY7861| TATAAGGCCATTAGAGACGG                                |
|           | ZY7866| GAAATTGGTCCAGAAACTGAGCGAT                          |
| nrpb2-1/+  | ZY7857| TGGGAATTCGAATTATATGTC                              |
| nrpb2-2/+  | ZY7858| CATTCACAGGCTGACAGG                                 |
| nrpb10     | ZY7892| TGGAAGAGACTATGTCGTCGAAAC                           |
|           | ZY7893| TATGTTCCCTTAAAGAACGAAAA                            |
| nrpb11/+   | ZY7894| GAGAATATGGGGCTGTTGATTG                             |
|           | ZY7895| AGAGCCCTGTGCTTTGAATG                               |
| wox2-4     | ZY8654| CTTACTTCAATCGGCTCCTCC                              |
|           | ZY8655| AGAATGTCTATGTCGCGCCTCC                             |
| pin7-1     | ZY2931| TGCCCTAAACGACTACAAAGC                              |
|           | ZY2932| ACCGCCATCGCAAAAGTC                                 |
| Cloning    |       |                                                     |
| JANUS      | ZY7639| CACCATGACGACTCGAATCGCTCCTCTG                        |
|           | ZY7642| TACACCTTGGTGTTGGGTGTTG                             |
| nJANUS     | ZY7639| CACCATGACGACTCGAATCGCTCCTCTG                        |
|           | ZY7640| TACACCTTGGTGTTGGGTGTTG                             |
| cJANUS     | ZY7641| CACCATGAAAGACACAAAGGAAGAG                          |
|           | ZY7642| TCAACCTTGGTGTTGGGTGTTG                             |
| JANUS-RR   |       |                                                     |
| M1         | ZY8676| CACCATGGCCACTGTTTACGTCGCTG                         |
|           | ZY8677| TTATGAGATGCTTCTTTACAGC                              |
| JANUS-RR   |       |                                                     |
| M2         | ZY8678| CACCATGGCTAACCTTTTTCATTGCAAAACC                    |
|           | ZY8679| TTATCTGTATCGTGAAAGACTGTG                           |
| NRPB2      | ZY7373| TAAGAAGGAGCGCCCCTCAGGCTATTGAACTACAGAAGGAAC         |
|           | ZY7374| GGTCGGCGCGCACCCTTGTGACTGCTTG                      |
| NRPB3      | ZY7499| CACCATGGACGTCGCGCACATAACAAAG                       |
|           | ZY7500| TCAATCCTCAGGCAATAGGGCA                             |
| NRPB4      | ZY7479| CACCATGGCTCCGAGAAAGAAAGAAGAAG                     |
|           | ZY7480| CTACTCGAATCTTGTGACAGAAAGAAGAAG                   |
| NRPB5      | ZY7481| CACCATGTGACAGAAGAAGAAGAAGAAGAAG                   |
|           | ZY7482| GGCTTTAACAAATAAAGAAGAAGAAGAAGAAG                   |
| NRPB6a     | ZY7483| CACCATGGCTAGCGAAGATTACAACAC                       |
|           | ZY7484| TCAATCACCACAACACTTGCG                              |
| NRPB7      | ZY7485| CACCGTGGAGATTTTTCCTACATAG                         |
|           | ZY7486| TTATGCGGGCAGGCGGTCTTTGATTG                          |
| NRPB8a     | ZY7487| CACCATGGGGAGCAATAATCATCCTTG                        |
|           | ZY7488| TCCAGGGTCTCTATGAGTA                               |
| NRPB8b     | ZY7489| CACCATGGGAGCAATAATCATGATTTTTCG                    |
