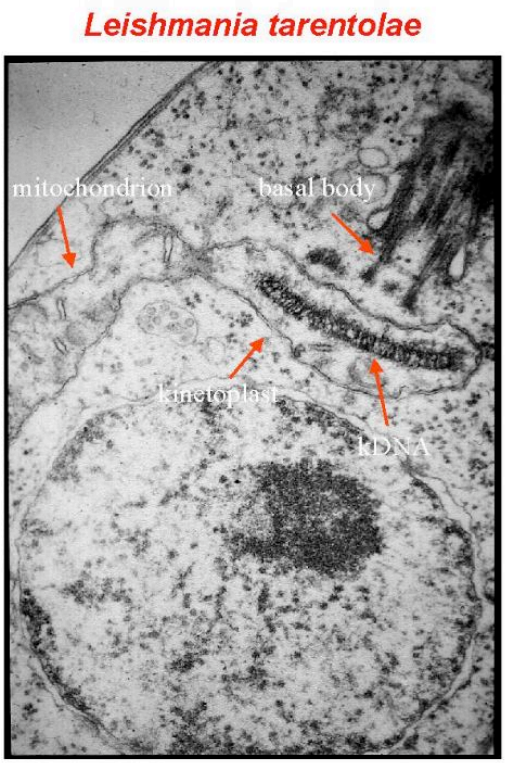
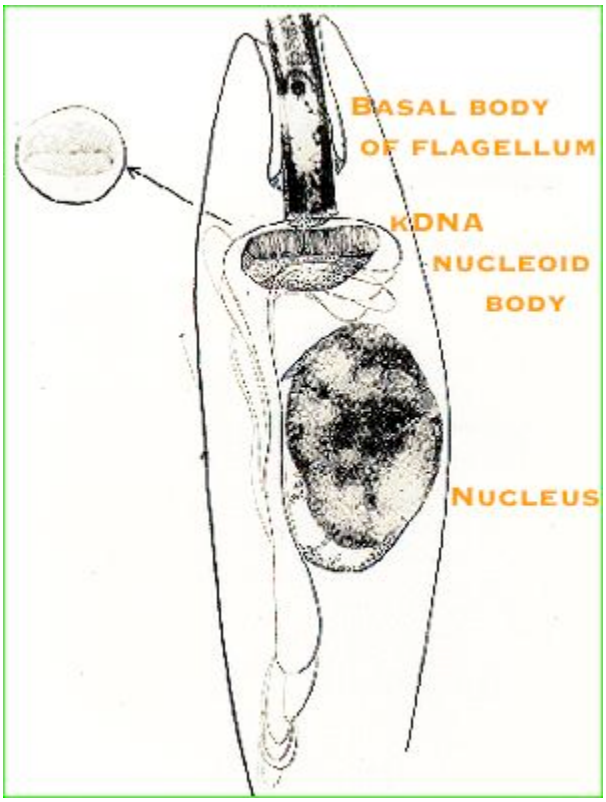
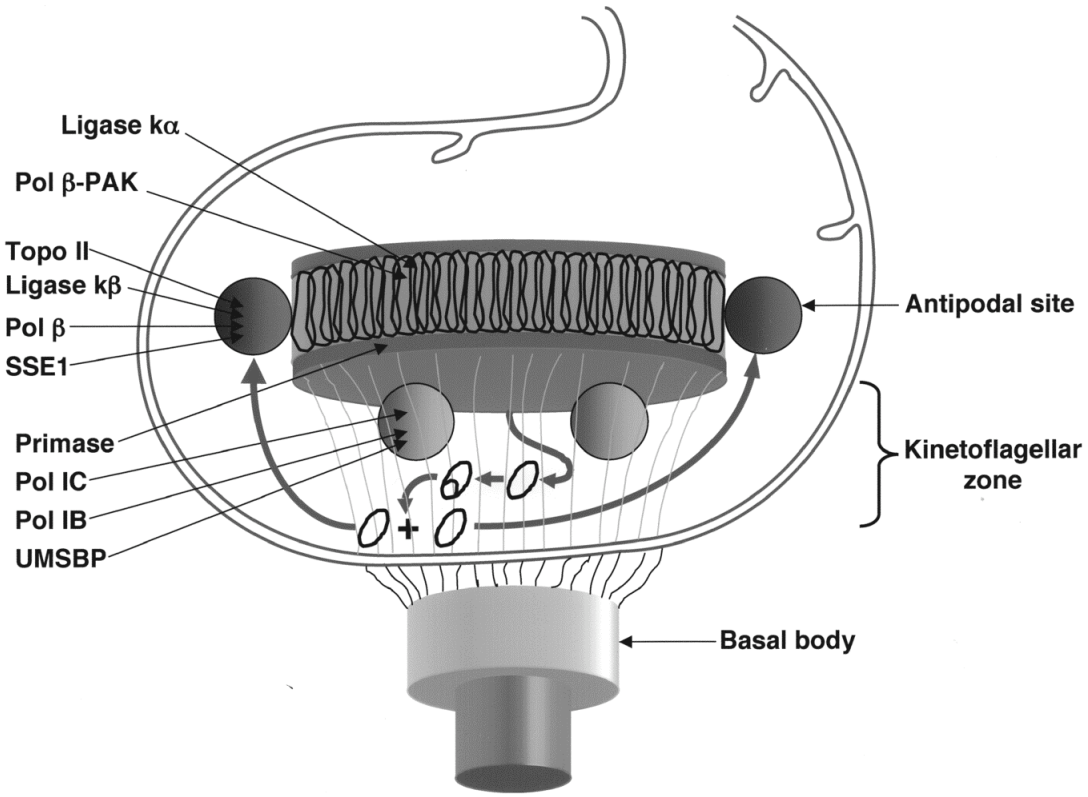
Schematics of the kinetoplastid mitochondrial kDNA organization







