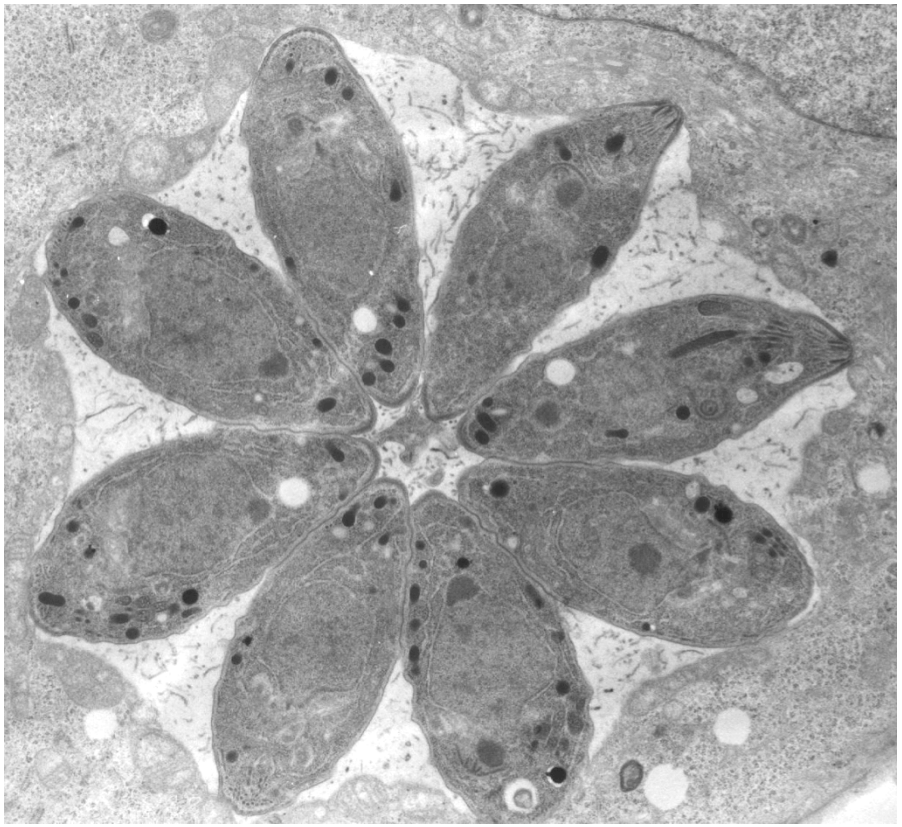
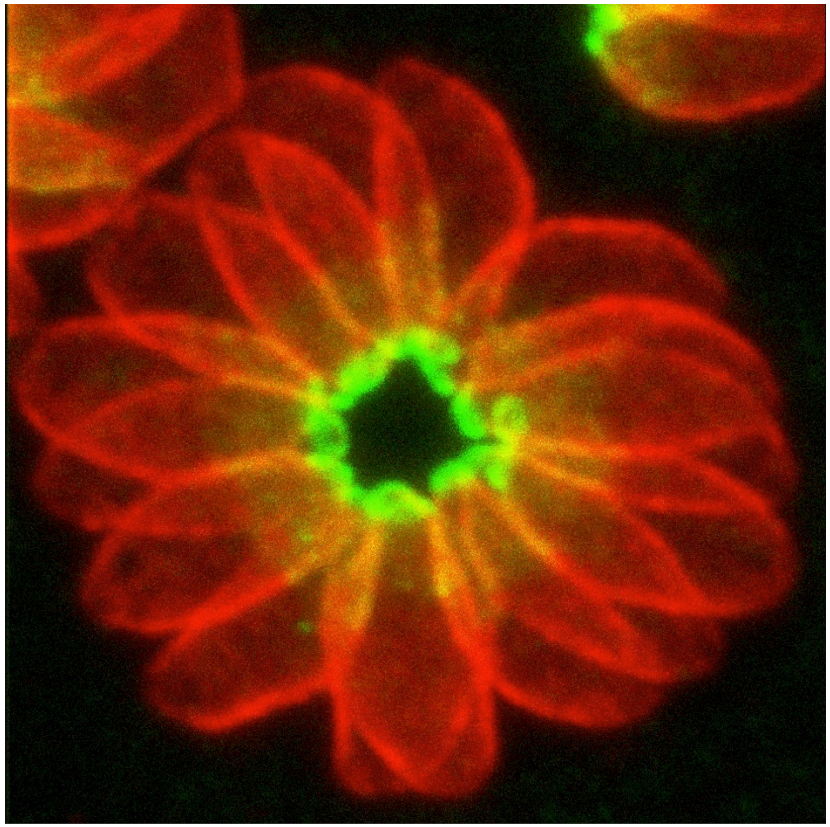
Toxoplasma gondii tachyzoites

Phenotypic analysis of mutants



From J-F.Dubremetz



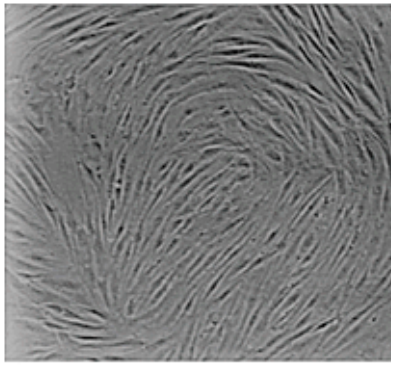
Toxoplasma gondii tachyzoite culture

Incubator

- 37°C
- 5% CO_2

Host cell

- HFF Human foreskin fibroblasts
- VERO cells

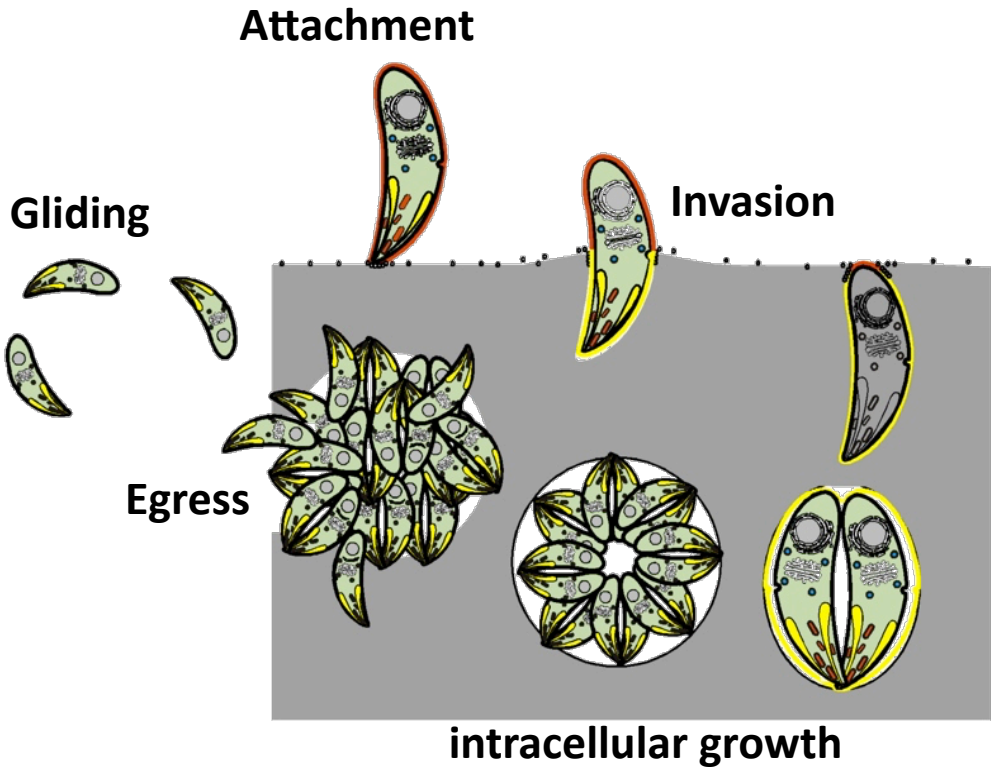


Medium

- DMEM (Dulbecco's Modified Eagle Medium), high glucose
- FCS: fetal calf serum
- L-Glutamine
- Gentamycin

