Activation mechanism of mitochondrial translation by LRPPRC-SLIRP

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In mammalian mitochondria, mRNAs are co-transcriptionally stabilized by LRPPRC. Here we show that LRPPRC forms a stable complex with SLIRP and mitoribosome to act as a carrier of genetic information, thus linking the gene expression system. The structure of translating human mitoribosome in complex with LRPPRC-SLIRP and mRNA shows how the mRNA is delivered for translation along a corridor formed by LRPPRC with proteins of the mitoribosomal small subunit mS31-mS39.

The mechanism of translation starts with a key step of binding mRNA that is delivered upon transcription by RNA polymerase (RNAP). In bacteria, physical transcription-translation coupling has been characterised[1-3]. However, in mammalian mitochondria, nucleoids are not compartmented with protein synthesis, and mitoribosomes are independently tethered to the membrane[4,5]. Thus, nascent mRNAs are thought to be protected from degradation by a large 130-kDa protein factor LRPPRC (leucine-rich pentatricopeptide repeat-containing protein), a member of the metazoan-specific protein kinase G tetratricopeptide repeat family. LRPPRC acts as an mRNA chaperone that binds co-transcriptionally, prevents secondary structure formation, and stabilizes mRNA structure by stimulating the activity of mitochondrial poly(A)polymerase[6-9]. Mutations in LRPPRC lead to a paediatric neurodegenerative disorder caused by damage in mitochondrial energy conversion that has no treatment[10]. LRPPRC has also been implicated in coordination of mitochondrial translation[6,11,12]. It has been further reported to interact with a small 11-kDa protein cofactor SLIRP (SRA stem-loop-interacting RNA-binding protein) that plays roles in LRPPRC stability and maintaining steady-state mRNA levels[13]. *SLIRP* variants also cause a respiratory deficiency that leads to mitochondrial encephalomyopathy[14]. *SLIRP* knockdown results in increased turnover of LRPPRC[13-15], and *in vivo* co-stabilisation has been shown[13,16], suggesting that the two entities might have interdependent functions. However, there are no structures available for LRPPRC, SLIRP or any complexes containing them, and thus although mitoribosomal models have been determined[17,18], the molecular mechanisms of mRNA binding and association with interacting partners remained unknown.

To explore the molecular basis for translation activation in human mitochondria, we used low salt conditions to isolate a mitoribosome:LRPPRC-SLIRP-mRNA complex for cryo-EM. We merged particles containing tRNAs in A- and P-site as well as an extra density in the vicinity of the mRNA channel, and applied iterative masked focused refinement and classification with signal subtraction that resulted in a 2.9 Å resolution map of the mitoribosome:LRPPRC-SLIRP complex (3.4 Å local resolution) trapped during mRNA delivery to the mitoribosomal small subunit (SSU) (Extended Data Fig. 1). The reconstruction showed a clear density for the LRPPRC N-terminal domains bound to the mitoribosome, while the C-terminal domains were too flexible for modeling. It allowed us to model 34 α-helices, 9 of which form a superhelical ring-like architecture (head), and the rest form an extended tail that adopts a 90° curvature and outspreads 110 Å from the SSU body in parallel to the L7/L12 stalk (Fig. 1a,b). The LRPPRC head in our structure corresponds to the nuclear export signal (NES) domain, whereas the tail comprises Epsin N-terminal homology (ENTH) domain (Fig. 1c). Upon completing of the LRPPRC building, the remaining associated density was assigned as SLIRP that is close to the LRPPRC tail. When LRPPRC-SLIRP is bound to the mitoribosome, a fragmented density of mS31 extending from the core also becomes ordered, revealing its N-terminal region. It is arranged in two helix-turn-helix motifs, offering 1930 $Å^2$ surface area for direct interactions with LRPPRC. This singled out a density for mRNA, into which we modeled 42 residues, numbered from the E-site codon. Thus, in the complete mitoribosome:LRPPRC-SLIRP-mRNA model, LRPPRC performs three functions: coordination of SLIRP, which plays a key role in the process

of mRNA recruitment, association with the SSU, and handing-off the mRNA for translation (Fig. 1, Extended Data Fig. 2a-c, Supplementary Video 1).

The binding of SLIRP in our model is enabled via LRPPRC helices α20 and 22, which is consistent with crosslinking mass-spectrometry data[19]. The structure reveals that SLIRP links the head with the curved region of the LRPPRC tail that induces its specific shape (Fig. 1b). This binding of SLIRP coincides with the start of a corridor formed by mS31 and mS39 (Fig. 1b, Supplementary Video 1). Moreover, the SLIRP density is continuous with that of mRNA, which extends through the tunnel, and further over more than 180 Å through the SSU mRNA channel all the way to the decoding center. Thus, SLIRP contributes to LRPPRC specific scaffold, and also accounts for a role in binding the mRNA. Our findings that SLIRP is involved in coupling to the mitoribosome provide a mechanistic explanation for results from biochemical studies showing that SLIRP affects LRPPRC properties *in vitro*[16,19], and the presentation of mRNA to the mitoribosome *in vivo*[13].

The association of LRPPRC with the mitoribosome involves the helices α1, 2, 4, 7-14 that form a mitoribosome-binding head (Fig. 1b,c). The binding is mediated by four distinct contacting regions (Fig 1c and Extended Data Fig. 5c): 1) α1-2 (residues 64-95) is flanked by a region of mS39, a PPR domain-containing protein, that consists of four bundled helices (α11-14); 2) α7 and 9 form a shared bundle with two N-terminal helices of mS31 (residues 175-208, stabilized by C-terminal region of mS39) that encircle the head to complement the PPR domain; 3) α10-12 are capped by a pronounced turn of mS31 (residues 209-232) acting as a lid that marks the LRPPRC boundary, and it is sandwiched by the mS39 helix α19 and C-terminus from the opposite side; 4) in addition, α11 is also positioned directly against the helix α23 of mS39. Thus, LRPPRC docks onto the surface of the mitoribosome via mS31-mS39, which are tightly associated with each other, and each provides two contact patches to contribute to stable binding.

