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### Journal
AJP Gastrointestinal and Liver Physiology, 325(4)

### ISSN
0193-1857



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### Publication Date
2023-10-01

### DOI
10.1152/ajpgi.00106.2023

Peer reviewed

AMERICAN JOURNAL OF PHYSIOLOGY
GASTROINTESTINAL AND LIVER PHYSIOLOGY

*Am J Physiol Gastrointest Liver Physiol* 325: G347–G355, 2023.
*First published August 2, 2023; doi:10.1152/ajpgi.00106.2023*

RESEARCH ARTICLE

# miR-122-5p is involved in posttranscriptional regulation of the mitochondrial thiamin pyrophosphate transporter (*SLC25A19*) in pancreatic acinar cells

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## Abstract

Thiamin (vitamin B1) plays a vital role in cellular energy metabolism/ATP production. Pancreatic acinar cells (PACs) obtain thiamin from circulation and convert it to thiamin pyrophosphate (TPP) in the cytoplasm. TPP is then taken up by the mitochondria via a carrier-mediated process that involves the mitochondrial TPP transporter (MTPPT; encoded by the gene *SLC25A19*). We have previously characterized different aspects of the mitochondrial carrier-mediated TPP uptake process, but nothing is known about its possible regulation at the posttranscriptional level. We address this issue in the current investigations focusing on the role of miRNAs in this regulation. First, we subjected the human (and rat) 3′-untranslated region (3′-UTR) of the *SLC25A19* to three *in-silico* programs, and all have identified putative binding sites for miR-122-5p. Transfecting pmirGLO-h*SLC25A19* 3′-UTR into rat PAC AR42J resulted in a significant reduction in luciferase activity compared with cells transfected with pmirGLO-empty vector. Mutating as well as truncating the putative miR-122-5p binding sites in the h*SLC25A19* 3′-UTR led to abrogation of inhibition in luciferase activity in PAC AR42J. Furthermore, transfecting/transducing PAC AR42J and human primary PACs with mimic of miR-122-5p led to a significant inhibition in the level of expression of the MTPPT mRNA and protein as well as in mitochondrial carrier-mediated TPP uptake. Conversely, transfecting PAC AR42J with an inhibitor of miR-122-5p increased MTPPT expression and function. These findings show, for the first time, that expression and function of the MTPPT in PACs are subject to posttranscriptional regulation by miR-122-5p.

**NEW & NOTEWORTHY** This study shows that the expression and function of mitochondrial TPP transporter (MTPPT) are subject to posttranscriptional regulation by miRNA-122-5p in pancreatic acinar cells.

*mitochondrial TPP transporter; miRNA; posttranscriptional regulation; SLC25A19*

## INTRODUCTION

Thiamin (vitamin B1; also known as the "energy vitamin") is an indispensable micronutrient for normal cell physiology and health due to its involvement (mainly in the form of thiamin pyrophosphate; TPP) in multiple vital cellular metabolic processes, including oxidative energy metabolism, ATP production, and maintenance of normal cellular redox state (1–5). Low cellular level of thiamin leads to impairment in energy metabolism and to oxidative stress (6); it also negatively impacts the function of mitochondria, an organelle that contains/utilizes ~90% of cellular TPP (7).

Like all other mammalian cells, pancreatic acinar cells (PACs) obtain thiamin from the blood circulation and enzymatically converted it to TPP in the cell cytoplasm (8, 9). The generated TPP is then transferred to the mitochondria via a carrier-mediated process that involves the mitochondrial TPP transporter (MTPPT; which is encoded by the gene *SLC25A19*) (10–12). We have previously characterized different physiological/pathophysiological, cell biological, structure, and functional aspects of the mitochondrial carrier-mediated TPP uptake process in PACs (13–15). This includes characterization of the basic transcriptional regulation of the *SLC25A19* gene, identification of its minimal promoter and demonstrating the involvement of the NF-Y nuclear factor in its basal activity (16), how expression of the MTPPT is adaptively regulated (17), as well as examination of the effect of clinical mutations found in the human MTPPT on its function and cell biology (18), and effect of chronic exposure to alcohol and to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (a component of cigarette smoke) on its activity and expression (15, 19). To date, however, there has been nothing

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Submitted 17 May 2023 / Revised 19 July 2023 / Accepted 26 July 2023

known about posttranscriptional regulation of the MTPPT expression and functioning in any cell type. We address this issue in the current investigation using PACs and focused on the possible role of microRNAs (miRNAs) in this mode of regulation. miRNAs represent a class of small (~20–22 nucleotides) noncoding single-stranded RNAs that play important roles in regulating gene expression at the posttranscriptional level. They do so via base pairing to complementary sequences in the 3′-UTRs of the target mRNAs, which leads to the degradation of such mRNAs and/or to suppression in their translation (20, 21). miRNAs are highly conserved across species and their expression is tissue-specific in nature (22). Also, a single miRNA can act on multiple and different target mRNAs, and thus, can affect a variety of cellular pathways and processes, including transport events across biological membranes (23–25). Furthermore, dysregulation in the expression of miRNAs has been implicated in a variety of diseases (26–29). Moreover, a variety of pathological conditions, including those that affect the pancreas (e.g., severe acute pancreatitis, pancreatic adenocarcinoma, and chronic pancreatitis), appear to be associated with altered patterns of expression of miRNAs (30–32). It is also interesting to mention here that different external/environmental (e.g., chronic alcohol exposure, bacterial LPS) and internal (e.g., proinflammatory cytokines) factors that affect the pancreas (33–35) are associated with alterations in miRNA expression pattern, which may contribute to the effects of these factors on pancreatic physiology/health (36, 37).

Our results in this investigation show, for the first time, that expression and function of the MTPPT are subject to posttranscriptional regulation by the miRNA-122-5p.

