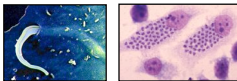


Introduction to Kinetoplastid Organisms

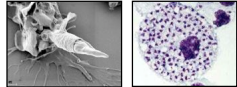
Trypanosoma brucei,
Trypanosoma cruzi and
Leishmania sp.

Trypanosomatidae Characteristics

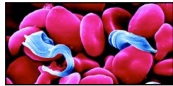
Trypanosoma cruzi - Chagas Disease



Leishmania species - Leishmaniasis



Trypanosoma brucei - African Sleeping Sickness

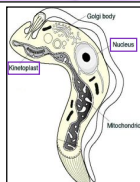
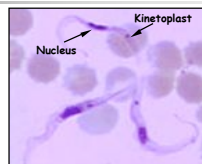


- Early diverging eukaryotes
- Flagellated parasitic protists
- Vector borne pathogens
- Complex life cycles
- *Trypanosoma brucei*

Model parasite
Two culture forms
Divergent eukaryotic branch with
demonstrated RNA interference

Kinetoplastid organisms

- Diverse group of flagellated protists
- Free-living & parasitic
 - Family Bodonidae
 - Family Trypanosomatidae
- Parasitic
 - Animals (fish - humans)
 - Insects
 - Plants
- Unifying feature - kinetoplast
 - Giemsa staining structure
 - Contains mitochondrial DNA network (kDNA)
 - Defining feature - unique in nature



kDNA - organized in a disk structure

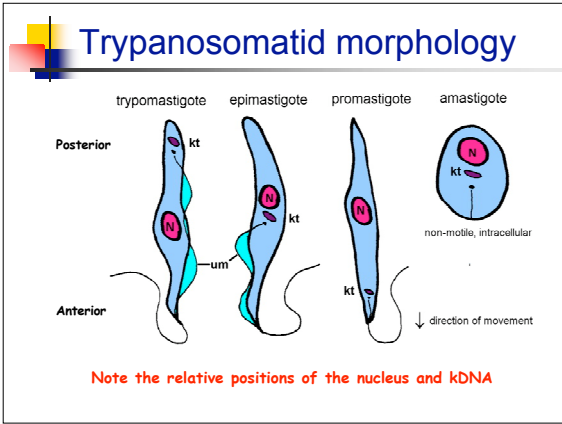
Kinetoplast is always associated with the flagellar basal body

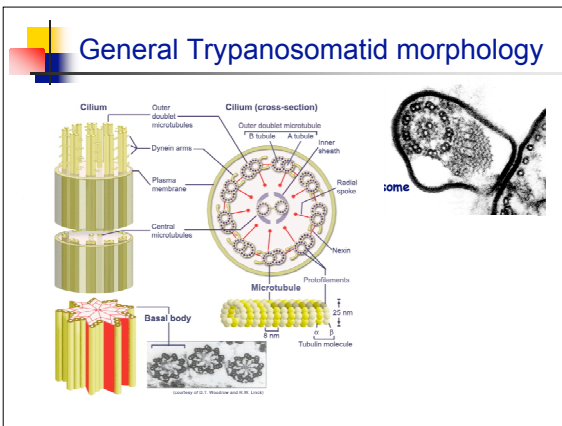
Characteristics of kDNA networks

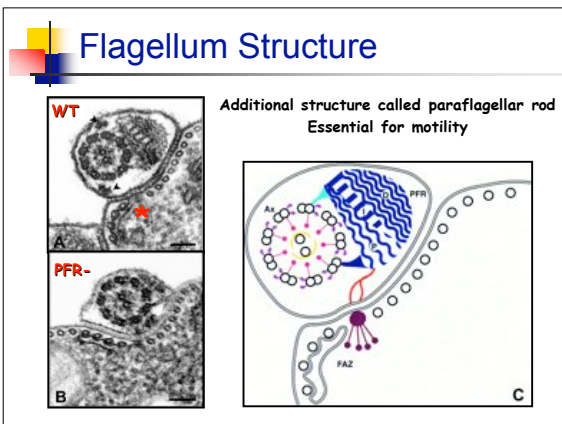
- Elliptical catenated monolayer: ~ 10 μm
- 2 types of circular molecules
 - Maxicircles: 20 kb**
 - Homologs of mitochondrial DNA
 - Encode electron transport subunits, rRNA, but no tRNAs
 - Require editing process for a functional transcript
 - Minicircles: 1 kb**
 - Heterogeneous minicircle classes
 - Encode guide RNAs that govern the specificity of editing

Unique Molecular Properties

- Polycistronic transcription
- Trans-splicing of mRNAs
 - SL RNA at 5' end of each ORF
- Packaging of glycolytic enzyme into glycosomes
 - Unique to kinetoplasts
- Acidocalcisomes
- Mitochondrial RNA editing
- kDNA network
 - Unique to kinetoplasts







Trypanosomatid flagellum: a multifunctional organelle

- **Cell motility**
 - Vigorous swimmers - 10-30 $\mu\text{m/s}$ in cell culture
 - Highly directional cell motility
 - "Run-and-tumble" motility to change direction
- **Cell morphogenesis**
 - Positioning and segregation of mitochondrial genome
- **Pathogenicity** (on a later slide)
 - Attachment to host surfaces

Cell Motility

Trypanon - auger cell
Trypanosoma

Role in the Cell Cycle

The trypanosome cell cycle

A Earliest point in cell cycle. Single mature flagellum subtended from a basal body.