The handing-off the mRNA for translation is mediated by four of the LRPPRC helical repeats: α1, 2, 16 and 18 (Fig. 1b,c, Supplementary Video 1). The mRNA nucleotides 33-35 are stabilized in a cleft formed by α1-2 from one side of the head and α16, 18 from the other. Nucleotides 31 and 32 are recognized by several arginine residues from the respective helices. The involvement of the NES-rich domain of LRPPRC in mRNA binding in our structure is consistent with a biochemical analysis of a recombinant protein where the N-terminal PPR segments were systematically removed, which showed a reduced formation of protein-RNA complexes[20]. The rest of the mRNA is situated too far to interact with LRPPRC. Here, the mRNA is handed to mS31-mS39, and the three proteins together form a 60-Å long corridor that channels the mRNA from SLIRP towards the mitoribosomal core (Fig. 1b, Supplementary Video 1). Nucleotides 26-30 run along with mS39 PPR domain 5, and nucleotide 26 connects to contact-2, where R219 of mS31 extends towards the base. Thus, the mRNA handing-off is achieved through a functional cooperation between LRPPRC-SLIRP and mS31-mS39.

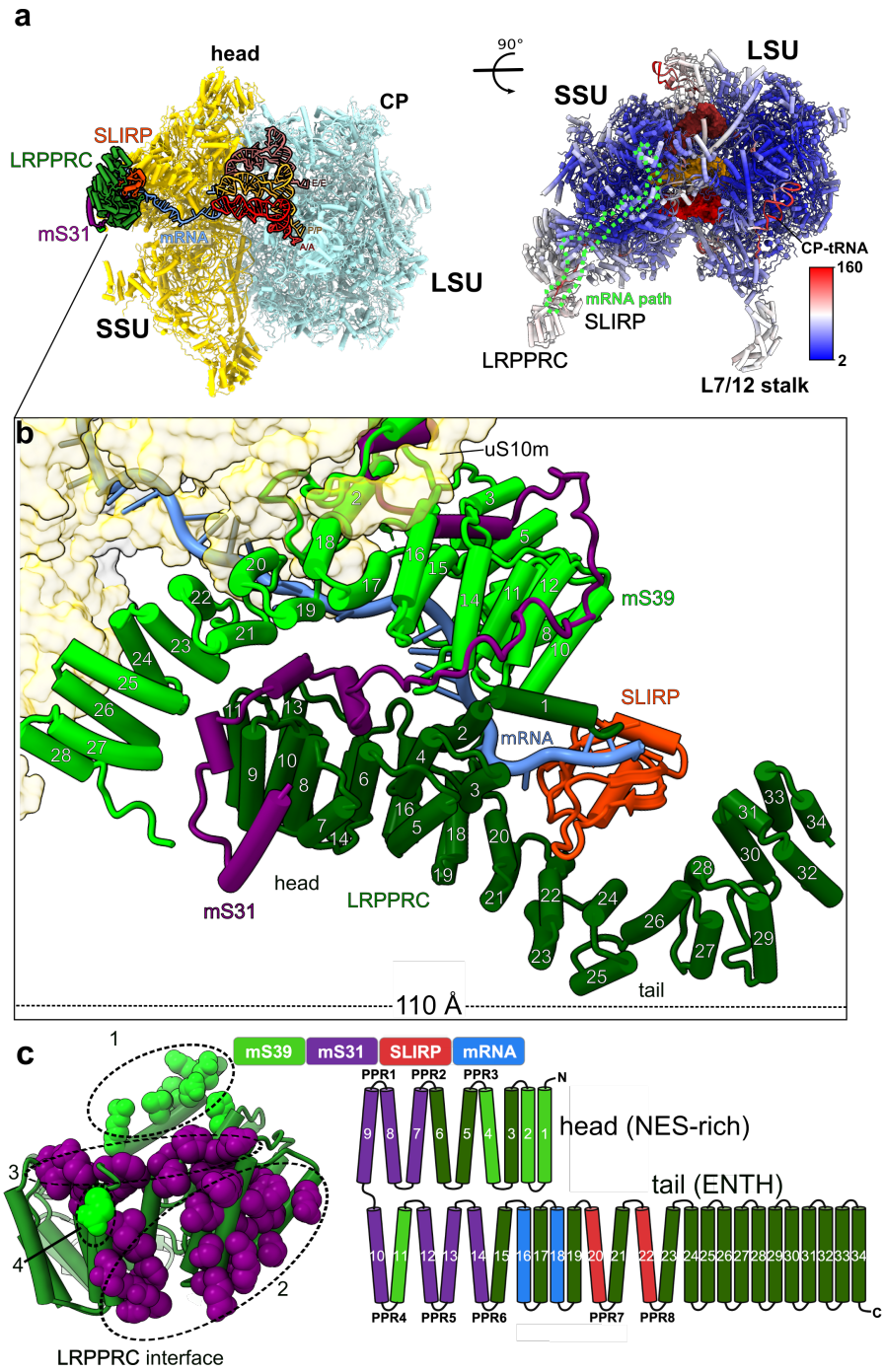

Fig. 1. Structure of mitoribosome with LRPPRC-SLIRP bound to mRNA.
a, Overview of the mitoribosome:LRPPRC-SLIRP model. Right panel, top view of the model colored by atomic *B*-factor (Å^2), tRNAs in surface, mRNA path (light green) is shown. **b,** A close-up view of the mitoribosome:LRPPRC-SLIRP-mRNA interactions. LRPPRC associates with mS31-39 via a superhelical ring-like structure (head) that together form a corridor for handing-off the mRNA from SLIRP. **c,** Contact sites between LRPPRC and mS31-mS39 (within 4 Å distance), view from the interface. Right panel, schematic diagram showing the topology of LRPPRC consisting of 34 helices arranged in head and tail domains. Colours represent engagement in interactions with mS39, mS31, SLIRP, mRNA.

