# Lecture 7

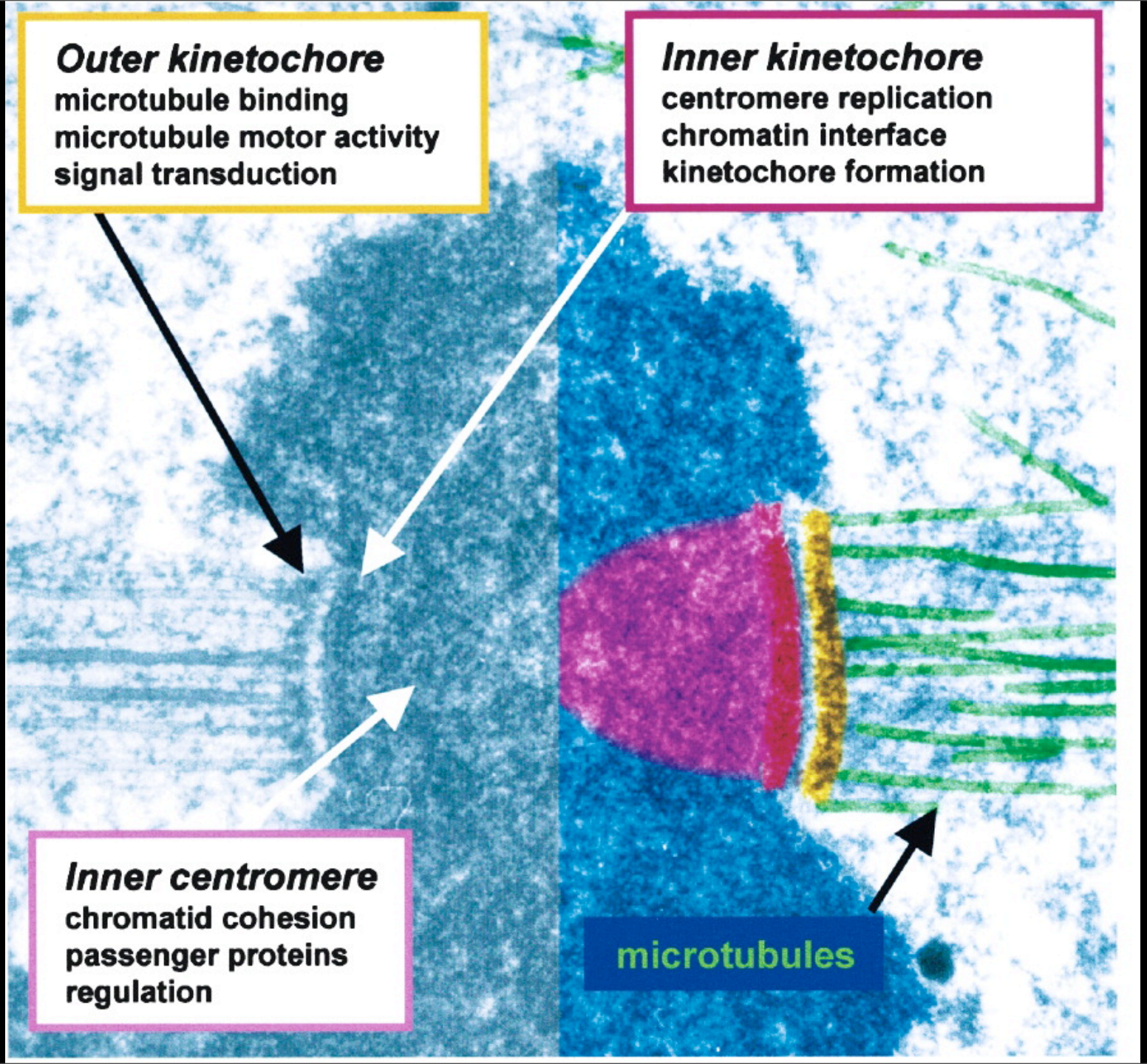
## Chromosome Structure and Function in Mitosis

**Outline:**

**Chromosomes in Mitosis**

- **Centromeres and Kinetochores**
- **Chromosomes, Kinetochores and the Spindle**

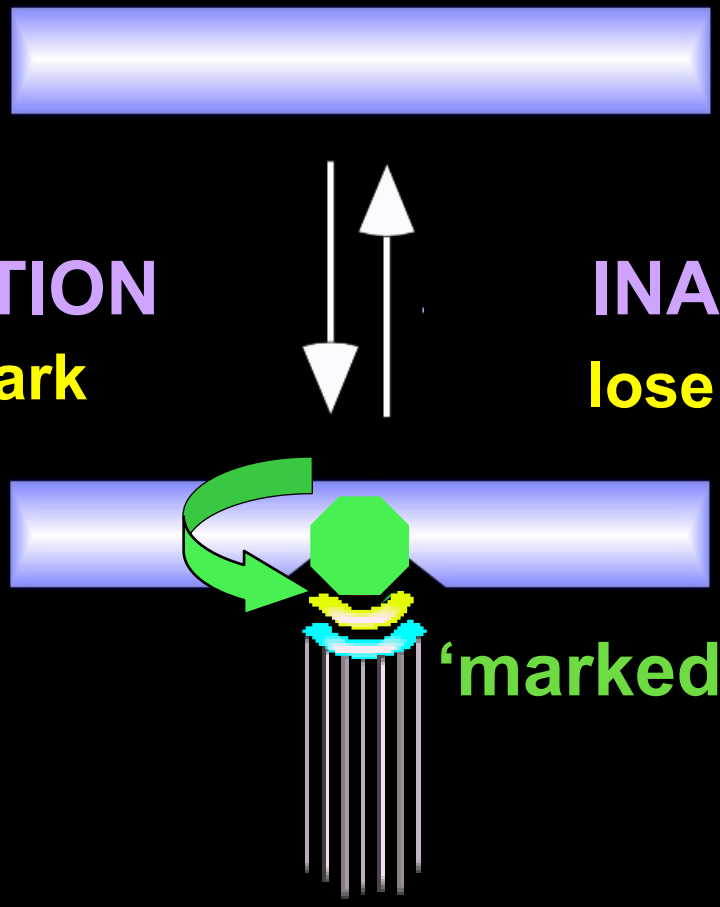
**Paper:**


Outer kinetochore
microtubule binding
microtubule motor activity
signal transduction
Inner kinetochore
centromere replication
chromatin interface
kinetochore formation
Inner centromere
chromatid cohesion
passenger proteins
regulation
microtubules

# Epigenetic Model for CEN Identity

primary sequence is not sufficient (dicentrics)

non-centromeric sequence can acquire and propagate centromere function (neocentromeres)



