The Leucine-rich Pentatricopeptide Repeat-containing Protein (LRPPRC) Does Not Activate Transcription in Mammalian Mitochondria*§

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Regulation of mtDNA expression is critical for controlling oxidative phosphorylation capacity and has been reported to occur at several different levels in mammalian mitochondria. LRPPRC (leucine-rich pentatricopeptide repeat-containing protein) has a key role in this regulation and acts at the post-transcriptional level to stabilize mitochondrial mRNAs, to promote mitochondrial mRNA polyadenylation, and to coordinate mitochondrial translation. However, recent studies have suggested that LRPPRC may have an additional intramitochondrial role by directly interacting with the mitochondrial RNA polymerase POLRMT to stimulate mtDNA transcription. In this study, we have further examined the intramitochondrial roles for LRPPRC by creating bacterial artificial chromosome transgenic mice with moderately increased LRPPRC expression and heterozygous Lrpprc knock-out mice with moderately decreased LRPPRC expression. Variation of LRPPRC levels in mice in vivo, occurring within a predicted normal physiological range, strongly affected the levels of an unprocessed mitochondrial precursor transcript (ND5-cytochrome b) but had no effect on steady-state levels of mitochondrial transcripts or de novo transcription of mtDNA. We further assessed the role of LRPPRC in mitochondrial transcription by performing size exclusion chromatography and immunoprecipitation experiments in human cell lines and mice, but we found no interaction between LRPPRC and POLRMT. Furthermore, addition of purified LRPPRC to a recombinant human in vitro transcription system did not activate mtDNA transcription. On the basis of these data, we conclude that LRPPRC does not directly regulate mtDNA transcription but rather acts as a post-transcriptional regulator of mammalian mtDNA expression.

Members of the pentatricopeptide repeat (PPR)² protein family play important roles in mitochondrial RNA metabolism in metazoans, plants, and yeast (1). They are RNA-binding proteins characterized by a 35-amino acid long motif, which can be repeated between 2 and 30 times. There are several hundred known PPR protein members in plants (localized to chloroplasts or mitochondria) that have been shown to have important roles in RNA editing, RNA stability, guidance of RNA modification, and initiation of translation (2, 3). In contrast to plants, mammals contain only seven PPR proteins, all localized mainly to mitochondria, with roles in RNA metabolism. The mammalian PPR proteins include POLRMT (4), PTCD1–3 (pentatricopeptide repeat domain-containing proteins 1–3) (5–7), MRPS27 (mitochondrial ribosomal protein S27) (8), MRPP3 (mitochondrial ribonuclease P protein 3) (9), and LRPPRC (leucine-rich pentatricopeptide repeat-containing protein) (10), which have been reported to play different roles in transcription, processing of polyadenylated RNAs, biogenesis of the small ribosomal subunit, mRNA stability, and mRNA polyadenylation.

LRPPRC forms a complex with SLIRP (stem-loop-interacting RNA-binding protein) and controls mRNA stability, mRNA polyadenylation, and coordination of translation (11–13). A recessive mutation creates an A354V amino acid substitution in LRPPRC that causes a neurodegenerative disease called Leigh syndrome French Canadian variant, which is characterized by cytochrome c oxidase deficiency, decreased mitochondrial oxidative phosphorylation capacity and has been reported to occur at several different levels in mammalian mitochondria.

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² The abbreviations used are: PPR, pentatricopeptide repeat; Cytb, cytochrome b; LSP, light strand promoter; HSP, heavy strand promoter; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

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mRNA levels, and reduced mitochondrial translation in liver and brain (11, 14). There are many reports that describe roles for LRPPRC in RNA transport from the nucleus to the cytoplasm (10), in regulation of cytoplasmic translation (15), and in nuclear transcription (16). However, the main part of LRPPRC is found in mitochondria (17), and RNAi knockdown of LRPPRC expression in cell lines (11) and conditional knock-out of Lrpprc in mice (12) have both shown a strong reduction in mtDNA expression. Homozygous knock-out of Lrpprc in mice is embryonic lethal, and tissue-specific disruption in heart creates a strong mitochondrial phenotype with decreased steady-state levels of mRNAs, defective polyadenylation, impaired coordination of translation, and cytochrome c oxidase deficiency (12). LRPPRC is thus important for post-transcriptional regulation of mtDNA expression in mammals (12). Forced expression of LRPPRC in mouse liver has been reported to cause cristae compaction and stimulation of oxidative phosphorylation (18). This effect has been attributed to a role for LRPPRC as a transcriptional activator, mediated by direct interactions with POLRMT (18).

