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Evolutionary compaction and adaptation visualized by the structure of the dormant microsporidian ribosome

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Abstract

Microsporidia are eukaryotic parasites that infect essentially all animal species, including many of agricultural importance^{1–3}, and are significant opportunistic parasites of humans⁴. They are characterized by having a specialized infection apparatus, an obligate intracellular lifestyle⁵, rudimentary mitochondria and the smallest known eukaryotic genomes^{5–7}. Extreme genome compaction led to minimal gene sizes affecting even conserved ancient complexes such as the ribosome^{8–10}. In the present study, the cryo-electron microscopy structure of the ribosome from the microsporidium *Vairimorpha necatrix* is presented, which illustrates how genome compaction has resulted in the smallest known eukaryotic cytoplasmic ribosome. Selection pressure led to the loss of two ribosomal proteins and removal of essentially all eukaryote-specific ribosomal RNA (rRNA) expansion segments, reducing the rRNA to a functionally conserved core. The structure highlights how one microsporidia-specific and several repurposed existing ribosomal proteins compensate for the extensive rRNA reduction. The microsporidian ribosome is kept in an inactive state by two previously uncharacterized dormancy factors that specifically target the functionally important E-site, P-site and polypeptide exit tunnel. The present study illustrates the distinct effects of evolutionary pressure on RNA and protein-coding genes, provides a mechanism for ribosome inhibition and can serve as a structural basis for the development of inhibitors against microsporidian parasites.

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Author contributions

J.B., C.R.V. and M.H. conceived the study. C.R.V. cultivated microsporidia and purified ribosomes. J.B. and M.H. performed all EM work, determined the cryo-EM structures and built the model. S.K., J.B., C.R.V. and M.H. interpreted the results and wrote and edited the manuscript.

Competing interests

The authors declare no competing interests.

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Microsporidian ribosomes were purified from spores of *Vairimorpha necatrix* grown in the corn earworm, *Helicoverpa zea*, using previously described procedures¹¹, and analysed by negative stain and cryo-electron microscopy (cryo-EM) (Fig. 1a,b and see Supplementary Fig. 1). Extensive three-dimensional classification resulted in two reconstructions of the ribosome in different conformational states (see Supplementary Fig. 1d). Using a combination of high-resolution density maps and mass spectrometry analysis (see Supplementary Table 1), a microsporidia-specific ribosomal protein and two microsporidian dormancy factors (MDF1 and MDF2), which define the two states, were identified. State 1 contains both factors and serves as the basis for the subsequent structural description whereas state 2 contains only MDF2 (see Supplementary Fig. 1d). By using multibody refinement, high-resolution maps were obtained for the large ribosomal subunit (LSU, 3.26Å), small ribosomal subunit (SSU) body (3.3Å) and SSU head (3.64 Å), which allowed for atomic model building in state 1 (see Supplementary Fig. 2 and Supplementary Table 2). The dynamic L1 and L7/L12 rRNA stalk and associated proteins are disordered and not included in the final model.

Early analysis of the *Vairimorpha necatrix* SSU rRNA sequence suggested that microsporidia are ancient eukaryotic organisms⁹. Phylogenetic analyses have now shown that they are related to fungi (see Supplementary Fig. 3a), as either a basal branch or a sister group^{12,13}, with their highly divergent features attributed to their extremely compacted genomes^{5–7}. The reduction of the microsporidian genome has affected the RNA and protein components of the ribosome differently. The rRNA moiety is the smallest found in any eukaryotic cytoplasmic ribosome to date, with total rRNA lengths even shorter than those in many prokaryotes¹⁴ (Fig. 1c). Essentially all eukaryotic expansion segments (ESs) have been compacted or removed from the *V. necatrix* rRNA (Fig. 2a,b and see Supplementary Fig. 3). Both the SSU and LSU rRNAs are approximately 30% shorter than the corresponding yeast rRNAs and 15% (LSU) to 20% (SSU) shorter than the *Escherichia coli* rRNAs, effectively reducing the rRNA to a minimal functional core for cytoplasmic ribosomes (Fig. 1c). Even the inter-domain base-pairing region between the co-localized expansion segments 3 and 6 in the SSU rRNA (16S) has been removed (Fig. 2a,c). Although the 5.8S rRNA and 25S rRNA of the LSU in *Saccharomyces cerevisiae* are separate molecules, their microsporidian counterparts are, through the loss of the internal transcribed spacer 2 (ITS2), covalently linked to form a single 23S rRNA^{10,14}. The selective loss of ITS2 and its associated assembly factors, which feature heavily in eukaryotic large subunit assembly^{15,16}, highlights an evolutionary convergence with bacterial 23S rRNA.

