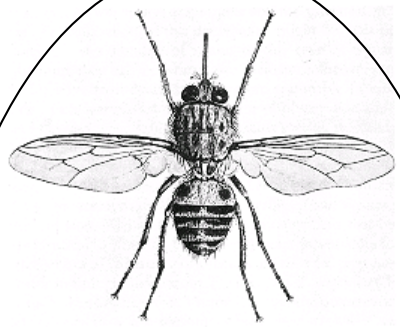
African trypanosome Life Cycle

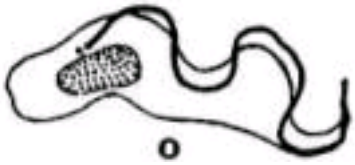
epimastigote
salivary gland

metacyclic trypomastigote
non-dividing
infection for vertebrate host
salivary gland

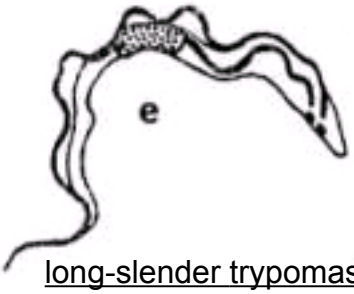
procyclic trypomastigote
fly midgut
dividing form



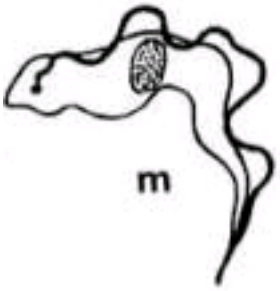
metacyclic replication
inflamation (chancre)
to lymph nodes
to bloodstream



short-stumpy trypomastigote
non-dividing (G0)
infectious for flies
preadaption for tsetse

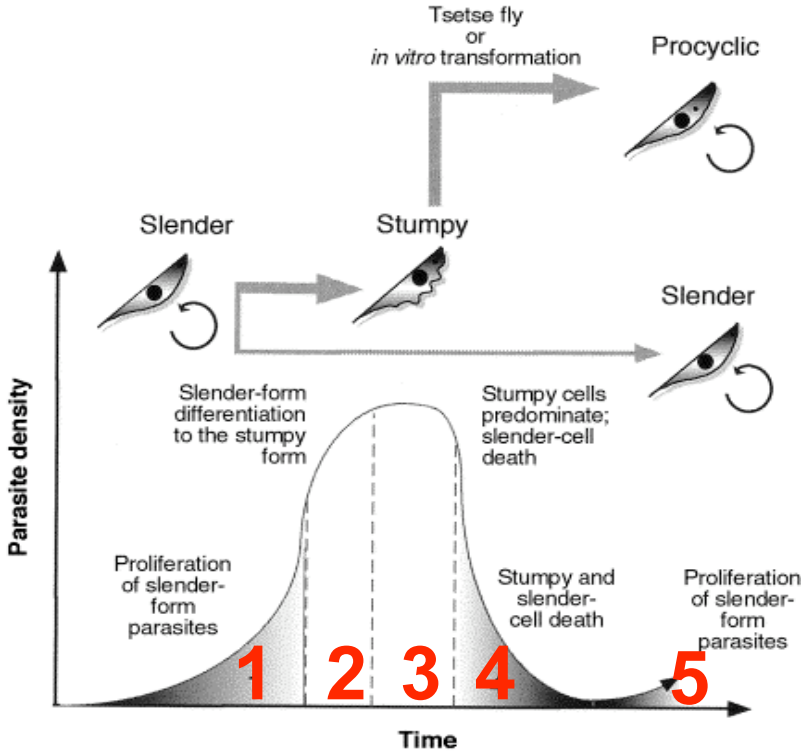


long-slender trypomastigote
dividing form
empty mitochondria



Intermediate blood form

Stages of T. brucei Bloodstream Infection



Stage 1 – Long-slender trypomastigotes actively divide

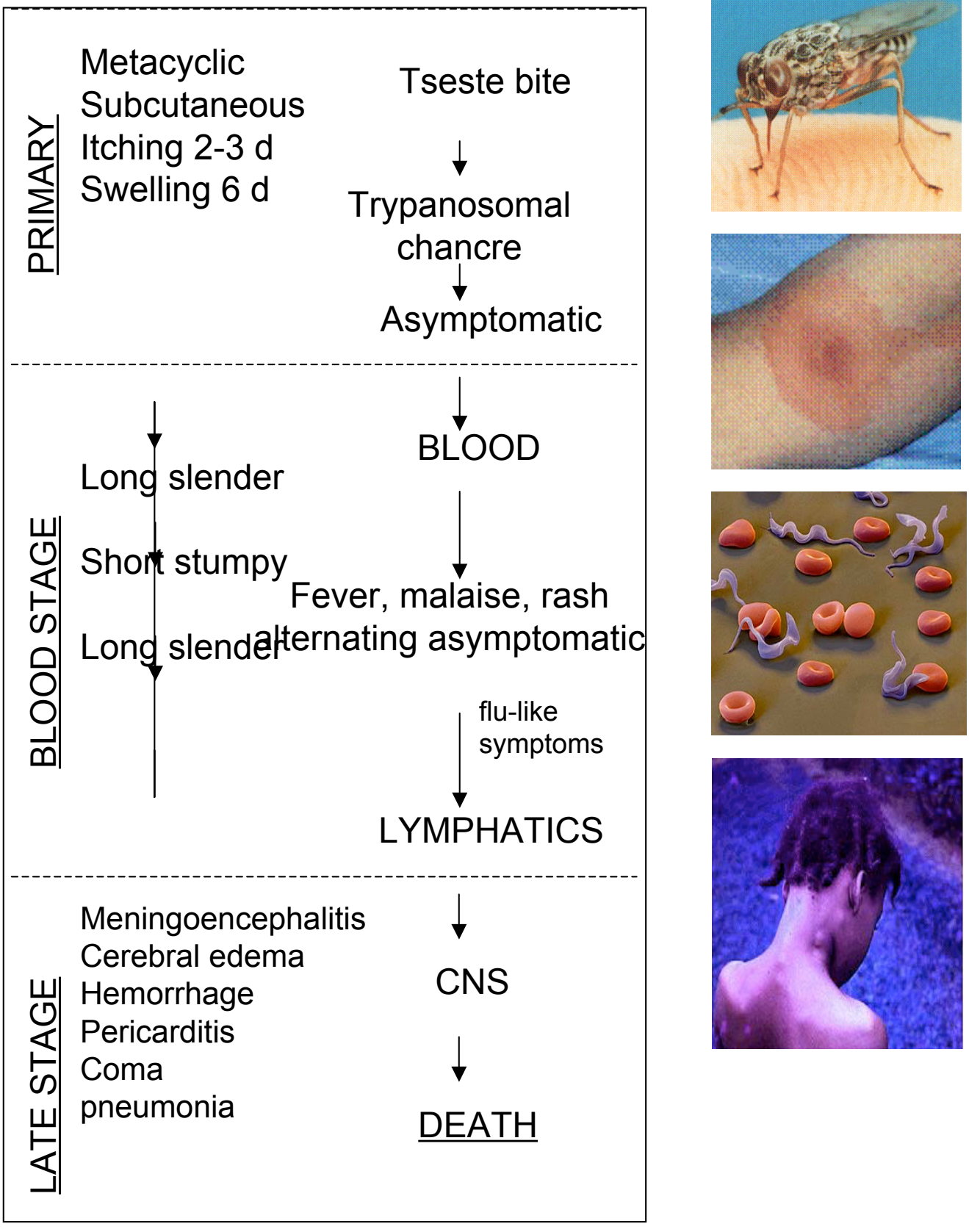
Stage 2 – At high cell densities, long-slenders differentiate into short-stumpys
(able to differentiate further into procyclics)