In this study, we have further characterized a putative role for LRPPRC in mitochondrial transcription by manipulating the in vivo expression of LRPPRC in mice, by biochemical fractionation of mitochondrial extracts, and by performing in vitro transcription reactions. We report a novel role for LRPPRC in mitochondrial RNA processing, but the in vivo and in vitro findings we present here do not support the hypothesis that LRPPRC also stimulates mtDNA transcription. LRPPRC rather seems to have a specific role in post-transcriptional regulation of mtDNA expression.

**EXPERIMENTAL PROCEDURES**

*Generation of Lrpprc-overexpressing and Heterozygous Lrpprc Knock-out Mice*—A bacterial artificial chromosome (BAC) clone of 241 kb (RP24-100M10) containing the whole mouse Lrpprc gene was obtained from the Children’s Hospital Oakland Research Institute BACPAC Resources Center. The BAC was modified by RecE and RecT protein mediated recombination to allow discrimination between transcripts expressed from the endogenous Lrpprc gene and the introduced BAC clone. A silent mutation that did not alter the encoded amino acid but did eliminate a BglII site was introduced in exon 3. The modified BAC was purified by cesium chloride gradient centrifugation and injected into the pronuclei of fertilized oocytes. Founders (+/BAC-LRPPRC) were identified by PCR and restriction enzyme analysis of genomic DNA to detect loss of the BglII site in the Lrpprc site. Tail DNA from offspring was genotyped for the presence of the BAC transgene by analyzing tail DNA from offspring was genotyped for the presence of the BAC transgene by analyzing the BglII site in the Lrpprc gene and the introduced BAC transgene by PCR with an initial denaturation for 3 min at 95 °C, followed by 35 cycles for 30 s at 95 °C, 30 s at 53 °C, and 45 s at 73 °C. The reaction was ended with extension for 5 min at 72 °C. Breeding and genotyping of Lrpprc overexpressing and heterozygous mice were performed as described previously (12). Mice were maintained on an inbred C57BL/6N background.

*RNA Isolation and Northern Blot Analysis*—RNA from mouse heart and liver was extracted with TriZol Reagent (Invitrogen) and treated with DNase (Invitrogen). Northern blotting was performed using standard protocols. Mouse monoclonal antibodies detecting nucleus-encoded subunits of complex I (NDUFA9 subunit; 1:1000; Invitrogen), complex II (SDHA subunit; 1:1000; Invitrogen), complex III (UQCRC2 subunit; 1:1000; Invitrogen), complex IV (COX1 and COX3 subunits; 1:1000; Invitrogen), and complex V (ATP5A1 subunit); MitoProfile total oxidative phosphorylation antibody mixture (1:1000; MitoSciences); and porin (1:1000; MitoSciences) were used for analysis of levels of mitochondrial respiratory chain complexes. Mouse and human FLAG-tagged LRPPRC proteins were detected with anti-FLAG monoclonal antibody M2 (1:250; Sigma). Human POLRMT and SLIRP were detected with polyclonal antibodies (1:1000; Abcam). Rabbit polyclonal antisera generated in-house were used to detect mouse LRFPPRC (1:250), mouse TFAM (transcription factor A, mitochondrial; 1:500), mouse POLRMT (1:500), and mouse TFB2M (transcription factor B2, mitochondrial; 1:50).

