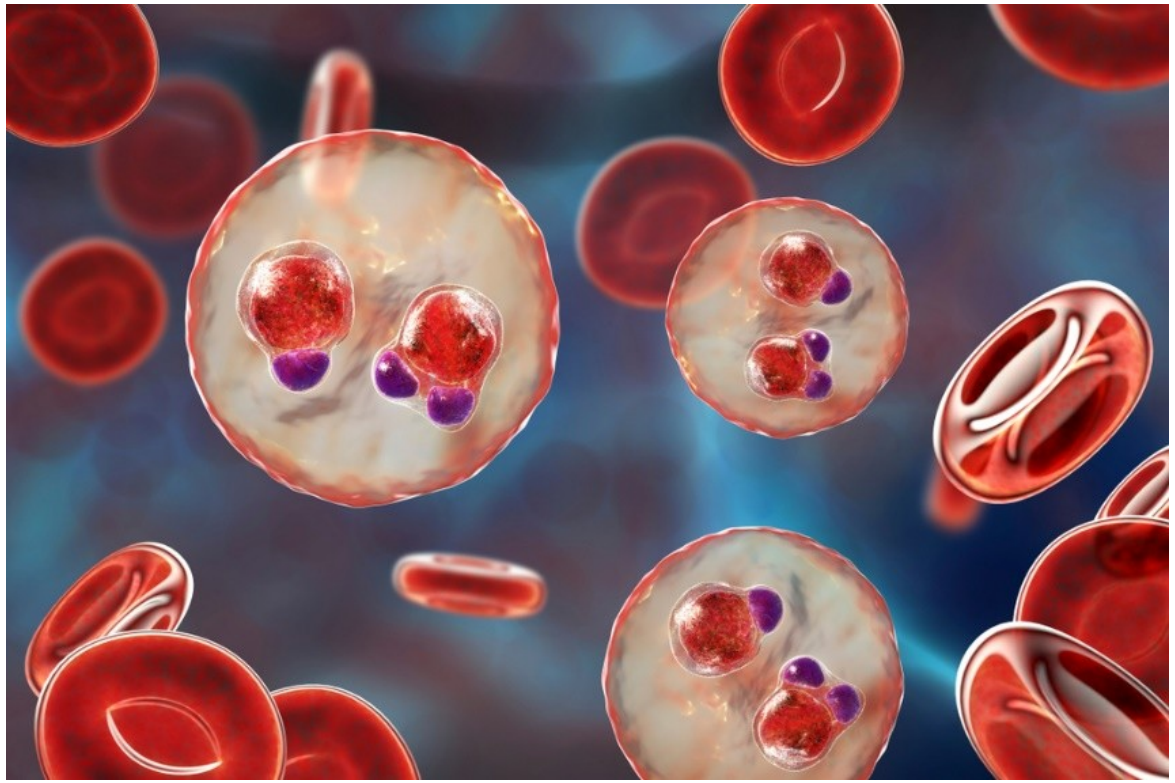
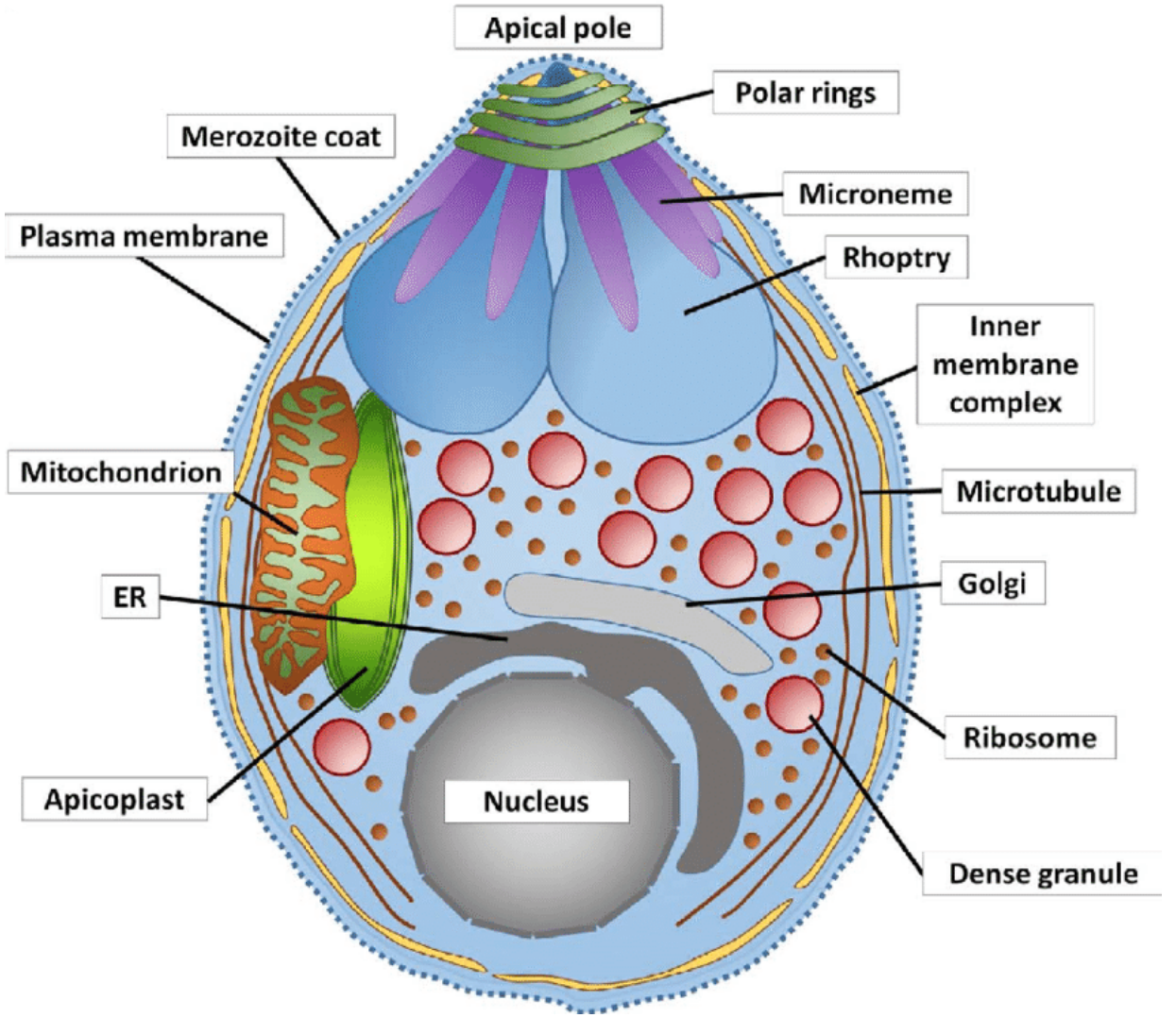
Apicomplexa
their genomes
mechanism of invasion



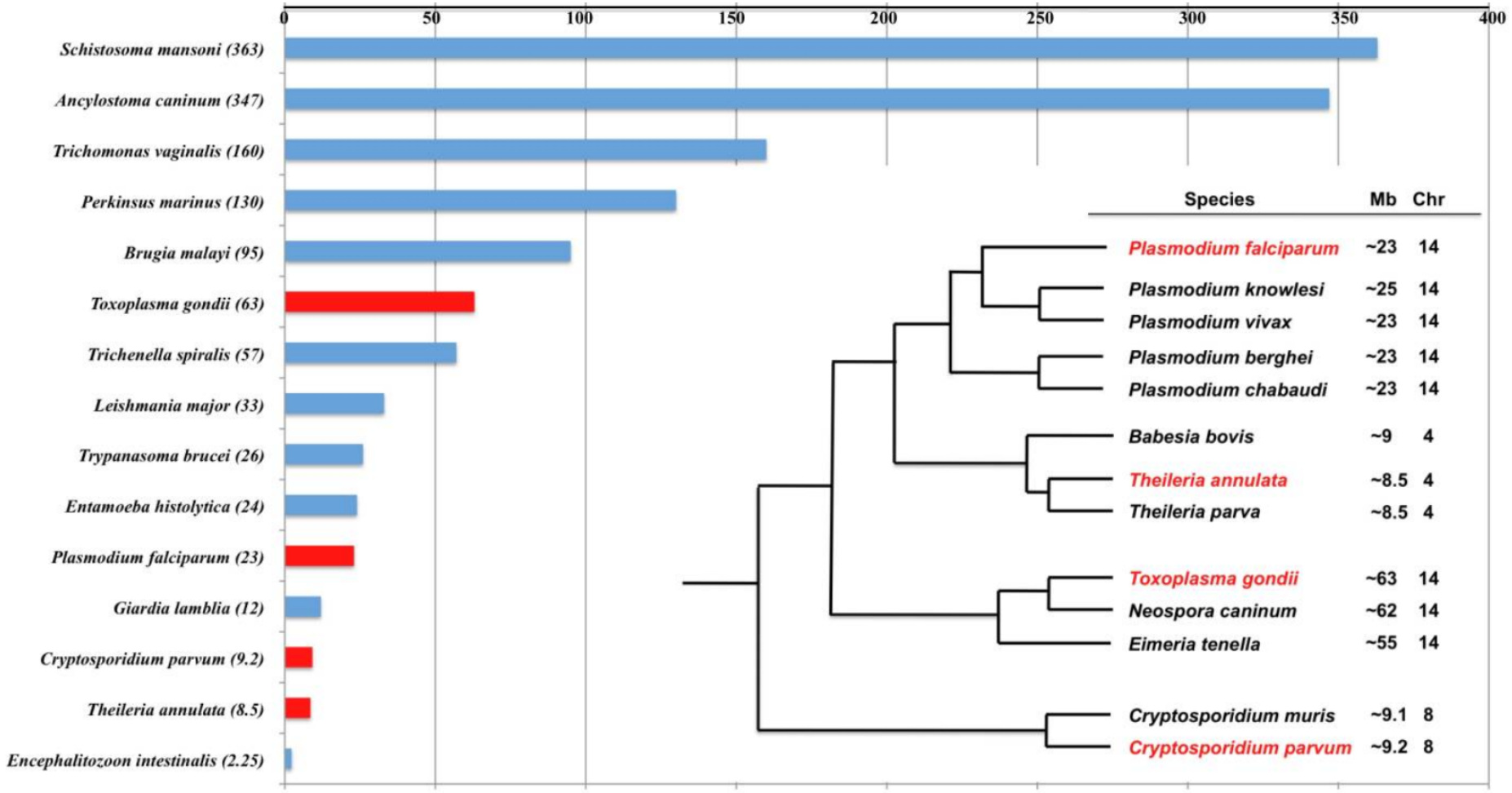
Apicomplexa – 3 genomes

- three genomes
  - nucleus
  - mitochondria
  - apicoplast



Apicomplexa – 3 genomes

- three genomes
  - **nucleus**
  - mitochondria
  - apicoplast



Apicomplexa – 3 genomes

- three genomes
  - **nucleus**
  - mitochondria
  - apicoplast

Apicomplexa

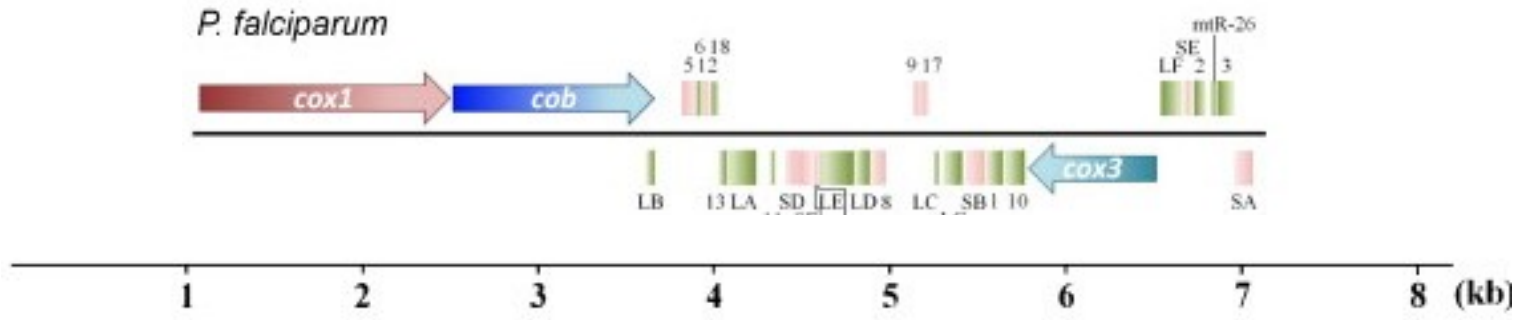
Organism	Relevance	Genome size	Number of genes predicted
Babesia bovis	Cattle pathogen	8.2 Mb	3,671
Cryptosporidium hominis	Human pathogen	10.4 Mb	3,994
Cryptosporidium parvum	Human pathogen	16.5 Mb	3,807
Eimeria tenella	Intestinal parasite of domestic fowl	55-60 Mb	
Neospora caninum	Pathogen for cattle and dogs	62 Mb	
Plasmodium berghei	Rabbit malaria	18.5 Mb	4,900
Plasmodium chabaudi	Rodent malaria	19.8 Mb	5,000
Plasmodium falciparum	Human pathogen (malaria)	22.9 Mb	5,268
Plasmodium knowlesi	Primate pathogen (malaria)	23.5 Mb	5,188
Plasmodium vivax	Human pathogen (malaria)	26.8 Mb	5,433
Plasmodium yoelii yoelii	Rodent pathogen (malaria)	23.1 Mb	5,878
Theileria annulata	Cattle pathogen	8.3 Mb	3,792
Theileria parva	Cattle pathogen (African east coast fever)	8.3 Mb	4,035
Toxoplasma gondii	Mammal pathogen	63 Mb	8,100