And how were these networks labeled

In contrast to the ones before, these were labeled in vitro, after the networks were isolated.
DNA polymerase or Terminal deoxynucleotidal transferase (TdT) were added to the isolated networks. Both will be active on Nicked or Gapped substrates (DNA Polymerase can add Labeled nucleotides to the 3'OH and so can TdT.

Here, the nucleotide is radio active

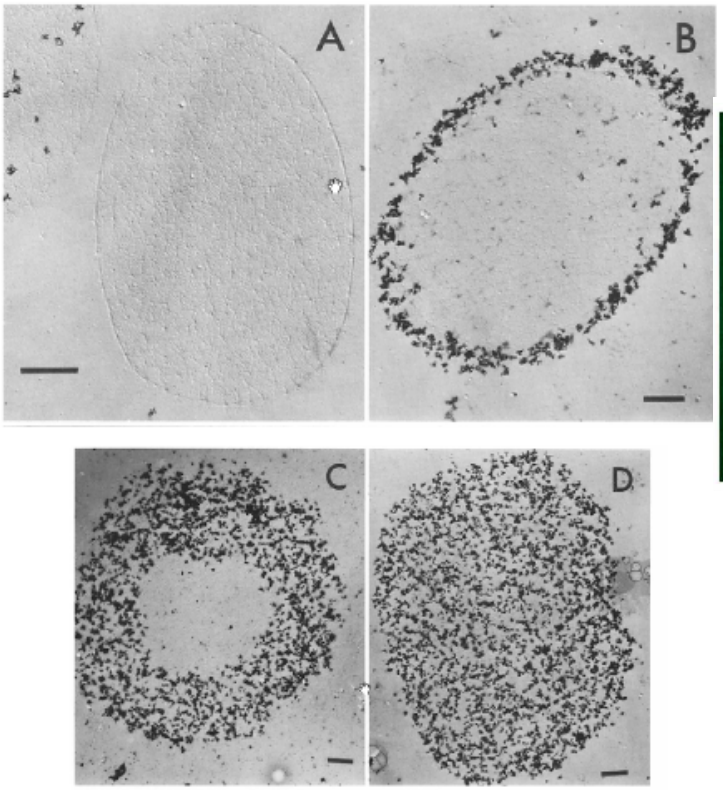
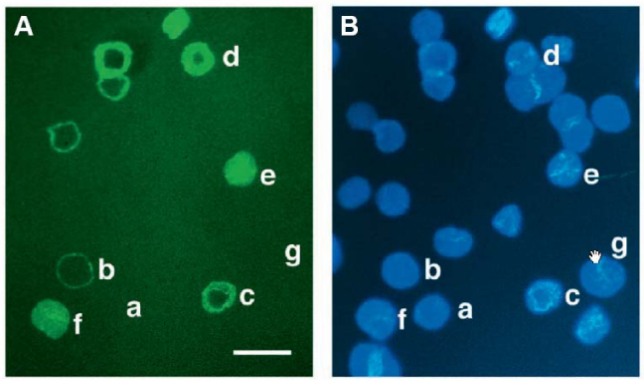