Despite the extreme rRNA reduction, the microsporidian ribosome contains all the ribosomal proteins found in fungi except for eL38 and eL41 (see Supplementary Table 1). The rRNA-binding site of eL38 has been lost due to removal of ES24 (Fig. 2d,e and Supplementary Fig. 3a) and, despite an intact rRNA-binding site, no density was observed for eL41. The ribosomal protein eL38 is present in *Rozella allomyces* and *Mitosporidium daphniae*. *R. allomyces* is an organism that has been placed close to the microsporidia within the basal branch of the fungi^{12,13}, while *Mitosporidium daphniae* represents an early diverging microsporidian species that has not undergone genome compaction¹². The presence of eL38 in earlier branching organisms (see Supplementary Fig. 3) and its absence in all but two sequenced microsporidian species (*M. daphniae*, *Amphiblysis* sp.) suggests

that this protein was present in a common ancestor. The presence of most of the eukaryotic ribosomal proteins in *V. necatrix* is contrary to previous *in silico* predictions and mass spectrometry analyses, which have suggested an absence and repurposing of several additional ribosomal proteins in microsporidia¹⁷. Unlike the recently described mitochondrial trypanosomal ribosome¹⁸, in which rRNA reduction has led to an increase in the size and number of ribosomal proteins, loss of rRNA in microsporidia has resulted in a different evolutionary strategy. Genome compaction has led to the truncation of tails and loops in several ribosomal proteins and rRNA loss has been compensated for by structural changes in ribosomal proteins to create specific protein-protein interactions or to serve as placeholders for removed expansion segments (see Supplementary Fig. 4). The retained ribosomal proteins still occupy the same positions as in yeast even in the absence of their rRNA-binding sites (see Supplementary Fig. 5). In the most extreme examples, which include eL14 and eL6 in the LSU, ribosomal proteins are exclusively bound through protein-protein interactions (see Supplementary Fig. 5d). Similarly, eS7 has only minor contacts with the 16S rRNA and is held in place by uS8, uS15 and eS27 (see Supplementary Fig. 5c). A previously uncharacterized conserved microsporidia-specific ribosomal protein (msL1), together with repurposed existing ribosomal protein tails, compensates for the extensive rRNA loss (Fig. 2d,e and Supplementary Fig. 4b). The fusion of two LSU rRNAs into a single molecule and the removal of the co-localized LSU expansion segments 5.8S-ES3, 5.8S-ES4, ES 19 and ES31 has created a binding site for msL1 (Fig. 2d). The key function of this ribosomal protein is to bridge uL23 (ScL25, HsL23A), eL37 and uL2 (ScL2, HsL8), and as a consequence to functionally replace the lost rRNA segments. Expansion segment removal in this region has also affected the structure and size of the ribosomal proteins. The N-terminal extensions of uL23 and eLS, which in *S. cerevisiae* weave through expansion segments, have been lost. The remaining N-terminal end of eL8 has been repurposed and, instead of binding to ES31 and contacting eL27, it now contacts the LSU rRNA fusion site and further serves as a placeholder for the removed helix 52 at the base of ESI 9 (Fig. 2d,e).

