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ORIGINAL RESEARCH

Translation Efficiency in Upstream Region of microRNA Targets in *Arabidopsis thaliana*

Wanjun Gu[1], Chuanying Zhai[1], Xiaofei Wang[1], Xueying Xie[1], Gurunadh Parinandi[2,3] and Tong Zhou[2,3]

[1]Key Laboratory of Child Development and Learning Science of Ministry of Education of China, Southeast University, Nanjing, Jiangsu 210096, China. [2]Institute for Personalized Respiratory Medicine, The University of Illinois at Chicago, Chicago, IL 60612, USA. [3]Section of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, The University of Illinois at Chicago, Chicago, IL 60612, USA. Corresponding authors email: wanjungu@gmail.com; tongzhou@uic.edu

Abstract: With respect to upstream regions of microRNA (miRNA) target sites located in protein coding sequences, experimental studies have suggested rare codons, rather than frequent codons, are important for miRNA function, because they slow down the local translational process. But, whether there is a trend of reduced translation efficiency near miRNA targets is still unknown. Using *Arabidopsis thaliana*, we perform genome-wide analysis of synonymous codon usage in upstream regions of miRNA target sites. At the whole genome level, we find no significant selection signals for decreased translational efficiency. However, the same genome analyses do show substantial variations of translation efficiency reduction among miRNA targets. We find that miRNA conservation level, gene codon usage bias, and the mechanism of miRNA action can account for the differences in translation efficiency. But gene's GC content, gene expression level, and miRNA target's conservation level have no effect on local translation efficiency of miRNA targets. Although local translation efficiency in the upstream region of miRNA targets is related to miRNA function in *A. thaliana*, the selection signal of rare codon usage in that region is weak. We propose some other biological factors are more important than local translation efficiency in miRNA action when miRNA targets are located in protein coding sequences.

Keywords: translation efficiency, miRNA binding, synonymous codons

Evolutionary Bioinformatics 2012:8 565–574

doi: 10.4137/EBO.S10362

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Introduction

microRNAs (miRNAs) are small, non-coding RNAs that are between nineteen to twenty-five nucleotides in length. miRNAs function as post-transcriptional regulators by complementarily binding to sequences on target mRNAs.[1] After specific binding of miRNAs to their target sites, targeted mRNA transcripts are usually silenced by translational repression, mRNA degradation, or gene silencing.[2,3] miRNAs have been widely found in many eukaryotes.[4] For example, more than 1,000 miRNAs which can target more than half of the human genes have been identified in the human genome. Since they have important functions in gene expression regulation, miRNAs are involved in many biological processes such as cell differentiation, development, and metabolism.[1] Aberrant miRNA expression, or miRNA activity, has been associated with many diseases,[5] such as cancer,[6] schizophrenia,[7] and cardiomyopathy.[8]

In genome evolution, various sequence features have been selected to ensure proper miRNA activity.[4] For example, miRNA genes, especially those in the seed region, are well conserved in plants[9] and animals.[1] Low level of nucleotide polymorphisms in miRNA genes has also been observed in many species.[10] Similar to miRNA genes, less nucleotide polymorphisms within species and lower nucleotide diversity among species have been observed in miRNA target regions.[11] In plants, miRNA target regions are mostly located in protein coding sequences. Unlike plants, animal miRNAs have their target sites in 3′-UTR region of mRNA transcripts.[1] However, many recent studies have suggested that animal miRNAs have functional target sites in protein coding regions as well as in non-coding regions.[12–17] Protein coding sequences may code extra information for proper miRNA activity.

Many studies have suggested protein coding sequences have the ability to code regulatory information by using specific synonymous codons.[18] Increasing evidence has shown that synonymous codons are widely selected for various biological functions in both prokaryotes and eukaryotes.[19,20] These selections can then be divided into three layers. First, biased usage of synonymous codons is related to biological features at the DNA level (such as DNA secondary structures[21] and nucleosome positioning).[22] Second, it is related to some features at the RNA level. These include mRNA stability,[23,24] mRNA splicing,[25,26] translation initiation,[27–29] and translation efficiency and accuracy.[20,30–33] Third, at the protein level, synonymous codons at specific positions are selected for proper co-translational protein folding.[34,35] Some recent analyses have suggested that synonymous codon usage is also related to miRNA function.[36–39] A computational analysis has observed low synonymous substitution rates in several miRNA target regions in humans.[36] By comparing orthologs of 29 mammals, Lin et al has suggested some genes are experiencing negative selection at synonymous codon sites in order to maintain proper miRNA function.[38,39] Silent mutations in some miRNA coding targets have been observed to eliminate miRNA activity, as well as delay the induced phenotype in mice.[17] Additionally, a synonymous mutation at the binding site of miR-196 in *IRGM* has been associated with Crohn's disease.[40] All these observations have suggested that synonymous codons directly targeted by miRNAs are selected for proper miRNA activity and function.

