Lecture 8

Integrating spindle dynamics and chromosomes

Outline:

- **Maintenance of the metaphase spindle**
- **Anaphase initiation and progression**
- **Cytokinesis**

Paper: Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1

Establishment of the bipolar spindle

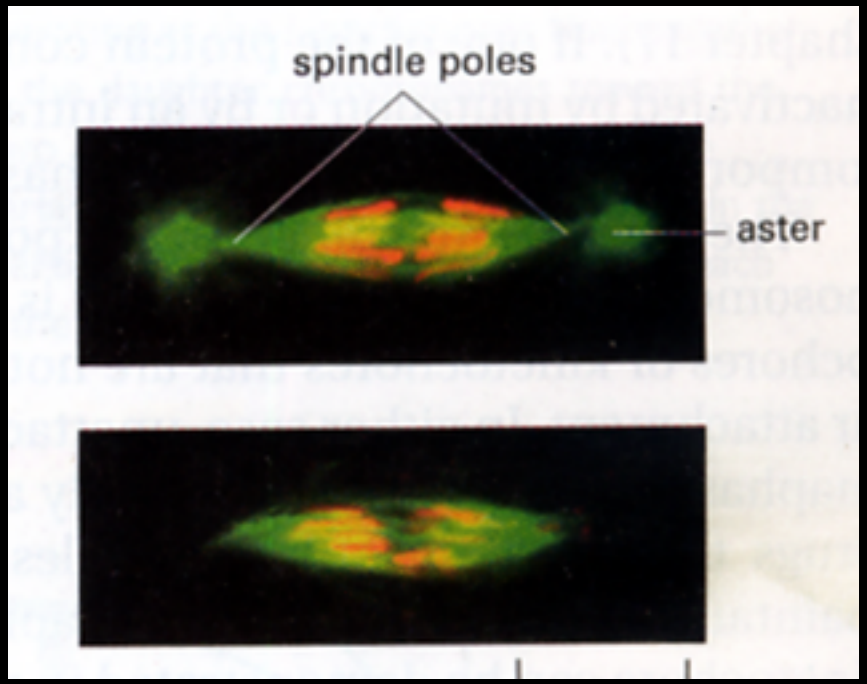
Microtubules become highly dynamic at the onset of mitosis

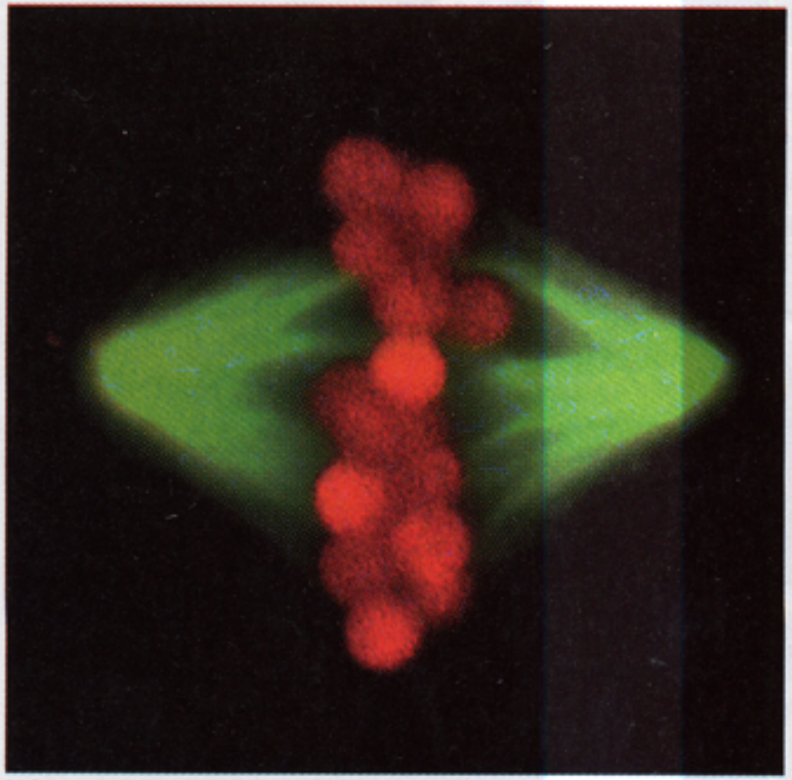
localized activity of MAPs and catastrophe promoting factors

Both kinetochores and chromosome arms contribute to spindle stabilization and congression

some spindles form without centrosomes (eg meiosis)

Bipolar spindles without centrosomes ?