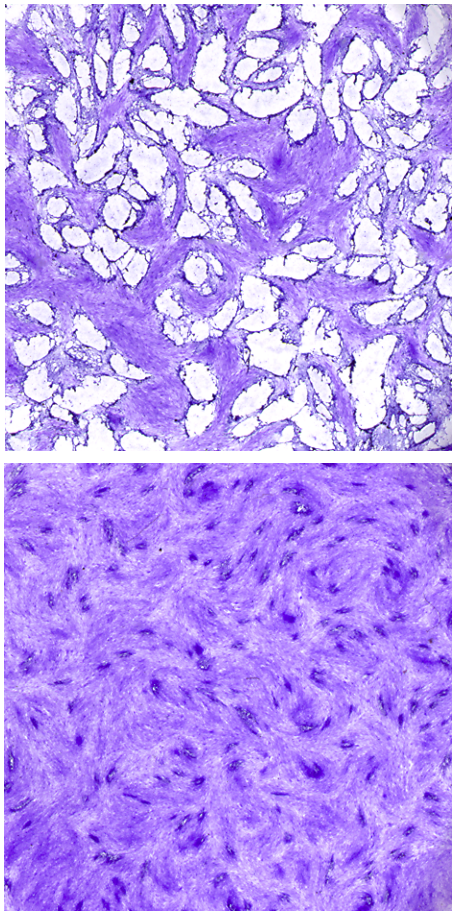
P2 lab !!

