A network of transcriptional repressors modulates auxin responses

The regulation of signalling capacity, combined with the spatiotemporal distribution of developmental signals themselves, is pivotal in setting developmental responses in both plants and animals. The hormone auxin is a key signal for plant growth and development that acts through the AUXIN RESPONSE FACTOR (ARF) transcription factors. A subset of these, the conserved class A ARFs, are transcriptional activators of auxin-responsive target genes that are essential for regulating auxin signalling throughout the plant lifecycle. Although class A ARFs have tissue-specific expression patterns, how their expression is regulated is unknown. Here we show, by investigating chromatin modifications and accessibility, that loci encoding these proteins are constitutively open for transcription. Through yeast one-hybrid screening, we identify the transcriptional regulators of the genes encoding class A ARFs from Arabidopsis thaliana and demonstrate that each gene is controlled by specific sets of transcriptional regulators. Transient transformation assays and expression analyses in mutants reveal that, in planta, the majority of these regulators repress the transcription of genes encoding class A ARFs. These observations support a scenario in which the default configuration of open chromatin enables a network of transcriptional repressors to regulate expression levels of class A ARF proteins and modulate auxin signalling output throughout development.

Transcriptional regulation of class A ARF genes

Class A ARF proteins are encoded by genes with 11–14 introns, with the first introns of ARF6 and ARF19 being around three times larger than the other introns. We tested the role of upstream sequences in determining the expression of class A ARF genes by comparing the patterns in meristems from transcriptional reporter lines (Fig. 1a, b, Extended Data Fig. 1a–j) using either the sequences 3–5 kb 5′ of the ATG and 3′ of the ATG up to the end of the first intron of ARF6, ARF7 and ARF19 or the 5′ sequences alone (designated respectively pARF and pARF exon). We observed a difference between the two reporters only for ARF7 (Fig. 1a, b, Extended Data Fig. 1c, h): the ARF7 transcriptional reporter including the first intron, but not the version lacking it, showed strong expression in the root apical meristem (Fig. 1b), implying that the 3′ sequence contains regulatory information required for ARF7 expression in the root. Furthermore, comparison with the patterns of class A ARF expression seen when using reporters with shorter (2-kb) promoters (Extended Data Fig. 1k–o) and with those observed through RNA in situ hybridization (Extended Data Fig. 1p–r) showed that sequences upstream of the first 2 kb 5′ of the ATG codon are necessary for the regulation of class A ARF expression.

Chromatin status of class A ARF loci

Specific expression patterns of class A ARF genes could be due to tissue-specific differences in the chromatin accessibility of these loci. We analysed the chromatin status of each class A ARF locus by scoring the presence of the histone H3 lysine 27 and lysine 4 trimethylation...
Meta-analysis of published datasets covering a range of tissues and loci allows them to be actively transcribed at different tissues and developmental stages, implying that the spatial expression pattern specific to class A ARF genes does not result primarily from alternate chromatin states with contrasting accessibility.

Repressors as regulators of class A ARF genes

Alternatively, specific spatiotemporal transcription of class A ARF loci could arise from regulatory networks made up of transcription factors (TFs). To identify TFs that could regulate the transcription of class A ARF genes, we used a semiautomated enhanced yeast one-hybrid (eY1H) assay with baits consisting of promoter sequences identical to those from the transcriptional reporter lines described above. The assay yielded 42 previously unrecognized putative transcriptional regulators of class A ARF genes (Fig. 2, Extended Data Fig. 3a, b, Supplementary Table 2). Analysis of this candidate gene-regulatory network indicated that individual class A ARF loci are likely to be regulated by specific sets of TFs, as only four TFs were identified as binding multiple class ARF sequences. Based on the expression of these TFs, the network may contain proteins that mediate either root- or shoot-specific responses (Extended Data Fig. 3c).

Most TFs in the network are involved in development, but many putative regulators of ARF8 are associated with biotic and abiotic stress (Extended Data Fig. 3a, d, Supplementary Table 2). ARF8 may therefore act as an environmental hub mediating auxin responsiveness, and indeed it has been shown to be involved in plant responses to both biotic and abiotic stresses.

To validate this regulatory network, we searched the class A ARF promoters for the presence of binding sites for the TFs identified by eY1H. We predicted the presence of many of these TF-binding sites within the ARF promoters and found that a small proportion of the inferred bindings have been confirmed experimentally (Extended Data Fig. 3e–g, Supplementary Table 3). Next, we systematically tested the regulatory activity of each TF through transient expression analysis using the TFs either alone or fused to the VP16 transactivation domain (Extended Data Fig. 4a, b, Supplementary Table 4). Thirty-four of 42 (81%) TFs induced a significant change in expression of their class A ARF target(s), corresponding to a decrease in mRNA transcript level for 32 of the 34 class A ARF genes (94%, or 76% of the total TFs; Fig. 2, Supplementary Table 4, Supplementary Note 1). We observed transcriptional repression of class A ARF genes in the majority of cases, both for TFs alone and for TF-VP16 fusions, indicating a strong repressive activity (Extended Data Fig. 4c, d, Supplementary Table 4).

Together, our data reveal a functional regulatory network controlling the transcription of class A ARF genes and demonstrate that this is regulated by TF-mediated repression.

Expression of class A ARF regulators

If the expression of class A ARF proteins is controlled by tissue-specific transcriptional repression, we would expect many of the repressors involved to have expression patterns complementary to those of their target ARF gene. To test for complementarity of expression with a high spatial resolution, we generated transcriptional reporters for six TFs and investigated them in seven combinations with class A ARF reporters in both root and shoot apical meristem (Fig. 3a, b, Extended Data Fig. 5). We observed complementary expression patterns in the root in five of the seven cases (Fig. 3b, Extended Data Fig. 5a, b). In the shoot, we assessed two combinations involving WRKY11 and At2g26940. We detected WRKY11 only in meristem layers L2/3, whereas its target ARF8 is expressed specifically in layer L1 (Fig. 3a). In the shoot apical meristem, At2g26940 is expressed weakly in the centre of the meristem, whereas ARF19 is expressed, also weakly, in flower primordia (Extended Data Fig. 5c). Hence, repressors and their target ARFs have mostly complementary expression patterns in both shoot and root tissues, although repressors and their targets co-localize in some cells, as for other TFs.

Mutants of class ARF regulatory genes

To further test the significance of our results in planta, we characterized mutants of 24 TFs from the regulatory network, representing regulators of all five class A ARFs (Supplementary Table 5). We measured the expression of the target class A ARF genes using qRT–PCR in whole root and shoot tissues (Extended Data Fig. 6, Supplementary Table 6). We
detected changes in the expression of target class A ARF genes identified in our network in 11 of the 24 mutants (46%). Four showed upregulation of their target ARFs, compatible with a repressive activity. The other seven, of which six are ARF8 regulators, showed downregulation of their target ARF. In the case of ARF8, this could be explained by complex, nonlinear regulation of ARF8 expression by multiple TFs. Indeed, the ARF8 regulators tested are themselves directly or indirectly regulated transcriptionally by ARF8 both negatively and positively, thus establishing a network structure that could result in upregulation of ARF8 in mutants (Extended Data Fig. 7, Supplementary Note 2). The low

Fig. 2 | Class A ARF transcription is regulated by repressors. eY1H promoter–transcription factor interaction network for class A ARF genes. Interactions between class A ARF promoters (green boxes) and the regulatory TFs listed were tested using transient protoplast assays. Solid lines, confirmed repression; dashed lines, confirmed transcriptional activity; thin grey lines, unconfirmed interaction. Light red background indicates TFs for which binding has been shown by DNA affinity purification sequencing (DAP-seq) or chromatin immunoprecipitation with sequencing (ChIP-seq; see Supplementary Table 3).