*De Novo Transcription Assays*—Isolated mitochondria (2 mg) from heart and liver tissue were pelleted and resuspended in 500 μL of transcription buffer containing 25 mM sucrose, 75 mM sorbitol, 100 mM KCl, 10 mM K2HPO4, 50 mM EDTA, 5 mM MgCl2, 1 mM ADP, 10 mM glutamate, 2.5 mM malate, and 10 mM Tris-HCl (pH 7.4) with 1 mg of BSA/ml. The mitochondrial suspension containing 50 μCi of [α-32P]UTP (Amersham Biosciences) was incubated by rotating the mixture for 1 h at 37 °C. After the incubation, the mitochondria were pelleted and

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washed twice with resuspension buffer containing 10% glycerol, 10 mM Tris-HCl (pH 6.8), and 0.15 mM MgCl₂. Mitochondrial RNA was isolated from the final pellet using the ToTALLY RNA kit and resuspended in 30–50 μl of glyoxal loading buffer with dye (Ambion). Samples were separated in 1.2% agarose gel containing formaldehyde at 120 V for 2 h. Additional procedures were as described under “RNA Isolation and Northern Blot Analysis.”

Quantitative PCR—Total RNA from mouse liver was extracted using the ToTALLY RNA kit. Reverse transcription and quantitative RT-PCR were performed using the High Capacity RNA-to-cDNA kit (Applied Biosystems) and TaqMan® 2× Universal PCR Master Mix, No AmpErase® UNG (Applied Biosystems). The following custom-made TaqMan® probes against mouse mitochondrial transcripts were obtained from Applied Biosystems: cytochrome b (Cytb), ND6, and COXI. 18 S rRNA was used as a probe to detect this nuclear transcript.

Immunoprecipitation—Mitochondria from stably transfected HeLa Tet-On cells lines expressing human LRPPRC-FLAG and transgenic mice expressing mouse LRPPRC-FLAG in a homozygous Lrpprc knockout background (genotype Lrpprc<sup>−/−</sup>, +/BAC-LRPPRC-FLAG) were used for immunoprecipitation (12). Human or mouse mitochondria were isolated by differential centrifugation in buffer A (320 mM sucrose, 1 mM EDTA, and 10 mM Tris-HCl (pH 7.4)) containing 1× Complete protease inhibitor mixture (Roche Applied Science). Mitochondria (1 mg) were incubated in lysis buffer B (50 mM Tris-HCl (pH 7.4), 1 mM EDTA, 150 mM NaCl, 5% glycerol, and 0.5% Triton X-11) and 1× Complete protease inhibitor mixture for 20 min on ice, followed by centrifugation at 13,000 × g for 45 min at 4 °C. Next, the lysate was incubated with anti-FLAG M2 agarose (Sigma), and protein partners were purified according to the recommendations of the manufacturer.

Size Exclusion Chromatography—Size exclusion chromatography was performed as described previously (12) with some modifications. Human mitochondria were isolated from HeLa cells by differential centrifugation in isolation buffer A containing 1× Complete protease inhibitor mixture. Mitochondria were lysed at a concentration of 5 mg/ml in lysis buffer B and 1× Complete protease inhibitor mixture for 20 min on ice, followed by centrifugation at 13,000 × g for 45 min at 4 °C. Next, 1 mg of the precleared lysate was subjected to size exclusion chromatography on a Superose 6 column (GE Healthcare) that had been pre-equilibrated with lysis buffer B. Fractions of 1 ml were collected, precipitated with TCA, and analyzed by SDS-PAGE and immunoblotting.

