Autoregulation of the gene for cystathionine γ-synthase in Arabidopsis: post-transcriptional regulation induced by S-adenosylmethionine

H. Onouchi, I. Lambein \(^1\), R. Sakurai, A. Suzuki, Y. Chiba \(^2\) and S. Naito \(^3\)

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Abstract

Cystathionine γ-synthase (CGS) catalyses the first committed step of methionine biosynthesis in higher plants. CGS is encoded by the CGS1 gene in Arabidopsis. Stability of CGS1 mRNA is down-regulated in response to methionine application and the exon 1-coding region of CGS1 itself is necessary and sufficient for this regulation. mto1 (for methionine overaccumulation) mutants of Arabidopsis, which carry single-amino-acid sequence alterations within CGS1 exon 1, are deficient in this regulation and overaccumulate methionine. Since CGS1 exon 1 acts in cis during this regulation, we have proposed a model that the regulation occurs during translation of CGS1 mRNA when the nascent polypeptide of CGS and its mRNA are in close proximity. In fact, application of the translation inhibitor cycloheximide abolished this regulation in vivo. This model predicts that the regulation can be reproduced in an in vitro translation system. Studies using the in vitro translation system of wheatgerm extract have indicated that S-adenosylmethionine, a direct metabolite of methionine, is the effector of this regulation. A S′-truncated RNA species, which is a probable degradation intermediate of CGS1 mRNA in vivo, was also detected in vitro, suggesting that the wheatgerm in vitro translation system reflects the in vivo regulation.

Introduction

Methionine is a sulphur-containing amino acid, which, in addition to its role in protein synthesis, is the direct precursor of AdoMet (S-adenosylmethionine). AdoMet is the primary methyl donor in transmethylation reactions, and is also used in the biosynthesis of polyamines and phytohormone ethylene. Cystathionine γ-synthase (CGS; EC 2.5.1.48; formerly EC 4.2.99.9) catalyses the first committed step of methionine biosynthesis (Figure 1) [1], which is considered to be the key regulatory step in the pathway [2–4]. CGS is encoded by the CGS1 gene in Arabidopsis (GenBank\(^\circ\) accession no. AB010888; Figure 2A) [5,6].

Unlike many of the key-step enzymes in metabolic pathways, CGS is not an allosteric enzyme. In the aquatic plant Lemna, CGS activity was repressed when the plant was grown in the presence of methionine, whereas the addition of methionine or related compounds, such as AdoMet, to the cell-free extract did not inhibit CGS activity [2]. Therefore CGS activity is regulated at some step during the gene expression, although its mechanism is still not known. In this review, we summarize our recent results on the regulation of CGS expression in Arabidopsis.

mto1 mutants of Arabidopsis carry single-amino-acid sequence alterations in CGS

The mto1 (for methionine overaccumulation) mutants of Arabidopsis [7–9] were isolated on the basis of their resistance to ethionine, a toxic analogue of methionine. The toxicity of ethionine is evident from the fact that it is used instead of methionine in many cellular reactions [10]. All mto1 mutants accumulated 10–40-fold higher concentrations of soluble methionine and the steady-state levels of CGS1 mRNA were 2–4-fold higher [8,9,11]. Consistent with the elevated level of CGS1 mRNA, levels of CGS protein and CGS enzyme activity were also higher in mto1-1 mutant plants than in wild-type [8], suggesting that the elevated level of CGS1 mRNA is the primary cause for the overaccumulation of soluble methionine in mto1 mutant plants. Genetic mapping and sequence analysis revealed that all the independently isolated mto1 mutants carry single-base changes in a small region within the exon 1-coding region of the CGS1 gene. In each case, the nucleotide change resulted in an alteration in the amino acid sequence (Figure 2C) [8,9].

Alignment of amino acid sequences of CGS from different plant species revealed that the amino acid sequence coded by exon 1 is not well conserved as a whole. However, there is an approx. 40-amino-acid region that is highly conserved among plant species (referred to as ‘conserved region’) [9].

Key words: cystathionine γ-synthase, feedback regulation, methionine biosynthesis, mRNA stability, nascent polypeptide, S-adenosylmethionine.

Abbreviations used: AdoMet, S-adenosylmethionine; CGS, cystathionine γ-synthase; LUC, luciferase.

\(^1\)Present address: Center for Plant Molecular Genetics and Breeding Research, Seoul National University, Seoul 151-742, South Korea.

\(^2\)Present address: Delaware Biotechnology Institute, University of Delaware, 15 Innovation Way, Newark, DE 19711, U.S.A.

\(^3\)To whom correspondence should be addressed (email naito@abs.agr.hokudai.ac.jp).