Fig. 3 | Expression levels and patterns of class ARF genes are altered when upstream transcription factors are modulated. a, b, ARF8 and WRKY11 show complementary expression patterns in the SAM (a) and RAM (b). c, d, pARF7–driven patterns are altered in the SAM of nf-yb13 mutant. Experiments were done twice (a–d). Scale bars, 40 μm (a), 60 μm (b), 45 μm (c, d). For SAM images, orthogonal projections are shown below. (e) Quantification of auxin response in mutant lines. Graph shows percentage change in root elongation at 15 d for plants with mutations in the indicated genes grown on 10 μM indole-3-acetic acid (IAA, the most common natural auxin) as compared to those grown without IAA. All values normalized to those for wild-type controls (WT). n of WT/mutant plants with and without IAA (P-values), left to right: 26/31 and 29/27 (0.002), 24/28 and 28/29 (0.003), 22/31 and 26/23 (0.015), 27/30 and 30/32 (0.03), 30/32 and 29/31 (0.0003), 24/30 and 31/32 (0.61), 31/31 and 29/30 (0.80), 24/31 and 31/32 (0.98), 18/30 and 30/25 (0.72), 30/30 and 31/28 (0.37), 29/30 and 28/27 (0.28), 30/30 and 29/30 (0.07), 29/30 and 31/25 (0.05), 28/28 and 32/30 (0.24), 19/30 and 27/28 (0.19), 22/25 and 29/28 (0.048), 23/27 and 29/32 (0.016), 21/27 and 27/30 (0.003), 28/30 and 31/27 (0.00002), 15/29 and 28/27 (0.004), 24/25 and 31/28 (3 × 10⁻⁵), 15/28 and 29/30 (0.002), 28/31 and 29/29 (1 × 10⁻⁶) and 28/28 and 27/28 (1 × 10⁻⁶). Statistical analyses: two-sided t test comparing variation in the rate of elongation on IAA against that of the WT control; *P ≤ 0.05, **P ≤ 0.01.
sensitivity of expression analysis on whole tissues could also explain our results. This prompted us to determine at higher spatial definition how TF mutations affect class A ARF expression. We first crossed pARF7::mVENUS and pARF19::mVENUS transcriptional reporters into various TF mutants. For the crf10 and wryk38 mutants, in which our qRT–PCR results had not revealed changes in ARF7 mRNA levels, we observed a significant increase in expression and an expansion of the expression pattern for pARF7::mVENUS in the root apical meristem (Extended Data Fig. 5a, b, h). We also observed enhanced expression of pARF7::mVENUS in the root apical meristem of nfyb13, in this case in agreement with the qRT–PCR results (Extended Data Fig. 5c, h). However, we saw no changes in the expression of pARF19::mVENUS in the root of three mutants we analysed (Extended Data Fig. 5d–f, h). In the shoot apical meristem, pARF7-driven fluorescence in the nfyb13 mutant was identical to that in the wild type in layer L1 but elevated in layers L2 and L3, indicating a change in the spatial pattern of pARF7 expression (Fig. 3c, d, Extended Data Fig. 8h). We also detected expression pattern changes for pARF7::mVENUS in the shoot apical meristem of the wryk38 mutant (Extended Data Fig. 8g, h). In addition, inducible constitutive overexpression of AL3 or CRF10 in the pARF7::mVENUS background triggered a decrease in mVENUS signal (Extended Data Fig. 8i, j). These results confirm in planta that four TFs are repressors and provide examples of how such repressors shape the expression level or pattern of class A ARF genes.

To investigate the functional role of this network, we scored the 24 TF mutants for defects in auxin-regulated root processes (Fig. 3e, Extended Data Fig. 9, Supplementary Table 7). Although none of these mutants had previously been implicated in auxin-dependent responses, 58% (14/24) showed altered root length in response to auxin and 29% (7/24) showed altered gravitropism. Among mutants with altered root length response, 64% (9/14) showed an enhanced response, and all mutants with changes in gravitropism had a faster response. Thus, for both traits, a majority of the TF mutants with altered auxin response show effects opposite to those observed for mutants in loci known to promote auxin signalling,22,23, consistent with a repressive role of the TF. We selected two genes with high auxin responsiveness in the root, IAA13 and IAA19, and tested their expression in the TF mutants. Although we mutated only one TF at a time, we found a small but significant increase in the expression of IAA19 in the roots of seven mutants (+28%), two of which also showed elevated levels of IAA13. A reduction in either IAA13 or IAA19 was observed in a further three mutants (+12%; Supplementary Table 8). A significant number of the mutants also had altered shoot phenotypes, further demonstrating that these TFs have important roles in development (Extended Data Fig. 10, Supplementary Table 9). Taken together, our results support a negative regulation of auxin responses by the corresponding TFs. That mutation of single genes in the class A ARF regulatory network can significantly affect auxin-dependent developmental responses further demonstrates the functional importance of individual nodes of this network.

Discussion

Although gene repression mediated by polycomb repressive complex 2 (PRC2) proteins plays a broad role in tissue-specific expression,35, the general absence of H3K27me3, a hallmark of PRC2 activity, at class A ARF loci indicates that their regulation does not rely on this epigenetic mechanism. This may be because such a system would not allow rapid changes in signalling output. Instead, our data suggest a regulatory system based on the use of transcriptional repressors that modulates expression of constitutively active loci and, in combination with post-translational modifications of class A ARF proteins36,37, constantly adjust auxin responsiveness during development. Other transcriptional regulation networks defined in eukaryotes involve both transcriptional activators and repressors38. Instead, the network we characterize resembles the early scenario proposed by Jacob and Monod39 for transcriptional regulation by repressors only, indicating that there may be a place for the concept that the expression of key developmental regulators may be controlled via transcriptional repression.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2940-2.
Plant material and growth conditions

All transgenic lines were generated in the Col-0 accession of Arabidopsis thaliana. T-DNA insertion mutants in transcription factor–coding genes and the arf8-1 mutant were obtained from NASC. All T-DNA lines were genotyped to confirm that they were homozygous, and qRT–PCR was used to confirm alterations in transcript levels (Supplementary Table S). The accession numbers of T-DNA lines and further details are listed in Supplementary Table S.

For root microscopy and in situ hybridization of ARF transcriptional reporter lines, plants were grown on half-strength Murashige and Skoog (1/2 MS) medium supplemented with 1% sucrose and 1% agar in 24 h light conditions (microscopy) or 12 h light/12 h dark conditions (in situ hybridization). For shoot microscopy, plants were grown in 8 h light/16 h dark conditions for 6 weeks and then transferred to 16 h light/8 h dark conditions for 2 weeks to induce bolting. For the qRT–PCR experiments, the seedlings were grown in 24 h light conditions on 1/2 MS plates containing 1% sucrose and 1% agar for 7 d. For the root imaging of crosses between ARF transcriptional reporter lines and TF mutants and for the co-expression analysis of ARF transcriptional reporter lines with TF transcriptional reporter lines, the plants were grown on 1/2 MS medium supplemented with 0.8% agar and 16 h light/8 h dark light. TF overexpression lines were grown for 12 h light/12 h dark light on 1/2 MS medium supplemented with 1% agar.

