Original Paper

Tissue Distribution and Regulation of the Small Sar1b GTPase in Mice

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Key Words

Sar1b GTPase • Transgenic mice • Coat protein complex II vesicles • Gene expression

Abstract

Background/Aims: Sar1b GTPase (Sar1b) represents an obligatory component of COPII vesicles that bud from the endoplasmic reticulum to transport proteins to the Golgi apparatus. Its genetic mutations lead to a severe disorder known as chylomicron retention disease. Despite growing knowledge on Sar1b, little is known about its tissue distribution and regulation, which constitute the aims of the present study. We aimed to determine the Sar1b tissue distribution and modulation by a high-fat diet and gene forcing using transgenic mice in comparison to wild-type mice. **Methods:** The expression pattern of Sar1b was studied in different organs of wild-type and *Sar1b* transgenic mice by qRT-PCR and Western blot. The effect of transgenesis and insulin resistance induced by a 12-week high-fat diet on *Sar1b* gene expression was also assessed by qRT-PCR. **Results:** Evaluation of *Sar1b* mRNA revealed the skeletal muscle as the tissue with the highest *Sar1b* expression, followed by the heart and liver, the organs composing the digestive tract, the brain and finally the lung and the adipose tissue. Sar1b protein expression levels follow a similar pattern among the organs, except for its lower expression in the heart. While the high-fat diet did not exert any significant alterations, *Sar1b* transgenic mice displayed higher gene expression in the liver, ileum, jejunum, proximal and distal colon compared to wild-type mice. **Conclusion:** Our study supports the importance of Sar1b in organs involved in lipid transport and/or calcium trafficking such as the liver, intestine, skeletal muscle and heart.

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Introduction

In eukaryote cells, intracellular delivery to downstream compartments requires that newly synthesized and properly folded proteins are transported from the endoplasmic reticulum (ER) to the Golgi network via coat protein complex (COP) II vesicles [1-3]. As revealed by numerous studies [4], COPII polymerizes on the membrane surface, captures cargo and SNARE molecules, and deforms the membrane to sculpt out vesicles [5]. Importantly, COPII vesicle biogenesis is initiated by the activation of a small Sar1 GTP-binding protein through Sec12, a guanine nucleotide exchange factor [6, 7]. The presence of Sar1b-GTPase (Sar1b) stimulates the recruitment of the inner coat components Sec23 and Sec24, which are responsible for capturing cargo proteins into the nascent vesicles [8, 9]. Then, the Sec13/31 heterotetramer is recruited to form the outer coat and stabilize the cargo-bound pre-budding complexes [3, 7, 10].

The Sar1 protein has two paralogs, Sar1a and Sar1b. In humans, the *SAR1B* gene (*SARA2*, HGNC: 10535; MIM *607690), not *SAR1A*, was identified as the specific gene undergoing molecular defects present in Anderson's disease or chylomicron (CM) retention disease, a rare autosomal recessive disorder characterized by an inability of the enterocytes to export dietary lipids via CMs. To date, 16 *SAR1B* mutations have been described in patients with CM retention disease [11-19]. No mutation in the *SAR1A* gene has been detected in patients with this specific disease. Accordingly, it has been suggested that, in enterocytes, the Sar1b paralogue would be used specifically for pre-CM mobilization by transport vesicles while the Sar1a paralogue would be employed for the transport of small soluble and membrane bound proteins by transport vesicles [15].

Patients with CM retention disease present with steatorrhea, chronic diarrhea and failure to thrive, as well as low plasma lipid and liposoluble vitamin levels, causing neurological impairment [20-23]. It appears that Sar1b is involved in multiple aspects of the cargo sorting process, e.g. the regulation of COPII coat dynamics and membrane deformation during vesicle formation, which dictates routing of numerous proteins and triglyceride-rich lipoproteins, including CM in the small intestine and very-low-density lipoproteins in the liver [11, 24]. Additional convincing evidence has recently been obtained by genetic manipulation; our previous study clearly demonstrated that Sar1b overexpression mediates stimulation of intestinal lipid transport, which was likely induced by the efficient assembly of CMs [25].

Despite the importance of Sar1b in COPII vesicle elaboration, there is an important gap of knowledge on the status and regulation of Sar1b in different organs. In this study, we assessed the tissue distribution and regulation by a high-fat diet and gene forcing of *Sar1b* gene expression in wild-type (WT) and transgenic (TG, Sar1b^{+/+}) mice.