Recombinant Proteins—For mitochondrial in vitro transcription, recombinant human TFAM and TFB2M were expressed and purified from insect cells as described previously (20). POLRMT was expressed and purified from Escherichia coli ArcticExpress cells (Stratagene). A DNA fragment encoding LRPPRC fused to a His<sub>6</sub> tag at the C terminus was cloned in the vector pJexpress 401 and expressed and purified from insect cells as described previously (20). LRPPRC was eluted with a linear gradient (10 ml) containing 0.2–1.2 M NaCl, and the peak fractions were dialyzed three times with buffer C containing 0 mM NaCl, followed by further purification on a 1-ml HiTrap SP column (Amersham Biosciences) equilibrated with buffer C containing 0.2 mM NaCl. After washing the column with 3 column volumes of buffer C containing 0.2 mM NaCl, LRPPRC was eluted with a linear gradient (10 ml) of buffer C containing 0.2–1.2 M NaCl, and the protein peak eluted at 600 mM NaCl. The peak fractions were dialyzed against buffer C containing 0.2 mM NaCl. The estimated purity of the purified LRPPRC was at least 95% as estimated using Coomassie Blue-stained SDS-polyacrylamide gels. For absolute quantification, codon-optimized (DNA2.0) DNA encoding the mature form of mouse LRPPRC fused to a His<sub>6</sub> tag at the N terminus was cloned in the vector plexpress 401 and heterologously expressed in ArcticExpress(DE3) cells (Stratagene) after induction with 0.2 mM isopropyl 1-thio-β-D-galactopyranoside at 16 °C for 20 h. LRPPRC was purified following the procedure for the MTERF4-NSUN4 complex as described previously (21). Mouse POLRMT, TFB2M, and TFAM were purified as described previously (22).

In Vitro Transcription—Plasmid constructs with human mtDNA sequences corresponding to bp 1–741 (light (LSP) and heavy (HSP) strand promoters), 1–477 (LSP), and 499–741 (HSP) were used as templates as described previously (20). In vitro transcription reactions contained 100 fmol of the indicated template, 20 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 1 mM DTT, 100 μg/ml BSA, 400 μM ATP, 150 μM CTP, 150 μM GTP, 10 μM UTP, 0.2 μM [α-<sup>32</sup>P]UTP (3000 Ci/mmol), 4 units of RNasin (AP-Biotech), 400 fmol of POLRMT, 400 fmol of TFB2M, and 5 pmol of TFAM (15 pmol of TFAM was added when the LSP/HS template was used). The reaction volume was 25 μl, and the final concentration of NaCl was adjusted to exactly 80 mM NaCl in all reactions. The concentrations of LRPPRC are indicated in the figure legends. Reactions were stopped after 30 min at 32 °C by the addition of 200 μl of stop buffer (10 mM Tris-HCl (pH 8.0), 0.2 M NaCl, 1 mM EDTA, and 0.1 mg/ml glycogen). The samples were treated with 0.5% SDS and 100 μg/ml proteinase K for 45 min at 42 °C and precipitated by the addition of 0.6 ml of ice-cold ethanol. The pellets were dissolved in 10 μl of gel loading buffer (98% formamide, 10 mM EDTA (pH 8.0), 0.025% xylene cyanol FF, and 0.025% bromphenol blue) and heated at 95 °C for 5 min. Transcription reaction products were analyzed in a 6% denaturing polyacrylamide gel with 1× Tris borate/EDTA buffer.

Electrophoresis Mobility Shift Assay—The RNA-binding activity of LRPPRC was assayed by EMSA using the HSP (bp 499–741) run-off transcript as a template. A 10× in vitro transcription reaction (250 μl) was performed as described above. After 30 min, 2 μl of DNase I (1 unit/μl) was added to the reaction, which was placed on a benchtop at room temperature for 10 min before the radioactive labeled RNA was purified using the RNeasy mini kit (Qiagen). The RNA was eluted in 50 μl of RNase-free water. The RNA-binding reactions were per-
LRPPRC Does Not Regulate the Amount of mtDNA and Respiratory Chain Complexes—It has previously been shown that LRPPRC is nonessential for mtDNA maintenance (12, 18), despite being identified as a component of the mitochondrial nucleoid (25). We assessed mtDNA levels in mice with moderately decreased and increased LRPPRC expression, but we found no differences in liver from mutant and wild-type mice as determined by Southern blot (Fig. 2, A and B) and quantitative PCR (Fig. 2C) analyses of mtDNA levels. Loss of LRPPRC is known to cause a profound complex IV (cytochrome c oxidase) deficiency and to decrease steady-state levels of complexes I and V (11, 12). Moreover, forced expression of LRPPRC in liver has been reported to increase the levels of subunits of the respiratory chain complexes and to remodel mitochondria by increasing cristae density (18). Forced expression of mitochondrial proteins sometimes create artifacts unrelated to the normal physiological function of the studied protein (26). We therefore decided to study Lrpprc−/− and Lrpprc+/+/T mice to determine whether moderately altered LRPPRC levels have any effect on steady-state levels of oxidative phosphorylation...
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enzyme complexes. Western blot analysis of respiratory chain subunits showed normal levels of NDUFB8 (complex I), SDHA (complex II), UQRC2 (complex III), COXI (complex IV), and ATP5A1 (complex V) in LRPPRC knock-out (+/-) and LRPPRC-overexpressing (+/T) mice at 10 weeks of age. The number of animals studied was as follows: n = 3 (control), n = 3 (LRPPRC+/-), and n = 3 (LRPPRC+/T).