Other than codons directly bound to miRNAs, nucleotides near miRNA binding sites present an important part in miRNA function. miRNAs are required to bind to their target sites in order to initiate the regulatory process.[2] Surrounding nucleotides of miRNA target sites may affect miRNA binding, which is caused by local mRNA secondary structure[41–43] and/or upstream translation efficiency.[44,45] Therefore, synonymous codons near miRNA target sites may be selected for efficient miRNA binding. Gu et al has shown experimentally that rare codons, located within upstream miRNA target sites, can slow down local translational process and increase miRNA activity in mammalian cells.[44] Another computational analysis did not reveal any consecutive rare codons within the upstream region of a virus miRNA target.[45] It is still unknown whether or not there is a biased usage of rare codons in the upstream region of miRNA target.

In this study, we performed a genome-wide analysis of local translation efficiency in the upstream region of all miRNA coding targets in *Arabidopsis thaliana*. We chose to use *A. thaliana* since plants have more target sites in protein coding sequences than animals, and *A. thaliana* is itself a well-studied plant organism. Although plant miRNAs are different from animal miRNAs in miRNA action, the requirement of opening

miRNA target sites for miRNA binding is largely the same. Decreased local translation efficiency is related to miRNA function by making miRNA target sites more accessible in animal genomes.[44] Therefore, if translation efficiency is important for miRNA activity, local translation efficiency should be selected in plant genomes as well. For each miRNA target region, we computed the local translation efficiency in the upstream region of miRNA target sites. To estimate the significance of synonymous selection for translation efficiency, we permuted mRNA sequences and assessed the deviation from random expectation. We addressed the following problems: (1) Is there any selection pressure acting on synonymous codons in upstream region of miRNA target sites for decreased translation efficiency? (2) Is there any variation of local translation efficiency among miRNA targets in the genome? (3) If there is within-genome variation, what are the possible factors that may affect local translation efficiency?

Materials and Methods

Data

We first downloaded all the protein coding sequences for *A. thaliana* from Ensembl[46] (http://plants.ensembl.org, release 9, April 2011) using BioMart.[47] In the case where a gene has multiple transcripts, we chose the longest transcript for our analysis. Next, we downloaded all miRNAs for *A. thaliana* from miRBase[48] (http://www.mirbase.org, release 16, September 2010). We then used the psTarget server[49] (http://plantgrn.noble.org/psRNATarget) to parse all of the putative miRNA target regions in protein coding sequences. All predicted miRNA target regions were available in the Supplementary Table 1.

To evaluate possible factors on local translation efficiency near miRNA target region, we downloaded the conservation data and expression data for both miRNAs and their target genes. miRNAs have been dynamically gained and lost throughout the evolutionary history of *A. thaliana.*[10] Some miRNAs are well conserved in all land plants, while some are specific to a single species or lineage. We classified miRNAs in *A. thaliana* into three conservation groups (*A. thaliana* specific miRNAs, *Arabidopsis* lineage specific miRNAs, and conserved miRNAs). The conservation category for each miRNA was obtained from Fahlgren et al.[10] Similarly, miRNA target regions have experienced dynamic gain or loss as well.[10] Hence, we also classified miRNA target regions in *A. thaliana* into two conservation groups (*A. thaliana* specific target regions and conserved target regions in *Arabidopsis* lineage). The conservation category for each miRNA target region was also parsed from Fahlgren et al.[10] Then, we downloaded Massively Parallel Signature Sequencing (MPSS) data of miRNAs and their target transcripts for *A. thaliana* from plant MPSS database[50] (http://mpss.udel.edu/at/mpss_index.php). Since there are not enough expression data for each specific tissue, we used two different measures to estimate the expression level from MPSS data across different tissues. We first summed the number of sequenced short tags for each mRNA or miRNA over all tissues (Exp_{sum}), and used it to estimate its expression level. Next, we counted the number of tissues with sequenced tags for each mRNA or miRNA (Exp_{count}) and used this number as another measure of expression level. We only considered mRNAs or miRNAs with 4 or more tags in a tissue as valid MPSS data.[51]

Plant miRNAs can suppress its target gene by translational repression or RNA degradation.[3] miRNAs with central mismatches to its target mRNA tend to suppress gene expression by translational repression, while miRNAs with perfect central matches to its target mRNA tend to cleave target mRNA.[49] Hence, all miRNA target regions in *A. thaliana* are then classified into two groups: miRNA targets with action mechanism of translational repression and miRNA targets with action mechanism of RNA degradation.