**ACTIVATION**

gain epigenetic mark

**INACTIVATION**

lose epigenetic mark

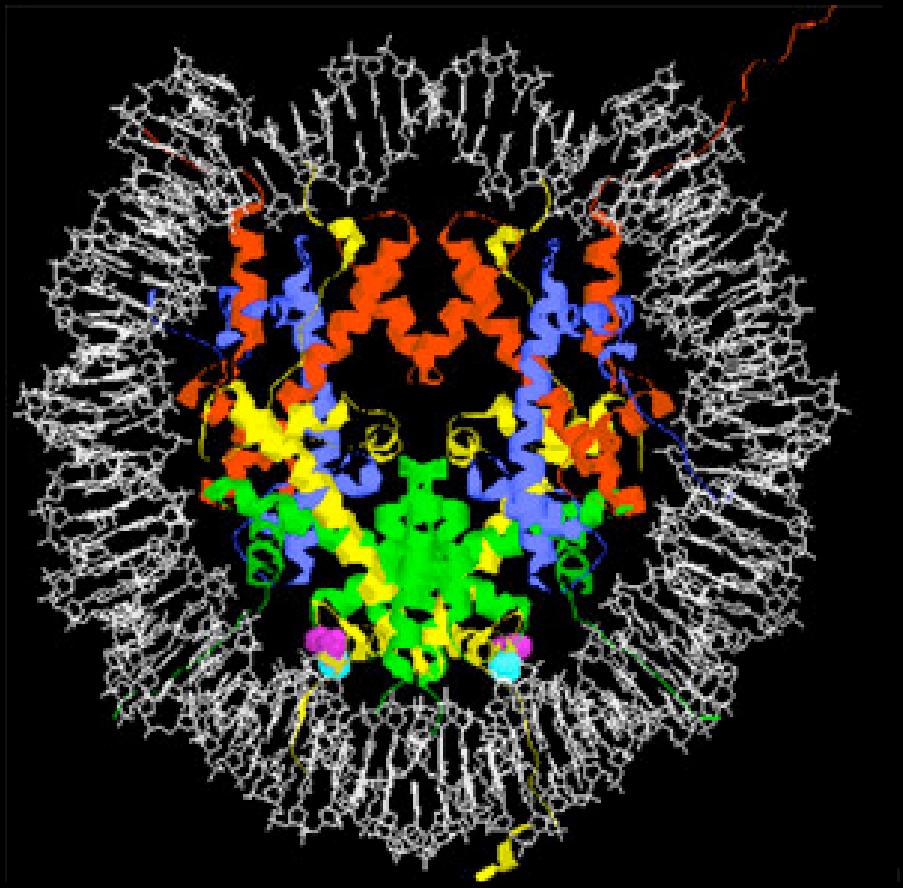
‘marked’ DNA/chromatin

# CENP-A: An epigenetic mark for centromere identity?

H3-like histone variant

H2A/B

H4

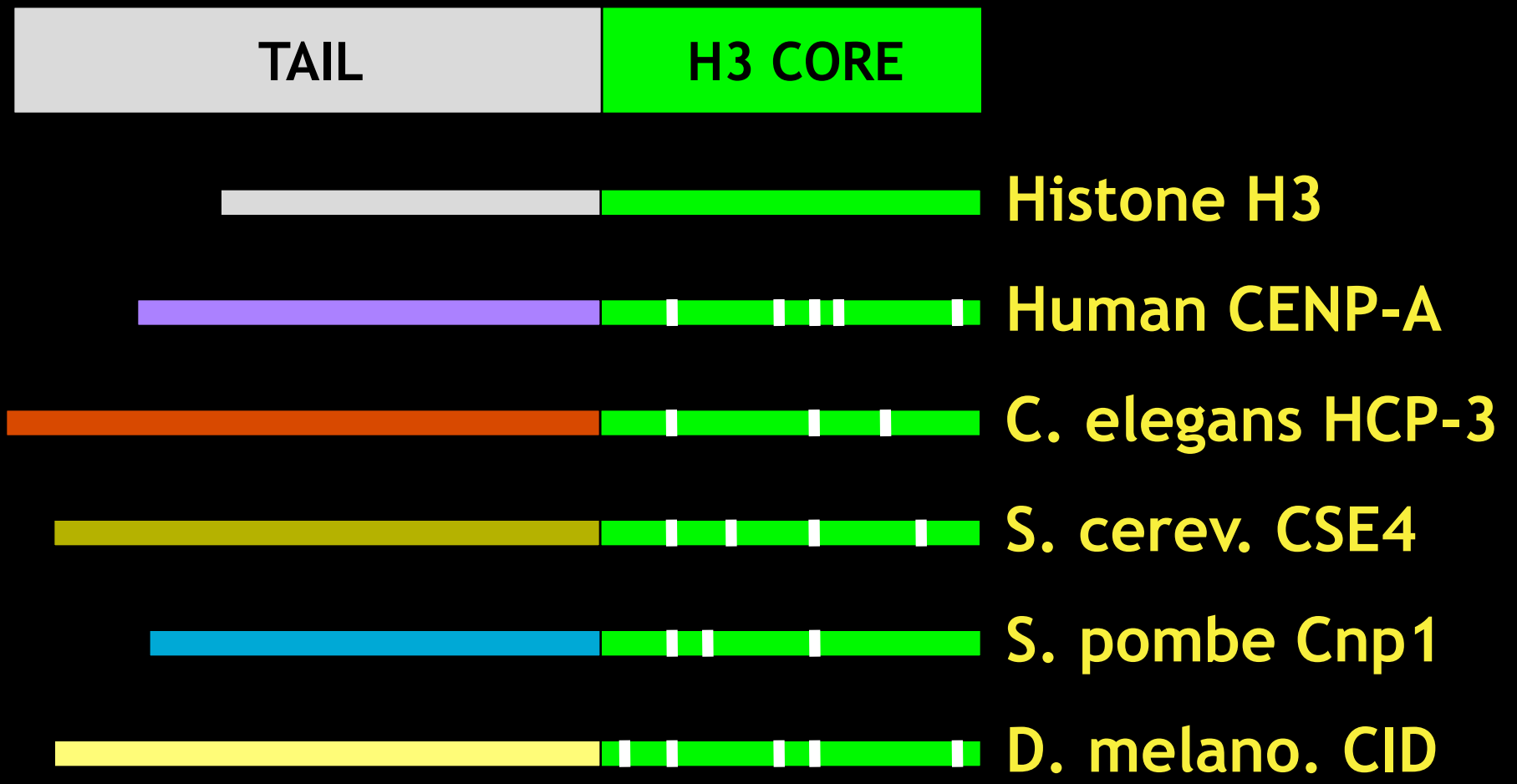


H3 dimer

↑

CENP-A dimer

# CENP-As are Conserved H3-like Proteins

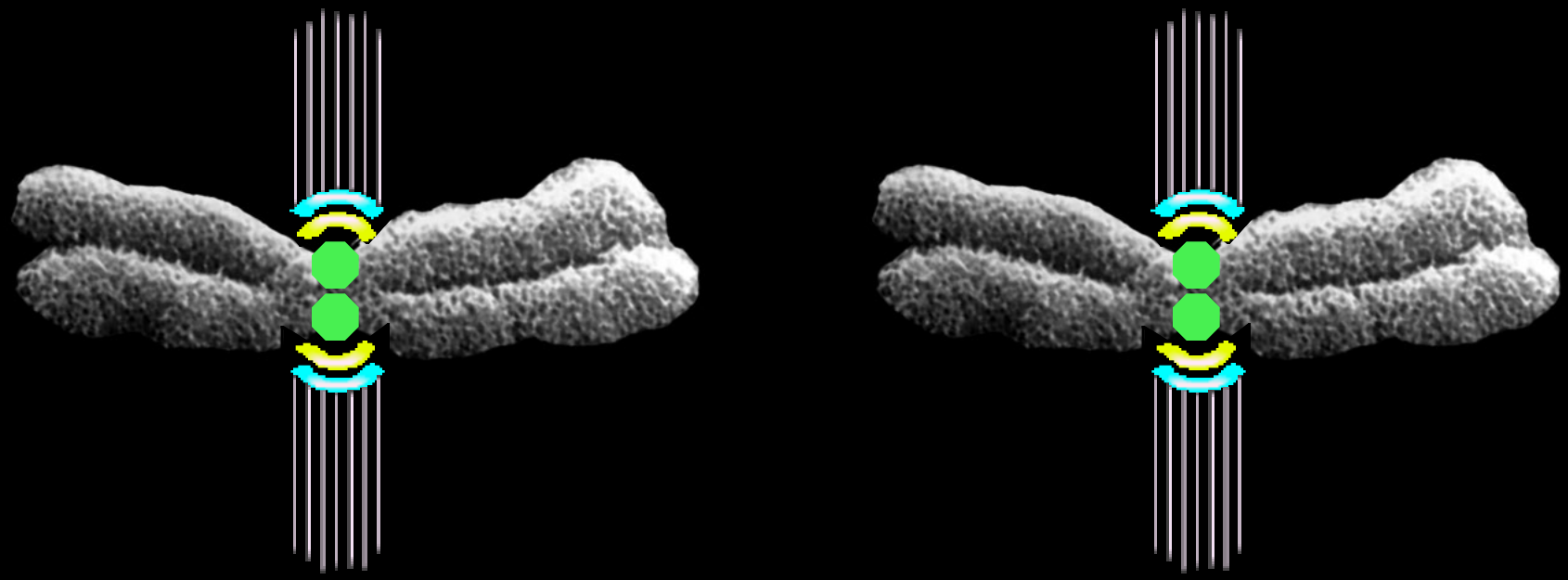



conserved CEN-specific histone, but highly divergent

# CENP-A: Functional Foundation for the Kinetochore

required for

**kinetochore formation, segregation, mitotic progression, in flies,worms, mammals, yeasts**

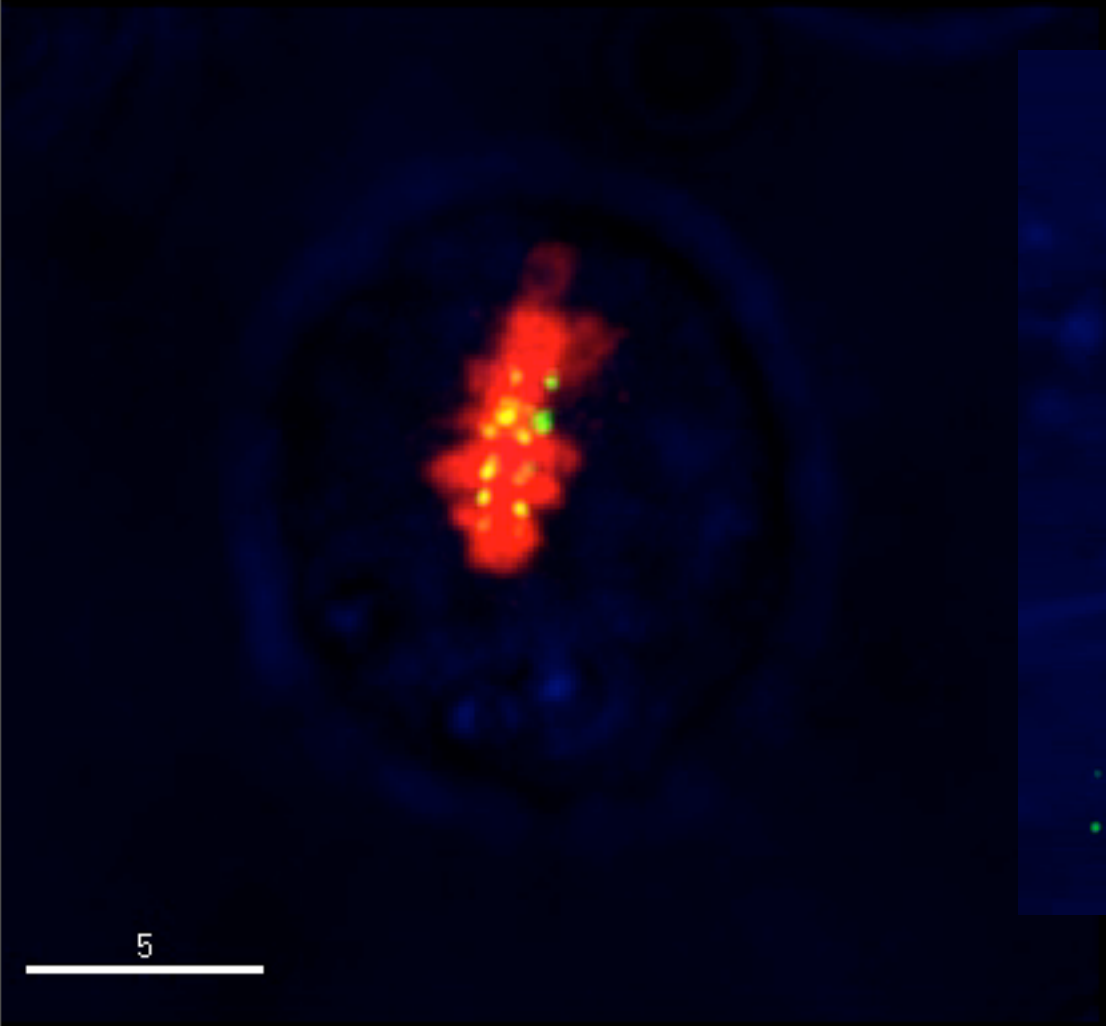


**high (est?) in kinetochore assembly and function pathway**

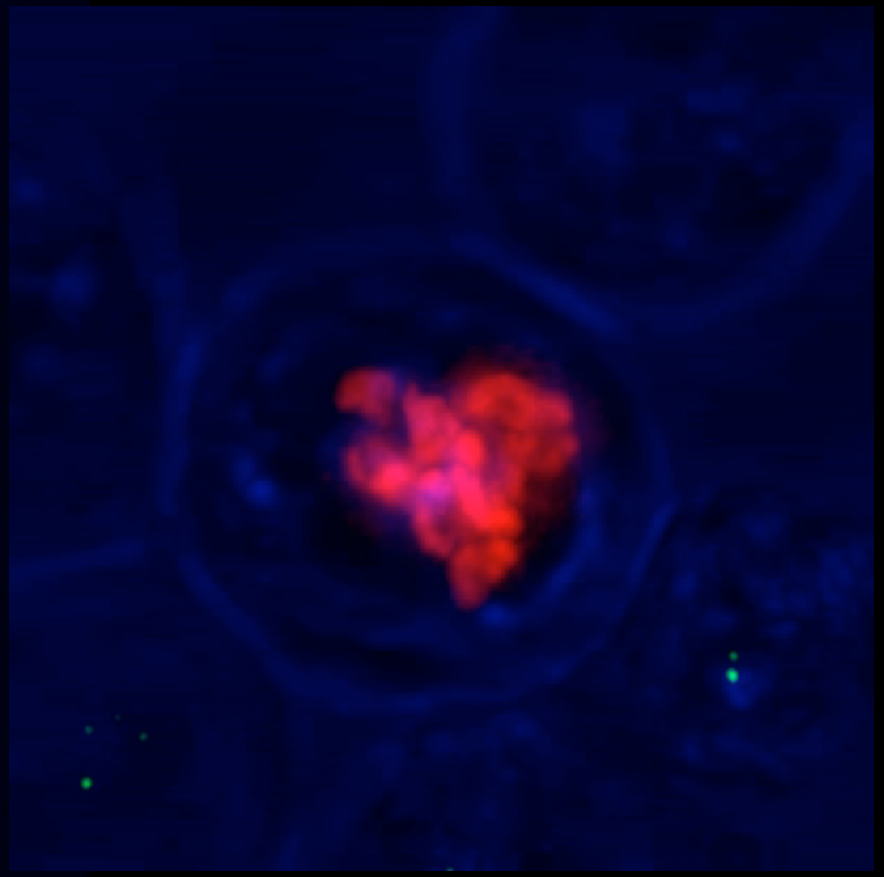
acting like a key mark for CEN identity
absence of CENP-A/kinetochore
leads to chromosome loss/aneuploidy

# Mitotic Defects caused by CENP-A Depletion

H2B-RFP CENP-A-GFP



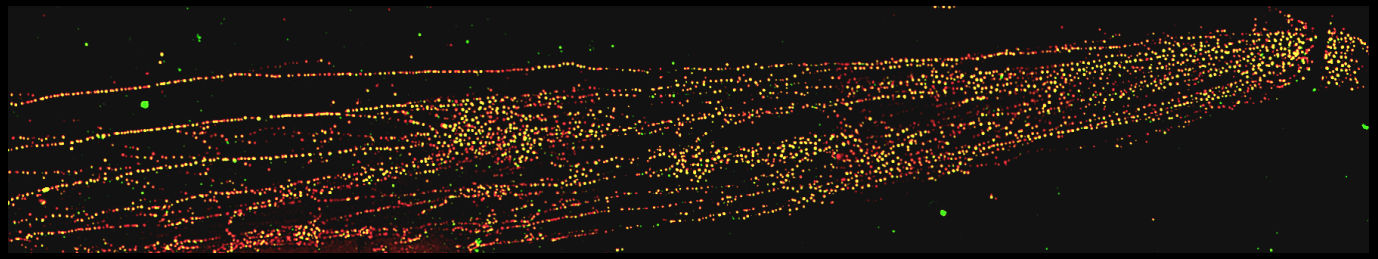

CENP-A RNAi



poor condensation / congression
no anaphase movement
cytokinesis cuts chromosome mass

# Blocks of H3 and CENP-A Nucleosomes are Interspersed in CEN Chromatin

## extended chromatin fibers



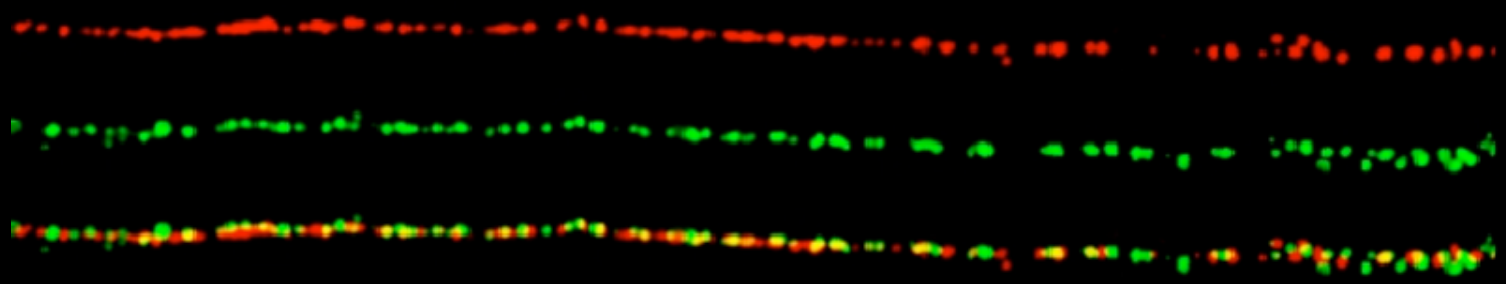
CORE H4-Ac

## human and fly

CENP-A

H3

MERGE



CENTRIC HET — CEN — CENTRIC HET

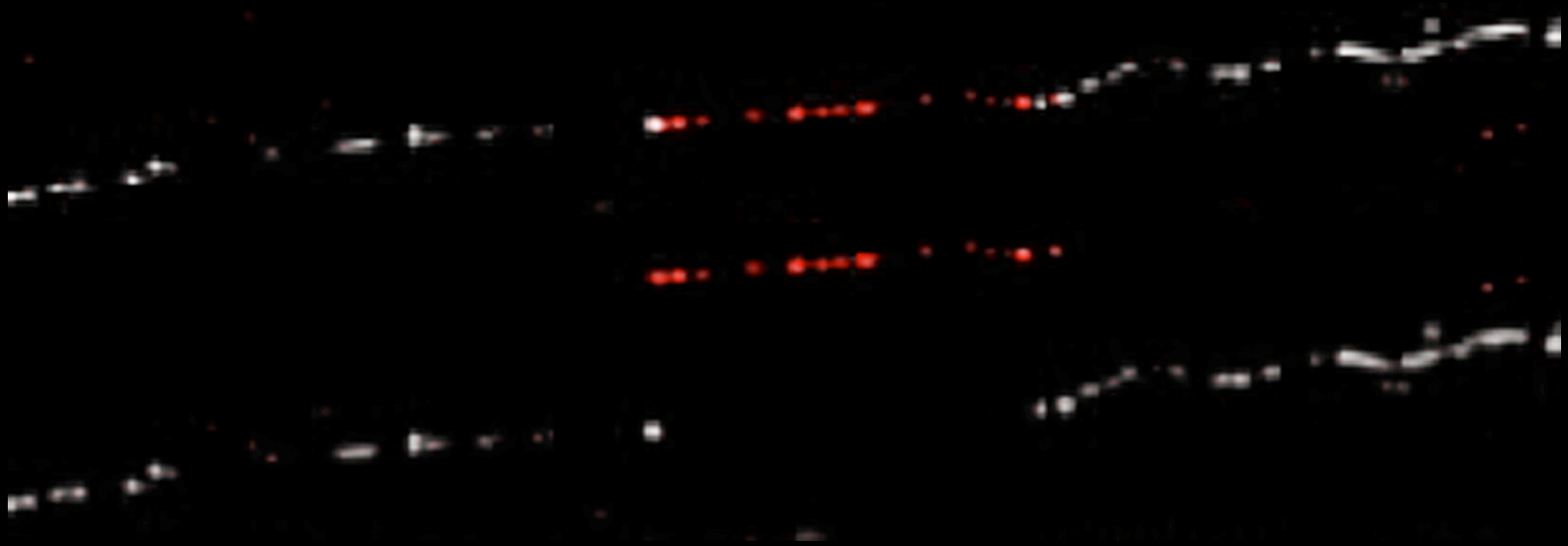
0.3-1.5 Mb

## Are there other epigenetic ‘marks’ at centromeres besides CENP-A?

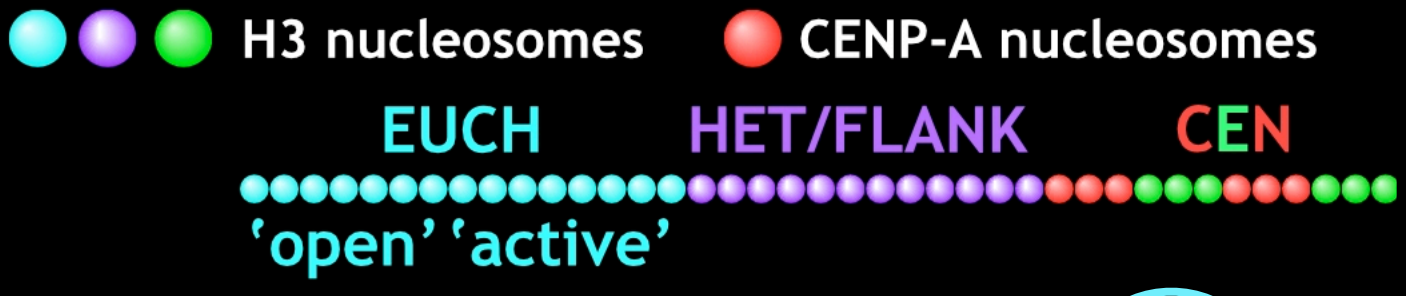
**CENs are embedded in heterochromatin-**
**but are they modified like heterochromatin ?**


| | H3 | H3 CENP-A |
|---|---|---|
| H3 diMeK9 (heterochromatin) | + | - |
| H3 diMeK4 (euchromatin) | - | + |

Human and fly



# CEN, EUCH, & HET Contain Distinct Modifications



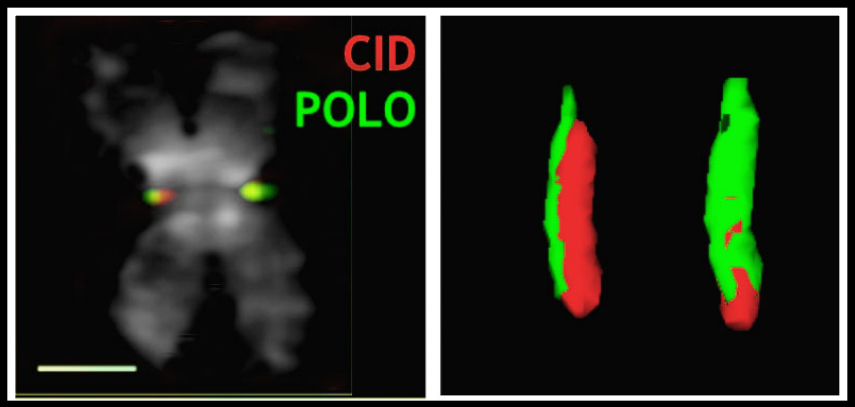

| | | | | |
|---|---|---|---|---|
| H3 K9 di-Me (heterochromatin) | - | - | + | - |
| H3 K4 di-Me (euchromatin) | + | - | - | + |
| H3 K4 tri-Me (active genes) | + | + | +/- | - |
| H3 K9,14 Ac (active genes) | + | + | - | - |
| H4 K5,8,12 Ac (active genes) | + | + | - | - |
| H4 K16 Ac (active genes) | + | + | +/- | - |
| H3 Ser10 Ph (mitosis) | + | + | + | + |

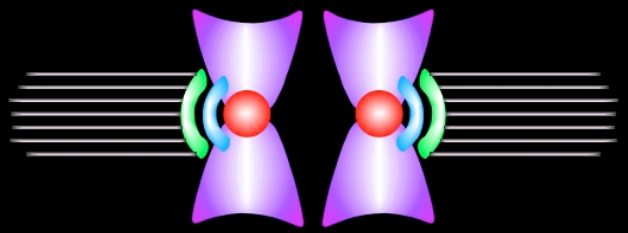
distinct from 'classical' Euchromatin & Heterochromatin

