

Supplemental Figure 1. Genomic DNA Sequence Contexts of TPP Riboswitches in *THIC* Genes from Different Plant Species.

TAA identifies the stop codon of the *THIC* open reading frame; **GT** and **AG** designate 5' and 3' splice sites of the first intron (shown in gray letters). **GT** and **AG** identify the splice sites used for generation of type III RNAs. The 3' UTR of type II RNAs is displayed in turquoise letters, the aptamer sequence is highlighted in yellow. The displayed 3' ends of the sequences correspond to the gene annotations for *Arabidopsis thaliana* and *Oryza sativa*. For the other plant species the displayed sequences comply with 3' ends identified by RT-PCR.

Arabidopsis thaliana

AGCTGCTCAGAAAT**TAA**AAAG**GT**CAGTATGTTTAGACTGTTAGTCGTTGCTTTCTCAACAAA
CATGTTAGTTACTGCATGCTAGTATAAAATCATT**CAGGTTTATAATCTTTTCTTAAATCT**
GCAACATATGGTCAACTCTTAAATGAGTCCTTACTGTGATCTTTGTTTTTATCGTGTTT
CTTTTCTTCTGCTGCATC**AGGCAAATGTTTTAAACAAGACCTTGCTTACCCAAGTCTTG**
GTGCTGTTGGACTATACCTGGATAAAGGCACAACTGTTGGT**AAAGCTTAGTAGTCTCTA**
TGTCATGTTACTTTTAGAACTATCTATGTTGTCTGTTTCATTTGAGTCAGAGTCAGCAATA
AAGACAATCTAAGTTGATGTTTCAATACTTTTTTGTGTGATTTGGTTGGTGAATTGACAT
GCAAAAGCACC**AGGGGTGCTTGAACCAGGATAGCCTGCGAAAAGGCGGGCTATCCGGGAC**
CAGGCTGAGAAAGTCCCTTTGAACCTGAACAGGGTAATGCCTGCGCAGGGAGTGTGCAGT
TTTTTTTTTTTCTGTAGCTTTCTAAAGGAGAAGAAGCTACTGTTGCCGCTCGAGTCTCG
TTCCACGGTTTTCAACAGTTAGTTTCTTATGAGCTAAGAGATTCAGCTTAATTGGCTTAC
AGCCATAAAAGAAGTCTTTAACTGATGACTAAGTCACTAACAGTAGGGAATAATTCAAT
CAAAAATCATCCAGATTGATAAAAATGCATTTGCACC

Raphanus sativus

GCCAGAGAGCTATGTCAAGGCTGCTAAGAAG**TAA**AAAG**GT**CAGTATCCTTAGTGGTTATT
ACTTATACAAACATGTTAGTTACTCACATATGGTCAAATGTGTCTTTTTCTGTGAGCTC
TGCCTGTTGTCATCTTTCTTTTCTTATGCTTCTT**AGGGAAAGGCTGGGAACAAGACCT**
CGCTTACACAAGTCTTGGTGCCTGTTGGACTATACCTGAATAAGGCACAAATGTTGGT**A**
AGCTTTAGTAGTCTCTCTGTCTGTTAACTTTAGAACTATCTATGGTTTTATGTTTTCTTCT
GTTTGTTCCTTTGAGTCTGAGTCAGTAATAAAAAAGACAACTGAGTTGATGTTTTAAAT
ACTTTACATGTGATTTGGATTGTGAATTGACATAGAAAGCACCAGGGGTGCTTGAACCAG****
GCTAGCCTGCTAAAGAGCGGGCTATCCTGGGAACAGGCTGAGAAAGTCCCTTTGAACCTG
AACAGGGTAATGCCTGCGCAGGGAGTGTGCAGTTTTCTTTTTTTCTTTTCTATAGGAGA
TGAAGCTTTTCTGCT

Nicotiana tabacum

ACCGGAAAATTACATCAACTCTATGAAAAGC**TAA**AG**GT**GAGTGTTACATTGGATTTTCTC
TTGACATTGTTGTTTTTGCAAGAAGGGGAGCCTTGGCGTAACTGGTAAAGTTGTTGTCAT
GTGACCAGGAGGTCACGGGTTTCGAGCCGCGCAAACAACCTCTTGTAGAAATGCAGGGTAA
GGCTGCGTACAGTAGACCCTTGTGGTCCGGCCCTTCCCTAGACACCGCACATAGCGGGAA
CTTAGTGATCGGGCTGCCCTTTATTATTGTTTCTGCAAGTTCTTTAAAGGTAGAGATAT
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TTCTTTTCTCGACGTCGGGTTATCCAATAGGAGTTGTTGTAGTTTTTCGTAGTTGCAGGAA
CCTTTTTTTTCCCTCCGCAACGTGTTACTTACTGAATGCAATTGC**AGGACTTCATAGAT**
TGAAAACCTGGATCAAGAGCTGTCTGAAGTGATCACGCTCCATCGGCCAGTCAAGAAAGGA

GGGTGTAGGAGCTTCATTATCAAGTGTTAGGTAAAGTATCTAGGTTTGGTATGTTTAAATAA
CTTGAAAACAACAGCCTTGTTTCAGAAAAGTACTCCTTATCAGTAATAAGAAATCACGTAT
TTTATTGTAATTCTGTCTCTTATATCTTTGACAATTTATTGACACGAGAAAAGCACCAGGGG
TGCCTGTGTGAGCTTCAAAAACCTGGCCTTATTAGCCAGGTTATACGCTGACTGAACAGG
CTGAGAAAAGTCCCTTTGAACCTGAACAGGATAAATTCCTGCGTAGGGAGTGTGCATTTTTT
TGTTGCTTGCACAGGGATGGAGATTCTTCCATGGTTCAAATTAAGCTGATCCTGCCCTTT
GCTAGGACCAGTTATACATTATATTCAAGATCAGGGGTGTATTAGTAGTAGAGTTATGG
GTTCAATTTGAAGTTTTGAACCTAACTTTTAGCCTGAATTTATCTGTGTTAGGCGCATGGC
CTTCTTCCGATTAGTCCGGGGCACATTTCCGGCGTGTACAATTTTTGACCACTGC