(A)

10 μm

Xenopus egg extract and DNA-coated beads ( )

MICROTUBULES ARE NUCLEATED IN REGION AROUND BEADS

microtubules

PLUS-END-DIRECTED MOTORS (•—•) BUNDLE AND SORT MICROTUBULES

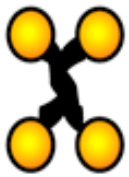
PLUS-END-DIRECTED MOTORS PUSH MINUS ENDS OF MICROTUBULES AWAY FROM BEADS AND MOVE ANTIPARALLEL MICROTUBULES APART

MINUS-END-DIRECTED MOTORS (•) FOCUS SPINDLE POLES

(B)

How is bipolar spindle maintained in metaphase?

Three Spindle Motors



KIN N bipolar
tetrameric plus end-directed

Kinesin 5

cross-links MTs
pushes poles apart
promotes bipolarity

KIN C
dimeric minus end-directed

Kinesin 14

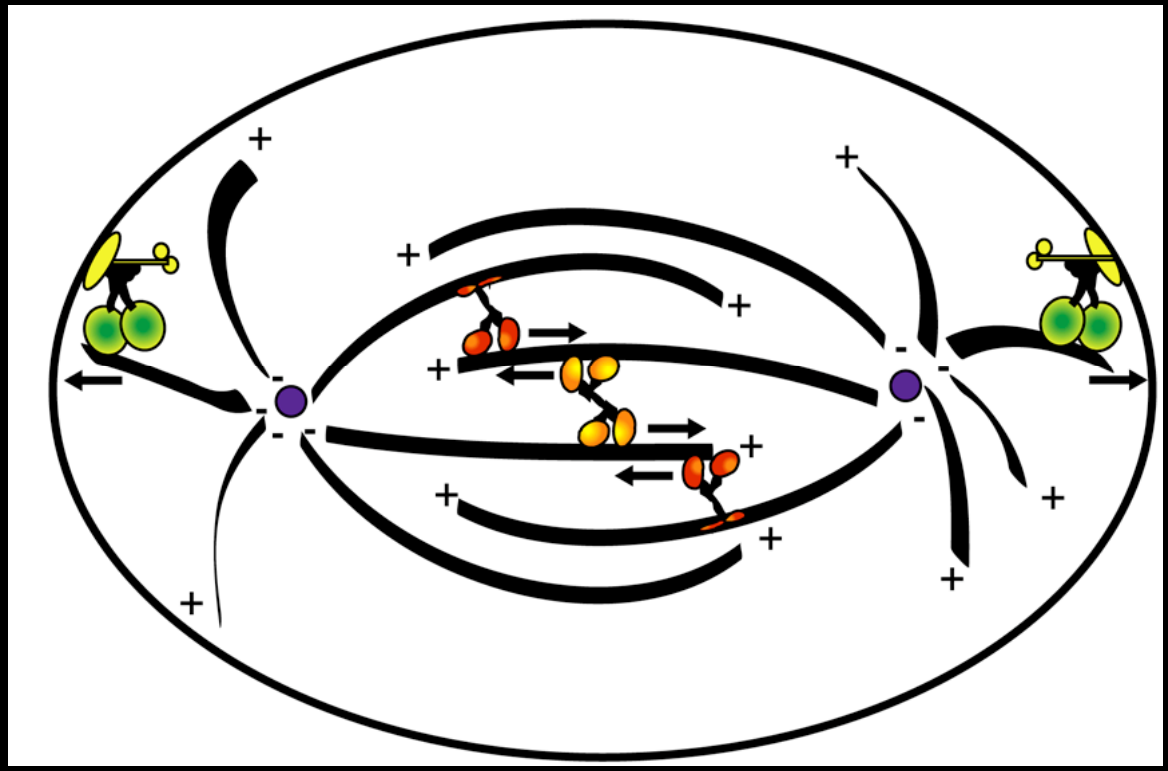
provides opposing force
pushes poles together

