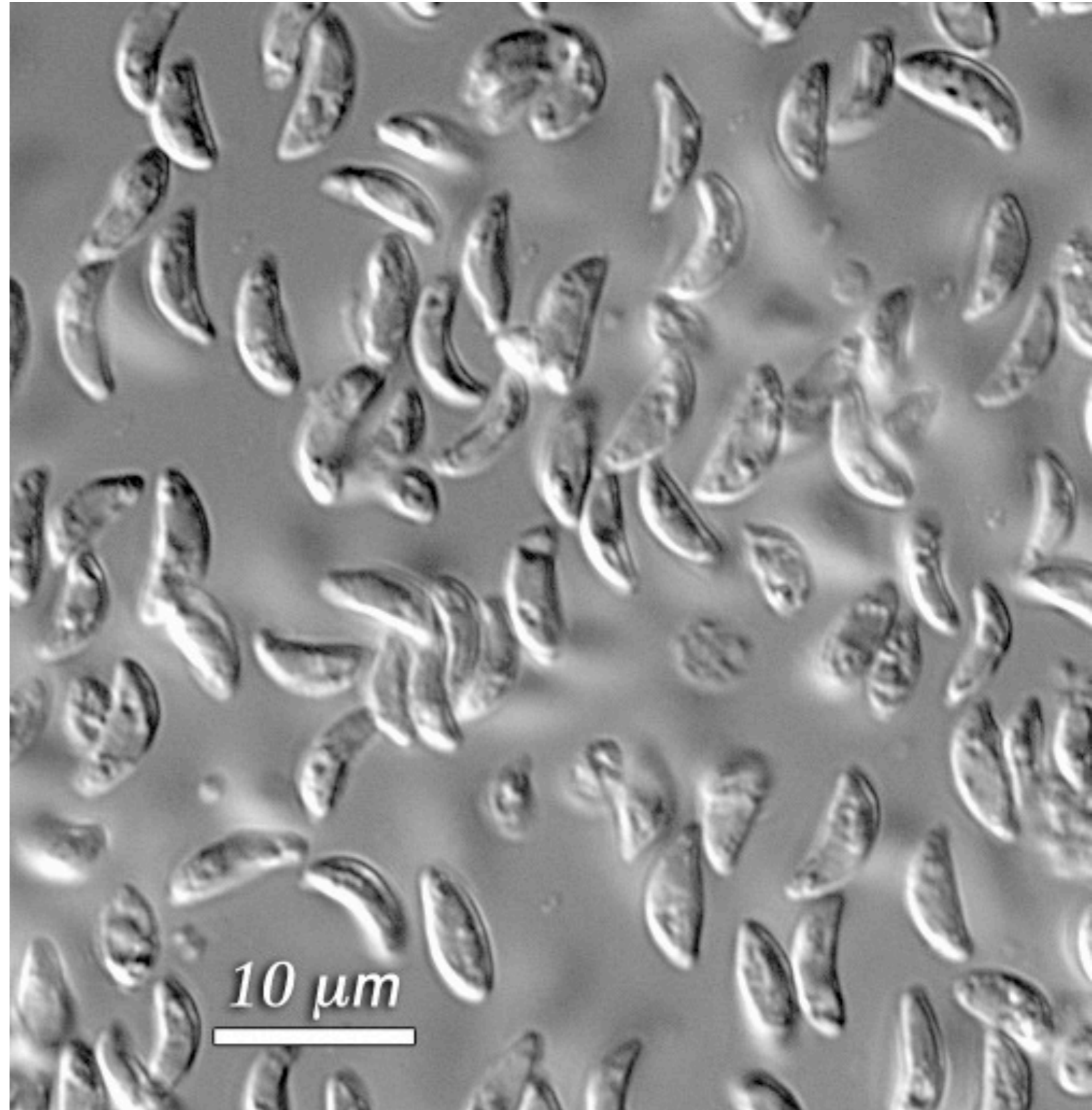


Toxoplasma gondii

a small, eukaryotic, single-celled organism, shaped like a fat banana

Toxoplasma cells,
free in suspension



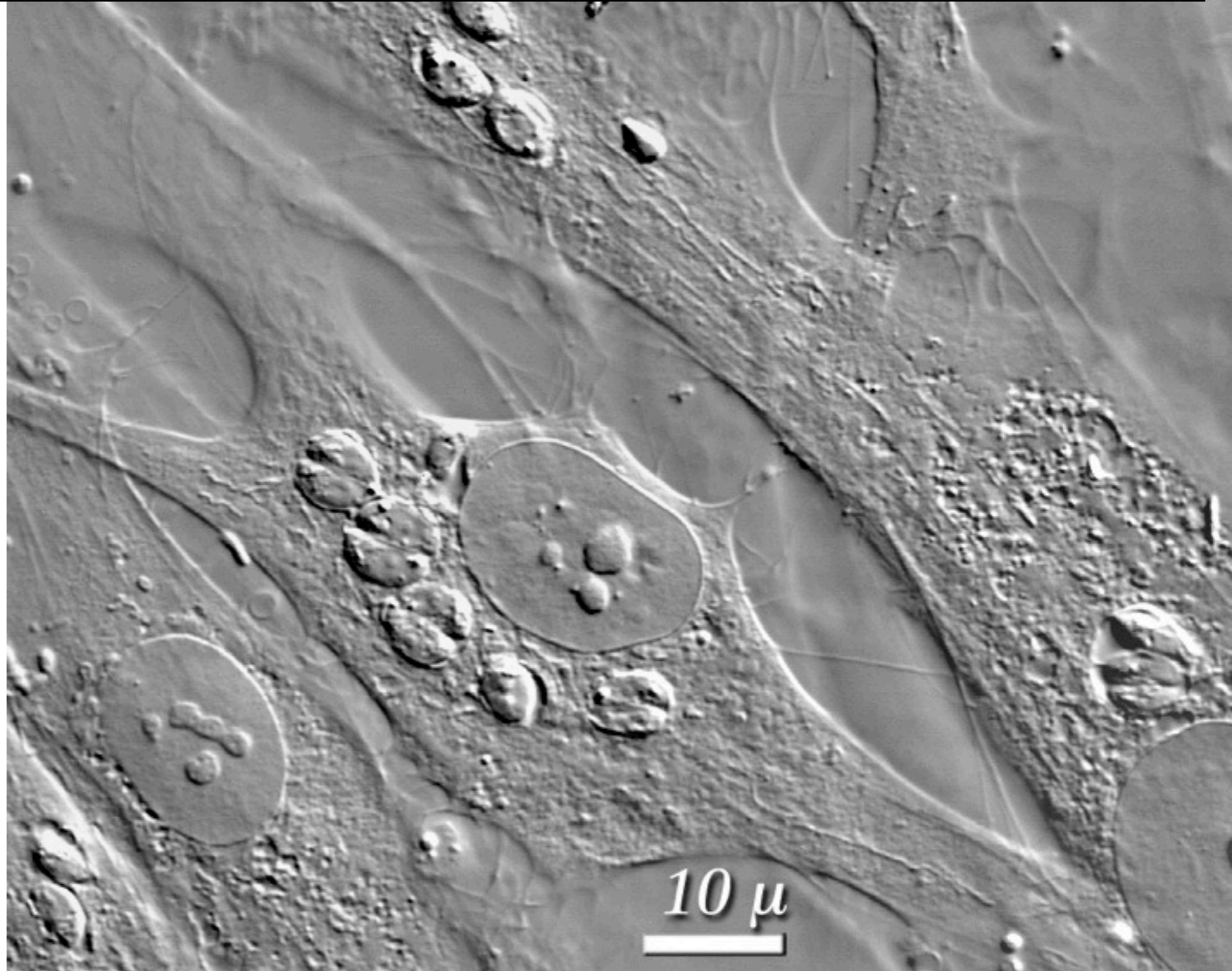
Toxoplasma gondii

a small eukaryotic single-celled organism

an obligate intracellular parasite, haploid

replicates asexually inside the host cell every 6-8 hours

a culture of human fibroblasts, infected with *Toxoplasma*

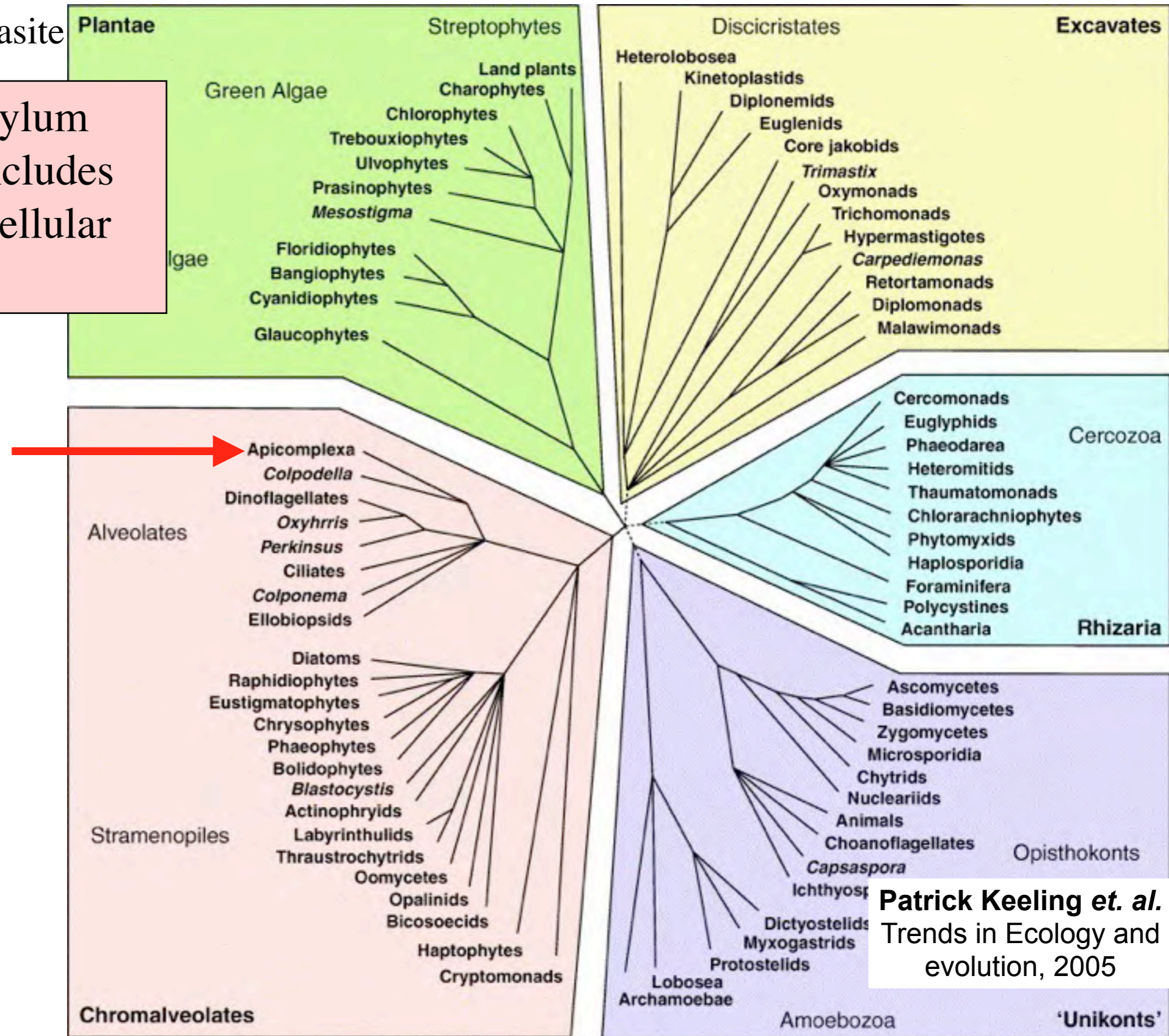


Toxoplasma gondii

a small eukaryotic single-celled organism

an obligate intracellular parasite

an Alveolate in the phylum Apicomplexa, which includes ~5000 species of intracellular parasites



Toxoplasma gondii

a small eukaryotic single-celled organism

an obligate intracellular parasite

an Alveolate in the phylum Apicomplexa

a ubiquitous parasite

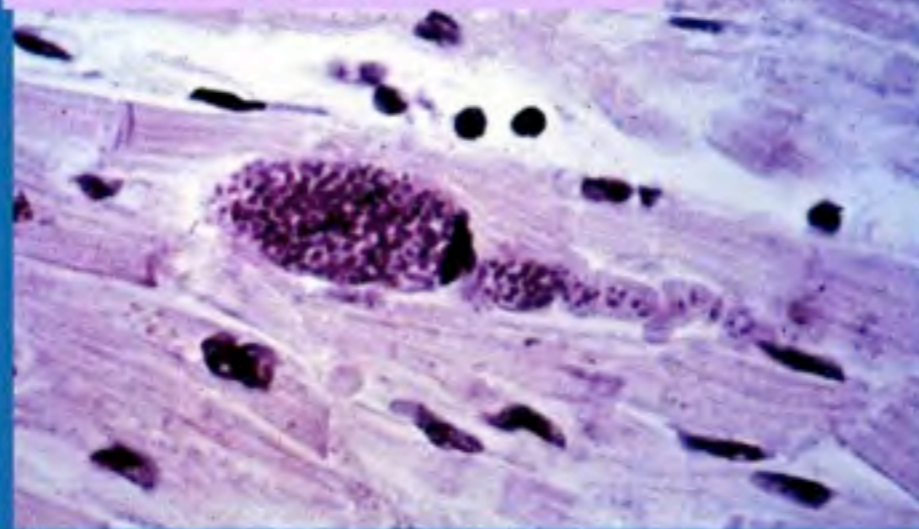
can infect any nucleated cell in any tissue, in any warm-blooded animal, from birds to humans

worldwide, ~25% of the human population is permanently infected.

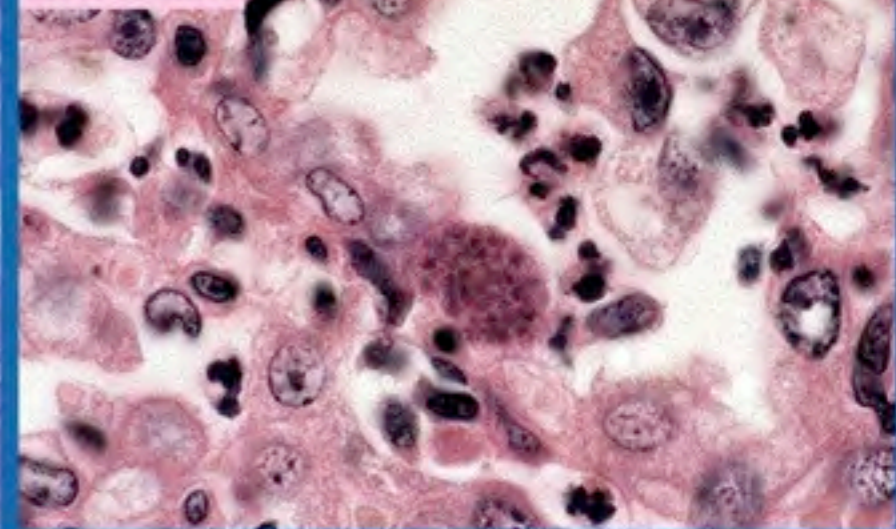
10-90% of domestic and wild animals are permanently infected

1/5 people in this room are permanently infected.

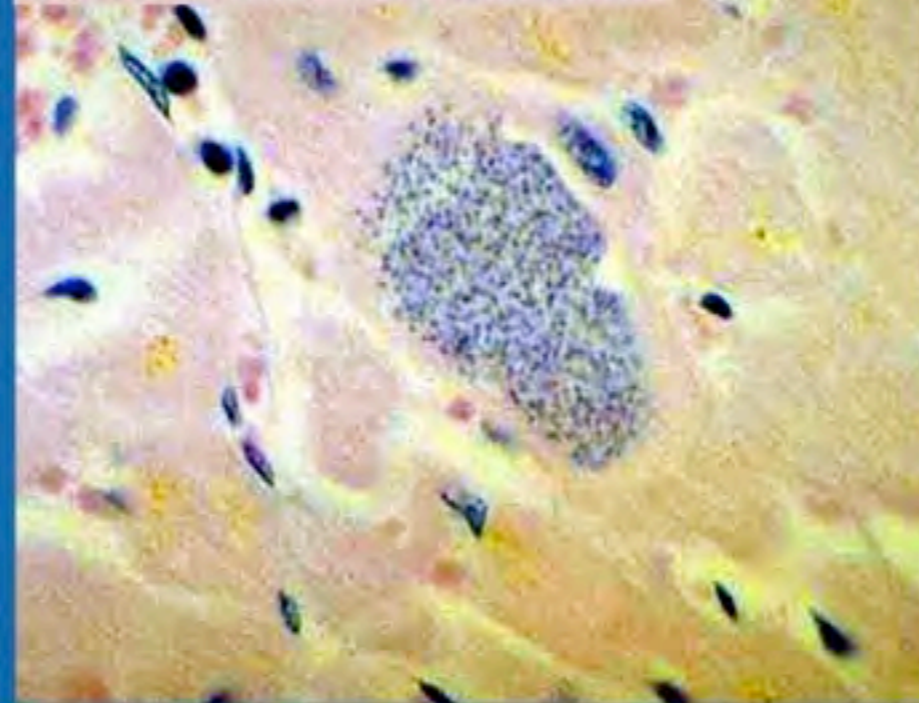
skeletal muscle



brain



cardiac muscle



live cyst mouse brain



Sub-clinical toxoplasmosis

Toxoplasma gondii

a small eukaryotic single-celled organism

an obligate intracellular parasite

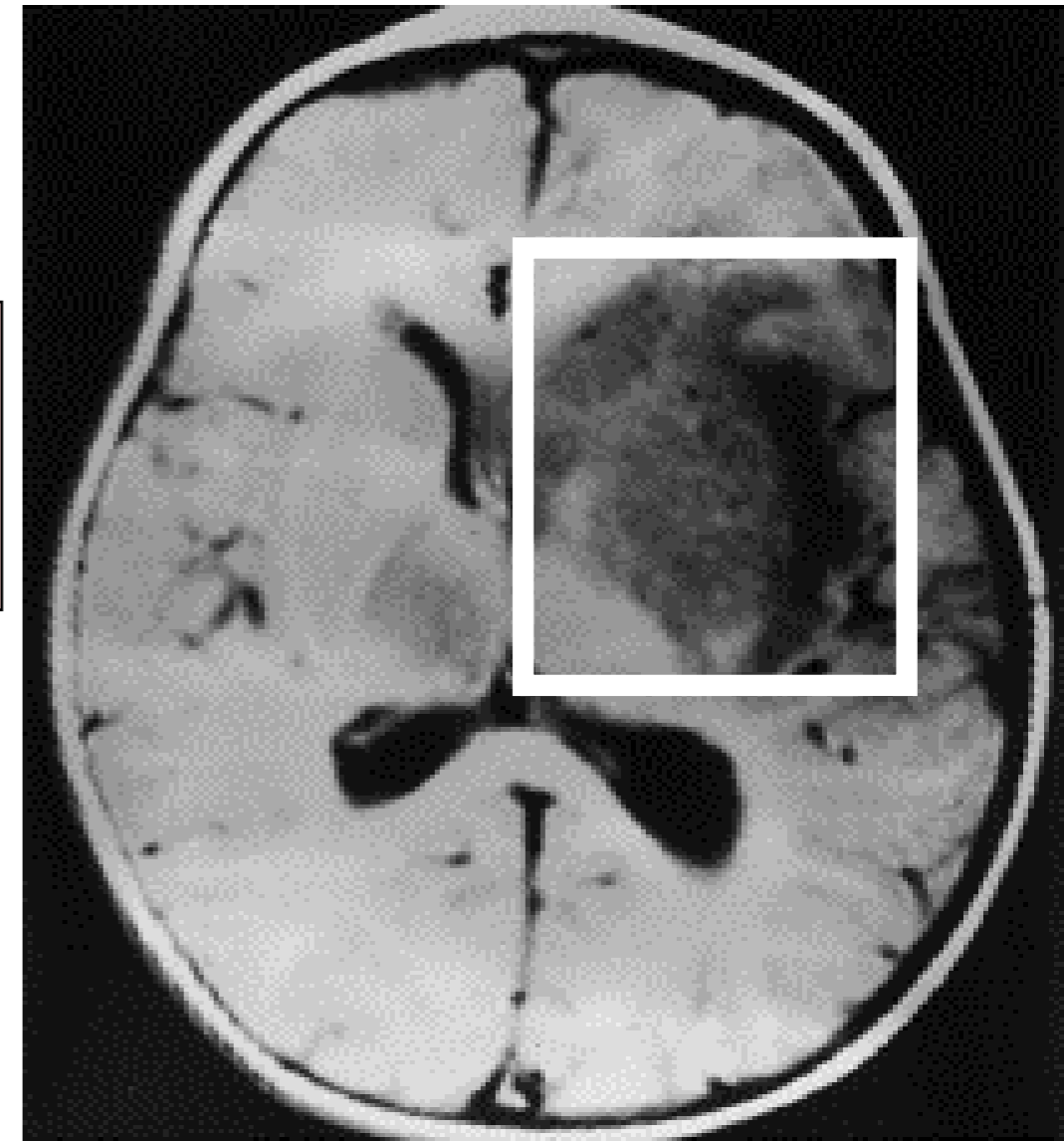
an Alveolate in the phylum Apicomplexa

a ubiquitous parasite

a human pathogen: lethal encephalitis in immunocompromised adults; severe congenital neurological defects (the #1 cause in most countries)



A leading cause of congenital neurological defects

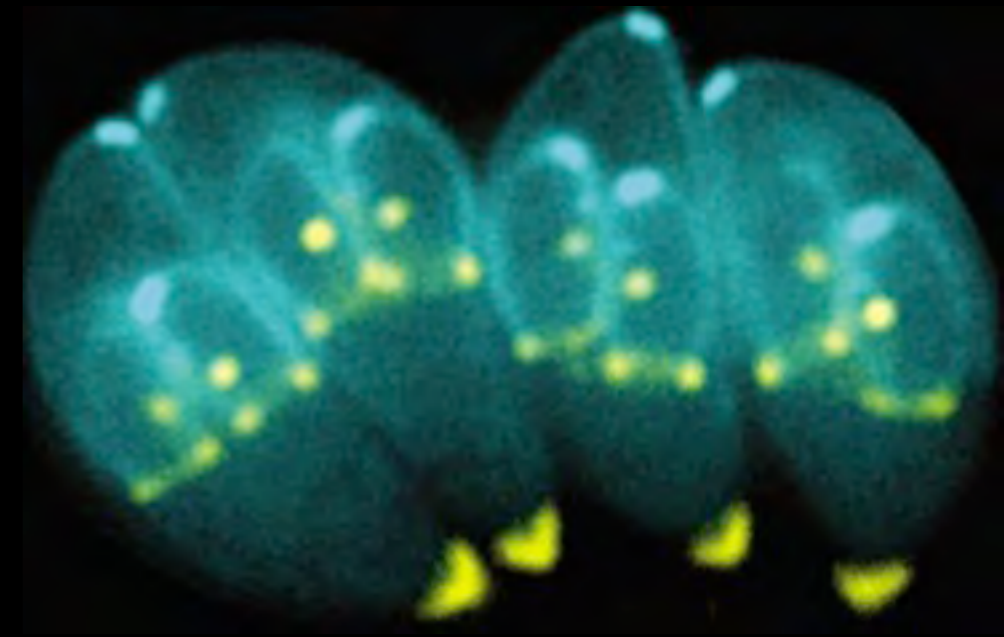
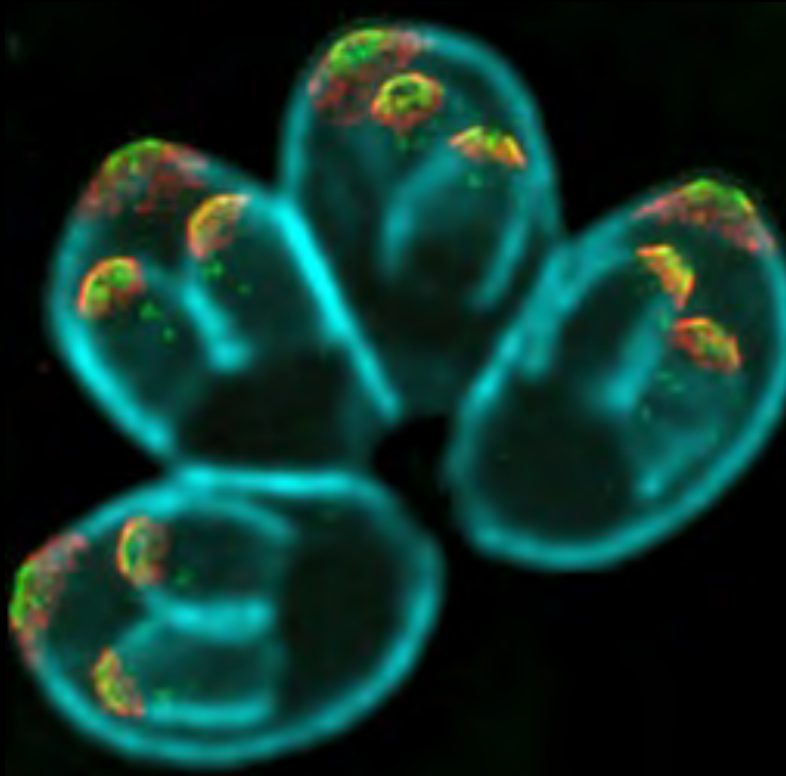


Opportunistic infection in immuno-compromised patients

How to build a parasite?

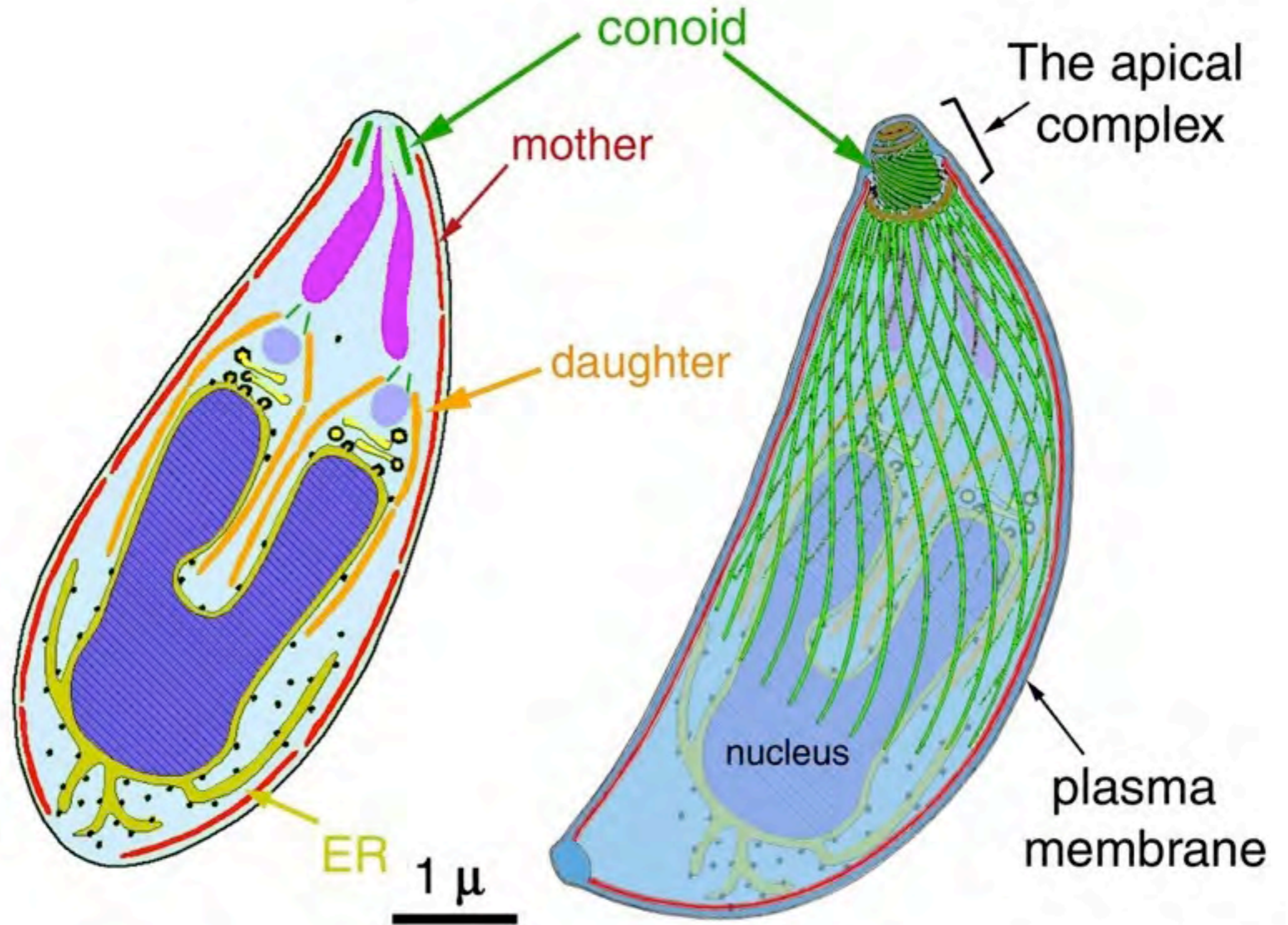
I

- how to make 2 copies?



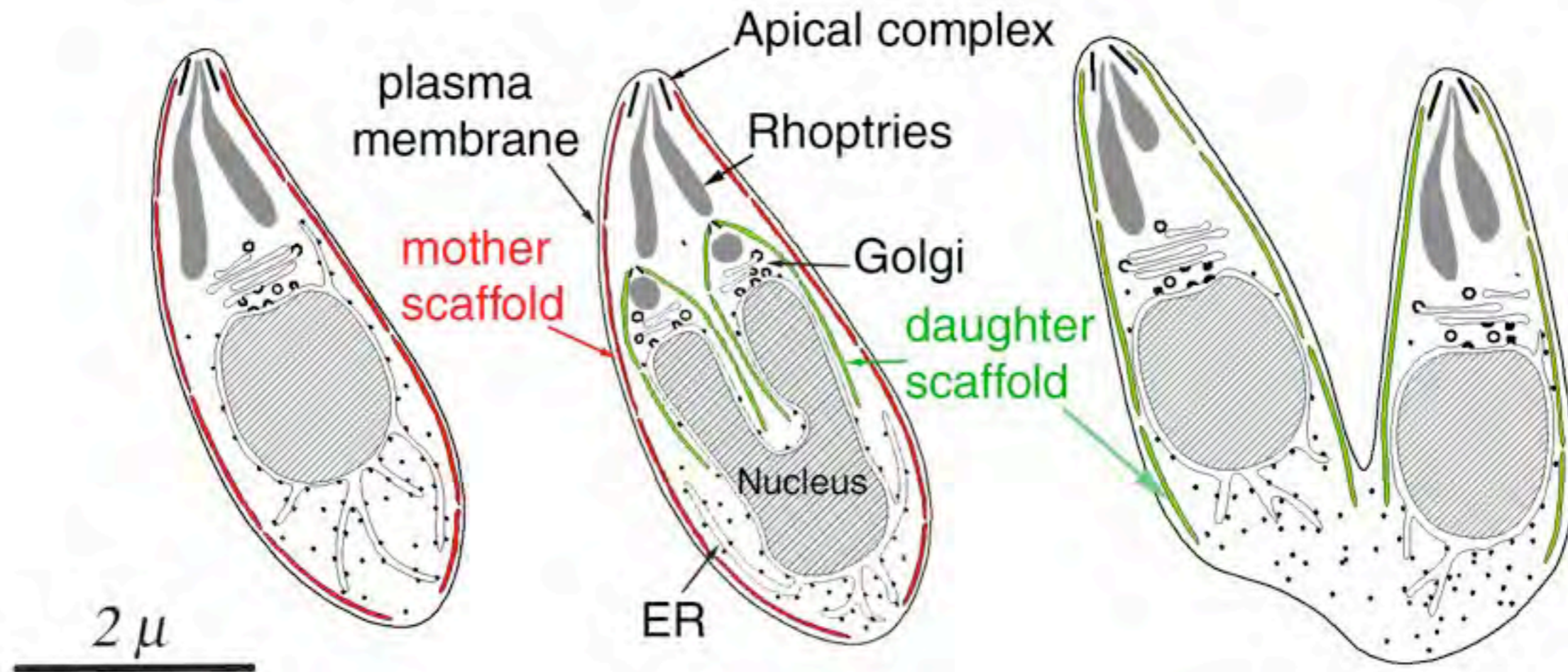
Apicomplexan Cell Division: The Inside View

Apical end



Basal end

T. gondii constructs daughters internally



divided by fission

Membrane-bound components

Invasion organelles (rhoptries)