Nicotiana benthamiana

GCCAAAGTTTTGTTCTATGAAGATAACTGAAGATATAAGGAAGTATGCTGAAACTCACGG
TTATGGAAGTGCAGAGGAAGCAATCCTCCGCGGCATGGATGCTATGAGTGCAGAAATTTCA
AGCTGCAAAGAAAACCATTAGCGGGGAACAACATGGTGAGGTTGGTGGTGAAATCTACTT
GCCGAAAATTACATCAACTCTTTGAAGAGCTAAAGGTGAGCGTTCAATCGGATTTTTCTC
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TTTGTGAACCTGCAACTTAACTCAACAACTCTCTATTTCCGACTACCTATATTTAAATC
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TATATCTTCAACAATTTGTTGACACGAGAAAAGCACCAGGGGTGCCTGTGTCAGCTTCAA
AACCTGGCCTTATTAGCCAGGTTATATGCTGACTGAACAGGCTGAGAAAAGTCCCTTTGAA
CCTGAACAGGATAAATTCCTGCGTAGGGAGTGTGCATTTTTTTTTTTTTGTTGCTTGCACAGG
GATGGAGATTCTTCCATGCTTCAAATTAAGCTGATCCTGCCCTTTGCCAGGACCAGTAAT
ACATTATATTCAAGATCAGGGGTGTATTAGTAGTAGAGTTATGGGTTCAATTTGAAGTTT
TGAACCTATAACTTTTAGCCTGAATTTATTTGTTAGGCGCATGGCCTTTCTTCCGATTAG
GTCCGGGGACTGTGTGTCCGGGGCACATTTCCGGC

Lycopersicon esculentum

GCCAGAGAATTACATCAACTCTTTGAAGAGCCAAAGGTGAGTATTACATTCTGCAAGTCC
TTTGAAGACACTCGTATAATGCAGAGTCTTTGTGTATTCCGATTAAAGCTCCTTTCACTT
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GTATTTATCACTCCATCACGACCCTTGTGGTAAGGCATATTCGTGTATGCCTCTGAGAC
GCGATTGGTTACTTACAATTGACCAATTTTCTCATGGTTAGTCTAGCTTTGGTTGGACTA
TTGAAACATTGAGTAATATGAATGACTCAATTGCATATATACCTTCATATTTTGAATCA
ATATTACGAATTTAAGATCTGG

Oryza sativa

CTATACAGCTCGCAAATAAAGTTGGTCTTTTTCTTATCATGTATAGTTCACCTTGTGGGA
GATGTTTGGACTCTTTTGTCTGAATATGTAGGGTTTTTAACCTCAGTATGAAGCAATTAG
TCATCTGGAGTAAAATGGTCTAGTGCCGTATTTCAATCAGTTTTACATAAATAAGCCATT
AATGTGAGCTTACCGTCTTGAAACAGCTTTTCAGATGCCTTCTCTAGCACTTTTGTGAC
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TAATTCGTGCTGGCTTGTAAATAAAATTAACCTCTATATGTGATCCTTCCGTGTCATATT
TTCTACTCTTTGCATCACTGTGGGTGTTAGTAATGAAAGTTGCACCAGGGGTGCCTGTA
TTCTCAACGATCTGAAGGCCTCTTGGCCTGGATTGTTGTGAATTGGGCTGAGAAAGTCCC
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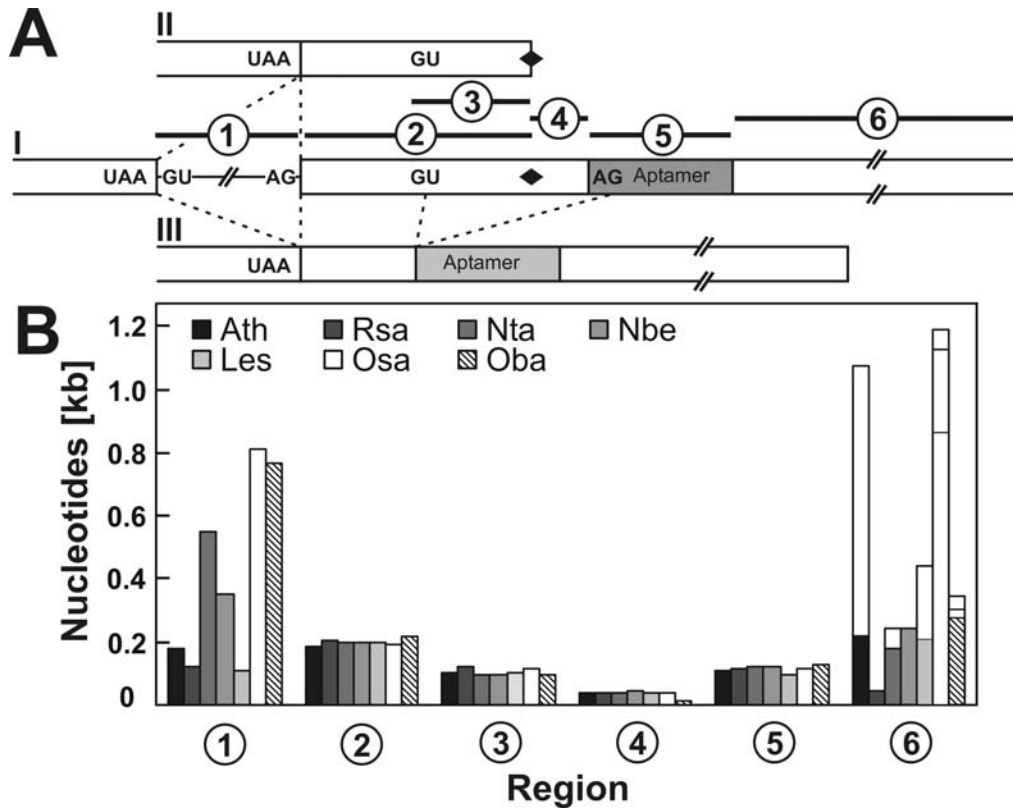
Ocimum basilicum

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ATTATCCTATTGTTTCACTAATTAAGGGATTTTATTCCATAATTTGAAAGTTAAGGTA
TTATCCTTCATTTACTTCTAGTAGGGTATTTTTACCCAAACGGTAGGGTAAAATACCCTA
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TACACATGACACAAAACGTCTTTCTGTATTGTGTATAATAAAATGTAATAATTACGTGTG
CACGTCCCCGTCAGCATCCACGTGGATTTAACGGTTGAATAAACACATTTTGAACACTAT
TAGCGTAAAGTTTGGGATACTTTTTCTAATAAAAATACTATGAGATAGATTTCCAAGCT
GACCGAAAGATATGTCATGTTTTCCCTTCTTTCTATTTCGTTTCGATCTTCAACTTCTAACG
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ATCCGCTCCGTCGGGGCGGTGCAGCTGAGAAAGTCCCTTTGAACCTGAACAGGATAATG
CCTGCGTAGGGAGTGTGCTATCTTTTCTGTTTTCACAGGGTTGGAGTTCGAATTCAAATG
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AAGTGGTGTGGGGTTAATAACTTAATATGGGCCCACTACTTGTGCGTATGCCGTGGGCAA
TATCCCACTGACATTATCGGAATTTTTTATATAAAAAACAAGGCTCTTAAAGATTTAG
AAGAAAAGTTAGAATTTTGCTTATTCTTTTAAATACTTTTTTAAATGAGGTTTCAGTTTC
AAGGAC

Supplemental Figure 2. The Architectures of *THIC* 3' UTRs are Conserved and Relative Distances of Key Features are Similar among Different Species.

