Dissection of the network of transcriptional responses to ethylene in *Arabidopsis thaliana* roots using a yeast system

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The volatile plant hormone ethylene is an essential growth regulator directing many developmental processes and stress responses. Ethylene signaling leads to substantial changes in the transcriptome which drive these developmental responses. To identify gene regulatory networks (GRNs) that drive ethylene responses, we examined the transcriptional responses to ethylene and its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), using microarray time-course studies and RNA-seq analyses in *etr1* mutants to map the GRN driving *Arabidopsis thaliana* root development. These analyses revealed a group of genes that were ethylene-regulated, including 60 genes predicted to encode transcription factors (TFs). We are currently studying the ability of these TFs to turn on transcription of more slowly induced target genes that play an integral role in the ethylene biosynthesis and response pathways. We are developing a yeast system which allows us to demonstrate direct TF binding to promoter regions of potential target genes driving synthesis of a fluorescent reporter. This system allows for a more elaborate study of the transcriptional machinery by allowing us to test temporally predicted relationships and to gradually introduce other genes whose products regulate the transcriptional response. To assess the viability of the yeast system, we are testing the ability of EIN3, EIL1, and EIL2 to turn on *ERFs,* as early steps in the ethylene GRNs and to identify targets for the 60 ethylene-induced TFs in roots. We are testing 15 TFs predicted to bind to the promoter regions of genes encoding one of the rate limiting enzymes in ethylene synthesis*,* ACC Oxidase (ACO), to better understand the mechanisms that drive the autocatalytic biosynthesis of ethylene. In parallel, we are screening T-DNA insertion lines of these TFs for aberrant ethylene-regulated phenotypes to gain a better understanding of where these TFs might fit in the ethylene response pathway. (Supported by NSF-MCB 1716279)