ISSN 2250-3153

Cloning and promoter activity of microRNA858 in Arabidopsis thaliana

Andrea Tovar-Aguilar*, Jesus Agustin Badillo-Corona and Noé Durán-Figueroa*

* Departamento de Bioprocesos, Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología

Abstract
MicroRNAs are RNAs of 21-22 nucleotides in length that are known as negative regulators of gene expression. By its molecular mode of action, have been described important biological roles in the development and physiology of plants, animals and fungi. In the model plant Arabidopsis thaliana, the miRNAs perform essential functions such as juvenile-adult transition, morphogenesis of leaves and response to biotic and abiotic factors. At genomic structure, sequencing studies demonstrate that miRNA genes are similar to genes encoding proteins. Thus, the MIR genes have promoters directing their expression in specific tissues. In this work, we cloned and characterized the promoter activity of miRNA858 in Arabidopsis. The transgenic lines generated show that miRNA858 promoter is active mainly in flower, this expression activity suggest that miRNA858 has functions in this tissue. This result is a first approximation for to know the biological role of miRNA858 in Arabidopsis thaliana.

Index Terms- microRNAs, promoter, Arabidopsis.

I. INTRODUCTION

MicroRNAs are genome-encoded noncoding RNAs of 21-22 nucleotides (nt) in length that act as repressors of target genes. MiRNAs act at transcriptional (TGS) and posttranscriptional (PTGS) level, where its partners, the ARGONAUTA (AGO) proteins interact with them to guide the gene repression. The AGO proteins together to miRNA form a protein-RNA complex called *RNA Induced Silencing Complex* (RISC), this complex recognizes the target gene and the repression could be through translational inhibition, accelerated exonucleolytic mRNA decay, or slicing within miRNA-mRNA base pairing (Voinnet 2009). These silencing mechanisms, are conserved across the kingdoms for to keep genome integrity and for most important, for to regulate many key developmental and physiological processes in animals and plants (Li et al., 2014).

In Arabidopsis thaliana, the biogenesis of miRNA has been well studied. Like a protein genes, the MIR genes are mostly transcribed by RNA polymerase II and their transcript is highly heterogeneous in length, between 70-120 nt; this primary stem-loop miRNA (pri-miRNA) is processed in the nucleus by a DICER-LIKE 1 (DCL1) enzyme and generates a miRNA duplex of 21-22 nt. In the cytoplasm, miRNAs are loaded onto cytoplasmic AGO proteins (Rogersa and Chen, 2013). Arabidopsis have 10 AGO proteins of which AGO1 is the major player for the miRNA pathway (Ha and Kim, 2014). However has been demonstrated that AGO proteins act at specific developmental stages; for example, AGO9 and its miRNAs have important functions in flower development and in female gametophyte identity (Olmedo-Monfil et al., 2010).

Despite having a broad characterization of miRNA at genome level, little is known about their biological function. So, one of the major challenges in field of the plant molecular biology is understand their biological function in specific tissues, developmental stages and ambient contexts. In order to understand the function of MiRNAs, them can be overexpressed or driven by a specific reporter gene. Thus, transcriptional fusions are the first step to discover where and when the miRNA is expressed; therefore, one way to discover their function is with the activity of their promoter. Because MIR genes are transcribed by RNA Pol II and according to Zhou et al., 2007, 500-bp upstream regions have enough motifs to determinate the possible promoter region, like the TATA-box.

In order to know, where are expressed the miRNAs in Arabidopsis, in this work we select a miRNA858 which interact with AGO9 protein. We performed a transcriptional fusion by cloning promoter of miRNA858 and using *uidA* gen (GUS) like reporter. We generate transgenic lines showing promoter activity mainly in the flower, suggesting that miR858 could have a function in this tissue.

II. MATERIALS AND METHODS

Plant material and grow conditions

Arabidopsis thaliana ecotype Columbia (Col-0) was used in this work. Seeds were surface sterilized with 100% etanol, and germinated in a growth chamber at 22ºC under a 16 h light/8 h dark photoperiod, in Murashige and Skoog (MS) medium. Seedlings were transferred to soil, and grown in the greenhouse under long day 16 h ligth/8 h dark controlled conditions.

Constructs

Transcriptional fusion was generated by amplifying different segments of the regions corresponding to MIR gene, promoter region was selected according Zhou et al., 2007. Annotation of *MIR* gene was performed using genomic information from TAIR10. This promoter was cloned into plasmid vector pBluescript KS (-) and digested with HindIII and XbaI, DNA fragment was subcloned into binary pBI101.3 to obtain the GUS fusions (Figure 1A and 1B). The resulting construct is referred to as pmiR858::GUS, oligonucleotides used for amplification of the

promoter are: 5' GGTTCAAATTAGTCAGTGCACAGTTTC and 3' GCTCACTTTGTCAAGCCTAGCATCG.

Plant transformation

Confirmed correct construct was transformed into wild-type plants by the floral dip method (Zhang et al 2006). *Agrobacterium tumefaciens* strain C58C1 was used to transformed Arabidopsis, all the positive colonies were confirmed by PCR.