Lytic cycle of *Toxoplasma gondii* tachyzoites



48 h for one lytic cycle

Lysis plaques

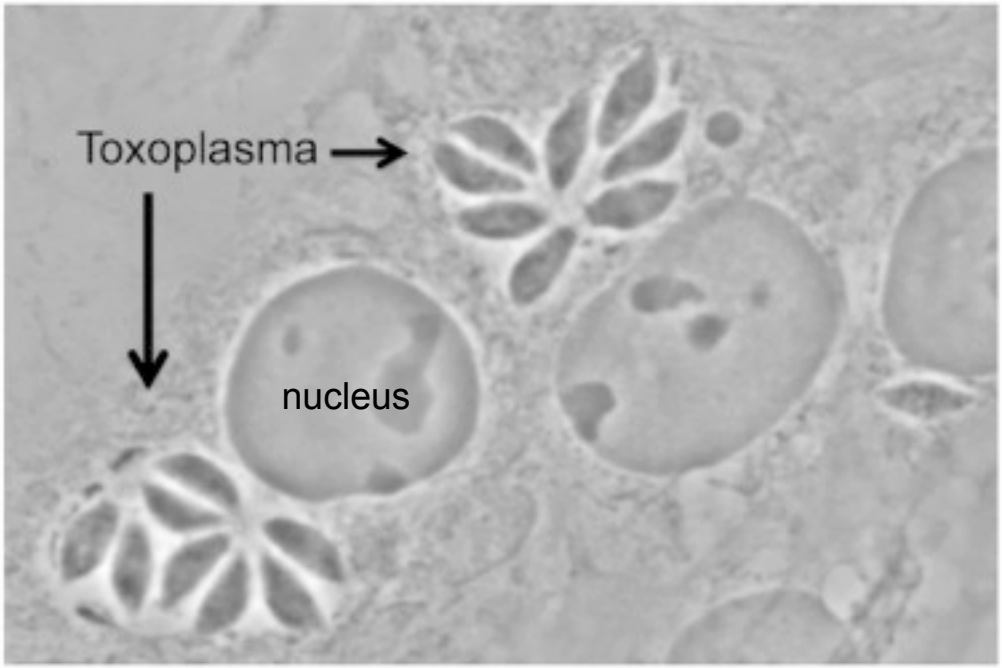


fixed after 7 days
stained with crystal violet

Soldati and Meissner, Current Opinion in Cell Biology, 2004

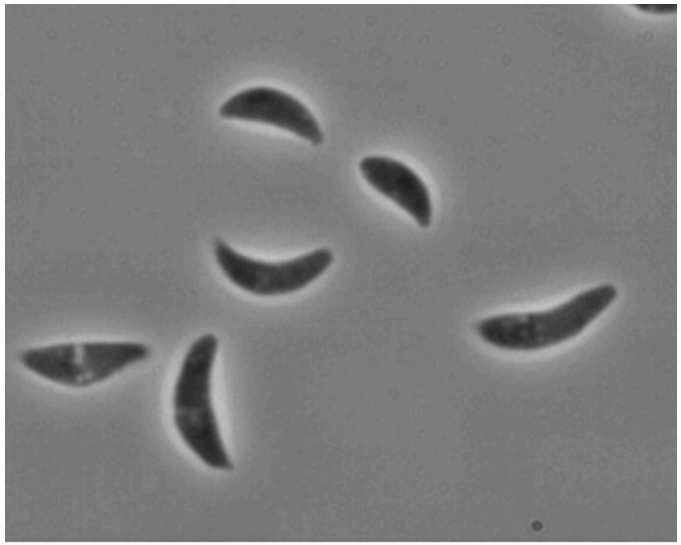
Toxoplasma gondii tachyzoites

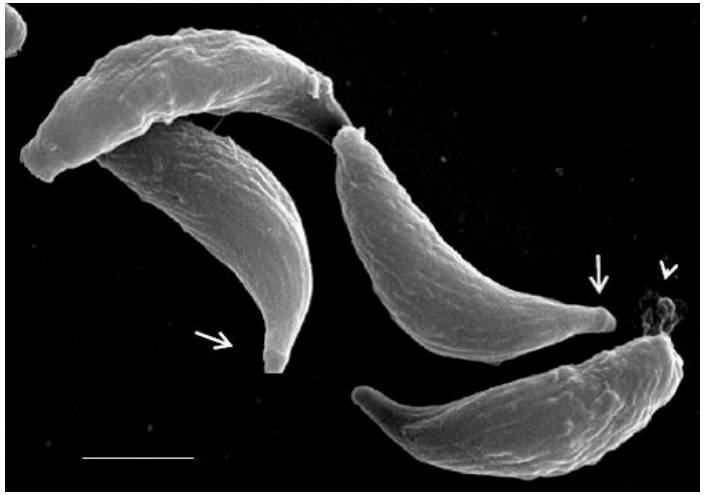
Intracellular



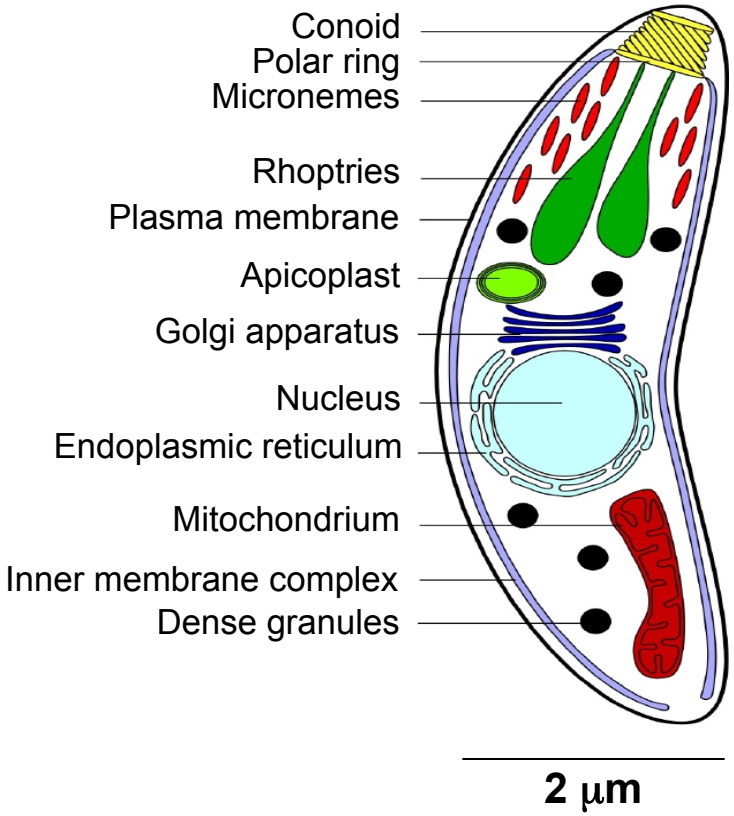
From M-A. Hakimi

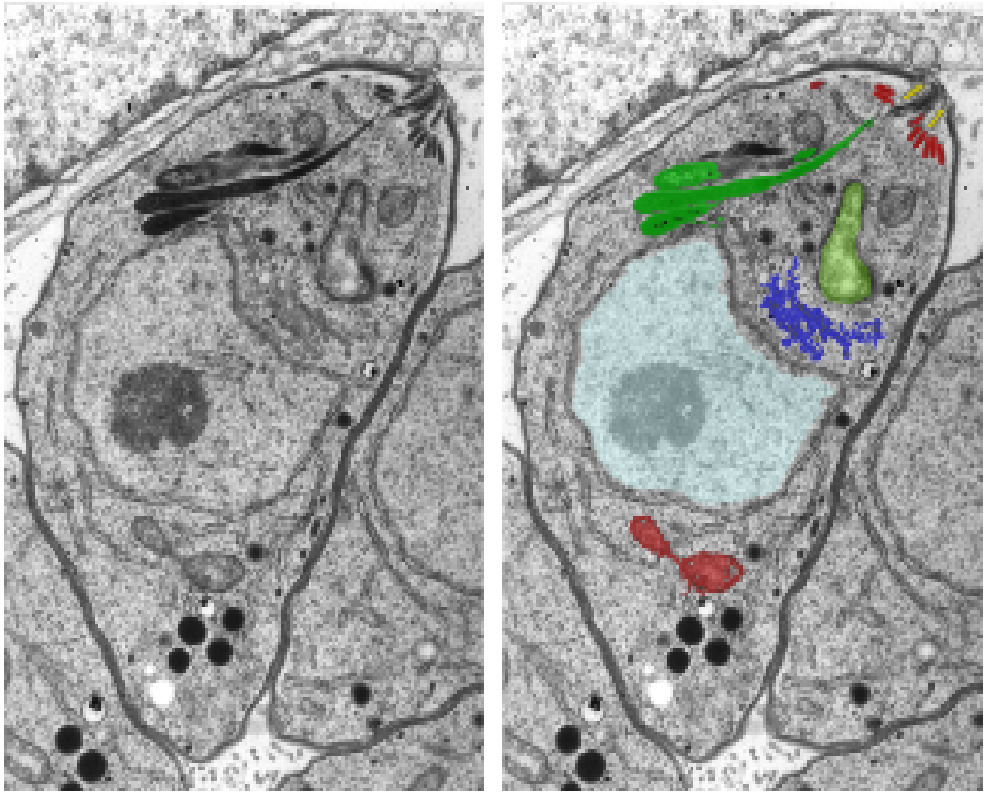
Extracellular





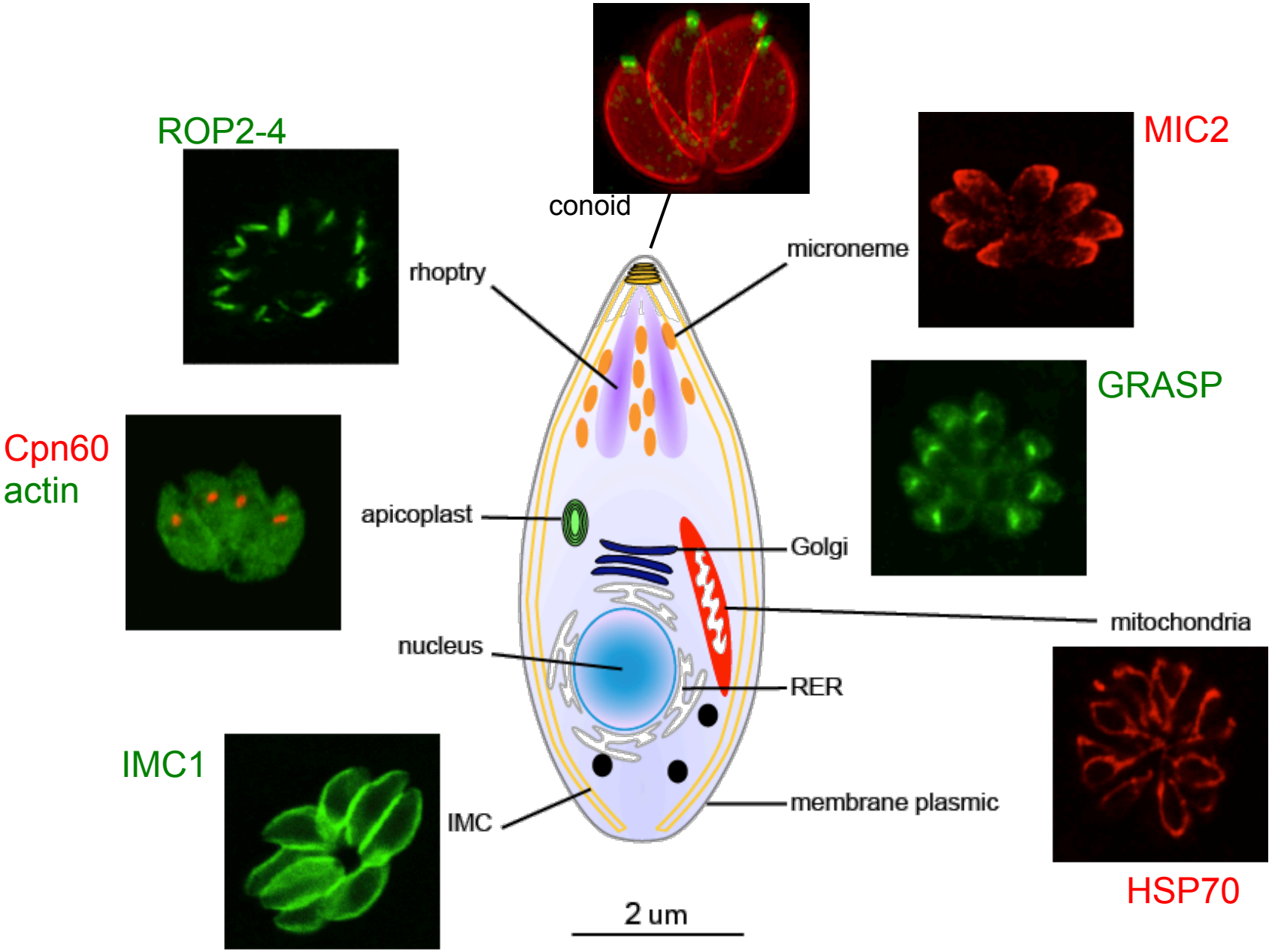
Ultrastructure of *Toxoplasma gondii* tachyzoite: organelles