Golgi

Nucleus, ER

Plastid

Mitochondrion

constructed *de novo*

Cytoskeletal components

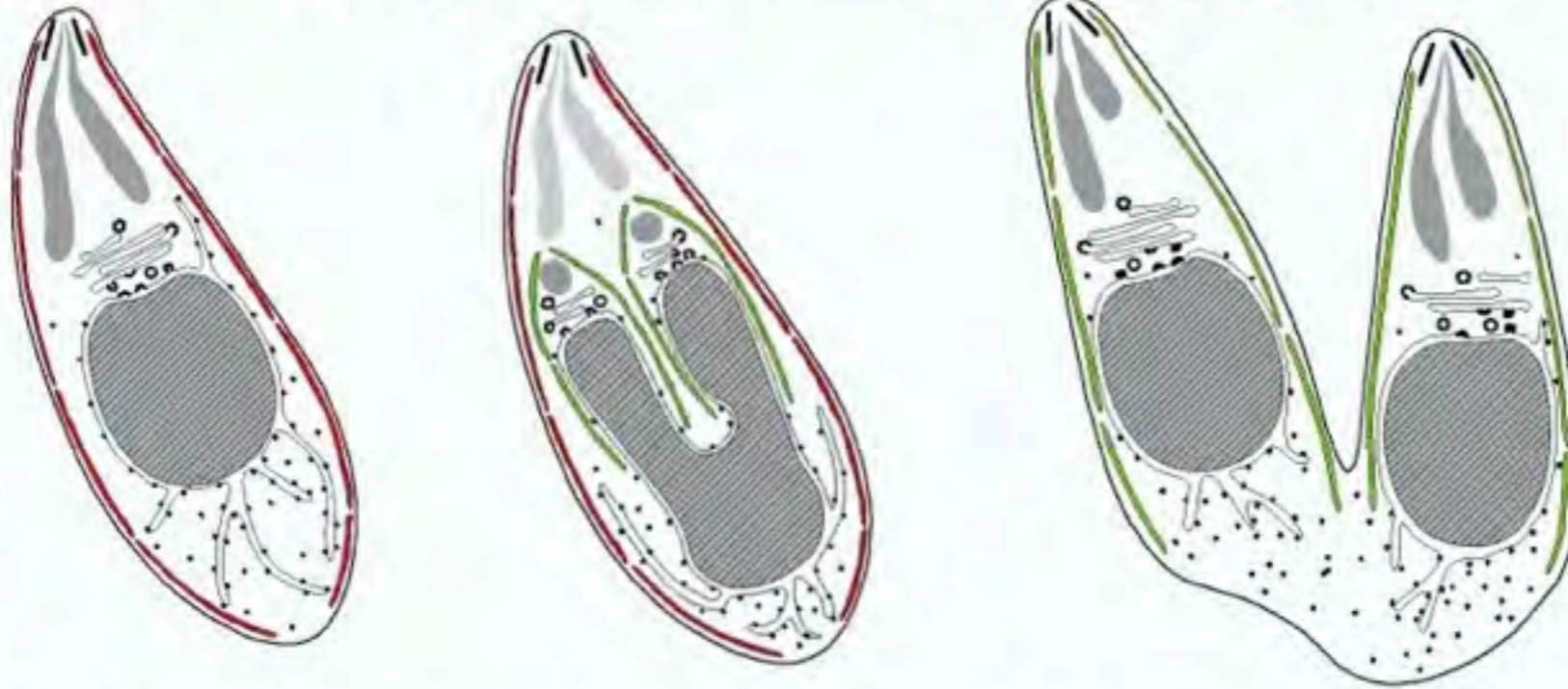
Apical complex

Cortical microtubules

Intermediate filament network

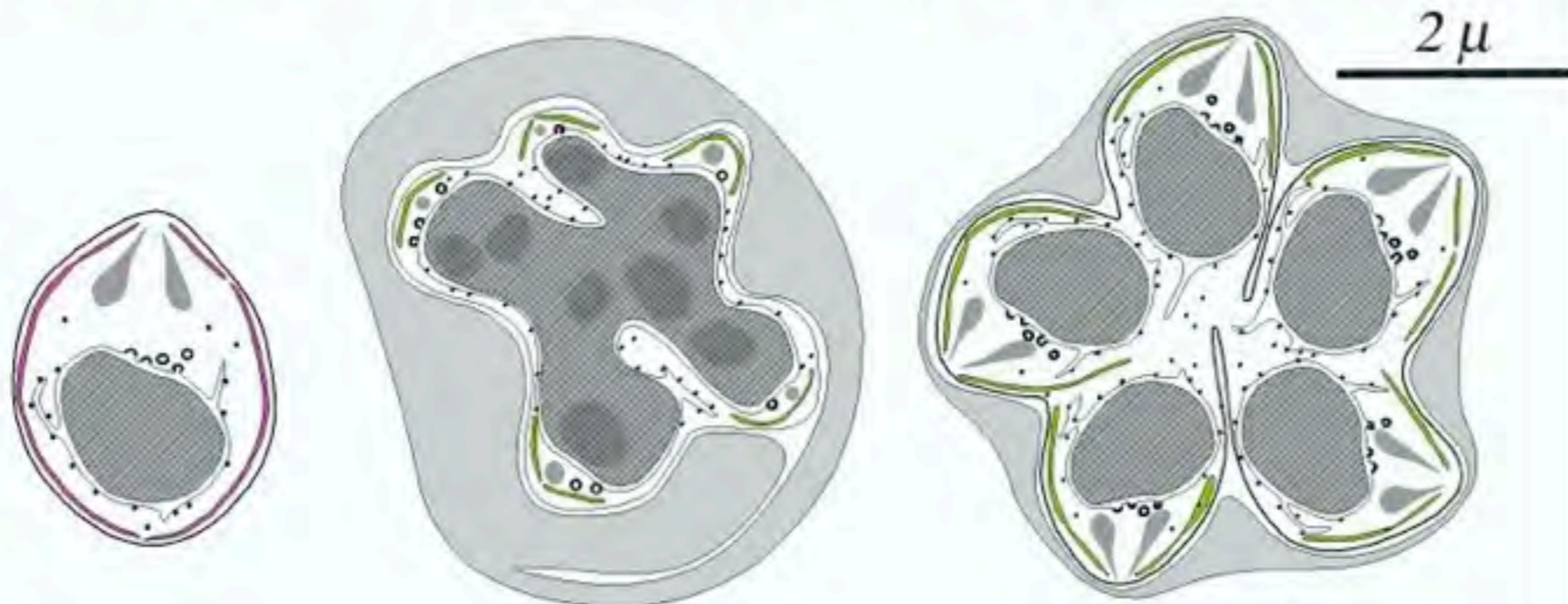
Flattened vesicles

Toxoplasma tachyzoites divide by endodyogeny



Tachyzoite (parasitophorous vacuole and host cell are not shown)

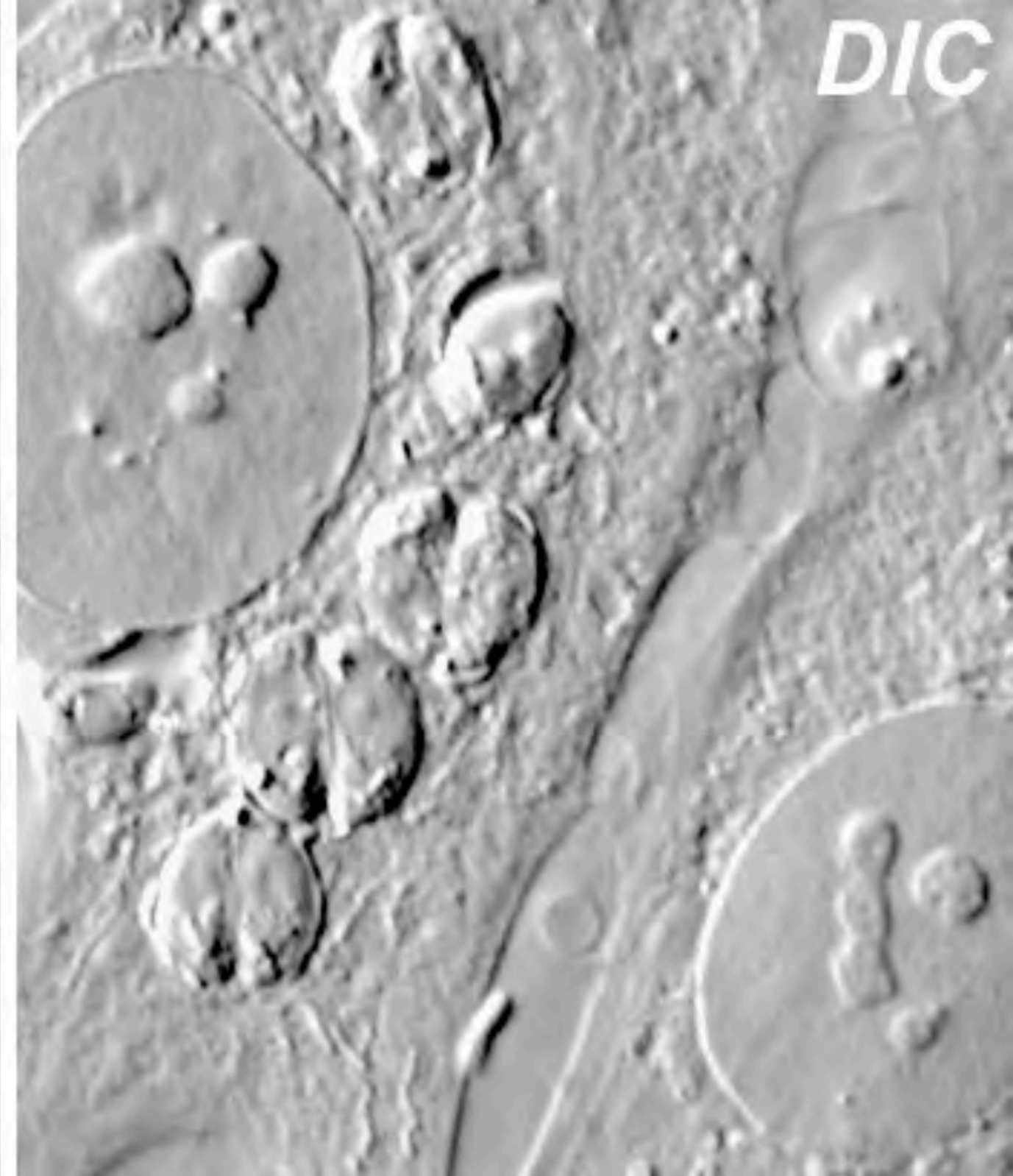
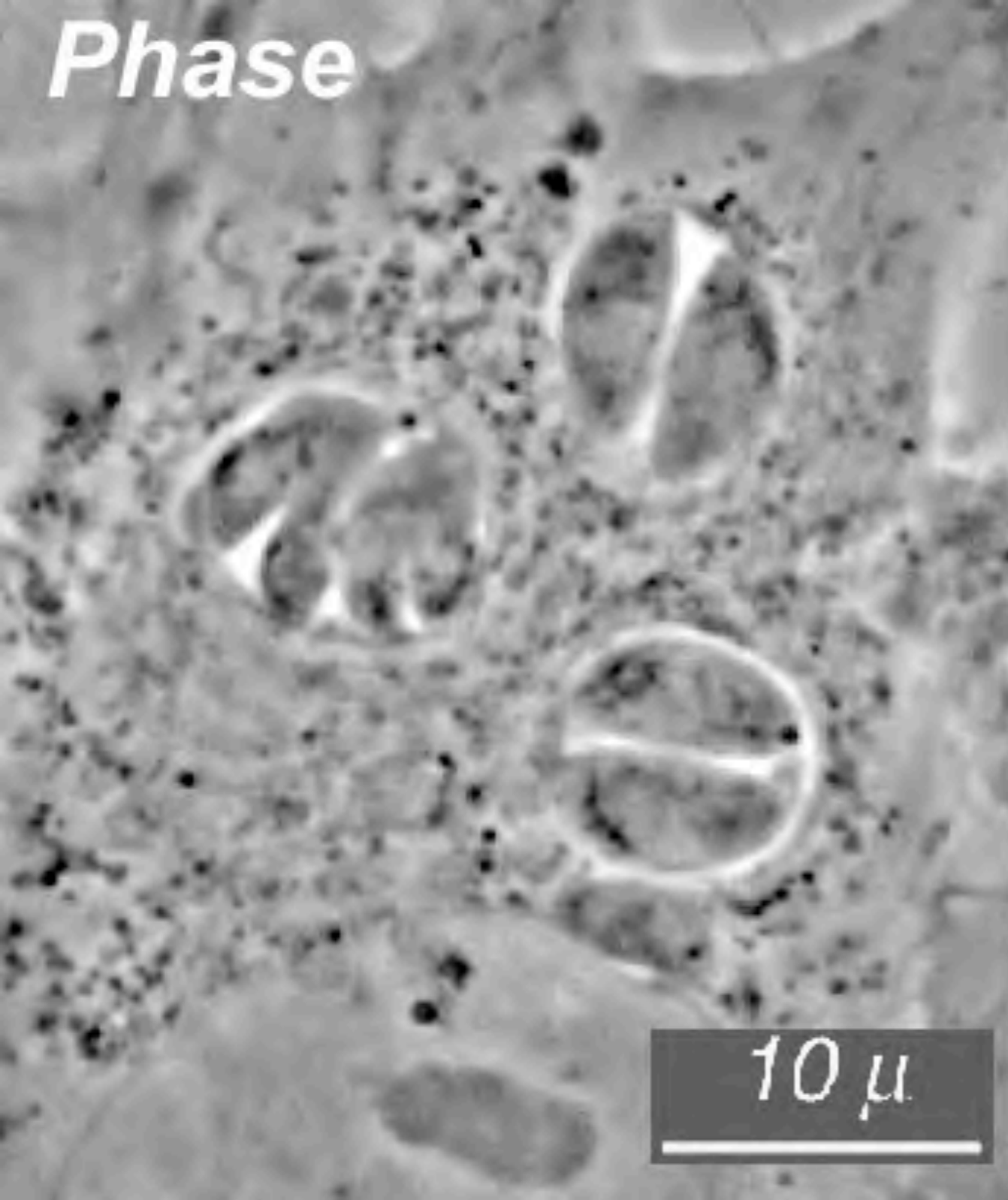
Plasmodium produces multiple daughters by schizogony



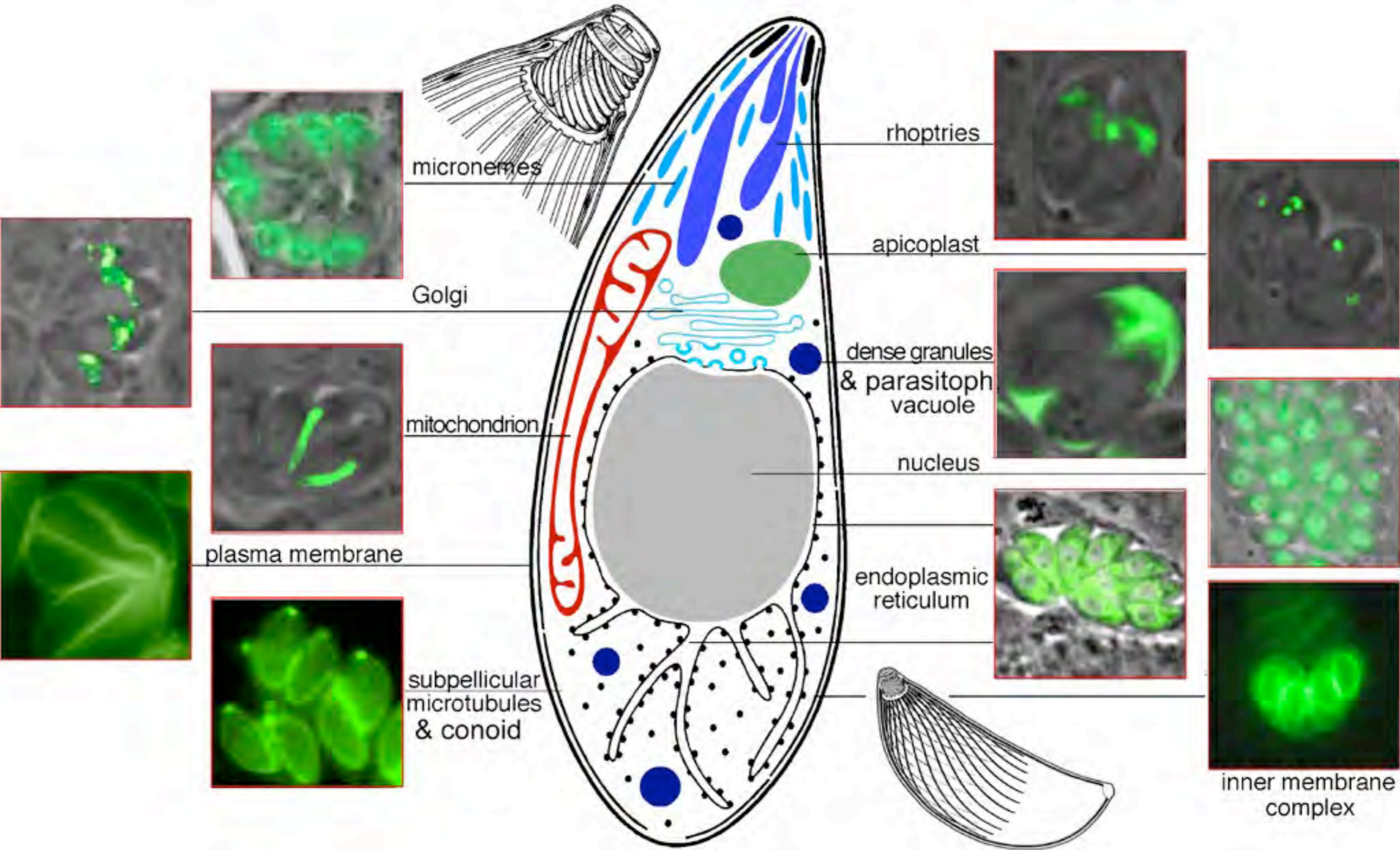
merozoite

early schizont
(intraerythrocytic)

late schizont
(intraerythrocytic)



Phase contrast or DIC microscopy shows little more than the cell outline until the daughters bud out.



Phase contrast or DIC microscopy shows little more than the cell outline, but tagging specific proteins with GFP makes each of the pieces visible.

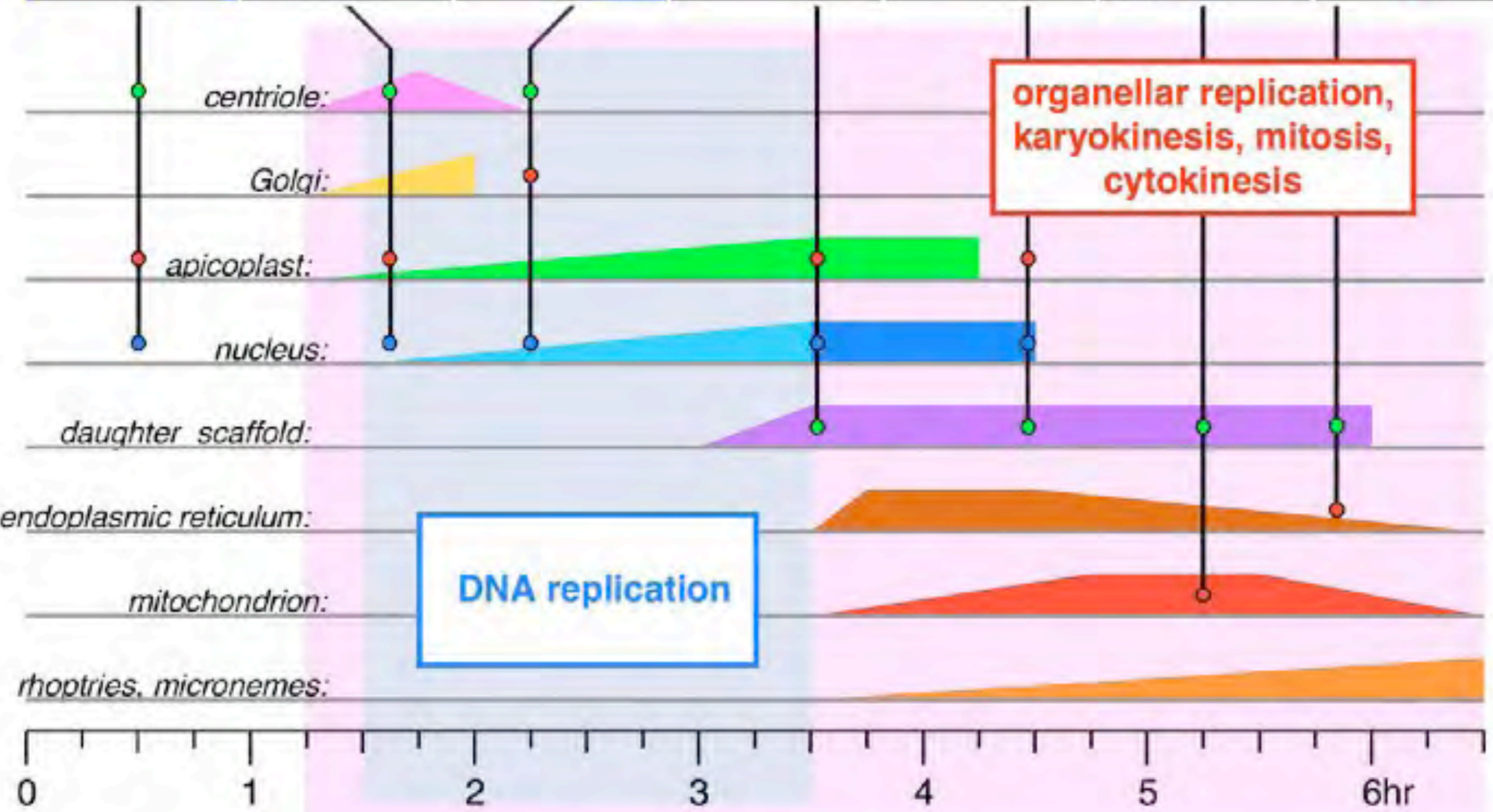
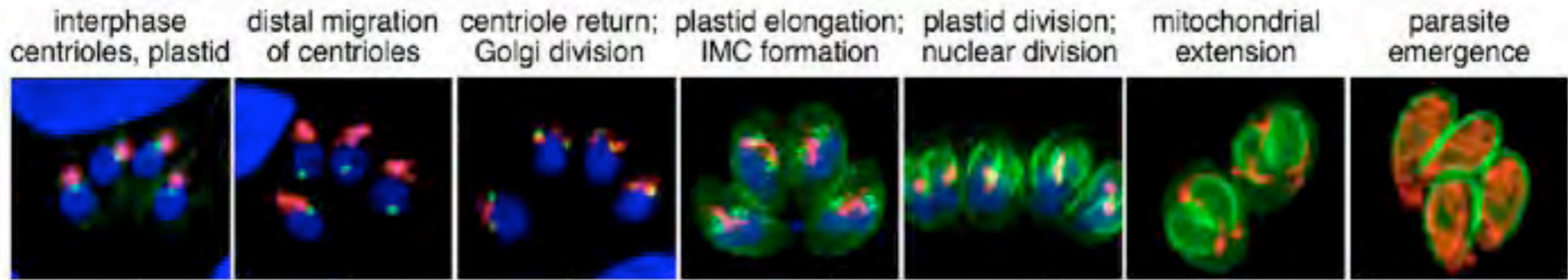


Developing daughter scaffolds visualized with a cytoskeletal protein tagged with GFP.

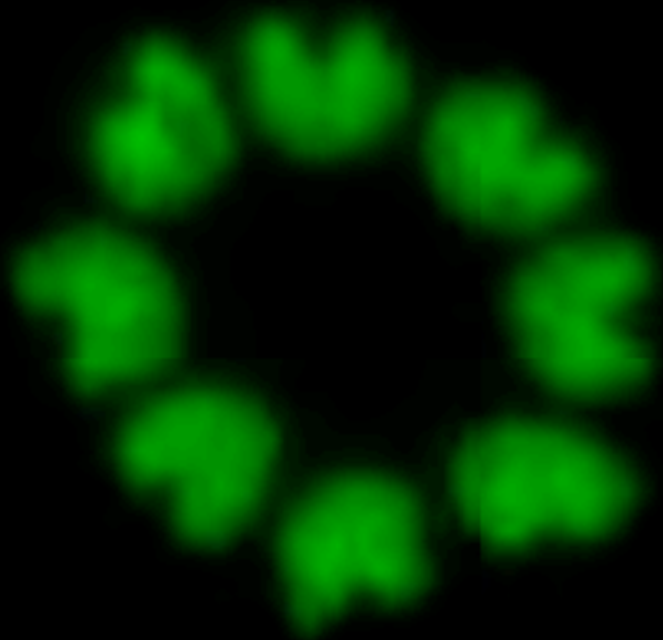
Daughters can be arranged side-by-side, at right angles, or with basal ends opposed.

As always, these parasites are in a vacuole inside a host cell, but the host cell is invisible here, as it contains no GFP.

The choreography of cell division in *T. gondii*

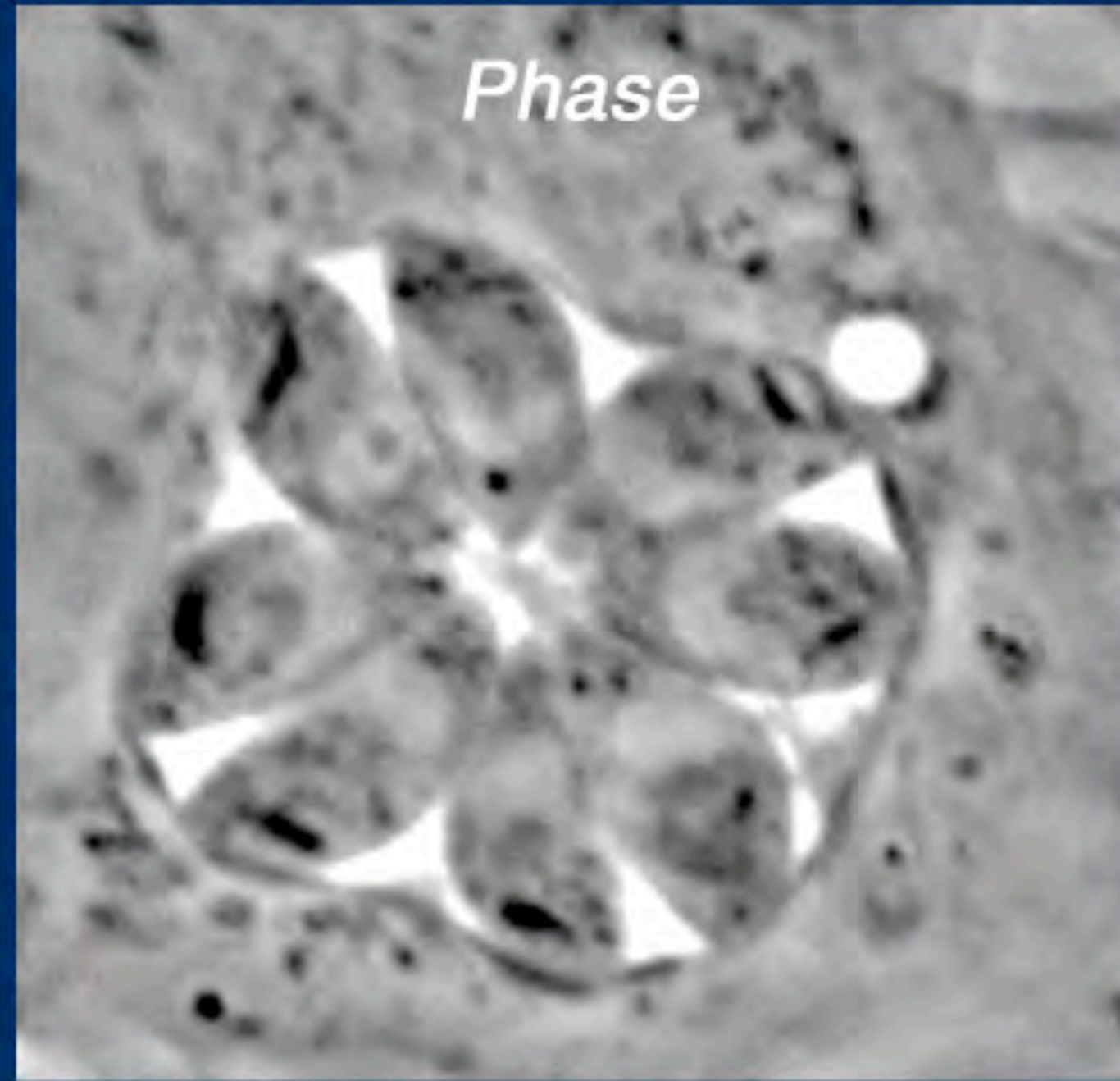


Histone2B-YFP



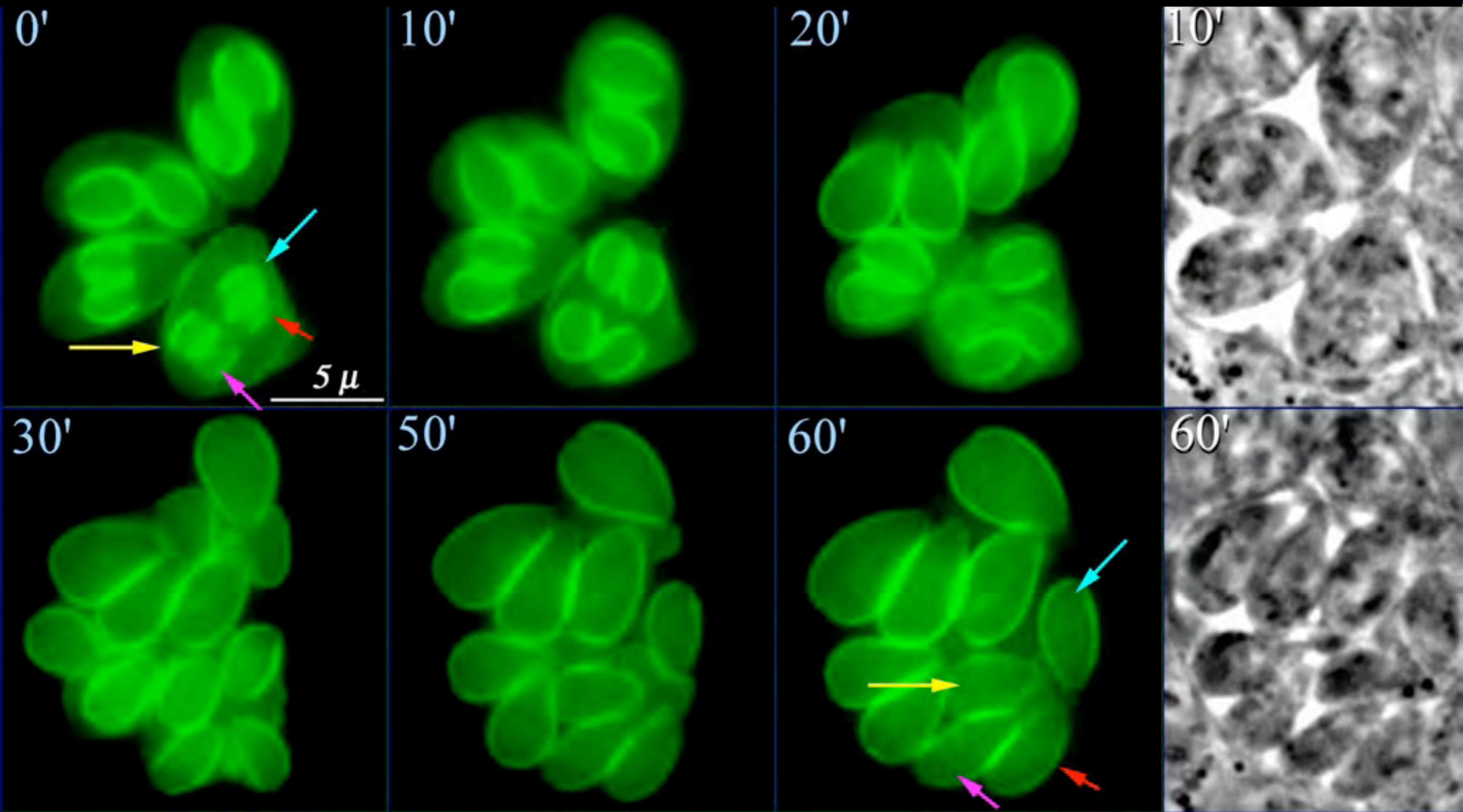
5 μ

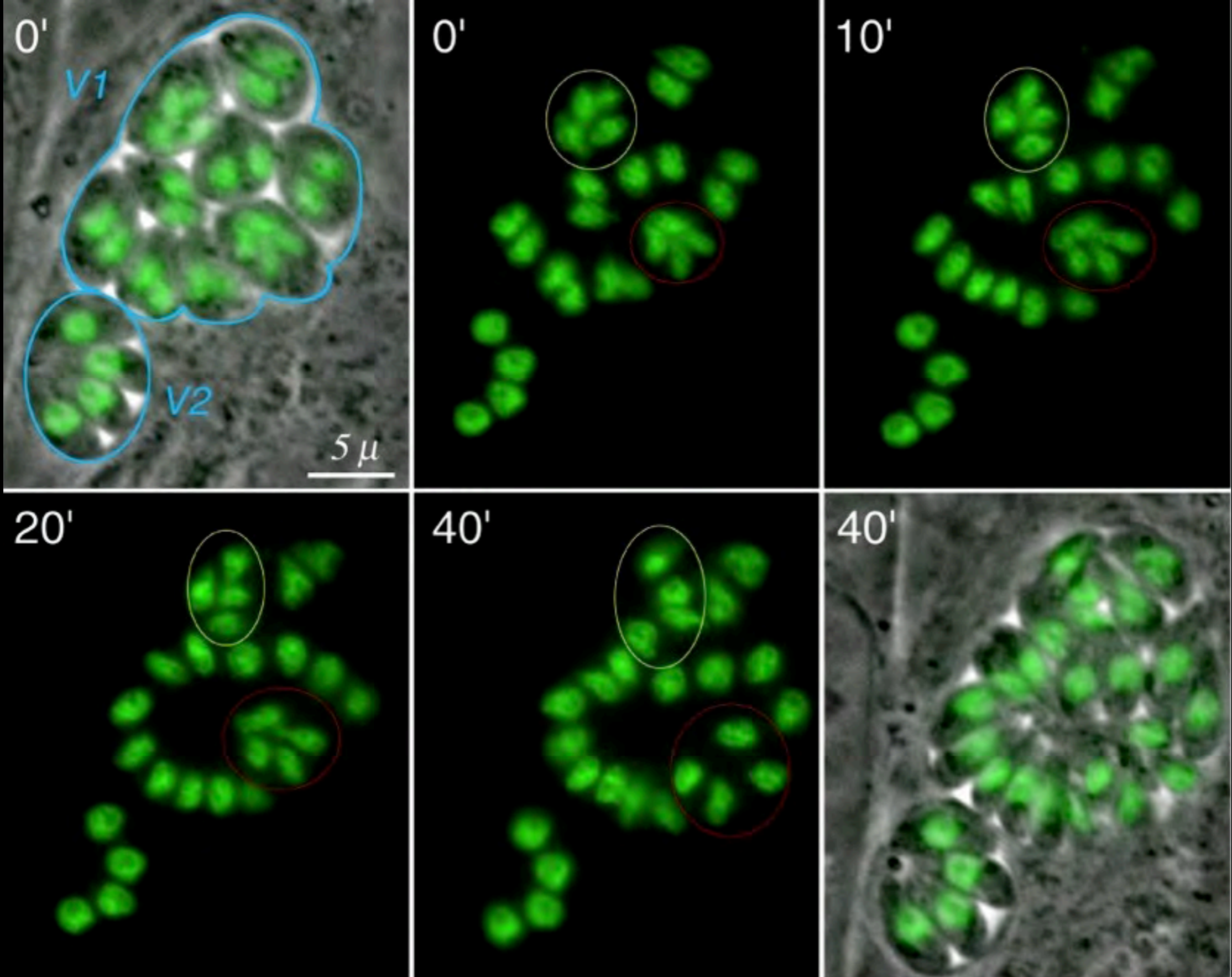
Phase



An FP-tagged histone makes the nucleus visible

In rare instances, *Toxoplasma* makes more than 2 daughters at once





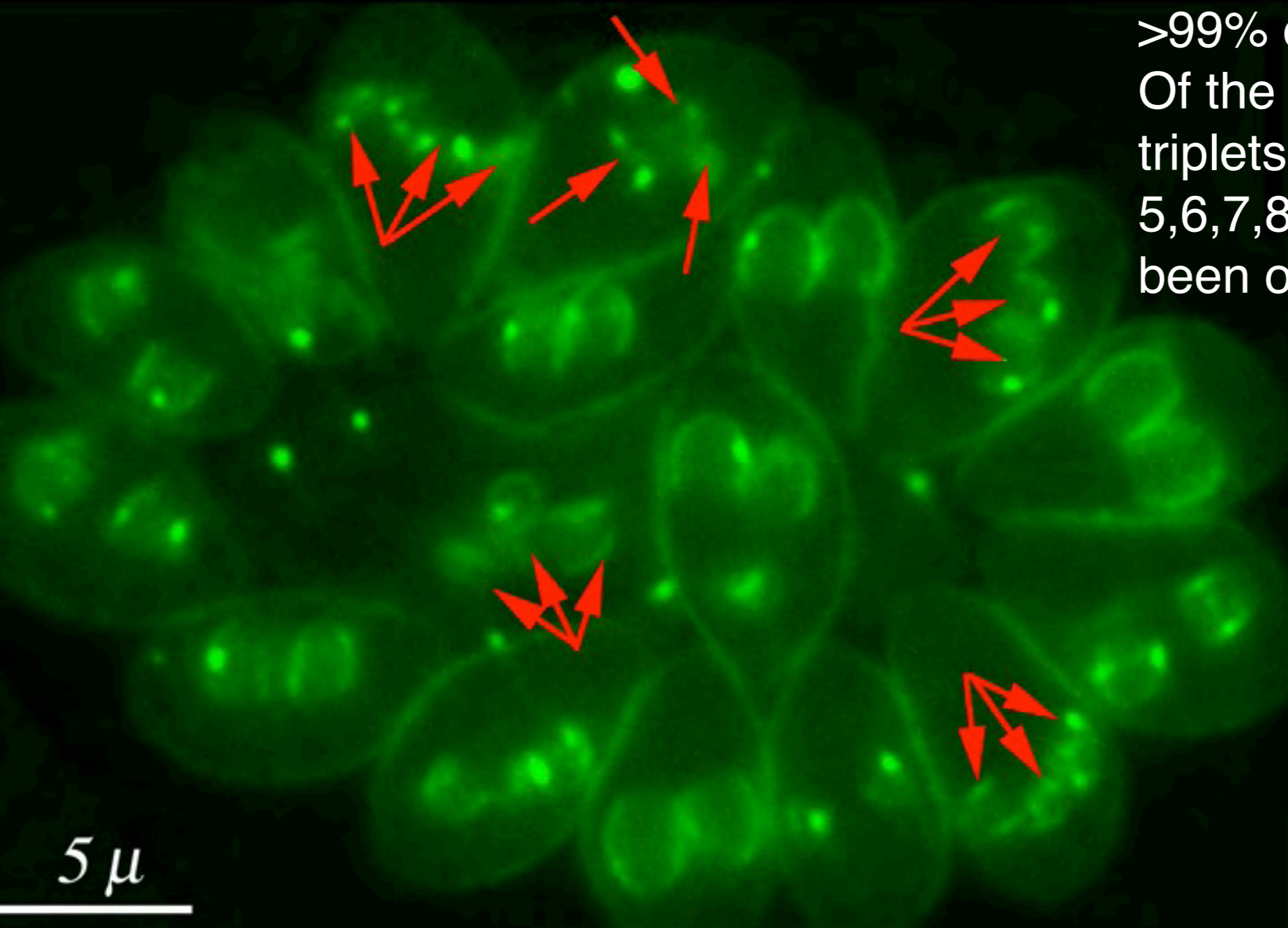
Sometimes *Toxoplasma* makes more than 2 daughters in a single round of cell division. In this vacuole (*v1*) of 9 parasites, 2 are producing quadruplets.

