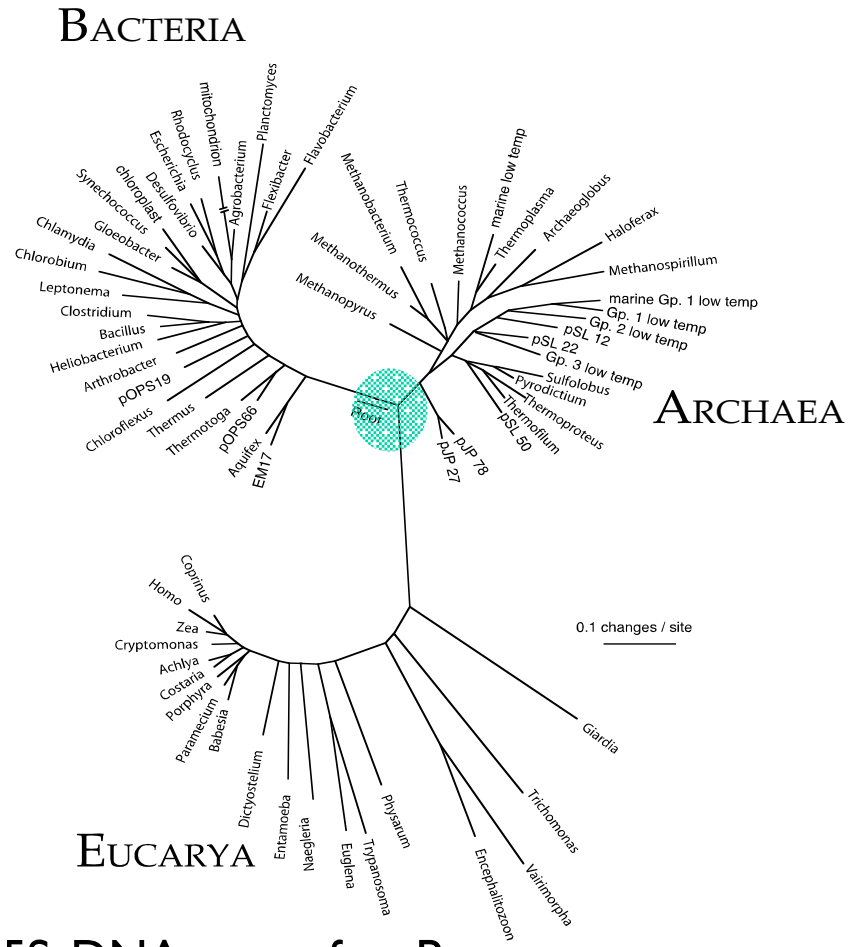
What is the meaning of life?

Zac Cande, UC Berkeley

KITP Evo Cell, Feb 17, 2010

What is the meaning of life and the evolutionary origi of mitosis?

The “Big Tree” and Eukaryotes



5SrDNA tree, after Pace

Not related to any specific bacterial or archaeal group

Eukaryotic lineage is old, thus NO prokaryote --> eukaryote transition

(no spindles in bacteria or archae, but cytoskeleton involved in nucleoid dispersion!)

A “few” gaps in our understanding of the evolution of Eucarya…

Map of evolutionary relationships (Big Tree of Eukaryotes) is incomplete and inaccurate:

1.) Controversies over where to root tree

2.) Relationships among and within 6 major groups

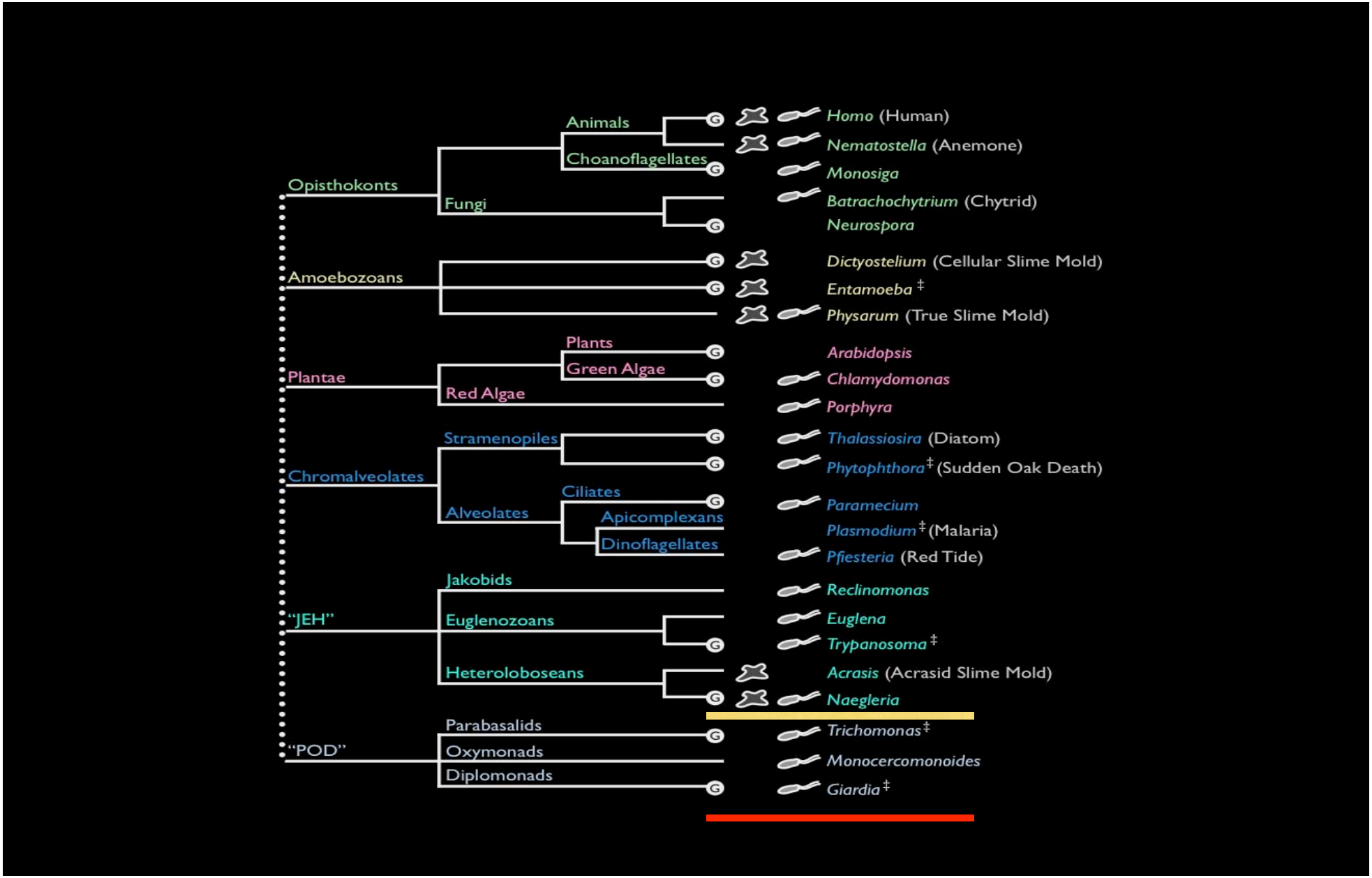
A “few” gaps in our understanding of the evolution of Eucarya…

Map of evolutionary relationships (Big Tree of Eukaryotes) is incomplete and inaccurate:

1.) Controversies over where to root tree

2.) Relationships among and within the 6 major groups

- Lack full comprehension of diversity of microbial eukaryotes in the natural world -need more genomes.


Opisthokonts
Animals
Choanoflagellates
Fungi
Amoebozoans
Plantae
Plants
Green Algae
Red Algae
Chromalveolates
Stramenopiles
Alveolates
Ciliates
Apicomplexans
Dinoflagellates
"JEH"
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Parabasalids
Oxymonads
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Homo (Human)
Nematostella (Anemone)
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Physarum (True Slime Mold)
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Thalassiosira (Diatom)
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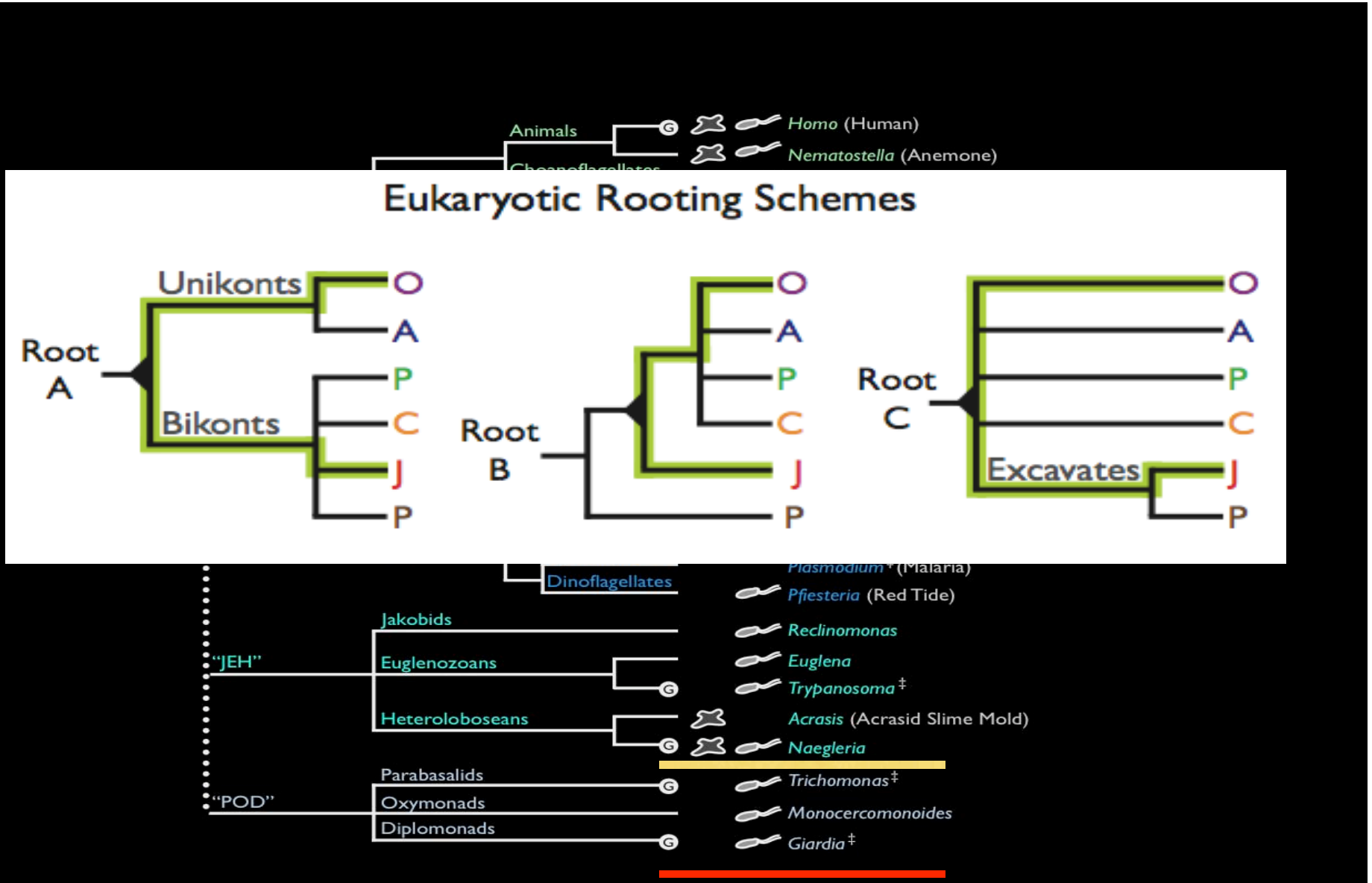
Naegleria
Trichomonas ‡
Monocercomonoides
Giardia ‡


Animals
Homo (Human)
Nematostella (Anemone)
Eukaryotic Rooting Schemes
Root A
Unikonts
Bikonts
O
A
P
C
J
P
Root B
O
A
P
C
J
P
Root C
O
A
P
C
Excavates
J
P
Dinoflagellates
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A “few” gaps…

Eukaryotic origins and subsequent evolution of unique features (e.g. organelles, cytoskeleton, molecular machines)

By studying extant microbial eukaryotes, can we discern evolutionary intermediates? (Has most of our analysis of cellular evolution been distorted by studying parasites which are missing many parts?)

A "few" gaps…

Eukaryotic origins and subsequent evolution of unique features (e.g. organelles, cytoskeleton, molecular machines)

Need to know more about Biology of "non-model" eukaryotes

Today: talk about the mitosis, cell cycle regulation

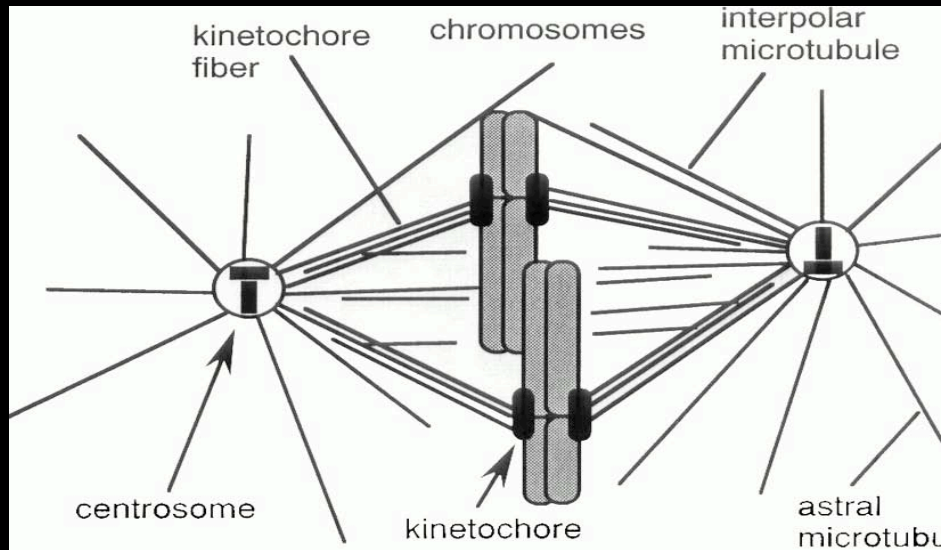
in Giardia (a Diplomond in POD, an intestinal parasite)

Mitosis- need

- Equal partitioning of sister chromatids by establishing bipolarity
- Force generation

A canonical metazoan spindle

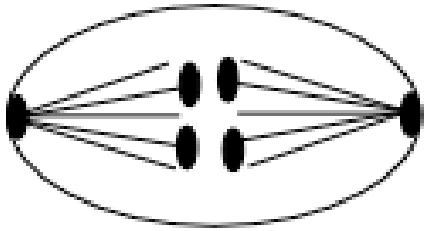
- mitosis is "open"
- bipolar, anti-parallel MT array with overlap zone
- MT attach to chromosomes at kinetochore
- equivalent centrosomes nucleate



Evolutionary variation in spindle morphology

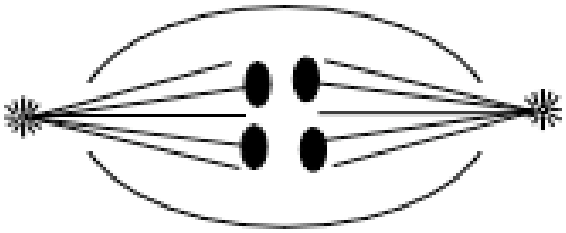
1. Spindle poles
2. Nuclear envelope behavior
3. Kinetochore morphology
4. Microtubule distribution (polar vs kinetochore mt organization)

Diversity of spindle structure



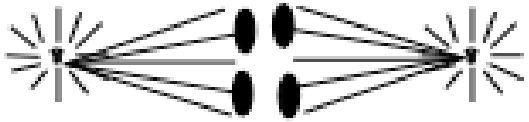
closed intranuclear orthomitosis
(*S. cerevisae, S. pombe, trypanosomes, ciliates*)

closed intranuclear pleuromitosis
(microsporidians, some fungi, Acantharea)

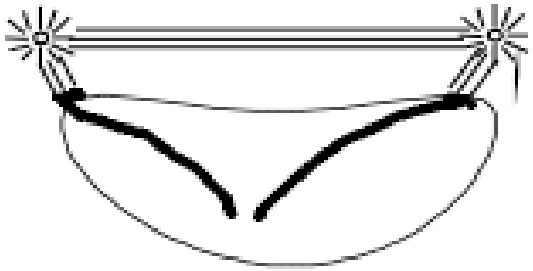


semiopen orthomitosis
(*Volvox, Giardia?*)

semiopen pleuromitosis
(*Plasmodium*)



open orthomitosis
(metazoans, stramenopiles, cryptomonads)



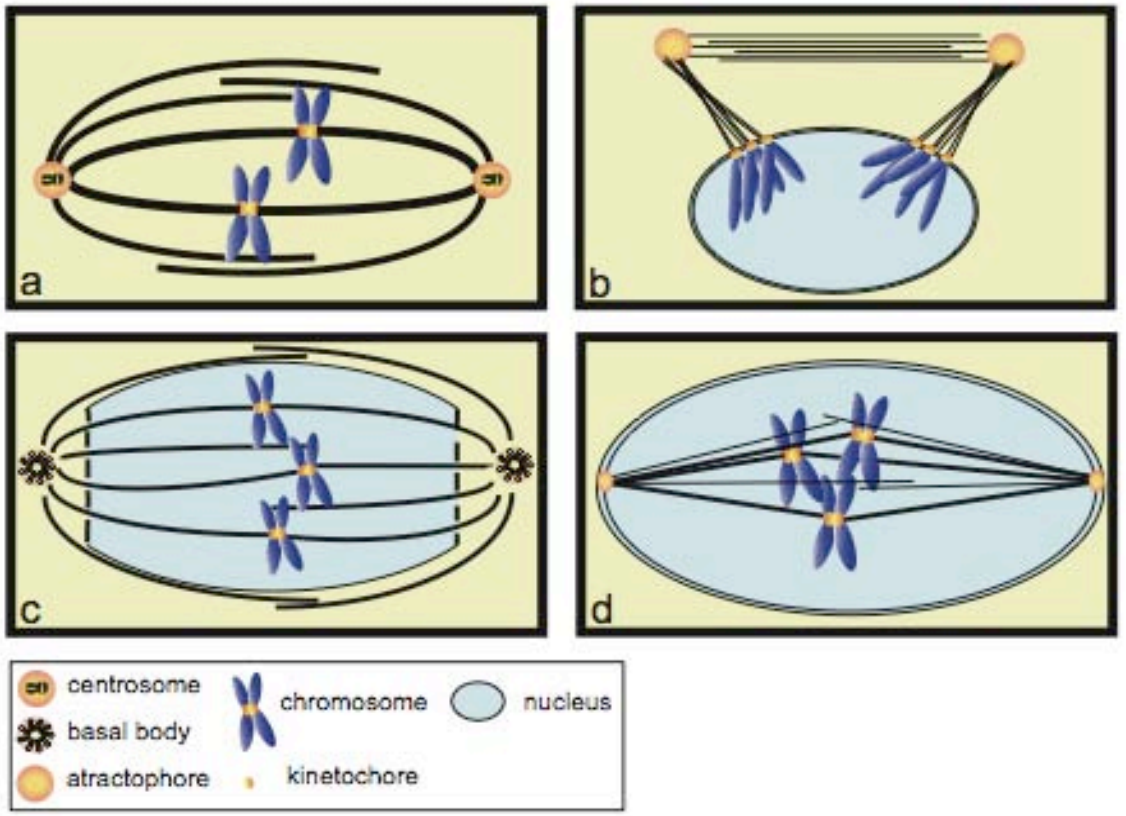
closed extranuclear pleuromitosis
(*Trichomonas vaginalis*)