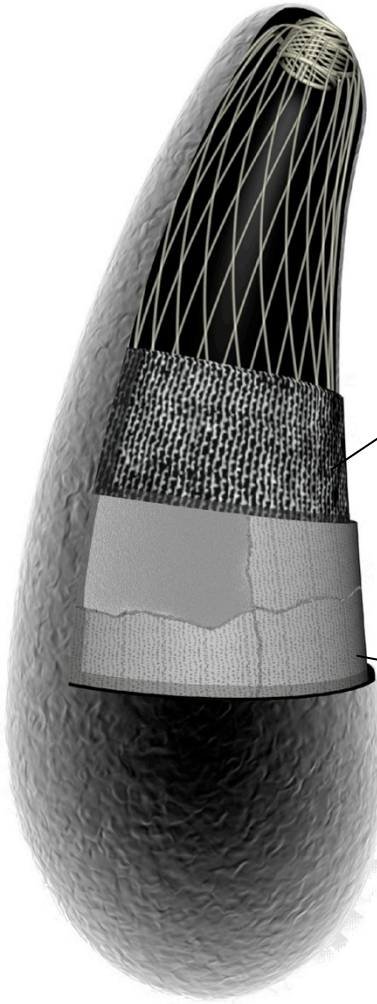


From J-F.Dubremetz and T. Dowse

Integrity and positioning of organelles by immunofluorescence

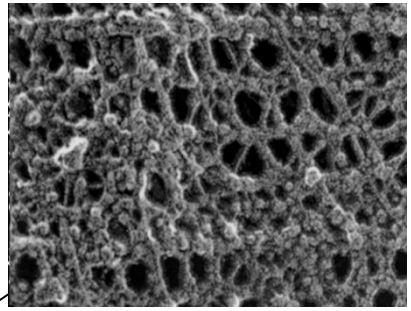


Ultrastructure of *Toxoplasma gondii* tachyzoite: cytoskeleton



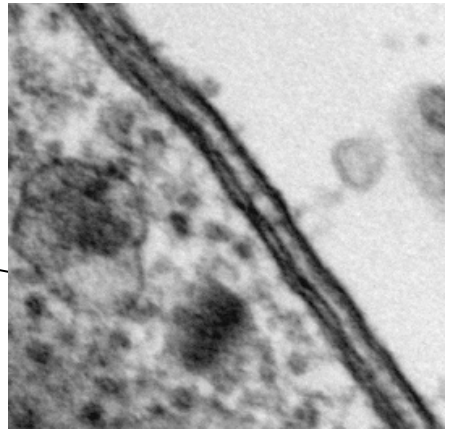
2 µm

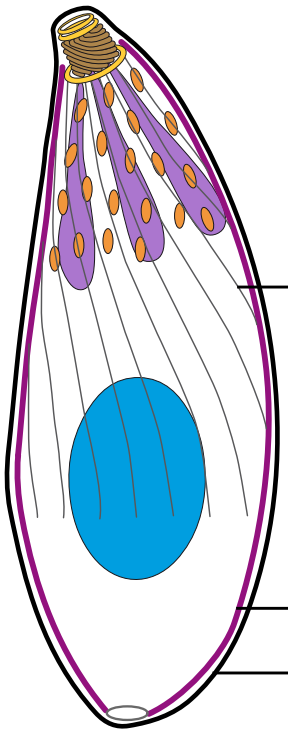
Alveolin network

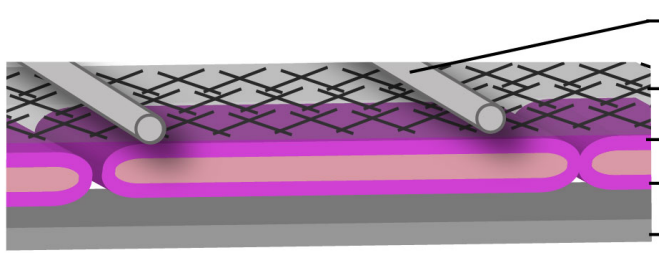


Morrissette et Sibley, 2002

Pellicle

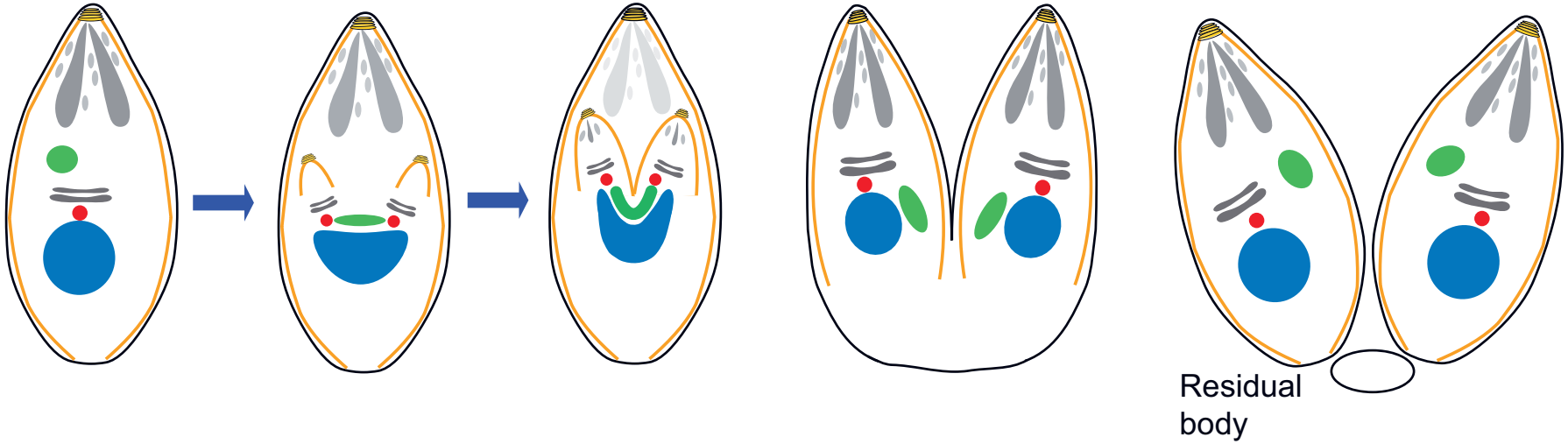




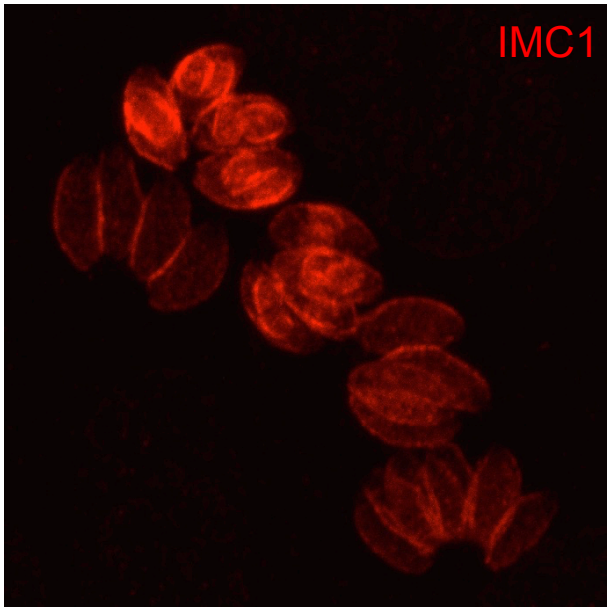


Division of *Toxoplasma gondii* tachyzoite

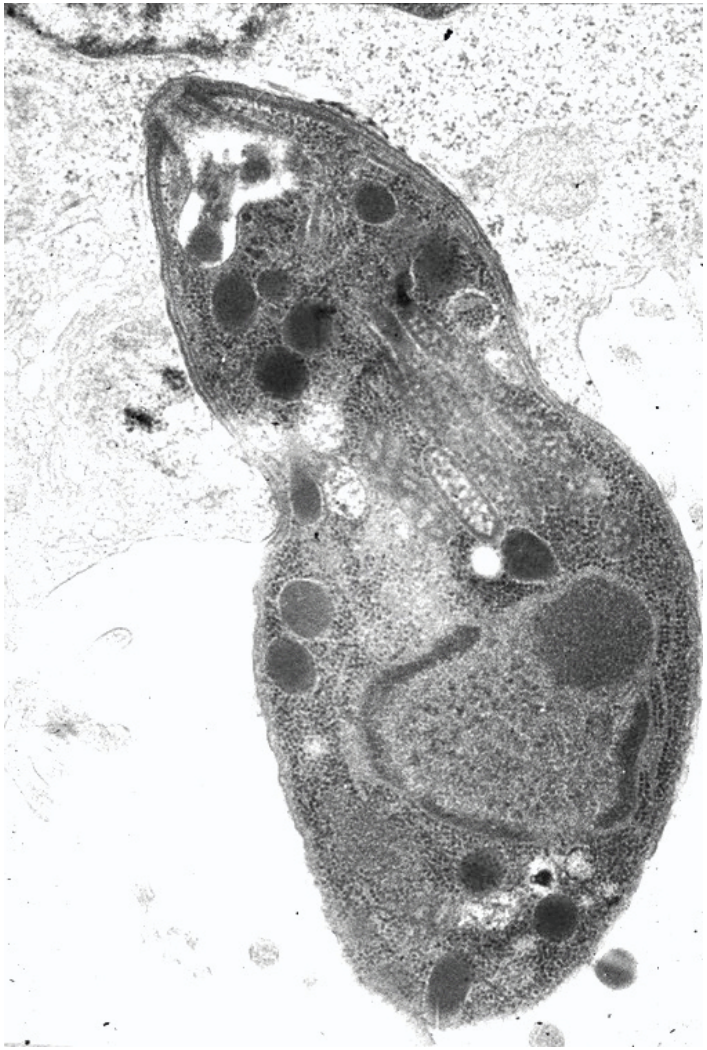
- Replication by endodyogeny and organization in rosettes