Ciliophora

Tetrahymena thermophila	Model organism of ciliates	104 Mb	27,000

Plasmodium spp. – mitochondrial genome

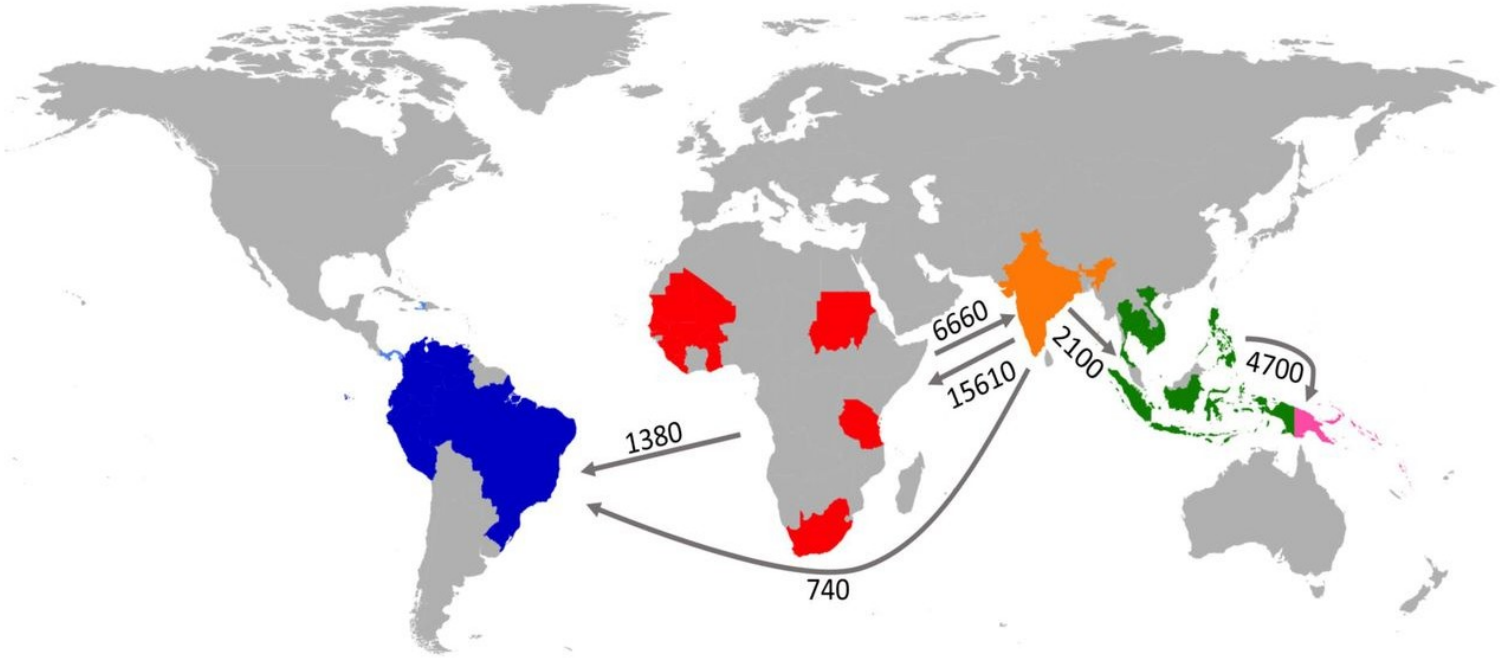
- **the smallest mitochondrial genome sequenced**
- the 5,967 bp mtDNA
- the form of a circular and/or tandemly repeated linear element
- encodes **only three mt protein-coding genes** (cox1, cox3 and cob) in addition to the large subunit (LSU) and small subunit (SSU) **ribosomal RNA (rRNA) genes**



- the two rRNA genes are highly fragmented with 20 rRNA pieces having been identified
- **the mt-genome organization is perfectly conserved among *Plasmodium* species**
- pairwise **sequence similarity** of complete mt-genome sequences between these species is very high at 84%–99%

Plasmodium spp. – mitochondrial genome

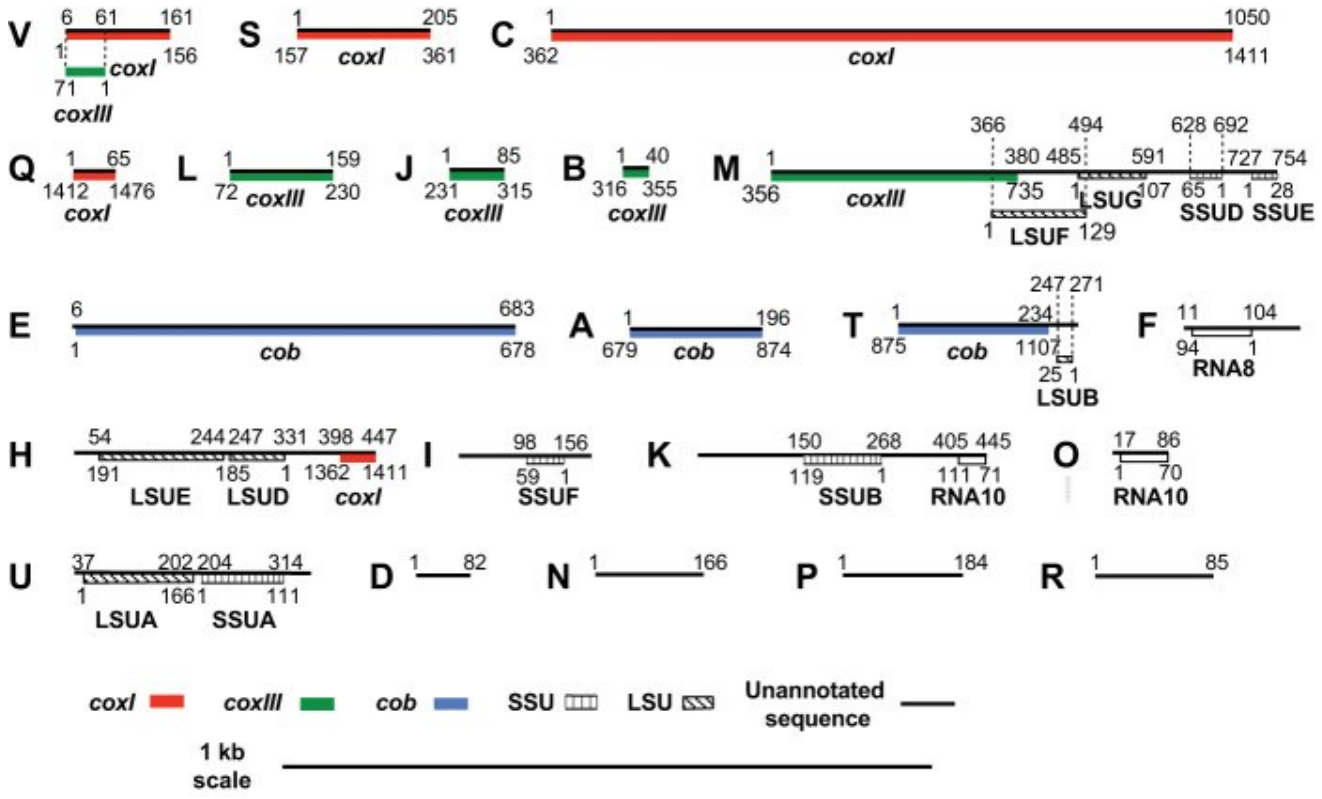
› phylogenetic analysis and coalescent-based gene flow modeling to a global collection of *Plasmodium falciparum* **mitogenome** to infer the **demographic history and geographic origins of malaria parasites**



enslaved Africans were likely the main carriers of *P. falciparum* mitochondrial lineages into the Americas after the conquest,
additional parasites carried by Australasian peoples in pre-Columbian times may have contributed to the extensive diversity of extant local populations of *P. vivax*.

Toxoplasma – mitochondrial genome

- evolution of a novel genome architecture consisting minimally of 21 sequence blocks (SBs)
- totaling 5.9 kb that exist as nonrandom concatemers

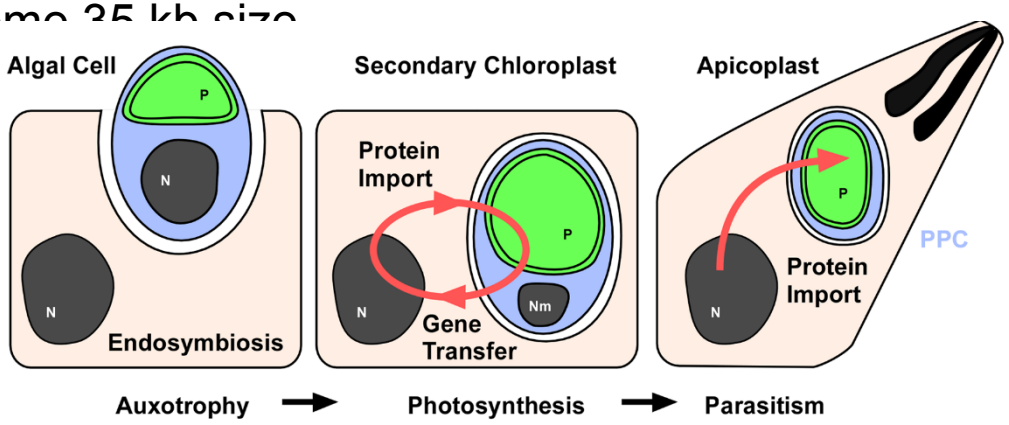


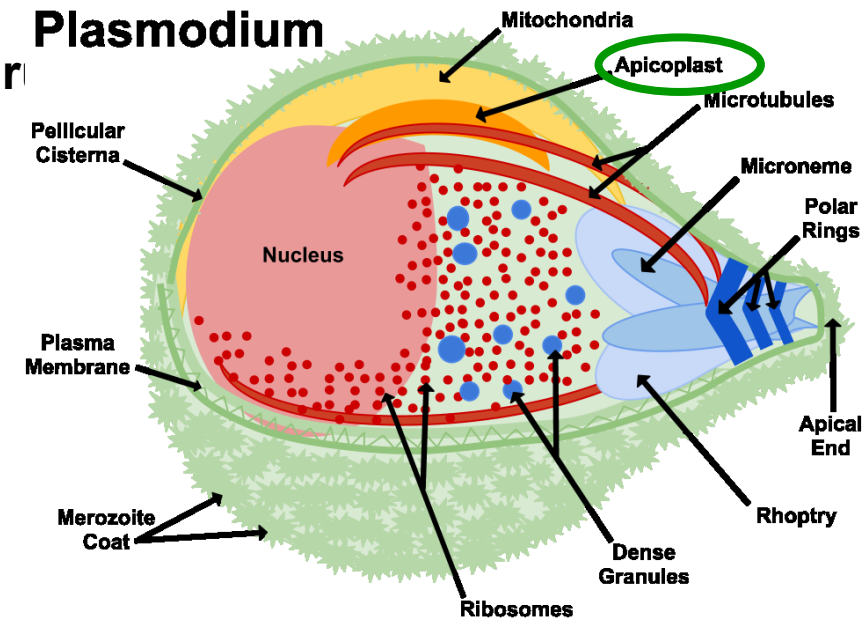
The 21 minimal *T. gondii* mtDNA sequence blocks.