To place the structural data into an evolutionary context, we performed comparative phylogenetic analysis of the proteins involved in the mRNA handing-off process. Since the mitochondrial rRNA has been generally reduced in metazoa[21], we examined whether the loss might coincide with the origin of LRPPRC and its interactors. The orthology database EGGNOGG[22] and previous analysis[23] indicated that LRPPRC and mS31 are only present in Bilateria, while mS39 in metazoa. We then confirmed the results with more sensitive detection[24] followed by manual sequence analysis examining domain composition, which put the origin of LRPPRC and mS31 at the root of the metazoan. Thus, the appearance of the proteins therewith coincides with the loss of parts of the rRNA (Fig. 2a). The data for SLIRP is less conclusive, probably due to its small size.

The correlation between rRNA reduction and protein aquisition is important, because the rRNA regions H16 (410-432) and H33-37 (997-1118) that bridge the mRNA to the channel entrance in bacteria[1] are either absent or reduced in the mitoribosome. However, a superposition of the mitoribosome:LRPPRC-SLIRP-mRNA complex with *E. coli* expressome[1] shows not only that the nascent mRNA follows a comparable path in both systems, but also the mRNA delivering complexes bind in a similar location with respect to their ribosomes (Fig. 2b). To test whether homolog protein interactions can explain the conservation, we compared the interfaces. Indeed, in the NusG-coupled expressome, NusG binds to uS10 and restrains RNAP motions[1], and in our structure uS10m has a related interface between helix α2 with mS31-mS39, which induces association of these two proteins (Fig. 2c, Extended Data Fig. 3). Yet, most of the interactions rely on mito-specific N-terminal extension of uS10m engaging in a shared sheet with mS39 via strand β1, and helices α1,16 and 18 are further involved in the binding (Extended Data Fig. 3). Together, this suggests that specific protein-based mechanisms must have evolved in the evolution of the mammalian mitoribosome for mRNA recognition and protection.

Overall, these findings redefine the mitoribosome-LRPPRC-SLIRP as the central regulator of mitochondrial gene expression and explain how the newly identified components modulate the function of translation via mRNA binding. This represents a step that follows a progression of the SSU through the assembly pathway[25] and preinitiation[26] that have been previously determined. Since the presence of large RNA-binding moieties was also reported in association with mitoribosomes in other species[27-31], the regulation principle of transcription-translation with mitochondrial molecular connects might be a general feature.