LRPPRC Strongly Influences Levels of the ND5-Cytb Precursor Transcript—Down-regulation of LRPPRC expression causes severe reduction in the levels of all mRNAs encoded on the heavy strand of mtDNA (11, 12), whereas forced expression of LRPPRC has been reported to lead to accumulation of the same transcripts (12, 27). We further investigated the role of LRPPRC in mtDNA transcription by performing Northern blot analyses of steady-state levels of mitochondrial transcripts in heart and liver from LRPPRC+/- and LRPPRC+/T mice at 10–12 weeks of age (Fig. 3, A–D). The levels of mature mRNAs, rRNAs, and tRNAs.
were normal in both types of mutant mice (Fig. 3, A–D). The finding of normal levels of tRNAs indicates that LRPPRC does not stimulate transcription because we have shown previously that steady-state levels of tRNAs are good indicators of de novo transcription activity (12, 28–30). However, we found an RNA-processing defect with a clear change in steady-state levels of a fusion transcript containing the ND5 and Cytb mRNAs, with decreased levels of this precursor RNA species in Lrpprc/H11001/H11002 mice (Fig. 3, A and C) and increased levels in Lrpprc/H11001/T mice (Fig. 3, B and D). The finding of an RNA-processing defect is interesting, as knockdown of the fly homolog to LRPPRC, denoted BSF (bicoid stability factor), also leads to impaired mitochondrial RNA processing (31). Thus, a moderate increase or decrease in the expression of LRPPRC strongly affects the levels of the unprocessed ND5-Cytb transcript, whereas the levels of processed mRNAs, rRNAs, and tRNAs are unaffected.

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We isolated mitochondria from heart and liver of Lrpprc+/− and Lrpprc+/T mice and performed in organello transcription reactions. The production of most transcripts was unaffected by the moderately decreased or increased levels of LRPPRC in the Lrpprc−/− and Lrpprc−/T mice, respectively (Fig. 4A and supplemental Fig. S1A). However, we observed some changes in the abundance of high molecular weight transcripts (Fig. 4A,

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**FIGURE 3. Steady-state levels of mitochondrial transcripts.** A, Northern blot analysis of RNA isolated from heart and liver of control and heterozygous Lrpprc knock-out (+/−) mice at 10 weeks of age. A separate autoradiograph is shown for every analyzed transcript. Nucleus-encoded 18 S rRNA (18S) was used as a loading control. B, Northern blot analysis of RNA isolated from heart and liver of control and Lrpprc-overexpressing (+/T) mice at 10 weeks of age. A separate autoradiograph is shown for every analyzed transcript. The nucleus-encoded 18 S rRNA was used as a loading control. The reiterated small artifacts in the panels showing different tRNAs (tF, tA, and tQ) are due to reprobing of a membrane derived from a single gel containing the depicted artifacts. C, quantification of steady-state levels of the transcripts from control (+/+; n = 5) and heterozygous Lrpprc knock-out (+/−; n = 5) mice at 10 weeks of age. Error bars indicate S.E.M. *** p = 0.001 (Student’s t test). D, quantification of steady-state levels of mitochondrial mRNAs, tRNAs, and rRNAs from control (n = 6) and Lrpprc-overexpressing (+/T; n = 6) mice at 10 weeks of age. Error bars indicate S.E.M. *** p = 0.001 (Student’s t test).
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We therefore analyzed the effects of LRPPRC on transcription in vitro, light; H, heavy. Recombinant LRPPRC has been reported to stimulate in vitro transcription of mtDNA in a recombinant system (18). However, we have recently reported that in vitro transcription reactions are very sensitive to even small alterations in salt concentrations (24), which may give the false impression that an experimentally investigated transcription system is sensitive to salt concentration. To this end, we purified recombinant LRPPRC protein to near homogeneity using affinity and ion exchange chromatography (supplemental Fig. S1A), consistent with the previously observed role for LRPPRC in RNA processing (Fig. 3, A–D). There were no changes in the levels of the mitochondrial transcription factors TFAM (32) and TFB2M (20) in protein extracts from heart and liver of Lrpprc+/− and Lrpprc+/+ mice (Fig. 4B), consistent with the largely normal production of mitochondrial transcripts in in organello transcription assays (Fig. 4A).