Dynein/dynactin
minus end-directed

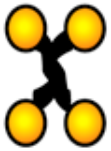
Dynein

at cortex - pulls poles apart
at pole - focuses minus ends

Motors create balance of forces independent of kinetochore



Kinesin 5



KIN N bipolar
tetrameric plus end-directed

push poles apart

Kinesin 14

KIN C
dimeric minus end-directed

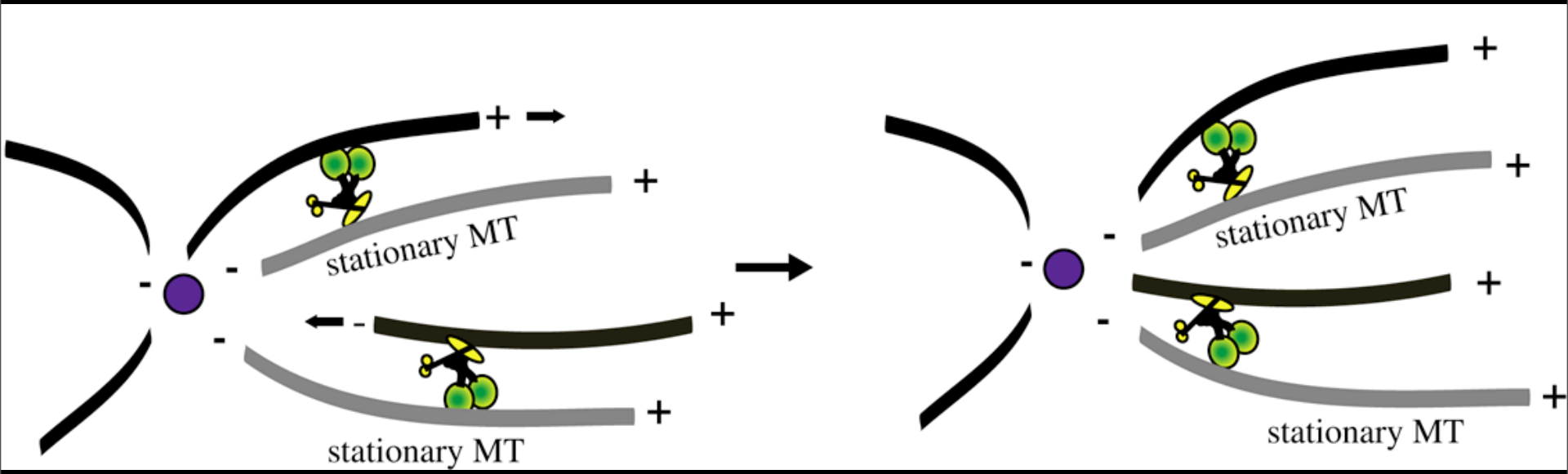
push poles together

Dynein

Dynein/dynactin
minus end-directed

pull poles to cortex
focus -ends at poles

Dynein pulls poles to cortex



Dynein/dynactin
minus end-directed

Useful system to explore balance of forces: Drosophila syncytial embryo

many nuclei in common cytoplasm synchronous divisions (8-10 min!!)