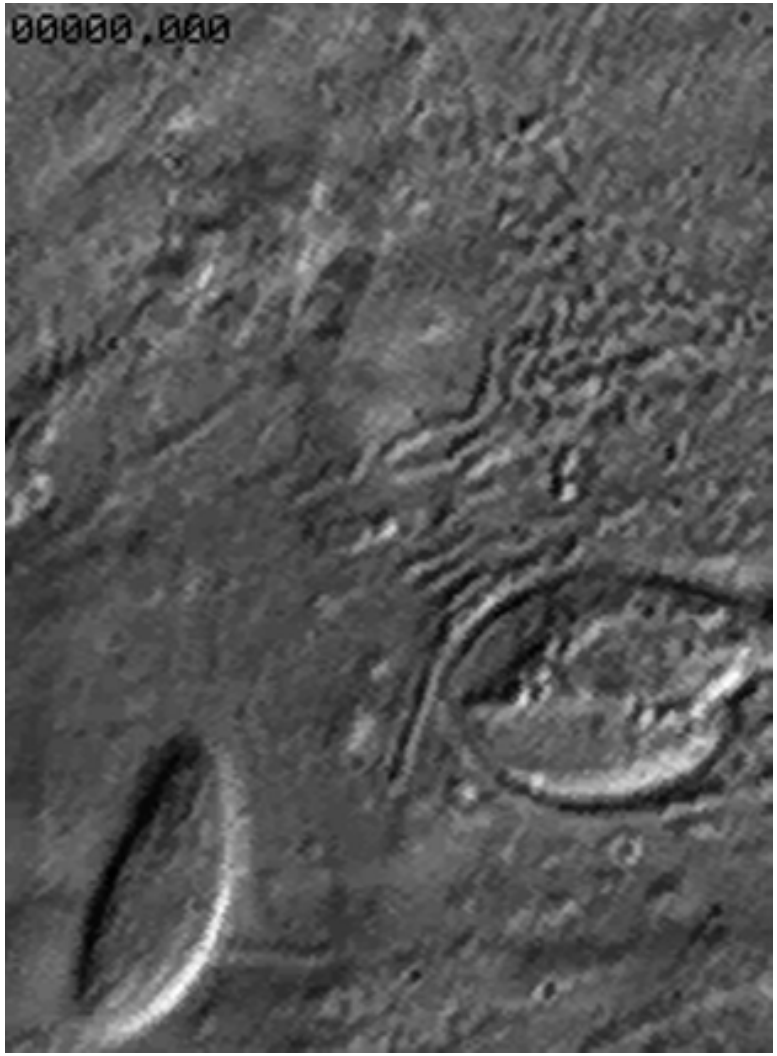
- Asynchronous culture but synchronized replication within each parasitophorous vacuole