B Two basal bodies, daughter flagellum elongates.

C Daughter flagellum continues to elongate and move posterior. The nucleus straddles axis of mature flagellum and starts dividing.

D Two daughter cells each with duplicate organelles. Segregation progressing.

Disease Causing Kinetoplastids



- *Trypanosoma brucei* complex
 - African sleeping sickness - Humans
 - Nagana - livestock
- *Trypanosoma cruzi*
 - Chagas' disease
- *Leishmania species*
 - Cutaneous leishmaniasis
 - Visceral leishmaniasis
 - Mucocutaneous leishmaniasis

All are called **hemoflagellates**
some stage of laboratory growth requires blood (serum)

Types of Vector Transmission

- **Salivarian**
 - Transmission via mouthparts
 - Very efficient
 - Infection rate in vector is LOW

- **Stercorarian**
 - Transmission through hindgut
 - Acquired from insect feces or eating the vector
 - Very inefficient
 - Infection rate in vector is HIGH


Six degrees of separation

BROUGHT BY FAITHFUL HANDS
OVER LAND AND SEA HERE RESTS
DAVID LIVINGSTONE,
MISSIONARY,
TRAVELLER,
PHILANTHROPIST.
BORN MARCH 19, 1813
AT BLANTYRE, LANARKSHIRE,
DIED MAY 1, 1873
AT CHITAMBO'S VILLAGE, ULALA.

FOR 30 YEARS HIS LIFE WAS SPENT
IN AN UNWEARIED EFFORT
TO EVANGELIZE THE NATIVE RACES,
TO EXPLORE THE UNDISCOVERED SECRETS,
TO ABOLISH THE DESOLATING SLAVE TRADE,
OF CENTRAL AFRICA.

WHERE WITH HIS LAST WORDS HE WROTE,
"ALL I CAN ADD IN MY SOLITUDE, IS,
MAY HEAVEN'S RICH BLESSING COME DOWN
ON EVERY ONE, AMERICAN, ENGLISH, OR TURK,
WHO WILL HELP TO HEAL
THIS OPEN SORE OF THE WORLD"



African Trypanosomiasis History

- In 1895 David Bruce discovered in South Africa that trypanosomes are transmitted by the tsetse fly and cause nagana in livestock. Several years later, in Uganda, he discovered that trypanosomes cause sleeping sickness.



Sir David Bruce
1855-1931

Trypanosoma brucei brucei

- Definitive Host
 - Ruminants - antelope, livestock (cattle, sheep goats)
- Non-pathogenic to humans
- Causes **Nagana** - very similar to human disease
 - 1890's British colonial farmers were losing their European bred cattle to a wasting disease
 - native cattle were more tolerant
 - "in low or depressed spirits" (Zulu)
- No treatment - drugs are expensive and used to treat human sleeping sickness

