Computational Prediction of miRNA Targets in three different Asian Citrus Psyllid (*Diaphorina citri*) Life stages

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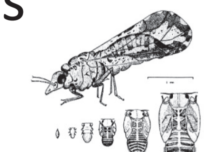

Abstract

MicroRNAs (miRNA) are small RNA molecules involved in the post-transcriptional regulation mechanism, RNA interference (RNAi). Unlike other small RNAs such as small interfering RNA's (siRNA), miRNAs are encoded in genomes. For this reason it is theorized that miRNAs play a key role in developmental regulation. In this study the targets of 103 pea aphid (*Acyrthosiphon pisum*) in transcriptomes of 3 asian citrus psyllid (*Diaphorina citri*) life stages were determined using the miRanda algorithm. The predicted targets were then identified using BLAST2GO annotation. It appears that no trend between life stages is true for all miRNAs. Implying variable importance of miRNAs. Predicted miRNA targeting is broad, and the methods mentioned most likely will yield viable target prediction and identification. This process may be very useful in the field of pest-management.

Introduction

The asian citrus psyllid is a Hemipteran that feeds on citrus plants. It is a know vector of the pathogen responsible for Huanglong Bing (HLB). HLB is a serious disease of citrus and is currently the single greatest threat to the citrus industry.

miRNAs are short RNAs involved in post transcriptional regulation. By forming RNA-induced silencing complexes (RISCs), miRNAs are used to block translation or destroy messenger RNAs (mRNA)*(Jones et al.)*. These RISCs form a major component of the RNA interference (RNAi) mechanism (figure 1). miRNAs are used through the RNAi mechanism to regulate the expression of key developmental genes. It is predicted that miRNAs can be used to modify aspects of psyllid physiology or inhibit mechanisms essential for life. They can also be used to sterilize a given population, thereby slowing their rate of expansion.

MiRanda is a miRNA target prediction algorithm. It can be used to determine the alignment, alignment score, and minimum free energy of RNA duplexes. It works by first aligning given miRNAs against some given cDNAs. It keeps the alignments that result in an alignment score greater than 100 by default. In this study a score cutoff of 110 was used instead. The algorithm then calculates the minimum free energy(ΔG) in Kcal/mol of the RNA duplexes. This second stage of the process uses RNAlib library routines from the ViennaRNA package. The duplexes with a ΔG less than -20 are kept by default. In this study a ΔG of less than -30 was used instead. Practical Extraction and Report Language (PERL) is a computer programming language that can be used to perform a wide range of task. Using this an E:SC score can be calculated where E:SC = (ΔG*-1)*(Score)*(1000^-1). E:SC scores can then be used to find duplexes with high alignment and low ΔG. Blast2GO is an all in one tool for functional annotation of sequences and the analysis of annotation data. It takes advantage of the Basic Local Alignment Search Tool (BLAST).

Materials and Methods

In this study complimentary DNA (cDNA) libraries were prepared, sequenced, and assembled. cDNAs that were shared between all three life stages by using the central 100 base pairs of the transcripts as a reference.

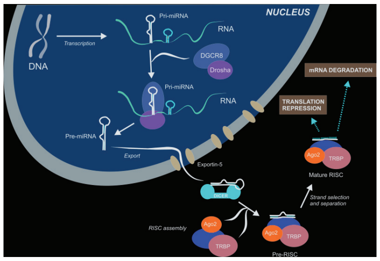

Figure 1. The miRNA life cycle and mechanisms observed in eukaryotic cells.

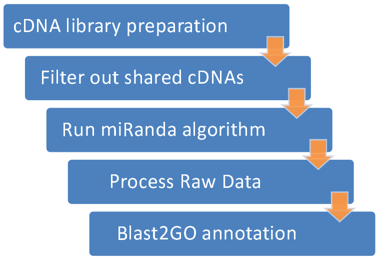

Figure 2. Prediction and analysis process performed in this study.

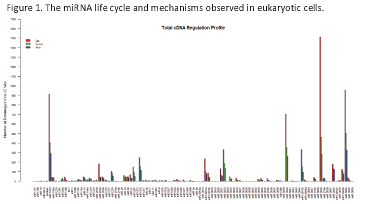

Figure 3. Total cDNA regulation profile. Y axis corresponds to the number of predicted miRNA duplexes. miRNA are represented on the X-axis. Red bars are egg. Nymph bars are green. Adult bars are blue.

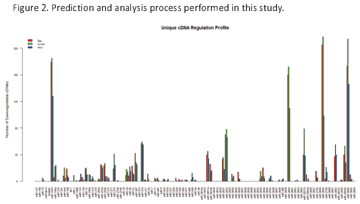

Figure 4. Unique cDNA regulation profile. Y axis corresponds to the number of predicted miRNA duplexes. miRNA are represented on the X-axis. Red bars are egg. Nymph bars are green. Adult bars are blue.

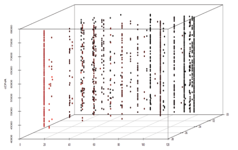

Figure 5. Nymph life stage miRNA-Interactome. Y axis corresponds to cDNA coordinate values. X axis corresponds to miRNA coordinate number. Z axis corresponds to ΔG.