Materials and Methods

Generation of *Sar1b* transgenic mice

The pCMV6-XL4 vector (OriGene Technologies, Rockville, MD), harbouring full length cDNA of *SAR1B*, was digested with *NotI* to excise a 1960 bp fragment. This fragment was subsequently cloned into a pBROAD3-mcs vector (InvivoGen, San Diego, CA) restricted with *NotI* to create pBROAD3-*SAR1B*. pBROAD3 features the ubiquitous mouse ROSA26 promoter; a high CpG content promoter that directs *in vivo* expression of the transgene [26]. Chicken β -globin gene insulator was subcloned upstream the ROSA26 promoter at the *NdeI* site to enhance transgene expression. Cloning of the β -globin gene insulator created pBROAD3- β -*SAR1B* vector. This 2 kb insulator fragment was derived from PCR amplification of chicken DNA with insulator specific primers containing *NdeI* site (primer 1: CATATGGCGCGCTCTAGACT; primer 2: CATATGGGATCCGTCGACGC). The capability of this construct to allow *SAR1B* expression in enterocytes was tested by transient transfection of Caco-2/15 cells. The DNA transgene vector, pBROAD- β -*SAR1B*, was then injected in fertilized eggs from 129/Sv mice at the Réseau de Recherche en Transgénèse du Québec facility.

Genomic DNA was prepared from founder mice and their offspring tail, and screened for the presence of *SAR1B* DNA by PCR amplification, using specific primers (primer 1: ATGTCCTTCATATTTGGATT; primer 2: ATCAATGTACTGTGCCATCC). Experiments were performed in male mice of mixed 129/Sv x C57BL6 backgrounds.

Animals and diets

Three-week-old TG for Sar1b and WT mice were housed in a temperature-controlled room at 24°C with a 12-h light-dark cycle and maintained on a standard laboratory chow normal diet (2018 Teklad Global 18% protein rodent diet, Harlan Laboratories, Indianapolis, IN) or a high-fat diet (BioServ F3282 60% fat Calories, BioServ, Frenchtown, NJ) and double distilled water *ad libitum* for 12 weeks. Animal experiments were conducted according to the Canadian Council on Animal Care guidelines for the care and use of experimental animals and were approved by the institutional animal care committee of Sainte-Justine Research Center.

Biochemical Analyses

After 12 weeks of feeding, mice were fasted overnight, weighed and sacrificed. Blood was collected for insulin and lipid assays. Plasma triglycerides and cholesterol levels were measured enzymatically (Boehringer-Mannheim, Mannheim, Germany). Blood glucose levels were determined using a OneTouch Ultra Meter (LifeScan, Milpitas, CA) and plasma insulin was measured using a rat/mouse insulin ELISA kit (Millipore, Billerica, MA). Plasma fatty acid composition was determined by gas chromatography as described previously [25]. Insulin sensitivity was determined using the HOMA-IR index. The score was calculated using the formula: (Insulinemia (mIU/L) x Glycemia (mmol/L)) / 22.5.

Cell Culture for Antibody Specificity

To test the specificity of our Sar1a/b antibody, we cultured Caco-2/15 cells for 14 days post-confluence as previously described [27]. We also established Caco-2/15 cell line overexpressing Sar1b and green fluorescent protein (GFP). To do so, the blunt-end PCR fragment encoding *SAR1B* gene was amplified from the human cDNA clone SC114457 (Origene Technologies, Rockville, MD) with ATGTCCTTCATATTTG as forward primer and CAGATCCTCTTCTGAGATGAGTTTTGTTTCATCAATGTTACTGTGCCAT containing myc epitope as reverse primer and TOPO Cloned into pLenti6/V5-D-TOPO (Invitrogen Corp., Carlsbad, CA 92008) to create an expression construct. The pLenti6/V5-D-TOPO expression plasmid of *SAR1B* was then transfected into Caco-2/15 cells with GenJet In Vitro Transfection Reagent (SignaGen Laboratories, Ijamsville, MD) according to the supplier's instructions. Generation of stable cell line was achieved using Blasticidin selection at a concentration of 1 µg/mL. Control Mock cells were obtained by transfection with pLentiV5/GFPtag (kindly provided by Dr. Jean-François Beaulieu, Université de Sherbrooke, Sherbrooke, Canada) harbouring the same features as the pLenti6/V5-D-TOPO system.