Translation efficiency

We used tRNA adaptation index (*tAI*) to quantify the translation efficiency of mRNA segments.[52] *tAI* is a measure of codon adaptation to the tRNA abundance in the genome, which has been proved to be a good indicator of translation efficiency and accuracy.[29] *tAI* was calculated using the codonR package.[52] In *tAI* calculation, tRNA copy numbers are required to estimate tRNA abundance. We downloaded tRNA copy numbers for *A. thaliana* from the Genomic tRNA Database[53] (http://lowlab.ucsc.edu/GtRNAdb, May 2011).

mRNA randomization

If selection acts on synonymous codons near miRNA target sites to slow down the local translational process

and facilitate miRNA binding, local translation efficiency in the upstream region of miRNA target sites in real mRNAs should be statistically different than that of randomized sequences. Therefore, for each mRNA transcript that is targeted by miRNAs in the protein coding region, we randomly shuffled synonymous codons among sites. mRNA sequences were shuffled after randomization while the encoded protein sequences, gene's codon usage bias, and gene's GC composition were kept the same. Since miRNA target sites are important for miRNA function, codons directly targeted by miRNAs were not shuffled during the mRNA randomization process. We generated 1,000 such permuted mRNA sequences for each miRNA target gene.

Since we were interested in local translation efficiency in the upstream region of miRNA targets, we calculated *tAI* in a window of nine consecutive codons right before the nucleotides targeted by miRNAs. A window of nine consecutive codons was chosen as experimental analysis had validated that nine upstream rare codons could increase target site accessibility and miRNA activity.[44] We also performed the analysis using several different window sizes (7, 8, 10, 11 and 12 consecutive codons) and confirmed that the choice of window size would not change our conclusions (data is not shown). To determine the deviation of the real sequence from randomized sequences, we calculated the *Z*-score of the local translation efficiency (Z_{tAI}) for each miRNA target region by:

$$Z_{tAI} = \frac{tAI_N - \overline{tAI_P}}{\sqrt{\sum_{i=1}^{n} \frac{(tAI_{P_i} - \overline{tAI_P})^2}{n-1}}}$$

Here, tAI_N is the local translation efficiency for the naturally occurring target region under consideration. tAI_{Pi} is the local translation efficiency for the target region in *i*th permuted sequence, and $\overline{tAI_P}$ is the mean of tAI_{Pi} over all permuted sequences. The variable *n* represents the total number of permuted sequences, which is equal to 1,000 in our analysis.

Unlike *tAI*, Z_{tAI} measures the deviation of the local translation efficiency in real mRNA from randomized sequences. A negative Z_{tAI} means lower translation efficiency is selected within upstream region of miRNA target sites, while a positive Z_{tAI} means higher translation efficiency is preferred. Hence, we used Z_{tAI} as the indicator of synonymous selection of local translation efficiency in the upstream region of miRNA target sites.

Results

Overall local translation efficiency upstream miRNA targets in *A. thaliana*

In *A. thaliana*, we parsed 1,000 miRNA target sites in protein coding region of mRNA transcripts (Supplementary Table 1). For each miRNA target region, we permuted mRNA sequences and calculated the *Z*-score of local translation efficiency (Z_{tAI}). The mean Z_{tAI} value of all miRNA targets was slightly above zero ($Z_{tAI} = 0.048$). Statistical analysis showed the overall Z_{tAI} did not deviate significantly from zero (Supplementary Fig. 1; *t*-test, $P = 0.15$). Therefore, there was no selection signal for decreased translation efficiency on synonymous codon sites with respect to upstream miRNA targets when all miRNA coding target regions in *A. thaliana* were taken into account.

Within genome variation of Z_{tAI} upstream miRNA target sites and potential factors

Although Z_{tAI} was not significantly different from zero at the genome level, there was a substantial variation of Z_{tAI} values among miRNA target regions (Supplementary Fig. 1). It is still possible that a subset of miRNA target regions may have signals of reduced local translation efficiency. Next, we tried to determine if we could find a subset of miRNA target regions that are selected for reduced local translation efficiency in the upstream region. We investigated several potential factors that might affect local translation efficiency in upstream miRNA target regions. These included gene's GC content, gene's codon usage bias, conservation level of miRNAs and miRNA target regions, expression level of miRNA and miRNA target gene, and mode of miRNA action.