The DNA sequence is represented by a black line, drawn to scale and named with 21 alphabet characters, A to V (there is no “G”). The coordinates of an SB that encodes a cytochrome or rRNA gene fragment are indicated *above* the black line and the corresponding coordinates of the assembled gene or rRNA fragment are indicated *below* the gene fragment; the fragments are colored as defined in the key. Portions of sequence block V contribute to both *coxI* and *coxIII* but in different orientations.

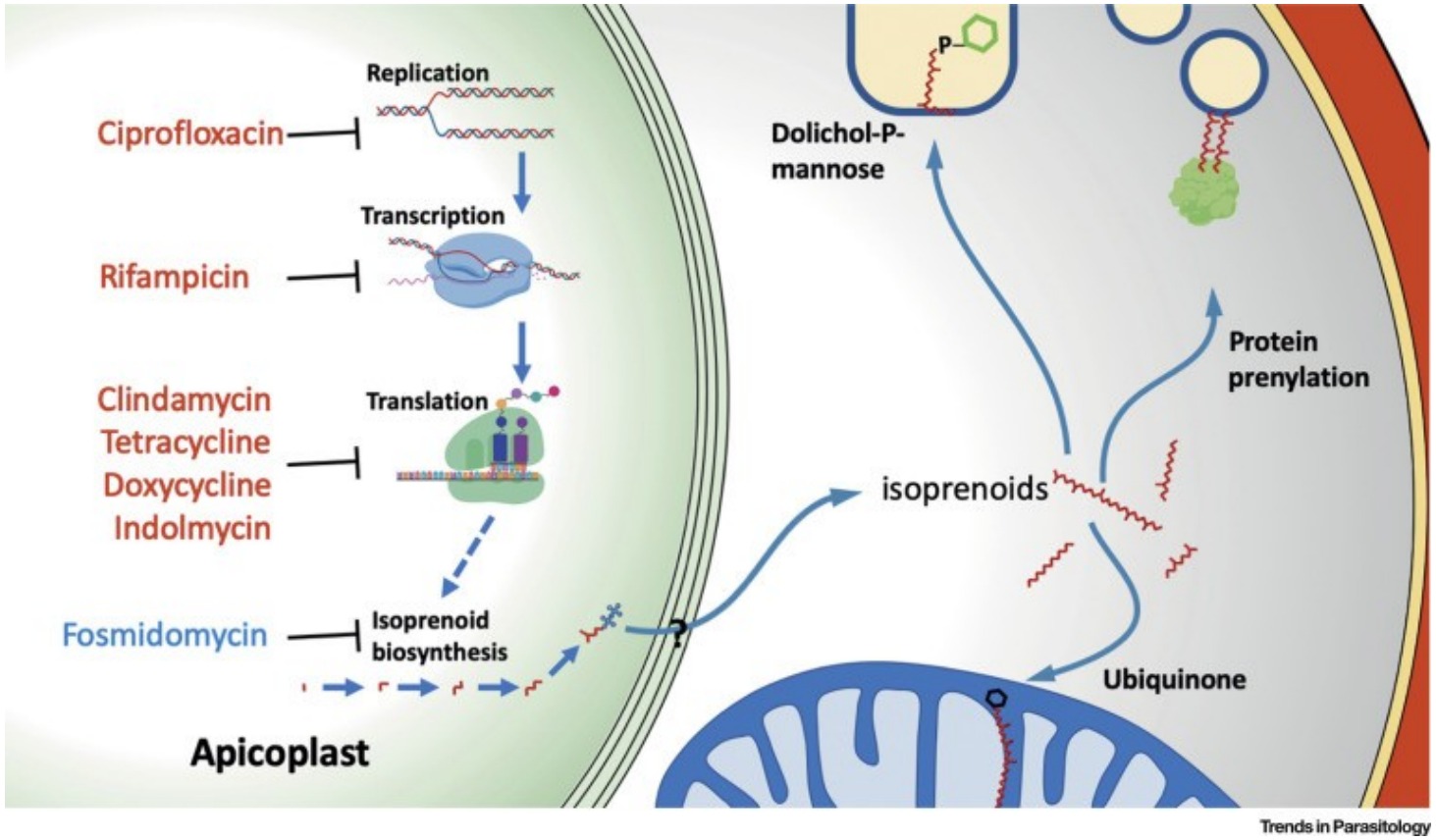
APICOMPLEXA: apicoplast

- **non-photosynthetic plastid** found in most Apicomplexa
- originated from an alga through **secondary endosymbiosis**
- there is debate as to whether this was a green or red alga
- surrounded by **four membranes** within the outermost part of the endomembrane system
- **vital to parasite survival**
- hosts **important metabolic pathways** like fatty acid synthesis, isoprenoid precursor synthesis and parts of the heme biosynthetic pathway
- **absent in some of apicomlexans** (eg. *Cryptosporidium*)
- apicoplasts' plant-like properties provide **a target for herbicidal dr**
- enticing **target for antimalarial drugs**
- ancestral genome 35 kb size

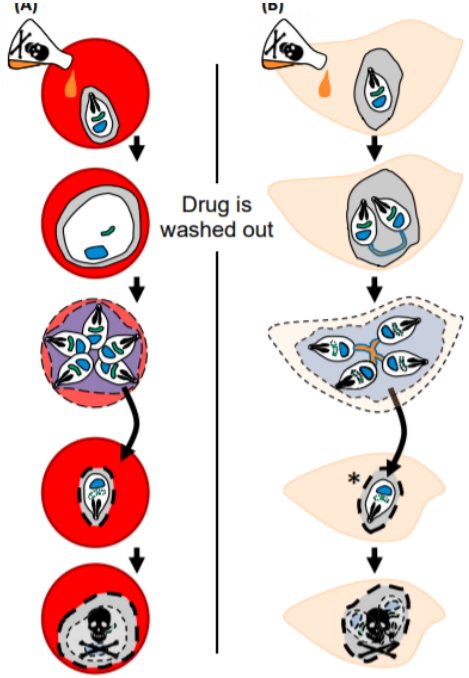




Apicoplast as a drug target



„delayed death“



Kennedy et al., Trends in Parasitol

Apicoplast genome

- **Apicoplast genomes are quite similar**, suggesting that much of the reduction in coding capacity happened prior to splitting the apicomplexan lineages

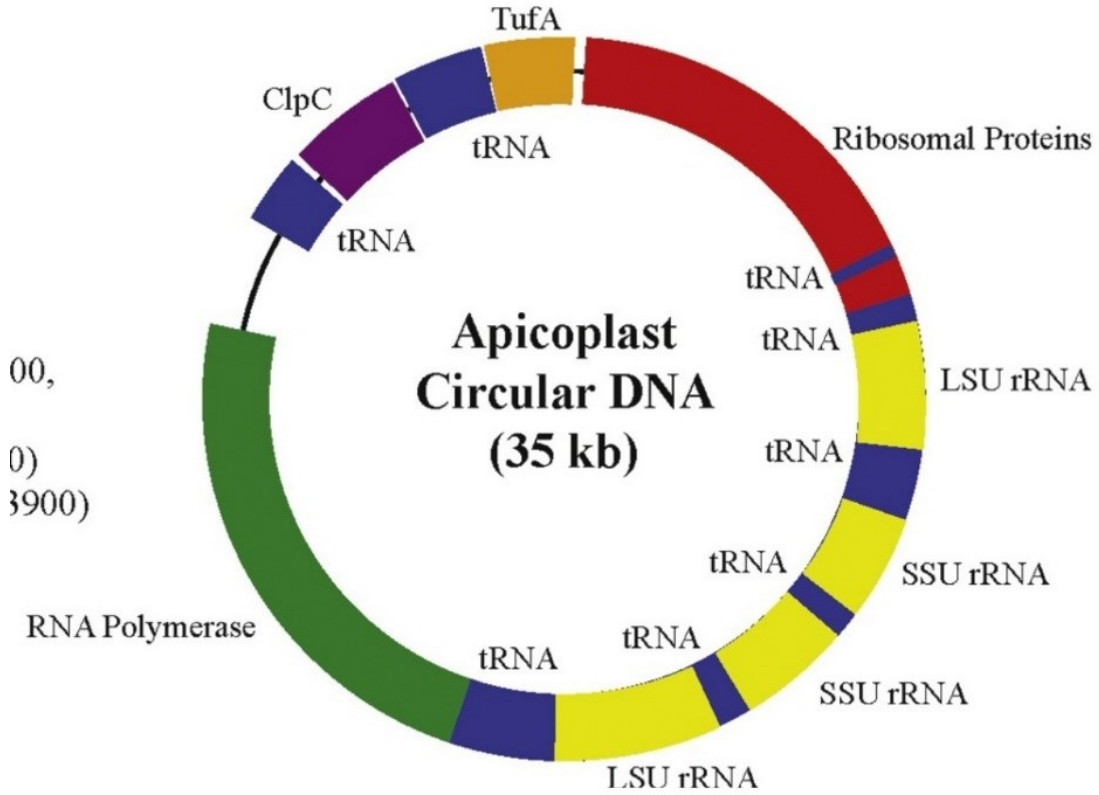
- the apicoplast genome has been under h**igh selective pressure** - **reducing the genome size**
- (chloroplast genomes average 150–200 kb in size, those of non-photosynthetic plants are ~70 kb)
  - *P. falciparum* (Wilson *et al.,* 1996)
  - *T. gondii* (ToxoDB)
  - *E. tenella* (Cai *et al.,* 2003)
    - **all ~35 kb in size**
- small subunit (SSU) and large subunit (LSU) rRNAs encoded head to head
- separated by seven tRNA genes
- single tRNA gene is found at the 3′ ends of both rRNAs
- this organization is highly reminiscent of chloroplast genomes

25 copies of the apicoplast genome in *T. gondii* and 15 in *P. falciparum*
multiple copies of a genome would facilitate repair of mutations by gene conversion

Apicoplast genome of *Plasmodium*

- low complexity and primarily **encodes genes involved in its own expression**
- one of the most **A/T-rich genomes** known to date with **86.9% A/T**
- contains **68 genes** coding for the large and small subunit rRNAs, a minimal but complete set of tRNAs, ribosomal proteins, three subunits of a bacterial-like RNA polymerase, and several protein chaperones

- **the gene content of the apicoplast genomes is highly conserved** apart from a few lineage specific genes
- **SSU and LSU rRNAs** (*rrs* and *rrl*)
- three subunits of the bacteria-type **RNA polymerase** (*rpoB*, *rpoC1*, *rpoC2*)
- 16 **ribosomal proteins**, an EF-Tu, a ClpC-like protein
- 24 **tRNA** species, the minimum sufficient for translation without importing a tRNA from the cytosol