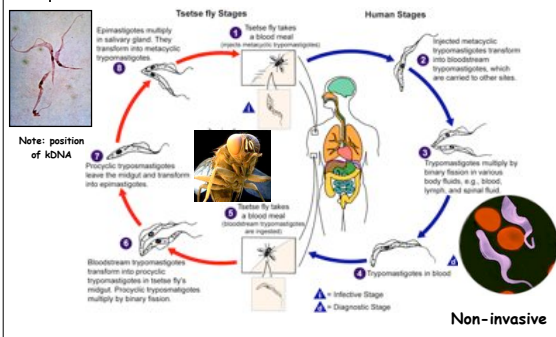
Zebu - sensitive

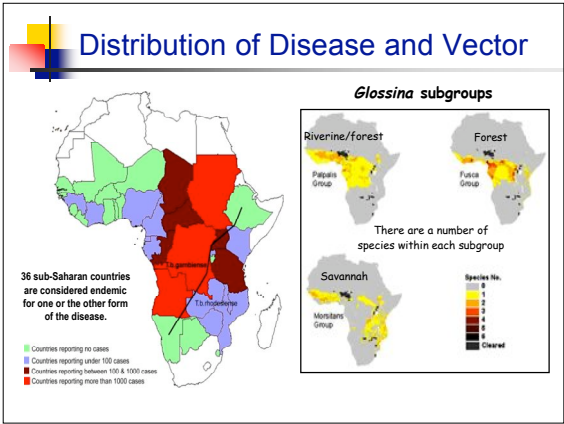


N'Dama - tolerant



Trypanosoma brucei Life Cycle



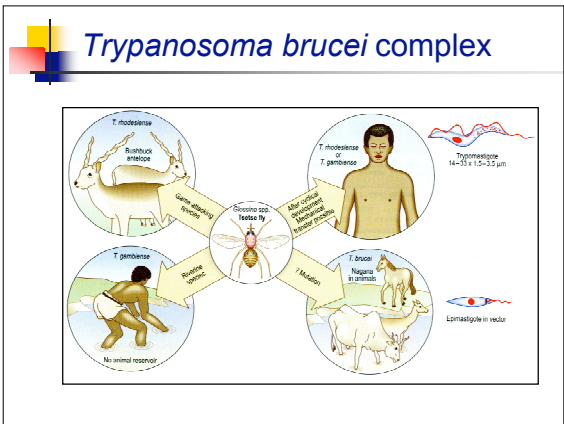


Trypanosoma brucei complex

<i>T. b. brucei</i>	game animals/livestock (nagana)
<i>T. b. rhodesiense</i>	E. African trypanosomiasis
<i>T. b. gambiense</i>	W. and Central African sleeping sickness

Morphologically indistinguishable species
All are transmitted via the bite of the Tsetse fly

- Trypanosome lytic factor (TLF) found in human sera
- component of HDL (high density lipoprotein) fraction
- human parasites resistant to TLF
- resistance associated with decreased uptake of HDL



Reservoirs and Ecology

Wild animal reservoirs

Wildebeest

Warthog

Bush buck

Host range of *Trypanosoma brucei*

Spotted hyena Waterbuck Wild dog Buffalo Coke's Hartbeest

Eland Lion Giraffe Zebra Reedbuck

Warthog Bushbuck Impala Hippo Cattle

Comparison of *T. brucei* subspecies

	East	West
	<i>rhodesiense</i>	<i>gambiense</i>
tse-tse vector	<i>G. morsitans</i>	<i>G. palpalis</i>
ecology	dry bush or woodland	rainforest, riverine, lakes
transmission cycle	ungulate-fly-human	animal-fly-human, human-fly-human
non-human reservoir	wild animals	domestic animals
epidemiology	sporadic, safaris	endemic, some epidemics
disease progression	rapid, often fatal	slow (~1 yr) acute chronic
parasitemia	high	low
asymptomatic carriers	rare	common

TLF-mediated Lysis

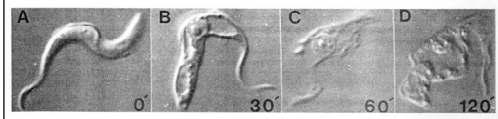


Fig. 2. Morphological changes in *T. b. brucei* during incubation with TLF for (A) 0, (B) 30, (C) 60 and (D) 120 min at 37°C, shown by the cells at 0 and 30 min of incubation retaining motility. Nomarski interference contrast microscopy of cells fixed with 1% glutaraldehyde.

1992 Parasitology Today

- In susceptible *T. brucei (brucei)*, TLF is taken up by receptor-mediated endocytosis, targeted to the lysosome where it causes lysosomal breakdown and autodigestion of the cell.
- Resistant *T. brucei (gambiense, rhodesiense)* bind TLF, but do not endocytose it.

Tsetse fly bite

- All 23 species of *Glossina* are potential vectors
- Both male and females take blood meals
- Metacyclic trypomastigotes in saliva enter bite wound
 - ~40,000 parasites/bite
 - Only 400 needed
 - Experimentally infected animal with a single parasite
- Parasite replication at bite site



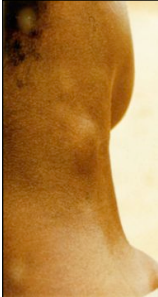
Acute Symptoms

- 1-2 week asymptomatic incubation period
- sometimes a local inflammation
 - 'trypanosomal chancre'
 - parasite replication at bite site
 - Can be confused with a simple boil
- invasion of blood characterized by irregular fever and headache
- *T. rhodesiense* can develop into full infection quickly
- *T. gambiense* can be self-limiting (rare) or slowly progressing to more serious disease




Lymphatic stage

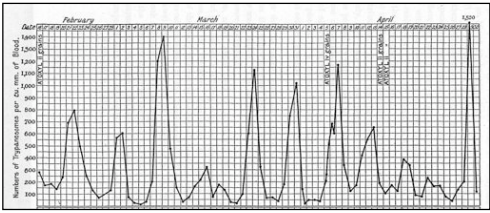
- Disease progression often involves invasion of lymphatics
 - Winterbottom's sign
 - Swelling of cervical lymph nodes
 - Rash
 - Itching
 - Edema
 - Continued febrile attacks
 - Weight loss (wasting)
 - weakness



Winterbottom's sign



A Human Trypanosome Infection



Ross and Thomson, 1910

Cyclical pattern of fever accompanied by increase in parasitemia

Antigenic Variation

Late Stage HAT

- parasites crossing blood-brain barrier result in CNS involvement and nervous impairment
 - described as meningoencephalitis
 - increased apathy and fatigue
 - confusion and somnolence
 - motor changes including tics, slurred speech, incoordination
 - convulsions, coma
- progression to CNS involvement is rapid (weeks) in *rhodesiense* and slow (6-12 months) in *gambiense*
- Death