Western Blot Analysis

Sections of different organs from a total of 12 mice (3 mice/group for the 4 groups: WT-normal diet; WT-high-fat diet; TG-normal diet; TG-high-fat diet) were homogenized in ice-cold T-PER Tissue Protein Extraction Reagent (Thermo Scientific, Rockford, IL). Protease inhibitors were added to tissues prior to homogenization. As for Caco-2/15 cells, they were homogenized and adequately prepared for Western blotting as described previously [27]. The Bradford assay was used to estimate protein concentration. Supernatant proteins (20 µg) were denatured at 95°C for 5 min in a sample buffer containing sodium dodecyl sulfate (SDS) and dithiothreitol (Thermo Scientific, Rockford, IL), separated on a 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electroblotted onto Hybond-C extra nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ) in 25 mM Tris and 192 mM glycine. Nonspecific binding sites of the membranes were blocked with Tris-buffered saline [20 mM Tris-HCl (pH 7.5) plus 137 mM NaCl] containing 0.1% Tween 20 and 5% non fat dry milk for 60 min at room temperature. The blots were then incubated overnight at 4°C in blocking solution with the primary antibodies directed against the targeted proteins Sar1b/a (1:20,000) (kindly provided by Dr Randy Schekman, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA), β-actin 1:250,000 (Sigma Aldrich) and myc (1:250) (provided by Dr Mounib Elchebly, Sainte-Justine Hospital Research Center, Montreal, Canada). The relative amount of primary

antibody was revealed with a species-specific horseradish peroxidase-conjugated secondary antibody, e.g. anti-mouse IgG-POD/anti-rabbit IgG-POD (1:20,000) (Roche Diagnostics, Indianapolis). Blots were developed and the protein mass was quantified using an HP Scanjet scanner equipped with a transparency adapter and the UN-SCAN-IT gel 6.1 software.

RNA Extraction and Reverse Transcription

Tissue specimens obtained from a total of 24 mice (6 mice/group for the 4 groups: WT-normal diet; WT-high-fat diet; TG-normal diet; TG-high-fat diet) were immediately conserved in RNA-Later according to the manufacturer's protocol. They were homogenized in TRIzol reagent (Ambion by Life Technologies Corporation, Carlsbad, CA) and total RNA was extracted. RNA concentration and quality were tested by absorbance at 260 nm while the absorbance ratio of 260/280 (using a Nanodrop® ND-1000 Spectrophotometer from Thermo Scientific, Wilmington, DE) and RNA analysis by agarose gel electrophoresis served to assess integrity. Complementary DNA was obtained by reverse transcribing 1 µg of RNA with the qScript cNDA Supermix (Quanta Biosciences, Gaithersburg, MD) according to the manufacturer's instructions. Negative controls without enzyme were also prepared.

Quantitative PCR

The TaqMan® Express Human Endogenous Control Plate (Applied Biosystems, Foster City, CA) was used to select the most stable reference gene amongst organs according to the manufacturer's instructions. Using this technique, polymerase (RNA) II (DNA directed) polypeptide A1 (*Polr2a*) was chosen as an endogenous control to normalize the different cDNA sample amounts. Quantitative PCRs (qPCR) were performed in 96-wells microtiter plates using Taqman® Fast Advanced Master Mix (Applied Biosystems) in an Applied Biosystems Step One Plus™ Real-Time PCR System (Applied Biosystems). The qPCRs were carried in 96-well plates with a final volume of 10 µl per well. A volume of 5 µl Taqman® Fast Advanced Master Mix (1 X) was added to a well containing 1 µl of Custom Taqman® Gene Expression Assay (Applied Biosystems) (1 X) for *Sar1b* or for *Polr2a* comprising 1 µl of cDNA previously diluted 1:5 and 3 µl of diethylpyrocarbonate H₂O. Taqman® hydrolysis probes used were Mm01294633_m1 for *Sar1b* and Mn00839493_m1 for *Polr2a* (Applied Biosystems). Subsequently, negative controls without cDNA were prepared. The amplification reaction was carried out using 40 cycles and was performed in duplicate for each gene in the same plate under the following conditions: 95°C 20 s, 95°C 1 s and 60°C for 20 s.