The microsporidian ribosome characterized in the present study is bound by two dormancy factors, MDF1 and MDF2, which represent two distinct classes of proteins that may inactivate eukaryotic ribosomes (Fig. 3 and see Supplementary Fig. 6c–h). MDF1 is a conserved eukaryotic β -strand-rich protein and, although structures of the human and *Plasmodium falciparum* homologues have been determined^{19–21}, their functional role has so far remained elusive. MDF1 is located in the E-site of the small ribosomal subunit, where it stabilizes a rotated and swivelled conformation (Figs. 3a,b and 4). Binding of this factor is incompatible with SSU movement, which is essential during the catalytic cycle²². One end of the elongated structure of MDF1 contains a zinc-binding site and contacts the E-site of the SSU, whereas the other end of the protein points towards the central protuberance of the LSU. The zinc-binding site is present in essentially all eukaryotic homologues except for the *Plasmodium falciparum* protein¹⁹ (Fig. 4b). The main interaction partner of MDF1 is the N-terminal tail of eS25, which becomes ordered, forms a shared secondary β -sheet structure and runs along the surface of the dormancy factor (Fig. 4a,c). In the E-site, the zinc-binding motif of MDF1 contacts the β -hairpin of uS7 (ScS5), a structural element interacting with the E-site codon of the messenger RNA during translation²³. Adjacent to uS7, additional contacts with the 16S rRNA and uS11 contribute to the interaction network of this dormancy

factor (Fig. 4a). A unique feature of all microsporidian MDF1 homologues, and that of *S. cerevisiae*, is an insertion of two β -sheets ($\beta 9$ – $\beta 10$, Fig. 4b–d and see Supplementary Fig. 6d), pointing towards the central protuberance and contacting uL5 (ScL11). Variability in this region among species suggests adaptation to changes in the structure of the ribosome. Structural similarity, charge distribution and conservation of the human homologue (C1orf123) point towards a similar function for MDF1 in other eukaryotic cells (Fig. 4b,d and see Supplementary Fig. 6e).

The second microsporidian dormancy factor, MDF2, occupies two critical functional regions of the ribosome. The C-terminal segment of MDF2 binds to the P-site of the large subunit (Fig. 3), whereas the less well-resolved N-terminus occupies the entire polypeptide exit tunnel and interacts with uL22 (ScL17) at the exit of the tunnel (see Supplementary Fig. 6f–h). The C-terminal segment completely blocks the peptidyl-transferase centre, occupies the transfer RNA (tRNA) acceptor arm-binding site on uL16 (ScL10) and ends with a short helix that contacts H69 (Fig. 3b). This arrangement distorts A2240 (ScA2971, Ec2602) (Fig. 4a), a catalytically important residue associated with nascent peptide release during translation termination²⁴. Binding of MDF2 sterically blocks the active site of the ribosome, which is incompatible with active translation. Homologues of MDF2 were identified in only two other organisms, the microsporidia *Nosema ceranae* and *Nosema apis*, both pathogens associated with sudden hive collapse in honeybees³. This could be due to the divergent nature of microsporidia and low sequence conservation of MDF2 or a clade-specific role for this factor.

As microsporidia have lost genes for many biosynthetic pathways, reduced their mitochondria to rudimentary mitosomes and lost the ability to synthesize their own nucleotides, they depend on their host for most of their metabolic activity⁵. An efficient shutdown of cellular processes would be advantageous during the spore stage to conserve energy. Translation of dormancy factors such as MDF1 and MDF2 at the end of sporulation could serve as an inhibitory mechanism to shut down translation within the spore until the infection of a new host begins. Increased mRNA levels of *mdf1* in the spore stage of *Vavraia culicis*²⁵, a microsporidium with a simple life cycle, are consistent with this hypothesis, whereas transcriptional analysis of *mdf1* in *Edhazardia aedis*²⁵, a microsporidium with multiple stages in its life cycle, points towards additional layers of regulation that might be required to activate MDF1 in such organisms (see Supplementary Fig. 7). As many mRNAs are still present in spores²⁶, ribosome dormancy factors could prevent their translation while allowing for the rapid reactivation of the ribosome on germination. Similar to other eukaryotic translation suppressors such as Stm1 in yeast^{27–28} and IFRD2 in mammals²⁹, which occupy mRNA- or tRNA-binding sites, MDF1 and homologues may block the ribosome via steric hindrance. Future work on mammalian and other eukaryotic homologues of MDF1 and MDF2 will be required to experimentally define their roles in these organisms.