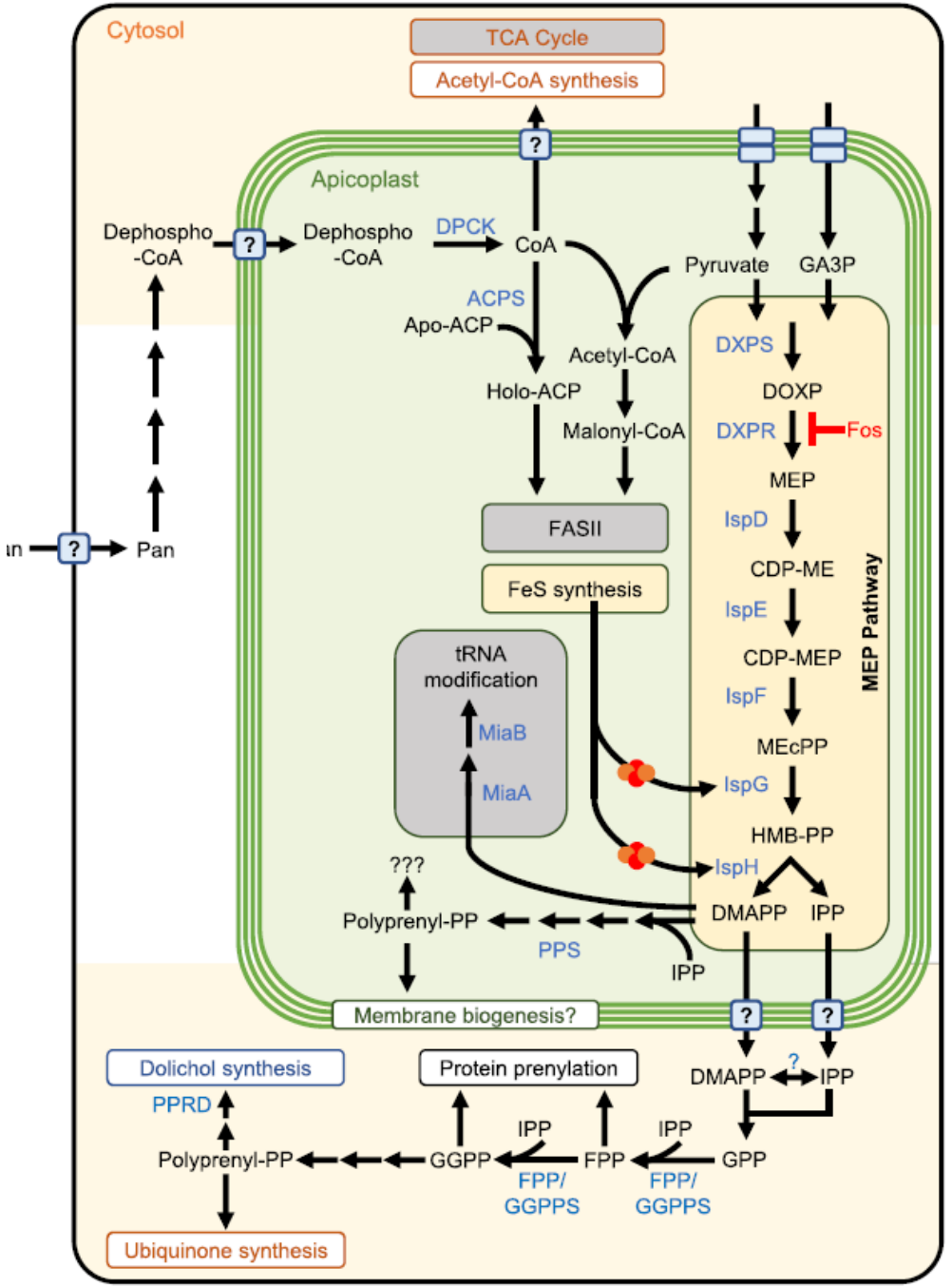


Apicoplast function

apicoplast proteins encoded by the nuclear genome

- **a bipartite organellar targeting sequence at the N terminus**
  - more than 500 proteins encoded by the nuclear genome of *P. falciparum* have an apicoplast targeting sequence

- enzymes involved in:
  - de novo biosynthesis of **isoprenoid, fatty acid and heme**
  - housekeeping proteins such as DNA polymerase, DNA gyrase subunits, ribosomal proteins, molecular chaperones
  - components of a Suf type **Fe–S cluster assembly system**



