MicroRNA target prediction and verification in the green alga
*Chlamydomonas reinhardtii*
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MicroRNAs (miRNAs) are a component of the RNA interference (RNAi) machinery, which provides cells with a precise and delicate means of regulating gene expression by a variety of mechanisms. MicroRNA-mediated gene silencing can occur by mRNA cleavage and degradation (found mainly in plants) or by translational repression (found mainly in animals). While it has been established that the green alga *Chlamydomonas reinhardtii* contains functional miRNAs, little is known about what genes these miRNAs target for silencing. In this study, we developed an in-silico method to predict miRNA targets in the *C. reinhardtii* genome.

Five potential miRNAs were identified from the sequences of expressed small RNAs isolated from *C. reinhardtii* strains. They were chosen based on folding patterns characteristic of miRNA precursors. Computational prediction of the targets of these five miRNAs proceeded in multiple steps. First, we identified potential binding-sites using the PITA target prediction program. This program determines the change in free energy (ddg) necessary for the binding to occur [1]. Next, we matched the miRNA sequences against all mRNAs 3' UTRs predicted by version 6.8 of the Augustus gene modeling program [2]. We also matched them separately against all mRNA transcripts in the Joint Genome Institute (JGI) frozen gene catalog, version 20080828 [3]. Only the most energetically favorable interactions (those with a ddg less than -20 for targets predicted from 3' UTRs and -15 for those predicted from transcripts) were saved for further analysis. We then used the RNAhybrid program to find the most likely binding site within the 3' UTR or transcript, requiring a perfect match for the entire seed region (nucleotides 2-8) [4]. For targets predicted from both 3' UTRs and transcripts, the results were filtered based on gap length and location, whereas those predicted from transcripts were additionally filtered to keep only predictions with one or no mismatches. Finally, we examined all remaining putative targets on the JGI genome browser, filtering out any predictions that did not correspond to a known gene, had no expressed sequence tag data, matched to a transposable element, or for which the target gene was not conserved in the genome of another green alga, *Volvox carteri*. As a result, we identified six potential targets with matches to their 3'UTRs and another seven with matches to their coding regions.

From the final list, we chose five predicted targets (three from 3'UTRs and two from coding regions) for experimental verification. We are currently examining the transcript levels of these target genes by quantitative real-time PCR in wildtype cells and corresponding RNAi-defective mutants. If the target genes were correctly identified, we expect to see higher mRNA levels in the mutants. We will also test protein-levels by immunoblotting on the same sets of strains. Finally, our prediction strategy will be refined and improved based on the results of this verification.