Sometimes an odd number of daughters is produced in a single cycle.

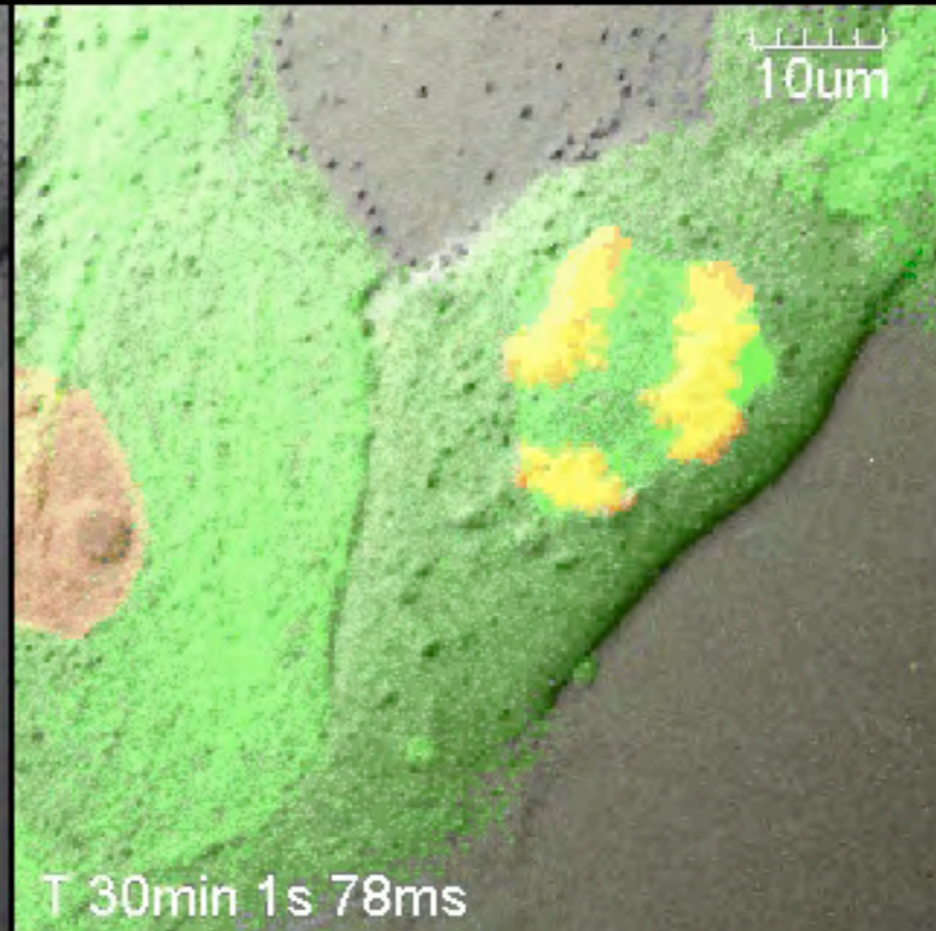
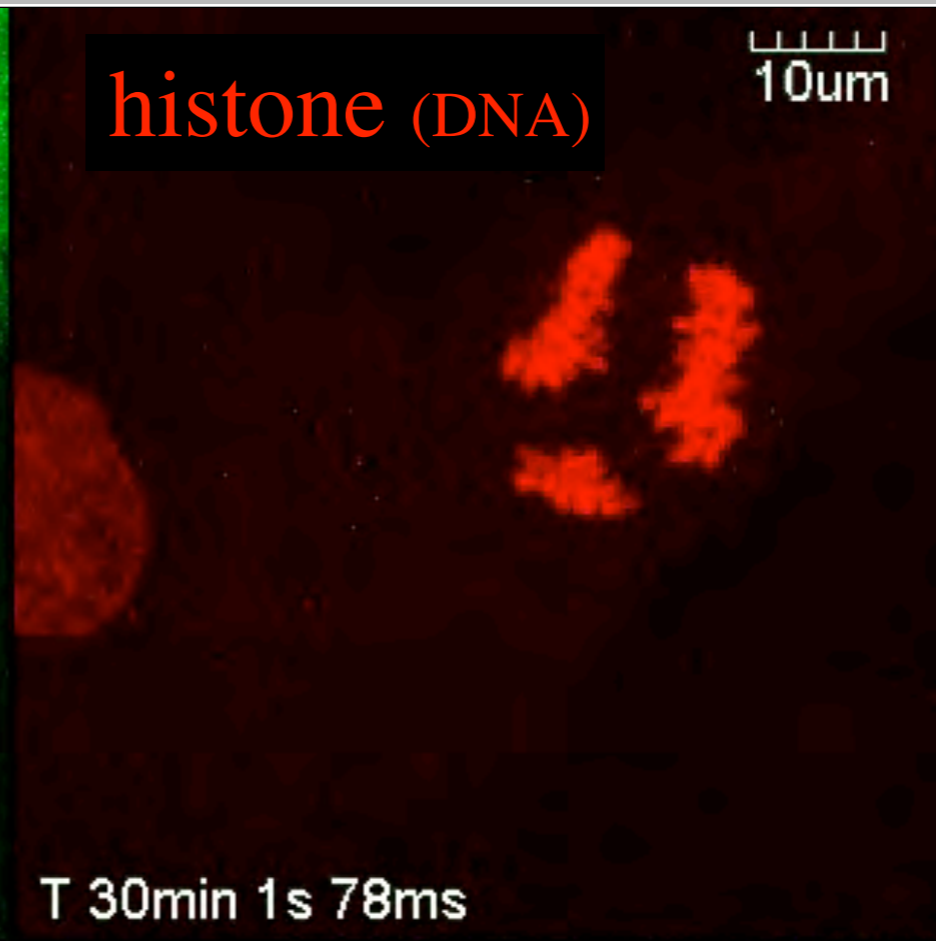
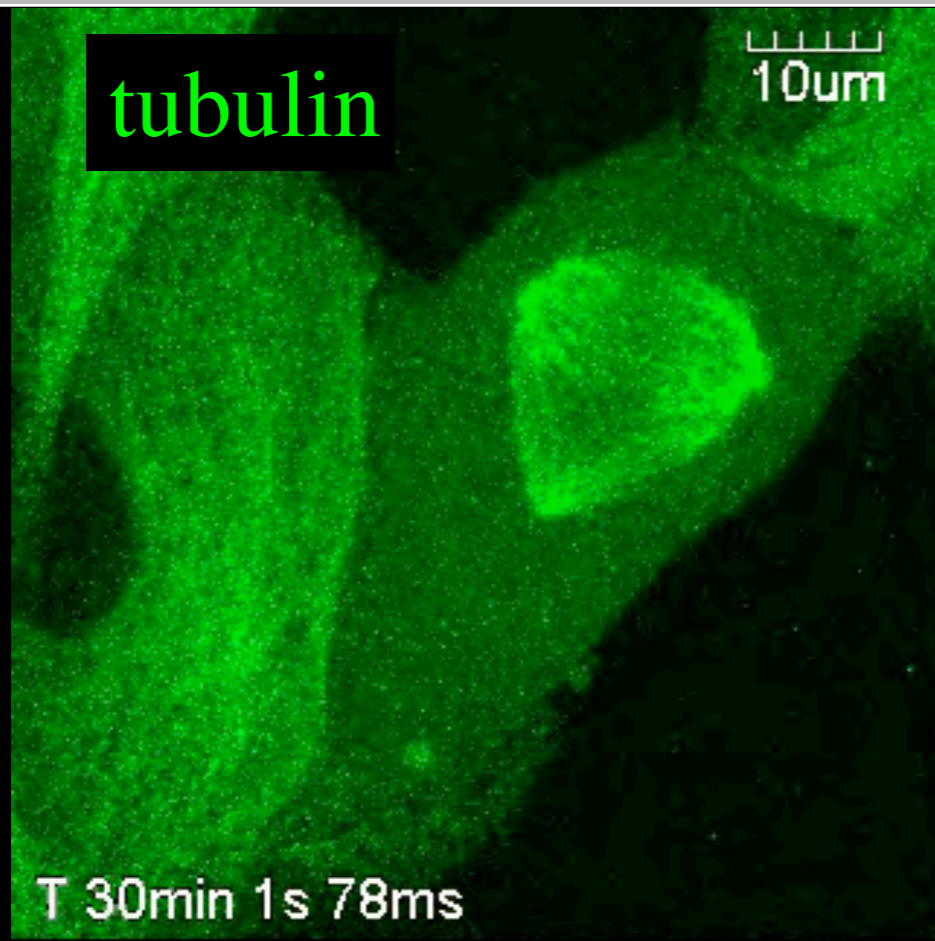
Of the 17 parasites in this vacuole,
ALL are producing daughters
12 are producing twins,
5 are producing triplets

In a typical lab culture, 20-30%
of vacuoles will contain
parasites with visible
daughters.

>99% of those are twins.
Of the other 1%, most are
triplets or quadruplets.
5,6,7,8 daughters have all
been observed.

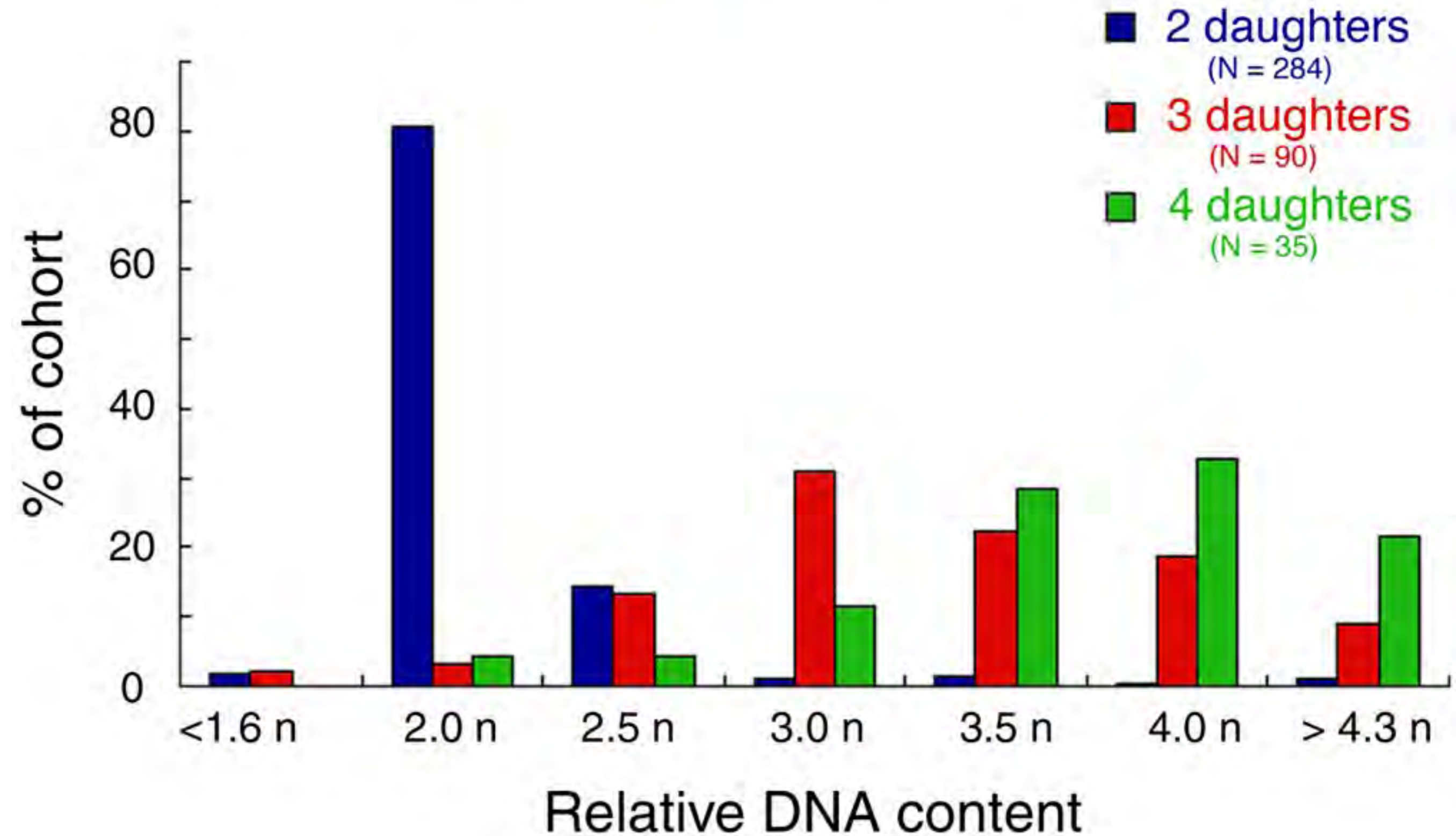


Tripolar mitosis: a construction accident, probably lethal for normal cells



LLC-PK1 cells

DNA content distribution among *T. gondii* having 2, 3, or 4 daughters



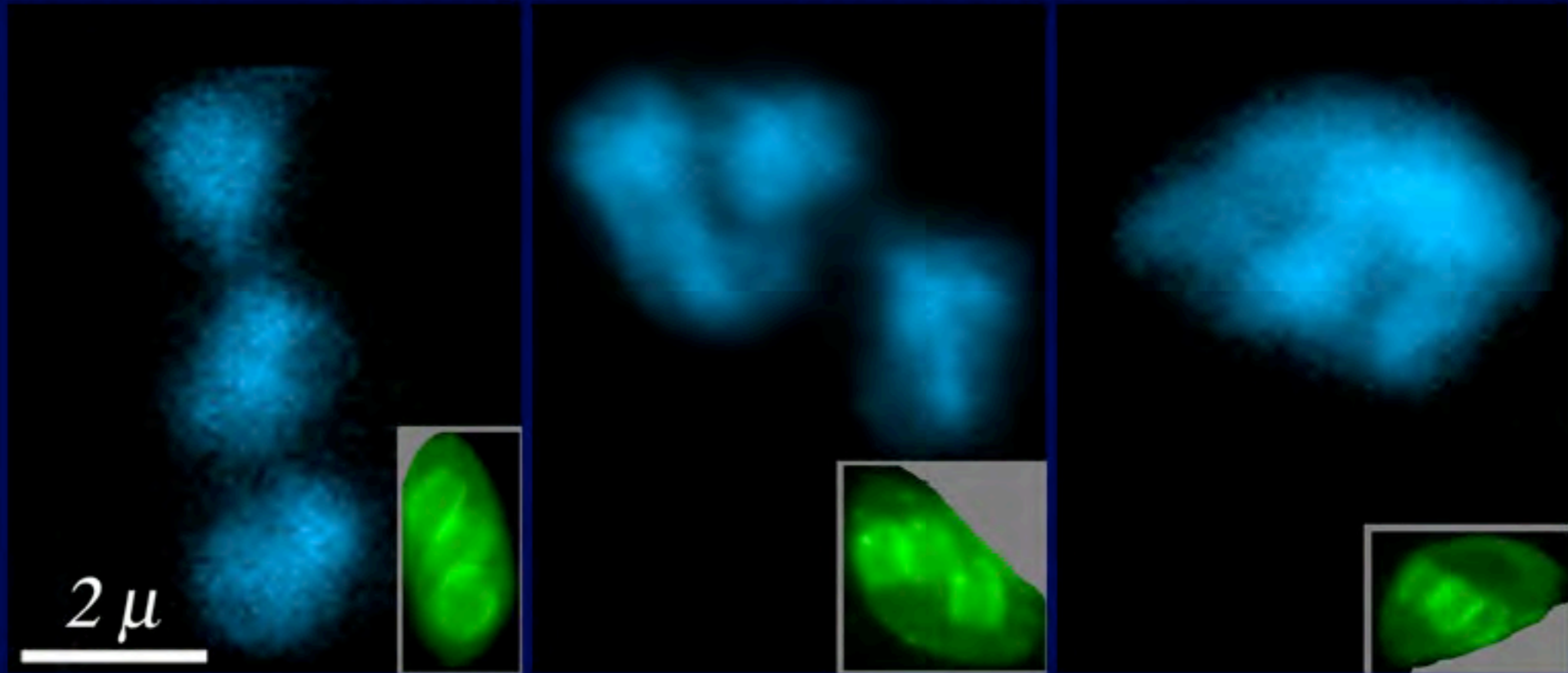
DNA content and nuclear morphology

3 daughters

$3 \times 1n$

$1n + 2n$

$4n$

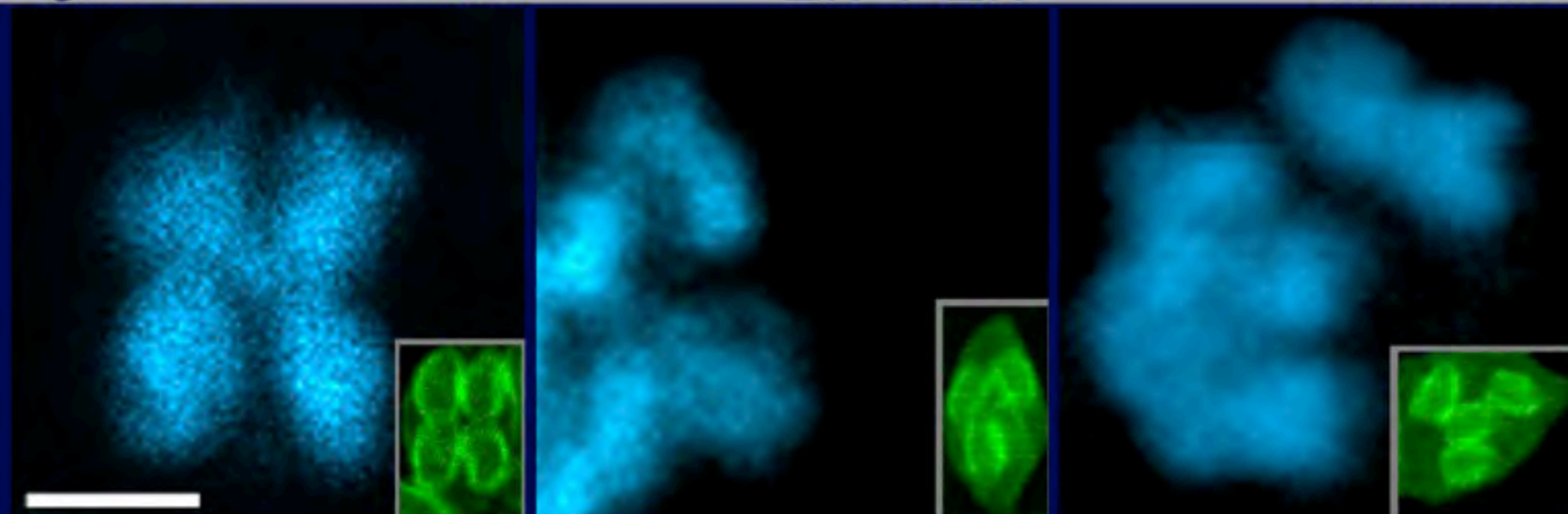


4 daughters

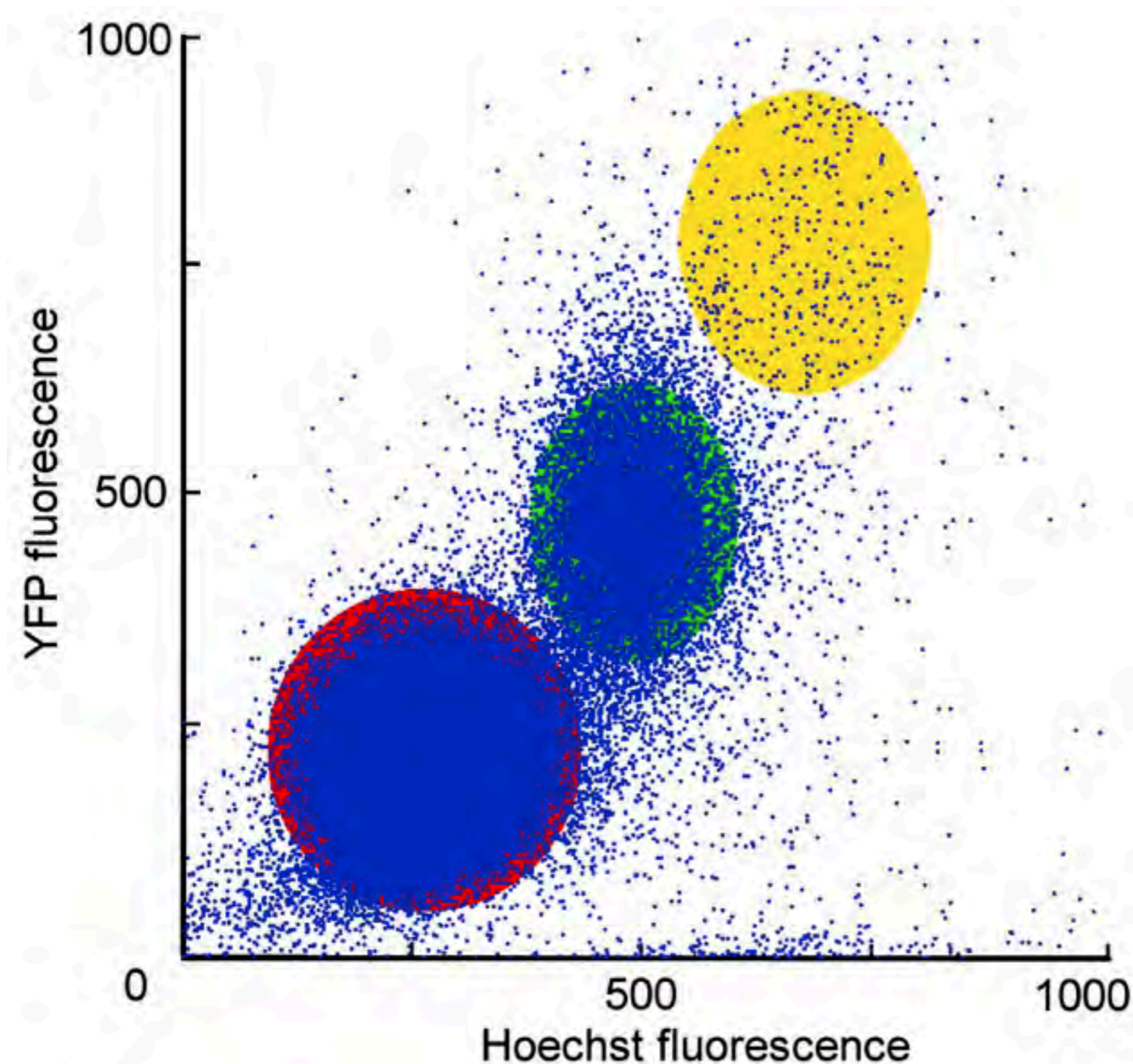
$4n$

$2n + 2n$

$1n + 3n$



Multiple-daughter formation by populations after FACS sorting by DNA content

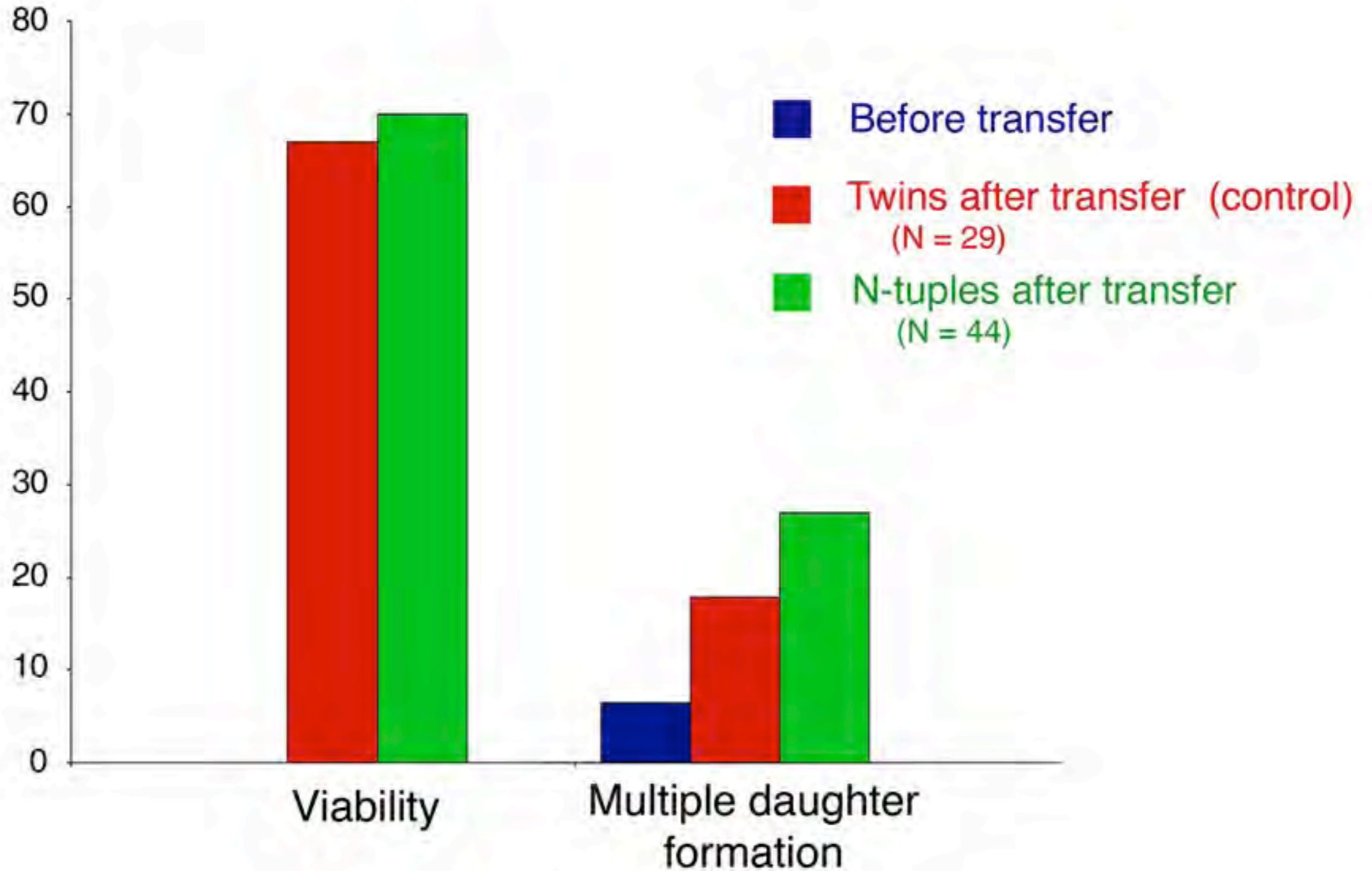


normal haploid DNA content is defined as “1.0 N”

	<i>% multi-daughter formation (s.d.)</i>
pre-sort	8.9 (1.5)
DNA = 1N	12.3 (2.5)
DNA = 2N	13.3 (2.5)
DNA \geq 3N	13.2 (4.7)

Cloning of single *Toxoplasma* cells by micro-needle transfer

Percentage (%)



Daughter assembly in *Toxoplasma gondii*

Hu et al., Mol. Biol. Cell 13: 593 (2002)

Hu et al., J. Cell Sci. 117: 5697 (2004)

T. gondii can form multiple (3,4,...) daughters in one mother.

Multiple rounds of nuclear division can occur during one round of cell division.

DNA replication is autonomous to each individual patch of DNA in a multi-DNA-patch cell.

Daughter scaffold formation is synchronous for all daughters in a multi-daughter mother, but is not tightly coupled to DNA replication.

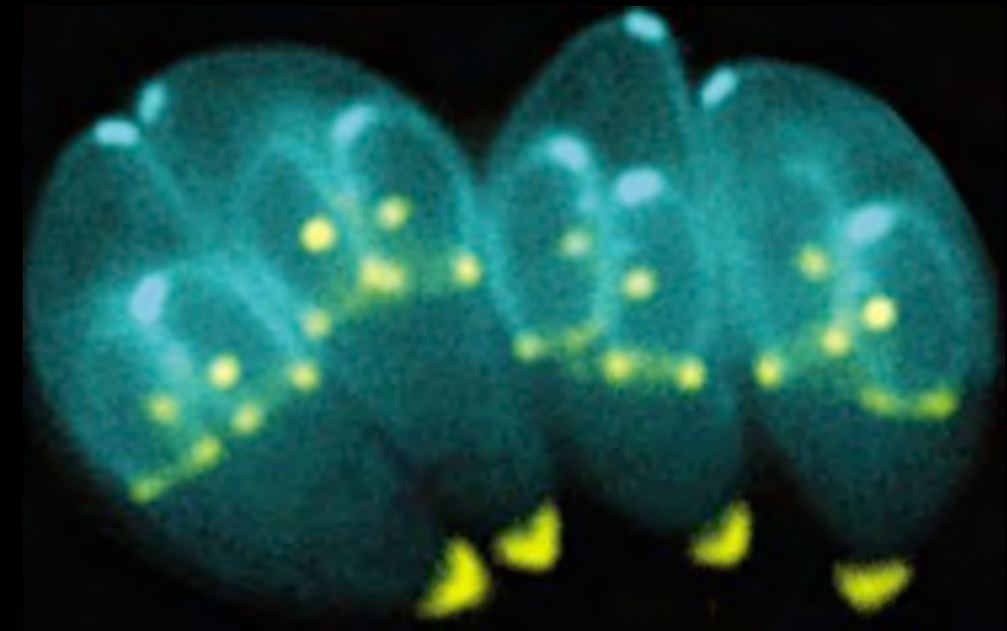
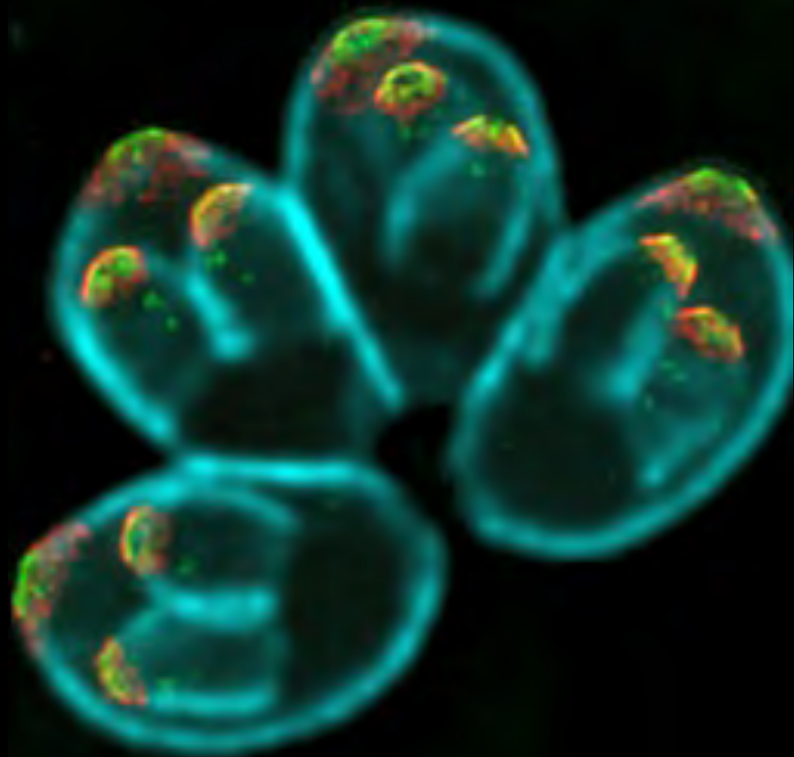
Organelle duplication is coordinated with daughter scaffold formation.

Formation of multiple daughters is a wild-type trait, sensitive to environmental conditions.

How to build a parasite?