Raikov1994

Diversity of spindle structure

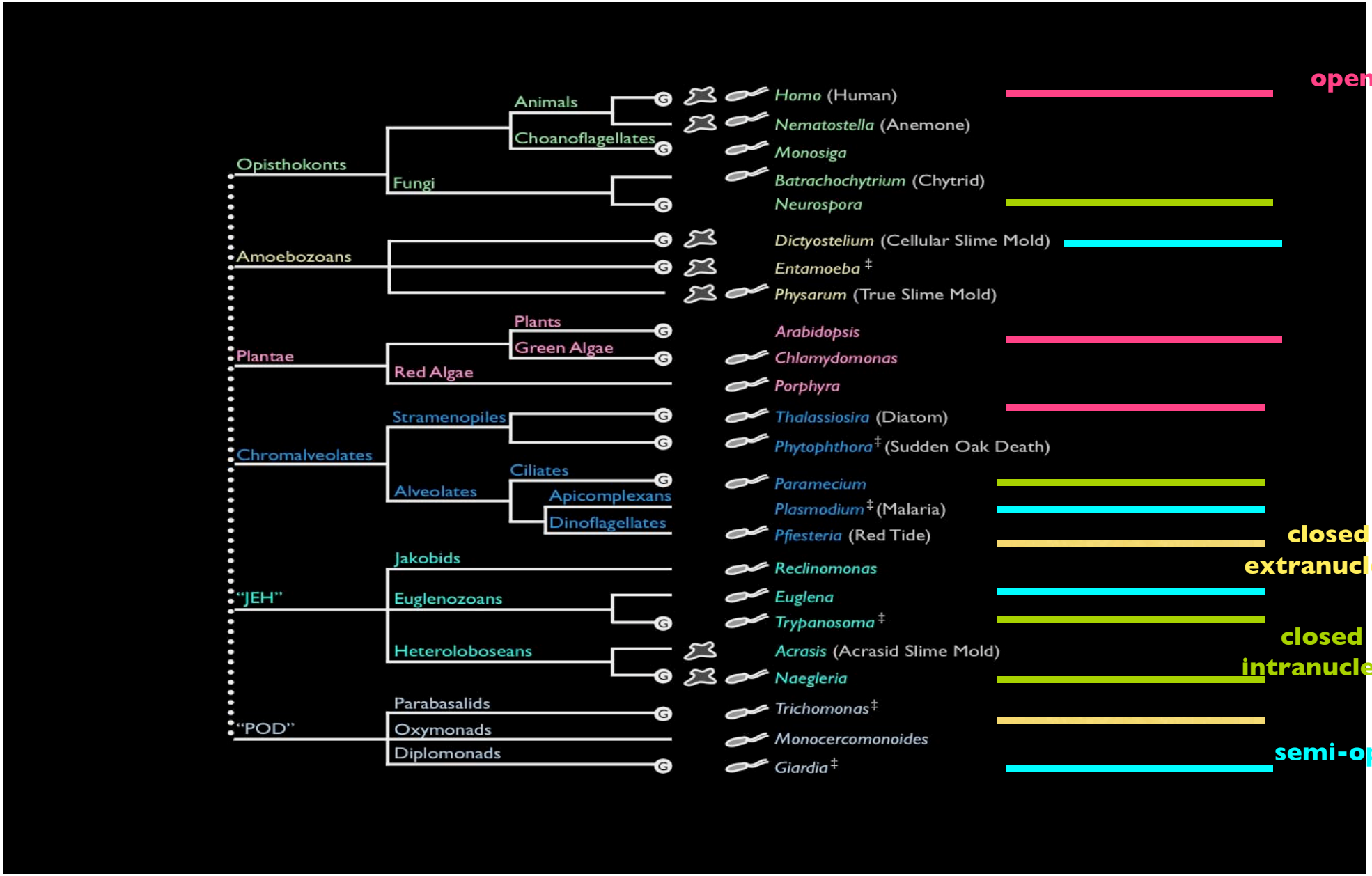
open

semi-open



closed extranuclear

closed intranuclear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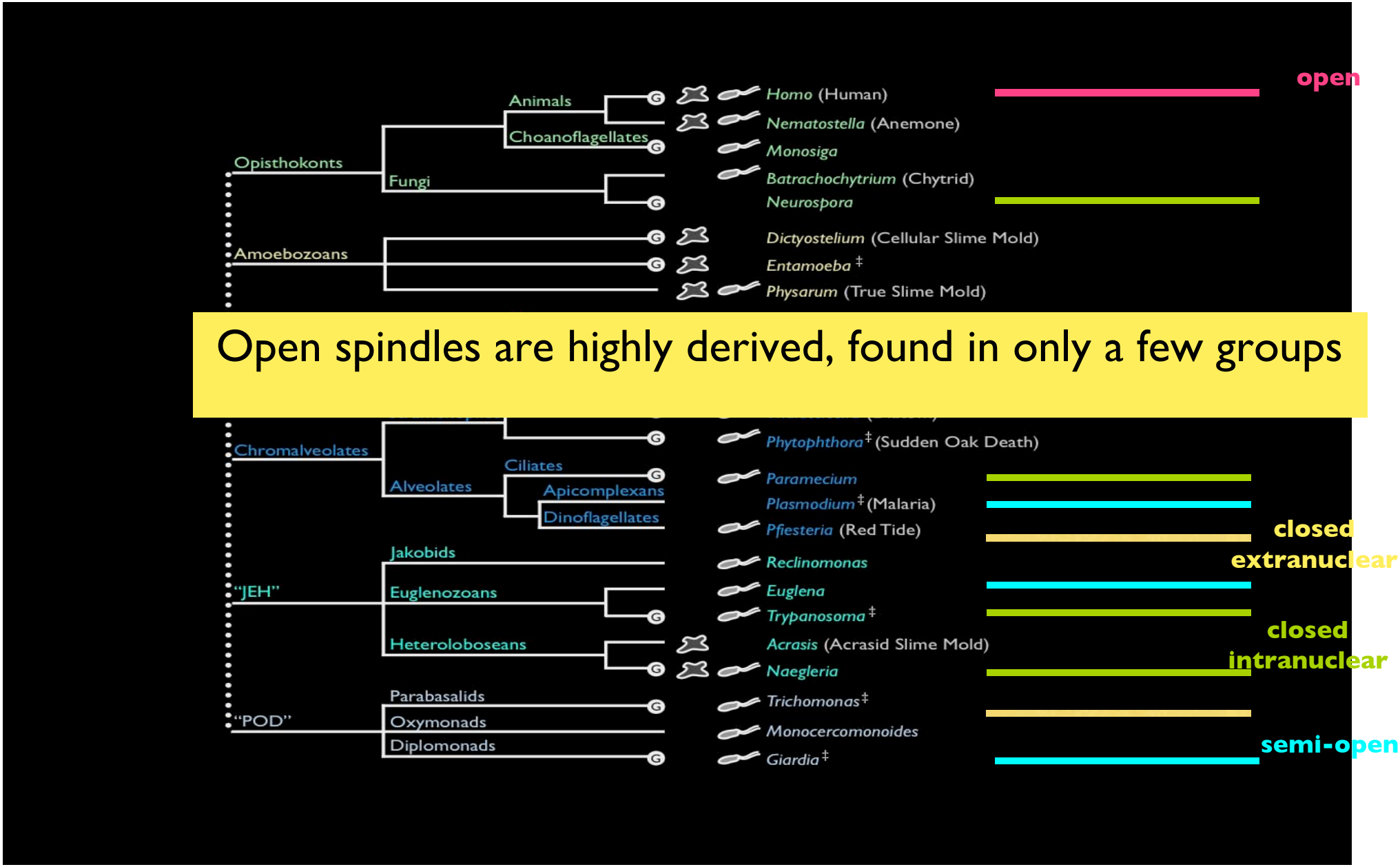
Naegleria
Trichomonas ‡
Monocercomonoides
Giardia ‡
open
closed
extranuclear
closed
intranuclear
semi-open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open
Open spindles are highly derived, found in only a few groups
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Alveolates
Ciliates
Apicomplexans
Dinoflagellates
"JEH"
Jakobids
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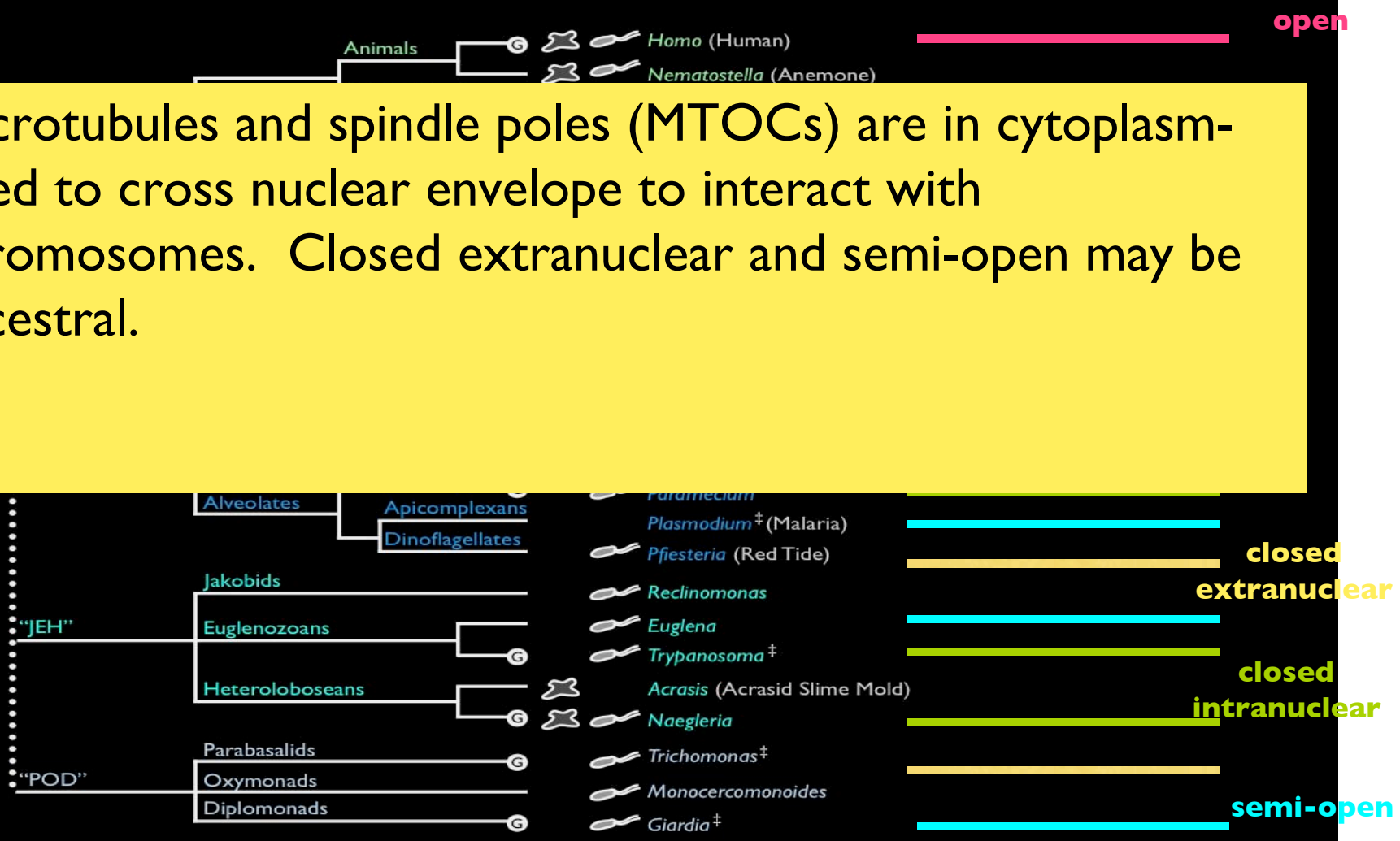
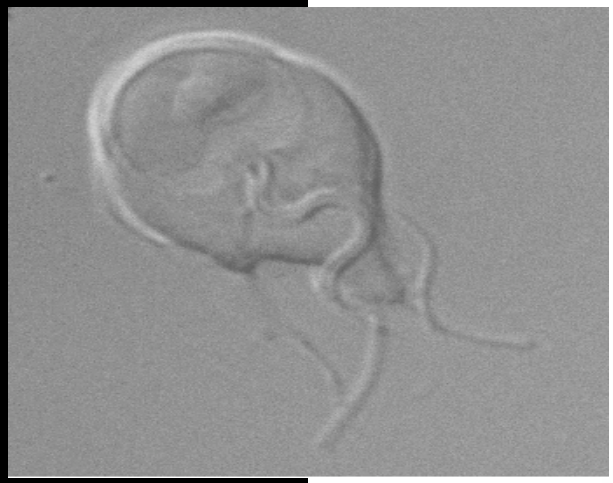
Monocercomonoides
Giardia ‡
closed
extranuclear
closed
intranuclear
semi-open

Microtubules and spindle poles (MTOCs) are in cytoplasm- need to cross nuclear envelope to interact with chromosomes. Closed extranuclear and semi-open may be ancestral.

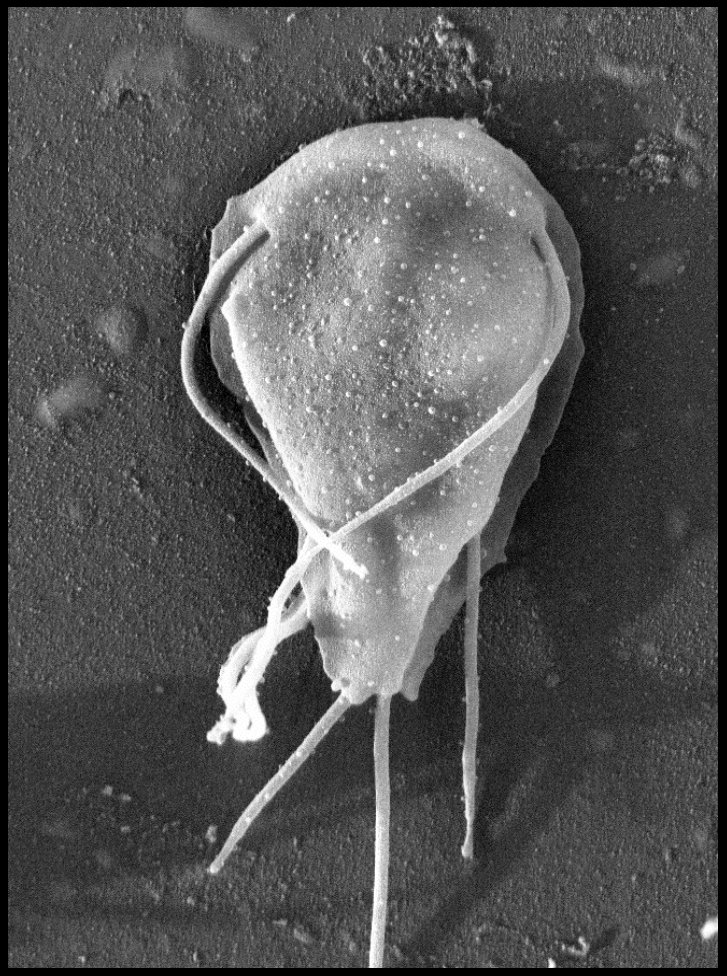


Giardia is a divergent eukaryote



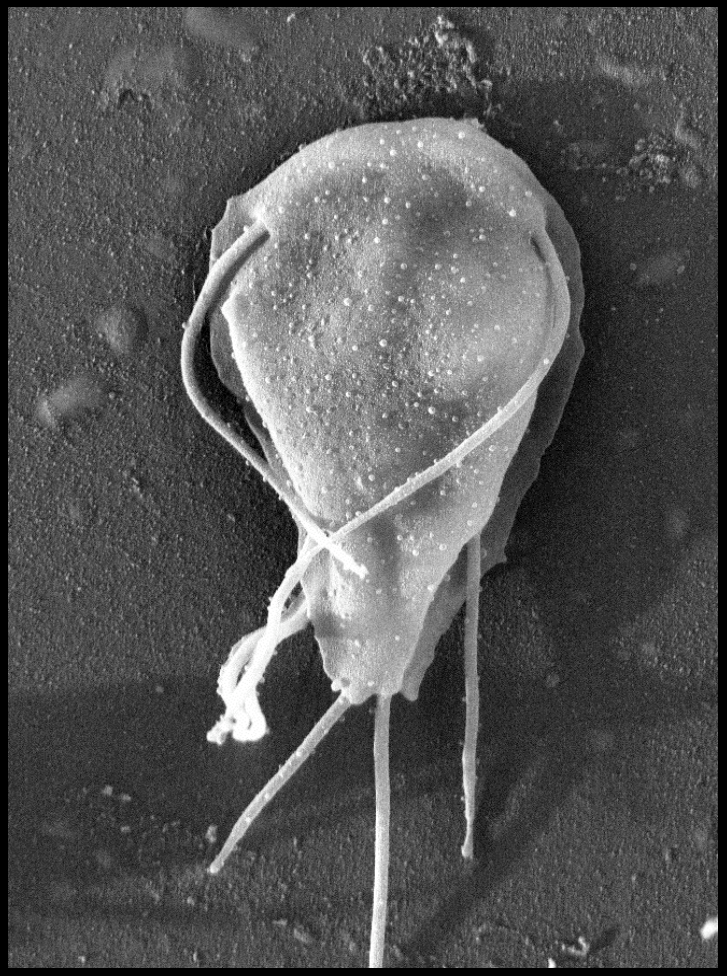
Adapted from Ciccarelli et al., 2006

Giardia intestinalis



Mitosis
1.Background on organism
2. How divide-spindle morphology
3. Kinetochore proteins
4. Kinesin motors
Cell Cycle regulation

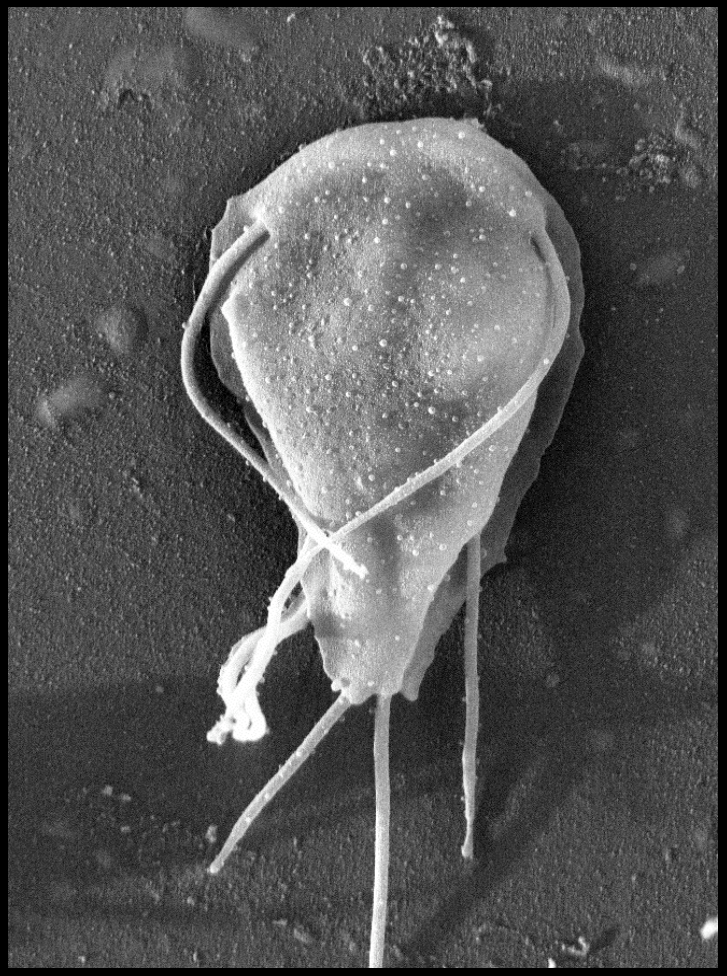
Giardia intestinalis



Biology

-binucleate (diploid and equivalent)=tetraploid
-small ribosomes,
-archae like transcription factors
-lack mitochondria (?)
-no peroxisomes (facultative anaerobe)
-rudimentary Golgi
-few (three?) introns
-asexual, lack meiosis?
-intestinal parasite (major cause of diarrhea)