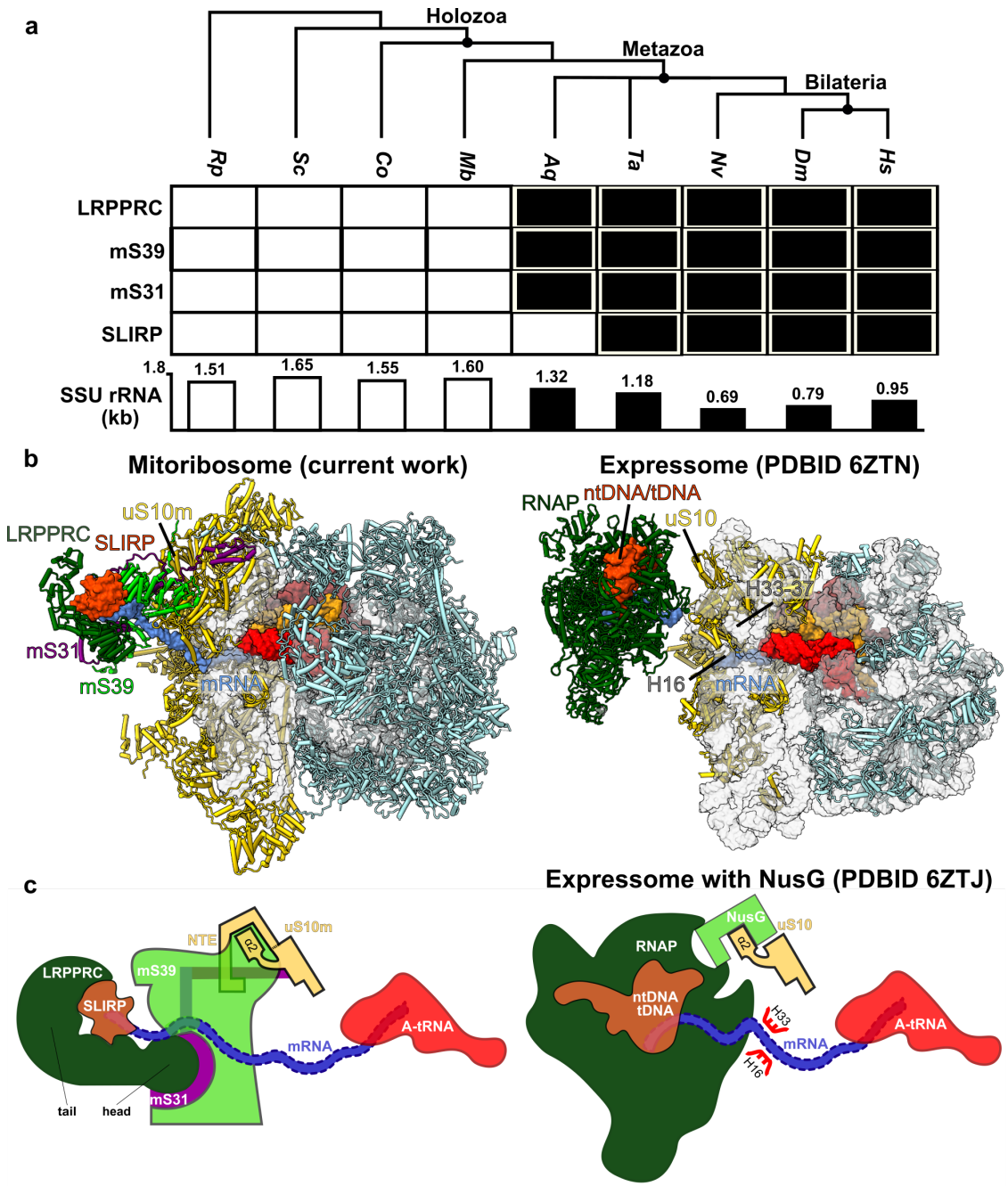

Fig 2. Formation of mitoribosome:LRPPRC-SLIRP and 70S-RNAP complexes.
a, Phylogenetic analysis shows correlation between acquisition of LRPPRC, SLIRP, mS31, mS39 and reduction of rRNA in metazoans. Black rectangles indicate the presence of proteins. *Hs*: *Homo sapiens, Ds: Drosophila melanogaster, Nv*: *Nematostella vectensis, Ta: Trichoplax adhaerens, Aq*: *Amphimedon queenslandica* (sponge - diverged from other metazoans ~600 mya), *Mb*: *Monosiga brevicollis* (unicellular choanoflagellate), *Co*: *Capsaspora owczarzaki* (protist), *Sc*: *Saccharomyces cerevisiae* (fungi), *Rp*: *Rickettsia prowazekii* (alpha-proteobacterium). **b,** Model of the mitoribosome:LRPPRC-SLIRP complex compared with uncoupled model of the expressome from *E.coli*[3]. **c,** Schematic representation indicating a more stable association of mRNA-delivering proteins in the mitoribosome compared to NusG-coupled expressome[1].

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Methods

Experimental model and culturing

HEK293S-derived cells (T501) were grown in Freestyle 293 Expression Medium containing 5% tetracycline-free fetal bovine serum (FBS) in vented shaking flasks at 37°C, 5% CO_2 and 120 rpm (550 x g). Culture was scaled up sequentially, by inoculating at 1.5 x 10^6 cells/mL and subsequently splitting at a cell density of 3.0 x 10^6 cells/mL. Finally, a final volume of 2 L of cell

culture at a cell density of 4.5 x 10^6 cells/mL was used for mitochondria isolation, as previously described[32].

Mitoribosome purification

HEK293S-derived cells were harvested from the 2 L culture when the cell density was 4.2 x 10^6 cells/mL by centrifugation at 1,000 g for 7 min, 4 °C. The pellet was washed and resuspended in 200 mL Phosphate Buffered Saline (PBS). The washed cells were pelleted at 1,000 g for 10 min at 4 °C. The resulting pellet was resuspended in 120 mL of MIB buffer (50 mM HEPES-KOH, pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, cOmplete EDTA-free protease inhibitor cocktail (Roche), and allowed to swell in the buffer for 15 min in the cold room by gentle stirring. About 45 mL of SM4 buffer (840 mM mannitol, 280 mM sucrose, 50 mM HEPES-KOH, pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1X cOmplete EDTA-free protease inhibitor cocktail (Roche) was added to the cells in being stirred in MIB buffer and poured into a nitrogen cavitation device kept on ice. The cells were subjected to a pressure of 500 psi for 20 min before releasing the nitrogen from the chamber and collecting the lysate. The lysate was clarified by centrifugation at 800 x g and 4 °C, for 15 min, to separate the cell debris and nuclei. The supernatant was passed through a cheesecloth into a beaker kept on ice. The pellet was resuspended in half the previous volume of MIBSM buffer (3 volumes MIB buffer + 1 volume SM4 buffer) and homogenized with a Teflon/glass Dounce homogenizer. After clarification as described before, the resulting lysate was pooled with the previous batch of the lysate and subjected to centrifugation at 1,000 x g, 4 °C for 15 minutes to ensure complete removal of cell debris. The clarified and filtered supernatant was centrifuged at 10,000 x g and 4 °C for 15 min to pellet crude mitochondria. Crude mitochondria were resuspended in 10 mL MIBSM buffer and treated with 200 units of Rnase-free Dnase (Sigma-Aldrich) for 20 min in the cold room to remove contaminating genomic DNA. Crude mitochondria were again recovered by centrifugation at 10,000 g, 4 °C for 15 min and gently resuspended in 2 mL SEM buffer (250 mM sucrose, 20 mM HEPES-KOH, pH 7.5, 1 mM EDTA). Resuspended mitochondria were subjected to a sucrose density step-gradient (1.5 mL of 60% sucrose; 4 mL of 32% sucrose; 1.5 mL of 23% sucrose and 1.5 mL of 15% sucrose in 20 mM HEPES-KOH, pH 7.5, 1 mM EDTA) centrifugation in a Beckmann Coulter SW40 rotor at 28,000 rpm (139,000 x g) for 60 min. Mitochondria seen as a brown band at the interface of 32% and 60% sucrose layers were collected and snap-frozen using liquid nitrogen and transferred to −80 °C.