Cloning

Multisite Gateway cloning technology was used to generate ARF transcriptional reporter lines harbouring DNA sequences both upstream and downstream from the start codon. The promoter fragments were amplified by PCR with the following sequences: pARF5: bp –5418 to +143; pARF6: bp –3255 to +197; pARF7: bp –2973 to +374; pARF8: bp –5091 to +42; pARF19: bp –4906 to +457. For pARF5, pARF6, pARF8 and pARF19, the fragments were inserted into pDONR P4–P1R and recombined with 3× mVen–N7 pDONR211 (containing triple mVen–coding sequences and an N7 nuclear localization signal), OCS terminator pDONR P2–P3 and pK7m34GW destination vector to yield pARF-3xmVenusN7 constructs.

For pARF7, the fragment was cloned into pCR8/GW/TOPO and recombined with a nuclear-localized mVen–N7, OCS terminator and pK7m34GW to produce the pARF7-mVen–N7 construct. Similarly, the shorter promoter fragments were amplified by PCR based on primers designed at the following locations: pARF5: bp –5418 to –1; pARF6: bp –3255 to –1; pARF7: bp –2973 to –1; pARF8: bp –5091 to –1; pARF19: bp –4906 to –1. The fragments were inserted into pDONR P4–P1R and recombined with 3× mVen–N7 pDONR211, OCS terminator pDONR P2–P3 and pK7m34GW destination vector to yield pARF-3xmVenusN7 shorter transcriptional reporter lines.

All constructs were transformed into Agrobacterium tumefaciens C58PMP90 strain by electroporation and then transformed into Col-0 plants by the floral dip method.

The ARF promoter sequences screened in the eY1H assay were amplified by PCR and sequenced to confirm absence of mutations. The overall promoter sequences screened correspond in length and content to those used in the construction of the transcriptional reporter lines except that the longer promoters were split into two fragments: pARF5 fragment 1: bp –2796 to +134; pARF5 fragment 2: bp –5418 to –2481; pARF6: bp –3255 to +197; pARF7: bp –2973 to +374; pARF8: fragment 1: bp –2899 to +42; pARF8 fragment 2: bp –5091 to –2121; pARF19 fragment 1: bp –2399 to +457; pARF19 fragment 2: bp –4906 to –1992. The amplified fragments were cloned into either pDONR P4–P1R or pENTR 5′ TOPO plasmids by the Gateway BP reaction or using the pENTR 5′-TOPO kit, respectively. The resulting plasmids were recombined with the Gateway LR reaction into both pMW2 and pMW3 Gateway destination vectors designed for yeast expression and containing respectively HIS3 or LacZ reporter genes. The resulting plasmids were transformed into the yeast strain YM4271.

Additional transcription factors were cloned and added to the collection of existing root-specific transcription factors (Supplementary Table S). The transcription factors were amplified by PCR from cDNA collections obtained by isolating total RNA from various tissues. Each full-length transcription factor cDNA PCR product (without a stop codon) was inserted into a pENTR-Zeo plasmid by the Gateway BP reaction and then recombined into the pDEST-AD-2μ destination vector designed for yeast expression and containing a GAL4 activation domain. The vectors were transformed into the yeast strain Yai1687.

To produce the reporter plasmid for the protoplast assays, the promoter fragment of the respective ARF corresponding to the one used in the eY1H assay, and the ARF transcriptional reporter lines described above, were amplified by PCR and cloned into the plasmid pDONR P4–P1R. For the pARF8 promoter, a short part of the 35S promoter (bp –107 to +1) was inserted at bp –115. Separately, a construct containing an NLS followed by the mVen–coding sequence and an OCS terminator was cloned into the plasmid pDONR211. Third, a construct containing the promoter of RPS5a (encoding ribosomal protein S5A), driving TagBFP following by an NLS and a nosT terminator was cloned into the plasmid pDONR P2–P3. These three plasmids were recombined using a multisite Gateway method to yield the final reporter plasmid pARF-NLS-mVen–term-pRPS5a-TagBFP-NLS-term. An alternative reporter plasmid contained a shorter ARF promoter fragment that contained sequences upstream and lacked sequences downstream of the start codon (corresponding to the transcriptional reporter lines with shorter promoters described above). To create the effector plasmid for the protoplast assays, the RPS5a promoter was cloned into pDONR P4–P1R; the cDNA of the respective transcription factor without the stop codon was cloned into pDONR211; and the construct, containing the self-cleaving 2A peptide, followed by mCherry coding sequence, an NLS and a nosT terminator, was cloned into pDONR P2–P3. Finally, these three plasmids were recombined with a multisite Gateway reaction to yield pRPS5a-cDNA 2A-mCherry-NLS-term. An alternative effector plasmid included an activator VP16 domain from the herpes simplex virus fused to the TF cDNA.

Microscopy

Roots of ARF transcriptional reporter lines were imaged 5 d after germination. Plant cell walls were visualized by staining with 15 μg ml−1 pro-pidium iodide solution. Roots were examined using a TCS-SP5 confocal microscope (Leica) with excitation at 514 nm and emission at 526–560 nm for mVen and 605–745 nm for propidium iodide.

For analysis of shoot apical meristems, bolted shoots were dissected under a stereomicroscope and transferred to an apex culture medium (1/2 MS medium supplemented with 1% sucrose, 0.8% agarose, 1× vitamin solution (myoinositol 100 mg l−1, nicotinic acid 1 mg l−1, pyridoxine hydrochloride 1 mg l−1, thiamine hydrochloride 10 mg l−1, glycine 2 mg l−1)), for overnight incubation. Before microscopy, cell walls were stained with 100 μg ml−1 propidium iodide solution. The shoot apices were then examined using a TCS-SP5 confocal microscope (Leica) with excitation at 514 nm and emission at 526–560 nm for mVen and 605–745 nm for propidium iodide.

eY1H assay

The eY1H assay was conducted according to. The ARF promoters screened correspond in length and content to those used in the construction of the transcriptional reporter lines except that the longer promoters (pARF5, pARF8 and pARF19) were split into two fragments (see Cloning section). With the longer promoters, only 1 out of 39 TFs was identified using the distal fragment of the pARF8 promoter. This suggests that the other 38 TFs bind in a region of the promoter from bp −2480 to +134 for pARF5, bp −2120 to +42 bp for pARF8 and bp −1991 to +457 for pARF19.
We used a TF collection enriched in root-expressed TFs expanded with additional TFs involved either in development of the shoot apical meristem or in hormonal regulation (see Supplementary Table 10).

**Transient expression analysis in Arabidopsis protoplasts**

For the protoplast assay Col-0 seedlings were grown in short-day conditions (8 h light/16 h dark) for 37–45 d. Leaves of similar size from the second or third pair were collected and digested in an enzyme solution (1% cellulose R10, 0.25% macerozyme R10, 0.4 M mannitol, 10 mM CaCl2, 20 mM KCl, 0.1% BSA, 20 mM MES at pH 5.7) overnight at room temperature. Protoplasts were collected through a 70-µm mesh, washed twice with ice-cold W5 solution (154 mM NaCl, 125 mM CaCl2, 5 mM KCl, 5 mM glucose, 2 mM MES at pH 5.7) and incubated on ice for 30 min. The protoplasts were then resuspended in MMG solution (0.4 M mannitol, 15 mM MgCl2, 4 mM MES at pH 5.7) at a final concentration 150,000 cells per ml. 10 µl of each the effector and the reporter plasmid DNA (concentration 3 mcg µl−1) were mixed with 200 µl of the protoplasts. Immediately, 220 µl of PEG solution (40% PEG 4000, 0.2 M mannitol, 0.1 M CaCl2) was added and the protoplasts were incubated for 5 min at room temperature and then washed twice in W5 solution. The protoplasts were resuspended in 800 µl of the W5 solution and incubated for 24 h in 16 h light/8 h dark growth chamber. Before imaging, the protoplasts were resuspended in 40 µl W5 solution and subsequently transformed into an 8-well imaging chamber.