Unlike the evolution of the mitochondrial ribosome, in which the erosion of the mitochondrial genome resulted in unstable intermediates prone to acquisition of stabilizing elements in a species-specific manner³⁰, genomic reduction in microsporidia has not led to a notable compositional diversification of the ribosome. In contrast, the extreme genomic reduction in microsporidia seems to rewind the evolutionary expansion seen in other

eukaryotic rRNAs (see Supplementary Fig. 3a). The structure of the microsporidian ribosome shows that, although compaction took place through a loss of rRNA expansion segments and the truncation of some of the ribosomal proteins, only two ribosomal proteins were lost. This would indicate that, although the evolutionary compaction of rRNA is feasible to an essential core that is smaller than the bacterial rRNA, the loss of eukaryotic ribosomal proteins poses an evolutionary hurdle. The high degree of protein-protein interactions within eukaryotic ribosomes²⁸, as well as the intricate assembly pathway of the eukaryotic ribosome¹⁵, may put severe constraints on the elimination of ribosomal proteins.

Structural and experimental biochemical data of endogenous microsporidian protein complexes have not been previously available. With the structure of the microsporidian ribosome presented in this study, an atomic view is provided of an essential machine affected by the rapid evolution and extreme genome compaction of these unique organisms.

Methods

Cultivation of *V. necatrix* in *Helicoverpa zea* and isolation of spores.

V. necatrix spores were produced by feeding approximately 100,000 spores to fourth and fifth instar corn earworm (*H. zea*) larvae grown on a defined diet (Benzon Research). After 3 weeks at 21–25 °C the spores were harvested by homogenizing the larvae in water. The homogenate was then filtered through two layers of cheese cloth followed by filtration through a 50-µm nylon mesh and centrifugation on a 50% Percoll cushion in a 2-ml microcentrifuge tube.

Purification of the *V. necatrix* ribosome.

Ribosomes were liberated by bead beating 100 mg of spores for 30 s in a 2-ml conical microfuge tube with 0.5 g of 0.5 mm zirconium beads in buffer A (30 mM Tris-HCl, pH 7.5, 5 mM magnesium acetate, 25 mM KCl, 1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid). The mixture was spun at 10,000g for 15 min in a microcentrifuge. The supernatant was then layered on top of a 1M sucrose cushion prepared by dissolving 17.1 g sucrose in 50 ml buffer A. The layered material was spun at 4°C for 4 h at 105,000g. The supernatant was removed, and the pellet was resuspended in 20 µl buffer A. Of the ribosome preparation, 5 µl was added to 95 µl buffer A and scanned between 240 and 300 nm to determine purity and concentration. Sample homogeneity was assessed by sodium dodecylsulfate/polyacrylamide gel electrophoresis and negative stain EM (Supplementary Fig. 1a).

Cryo-EM grid preparation.