GC content

We first considered gene's GC content. We did not observe a significant correlation between the gene's GC content and local Z_{tAI} in the upstream region of miRNA target sites (Supplementary Fig. 2A; Pearson's correlation test, $P = 0.39$). We further

separated miRNA targets in genes with the highest 5% and the lowest 5% GC content, and compared Z_{tAI} of these two groups. miRNA target regions in genes with the highest 5% GC content and the lowest 5% GC content had similar local Z_{tAI} values (*t*-test, $P = 0.85$; Supplementary Fig. 2B). For each group of miRNA target regions, neither miRNA targets in genes with higher GC content (*t*-test, $P = 0.31$) nor those in genes with lower GC content (*t*-test, $P = 0.21$) had Z_{tAI} values that deviated significantly from zero (Supplementary Fig. 2B).

Gene codon bias

Next, we considered the gene codon usage bias. We used ENC (Effective Number of Codons) to measure a gene's codon usage bias.[54] The higher a gene's codon usage bias, the lower the gene's ENC. We compared Z_{tAI} of target regions in genes with the top 5% ENC to genes with the bottom 5% ENC. There was a significant difference (*t*-test, $P = 0.03$) of Z_{tAI} values between miRNA targets in genes with different codon usage bias (Fig. 1). Notably, miRNA target regions in genes with higher codon bias had lower Z_{tAI} values. The mean value of Z_{tAI} was weakly significant (Fig. 1; *t*-test, $P = 0.09$), and was less than zero when miRNA target regions in genes with the bottom 5% ENC were separately considered. In contrast, the mean value of Z_{tAI} of miRNA target regions in genes with the top 5% ENC was positive (Fig. 1), but not significant (*t*-test, $P = 0.17$).

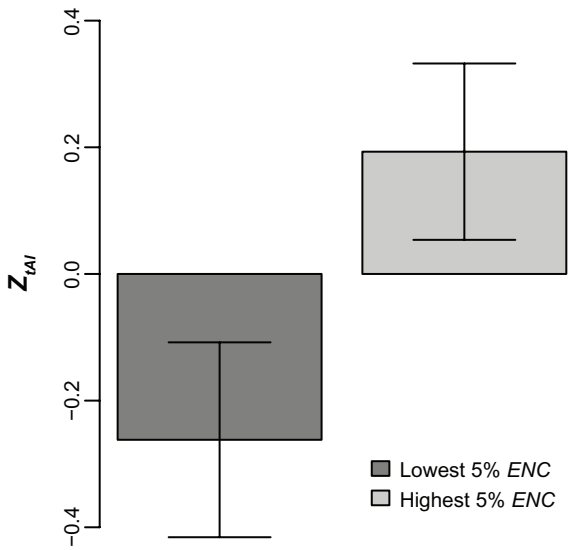

Figure 1. Comparison of Z_{tAI} values of miRNA targets with the top 5% and bottom 5% codon usage bias in *A. thaliana*.

Conservation level of miRNA and its target region

We further considered the conservation level of miRNA and its target regions. First, we compared the mean value of Z_{tAI} for miRNA target regions with different miRNA conservation levels (Fig. 2A). Target regions with *Arabidopsis* lineage-specific miRNAs had significantly lower Z_{tAI} values, when compared to those with *A. thaliana* specific miRNAs (*t*-test, $P = 0.02$). However, the mean value of Z_{tAI} in target regions with *Arabidopsis* lineage-specific miRNAs, when compared to those with deep conserved miRNAs, showed no statistical difference (*t*-test, $P = 0.62$). Notably, the Z_{tAI} values of target regions with *A. thaliana* specific miRNAs were statistically positive (*t*-test, *P*-value = 0.03; Fig. 2A). Second, we compared Z_{tAI} values between target regions with different conservation levels. Target regions that specific to *A. thaliana* and those conserved in *Arabidopsis* lineage had similar Z_{tAI} values (Fig. 2B; *t*-test, $P = 0.98$). When miRNA target regions were separated by target region conservation level, none of the subgroups showed Z_{tAI} values to be statistically deviated from zero (Fig. 2B).