inject labeled tubulin

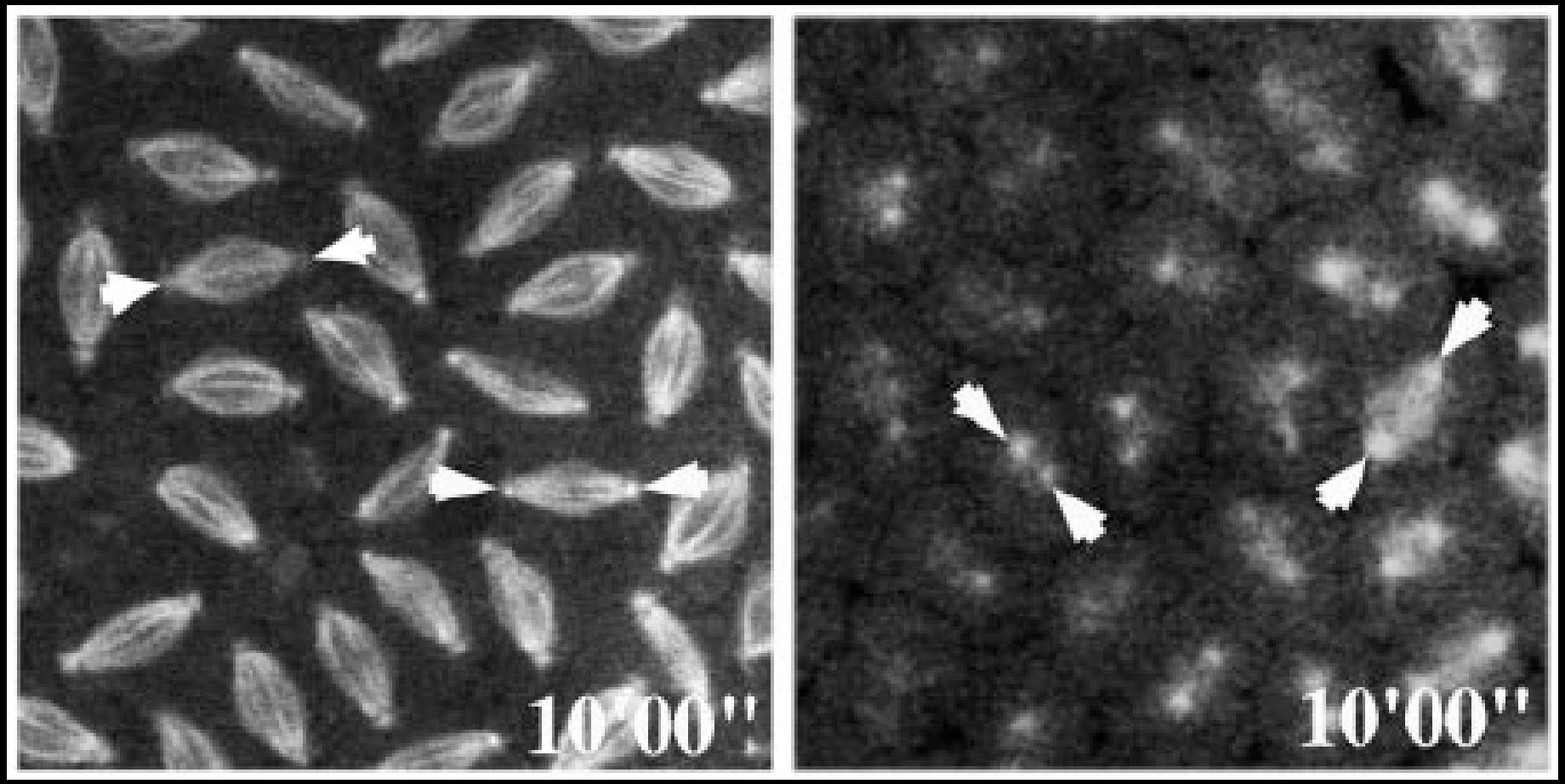
disrupt motor function:

mutants

microinjection of antibodies

control

injected with antibody to bipolar Kinesin 5



spindle collapse

Poleward Flux

another kind of movement in the spindle

MTs turnover

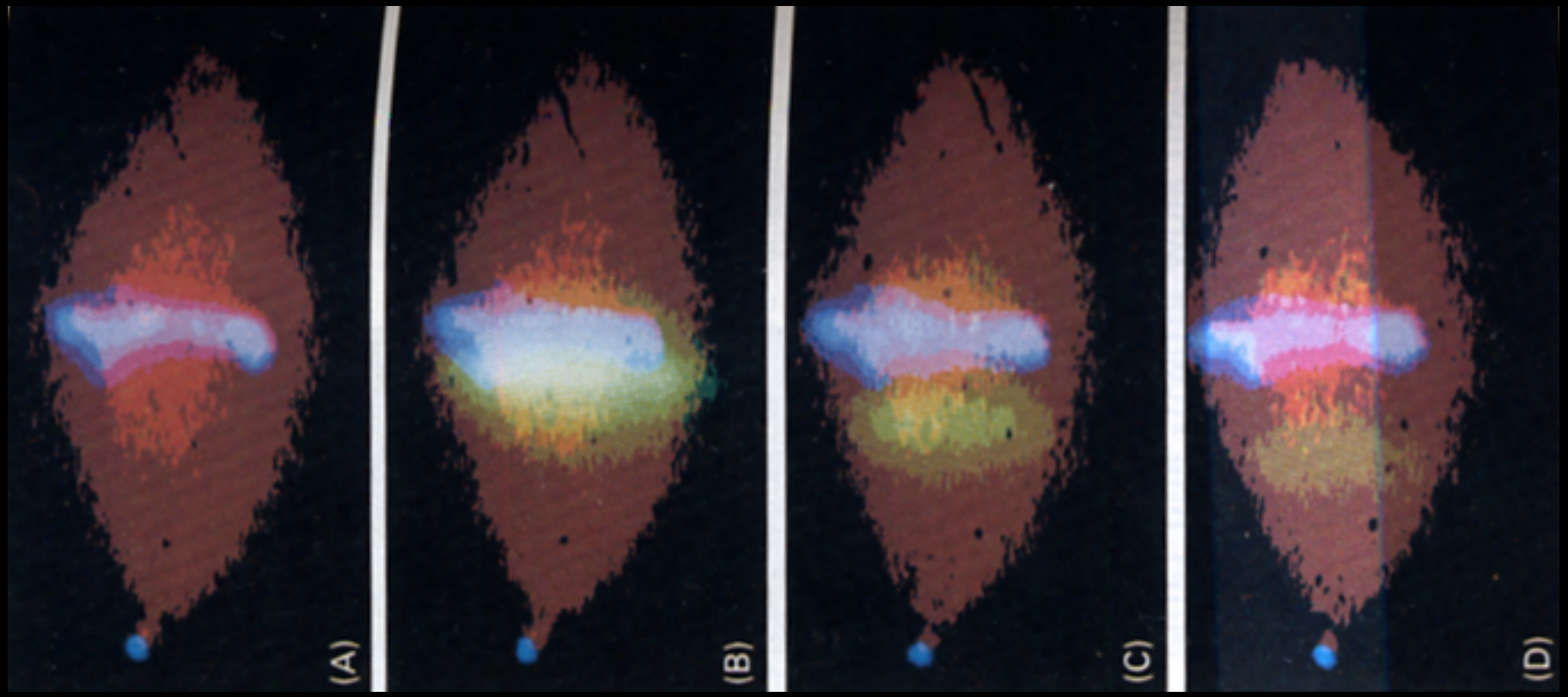
requires ATP hydrolysis

Fluorescence Speckle Microscopy

spike with fluorescent tubulin (low concentration)

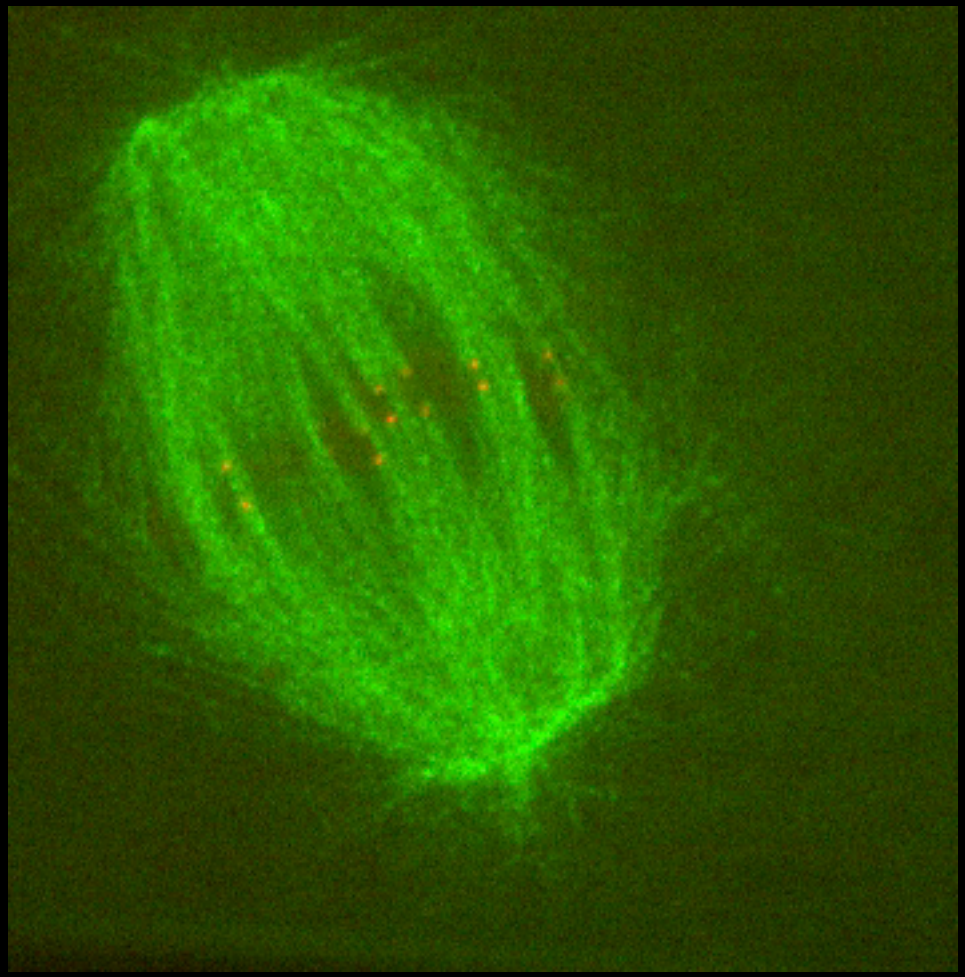
Photobleaching of ‘Caged’ Fluorescent Tubulin