Ribosome preparations were analysed for homogeneity by negative stain EM, and cryo-EM grids were prepared from the most homogeneous sample (see Supplementary Fig. 1a,b). Then, 3.5 µl of sample in buffer A (30 mM Tris-HCl, pH 7.5 (4°C), 5 mM magnesium acetate, 25 mM KCl, 1 mM dithiothreitol and 1 mM ethylenediaminetetraacetic acid) at an absorbance of 11 mAU (AU is arbitrary units) at 260 nm (Nanodrop 2000, Thermo Scientific) were applied to glow-discharged (30 s at 50 mA), lacy-carbon grids (TED

PELLA, Inc., product no. 01824) and flash frozen in liquid ethane using a Vitrobot Mark IV (Thermo Fisher Scientific) at 100% humidity, blot force of 0 and blot time 2.5 s.

Cryo-EM data collection and image processing.

Micrographs, 3,284, were collected from two independent cryo-EM grids in three sessions on a Talos Arctica (Thermo Fisher Scientific), operated at 200 kV, mounted with a K2 Summit detector (Gatan, Inc.). Serial EM³¹ was used for automated data collection of movies with a defocus range of 0.5–4 (μm at a pixel size of 1.2 $\text{\AA}$ (0.6 $\text{\AA}$ super-resolution).

Movies with 32 frames were collected at a dose of 8 electrons per pixel per second over an exposure time of 8 seconds, resulting in a total dose of 44.4 e⁻ $\text{\AA}^2$. Data collection parameters can be found in Supplementary Table 2. All 32 movie frames were initially aligned, drift corrected and dose weighted with MotionCor2(ref.³²) and later for the final refinement with RELION-3 (ref.³³). CTFIND-4.1.5 (ref.³⁴) was used to estimate the contrast transfer function. Manual inspection and removal of micrographs with poor CTF fits or drift reduced the number of micrographs to 3,007 used for reference-free particle picking with gautomatch (<http://wxvw.mrc-lmb.cam.ac.uk/kzhang/>). A total of 471,121 particles were extracted with a box size of 340 pixels (408 $\text{\AA}$) and subjected to an initial 2D classification to remove picking contaminations. Selection of class averages with high-resolution features resulted in 390,394 particles that were subjected to dsTEM³⁵ for 3D auto-refinement with 3 classes, using an initial model generated with cryoSPARC³⁶. This resulted in two classes representing the complete ribosome in different conformational states and one class consisting only of the large ribosomal subunit. Particles from the two classes of the complete ribosome were combined and refined in RELION-3 to a resolution of 3.7 $\text{\AA}$. In this overall map, the small subunit was less well resolved than the large subunit due to conformational heterogeneity. Multibody refinement with 3 masks (LSU, SSU body, SSU head) resulted in improved density for the small subunit 3D classification with two classes, followed by a similar multibody refinement procedure, improved the density of the E-site factor (MDF1) and permitted model building. The cryo-EM processing strategy is summarized in Supplementary Fig. 1.

Mass spectrometry analysis.

The purified ribosome sample was dried and resuspended in 8M urea/10mM dithiothreitol/0.1 M ammonium bicarbonate. Cysteines were alkylated (30 mM iodoacetamide) and digested first using LysC in <4M urea and second using trypsin in <2M urea. After solid phase extraction³⁷, peptides were analysed by nano-liquid-chromatography-tandem mass spectrometry (LC1200 coupled to a Fusion Lumos, Thermo Scientific operated in high/high mode). Peptides were separated using a 12cm $\times$ 75 μm C18 column (3 μm particles, Nikkyo Technos Co., Ltd) at a flow rate of 300nlmin⁻¹, with a 2–35% gradient over 70min (buffer A: 0.1% formic acid; buffer B: 80% acetonitrile/0.1% formic acid).

Using ProtomeDiscoverer (v.1.4) the data were searched against an in-house *V. necatrix* database (obtained from a preliminary assembled and annotated genome (manuscript in preparation)), concatenated with common contaminants. Oxidation of methionine and protein N-terminal acetylation were allowed as variable modifications, cysteine

carbamidomethyl was set as a fixed modification and two missed cleavages were allowed. Identified proteins are listed in Supplementary Table 1. Area³⁸ was used to rank different protein abundances in the sample.