# Forms HIgher-Order ‘Cylindrical’ Structure in Mitotic Chromosomes

## inner and outer kinetochore proteins ‘wrapped’ around CENP-A cylinder

Drosophila



CENP-A

CENP-C

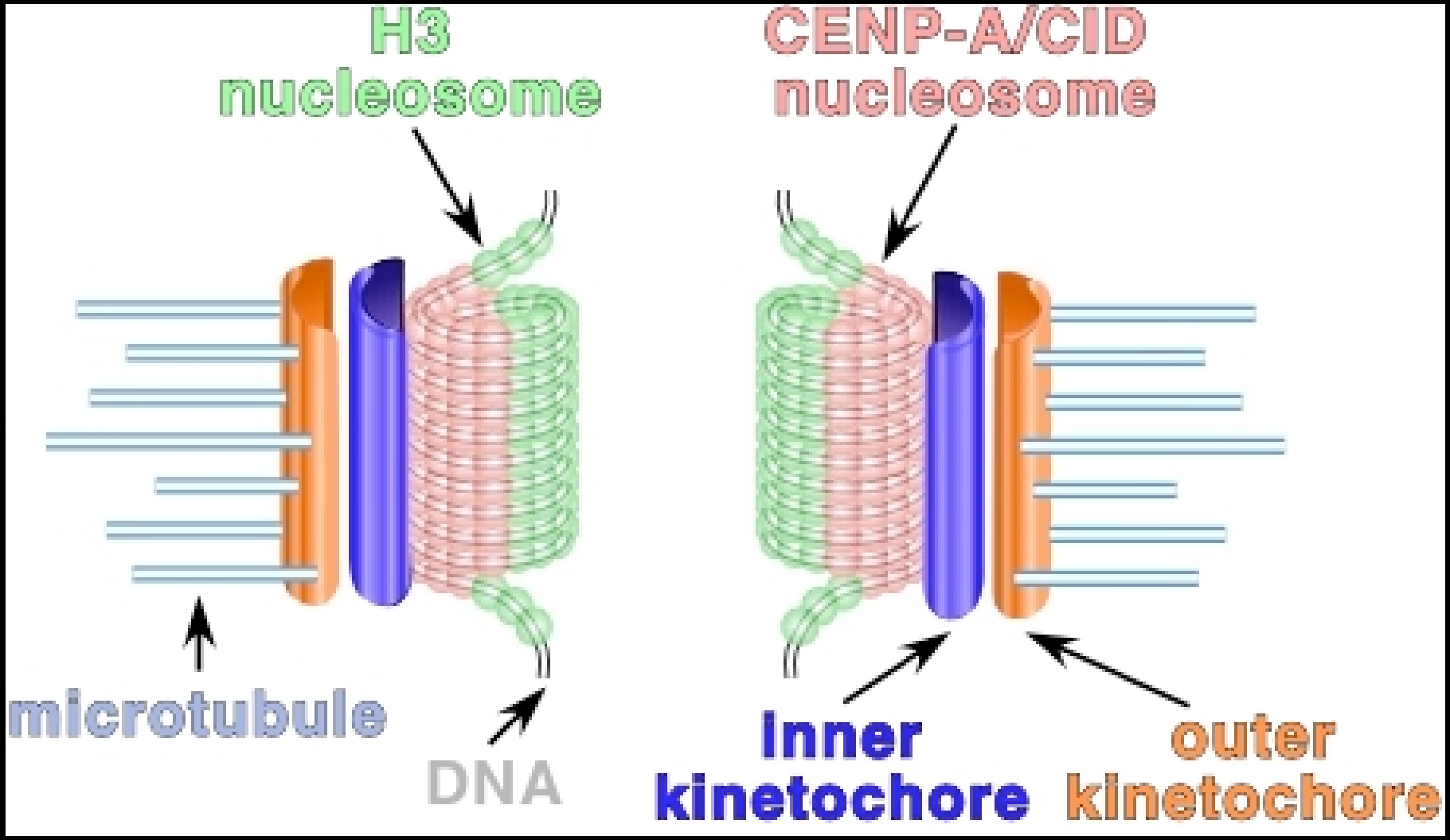
OUTER

in the right place to
nucleate the kinetochore

# Spiral/Loop Model for 3D Structure of CEN Chromatin

**little or no H3 in cylinders, no mixing in mononucleosomes**

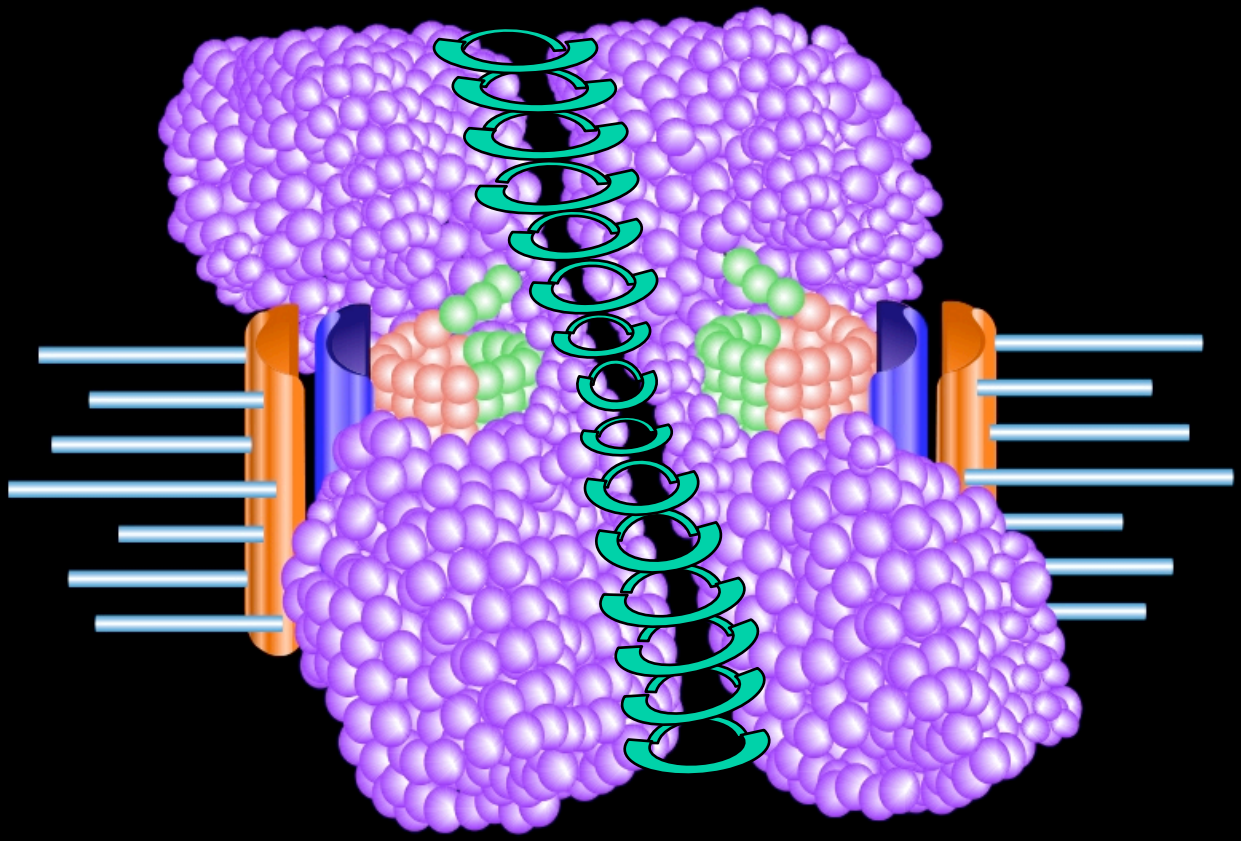
**how reconcile 3D exclusion and 2D interspersion of H3?**




**'presentation' of CEN chromatin for attracting kinetochore proteins**

# Centromere region contains distinct domains

'stacking' of similar nucleosomes ? HET H3, CEN H3, CENP-A



- role of distinct modifications and flanking HET:

3D structure ?

concentrate cohesin?

epigenetic propagation of CEN identity (w/CENP-A)?

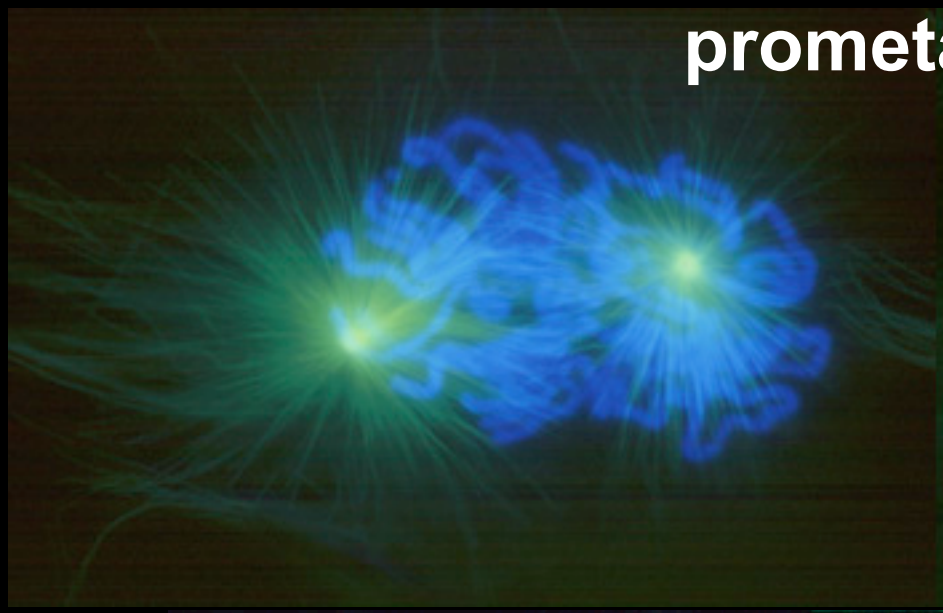
## CENP-A and Centromeric Chromatin:

**Functional & Structural Foundation for the Kinetochore**

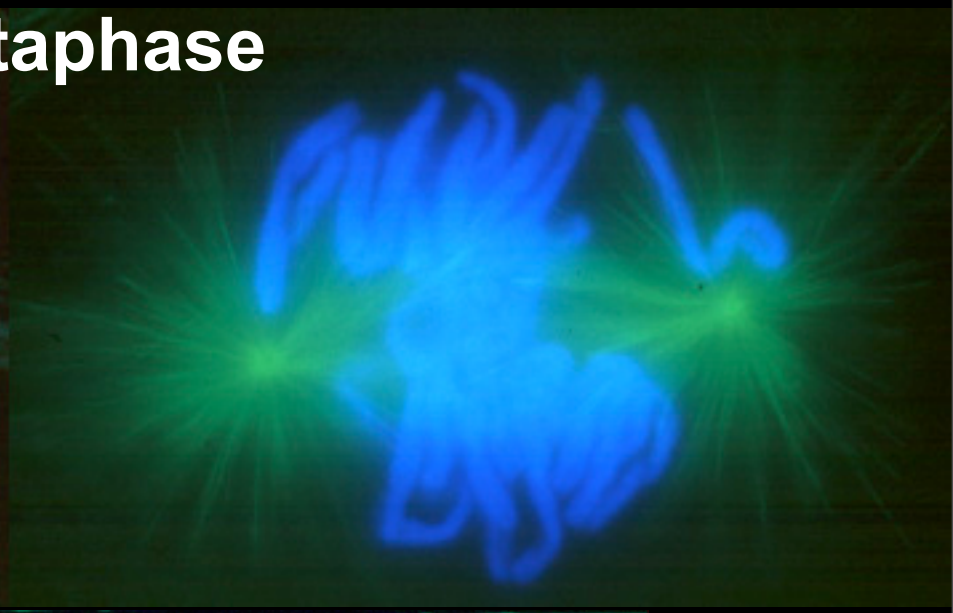
**unique chromatin composition and organization provides ample components for epigenetic regulation**

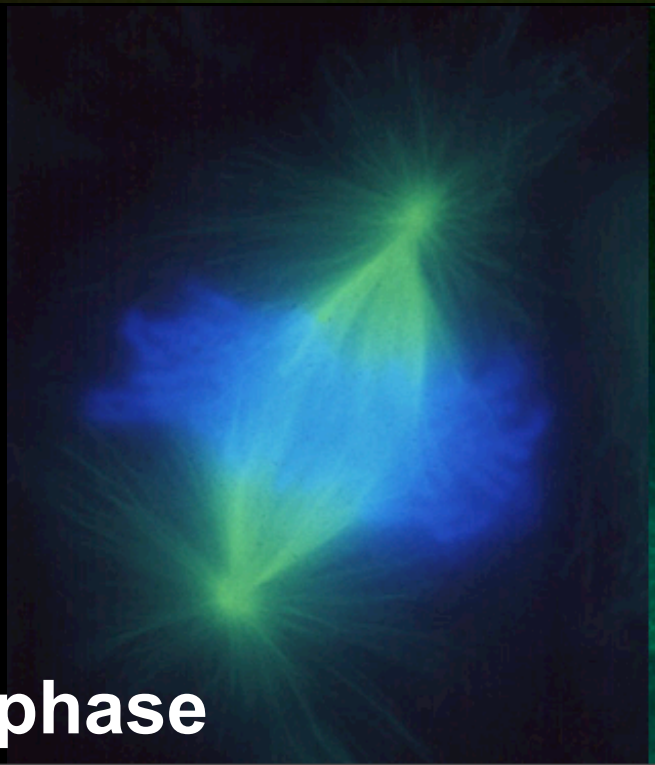
**but how is CENP-A loaded only at centromeres ?**

**How does the kinetochore function during mitosis ?**

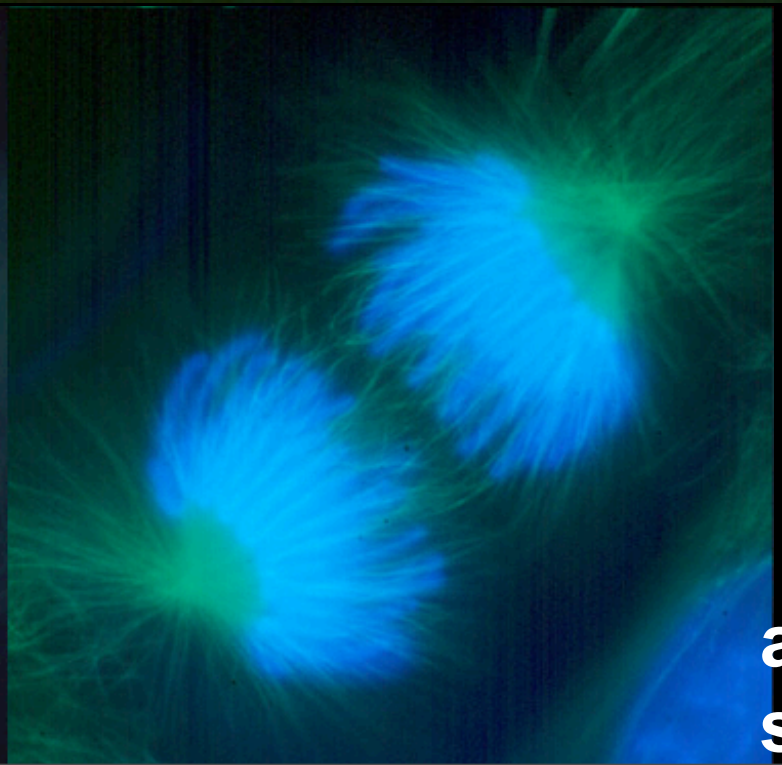


prometaphase



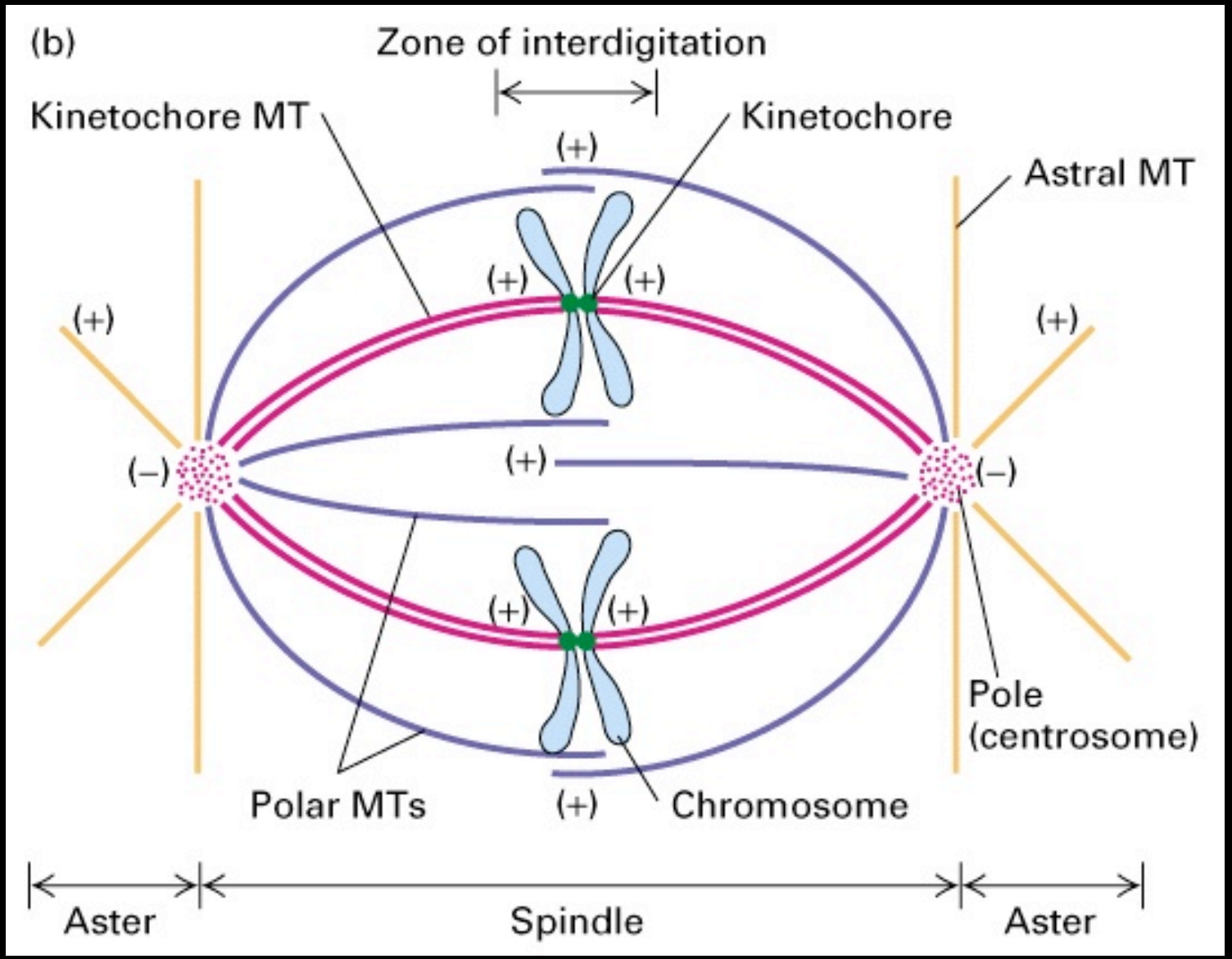


metaphase



anapha
se

# Spindle Anatomy




**how is the spindle formed?**

# Review: microtubule dynamics

microtubules grow from MTOC (centrosome)

undergo dynamic instability

parameters:

- growth rate ($V_g$)
- shrinkage rate ($V_s$)
- catastrophe frequency ($f_{cat}$)
- rescue frequency ($f_{res}$)

modified by many cellular factors

# Mitotic microtubule dynamics and spindle assembly

1) global changes in MT dynamics

**Concept:**
**Factors with opposing activities modulate MT dynamics**

2) local modulation of MT dynamics:
chromosome attachment and congression

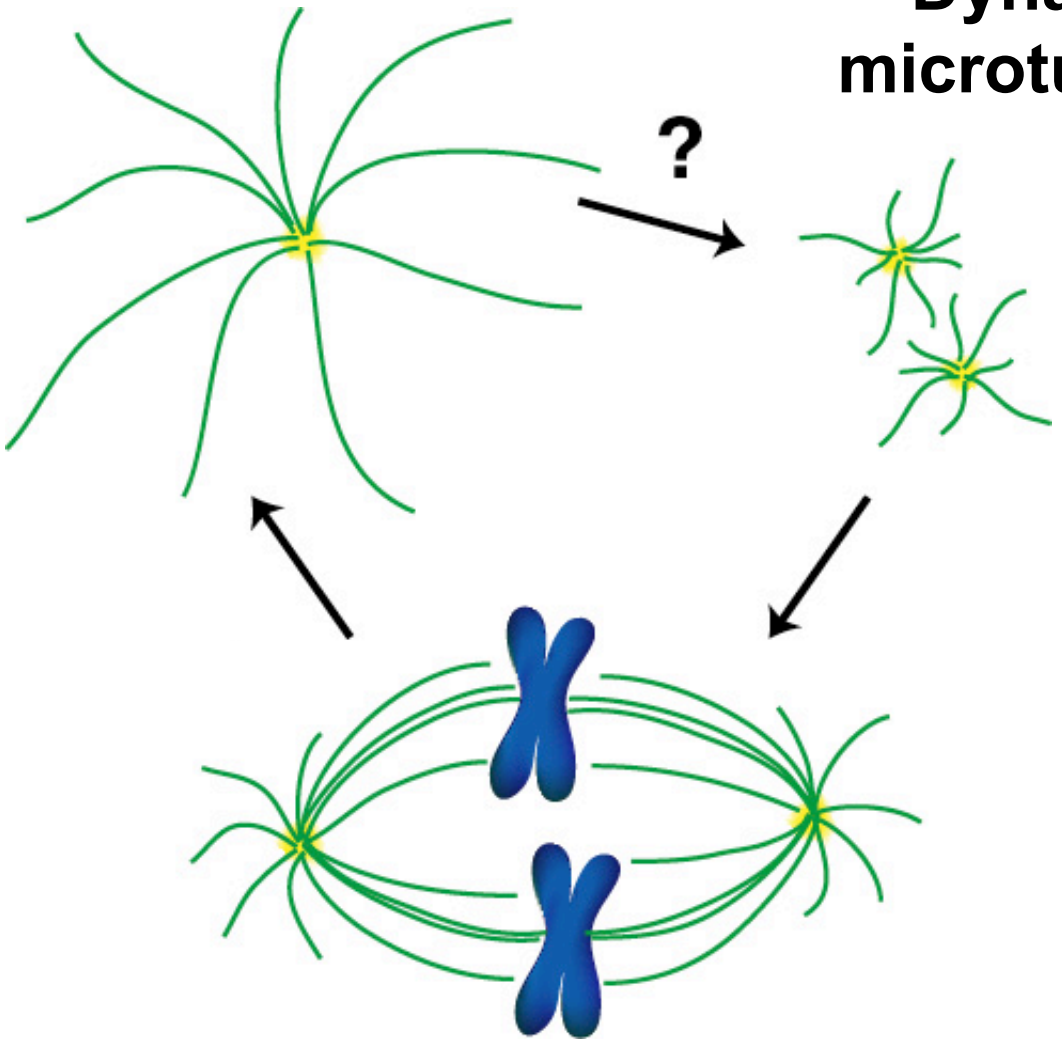
**Concept:**
**chromosome movements are linked to MT dynamics and motor proteins**

3) organization into a spindle

**Concept:**
**Motors with opposing activities arrange MTs**

Stable microtubules

Dynamic microtubules




## Questions:

1) what MT parameters change?

2) what generates this global change?

**Photobleaching studies:**

interphase MTs $t_{1/2}$ ~ 5 min

mitotic MTs $t_{1/2}$ ~ 0.5 min

**Approach:**

use a system in which dynamics are easily measured

Xenopus egg extracts

identify and deplete different factors

reconstitute dynamics with pure tubulin and factors

**primary parameter affected:**

$f_{catastrophe}$ increases

**Possible regulatory mechanisms:**

stabilizing MAPs inactivated

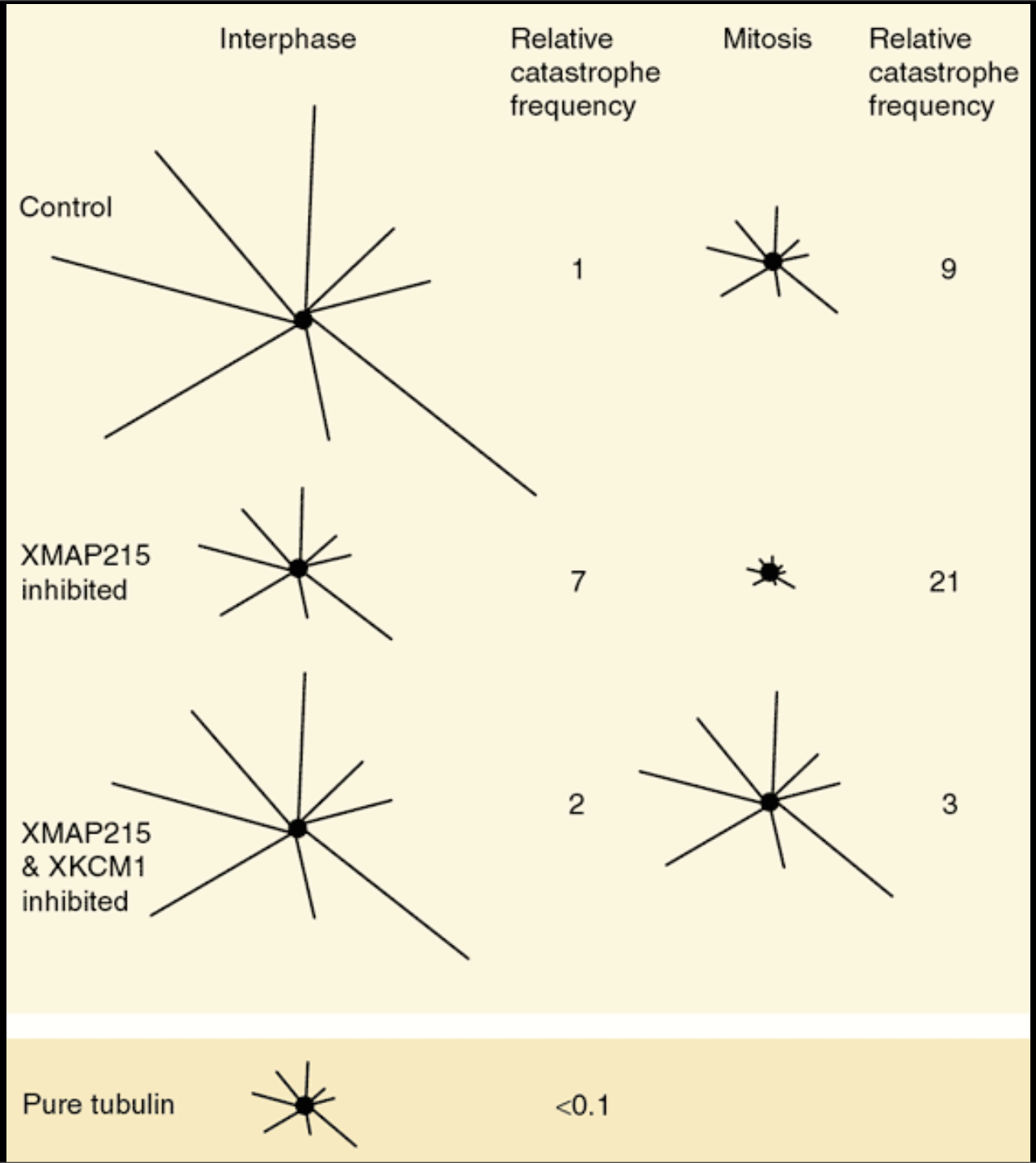
and/or

catastrophe factors activated

**Opposing factors identified:**

XMAP215 & XKCM1

**MTs polymerized from centrosomes**




Tournebize et al., Nature Cell Biol. 2, 13 (2000)

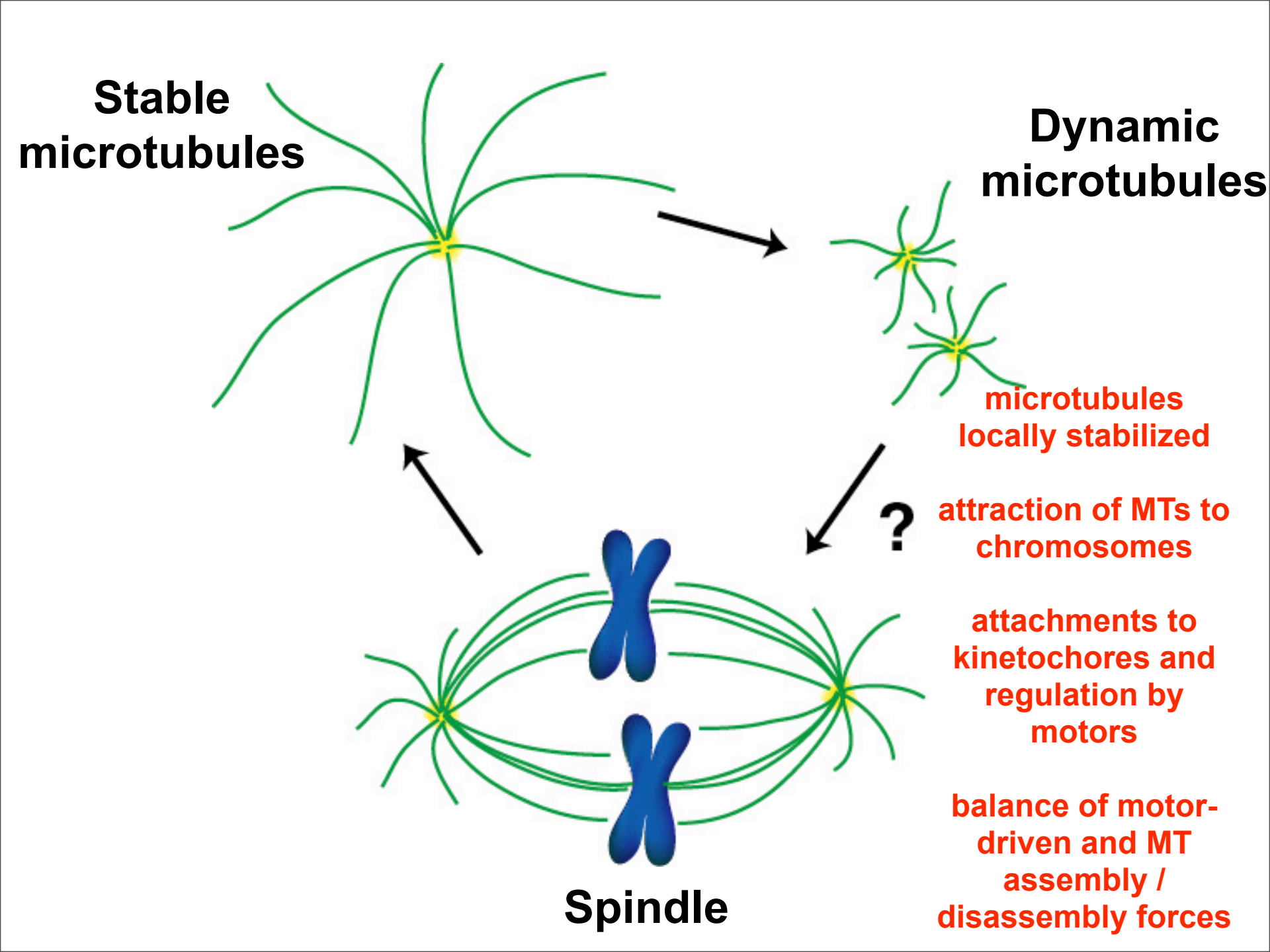
## Conclusions:

XMAP215 stabilizes MTs in interphase and mitosis

**Model:**

XKCM1 constitutively active
XMAP215 activity varies during cell cycle

other cellular factors must also contribute to MT dynamics

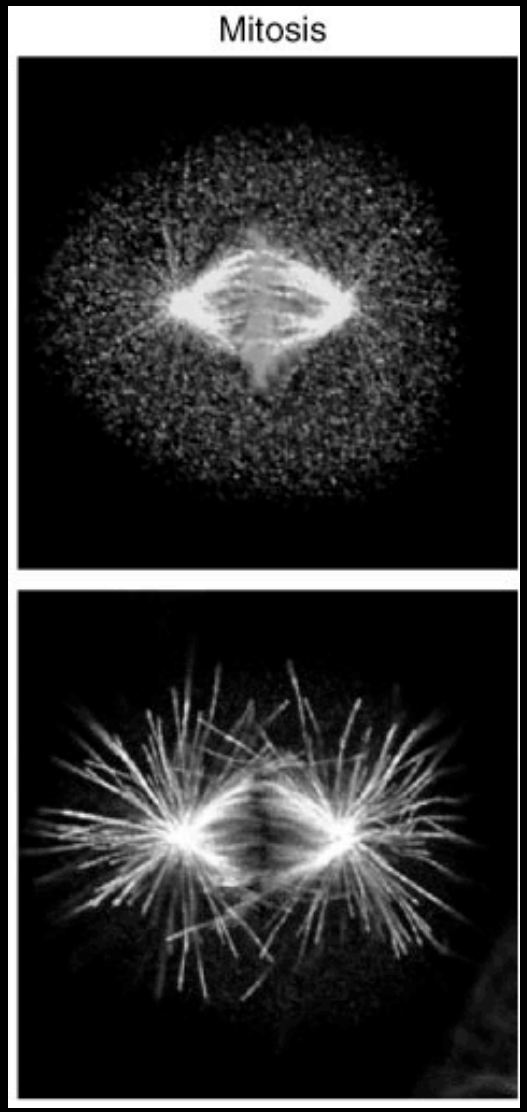

Stable microtubules
Dynamic microtubules
microtubules locally stabilized
?
attraction of MTs to chromosomes
attachments to kinetochores and regulation by motors
balance of motor-driven and MT assembly / disassembly forces
Spindle

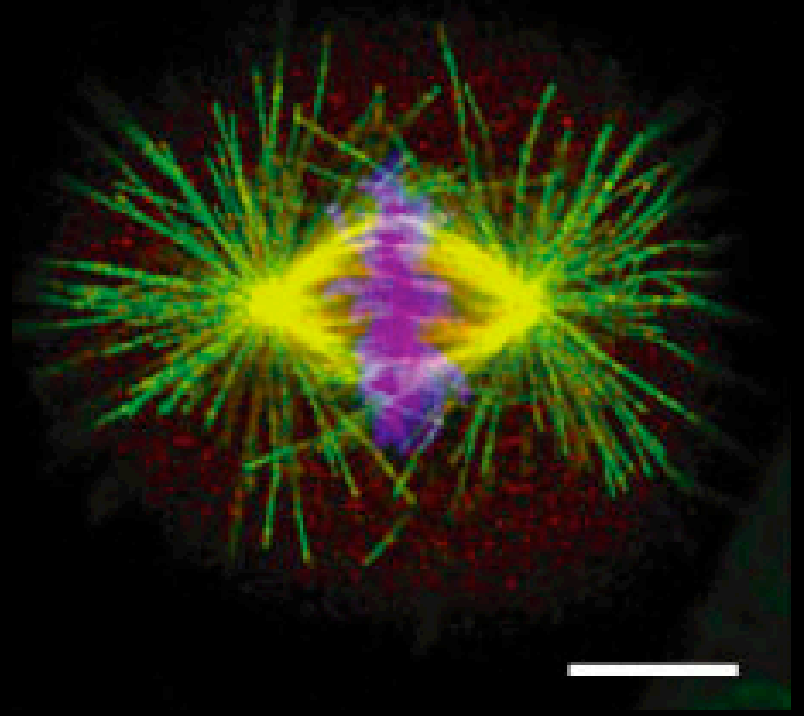
# MAP distribution is locally regulated

## XMAP215 is on spindle but not astral microtubules

XMAP215

tubulin



yellow = XMAP215
green = tubulin
blue = DNA

get stabilization of spindle but not astral microtubules

# Chromosome Attachment and Making a Bipolar Spindle

**2 models:**

1) biochemical signal on chromosomes - diffusible molecules stabilize MTs

2) search and capture - kinetochores capture MTs

not mutually exclusive

# Biochemical signals on chromosomes

**MT growth toward chromosomes**

due to diffusible factors, independent of kinetochores

**best evidence:**

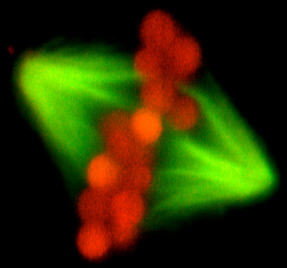
micromanipulation in insect spermatocytes (Nicklas lab)