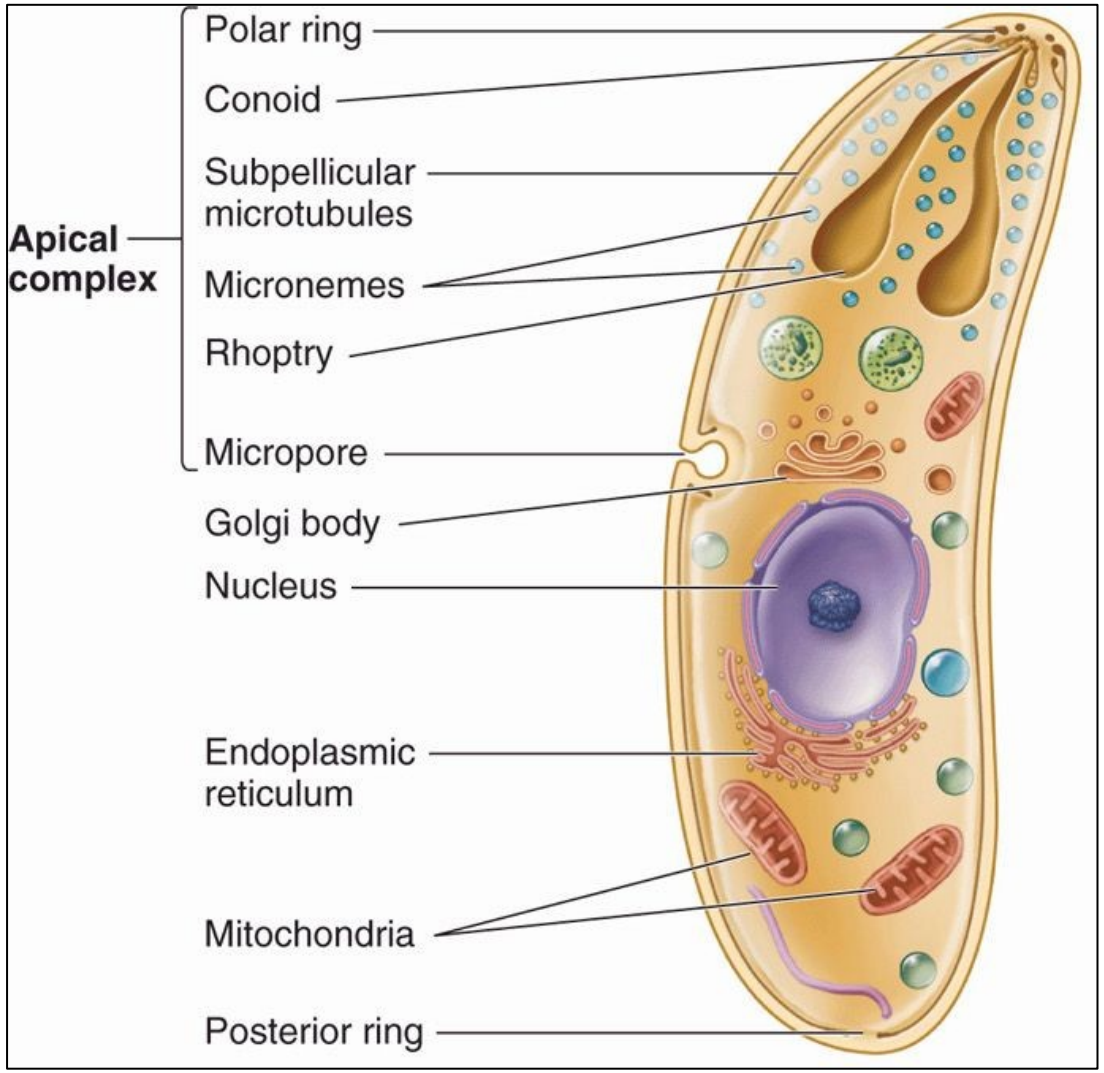
APICAL COMPLEX

only in invading stages

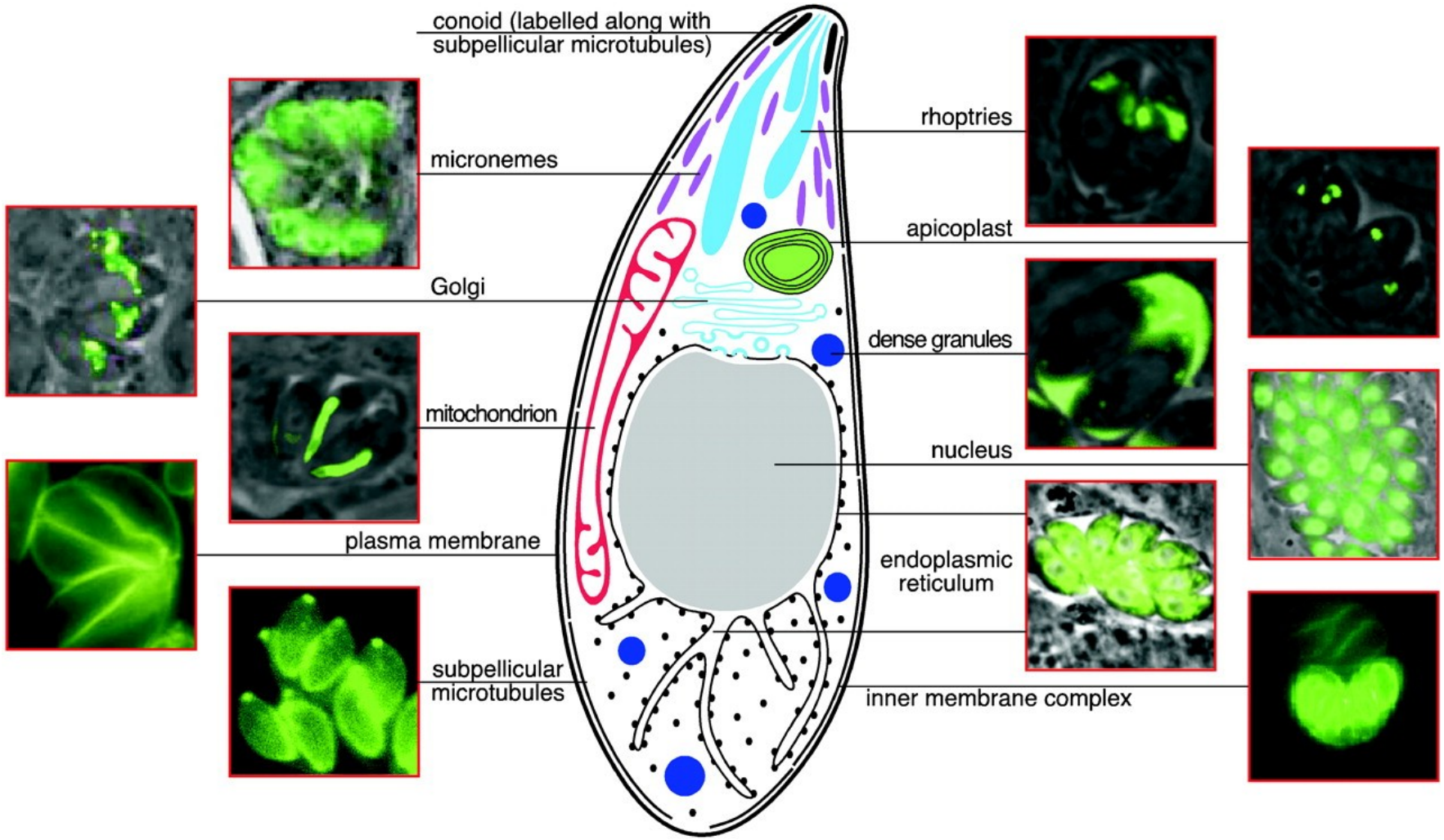
„zoit“

repeated dedifferentation and differentation in Apicomplexa life cycle

2 components
skeletal and secretory

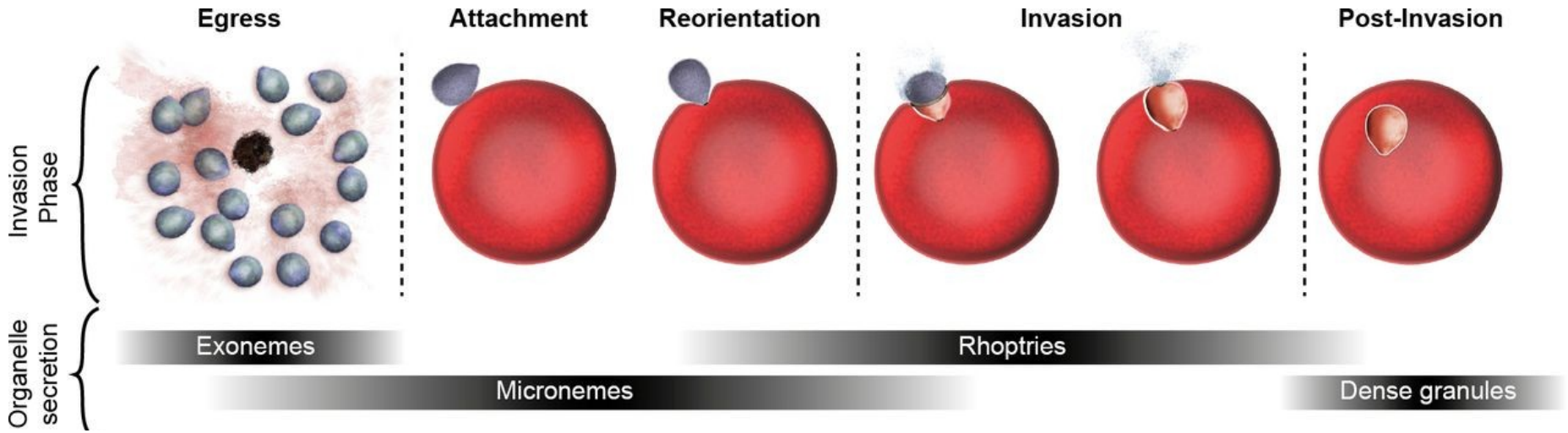


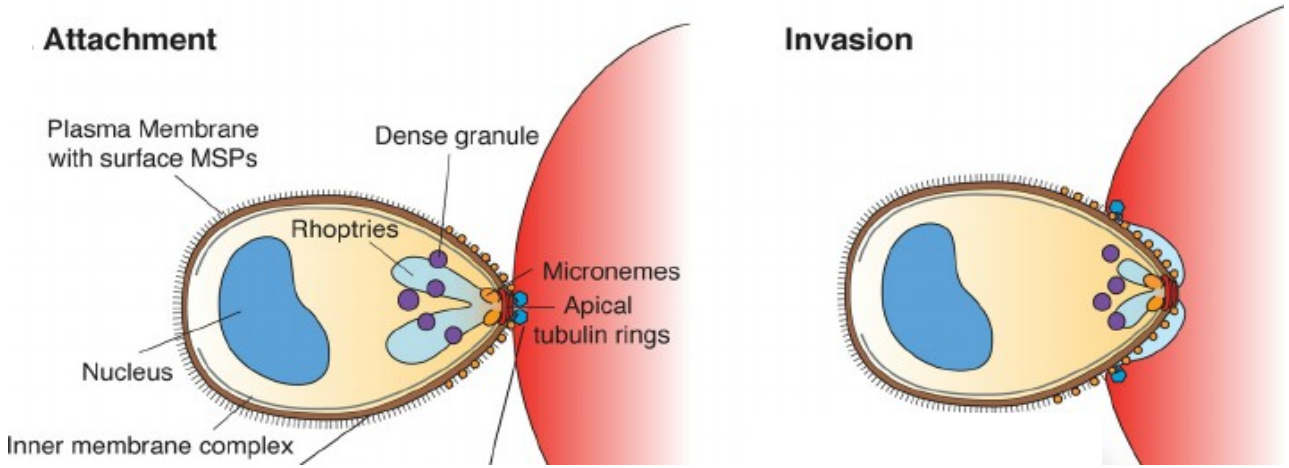
ZOIT = infecting stage



INVASION into host cells

proteins secreted from apical complex
required apical-end zoite orientation

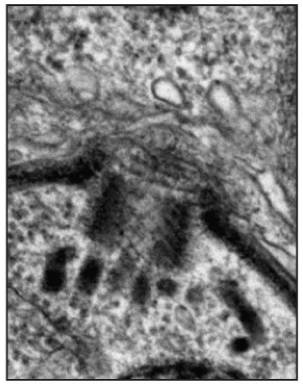




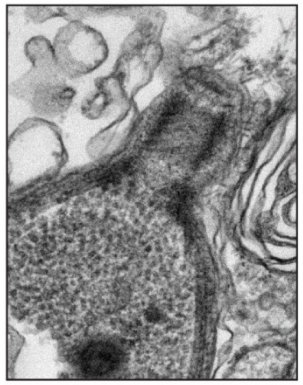
SKELETAL PART OF APICAL COMPLEX

The alveoli and proteinaceous skeleton form a structure called the **inner membrane complex (IMC),** which, together with the subpellicular microtubules, provides the shape and stability of the cell.

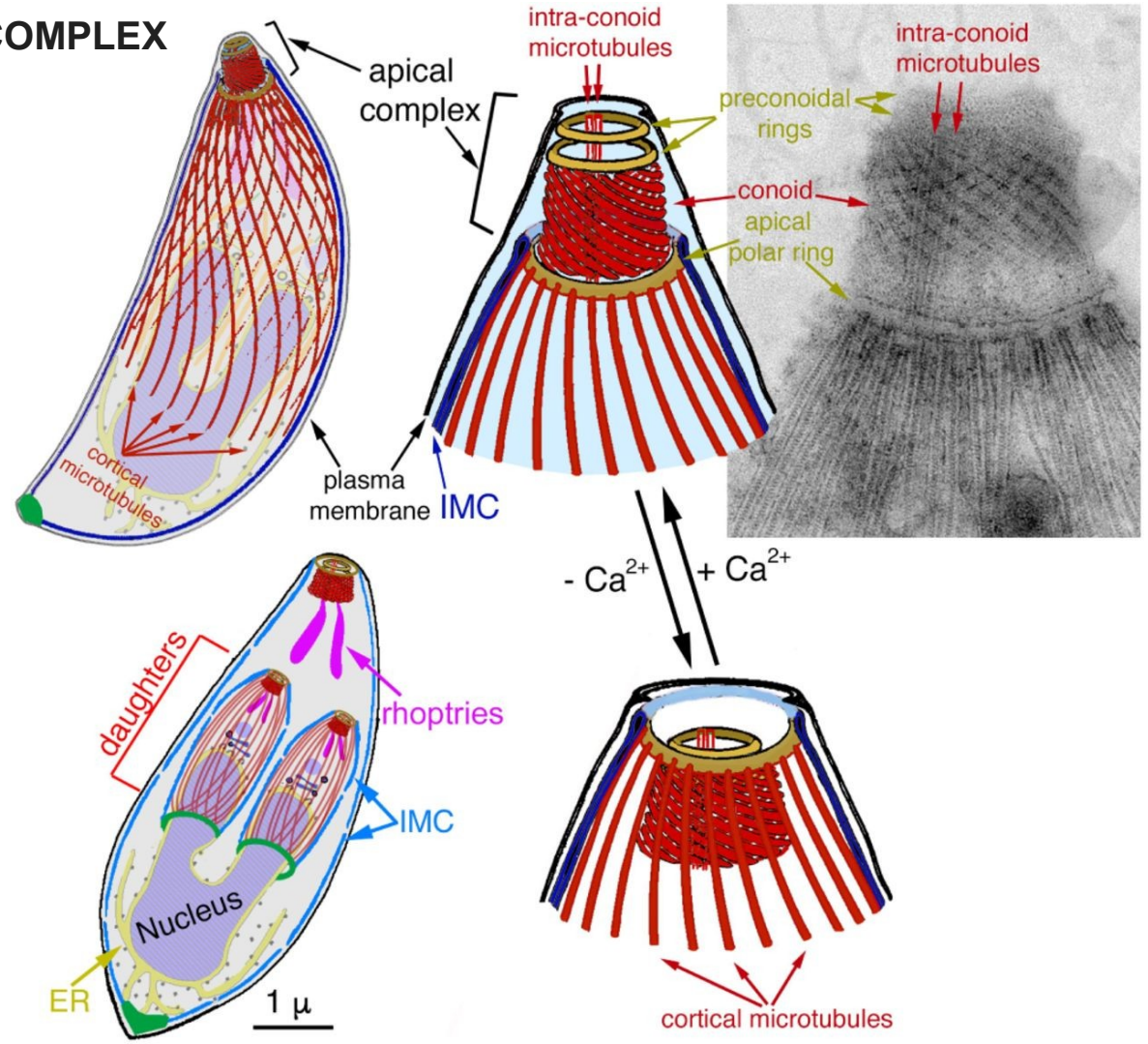
The most apical portion of IMC is APICAL COMPLEX



non-protruded



protruded

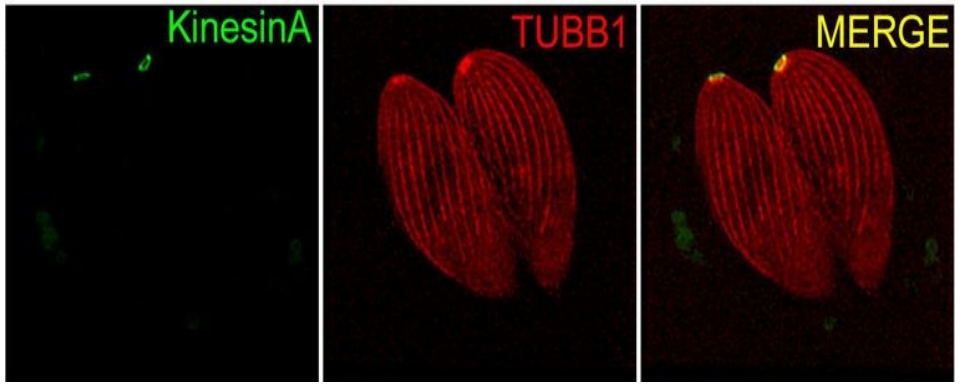


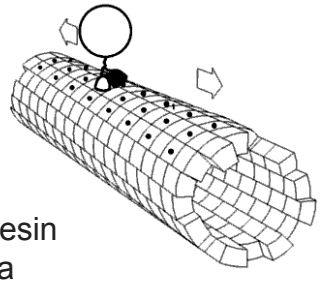
conoid is absent in *Plasmodium*, *Babesia* and *Theileria*

SKELETAL PART OF APICAL COMPLEX

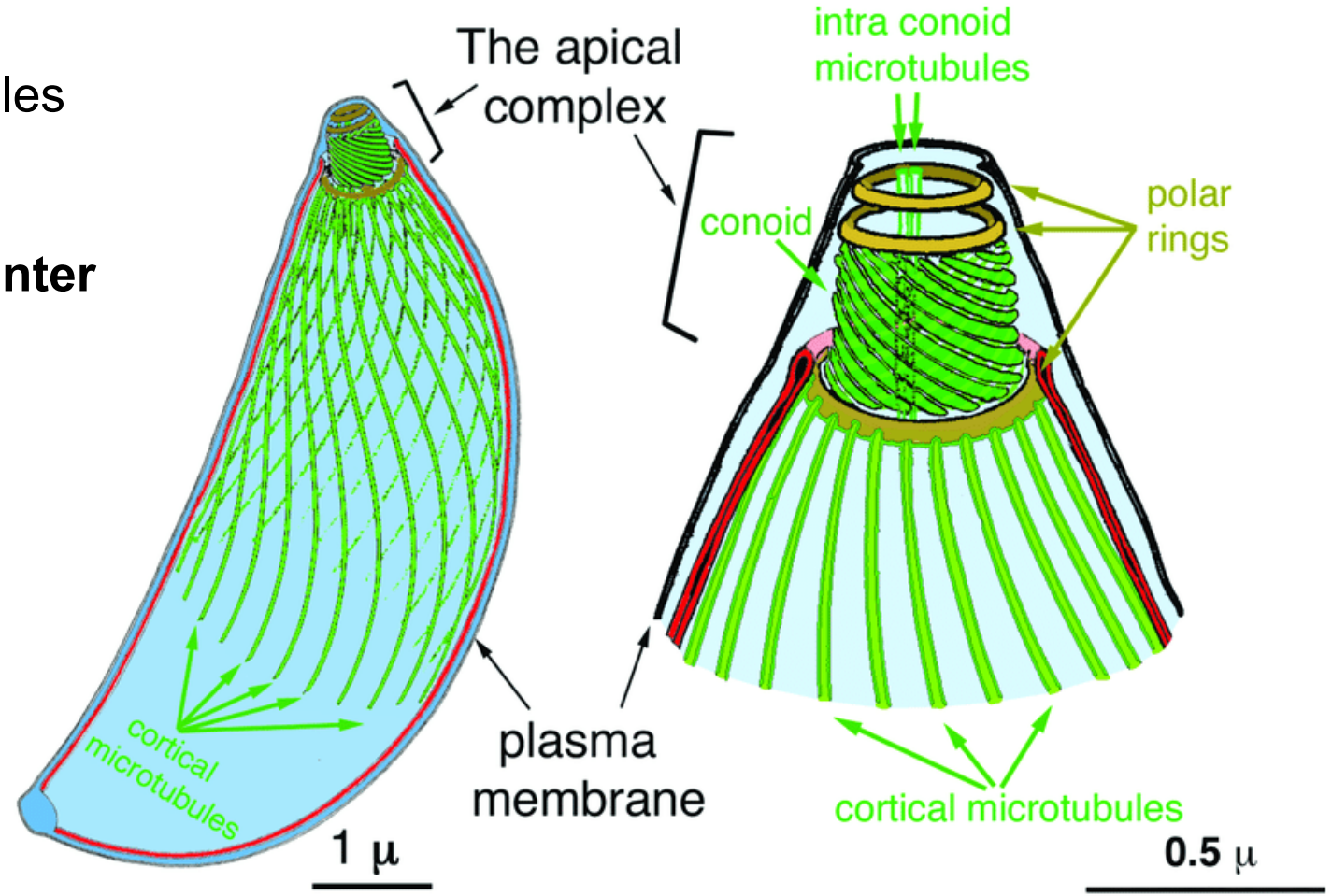
- **preconoidal rings** (= apical rings)
- **conoid** = spirally arranged microtubules
- one or more **polar rings**