Frozen mitochondria were transferred on ice and allowed to thaw slowly. Lysis buffer (25 mM HEPES-KOH, pH 7.5, 50 mM KCl, 10 mM $Mg(Oac)_2$, 2% polyethylene glycol octylphenyl ether, 2 mM DTT, 1 mg/mL EDTA-free protease inhibitors (Sigma-Aldrich) was added to mitochondria and the tube was inverted several times to ensure mixing. A small Teflon/glass Dounce homogenizer was used to homogenize mitochondria for efficient lysis. After incubation on ice for 5-10 min, the lysate was clarified by centrifugation at 30,000 x g for 20 min, 4 °C. The

clarified lysate was carefully collected. Centrifugation was repeated to ensure complete clarification. A volume of 1 mL of the mitochondrial lysate was applied on top of 0.4 mL of 1 M sucrose (v/v ratio of 2.5:1) in thick-walled TLS55 tubes. Centrifugation was carried out at 231,500 x g for 45 min in a TLA120.2 rotor at 4 °C. The pellets thus obtained were washed and sequentially resuspended in a total volume of 100 µl resuspension buffer (20 mM HEPES-KOH, pH 7.5, 50 mM KCl, 10 mM $Mg(Oac)_2$, 1% Triton X-100, 2 mM DTT). The sample was clarified twice by centrifugation at 18,000 g for 10 min at 4 °C. The sample was applied on to a linear 15-30% sucrose (20 mM HEPES-KOH, pH 7.5, 50 mM KCl, 10 mM $Mg(Oac)_2$, 0.05% n-dodecyl-β-D-maltopyranoside, 2 mM DTT) gradient and centrifuged in a TLS55 rotor at 213,600 x g for 120 min at 4 °C. The gradient was fractionated into 50 µL volume aliquots. The absorption for each aliquot at 260 nm was measured and fractions corresponding to the monosome peak were collected. The pooled fractions were subjected to buffer exchange with the resuspension buffer.

Cryo-EM data acquisition

3 µL of ~120 nM mitoribosome was applied onto a glow-discharged (20 mA for 30 sec) holey-carbon grid (Quantifoil R2/2, copper, mesh 300) coated with continuous carbon (of ~3 nm thickness) and incubated for 30 sec in a controlled environment of 100% humidity and 4 ºC. The grids were blotted for 3 sec, followed by plunge-freezing in liquid ethane, using a Vitrobot MKIV (FEI/Thermofischer). The data were collected on FEI Titan Krios (FEI/Thermofischer) transmission electron microscope operated at 300 keV, using C2 aperture of 70 µm and a slit width of 20 eV on a GIF quantum energy filter (Gatan). A K2 Summit detector (Gatan) was used at a pixel size of 0.83 Å (magnification of 165,000X) with a dose of 29-32 electrons/$Å^2$ fractionated over 20 frames.

Cryo-EM data processing

The beam-induced motion correction and per-frame B-factor weighting were performed using RELION-3.0.2[33,34]. Motion-corrected micrographs were used for contrast transfer function (CTF) estimation with gctf[35]. Unusable micrographs were removed by manual inspection of the micrographs and their respective calculated CTF parameters. Particles were picked in RELION-3.0.2 , using reference-free followed by reference-aided particle picking procedures. Reference-free 2D classification was carried out to sort useful particles from falsely picked objects, which were then subjected to 3D classification. 3D classes corresponding to unaligned particles and LSU were discarded and monosome particles were pooled and used for 3D auto-refinement yielding a map with an overall resolution of 2.9-3.4 Å for the five datasets. Resolution was estimated using a Fourier Shell Correlation cut-off of 0.143 between the two reconstructed half maps. Finally, the selected particles were subjected to per-particle defocus estimation, beam-tilt correction and per-particle astigmatism correction followed by Bayesian polishing. Bayesian polished particles were subjected to a second round per-particle defocus correction. A total of

994,919 particles were pooled and separated into 86 optics groups in RELION-3.1[36], based on acquisition areas and date of data collection. Beam-tilt, magnification anisotropy and higher-order (trefoil and fourth-order) aberrations were corrected in RELION-3.1[36]. Particles with A-site and/or P-site occupied with tRNAs which showed comparatively higher occupancy for the unmodeled density potentially corresponding to LRPPRC-SLIRP module were pooled and re-extracted in a larger box size of 640 Å. The re-extracted particles were subjected to 3D autorefinement in RELION3.1[36]. This was followed by sequential signal subtraction to remove signal from the LSU, and all of the SSU except the region around mS39 and the unmodeled density, in that order. This was followed by masked 3D classification (T=200) to enrich for particles carrying the unmodeled density. Using a binary mask covering mS39 and all of the unmodeled density, we performed local-masked refinement on resulting 41,812 particles within an extracted sub-volume of 240 Å box size leading to 3.37 Å resolution map.

Model building and refinement

At the mRNA channel entrance, a more accurate and complete model of mS39 could be built with 29 residues added to the structure. Improved local resolution enabled unambiguous assignment of residues to the density which allowed us to address errors in the previous model. A total of 28 α-helices could be modeled in their correct register and orientation. Further, a 28-residue long N-terminal loop of mS31 (residues 247–275) along mS39 and mito-specific N-terminal extension of uS9m (residues 53–70) approaching mRNA were modeled by fitting the loops in to the density maps.