Giardia intestinalis



Model organism

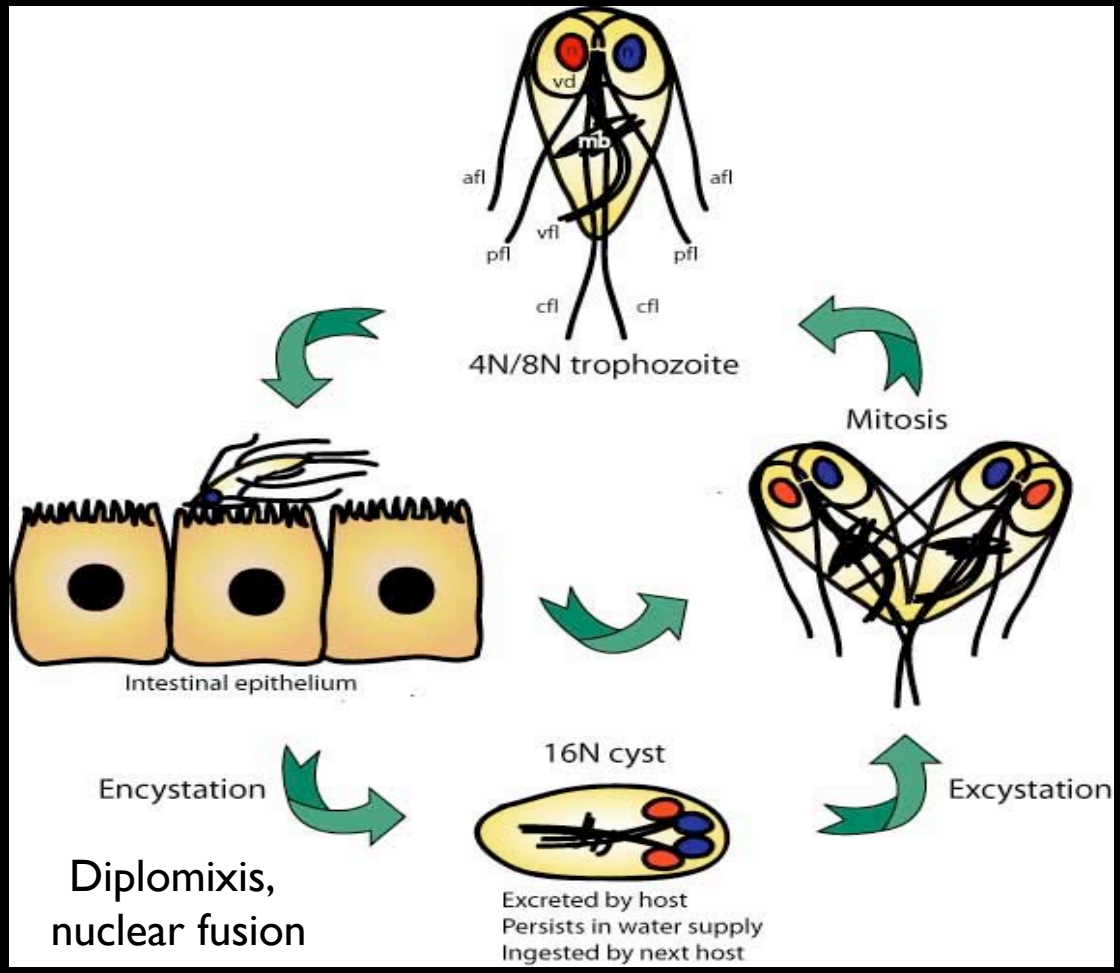
-Easy to grow , all stages of life cycle in culture

-Sequenced genome (12MB)

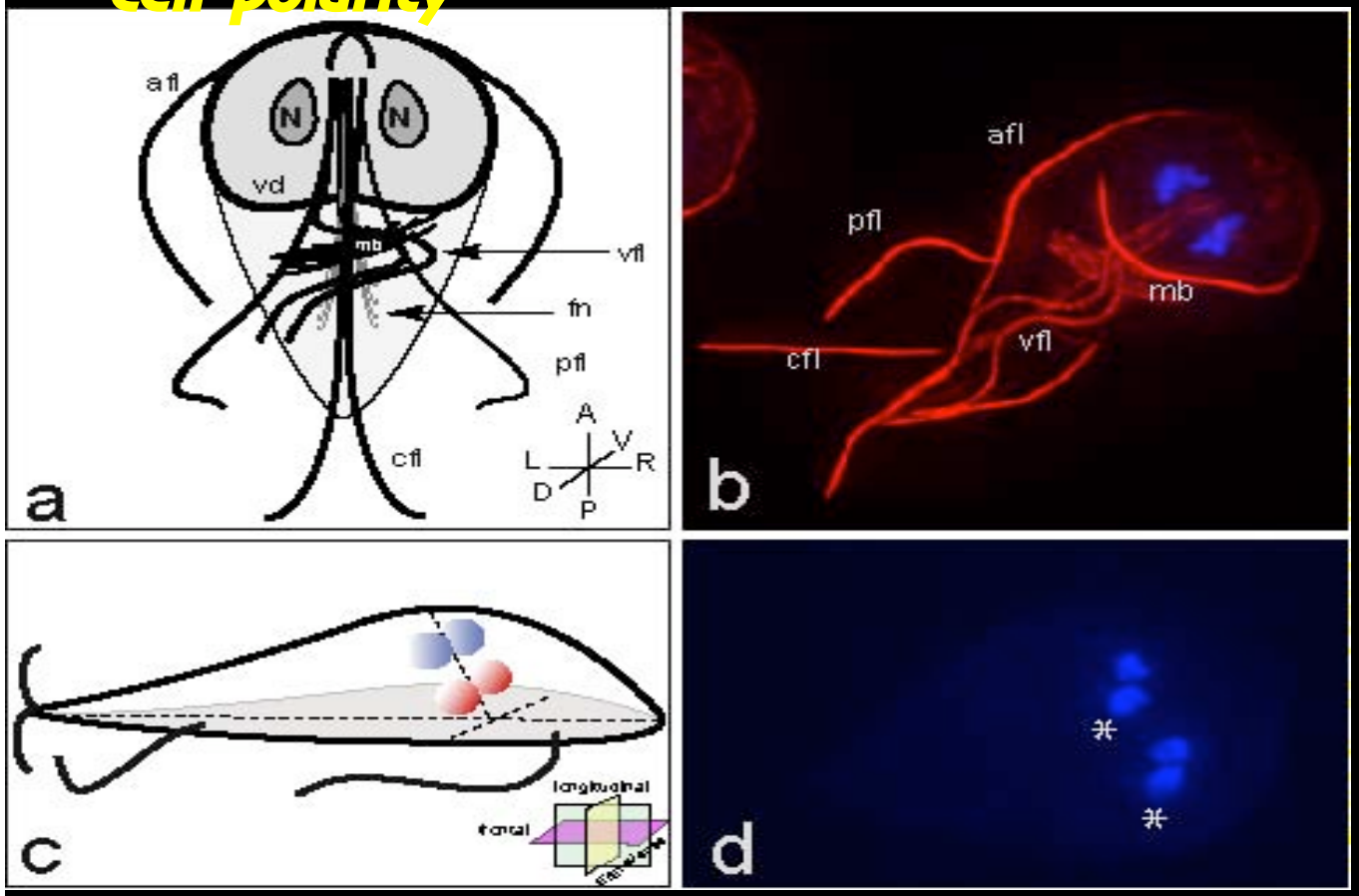
-Transformable with episomes and integratable markers (endogenous tagging)

-Some reverse genetic tools – dominant negatives and morpholinos

The *Giardia* life cycle



Trophozoite microtubule cytoskeletal organization and cell polarity

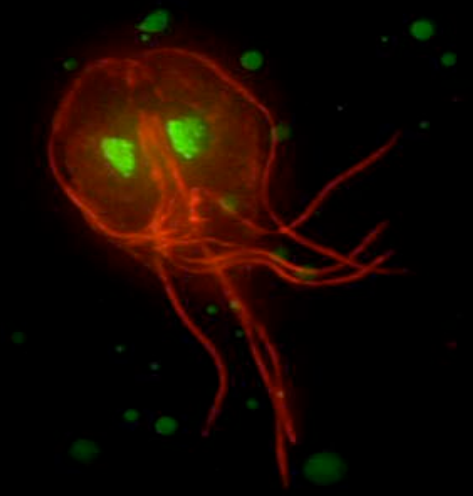


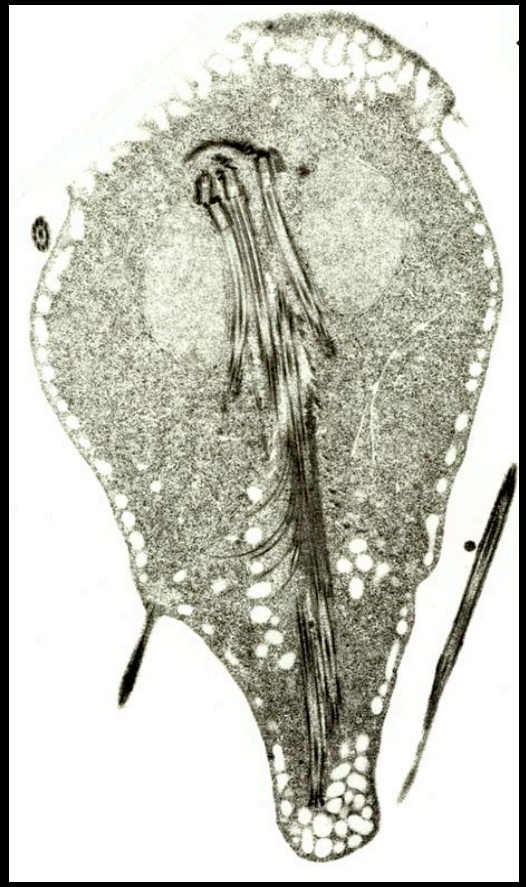
Mt structures
Ventral disc
4 pairs of flagella
& paraflagellar rods
Funis
Median body

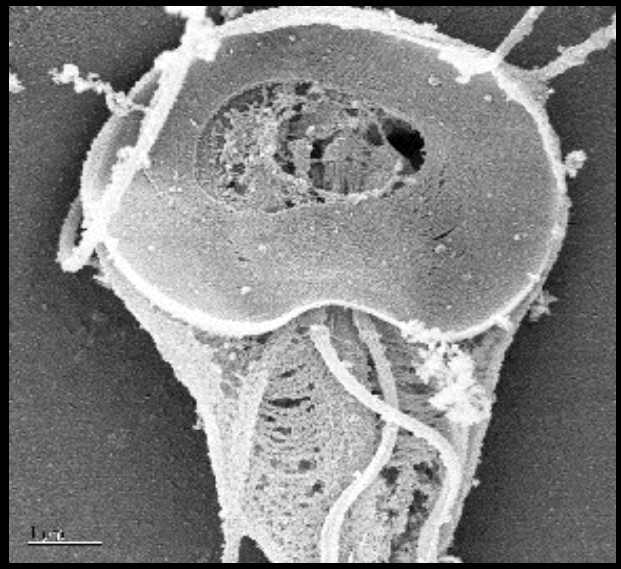
Mt cytoskeleton
is essential for
pathogenesis.

Trophozoite Microtubule organization

Ventral disc

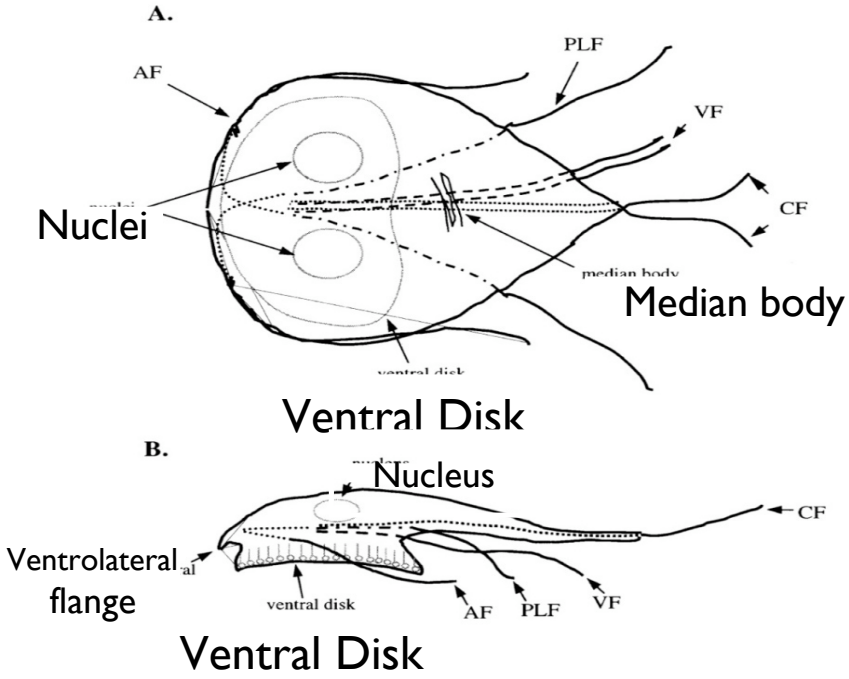






The ventral disc enables attachment to microvilli and other substrates!

Giardia has a divergent cytoskeleton



Elmendorf et al., 2002

Cytoskeleton

Tubulin:

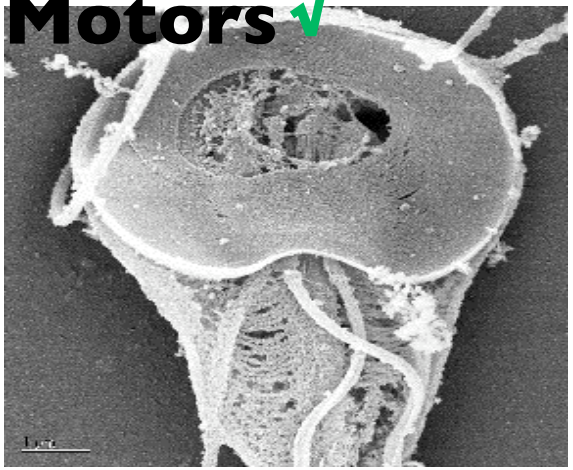
Nucleators √

Severing √

Bundling √

Tip tracking √

Motors √



Actin:

~~**Nucleators**~~

Severing

Bundling

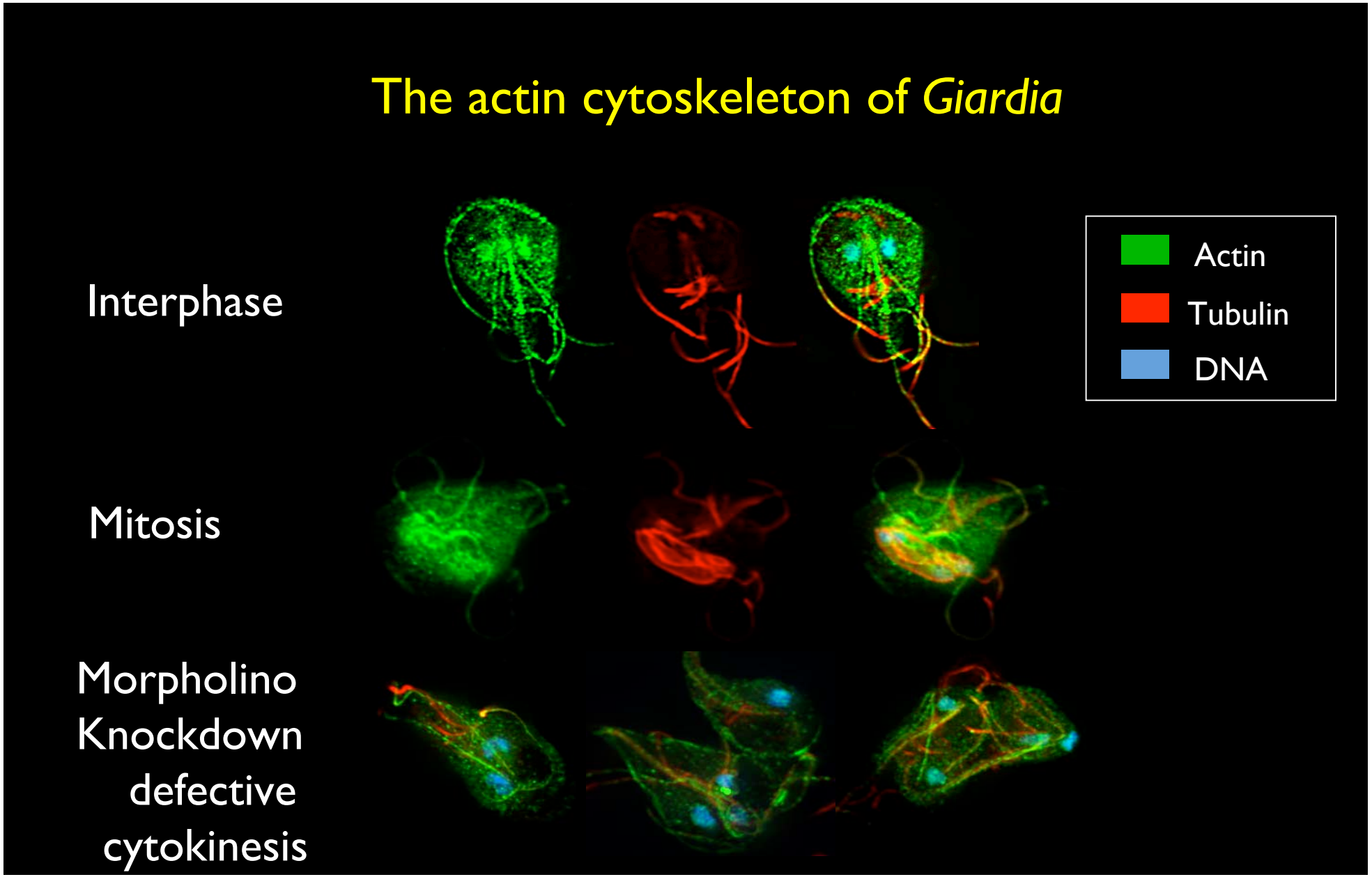
Capping

Motors

Localization and Function?

Alex Paredez

The actin cytoskeleton of *Giardia*



How do you divide if you have two nuclei?

Does each daughter cell inherit one of each parental nuclei?

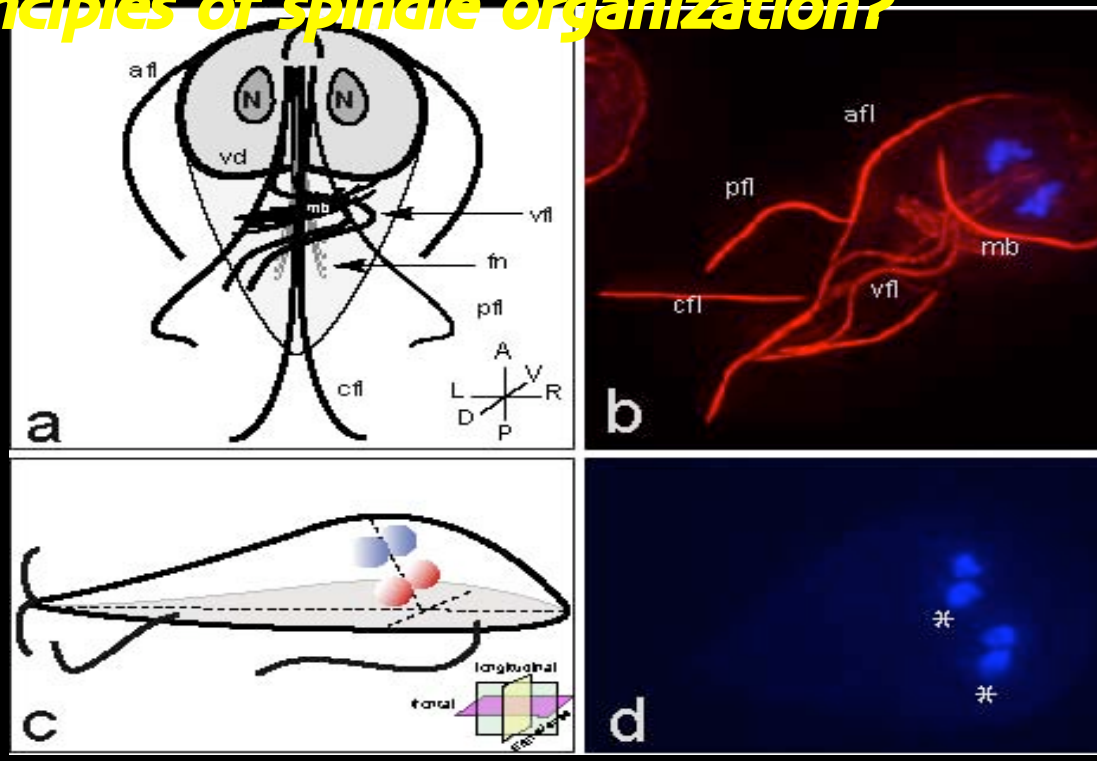
Do you have one or two spindles?

What is the plane of chromosome segregation?

How conserved are the principles of spindle organization?

Are spindle kinesins conserved in function?