Stage 3 – Mostly short-stumpys, immune clearance of long-slenders

Stage 4 – Immune clearance of short-stumpys

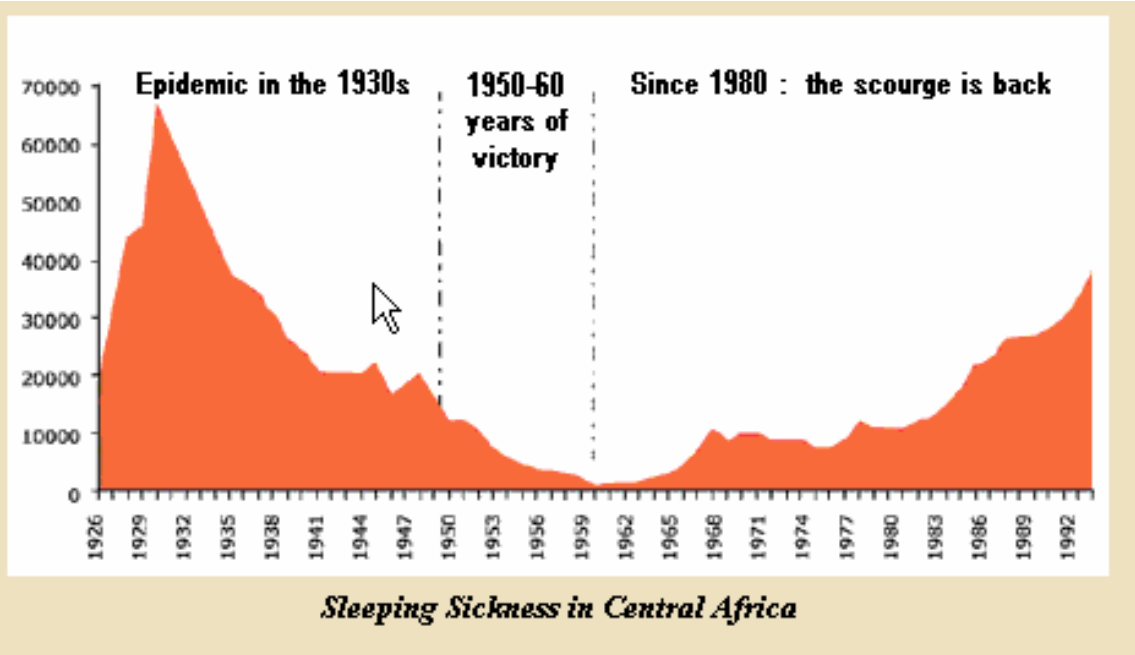
Stage 5 – Next proliferation of long-slenders which can evade host immune response (How? Antigenic Variation)

Pathogenesis of African Trypanosomiasis



Invention of DDT

Widespread spraying campaign



Sleeping Sickness in Central Africa

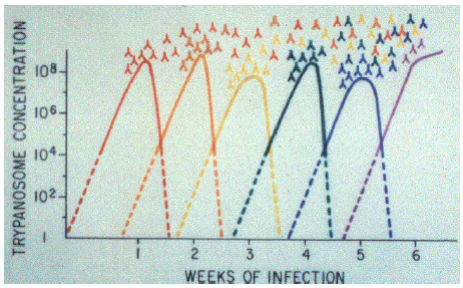
Drug treatment: Painful, expensive, and potentially ineffective

Table 63.9 Chemotherapy of African trypanosomiasis.

Drug	*Pentamidine*	*Suramin*	*Metarsoprol*	*Eflornithine*	*Nifurimox‡*
Introduced	1937	1922	1949	1990	–
Chemical status	Diamidine	Sulphated naphthylamine	Arsenical	Difluoromethyl-ornithine	Nitrofuran
Route of administration	Intramuscular	Intravenous	Intravenous	Intravenous	Oral
Effective in relation to disease stage	Early-stage *T. b. gambiense*	Early-stage *T. b. gambiense* and *T. b. rhodesiense*	Early or late *T. b. gambiense* and *T. b. rhodesiense*	Early or late *T. b. gambiense*	Arsenical-resistant *T. b. gambiense*
Dosage regimen	4 mg/kg body weight > 10 daily injections	20 mg/kg body weight 5-7 injections every 5-7 days	3.6 mg/kg body weight 3-4 series of 4 injections separated by 1 week	400 mg/kg body weight 100 mg every 6 hours for 14 days	10 mg/kg body weight daily 60-90 days
Resistant strains	+	+	+	*T. b. rhodesiense* refractory	Unknown
Side-effects	Vomiting, hypotension, hypoglycaemia	*Pyrexia, joint pains, rash, desquamation	Encephalopathy, diarrhoea	Diarrhoea, anaemia, thrombocytopenia	Convulsions, psychosis, vomiting, neuralgia, polyarthritis, paraesthesia
Cost of drug/ treatment ($US)	100†	15	47	266	60-100
Costs of complementary drugs ($US)	–	–	130 if encephalopathy occurs	200, i.v. fluid, perfusion kits, etc.	–
Hospitalization costs ($US)	60 (12 days)	120 (30 days)	150 (30 days)	70 (14 days)	Not available
Total costs $US	160	175	197 (or 327)	536	–

