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Data in brief

Analyzing the temporal regulation of translation efficiency in mouse liver

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ABSTRACT

Mammalian physiology and behavior follow daily rhythms that are orchestrated by endogenous timekeepers known as circadian clocks. Rhythms in transcription are considered the main mechanism to engender rhythmic gene expression, but important roles for posttranscriptional mechanisms have recently emerged as well (reviewed in Lim and Allada (2013) [1]). We have recently reported on the use of ribosome profiling (RPF-seq), a method based on the high-throughput sequencing of ribosome protected mRNA fragments, to explore the temporal regulation of translation efficiency (Janich et al., 2015 [2]). Through the comparison of around-the-clock RPF-seq and matching RNA-seq data we were able to identify 150 genes, involved in ribosome biogenesis, iron metabolism and other pathways, whose rhythmicity is generated entirely at the level of protein synthesis. The temporal transcriptome and translatome data sets from this study have been deposited in NCBI's Gene Expression Omnibus under the accession number GSE67305. Here we provide additional information on the experimental setup and on important optimization steps pertaining to the ribosome profiling technique in mouse liver and to data analysis.

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Specifications	
Organism/cell line/tissue	Mus musculus/liver
Sex	Male
Sequencer or array type	Illumina HiSeq 2500
Data format	Raw and processed data
Experimental factors	Livers were collected every 2 h during the 24-h daily cycle (with 2 replicate time series)
Experimental features	RNA-seq and RPF-seq were performed in parallel on the same liver lysates to identify mRNA subject to rhythmicity at the translational level
Consent	Data are publicly available at NCBI GEO
Sample source location	Lausanne, Switzerland

1. Direct link to deposited data

Direct link to deposited files: http://datalink.elsevier.com/midas/datalink/api/downloadfiles?items=18934-18935-18936

Direct link to deposited genomic data: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=etmbssamttcnzsb&acc=GSE67305

2. Experimental design, materials and methods

2.1. Experimental design

To investigate daily rhythms in translation, we recently performed ribosome profiling in mouse liver (Janich et al., 2015 [2]), which is the most commonly used peripheral organ in circadian research due to its easy dissectability, its relatively homogenous cellular composition and its abundant, high-amplitude rhythms [3]. Ribosome profiling is based on the deep sequencing of ≈30 nucleotide mRNA fragments that are protected by translating ribosomes upon nuclease digestion [4]. The sequence information contained in the footprints allowed us to perform transcriptome-wide, quantitative analyses of protein synthesis rhythms in mouse liver. Parallel RNA-seq data was used to quantify RNA abundance around-the-clock, allowing the identification of those genes whose rhythmicity was exclusively translational. Livers were collected at 2 h intervals around-the-clock in order to have sufficient temporal resolution for reliable rhythmicity detection. For each time point, two replicate samples were generated. Each replicate consisted of a pool of 2 individual livers.

2.2. Mice

Wild type C57BL/6J mice were purchased from Janvier Labs. Animal housing and experimental procedures were in agreement with the veterinary law of the Canton Vaud, Switzerland (authorization to DG: VD2376). For all experiments, mice were entrained to 12-h-light/12-

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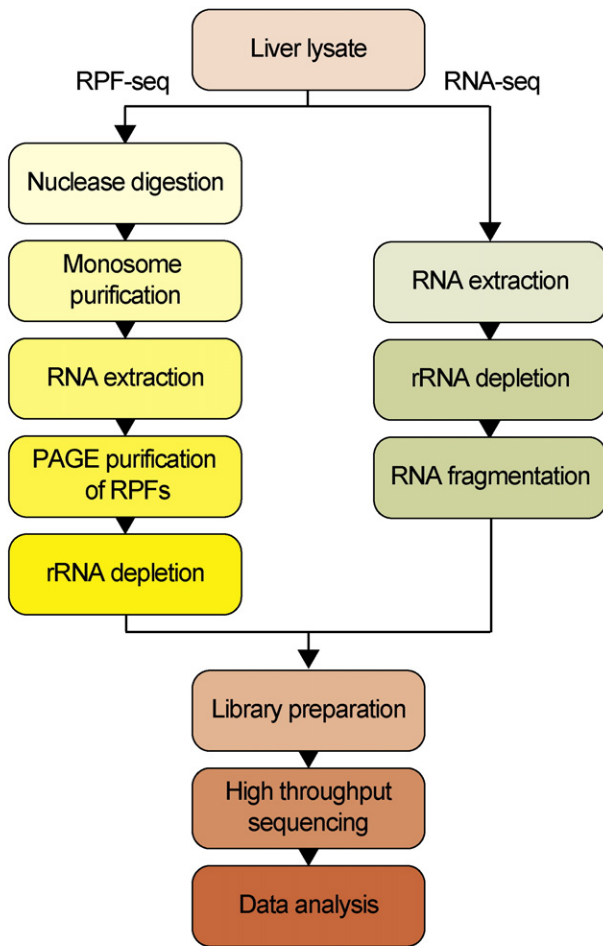

Fig. 1. Overview of the experimental workflow used for ribosome profiling (RPF-seq) and for RNA-seq in mouse liver.

h-dark cycles for 2 weeks with water and normal chow available ad libitum. Prior to organ collection, mice were anesthetized with isoflurane and sacrificed by decapitation. Mice were sacrificed at the indicated Zeitgeber times (ZT), with ZT00 corresponding to "lights on" and ZT12 to "lights off" in the animal housing facility. Livers were rapidly excised and immediately processed to lysate.