(Sagolla etal, JCS 2006)

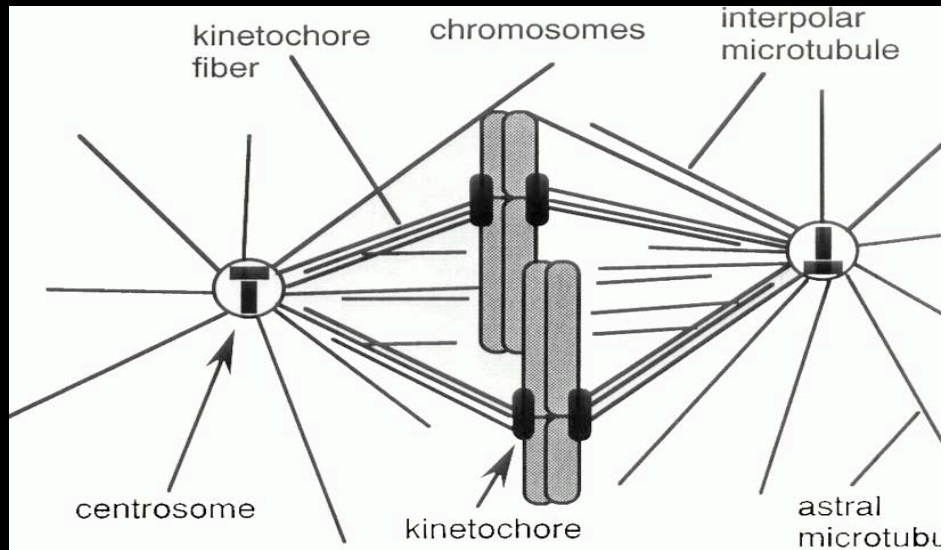


Need:

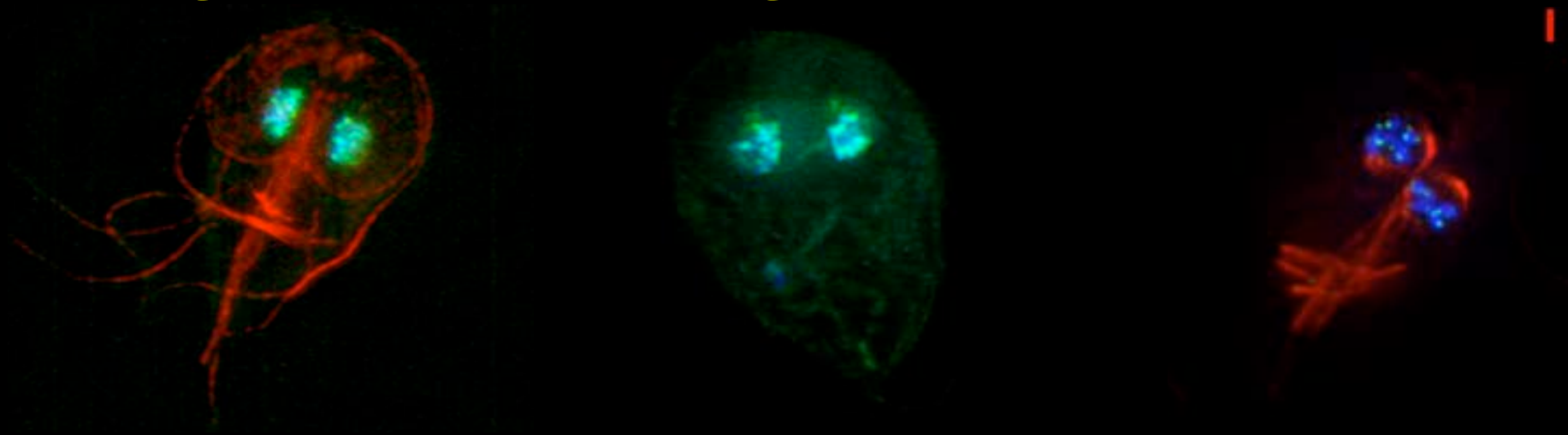
- Equal partitioning of sister chromatids by establishing bipolarity
- Force generation

A canonical metazoan spindle

- mitosis is "open"
- bipolar, anti-parallel MT array with overlap zone
- MT attach to chromosomes at kinetochore
- equivalent centrosomes nucleate

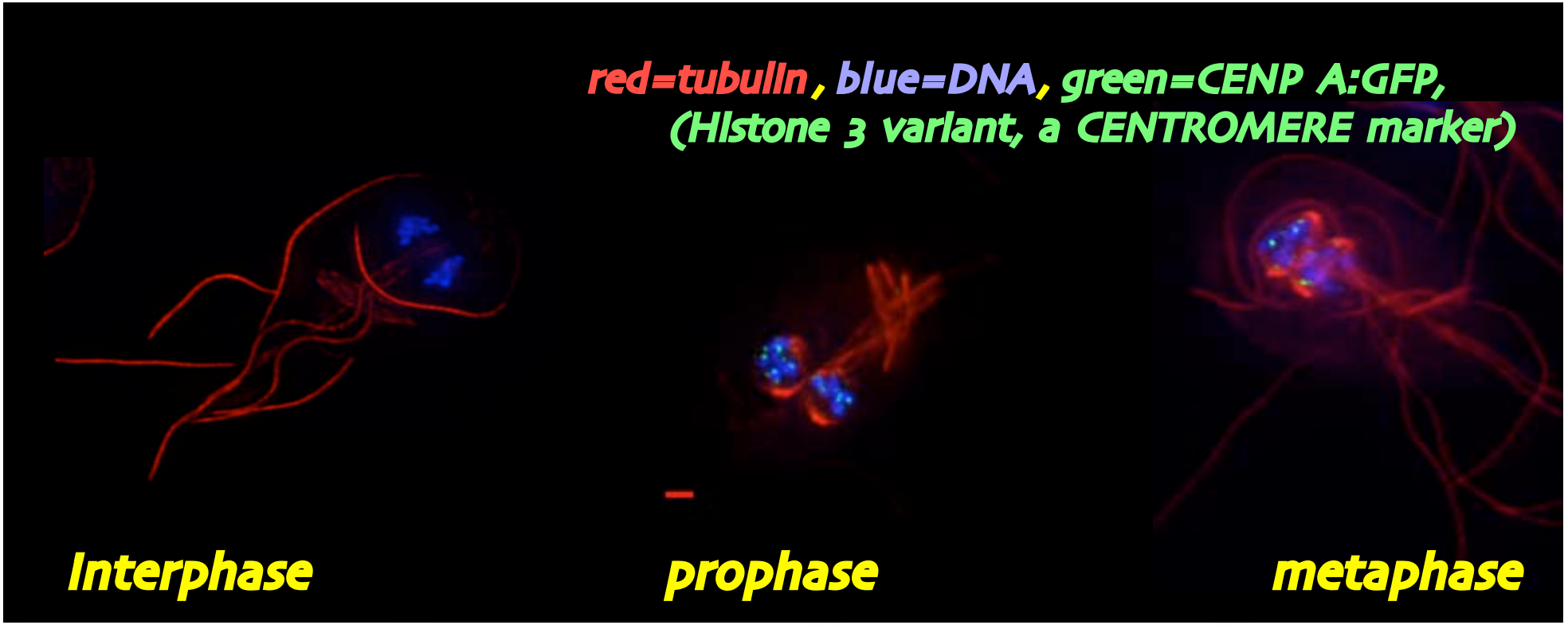


A variant H3 histone defines the **centromere** *In eukaryotes (CENP-A)*

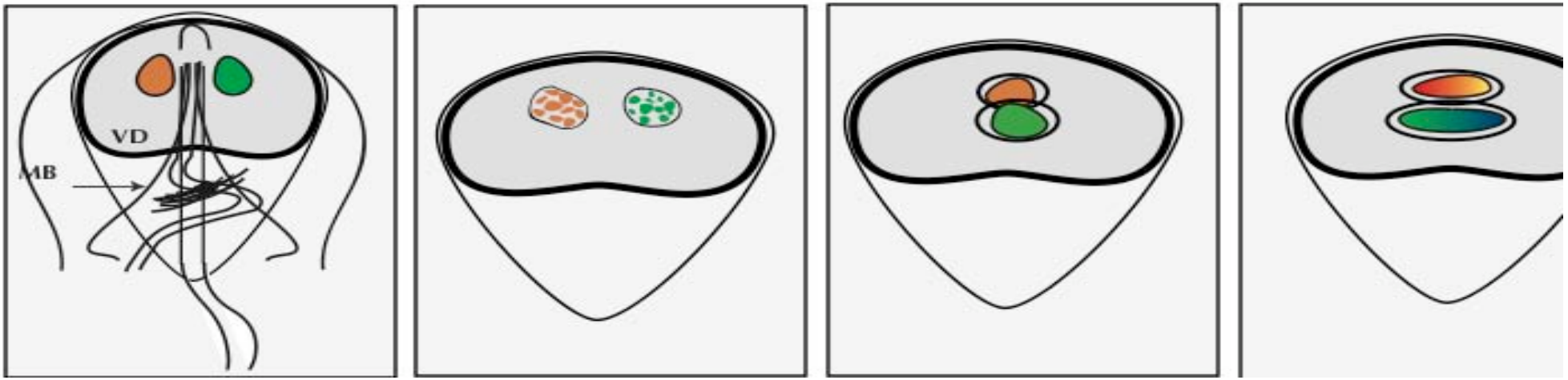


Giardia has two histone H3 variants; one is CENP-A. It is a centromere marker. (Dawson et al 2007)

red=tubulIn , blue=DNA, green=CENP A:GFP,
(HIstone 3 varIant, a CENTROMERE marker)



Interphase prophase metaphase



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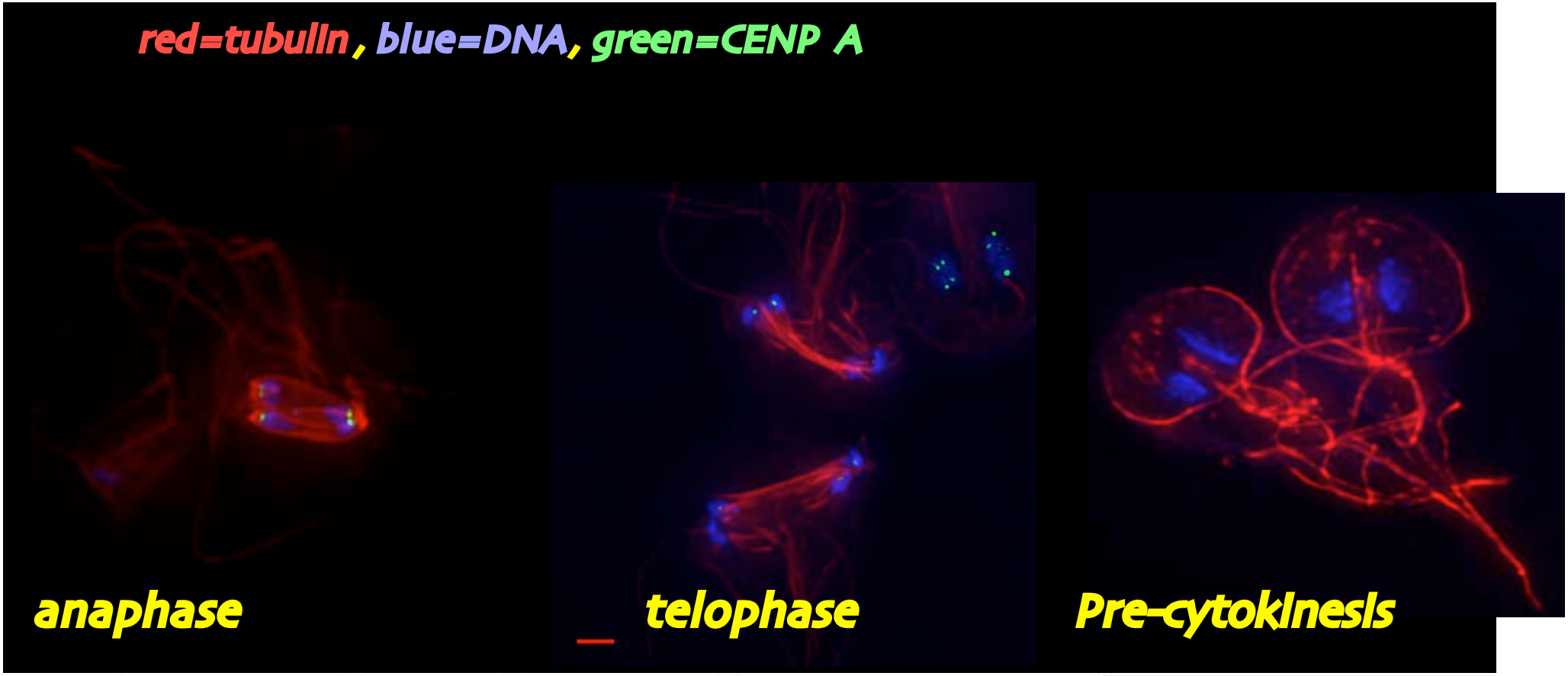
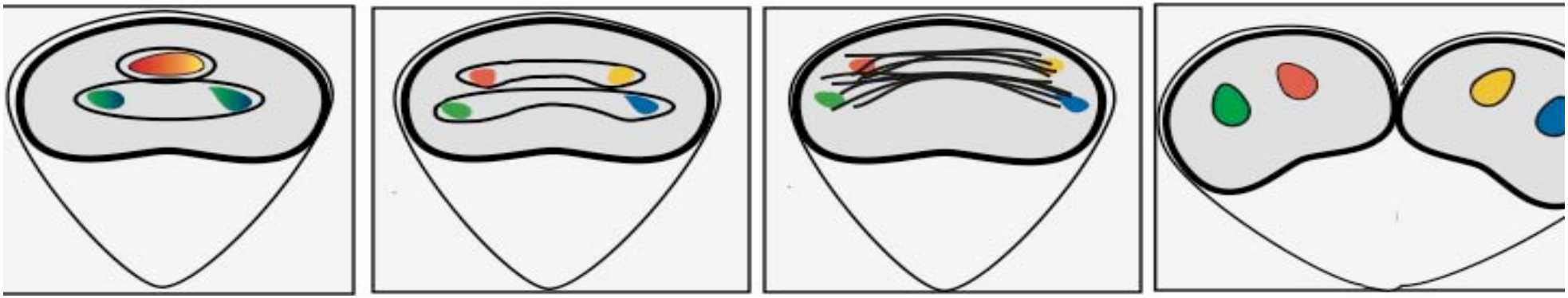
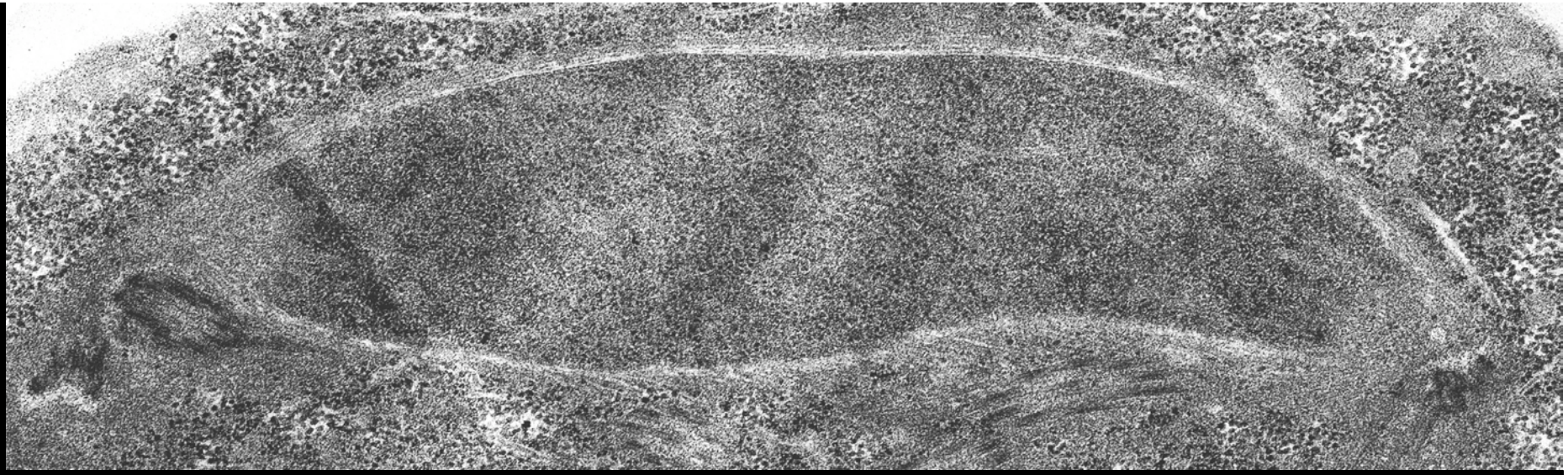