*Test dose in onchocerciasis areas.
†Pentamidine is presently being supplied by WHO at a nominal cost through the courtesy of Rhône Poulenc–the manufacturer.
‡Nifurtimox has not been widely used in African trypanosomiasis. Figures are based on best estimates from use in Chagas' disease.
+Resistance to drugs exists.

Variant Surface Glycoprotein



-Episodes of relapsing parasitemias

-Variant Antigenic Type

-switching rate 1:10,000 to 1:1,000,000 per division
-300-1000 VSG genes per tryp.
-5-10% of the genome

-serodemes – lineages of antigenically different tryps. w/ common ancestor

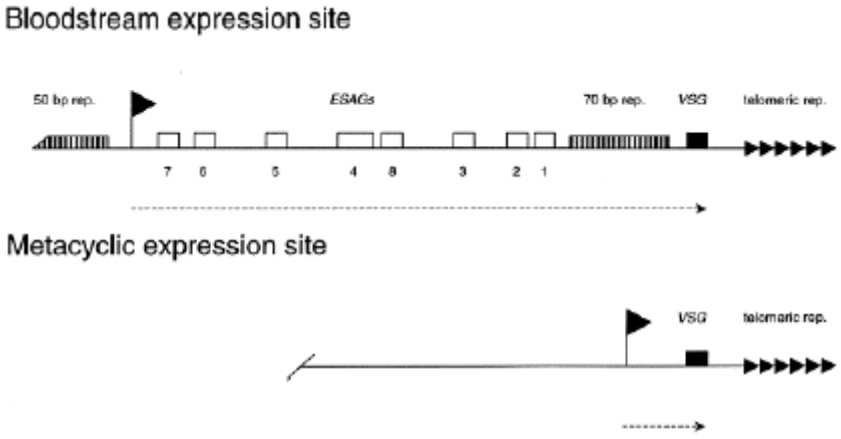
-isogenic mutants – differ by the expression/function of single gene (VSG)

-VSG cDNA - N-term. cleaved during membrane transport
C-term. removed, GPI anchor

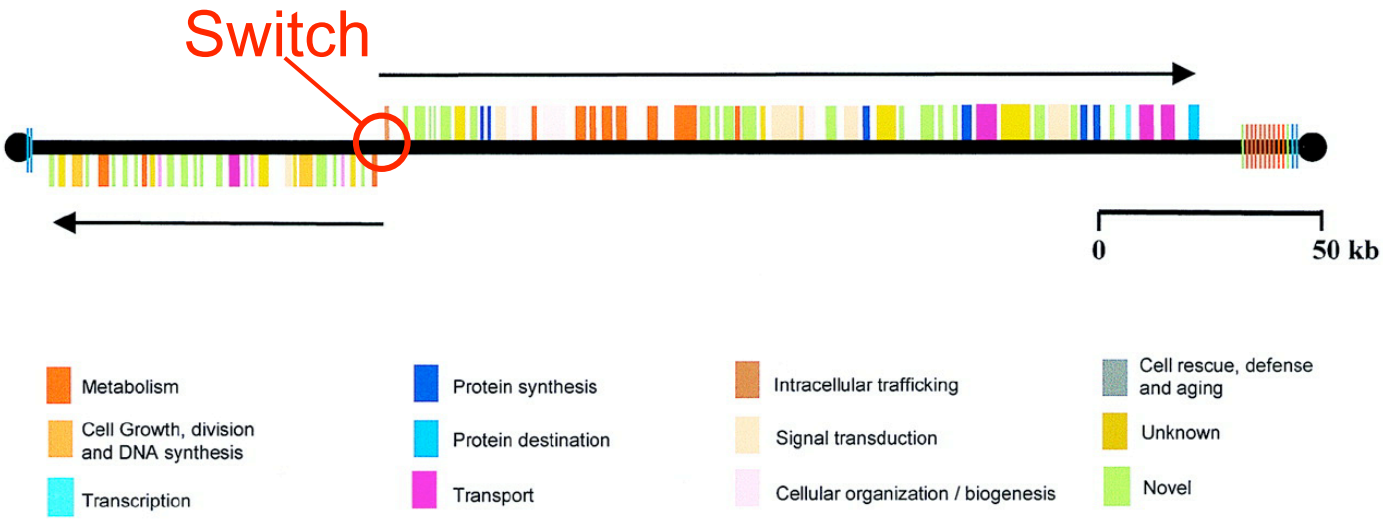
-One VSG gene expressed per tryp., no mixing

-Variation of expressed VSG is random

-~20 different ES (10-20 for metacyclic VSG expression)

