PIF4 and CDF2 co-operate to regulate cell elongation in *Arabidopsis thaliana*

Cellular responses to environmental and developmental signals depend on the recruitment of transcription factors to specific genes. Although PIF4 and CDF2 belong to different transcription factor families in *Arabidopsis thaliana*, data suggest that they act co-operatively to bind to specific target genes in the gene network that regulates hypocotyl cell elongation.

This is a summary of:
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The question

Transcription factors (TFs) containing different DNA-binding domains, namely PHYTOCHROME INTERACTING 4 (PIF4; a bHLH family member) and CYCLING DOF FACTOR 2 (CDF2; a member of the plant-specific DOF family), have been implicated in the regulation of hypocotyl growth in *Arabidopsis thaliana*. PIF4 promotes growth of *Arabidopsis* in response to light and high temperature[1], and the functions of DOF transcription factors have been explored in many biological contexts since their identification in maize in 1993[2,3]. However, as DOF transcription factors contain only a single zinc finger domain and bind to a very simple consensus motif[4], how they recognize and are recruited to specific genes is poorly understood.

The discovery

PIF4 and CDF2 are known to promote hypocotyl growth of *Arabidopsis*. Here, confocal microscopy revealed that mutations in the genes encoding these TFs reduce cell elongation in the middle portion of the hypocotyl, and that these TFs act in the same genetic pathway (Fig. 1a). PIF4 and CDF2 were also found to be expressed in the same temporal patterns and in the same epidermal cells, and to physically interact.

Next, to identify the genome-wide DNA-binding sites of CDF2 and to compare these with previously identified PIF4 binding sites, we performed chromatin immunoprecipitation with sequencing (ChIP–seq) for CDF2 in wild-type and *pif4* mutant seedlings as well as RNA sequencing (RNA-seq) using cotyledons and hypocotyls of *pif4* and *cdf* mutants and wild-type seedlings. Analysis of the data identified over-representation of common target genes for PIF4 and CDF2, supporting the idea that these proteins are functionally related. Furthermore, the RNA-seq data revealed genes that are transcriptionally regulated by both TFs. Finally, the binding strength of CDF2 to many genes was reduced in the *pif4* mutant, suggesting that PIF4 enhances CDF2–DNA binding.

By cross-referencing the ChIP–seq and RNA-seq data, we identified a set of genes that are bound by both PIF4 and CDF2 and to which CDF2 binding is strengthened by PIF4; the expression of these genes was reduced in the *pif4* and *cdf* mutants. One of these genes encodes YUCCA8, an enzyme that contributes to biosynthesis of the growth-regulatory hormone auxin. Using protein structure modelling combined with extensive mutagenesis to understand the molecular basis for binding of CDF2 (Fig. 1b) and PIF4 (Fig. 1c, d) to the *YUCCA8* promoter in vitro, we found that PIF4 enhances CDF2–DNA binding. We propose that this enhancement occurs via direct PIF4–CDF2 protein interactions and by PIF4-mediated alteration of DNA conformation (or DNA allostery[5]) that strengthens CDF2–DNA binding. Therefore, a combination of imaging, genome-wide genomics and in vitro analysis enabled us to demonstrate how these two previously unrelated classes of TF mechanistically combine to increase auxin biosynthesis and cell elongation.

The implications

We identify an unexpectedly close functional relationship between the bHLH factor PIF4 and the DOF factor CDF2, and show how they combine to regulate transcription of the growth-regulatory gene *YUCCA8*. Furthermore, in vitro experiments showed that PIF4 forms tetramers rather than the previously assumed dimers, and that by binding to DNA it enhances CDF2–DNA binding. Thus, novel functional interactions are defined between two TFs that are of wide interest, and the data suggest that PIF4 might promote hypocotyl growth by recruiting CDF2 to specific genes.

PIF4 is proposed to increase the strength and specificity of CDF2–DNA binding through direct PIF4–CDF2 protein interactions that enhance sequence-specific DNA binding and by altering local DNA topology, but whether CDF2 influences PIF4–DNA binding in vivo is unclear. Furthermore, as the in vitro experiments used only the DNA-binding domains of the TFs, whether the other domains in these proteins regulate gene transcription in cells is unknown. Studies using full-length proteins are planned to answer these questions. Obtaining structural information on the $CDF2^{DOF}$–$PIF4^{bHLH}$–DNA complex would also increase our understanding of the interactions between its components. Combining genome-wide analysis of DNA binding and gene expression in vivo with extensive mutagenesis of proteins and DNA in vitro, as we did here, could be used to study interactions between other TFs in plants.