I

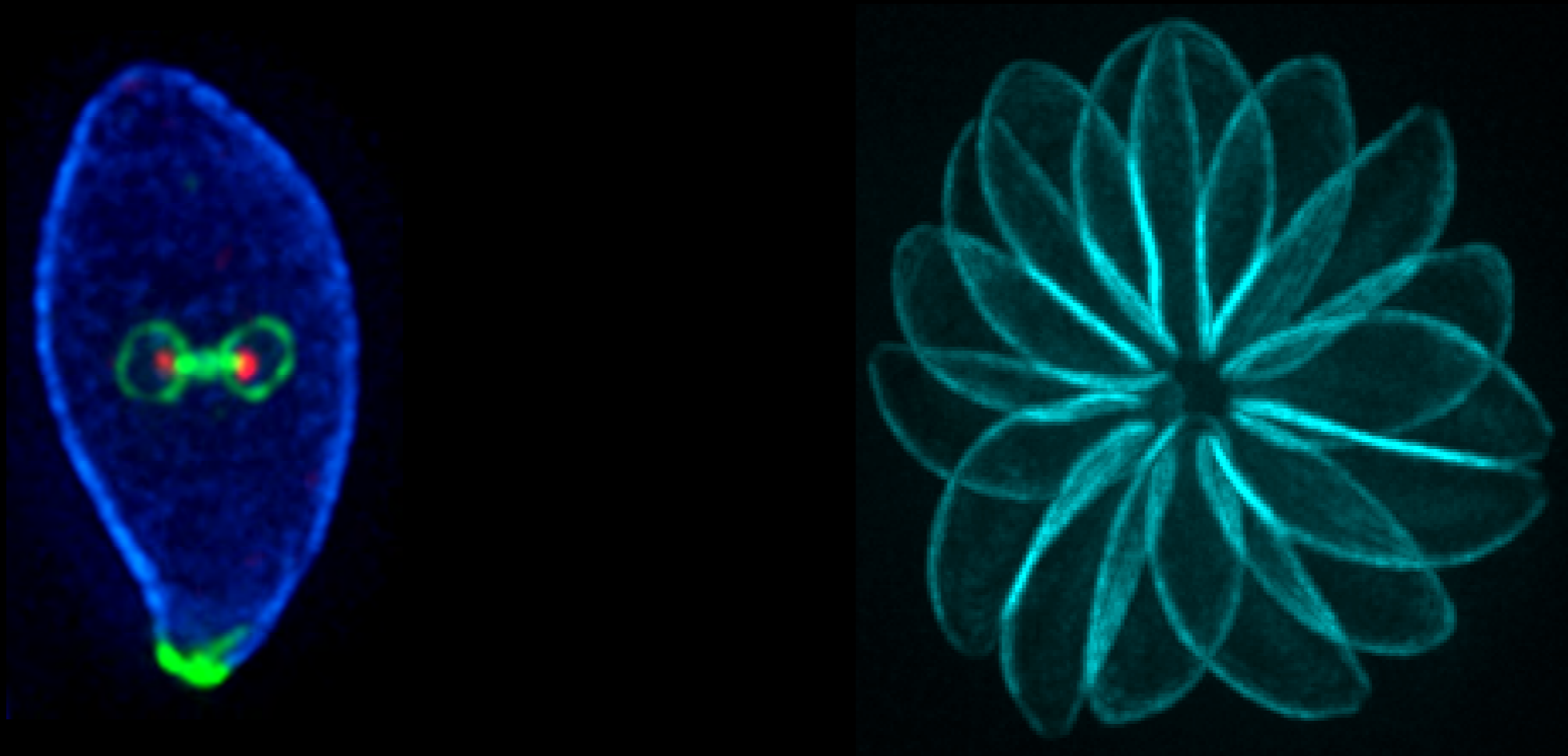
- *how many copies to make?*
- *how to make a variable number?*
- *why allow for different numbers?*



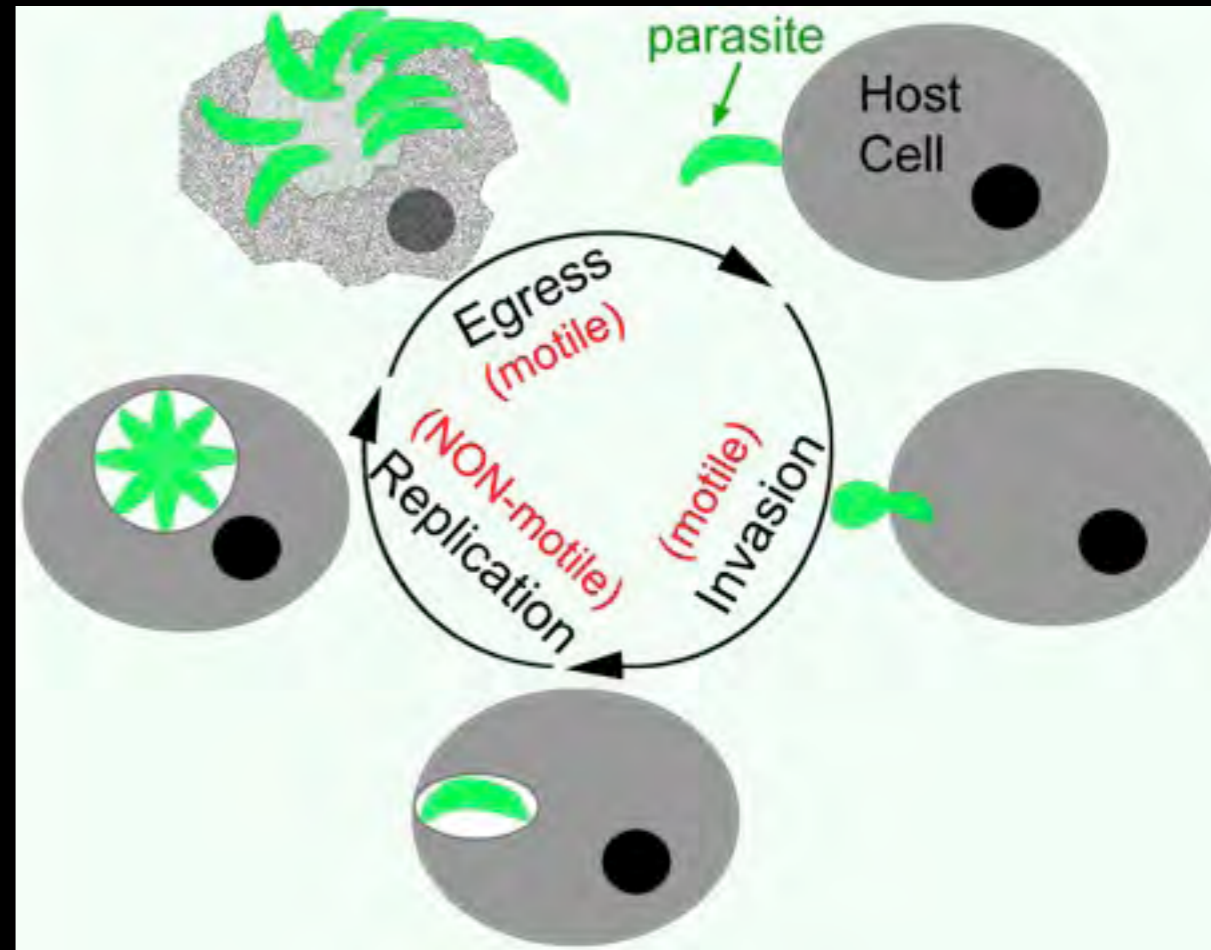
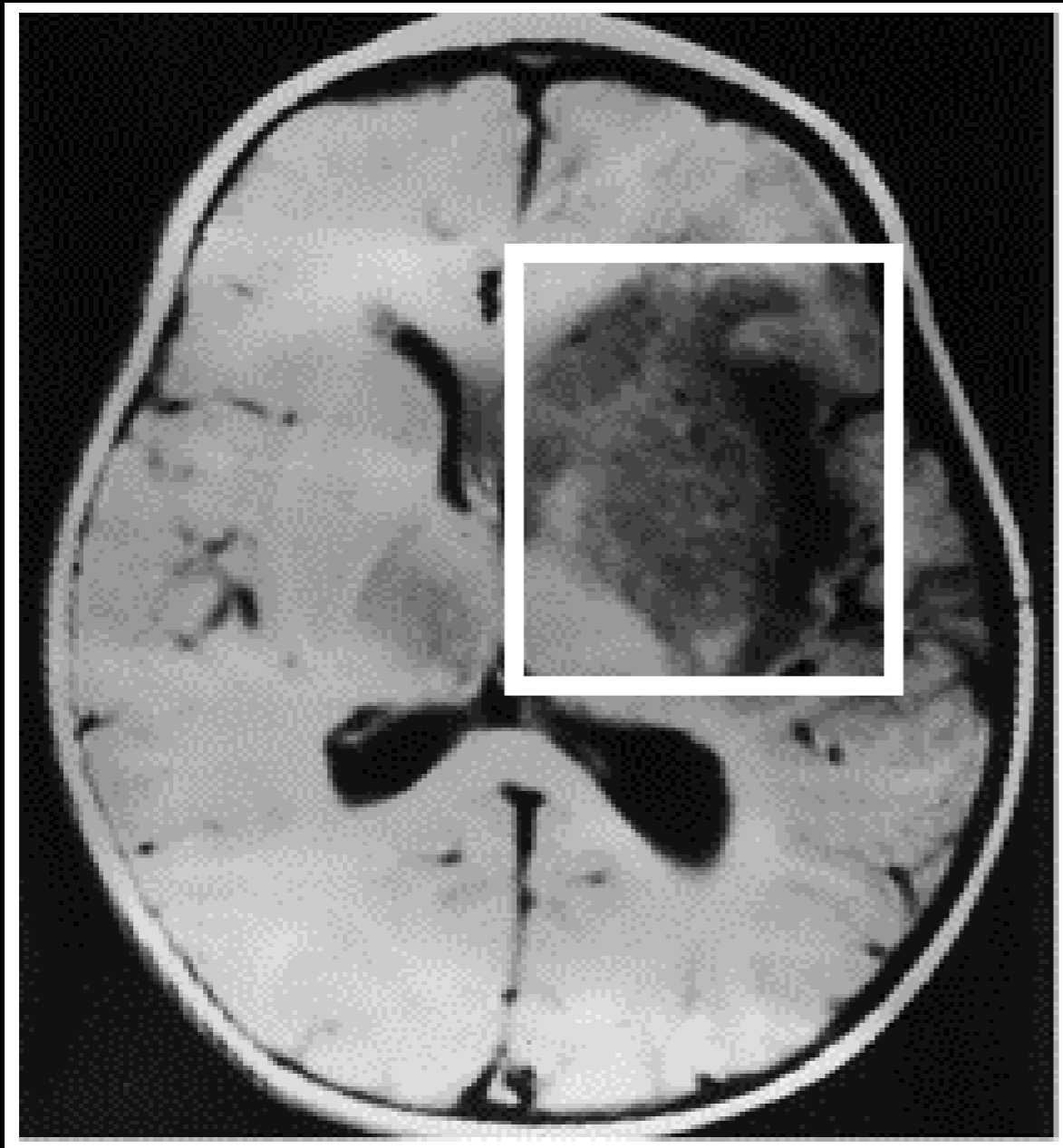
How to build a parasite?

II

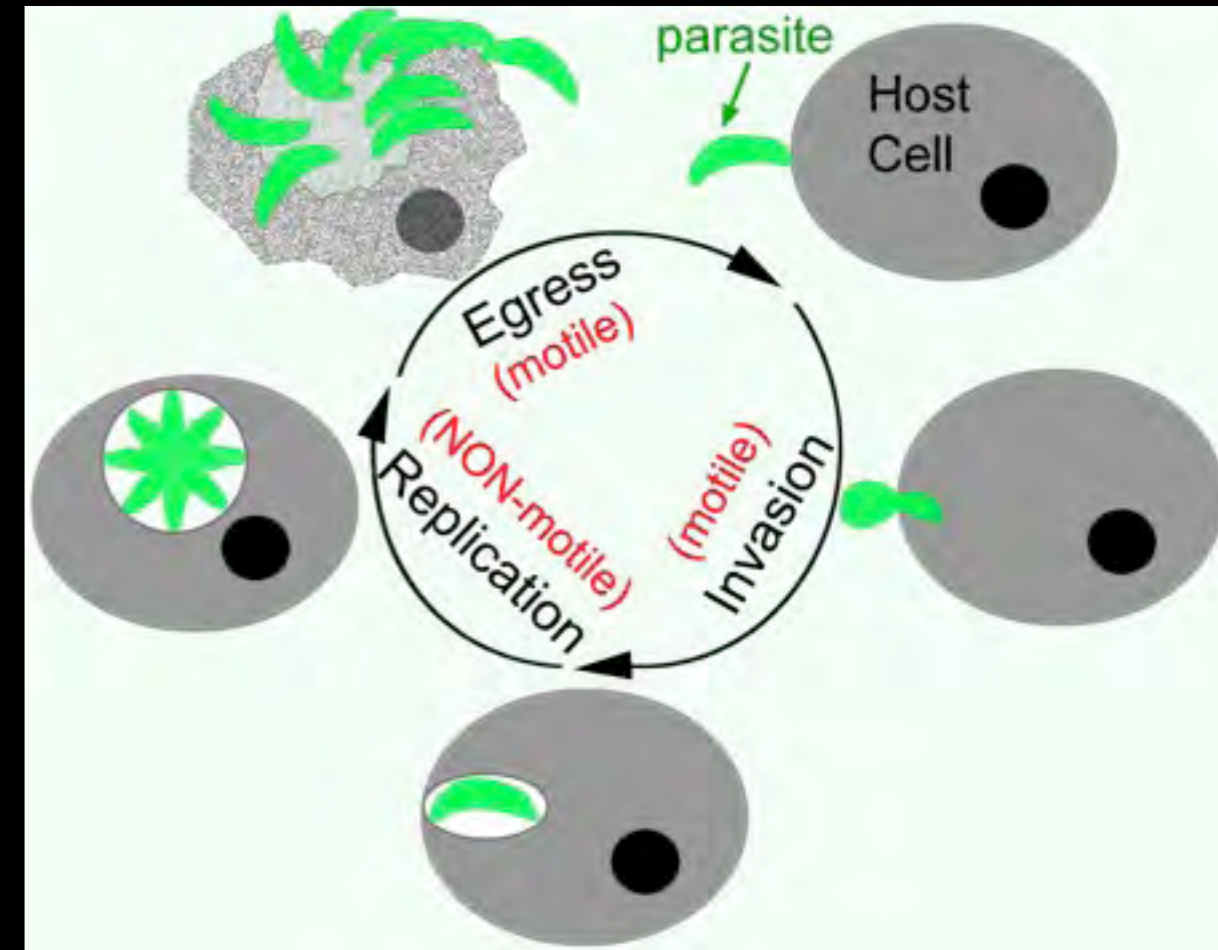
- how do molecules get to the right place?



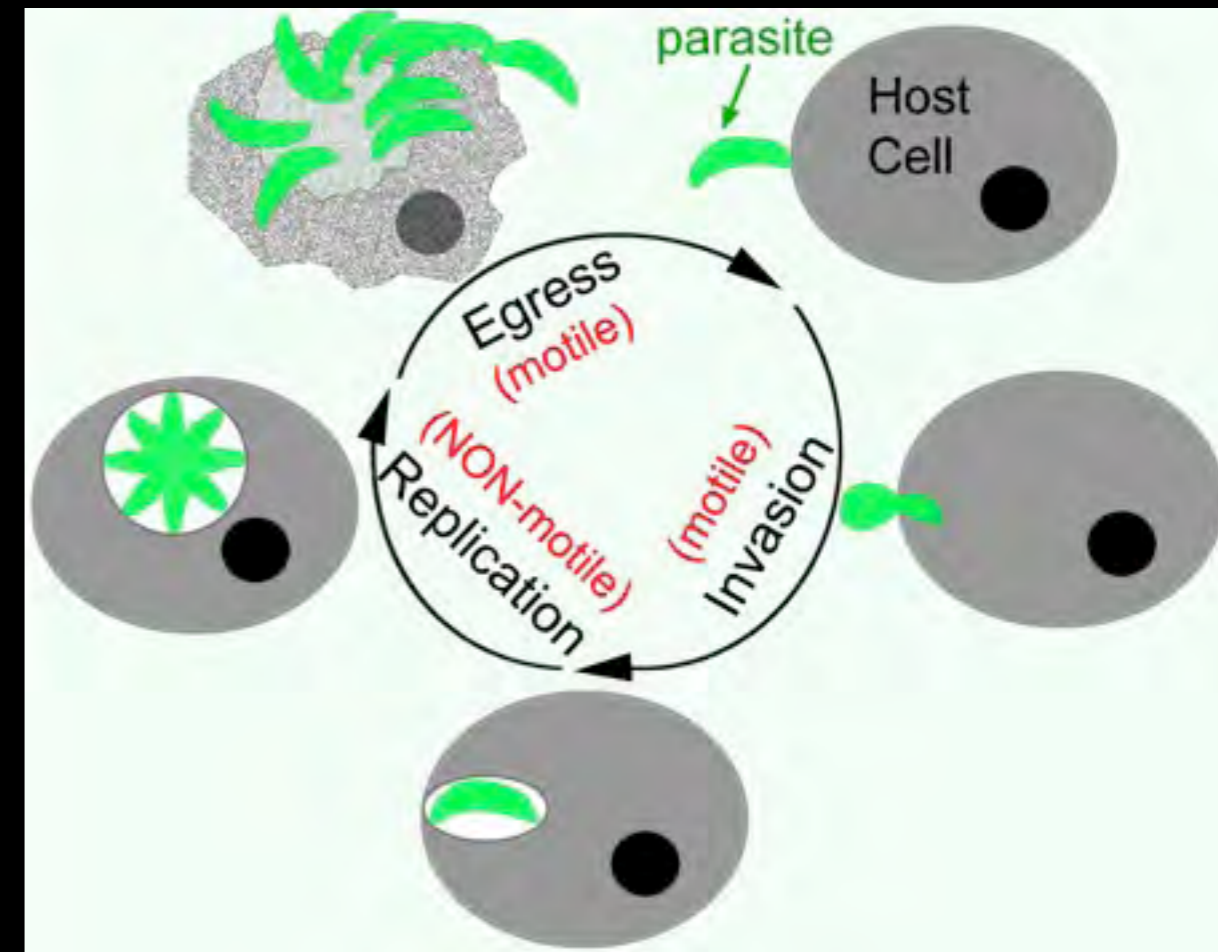
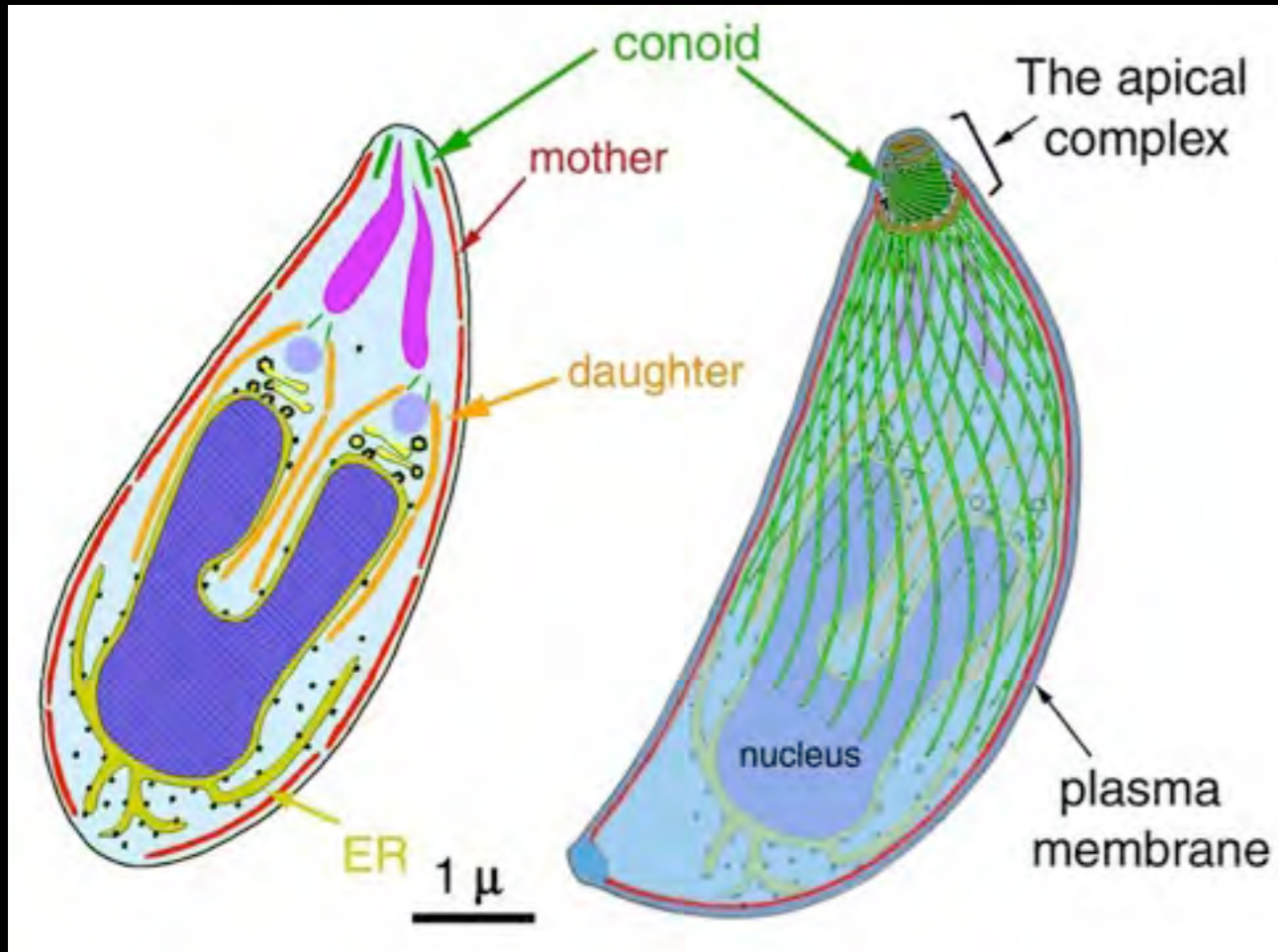
To cause disease, *T. gondii* needs to complete and reiterate the lytic cycle



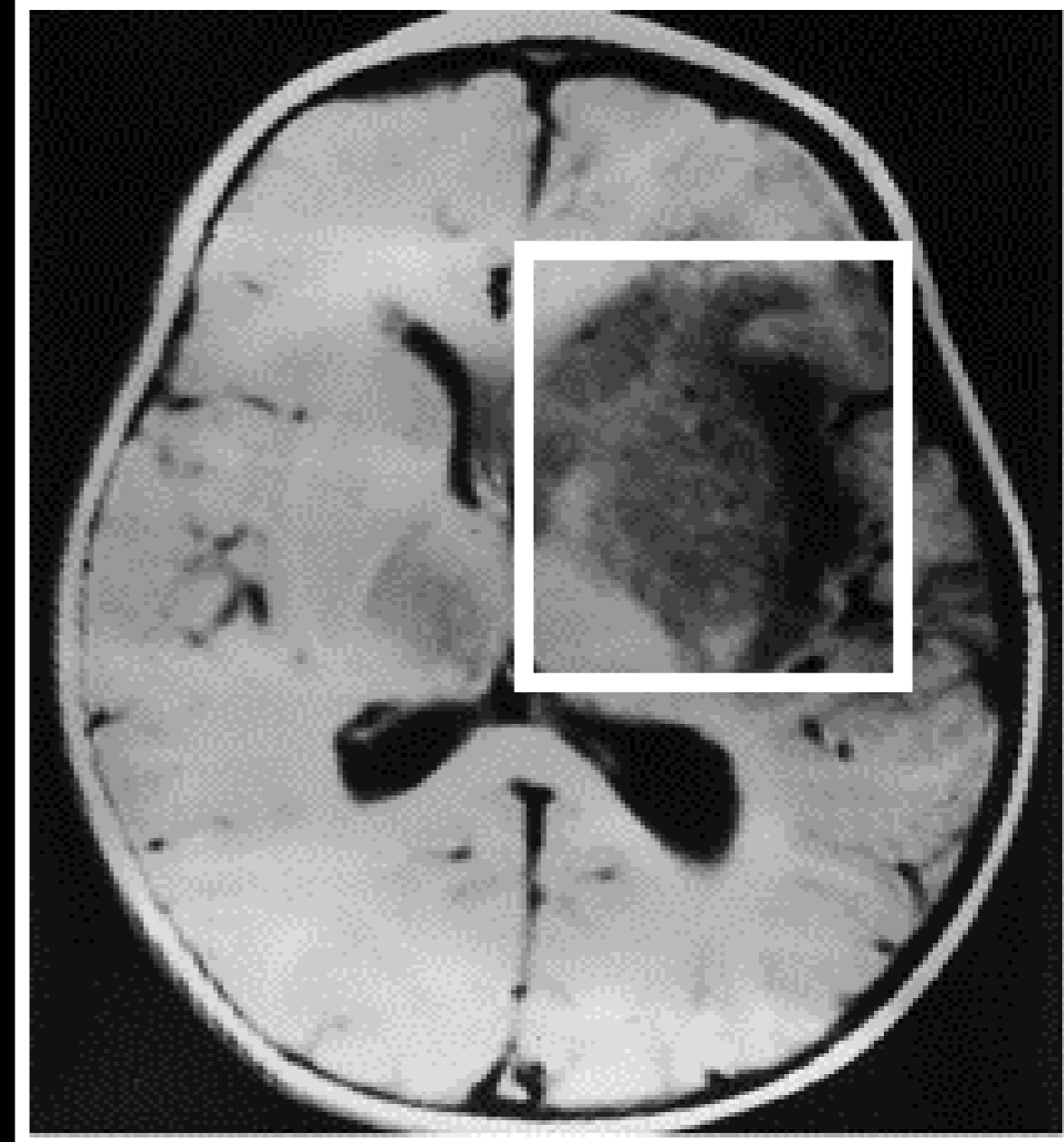
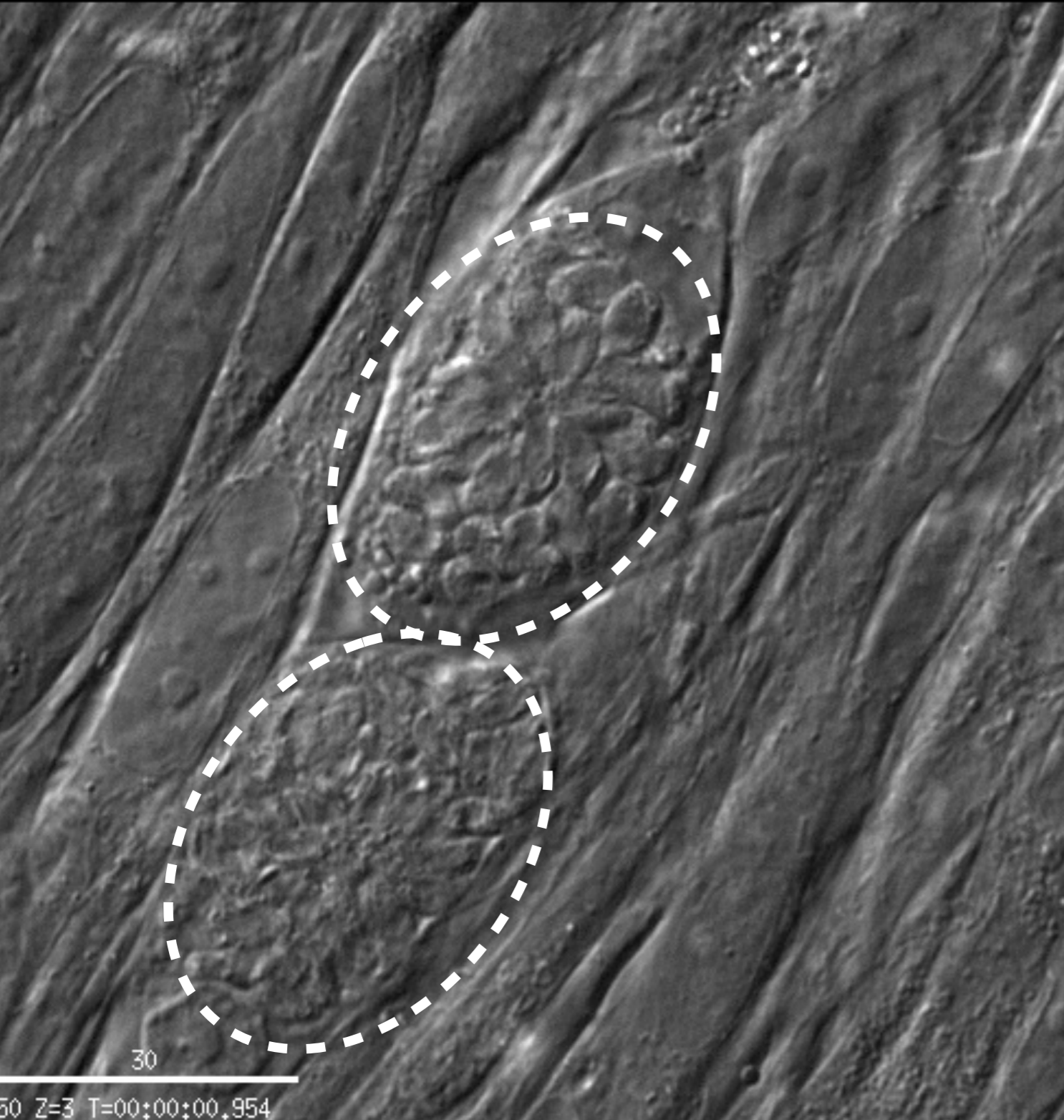
The cortical cytoskeleton allows the parasite to withstand mechanical stress as it moves in and out of the host cell



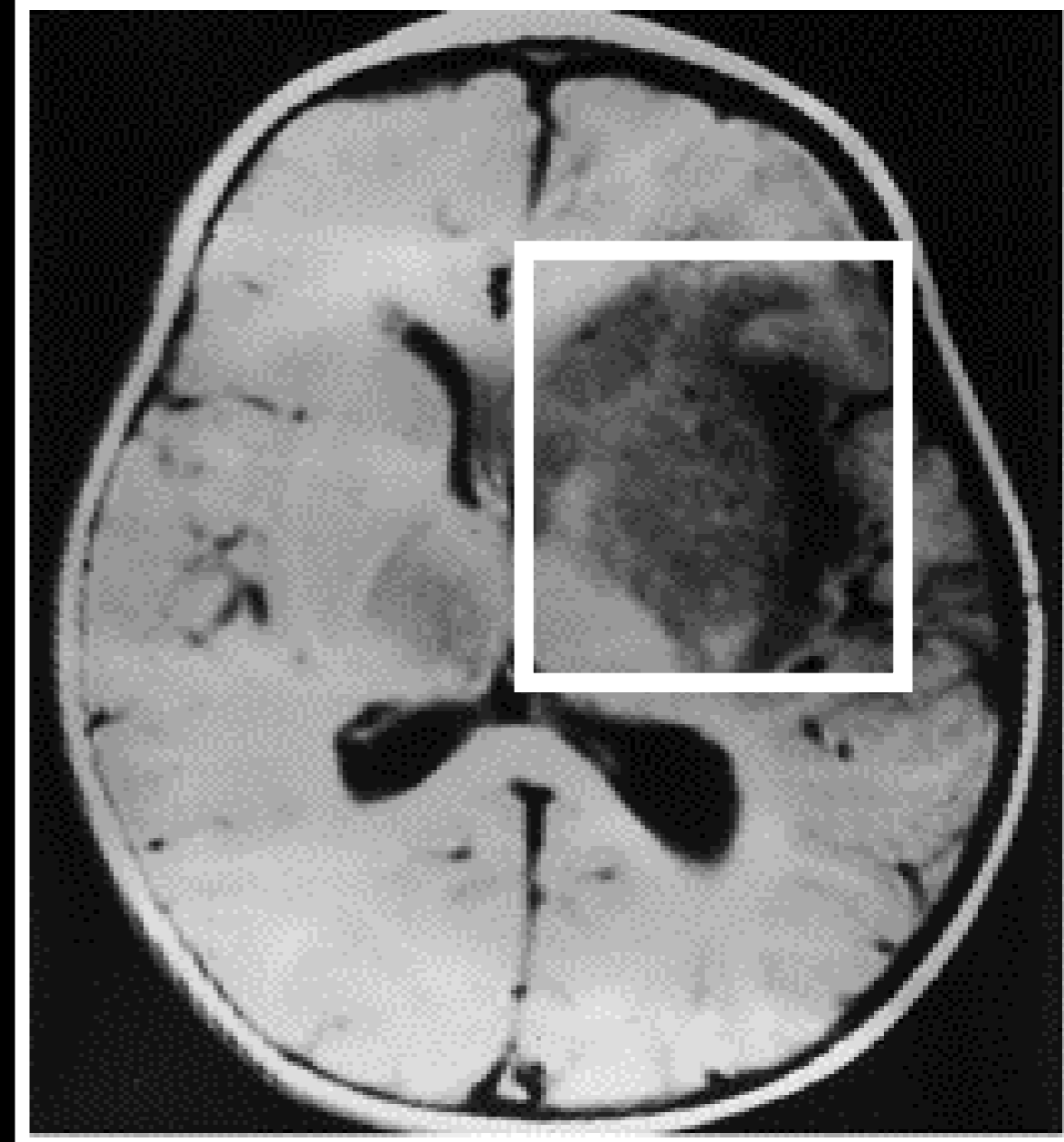
The cortical cytoskeleton provides the framework for making new parasites



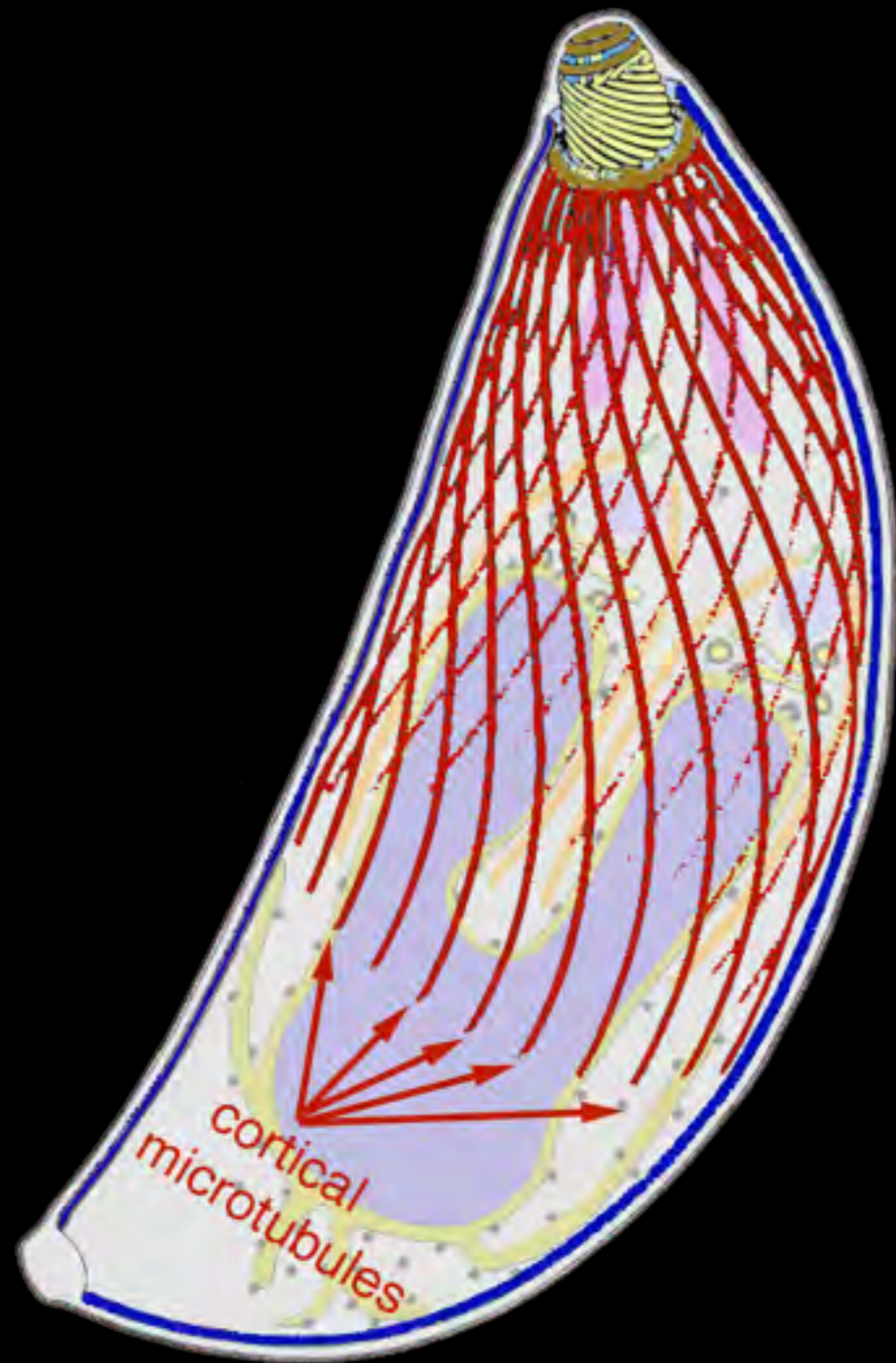
The reiteration of the lytic cycle of the parasite is the basis for the pathogenesis of Toxoplasmosis