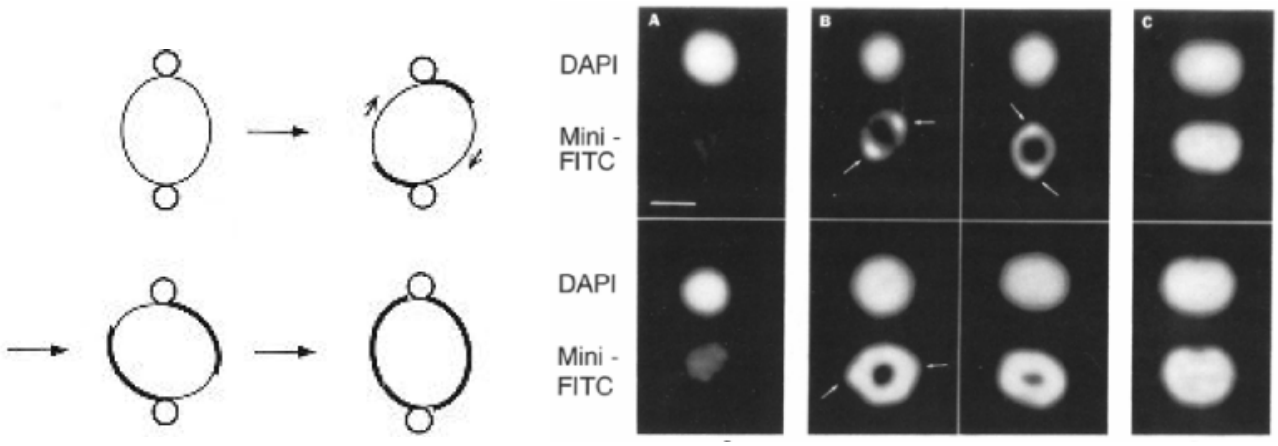
Figure 1. EM autoradiography of isolated kDNA networks radiolabeled by nick translation at endogenous minicircle nicks. The networks in *A*, *B*, and *D* had been fractionated according to stage of replication using a CsCl-propidium diiodide gradient, and that in *C* was from a solution of unfractionated networks. The nick translation reactions (50 μl) contained 100–200 ng of kDNA networks, 50 mM Tris-HCl, pH 7.5, 10 mM $MgSO_4$, 20 μM each of dCTP, dATP, and dGTP, 37.5 μCi of [^{3}H]dTTP (105 Ci/mmol; New England Nuclear), and 1 U of *E. coli* DNA polymerase I (GIBCO BRL, Gaithersburg, MD). The reactions were conducted at 16°C for 60 min and unincorporated nucleotides were removed by overnight dialysis against TE at 4°C. The radioactive kDNA was then spread on EM grids (in the presence of 100 μg/ml ethidium bromide) and autoradiographed. Exposure was 4 mo for *A*, 11 d for *B* and *D*, and 3 d for *C*. *A* shows a Form I network, *B* and *C* show replicating networks, and *D* shows a Form II network. Bars, 2 μm.

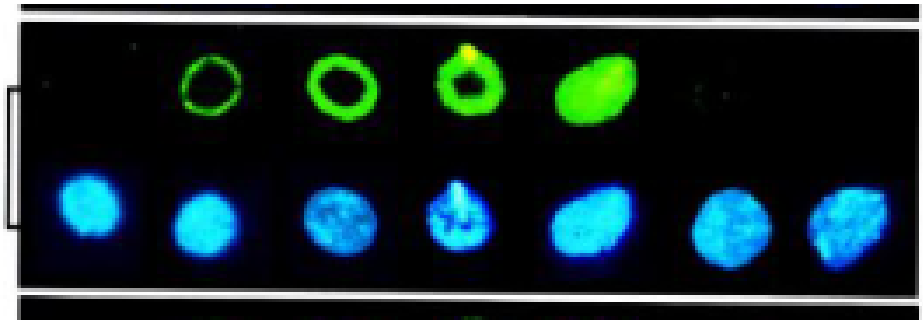
Here, the nucleotide is fluorescent



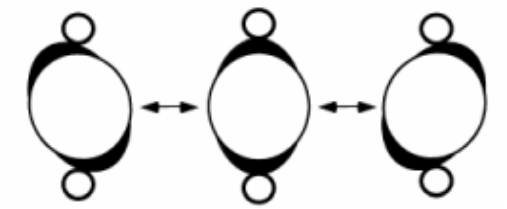
Same experiment, different label

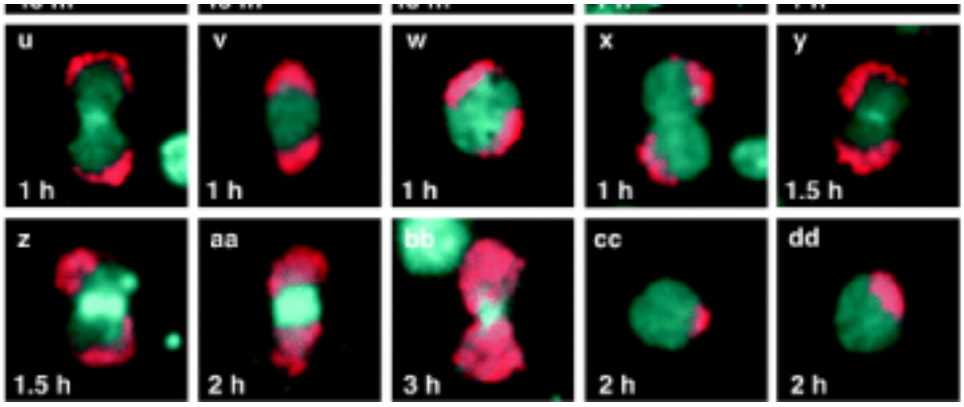
kDNA rotates during replication





…except in T brucei, where it oscillates





How are these kDNA networks labeled?

