IDENTIFICATION AND PRELIMINARY CHARACTERIZATION OF miRNAs IN MAIZE

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MicroRNAs (miRNAs) are short RNAs, 21-24 nt in length, which play an important role in post-transcriptional gene regulation. The mechanism of action is similar, but not identical, to RNA-silencing: miRNAs are part of a ribonucleic complex that degrades the mRNA of a gene, or regulates its translation level upon base paring between the sequence of the small RNA and target mRNA. A single miRNA is produced from one to several longer primary transcripts (pre-miRNA), that assume a stem-loop structure, originating from MIR genes. Plant miRNAs are very similar to those characterized in animals, with some peculiarities. Plant miRNAs are homologous to the coding sequence of target mRNAs, but the region of homology can tolerate up to 2-3 mismatches.

To-date MIR genes and miRNAs have been identified in Arabidopsis thaliana and Oryza sativa. By comparison analysis between these 2 species, it’s clear that the 21-24 nt sequence and stem-loop structure are highly conserved (Bartel and Bartel, 2003). Eight miRNAs out of the 19 sequences found in A. thaliana are also present in O. sativa. Target genes of these miRNA seem to be transcription factors.

Recently is has been shown in maize the involvement of a miRNA in the regulation of leaf development (Juarez et al., 2004).

Here we present our results in identifying miRNAs sequences in Zea mays L and their corresponding MIR genes, evolutionary related to those of rice. The maize EST data base was screened for maize expressed sequences containing complementary regions to rice miRNAs. BLAST algorithm, with a maximum of 3 mismatches allowed, was used to investigate for potential miRNA target in maize. Seven of the 8 rice miRNAs tested produced at least one significant blast hit in maize. These maize ESTs have putative functions often similar to those supposed for target genes in rice.

Starting from rice pre-miRNAs sequences we designed primers to amplify correspondent maize genomic regions. As expected every pair of primers gave rise to several PCR products. These products were cloned and sequenced. Based on sequence homology and on the determination of their RNA most stable secondary structure, among the 8 miRNAs tested we have identified 5 potential miRNAs in maize. These 5 sequences have one, or more, pre-miRNA sequences with a stem-loop folding structure quite linear and with few bulges, compatible with the expected structure of pre-miRNAs. These sequences have, with few exceptions, no similarity with any annotated EST. The sequence of all of them was found within the hypomethylated maize genomic sequences annotated in the TIGR database. Expression pattern was determined by Northern blot analysis of low-weight RNAs purified from
different maize tissues in three different genotypes. This analysis showed tissue and genotypic variation relative to expression level for all the identified miRNAs.

References