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# Kinetoplast RNA of Leishmania tarentolae

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## Summary

**RNA has been isolated from highly purified kinetoplast-mitochondrial fractions of Leishmania tarentolae, and shown to consist of two major species that sediment at 9S and 12S in sucrose and also several additional low molecular weight species which were visualized by gel electrophoresis. The in vivo transcription of 9S and 12S RNAs was inhibited by ethidium bromide and rifampin, and was fairly insensitive to low actinomycin D and camptothecin. The 9S and 12S RNAs were isolated by acrylamide gel electrophoresis or by sedimentation in sucrose. Both RNAs contained approximately 80% A + U and did not contain long stretches of poly(A). The 9S and 12S RNAs were found to hybridize selectively to the maxicircle sequences of the kinetoplast DNA, implying that the maxicircle, and not the minicircle, represents the informational mitochondrial DNA in the kinetoplast.**

## Introduction

Although there is indirect evidence that the kinetoplast DNA in the hemoflagellate protozoa is essential for the continued synthesis of mitochondrial respiratory proteins (Simpson, 1972), there is yet little direct evidence for in vivo transcriptional activity of this unusual mitochondrial DNA. Presumptive kinetoplast ribosomes have been visualized in Trypanosoma brucei culture forms (Hanas, Linden and Stuart, 1975) by in vivo labeling of nascent polypeptides in the presence of cycloheximide, an inhibitor of cytoplasmic protein synthesis, but nothing is known about kinetoplast ribosomal RNA, transfer RNA or messenger RNA.

Nichols and Cross (1977) have recently described several presumptive kinetoplast RNA species sedimenting at 6.9S, 10.1S, 11.4S and 14.6S from a mitochondrial fraction of Crithidia fasciculata. The synthesis of these RNAs was inhibited by 10 $\mu$g/ml ethidium bromide (EthBr).

In HeLa cells and Xenopus, the identified stable mitochondrial transcripts consist of the large and small ribosomal RNAs (Aloni and Attardi, 1971a; Dawid, 1972a, 1972b), 19–22 transfer RNAs (Wu et al., 1972; Dawid et al., 1976; Angerer et al., 1976) and approximately eight polyadenylated messenger RNAs (Perlman, Abelson and Penman, 1973; Hirsch, Spradling and Penman, 1974; Ojala and Attardi, 1974). Pulse-labeled HeLa mitochondrial RNA apparently is transcribed symmetrically from both mitochondrial DNA strands (Aloni and Attardi, 1971b, 1971c), whereas the stable RNA species are mostly transcripts of the H strand.

We have examined the question of the in vivo transcriptional activity of the kinetoplast DNA of Leishmania tarentolae by isolation of the major stable RNA species from a highly purified kinetoplast fraction (Braly, Simpson and Kretzer, 1974). This paper describes the isolation, physical properties, labeling characteristics and transcriptional origin of the two major kinetoplast RNA species.

## Results

### Cytoplasmic Ribosomal RNA

Cytoplasmic ribosomes were isolated from L. tarentolae culture forms and subjected to band sedimentation in SDS-sucrose gradients. The rRNA sedimented at 18S and 25S with a shoulder at 14S. There was also a 5S peak and a 10S peak. The 14S peak may represent a breakdown product. The 10S peak may represent contaminating 10S kinetoplast DNA minicircles (Wesley and Simpson, 1973).

Cytoplasmic rRNA runs in 3.5% acrylamide as three major bands and several minor bands (Figure 1a), indicating that some breakdown has occurred. Several low molecular weight bands are separated in 10% acrylamide (Figure 1a), three of which do not co-migrate with E. coli 4S or 5S RNA. These bands will be designated the "5.8S," "5.9S" and "6.2S" RNAs.

### Total RNA Isolation from Purified Kinetoplast Fraction

A highly purified kinetoplast mitochondrion fraction was isolated from late log-phase cells by isopycnic centrifugation in Renografin density gradients (Braly et al., 1974), and total RNA was isolated by SDS lysis and phenol deproteinization. Band sedimentation of this preparation in a sucrose gradient yielded three major peaks, sedimenting at 4–5S, 9S and 12S.

The 9S peak was contaminated with free 10S K-DNA minicircles, which are known to be released to a small extent upon lysis of the kinetoplast (Wesley and Simpson, 1973). The extent of this contamination was evidenced by the gel electrophoresis pattern of $^{32}$P-labeled K-RNA before and after DNAase treatment, shown in Figure 1d, slots 1 and 2. Identification of the position of free monomeric minicircles in this gel was achieved by co-running $^{32}$P-labeled, closed monomeric minicircles in slot 3. It is clear that the use of gel electrophoresis for RNA isolation eliminates the necessity for