2.3. Lysate preparation

Freshly extracted liver tissue from each individual mouse was weighed and subsequently lysed with 8 strokes in a Teflon homogenizer containing 3 volumes of ice-cold lysis buffer (20 mM Tris–HCl pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 5 mM DTT, 100 μg/ml cycloheximide, 1% Triton X-100, and 0.5% sodium deoxycholate) supplemented with complete EDTA-free protease inhibitors (Roche) and 40 U/ml RNasin plus (Promega). Of note, RNasin plus inhibits RNase A, B and other RNases present in liver extracts, but not RNase I, which will be used at a later stage of the protocol to generate ribosome protected mRNA fragments (RPFs). The liver homogenates were transferred to microcentrifuge tubes and incubated for 10 min on ice. Cellular debris was pelleted by centrifugation at 1000 ×g for 3 min at 4 °C. The supernatant was removed, aliquoted, and snap-frozen and stored under liquid nitrogen until further processing. For absorbance measurements at 260 nm, lysates were gently thawed on ice, diluted 1:10 and 1:20 in water, the absorbance determined by Nanodrop and the average value from the two dilutions was determined. In general, the lysates ranged between 100 and 200 OD260 per 1 ml lysate. Equal amounts of lysate (OD260) from 2 mice, collected at the same time point, were pooled and diluted with lysis buffer to a final concentration of 15 OD260/100 μl. Lysates were processed separately for RPF-seq and RNA-seq (Fig. 1).

2.4. RNA extraction and RNA-seq library preparation

For the isolation of total (cytoplasmic) RNA, 100 μl pooled lysate was mixed with 1 ml Trizol and incubated for 5 min at room temperature. RNA was isolated using the miRNeasy kit (Qiagen) according to the manufacturer's protocol and the concentration determined by Nanodrop. Prior to library preparation, a total of 5 μg RNA was subjected to ribosomal RNA depletion (Ribo-Zero magnetic kit, Epicenter) and subsequently purified using a RNA purification kit (RNA Clean & Concentrator-5, Zymo Research). RNA-seq libraries were generated following the instructions for total RNA library preparation of the ARTseq ribosome profiling kit (Epicenter).

2.5. Preparation of ribosome protected mRNA fragments (RPFs)

In preparation to processing the samples from the large-scale time series, we optimized the conditions for nuclease digest in order to ensure efficient and reproducible generation of RPFs of ≈30 nucleotides in length. To this end, liver lysates were incubated with different amounts of RNase I ranging from 0 to 1000 units (Ambion). The digested mRNA fragments were purified using Trizol extraction and analyzed by northern blot as described previously [5]. Northern blot hybridization was performed using 2 different probes recognizing two highly expressed liver mRNAs (Alb, albumin and Mup, major urinary protein). Analysis of the autoradiographs showed that the optimal concentration for obtaining mRNA fragments of 30 nucleotides in lysates prepared from mouse liver was in the range of 600 to 1000 units RNase I (Fig. 2).

Thus, for our time course experiment, lysates of a concentration of 15 OD260 in a volume of 100 μl were incubated with 650 units RNase I and 2.5 μl DNase I for 45 min at room temperature. After the incubation, samples were placed on ice and 8.7 μl Superasin RNase inhibitor (Ambion) were added to inactivate the RNase I enzyme. In the meantime, size exclusion spin columns (S-400, GE Healthcare Life Sciences), that would subsequently serve to purify the nuclease-generated monosomes, were prepared. To this end, spin columns were washed 3 times with 700 μl lysate buffer containing 20 U/ml Superasin in a microcentrifuge at 600 ×g for 1 min. Between each washing step the matrix of the spin column was gently resuspended by vortexing. After the washing steps, lysates were applied to the matrix and the spin columns were centrifuged for 2 min at 600 ×g. 1 ml of Trizol was added

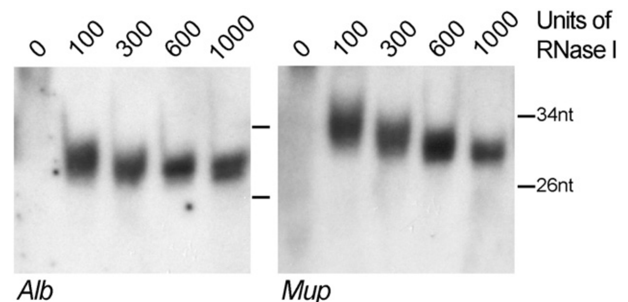

Fig. 2. Optimization of RNase I concentration. Autoradiographs of RNase I-digested liver RPFs probed for two highly expressed liver mRNAs, albumin (Alb, probe: cgatgggcgatctcactcttgtgtgcttctc) and major urinary protein (Mup, probe: gttccttcccgtagaactagcttc). RPFs of 30 nucleotides in length were obtained when 600–1000 units of RNase I were used for digestion.

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