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EXPERT OPINION

"The experiments in this study were thoughtfully designed and carried out and have answered many questions regarding how transcription factors bind to each other, as well as to DNA, and on their impact on gene expression. This study will be of broad interest to many readers, including scientists not working on plants." **Enamul Huq, The University of Texas at Austin, Austin, TX, USA.**

FIGURE

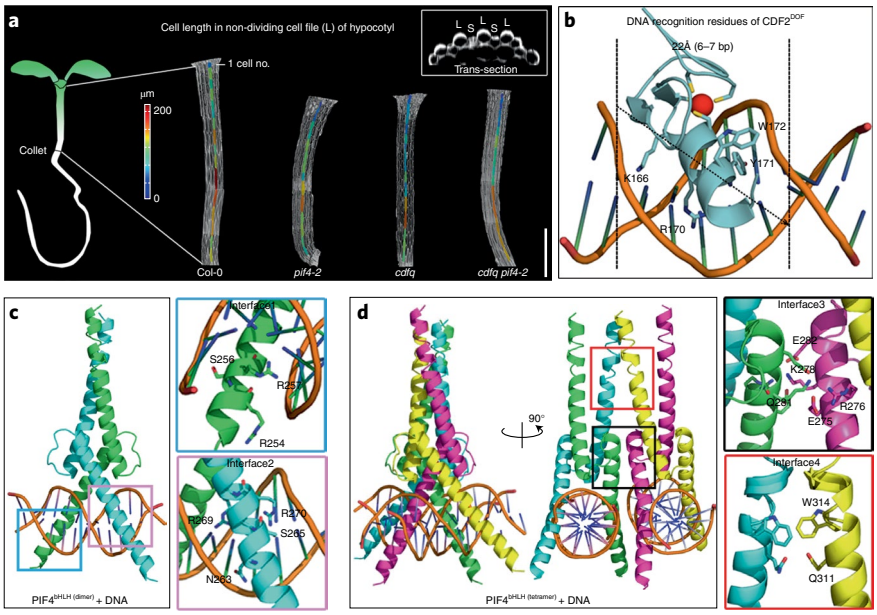

Fig. 1 | Analysis of epidermal cells of *Arabidopsis* hypocotyls and structural modelling of TF–DNA complexes. a, Heat-map quantification of cell length in the non-dividing cell files (L) of hypocotyls from *Col-0*, *pif4*, *cdfq* and *cdfq pif4* mutants grown under short-day photoperiods for 7 days. S indicates small dividing cell file. Scale bar, 500 μm. **b**, Modelled structure of the CDF2^{Dof}–DNA complex, with CDF2^{Dof} in blue. Cysteines in yellow bind the metal ion (red ball) to maintain protein conformation, and residues in dark blue interact with the DOF binding site from the *YUCCA8* promoter (orange). **c**, Modelled structure of a DNA-bound PIF4^{bHLH} homodimer. The PIF4^{bHLH} monomers (green and blue) interact with DNA containing the G-box of *YUCCA8* (orange) via the indicated residues. **d**, A DNA-bound PIF4^{bHLH} homotetramer. As for **c**, with extra PIF4^{bHLH} monomers in yellow and purple.

BEHIND THE PAPER

When I joined the Coupland laboratory, I became interested in DOF transcription factors because of their role in regulating environmental responses and repressing floral transition. I realized that one of the most important biological questions in studying these transcription factors is how they achieve DNA-binding specificity in vivo. One of my goals since then has been to solve this problem in the model plant *Arabidopsis*. However, efforts to use the ChIP–seq method were hampered by technical difficulties. For example, DOF proteins show low affinity for DNA because they contain only one zinc finger. The discovery that PIF4 and CDF2 act in combination changed our perspective on how DNA sequences are recognized by DOF transcription factors. By employing many different layers of analysis with my collaborators, we uncovered an important mechanism by which CDF2 binds to DNA sequences in *Arabidopsis*. **H.G.**

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FROM THE EDITOR

"This is an interesting study about the molecular co-operation of two transcription factors from different families, and how this interaction has consequences for growth. It is a multi-scale analysis, from structures to phenotypes. PIFs and CDFs mediate responses to light and temperature. The authors show, by using genome-wide approaches, that PIF4 and CDF2 physically interact to activate target genes and induce hypocotyl elongation, notably through enhanced auxin biosynthesis." **Guillaume Tena, Senior Editor, *Nature Plants*.**