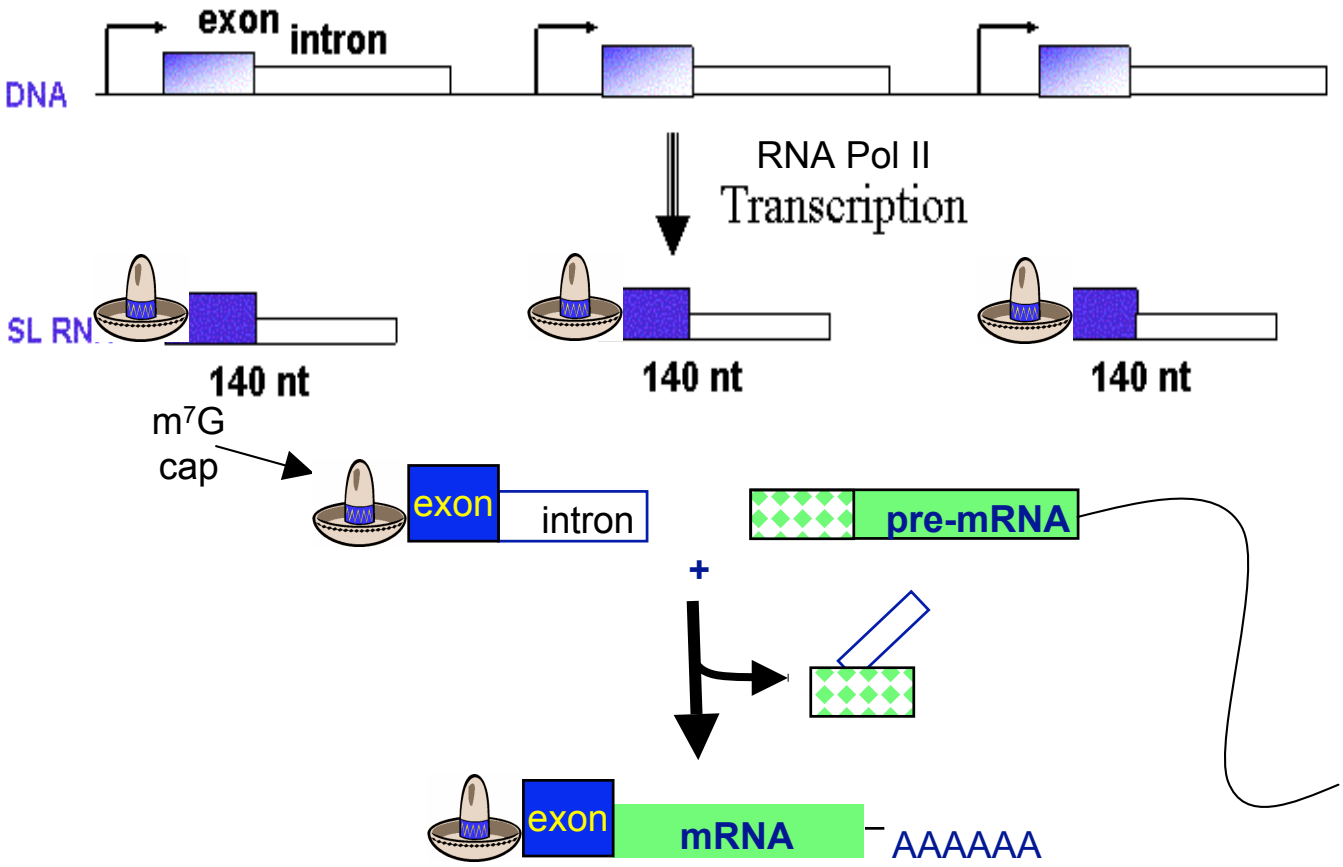


Leishmania major chromosome 1 gene organization: polycistronic transcription



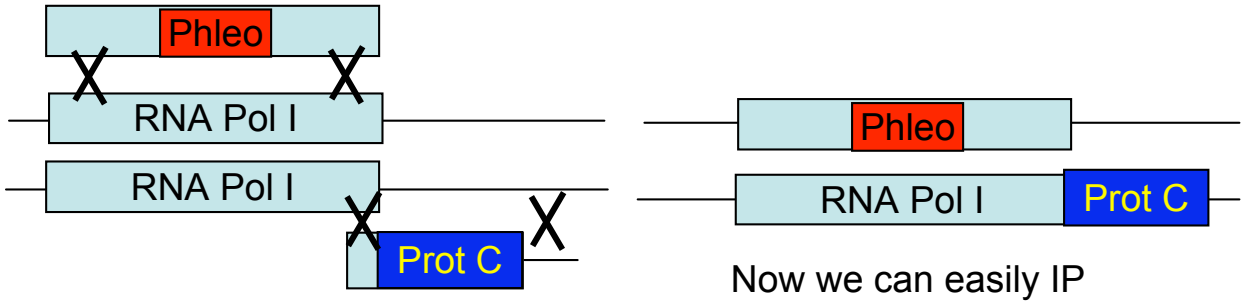
- No transcriptional regulation of mRNAs
- Adjacent genes are frequently not related in function
- *trans*-splicing resolves individual mRNAs from polycistronic transcripts

Spliced Leader and the Processing of Polycistronic pre-mRNAs into discreet messages



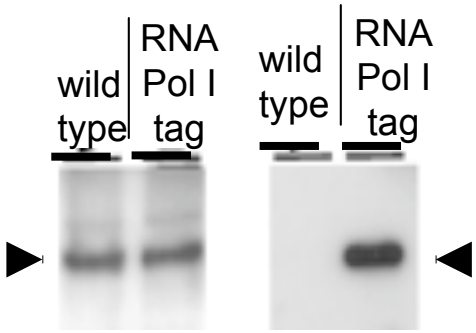
Epitope Tagging

Adding a sequence coding a peptide fragment that you already have Ab for.Protein C, HA, c-Myc, Strep, Flag, BB2….the list goes on and on.



Now we can easily IP and detect RNA Pol I

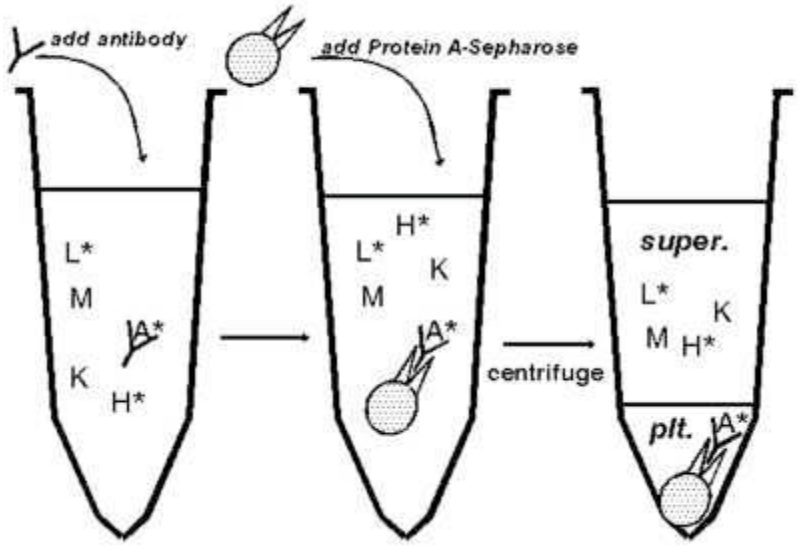
Cell extract from:



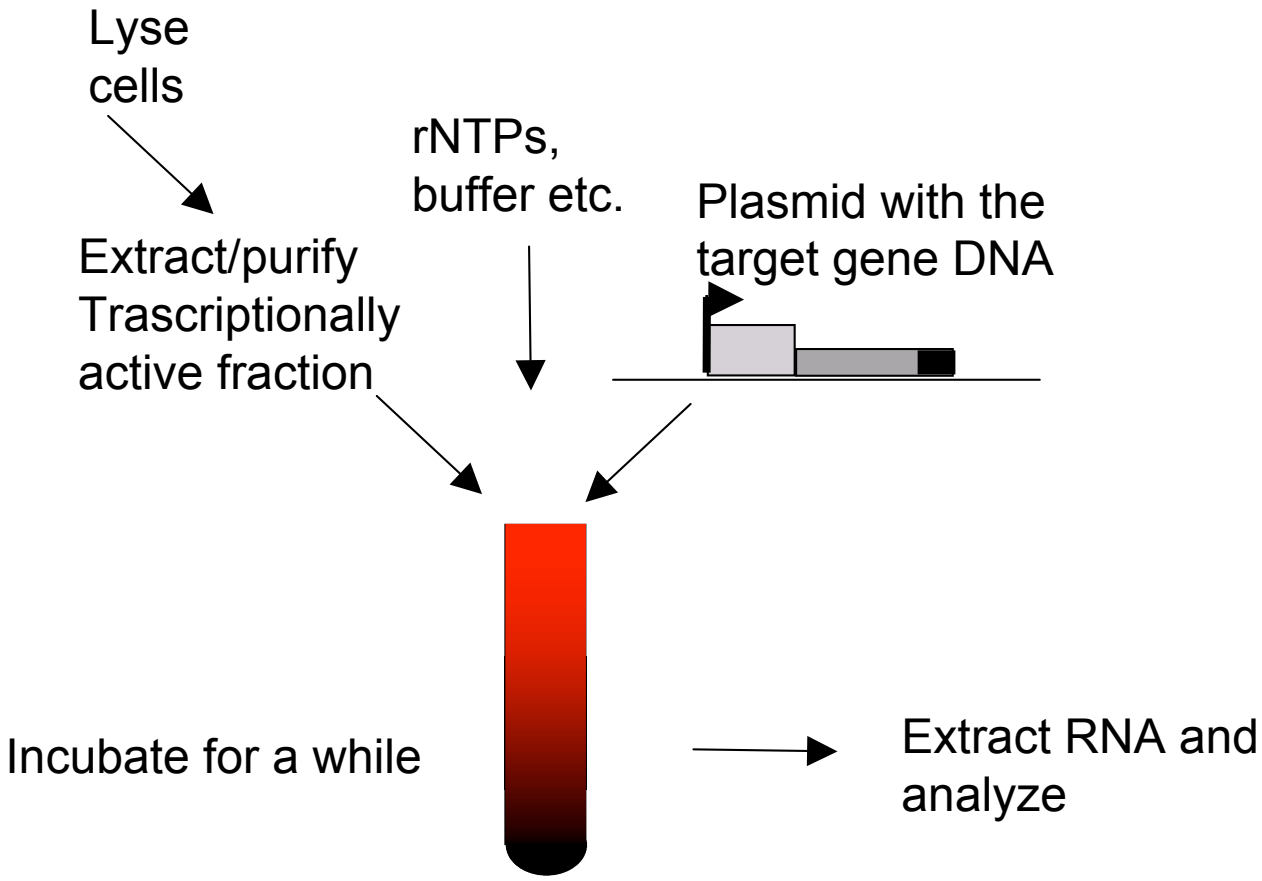
Anti-RNA pol I Anti-Protein C

Immunoprecipitation

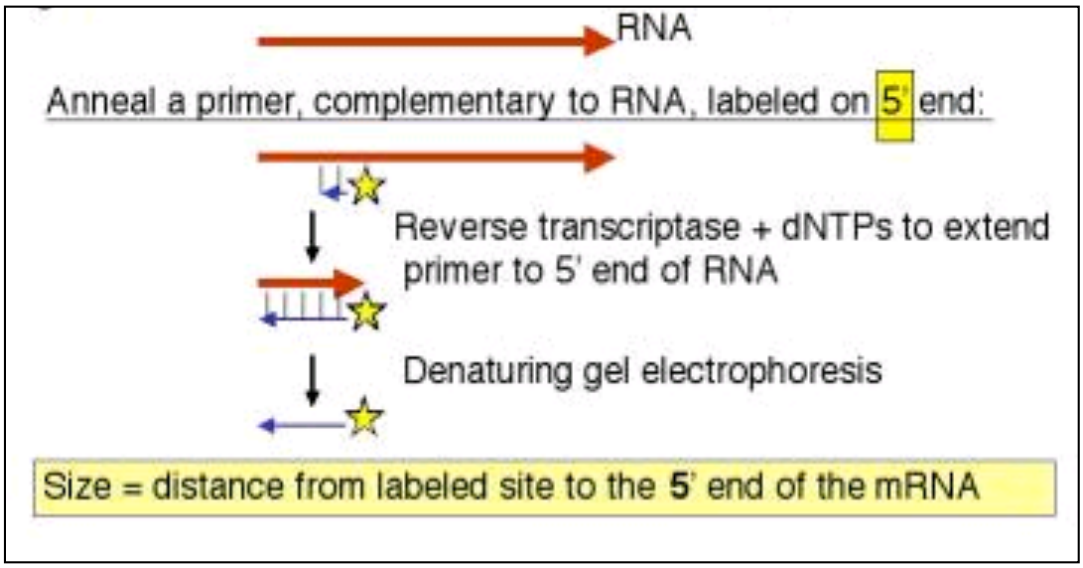
Protein A binds the Fc region of Abs



Invitro trancription



Primer Extension – measures RNA quantity



The Gunzl RNA POL I experiment

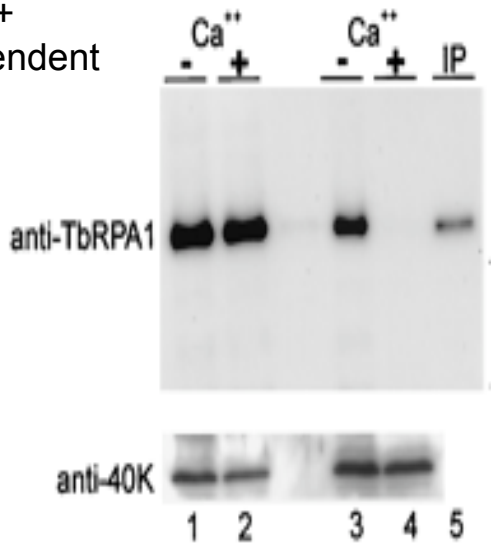
Immuno depletion

Invitro transcription