|           | ZY7490| TCAAGAGCTTCTCTTAGAAGAAGAAGAAG                      |
| NRPB9a     | ZY7192| CACCATGAGTACTATGAAATTTTGGCGCG                     |
|           | ZY7193| TATTCTGCTGACCAGGACTACAA                           |
| NRPB9b     | ZY7491| CACCGATTTCTCGCGAGATGATGACTATG                     |
|           | ZY7492| CTATTCTCAGGCGACTACAC                              |
| NRPB10     | ZY7497| CACCATGATCATCCCTTGTTGTCG                           |
|           | ZY7498| CTACGGTTGCTGATTTCCTCCTCG                          |
| NRPB11     | ZY7493| CACCATGAAATGCTCCGAAAGACTGAC                       |
|           | ZY7494| ACTGCCAGTGCTTTAAGAAGACAGTTGCG                     |
| NRPB12     | ZY7495| CACCATGGATCGCGCGCCAAGACC                         |
|           | ZY7496| TCACGGAGCTCTGATGAGA                                |
| PIN7 CDS | ZY7986 | CACCATGATCACATGGCACGACCTCTAC |
| JANUS genomic | ZY6987 | ACCCTTGTTGTTGTTTGGTTG |
| JANUS RNAi | ZY7194 | GGGGACAAGTTTGTAACAAAAAGCGCTATGACGACCTCTCTCT |
| ZY7195 | GGGGACCACTTTTTGTAACAAAAAGGTGATGGATGAAACCA |
| ProPIN7 | ZY7984 | CACCGTGAAGCGGTTAGACACACAA |
| ZY7985 | ATTGTGCTGCGCTGAGTGGAC |
| ProNRPB2 | ZY7477 | CACCTAGCGCCACAGGGTTAGACAGAAT |
| ZY7478 | TCTCAGTCTCTGAAGTTAGAGTTA |
| ProNRPB11 | ZY7575 | CACCCGCTTGACAACTCTAGTCACGTTAG |
| ZY7576 | GCCTAAATCGGAGATGGAGC |
| NRPB7g | ZY7571 | CACCCATACCCGAGGAGGGAATAT |
| ZY8235 | TGCGTGCTGAGGTGTTTATAG |
| NRPB10g | ZY7573 | CACCTCTGAAAGCTGACAGTCA |
| ZY8236 | TGCCTGAGGTGTTTATAG |
| SF3B4 (Mice) | ZY9179 | CACCATGGCTGCAGAACAGAAG |
| ZY9180 | TTACCTGAGGAGGTGACGAGAG |
| SF3B4 RRM2 (Mice) | ZY9183 | CACCATGGCTGCAGAACAGAAG |
| ZY9184 | TCAGCTGAGGAGGTGACGAGAG |
| RPB7 (Mice) | ZY9185 | CACCATGGCTGCAGAACAGAAG |
| ZY9186 | TCGCTGAGGAGGTGACGAGAG |
| RPB10 (Mice) | ZY9187 | CACCATGGCTGCAGAACAGAAG |
| ZY9188 | TCAGCTGAGGAGGTGACGAGAG |
| RPB11 (Mice) | ZY9189 | CACCATGGCTGCAGAACAGAAG |
| ZY9190 | TCAGCTGAGGAGGTGACGAGAG |
| RT-PCR PIN7 | ZY7986 | CACCATGATCACATGGCACGACCTCTAC |
| ZY7987 | TATAGCCGAGTAAATTGAAG |
| WOX2 | ZY7880 | CACCATGGAAGGGGGAACCTCTCT |
| ZY7881 | TTACTGAGAGCTGCTGAGAT |
| TUBLLIN2 | Tub2 U | GTGATTCCGAGGGAACAGAT |
| Tub2 D | TCCGATGCAACAGAAGAAT |
| qRT-PCR ChIP PIN7 Region a | ZY8596 | TGAGTGAGGTGCTTAAAGAAGTGAGA |
| ZY8597 | TGGCAGAGGTGCTTAAAGAAGTGAGA |
| ChIP PIN7 Region b | ZY8598 | TTTCCTCCTCTCTCTCTCTCT |
| ZY8599 | ATGTGTGGCTCGAGGAGGTG |
| WOX2 | ZY8592 | AGGAGCGCATGGGCTTACCTCA |
| ZY8593 | TTTCCTGAGCCACCCCACTT GAG |
| WOX8 | ZY9725 | AACCTGATCTCCTTCTCCTCCTCA |
| ZY9727 | AGGTTGCAAATATAGTACCCGGAAT |
| SCR | ZY9594 | GATGCTACTGGTCTGTGCAACAC |
| ZY9595 | TGCTGATAATGCTGCACTGAGGAG |
| PIN7 | ZY8594 | GTGGGTGAAGCTGAAAT |
| ZY8595 | TGCTGATAATGCTGCACTGAGGAG |
| JANUS | QRT U | TTGGGAGCCCAACATGCAACTGCA |
| QRT D | GCTGCTGTTGCTGTTGGGCTGAGGAG |
| GAPDH | ZY6877 | TTGATCTTTTGTGTATTCCCTCT |
| ZY6888 | CATATCCTGAGGTGATAAG |
| ACT2 | ZY313 | CGTGCATTACTGATTAC |
| ZY314 | TCTTCCTTGATGTCTTCTT |
| Splicing of PIN7 and WOX2 | PIN7 intron1 | F1 | CAAGTTGATAATGGGAGCCCAATG |
| R1 | CAGTTTGAGATTTGCTGCTCATAG |
| R1’ | GTAGGCGACTTCCTTACCTCCT |