For building LRPPRC-SLIRP module, the initial model of the full length LRPPRC was obtained from *AlphaFold2*[37] Protein Structure Database (Uniprot ID P42704). Based on the analysis, three stable domains were identified that are connected by flexible linkers (673-983, 1035-1390). We then systematically assessed the domains against the map, and the N-terminal region (77-660) could be fitted into the density. The initial model was real space refined into the 3.37 Å resolution map of mS39-LRPPRC-SLIRP region obtained after partial signal subtraction using reference-restraints in *Coot* v0.9[38]. The N-terminal region covering residues 64-76 was identified in the density map and allowed us to model 34 helices of LRPPRC (residues 64-644). Helices α1-29 could be confidently modeled. An additional five helices, as predicted by *Alphafold2*[37], could be accommodated into the remaining density. After modelling LRPPRC into the map, there was an unaccounted density that fits SLIRP. The initial model of SLIRP was obtained from *Alphafold2*[37] Protein Structure Database (Uniprot ID Q9GZT3). The unmodeled density agreed with the secondary structure of SLIRP. The model was real space refined into the density using reference-restraints as was done for LRPPRC in *Coot* v0.9[38]. Five additional RNA residues could be added to the 3' terminal of mRNA to account for tubular density extending from it along the mRNA binding platform. The A/A P/P E/E state model was rigid body fitted into the corresponding 2.85 Å resolution consensus map. Modeled LRPPRC was merged with the rigid body fitted monosome model to obtain a single model of the mitoribosome bound to LRPPRC

and SLIRP. The model was then refined against the composite map using PHENIX v1.18[39] (Supplementary Information Table 1).

Data availability statement

The atomic coordinates were deposited in the RCSB Protein Data Bank, and EM maps have been deposited in the Electron Microscopy Data bank under accession numbers: XXXX and EMD-XXXXX.

The atomic coordinates that were used in this study: 6ZTJ (*E.coli* 70S-RNAP expressome complex in NusG); 6ZTN (*E.coli* 70S-RNAP expressome complex in NusG).

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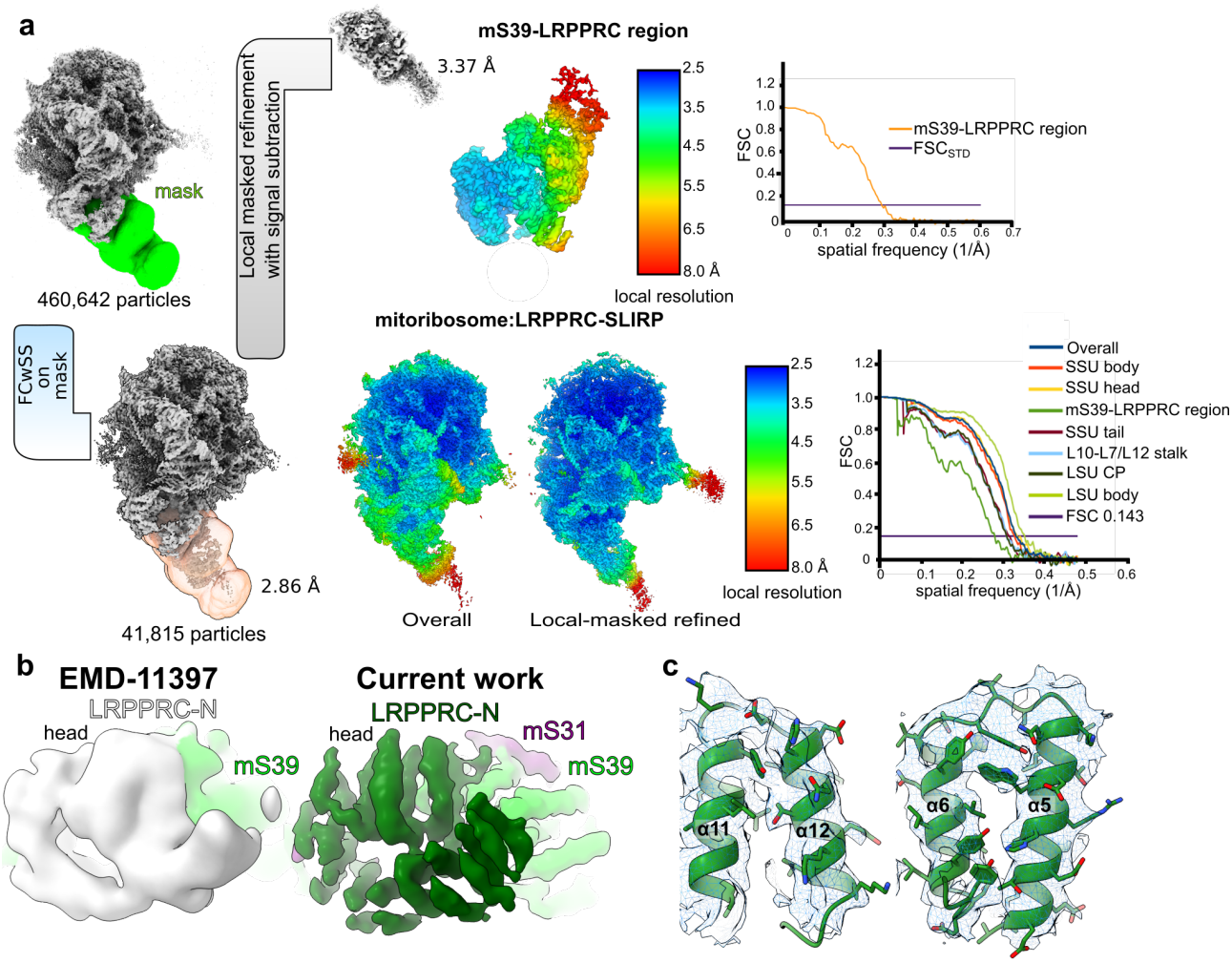
Author contributions