## MATERIALS AND METHODS

### Materials

#### *Rat and human PACs.*

Rat-derived pancreatic acinar (AR42J) cells were obtained from American Type Tissue Collection (ATTC; Rockville, MD). AR42J cells were cultured as described by us/others previously (33, 38) using complete DMEM growth medium supplemented with 10% FBS as well as streptomycin and penicillin in a 5% $CO_2$ atmosphere at 37°C. Human pancreases were obtained from brain-dead donors (kindly provided to us by the Islet Laboratory for Transplantation and Beta Cell Biology; Nationwide Children's Hospital/Ohio State University, Columbus, OH). Isolated hPACs (received between 24 and 48 h postisolation) were cultured in Ham's F-12K growth medium (supplemented with 10% FCS, 5% BSA, 10 ng/mL epidermal growth factor, and 0.1 mg/mL soybean trypsin inhibitor) and used (within 48 h after receiving) for uptake investigations, as described by us/others previously (25, 39). Protocols for the use of hPACs have been approved by the institutional review board of the University of California, Irvine, CA (Institutional Review Board: 2017-1593).

#### *Chemicals and reagents.*

[$^3$H]TPP (specific activity >1.4 Ci/mmol; radiochemical purity: >9 8.2%) from Moravek, Inc. (Brea, CA); anti-MTPPT affinity-purified rabbit polyclonal antibodies (Cat. No. AP5269b) was purchased from ABCEPTA (San Diego, CA); anti-PDHA1 (8D10E6) monoclonal antibody (Cat. No. 45-6600) was purchased from Thermo Fisher; Lenti micro RNA mimic transduction particles has-miR-122-5p (HLMIR1002), negative control ath-miR-416 (NCLMIR001) as well as the mimic (HMI1002) inhibitors (HSTUD1002) of miR-122-5p and their corresponding negative controls (HMC0002) (NSTUD001) were procured from Sigma-Aldrich (St. Louis, MO); mitochondrial isolation kit was from Thermo Scientific; and PCR primers were from Integrated DNA Technologies. Nylon filters (0.45-μm pore size) were from Millipore (CA, USA) other chemicals and molecular biology reagents, kits were from commercial vendors and were of analytical grade.

### Methods

#### *In silico analysis.*

We used three widely used in silico algorithms [TargetScan, (http://www.targetscan.org/), miRWalk (http://mirwalk.umm.uni-heidelberg.de), and miRcode (http://www.mircode.org/)] (25, 40, 41) to predict putative miRNA(s) binding sites in the human (and rat) *SLC25A19* 3′-UTRs (Table 1).

### Cloning of the 3′ UTR of Human *SLC25A19* (MTPPT) into pmirGLO Vector

The full-length (458 bp) 3′-UTR of the human *SLC25A19* was amplified (by PCR) using cDNA reverse transcribed from human total RNA (Takara Bio). The purified PCR product (h*SLC25A19*-3′-UTR) was then ligated into the multiple cloning sites (MCS) of pGEM-T easy vector (Promega, Madison, WI). The primer sequences flanked by *SacI* and *XhoI* sites used for PCR reactions are presented in Table 2. The cloned insert (h*SLC25A19*-3′-UTR) was then ligated into the MCS of pmirGLO miRNA targeting expression vector (Promega), following the manufacturer's instructions. The resulting ligation mix (pmirGLO-h*SLC25A19* 3′-UTR) was then transformed into competent *Escherichia coli* (JM109, Promega), followed by selection of clones on ampicillin agar plates. DNA sequencing was performed to verify the sequence of the cloned constructs (Genewiz).

### Site-Directed Mutagenesis and Truncation of Putative miRNA Binding Sites

To introduce mutations in the putative miR-122-5p-binding sites of the h*SLC25A19* 3′-UTR, we utilized the Quick-change XL site-directed mutagenesis kit (Agilent, CA), following the manufacturer's instructions. Sequences of the primers used in the site-directed mutagenesis studies are shown in Table 2. Sequences of the mutated

**Table 1.** *Context score percentile of miR-122-5p predicted for SlC25A19 3′-UTRs based on TargetScan program*

| miRNA | Position in the UTR | Context ++ Score Percentile |
|---|---|---|
| Human | | |
| hsa-miR-122-5p | 269–276 | 99 |
| hsa-miR-122-5p | 332–338 | 92 |
| Rat | | |
| rno-miR-122-5p | 286–292 | 64 |

**Table 2.** *List of PCR primers used in the study*

| | |
|---|---|
| Primers used for cloning 3′-UTR *SLC25A19* (restriction sites underlined) | |
| Forward: | 5′-ATTGAGCTCGTGCAGGAAGGAC-3′ |
| Reverse: | 5′-TAACTCGAGGTGTGTTTAGCTTTAATGTCT-3′ (full-length) |
| Reverse: | 5′-TAACTCGAGGTAGGATGGCGTCTGTTTC-3′ (298 bp) |
| Reverse: | 5′-TAACTCGAGTTCAAGGCTGCTACCCCG-3′ (129 bp) |
| Real-time PCR primers human-MTPPT | |
| Forward: | 5′-AGCATGAGCGCCTGTCGC-3′ |
| Reverse: | 5′-TGAGCTGGGACTGTCCTTTCCA-3′ |
| Human-β-actin | |
| Forward: | 5′-CATCCTGCGTCTGGACCT-3′ |
| Reverse: | 5′-TAATGTCACGCACGATTTCC-3′ |
| Rat-MTPPT | |
| Forward: | 5′-GGCCATACGCACCATG-3′ |
| Reverse: | 5′-GGGTCTTGCTGATGACTC-3′ |
| Rat-18S rRNA | |
| Forward: | 5′-GCAGAATTCCCACTCCCGACCC-3′ |
| Reverse: | 5′-CCCAAGCTCCAACTACGAGC-3′ |
| Primers used for site directed mutagenesis for miR-122-5p binding sites (mutated sites are underlined) | |
| Site 1 | |
| Forward: | 5′-CTGTTTCTCCTCTGACCAGCCAATAGTTCAAAGGAAACAGACGCCATCC-3′ |
| Reverse: | 5′-GGATGGCGTCTGTTTCCTTTGAACTATTGGCTGGTCAGAGGAGAAACAG-3′ |
| Site 2 | |
| Forward: | 5′-GCCCTGCCTGCCAGGAGAACAGAAAATAGTTCTGGTCTGGATGG-3′ |
| Reverse: | 5′-CCATCCAGACCAGAACTATTTTCTGTTCTCCTGGCAGGCAGGGC-3′ |

h*SLC25A19* 3′-UTR constructs were then verified by DNA sequencing (Genewiz).