(A) Thick lines numbered 1 through 6 designate six regions of RNA transcripts whose lengths were analyzed as described in (B). Dashed lines indicate splicing events and the diamond symbol represents the transcript processing site (see legend to figure 2 for more details).

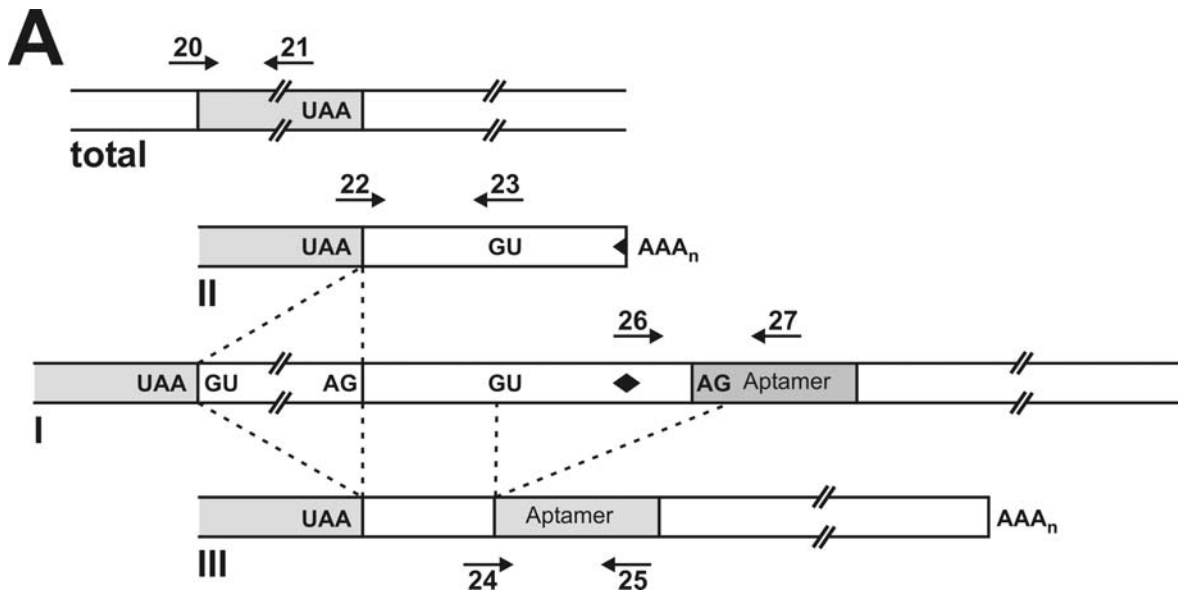
(B) Numbers of nucleotides in the regions defined in (A) are similar amongst seven plant species. The stacked bars for region 6 indicate the identification of transcripts of different lengths.



Supplemental Figure 3. Binding Sites and Sequences of DNA Primers used for qRT-PCR Analysis of the Various *THIC* RNAs from *A. thaliana*.

(A) Schematic representation of the binding sites of primers used for quantitation of total *THIC* transcripts or for separate analysis of *THIC-II*, *THIC-I*, or *THIC-III* RNAs, respectively. Several primers are covering an exon-exon border to avoid detection of the unspliced transcript. DNA primers 20 and 21 are binding to the coding region of *THIC* RNAs and thereby detect all 3' UTR variants.

(B) Sequences and binding sites in the 3' UTR of the forward primers (highlighted in yellow) and reverse primers (highlighted in blue) used for selective detection of the three different *THIC* RNAs from *A. thaliana* by qRT-PCR.



B

***THIC-II* (DNA22/DNA23)**

AGCTGCTCAGAAATAAAGGT CAGTATGTTTAGACTGTTAGTCGTTGCTTTCTCAACAAA
 CATGTTAGTTACTGCATGCTAGTATAAAATCATT CAGGTTTATAATCTTTTCTTAAATCT
 GCAACATATGGTCAACTCTTAAATGAGTCCTTACTGTGATCTTTGTTTTTTATCGTGTTT
 CTTTTTCTTCTGCTGCATCAGGCAATGTTTTAAACAAGACCTTGCTTACCCAAGTCTTG
 GTGCCTGTTGGACTATA CCTGGATAAAGGCACAAACTGTTGTAAGCTTAGTAGTCTCTA
 TGTCATGTTACTTTTAGA ACTATCTATGTTGTCTGTTCAFTTGAGTCAGAGTCAGCAATA
 AAGACAATCTAAGTTGATGTTTCAATACTTTTTTGTGTGATTTGGTTGGTGAATTGACAT
 GCAAAAGCACCAGGGTGCTTGAACCAGGATAGCCTGCGAAAAGGCGGGCTATCCGGGAC
 CAGGCTGAGAAAGTCCCTTTGAACCTGAACAGGGTAATGCCTGCGCAGGGAGTGTGCAGT
 TTTTTTTTTTCTGTAGCTTTCTAAAGGAGAAGAAGCTACTGTTGCCGCTCGAGTCTCG
 TTCCACGGTTTTCAACAGTTAGTTTCTTATGAGCTAAGAGATT CAGCTTAATTGGCTTAC
 AGCCATAAAAGAAGTCTTTAACTGATGACTAAGTCACTAACAGTAGGGAATAATTCAAT
 CAAAAATCATCCAGATTGATAAAAATGCATTTGCACC

THIC-III (DNA24/DNA25)

AGCTGCTCAGAAA**TAA**AAGGT CAGTATGTTTAGACTGTTAGTCGTTGCTTTCTCAACAAA
CATGTTAGTTACTGCATGCTAGTATAAAATCATT CAGGTTTATAATCTTTTCTTAAATCT
GCAACATATGGTCAACTCTTAAATGAGTCCTTACTGTGATCTTTGTTTTTTATCGTGTTT
CTTTTCTTCTGCTGCATCAGGCAAATGTTTTAAACAAGACCTTGCTTACCCAAGTCTTG
GTGCTGTTGGACTATACCTGGATAAAGCCACAACTGTTGTAAGCTTAGTAGTCTCTA
TGTCATGTTACTTTTAGAACTATCTATGTTGTCTGTTCAATTTGAGTCAGAGTCAGCAATA
AAGACAATCTAAGTTGATGTTTCAATACTTTTTTGTGTGATTTGGTTGGTGAATTGACAT
GCAAAAGCACCAGGGTGTCTTGAACCAGGATAGCCTGCGAAAAGGCGGGCTATCCGGGAC
CAGGCTGAGAAAGTCCTTTGAACCTGAACAGGGTAATGCCTGCGCAGGGAGTGTGCAGT
TTTTTTTTTTTCTGTAGCTTTCTAAAGGAGAAGAAGCTACTGTTGCCGCTCGAGTCTCG
TTCCACGGTTTTCAACAGTTAGTTTCTTATGAGCTAAGAGATTCAGCTTAATTGGCTTAC
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CAAAAATCATCCAGATTGATAAAAATGCATTTGCACC