A Zeiss 710 LSM confocal microscope was used for imaging the protoplasts (Extended Data Fig. 4). Sequential scanning was performed with mVenus (excitation at S14 emission at 520–539), TagBFP (excitation at 405 and emission at 423–491), mCherry (excitation at 561, emission at 598–636) and bright-field channels. 2 stacks of several protoplasts were taken. The data were analysed using ImageJ software (imagej.net/Fiji). The image with the best focus for each protoplast was selected from the 2 stack. The nucleus was selected and the mean fluorescence was measured as illustrated in Extended Data Fig. 4. The number of replicates was between 15 and 34 protoplasts with a majority of experiments including at least 20 protoplasts. For most ARF-TF interactions, 4 or 5 independent experiments were performed (Supplementary Table 4): 2 or 3 experiments with the standard effector plasmid and 2 experiments with alternative effector plasmid containing VP16 domain. For the statistical analysis, we first run a Kruskal–Wallis H-test on all controls and subsequently transformed into a 8-well imaging chamber.

**Expression analysis with qRT–PCR**

The whole root and the whole shoot parts of the seedlings were collected separately. For one root sample, roots from 30 seedlings grown on the same plate were pooled together. For one shoot sample, 8 shoots from seedlings grown on the same plate were pooled together. Three independent replicates per genotype were collected. RNA was extracted using Spectrum Plant Total RNA kit (Sigma-Aldrich). The DNA was removed using TURBO DNA-free kit (Invitrogen). The cDNA was produced using SuperScript VILO CDNA Synthesis kit (Thermo Fischer) with 500 ng RNA. The cDNA was diluted 1:1000 before use. The qRT–PCR was performed using Applied Biosystems Fast SYBR Green Master Mix. Expression of TUB4 gene was used as standard. The statistical analysis was performed with a one-sided Mann–Whitney test, with P < 0.1 considered as statistically significant. IAAt3 and IAAt9 were chosen as auxin-responsive genes for qRT–PCR analysis in roots from ref. 35.

**Expression analysis of crosses between ARF transcriptional reporter lines and TF mutants**

Mutants of the regulatory transcription factors were crossed with pARF7:mVenus transcriptional reporter line described above. The crosses were selected for the presence of homozygous pARF7:mVenus reporter construct. The F3 generation wild-type and mutant plants were compared.

The roots of 5 d-old plants were stained with 15 µg ml−1 propidium iodide and imaged using the TCS-SP8 (Leica) confocal microscope with excitation at 514 nm and emission at 526–560 nm for mVenus and 605–745 nm for propidium iodide.

For the shoot microscopy the images were taken with a Zeiss 710 LSM confocal microscope. mVenus intensity was measured separately in L1 and in L2/L3 layers in each of the 8 cross-sections with 50 nm distance between each cross-sections. Number of replicates: 7 WT and 7 mutant plants for nf-yb13, 12 WT and 12 mutant plants for wrky38.

**Inducible overexpression of TFs**

Multisite Gateway cloning technology was used to generate TF transcriptional reporter lines. The promoter fragments of TFs were amplified by PCR with sequences: pWRKY11: bp –3626 to –1 bp; pDFOF1.8: bp –4389 to –1; pAt2g26940: bp –3179 to –1 bp; pAt2g44730: bp –2738 to –1 bp; pCRF10: bp –4060 to –1 bp; pZFP6: bp –2117 to –1. The fragments were inserted into pDONR P4-P1R and recombined with 2× mCherry pDONR211 (containing double mCherry coding sequences) and N7 pDONR P2R-P3 (containing a nuclear localization signal) and pB7m34GW (the destination vector containing basta resistance gene for in planta selection) to produce pTF-2xmCherryN7 constructs. These constructs were transformed into the pARF7:mVenus transcriptional reporter line backbones by the floral dip method.

Roots of the plants grown for 5–10 d were imaged using the TCS-SP8 (Leica) confocal microscope, with excitation at S14 nm and emission at 526–560 nm for mVenus and excitation and emission at 587 nm and 610–670 nm respectively for mCherry. Total fluorescence was calculated for individual nuclei from two or three individual roots using a 6-px circular selection in ImageJ. These values were then normalized for each channel based on a scale of 0–1 with the brightest nuclei in each root being set to a value of 1. The shoots were examined using the TCS-SP8 (Leica) confocal microscope, with excitation at S14 nm and emission at 526–560 nm for mVenus and excitation and emission at 587 nm and 610–670 nm respectively for mCherry.

**Expression analysis of crosses between ARF transcriptional reporter lines and TF mutants**

Mutants of the regulatory transcription factors were crossed with pARF7:mVenus transcriptional reporter line described above. The crosses were selected for the presence of homozygous pARF7:mVenus reporter construct. The F3 generation wild-type and mutant plants were compared.

The roots of 5 d-old plants were stained with 15 µg ml−1 propidium iodide and imaged using the TCS-SP8 (Leica) confocal microscope with excitation at 514 nm and emission at 526–560 nm for mVenus and 605–745 nm for propidium iodide.

For the shoot microscopy the images were taken with a Zeiss 710 LSM confocal microscope. mVenus intensity was measured separately in L1 and in L2/L3 layers in each of the 8 cross-sections with 50 nm distance between each cross-sections. Number of replicates: 7 WT and 7 mutant plants for nf-yb13, 12 WT and 12 mutant plants for wrky38.

**Co-expression analysis of ARF transcriptional reporter lines and TF transcriptional reporter lines**

Multisite Gateway cloning technology was used to generate TF transcriptional reporter lines. The promoter fragments of TFs were amplified by PCR with sequences: pWRKY11: bp –3626 to –1 bp; pDFOF1.8: bp –4389 to –1; pAt2g26940: bp –3179 to –1 bp; pAt2g44730: bp –2738 to –1 bp; pCRF10: bp –4060 to –1 bp; pZFP6: bp –2117 to –1. The fragments were inserted into pDONR P4-P1R and recombined with 2× mCherry pDONR211 (containing double mCherry coding sequences) and N7 pDONR P2R-P3 (containing a nuclear localization signal) and pB7m34GW (the destination vector containing basta resistance gene for in planta selection) to produce pTF-2xmCherryN7 constructs. These constructs were transformed into the pARF7:mVenus transcriptional reporter line backbones by the floral dip method.

Roots of the plants grown for 5–10 d were imaged using the TCS-SP8 (Leica) confocal microscope, with excitation at S14 nm and emission at 526–560 nm for mVenus and excitation and emission at 587 nm and 610–670 nm respectively for mCherry. Total fluorescence was calculated for individual nuclei from two or three individual roots using a 6-px circular selection in ImageJ. These values were then normalized for each channel based on a scale of 0–1 with the brightest nuclei in each root being set to a value of 1. The shoots were examined using the TCS-SP8 (Leica) confocal microscope, with excitation at S14 nm and emission at 526–560 nm for mVenus and excitation and emission at 587 nm and 610–670 nm respectively for mCherry.