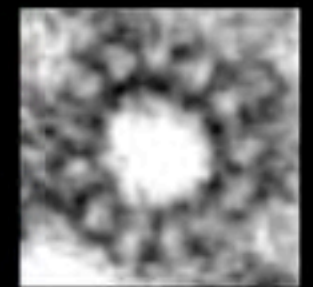
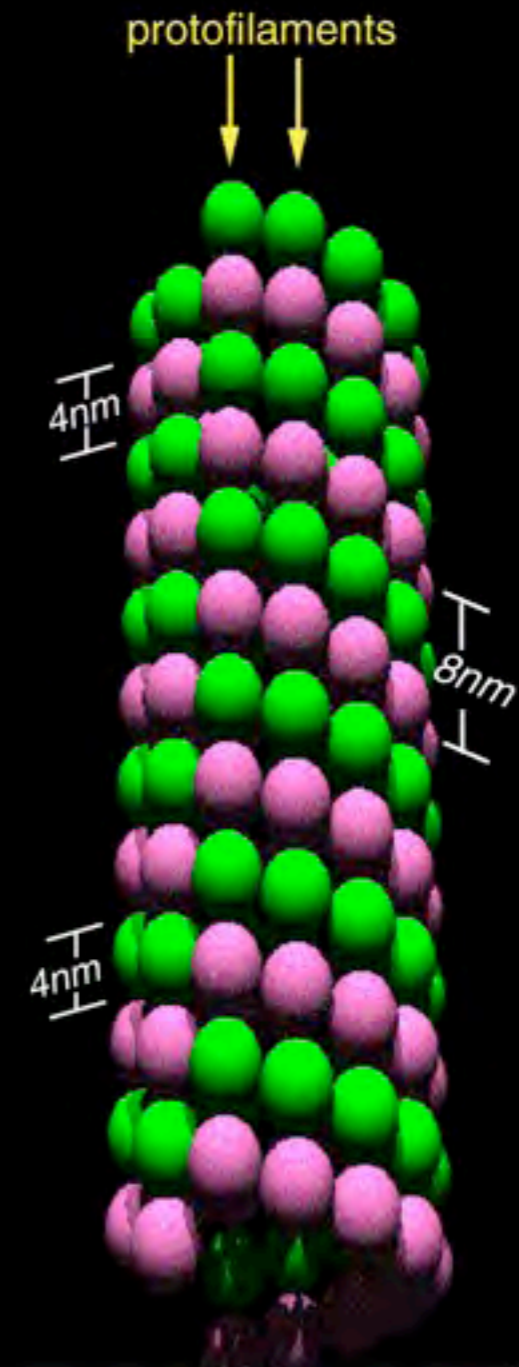
The reiteration of the lytic cycle of the parasite is the basis for the pathogenesis of Toxoplasmosis



T. gondii has 22 microtubules associated with the membrane cortex



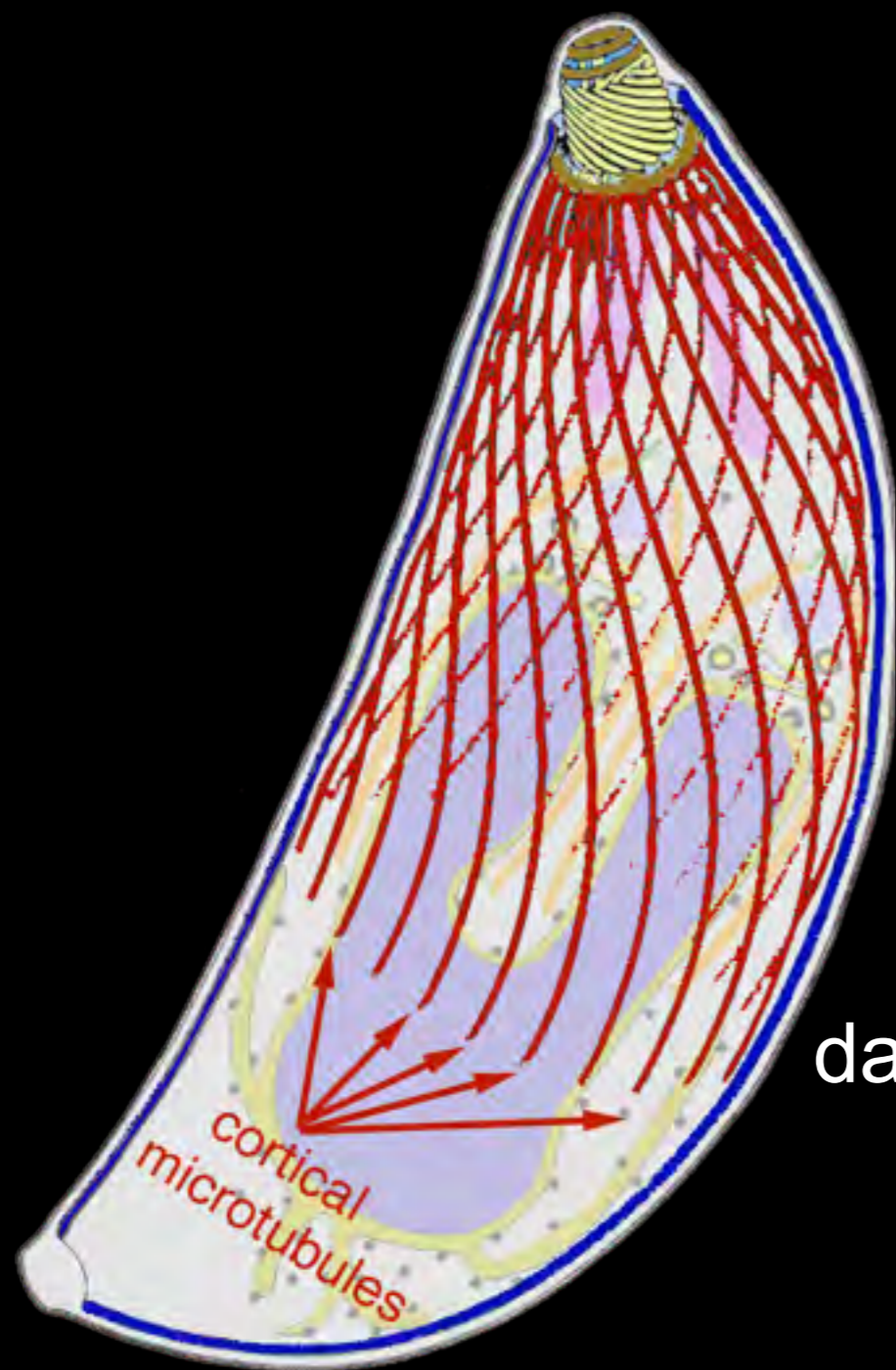
● α -tubulin ● β -tubulin



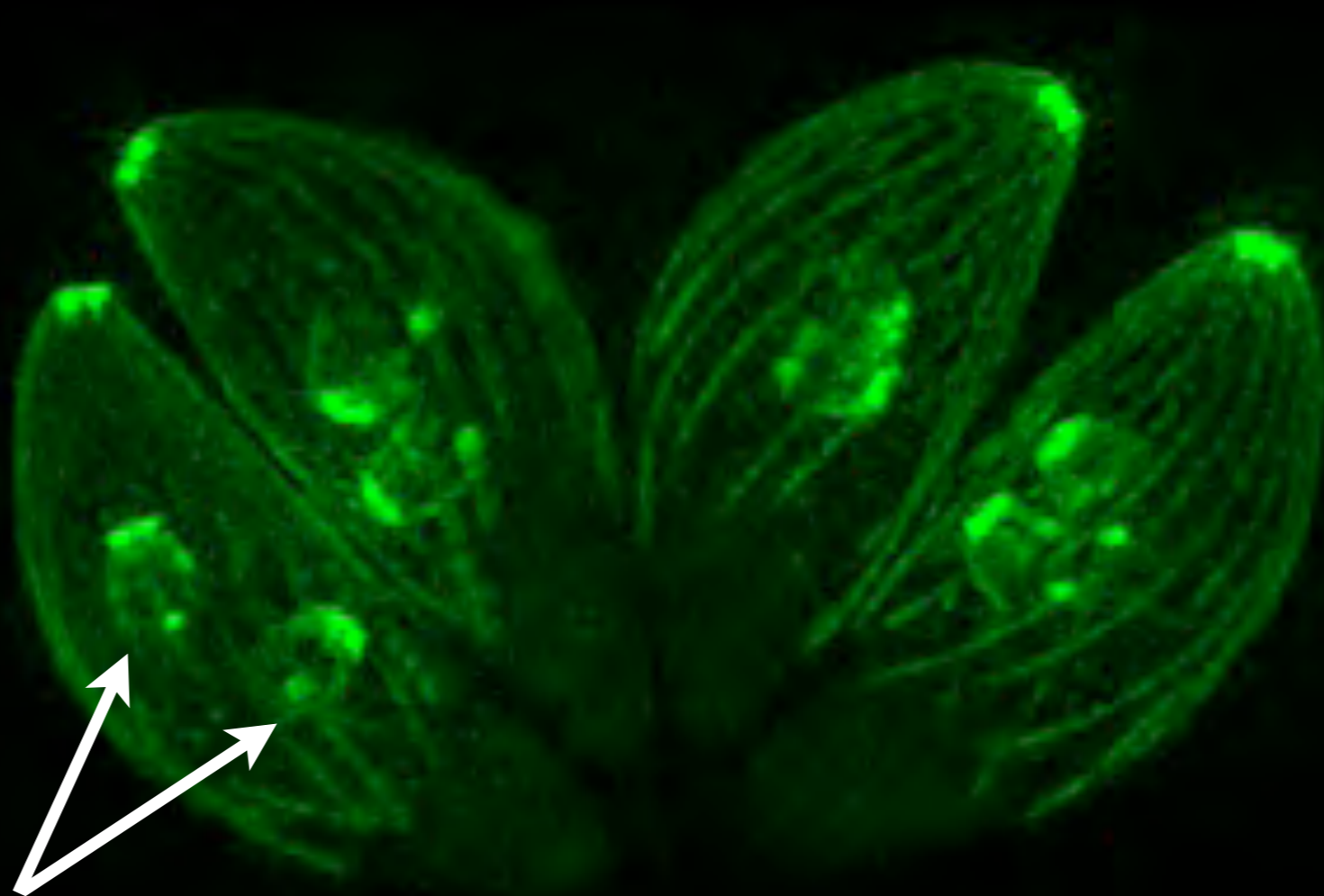
20 nm

Created by
Craig Amundsen

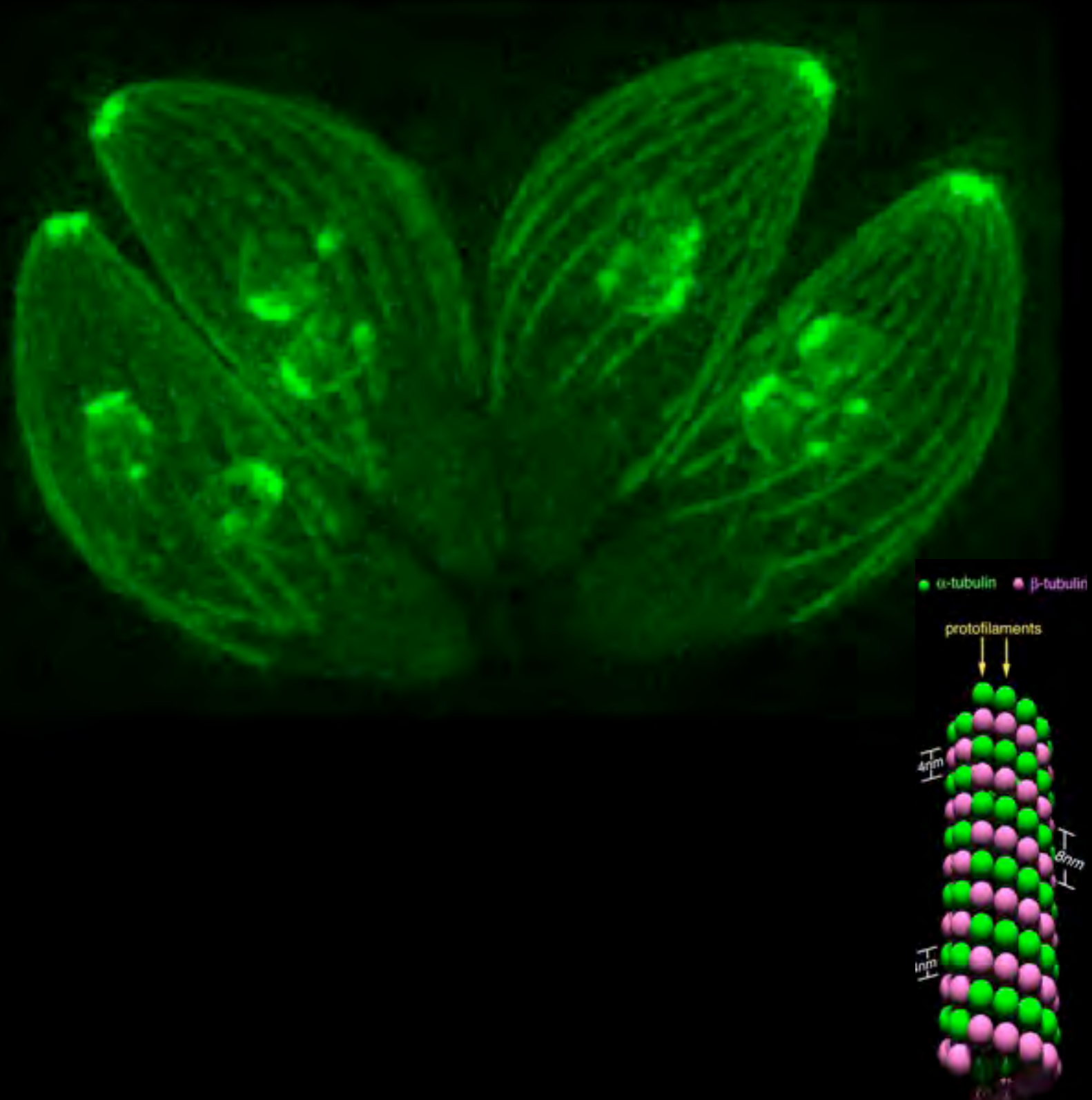
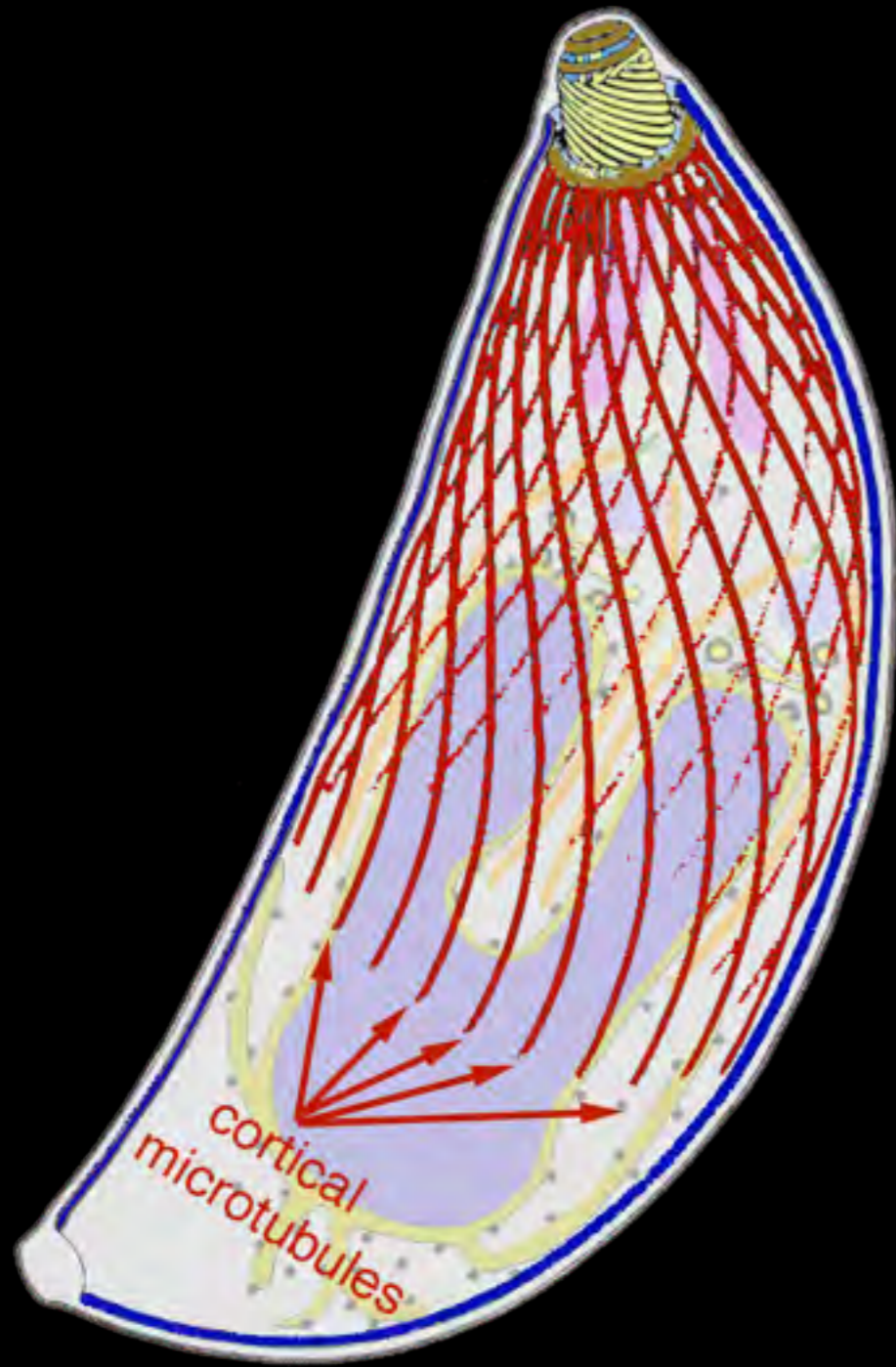
T. gondii has 22 microtubules associated with the membrane cortex



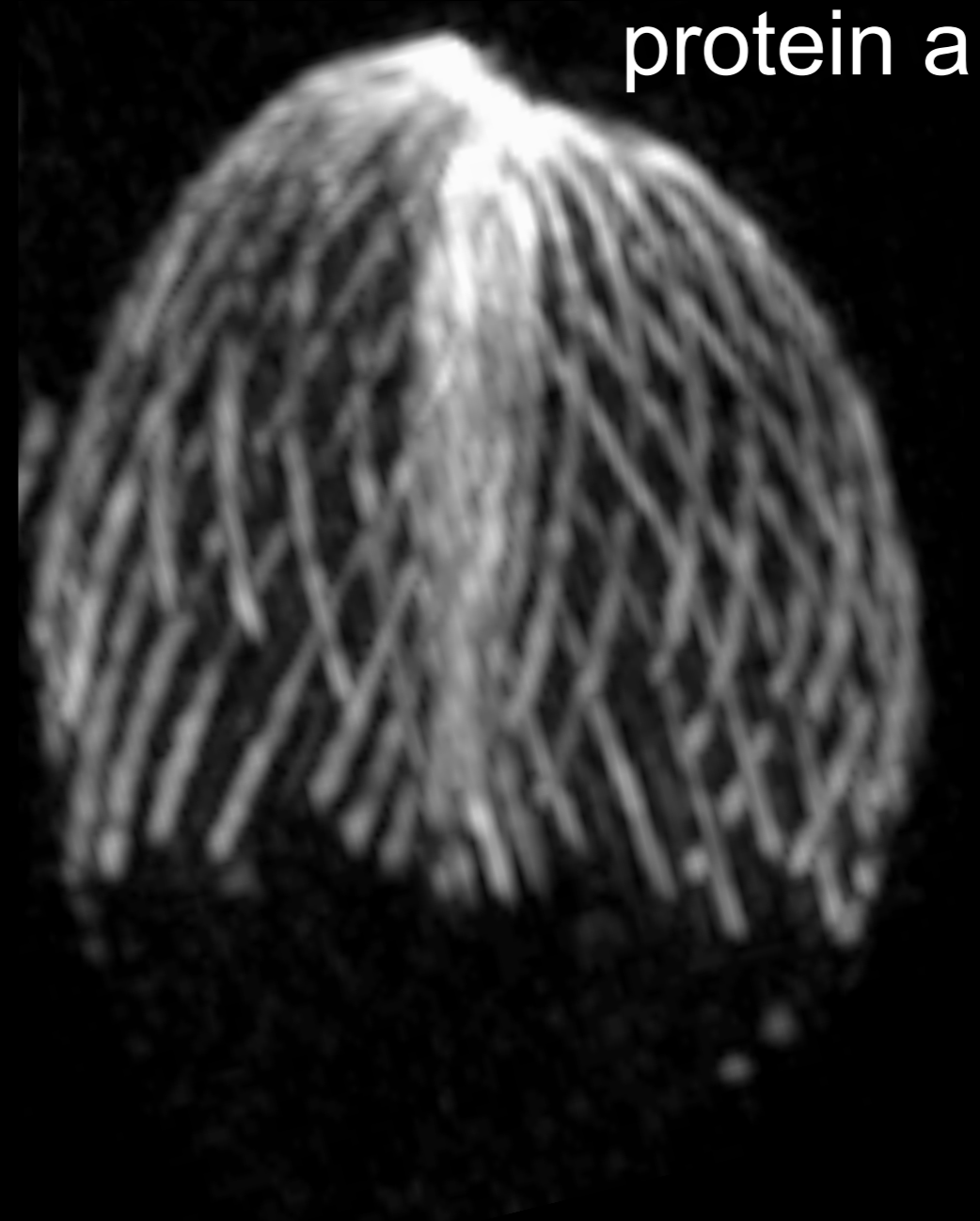
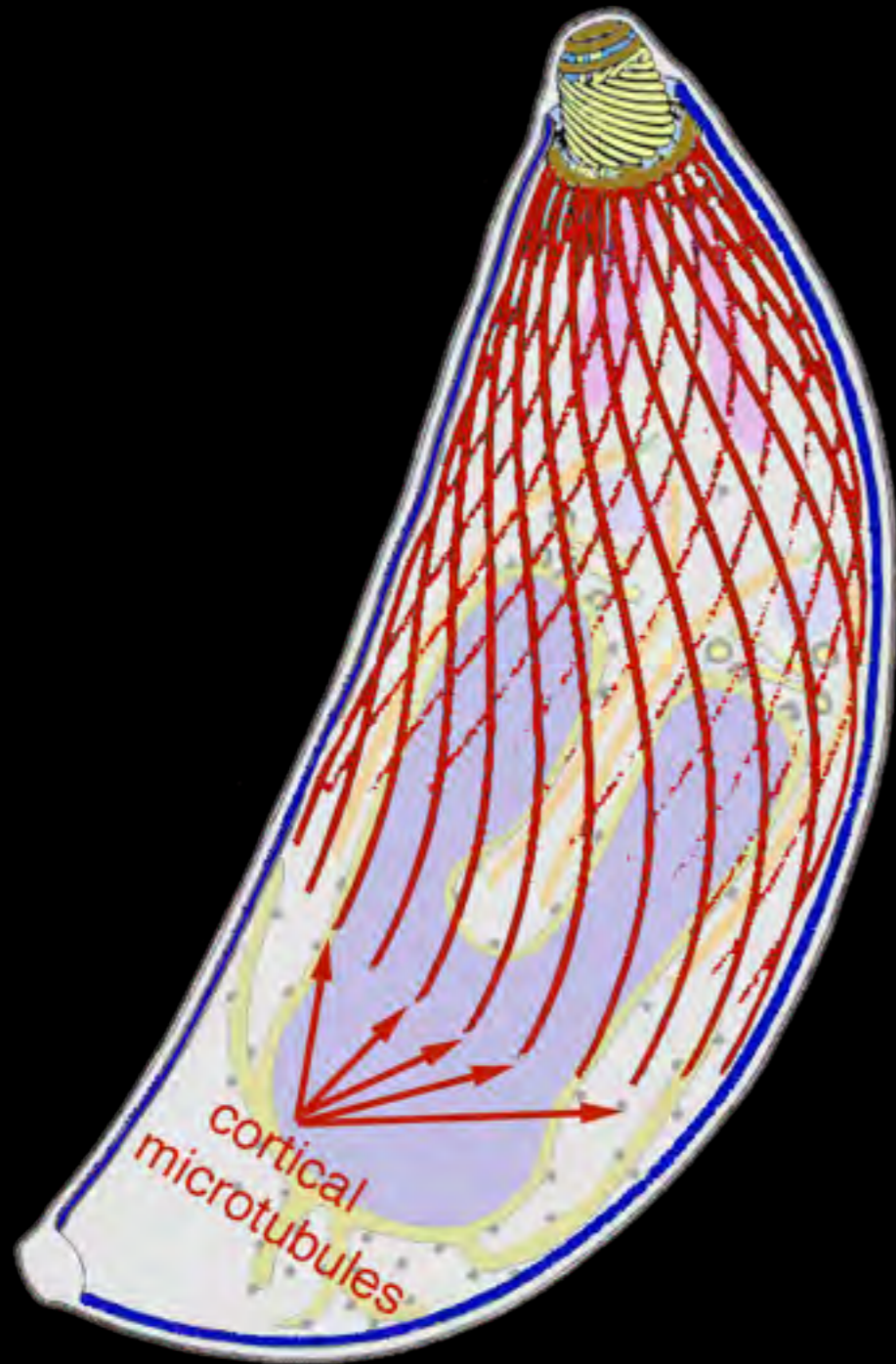
daughters



T. gondii has 22 microtubules associated with the membrane cortex

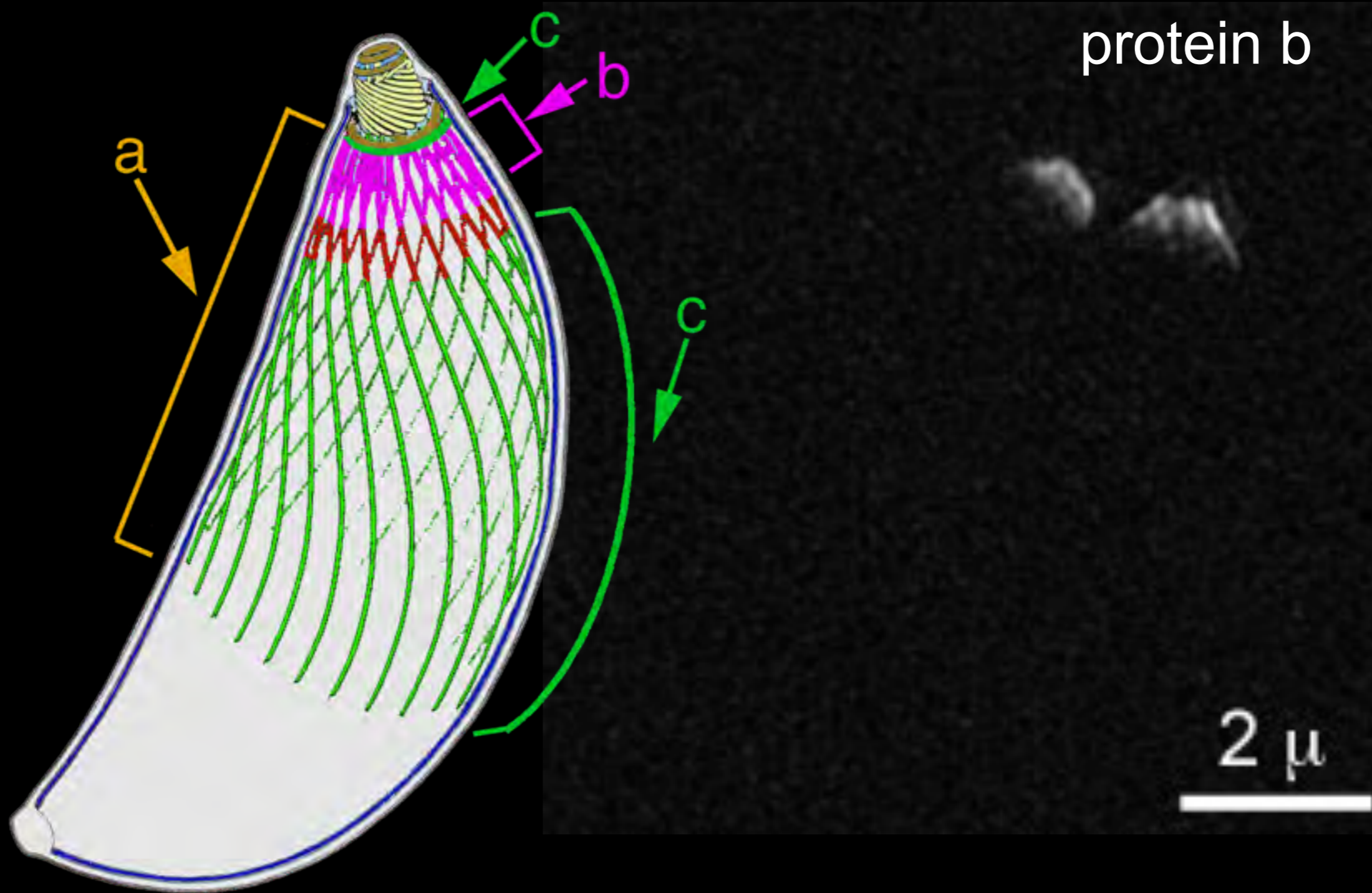


The cortical microtubules are heavily decorated with associated proteins



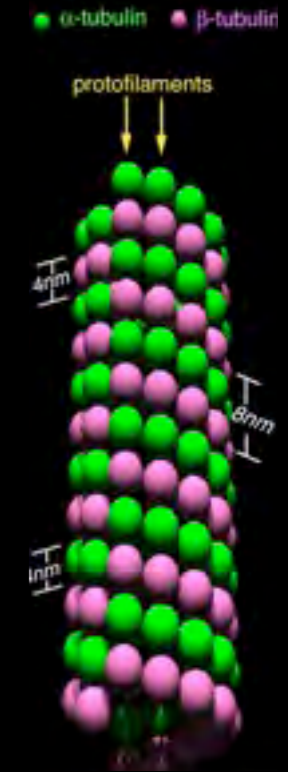
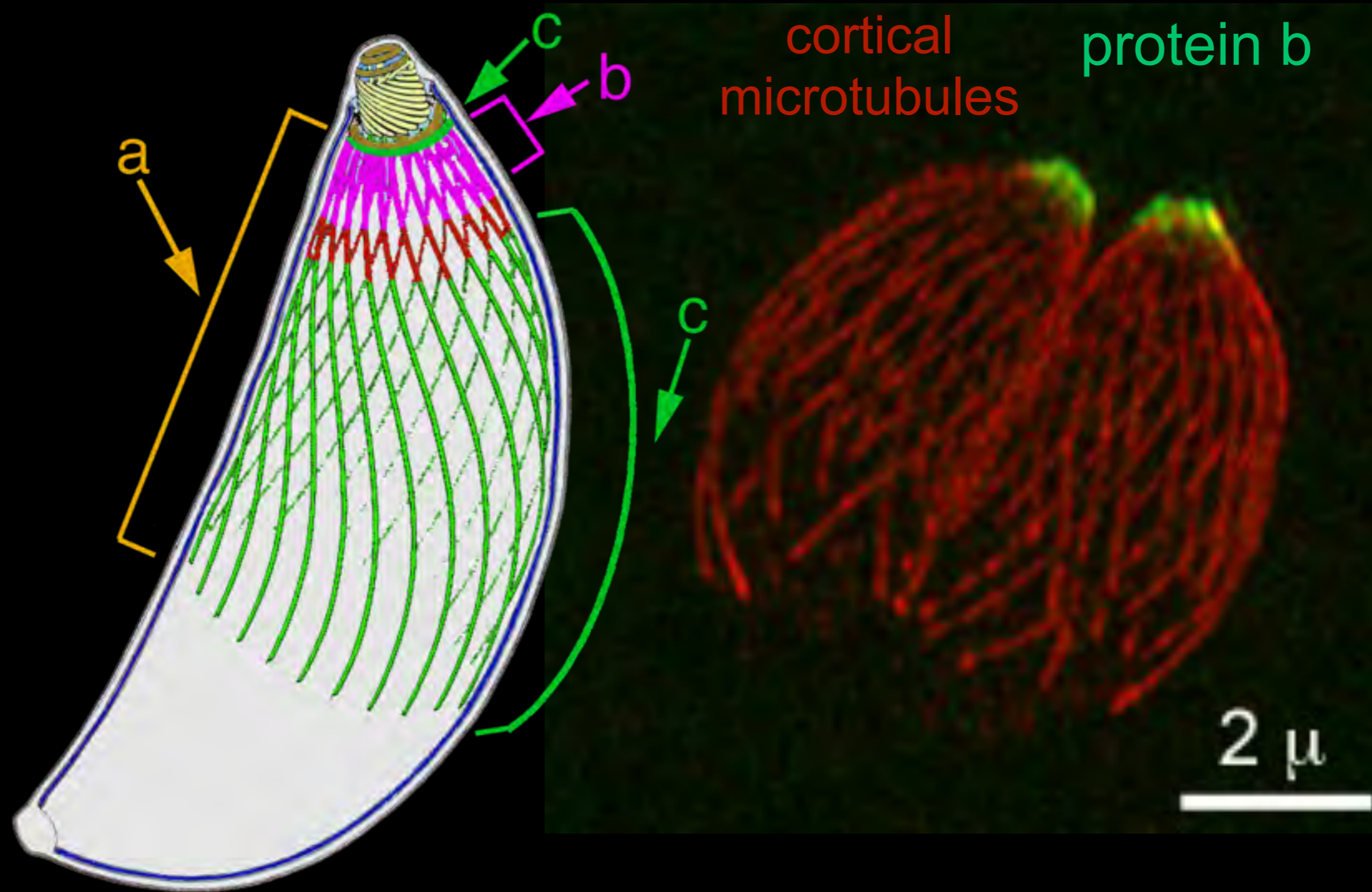
Jun Liu

Some proteins differentially decorate the microtubules



Phoebe He

Some proteins differentially decorate the microtubules

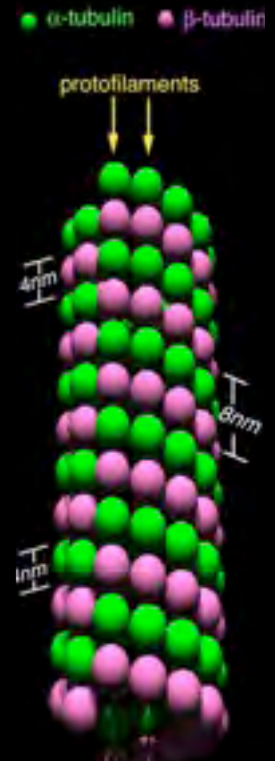
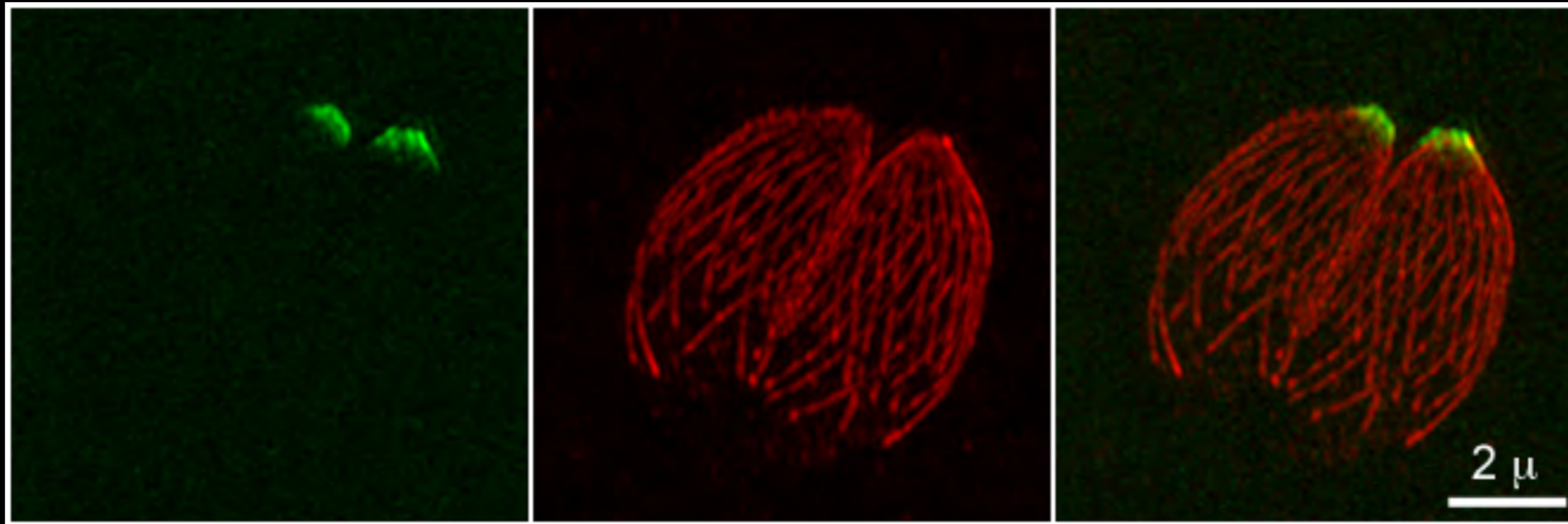


Phoebe He

protein b

cortical
microtubules

merge



How is a protein targeted to a specific region of a polymer?

a. When is it targeted (i.e. during or after the polymerization of the microtubules)?

