FEBS Letters 582 (2008) 2532–2536

Regulation of the human tRNase Z^S gene expression

Masayuki Takahashi, Hiroaki Takaku, Masayuki Nashimoto*

Department of Applied Life Sciences, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Niigata 956-8603, Japan

Received 9 April 2008; revised 11 May 2008; accepted 13 June 2008

Available online 23 June 2008

Edited by Horst Feldmann

Abstract There are two types of tRNA 3′ processing endoribonucleases (tRNase Z): a short form (tRNase Z^S) and a long form (tRNase Z^L). Although the human genome contains both genes, little is known about the physiological role of tRNase Z^S. We found that the human tRNase Z^S gene expression appears to be post-transcriptionally regulated. Additionally, analyses for *cis*-regulatory elements for the tRNase Z^S gene transcription suggested that transcription factors that bind to five different sites on the promoter work together to potentiate the transcription initiation. Furthermore, we found that tRNase Z^S is predominantly present in the cytosol and hardly in the nucleus.

Keywords: Promoter; Subcellular localization; Transcription; tRNase Z^S; tRNA 3′ processing

1. Introduction

tRNase Z (EC 3.1.26.11) is one of the tRNA processing enzymes, which removes a 3′ trailer from pre-tRNA [1–5], and can be categorized into two groups: a short form (tRNase Z^S) that consists of 300–400 amino acids and a long form (tRNase Z^L) that contains 800–900 amino acids [6]. Bacteria and archaea genomes contain a tRNase Z^S gene only, while eukaryotic genomes encode either only tRNase Z^L or both forms. The human genome contains both tRNase Z^S and tRNase Z^L genes. This raises an interesting question whether the short and long forms of tRNase Z play different roles in the cells.

The long form of tRNase Z has the following interesting physiological properties. *Caenorhabditis elegans* tRNase Z^L has been shown to play a role in germline proliferation [7], while human tRNase Z^L has been suggested to play a role in mitosis as a modulator of centrosome through physically interacting with the γ-tubulin complex [8]. Three missense changes in the human tRNase Z^L gene have been reported to be significantly associated with the occurrence of prostate cancer [9], although this conclusion is controversial [10]. Human tRNase Z^L can function as a four-base-recognizing RNA cutter (termed RNase 65) through a relatively stable complex with a 3′-truncated tRNA [6,11].

In contrast, little is known about the physiological role of human tRNase Z^S, although the recombinant enzyme has been shown in vitro to have the tRNA 3′ processing activity and the endoribonuclease activity that cleaves unstructured RNAs [2,12]. Notably, recombinant human tRNase Z^S shows a weaker activity on pre-tRNA substrates than recombinant human tRNase Z^L [13]. In addition, the human tRNase Z^S gene is not essential for a cell to survive because the genome of the lung cancer cell line Ma29 has a region of homozygous deletion on chromosome 18 that contains the whole tRNase Z^S gene [14]. On the other hand, the *Drosophila melanogaster* tRNase Z^L gene, which is juvenile hormone-inducible, appears to be indispensable because its knockdown by RNA interference impairs the tRNA 3′ processing in both nuclei and mitochondria [15,16]. This implies that the human tRNase Z^L gene on chromosome 17 would be also indispensable.

Here, we began a voyage to elucidate the physiological role of human tRNase Z^S. We first examined if the tRNase Z^S gene is really expressed at the protein level, and then analyzed *cis*-regulatory elements for the tRNase Z^S gene transcription. Furthermore, we examined the subcellular localization of human tRNase Z^S.

2. Materials and methods

2.1. Cell culture and luciferase assay

Cell culture and the luciferase reporter assays were performed basically as described before [17].

2.2. Western analysis

Whole cell extracts dissolved in a buffer (50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 100 mM dithiothreitol) or the subcellular fractions described below were separated by SDS/7.5–15% polyacrylamide gel electrophoresis, and transferred to a nitrocellulose membrane. The membrane was probed with antibodies raised to recombinant human tRNase Z^S, or antibodies against the standard proteins calpain, porin, c-jun, and vimentin using the ECL Western Blotting Detection System (GE Healthcare).