Motility and invasion

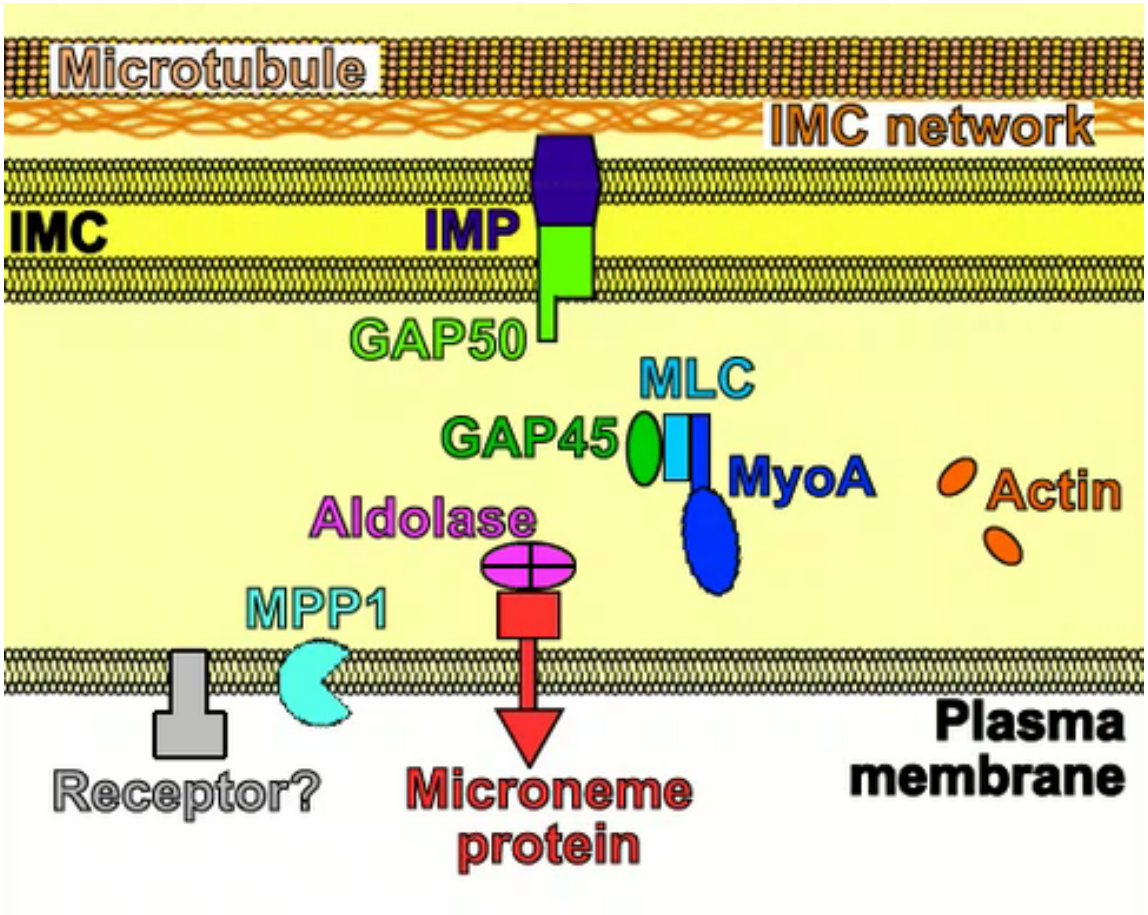


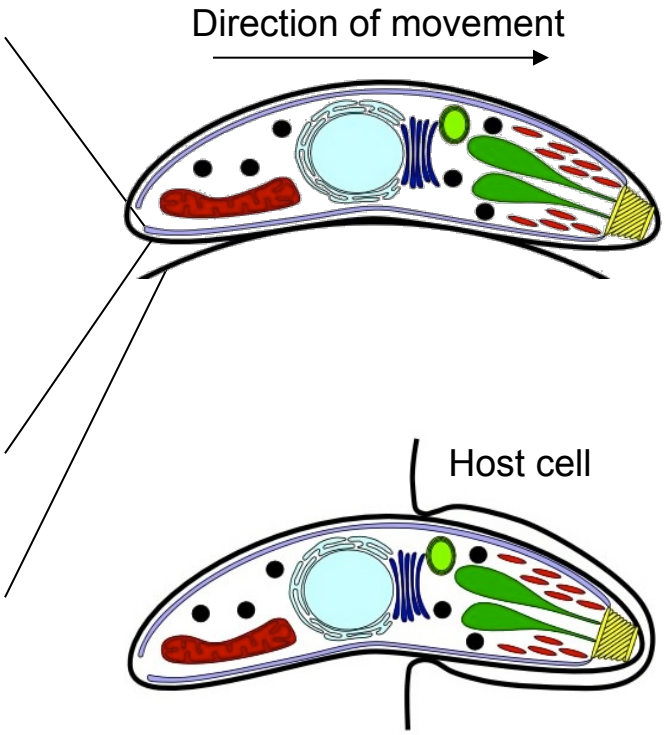
From J-F.Dubremetz



Movie from G. Ward
http://sbb.uvm.edu/~gward/Movies.html

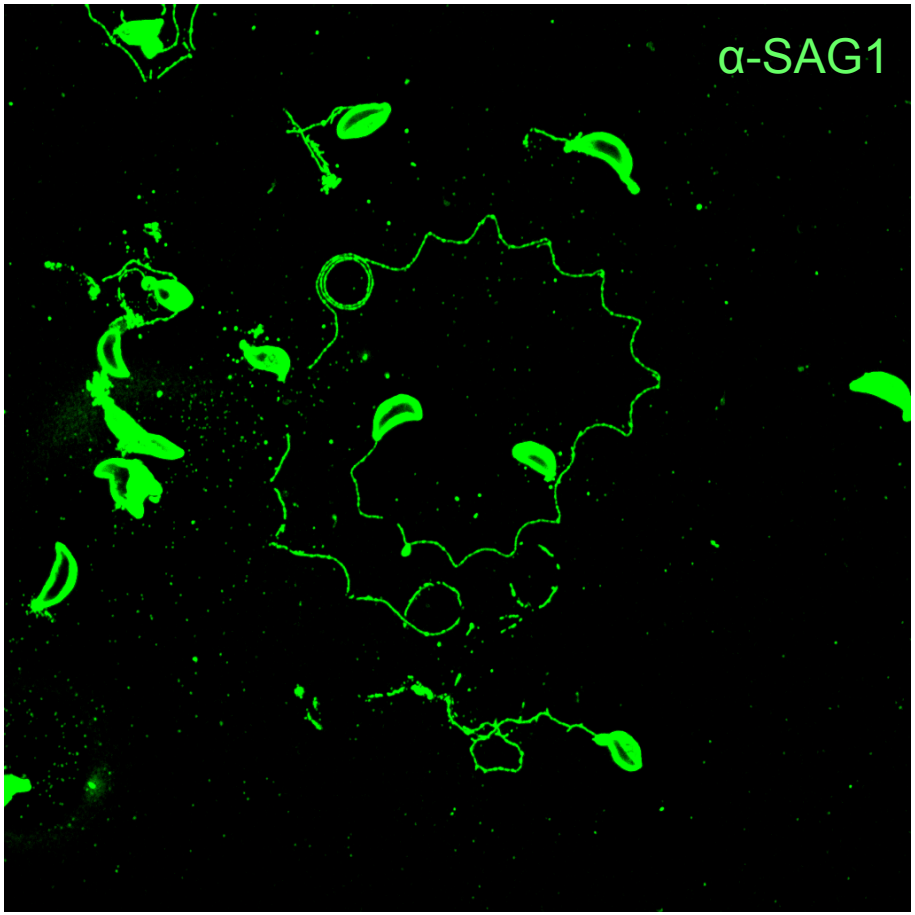
The gliding motility relies on the glideosome

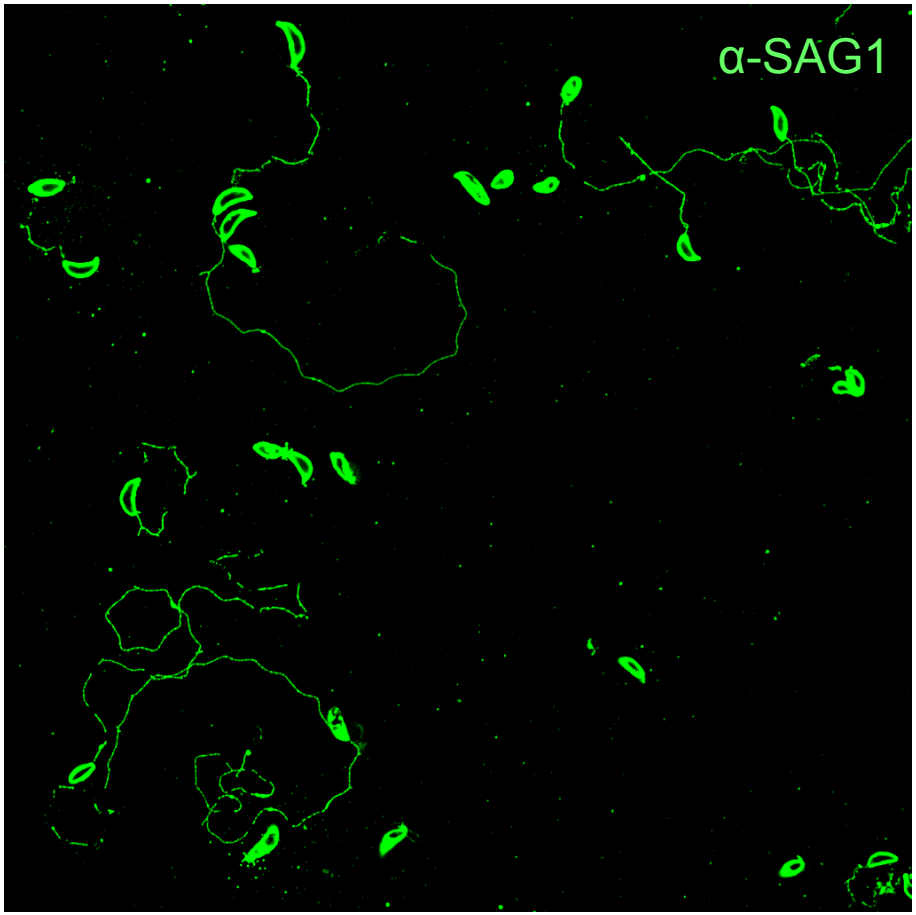




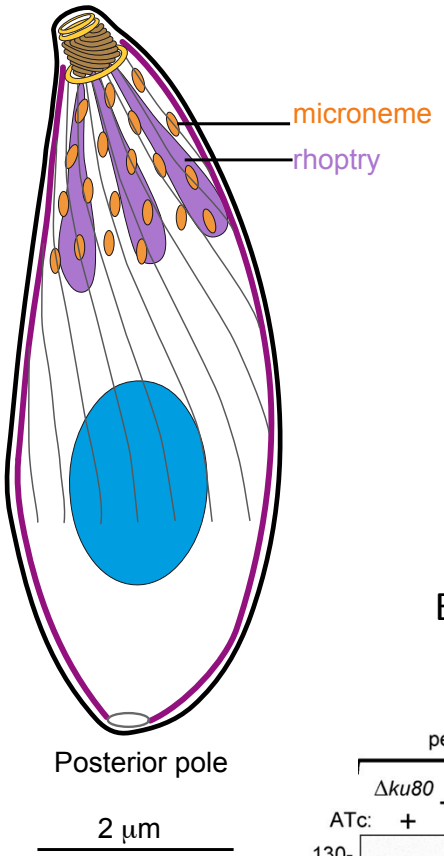
Movie from Soldati *et al*., Trends in Parasitology, 2004

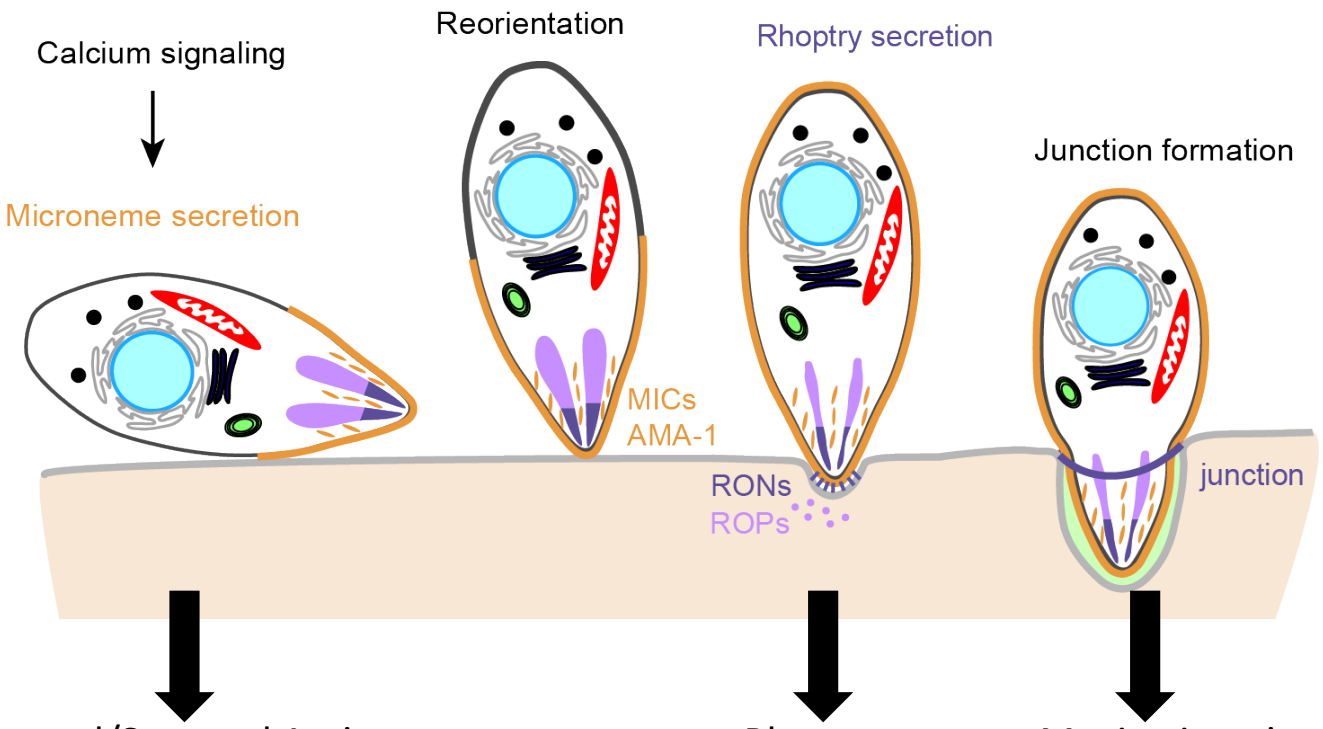
Gliding assay on poly-L-lysine coated coverslip