miRNA expression and target gene expression

We also considered the expression level of miRNA and its target mRNA. When comparing Z_{tAI} values of miRNA targets with the highest 5% expression level to those with the lowest 5% gene expression level, we did not observe any significant difference. No method of quantifying the gene expression affected local translation efficiency (Exp_{sum}: *t*-test, $P = 0.99$, Exp_{tissue}: *t*-test, $P = 0.48$; Supplementary Fig. 3). Other than the gene expression level, we compared Z_{tAI} in miRNA targets with the highest 5% mRNA expression level to those with the lowest 5% expression level. We did not find any obvious difference between these two groups of targets (Exp_{sum}: *t*-test, $P = 0.85$, Exp_{tissue}: *t*-test, $P = 0.90$; Supplementary Fig. 4). When miRNA targets were separated by expression level from miRNA or its target gene, Z_{tAI} showed no significant deviation from zero in all subgroups (Supplementary Figs. 3 and 4).

Mechanism of miRNA regulation

We finally considered the mechanism that miRNAs use to regulate target gene's expression.

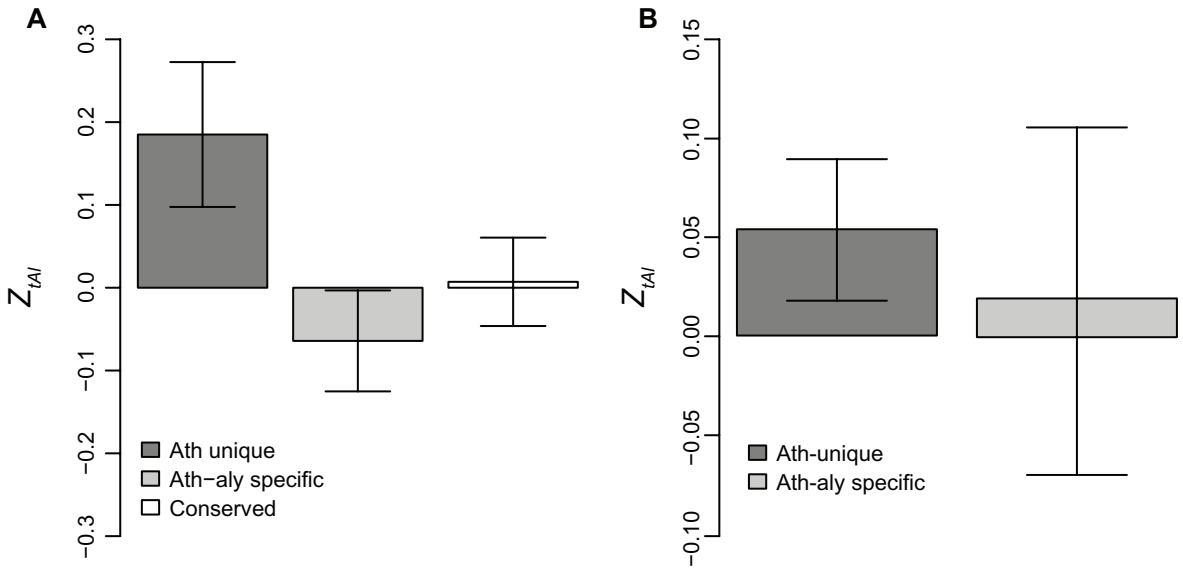

Figure 2. Comparison of Z_{tAI} values in upstream region of miRNA targets with (**A**) miRNA conservation level and (**B**) different conservation level of miRNA target sites.

We compared Z_{tAI}values of miRNA targets with these two mechanisms of miRNA regulation. Z_{tAI} values in these two groups of miRNA targets showed significant difference (*t*-test, $P = 0.02$; Fig. 3). miRNA targets with the action mechanism of translational repression showed significant positive Z_{tAI} values (*t*-test, $P = 0.006$). Those miRNA targets with the action mechanism of RNA degradation showed no significant deviation (*t*-test, $P = 0.91$).