2.3. Northern analysis

Total RNA (20 μg per lane), extracted from each cell line with ISOGEN (Nippon Gene), was separated by formaldehyde/1% agarose gel electrophoresis, and transferred to a nitrocellulose membrane. Hybridization with digoxigenin-labeled RNA probes and detection were performed according to the manufacturer's protocol (Roche Diagnostics). The glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mRNA level was used as an internal standard.

2.4. Plasmid construction

A 2 kbp putative promoter DNA region for the human tRNase Z^S gene transcription was PCR-amplified from a human genomic BAC clone (Invitrogen), and cloned between the NheI and HindIII sites of the luciferase reporter plasmid pGL3-Basic (Invitrogen) to generate

*Corresponding author. Fax: +81 250 25 5021.
E-mail address: mnashimoto@nupals.ac.jp (M. Nashimoto).

Abbreviations: tRNase Z^S, a short form of tRNase Z; tRNase Z^L, a long form of tRNase Z

0014-5793/$34.00
doi:10.1016/j.febslet.2008.06.020

pGL/trzs/full. The downstream deleted promoters were generated by PCR from pGL/trzs/full, and cloned between the NheI and XhoI sites of pGL3-Basic to generate pGL/trzs/Δd1–pGL/trzs/Δd7. For example, pGL/trzs/Δd4 contains a promoter that lacks the region from the proximal end to the putative HNF-1 binding site inclusive (see Section 3). The upstream deleted promoters were PCR-generated from pGL/trzs/full, and cloned between the XhoI and HindIII sites of pGL3-Basic to generate pGL/trzs/Δu1–pGL/trzs/Δu7. The plasmids pGL/trzs/mp1–pGL/trzs/mp7 containing the multipoint mutant promoters were constructed based on the pGL/trzs/Δd and pGL/trzs/Δu series.

2.5. Subcellular fractionation

The cytosolic, membrane/organelle, nuclear, and cytoskeletal fractions were prepared from each cell line by using a ProteoExtract Subcellular Proteome Extraction Kit (Merckbiosciences).

3. Results and discussion

3.1. Human tRNase Z^S gene expression and post-transcriptional regulation

To examine whether tRNase Z^S is really expressed in human cells, we performed western analysis for tRNase Z^S in various human culture cells. The Western blot for total protein samples from A549 epithelial lung adenocarcinoma cells, HeLa cells, IMR90 lung fibroblasts, and embryonic kidney 293 cells was probed with polyclonal antibodies raised to recombinant human tRNase Z^S. All these cells expressed tRNase Z^S with one exception of 293 cells (Fig. 1A).

To see a possibility of post-transcriptional regulation in 293 cells, we also carried out Northern analysis for the tRNase Z^S mRNA in the four human cells. The mRNA was detected in all cell lines including the 293 cells, and its level in HeLa cells was the lowest although the tRNase Z^S protein level was the highest (Fig. 1B). Together, these observations suggest that the human tRNase Z^S gene expression may be regulated post-transcriptionally. Several miRNAs such as miR-136, miR-422a, miR-488, and miR-802 may be involved in the post-transcriptional regulation, which were predicted to bind to the 3′ untranslated region of the human tRNase Z^S mRNA (http://microrna.sanger.ac.uk).