Two methods: In vivo: PULSE/CHASE
In vitro: Labeling nicked and gapped Minicircles

In this figure, the in vivo labeling trick was used:

Cells are grown in the presence of a radioactive Thymidine for A) 3 min B) 6 min C) 30 min and D) 60 min (That's the Pulse). Any DNA replicated during the pulse will incorporate the radioactive Thymidine. A Pulse/chase experiment is when you grow the cells in the presence of the labeled substrate (pulse) and then grow the cells in the presence of unlabeled substrate for a defined period of time after (chase)

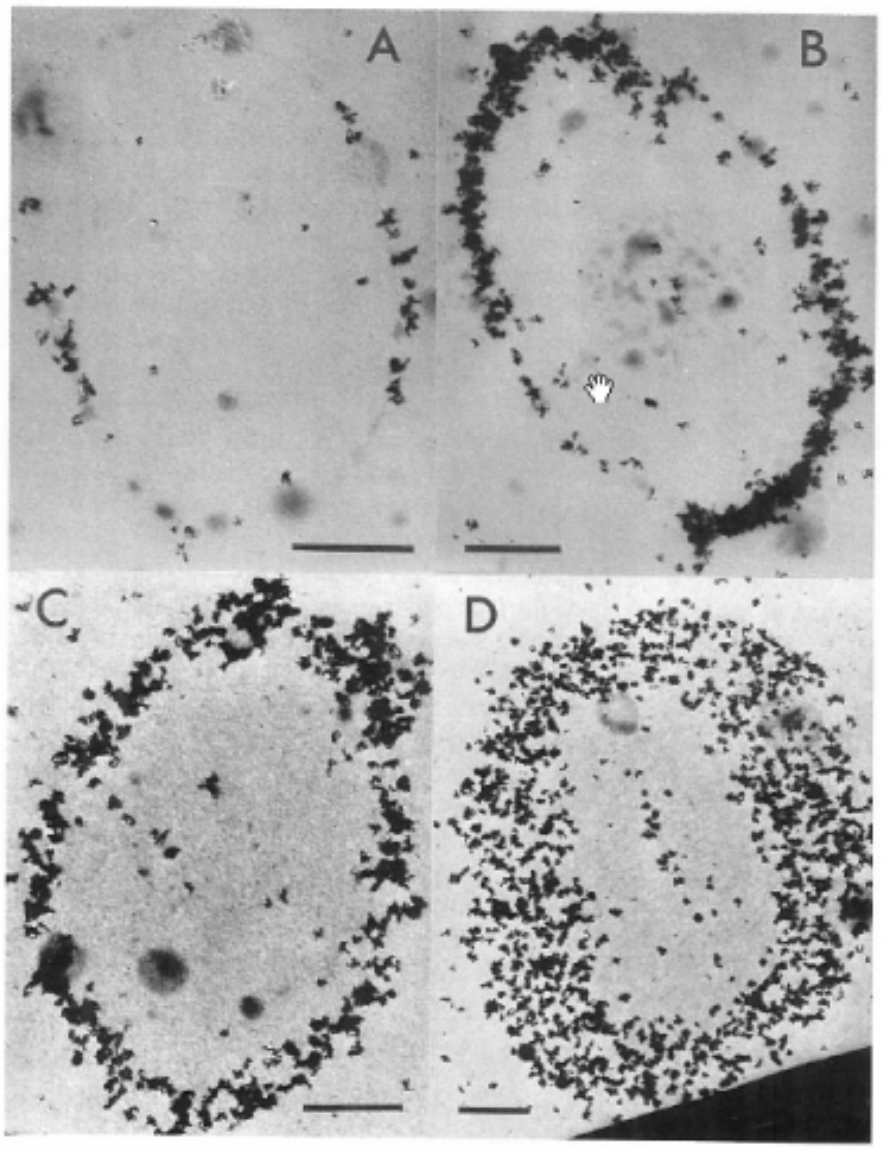


Figure 1. EM Autoradiographs of kDNA Networks Labeled In Vivo with [^{3}H]Thymidine

Labeling periods were: (A), 3 min; (B), 6 min; (C), 30 min; (D), 60 min. Propidium diiodide, used to distinguish nicked or gapped minicircles from colvalently closed minicircles, was present in the hypophase and spreading solution at 10 μg/ml. Autoradiographic exposure times were: (A and B), 11 weeks; (C and D), 40 hr. Bars, 3 μm.