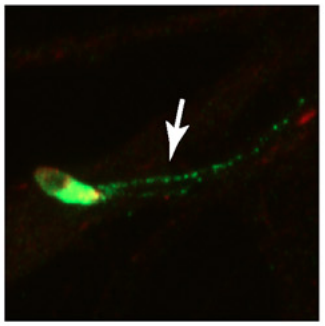
Apical secretory organelles and invasion



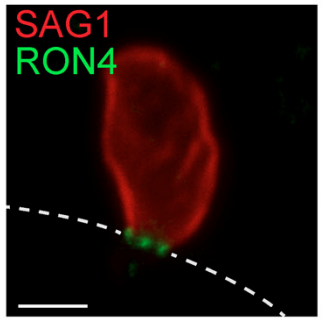


Excreted/Secreted Antigen (WB)

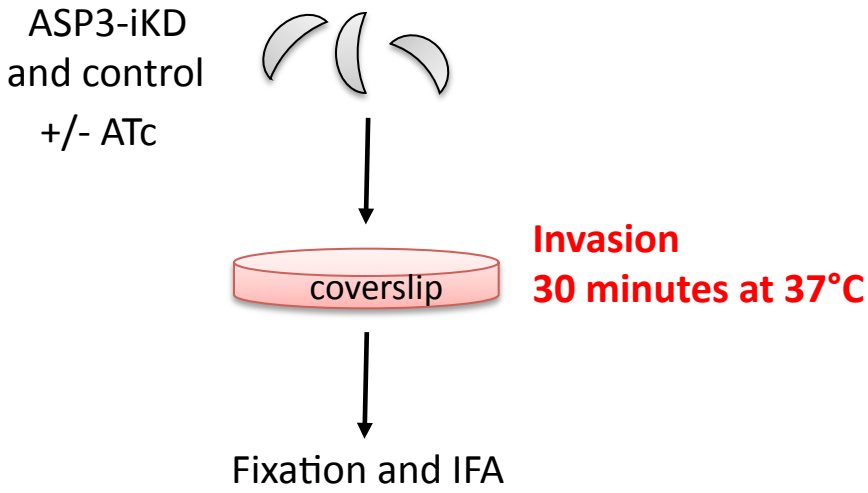
Rhoptry secretion assay or e-vacuole assay (IFA)

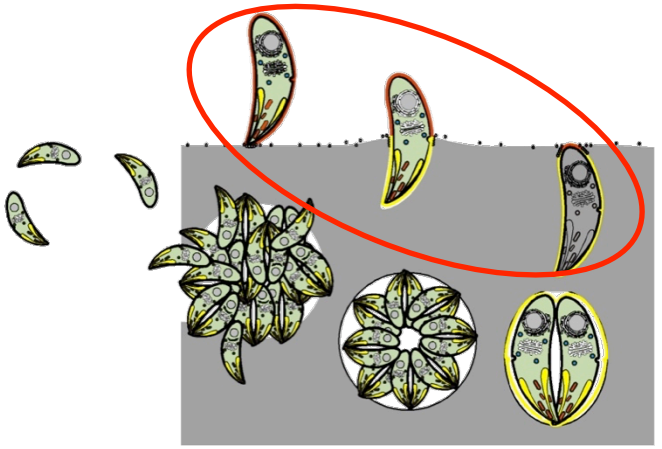


Moving junction formation (IFA)



Invasion assay (red/green)

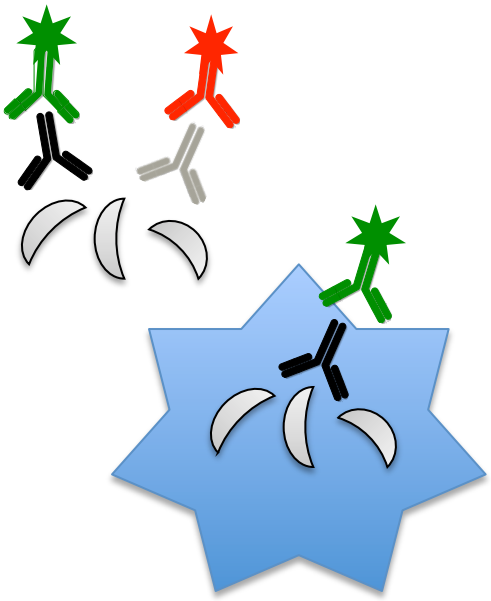




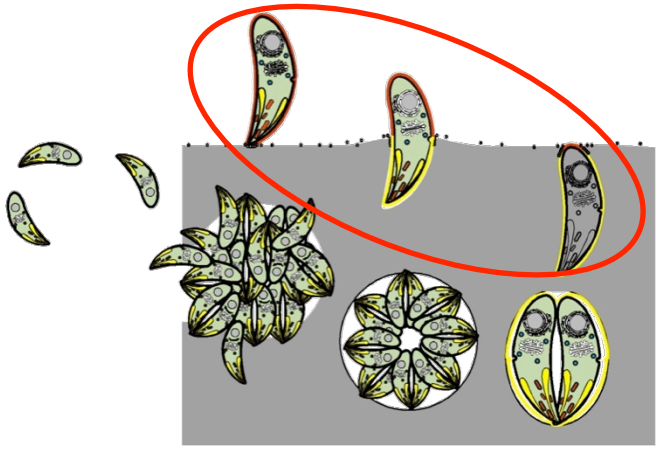
Immunofluorescence assay or IFA

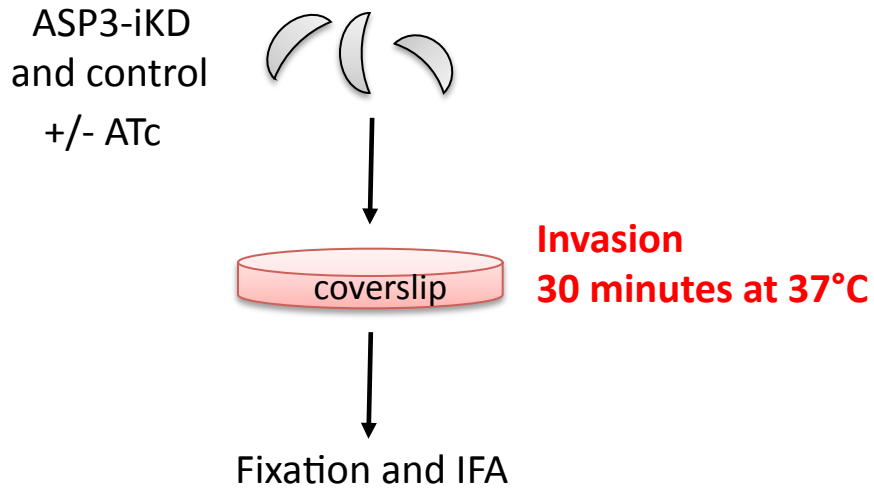
Step 1: **No permeabilization**!!
Staining of the extracellular parasites
(primary Ab: surface marker, ex: anti-SAG1)