3.2. Cis-regulatory elements for the human tRNase Z^S gene transcription

To elucidate *cis*-regulatory elements for the human tRNase Z^S gene transcription, which appears to be activated in every cell, we cloned a 2 kbp region upstream of the putative transcription initiation site into the luciferase reporter plasmid pGL3-Basic and dissected this region, which we tentatively call transcriptional promoter (Fig. 2; http://dbtss.hgc.jp). Notably, this promoter does not contain the TATA box. Based on pGL/trzs/full containing the full-length promoter region, we constructed derivatives that contain seven downstream deletions (pGL/trzs/Δd1–pGL/trzs/Δd7), seven upstream deletions (pGL/trzs/Δu1–pGL/trzs/Δu7), or seven multipoint mutations (pGL/trzs/mp1–pGL/trzs/mp7). pGL/trzs/Δd1–pGL/trzs/Δd7 contain promoters that lack the regions from the proximal end to the putative binding sites for transcription factors, Nkx2-5, FOXD3 (distal), FOXD3 (proximal), HNF-1, Pax-4, Hand1/E47, and HNF-3β, respectively (Fig. 3A; http://www.gene-regulation.com). For example, pGL/trzs/Δd4 contains a promoter (−2000 to −1082) that lacks the region from the proximal end to the putative HNF-1 binding site inclusive (Figs. 2 and 3A). pGL/trzs/Δu1–pGL/trzs/Δu7 contain promoters that lack the regions from the distal end to the putative binding sites for transcription factors, Nkx2-5, FOXD3 (distal), FOXD3 (proximal), HNF-1, Pax-4, Hand1/E47, and HNF-3β, respectively (Fig. 3A). pGL/trzs/mp1–pGL/trzs/mp7 have full-length promoters that contain 2–5 point mutations in the putative binding sites for transcription factors, Nkx2-5, FOXD3 (distal), FOXD3 (proximal), HNF-1, Pax-4, Hand1/E47, and HNF-3β, respectively (Fig. 3A).

We performed luciferase reporter assays for these plasmids in HeLa cells. None of the downstream or upstream deleted promoters led to transcription with two exceptions of Δu4 and Δu7 (Fig. 3B). The deletion promoter Δu7 was 1.6-fold more active than the full-length one, while the Δu4 activity was only 40%. In the multipoint mutant series, only two promoters, mp4 and mp7, showed the transcription activity, which were nearly the same as and 20% higher than that of the full-length intact promoter. Similar results of the luciferase assays were obtained with respect to 293 cells, but the relative transcription activities of Δu4, Δu7, mp4, and mp7 were weaker than those in HeLa cells and the downstream deletion promoters Δd1 and Δd2 showed 5% and 8% activities, respectively (Fig. 3C).

Taken together, these observations indicate that the ~430 bp proximal region is essential for a promoter activity, that the putative HNF-3β binding site is dispensable, and that the putative HNF-1 binding site is dispensable in HeLa cells but important in 293 cells. The substantial activity of the deletion promoter Δu7 suggests that there may be the equivalent of a TATAA element within the proximal region. Indeed, we found similar sequences, TATTA (−256 to −252), TATGA (−296 to −292), and TATGA (−384 to −380), which might work as a minimal promoter. More interestingly, each one of the five putative factor binding sites (Nkx2-5, FOXD3 (distal), FOXD3 (proximal), Pax-4, and Hand1/E47) is indispensable for a strong promoter activity in the context of the full-length promoter, suggesting that transcription factors that bind to these five sites work together to potentiate the transcription initiation.

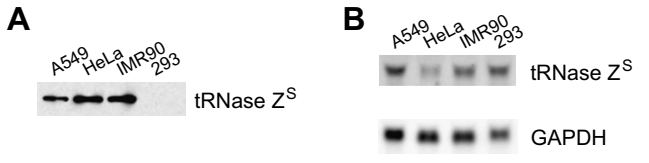

Fig. 1. Human tRNase Z^S gene expression in A549 epithelial lung adenocarcinoma cells, HeLa cells, IMR90 lung fibroblasts, and embryonic kidney 293 cells. (A) Western analysis. (B) Northern analysis.

3.3. Human tRNase Z^S does not exist in the nuclei

To elucidate a subcellular distribution of human tRNase Z^S, we fractionated the HeLa cells to their components through differential centrifugation and performed western analysis for tRNase Z^S in each subcellular component. We separated the cells into cytosolic, membrane/organelle, nuclear, and cytoskeletal fractions, and assessed the integrity of the fractionation by western assays for subcellular specific standard proteins, calpain for the cytosolic fraction, porin for the membrane/organelle fraction, c-jun for the nuclear fraction, and