removal of chromosomes decreases MT mass

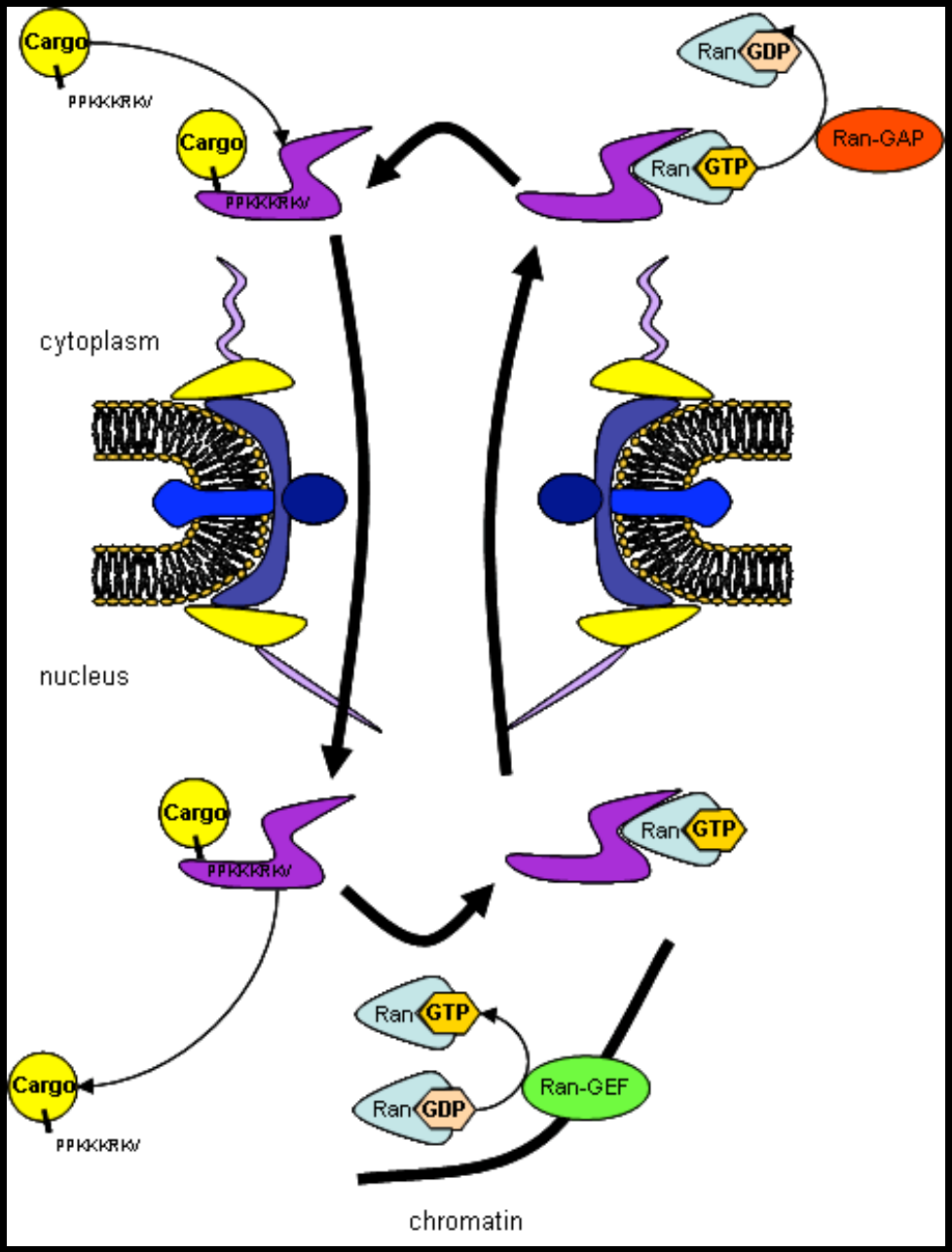
Xenopus:

plasmid DNA coated beads

chromatin enzyme generates signal



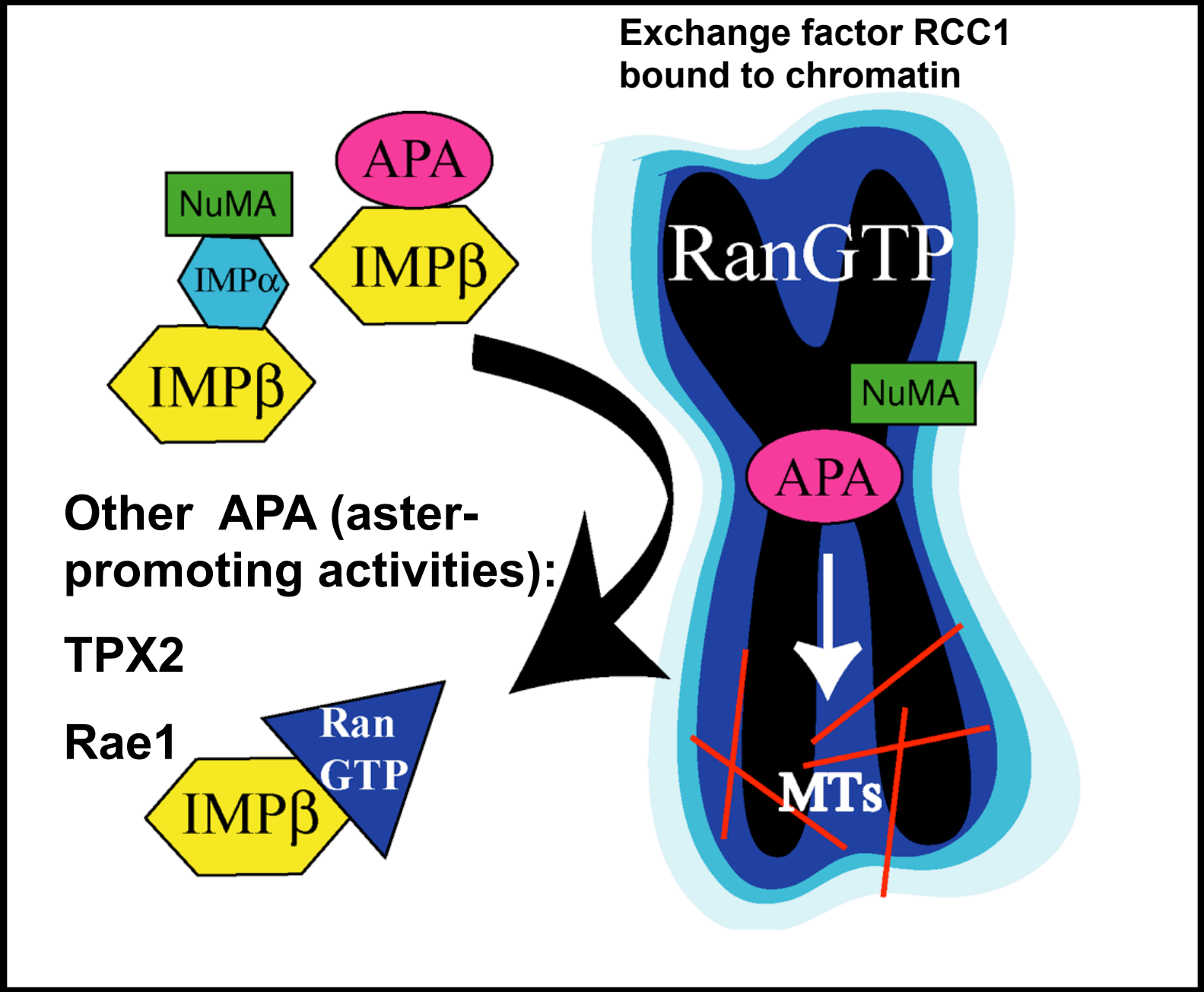
# RanGTP pathway



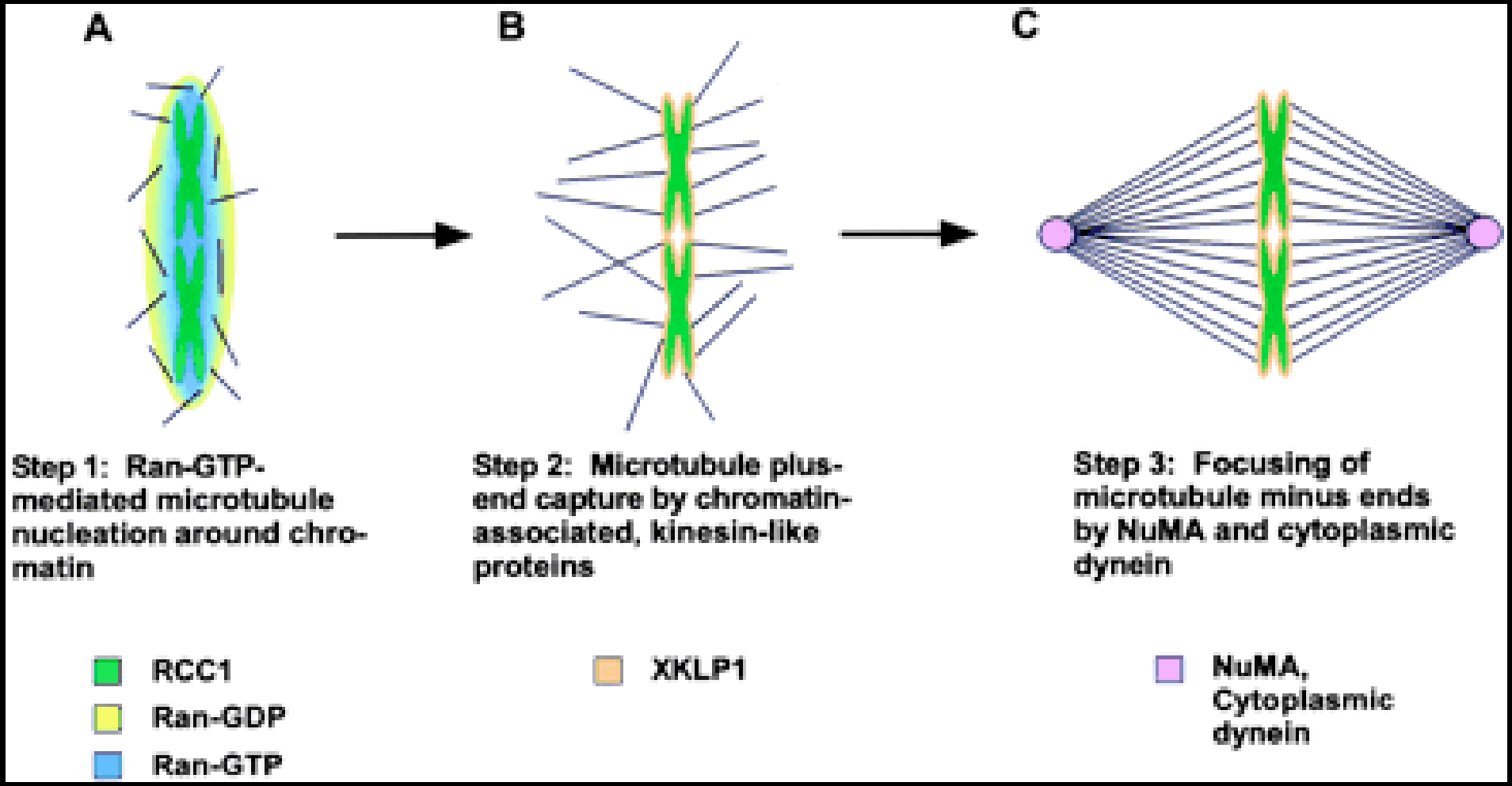

**nuclear import/ export**

**chromatin bound**

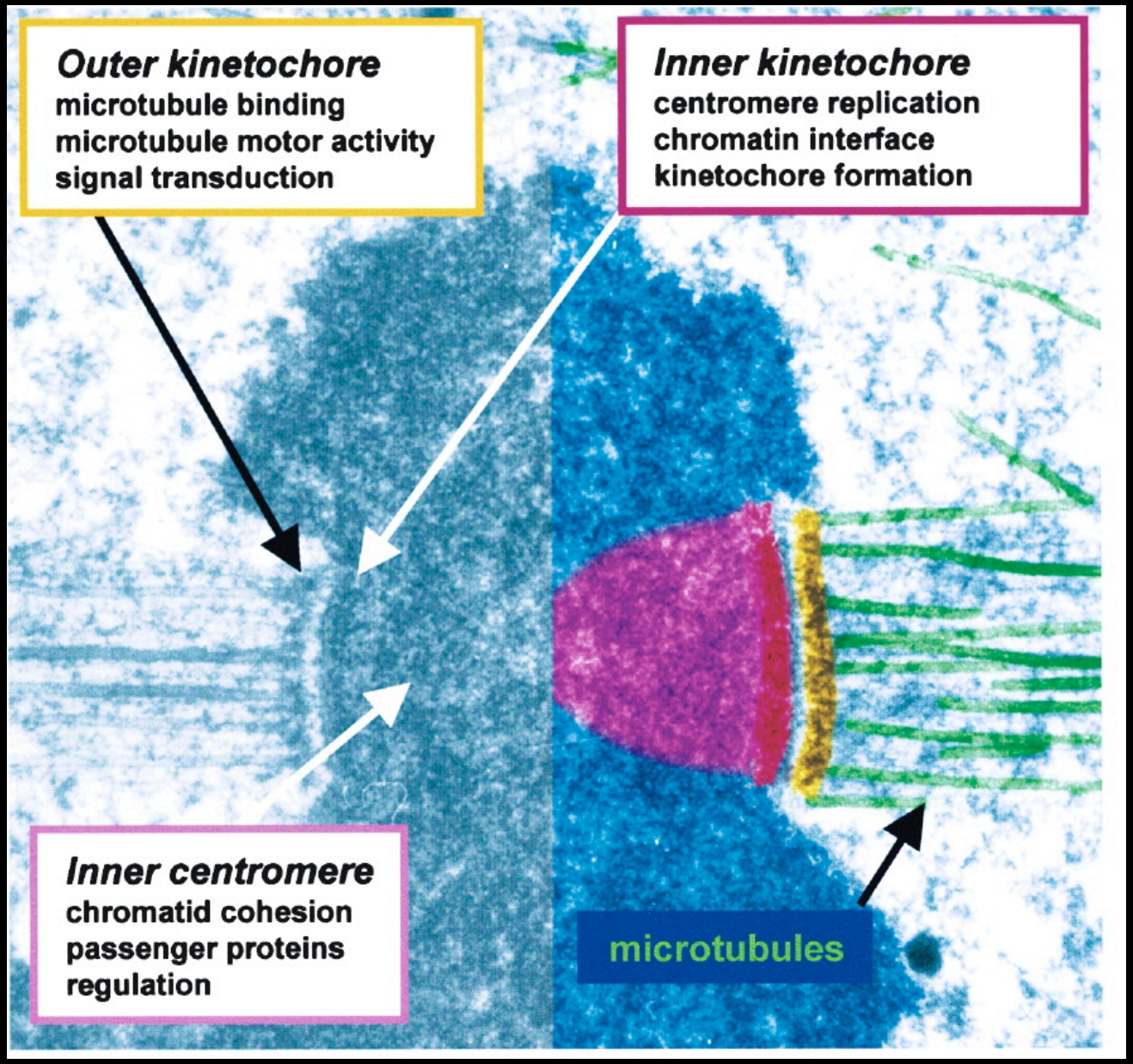
# Chromatin - RanGTP pathway



# Chromatin - RanGTP recruitment of MTs

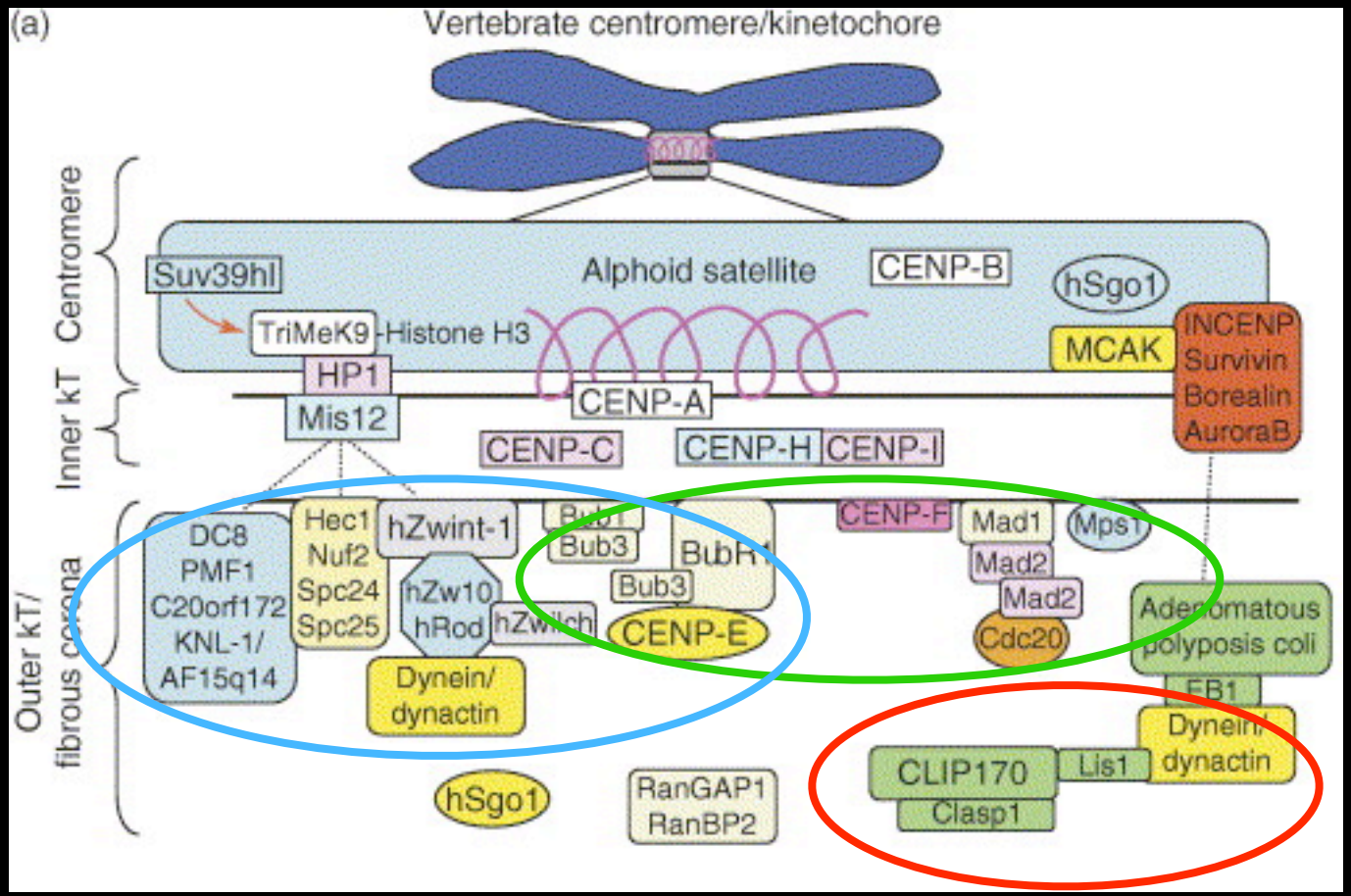


# Search and capture: Kinetochores and MTs



# The Metazoan Kinetochore

**large- Mbs of DNA, kinetochore binds ~30 MTs**




**~80-90 proteins**

**organized as subcomplexes**

**with different and overlapping functions**

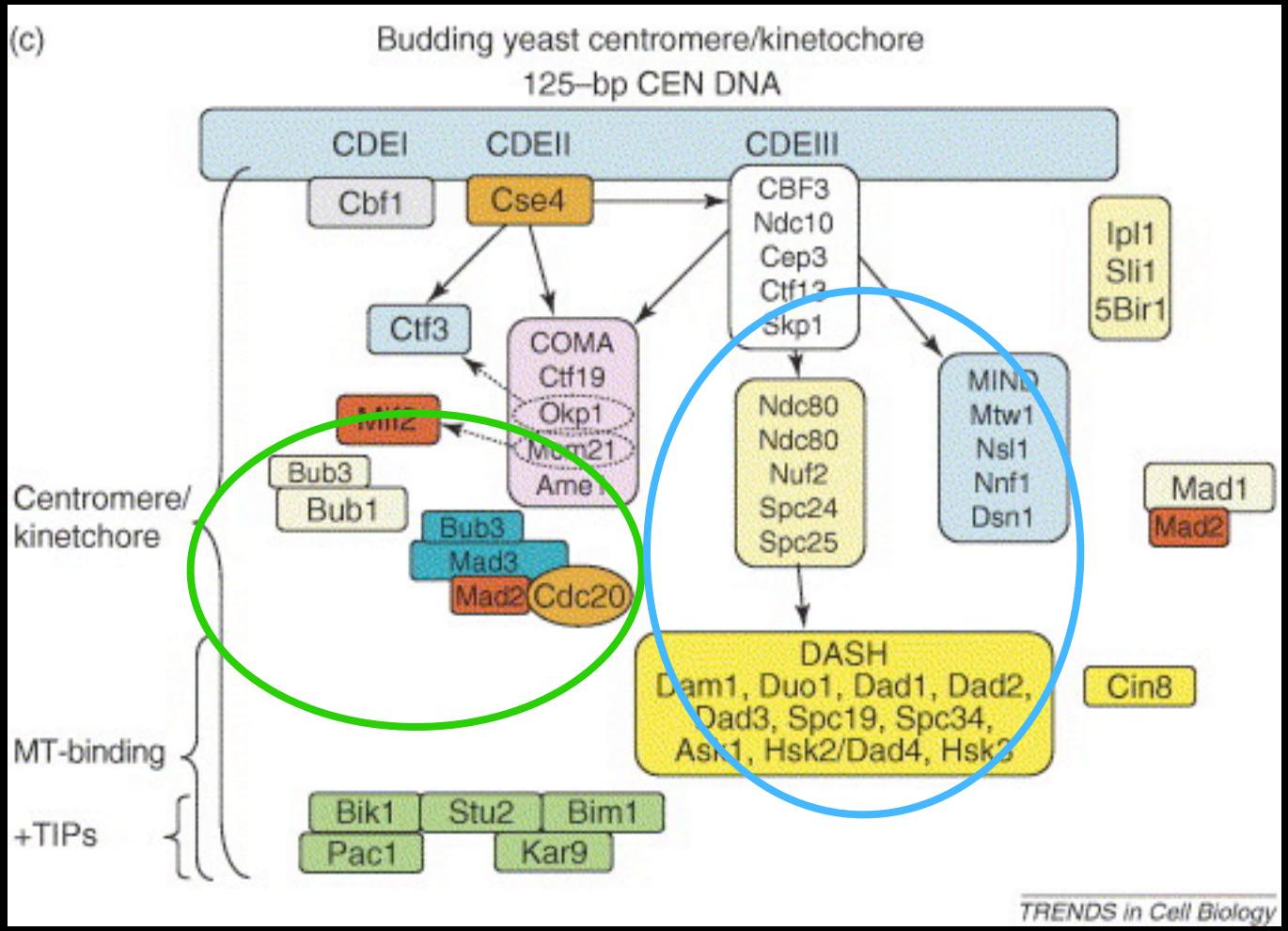
**MT attachment and movement**

**CENP-E, dynein = motor proteins** **MT plus end binding**

**checkpoint signaling**

# Marginally more simple in yeast

**small- 125 bp DNA, kinetochore binds single MT**




**~70 proteins**

**many homologous to metazoan kinetochore proteins**

**similar functional complexes**

**MT attachment and movement**

**checkpoint signaling**

# Search and capture

dynamic MTs act as searching devices

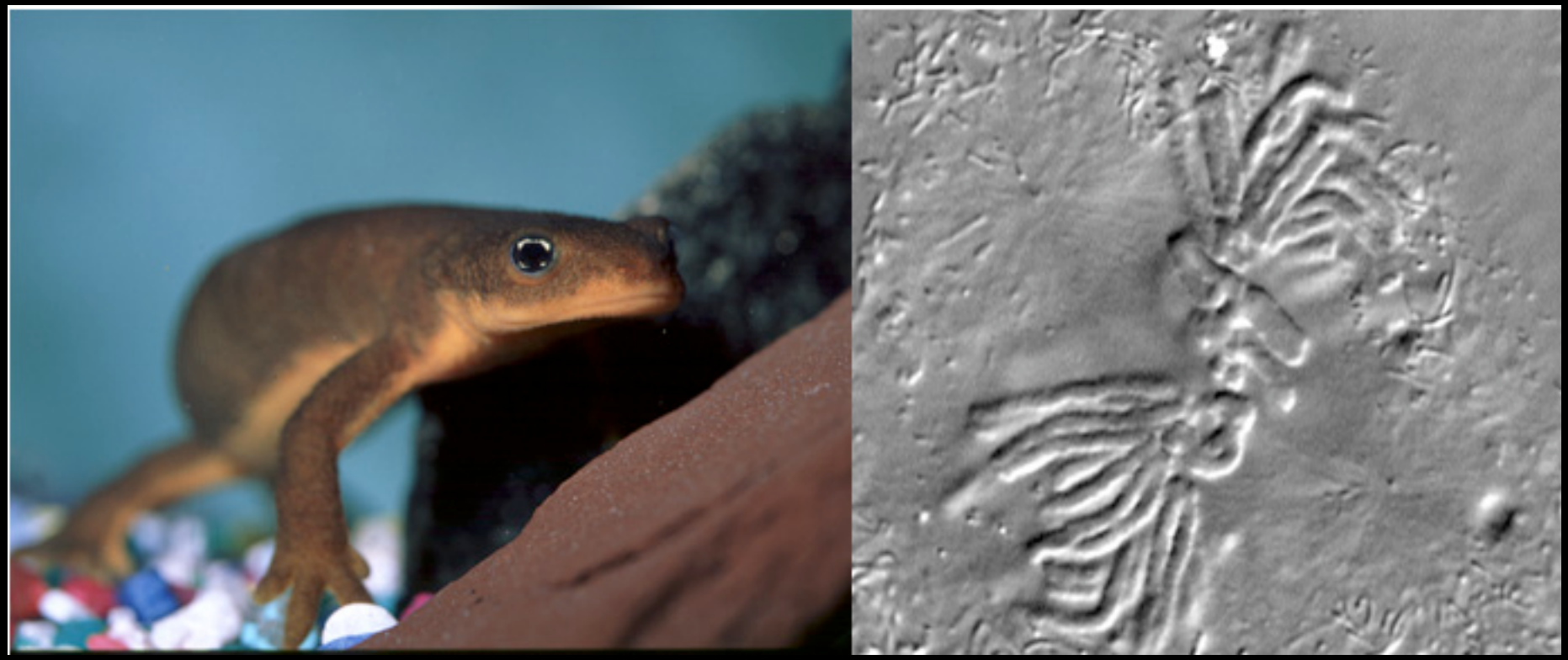
once captured by kinetochore, MT is stabilized

**best evidence:**

1) video recordings of Newt lung cells

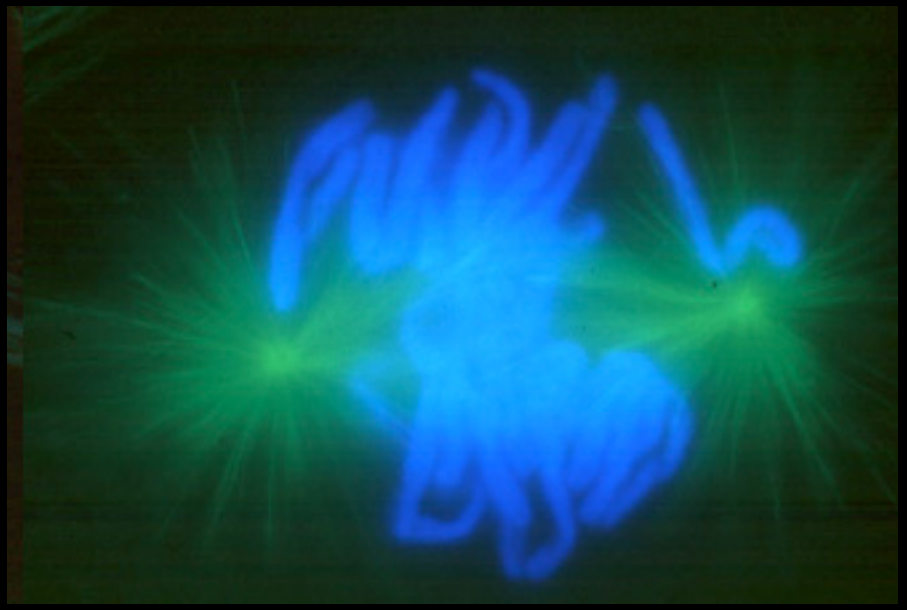
2) physical interaction between MTs and kinetochores in vitro

# kinetochore capture in Newt lung cell

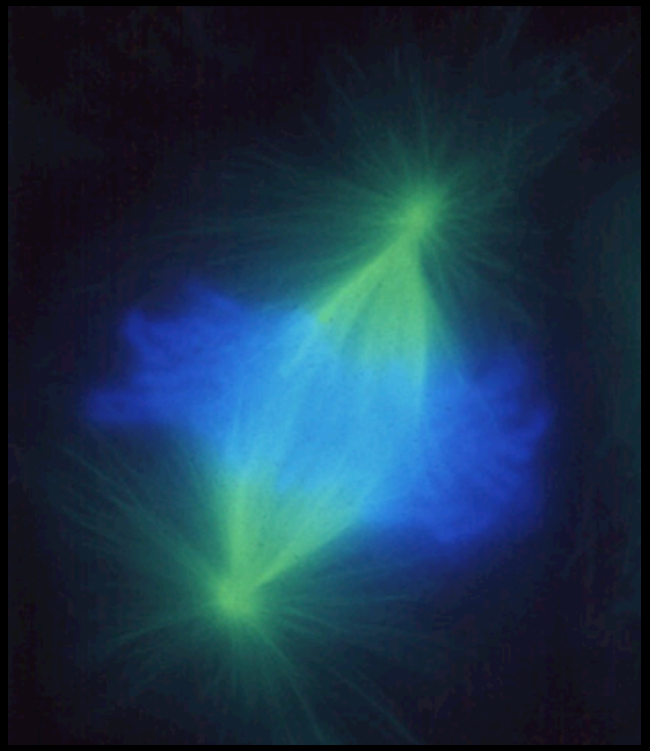


# Search and capture

# How do chromosomes gain bipolar attachments and congress ?

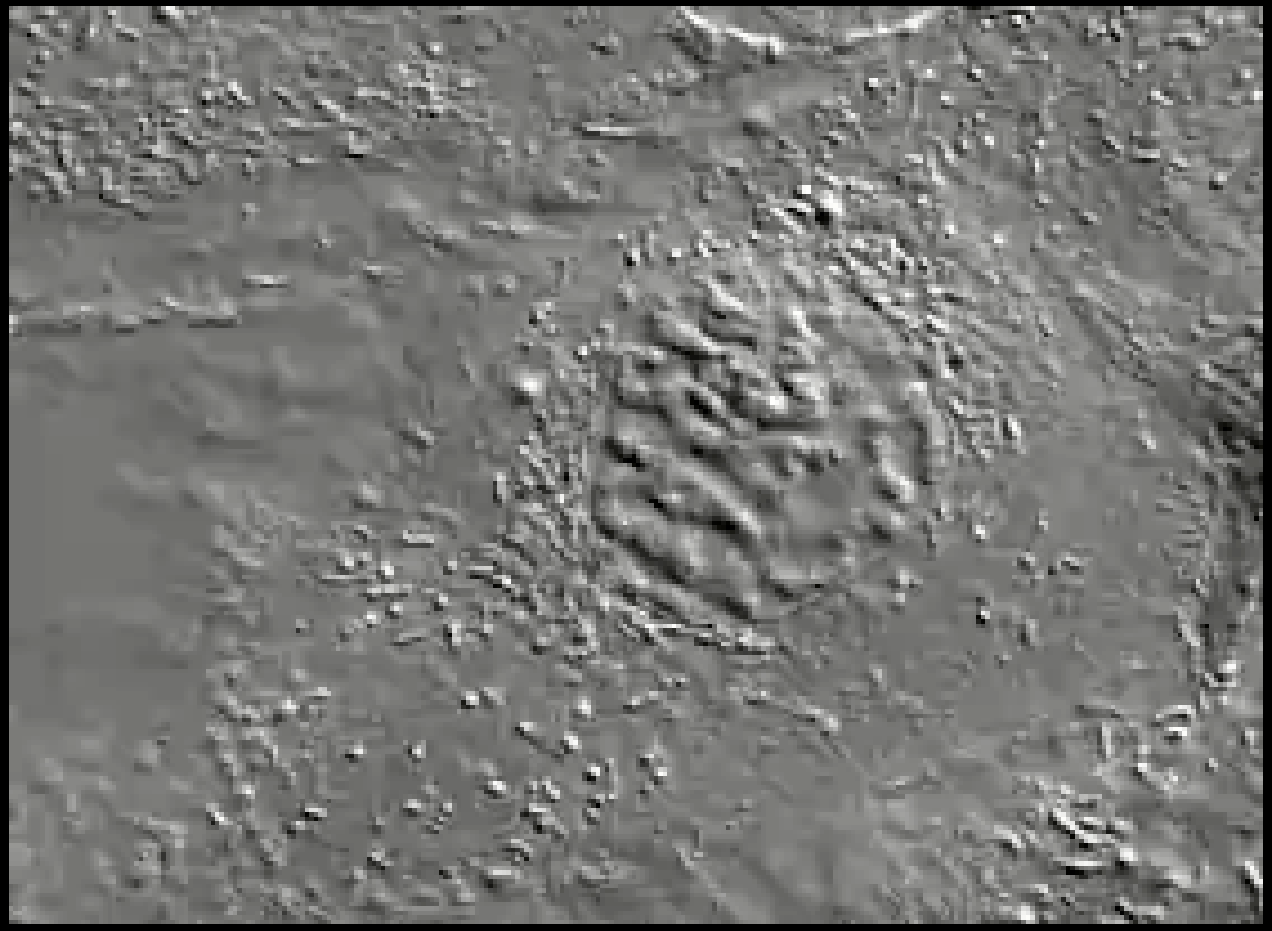


prometaphase



metaphase

# Sister chromatid pairs move to poles, congress to plate



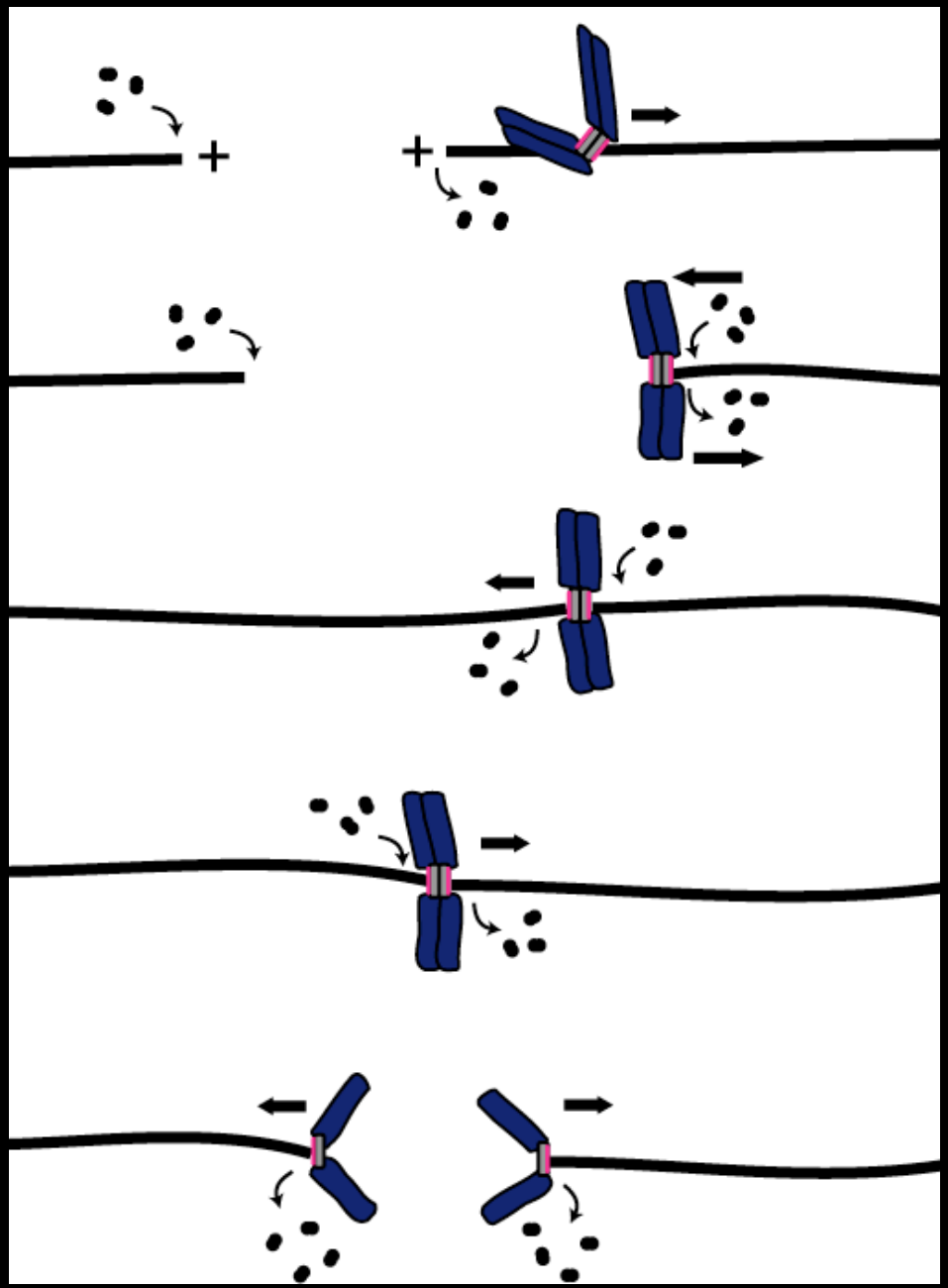
# Chromosome congression

Microtubules attach to kinetochores and chromosomes oscillate

Kinetochore MTs (K-MTs) polymerize/depolymerize at their plus ends
kinetochore stays attached!!!

