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FindTFnet: a tool for reconstruction of Arabidopsis transcription factor networks from transcriptome data

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Motivation and Aim: Transcription factors (TFs) are proteins that bind to regulatory DNA sequences mainly in gene promoters and regulate transcription of these genes [1]. TFs directly regulating transcription of genes encoding other TFs compose transcription factor regulatory networks (TFRNs) [2]. In actions of exogenous or endogenous factors, TFRNs are intermediate between signaling pathway, which they activate and the processes they affect. Nevertheless, TFRNs are the less studied elements in these biological chains. Here we present FindTFnet – a tool for the reconstruction of TFRNs in *Arabidopsis thaliana* from transcriptome data on differentially expressed genes (DEGs) and a list of their upstream regulators predicted with the CisCross web service [3]. We illustrate the efficiency of FindTFnet by its application for the reconstruction of mechanisms underlying auxin response.

Methods and Algorithms: The lists of DEGs down- and upregulated by auxin (dDEGs and uDEGs, respectively) were compiled based on the analysis of microarray data on auxin-induced changes in the *A. thaliana* root [4]. First, we identified the lists of TFs, for which DAP-seq peaks were enriched in 1000 bp-long upstream regions of DEGs. According to the enrichment status, TFs were subdivided into three following groups: overrepresented in dDEGs, in uDEGs, and in both dDEGs and uDEGs (Table 1). The corresponding TF-coding genes can be dDEG, uDEG or not transcriptionally regulated (NTRs).

Table 1. The scheme for determining TF functions in a TFRN reconstruction

	TF is enriched in dDEG promoters	TF is enriched in uDEG promoters	TF is enriched in both dDEG and uDEG promoters
TF gene is dDEG	Downregulated activator – DA	Downregulated suppressor – DS	Downregulated Regulator (DR)
TF gene is uDEG	Upregulated suppressor – US	Upregulated activator – UA	Upregulated Regulator (UR)
TF gene is not DEG	NTR	NTR	NTR

DRs, URs and NTRs are out of scope of this research. If a TF is encoded by a dDEG, it is an auxin downregulated regulator. If this TF's binding loci are enriched in dDEG promoters, it should be a Downregulated Activator (DA), if they are enriched in uDEG promoters, this TF should be a Downregulated Suppressor (DS) (Table 1). Similarly, the functions in the TFRN are defined for TFs encoded by auxin-activated genes: enrichment of TF binding loci in dDEGs or uDEGs testifies that it should be an Upregulated

Suppressor (US) or an Upregulated Activator (UA), respectively. It is clear that downregulated TFs are active before auxin treatment, and since the amount of DAs and DSes is decreased upon auxin treatment, their targets are downregulated and upregulated passively. In this downregulated subnetwork (D-subnetwork) DAs activate other DAs and DSes, and DSes suppress UAs and USes. In opposite, auxin application switches on USes and UAs (U-subnetwork). USes actively suppresses DAs and DSes, and UAs actively activate other UAs and USes.

Results: FindTFnet is available as a part of CisCross web service [3]. Its application for the analysis of auxin-sensitive DEGs identified 60 TFs, which mediate response to auxin: 23 DAs, two DSes, eight USes and five UAs. Of them, 54 TFs constitute a connected transcriptional network (Fig. 1). The rest of TFs, which do not participate in any "TF regulator–TF target" pair, include one DA (LCL1), one DS (RAP2.12), two UAs (MYB3R1 and DEL2), and two USes (HB18 and NAM).

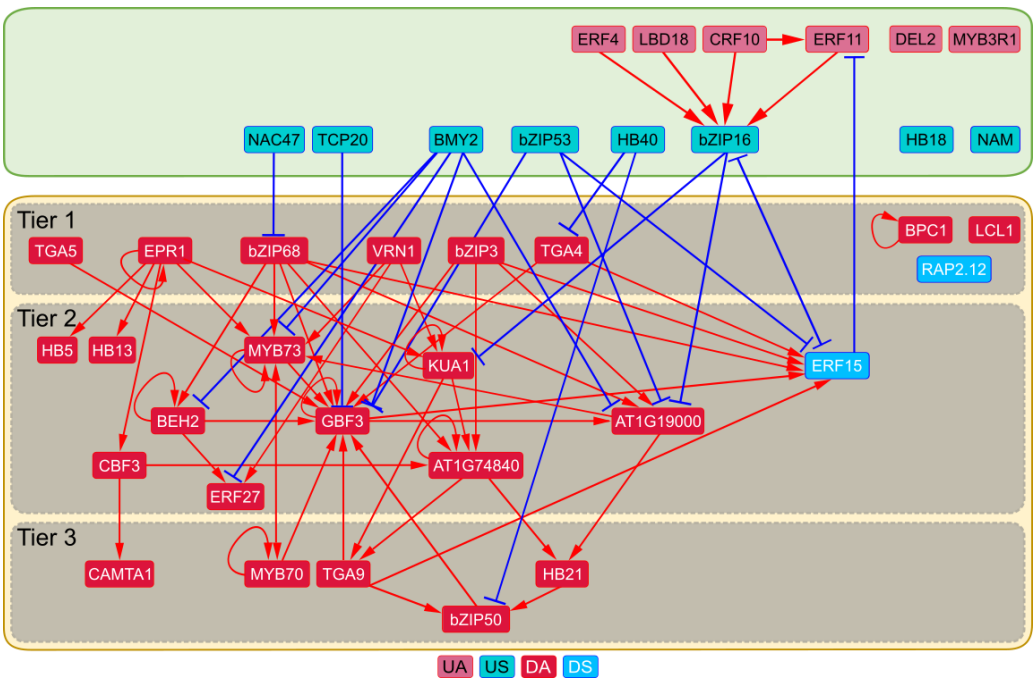

Fig. 1. Auxin depended TFRN in Arabidopsis roots inferred by FindTFnet. Three tiers of the downregulated subnetwork (D-subnetwork) denoted by grey boxes are encircled by yellow contours, an activated subnetwork (U-subnetwork) is enclosed in a green box

Of 60 TFRN TFs, the role in auxin response was previously demonstrated only for 12 TFs (25 %), namely, DAs: CAMTA1 [5]; HB5 [6], KUA [7], MYB70 [8], MYB73 [9], DS: RAP2.12 [10]; USes: BMY2 [11], HB40 [12], TCP20 [13]; UAs CRF10 [14], DEL2 [15], LBD18 [16].

Conclusion: FindTFnet (https://plamorph.sysbio.ru/ciscross/FindTFnet_index.html) reconstructs TF regulatory networks guiding gene expression changes in response to endogenous or environmental factors. The analysis of auxin-sensitive DEGs with FindTFnet allowed inferring the TFRN acting in auxin response, in which only for one fifth of TFs this role was previously recognized.

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