- **MTOC – microtubule organizing center**



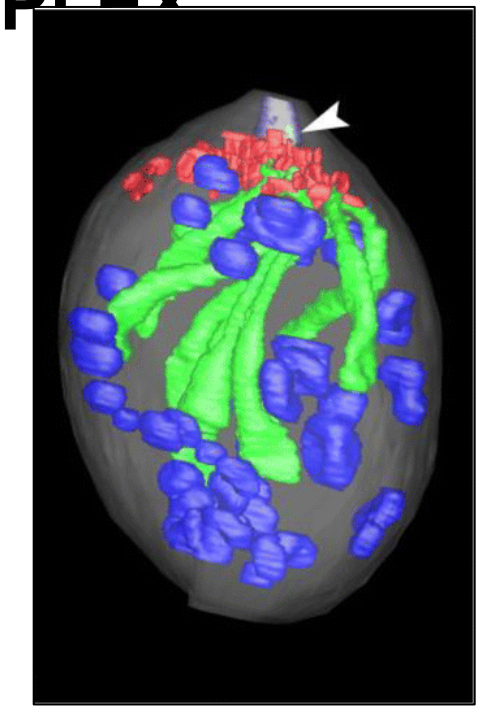


Animation of kinesin "walking" on a microtubule



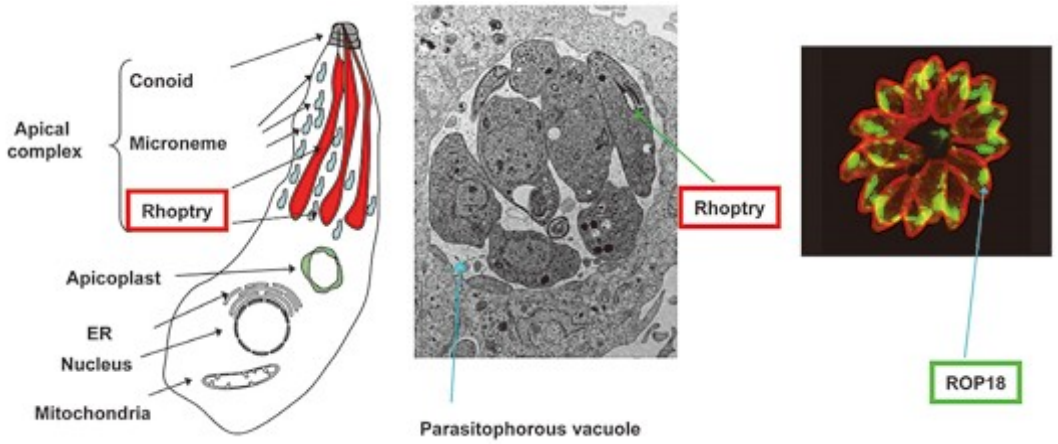
SECRETORY PART OF APICAL COMPLEX

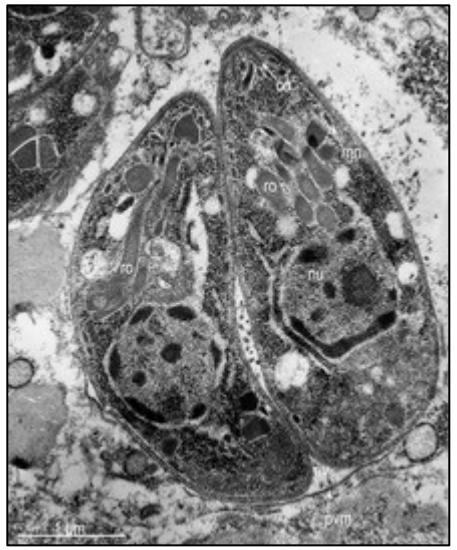
Rhoptries + Micronemes + Dense Granules



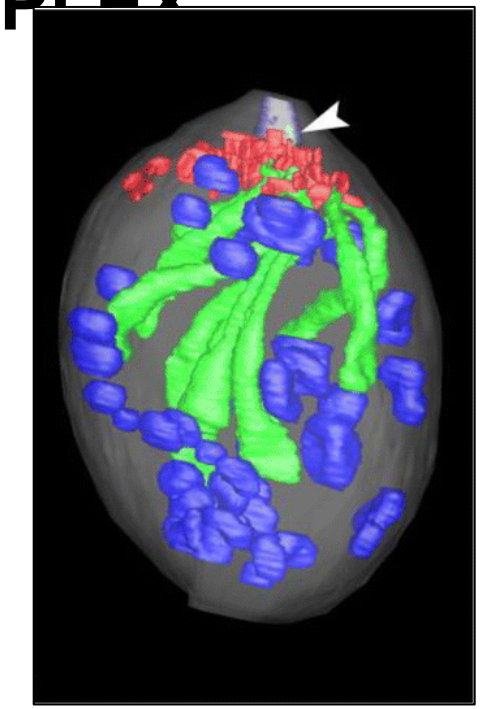
Rhoptries

- club-shaped organelles connected by thin necks to the extreme apical pole of the parasite
- enzymes that are released during the penetration process → internalization into the host cell
- egress from the host cell
- proteins to create parasitopholous vacuole and establishment the parasite inside
- modification of the surface of the host cell





SECRETORY PART OF APICAL COMPLEX



Micronemes

› proteins specialized in attachement onto host cell surface receptors and facilitating erythrocyte entry
› only by this initial chemical exchange can the parasite enter into the erythrocyte via actin-myosin motor complex
› motility → TRAP protein

Dense granules

› secretion takes place after parasite invasion and localization within parasitophorous vacuole
› persists for several minutes

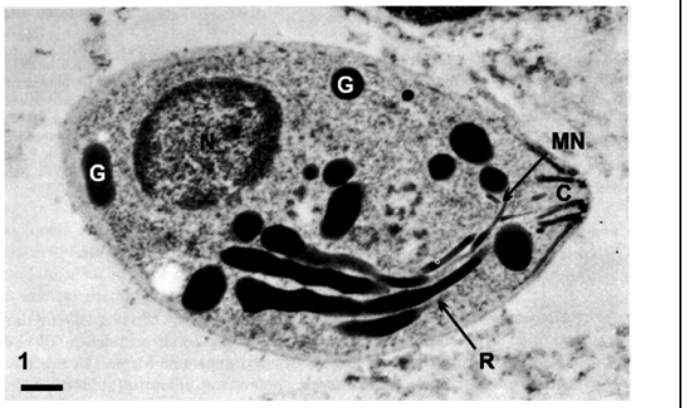
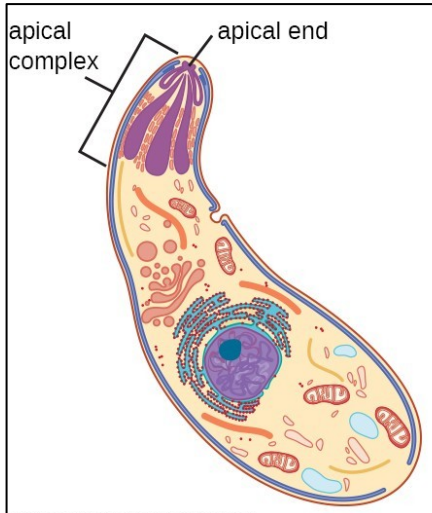
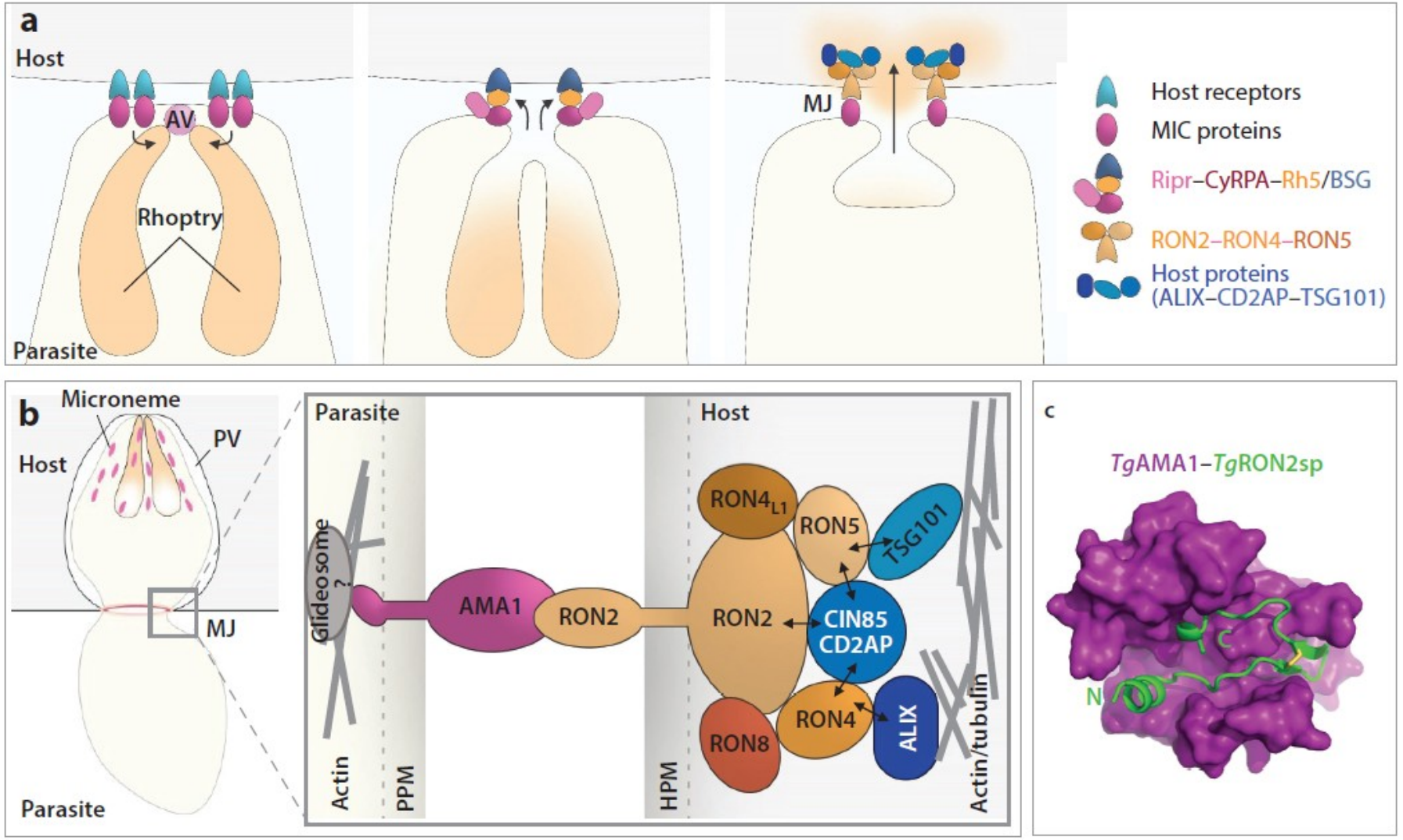


Fig. 1 – Transmission electron microscopy of a tachyzoites of *Toxoplasma gondii* submitted to the ethanolic phosphotungstic acid technique, which labels structures containing basic proteins. In addition to the nucleus (N), staining of the dense granules (G), Rhoptries (R), Micronemes (M) and the Conoid (C) is observed. Bar, 0.3μm. After De Souza and Souto-Padrón 1978.