Histochemical Analysis

Histochemical localization of GUS activity was performed by incubating inflorescences in GUS staining solution (10 mM EDTA, 0.1% Triton X-100, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 1 mg mL21 5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid in 50 mM sodium phosphate buffer, pH 7.4) for 48 h at 37°C.

III. RESULTS AND DISSCUSION

MicroRNAs expression was determined across the activity of his promoter; transcription fusion was performed to known where was the promoter expression. According with the Figure 1C, the colonies with the vector pBluescript::promoter was digested with HindIII and XbaI and the promoter were identified on 1% agarose gel. The second construction, pBI101.3 + promoter (figure 1B) was digested with the same enzymes to obtain the final vector pmiR858::GUS (Figure 1C). In all cases the result was confirmed with PCR and sequencing corresponding the correct region.

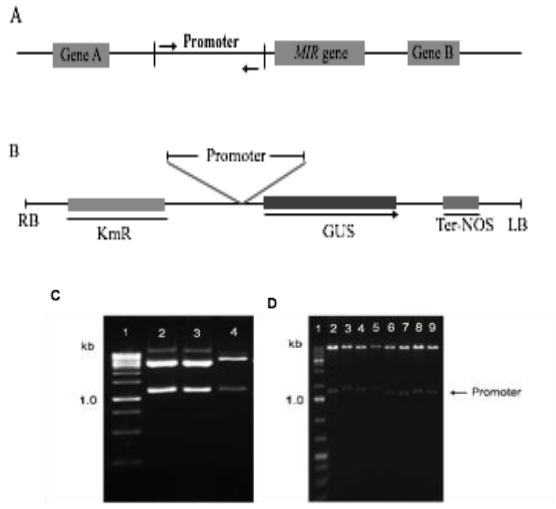

Figure 1. Transcriptional fusions. (A), (B) Schematic diagrams of the constructions to the transcriptional fusions pmiR858::GUS. (C) Double digestion on a 1% agarose gel. Lane 1 corresponds 1kb ladder, lane 2,3 and 4 promoter (1000 pb) + pBluescript KS (-). (D) Lane 1 corresponds 1kb ladder, lane 2-7 clonal transcriptional fusions, promoter region + pBI101.3 (pmiR858::GUS).

Selection of transgenic lines with fusion pmiR858::GUS

Once confirmed the transcriptional fusion pmiR858::GUS, we transformed *Arabidopsis thaliana* ecotype Columbia (Col-0) by floral dip method. Then, we plated seeds from plants previously transformed with Agrobacterium in medium with kanamycin; the resistant seedlings were selected for further experiments. In Figure 2, is observed that in resistant seedlings, the grow is normal and the true leaves emerge two weeks after germination while non resistant seedlings, have a yellow color and do not have true leaves.

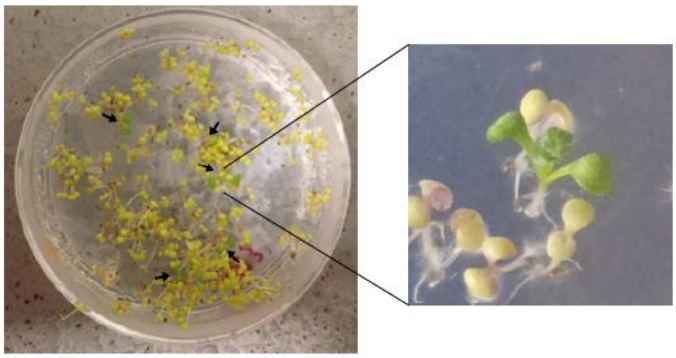

Figure 2. Selection of resistant seedlings in F_1 plants. Seedlings of pmiR858::GUS were plated in selective media after two weeks of germination. Resistant seedlings presents true leaves and growth normal in selective media (dark arrows)

Promoter activity of miRNA858

Like described in materials and methods, the transgenic lines were grown in controlled greenhouse conditions and the promoter activity was tested by GUS stain. Figure 3 shows GUS staining in both vegetative and reproductive tissues. In 3A, like negative control of stain, shown GUS staining in a flower of wild type plant treated with a staining solution. In 3B we see little GUS activity in the fruit of Arabidopsis (silique), activity is observed in the region called "receptacle". In leaves, promoter activity is reduced at the beginning of the pedicel. Unlike this low activity, before fecundation, the flowers had shown high GUS staining in the filaments (Figure 3D) and receptacle but not in anther or carpel (gynaeceum). However, after fertilization (about 4 days), miRNA858 promoter has strong activity in the anther and in the filament of the flower (Figure 3E). This indicates that before fertilization, the promoter activity of miRNA858 is in flower but not in male (pollen) or female reproductive tissue (gametophyte). Nevertheless, once fertilization occurred, the organ that contains the mature pollen has activity, suggesting that the miRNA858 has a biological role in vegetative level before fertilization and other role in male reproductive level after fertilization. This result correlates with the function demonstrated for other miRNAs studied in Arabidopsis thaliana (Zhou and Wang, 2013).