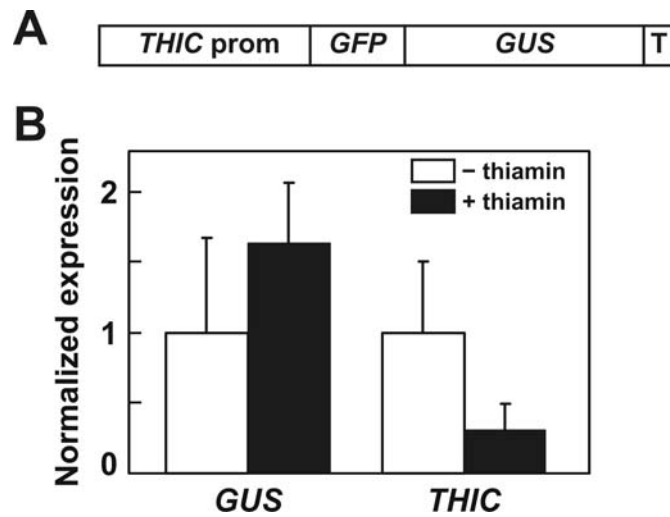
THIC-I (DNA26/DNA27)

AGCTGCTCAGAAA**TAA**AAGGT CAGTATGTTTAGACTGTTAGTCGTTGCTTTCTCAACAAA
CATGTTAGTTACTGCATGCTAGTATAAAATCATT CAGGTTTATAATCTTTTCTTAAATCT
GCAACATATGGTCAACTCTTAAATGAGTCCTTACTGTGATCTTTGTTTTTTATCGTGTTT
CTTTTCTTCTGCTGCATCAGGCAAATGTTTTAAACAAGACCTTGCTTACCCAAGTCTTG
GTGCTGTTGGACTATACCTGGATAAAGGCACAACTGTTGTAAGCTTAGTAGTCTCTA
TGTCATGTTACTTTTAGAACTATCTATGTTGTCTGTTCAATTTGAGTCAGAGTCAGCAATA
AAGACAATCTAAGTTGATGTTTCAATACTTTTTTGTGTGATTTGGTTGGTGAATTGACAT
GCAAAAGCACCAGGGTGTCTTGAACCAGGATAGCCTGCGAAAAGGCGGCTATCCGGGAC
CAGGCTGAGAAAGTCCTTTGAACCTGAACAGGGTAATGCCTGCGCAGGGAGTGTGCAGT
TTTTTTTTTTTCTGTAGCTTTCTAAAGGAGAAGAAGCTACTGTTGCCGCTCGAGTCTCG
TTCCACGGTTTTCAACAGTTAGTTTCTTATGAGCTAAGAGATTCAGCTTAATTGGCTTAC
AGCCATAAAAGAAGTCTTTAACTGATGACTAAGTCACTAACAGTAGGGAATAATTCAAT
CAAAAATCATCCAGATTGATAAAAATGCATTTGCACC

Supplemental Figure 4. The *THIC* Promoter from *A. thaliana* Does Not Appear to be Responsible for Down Regulation of *THIC* Expression After Thiamin Supplementation.

(A) A construct consisting of a 1595 bp fragment of the *THIC* promoter from *A. thaliana* fused to the reporter genes *GFP* and β -glucuronidase (*GUS*) and the 35S terminator (T) from cauliflower mosaic virus was transformed into *A. thaliana*.

(B) Amounts of *GFP-GUS* and *THIC* transcripts were analyzed by qRT-PCR and normalized to the expression of the reference transcript *eEF-1 α* in 9 day old seedlings grown on medium without thiamin or supplemented with 100 μ M thiamin. Data are mean values from three different transgenic lines and from three independent experiments. Error bars represent standard deviation.

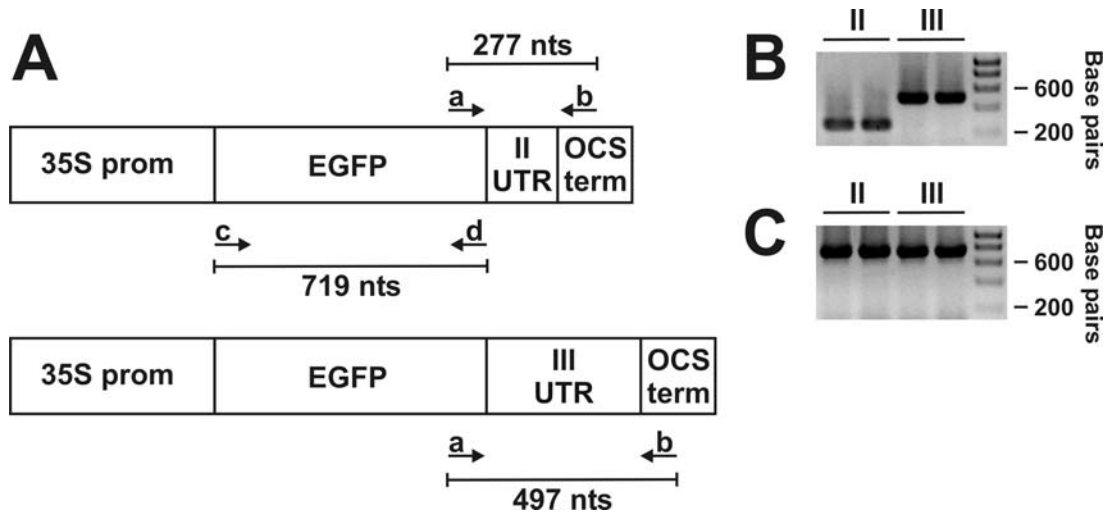


Supplemental Figure 5. Schematic Representation of Reporter Constructs and RT-PCR Detection of RNA Products Containing Two Different 3' UTRs of *AtTHIC*.

(A) Reporter constructs were expressed under the control of the constitutive cauliflower mosaic 35S promoter. Constructs carried the *EGFP* coding region fused either to the 3' UTR of *THIC-II* or *THIC-III* from *A. thaliana* and the octopine synthase (OCS) terminator sequence. Arrows with letters indicate binding sites for primers used for PCR amplification of the respective fragments from cDNA shown in (B) and (C).