Chromosome arms are pushed to the metaphase plate

# MT assembly/disassembly promotes bipolar attachment and congression



# How is kinetochore movement coupled to MT polymerization/depolymerization?

**motor proteins:**

| | |
|---|---|
| CENP-E | (+) end-directed |
| dynein/dynactin | (-) end-directed |
| Kinesin 13 | depolymerase |

**+TIPs/other MAPs??-retain attachment!!!**

- CLASP
- Dam1 complex?

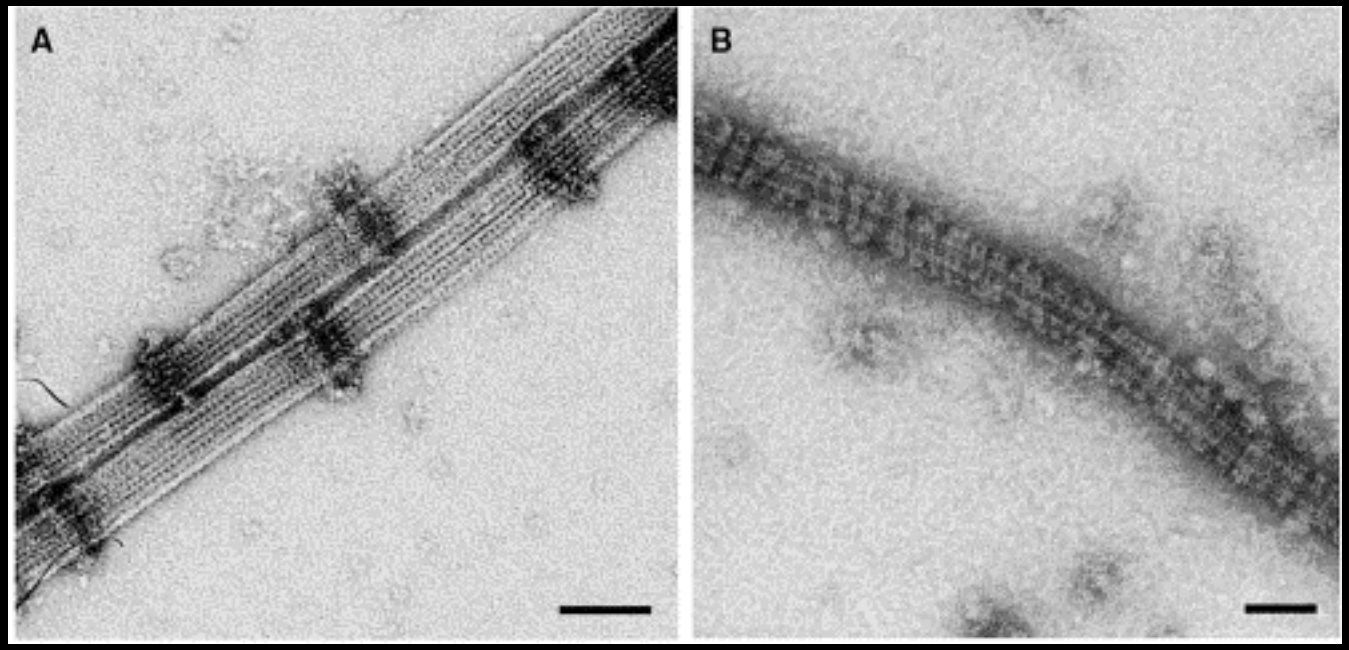
**pushing forces generated by motors on chromosome arms:**

- chromokinesins

# How maintain kinetochore attachment to microtubules ?

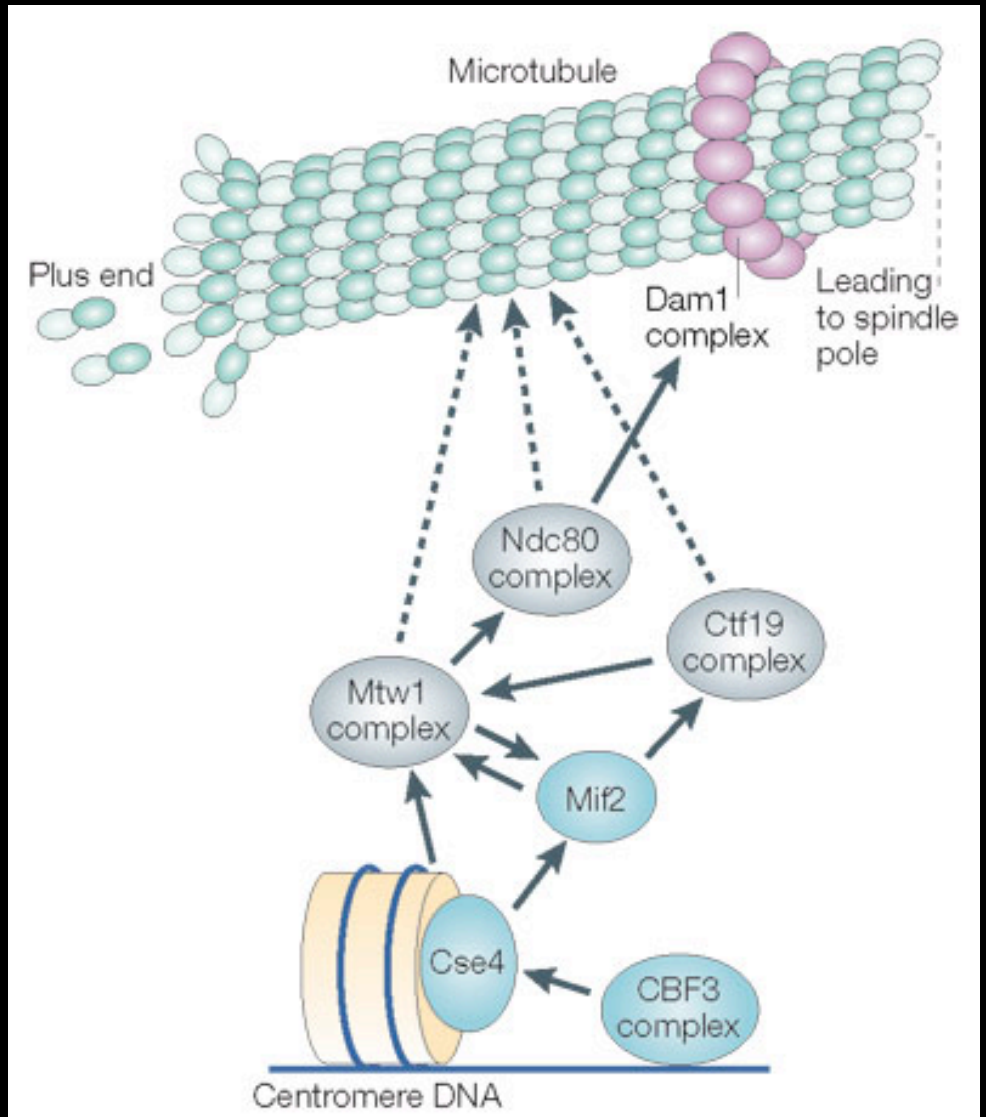
**Dam1 mediated MT-kinetochore attachment**

**complex can form rings around microtubules**



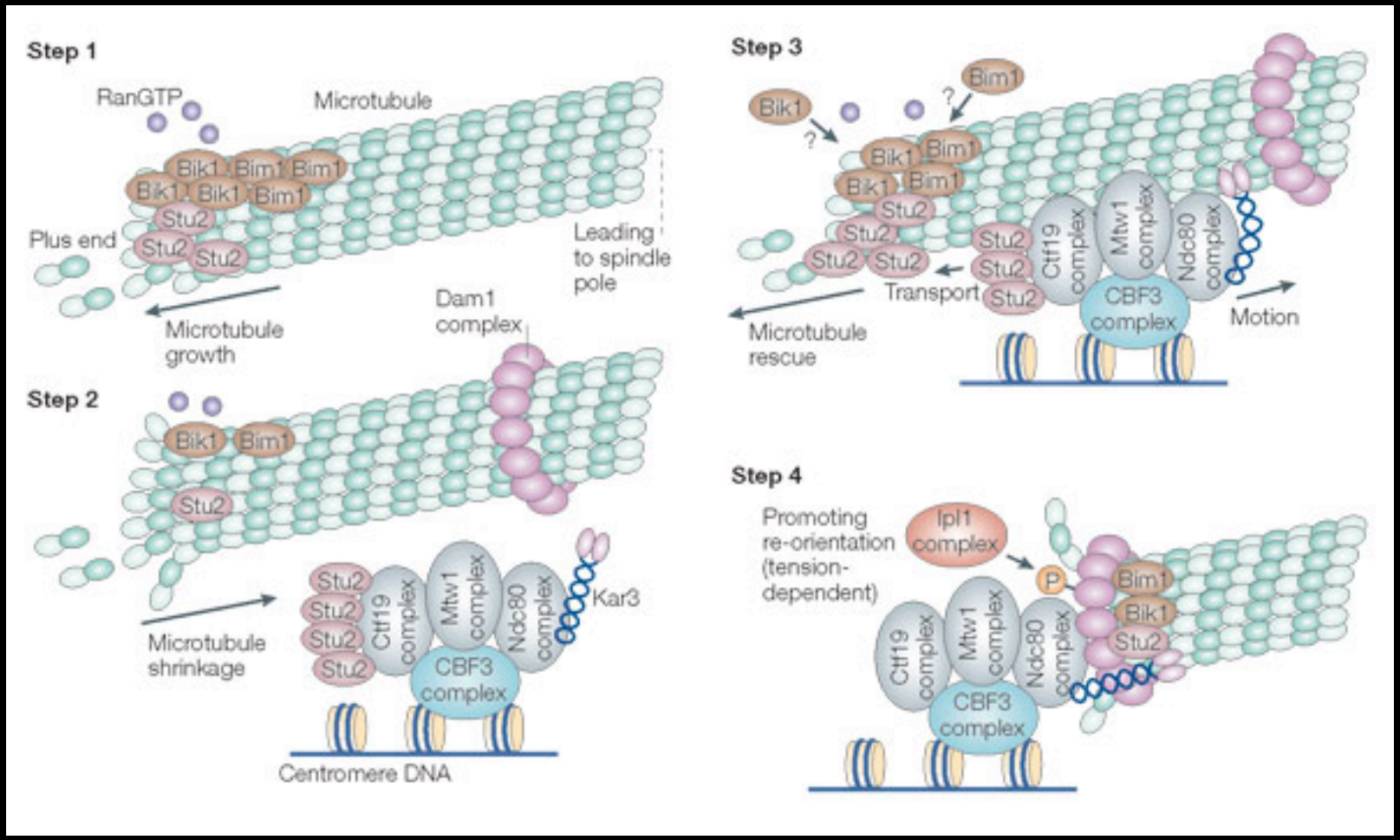

Barnes/Drubin
Nogales

**not present in organisms other than cerevisiae?**

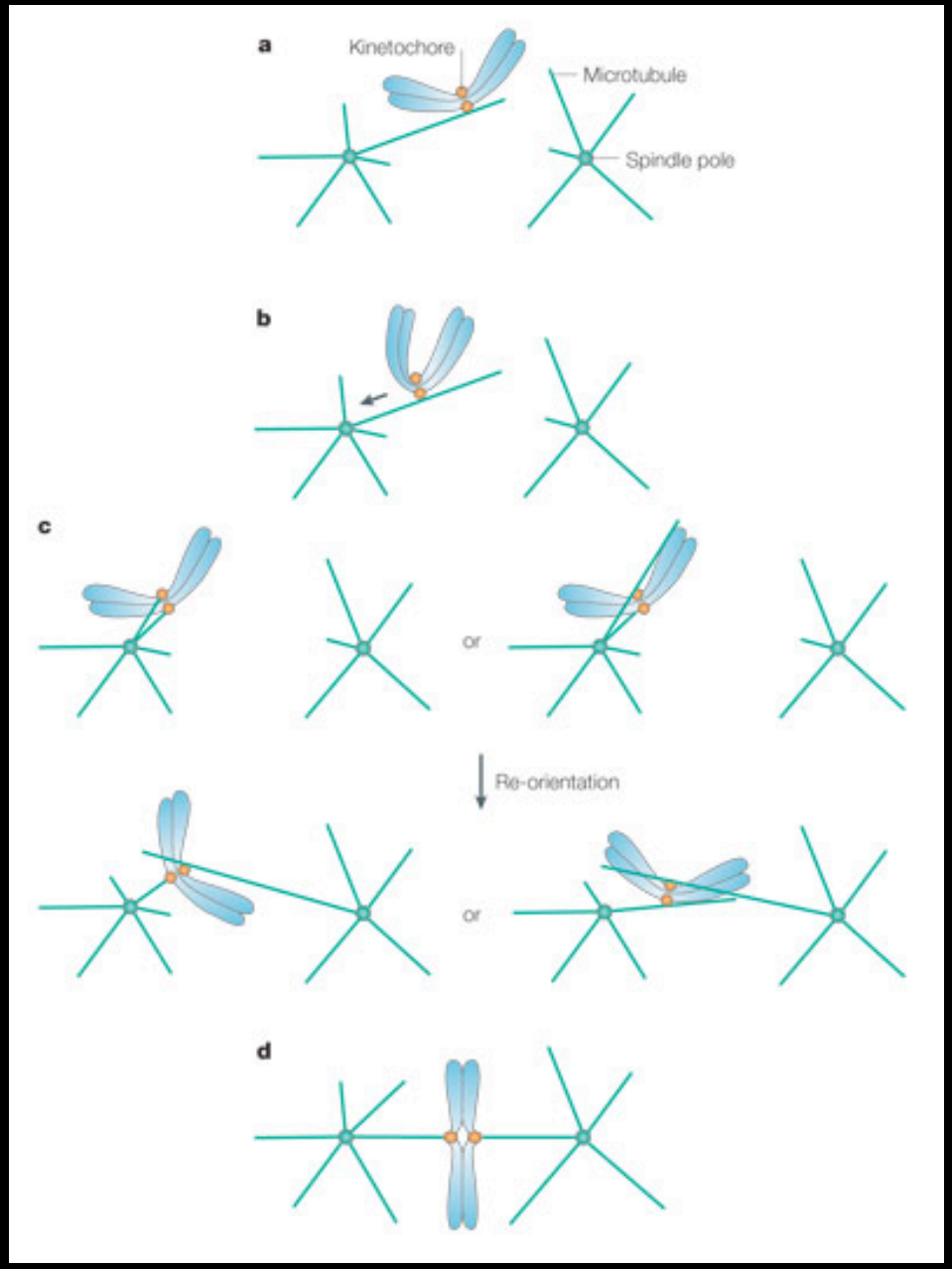




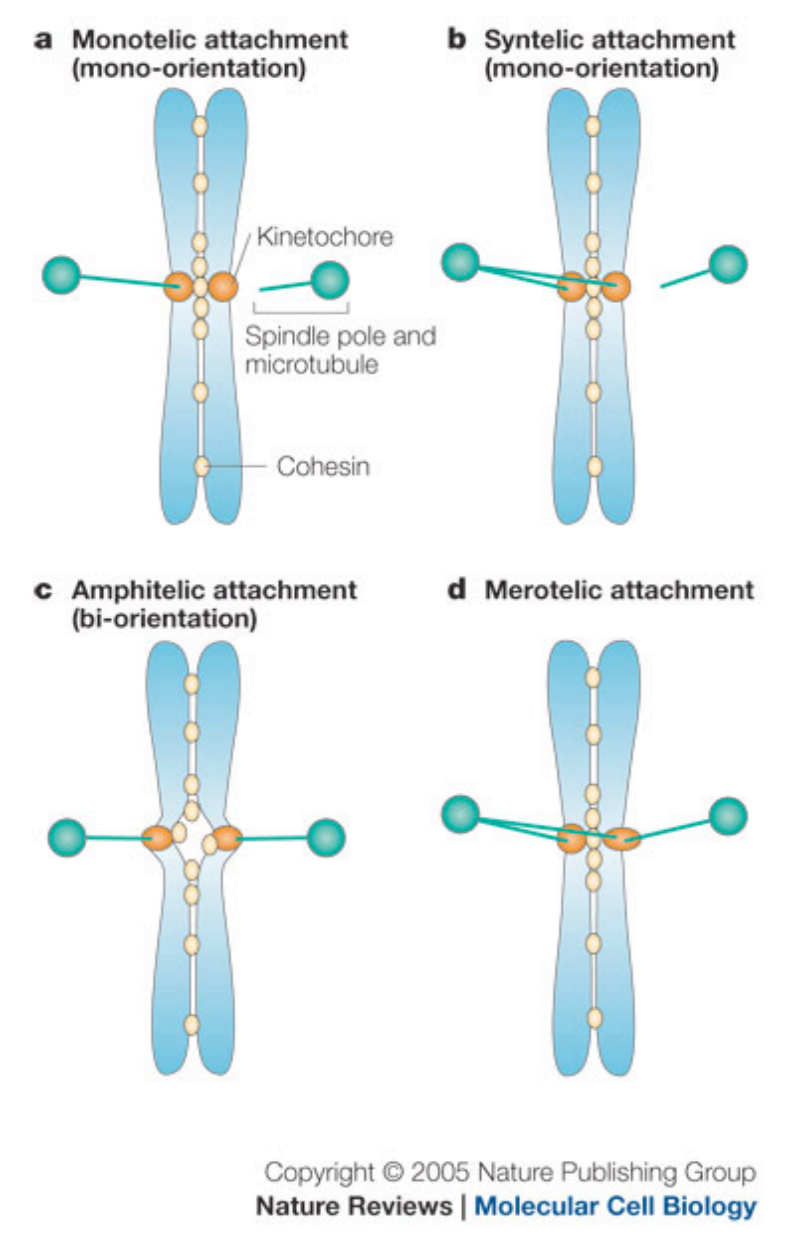
**Nature Reviews | Molecular Cell Biology**