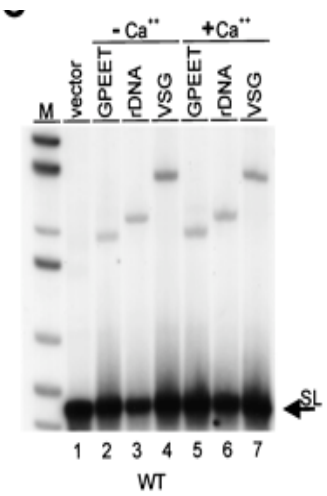
WT

Prot C

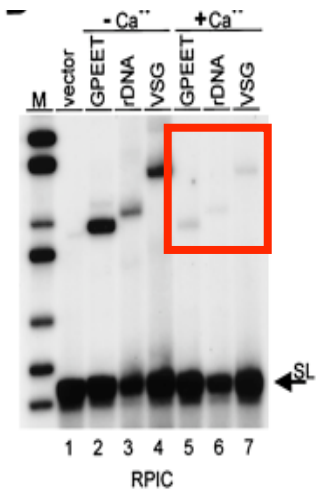
Ab binding is Ca++ dependent



WT



Prot C



Western blot detects the presence of RPA1

Primer extension to detect invitro trxn RNA

This shows that the extract is clear of tagged Pol I

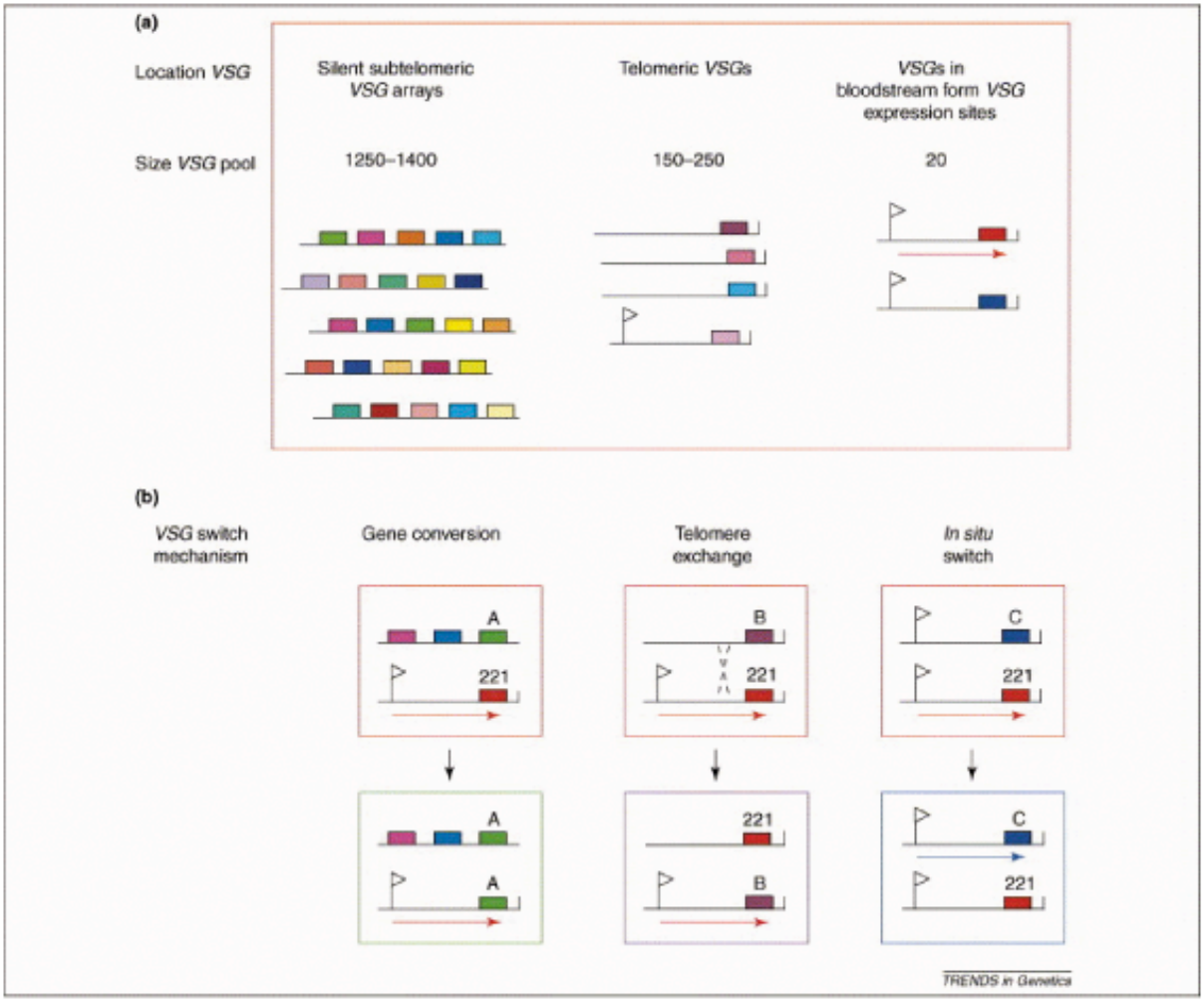
<u>One of these things is not like the other…</u>