(B) PCR amplification with DNA primers a (end of EGFP) and b (start of OCS terminator) yields expected product sizes from cDNAs derived from plants transformed with constructs containing the 3' UTR of *THIC-II* or *THIC-III*, respectively. The two lanes for each reaction represent two independent experiment replications. cDNAs were generated with a polyT primer, which reveals that transcripts extend to and presumably end within the OCS terminator sequence. RT-PCR analysis with primer a and a polyT primer as reverse primer indicates for both constructs that transcripts can extend approximately 150 nts downstream of the end of the *THIC* portion of the construct (data not shown). RT-PCR products were separated using 2% agarose gel electrophoresis and visualized by ethidium bromide staining and UV illumination. "M" designates the marker lane containing DNAs of 200 base-pair increments.

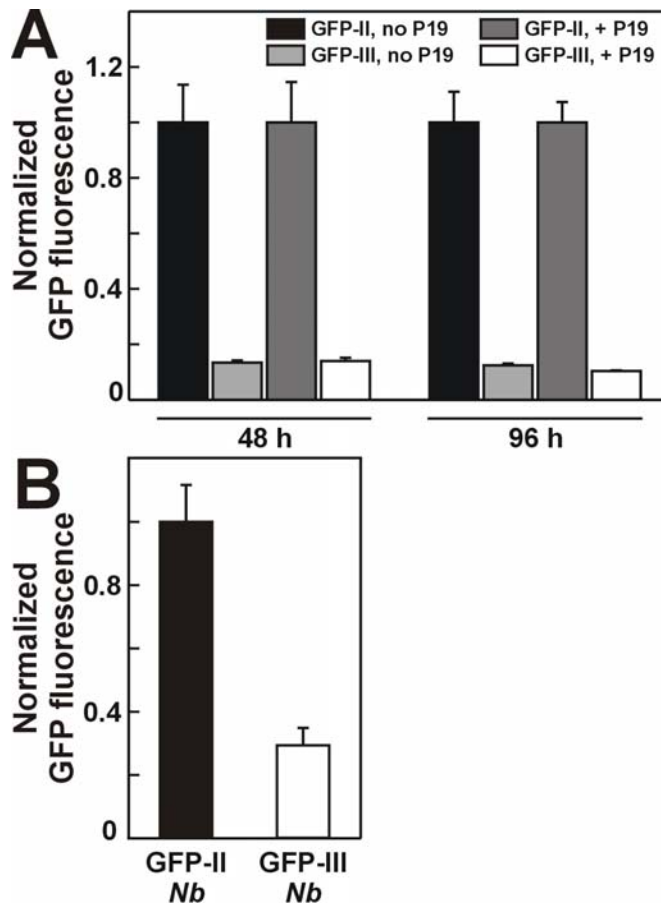
(C) PCR amplification of the coding region of *EGFP* as control reaction with DNA primers c and d from the same cDNAs used in (B).



Supplemental Figure 6. Effect of 3' UTRs from Different Types of *THIC* Transcripts on Reporter Gene Expression.

(A) Reporter fusion constructs consisting of *EGFP* and the 3' UTRs from *THIC*-II or *THIC*-III RNAs from *A. thaliana* were expressed using a transient leaf infiltration assay and fluorescence was measured after 48 h and 96 h. Results were comparable to those observed with the luciferase reporter constructs. It is known that transient expression systems can lead to post-transcriptional gene silencing (PTGS) (Johansen and Carrington, 2001; Voinnet et al., 2003). To assess the possible effects of PTGS, the relative expression of the two 3' UTR variants was determined in the absence or presence of P19, a known suppressor of gene silencing. Fluorescence was normalized relative to the value for the construct containing the 3' UTR of *THIC*-II. Data are averages from four independent experiments and error bars represent standard deviation. The ratio of the activity for the two constructs remained unchanged after coexpression of P19, indicating that PTGS is not involved in the observed differences.

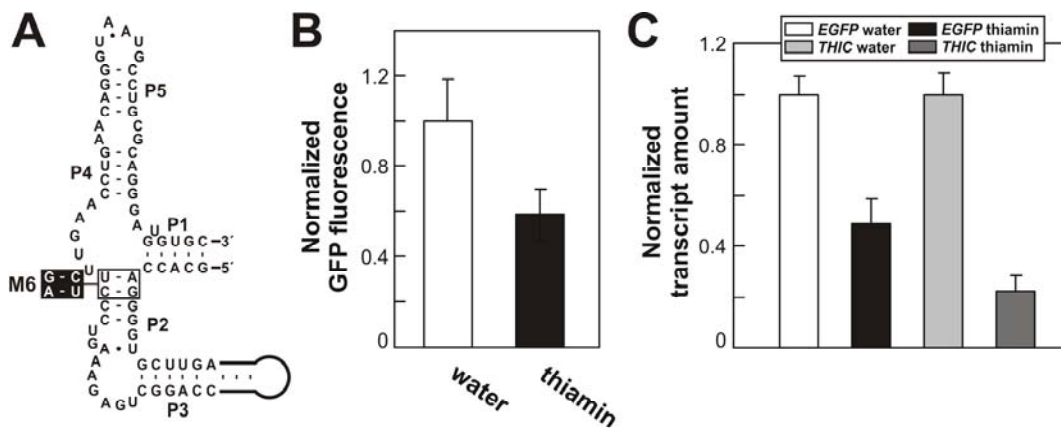
(B) Relative fluorescence of *EGFP* reporter constructs containing the 3' UTRs from *N. benthamiana* *THIC* type II and III RNAs after expression in a leaf infiltration assay. Expression was normalized relative to the value for the construct containing the 3' UTR of *THIC* type II RNAs. Values are averages from two independent experiments and error bars represent standard deviation. The results are equivalent to those observed with constructs based on the 3'UTRs from *A. thaliana*.



Supplemental Figure 7. The Relative Position of the 3' Splice Site for Formation of Type III RNAs can be Variable.

(A) Secondary structure model and sequence of the WT TPP aptamer from *A. thaliana* located in the 3' region of *THIC* that was fused to *EGFP*. Black boxed nucleotides were altered as indicated to generate mutant M6 that is altered at the 3' splice site involved in formation of type III RNAs, and includes additional mutations to permit the formation of pairing stem P2. **(B)** Quantitation of EGFP fluorescence in leaves from *A. thaliana* transformants expressing a reporter construct containing the M6 mutant aptamer sequence. Leaves were excised and incubated with their petioles in water or 0.02% thiamin for 72 h before fluorescence analysis. Values are averages from three independent experiments using different transgenic lines. Error bars represent standard deviation. **(C)** qRT-PCR analyses of *EGFP* and *THIC* transcript amounts in *A. thaliana* transformants. Thiamin treatment was performed as described in (B). Transcript amounts (standardized using a reference transcript) were normalized to transcript abundance in water treated samples. Values are averages from three independent experiments using different transgenic lines. Error bars represent standard deviation.