Analyses

The relative mRNA fold-changes (relative quantification) between the animal groups were calculated using the $2^{-\Delta\Delta C_t}$ method [28]. For each sample, the first ΔC_t was calculated using the corresponding endogenous control. The second ΔC_t was calculated using the defined control group. For the comparison of *Sar1b* gene expression in the different organs, the average ΔC_t of the lung group was used as reference. Similarly, the average ΔC_t was employed for the comparison of *Sar1b* gene expression between WT and TG mice under the normal chow and high-fat diets, using the WT/normal diet mice as reference. Exponential values were then transformed in linear values and expressed as relative fold change in gene expression. The analyses and statistics were performed using the Expression Suite Software Version 1.0 (Applied Biosystems) and the Prism 5 for Mac Software Version 5.0d (GraphPad Software Inc., La Jolla, CA). Data are expressed as means $\pm$ SEM. For statistical analysis, one-way ANOVA followed by Bonferroni's multiple comparison test or t-tests were used and a *P* value < 0.05 was considered significant.

Results and Discussion

At first, we characterized the metabolic and lipid profile of mice from each group at sacrifice, following a 12-week diet. Results are presented in Table 1. To determine whether the overexpression of *Sar1b* influenced diet-induced obesity, male TG mice were maintained with *ad libitum* access to a pelleted high-fat diet. Then anthropometric and biochemical parameters were assessed. Although high-fat diet increased body weight in TG and WT mice, the mean body weight of TG carriers was slightly higher compared to corresponding WT controls. Similarly, the high-fat diet led to a rise in glucose and insulin in WT and TG mice,

Table 1. Metabolic characteristics of mice at sacrifice. Mice were sacrificed after being fed a normal or high-fat diet for 12 weeks. Results represent the means $\pm$ SEM of 6 animals per group. One-way ANOVA were performed ($P < 0.0001$) followed by Tuckey's multiple comparison tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. Wild-type, normal diet mice. # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$ vs. Transgenic, normal diet mice

	Wild-type Normal Diet	Wild-type High-fat Diet	Transgenic Normal Diet	Transgenic High-fat Diet
Weight (g)	29.35 $\pm$ 2.14	34.90 $\pm$ 2.13	37.67 $\pm$ 2.74*	50.70 $\pm$ 0.72***,##
Blood glucose (mmol/L)	6.00 $\pm$ 0.20	6.70 $\pm$ 0.20	7.00 $\pm$ 0.20**	7.80 $\pm$ 0.10***,##
Insulin (ng/mL)	0.71 $\pm$ 0.06	1.20 $\pm$ 0.08**	1.60 $\pm$ 0.12***	2.41 $\pm$ 0.17***,###
HOMA-IR (mIU/L*mmol/L)/22.5	5.00 $\pm$ 0.60	9.30 $\pm$ 0.60	13.00 $\pm$ 1.10***	22.40 $\pm$ 1.60***,###
Triglycerides (mmol/L)	0.68 $\pm$ 0.07	1.23 $\pm$ 0.12**	1.00 $\pm$ 0.09	1.50 $\pm$ 0.13##
Total cholesterol (mmol/L)	3.80 $\pm$ 0.40	4.70 $\pm$ 0.50	5.20 $\pm$ 0.50	5.90 $\pm$ 0.50*

Fig. 1. Protein expression Sar1 in Caco-2/15 cells. (A) Proteins from cell homogenates were extracted and protein expression was measured by Western blot as described in the Material and Methods section. A representative blot is shown. (B) After transfection with GFP and Sar1b constructs, Caco-2/15 cells were allowed to differentiate for 14 days before being tested for Sar1b protein expression by Western blot using anti-Sar1 and anti-myc antibodies. Results represent the means $\pm$ SEM of 3 experiments and were calculated as densitometric ratios of Sar1b/a to Sar1b-myc and are illustrated as % of Sar1a/b. A representative blot is shown. *** $P < 0.0001$ vs. Sar1a/b.