| Gene  | Exon | Primer F | Primer R | Primer R' |
|-------|------|----------|----------|-----------|
| PIN7  | intron2 | F2 | CTGATTTGATAATGCGATGGGAG | R2 | TCACTCCTACATAACATCTCAACAA |
|       |       |          |          | R2' | CCAACTGAACATTTGACATACCAAG |
| PIN7  | intron3 | F3 | CATGTATTGCATCATCATTGACGACCTTG | R3 | CTGGTCCAGTAAAGAATCTCACC |
| PIN7  | intron4 | F4 | CCAATCTTCTGCTGTAACGTTGGAG | R4 | CTGGTCCAGTAAAGAATCTCACC |
| PIN7  | intron5 | F5 | GTTGATTCTATGTGATTATTGCAG | R5 | GCGTTCCACTAATCTTGGAATTATC |
| WOX2  | intron | F6 | CCAGTTTTGGCCATTTATCTCCTGAG | R6 | GTAATAGTACGGACTGACACCAACCCA |
|       |       | F6' | TGGTTCCAGAACCATAAGGCTAGG |

Related to Figure 1-5.
Transparent Methods

Arabidopsis thaliana ecotype Columbia-0 (Col-0) was used as wild type for all experiments in this study. T-DNA insertion mutants of JANUS (SALK_008993 as janus), NRPB2 (CS16201 as nrpb2-1), CS16202 as nrpb2-2), NRPB10 (SALK_114301C as nrpb10-1), and NRPB11 (SALK_100563 as nrpb11-1), WOX2 (SALK_114607 as wox2-4), PIN7 (SALK_044687 as pin-7-1) were verified by PCRs. Plant marker lines carrying DR5rev::GFP (Ulmasov et al., 1997), PIN7::GFP (Biliou et al., 2005), ProWOX3::GFP (Biliou et al., 2005), ProWOX2::DsRed2/ProWOX8b::NLS-vYFP3 (Yu et al., 2016), ProNRPB1::H2B-YFP (Heidstra et al., 2004), ProWOX2::H2B-GFP (Gooh et al., 2015) were described. The marker lines were crossed with janus+, nrpb10-1, or nrpb2-1/+ and verified by PCRs and by fluorescence imaging. Seedlings and plants were grown in a growth chamber at 20 ± 2 °C under long-day condition (16 h light/8 h dark).

DNA manipulation

All constructs were generated using the Gateway technology (Invitrogen) except where noted. Entry clones were generated in the pENTR/D/TOPO vector (Invitrogen). The coding sequences were cloned with the following primers: ZY7639/ZY7642 for the respective gene was amplified with primer pairs, ZY7571/ZY8235 for the expression analysis of NRPB7 and NRPB10, a 2924 bp or a 3277 bp genomic sequence of JANUS, ZY8676/ZY8677 for JANUS-RNAi without the stop codon was amplified with the primer pair ZY7194/ZY7195. The fragment was cloned intoDestination vectors used for expressing GFP-translational fusions in planta are ProUBQ10::GFP-GW and ProUBQ10::GW (Zhang et al., 2018), for Y2H are pDEST-GBKT7 and pDEST-GADT7 (Invitrogen), for BiFC are pSITE::cEYFP-C1 and pSITE-nEYFP-C1 (Martin et al., 2009).

A 3875 bp sequence downstream of the 1489 bp upstream of the JANUS translation start codon and the genomic fragment of JANUS without the stop codon was amplified with the primer pair ZY6987/ZY6988. The entry vector containing JANUSg was used in an LR reaction with the destination vector GW::GFP (Zhou et al., 2013) to generate JANUSg-GFP.