©2004 Biochemical Society
Figure 1: Biosynthetic pathway of methionine and AdoMet
Broken lines with an arrow head represent multiple reaction steps.

is within this conserved region that all the mto1 mutations were located (Figure 2B).

Feedback regulation of CGS1 gene expression at the step of mRNA stability
Expression of the CGS1 gene was analysed using liquid callus cultures. Application of exogenous methionine to wild-type calli down-regulated the level of CGS1 mRNA, whereas the level remained high independent of methionine application to mto1-1 mutant calli. This result suggests that CGS1 mRNA accumulation is subject to feedback regulation by methionine or one of its metabolites, and this regulation is impaired in mto1 mutant plants [8].

The fact that mto1 mutations were found within the coding region of the CGS1 gene suggests a post-transcriptional regulation rather than a transcriptional regulation. To test this possibility, we measured the stability of CGS1 mRNA with and without added methionine. Samples were withdrawn at different time points after the addition of the transcriptional inhibitor actinomycin D, and the amount of CGS1 mRNA was determined by Northern hybridization. Application of methionine reduced the half-life of wild-type CGS1 mRNA from approx. 150 to 80 min. In contrast, mto1-1 mutant CGS1 mRNA was very stable, with a half-life of >10 h irrespective of methionine treatment (Figure 3) [12]. Therefore the stability of CGS1 mRNA is down-regulated in response to methionine application.

When wild-type calli were treated with methionine, the amount of full-length CGS1 mRNA was decreased and a short CGS1 RNA species that is truncated at its 5′ region emerged, whereas no such RNA was detected in the mto1-1 mutant. This 5′-truncated RNA species is a probable intermediate of CGS1 mRNA degradation [8].

Figure 2: CGS1 gene and location of the mto1 mutations
(A) Structure of the CGS1 gene. Filled and open boxes represent the coding and non-coding regions respectively.
(B) Amino acid sequence of the conserved region. Identical (reversed) and similar (shaded) amino acids of seven plant species (Arabidopsis, wild strawberry, soya bean, ice plant, tobacco, potato and maize) are indicated. The MTO1 region is indicated below the sequence. (C) Nucleotide and amino acid changes (underlined) in mto1 mutants are indicated below the wild-type sequence. mto1-3 and mto1-5 mutations were identical, although they were isolated independently [8]. Arrowheads indicate the 5′-end points of the 5′-truncated RNA species, with the larger arrowhead representing the more prominently detected end points [14].
Figure 3 | Time course of CGS1 mRNA decay in the presence (filled symbols) or absence (open symbols) of 1 mM methionine, as determined after the addition of actinomycin D alone (●, ○) or both actinomycin D and cycloheximide (●, ○) to liquid callus cultures [12]

Functional analysis of exon 1-coding region of CGS1
The exon 1-coding region of CGS1 was fused in-frame with the coding region of a β-glucuronidase reporter gene, and placed under the control of a cauliflower mosaic virus 35 S RNA promoter. A LUC (firefly luciferase) reporter gene, placed directly under the control of cauliflower mosaic virus 35 S RNA promoter, was used as an internal control. Protoplasts prepared from wild-type calli were transfected with these plasmids by electroporation and incubated for 48 h in the presence or absence of added methionine. β-Glucuronidase activity (normalized with LUC activity) of the plasmid carrying wild-type exon 1 was repressed when the protoplasts were incubated in the presence of methionine, whereas reporter activity remained high irrespective of methionine treatment if the plasmid carried mto1-1 mutant exon 1 [8]. To address whether the down-regulation observed by reporter assays reflects the mRNA level, we constructed transgenic Arabidopsis plant lines carrying essentially the same DNA as used in transfection experiments. Methionine feeding experiments showed that both reporter activity and accumulation of the trans-gene mRNA were down-regulated if the trans-gene carried wild-type exon 1, whereas the levels remained high irrespective of methionine treatment if the trans-gene carried mto1-1 mutant exon 1 [13]. Taken together, these results indicated that the exon 1-coding sequence of CGS1 is necessary and sufficient for its post-transcriptional down-regulation in response to methionine application.

Amino acid sequence in CGS has a role in the regulation
All the mto1 mutants carried single-base changes that resulted in single-amino-acid sequence alterations. To find out whether the nucleotide sequence or the amino acid sequence is important for the regulation, we introduced synonymous codon changes to those codons that were altered in mto1 mutants. The results of transfection experiments showed that these silent mutants behaved similar to the wild-type, indicating that it is the amino acid sequence that has a role in the down-regulation [8].