Immunodepleting the extract abolishes Pol I transcription (Gpeet, rRNA, VSG) but does not change Pol II transcription(SL RNA)

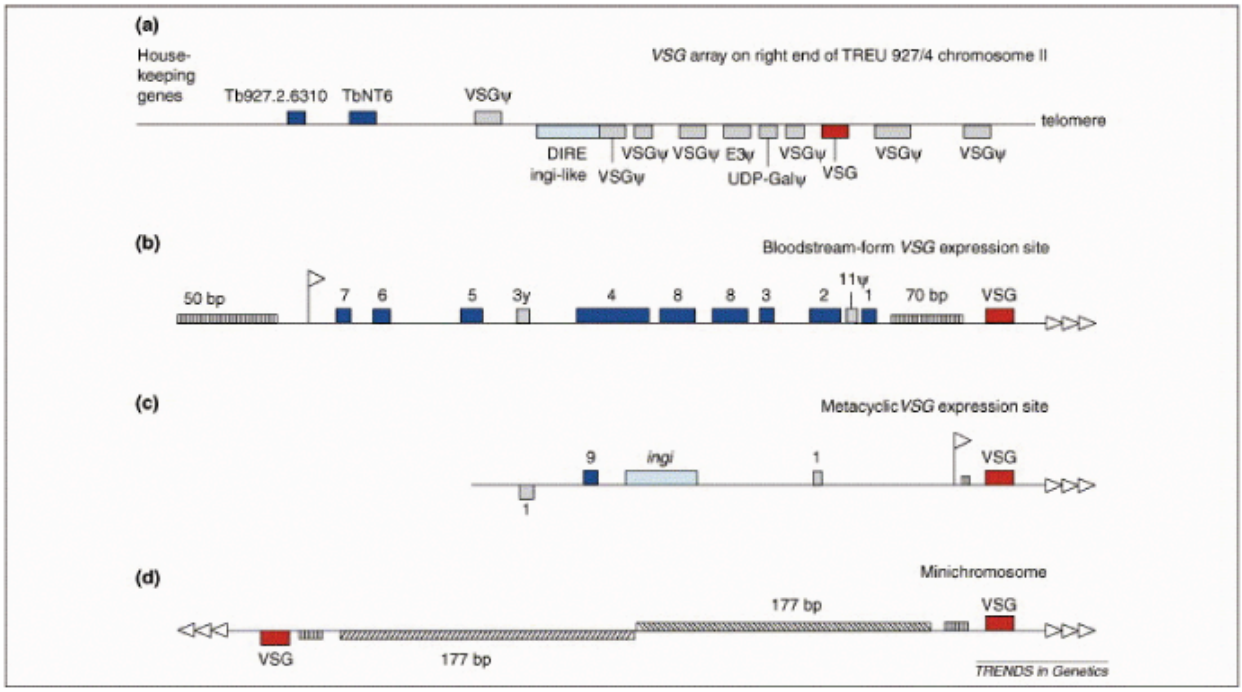
Compare the + CA with the - CA

VSG genes and VSG switching in African trypanosomes

(Taylor and Rudenko, Trends in Genetics, 22, 614-620)



Genomic location of *VSG* genes in *T. brucei* at **(a)** *VSG* gene arrays, **(b) bloodstream-form** *VSG* expression sites, **(c) metacyclic** *VSG* expression sites or **(d) minichromosomes.**



Progression VSG switching

Early:

Full genes stored generally in the minichromosomes

Late:

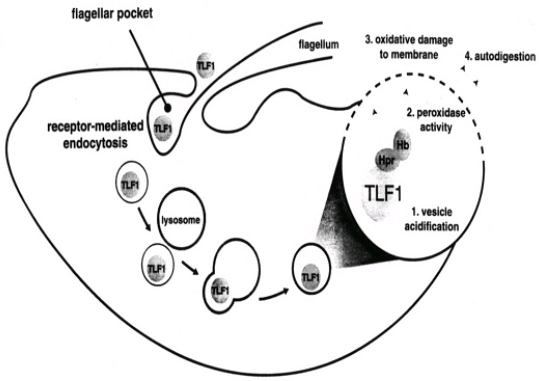
Mosaic VSG's made from multiple recombined pieces of sub telomeric psuedo genes

Trypanosome Lytic Factor 1

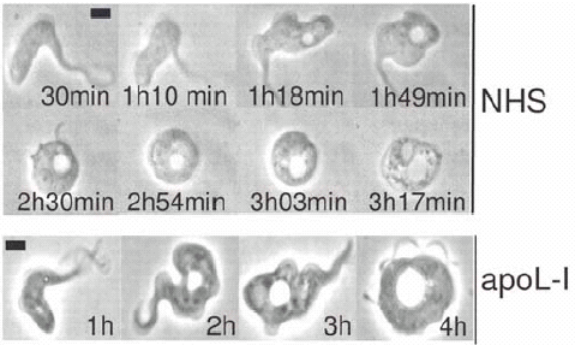
High density lipoprotein complex
(phospholipids, cholesterol, cholesterol ester, Apo-proteins)

ApoL-1 is the lytic factor of TLF1

TLF1 complex is endocytosed by the trypanosome



There is a fusion with the lysosome, swelling of lysosome, cell lysis



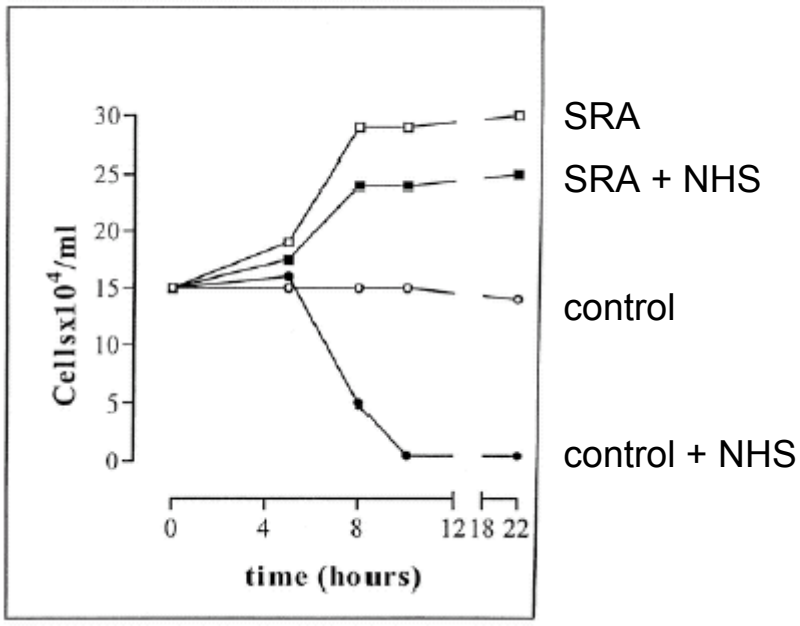
Serum Resistance-Associated Gene (SRA)