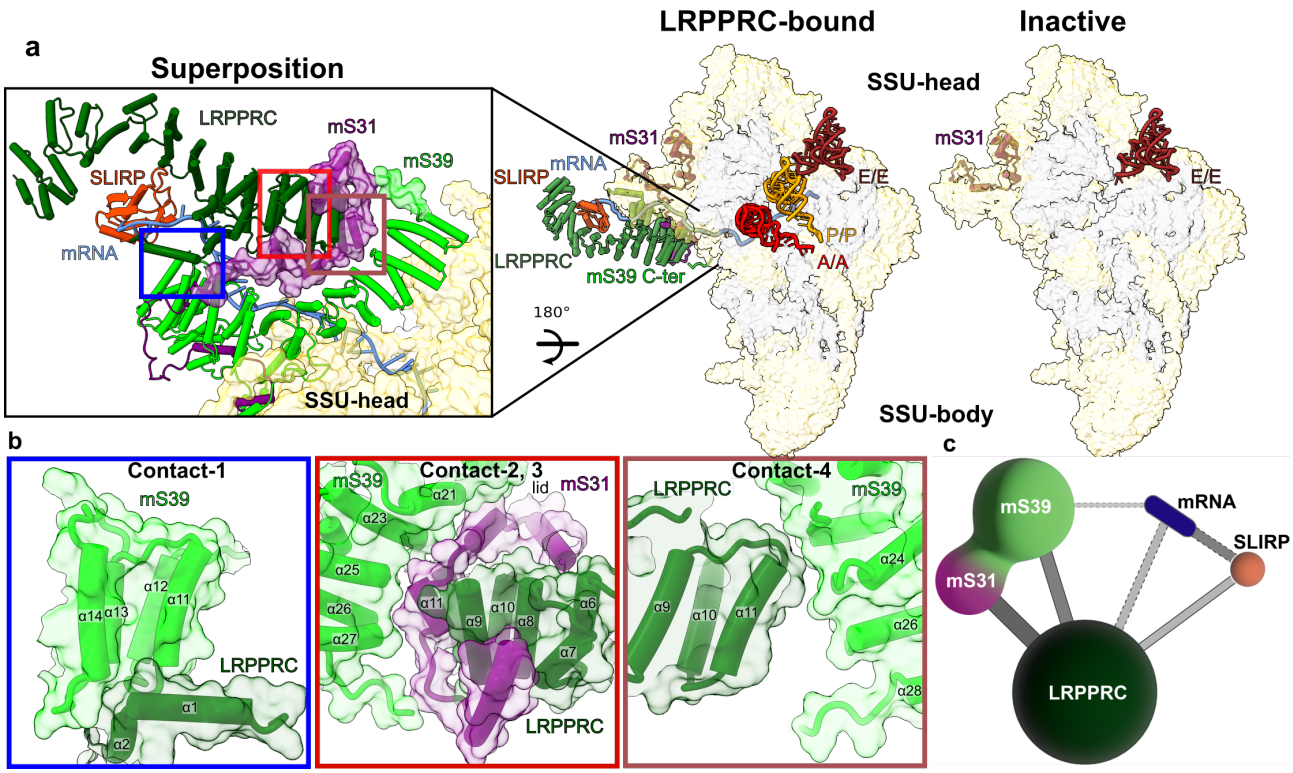
V.S. and Y.I. collected cryo-EM data, processed the data, and built the models. V.S., Y.I. and A.A. carried out structural analysis. V.S., M.H. and A.A. performed evolutionary analysis. A.A. wrote the manuscript. All authors contributed to data interpretation and manuscript writing.

Competing Interests Statement

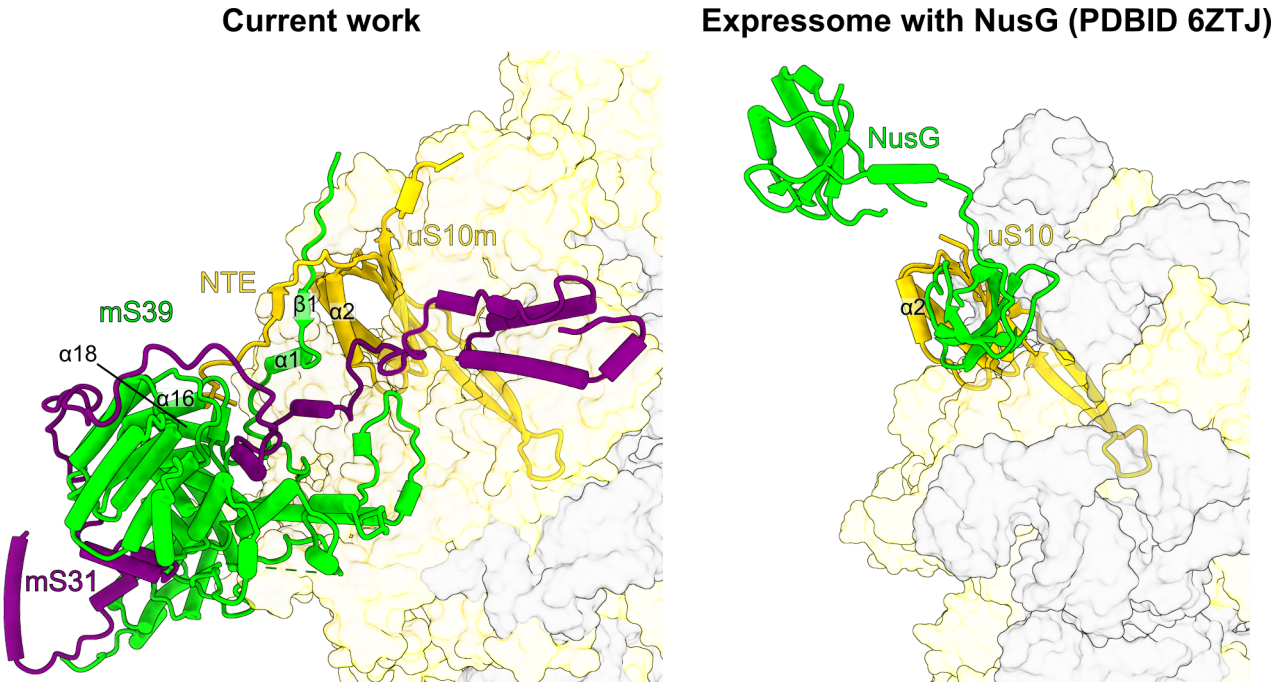
The authors declare no competing interests.



