Universidad Miguel Hernández de Elche

Generation of bait constructs for yeast-one-hybrid assays with the Arabidopsis *ARGONAUTE1* promoter

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Master Thesis

Elche, 2012

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HEREBY CERTIFIES

that this document faithfully reproduces the research conducted by Miss Zeynep Eren under my supervision in the Unit of Genetics of the Institute of Bioengineering of the Universidad Miguel Hernández de Elche, and that the candidate is qualified to submit this thesis in application for the Degree of Master in Bioengineering.

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Elche, June 22, 2012.

I.- SUMMARY AND CONCLUSIONS

Until only two decades ago, the development of multicellular organisms was thought to be controlled almost exclusively by the hierarchical action of different transcription factors, activator and/or repressor proteins that bind to specific, regulatory DNA sequences. An additional regulatory mechanism was discovered in 1993: silencing by microRNAs (miRNAs). These noncoding, single-stranded RNA molecules of about 22 nt induce the degradation and/or attenuation of the translation of its target mRNAs, to which they bind by complementary base pairing. Repression by miRNAs is a post-transcriptional gene silencing process that occurs in the cytoplasm, in ribonucleoprotein complexes called RISC (RNA-Induced Silencing Complexes). miRNA-mediated silencing has been demonstrated or predicted to participate in the control of hundreds or even thousands of eukaryotic genes.

ARGONAUTE (AGO) endoribonucleases are the key RISC component in all eukaryotic organisms. The Arabidopsis AGO family consists of 10 proteins, of which AGO1 plays a pivotal role in the miRNA pathway and is essential for the proper development of this model plant. The laboratory of Prof. M.R. Ponce is contributing to the understanding of the mechanisms of gene expression regulation mediated by AGO1. Her group has focused on the large-scale identification of mutations suppressing the morphological phenotype caused by *ago1-52*, a viable mutant allele of the *AGO1* gene.

There is a wealth of information on the regulation by AGO1 of miRNA-target genes. However, an understanding of the genes regulating *AGO1* is lacking. To functionally dissect the regulation of *AGO1*, we have taken an experimental approach based on the identification of transcription factors binding to the promoter of *AGO1*, by means of yeast-one-hybrid (Y1H) assays. Previously to this work, the promoter of Arabidopsis *AGO1* and several of its orthologs was analyzed *in silico*, finding several regions that were considered putatively regulatory because of their conservation among monocot and dicot species. Here, we present our results on part of these Y1H assays.

We generated four $AGO1_{pro}$ constructs that represent different segments of the promoter of the *AGO1* gene. They were designed to be used as baits in Y1H screens aimed to the identification of transcriptions factors that regulate *AGO1*. The constructs were transferred into *Saccharomyces cerevisiae* cells and their activation by a control prey was found adequate. We also determined the optimal concentration of 3-AT required for each construct in screens for preys. The $AGO1_{pro}$ bait plasmids obtained in this work will be instrumental for the functional dissection of the regulation of the *AGO1* gene.