To generate ProSSS::JANUS-RNAi, a 500 bp fragment from the N-terminus of JANUS was amplified with the primer pair ZY7194/ZY7195. The fragment was cloned into ProSSS::GW-RNAi vector (Wang et al., 2017) via a BP reaction (Invitrogen) to generate ProSSS::JANUS-RNAi.

For the promoters of PIN7, NRPB2, and NRPB11, a 2175 bp, a 1904 bp, or a 1973 bp sequence upstream of the translational start codon of the respective gene was amplified with primer pairs, ZY7984/ZY7985 for PIN7, ZY7477/ZY7478 for NRPB2 and ZY7575/ZY7576 for NRPB11. The entry vectors were used in LR reactions with the destination vector GW::NLS-YFP (Wang et al., 2017) to generate ProNRPB2::NLS-YFP or ProNRPB11::NLS-YFP. For the expression analysis of NRPB7 and NRPB10, a 2924 bp or a 3277 bp genomic sequence of the respective gene was amplified with primer pairs, ZY7571/ZY8235 for NRPB7 and ZY7573/ZY8236 for NRPB10. The entry vectors were used in LR reactions with the destination vector GW::GFP (Zhou et al., 2013) to generate NRPB7g-GFP or NRPB10g-GFP, respectively.

PCR amplifications were performed with Phusion hot-start high-fidelity DNA polymerase with the annealing temperature and extension times recommended by the manufacturer. All entry vectors were sequenced. All primers are listed in Supplemental Table 3.

PCRs, RNA extraction, RT-PCRs, and qPCRs

Genotyping PCRs of janus, nrpb2-1, nrpb2-2, nrpb10, nrpb11, wox2-4, and pin-7-1 were performed using the following primers: ZP7860/ZP7861 for the wild-type copy of JANUS, ZP1/ZP7861 for janus; ZP7857/ZP7858 for the wild-type copy of NRPB2, ZP4/ZP7858 for nrpb2-1 and nrpb2-2; ZP7892/ZP7893 for the wild-type copy of NRPB10, ZP1/ZP7893 for nrpb10; ZP7894/ZP7895 for the wild-type copy of NRPB11, ZP1/ZP7895 for nrpb11-1;
ZP8654/ZP8655 for the wild-type copy of WOX2, ZP1/ZP8655 for wox2-4; ZP2931/ZP2932 for the wild-type copy of PIN7, ZP1/ZP2932 for pin7-1. Genotyping PCRs of the JANUSg-GFP:janus were performed using the following primers: ZP7860/ZP7666 for the endogenous JANUS and ZP7190/ZP7191 for the transgene. ACT2 was amplified with the primer pair ZP16/ZP17.

For qRT-PCRs analyzing the expression of JANUS, SCR, and WOX8, total RNAs were isolated from siliques (2-3 days after anthesis). For qRT-PCRs analyzing the expression of WOX2 and PIN7, total RNAs were isolated from seedlings at 12 days after germination (DAG) of wild type and Pro35S::JANUS-RNAi plants. For RT-PCRs analyzing the splicing pattern of WOX2 and PIN7, total RNAs were isolated from siliques (2-3 days after anthesis) of wild type, janus/+; and Pro35S::JANUS-RNAi plants. Total RNAs were isolated using a Qiagen RNeasy plant mini kit according to manufacture’s instructions. Oligo(dT)-primed cDNAs were synthesized using Superscript III reverse transcriptase with on-column DNase II digestion (Invitrogen). The qRT-PCRs were performed with the Bio-Rad CFX96 real-time system using SYBR Green real-time PCR master mix (Toyobo) as described (Zhou et al., 2013). GAPDH and ACT2 were used as a quantitative control for qRT-PCR. All experiments were repeated in three biological replicates with similar results. All primers are listed in Supplemental Table 3.

Ovule clearing
Ovules were dissected from siliques and cleared with Hoyer’s solution following the protocol described previously (Yadegari et al., 1994). A Zeiss LSM880 laser scanning microscope with differential interference contrast (DIC) optics was used to capture the images of cleared embryos.