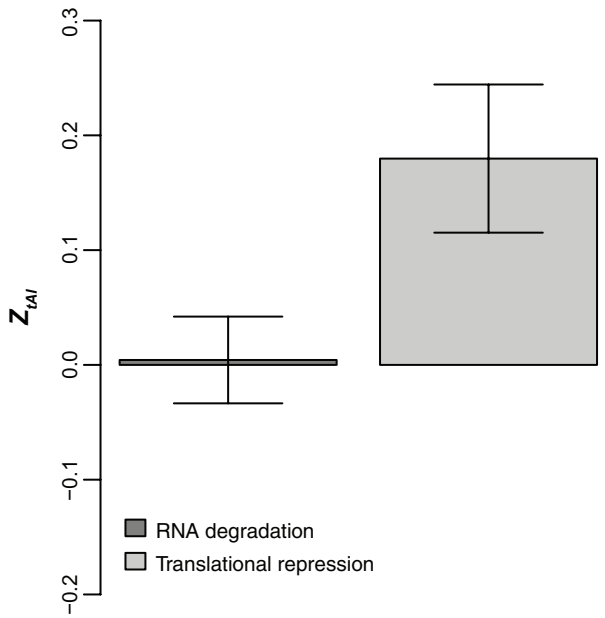

Figure 3. Comparison of Z_{tAI} values in upstream region of miRNA targets with different mechanisms of miRNA action.

Discussion

Here we have completed a genome wide survey of local translation efficiency in the upstream region of miRNA target sites in *A. thaliana*. We have observed no significant difference of local translation efficiency in upstream region of miRNA target sites in protein coding sequences (Supplementary Fig. 1). However, we have observed substantial variations of Z_{tAI} values among miRNA target regions (Supplementary Fig. 1). We have also identified several factors that may explain the local translation efficiency selection variation (Figs. 1, 2A and 3).

The factors that are related to selection of local translation efficiency include gene codon usage bias, miRNA conservation level, and mechanism of miRNA regulation. Since codon preference and tRNA abundance are highly correlated, synonymous codons are selected for efficient mRNA translation in *A. thaliana*.[51] If reduced local translation efficiency was important for proper miRNA function, biased usage of rare codons in upstream regions of miRNA target sites should be more obvious for genes with higher codon usage bias. Our results show Z_{tAI} values are significantly smaller for miRNA target regions in genes with higher codon usage bias (Fig. 1), which is consistent with the above inference.

Previous analysis has also suggested that younger miRNAs are experiencing less purifying selection than conserved miRNAs.[10,55] In our analysis, miRNA

target regions bound by conserved miRNAs have negative Z_{tAI} values, while those targeted by recently gained miRNAs in *A. thaliana* have positive Z_{tAI} values (Fig. 2A). This suggests selection of reduced local translation efficiency is smaller for target regions with younger miRNAs, which is similar to reduced purifying selection in younger miRNA genes.[10,55] In addition to gene codon usage and miRNA conservation level, we observed a significant difference of Z_{tAI} values in groups of miRNA targets using different mechanisms to regulate target mRNAs (Fig. 3). miRNA targets with the mechanism of RNA degradation have lower Z_{tAI} values, when compared to those with the mechanism of translational repression. The reason why these two groups of miRNA target regions have different Z_{tAI} values is unclear. It may be caused by different base pairing schemes of miRNA and mRNA within these two miRNA target groups, since target recognition can potentially affect miRNA binding.[2] We have also found some other factors, such as a gene's GC content, gene expression, miRNA expression, and conservation level of target region, do not affect local translation efficiency in the upstream region of miRNA target sites. Therefore, significant Z_{tAI} differences between subgroups of miRNA targets with different gene codon bias, miRNA conservation level, and mode of miRNA action, suggest local translation efficiency is related to proper miRNA function.

Although local translation efficiency is related to miRNA binding, the selective effect of reduced local translation efficiency is weak. When different subsets of miRNA targets are considered separately, only miRNA target regions in genes with the top 5% codon usage bias show a marginally significant signal of reduced local translation efficiency (Fig. 1). But, miRNA targets which are bound to *A. thaliana* specific miRNAs, as well as those with mechanism of translational repression, have a significant signal of increased local translation efficiency. This is contrary to the proposed reduced local translation efficiency in upstream region of miRNA targets. Previous analysis has suggested synonymous codons are selected for efficient mRNA translation in *A. thaliana*.[51] Hence, the increased local translation efficiency within these two groups of miRNA targets could be the signal of efficient translational selection for full-length mRNA. Compared to translational selection acting on full-length mRNA, a selective constraint to slow down the local translation process in the upstream region of miRNA target sites is probably too weak to be detected.

Our results are comparable with two previous experimental studies that examined the effects of translation on miRNA activity.[44,45] Both studies have proposed that translation may inhibit miRNA access to its target sites when they are located in protein coding regions.[44,45] Gu et al[44] has observed a loss in miRNA function when a miRNA target naturally located in 3′-UTR is modified to be situated in human protein coding regions. However, if the local translational process of miRNA upstream target sites were slowed down, miRNA activity can be restored. In contrast, Lin et al[45] suggested that translation can diminish, but not completely stop miRNA activity. This was accomplished through analysis of a naturally occurring viral miRNA target located in the coding region of one transcript, and in the 3′-UTR of an overlapping transcript. However, codon usage in the upstream region of that viral miRNA target is similar to randomly selecting cellular and viral coding sequences.