Model building and refinement.

The structure of the yeast ribosome (PDB 4V88 (ref.²⁸)) served as a starting model for the building of the microsporidian ribosome in Coot³⁹. A complete list of sequences and chains can be found in Supplementary Table 3. The model was refined against the state 1 overall map (3.4 Å) with PHENIX⁴⁰ using phenix.real_space_refine and manually defined secondary structure restraints for RNAs and zinc coordination (obtained from the *S. cerevisiae* structure PDB 4V88 (ref.²⁸)). Model statistics can be found in Supplementary Table 2.

Map and model visualization.

Structure analysis and figure preparation were performed using PyMOL (Schrödinger) and UCSF ChimeraX⁴¹.

Reporting Summary.

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available from the corresponding author on request. The cryo-EM density maps for the microsporidian ribosome have been deposited in the EM Data Bank with accession code EMD-4935 (overall state 1), EMD-4935—additional map 1—(overall state 2), EMD-4931 (LSU focused), EMD-4932 (SSU-body focused), EMD-4933 (SSU-head focused) and EMD-4934 (state 1, SSU-head focused). Coordinates for state 1 have been deposited in the Protein Data Bank under accession code 6RM3. Mass spectrometry data has been uploaded to PRIDE⁴² under project accession PXD013432.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Stentiford GD et al. Microsporidia—emergent pathogens in the global food chain. *Trends Parasitol.* 32, 336–348 (2016). [PubMed: 26796229]
2. Franzen C Microsporidia: a review of 150 years of research. *Open Parasitol. J* 2, 1–34 (2008).
3. Higes M et al. How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environ. Microbiol* 10, 2659–2669 (2008). [PubMed: 18647336]
4. Didier ES & Weiss LM Microsporidiosis: not just in AIDS patients. *Curr. Opin. Infect. Dis* 24, 490–495 (2011). [PubMed: 21844802]
5. Corradi N Microsporidia: eukaryotic intracellular parasites shaped by gene loss and horizontal gene transfers. *Annu. Rev. Microbiol* 69, 167–183 (2015). [PubMed: 26195306]
6. Katinka MD et al. Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi*. *Nature* 414, 450–453 (2001). [PubMed: 11719806]
7. Corradi N, Pombert J-F, Farinelli L, Didier ES & Keeling PJ The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. *Nat. Commun* 1, 1–7 (2010). [PubMed: 20975674]
8. Cury JJ, Vavra J & Vivares C Presence of ribosomal RNAs with prokaryotic properties in Microsporidia, eukaryotic organisms. *Biol. Cell* 38, 49–51 (1980).
9. Vossbrinck CR, Maddox JV, Friedman S, Debrunner-Vossbrinck BA & Woese CR Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature* 326, 411–414 (1987). [PubMed: 3550472]
10. Vossbrinck CR & Woese CR Eukaryotic ribosomes that lack a 5.8S RNA. *Nature* 320, 287–288 (1986). [PubMed: 3083262]
11. Rivera MC, Maguire B & Lake JA Isolation of ribosomes and polysomes. *Cold Spring Harb. Protoc* 2015, 293–299 (2015). [PubMed: 25734065]
12. Haag KL et al. Evolution of a morphological novelty occurred before genome compaction in a lineage of extreme parasites. *Proc. Natl Acad. Sci. USA* 111, 15480–15485 (2014). [PubMed: 25313038]
13. James TY et al. Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature* 443, 818–822 (2006). [PubMed: 17051209]
14. Peyretailade E et al. Microsporidian *Encephalitozoon cuniculi*, a unicellular eukaryote with an unusual chromosomal dispersion of ribosomal genes and a LSU rRNA reduced to the universal core. *Nucleic Acids Res.* 26, 3513–3520 (1998). [PubMed: 9671812]
15. Klinge S & Woolford JL Ribosome assembly coming into focus. *Nat. Rev. Mol. Cell Biol.* 20, 116–131 (2019). [PubMed: 30467428]
16. Ebersberger I et al. The evolution of the ribosome biogenesis pathway from a yeast perspective. *Nucleic Acids Res.* 42, 1509–1523 (2014). [PubMed: 24234440]
17. Melnikov SV et al. Muller's ratchet and ribosome degeneration in the obligate intracellular parasites microsporidia. *Int. J. Mol. Sci* 19, 1–12 (2018).
18. Ramrath DJFF et al. Evolutionary shift toward protein-based architecture in trypanosomal mitochondrial ribosomes. *Science* 362, eaau7735 (2018). [PubMed: 30213880]
19. Holmes MA et al. Structure of the conserved hypothetical protein MAL13P1.257 from *Plasmodium falciparum*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun* 62, 180–185 (2006).
20. Rahaman SNA et al. Crystal structure and functional analysis of human Clorf123. *PeerJ.* 6, 1–25 (2018).
21. Id YF et al. Identification of a novel zinc-binding protein, Clorf123, as an interactor with a heavy metal-associated domain. *PLoS ONE* 13, e0204355 (2018). [PubMed: 30260988]
22. Ratje AH et al. Head swivel on the ribosome facilitates translocation by means of intra-subunit tRNA hybrid sites. *Nature* 468, 713–716 (2010). [PubMed: 21124459]
23. Devaraj A, Shoji S, Holbrook ED & Fredrick K A role for the 30S subunit E site in maintenance of the translational reading frame. *RNA* 15, 255–265 (2009). [PubMed: 19095617]