IV. CONCLUSION

In order to know the function of a miRNA, the first step is understand where is expressed. In this work has been tested the promoter activity of miRNA858, we demonstrate that the miRNA858 has activity in flower, which suggest that the potential role of this miRNA in Arabidopsis can be associated with the development of the floral structure. The use of

Arabidopsis thaliana like model plant, allow demonstrate that through transcriptional fusions is possible to analyze where a miRNA is expressed.

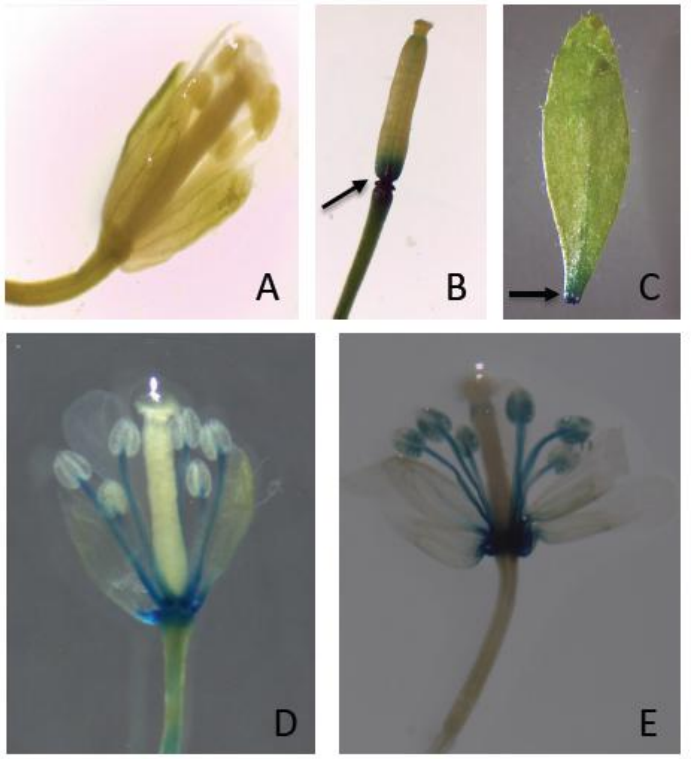

Figure 2. *Promoter activity of miRNA858*. (A) Wild type plant staining with GUS solution. (B) The siliques shows GUS activity in the receptacle. (C) The leaf shows GUS activity in the pedicel. (D) Before fertilization, the flower shows GUS activity only in the filament and receptacle. (E) After fertilization the flower shows GUS activity in the anther, the filament and receptacle. All photos were taken with stereoscopic microscope Leica EZ4 HD at 4x (A, D, E) and 8x (B, C).

ACKNOWLEDGMENT

This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACyT-Mexico project No.184245). Tovar-Aguilar has a PhD scholarship by CONACyT-Mexico.

REFERENCES

[1] Voinnet O. (2009) Origin, biogenesis, and activity of plant microRNAs. Cell 136:669-87.

[2] Li J, Reichel M, Li Y, Millar AA. (2014) The functional scope of plant microRNA-mediated silencing. Trends Plant Sci. 12:750-756.

[3] Kestrel Rogersa and Xuemei Chen (2013) Biogenesis, Turnover, and Mode of Action of Plant MicroRNAs. The Plant Cell 25, 2383–2399.

[4] Ha M, Kim VN. (2014) Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 8:509-24.

[5] Olmedo-Monfil V, Durán-Figueroa N, Arteaga-Vázquez M, Demesa-Arévalo E, Autran D, Grimanelli D, Slotkin RK, Martienssen RA, Vielle-Calzada JP. (2010) Control of female gamete formation by a small RNA pathway in Arabidopsis. Nature. 464(7288):628-32.

[6] Zhou X, Ruan J, Wang G, Zhang W. (2007). Characterization and Identification of MicroRNA Core Promoters in Four Model Species. Plos Computational Biology. 3:0412-0423.

[7] Zhang X, Henriques R, Lin S-S, Niu Q-W, Chua N-H. (2006) Agrobacterium-mediated transformation of Arabidopsis thaliana using the floral dip method. Nature Protocols, 1:2.

[8] Zhou CM, Wang JW. (2013) Regulation of flowering time by microRNAs. J Genet Genomics. 40(5):211-5.

AUTHORS

First Author – Andrea Tovar-Aguilar, PhD Student. Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología. México, D.F.
Email: andrea.tovar.aguilar@gmail.com

Second Author – Jesus Agustin Badillo-Corona, PhD. Principal Investigator in Algal and Plant Biotechnology. Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología. México, D.F.
Email: jbadilloc@ipn.mx

Correspondence Author – Noé Durán-Figueroa, PhD. Principal Investigator in Plant Molecular Biology. Instituto Politécnico Nacional, Unidad Profesional Interdisciplinaria de Biotecnología. México, D.F.
Email: nduranf@ipn.mx
Alternate email address: noeduranfigueroa@gmail.com