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Ribosomal RNA Genes in the Protozoan Parasite *Leishmania major* Possess a Nucleosomal Structure

Juan C. Vizuet-de-Rueda[a], Luis E. Florencio-Martínez[a], Norma E. Padilla-Mejía[a], Rebeca Manning-Cela[b], Rosaura Hernández-Rivas[b], and Santiago Martínez-Calvillo[a,1]

[a]Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Av. De los Barrios 1, Col. Los Reyes Iztacala, Tlalnepantla, Edo. de México, CP 54090, México
[b]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del IPN, Av. IPN 2508, México, D.F., CP 07360, México

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Little is known about nucleosome structure and epigenetic regulation of transcription of rRNA genes in early-branched eukaryotes. Here we analyze the chromatin architecture and distribution of some histone modifications in the rRNA genes in the parasitic protozoon *Leishmania major.* Southern blots of MNase-partially-digested chromatin with DNA probes spanning the whole rRNA gene repeat showed that the intergenic spacer presents a tight nucleosomal structure, whereas the promoter region is practically devoid of nucleosomes. Intermediate levels of nucleosomes were found in the rRNA coding regions. ChIP assays allowed us to determine that H3K14ac, H3K23ac and H3K27ac, epigenetics marks that are generally associated with activation of transcription, are enriched in the promoter region. In contrast, H4K20me3, which is generally related to transcriptional silencing, was absent from the promoter region and intergenic spacer and enriched in the coding region. Interestingly, the distribution pattern for H3K9me3, generally linked to heterochromatin formation, was very similar to the distribution observed with the euchromatin marks, suggesting that this modification could be involved in transcriptional activation of rRNA genes in *L. major*.

Key words: Pol I transcription; epigenetic regulation; rRNA genes; *Leishmania major*; histone modification; nucleosome structure.

[1]Corresponding author; fax +52 55 5623-1138
e-mail scalv@campus.iztacala.unam.mx (S. Martínez-Calvillo).

Abbreviations: Pol, RNA polymerase; rRNA, ribosomal RNA; PTM, post-translational modification; IGS, intergenic spacer; TSS, transcription start site; MNase, micrococcal nuclease.

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Introduction

In eukaryotes, ribosomal RNA (rRNA) genes are tandemly repeated at one or few chromosomal loci. The rRNA genes are transcribed by RNA polymerase I (Pol I), which produces a primary transcript (~35-47S) that is processed into the mature 18S, 5.8S and 28S rRNAs (Goodfellow and Zomerdijk 2012; Paule and White 2000). These three rRNA molecules, together with the Pol III-transcribed 5S rRNA, comprise the main structural and catalytic components of the ribosome. The number of rRNA gene repeats varies from ~100 to more than 10,000 among different organisms (Russell and Zomerdijk 2005; Sollner-Webb and Mougey 1991). Interestingly, only a subset of rRNA genes is transcribed at any given time (Grummt 2007), and it has been established that chromatin structure plays an important role in the silencing and activation of these genes (Hamperl et al. 2013; Lawrence and Pikaard 2004; McStay and Grummt 2008).

Eukaryotic DNA is folded into nucleosomes, the basic repetitive units of chromatin, that comprise ~150 bp of DNA wrapped around a histone octamer composed of two copies each of histones H2A, H2B, H3 and H4 (Kornberg and Lorch 1999). Chromatin structure is important in several biological processes, including transcriptional regulation, and DNA recombination and replication. Changes in chromatin architecture can be achieved by histone post-translational modifications (PTMs) (Kouzarides 2007), and by incorporation of distinct histone variants (Weber and Henikoff 2014). Histone PTMs include methylation, acetylation and phosphorylation, which occur at specific residues mainly within the N-terminal tail of histones (Kouzarides 2007; Rando 2007).

Each rRNA gene repeat contains regulatory sequences that include promoters, enhancers and terminators, which are located within an intergenic spacer (IGS) of variable length (Torres-Machorro et al. 2010). Analyses of the nucleosomal structure of rRNA genes have been complicated by the repetitive nature of rRNA genes and by the presence of both active and inactive genes at any particular time. Nevertheless, different lines of evidence indicate that transcriptionally active rRNA gene repeats show an open chromatin state characterized by acetylated histones (Goodfellow and Zomerdijk 2012; Grummt 2007; Zentner et al. 2011). In contrast, inactive rRNA gene repeats possess a packaged chromatin structure characterized by methylation of the DNA and repressive histone modifications, for instance, trimethylation of lysine 9 in histone H3 (H3K9me3) (Zentner et al. 2014).

The trypanosomatid parasite *Leishmania major* possesses atypical mechanisms of gene expression, including polycistronic Pol II transcription and maturation of mRNAs by trans-splicing (Martinez-Calvillo et al. 2010; Michaeli 2011). Little is known about chromatin structure and epigenetic regulation in *L. major* and other trypanosomatids. Histones in these organisms are divergent from those found in other organisms. Nevertheless, as in other organisms, nucleosomes constitute the basic structural unit of chromatin in trypanosomatids (Belli 2000; Figueiredo et al. 2009; Horn 2001). A genome-wide study showed the presence of histone H3 acetylated at K9/K14 at the origins of Pol II polycistronic transcription in *L. major* (Thomas et al. 2009). Other studies revealed that origins of Pol II transcription contain acetylated (H3K9/K14 and H4K10) and methylated (H3K4) histones in the related parasites *Trypanosoma cruzi* (Respuela et al. 2008) and *Trypanosoma brucei* (Siegel et al. 2009). Also, histone variants H2AZ and H2BV are enriched at Pol II transcription start sites in *T. brucei* (Siegel et al. 2009). Thus, post-translational histone modifications and histone variants might generate an open chromatin structure that is needed for initiating Pol II transcription in trypanosomatids.

The knowledge of transcription and chromatin structure in rRNA genes in *L. major* is scarce. It was estimated that the genome of this parasite contains only ~12 copies of the rRNA gene repeat per haploid genome, organized in head-to-tail tandem arrays on chromosome 27 (Ivens et al. 2005; Martinez-Calvillo et al. 2001). Repetitive elements of 63 bp are contained within the IGS, between the 18S and the 28S rRNA genes. As in other trypanosomatids, the *L. major* 28S rRNA gene is fragmented into six separate molecules: 28Sα, β, γ, δ, ε and ζ. However, some of the rRNA gene repeats in *L. major* possess two copies of the 28Sε rRNA gene (Martinez-Calvillo et al. 2001). The transcription start site (TSS) of the rRNA unit was localized to 1043 bp upstream of the 18S rRNA gene and 184 bp downstream of the 63-bp repeats. A 391-bp fragment from -184 to +207 (relative to the TSS) was shown to contain a Pol I promoter that can drive high levels of expression of a reporter gene. Transcription of the rRNA genes seems to terminate upstream of the 63-bp repeats (Martinez-Calvillo et al. 2001).

Here we report the analysis of chromatin architecture of rRNA genes in *L. major*. Nucleosomal ladders analyzed by Southern blots showed that the