but the increase was more substantial in the Sar1b^{+/+} mice. Noteworthy, differences in these variables in WT and TG mice were observed even on the regular chow diet, which depicts the separate influence of the two factors, i.e. transgenesis and diet. As a result from the glucose and insulin modifications, the HOMA-IR index showed a more significant increase in the Sar1b^{+/+} mice vs. controls, thereby indicating reduced insulin sensitivity. Finally, our data show that the high-fat diet caused an augmentation in plasma triglycerides and cholesterol levels in WT and TG mice, but their increment was more substantial in Sar1b^{+/+} mice. Hence, as expected, the HFD induced a state of insulin resistance and dyslipidemia in the animals, and these effects were exacerbated by Sar1b transgenesis. A study, recently published by our group, has described the complete metabolic profile of Sar1b^{+/+} mice under control and high-fat diets and corroborates the present findings [29]. When fed a HFD, Sar1b^{+/+} mice were found to develop obesity, insulin insensitivity, hepatic and plasma lipid abnormalities and CM hyperproduction. This increased triglyceride-rich lipoprotein production elicited by Sar1b^{+/+} mice is in line with our other previous results obtained in Caco-2/15 cells, demonstrating

Fig. 2. Protein expression Sar1a and Sar1b in transgenic mice. (A) Proteins from liver were extracted and protein expression was measured by Western blot as described in the Material and Methods section. A representative blot is shown. Results represent the means $\pm$ SEM of 3 experiments. Results were calculated as densitometric ratios of Sar1a to Sar1b and are illustrated as % of Sar1a. A representative blot is shown. *** $P < 0.0003$ vs. Sar1a.

that Sar1b overexpression can enhance intestinal lipoprotein trafficking and sorting [25]. According to the prevailing concept, intestinal CM assembly requires the association of apo B-48 with lipids, which is mediated by microsomal triglyceride transfer protein in the rough ER, resulting in a lipid-poor, small apo B-48-containing particles that serve as precursors of mature CM. The intracellular traffic of apo B-48-containing CM is dependent on the COPII coat complex, which buds vesicles from the ER membrane to transport newly synthesized proteins to the Golgi apparatus. COPII vesicle biogenesis and assembly necessitates Sar1b to trigger a sequence of recruitment events that assemble the COPII coat from its cytosolic components and ultimately drives vesicle budding [2]. Our TG mouse model shows that overexpressing Sar1b leads to increased insulin resistance and dyslipidemia, phenomena that were amplified by the high-fat diet. Although the mechanisms controlling these metabolic changes are still nebulous and deserve further exploration, we propose that the increased intestinal fat absorption associated with Sar1b profusion in Sar1b^{+/+} mice leads to greater triglyceride accumulation in the blood circulation and insulin-responsive tissues [29], which would impede glucose uptake and provoke insulin resistance.

We have conducted experiments to determine the specificity of our Sar1b/a antibody in WT and TG animals. First, in Caco-2/15 cells, the Western blots reveal two visible bands, previously described as Sar1b (the upper band) and Sar1a (the lower band) [30] (Fig. 1A). The experiments were repeated in Caco-2/15 cells in which a pLenti6/V5-D-TOPO expression plasmid of *SAR1B* was transfected and, as a result, Sar1b was overexpressed. In this step, not only the blots were incubated with the anti-Sar1b/a antibody, but also with an anti-myc antibody, permitting to distinguish between the endogenous Sar1 and the transfected Sar1b. As illustrated in Fig. 1B, even if the double band (endogenous Sar1a and Sar1b) is still visible, the exogenous form of Sar1b represented 222% of the Sar1a/b band. However, in mice, the band corresponding to the Sar1a protein was barely visible. In fact, the band was rarely observed since Sar1b was overexpressed compared to Sar1a. Fig. 2 shows an experiment where the Sar1a and Sar1b bands could be distinguished in the liver of TG animals. These results showed that the densitometric ratio of Sar1b was over 1,500% of Sar1a. Hence, we concluded that, although our antibody could recognize Sar1a and Sar1b, the important difference in protein expression, especially in mice, allowed us to study the regulation of Sar1b.