With regard to the truncations, two truncated constructs of 298 bp (deletion of the putative miR-122-5p binding site: S2) and 145 bp (deletion of both miR-122-5p binding sites: S1 and S2) in length were generated by standard PCR using primers (flanked with *SacI* and *XhoI* restriction sites) shown in Table 2. PCR-amplified DNA fragments were digested using the restriction enzymes *SacI* and *XhoI* and then loaded onto agarose gel. The DNA bands of appropriate size were excised, gel purified, and then cloned into pmirGLO dual-luciferase miRNA target expression vector (Promega). The generated truncated constructs were then verified by DNA sequencing (Genewiz).

### Transient Transfection and Luciferase Assay

Rat PAC AR42J cells were subcultured in 24-well plates and transiently transfected with 2 μg of pmirGLO vector (mock) or pmirGLO-h*SLC25A19* constructs [the latter refers to either full-length (WT), mutated (S1, S2 and S1 and S2), or truncated (pmirGLO-298 bp) constructs] using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), by following the manufacturer's instructions. After 48 h of posttransfection, the cells were lysed, and the luciferase activity was determined using a Dual Luciferase Assay Kit (Promega). The luciferase activity was calculated as a ratio of firefly luciferase relative to *Renilla* luciferase luminescence in each sample and is expressed as a percentage of luciferase activity relative to simultaneously performed controls.

### Mitochondrial [$^3$H]TPP Uptake

For carrier-mediated [$^3$H]TPP mitochondrial uptake, we first transfected PAC AR42J (grown in 24-well plates) with 200 nM of miR-122-5p mimic or inhibitor with their respective controls for 48 h followed by isolation of the mitochondria (using mitochondria isolation kit) and performed the carrier-mediated [$^3$H]TPP uptake studies, as described by us previously (14–16). In brief, isolated mitochondria were suspended in uptake buffer pH 7.4 (140 mM KCl, 0.3 mM EDTA, 5 mM MgCl2, 10 mM MES, 10 mM HEPES, and 10 mM succinate), and uptake studies were performed using a rapid-filtration technique. Uptake reaction was initiated by adding freshly isolated mitochondria (20 μL; containing ~15–20 μg/μL of protein) to the uptake buffer (80 μL) that contains [$^3$H]TPP (0.23 μM) at 37°C. One milliliter of ice-cold stop solution (in mM: 100 KCl, 100 mannitol, and 10 $KH_2PO_4$, pH 7.4) was added 5 min later to stop the reaction (initial rate of uptake), and the uptake mixture was then subjected to rapid filtration. Measurement of radioactivity levels in the individual filters was then determined using the liquid scintillation counter.

For carrier-mediated [$^3$H]TPP uptake by isolated mitochondria from hPACs, cultured cells were used for lentiviral transduction followed by mitochondria isolation and performed carrier-mediated [$^3$H]TPP uptake by the isolated mitochondria. Lentiviral transduction into hPACs was performed (for 48 h) using recombinant lentiviral particles, lenti-hsa-miR-122-5p mimic carrying the miR-122-5p sequence (miRBase accession no. MIMAT0000421), or lenti-negative control. PBS-washed posttransduced hPACs were then treated with trypsin (for the detachment of cells from culture flasks), and then the resulting cell pellet was used to isolate mitochondria. The freshly isolated mitochondria were then used for carrier-mediated [$^3$H]TPP uptake studies.

### Real-Time Quantitative PCR

Total RNA was isolated from both PAC AR42J and hPACs using QIAzol Lysis reagent (QIAGEN) and purified by using RNeasy Kit (QIAGEN), as described previously. Two micrograms of total RNA were reverse transcribed using the VersocDNA Synthesis Kit (Thermo Fisher Scientific), and the levels of expression of MTPPT mRNAs were quantified by real-

time quantitative PCR using iQ SYBER Green Supermix (Bio-Rad) in the CFX96 real-time PCR system (Bio-Rad). Gene-specific primers for MTPPT, β-actin, and 18S rRNA are shown in Table 2. Data were normalized relative to internal controls (for human samples, β-actin; for rat samples, 18S rRNA) (42) and calculated using a relative relationship method (43).

### Western Blotting

Protein lysates of freshly isolated mitochondria from control and microRNA mimic- or inhibitor-treated cells were prepared in radio-immunoprecipitation assay (RIPA) buffer (Sigma) containing protease inhibitor cocktail (Roche, Branchburg, NJ), following the manufacturer's instructions. Mitochondrial membrane protein fractions were separated (by centrifugation at 14,000 rpm for 20 min), followed by the determination of protein concentration using the Bio-Rad DC protein assay kit (14–16). To examine the level of expression of the human and rat MTPPT, equal amounts of protein (40 μg) were resolved using NuPAGE 4%–12% premade gel (Invitrogen), followed by blotting proteins to polyvinylidene difluoride (PVDF) membranes (Fisher Scientific). The membranes were then blocked using Odyssey blocking solution (LI-COR; Bioscience, Lincoln, NE), followed by probing with anti-MTPPT (1:1,000) and anti-PDHA1 (1:4,000). The immune-reactive bands for human and rat MTPPT as well as PDH were then detected using the corresponding secondary anti-rabbit IgG IRDye 800 and anti-mouse IRDye-680 (1:30,000) (LI-COR Bioscience). Identification and quantification of the identified specific bands were then performed using the Odyssey infrared imaging system (LI-COR Bioscience); data were then normalized relative to PDH.