24. Polacek N et al. The critical role of the universally conserved A2602 of 23S ribosomal RNA in the release of the nascent peptide during translation termination. *Mol. Cell* 11, 103–112 (2003). [PubMed: 12535525]
25. Desjardins CA et al. Contrasting host-pathogen interactions and genome evolution in two generalist and specialist microsporidian pathogens of mosquitoes. *Nat. Commun* 6, 1–12 (2015).
26. Gill EE et al. Splicing and transcription differ between spore and intracellular life stages in the parasitic microsporidia research article. *Mol. Biol. Evol* 27, 1579–1584 (2010). [PubMed: 20167610]
27. Van Dyke N, Baby J & Van Dyke MW Stim1p, a ribosome-associated protein, is important for protein synthesis in *Saccharomyces cerevisiae* under nutritional stress conditions. *J. Mol. Biol* 358, 1023–1031 (2006). [PubMed: 16580682]
28. Ben-Shem A et al. The structure of the eukaryotic ribosome at 3.0 Å resolution. *Science* 334, 1524–1529 (2011). [PubMed: 22096102]
29. Brown A, Baird MR, Yip MC, Murray J & Shao S Structures of translationally inactive mammalian ribosomes, *eLife* 7, 1–18 (2018).
30. Petrov AS et al. Structural patching fosters divergence of mitochondrial ribosomes. *Mol. Biol. Evol* 36, 207–219 (2019). [PubMed: 30517740]
31. Mastronarde DN Automated electron microscope tomography using robust prediction of specimen movements. *J. Struct. Biol* 152, 36–51 (2005). [PubMed: 16182563]
32. Zheng SQ et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat. Methods* 14, 331–332 (2017). [PubMed: 28250466]
33. Zivanov J et al. New tools for automated high-resolution cryo-EM structure determination in RELION-3, *eLife* 7, e42166 (2018). [PubMed: 30412051]
34. Rohou A & Grigorieff N CTFFIND4: fast and accurate defocus estimation from electron micrographs. *J. Struct. Biol* 192, 216–221 (2015). [PubMed: 26278980]
35. Grant T, Rohou A & Grigorieff N CisTEM, user-friendly software for single-particle image processing. *eLife* 7, e35383 (2018). [PubMed: 29513216]
36. Punjani A, Rubinstein JL, Fleet DJ & Brubaker MA CryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* 14, 290–296 (2017). [PubMed: 28165473]
37. Rappsilber J, Mann M & Ishihama Y Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using stagetips. *Nat. Protoc* 2, 1896 (2007). [PubMed: 17703201]
38. Silva JC, Gorenstein MV, Li G-ZZ, Vissers JPC & Geromanos SJ Absolute quantification of proteins by LCMSE: a virtue of parallel MS acquisition. *Mol. Cell. Proteom* 5, 144–156 (2006).
39. Emsley P & Cowtan K Coot: model-building tools for molecular graphics. *Acta Crystallogr. Sect. D Biol. Crystallogr* 60, 2126–2132 (2004). [PubMed: 15572765]
40. Adams PD et al. The Phenix software for automated determination of macromolecular structures. *Methods* 55, 94–106 (2011). [PubMed: 21821126]
41. Goddard TD et al. UCSF ChimeraX: meeting modern challenges in visualization and analysis. *Protein Sci.* 27, 14–25 (2018). [PubMed: 28710774]
42. Vizcaíno JA et al. 2016 update of the PRIDE database and its related tools. *Nucleic Acids Res.* 44, 11033–11033 (2016). [PubMed: 27683222]