Next, we defined Sar1b protein and mRNA expression in different organs in mice. As shown in Fig. 3A, the organ with the highest mRNA expression of *Sar1b* was the skeletal muscle, followed by the heart and the liver. The organs composing the digestive system and the brain had similar levels of expression. Finally, we found relatively low gene expression

Fig. 3. Differential Sar1b gene and Sar1 protein expression levels in mice organs. RNA (A) and protein (B) from tissues were extracted as described in the Material and Methods section. Gene and protein expression were measured by RT-qPCR and Western blot, respectively. The mean relative expression was calculated relative to lung. Results represent the means $\pm$ SEM of 24 (gene) and 12 (proteins) animals. One-way ANOVA were performed ($P < 0.0001$) followed by Tuckey's multiple comparison tests. A. *** $P < 0.001$ vs. lung, PAT, distal colon, proximal colon, ileum, duodenum, brain and jejunum; ## $P < 0.01$ vs. stomach, liver and heart. B. * $P < 0.05$ vs. lung, PAT and brain; *** $P < 0.001$ vs. lung, PAT, distal colon, proximal colon, ileum, duodenum, brain, jejunum, stomach and heart; # $P < 0.05$ vs. liver. PAT: perirenal adipose tissue. A representative blot is shown.

in the lung and perirenal adipose tissue. The skeletal muscle was the only organ for which post-hoc analyses revealed a significant difference with the others. The measurement of Sar1b at the protein level demonstrated a similar pattern among the organs, except for its lower expression in the heart (Fig. 3B). Western blots showed that the liver was the organ that mostly expressed Sar1b and confirmed the high expression in the skeletal muscle, followed by a roughly equivalent expression in the organs of the digestive tract. The gene and protein expression profile of Sar1b in the duodenum, jejunum and ileum, parts of the digestive system, was relatively equivalent, which corresponds to their respective function in lipid and bile acid handling. It is also important to specify that, since the pattern of Sar1b protein distribution was similar between WT and TG mice under chow and high-fat diet, all the tissue samples from the different groups were analyzed on the same Western blot for comparison needs.

Limited information is available concerning the distribution of Sar1b in various organs despite its crucial function in intracellular trafficking. Using the state-of-the-art technique for mRNA analysis, e.g. qPCR, we documented a preponderance of *Sar1b* mRNA content in the skeletal muscle. The surprisingly abundant expression of *Sar1b* in the muscle suggests highly specialized role of Sar1b in this particular tissue, including the regulation of calcium (Ca^{2+}) trafficking among multiple Ca^{2+} storage organelles, for instance the sarcoplasmic reticulum and the ER. In fact, the involvement of Sar1b in Ca^{2+} trafficking in skeletal muscle was demonstrated a decade ago when calsequestrin, an acidic, low-affinity, high-capacity Ca^{2+} -binding glycoprotein that plays a crucial role in Ca^{2+} routing to sarcoplasmic reticulum, was found sensitive to the Sar1-H79G mutant, resulting in its accumulation in COPII vesicles in rat skeletal muscle fibers [31]. Later, it was demonstrated that the molecular mechanism underlying the human Ca^{2+} receptor exit from the ER to the cell surface requires Sar1b and is dependent on COPII vesicles [32]. Likewise, the apoptosis-linked gene 2, a Ca^{2+} -binding protein that serves as a Ca^{2+} sensor, is dependant of the COPII vesicle formation process and components [33]. Hence, the role of Sar1b in Ca^{2+} trafficking can explain its high level of expression not only in the skeletal muscle, but also in the heart, in view of Ca^{2+} signaling and trafficking in cardiomyocytes [34, 35]. Accordingly, it was reported that patients with

Fig. 4. Effect of diets and transgenesis on the differential gene expression levels of *Sar1b* in organs in mice. Wild-type and transgenic *Sar1b* mice were fed a standard laboratory chow or high-fat diet for a 12-week period. RNA from tissues was extracted as described in the Material and Methods section. Gene expression was measured by RT-qPCR and mean relative expression was calculated for each organ to compare gene expression relative to wild-type mice fed a normal diet. Results represent the means $\pm$ SEM of 6 animals per group. T-tests were performed. WT-ND: wild-type mice, normal diet; WT-HFD: Wild-type mice, high-fat diet; TG-ND: *Sar1b*^{+/+} transgenic mice, normal diet; TG-HFD: *Sar1b*^{+/+} transgenic mice, high-fat diet; PAT: perirenal adipose tissue. * $P < 0.05$; ** $P < 0.01$ vs. wild-type mice; # $P < 0.05$ vs. wild-type/normal diet mice.

chylomicron retention disease suffer from myolysis, cardiac abnormalities and elevated creatine kinase levels [16].