Recombinant LRPPRC has been reported to stimulate in vitro transcription of mtDNA in a recombinant system (18). We decided to test this possibility further by using TFAM at a concentration of 200–600 nM for the in vitro transcription assays, which resulted in a TFAM ratio similar to that observed in vivo (19). The authors of a previous report showing that LRPPRC stimulates transcription (18) used TFAM concentrations much lower than those observed in vivo (19), which potentially could have affected the outcome of their experiments. In this previous study, His-tagged LRPPRC was purified from mitochondria from transfected cell lines (18), and contamination with other mitochondrial proteins can therefore not be excluded. Given the low TFAM concentrations, even small amounts of TFAM or other transcription factors contaminating purified recombinant LRPPRC may explain the strong stimulation of transcription they observed.

LRPPRC has been reported to directly interact with POLRMT and thereby modulate mitochondrial transcription (18). We decided to test this possibility further by using HeLa cells with doxycycline-inducible expression of human LRPPRC-FLAG. Immunoprecipitation followed by mass spectrometry revealed that LRPPRC-FLAG interacted with SLIRP, consistent with the previously observed role for LRPPRC in RNA processing (Fig. 5A). Forced expression is prone to generate protein-protein interaction artifacts, and we therefore decided to investigate whether LRPPRC interacts with other proteins under physiological in vivo conditions. To this end, we utilized BAC
transgenic mice expressing LRPPRC-FLAG (12). This BAC transgene fully rescues the germ-line Lrpprc knock-out (12), showing that the expressed FLAG-tagged LRPPRC protein is fully functional. Furthermore, the expression of LRPPRC-FLAG is at levels comparable to endogenous LRPPRC expression. We performed immunoprecipitation experiments in mitochondrial extracts from liver, kidney, and heart of LRPPRC-FLAG BAC transgenic mice, followed by mass spectrometry analysis, and again identified SLIRP as an interaction partner, but not POLRMT (supplemental Table S1).

As a further means to detect a possible interaction between LRPPRC and POLRMT, we performed size exclusion chromatography on mitochondrial extracts from HeLa cells (Fig. 5B). LRPPRC migrated at a higher apparent molecular weight than the one predicted for LRPPRC monomers (Fig. 5B). There was a clear co-migration between LRPPRC and SLIRP, thus confirming previous data that these two proteins form a complex (12). However, POLRMT did not co-migrate with LRPPRC, but it rather co-migrated at a high molecular weight with TFAM. This finding could indicate interaction with TFAM and other proteins localized to the mitochondrial nucleoids (34). Interestingly, a minor portion of SLIRP co-migrated at a high molecular weight with TFAM, supporting a model in which SLIRP binds newly transcribed mRNAs close to the nucleoid, prior to processing and translation. Taken together, these data suggest that LRPPRC and POLRMT do not interact to form a stable complex.