To identify the functional region, a number of deletions within CGS1 exon 1 were tested by transfection experiments. The results indicated that the conserved region is necessary and sufficient for the down-regulation of reporter activity in response to methionine application [9]. To identify further the functional amino acid sequence within the conserved region, alanine-substituted mutants were analysed by transfection experiments. The results showed a short stretch of the amino acid sequence, (A)RRNCSNIGVAQ(I), to be important for the regulation. This region, designated the ‘MTO1 region’, covers all the mto1 mutation sites (Figure 2C) [9]. The sequence is almost perfectly conserved among the CGS proteins of higher plants, but has not been found elsewhere in the public databases [9].

Regulation occurs during translation
To test if the CGS1 exon 1 sequence acts in cis or trans, we performed genetic cross experiments using transgenic Arabidopsis lines carrying wild-type or mto1-1 mutant exon 1 fused to different reporters. Assays of reporter activities in the F1 progenies indicated that the CGS1 exon 1 sequence acts in cis during the regulation [13].

Despite the fact that the amino acid sequence of exon 1 is crucial, the exon 1 sequence acts in cis. To account for this finding, we have proposed a model that the regulation occurs during the translation of CGS1 mRNA when the nascent polypeptide of CGS and its mRNA are in close proximity [8]. As a test for this model, we analysed the effect of the translation inhibitor cycloheximide on the down-regulation of CGS1 mRNA stability [12]. Simultaneous addition of actinomycin D and cycloheximide significantly stabilized wild-type CGS1 mRNA irrespective of methionine application (Figure 3). In addition, cycloheximide treatment inhibited the production of the truncated CGS1 RNA species. These results indicated that inhibition of translation abolishes the CGS1 mRNA-specific decay process, supporting the idea that translation is necessary for this regulation [12].

Studies using the in vitro translation system of wheatgerm extract
Establishment of an in vitro system would greatly facilitate the elucidation of the molecular mechanism behind the regulation. Our model that the regulation occurs during translation of CGS1 mRNA suggests that the regulation can be reproduced in an in vitro translation system. Chimaeric RNAs carrying the exon 1-coding region of CGS1 fused in-frame to the LUC reporter gene and a poly(A)+ (polyadenylated) tract were transcribed in vitro in the presence of a cap analogue, m7G[5′]ppp[5′]GTP. A similar RNA carrying
sea pansy luciferase reporter without CGS1 exon 1 was used as an internal control. Poly(A)+ RNAs were translated in wheatgerm extract, and reporter activities were analysed [14].

In the in vitro translation system, addition of up to 1 mM methionine did not repress reporter activity from the RNA carrying the wild-type CGS1 exon 1. However, AdoMet at concentrations higher than 0.1 mM did repress reporter activity. Neither methionine nor AdoMet had any effect if the RNA carried mto1-1 mutant exon 1. The results indicated that AdoMet or one of its metabolites is the effector of this regulation, and after testing many metabolites, we could conclude that AdoMet itself is the effector of this regulation [14]. Consistent with this result, transfection experiments showed that AdoMet also down-regulates reporter activity in vivo [14]. These results established that AdoMet is the natural effector of CGS1 expression. It should be noted that the mto1-1 mutant accumulates a 3-fold higher concentration of AdoMet than the wild-type [15].

We analysed the fate of RNA after in vitro translation. When RNA carrying wild-type exon 1 was translated in vitro, a 5′-truncated RNA species similar to the one we have observed in vivo was detected. Primer extension experiments revealed two major 5′-end points of the 5′-truncated RNA species and these two end points were detected both in vivo and in vitro [14]. It is interesting to note that the 5′-end points are located within or very close to the MTO1 region (Figure 2C).

The fact that the same 5′-truncated RNA species was observed in vivo and in vitro strongly suggests that the wheatgerm in vitro translation system reproduces the same RNA degradation event that occurs in vivo. However, the amounts of full-length RNA recovered after in vitro translation in the presence and absence of AdoMet were not appreciably different [14]. This is despite the fact that reporter activity is down-regulated by AdoMet. The results suggest a link between translational regulation and the control of CGS1 mRNA stability.

**Concluding remarks**

Stability determining sequences have been identified in a number of mRNAs, many of which are located in the 3′-untranslated region. There are cases where a cis determinant sequence is located within the coding region [16]. However, there are only a few mRNAs for which the importance of the amino acid sequence has been shown experimentally [8,17,18]. Although we do not yet fully understand the molecular mechanism of CGS1 regulation, studies with the in vitro translation system will help disclose the molecular mechanism of the CGS1 mRNA-specific decay process.

This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by grants from Japan Society for the Promotion of Science.

**References**


Received 29 March 2004