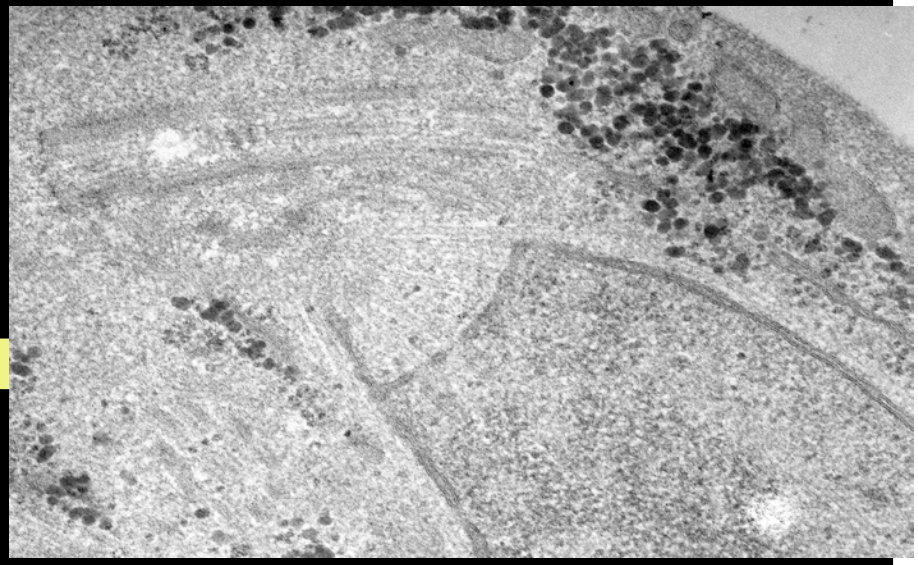




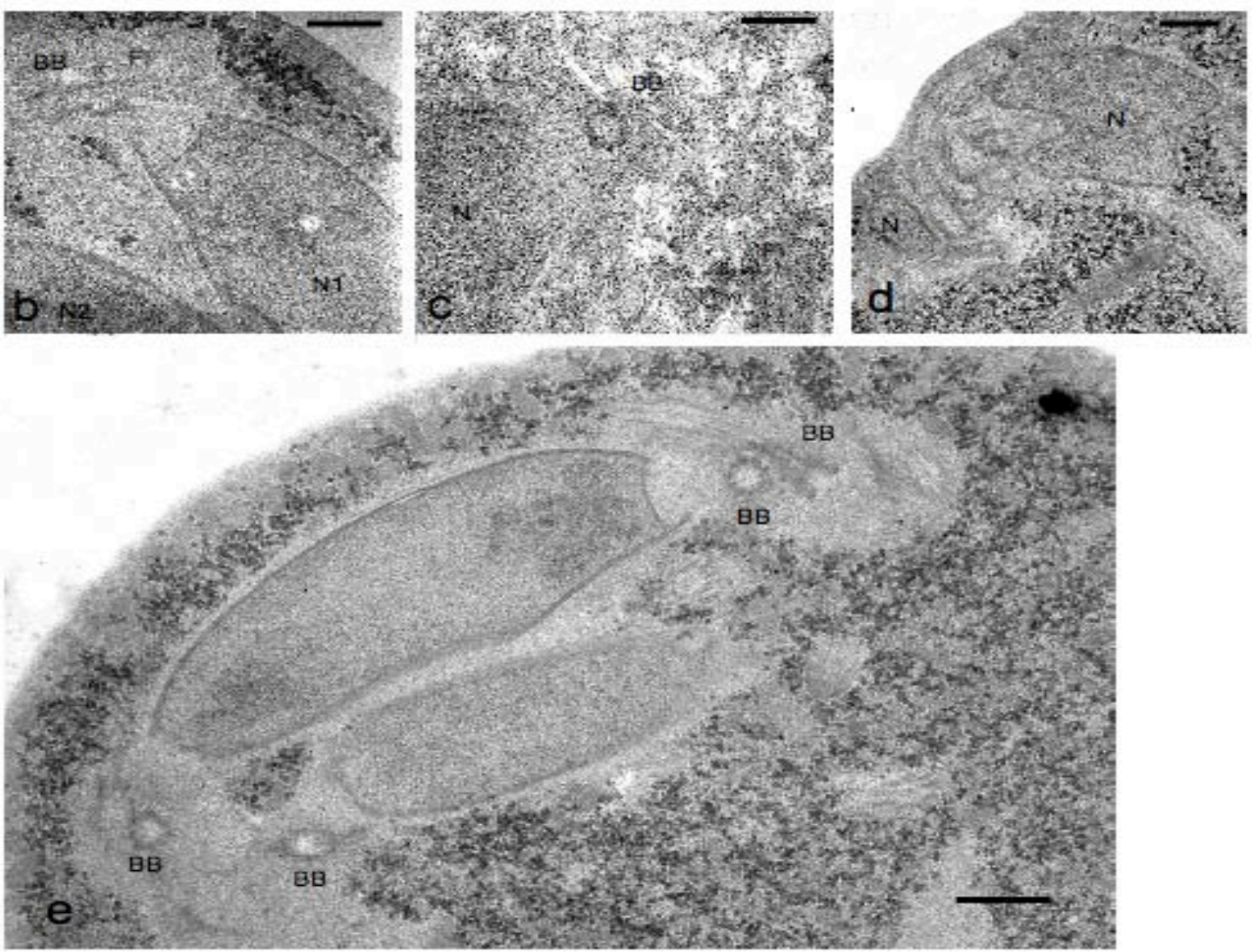
Mitosis is semi-open

- Extra-nuclear "basket" of MTs on surface of NE

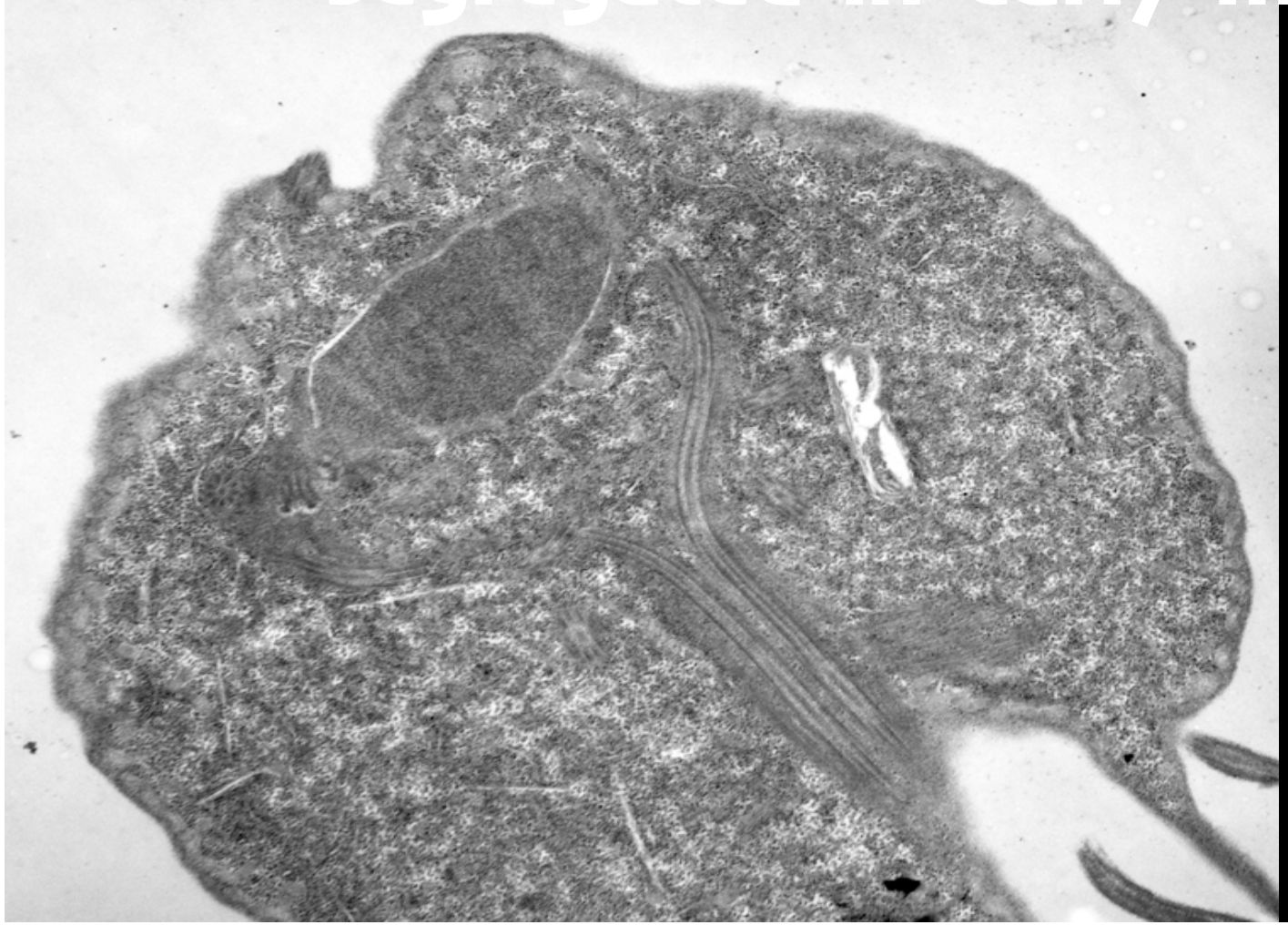
- MTs penetrate through open end of NE



Two autonomous spindles, each pole has two basal bodies and axonemes



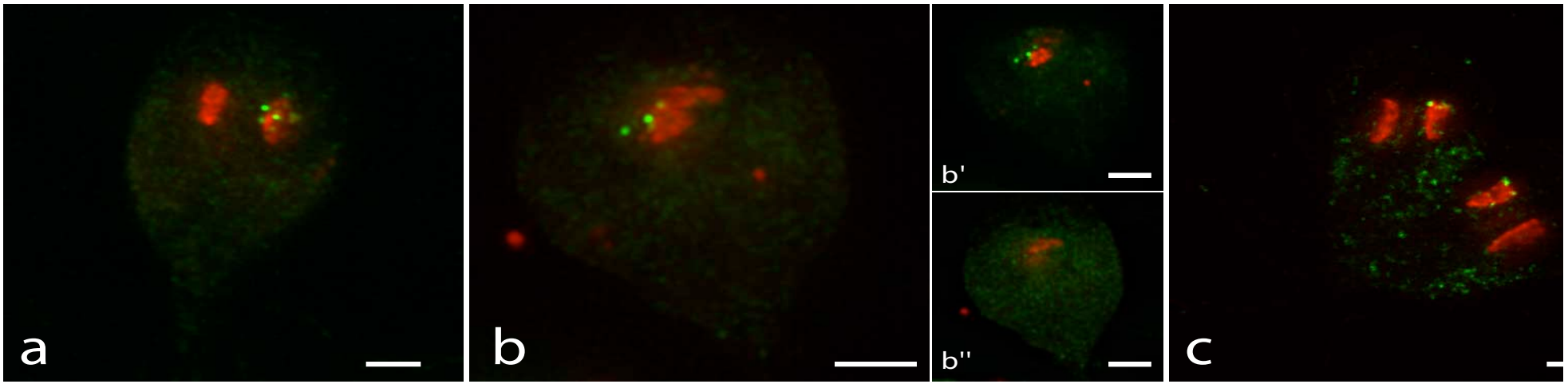
The basal bodies of internal flagella are segregated in early mitosis



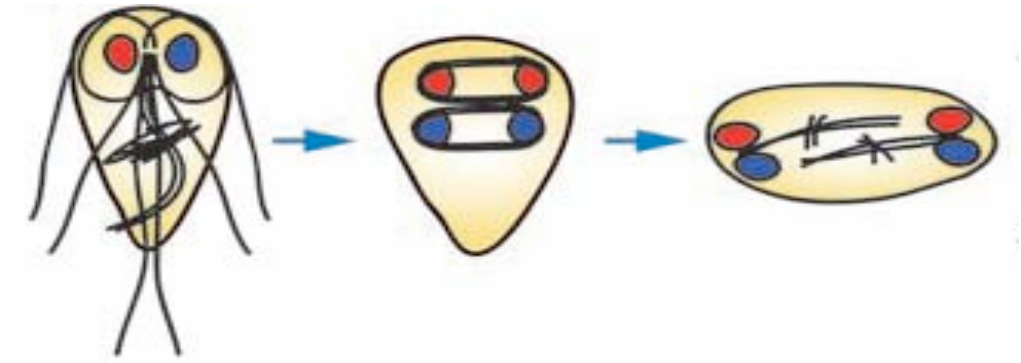
- different flagella associated with different spindle poles
- asymmetry of spindle poles
- cell polarity

The nuclei are autonomous

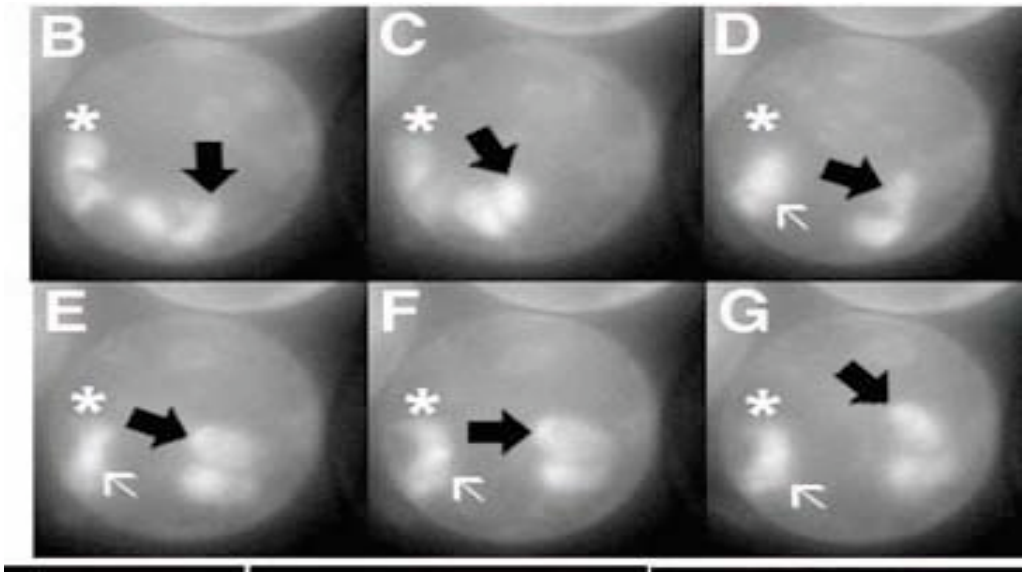
FISH probe against GFPk13 episome labels only one of two nuclei.
DAPI red, FISH probe yellow



Pre-cytokinesis, 4 nuclei



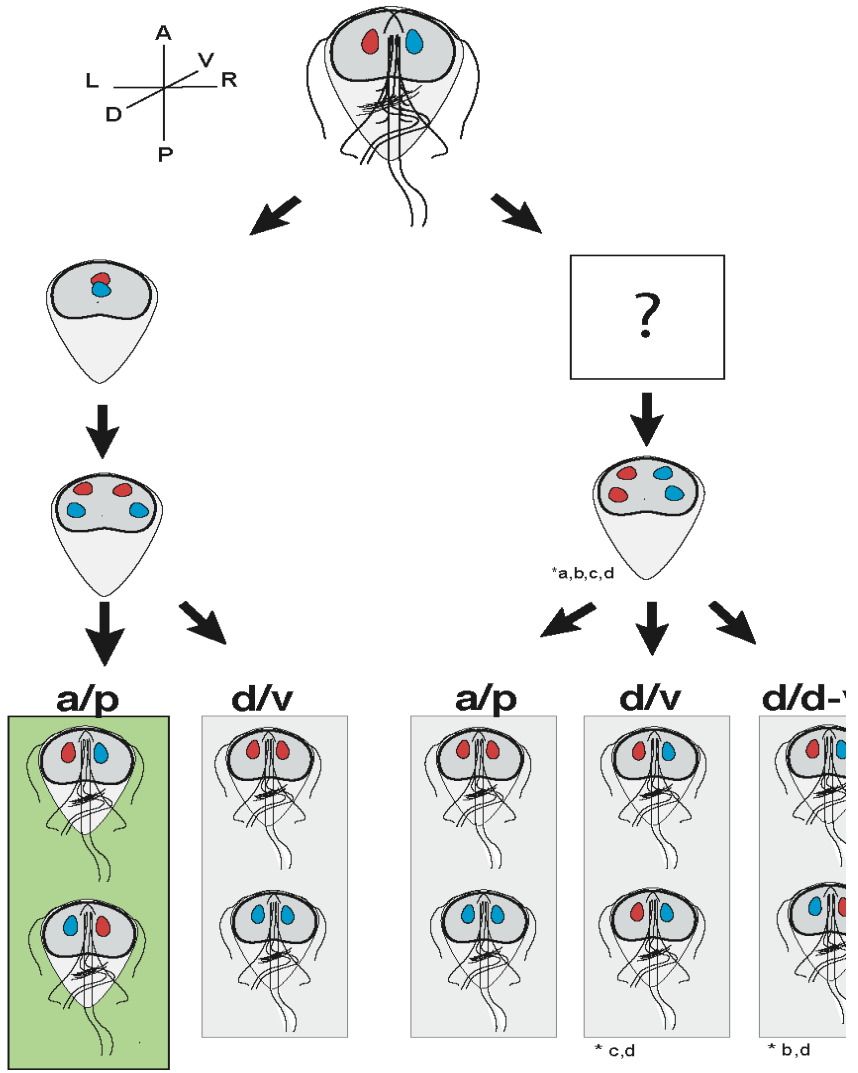
Encystation



Poxleitner et al, 2008

How two nuclei divide:

1. Nuclei migrate to cell center, stacked on top of each other
2. Two spindles segregate chromosomes on left-right axis.
3. Cytokinesis is anterior-posterior.
4. Each daughter cell gets one of each daughter nuclei.

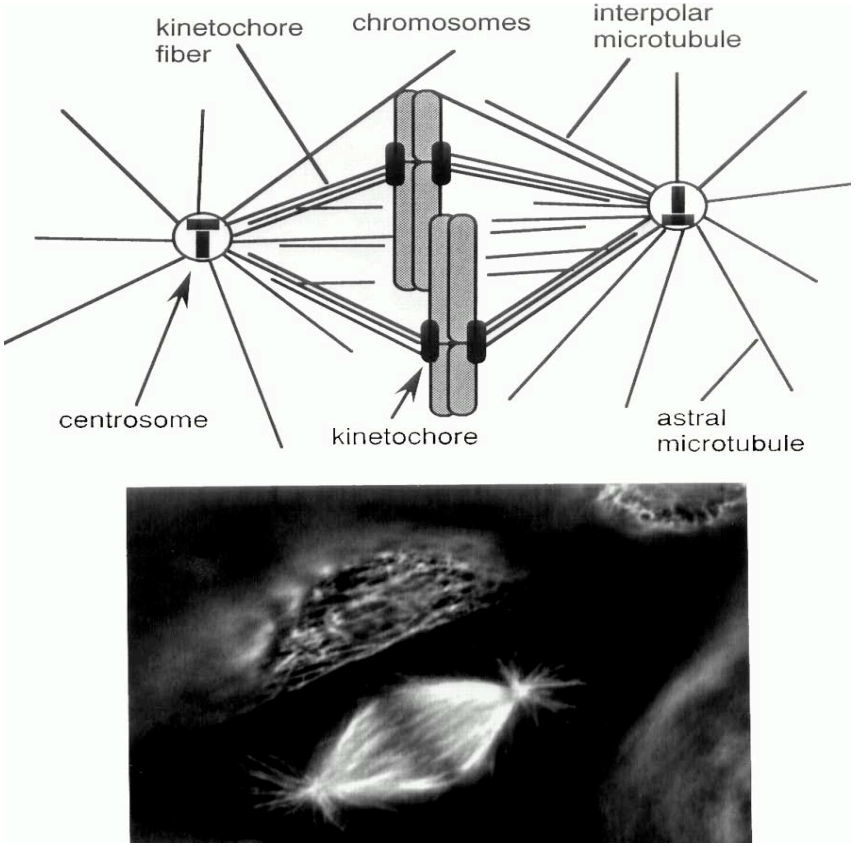


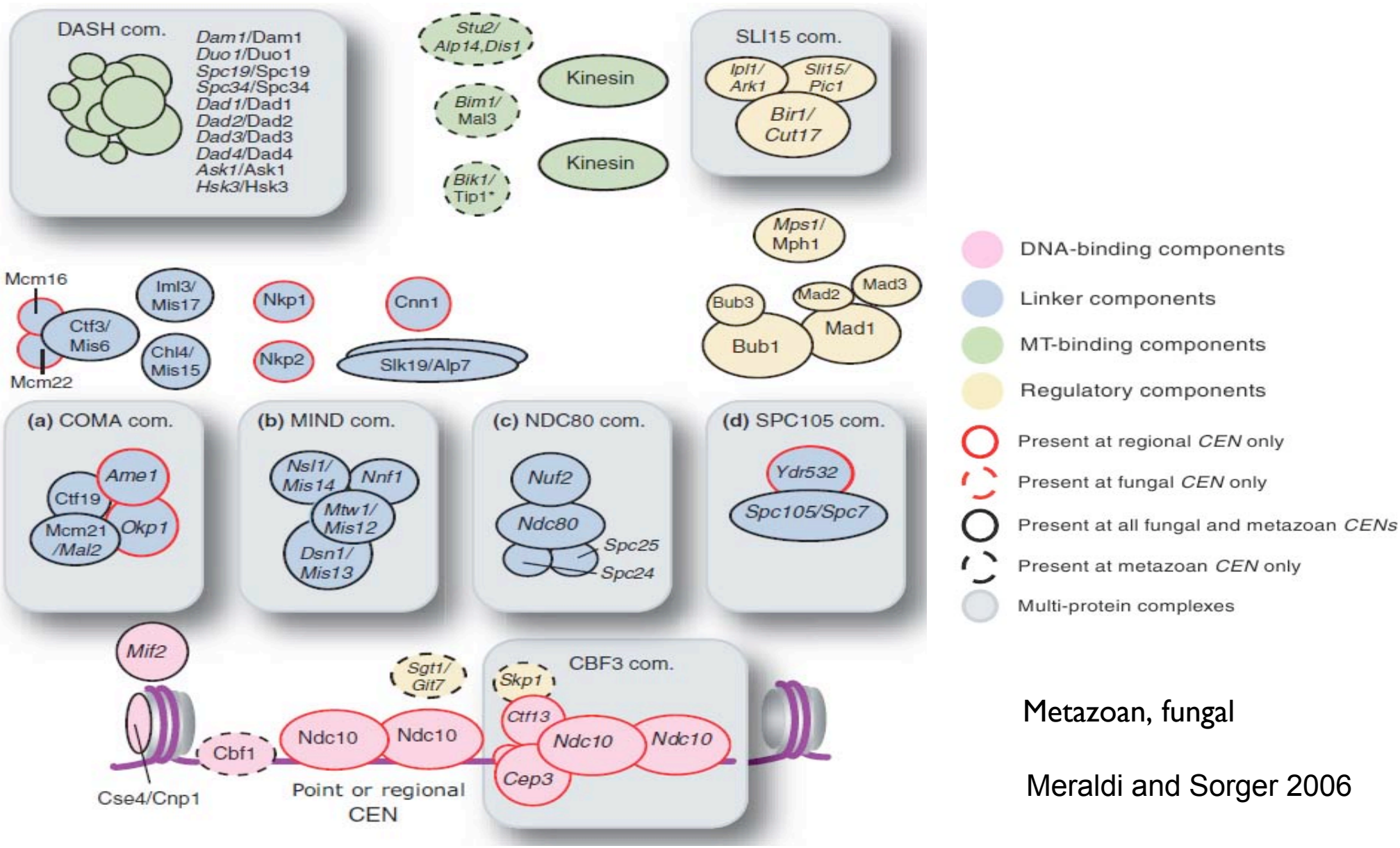
(Michael Cipriano and Scott Dawson, UC Davis)