(D) 3' UTRs of *EGFP* transcripts were analyzed as described in the legend to Figure 4, which resulted in similar RT-PCR products as the construct based on the WT aptamer (data not shown). Sequencing of 3' UTRs from type III RNAs showed the use of a new 3' splice site (**AG**) in the M6 mutant. The green highlighted letters indicate mutations in M6 compared to the wild type sequence and the rest of the sequence is displayed as described in the legend for Supplemental Figure 1.



D *Arabidopsis thaliana*, wildtype

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AGCTGCTCAGAAATAAAGGTCAGTATGTTTAGACTGTTAGTCGTTGCTTTCTCAACAAA
CATGTTAGTTACTGCATGCTAGTATAAAATCATTCAGGTTTATAATCTTTTCTTAAATCT
GCAACATATGGTCAACTCTTAAATGAGTCCTTACTGTGATCTTTGTTTTTTATCGTGTTT
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GTGCCTGTTGGACTATACCTGGATAAAGGCACAAACTGTTGTAAGCTTAGTAGTCTCTA
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CAGGCTGAGAAAAGTCCAGTGAACCTGAACAGGGTAATGCCTGCGCAGGGAGTGTGCAGT
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TTCCACGGTTTTCAACAGTTAGTTTCTTATGAGCTAAGAGATTCAGCTTAATGGCTTAC
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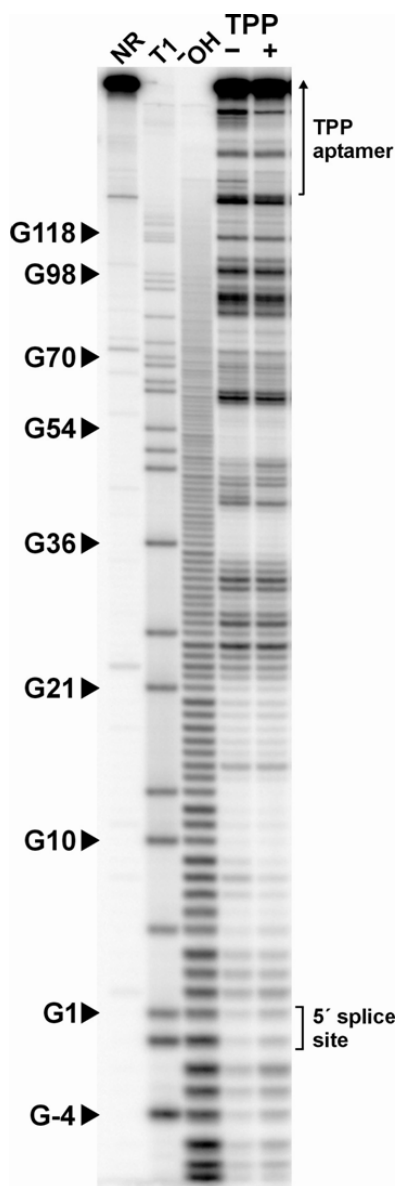
Arabidopsis thaliana, M6 mutations

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CAAAAAATCATCCAGATTGATAAAAAATGCATTTGCACC
    
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Supplemental Figure 8. TPP Induced Modulation in the 5' Flanking Sequence of the Aptamer.

An RNA starting 14 nts upstream of the 5' splice site and extending to the end of the aptamer (-14-261) from *A. thaliana* was produced by *in vitro* transcription and 5' end labeled with ^{32}P . After performing in-line probing reactions in the absence or presence of 10 μM TPP, cleavage products were separated by polyacrylamide gel electrophoresis. Markers were generated by RNase T1 treatment (T1) or partial alkaline digestion (^-OH). The G residue of the 5' splice site was defined as position 1 and the aptamer spans nts 146-256. TPP dependent modulation outside of the aptamer is mainly observed in the region next to the 5' splice site. However, additional structural changes reveal that ligand dependent modulation elsewhere in the 5' flank might be important for control of the 5' splice site structure.



Supplemental Table 1. Sequences of DNA primers.

RT-PCR analysis <i>THIC</i> from <i>Arabidopsis</i>		
DNA1	5'-GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTT TTTTTTTTTTTTTTTTTTTT	polyT
DNA2	5'-AGCT GTCGACA AAGGCCAAATGTTTTAAACAAGACC	<i>Sall</i> ; for 3' UTR
DNA3	5'-AGCT GTCGACG GGTGCAAATGCATTTTTATCAATC	<i>Sall</i> ; rev +221 nt
DNA4	5'- CAGTCACAAAGCCTACGATCAA	rev +882 nt
DNA5	5'-CGGTGAAGTAGGTGGAGAAA	for, end of coding region
RT-PCR analysis <i>EGFP</i>		
DNA6	5'-CGGGATCACTCTCGGCATG	for
RT-PCR analysis <i>THIC</i> from more plant species		
DNA7	5'-GCACAYTTYTGCTCNATGTGYGG	for, end of coding region
DNA8	5'-GGTTCAAAGGGACTTTCTCAG	rev; conserved aptamer region
DNA9	5'-CTGAGAAAGTCCCTTTGAACC	for; conserved aptamer region
Amplification of <i>THIC</i> 3' genomic fragment		
DNA10	5'-ACCGAAATTCTGCTCCATGAA	for; <i>Rsa</i>
DNA11	5'-AGCAGAAAAGCTTCATCTCC	rev; <i>Rsa</i>
DNA12	5'-GCCAAAGTTTTGTTCTATGAAAA	for; <i>Nta</i>
DNA13	5'-GCAGTGGTCAAAAATTGTACAC	rev; <i>Nta</i>
DNA14	5'-GCCAAAGTTTTGTTCTATGAAG	for; <i>Nbe</i>
DNA15	5'-GCAGTGGTCAAAAATTGTACAC	rev; <i>Nbe</i>
DNA16	5'-TCCTAAGTTTTGCTCCATGAAA	for; <i>Les</i>
DNA17	5'-CCAGATCTTAAATTCGTAATATT	rev; <i>Les</i>
DNA18	5'-TTGGCGGCGAAGAAGACG	for; <i>Oba</i>
DNA19	5'-AAATCTTTAAGAGCCTTGTTTTT	rev; <i>Oba</i>
qRT-PCR analysis		
DNA20	5'-ATGTGCAGGTGATGAATGAAGG	for; <i>THIC</i> total
DNA21	5'-GTAGAATGGTGCCTCGTTACACC	rev; <i>THIC</i> total
DNA22	5'-CTGCTCAGAAATAAAAGGCCAAATG	for; <i>THIC</i> II
DNA23	5'-CTACTAAGCTTACCAACAGTTTGTGCC	rev; <i>THIC</i> II
DNA24	5'-GCACAAACTGTTGGGGTGC	for; <i>THIC</i> III
DNA25	5'-CATTACCCTGTTCAAGTTCAAAGG	rev; <i>THIC</i> III
DNA26	5'-AATACTTTTTGTGTGATTTGGTTGG	for; <i>THIC</i> I
DNA27	5'-AGCCTGGTCCCGGATAGC	rev; <i>THIC</i> I
DNA28	5'-GGTAATAACTGCATCTAAAGACAGAGTTCC	for; <i>AT1G13320</i>
DNA29	5'-CCACAACCGCTTGGTGC	rev; <i>AT1G13320</i>
DNA30	5'-GTGTCTACCGACTTTGGTCAAGC	for; <i>At1G13440</i>
DNA31	5'-ACCCATTGCTTGTGCGTACC	rev; <i>At1G13440</i>
DNA32	5'-CTGCTGCCCGACAACCA	for; <i>EGFP</i>
DNA33	5'-GAACTCCAGCAGGACCATGTG	rev; <i>EGFP</i>
DNA34	5'-AGACCCACAAGGCCCTGAA	for; <i>DsRed2</i>
DNA35	5'-CAGCTGCACGGGCTTCTT	rev; <i>DsRed2</i>
Probes for RNA gel blot analysis		
DNA36	5'-CAAGCGTTTGACCGGGA	for; coding region
DNA37	5'-ATGCGTCGACTTATTTCTGAGCAGCTTTGAC	rev; coding region
DNA38	5'-GGGTGCTTGAACCAGGA	for; extended 3' UTR
DNA39	5'-AGCTGTCGACGGTGCAAATGCATTTTTATCAATC	rev; extended 3' UTR