Extended Data fig. 1. Cryo-EM data processing and map for LRPPRC-SLIRP-mS39 region. **a.** Focused classification with signal subtraction around mS39-LRPPRC-SLIRP region on A/A- or P/P-RNA containing particles to improve LRPPRC-SLIRP occupancy, followed by, focused masked refinement on mS39-LRPPRC-SLIRP region with signal subtraction to improve local resolution (left). Fourier shell correlation curves for the overall and masked maps (right). Binary masks used for local-masked refinements. Local-masked refinements are shown and colored by local resolution (middle). **b.** Map comparison for LRPPRC region between our work and EMD-11397. Map has been Gaussian filtered for better visibility. **c.** Density map around helices α11-12 and 5-6.



Extended Data fig. 2. LRPPRC-SLIRP contacts with SSU head and high resolution features identified in the structure. a. Comparison of SSU from mitoriboome:LRPPRC-SLIRP complex with SSU from E-site tRNA bound monosome. Zoom-in shows N-terminal region of mS31 and C-terminal loop of mS39 (in surface) stabilized by LRPPRC. **b.** Contact regions of LRPPRC with mS31 and mS39. **c.** Schematic depicting interface of LRPPRC with SLIRP, mS31, mS39 and mRNA. Size of circles corresponds to molecular weight of the protein.



Extended Data fig. 3. Close-up view of uS10m interactions with mS31-mS39.
Interface between uS10m with mS31-mS39 that serve as the platform for LRPPRC-SLIRP is similar to that formed between uS10 and NusG that binds RNA polymerase in bacterial expressome[1].

Activation mechanism of mitochondrial translation by LRPPRC-SLIRP

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SUPPLEMENTARY INFORMATION

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Supplementary Table 1. Data collection and model statistics

SI Video 1. Structure of LRPPRC-SLIRP module bound to monosome

Supplementary table 1. Data collection and model statistics.

	Monosome with LRPPRC:SLIRP PDB: xxxx EMD-xxxxx
Data collection and processing	
Electron microscope	Titan Krios
Camera	K2 Summit (counting mode)
Magnification	165,000
Voltage (kV)	300
Electron exposure ($e^-/Å^2$)	29-32
No. of frames	20
Defocus range (μm)	-0.6 to -2.8
Pixel size (Å)	0.83
Symmetry imposed	C_1
Final particle number (no.)	41,815
Map resolution (Å) (Overall/ LSU-body/ CP/ L10-L12-stalk/ L1-stalk/ SSU-body/ SSU-head/ mS39/ SSU-tail/ mS39-LRPPRC-SLIRP)	2.85/2.69/ 3.07/ 3.07/ −/ 2.89/ 2.84/−/ 3.02/3.37
FSC threshold	0.143
Map resolution range (Å)	2.5-8.0
Refinement	
Initial model used (PDB code)	6ZSG, 6RW4
Model resolution (Å)	2.7
Model to map CC (CC_{volume})	0.84
FSC threshold	0.5
Map-sharpening *B* factor ($Å^2$) (Overall/ LSU-body/ CP/ L10-L12-stalk/ L1-stalk/ SSU-body/ SSU-head/ mS39/	-33/ -28/ -58/ -49/−/ -39/ -39/−/-55/ -60
Model composition	
Non-hydrogen atoms	356138
Hydrogen atoms	160529
Protein chains	90
RNA chains	7
Protein residues (non-modified/ *N*-acetylAla/ *N*-acetylSer/ *N*-acetylThr O^1-methylisoAsp)	15631/3/1/1/1
RNA residues (non-modified/ mG/ mU/ m^1A/ m^2G/ψ /m^4C/ m^5C/ m^5U/ $m^6{}_2A$)	2822/2/ 1/ 2/ 1/ 2/ 1/ 1/ 1/ 2
Ligands (ATP/ GDP/ NAD/ 2Fe-2S/ spermine/ spermidine/ putrescine)	1/ 1/ 1/ 3 /1 /4/ 1
Ions (Zn^{2+}/ K^+/ Mg^{2+})	3/ 58/ 207
Waters	7,702
Mean atomic *B*-factor ($Å^2$)	
Protein	38.74
RNA	36.58
Ligand	20
Water	16.55
Validation	
Ramanchandran plot (%)	
Outliers	0.04
Allowed	1.84
Favored	98.12
Clash score	2.61
RMSD	
Bonds (Å)	0.002
Angles (°)	0.429
Rotamer outliers (%)	0.00
C_β outliers (%)	0.00
CaBLAM outliers (%)	0.89

Supplementary Video 1. Structure of LRPPRC-SLIRP module bound to monosome.

The video shows the structure of LRPPRC-SLIRP determined in this work and how docking of mRNA on SSU is achieved by LRPPRC-SLIRP together with mito-specific proteins mS31 and mS39.