Types of proteins - kinetochores

DNA -binding
Linker layer
MT-binding
Regulatory

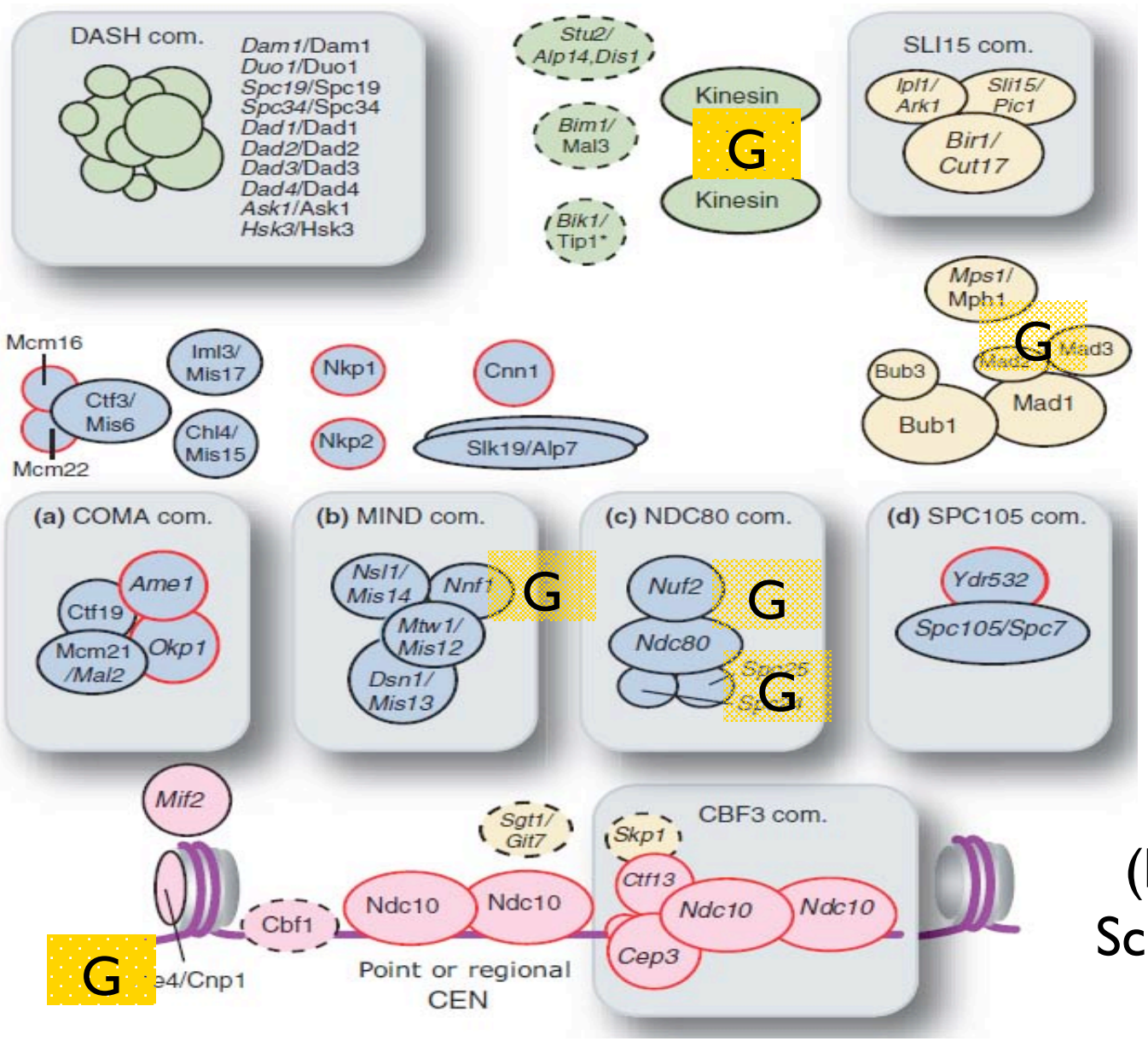
transient and persistent





Metazoan, fungal

Meraldi and Sorger 2006



Giardia only has a few known kinetochore proteins

DNA-binding components

Linker components

MT-binding components

Regulatory components

Present at regional *CEN* only

Present at fungal *CEN* only

Present at all fungal and metazoan

Present at metazoan *CEN* only

Multi-protein complexes

(Michael Cipriano and Scott Dawson, UCDavis)

Why so few proteins found

1. Genomic minimalism:

Other complexes in Giardia shown to have less members compared to metazoans and fungii.

2. Proteins too distant to find:

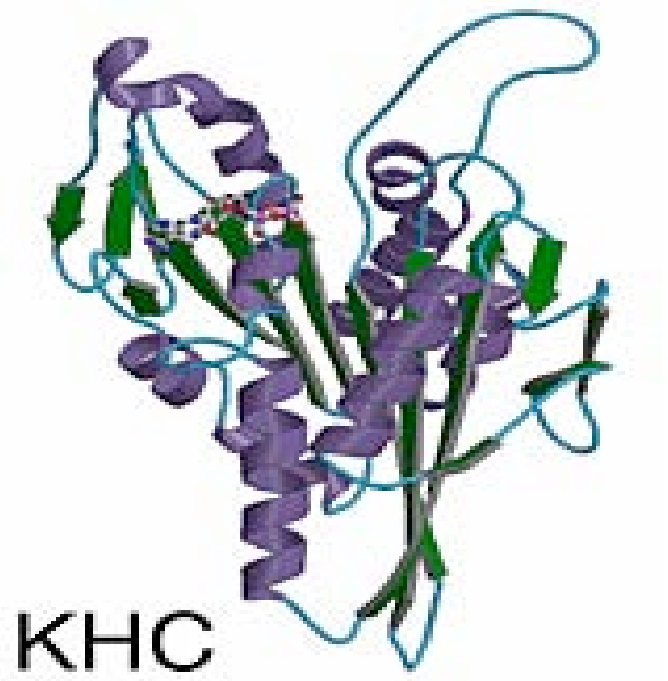
Kinetochore proteins have a large divergence, even in closely related species.

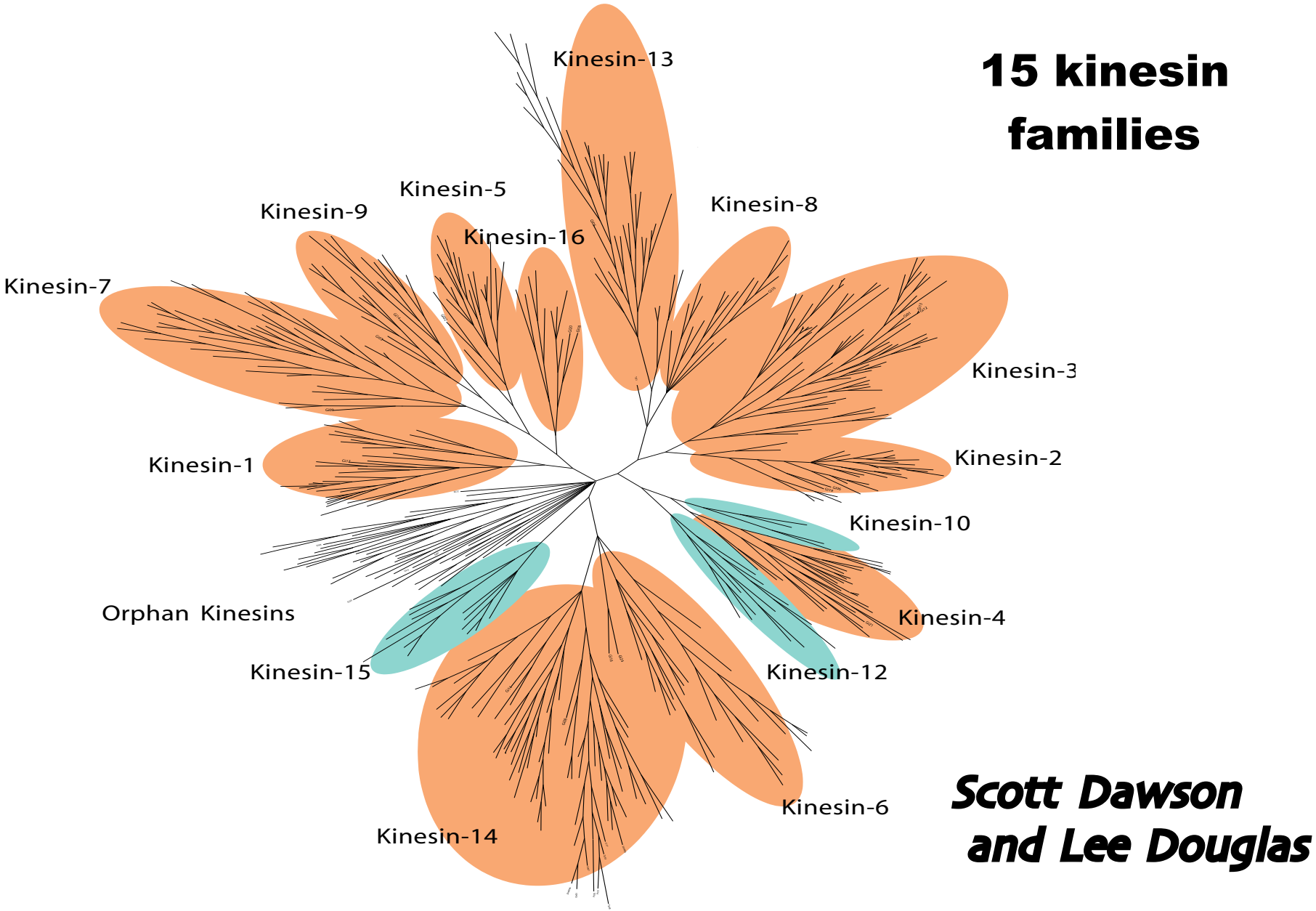
3. It never had them:

Many kinetochore proteins might have evolved after Giardia split from other eukaryotes.

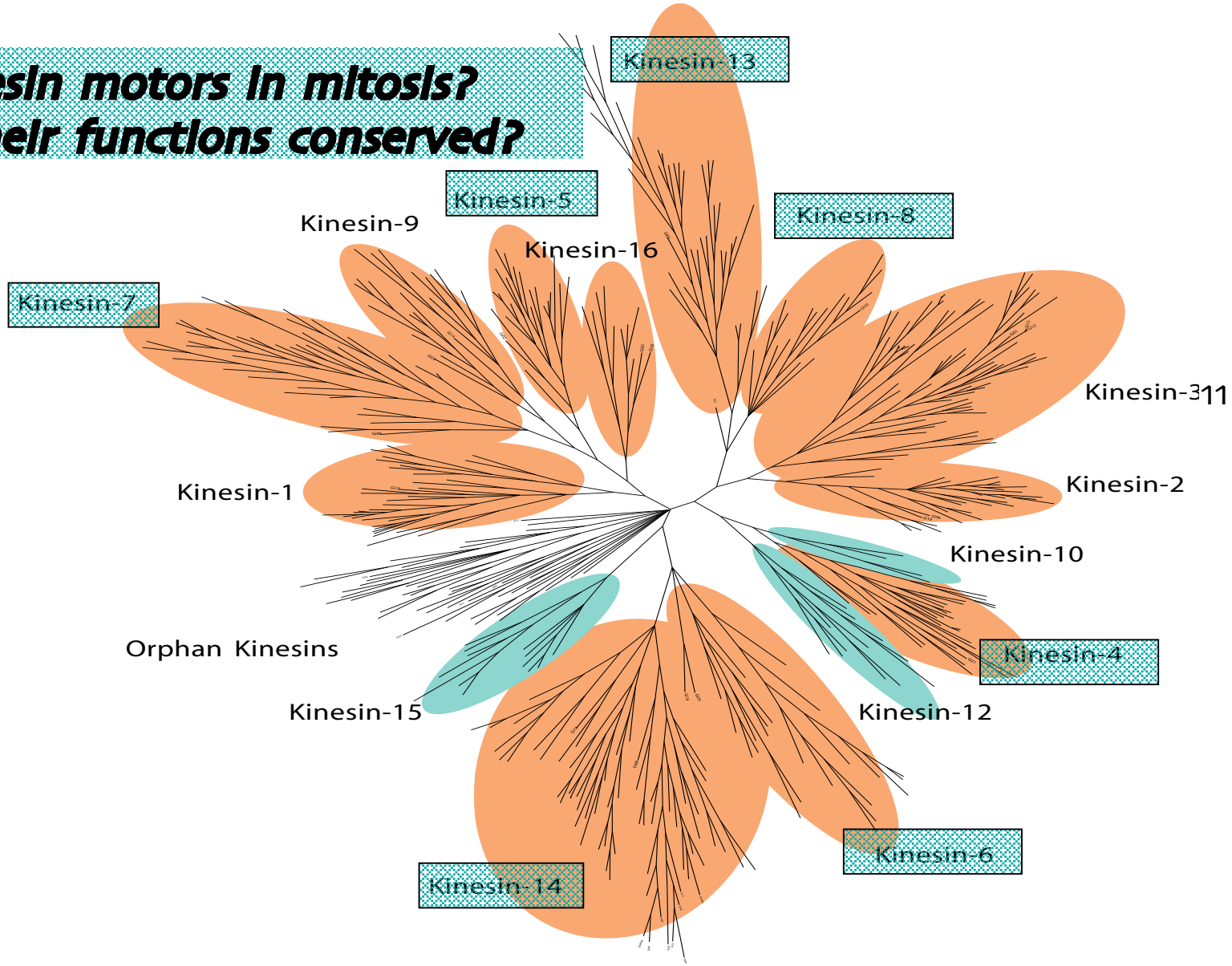
Kinesins (microtubule motors)

motor, neck, tail
motor domain used for phylogenetic analysis
-682 aligned motor domains
-emphasized kinesins in unicellular eukaryotic kingdoms
15 major families
multigene families: 6 to 40/organism

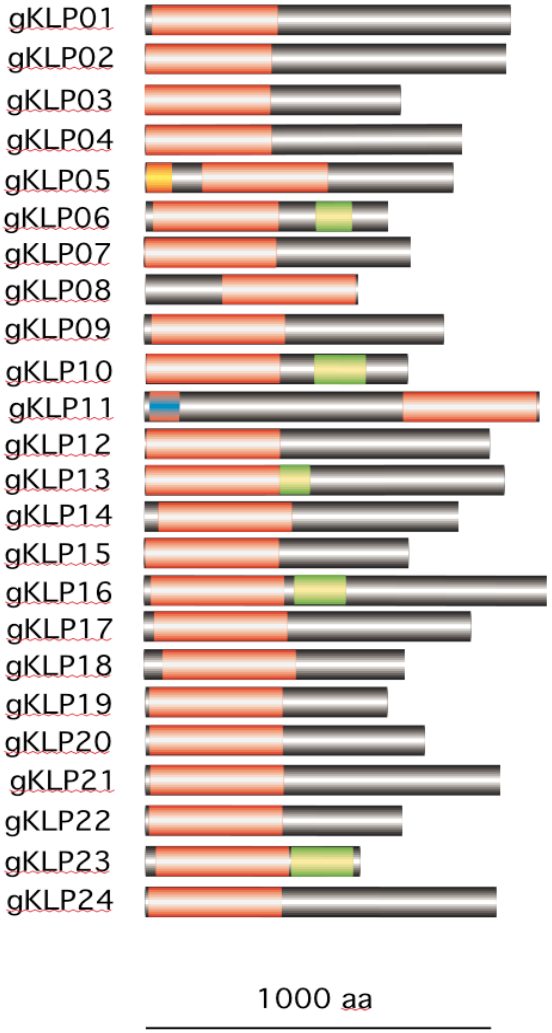



15 kinesin families
Kinesin-13
Kinesin-5
Kinesin-9
Kinesin-8
Kinesin-16
Kinesin-7
Kinesin-3
Kinesin-1
Kinesin-2
Kinesin-10
Orphan Kinesins
Kinesin-4
Kinesin-15
Kinesin-12
Kinesin-6
Kinesin-14
Scott Dawson and Lee Douglas

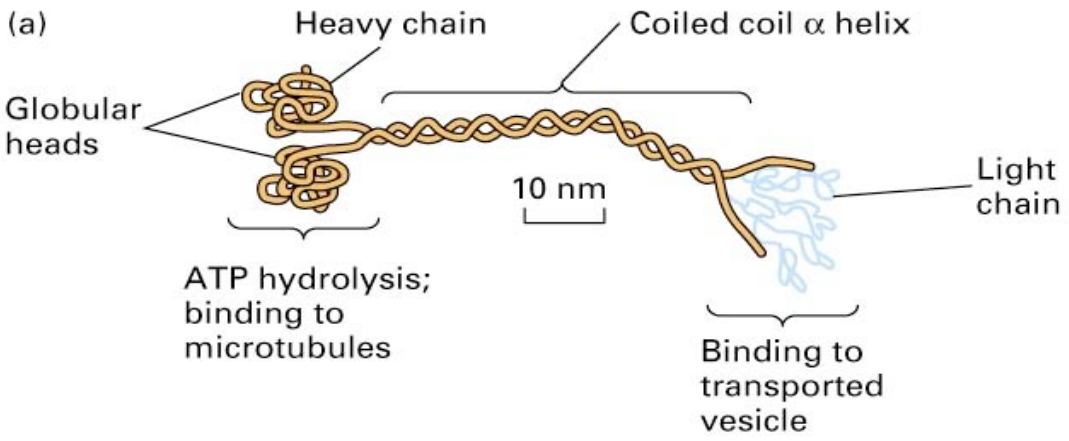
Kinesin motors in mitosis?
Are their functions conserved?