Next, we evaluated the effect of insulino-resistance induced by high-fat diet and the impact of *Sar1b* transgenesis on *Sar1b* gene expression in different tissues. The provision of a 12-week high-fat diet to mice did not result in significant changes in mRNA *Sar1b*

Fig. 5. Effect of transgenesis on Sar1 protein and Sar1b gene expression in liver and jejunum. Differential levels of Sar1 protein (A) and Sar1b mRNA (B) expression in the liver and the jejunum of wild-type (WT) and transgenic (TG) mice was assessed by Western Blot and by RT-PCR as described in the Material and Methods. A. Results represent the means $\pm$ SEM of 3 animals per group. Results were calculated as densitometric ratios of Sar1b to β -actin and are illustrated as % of WT mice. ** $P < 0.004$; * $P < 0.022$ vs. WT mice.

expression in the various tissues when compared to a normal diet in neither WT nor TG mice (Fig. 4). This is divergent from a previous study documenting *Sar1b* up-regulation by fructose diet and olive oil in Syrian golden hamsters [36], suggesting that the regulation of *Sar1b* expression by diet and insulin resistance may be species-dependent. Also, *in vitro* studies have suggested that Sar1b can be regulated by lipids, as demonstrated by the regulation of COPII vesicle formation by lipid composition of membranes [37]. Similarly, depletion of sterols or inhibition of sterol synthesis reduce the mobility of membrane cargo proteins within the ER and inhibit the dynamic interactions of COPII subunits with ER exit sites [38, 39]. Previous work from our laboratory demonstrated that, in Caco-2/15 cells, Sar1b overexpression promotes intestinal lipid transport in a process involving the COPII network [25]. In the present experimental conditions, the high-fat diet did not effectively regulate *Sar1b* expression, which allows us to speculate that diet could regulate *Sar1b* turnover without altering mRNA, thereby increasing recycling activity of COPII vesicle.

Our results also revealed that TG mice displayed enhanced mRNA level of *Sar1b* in several organs (Fig. 4). Significant enrichment was noted in the liver, ileum, jejunum, proximal colon and distal colon while there was also a higher, albeit not statistically significant, expression in the skeletal muscle, heart and lung. Interestingly, the enrichment was observed in the tissues endowed with functional roles in Ca^{2+} and lipid transport. In line with our results showing disparate enrichment between organs, various reports have emphasized that various TG mouse models equipped, genetically manipulated in different genes expressed variable amounts of mRNA in peripheral tissues [40]. More specifically, the pCAG promoter in the Rosa26 locus suffers from mosaic transgene expression among organs [41] and the responder gene expression was moderate and highly variable between and within different tissues [42].

We have evaluated the effect of transgenesis on Sar1b protein expression in the liver and jejunum, the two key organs involved in lipid transport and lipoprotein assembly. Fig. 5A shows that Sar1b in these two organs is elevated in TG compared to WT mice. In fact, we found 446% ($P < 0.004$) and 388% ($P < 0.022$) increases in Sar1b expression in the liver and jejunum, respectively. This corresponds to the elevation in gene expression in these organs presented in Fig. 4. Compared to WT animals, transgenesis led to elevated gene expression (459%, $P < 0.0015$) in the liver and in the jejunum (90%), although the differences in the latter were not statistically significant. Using standard RT-PCR method, we have also compared the endogenous and exogenous *Sar1b* gene expression in mice. Fig. 5B reveals that the exogenous gene expression in transgenic animals represents approximately 70% of the total expression in the liver and in jejunum.

A limit of the present work is that it was performed in mice, which does not necessarily reflect the expression profile in humans. However, previous investigation has shown that the presence of Sar1b in the digestive tract is consistent in different species: it was found in the human duodenum [15], in the human Caco-2/15 cell model [25], in the small intestine of hamsters [36] and in rat enterocytes [43]. Further studies are needed to evaluate the distribution and expression profile of Sar1b in the human digestive tract and in other tissues.

Conclusions

In conclusion, in our mouse model Sar1b was not regulated by insulin resistance or by high-fat content. However, the results obtained from tissue distribution pattern and regulation by transgenesis support the importance of Sar1b in organs involved in lipid transport such as the liver, jejunum, ileum and colon, which highlight its crucial role in organs transporting lipids. Interestingly our findings also support a significant role for Sar1b in organs necessitating major Ca^{2+} trafficking, namely the skeletal muscle and the heart.

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