Protein interaction assays
Y2H assays were performed as described (Park et al., 2014) with slight modifications. Briefly, different combinations of bait and prey vectors were co-transformed into the Y2HGold yeast strain (Clontech). Positive interactions were determined by the appearance of blue colonies after 3 days on YSD-WHLA supplemented with 80 mg/l X-α-Gal. BiFC by Agrobacterium infiltration was performed as described, in which a P19 protein was used to suppress gene silencing (Park et al., 2014). U1-70K-mCherry was used as the nuclear marker (Wang et al., 2012). Confocal imaging was performed 48 hours after infiltration.

Fluorescence imaging and quantification
Fluorescence images were captured using a Zeiss LSM880 laser scanning microscope with a 20 or 40/1.3 oil objective. Fluorescence of GFP, YFP, mCherry and PI staining was captured using the following excitation/emission settings: 488 nm/505-550 nm for GFP, 514 nm/530–590 nm for YFP, 561 nm/600–650 nm for mCherry and PI staining. Image processing was performed with the Zeiss LSM image processing software (Zeiss). Embryos of the ProWOX2:DsRed2;ProWOX8g:NLs-vYFP3 or the ProWOX2:DsRed2;ProWOX8g:NLs-vYFP3:janus+/+. Containing constructs were transformed into seedlings under the control of the ProWOX2::DsRed2;ProWOX8g:NLs-vYFP3 promoter. Embryos were imaged using a confocal microscope. Regions of interest (ROI) were defined as the nuclear region. Quantification of fluorescence intensity was performed using 20 roots involving 40 to 60 cells. Quantification of fluorescence intensity was performed with ImageJ. Statistical analyses were done by Student’s t-test.

Chromatin immunoprecipitation (ChIP)
ChIP assays were performed as described (Saleh et al., 2008). Wild-type and Pro35S::JANUS-RNAi seedlings at 12 DAG of 2 g were harvested in a cross-linking buffer (0.4 M sucrose, 10 mM Tris-HCl (pH8.0), 1 mM PMSF, 1 mM EDTA, 1% formaldehyde) for 10 min using vacuum infiltration and then halted in 2 M glycine. After the addition of 5 μg Pol II antibodies (Abcam) to the chromatin and incubation at 4°C overnight, the agarose beads of protein A and protein G were added and incubated at 4°C for 2.5 h. After reverse cross-linking, DNA was purified and dissolved in 20 μl water. The immuno-precipitated DNA was diluted and then quantified by real-time PCR. Real-time PCR data of Pol II were normalized to Actin. The
enrichment of Pol II at the genomic locus of PIN7 was given as the ratio of (Pol II PIN7/input PIN7) to (Pol II Actin/input Actin). ZY8596/ZY8597 for PIN7 fragment A; ZY8598/ZY8599 for PIN7 fragment B. Primers are listed in Supplemental Table 3.

Detection of alternative splicing
Primers designed within the introns were used in RT-PCR. Splicing efficiency was measured as described (Mahrez et al., 2016) where a primer in an exon was combined with a primer in a neighboring intron (for the unspliced transcript) or covering the splicing junction (for the spliced transcript).

Phylogenetic analysis and homology modeling
Protein homologs of JANUS were identified by performing BLASTP searches against the NCBI protein database (http://www.ncbi.nlm.nih.gov/) using default parameters. Multiple sequence alignment of JANUS homologs in various species was generated with ClustalX1.83. Then the alignment result was used to build the phylogenetic tree using MEGA5.1. The neighbor-joining method was used with a bootstrap (1000 replicates) test of phylogeny. The predicted structural models of JANUS were obtained by SWISS-MODEL (http://www.swissmodel.expasy.org/), while the crystal structure of human SAP49 was used as the template. The finished models were visualized using Swiss-Pdb Viewer 4.1.0.

Accession numbers
Sequence data in this article can be found in TAIR (The Arabidopsis Information Resource) under these accession numbers: JANUS (At2g18510), NRPB1 (At4g35800), NRPB2 (At4g21710), NRPB3 (At2g15430), NRPB4 (At5g09920), NRPB5 (At3g22320), NRPB6a (At5g51940), NRPB7 (At5g59180), NRPB8a (At1g54250), NRPB8b (At3g59600), NRPB9a (At3g16980), NRPB9b (At4g16265), NRPB10 (At1g11475), NRPB11 (At3g52090), NRPB12 (At5g41010), PIN7 (At1g23080), SCR (At3g54220), WOX2 (At5g59340), WOX5 (At3g11260), WOX8 (At5g45980).

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