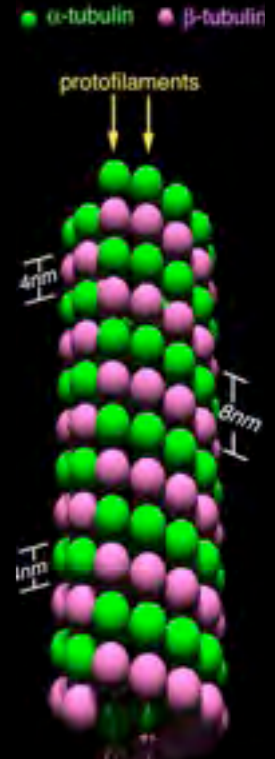
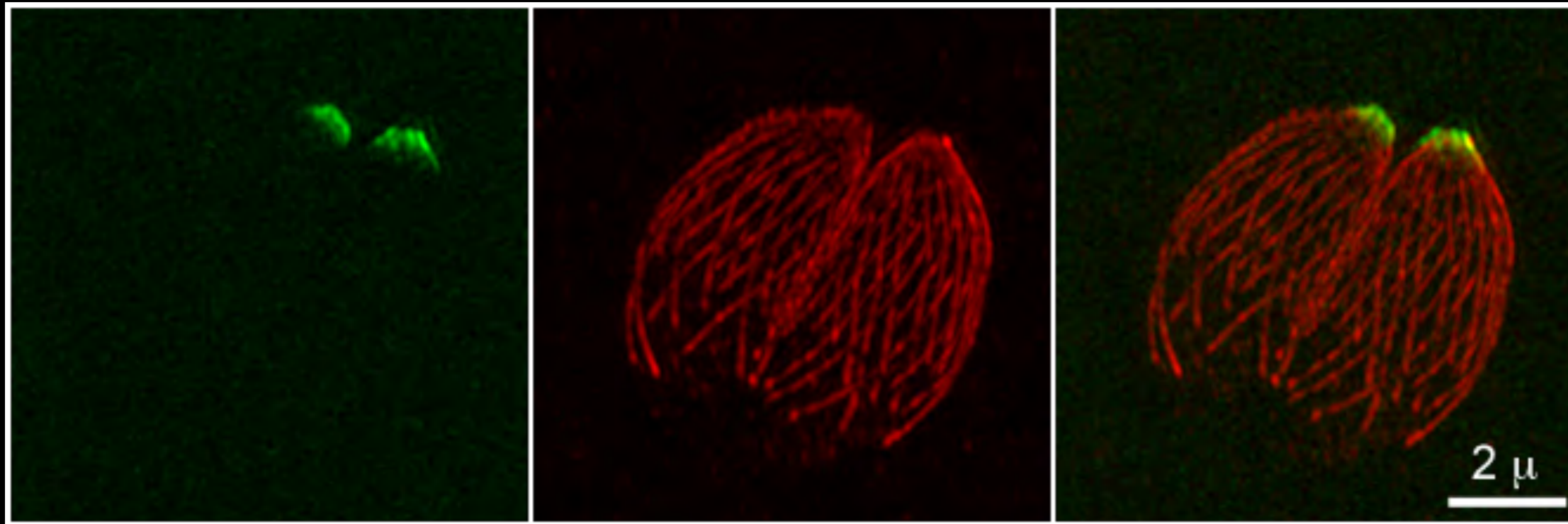
b. What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)



protein b

cortical
microtubules

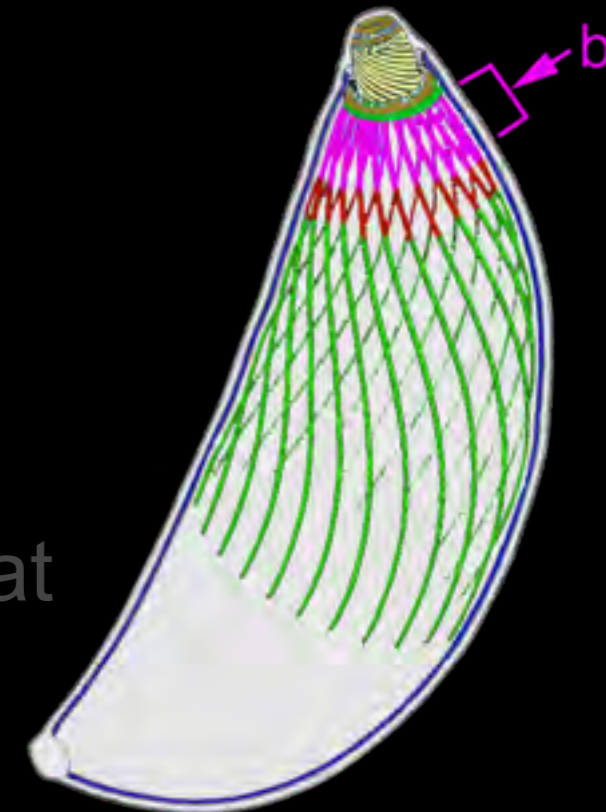
merge



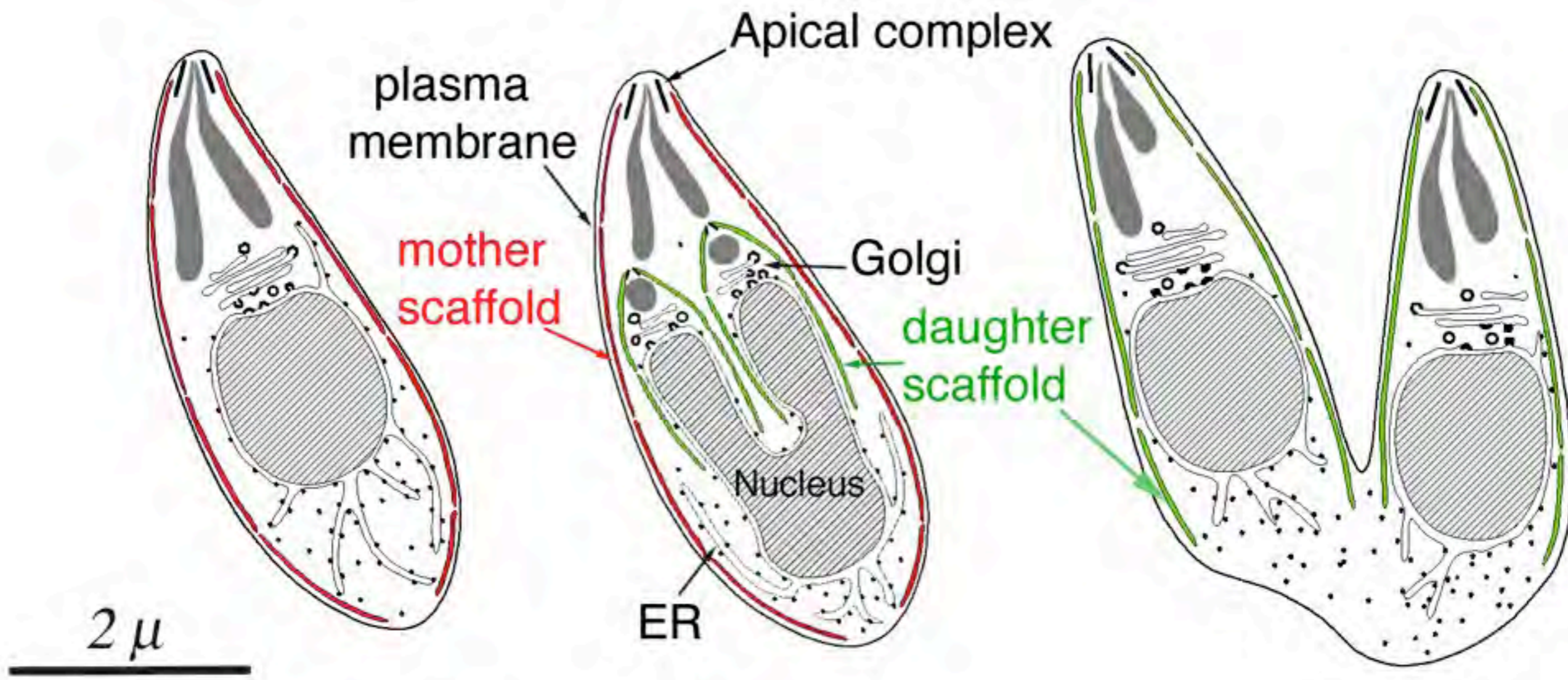
How is a protein targeted to a specific region of a polymer?

a. When is it targeted (i.e. during or after the polymerization of the microtubules)?

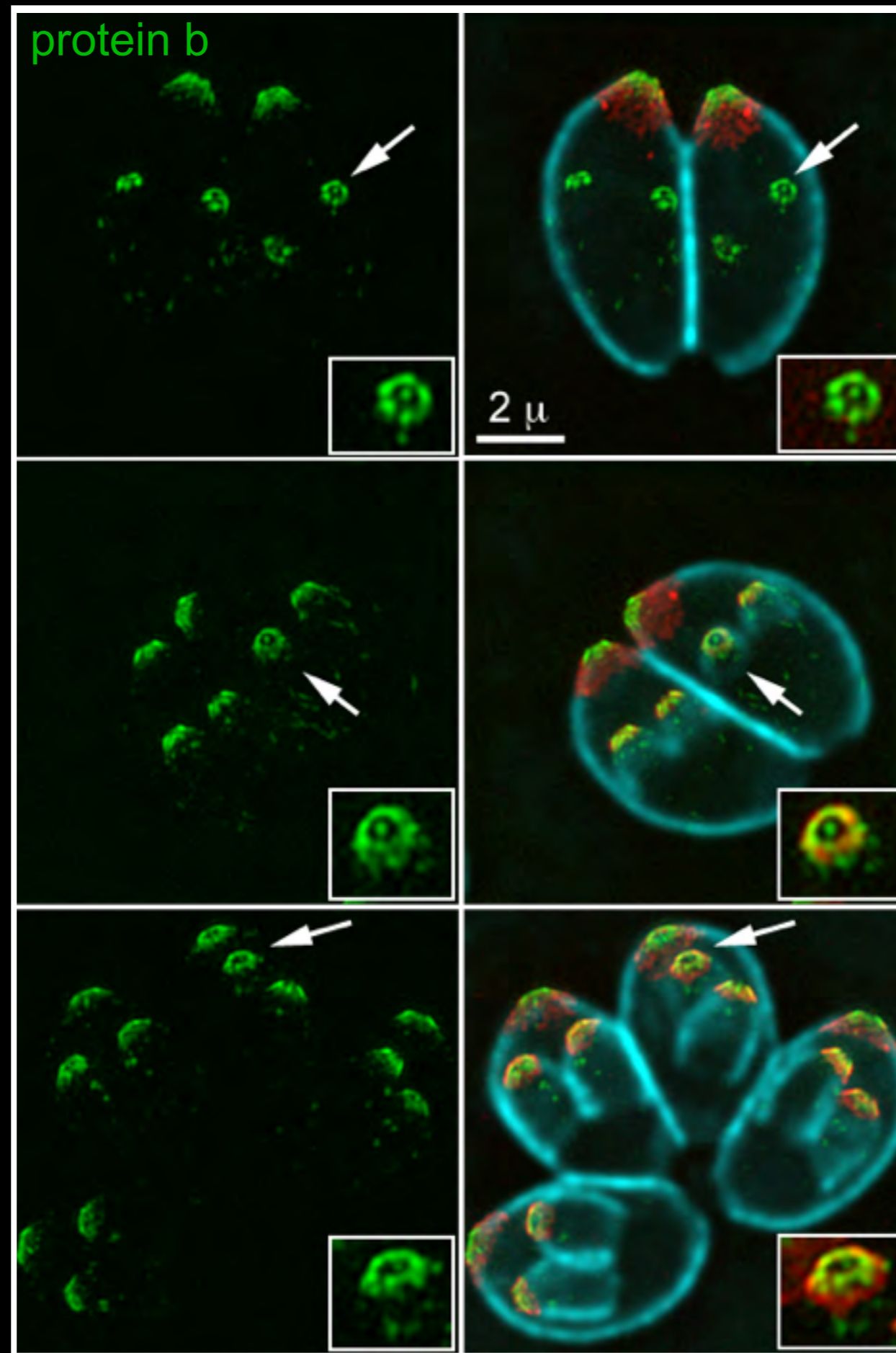
b. What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)



T. gondii constructs daughters internally, which makes it easy to track the construction of new microtubules



When is it targeted?-- while the microtubules are being made

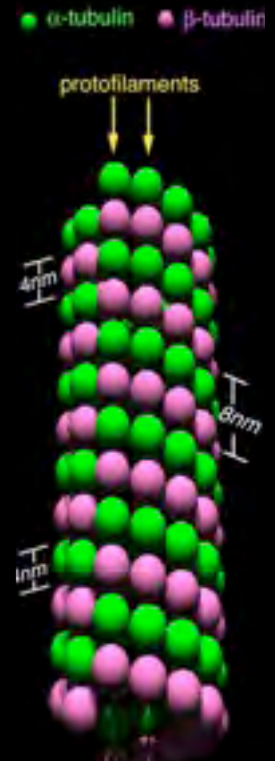
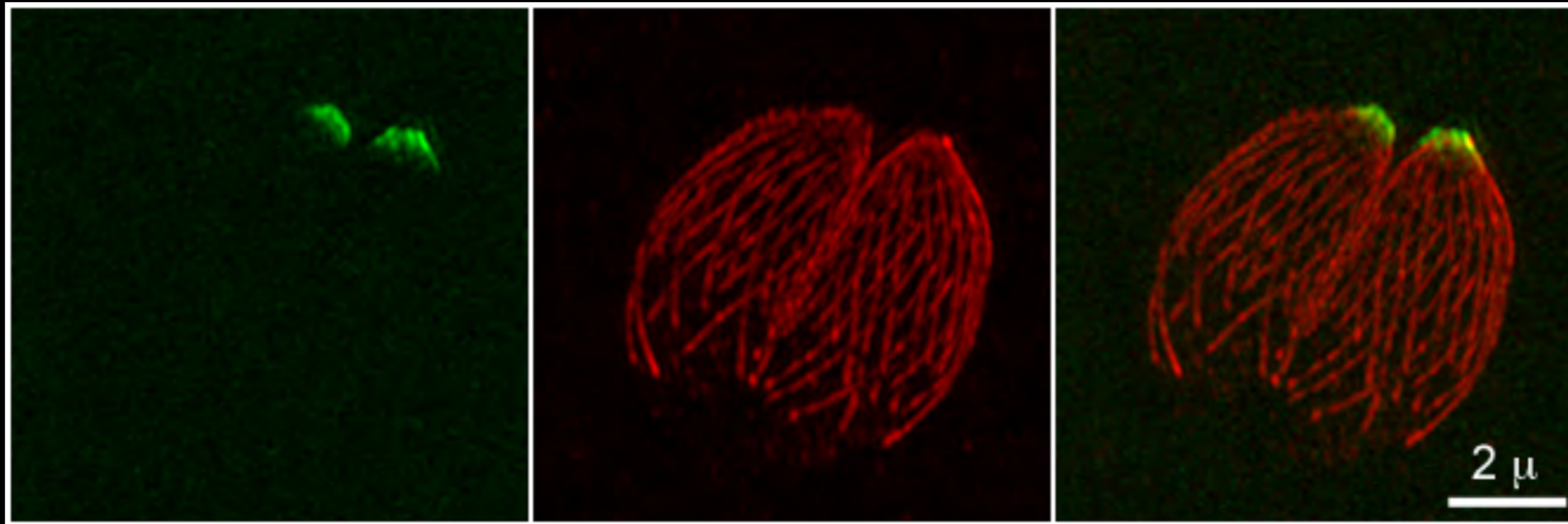


Phoebe He

protein b

cortical
microtubules

merge



How is a protein targeted to a specific region of a polymer?

a. When is it targeted (i.e. during or after the polymerization of the microtubules)?

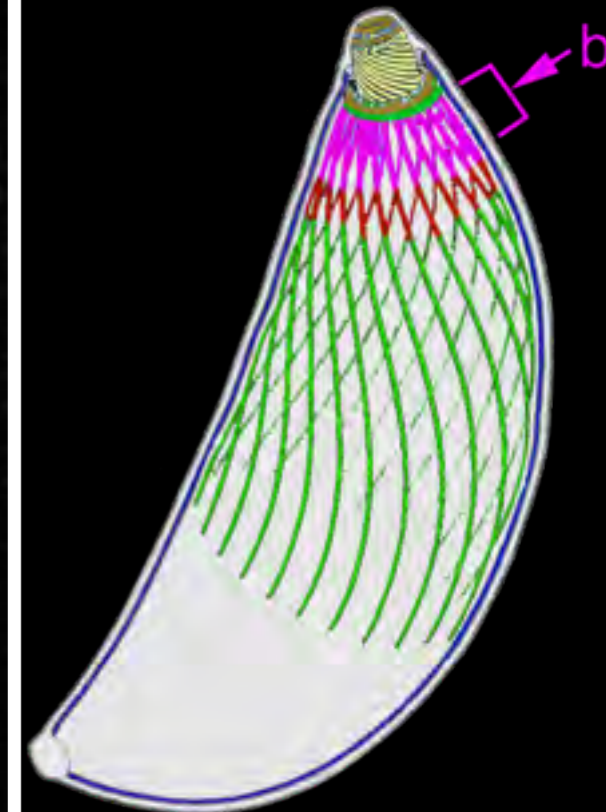
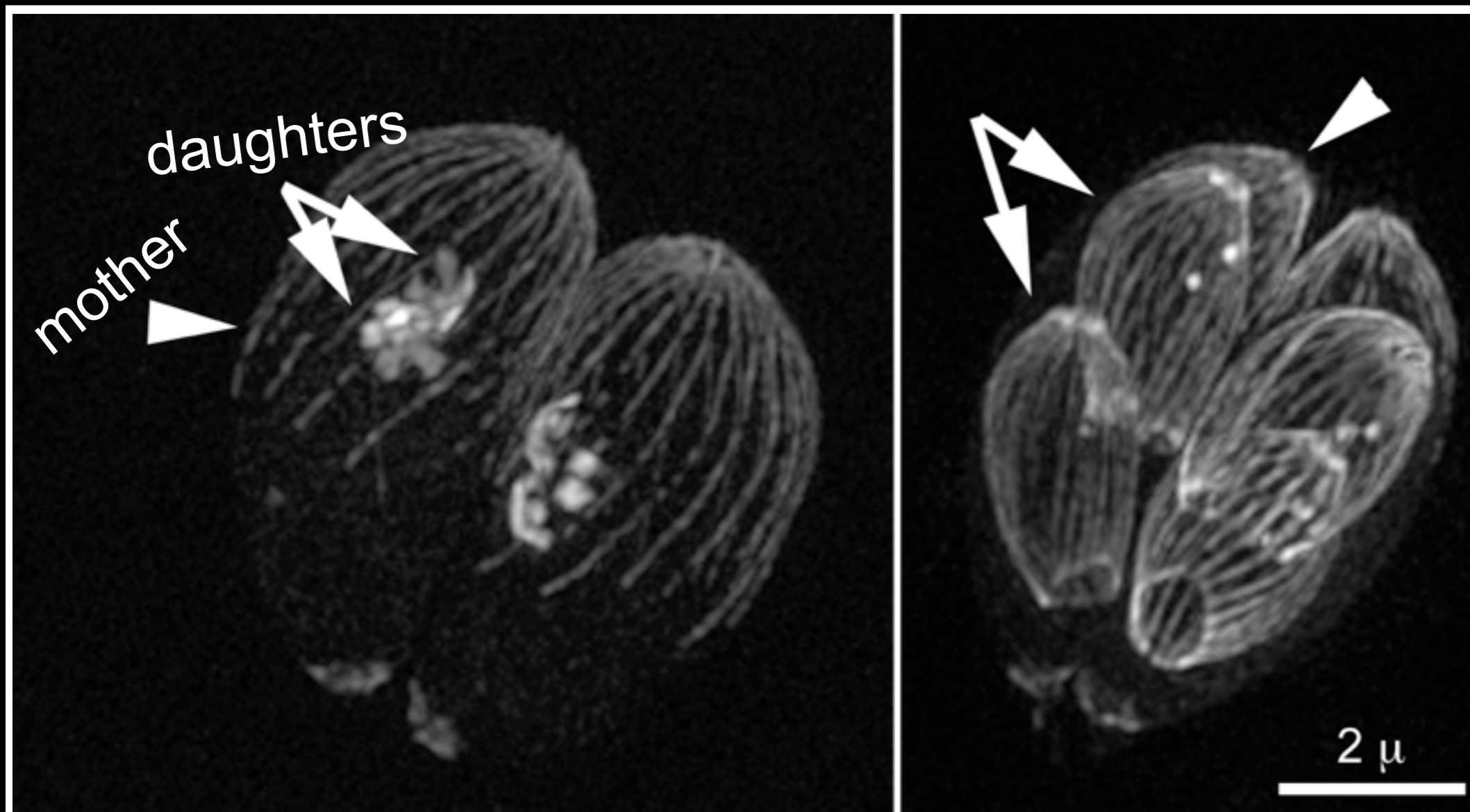
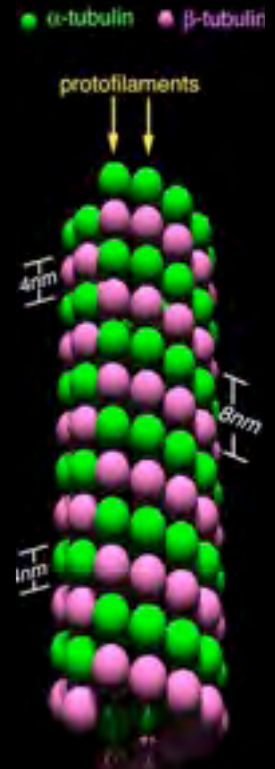
b. What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)



What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)

1. Binding sites only available in the "cap" region

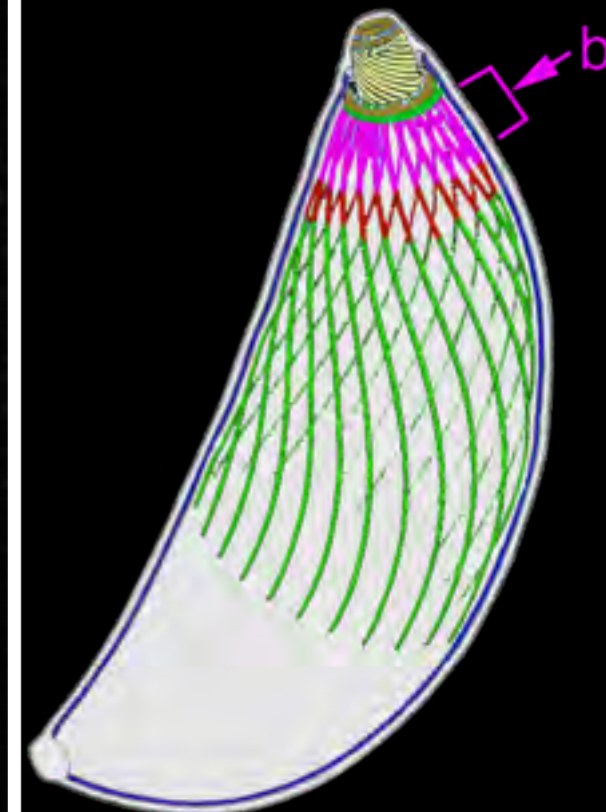
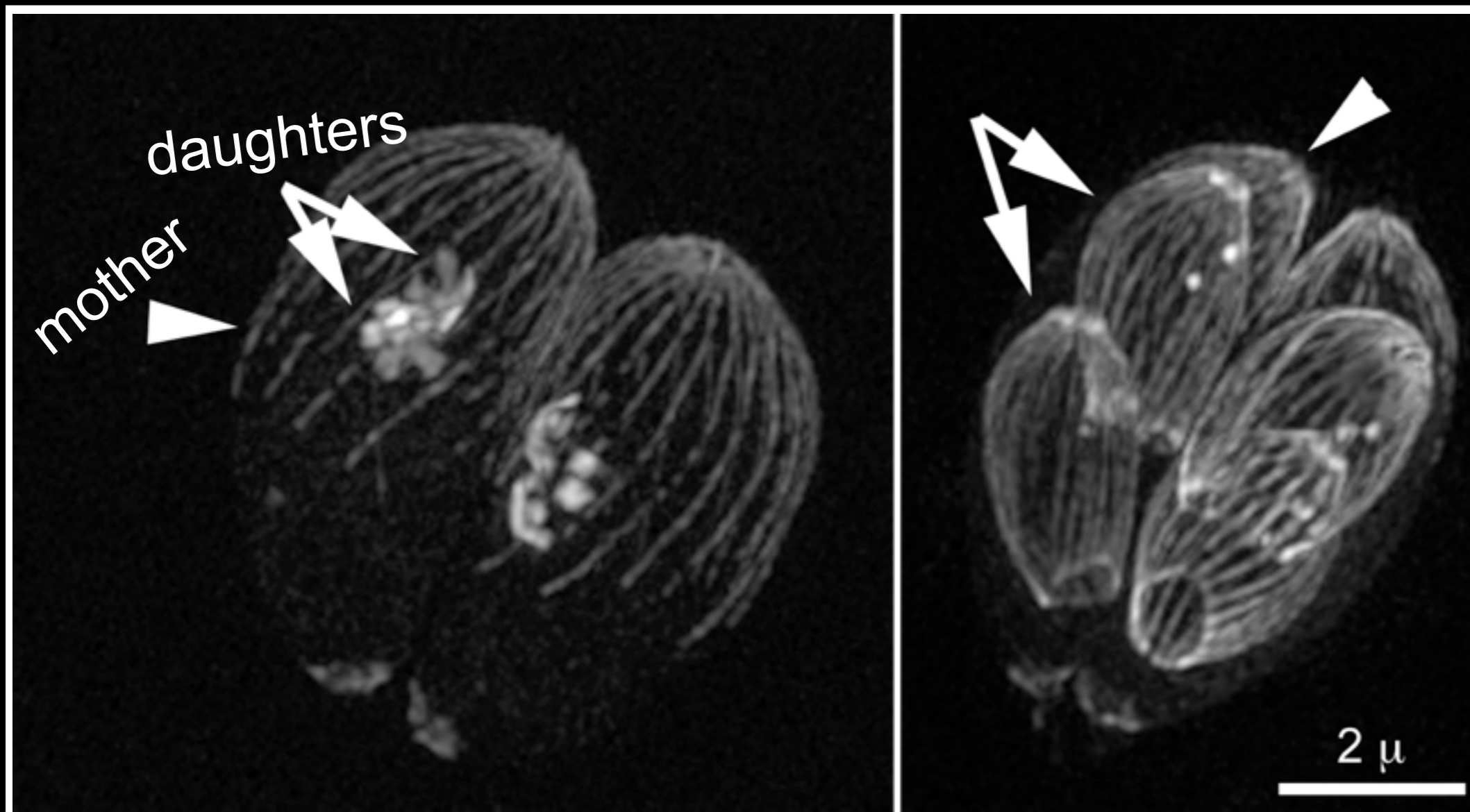
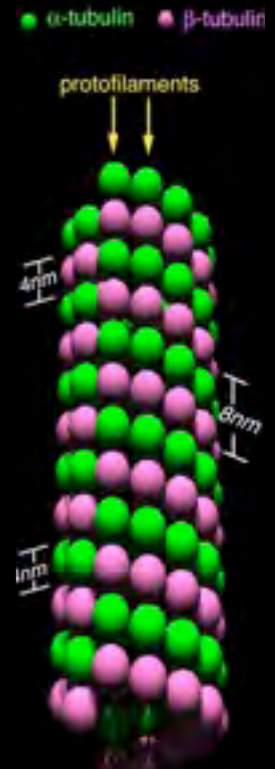
Protein b coats the entire length of the polymer when constantly expressed



What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)

1. ~~Binding sites only available in the "cap" region~~

Protein b coats the entire length of the polymer when constantly expressed

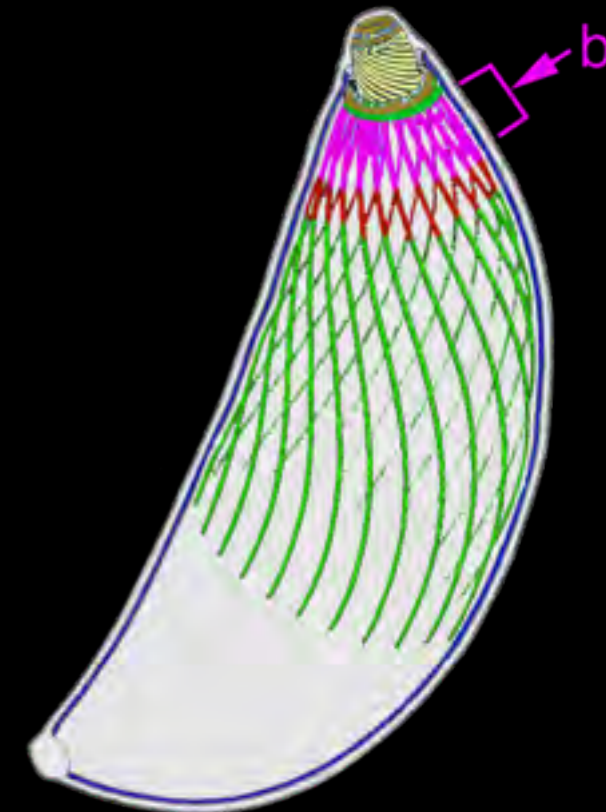
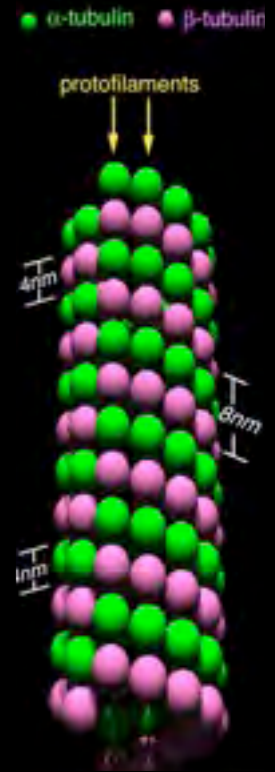


What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)

2. Coupling between protein availability and polymerization

a. The coating protein is only available when the apical section of the microtubules form

b. The coating protein associates with the microtubules irreversibly once bound

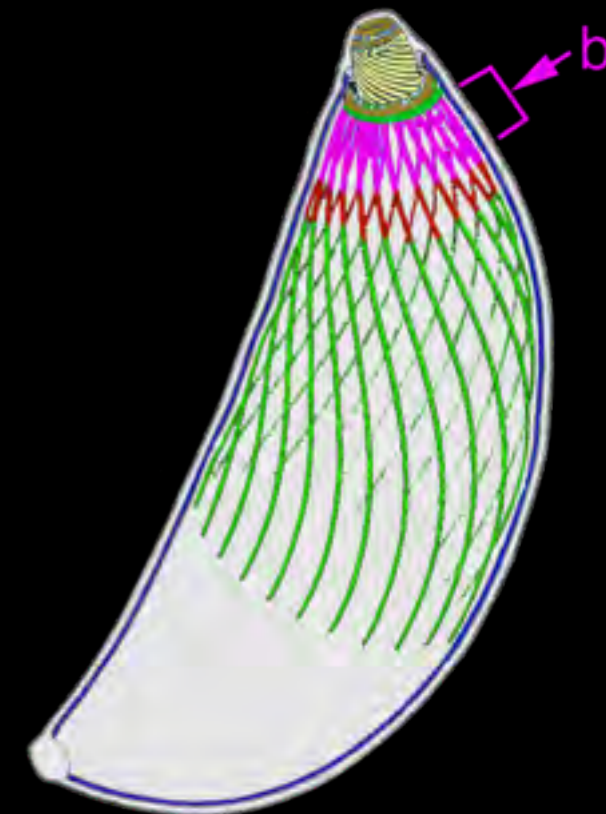
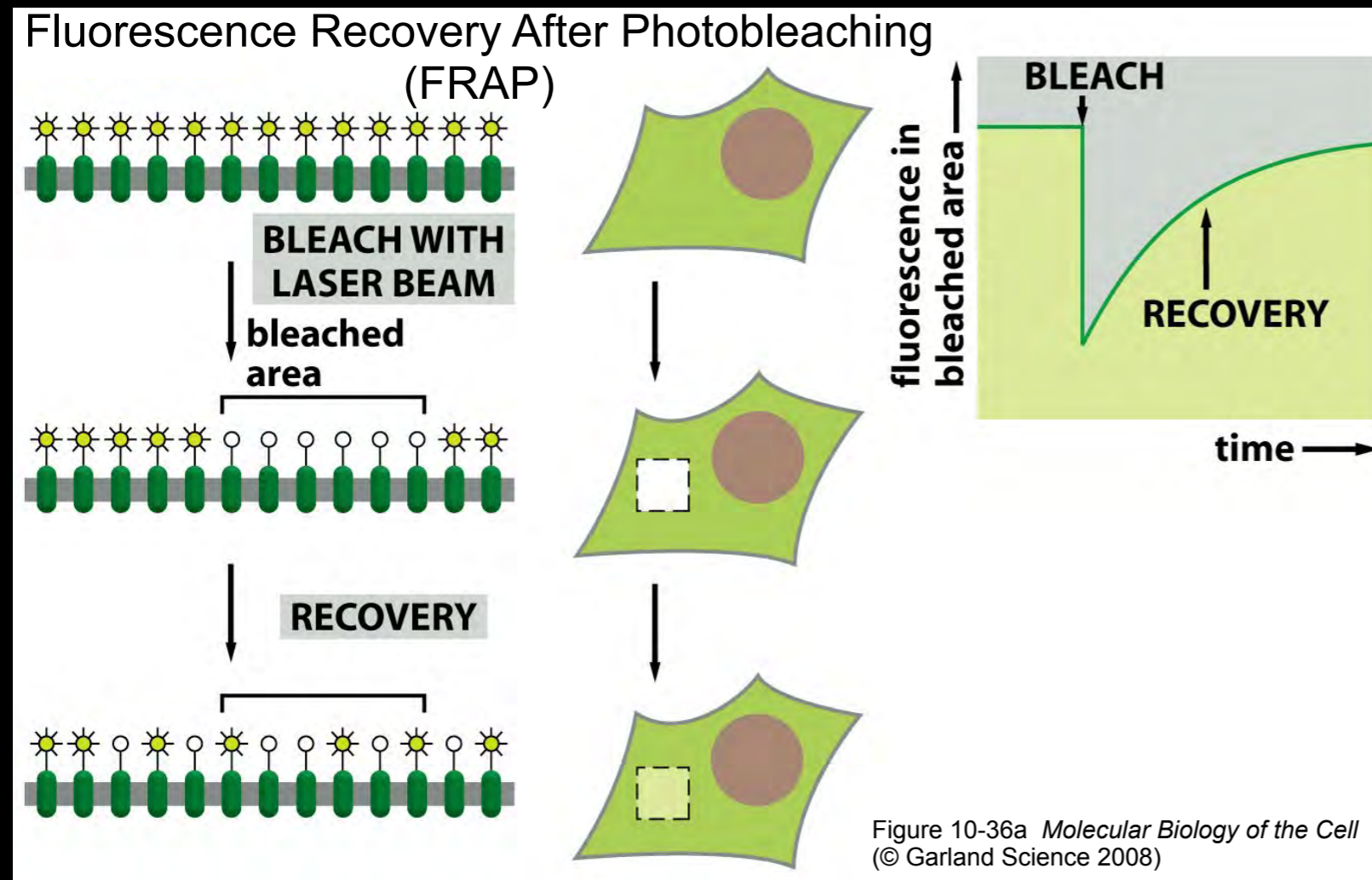
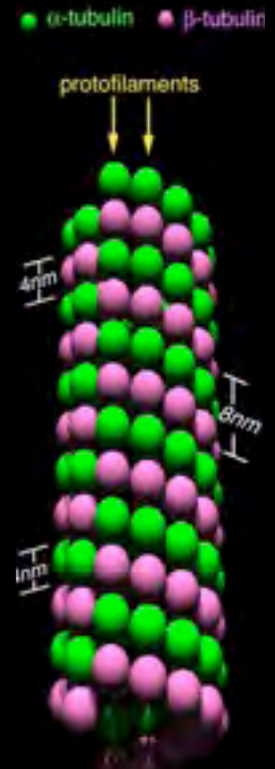