In our results, we have confirmed local translation efficiency is related to miRNA activity in *A. thaliana*. The selection constraint of reduced local translation efficiency is weak, however, which is consistent with the suggestions of Lin et al.[45] What might be the reason that a different magnitude of translation effect has been observed on miRNA activity? As indicated by Lin et al,[45] we have analyzed naturally occurring miRNA targets in *A. thaliana* protein coding sequences. In contrast, Gu et al[44] made their conclusions on model constructs. This may partly explain the different effects of local translation efficiency on miRNA activity observed in these studies.

Another notable difference is whether rare codons are preferred in upstream region of miRNA targets to facilitate miRNA binding. We have observed most miRNA targets have no preference to use rare codons in their upstream region. However, some miRNA target sites do prefer to use rare codons in the upstream region, such as those in genes with higher codon usage bias. This was observed because we have performed a genome wide analysis on miRNA targets. We have analyzed all miRNA targets in protein coding sequences for *A. thaliana*, compared to only one viral

miRNA target by Lin et al,[45] or several miRNA targets in human by Gu et al.[44] Therefore the conclusion could be biased when inferred from a single or several miRNA targets. Though local translational process is related to miRNA binding when miRNA targets are located in protein coding sequences, local translation efficiency is not strongly selected in *A. thaliana*. As suggested by Lin et al,[45] some other genomic features, such as local mRNA secondary structure, or fold energy near miRNA targets,[41,42] may be the potential factor to facilitate miRNA binding. Recently, we have found increased site accessibility is widely selected to ensure miRNA activity in plant genomes.[56] Although somewhat different, our results are largely comparable with those two previous studies.

In conclusion, we suggest synonymous codon usage in the upstream region of miRNA targets is related to local translation efficiency in *A. thaliana*. However, the selective constraints near most miRNA targets are too weak to be detected. In addition to local translation efficiency, other genomic features may be even more important to miRNA activity, when miRNA targets are located in protein coding sequences. By analyzing local translation efficiency of all miRNA targets in protein coding sequences of *A. thaliana*, we present a genomic view of selective effects on synonymous codon usage in the upstream region of miRNA targets for proper miRNA activity.

Funding

This work was supported by grants from the National Basic Research Program of China [2012CB316501 to WG and XX], the National High Technology Research and Development Program of China (863 Project) [2012AA020401 to WG], National Natural Science Foundation of China [30900836 and 61171143 to WG, and 61001175 to XX], and a SRF for ROCS, SEM, China.

Author Contributions

Conceived and designed the experiments: WG and TZ. Analyzed the data: WG, CZ, XW, XX and TZ. Wrote the first draft of the manuscript: WG. Contributed to the writing of the manuscript: GP and TZ. Agree with manuscript results and conclusions: CZ, XW and TZ. All authors reviewed and approved of the manuscript.

Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

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Supplementary Data

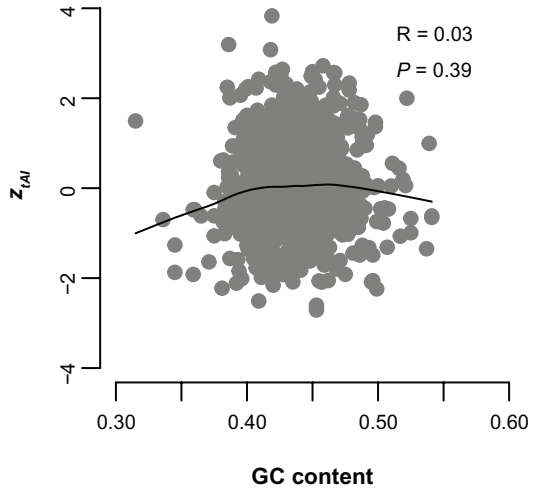

Figure S1. Histogram of Z_{tAI} values of all miRNA targets in protein coding sequences in *Arabidopsis thaliana*.
Note: The solid line represents a null normal distribution.