### Statistical Analysis

Data on mitochondrial carrier-mediated TPP uptake, Western blotting, real-time quantitative PCR (RT-qPCR), and luciferase assays are expressed as means ± SE of at least three to six separate determinations and are presented as percentage relative to simultaneously performed controls. Graphs shown in the figures are generated using GraphPad Prism 9 software (GraphPad Software, Inc.). For statistical analysis, the unpaired Student's $t$ test was used and a $P$ value of $< 0.05$ was considered as statistically significant.

## RESULTS

### Mapping the 3′-UTR of the Human *SLC25A19* for Putative miRNA(s) Binding Sites

In this study, we subjected the 3′-UTR of the human *SLC25A19* (458 bp) to three different and commonly used in silico programs [TargetScan, miRWalk, and miRcode; (25, 40, 41)] to predict putative binding sites for miRNA(s). Although each of these programs predicted several putative binding sites for different miRNAs, putative binding sites for miRNA-122-5p were predicted by all three programs and with high context score percentile (Fig. 1*A* and Table 1). Interestingly, putative binding site(s) for miRNA-122-5p was also predicted in the 3′-UTR of the rat *Slc25a19* (Fig. 1*B*). It is relevant to mention here that this miRNA is expressed, at a modest level, in normal pancreatic cells (44).

To directly examine the possible involvement of miRNAs in regulating the expression of *SLC25A19* at the posttranscriptional level in PACs, we cloned the full-length h*SLC25A19* 3′-UTR (458 bp) into the pmirGLO dual luciferase (miRNA target expression) vector (Fig. 2*A*), then transfected the reporter construct into PAC AR42J and determined luciferase activity. The results were compared with luciferase activity in cells transfected with pmirGLO vector alone (i.e., controls). A significant ($P < 0.01$) decrease in luciferase activity was observed in cells transfected with the pmirGLO-h*SLC25A19* 3′-UTR compared with those transfected with empty pmirGLO vector (Fig. 2*B*). This finding suggests that the h*SLC25A19* 3′-UTR is a potential target for regulation by miRNA(s) at the posttranscriptional level.

### Role of the Putative Binding Sites for miR-122-5p in the 3′-UTR of h*SLC25A19* in Regulating Expression in PACs

In this investigation, we aimed at determining the involvement of the putative binding sites for miR-122-5p in the 3′-UTR of h*SLC25A19* [located at position 268–275 (S1) and 331–338 (S2)] in regulating the expression of the MTPPT. For this we mutated the seed sequence of the putative miR-122-5p binding sites located at S1 and S2, both individually and together, and then determined the effects of these mutations on the degree of inhibition in luciferase activity following transfection of the mutated constructs

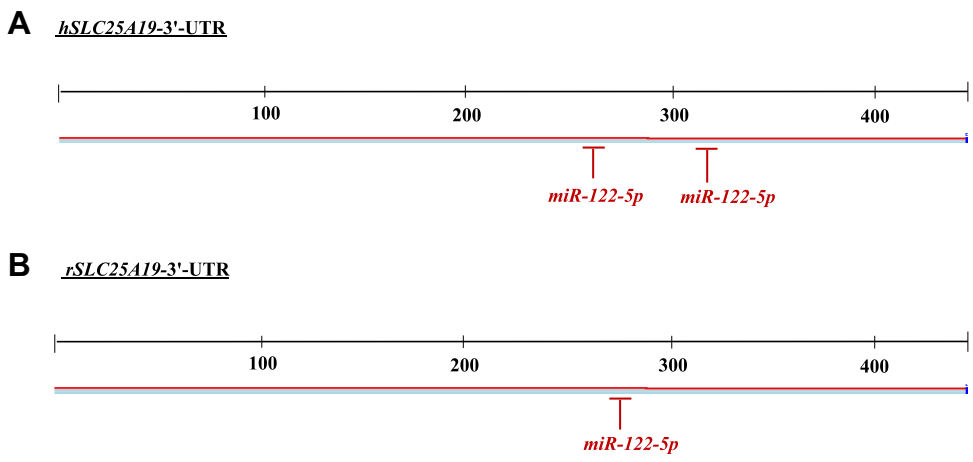


**Figure 1.** Prediction and localization of putative miRNA binding sites in the 3′-UTRs of *SLC25A19*. miRNA binding sites in human (*A*) and rat *SLC25A19* 3′-UTRs (*B*) as predicted by TargetScan (the depicted figure is based on http://www.targetscan.org).