What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)

2. Coupling between protein availability and polymerization

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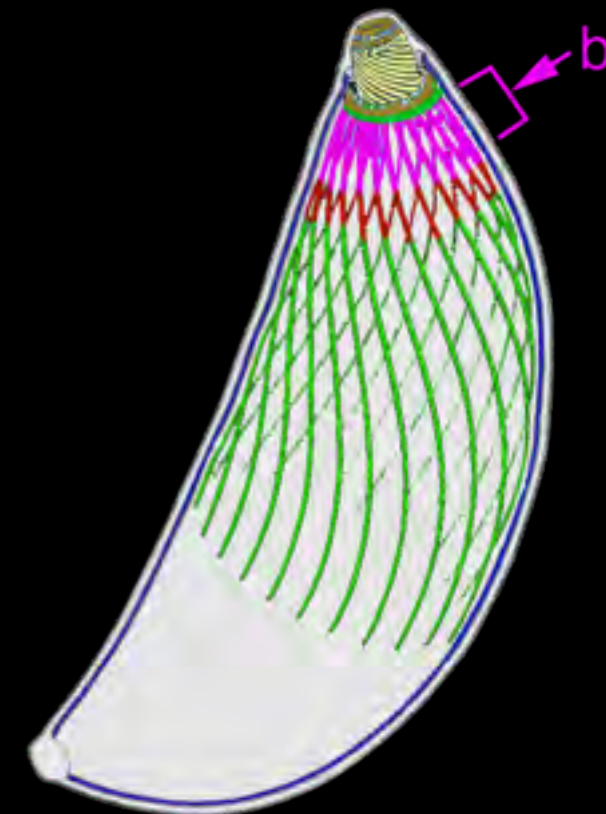
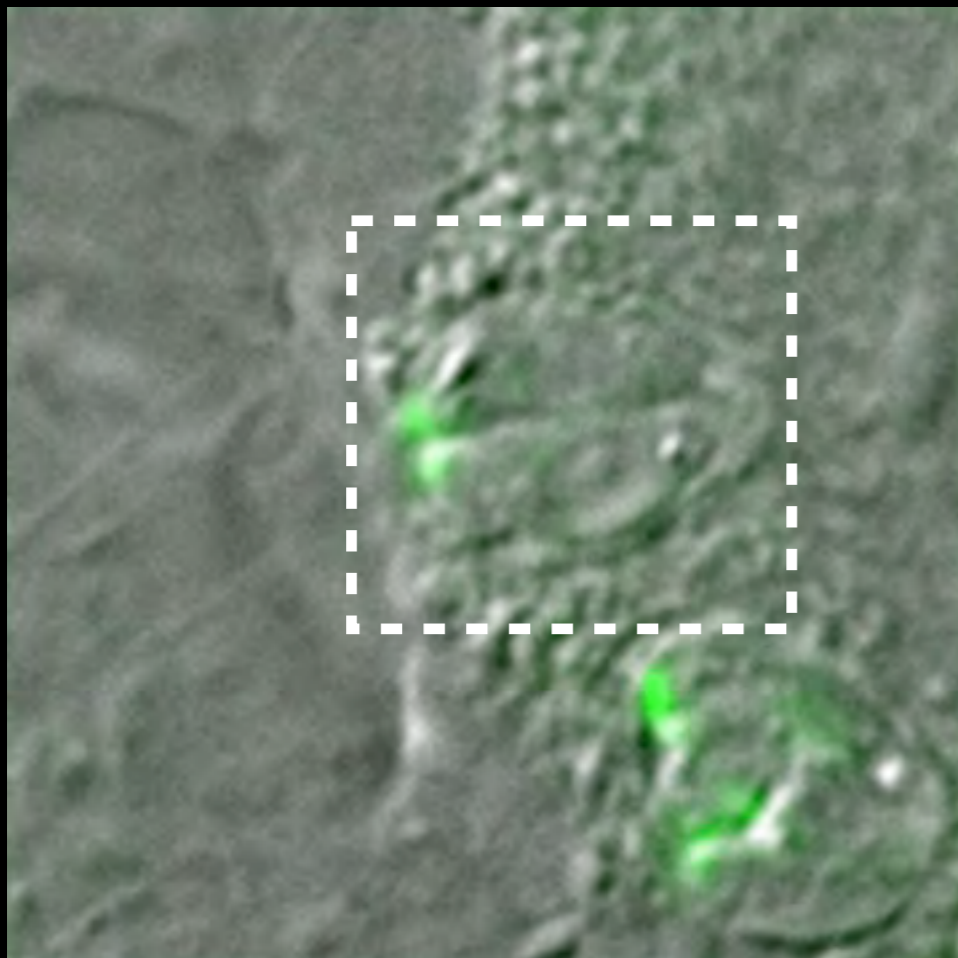
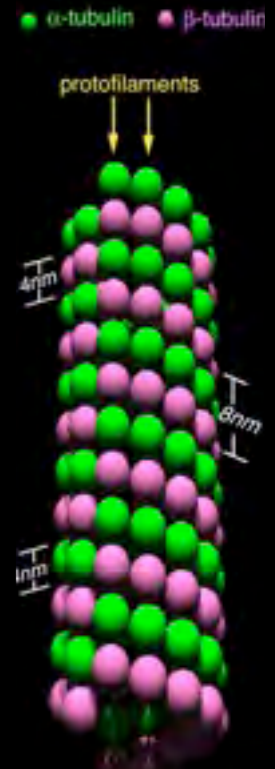


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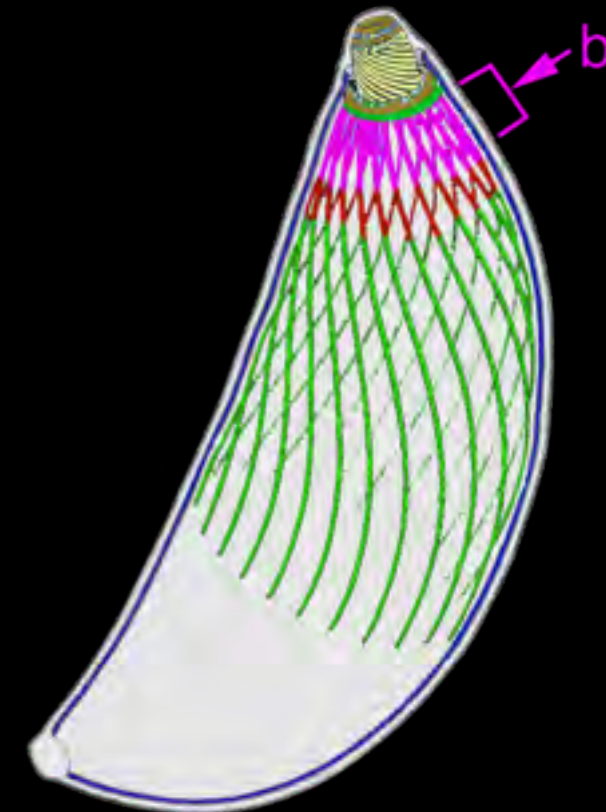
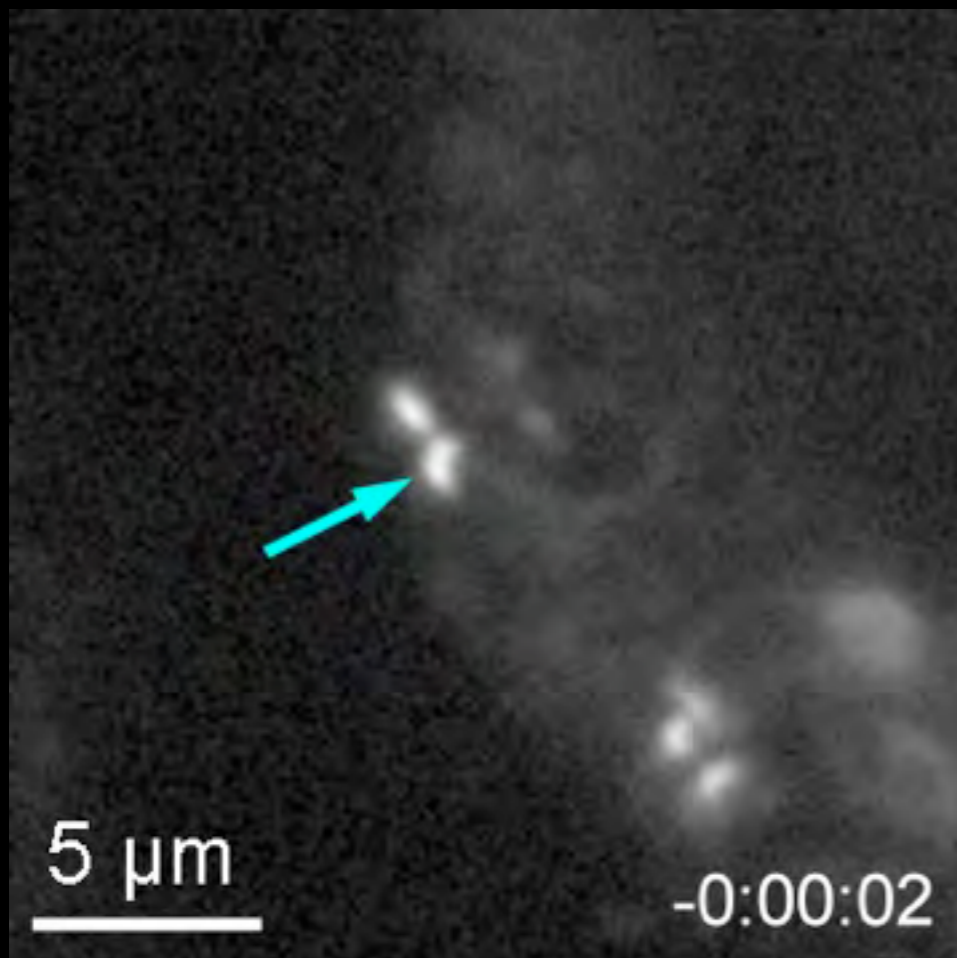
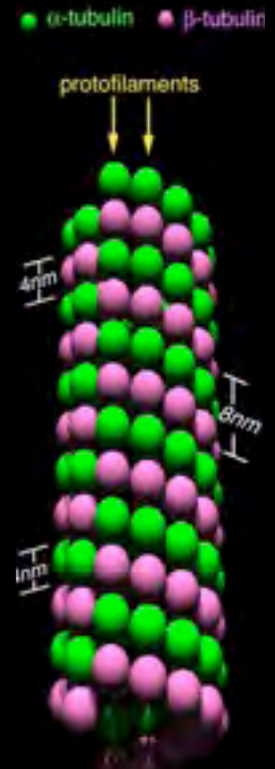


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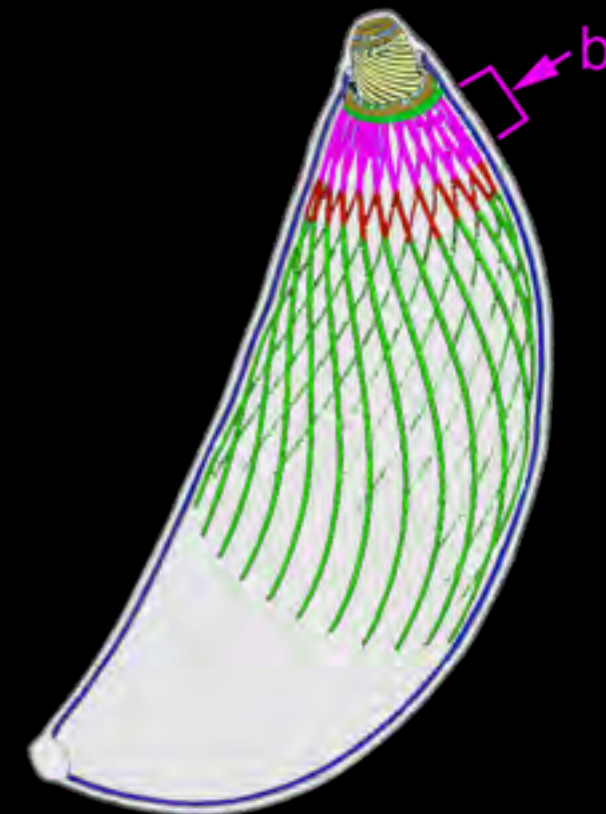
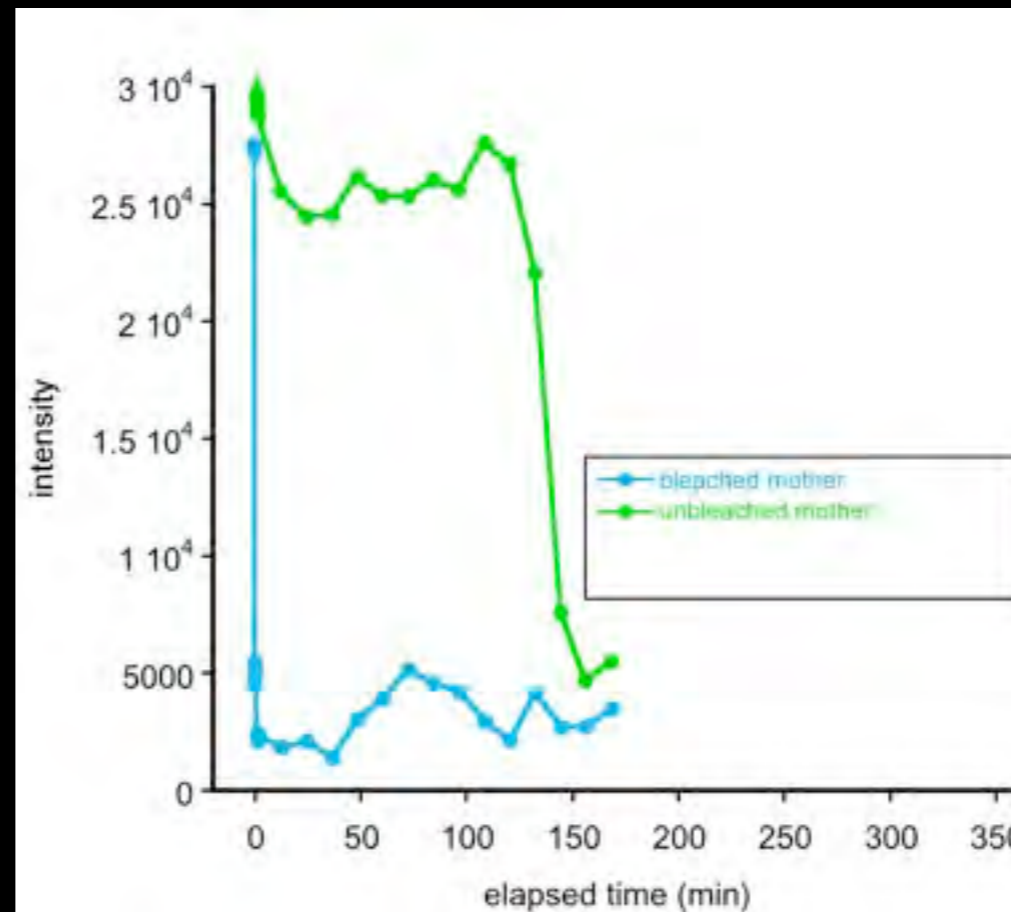
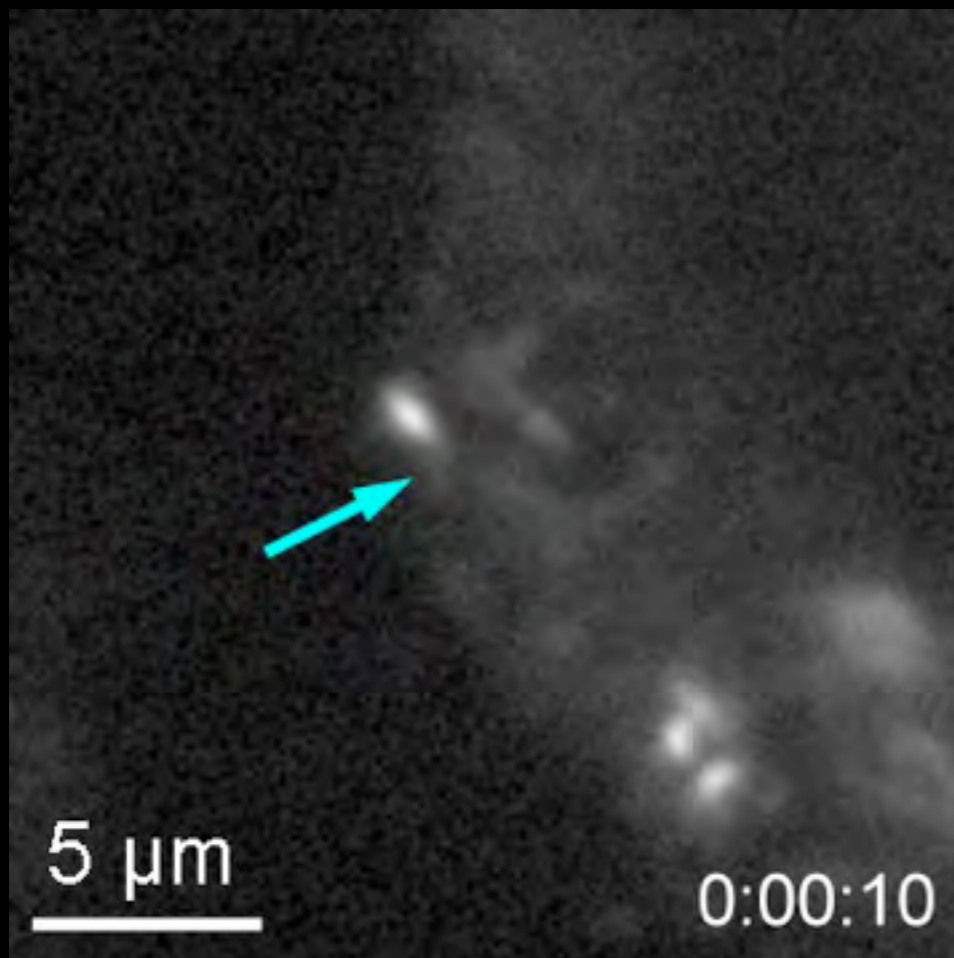
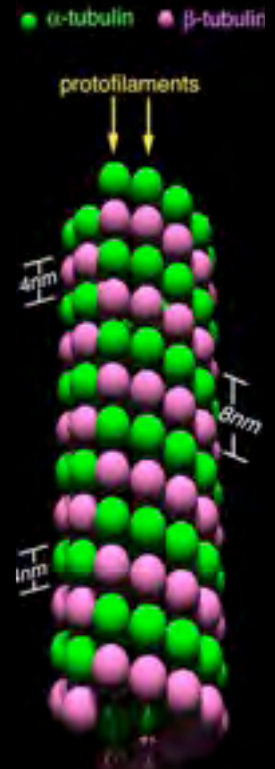


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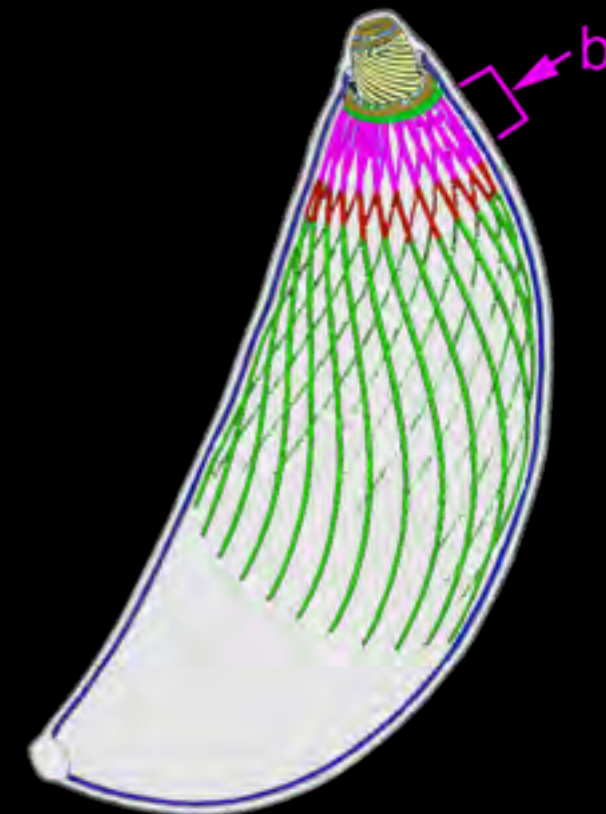
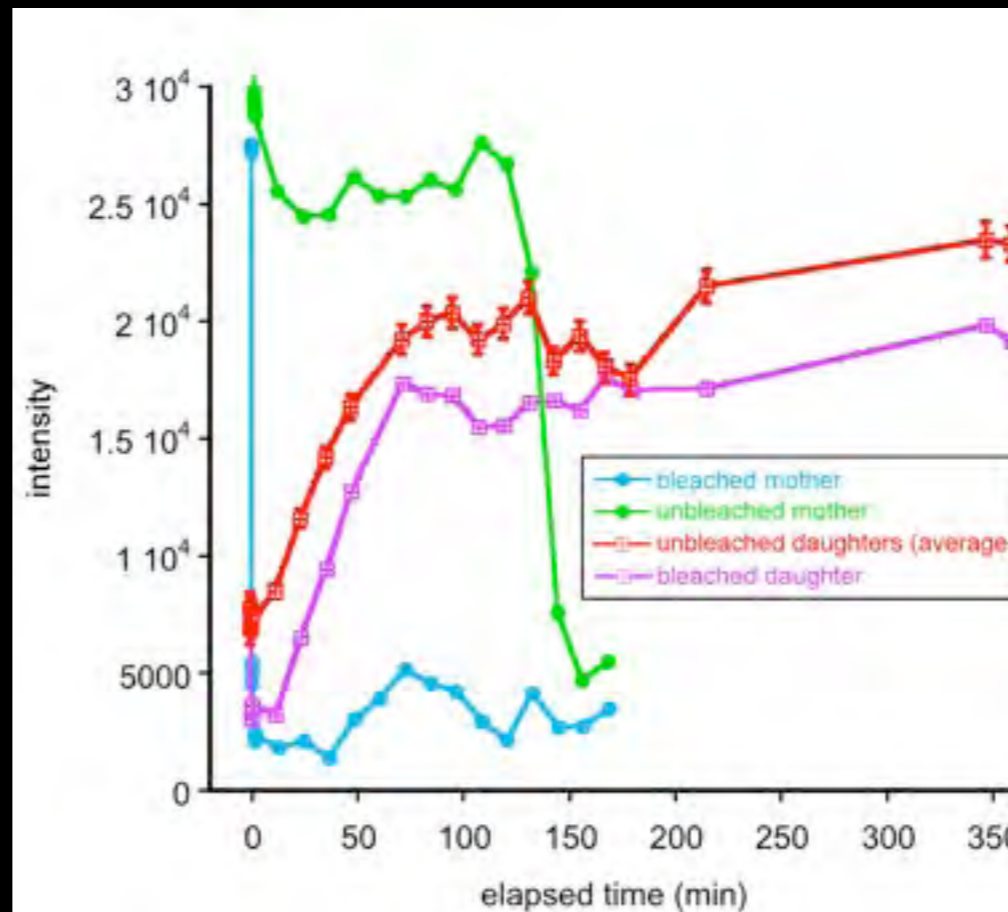
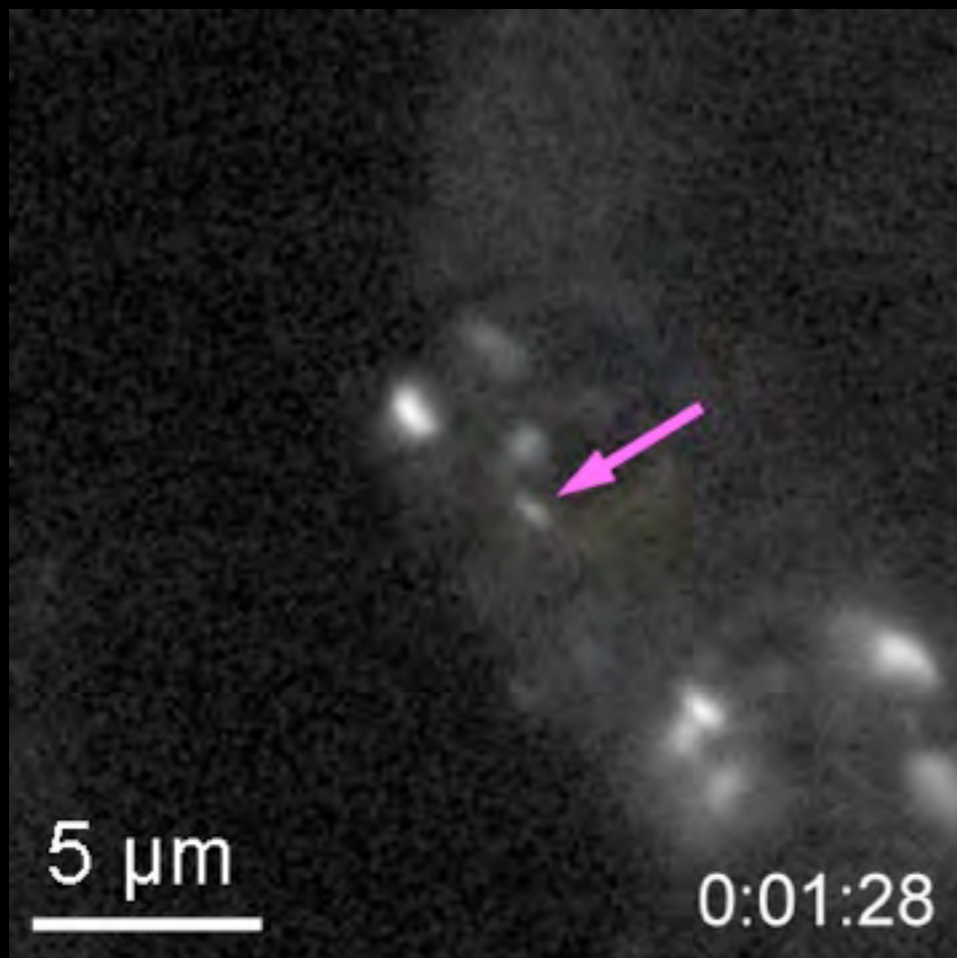
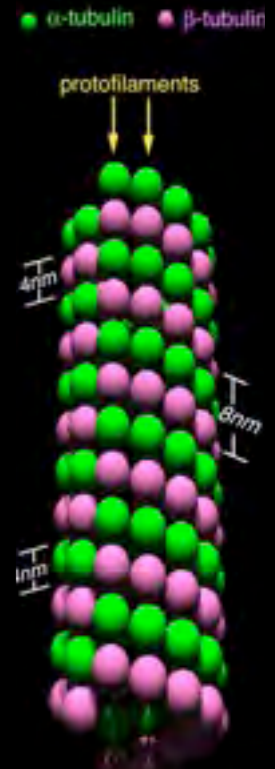


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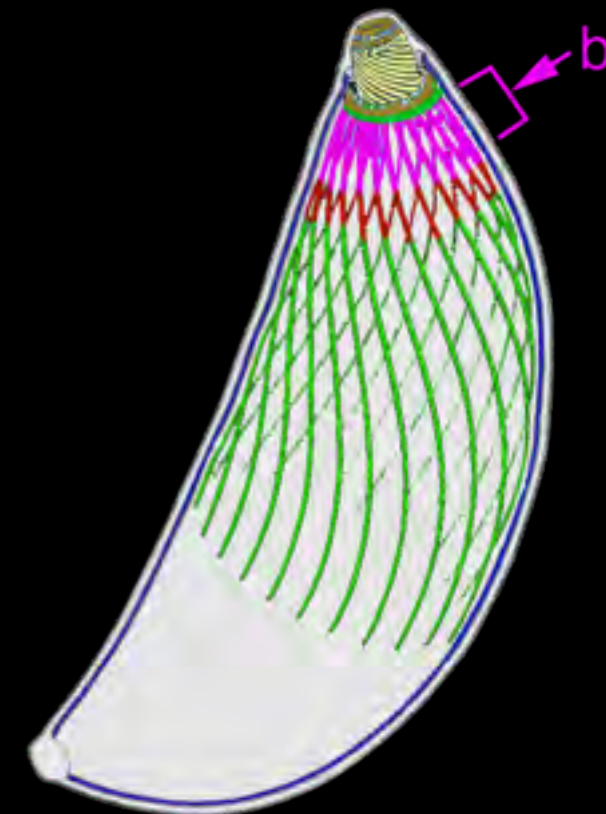
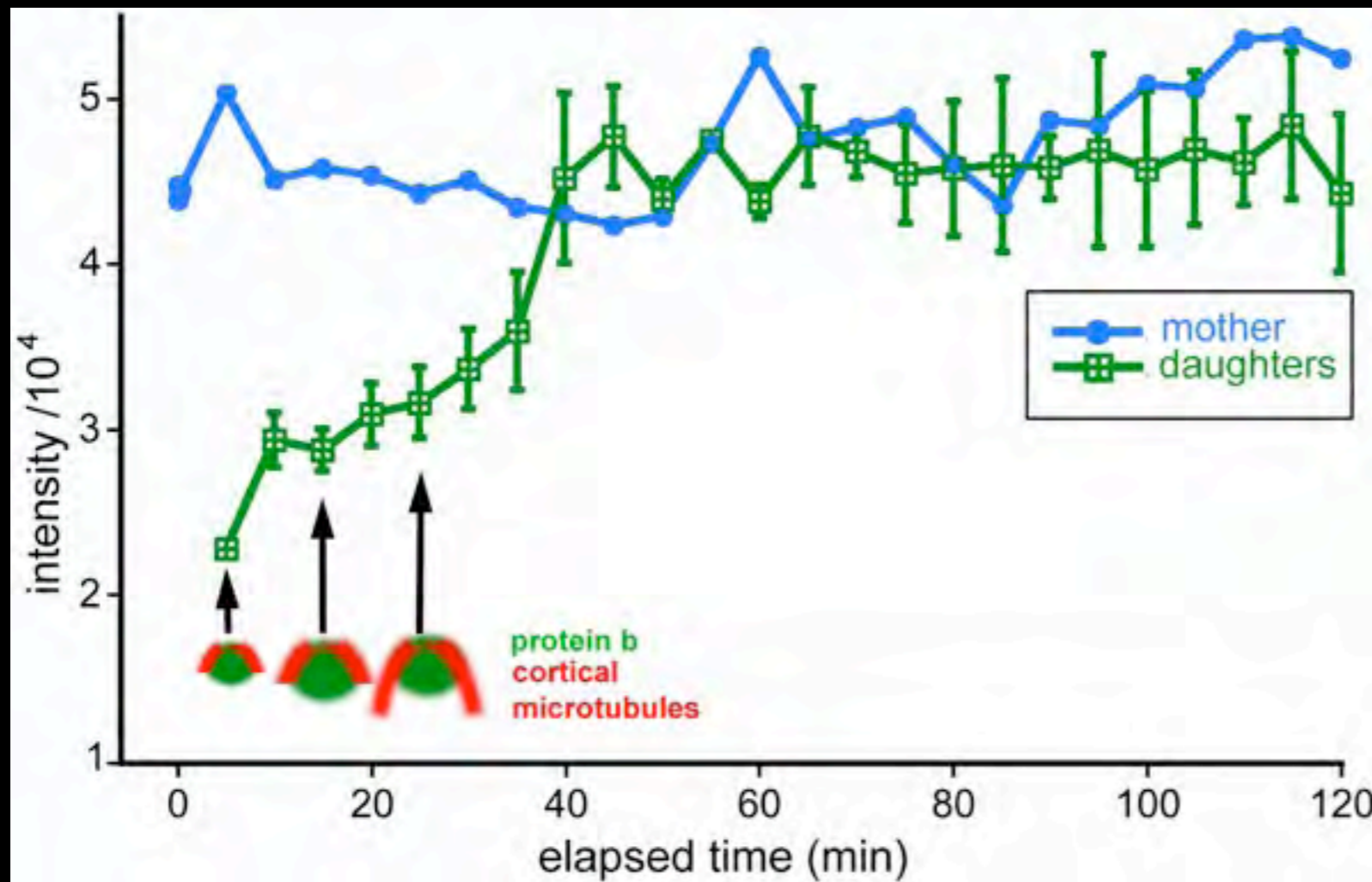
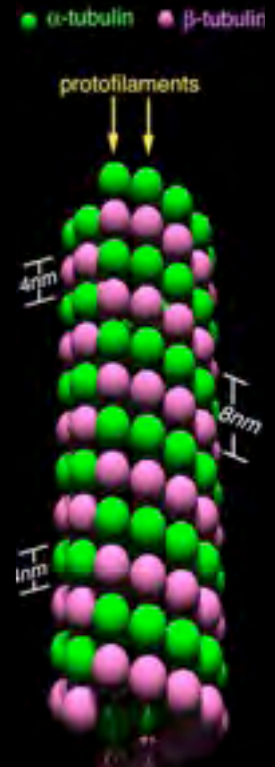


