J. Protozool. 21(2), 382-393 (1974).

Isolation of the Kinetoplast DNA of *Leishmania tarentolae* in the Form of a Network*

LARRY SIMPSON and JUDITH BERLINER†‡
Biology Department and the Molecular Biology Institute, University of California, Los Angeles, California 90024

SYNOPSIS. The major kinetoplast DNA complement of *Leishmania tarentolae* promastigotes has been isolated as a single sheet of interconnected molecules on the basis of its relative stability to shear forces and its high sedimentation coefficient. Two successive differential centrifugations were sufficient to recover 50 ± 10% of the total kinetoplast DNA free of nuclear DNA contamination. We use the term "network" to describe this unusual type of DNA configuration.

Leishmania networks have a molecular weight of $\sim 10^{10}$ daltons and an $s_{20,w}$ in neutral sucrose gradients of 1729 ± 189 [n = 19] and exhibit an extremely low specific viscosity due to the compactness of packing of the DNA. The networks were visualized in the electron microscope, and in the light microscope either by fluorescence in solution after staining with acridine orange or in dried smears after staining with Giemsa.

Purified networks from stationary phase cells banded in the position characteristic of closed monomeric minicircles in ethidium bromide-CsCl equilibrium gradients, and were stable in alkaline sucrose. Treatment of the closed networks with RNase and pronase had no effect on the ethidium bromide-CsCl banding pattern. However, treatment of closed networks with DNase I or II, X-irradiation or γ-irradiation changed the banding pattern by introducing single strand and double strand breaks, yielding an upper band and in some cases a intermediate band.

Index Key Words: *Leishmania tarentolae*; kinetoplast DNA; networks; ethidium bromide-CsCl; light and electron microscopy.

THE kinetoplast DNA (K-DNA) of several species of hemoflagellates previously has been isolated on the basis of buoyant density in CsCl or Cs_2SO_4, and in a few cases directly from kinetoplast-enriched cell fractions, and partially characterized (14, 15, 17, 18, 21). In the case of *Leishmania tarentolae* the K-DNA consisted of a complex series of molecular types, with the predominant species being large "associations" of topologically interlocked minicircular molecules and longer molecules. It was assumed that the observed free minicircles and small catenanes of minicircles were released from the larger units during the long purification procedure. In fact, Williamson et al. (25), Laurent et al. (11) and Renger & Wolstenholme (16) have now succeeded in obtaining possibly the entire K-DNA complements of several hemoflagellate species (*Trypanosoma brucei*, *Crithidia luciliae* and *Crithidia acanthocephali*) in an intact form. In line with these reports, we have developed a simple procedure for the isolation of the intact K-DNA genome of *L. tarentolae* in the form of a single sheet ("network") of interconnected molecules and have studied several physical properties of these unusual structures.[1]

The rapid isolation of K-DNA networks in good yield represents an essential starting point for the study of the replication and transcription of this unusual mitochondrial DNA. This report describes our isolation technic in detail and presents some preliminary data on the physical characterization of nonreplicating K-DNA networks.

MATERIALS AND METHODS

Culture of Cells

The strain of cells (Lt-C-1) was derived from a clone in September 1969. The culture conditions were as described previously (20). Cells were kept routinely in BHI (Brain Heart Infusion Medium, Difco) and transferred to modified defined medium C (CA) (22), containing 20 μg/ml of adenine in place of the original purine/pyrimidine mix, for at least 1

* Supported in part by NIH Research Grant No. AI09012 to L. Simpson, and by a UCLA Biomedical Science Support Grant. Dr. Berliner was a postdoctoral Trainee on NIH Training Grant No. AI0070.

† We thank Patricia Braly for expert technical assistance, June Balmer for assistance with analytical ultracentrifugation, Ron Wesley for discussions and suggestions, and Dr. Kay Noel for performing the viscosity measurements.

‡ **Present address of Dr. Berliner:** Dept. of Pathology, University of California Center for the Health Sciences, Los Angeles, CA 90024.

[1] We thank Dr. Ken Stuart for communicating to us his results before publication, thereby stimulating this project.