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EIN3 binding site architecture guides transcriptional response to ethylene in Arabidopsis

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***Abstract* — EIN3 transcription factor is the master regulator of gene expression in response to plant hormone ethylene. The structure of EIN3 binding site (EBS) was determined using *in vitro* binding assays. However, the role of EBS architecture in EIN3 binding at the whole-genome level is poorly understood. Here we demonstrate that an inverted repeat of EBS with the overlap of the motifs is a canonical EIN3 target in the Arabidopsis genome. We show that this EBS architecture provides a more pronounced response to ethylene than either a single EBS or EBS repeats with a spacer. These findings can be used to develop a new genetic sensor for highly sensitive detection of ethylene signaling in Arabidopsis.**

Keywords* — *bioinformatics, transcription factor, ETHYLENE-INSENSITIVE3, EIN3 binding site (EBS), ChIP-seq, RNA-seq

Motivation and aim

Motivation

ETHYLENE INSENSITIVE 3 (EIN3) transcription factor is the master regulator of gene expression in response to plant hormone ethylene that guides plant growth under stress conditions. EIN3 is a transcriptional activator that binds a short nucleotide sequence referred to as EBS to induce transcription [1]. *Arabidopsis thaliana* reporter *EBS:GUS* driven by such EIN3 binding site [2] is widely used as a sensor for detection of ethylene signaling. EIN3 tends to bind DNA as a homodimer and it has been shown recently that EBS inverted repeats with a spacer of 10 bp provide EIN3 binding *in vitro* with a higher affinity than a single EBS [3]. However, the role of EBS architecture in EIN3 functioning was not investigated on the whole genome level.

Aim

Here we accomplish a systematic bioinformatics analysis of EIN3 bound sequences in Arabidopsis genome to shed light on molecular mechanisms utilized for regulation of transcriptional response to ethylene in plants.

Methods

We used publicly available ChIP-seq data on EIN3 binding, RNA-seq data on ethylene-induced transcriptomes in Arabidopsis seedlings [4] and published DAP-seq data [5]. We used Homer [6] for *de novo* motif search in the peaks, and MCOT [7] for enrichment analysis of EBS repeats. Associations of EBS configurations with peaks and genes features were estimated with Fisher's exact test.

Results

We discovered a previously unknown EBS architecture that is enriched in EIN3 bound sequences to a much greater extent than a single EBS motif. This new configuration is a head-to-head inverted repeat of EBS-like sequences with 1 bp overlap referred to as 2EBS(-1). It is noteworthy that none of the EBS repeats with spaced motifs (including the one with 10 bp spacer) was enriched in EIN3 bound sequences compared to a random expectation. We also demonstrated that unlike a single EBS motif, 2EBS(-1) repeat was significantly associated with a sustained profile of EIN3 binding, i.e. it facilitated EIN3 binding regardless of the duration of ethylene treatment. Based on these findings we consider that 2EBS(-1) is a canonical EIN3 binding site in *A. thailana* genome. We further showed that of all EBS configurations under study only 2EBS(-1) was significantly associated with transcriptional response of EIN3 targets to ethylene treatment. Moreover, it tended to cause a more pronounced transcriptional response than other EBS configurations. These findings can be used to design a new genetic sensor for highly sensitive detection of ethylene signaling. Taken together, this work provides new insight on the molecular mechanisms utilized for regulation of transcriptional response to ethylene in plants.

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