<i>in vitro</i> transcription		
TPP aptamer present in <i>THIC</i> transcript type III		
DNA40	5'- TAATACGACTCACTATAGG CAAAGTGTGGGGTCTTG	for; T7 promoter
DNA41	5'-CACACTCCCTGCGCAGGC	rev
TPP aptamer with 5' flank (nts -14-261 relative to 5' splice site)		
DNA42	5'- TAATACGACTCACTATAGG CACAAAGTGTGGTAA	or; T7 promoter
DNA43	5'-AAACTGCACACTCCCTG	
Cloning of reporter constructs		
DNA44	5'-AGCT GGATCC GCATTCCGGTACTGTTGG	for; <i>Bam</i> HI
DNA45	5'-AGCT GTCGAC TTATACGGCTATTCGCCCTTCTTGCC TTTATG	rev; <i>Sal</i> I
DNA46	5'-AGCT GGATCC ATGACTTCGAAAGTTTATG	for; <i>Bam</i> HI
DNA47	5'-AGCT GTCGAC TTATTGTTCATTTTGAGAAC	rev; <i>Sal</i> I
DNA48	5'-AGCT GGATCC ATGGTGAGCAAGGGCGAGGAG	for; <i>Bam</i> HI
DNA49	5'-AGCT GTCGAC TTACTTGTACAGCTCGTCCATGC	rev; <i>Sal</i> I
DNA50	5'-AGCT GGATCC ATGGCCTCCTCCGAGAAC	for; <i>Bam</i> HI
DNA51	5'-AGCT GTCGAC CTACAGGAACAGGTGGTG	rev; <i>Sal</i> I
DNA52	5'-AGCT GTCGAC ATTGAAACATCAACTTAGATTGTC	rev; <i>Sal</i> I
DNA53	5'-AGCT GTCGAC AGGACTTCATAGATGGAAAA	for; <i>Sal</i> I
DNA54	5'-AGCT GTCGAC TAAAAAACGCGATTTCTTATTA	rev; <i>Sal</i> I
DNA55	5'-AGCT GTCGAC GCCCCGAAATGTGCCCCG	rev; <i>Sal</i> I
DNA56	5'-TCCGGGACCAGGCTGTCAAAGTCCCTTTGAAC	for; M5
DNA57	5'-GTTCAAAGGGACTTT GAC AGCCTGGTCCCGGA	rev; M5
DNA58	5'-CCTTTGAACCTGAACT CGG TAATGCCTGCGC	for; M1
DNA59	5'-GCGCAGGCATTACC GAG TTCAGGTTCAAAGG	rev; M1
DNA60	5'-AGCT GTCGAC AAGGTCAGTATGTTTAGACTGTTAG	for; <i>Sal</i> I
DNA61	5'-AGCT GTCGAC CTCTCCACCTAAACTCAGATTTTG	rev; <i>Sal</i> I
DNA62	5'-AGCT GTCGAC ACCGGTGAGCTCACTAGT AAGCT TAGCT	for; <i>Sal</i> I, <i>Hind</i> III
DNA63	5'-AGCT AAGCT TACTAGTGAGCTCACCGGT GTCGAC AGCT	rev; <i>Hind</i> III, <i>Sal</i> I
DNA64	5'-TCCGGGACCAGGCT CTCT AAGTCCCTTTGAAC	for; M2
DNA65	5'-GTTCAAAGGGACTT AGAG AGCCTGGTCCCGGA	rev; M2
DNA66	5'-GCACCAG CCG TGCTTGAAC	for; M3
DNA67	5'-GTTCAAGCAC GG CTGGTGC	rev; M3
DNA68	5'-CTGAGAAAGT GG CTTTGAACCT	for; M4
DNA69	5'-AGGTTCAAAG CC ACTTTCTCAG	rev; M4
<i>THIC</i> promoter- <i>GFP-GUS</i> expression analysis		
DNA70	5'-CACCTTCTCCTTCTAGTGAAT	for, <i>THIC</i> promoter
DNA71	5'-AGCTGGAGACAAACGAAA	rev, <i>THIC</i> promoter
DNA72	5'-ATGTGCAGGTGATGAATGAAG	for, qRT-PCR <i>THIC</i>
DNA73	5'-CAAAGGACCAAGGGTGTAGAA	rev, qRT-PCR <i>THIC</i>
DNA74	5'-TGGAGTGGTGTAAACGAG	probe, qRT-PCR <i>THIC</i>
DNA75	5'-GCGT*CAATGTAATGTTCT	for, qRT-PCR <i>GUS</i>
DNA76	5'-TCTCTGCCGT*TTCCAAATC	rev, qRT-PCR <i>GUS</i>
DNA77	5'-GATGTGCTGTGCCTGAA	probe, qRT-PCR <i>GUS</i>
DNA78	5'-GAGCCCAAGTTTTTGAAGA	for, qRT-PCR <i>eEF-1α</i>
DNA79	5'-CTAACAGCGAAACGTCCCA	rev, qRT-PCR <i>eEF-1α</i>
DNA80	5'-CCCCAACCAAGCCCAT	probe, qRT-PCR <i>eEF-1α</i>

“*” identifies nucleotides that were introduced to increase the efficiency of the combination of primers and probe in qRT-PCR. Forward and reverse primers are designated “for” and “rev”, respectively.

