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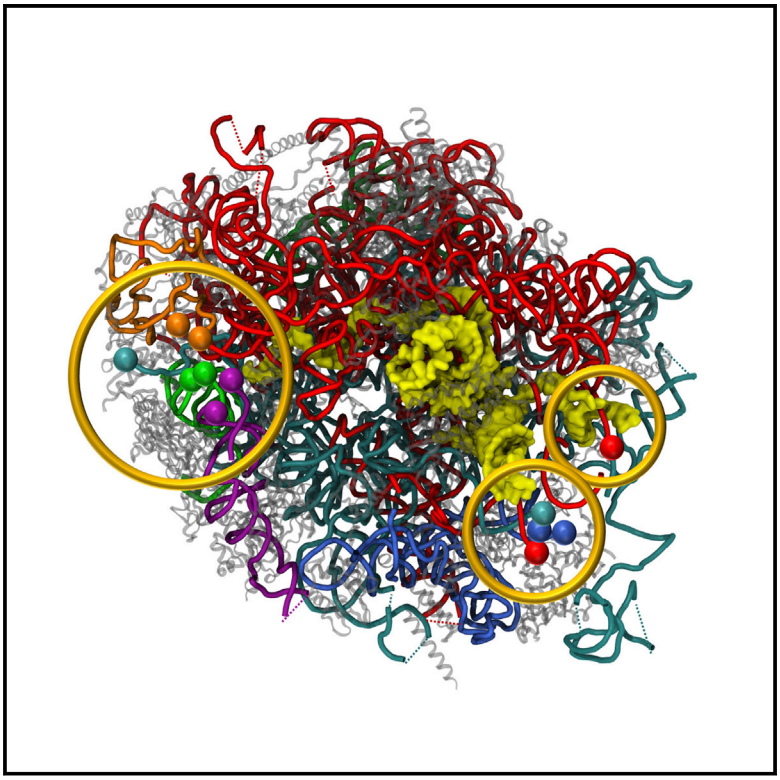
Report

2.8-Å Cryo-EM Structure of the Large Ribosomal Subunit from the Eukaryotic Parasite *Leishmania*

Graphical Abstract



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In Brief

Shalev-Benami et al. describe the structure of the *Leishmania donovani* large ribosomal subunit (LSU), obtained by cryo-EM at 2.8-Å resolution. The structure shows the fragmented nature of leishmanial rRNA and highlights the irregular distribution of rRNA modifications with implications for drug development against this protozoan parasite, which afflicts millions of people worldwide.

Highlights

- 2.8-Å cryo-EM map of *Leishmania* ribosome facilitates atomic resolution structure
- Direct observation of eukaryotic rRNA modifications
- Leishmanial rRNA is fragmented and hyper modified at unique positions
- Fragmented rRNA termini converge into three focal points involving 5.8S

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2.8-Å Cryo-EM Structure of the Large Ribosomal Subunit from the Eukaryotic Parasite *Leishmania*

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SUMMARY

Leishmania **is a single-cell eukaryotic parasite of the Trypanosomatidae family, whose members cause an array of tropical diseases. The often fatal outcome of infections, lack of effective vaccines, limited selection of therapeutic drugs, and emerging resistant strains, underline the need to develop strategies to combat these pathogens. The Trypanosomatid ribosome has recently been highlighted as a promising therapeutic target due to structural features that are distinct from other eukaryotes. Here, we present the 2.8-Å resolution structure of the *Leishmania donovani* large ribosomal subunit (LSU) derived from a cryo-EM map, further enabling the structural observation of eukaryotic rRNA modifications that play a significant role in ribosome assembly and function. The structure illustrates the unique fragmented nature of leishmanial LSU rRNA and highlights the irregular distribution of rRNA modifications in *Leishmania,* a characteristic with implications for anti-parasitic drug development.**

INTRODUCTION

L. donovani belongs to a distinct order of protozoan parasites called kinetoplastids, several of which are dangerous pathogens for humans and represent a major global health concern; these include *Trypanosoma cruzi* (*T. cruzi*), *Trypanosoma brucei* (*T. brucei*), and various *Leishmania* species that cause Chagas' disease, African sleeping sickness, and leishmaniasis, respectively. Kinetoplastids are markedly diverged from other species in the eukaryotic lineage and are characterized by numerous distinct features at the genetic, biochemical, and cytological levels (extensively reviewed in Fernandez-Rodrigez et al., 2014).

One particularly interesting peculiarity of kinetoplastids lies in their cytosolic ribosomes, which include an unusual segmentation of their large ribosomal subunit (LSU) 26S rRNA (Hernández and Cevallos, 2014), extensive number of rRNA modification sites (Eliaz et al., 2015), and enlarged rRNA expansions (Gao et al., 2005; Hashem et al., 2013). Many studies have focused on these unique features in trypanosomatid ribosomes, aiming at exploiting them as targets for the development of novel anti-parasitic drugs (Michaeli et al., 2012). Notwithstanding these efforts, the mechanism of rRNA segmentation and its biological role remain largely obscure. Electron cryo-microscopy (cryo-EM) investigations of ribosomes from *T. cruzi* and *T. brucei* at resolutions of 12 Å and 5.6 Å, respectively, have demonstrated the overall segmented architecture of kinetoplastid ribosomes (Gao et al., 2005; Hashem et al., 2013). However, the limited resolution of these earlier studies precluded the building of atomic models and understanding of functional aspects in detail. Here, we describe a high-resolution structure of the large ribosomal subunit from *L. donovani* promastigotes recently isolated from a spleen biopsy of a visceral leishmaniasis patient in northern Ethiopia. The structure is based on a 2.8-Å cryo-EM reconstruction of the leishmanial LSU that enabled determination of a nearly complete atomic model. The high-resolution cryo-EM map obtained in this study has also enabled the direct visualization of the unusual rRNA modification pattern in *Leishmania*. Some of these modification loci were recently predicted using bioinformatics tools (Eliaz et al., 2015), and our structure provides experimental evidence for the existence of these modifications on the leishmanial ribosome. The *L. donovani* LSU atomic model presented here will serve as a reliable template for future structure-based drug design.

RESULTS AND DISCUSSION

Cryo-EM Analysis and Model Building

We employed single-particle cryo-EM to obtain a 3D map of the *L. donovani* LSU at resolution of 2.8 Å. For these studies, we purified intact 91S leishmanial ribosomes and initially obtained a 3D map of the entire apparatus. However, the wide range of conformations of the small subunit (SSU) relative to the LSU limited the resolution in this region. This problem was compounded by internal flexibility within the SSU, evident from our further attempts of masked refinement with signal subtraction of the LSU resulting in SSU maps with resolution lower than 4 Å (Figures S1 and S2). As such, the 3D maps for the SSU were insufficient for fully reliable model building and refinement. Thus, we focused our efforts on obtaining the structure of the