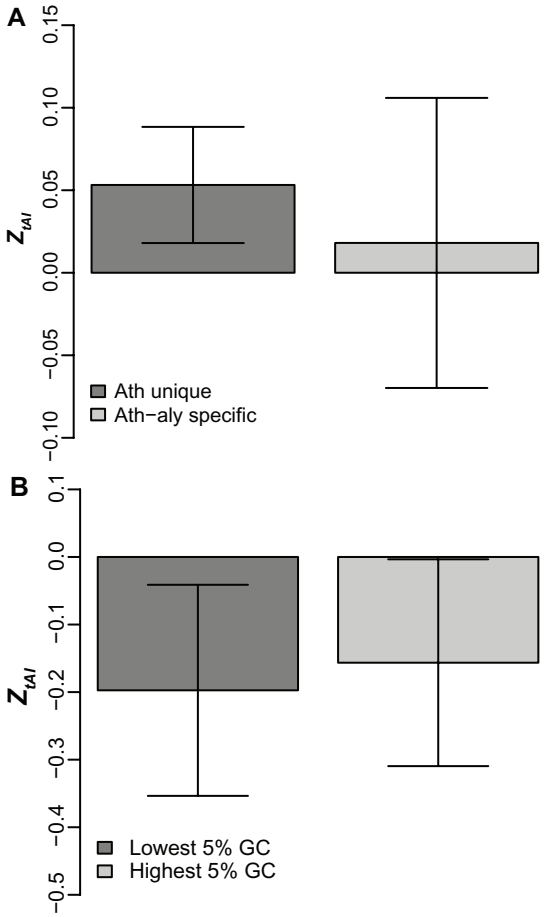

Figure S2. (**A**) The correlation between local Z_{tAI} and gene GC content of each miRNA target in *Arabidopsis thaliana*. Each point represents a miRNA target in the genome. (**B**) Comparison of Z_{tAI} values in upstream region of miRNA targets in genes with the highest 5% and lowest 5% GC content in *Arabidopsis thaliana*.

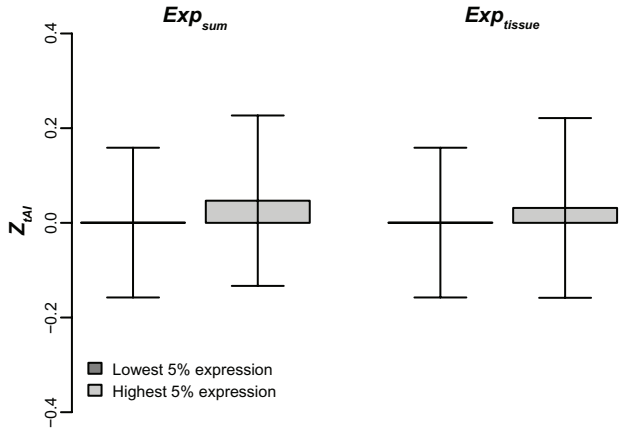

Figure S3. Comparison of Z_{tAI} of miRNA targets with the top 5% and bottom 5% expression level of miRNA gene in *Arabidopsis thaliana*.
Note: Both expressed tag counts (Exp_{sum}) and the number of expressed tissues (Exp_{count}) are used to quantify gene expression level.

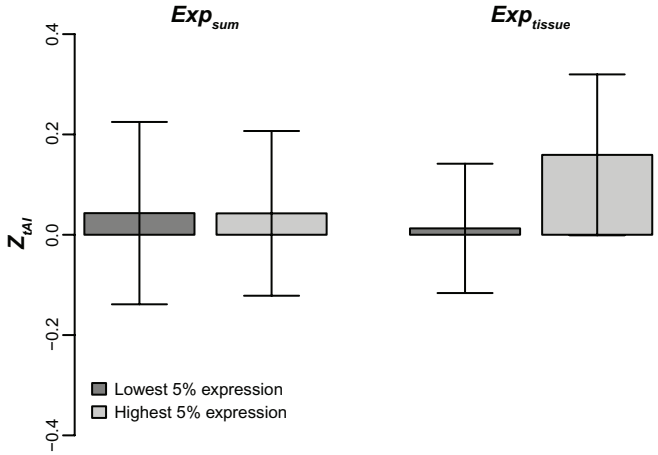

Figure S4. Comparison of Z_{tAI} of miRNA targets with the top 5% and bottom 5% expression level of miRNA target gene in *Arabidopsis thaliana*.
Note: Both expressed tag counts (Exp_{sum}) and the number of expressed tissues (Exp_{count}) are used to quantify gene expression level.

Table S1. miRNA targets in protein coding sequences in Arabidopsis thaliana.