**Inducible overexpression of TFs**

Multisite Gateway cloning technology was used to generate TF inducible overexpression lines. The chimaeric transcription activator pIR4:pGI090:XVE3 containing XVE followed by the rbs and nos terminators and LexA operon, expressed under UBQ10 promoter was recombined with TF coding sequence (lacking STOP codon) in pDONR211 and the 2A-mCherry-term pDONR P2R-P3 (containing the self-cleaving 2A peptide) followed by the mCherry coding sequence, a nuclear localization sequence (NLS) and a nos T terminator) and pB7m34GW (the destination vector containing basta resistance gene for in planta selection) to produce pUBQ10-XVE-TF-2A-mCherry oestriadiol-inducible constructs. These constructs were transformed in the pARF7-mVenus transcriptional reporter line background by floral dip method.

For the overexpression analysis, roots of the plants grown for 5 d were treated with 10 µM β-oestradiol for 24 h and imaged using the TCS-SP8 (Leica) confocal microscope, with excitation at 514 nm and emission at 526–560 nm for mVenus and excitation and emission at 587 nm and 610–670 nm, respectively, for mCherry.
Shoot phenotype analysis of the TF mutants

24 T-DNA insertion mutants and the wild-type Col-0 were grown in 8 h light/16 h dark conditions on soil for 43 d. Leaf number was counted every 3 d starting from day 24. Rosette diameter was measured at 43 d. After 43 d of growth in the above conditions, the plants were transferred to 16 h light/8 h dark conditions to induce bolting. The following parameters were measured after 21 and 27 d in the 16 h light/8 h dark conditions: length of the main stem, number of cauline branches growing from the main stem, number of axillary branches growing from rosette (the main stem not included). The number of replicates per genotype was 12 plants. For the statistical analysis, an unpaired two-tailed t test was conducted with \( P \leq 0.05 \) considered as statistically significant.

Root phenotype analysis of the TF mutants

For root length measurement and for gravitropic analysis plants were grown on 1/2 MS medium supplemented with 1% agar in 12 h light/12 h dark conditions. For root length analysis, plants were grown either on medium lacking IAA or supplemented with 10 \( \mu \)M IAA. To reduce plate-to-plate variation wild-type plants and mutants were grown on the same agar plate. Images were taken at 15 d and the root length was measured. The number of replicates per genotype was at least 26 plants from the main stem, number of axillary branches growing from rosette (the main stem not included). The number of replicates per genotype was at least 26 plants. Rootov n v.1.8 software (https://www.nottingham.ac.uk/research/groups/cvl/software/rootovn.aspx) was used for data analysis. Statistical analysis was done with unpaired two-tailed t test with \( P \leq 0.05 \) considered as statistically significant.

In situ hybridization

For RNA probe synthesis, 300–500 bp templates were amplified from a cDNA library adding the T7 RNA polymerase promoter sequence at the 5’ prime overhang. The products were gel purified and used directly as a template for transcription with DIG RNA Labelling Kit (SP6/T7, Roche). The following primers were used: 3’-ctgtgtgacctgcttcgatggtcgggagctgcgtgga-5’ and 3’-ggtcctgcacgctgactaatg-5’ (ARF5); 3’-gacggtcctgcacgctgactaatg-5’ and 3’-ggtcctgcacgctgactaatg-5’ (ARF6); 3’-gtggagggggtttgacattccgttcggcat-5’ (ARF8). Roots were cut from 4-d-old plants and vacuum-infiltrated in 500 \( \mu \)g ml\(^{-1}\) tRNA, 5 mM EDTA, 300 mM NaCl, 10 mM Tris pH 7.0, 10 mM sodium phosphate pH 7.0), denatured at 80 \( ^\circ \)C for 2 min and applied to the samples which were placed into the wet chamber aligned with paper towels soaked in the soaking solution (2× SSC in 1% formaldehyde). The samples were hybridized overnight at 50 \( ^\circ \)C. The samples were washed 4 times with 0.2× SSC at 55 \( ^\circ \)C for 30 min and then once each with 0.2× SSC at 37 \( ^\circ \)C for 5 min and 0.2× SSC at room temperature for 5 min, PBS 5 min. Detection was done by incubating the samples in 1% blocking solution (1% blocking reagent, 100 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.3% Triton X-100) for 45 min and then in a wet chamber with antibody solution (anti-Digoxigenin-AP 1:250 in 1% blocking solution) for 1.5 h, washing 3 times with buffer A (1% BSA in 100 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.3% Triton X-100) for 30 min, washing twice with detection buffer (100 mM Tris pH 9.5, 100 mM NaCl, 50 mM MgCl\(_2\)) for 5 min each, applying 200 \( \mu \)l of colour substrate solution (4.5 ml detection buffer + 90 \( \mu \)l NBT-BCIP) and incubating 24 h for ARF5 and ARF6 and overnight for ARF8 at room temperature. The reactions were stopped by washing the samples twice with TE buffer for 5 min each. The samples were then mounted in 50% glycerol and observed under the light microscope.

In silico analyses

Analysis of expression and function of regulatory TFs. Expression of TFs in the root and the shoot apical meristems was analysed using cell type-specific expression profiles from refs. 38–40. Overrepresentation of TF gene families was analysed for families represented by two or more members in the network. The number of gene family members in the network was compared to total number of genes from the same family in the TF library. Statistical analysis was done using a hypergeometric test, with \( P \leq 0.05 \) considered as statistically significant.

Involvement of TFs in specific developmental processes (development, biotic and abiotic stress) was analysed based on literature description.

Chromatin state analysis. Binary data on H3K27me3- and H3K4me3-marked genes and chromatin accessibility regions were retrieved from multiple datasets covering a range of tissues and developmental stages. For each dataset, at least two biological replicates were considered, and only the presence of a given ARF in both gene lists was scored as a positive association with a chromatin mark or an accessible region.

Datasets used for chromatin marking analysis were: H3K27me3, from refs. 17, 41–43 (GEO database GSE24657, GSE7907, GSE42450, GSE50636, GSE24657, GSE24710, GSE19654; ArrayExpress database E-MTAB-4680, E-MTAB-4684); H3K4me3, from refs. 17, 41–43 (GEO GSE24657, GSE50636, GSE24665, GSE19654; ArrayExpress E-MTAB-4680, E-MTAB-4684).

Datasets used for chromatin accessibility analysis were: DNase hypersensitive sites, from ref. 46 (GEO GSM2893358, GSM289362, GSM289374); FANS-ATAC-defined accessible regions, from ref. 47 (GEO GSM2260231, GSM2260232, GSM2260235, GSM2260236); ATAC-defined transposase hypersensitive sites, from refs. 48, 49 (GEO GSM2704255, GSM2704256, GSM2719200, GSM2719201, GSM2719203, GSM2719204, GSM2719205). For each chromatin accessibility dataset, the presence of at least one accessible region within the ARF gene and up to 1 kb upstream of its transcription start site was scored using ad hoc scripts.

Visualization of epigenomic data was carried out using the IGV software.