**DISCUSSION**

LRPPRC has been reported to have several functions in the nucleus (27), cytoplasm (35), and mitochondria (11, 12). However, there are several lines of evidence suggesting that LRPPRC has predominantly a mitochondrial role. First, LRPPRC belongs to a large family of PPR motif proteins that are widespread in mitochondria and chloroplasts, where they have important roles in RNA metabolism (1). Second, cell fraction-
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The endogenous levels of LRPPRC, POLRMT, TFAM, and TFB2M in mouse liver mitochondrial lysates were determined by Western blot analyses using purified standards of the corresponding mouse proteins. The asterisk indicates an unspecific cross-reacting band.

| ng/µg total mitochondrial protein | fmol/µg total mitochondrial protein |
|---------------------------------|-------------------------------------|
| LRPPRC                          | 1.1 ± 0.09                          | 7                                   |
| POLRMT                          | 0.02 ± 0.04                         | 0.15                                |
| TFAM                            | 0.4 ± 0.06                          | 14.3                                |
| TFB2M                           | 0.02 ± 0.02                         | 0.5                                 |

FIGURE 6. LRPPRC is an abundant protein. The endogenous levels of LRPPRC, POLRMT, TFAM, and TFB2M in mouse liver mitochondrial lysates were determined by Western blot analyses using purified standards of the corresponding mouse proteins. The asterisk indicates an unspecific cross-reacting band.

LRPPRC is found predominantly or even exclusively in mitochondria (17). Third, genetic studies of LRPPRC in a conditional knock-out mouse model show that LRPPRC has an essential intramitochondrial role in maintaining mtDNA gene expression (12). Fourth, BSF, the fly homolog of LRPPRC, was originally reported to be a cytoplasmic protein (36), but it is located predominantly in mitochondria, where it has a critical role in regulation of fly mtDNA gene expression (31). Several of the studies in which LRPPRC has been reported to have extramitochondrial roles were based on the finding of biochemical activities in cytoplasmic or nuclear extracts (10, 16, 37). However, it cannot be excluded that these extracts have been contaminated with proteins released from broken mitochondria, and studies of extramitochondrial functions of LRPPRC should therefore be repeated under more defined conditions by using nuclear or cytoplasmic extracts that are free from contaminating mitochondrial proteins.

There are different views on the intramitochondrial role of LRPPRC. We and others have presented strong genetic data showing that LRPPRC regulates mitochondrial mRNA stability, polyadenylation, and coordination of mitochondrial translation (11–13). However, there are also reports that LRPPRC is a transcriptional activator that forms a complex with POLRMT (18, 38). On the basis of the data we have presented in this work, we find it unlikely that LRPPRC is a mitochondrial transcriptional activator because (i) moderately decreased or increased protein levels of LRPPRC in vivo do not affect mitochondrial transcription, (ii) immunoprecipitation experiments do not show interaction between LRPPRC and POLRMT, (iii) size exclusion chromatography gives no support for complex formation between LRPPRC and POLRMT, and (iv) purified recombinant LRPPRC does not activate mitochondrial transcription when added to a purified recombinant in vitro transcription system.

The steady-state levels of the ND5-Cytb precursor RNA are strongly influenced by moderate alterations of the LRPPRC protein levels in vivo and are decreased in heterozygous Lrpprc knock-out mice and increased in LRPPRC-overexpressing mice. It was recently shown that LRPPRC binds to mitochondrial precursor mRNAs, such as ATP6-COX3, tRNA^{Met-ND2}, tRNA^{Lys-12S}, tRNA_{Val-16S}^{rRNA}, and tRNA^{Phe-12S} (13). It is thus possible that LRPPRC also has a function in maturation of precursor transcripts. Such a role is also suggested for BSF, the fly homolog of LRPPRC, as knockdown flies show processing aberrations in several mitochondrial transcripts (31). Taken together, our data suggest that LRPPRC has no role in mitochondrial transcriptional activation but rather functions as a regulator of mtDNA gene expression at the post-transcriptional level. We report here that the levels of a mitochondrial precursor transcript depend on LRPPRC levels, suggesting that LRPPRC may also have a role in RNA processing, in addition to the previously identified roles in regulation of mRNA stability, polyadenylation, and translational coordination in mammalian mitochondria.

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