Supplemental Methods

Cloning of DNA Constructs

All reporter constructs were based on the plasmid pBinAR (Höfgen and Willmitzer, 1992), which contains the constitutive CaMV 35S promoter. The coding sequence of luciferase from *Photinus pyralis* (firefly) was amplified with primers DNA44 and DNA45 and, after restriction with *Bam*HI and *Sal*I, was cloned into appropriate sites of pBinAR to obtain pBinARFLUC. In pBinARFLUC, the peroxisomal targeting sequence at the C-terminus of luciferase was replaced by the amino acid sequence “IAV” to prevent peroxisome localization. To prepare pBinARRiLUC, an intron-containing version of luciferase from the sea pansy *Renilla reniformis* (Cazzonelli and Velten, 2003) was amplified with primers DNA46 and DNA47 and, after restriction, cloned into *Bam*HI/*Sal*I sites of pBinAR. To prepare plasmids containing fluorescent proteins as reporters, the coding sequences of *EGFP* and *DsRed2* were amplified with primers DNA48/49 and DNA 50/51, respectively. After restriction with *Bam*HI/*Sal*I, products were cloned into appropriate sites of pBinAR.

3' UTR sequences from *A. thaliana* *THIC* type II and III RNAs were amplified with primers DNA2/52 and DNA2/3, respectively and cloned into the *Sal*I site of the pBinAR reporter plasmids. For cloning of corresponding constructs based on *THIC* sequences from *N. benthamiana*, 3' UTRs from type II and III RNAs were amplified with primers DNA 53/54 and DNA53/55, respectively. Sequences and orientation of *THIC* 3' UTRs in reporter fusion constructs were confirmed by sequencing.

For generation of the aptamer mutants M1 and M5 (in the context of type III RNAs), the wild-type 3' UTR sequence of *THIC*-III from *A. thaliana* was amplified with DNA2 and DNA3, and cloned using a TOPO TA cloning kit (Invitrogen). PCR mutagenesis was performed on the *THIC*-III 3' UTR in the TOPO TA vector and the nucleotide changes were confirmed by sequencing. Subsequently, the 3' UTR sequences were released from the vector by restriction with *Sal*I and cloned into the appropriate site of the reporter plasmid.

To prepare constructs containing the riboswitch in its genomic context, a fragment of 2242 bp starting from the translational stop codon of *THIC* was amplified from *A. thaliana* genomic DNA with primers DNA60 and DNA61 and cloned into the TOPO TA vector. As pBinAR contains an *Agrobacterium* derived octopine synthase (OCS) terminator that might interfere with riboswitch function, the OCS sequence was removed by restriction with *Sal*I and *Hind*III and the vector religated using a linker consisting of two complementary oligonucleotides (DNA62, DNA63) with the appropriate restriction sites resulting in vector pBinAR-term. This vector without the terminator sequence was used for subsequent cloning of reporter constructs containing the genomic *THIC* sequence and its variants. The coding sequence of *EGFP* was amplified with primers DNA48 and DNA49 and, after restriction with *Bam*HI and *Sal*I, was cloned into appropriate sites of pBinAR-term. In a second step, the genomic *THIC* fragment was released from the TOPO TA vector by *Sal*I digestion and cloned into the *Sal*I site of pBinAREGFP-term. Sequence and orientation of the *THIC* fragment were confirmed by sequencing. For generation of aptamer mutants M1, M2, M3, and M4, PCR mutagenesis was performed on the TOPO TA plasmid containing the *THIC* 3' fragment and, after sequence confirmation, the *Sal*I fragment was cloned into the appropriate site of pBinAREGFP-term. Again, sequence and orientation of the *THIC* fragment were confirmed by sequencing.

In-line Probing of RNA

The DNA template for *in vitro* transcription was obtained by PCR amplification from cDNA and a T7 promoter was introduced by inclusion in the forward primer. *In vitro* transcription, RNA

purification by denaturing polyacrylamide gel electrophoresis (PAGE), and 5' ³²P-labelling of the RNA were performed as described previously (Seetharaman et al., 2001). For in-line probing analysis, the labeled RNA was incubated at room temperature for 40 hours in 50 mM Tris-HCl (pH 8.3 at 23°C), 20 mM MgCl₂, and 100 mM KCl in the absence or presence of varying concentrations of TPP. Cleavage products were resolved by denaturing 10% PAGE, visualized by PhosphorImager (GE Healthcare), and quantitated using ImageQuant software. The apparent K_D value, reflecting the concentration of TPP needed to half-maximally modulate RNA structure, was determined by plotting the normalized fraction of RNA cleaved versus the logarithm of TPP concentration.

***Agrobacterium*-mediated Leaf Infiltration Assay Reporter Gene Quantitation**

Luciferase activity was measured using a dual-luciferase reporter assay system (Promega). Leaf material (~100 mg per sample) was typically harvested 60 h after infiltration and frozen in liquid nitrogen. After grinding, 100 µl 1 X Passive Lysis Buffer (Promega) was added and mixed with the sample vigorously. Samples were incubated for 1 h on ice followed by centrifugation for 20 min at 13,000 g. The resulting supernatant was diluted 1:40 and luciferase activity was measured by subsequent addition of the dual luciferase assay buffers in a plate-reading luminometer (Wallac). Activity of firefly luciferase was normalized to the activity of coexpressed luciferase from sea pansy (or vice versa) or relative to total protein amount determined by Bradford Protein Assay (BioRad).

For fluorescence quantitation, leaves were scanned at several time points after infiltration using a Typhoon Trio+ laser scanner (Amersham Biosciences). Settings for EGFP were excitation at 488 nm and detection at 520 nm BP 40. DsRed2 was excited at 532 nm and detected at 580 nm BP 30. Leaves were not significantly damaged by scanning and were incubated with the petioles in H₂O after excision.

References

- Cazzonelli, C.I., and Velten, J.** (2003). Construction and testing of an intron-containing luciferase reporter gene from *Renilla reniformis*. *Plant Mol Biol Rep* **21**; 271-280.
- Höfgen, R., and Willmitzer, L.** (1992). Transgenic potato plants depleted for the major tuber protein patatin via expression of antisense RNA. *Plant Sci* **87**: 45-54.
- Johansen, L.K., and Carrington, J.C.** (2001). Silencing on the spot. Induction and suppression of RNA silencing in the *Agrobacterium*-mediated transient expression system. *Plant Physiol.* **126**: 930-938.
- Voinnet, O., Rivas, S., Mestre, P., and Baulcombe, D.** (2003). An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant J.* **33**: 949-956.