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Using ChIP assays to study potential binding of NPH4/ARF7 on TSI promoters

Using ChIP Assays to Study Potential Binding of NPH4/ARF7 on TSI Promoters Amanda Tinsley1, Shiv Tiwari2, C. Alex Esmon1, Mannie Liscum1, Gretchen Hagen2, and Tom Guilfoyle2 1 Division of Biological Sciences, University of Missouri-Columbia 2 Department of Biochemistry, University of Missouri-Columbia Non-Phototropic Hypocotyl 4/ Auxin Response Factor 7(NPH4/ARF7), a potent transcriptional activator that has an important function in tropic growth and development. Earlier research has shown that when there is a lesion in the NPH4/ARF7 locus, there is a loss of phototropic and gravitropic responsiveness in the stem (Stowe-Evans et al., 1998; Harper et al., 2000). In addition to providing genetic support for the long held notion that tropic responses require the formation of, and response to, localized changes in auxin concentration, the dependence of tropic responses on NPH4/ARF7 further suggest that auxin-dependent changes in gene expression are critical to these responses. Previous global expression studies have implicated a set of Tropic stimulus-responsive (TSI) genes that are potentially under the transcriptional control of NPH4/ARF7 in tropically responsive plant stems (Esmon et al., 2006). We are further investigating the interaction between this set of TSI genes and NPH4/ARF7 by using chromatin immunoprecipitation (ChIP) assays to determine if NPH4/ARF7 is binding to the auxin response elements (AuxREs) located in the promoter regions of the TSI genes. We have also constructed RNA interference (RNAi) loss-of-function lines for each TSI gene and are in the process of screening for positive transformants. Our preliminary results from the ChIP assays suggest that NPH4/ARF7 does bind to certain AuxREs in GH3.5 and DFL1/GH3.6 in a preferential manner under auxin and blue-light stimulations. We believe this to be the first direct evidence of targets under the influence of NPH4/ARF7 transcriptional activation. We are currently testing the rest of the TSI genes for NPH4/ARF7 preferential binding.