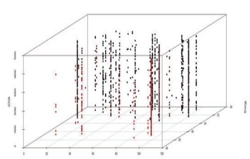

Figure 6. Egg life stage miRNA-Interactome. Y axis corresponds to cDNA coordinate values. X axis corresponds to miRNA coordinate number. Z axis corresponds to ΔG.

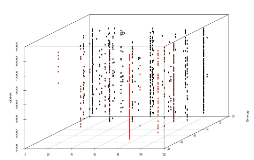

Figure 7. Adult life stage miRNA-Interactome. Y axis corresponds to cDNA coordinate values. X axis corresponds to miRNA coordinate number. Z axis corresponds to ΔG.

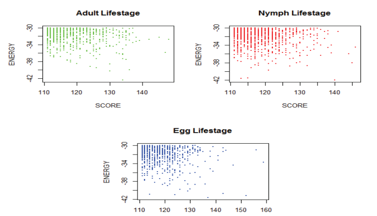

Figure 8. Plot showing relationship between score on x-axis and ΔG on Y-axis of predicted duplexes.

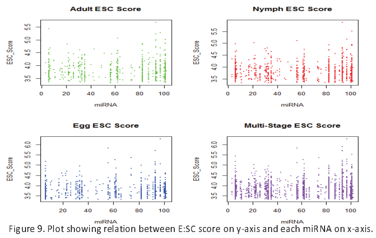

Figure 9. Plot showing relation between E:SC score on y-axis and each miRNA on x-axis.

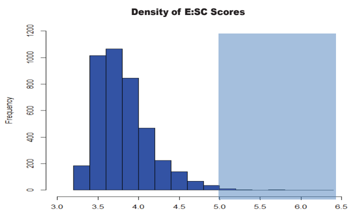

Figure 10. Density of E:SC score histogram all life stages. Shaded region is top 10 %.

Table 1. Predicted duplexes with an E:SC score greater than 5.1 and their respective attributes.

cDNA	Gene Name	Function	miRNA	Score	Minimum Free Energy	E:SC	Stage
8422	alpha 1c	protein metabolic process	Mir-3049	134	-42.41	5.7	Adult
21172	ubiquitin-protein ligase	catalytic activity	Mir-1923	136	-39.99	5.4	Adult
11463	inactivation-no-after-potential	cytoskeletal protein binding	Mir-3049	130	-40.25	5.2	Adult
26098	60s ribosomal protein l39	ribosome	Mir-3054	145	-38	5.5	Nymph
24140	cuticular protein 56f	structural constituent of cuticle	Mir-3049	137	-41.62	5.7	Egg
35093	cuticular protein 56f	structural constituent of cuticle	Mir-3049	137	-41.62	5.7	Egg
5272	homogentisate phytyltransferase	homogentisate phytyltransferase activity	Mir-3052	147	-34.44	5.1	Egg
39678	myosin-viia	imaginal disc-derived wing vein morphogenesis	Mir-3049	124	-40.98	5.1	Egg
17777	Protein	protein polymerization	Mir-3052	152	-41.19	6.3	Egg

Next The miRanda algorithm was run using 103 pea aphid (*Acyrthosiphon pisum)* miRNAs as reference sequences. The respective cDNAs unique to each life stage were used as the cDNA reference sequences. The score cutoff was set to 110 and the ΔG was set to -30. The resulting raw data was then mined for pertinent information. The format was changed and the original miRNA and cDNA sequences were attached to their respective predicted duplexes. The E:SC score was calculated and the top 10% of interactions were kept for further analysis. The cDNA sequences belonging to the kept duplexes were then run through BLAST via Blast2GO. The sequences that returned a meaningful value were then annotated for Gene Ontology(GO). The resulting data was then analyzed.

Results

The total cDNA regulation profile including non-unique cDNAs shows there is a trend where the number of miRNA duplexes decreases for each miRNA as the insect ages (figure 3). The unique cDNA regulation profile shows that the number of miRNA duplexes predicted for each miRNA does not follow a general trend (figure 4). The plot of ΔG vs Score shows that there is not an inherant correlation between alignment score and ΔG (figure 8). The E:SC score must then be used to find a cut off (figure 9). Keeping the top ten percent of E:SC score resulted in an E:SC cutoff of 5.1 (figure 10). The resulting predicted duplexes and the genes involved are shown in table 1. Five of the nine genes shown are predicted to be target by mir-3049.

Discussion

It has been determine that though most of the studied miRNAs target many genes there is no trend in the number of duplexes for each miRNA with respect to the age of the insect. Some miRNAs are active to the adult life stage, but less active in the egg life stage. This makes sense as key genes need to be turned on and then off during the development of the insect. miRNAs have been predicted to down regulate hundred or even thousands of genes. This makes sense in terms of broad regulation and is supported by the literature. It does indicate though that these miRNAs while useful for inducing a wide scale proteomic shift cannot be used for knockouts of key genes. Such key miRNAs such as mir-3049 could be useful for inducing such wide-scale shifts. Further stringencies that take into account miRNA specificity must be introduced. Overall this process may prove to be invaluable in the search for new and better techniques for the purposed of pest management.

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