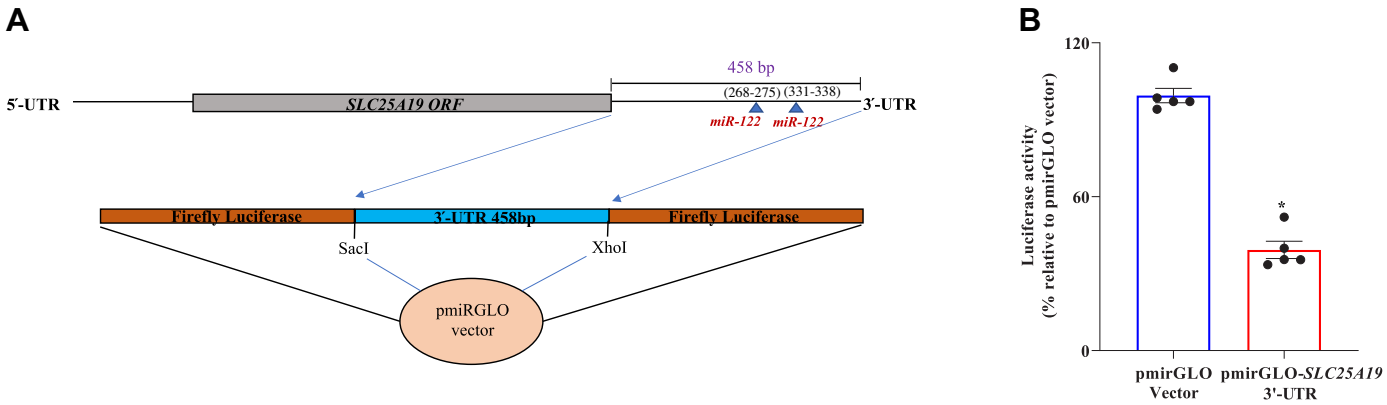


**Figure 2.** Determination of luciferase activity of pmirGLO-h*SLC25A19* 3′-UTR in PAC AR42J. Schematic representation of human *SLC25A19* gene showing the 3′-UTR (458 bp) cloned in pmirGLO vector (*A*). PAC AR42J were transiently transfected with pmirGLO vector or with pmirGLO-h*SLC25A19* 3′-UTR constructs for 48 h (*B*). Activity of luciferase was then determined then normalized relative to *Renilla* luciferase activity. Data were expressed as percentage relative to control (pmirGLO vector). Data are means ± SE of 3–5 independent determinations (*$P <$ 0.01).

of pmirGLO-h*SLC25A19* 3′-UTR into PAC AR42J; comparison was made relative to luciferase activity in cells transfected with wild-type pmirGLO-h*SLC25A19*-3′-UTR. The result showed a significant ($P < 0.01$) abrogation in the degree of inhibition in luciferase activity observed in cells transfected with mutated constructs compared with cells transfected with the wild-type construct (Fig. 3). We also examined the effect of truncating the putative binding sites (Fig. 4*A*) for miR-122-5p in the h*SLC25A19* 3′-UTR on luciferase activity in PAC AR42J; comparison was made relative to full-length pmirGLO-h*SLC25A19*-3′-UTR. The results again showed a significant decrease ($P < 0.01$) in the degree of inhibition in luciferase activity in cells expressing the truncated pmirGLO-h*SLC25A19* 3′-UTRs compared with the full-length construct (Fig. 4*B*).

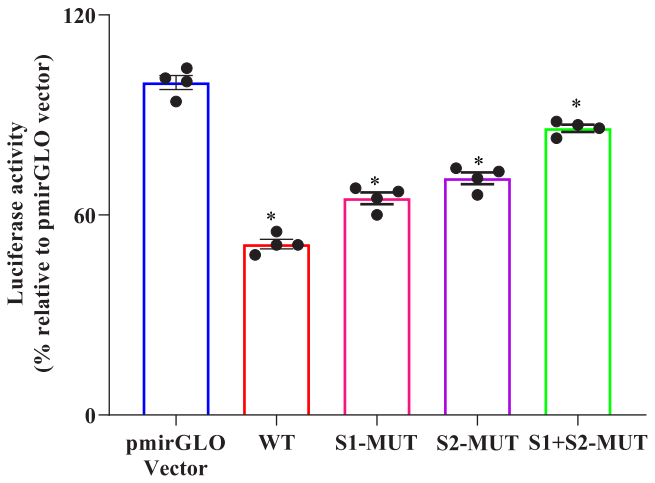


**Figure 3.** Effect of mutating the putative binding sites for miR-122-5p in the h*SLC25A19* 3′-UTR on luciferase activity in PAC AR42J. Cells were transfected with either pmirGLO empty vector or with pmirGLO-h*SLC25A19* 3′-UTR wild-type (WT) and mutated constructs (S1, S2, S1 and S2; see *Methods*) for 48 h. Activity of luciferase was then determined and normalized relative to *Renilla* luciferase activity. Data are means ± SE of four independent determinations (*$P <$ 0.01).

## Effect of Mimic of miR-122-5p on Level of Expression of the MTPPT mRNA and Protein and on Mitochondrial Carrier-Mediated TPP Uptake: Studies with Rat PAC AR42J, and Human Primary Pancreatic Acinar Cells

In this study, we aimed at further examining the role of miR-122-5p in posttranscriptional regulation of MTPPT expression and function. This was done by examining the effect of a mimic of miR-122-5p on the level of expression of MTPPT mRNA (by RT-qPCR) and protein (by western blotting), as well as on the initial rate of carrier-mediated mitochondrial TPP (0.23 μM) uptake. This study was performed using both rat PAC AR42J as well as human primary pancreatic acinar cells (hPACs). The results with PAC AR42J showed a significant decrease in expression of MTPPT at both the mRNA ($P < 0.01$) and protein ($P < 0.05$) levels in cells transfected with mimic miR-122-5p compared with cells transfected with negative control mimic (Fig. 5, *A* and *B*). Such treatment also led to a significant ($P < 0.01$) inhibition in mitochondrial carrier-mediated TPP uptake by isolated mitochondria from PAC AR42J treated with the miR-122-5p mimic compared with control (Fig. 5*C*). Similar findings were observed when hPACs were transduced (with the use of lenti-hsa-miR-122-5p mimic) in that significant inhibition in level of expression of the human MTPPT mRNA ($P < 0.05$) and protein ($P < 0.01$), as well as in carrier-mediated [$^3$H]TPP (0.23 μM) uptake ($P < 0.01$) by isolated mitochondria from mimic-treated cells compared with cells treated with lenti-negative control (Fig. 6, *A*–*C*). These findings provide further evidence for the involvement of miR-122-5p in the regulation of MTPPT expression and function in PACs.