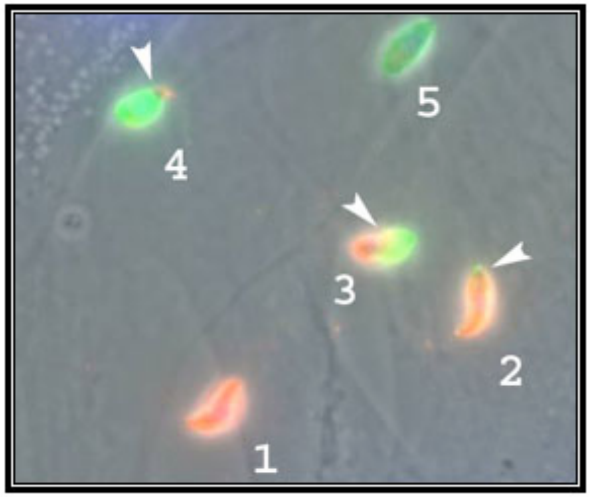
Step 2: **after permeabilization**!!
Staining of the intracellular and extracellular parasites
(primary Ab: inside marker, ex: anti-GAP45 then secondary Ab)



Invasion assay (red/green IFA)







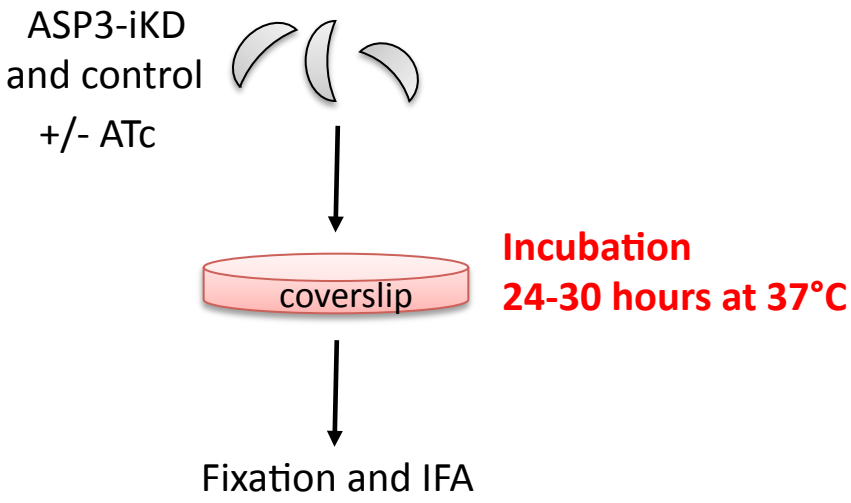
SAG1-red GAP45-green

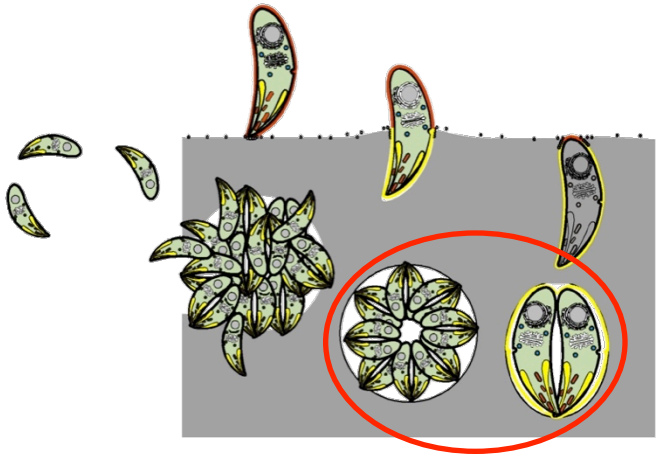
Immunofluorescence assay or IFA

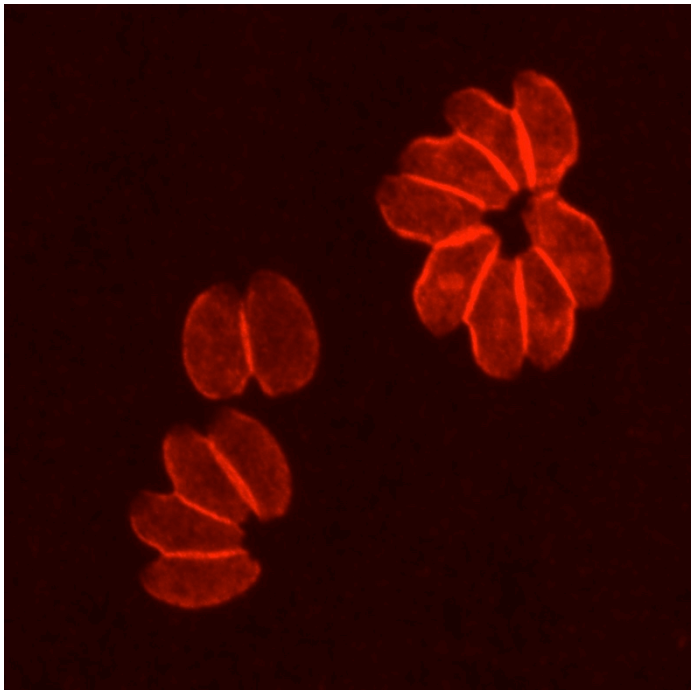
Step 1: **No permeabilization**!!
Staining of the extracellular parasites
(primary Ab: surface marker, ex: anti-SAG1)

Step 2: **after permeabilization**!!
Staining of the intracellular and extracellular parasites
(primary Ab: inside marker, ex: anti-GAP45 then secondary Ab)

Intracellular growth assay (IFA)



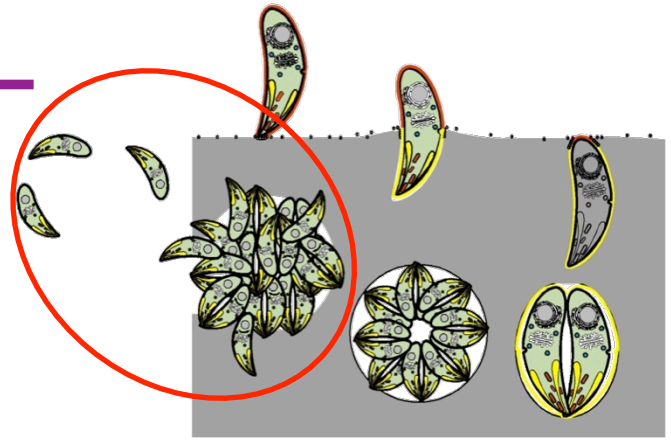




IFA

With **permeabilization**
(primary Ab: anti-GAP45, secondary Ab: anti-rabbit-Alexa594)

Ionophore-induced egress assay (IFA)

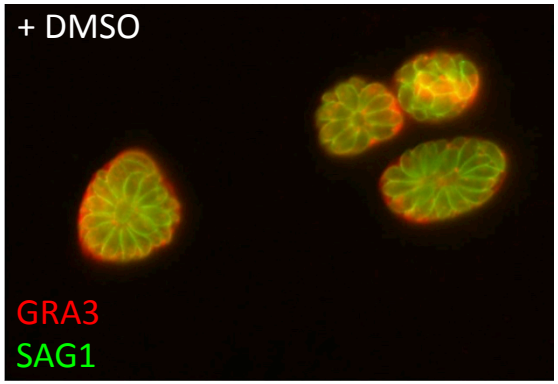


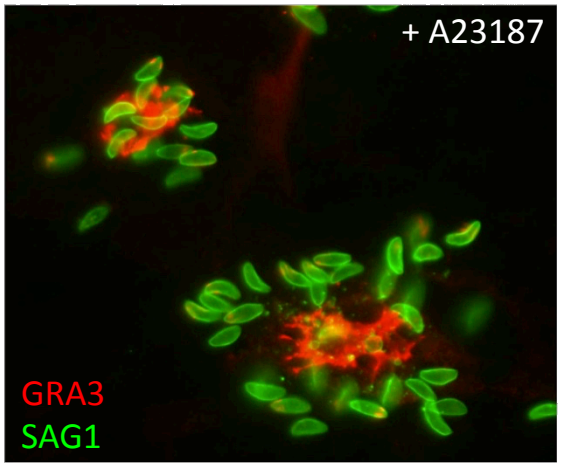
ASP3-iKD
and control
+/- ATc

coverslip

Incubation
30 hours at 37°C

Stimulation with A23187 or DMSO
for 7 minutes 37°C





IFA

With **permeabilization**
(primary Ab: anti-GAP45, secondary Ab: anti-rabbit-Alexa594)
(primary Ab: anti-GRA3, secondary Ab: anti-mouse-Alexa488)