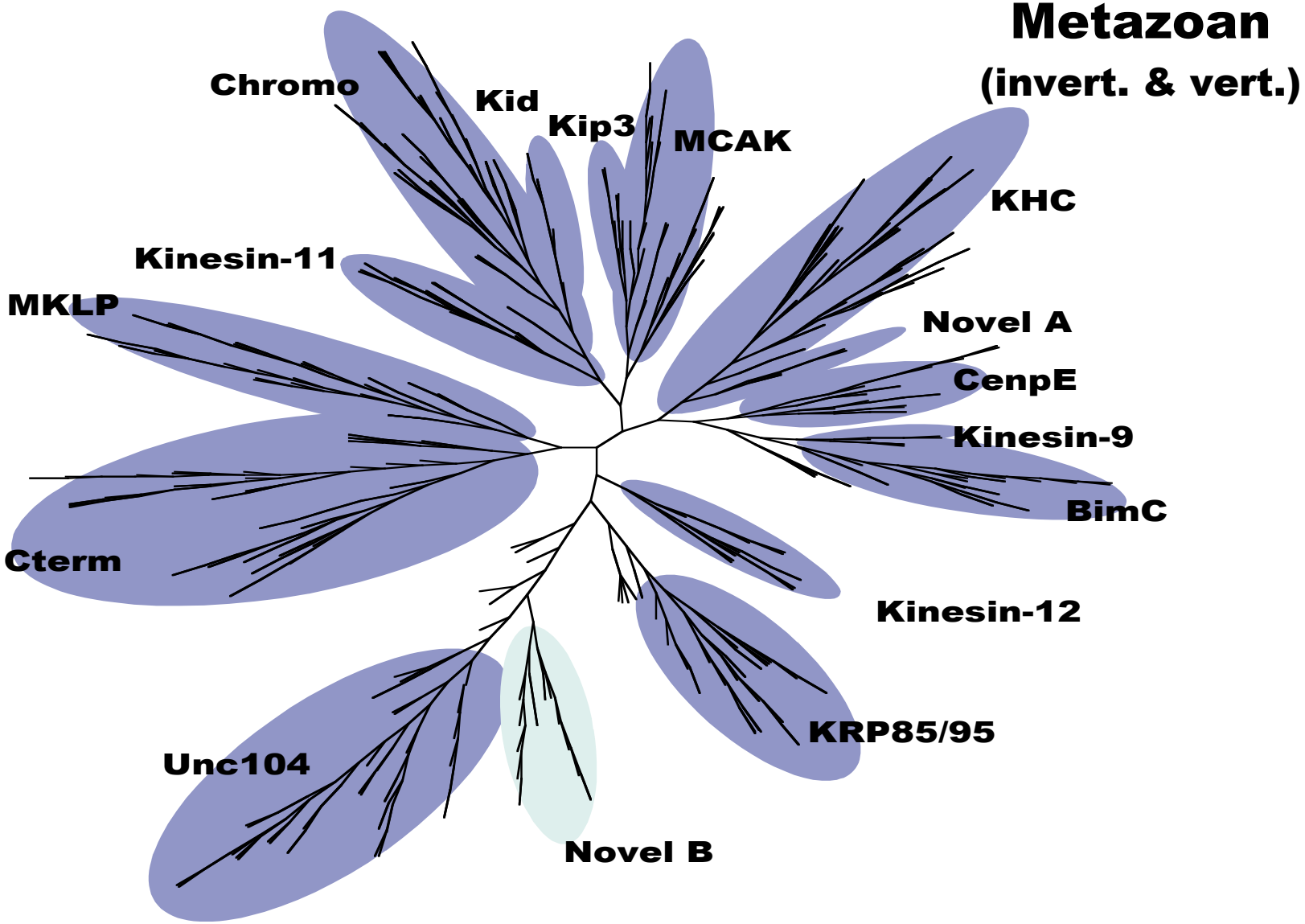
Giardia has 24 kinesins

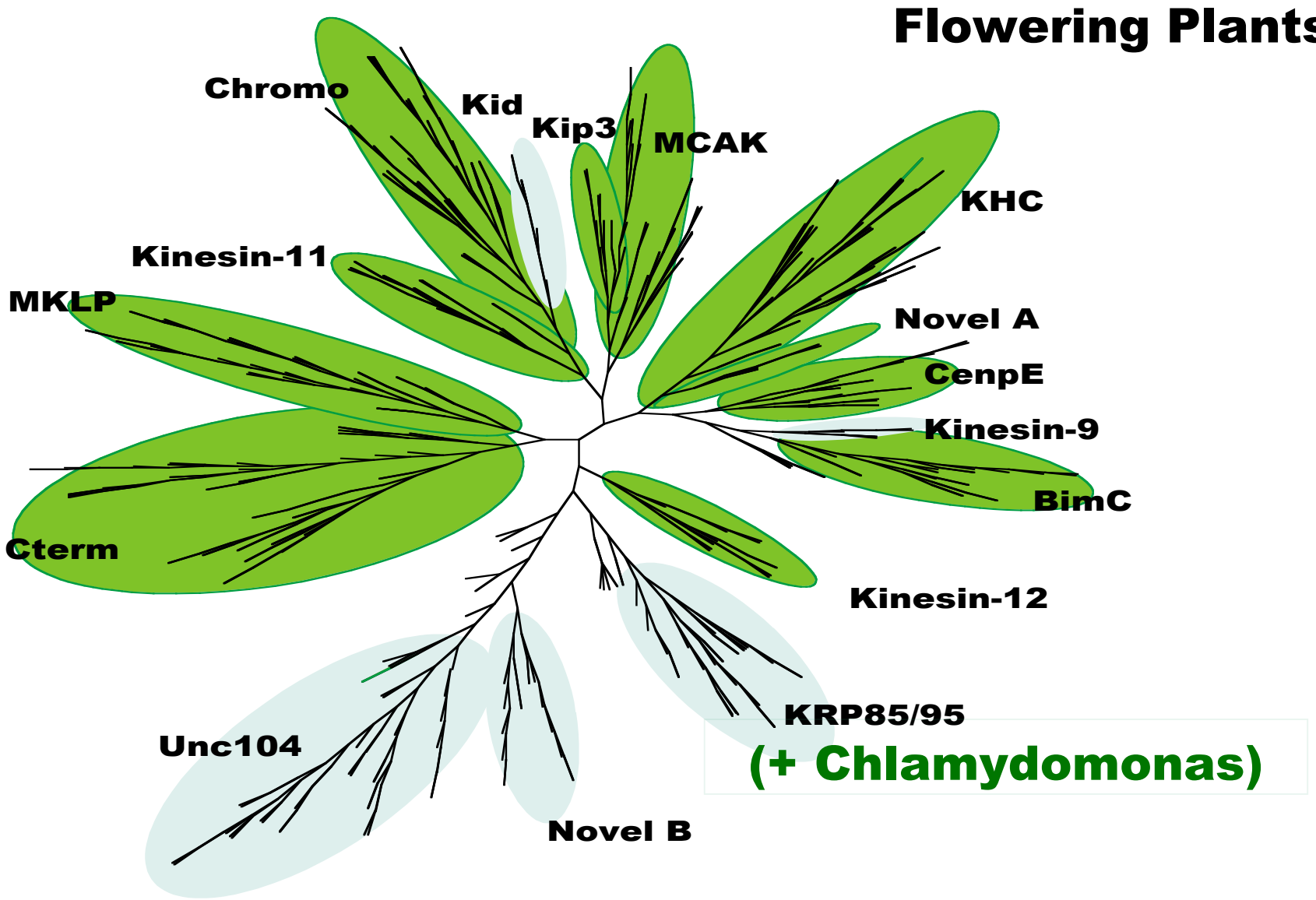


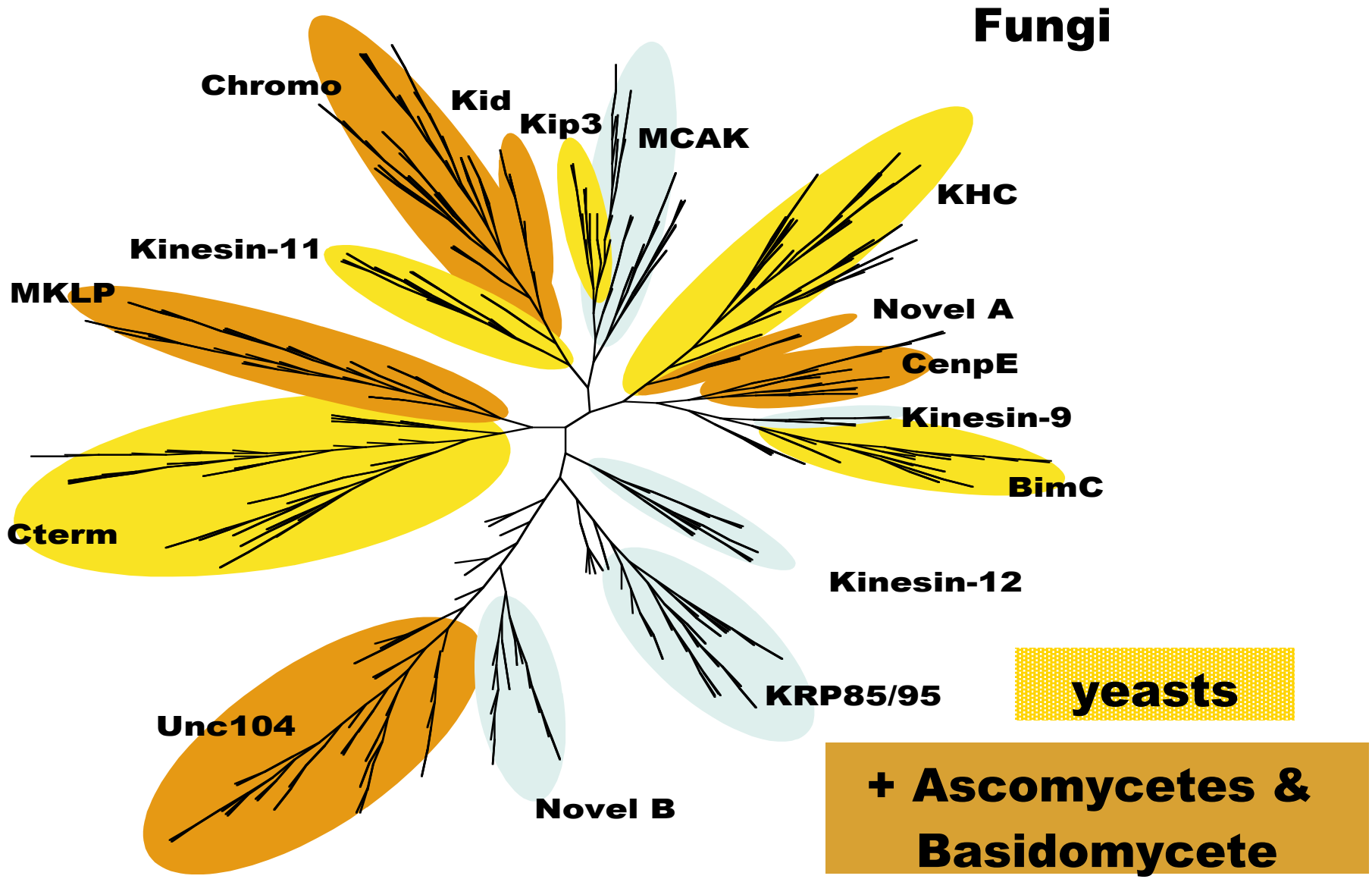
Dominant negative rigor mutant; can not hydrolyze ATP and can not release from MT

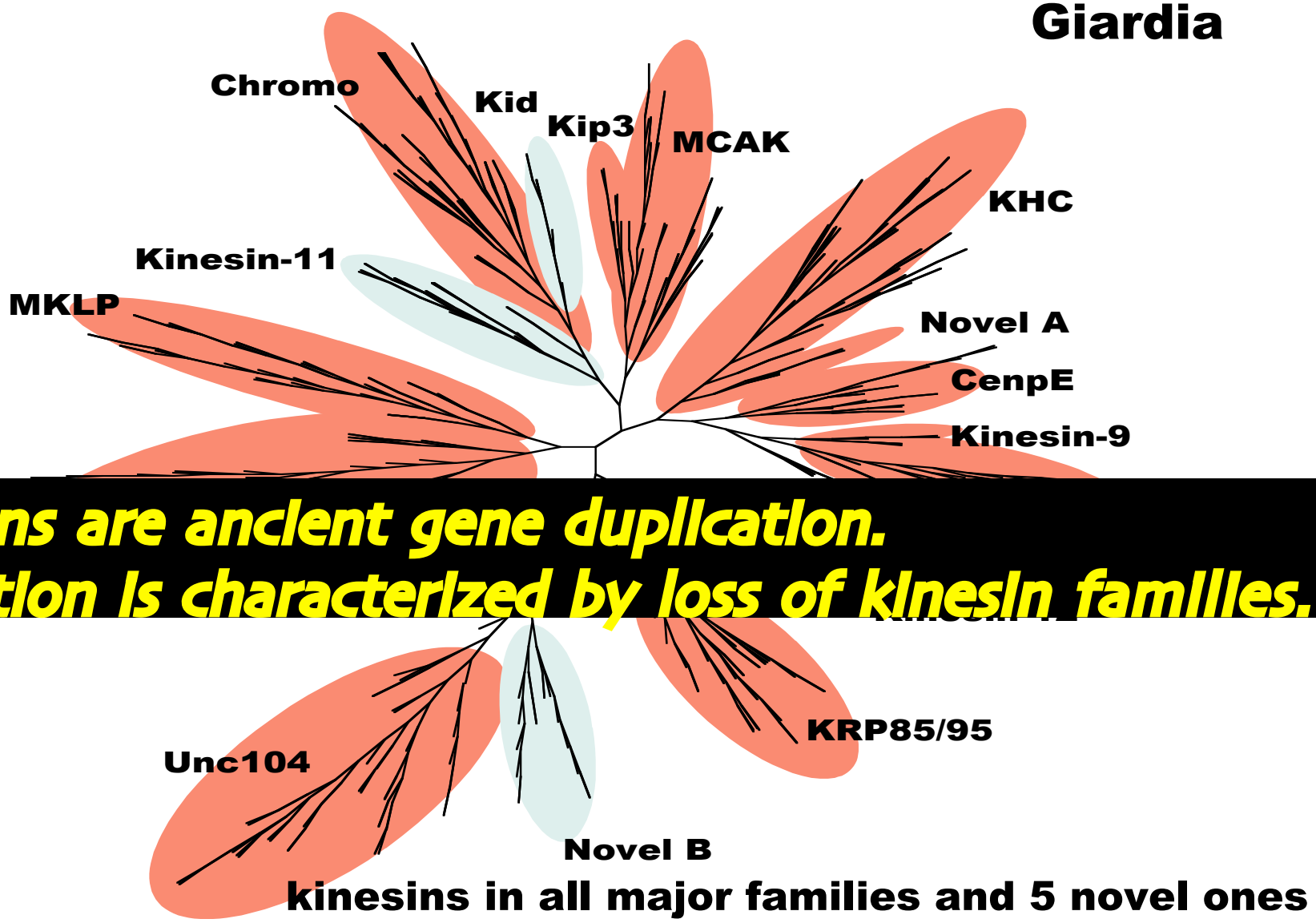


Red = motor domain


Metazoan
(invert. & vert.)
Chromo
Kid
Kip3
MCAK
KHC
Kinesin-11
MKLP
Novel A
CenpE
Kinesin-9
BimC
Cterm
Kinesin-12
KRP85/95
Unc104
Novel B


Flowering Plants
Chromo
Kid
Kip3
MCAK
KHC
Kinesin-11
MKLP
Novel A
CenpE
Kinesin-9
BimC
Cterm
Kinesin-12
KRP85/95
Unc104
(+ Chlamydomonas)
Novel B


Fungi
Chromo
Kid
Kip3
MCAK
KHC
Kinesin-11
MKLP
Novel A
CenpE
Kinesin-9
BimC
Cterm
Kinesin-12
KRP85/95
Unc104
Novel B
yeasts
+ Ascomycetes &
Basidomycete

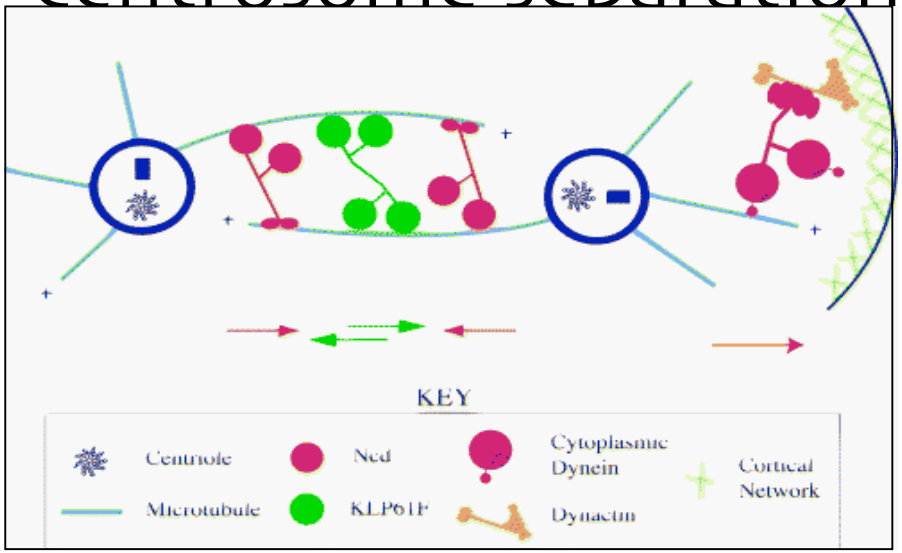


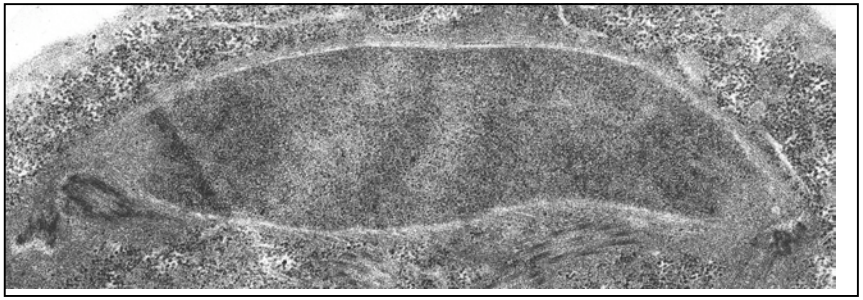
Kinesins are ancient gene duplication.
Evolution is characterized by loss of kinesin families.

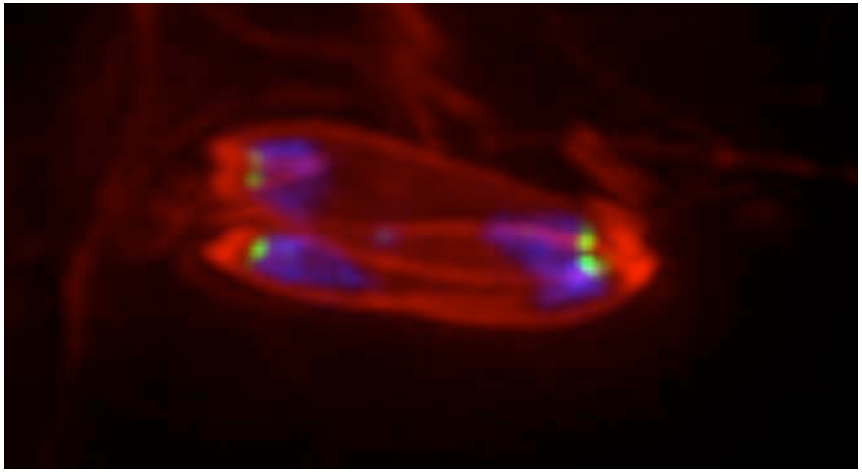
kinesins in all major families and 5 novel ones

Klnesln-5 (BlmC,Eg5)

- tetramer
- MT sliding
- spindle bipolarity
- centrosome separation

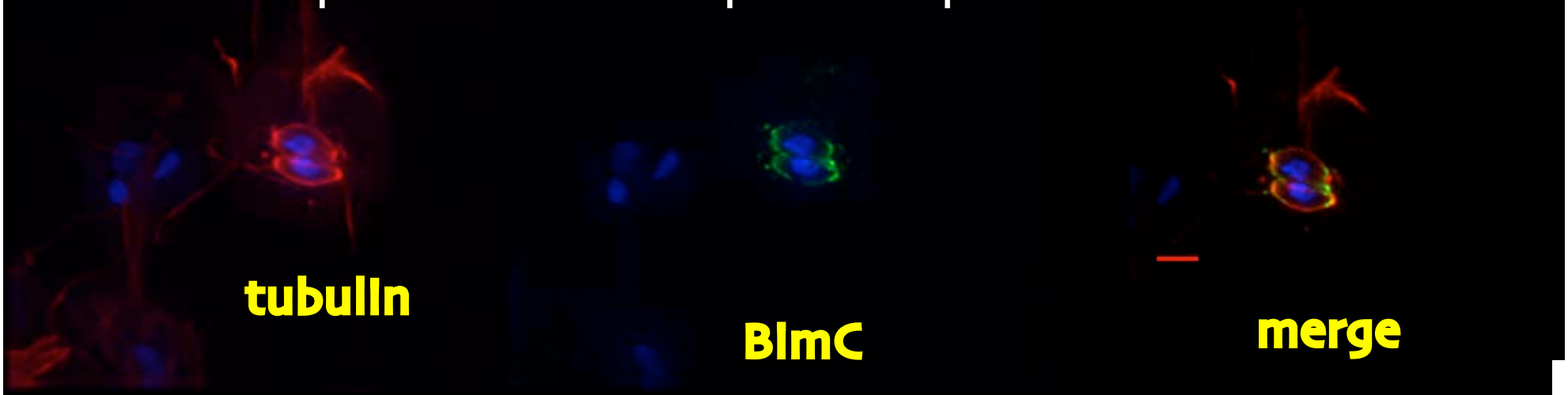






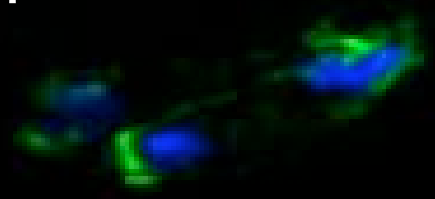
red=tubulin, green=cenP-A
blue=DNA

Kinesin-5 (BimC)::GFP localizes to the spindle poles of metaphase spindles



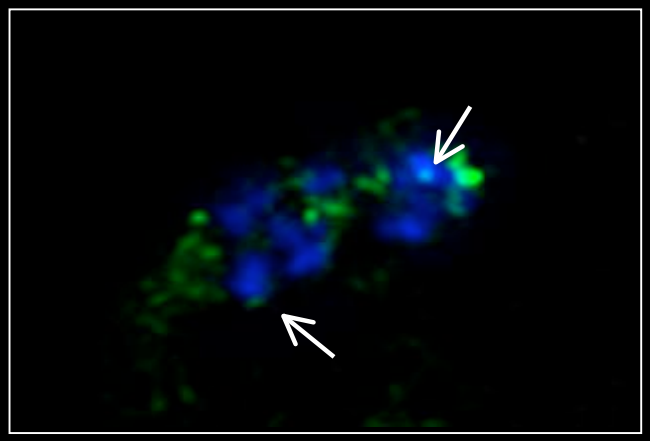
and spreads out along the spindle in anaphase