## Effect of Inhibiting miR-122-5p on Level of Expression of the MTPPT mRNA and Protein and on Mitochondrial Carrier-Mediated TPP Uptake

In this investigation, we examined the effect of transfecting PAC AR42J with an inhibitor of miR-122-5p (this inhibitor is an antagomir of ~22 nucleotides designed to inhibit miR-122-5p and has been used in similar studies previously; 45) on

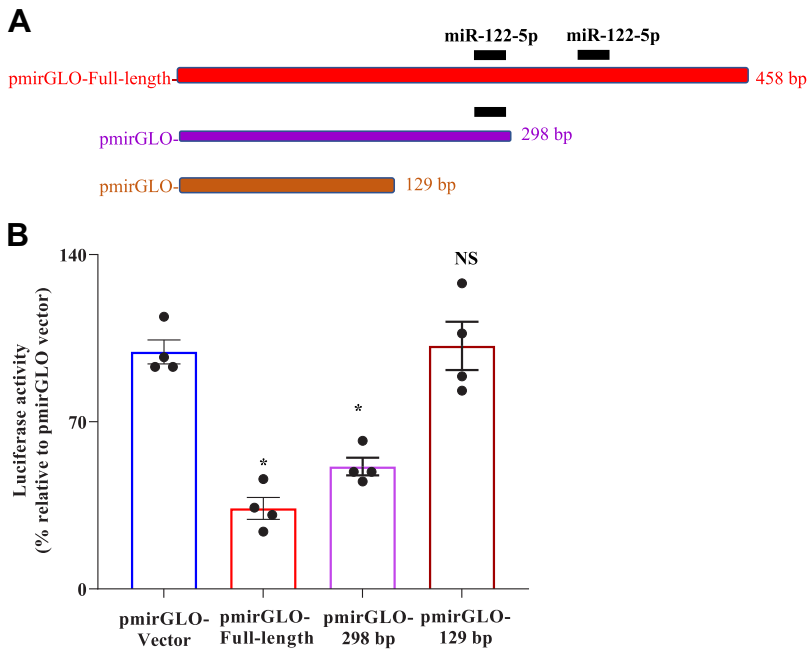


**Figure 4.** Effect of truncating the putative binding sites for miR-122-5p in the h*SLC25A19 3′-UTR* on luciferase activity in PAC AR42J. Schematic representation of different h*SLC25A19* 3′-UTR constructs [PCR amplified and cloned (see METHODS)] in pmirGLO vector (*A*). PAC AR42J were transfected with pmirGLO vector or with pmirGLO [full-length and truncated constructs (298 bp and 145 bp)] for 48 h (*B*). Activity of luciferase was then determined and normalized relative to *Renilla* luciferase activity. Data are means ± SE of 3–4 independent determinations (*$P <$ 0.01). NS, not significant.

level of expression of the MTPPT mRNA and protein as well as mitochondrial carrier-mediated TPP uptake. Comparisons were made relative to cells transfected with negative control of the inhibitor. The results showed a significant ($P < 0.05$ and $P < 0.01$) increase in the level of expression of MTPPT mRNA and protein in the miR-122-5p inhibitor transfected cells compared with negative control inhibitor transfected cells (Fig. 7, *A* and *B*). Similarly, a significant ($P < 0.05$) increase in carrier-mediated TPP uptake by mitochondria isolated from cells transfected with miR-122-5p inhibitor compared with those treated with negative control inhibitor was observed (Fig. 7*C*). These findings again strengthen the conclusion that miR-122-5p is involved in posttranscriptional regulation of MTPPT expression and function in PAC.

## DISCUSSION

Our aim in this investigation was to determine whether the expression and function of the human MTPPT are subject to regulation at the posttranscriptional level. We focused our effort on the possible role of miRNAs in this type of regulation, since these noncoding RNAs can exert profound