Fig. 2 |. Extensive rRNA expansion segment loss in microsporidia.

a, Four 90°-related views of the *V. necatrix* (top) and *S. cerevisiae* (bottom) ribosomes with LSU rRNAs coloured in light blue and SSU rRNA in pale yellow. Locations of expansion segments that have been lost in *V. necatrix* (top) but are present in *S. cerevisiae* (bottom) are coloured (SSU, shades of blue and green; LSU, shades of red and purple). **b,c**, Secondary structure diagram of the LSU (**b**) and the SSU (**c**) rRNAs. Lost expansion segments are coloured as in **a**. **d**, Comparison between the *S. cerevisiae* and the *V. necatrix* region around the LSU rRNA fusion site with lost expansion segments and selected ribosomal proteins coloured as in **a** and labelled. The msL1 is shown in light green, **e**, Same view as in **d** with selected proteins in isolation.

Fig. 3 |. Dormancy factors blocking the E-site, the peptidyl-transferase centre and the polypeptide exit tunnel.

a, Location of the peptidyl-transferase centre-blocking factor (MDF2, light pink) in the large ribosomal subunit and the E-site factor (MDF1, dark pink) in the small ribosomal subunit. Superimposed tRNAs (white, from PDB 4V6F) are shown in the A-, P- and E-site, **b,** Slab view and a zoom-in thereof showing the position of MDF1 in the E-site and MDF2 in the peptidyl-transferase centre, **c,** Binding of tRNAs in the P- and E-site is sterically hindered by the presence of MDF2 and MDF1, as indicated by the superimposed tRNAs shown as a transparent white surface. The location of a superimposed mRNA (grey) and the polypeptide exit tunnel (blue) is indicated.

Fig. 4 |. MDF1 is a conserved eukaryotic protein.

a, Detailed view of the E-site blocking factor MDF1 (pink). The N-terminus of eS25 (yellow) interacts with MDF1. The inset shows the cryo-EM density of eS25 and MDF1. **b**, Superposition of *V. necatrix* MDF1 (red) with the structure of the *H. sapiens* (PDB 5ZRT, green) and *P. falciparum* (PDB 1ZSO, light blue) homologues, **c**, Schematic secondary structure diagram of MDF1 with the main interactions indicated, **d**, Sequence alignment of MDF1 and eukaryotic homologues, with secondary structure elements indicated above. The following organisms in **d** are not otherwise mentioned: *Drosophila pseudoobscura*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Schizosaccharomyces pombe*, *Dictyostelium discoideum*, *Nematostella vectensis*, *Rattus norvegicus*, *Bos taurus*, *Mus musculus* and *Xenopus laevis*.