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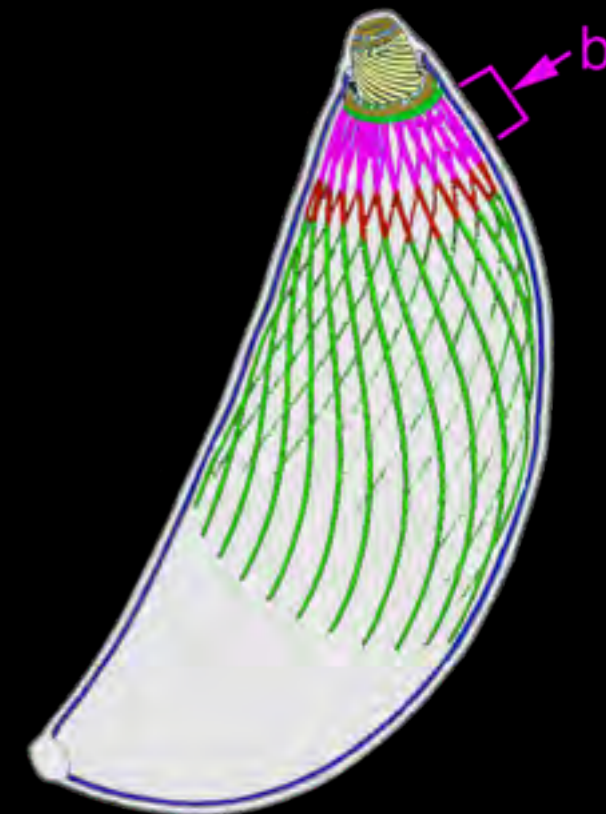
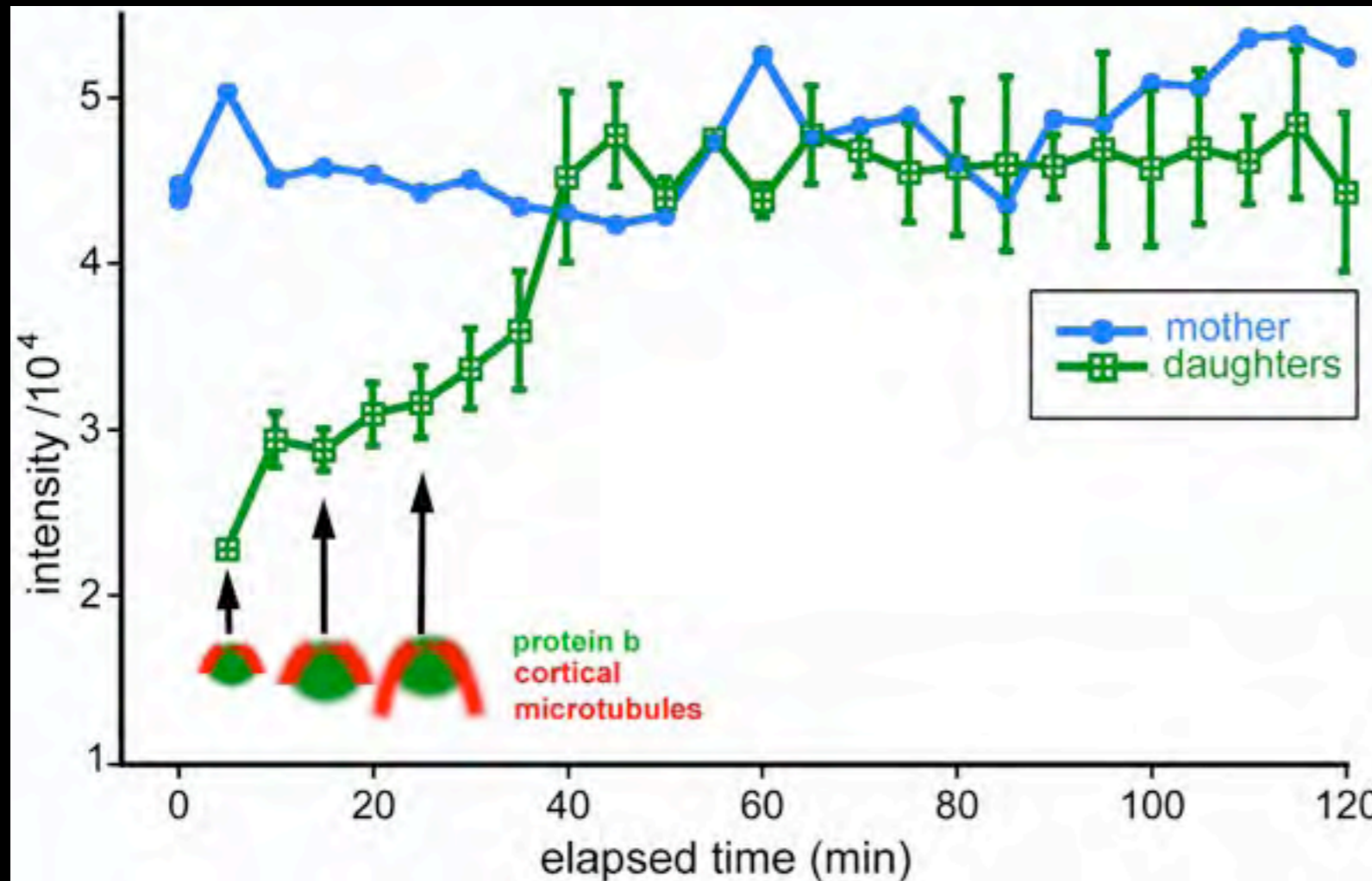
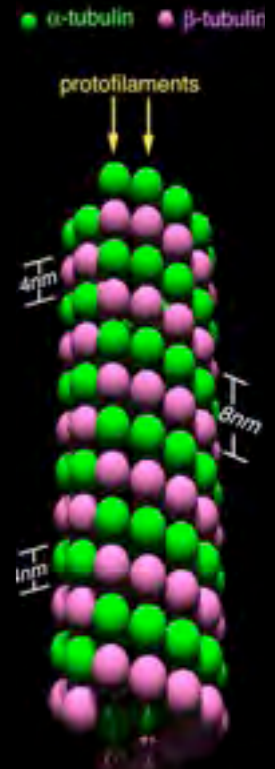
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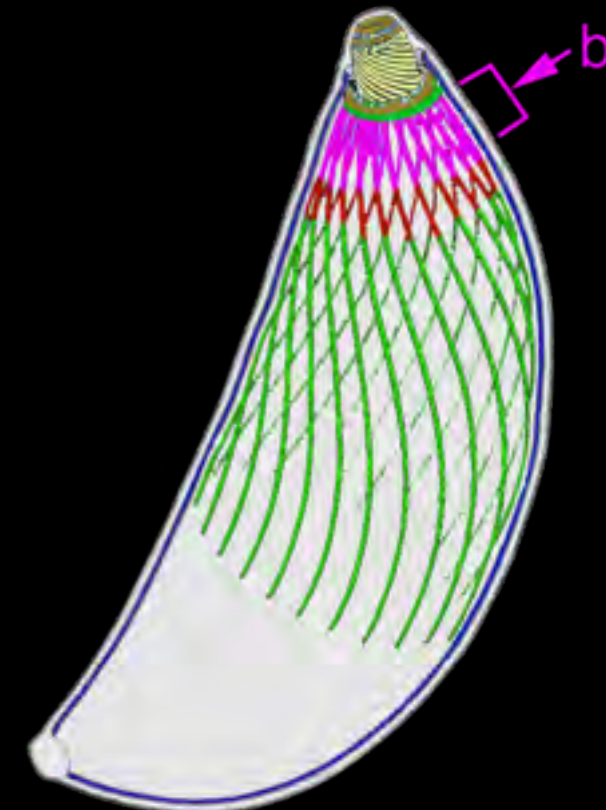
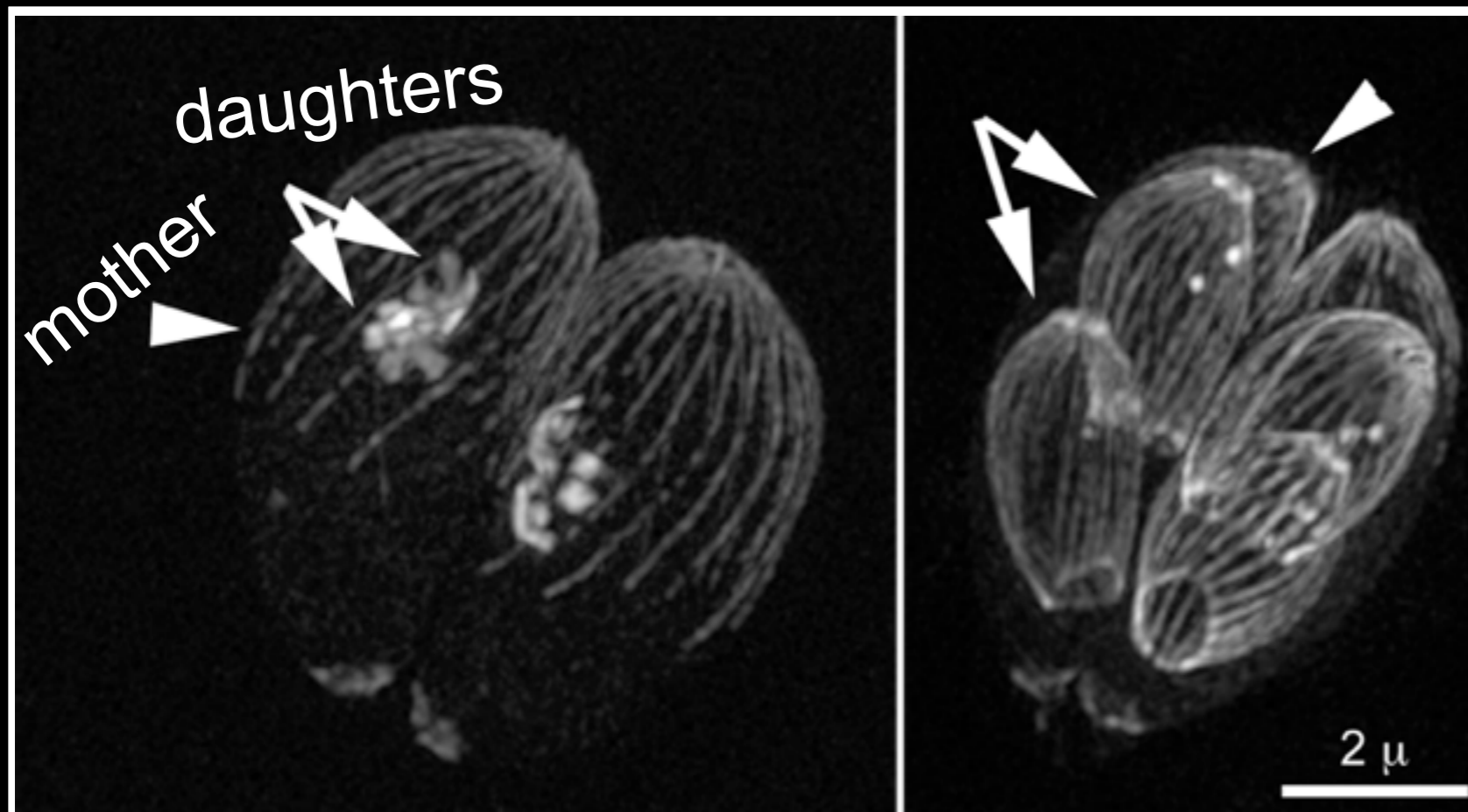
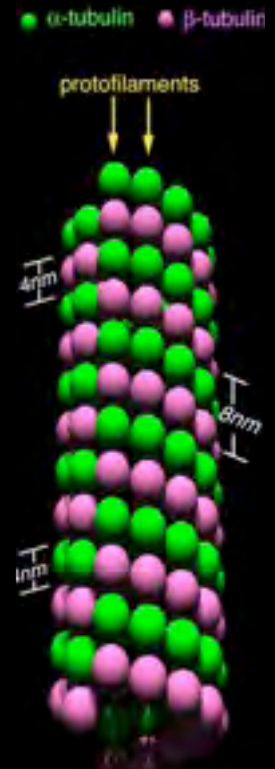
What restricts the localization (i.e. why doesn't it coat the entire length of the polymer?)

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a. X binds to microtubule much faster and/or better than protein b

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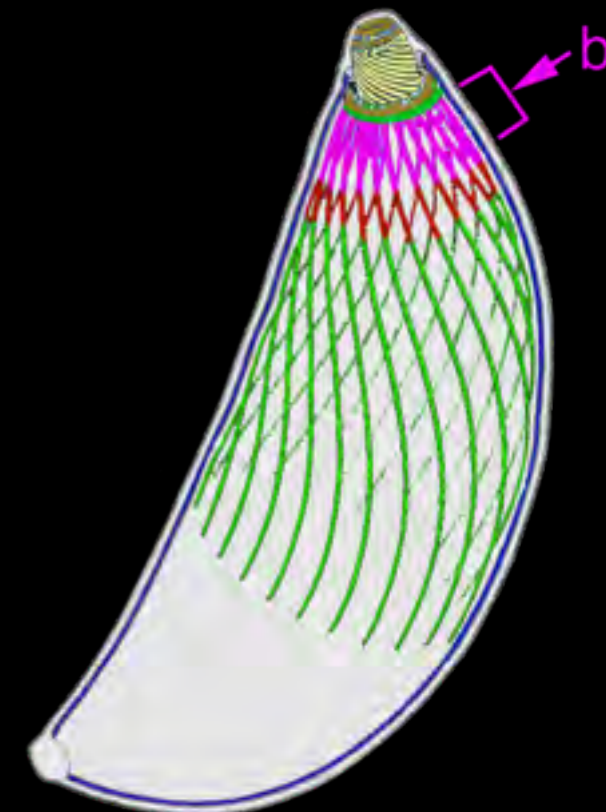
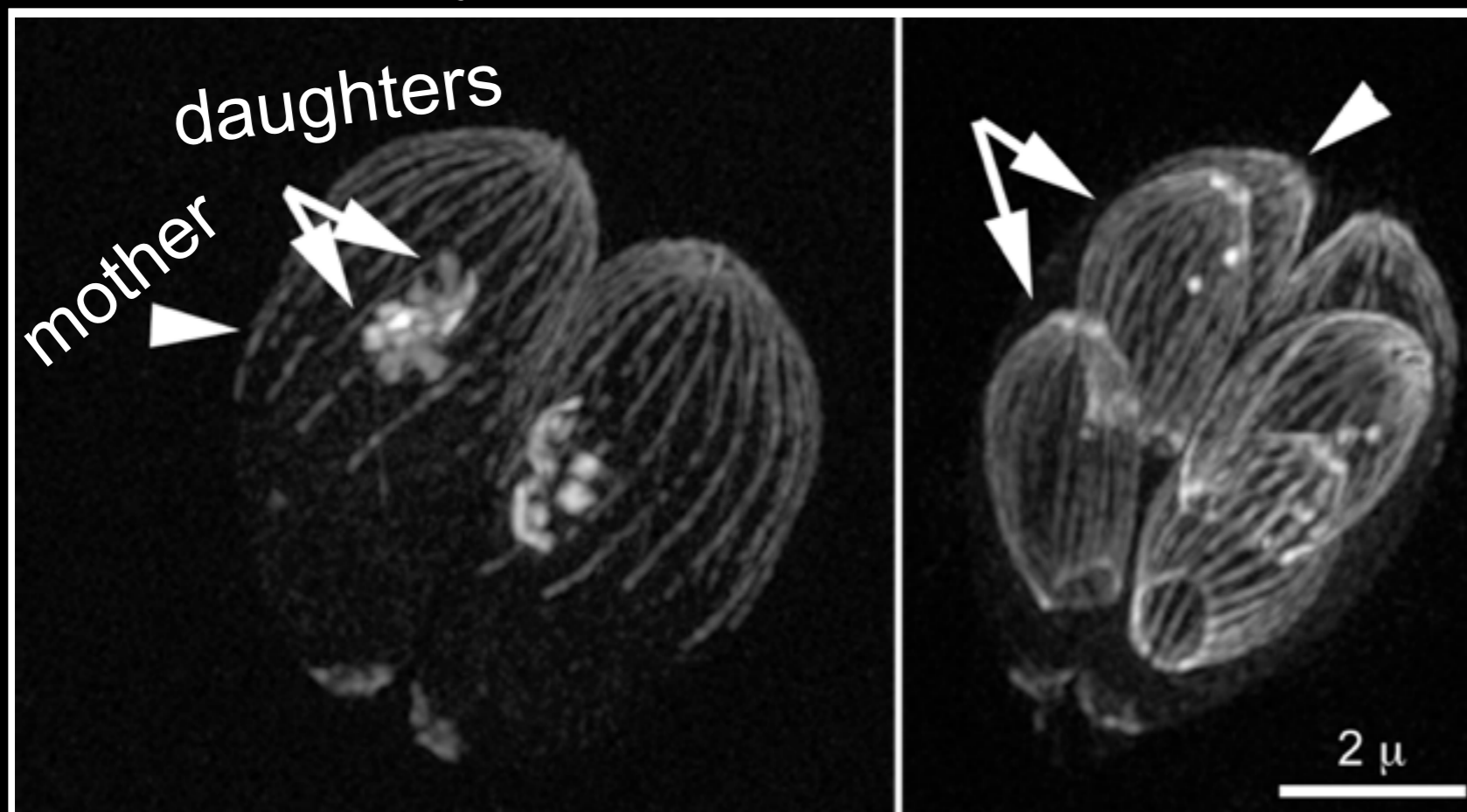
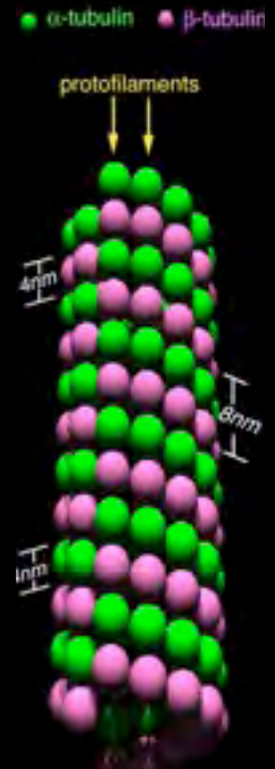
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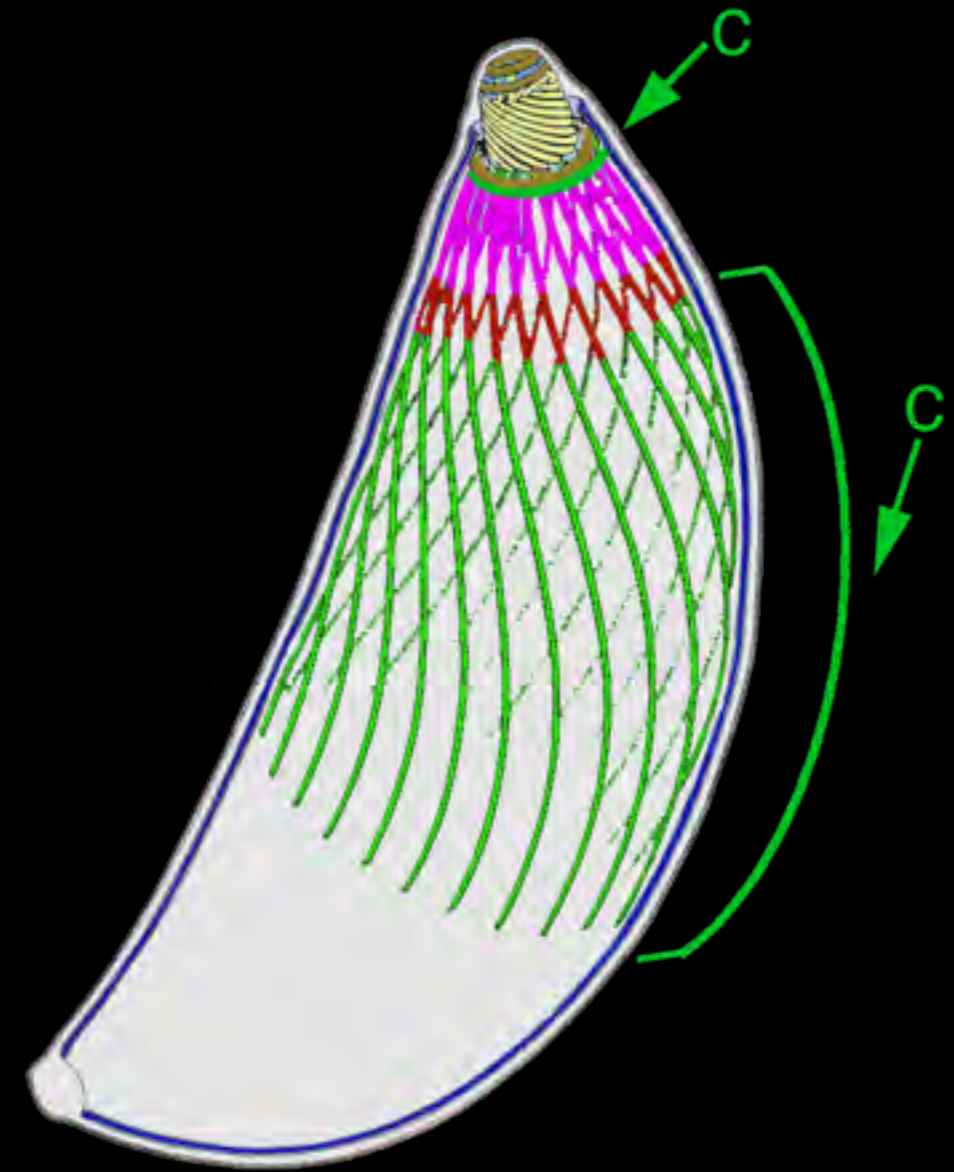
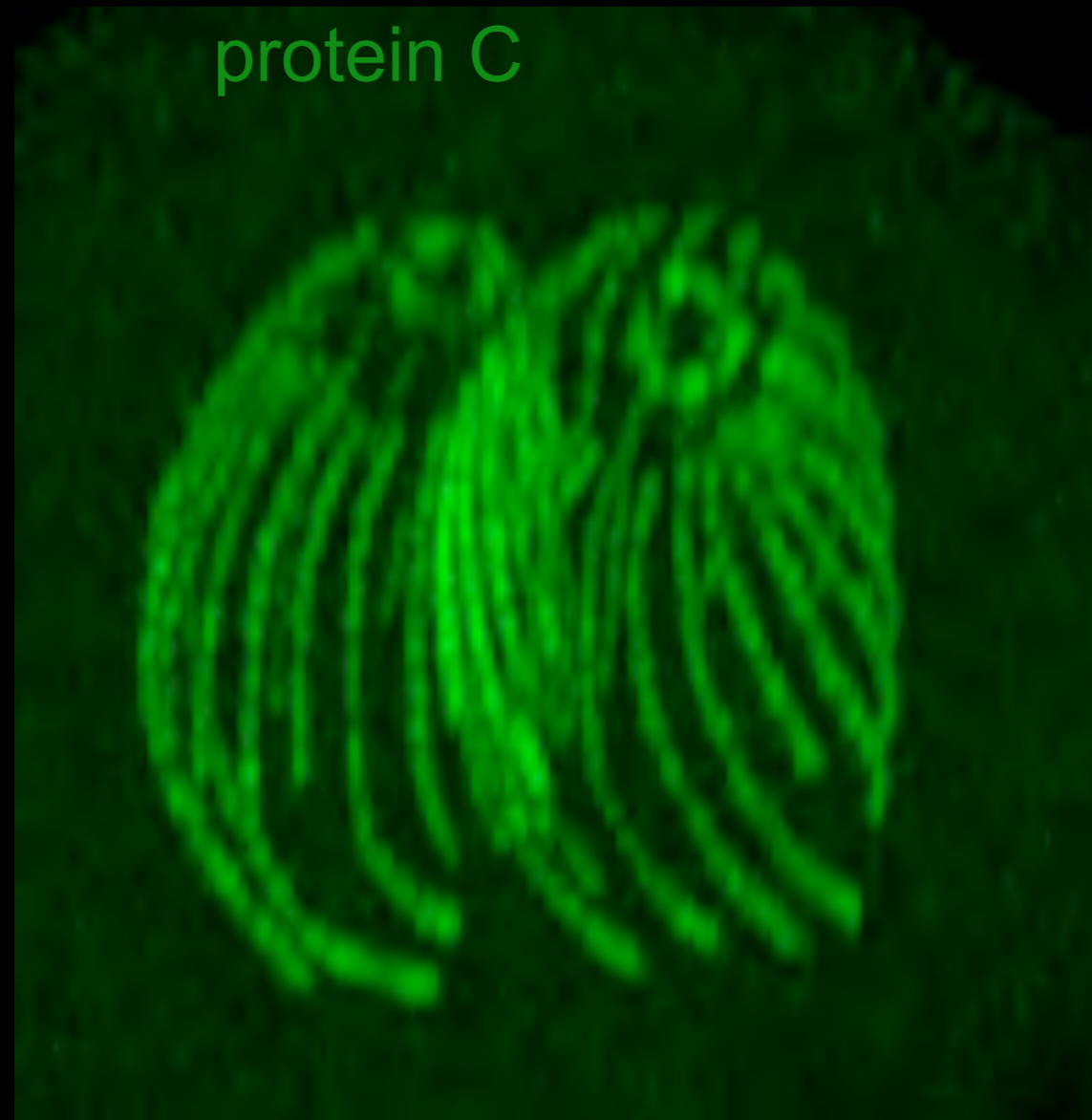
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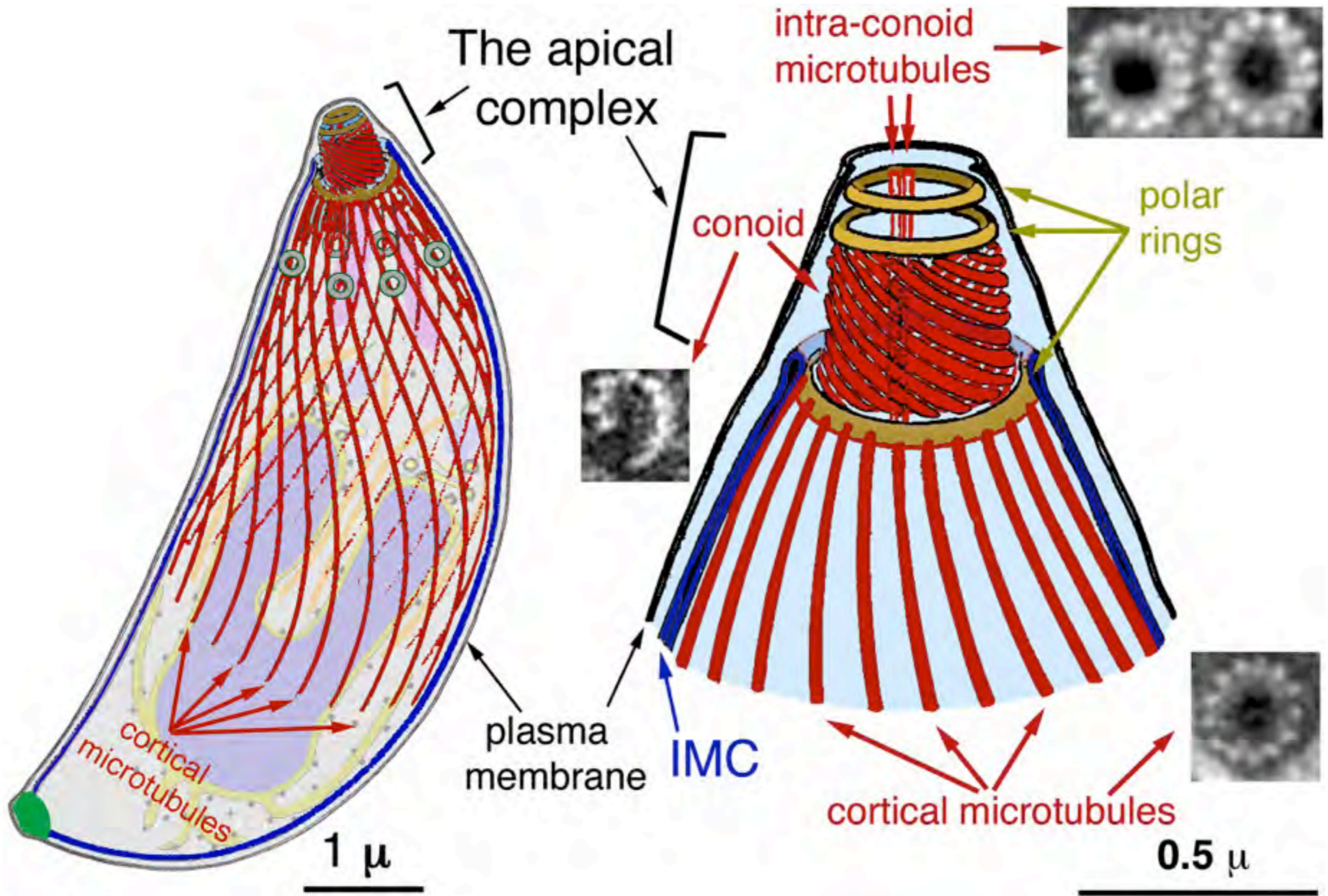
Protein C decorates cortical microtubules in multiple sections



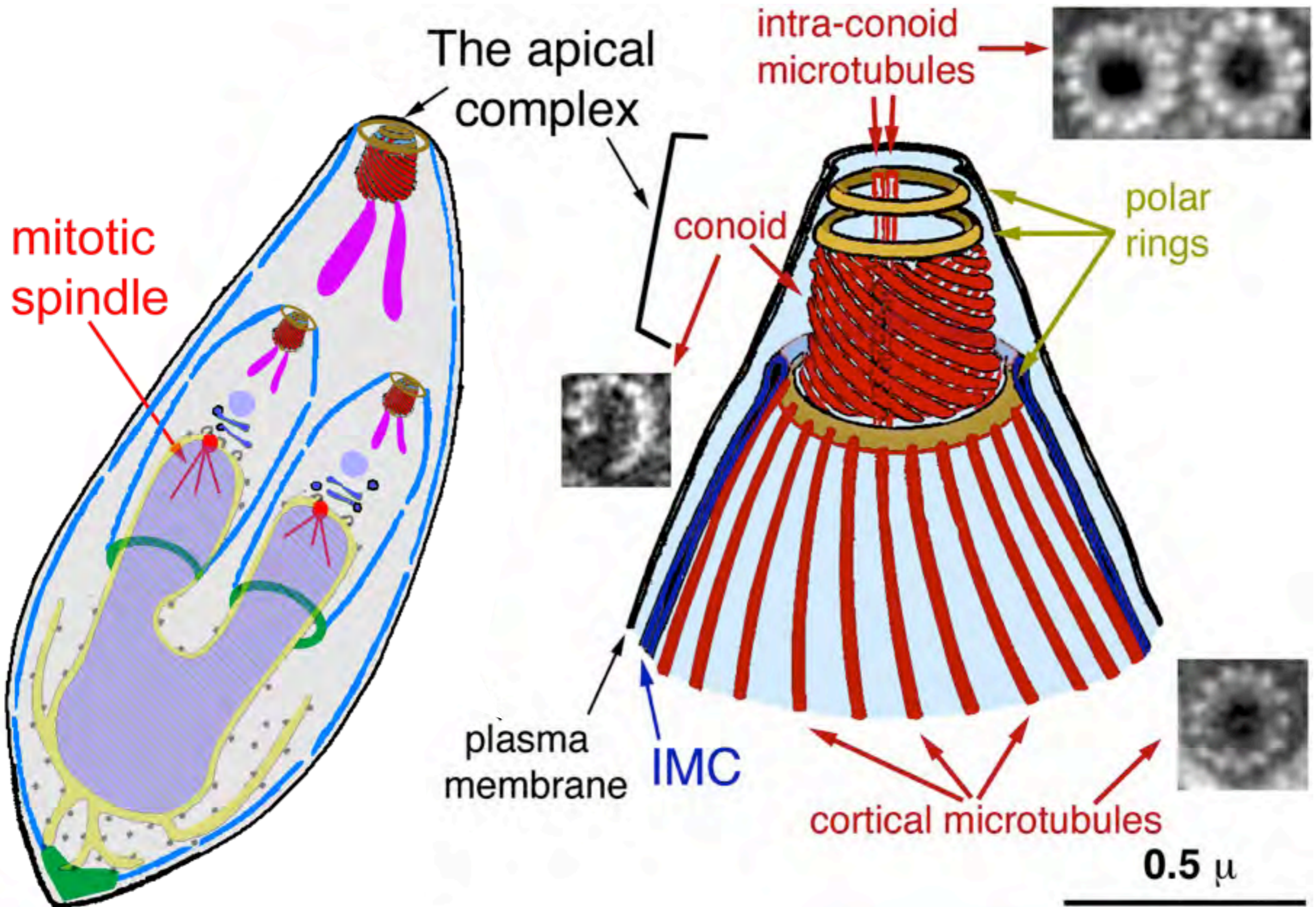
Jun Liu



There are multiple tubulin-containing structures in the same cell



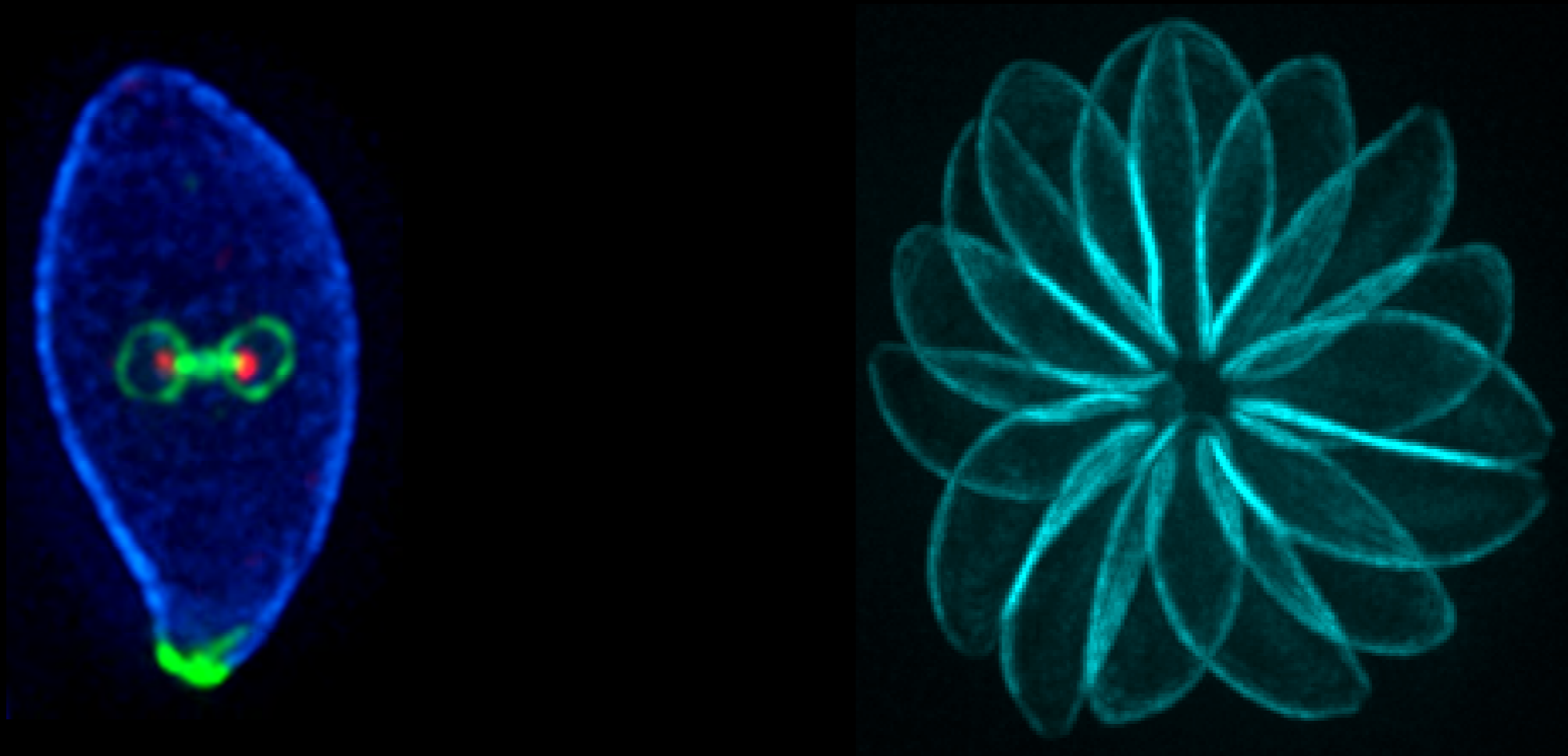
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How to build a parasite?

II

- how do molecules get to the right place?



Hu Lab

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Phoebe (Yudou) He

Jacqueline Leung

Chang Zeng

Amanda Rollins

Tiffany Fortney

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Christopher Connell

Dayana Arellano

Jane Stout

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Indiana University

Stephanie Ems-McClung

Barry Stein

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/Carleton University

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of Allergy and
Infectious Diseases



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