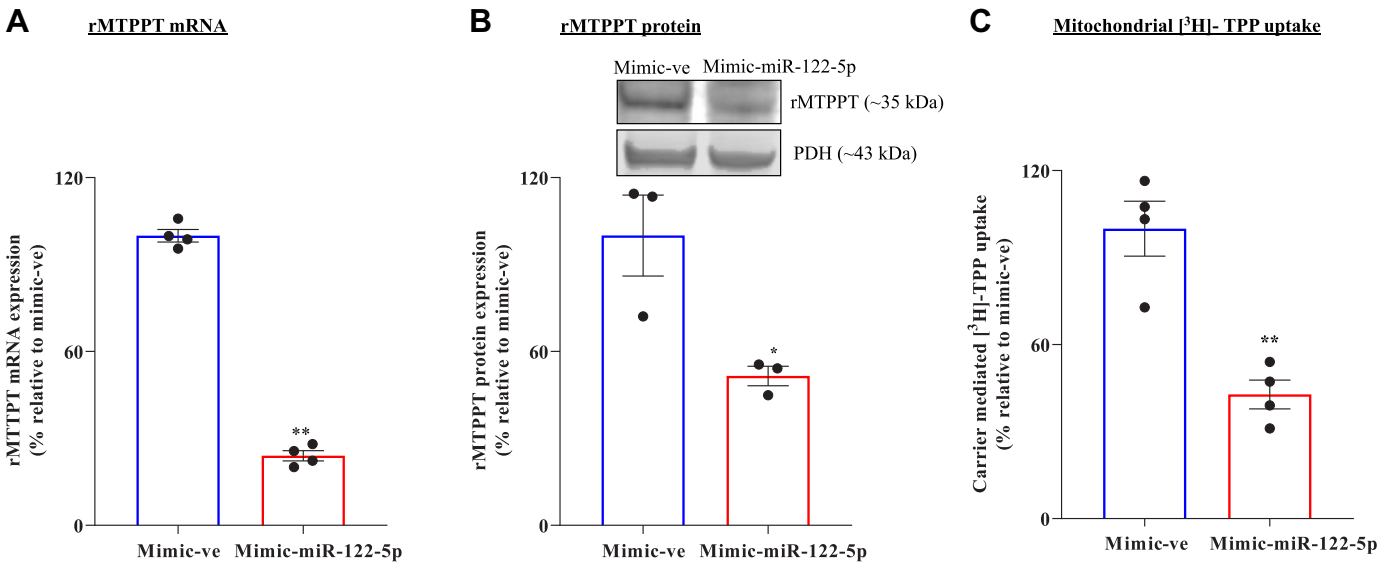


**Figure 5.** Effect of transfection of PAC AR42J with mimic miR-122-5p on MTPPT expressions and on mitochondrial carrier-mediated TPP uptake. Cells were transfected with mimic miRNAs [mimic negative control or miR-122-5p (200 nM; 48 h)] followed by determination of level of expression of MTPPT mRNA (by RT-qPCR) (*A*) and protein (by Western blotting) (*B*) as well as mitochondrial carrier-mediated uptake of [$^3$H]TPP (*C*). Data of RT-qPCR and Western blotting were normalized relative to internal controls (18S rRNA and PDH, respectively). Data are means ± SE of 3–4 independent determinations (**$P <$ 0.01; *$P <$ 0.05).

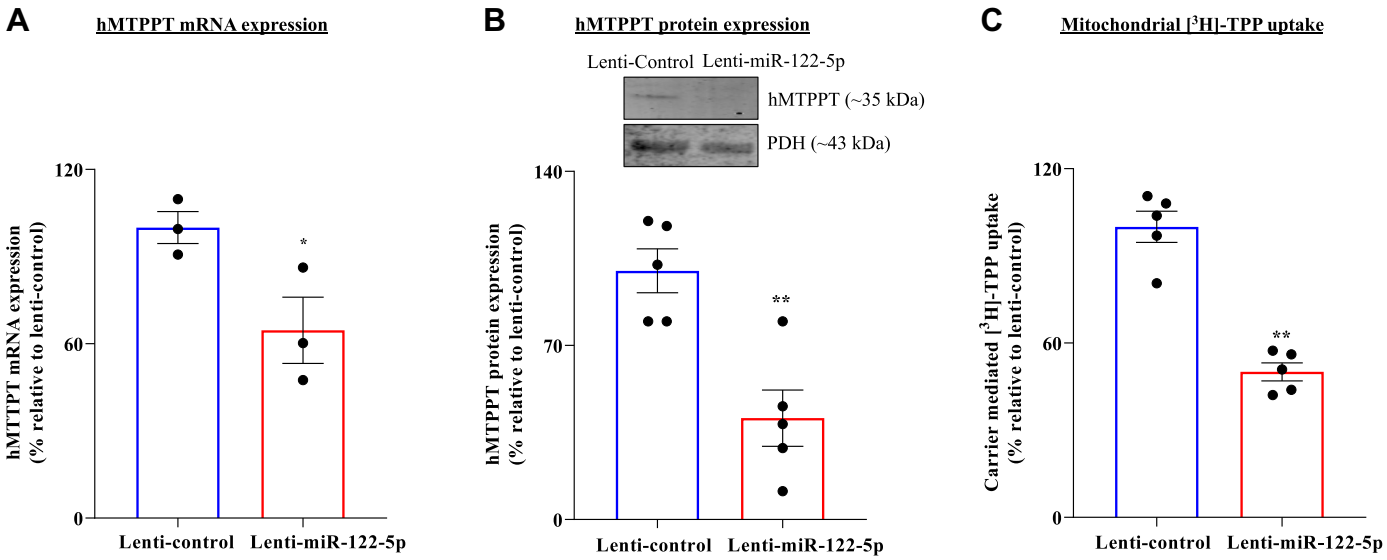


**Figure 6.** Effect on MTPPT expressions and mitochondrial carrier-mediated TPP uptake of transducing hPACs with lentiviral miR-122-5p mimic. Mimic miRNAs (lenti-control or lenti-122-5p, 48 h) were transduced into hPACs, followed by determination of MTPPT mRNA (by RT-qPCR) (*A*) and protein (Western blotting) expression (*B*), as well as mitochondrial carrier-mediated uptake of [$^3$H]TPP (*C*). Data of RT-qPCR and Western blotting were normalized relative to internal controls (β-actin and PDH, respectively). Data are means ± SE of 3–5 independent determinations (**$P < 0.01$, *$P < 0.05$).

effects on a variety of cellular processes, including transport events across biological membranes (23–25). Also, levels of miRNAs have been shown to be altered under a variety of conditions (30–32), which may affect mitochondrial physiology (46, 47). We addressed this issue using PACs as a model since these cells maintain a high rate of metabolic activity and that deficiency of thiamin affects their function (48, 49).

We began our investigation by subjecting the human (and rat) *SLC25A19* 3′-UTRs to three widely used bioinformatics programs to identify putative miRNAs binding sites in these regions. These programs predicted putative binding sites for miR-122-5p in both human and rat *SLC25A19* 3′-UTRs. Interestingly, this miRNA is expressed in the pancreas, and its level is induced in severe acute pancreatitis, chronic pancreatitis, and pancreatic adenocarcinoma (30, 44). We then obtained direct confirmation for the involvement of miRNAs in posttranscriptional regulation of MTPPT expression and function by demonstrating a significant reduction in luciferase