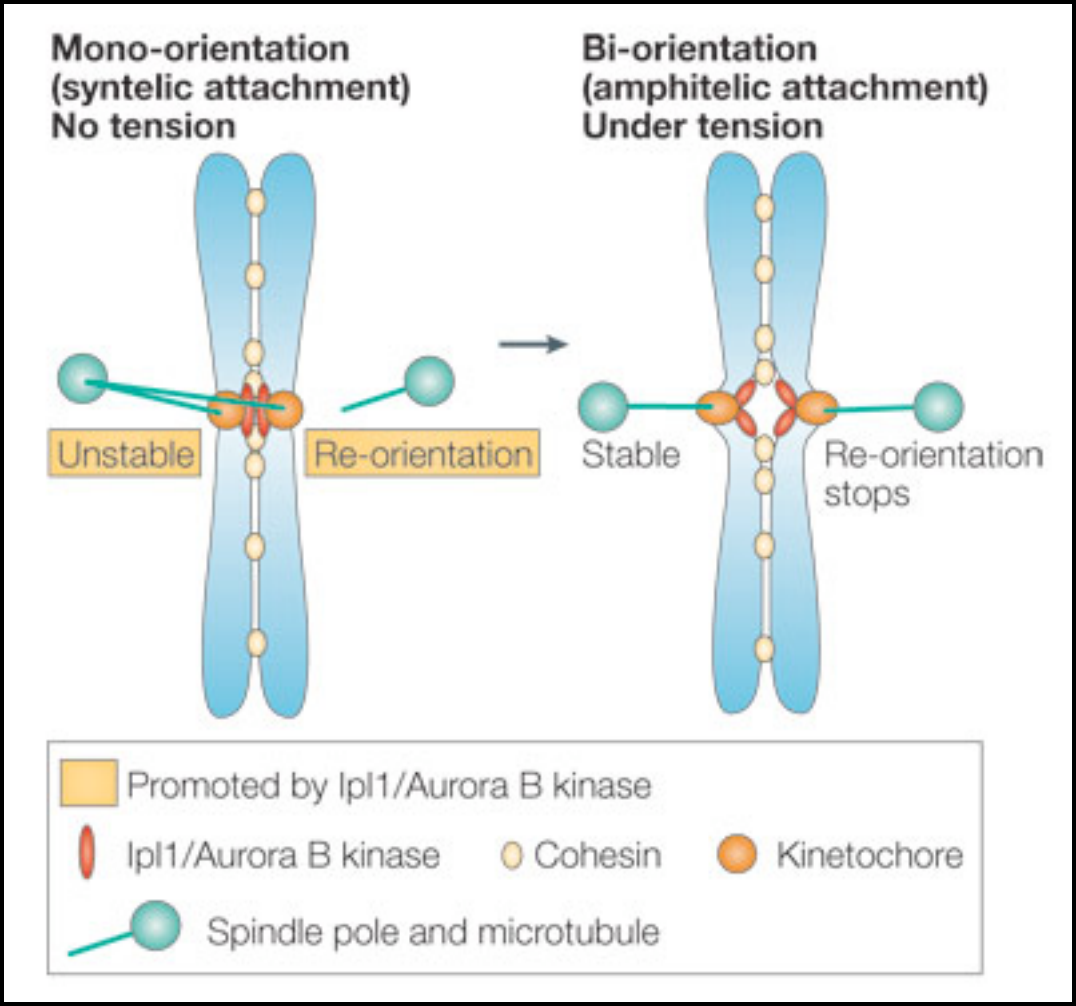

Step 1
RanGTP
Microtubule
Bik1
Bim1
Stu2
Plus end
Leading to spindle pole
Microtubule growth
Dam1 complex
Step 2
Microtubule shrinkage
Ctf19 complex
Mtw1 complex
Ndc80 complex
Kar3
CBF3 complex
Centromere DNA
Step 3
Transport
Microtubule rescue
Motion
Step 4
Promoting re-orientation (tension-dependent)
Ipl1 complex
P

# How are both sisters oriented properly?



# Four types of attachments during prometaphase

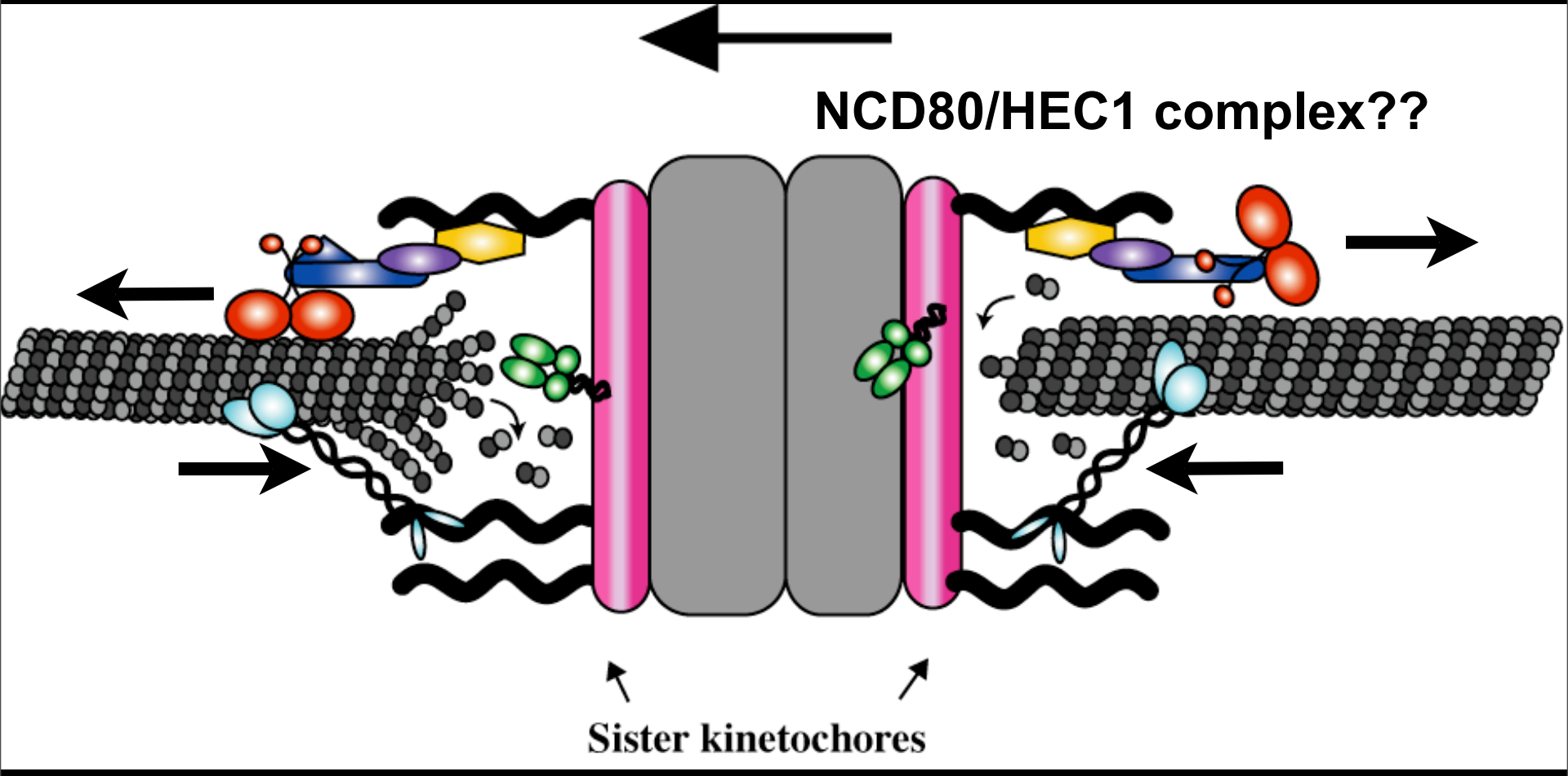





**bipolar attachment is stable**

# Model for motor function at the metazoan kinetochore

## attachment and movement




cytoplasmic dynein
minus end-directed

dynactin

ZW10

ROD

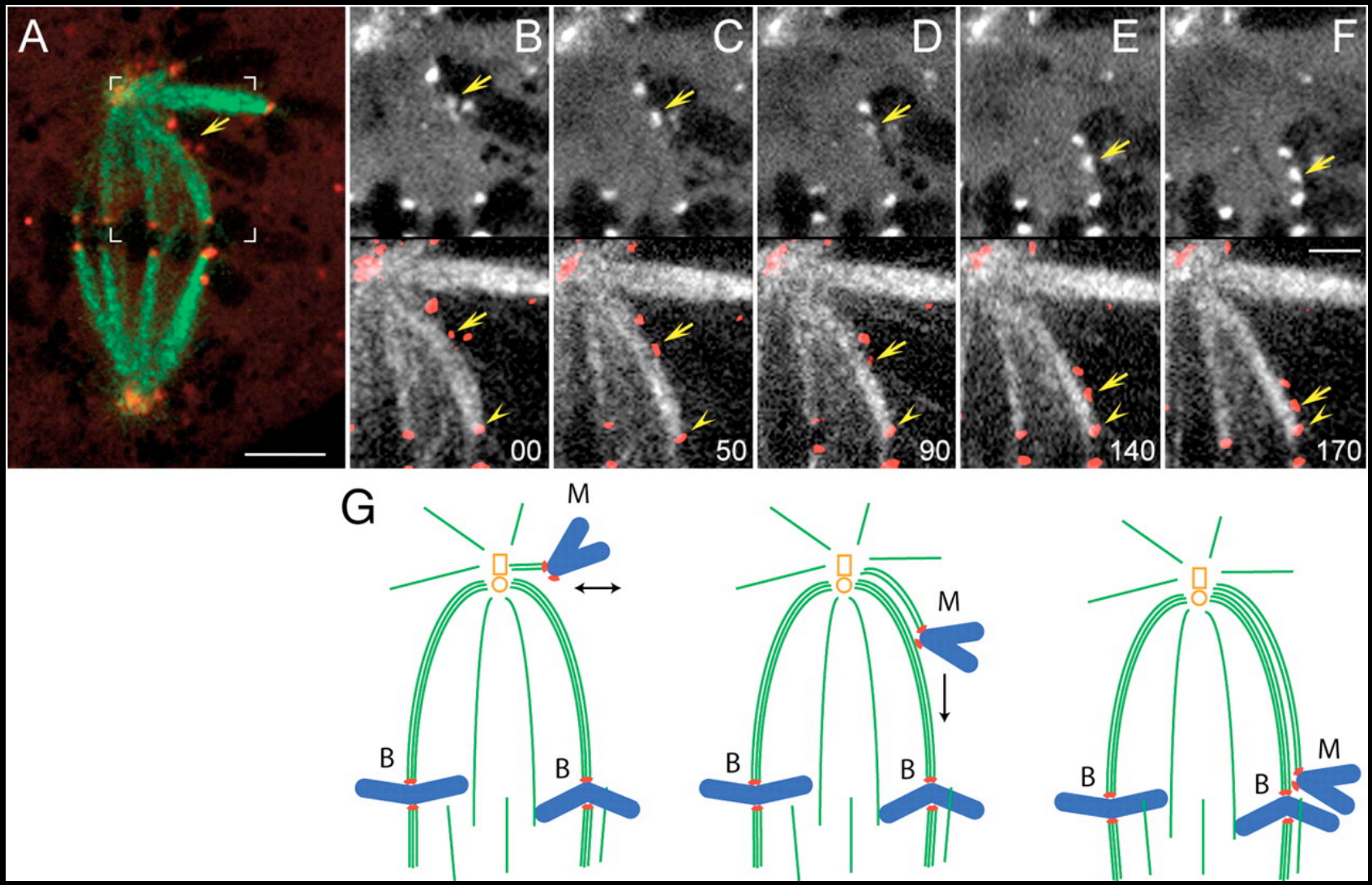
CENP-E
plus end-directed

MCAK/XKCM1
depolymerase

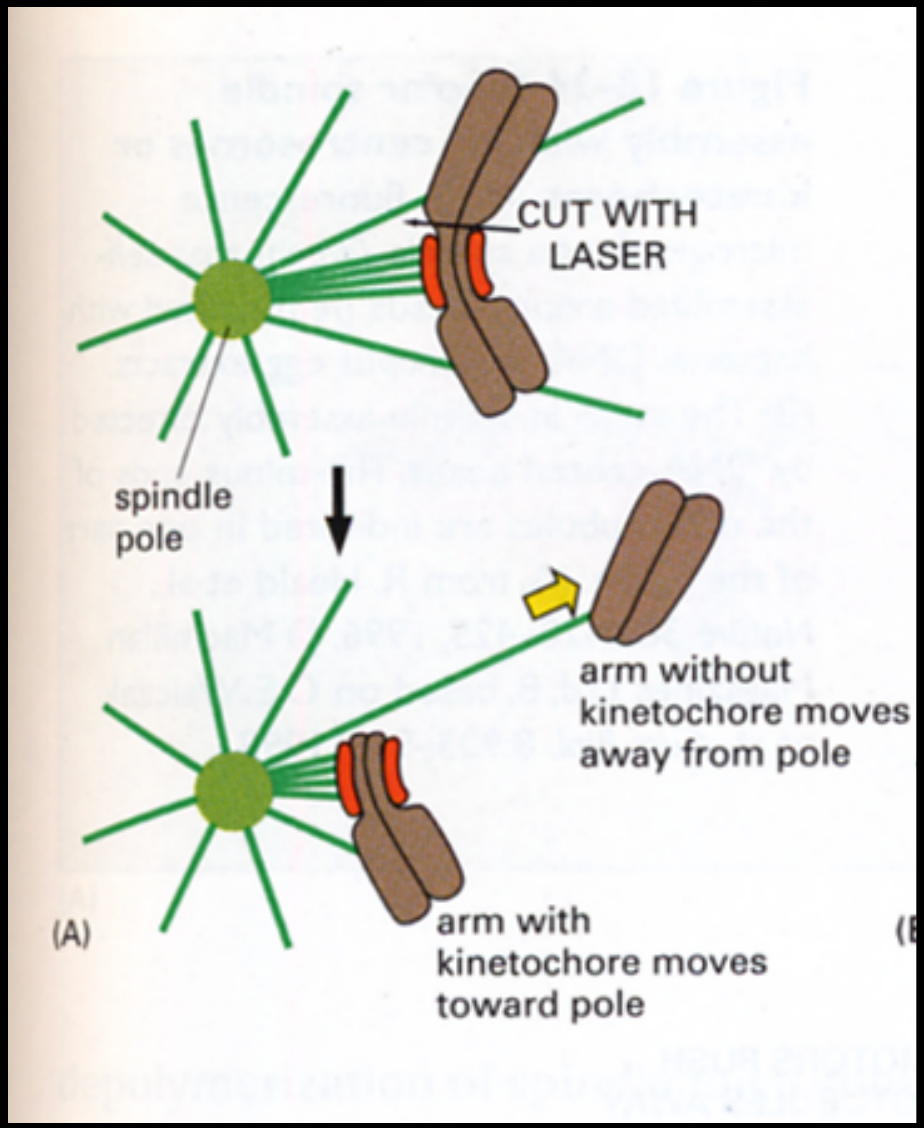
Search and capture inefficient if the chromosome is attached to one pole and far from the other pole

1) Unattached sister generates a microtubule bundle that can more easily be incorporated into the spindle

2) New role for CENP-E elucidated:
Motor transports unattached kinetochores to metaphase plate along K-fiber of bioriented chromosome


A
B
C
D
E
F
00
50
90
140
170
G
M
B
B
M
B
B
M
B
B

# Antipolar forces or ‘polar wind’ contributes to congression



# Model for motor function on chromosome arms

## chromokinesins