Conserved function

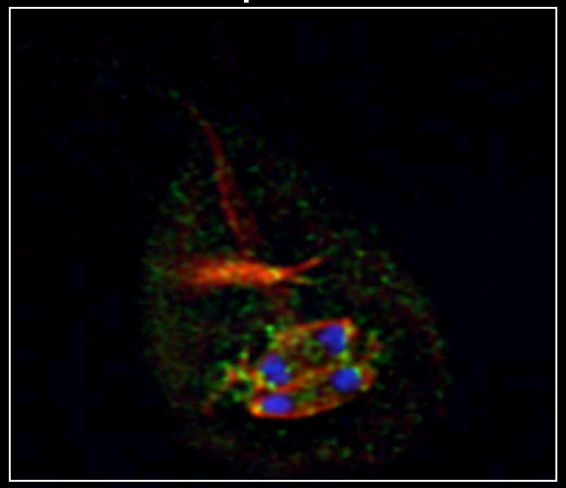


Kinesin-13 (MT destabilizer) localizes to kinetochores and the mitotic spindle

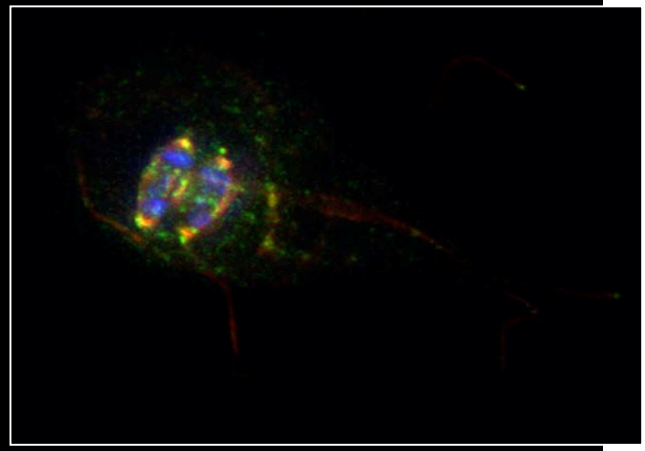
Prophase



Anaphase A



Anaphase A

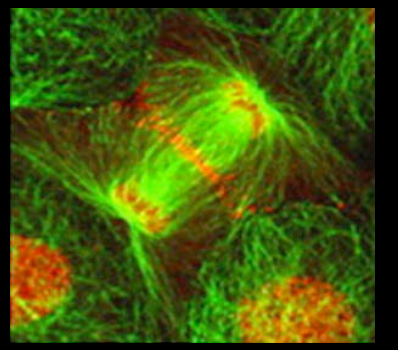


dominant negative

Lagging chromosomes in dominant negative

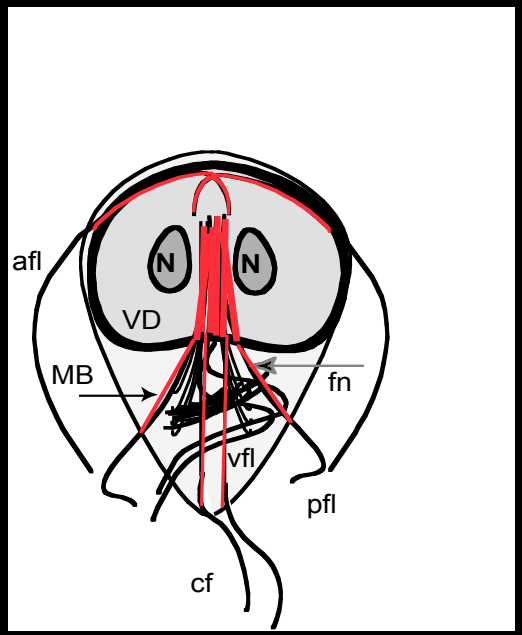
Green - K13:GFP Red - Tubulin Blue - DAPI

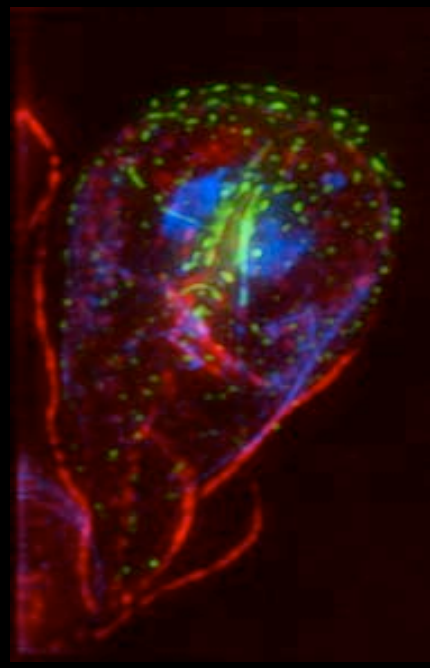
KInesIn-4::GFP "chromokInesIn"



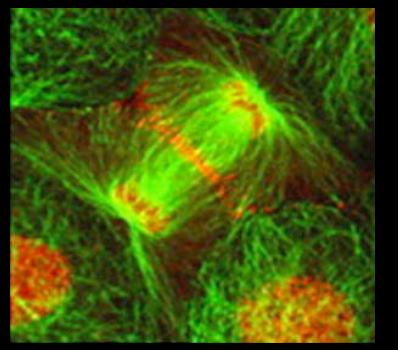
- Not mItotIc rather on Internal regIons of axonemes, In area of ventral dIsc

- Not a conserved mItotIc functIon





KInesIn-4::GFP "chromokInesIn"



- Not mItotIc rather on Internal regIons of axonemes, In area of ventral dIsc

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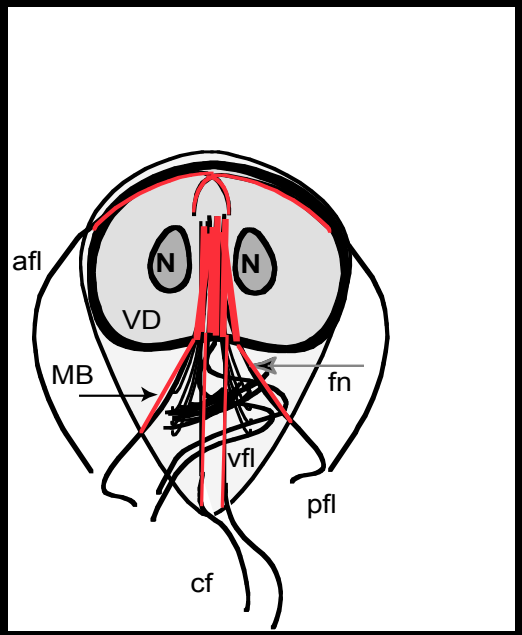
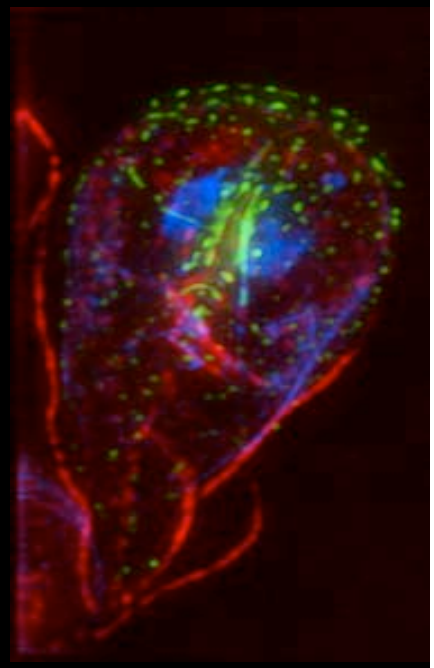




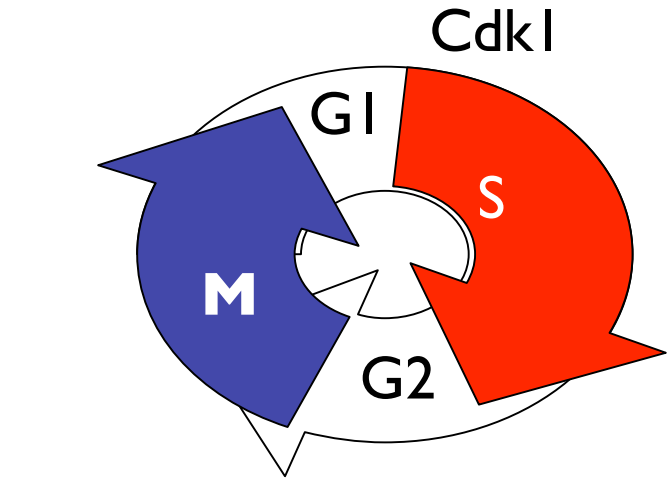
Conserved features of Giardia spindles:

- Bipolar MT array
- MT-dependent chromosome segregation
- Centromere is likely site of MT attachment, kinesin 13 is on kinetochore
- Kinesin-5 and kin13 shows conserved mitotic localization

Unique features of Giardia spindles:

- There are TWO!
- Extra-nuclear and intra-nuclear MT populations
- Intra-nuclear MTs penetrate through polar openings in NE
- Complex and unequal spindle poles?
- Kin4 does not have a mitotic function
- Missing many kinetochore components

Giardia cell cycle



Machinery:
1 Polo-like kinase
1 aurora kinase
2 CDKs
2 cyclins (A and B)
No Anaphase Promoting Complex)
(APC is E3 ubiquitin ligase regulating cyclin B turnover)

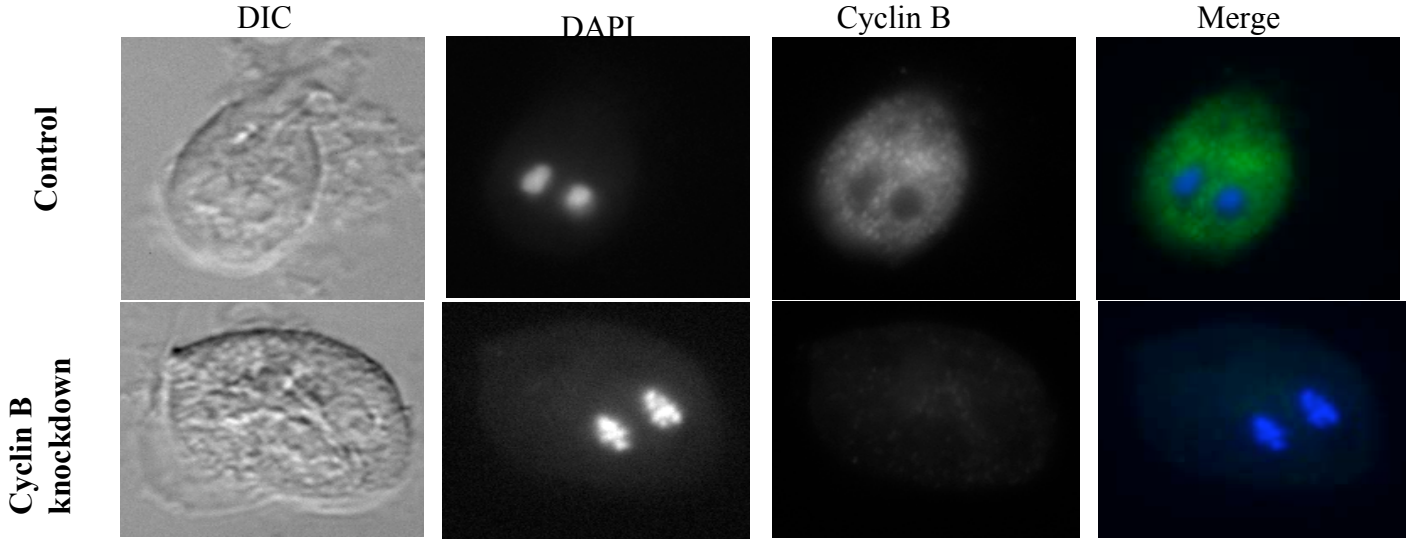
Stephane Gourguechon

How is cyclin

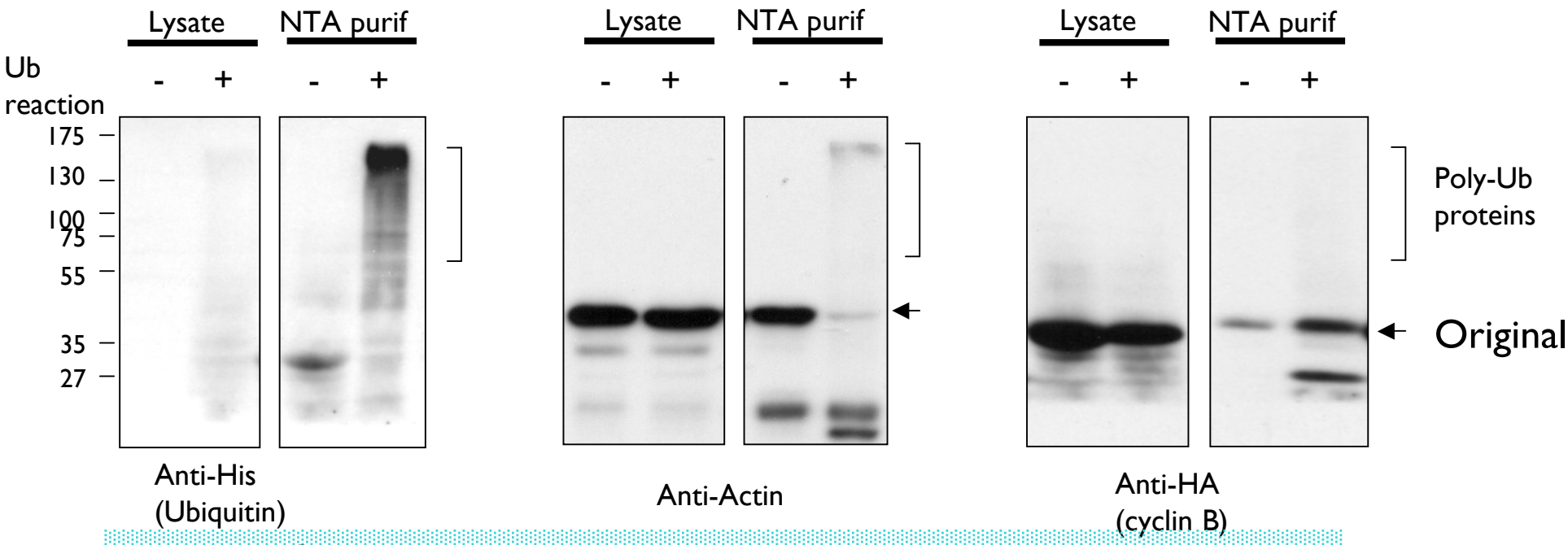
Giardia cyclin B is mitotic cyclin

Expression of *Gi* cyclin B in fission yeast is toxic leading to G2/M arrest.
Gi cyclin B has no N-terminal degradation motifs (PEST, D-box).
Gi cyclin B levels change in cell cycle, no transcriptional regulation.

Morpholino knockdown phenotype: block at G2/M



Cyclin B is not ubiquitinated *in vitro* using *Giardia* ubiquitin



Conclude: since cyclin B levels oscillate,
cyclin B must be degraded at mitosis
by an unknown mechanism, most likely proteolysis.

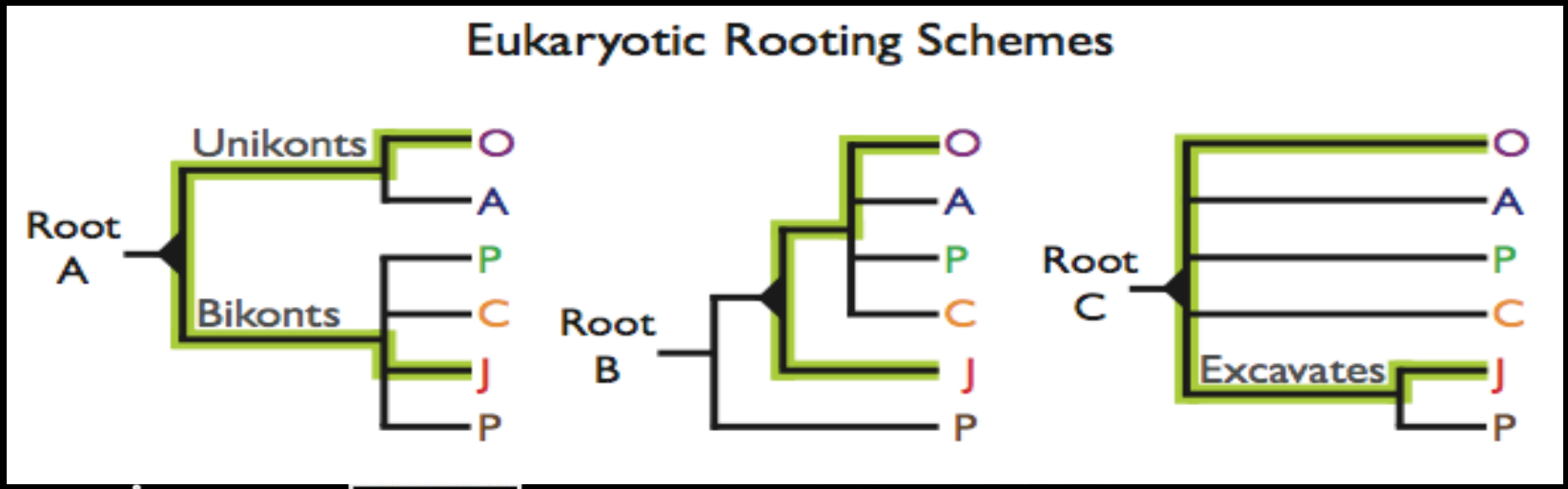
Evolution of cell cycle regulation

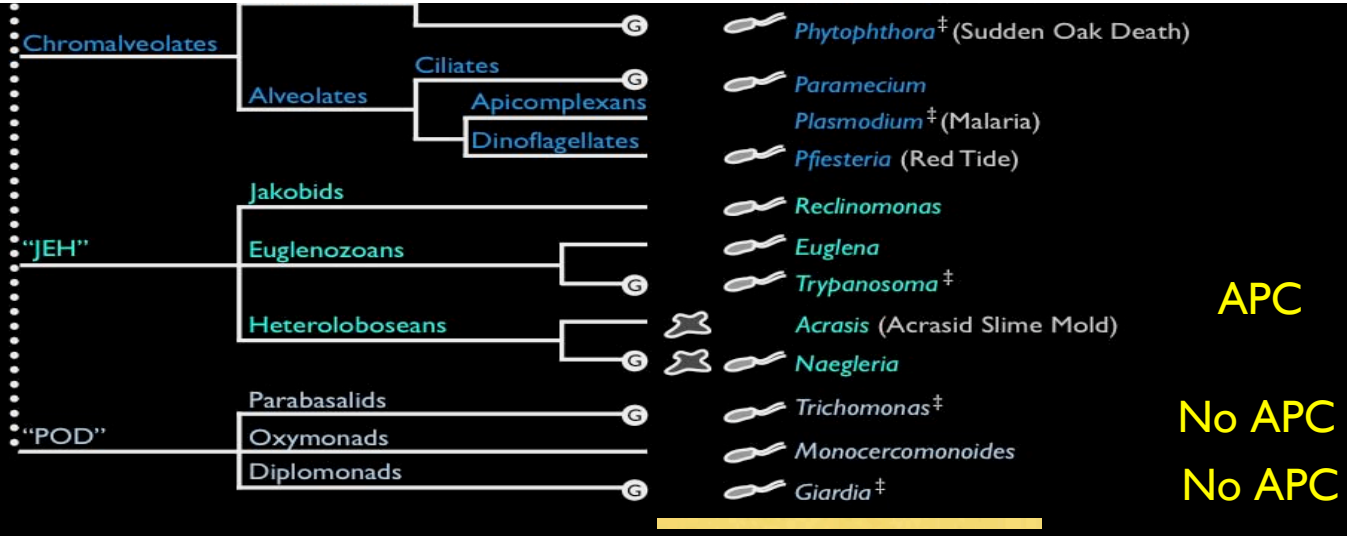
Dilemna: loss of components vs. evolutionary intermediate?

Cell cycle regulation: Sister group (Trichomonas) also lacks APC and their cyclins do not have PEST or D-box.

Role of ubiquination in cycle cycle may have evolved later than cdk/cyclin regulation.

Eukaryotic Rooting Schemes





APC

No APC

No APC

To gain insight: need to resolve branching order and group identity

Need more genomes and more cell biology of free living early branching eukaryotes


The Naegleria Genome Team
Giardial biology
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Stephane Gourguechon
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Zoe Assaf
Scott Dawson
(UCDavis)
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(JEOL)
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(Genetech)
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