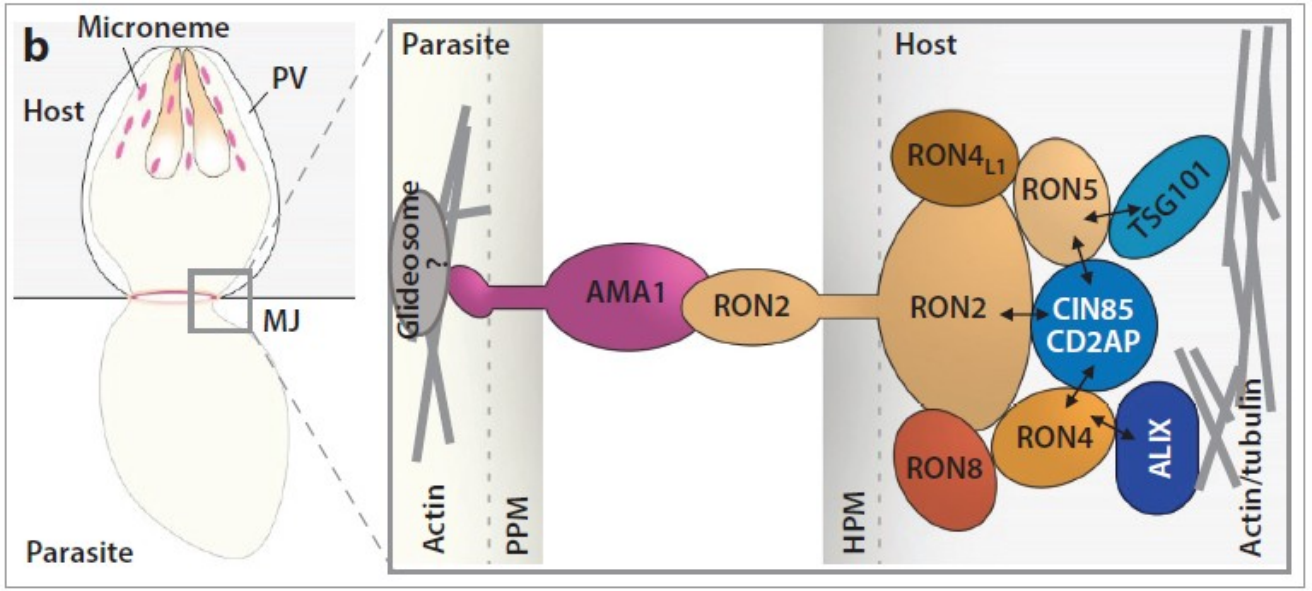
Cooperative role of microneme and rhoptry proteins for invasion

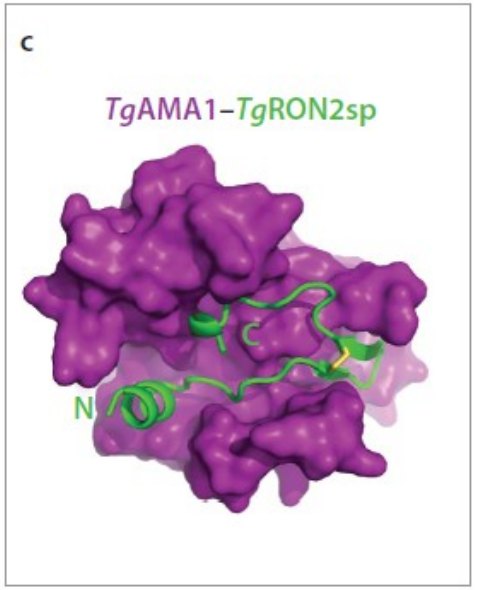


Cooperative role of microneme and rhoptry proteins for invasion

Moving junction (MJ)

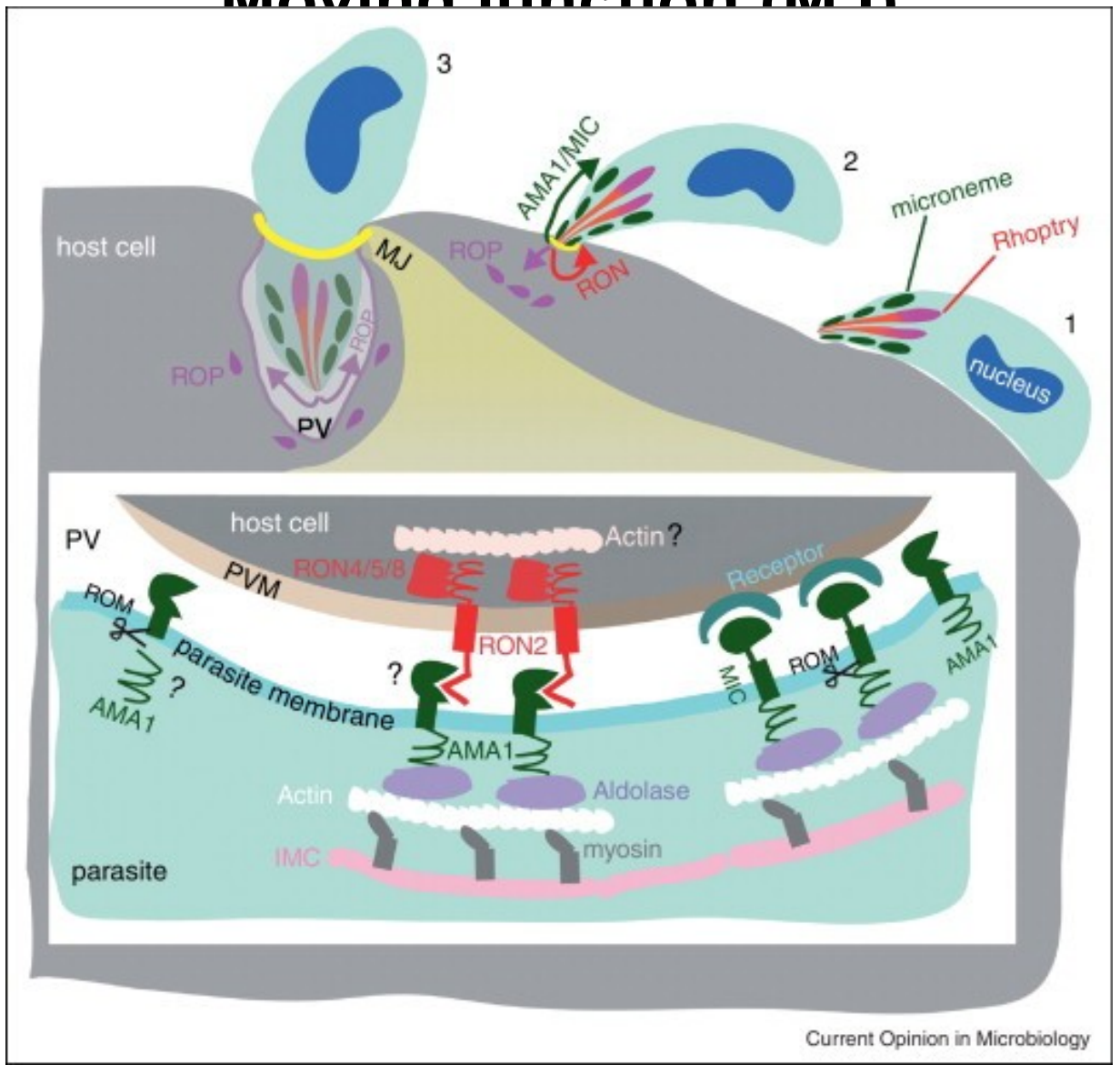
- a tight **connection between invading parasite and host cell membranes** through which the parasite passes to **enter into the host**
- **AMA1 = apical membrane antigen**
- **RON2= rhoptry neck protein**
- AMA1 binds to RON2 that is inserted into the host cell membrane at the site of invasion
- **the AMA1-RON2 complex** contribute to the **formation of moving junction (target for vaccines and drugs)**
- MJ assembles at the site of parasite invasion and provides **a site of traction** for **active penetration of the host cell** and coincident formation of the parasitophorous vacuole





Cooperative role of microneme and rhoptry proteins for invasion

Moving junction (M.J)

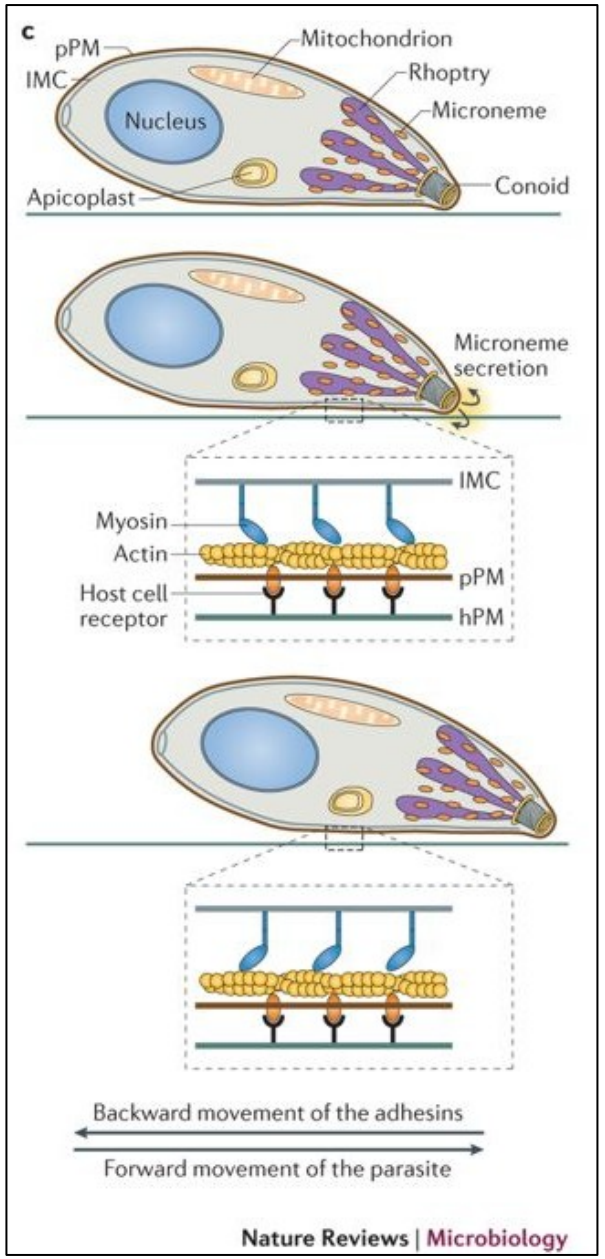


ZOIT MOTILITY → „gliding“ locomotion

- a unique machinery called the **glideosome**
  - composed of an actomyosin system that underlies the plasma membrane
  - glideosome promotes substrate-dependent gliding motility
  - active host cell entry and egress from infected cells
- carefully choreographed and regulated by both **internal and external factors**
- **calcium signaling pathways** playing an integral role