Binding motif search and reanalysis of DAP-seq data. Position weight matrices (PWM) available for TFs identified in the eY1H screen were retrieved from the Jaspar\(^{33}\) and CisBP\(^{37}\) databases. Using these PWMs, we computed the best score of the TF binding sites present in each Arabidopsis 2-kb promoter with an R script using the Biostrings library (https://bioconductor.org/packages/releases/biohtml/Biostrings.html) and ranked the class AARF gene promoter among all Arabidopsis promoters based on this score. As negative control, this operation was repeated identically five times for each class AARF promoter with 20 randomly selected TFs (excluding specific TF classes and families identified in the eY1H screen). The distributions of class AARF promoter ranks with eY1H-selected and randomly selected TFs were compared using a one-sided t-test.
DAP-seq files containing the peak list from ref. 20 were retrieved (GEO accession number GSE60141). Bedtools intersect (bedtools.readthedocs.io/en/latest/index.html) was then used with the --wb option to determine which DAP peak overlap with each promoter.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**

The data including the source data that supports the finding of this study are available within the paper, its supplementary information files or publicly available datasets. Publicly available position weight matrices were obtained from the Jaspar and CisBP databases. Publicly available chromatin marking and accessibility datasets were acquired from the GEO and ArrayExpress databases with the following accession numbers: GSE24645, GSE24658, GSE7907, GSE24507, GSE50636, GSE24467, GSE24710, GSE19654, GSM2260231, GSM2260232, GSM2260235, GSM2260236, GSM2704255, GSM2704256, GSM2719200, GSM2719201, GSM2719202, GSM2719203, GSM2719204, GSM2719205, GSM1289362, GSM1289374, E-MTAB-4680, E-MTAB-4684 and GSM128938.

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Author contributions A.B. and TV. designed the study and supervised the work; J.T., F.R., F.E., S.M.B., A.B. and TV. designed the experiments; J.T., A.M.B. and M.E.S. performed the eYIH screen with the help of S.P. and M.B.; JT, J.H., E.C., C.S.G.-A., S.L. and G.B. performed all experiments in relation to TF biological activity characterization; J.L. performed the statistical analysis of the protoplast experiment and participated in all statistical analysis; O.S. and A.F.M. performed the in situ hybridization experiments; A.S. and F.P. performed TF binding site analysis; S.B. and E.F. performed the modelling analysis; J.M. and F.R. performed the epigenetic data analysis; all authors were involved in data analysis; J.T., A.B. and TV. wrote the manuscript with inputs from all authors.

Competing interests The authors declare no competing interests.

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Extended Data Fig. 1 | Analysis of class A ARF expression in the RAM and the SAM using transcriptional reporter lines and in situ hybridization. 

a–j, Confocal images showing expression of ARF5 (a, f), ARF6 (b, g), ARF7 (c, h), ARF8 (d, i) and ARF19 (e, j) in the RAM and the SAM using promoters that lack sequences downstream of the start codon but contain the long upstream sequences (pARF\textsuperscript{−intron}\textsuperscript{::}mVenus) (~3 kb for ARF6 and ARF7; 5 kb for ARF5, ARF8 and ARF19) (see Methods). For SAM images (f–j) an orthogonal projection is shown below to provide information about expression in different layers. 
k–o, For comparison, the expression of each class A ARF gene in the SAM using the previously published pARF::GFP lines with shorter (~2 kb) promoters containing sequences upstream of the start codon is shown in panels k–o\textsuperscript{14}. 

ARF5 (k), ARF6 (l), ARF7 (m), ARF8 (n) and ARF19 (o). (p–r) In situ hybridizations through the RAM for ARF5 (p), ARF6 (q) and ARF8 (r). Note that expression patterns of the class A ARF reporters (a–j) differ from those with shorter (2 kb) promoters (k–o\textsuperscript{14}) and recapitulate the patterns observed with RNA in situ hybridization (p–r; ref. \textsuperscript{15}). This was particularly clear in the shoot for ARF5 and ARF6. Shorter promoters drive GFP expression mostly in flower boundaries for ARF5 and throughout the meristem for ARF6, in contrast with detection of both genes throughout the periphery of the meristem both with longer promoters (k–o; also Fig. If–j) or using in situ hybridization\textsuperscript{15}. Experiments were done three (a–e) and two times (f–r). Scale bars: 50 μm.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Distribution of the repressive chromatin marker H3K27me3, the active chromatin marker H3K4me3 and chromatin accessibility at class A ARF loci.  

**a.** Chromatin landscape of class A ARF and LEC2 in whole seedlings illustrating the chromatin status of class A ARF loci. Repressive H3K27me3 marker (top row), active H3K4me3 marker (middle row) and FANS-ATAC chromatin accessibility (bottom row; see Supplementary Table 1).  

**b, c.** Chromatin landscape of class A ARF and LEC2 loci showing distribution of the repressive chromatin marker H3K27me3 (a) and the active chromatin marker H3K4me3 (b) in various tissues. Seedling, whole seedlings45; leaf, rosette leaves46; root, whole roots45; seedling 2, whole seedlings44; SAM, shoot apical meristems after 0, 1, 2 or 3 d in long-day conditions44. Gene models are shown below with arrowheads indicating direction of transcription.  

**d.** The chromatin landscape of class A ARF and LEC2 loci showing chromatin accessibility in various tissues. DNaseI-seq seedling: DNaseI hypersensitive sites in whole seedling46; DNaseI-seq root: DNaseI hypersensitive sites in root46; FANS-ATAC seedling: FANS-ATAC accessible regions in whole seedling47; FANS-ATAC roots: FANS-ATAC accessible regions in roots46; INTACT-ATAC root tip: INTACT-ATAC transposase hypersensitive sites in root tips48. The LEC2 locus is included as a negative control for H3K4me3 marking and chromatin accessibility, and as a positive control for H3K27me3 marking45. The y axis scales (at right) show the minimum and maximum number of reads represented in each windows of the same row, except for the data set related to ref. 45, for which the data range corresponds to the IP/INPUT value of the ChIP-chip experiments. For the x axis the window size is fixed at 8.5 kb and centred on the gene of interest (gene model in blue below each column, 5′ sequences in green), with arrowheads by the gene name showing the direction of the locus.
Extended Data Fig. 3. See next page for caption.
**Extended Data Fig. 3 | Characterization of the TFs and TF binding sites that regulate class A ARF expression.**

**a**, Yeast one-hybrid promoter–transcription factor interaction network for class A ARF genes. Green boxes correspond to the class A ARF; pink boxes are transcription factors binding to the ARF promoters. TF-associated functions and expression analysis are indicated in the upper and lower small boxes and colour-coded as indicated in the key. Note that when two promoter fragments were used for the screen (see Methods), 35 out of 36 regulators bound to the more proximal fragment, supporting previous observations that the majority of transcription factor binding sites reside within a few kb of the transcriptional start site

**b**, Frequency of TF gene families in the Y1H library collection (black) and in the Y1H network (white). Only families represented by at least two members in the Y1H network were analysed. The network is overrepresented with members of the WRKY and SPL TF families. Statistical analysis: hypergeometric test significant to 5% (*; P = 4e-05 for WRKY family and P = 0.044 for SPL family). Sample sizes for TFs in Y1H library in black/Y1H network in white:

- WRKY: n = 29/8 TFs
- AP2/ERF: n = 68/6
- NAC: n = 91/6
- SPL: n = 7/2 TFs
- homeobox: n = 52/2 TFs
- bHLH: n = 52/2 TFs

**c**, TF expression in the RAM and the SAM. 50% of the identified TFs are expressed in both shoots and roots, whereas 24% and 14% are expressed specifically in roots or shoots respectively.