-occurs in natural human serum (NHS)

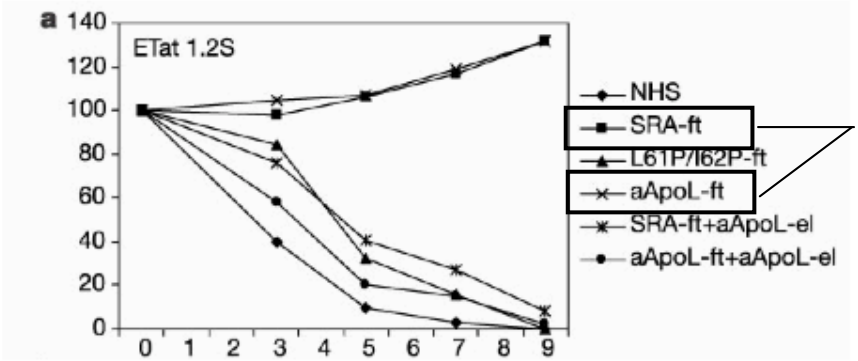
-structure similar to N-term. fold of VSG

-T.brucei lacks the SRA gene – lysed in humans

-addition of SRA to T.brucei confers resistance to lysis

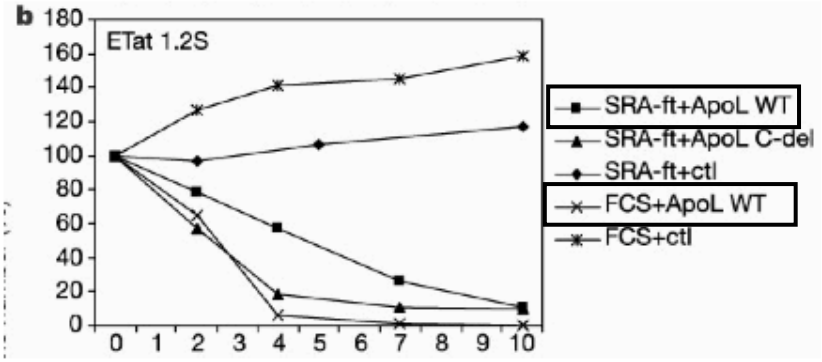


Removal of ApoL-I from NHS caused loss of lytic activity



These two columns bind ApoL-I, thus the flow through (FT) will be depleted for that protein. These depleted serums have lost lytic activity

Addition of recombinant ApoL-I restored lytic activity

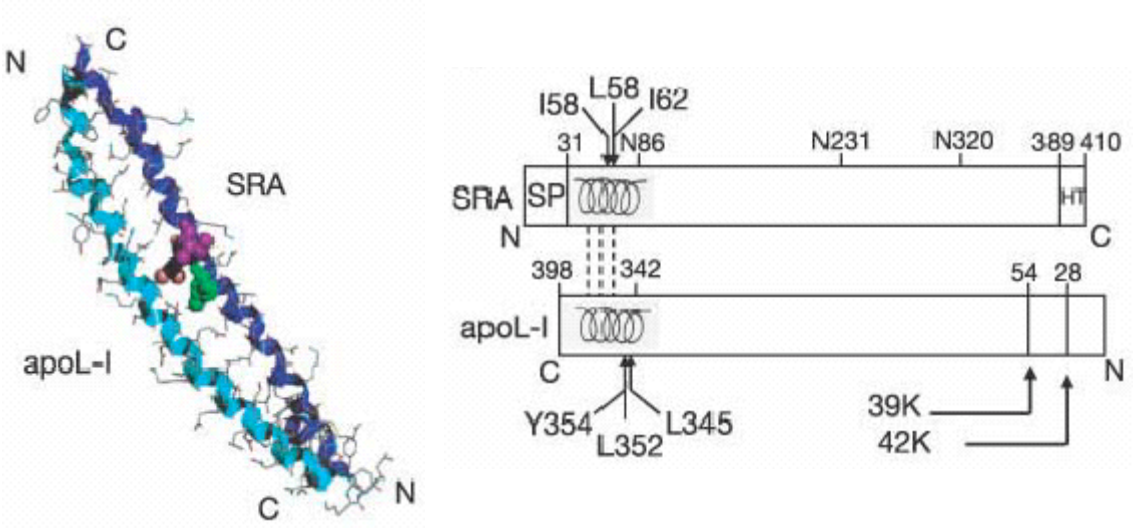


Adding Apol 1 (Apol-1 WT) back to either Apol-1 depleted serum (top box) or to FCS (bottom box) confers lytic activity to these serums

a, Incubation of ETat 1.2S with differently treated NHS (SRA-ft, L61P/I62P-ft, aApoL-ft indicate flow-through fractions from SRA–, L61P/I62P SRA– and anti-apoL-I–Sepharose, respectively; aApoL-el, eluate of the fraction bound to anti-apoL-I–Sepharose). b, Incubation of ETat 1.2S in either SRA-ft or FCS supplemented with recombinant apoL-I (C-del, lacking the C-terminal 343–398 peptide) or with the equivalent fraction from control CHO cells (ctl).

NHS = Normal Human Serum (lytic activity))
FCS=fetal calf serum (no lytic activity)

SRA Protein binds to apoL-1



Remember: ApoL-1 in NHS causes tryp. lysis
SRA confers resistance to lysis

Depletion of ApoL-1 (IP) from NHS removes lytic activity

Addition of ApoL-1 to depleted serum restores lysis