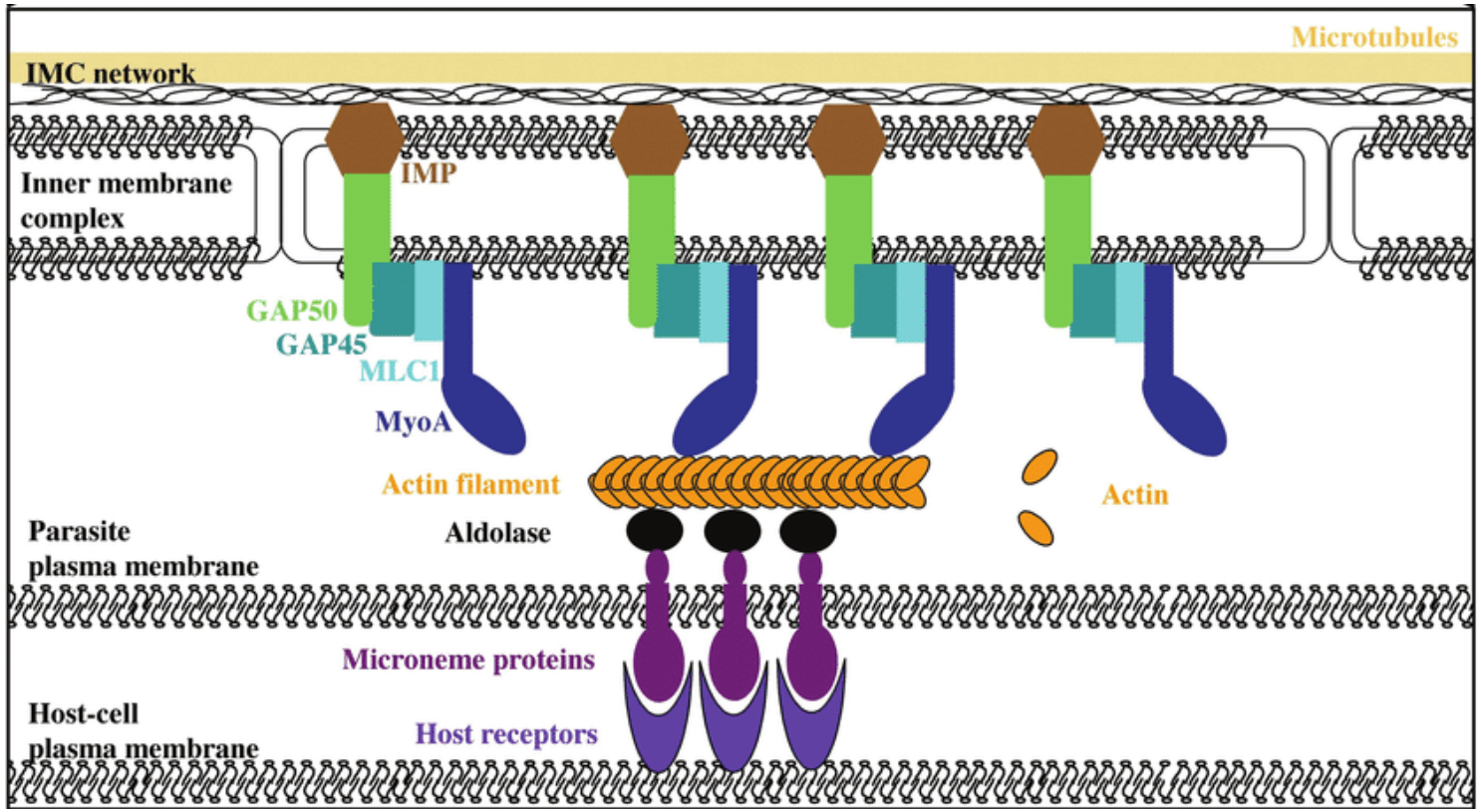
- anchoring of the motor complex internally so that when the motor is engaged, a locomotory force can be generated that propels the parasite over the substrate

- the proteins first implicated in **directly anchoring the motor** were termed **gliding associated proteins or GAPs**

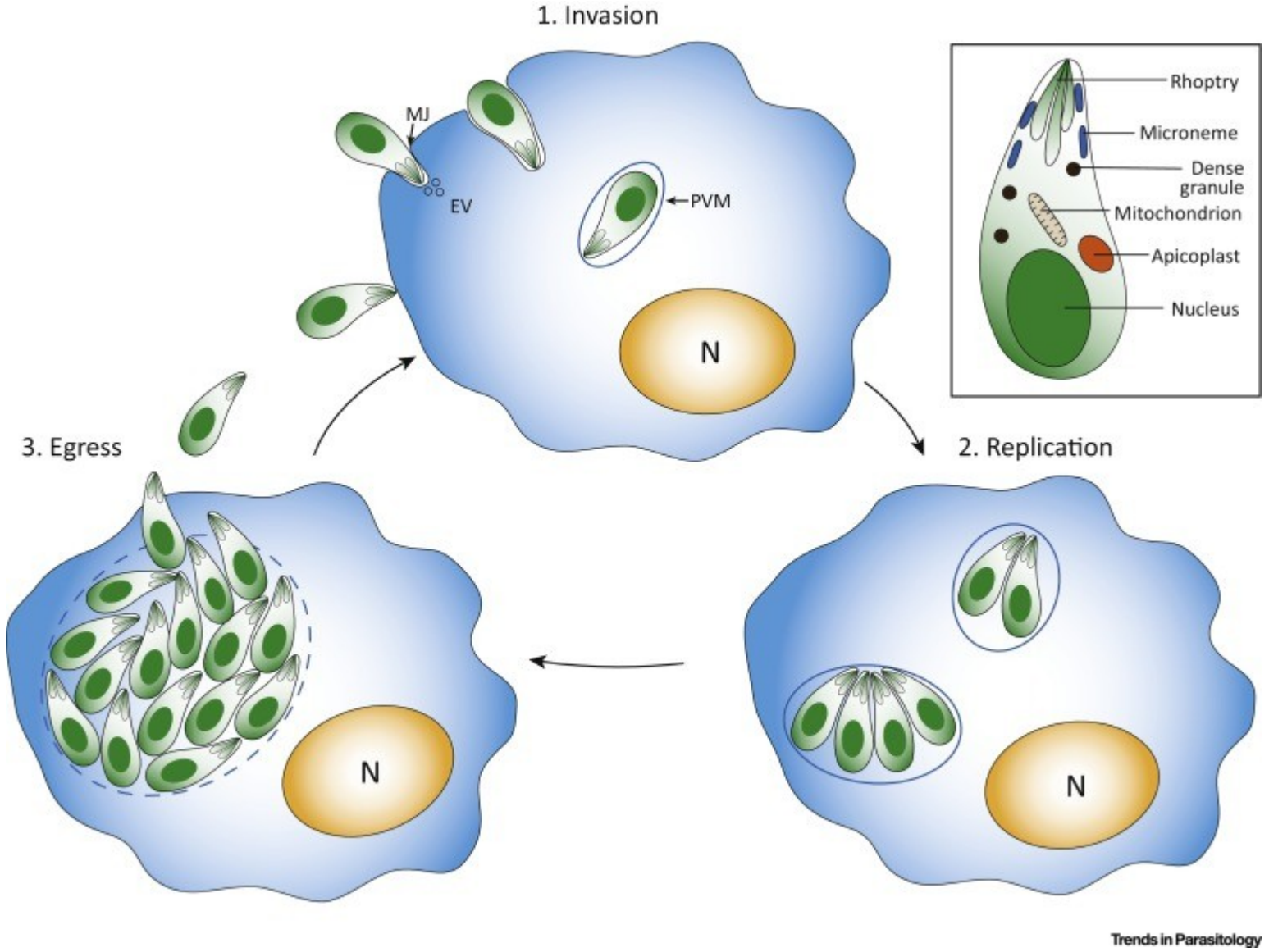


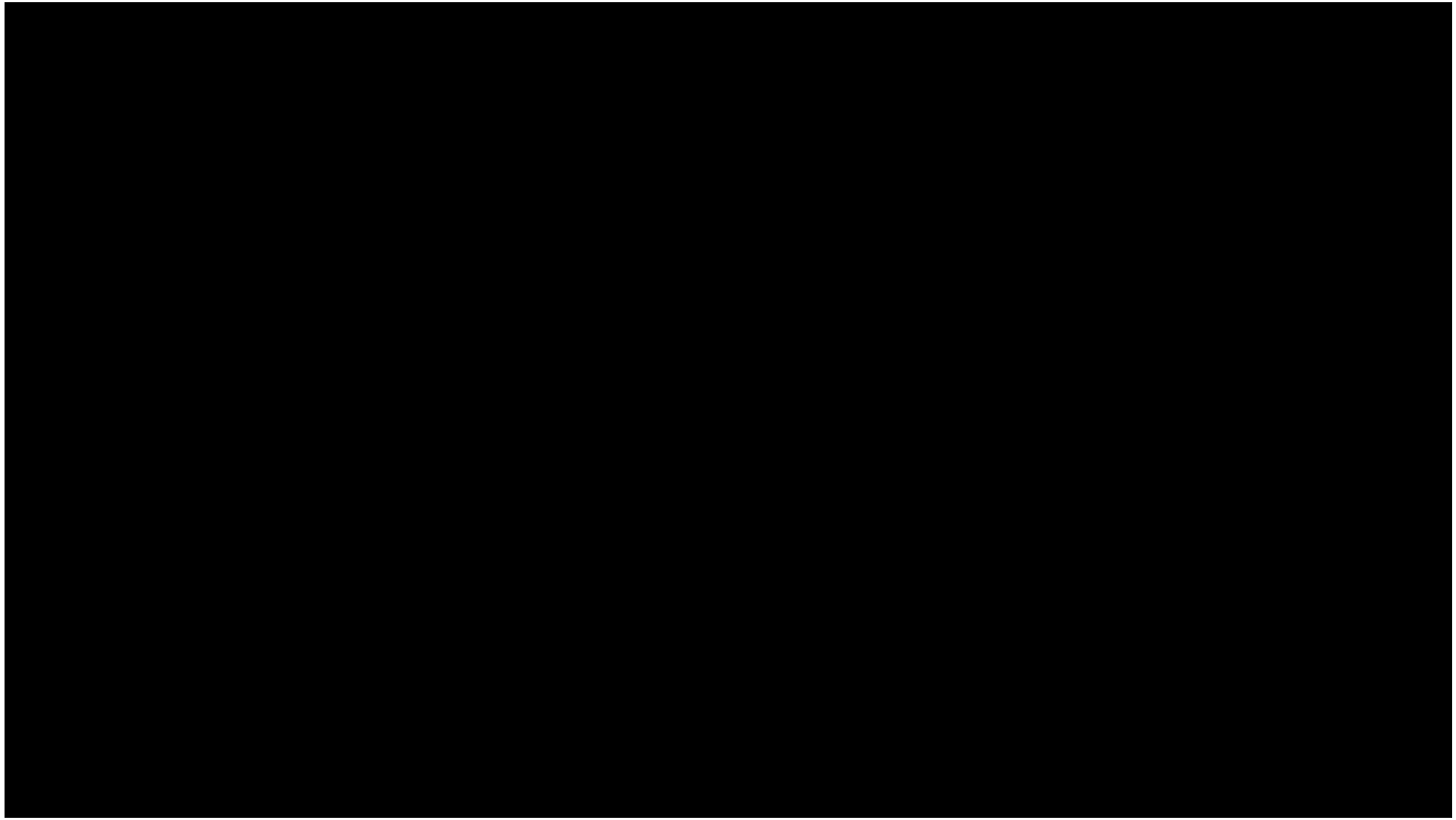
ZOIT MOTILITY → „gliding“ locomotion

- establishment of transient contacts with the substrate via molecules of an **adhesion complex**
  - **released from the apically positioned microneme organelles into the plasma membrane of the parasite**
  - most well-characterized of **adhesins** include the **apical membrane antigen-1 (AMA1)** protein, and members of the **thrombospondin-related anonymous protein (TRAP)** family - indirectly link the motor complex to the adhesion site
- **connection of the adhesins to the molecular motor** apparatus



HOST CELL INFECTION: invasion → replication → egress





https://www.youtube.com/watch?v=TIc6exbsH90

https://www.youtube.com/watch?v=JSuSsn4HwHI