**d**, Known functions of the TFs in the Y1H network based on a literature search (see also Supplementary Table 2).

**e**, Boxplot representation of the distribution of class A ARF promoter ranks. For TFs with established binding models, we ranked class A ARF promoters among all Arabidopsis promoters based on the score of the predicted TF binding sites. We repeated the same operation with a set of randomly chosen TFs from different families (see Methods). The comparison of rank distributions with those of a set of randomly chosen TFs from different families revealed significantly higher ranks for eY1H-identified TFs (see also Supplementary Table 3). Statistical analysis: one-sided t-test. Sample sizes: n = 29 for eY1H-selected TFs and n = 100 for randomly selected TFs. Data are represented as boxplots where the middle line is the median, the lower and upper hinges correspond to the first and third quartiles, the upper whisker extends from the hinge to the largest value no further than 1.5× interquartile range (IQR) from the hinge and the lower whisker extends from the hinge to the smallest value at most 1.5× IQR of the hinge. All the individual values are plotted.

**f**, Summary of the DAP-seq analysis for the 17 TFs (see also Supplementary Table 3). G, Example of DAP-seq data, here a DAP-seq peak for WRKY33 in the promoter of ARF8. DAP-seq (f, g) thus confirms experimentally inferred bindings (e) for 4 of the 17 (24%) TFs for which DAP-seq data are available (see also Supplementary Table 3). Note also that chromatin immunoprecipitation sequencing (ChIP-seq) confirms the binding of WUSCHEL to the ARF8 promoter.
Extended Data Fig. 4 | Methodology used for the transient protoplast assay. 

**a**  Design of the standard reporter plasmid containing sequences upstream and downstream of the ARF promoter including the first intron (1), the alternative reporter plasmid containing only sequences upstream of the ARF promoter (2), the standard effector plasmid (3), and an alternative effector plasmid containing the VP16 domain fused to the TF coding sequence (4).

**b**  Example of a nucleus of a transformed living protoplast imaged with confocal microscopy with channels for mVenus, TagBFP, mCherry and bright-field. The presence of TagBFP and mCherry specifically in the nucleus is used as a transformation control and as a test of viability of the protoplasts. Quantification: definition of the nucleus as a region of interest using ImageJ to quantify fluorescence (see also Methods). Measurements were conducted in at least 4 independent experiments for each TF (minimum of 2 experiments for TF alone and 2 experiments for TF fused to VP16 domain). Scale bars, 10 μm.

**c, d**  Example of results using the ARF5 reporter plasmid, with (c) and without (d) the VP16 activator domain fused to the TF coding sequence (left and right). Error bars, mean ± s.d.; statistical analysis, one-sided Mann–Whitney U-test with \( P \leq 0.05 \) (*); \( N \) of protoplasts (\( P \) values): (c) control, \( n = 35 \); DOF1.8, \( n = 38 \) (0.33); KNAT1, \( n = 37 \) (0.11); LBD3, \( n = 38 \) (6e-04); SMZ, \( n = 43 \) (3e-10); (d) control, \( n = 43 \) (1e-07); DOF1.8-VP16, \( n = 46 \); KNAT1-VP16, \( n = 44 \) (0.37); LBD3-VP16, \( n = 32 \) (1e-05); SMZ-VP16, \( n = 39 \) (0.015).
Extended Data Fig. 5 | ARF transcriptional regulators mostly show complementary expression patterns to their target ARFs. a, Plants carrying the ARF transcriptional reporters were transformed with transcriptional reporters for a subset of ARF regulators driving mCherry. For five out of seven constructs (see also Fig. 3), we saw complementary patterns of expression between transcriptional repressors and their ARFs in the root. b, To further quantify the complementarity of TF versus ARF expression, we quantified the red versus green fluorescence levels in individual nuclei from different cell types (root cap, blue diamond; columella, green triangle; epidermis, red square; vascular cells, purple cross). These values were normalized so that the brightest nucleus of each channel in each line was set to 1, and values were plotted onto scatter plots. Any value falling outside the reference lines shows a >4× bias for expression of either TF or ARF (n = 3 for pAT2G26940::mCherry and pAT2G44730::mCherry in pARF8::mVenus; n = 2 for the remaining genotypes). In some cases there was clear complementarity in some cell types but not others. For example, ZFP6 shows complementary expression patterns in the root cap, epidermis and columella but overlaps with ARF8 in the vascular tissues. c, Analysis of At2g26940 expression in the SAM, where it was found in organ primordia and weakly in the centre of the SAM; no clear expression was observed in roots. As previously observed with other developmental and hormonal regulators22,23, co-localization of repressors and their target ARF occurs in some cells as in the case of ZFP6/ARF8 in the root epidermis (a, b) and At2g26940/ARF19 in shoot organ primordia (c), suggesting potential regulatory interactions to modulate transcription levels. Scale bars, 60 μm (a) and 40 μm (c). Experiments were done twice (a, c).
Extended Data Fig. 6 | Expression of class A ARF in mutants for the regulatory transcription factors. Expression of class A ARF in 24 mutants of the regulatory TFs measured with qRT–PCR, in whole root and whole shoot tissue of 7-d-old seedlings. Green boxes indicate statistically significant upregulation of the corresponding ARF in the mutant background compared to wild-type control, and blue boxes indicate statistically significant downregulation. Statistical analysis was performed using a one-sided Mann–Whitney test and a threshold at $P \leq 0.1$. For simplicity, only the interactions predicted by the Y1H are shown, with other combinations shaded with a grey box. The full data set is available in Supplementary Table 6.

| ARF | Root | Shoot |
|-----|------|-------|
| ARF5 | [ ] | [ ] |
| ARF6 | [ ] | [ ] |
| ARF7 | [ ] | [ ] |
| ARF8 | [ ] | [ ] |
| ARF19 | [ ] | [ ] |