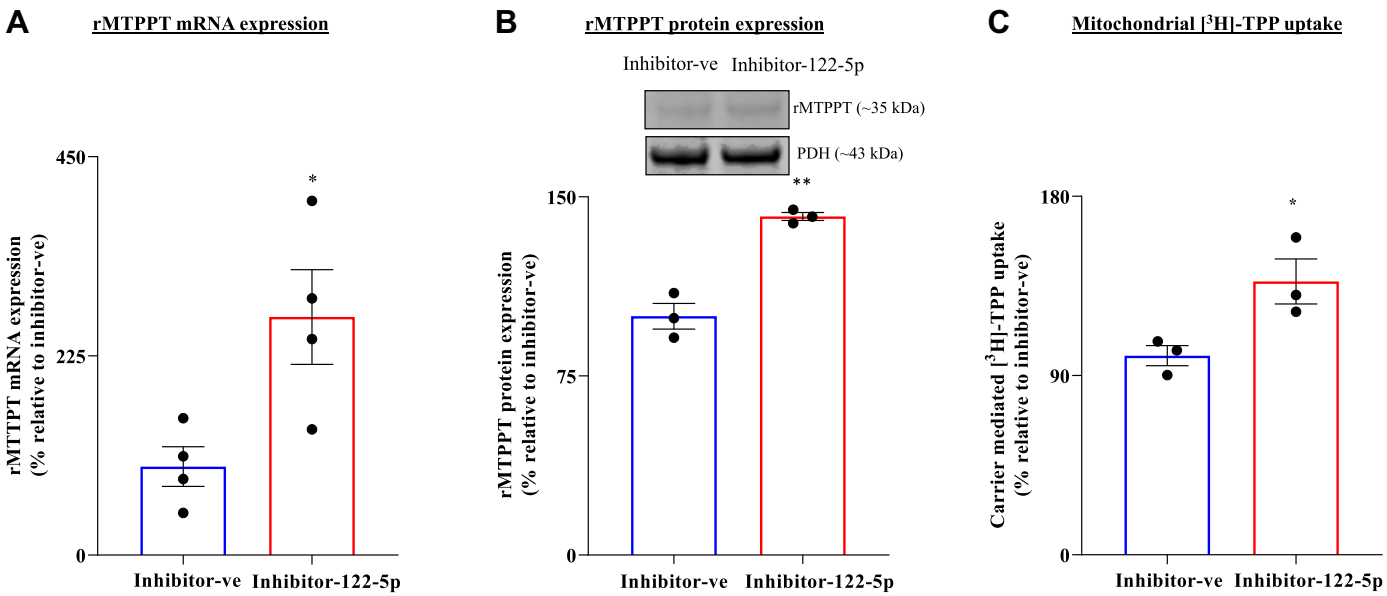


**Figure. 7.** Effect on MTPPT expressions and mitochondrial carrier-mediated TPP uptake of transfecting PAC AR42J with miR-122-5p inhibitor. Negative (control) inhibitor or miR-122-5p inhibitor (200 nM; 48 h) were transfected into PAC AR42J followed by determination of MTPPT mRNA (by RT-qPCR) (*A*) and protein (Western blotting) expression (*B*), as well as mitochondrial carrier-mediated uptake of [$^3$H]TPP (*C*). Data of RT-qPCR and Western blotting were normalized with internal controls of 18S rRNA and PDH, respectively. Data are means ± SE of 3–4 independent determinations (**$P < 0.01$; *$P < 0.05$).

activity in PAC AR42J expressing pmirGLO-h*SLC25A19* 3′-UTR luciferase reporter construct compared with those that expressed empty vector.

Focusing on the putative miR-122-5p bindings sites, we then confirmed their involvement by mutating these sites as well as by truncating them in the h*SLC25A19* 3′-UTR. Both manipulations were found to lead to a significant reduction in the level of inhibition in luciferase activity in cells expressing the mutated/truncated pmirGLO-h*SLC25A19* 3′-UTR luciferase reporter constructs. To further confirm the involvement of miR-122-5p in posttranscriptional regulation of MTPPT, we examined the effect of a mimic miR-122-5p on the level of expression of MTPPT mRNA and protein, as well as on mitochondrial carrier-mediated TPP uptake. We did the study using both rat PAC AR42J as well as human primary pancreatic acinar cells obtained from organ donors. The results showed that such treatment led to a significant inhibition in all three of these parameters in miR-122-5p mimic-treated rat and human PACs compared with control cells. We also examined the effect of inhibiting miR-122-5p of PAC AR42J with the use of a specific inhibitor (an antagomir) on MTPPT mRNA and protein expression, as well as on mitochondrial carrier-mediated TPP uptake and observed a significant increase in all of these parameters in PACs treated with the antagomir compared with cells transfected with negative control inhibitor.

Taken together, our findings demonstrate (for the first time) that the human MTPPT is subject to posttranscriptional regulation by miRNA (specifically by miR-122-5p) in PACs, and that this regulation impacts its level of expression as well as function. This may contribute to the observed pancreatic acinar organelles (e.g., mitochondrial) dysfunction that is associated with the progression of acute pancreatitis (50).

## DATA AVAILABILITY

Data will be made available upon reasonable request.

## ACKNOWLEDGMENTS

We extend our deepest gratitude to the courageous families who generously donated their loved one's organs and tissues for biomedical research. Such important research like this would not be possible without this selfless gift of hope. Thanks to the organ procurement organizations, Kentucky Organ Donor Affiliates (KODA), Louisville; LifeCenter, Cincinnati; and Lifeline of Ohio, Columbus for supporting these special families.

## GRANTS

This study was supported by National Institutes of Health Grants AA-018071, DK-56061, and DK056061-23S1 (to H.M.S.) and the Department of Veterans Affairs Grants 101BX001142 and IK6BX006189 (to H.M.S.).

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

H.M.S. conceived and designed research; K.R., S.S., and K.I.M. performed experiments; K.R., S.S., K.I.M., and H.M.S. analyzed data; K.R., S.S., and H.M.S. interpreted results of experiments; K.R. and S.S. prepared figures; K.R., S.S., and H.M.S. drafted manuscript; K.R., S.S., K.I.M., A.N.B., and H.M.S. edited and revised manuscript; K.R., S.S., K.I.M., A.N.B., and H.M.S. approved final version of manuscript.

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