- **DOF1.8 (5)**
- **lb3 (5 & 19)**
- **nf-yb13 (6&7)**
- **At2g26940 (6,8 & 19)**
- **crf10 (7)**
- **wrky38 (7)**
- **al3 (7)**
- **hfr1 (8)**
- **ntl4 (8)**
- **iaa30 (8)**
- **abs2 (8)**
- **asil1 (8)**
- **At1g61730 (8)**
- **wrky4 (8)**
- **wrky11 (8)**
- **wrky17 (8)**
- **wrky21 (8)**
- **At1g26610 (8)**
- **abf4 (8)**
- **At2g44730 (8)**
- **ddf1 (8)**
- **zfp6 (8)**
- **myb65 (19)**
- **nlp5 (19)**
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Feedback regulations between the transcription factors and auxin signalling. a, Expression of several TFs are regulated by auxin, which proves feedback regulation from auxin signalling output primarily on ARF8 expression. Expression was measured after treatment with 1μM IAA for 30 min, 1h or 3h56. Green boxes indicate upregulation, blue boxes indicate downregulation of gene expression compared with a mock treatment. b, Schematic representation of ARF8 regulation with feedbacks. Feedback from auxin signalling on regulatory TFs is expected to induce complex nonlinear regulation of ARF8 expression (see also Supplementary Note 2). c, Diagrammatic representation of the interactions taking place for different instances of model analysed in Supplementary Note 2. The two diagrams on the right (without feedback) are identical. However, for comparison with the models with feedback the parameters used for these differ (see Supplementary Note 2). d–g, left, bar chart displaying concentrations before and after knock out of transcription factor X, where Y is activated (d) or repressed (f) by ARF. Right, contour plot displaying ARF transcription rate before and after knock out of transcription factor X relative to Y and X populations, where Y is activated (f) or repressed (g) by ARF. Steady-state (SS) values corresponding to the bar plot are also reported. These results are discussed in Supplementary Note 2.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Modulating the levels of ARF transcriptional regulators regulates the expression of associated ARFs. a–f, Comparison of ARF expression in wild-type versus mutants in roots. g, Comparison of pARF7::VENUS expression in wild-type versus wrky38 shoot. For quantification (see f), fluorescence was measured in the central zone and primordia 2 (green circles). h, Quantification of fluorescence changes shown as relative changes in mean fluorescence level in mutant compared to wild type (single value). Quantifications are shown for a–g and for Fig. 3c, d. In roots, the total pARF7/19-driven fluorescent signal was quantified within a standardized zone covering the stele meristem zone and quantified relative to the wild-type controls. In the shoot, L1 and L2 correspond to quantification in the corresponding layers in the SAM of wild-type and nf-yb13 (see also Fig. 3c, d). Quantification demonstrated a significant change in pattern in wrky38 mutant SAMs (g), with an increase of pARF7 activity in the centre and a loss of the differential expression between the SAM centre and lateral organs. Statistical analysis: unpaired two-sided t test with P ≤ 0.01 (**). Number of samples observed and quantified: for mutant/wild type roots, 13/13 for crf10, 12/14 for wrky38, 9/9 for nf-yb13, 9/8 for At2g26940, 12/11 for myb65, 12/10 for nlp5; 7 shoots for nf-yb13 and wild-type controls; 7 shoots for wrky38 and 6 wild-type controls. P values from left to right: 0.003, 2e-05, 3e-08, 0.26, 0.57, 0.11, 0.84, 0.007, 0.009. Raw data are provided in Supplementary Table 11. I. Inducible constitutive overexpression of CRF10:mCherry and AL3:mCherry in the pARF7::VENUS line. pARF7::VENUS is shown in yellow and the transcription factors fused to mCherry in red following a 24h induction with β-oestradiol. J, Both lines shown in I show a significant reduction in pARF7::VENUS expression. Unpaired two-sided t test: P = 4e-10 (CRF10) and 2e-10 (AL3). Number of plants: wild-type control, n = 15; CRF10, n = 21; AL3, n = 20. Error bars: mean ± s.d.. Scale bars: 45 μm for root images; 50 μm for shoot images. For each analysis, the confocal settings were identical in the compared genetic backgrounds. All experiments were done two times.
Extended Data Fig. 9 | Mutations in transcriptional regulators of class A ARF genes accelerate the root gravitropic response. a–g, Kinetics of perturbed gravitropic responses of TF mutants (dashed line) compared to wild-type (solid line) over 12 h after application of the gravitational stimulus. Mutants with statistically significant difference in gravitropic response compared to the wild-type are shown: (a) nlp5, (b) zfp6, (c) al3, (d) at2g44730, (e) wrky11, (f) myb65 and (g) wrky38. Statistical analyses: unpaired two-tailed t-test with $P \leq 0.05$. $P$ values from 1 h to 12 h (left to right): (a) $0.86, 0.19, 0.37, 0.004, 0.01, 0.008, 0.0008, 0.001, 0.007, 0.004, 0.06, 0.07$; (b) $0.01, 0.02, 0.05, 0.009, 0.002, 0.007, 0.01, 0.01, 0.14, 0.1, 0.01, 0.04$; (c) $0.75, 0.25, 0.85, 0.12, 0.07, 0.16, 0.02, 0.1, 0.01, 0.02, 0.1, 0.06$; (d) $0.40, 0.50, 0.71, 0.95, 0.86, 0.23, 0.07, 0.36, 0.12, 0.01, 0.009, 0.04$; (e) $0.058, 0.97, 0.88, 0.27, 0.81, 0.16, 0.27, 0.04, 0.03, 0.01, 0.01, 0.01$; (f) $0.31, 0.07, 0.09, 0.10, 0.45, 0.26, 0.08, 0.04, 0.01, 0.24, 0.02, 0.11$; (g) $0.1, 0.26, 0.003, 0.003, 0.007, 0.0003, 0.0003, 0.0004, 8e-05, 0.0002, 0.001$ and $0.001$. Sample sizes (WT/mutant plants): (a) $n = 29/29$, (b) $n = 32/32$, (c) $n = 28/30$, (d) $n = 28/26$, (e) $n = 30/29$, (f) $n = 30/28$, (g) $29/30$. Raw data are provided in Supplementary Table 12. Error bars: mean ± s.d.
Extended Data Fig. 10 | Transcriptional regulation of class A ARF genes regulates shoot development. a, Phenotypic analysis of the shoot defects in TF mutants. Leaf nr, leaf number; rosette d., rosette diameter; C. branch nr, cauline branch number; A. branch nr, axillary branch number. Green boxes indicate statistically significant increases; blue boxes indicate statistically significant reductions in the indicated developmental parameter compared to Col-0. Statistical analyses: unpaired two-tailed t-test, \( P \leq 0.05 \) considered as statistically significant; number of plants \( n = 12 \) per genotype. b, Examples of shoot growth phenotypes: shoot growth during vegetative stage in the at2g26940 mutant alongside the control after growth for 43 d in short-day conditions. c, The dof1.8 mutant has a shorter inflorescence than control plants.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection | No software was used for data collection

Data analysis | RootNav 1.8 [http://www.nottingham.ac.uk/research/groups/cv/software/rootnav.aspx] for root phenotyping; Fiji/ImageJ 1.50d for image analysis; IGV 2.8.5 to visualize epigenomic data; Bedtools 2.25.0 and Python 3.6.9 to analyze DAP-seq data; biostrings 2.4.10 and Bioconductor 3.1 for binding motif search

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data generated and analysed during this study are included in this published article (and its supplementary information files). Publicly available datasets used in this study are JaspR, CSBP, GSE24655, GSE24658, GSE7907, GSE2407, GSE50636, GSE24657, GSE24710, GSE19654, GSM2260231, GSM2260232, GSE2255235, GSE2255236, GSE2704255, GSE2704256, GSE2719200, GSE2719202, GSE2719203, GSE2719204, GSE2719205, GSE1289362, GSE1289374, E-MTAB-4860, E-MTAB-4884, GSE1289358.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
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For a reference copy of the document with all sections, see https://nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- Sample size: No sample size calculation was performed. Sample size was chosen accordingly to the minimal sample size requirements for each statistical test.
- Data exclusions: No data were excluded.
- Replication: All experiments were performed 2 to 3 times. All replications were successful.
- Randomization: No randomization was used. Each statistical test uses two groups.
- Blinding: Blinding was not possible since we know the nature of all samples for each experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a                              | n/a     |
| Involved in the study            | Involved in the study |
| Antibodies                       | ChIP-seq |
| Eukaryotic cell lines            | Flow cytometry |
| Palaeontology                    | MRI-based neuroimaging |
| Animals and other organisms      |         |
| Human research participants      |         |
| Clinical data                    |         |

Antibodies

- Antibodies used: Anti-digoxigenin-AP, Fab fragments, Roche, Catalog number: 11 293 274 910, Lot number: 11266027; dilution 1:1250
- Validation: Validation of the antibody is available from the website of the company [https://www.sigmaaldrich.com/catalog/product/roche/11093274910?lang=en&region=GB]; see also e.g. Hejatko J et al. Nature Protocols 2006 1:1939-1946 and Rozier F et al 2014 Nature Protocols 3: 2464-2475