release fluorescence only in photobleached area
follow over time



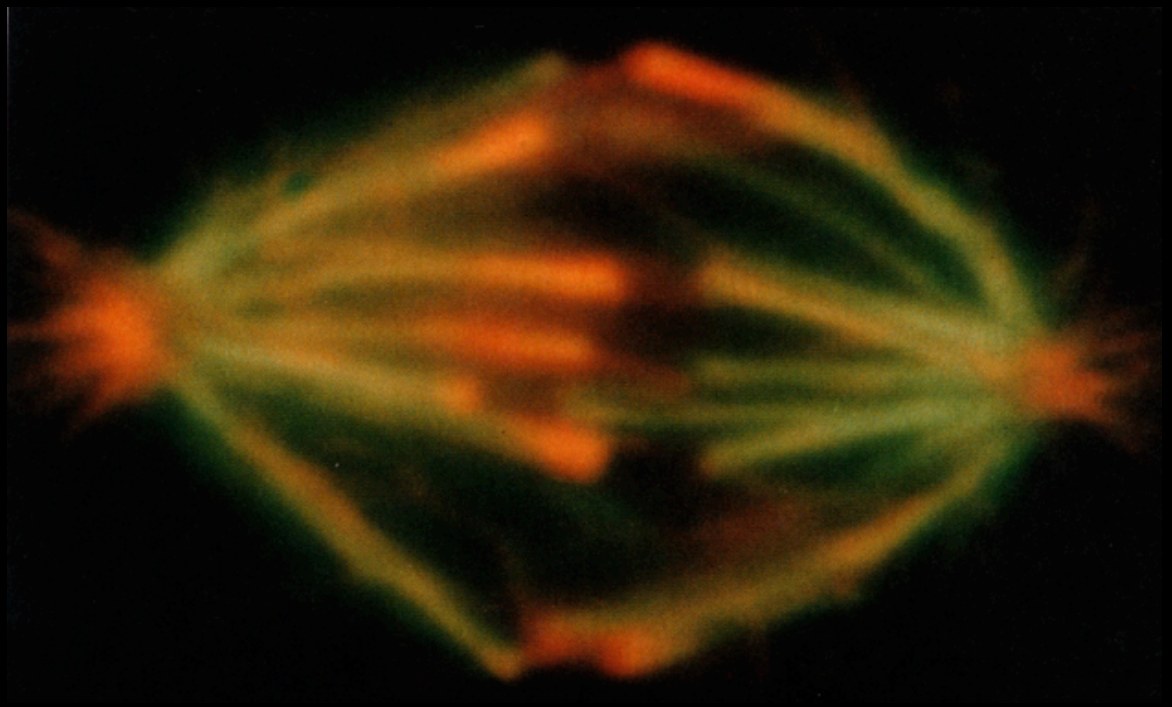
movement toward poles

but kinetochores remain at plate



how do kinetochores remain at the plate ?

add rhodamine-labeled tubulin to identify newly polymerization

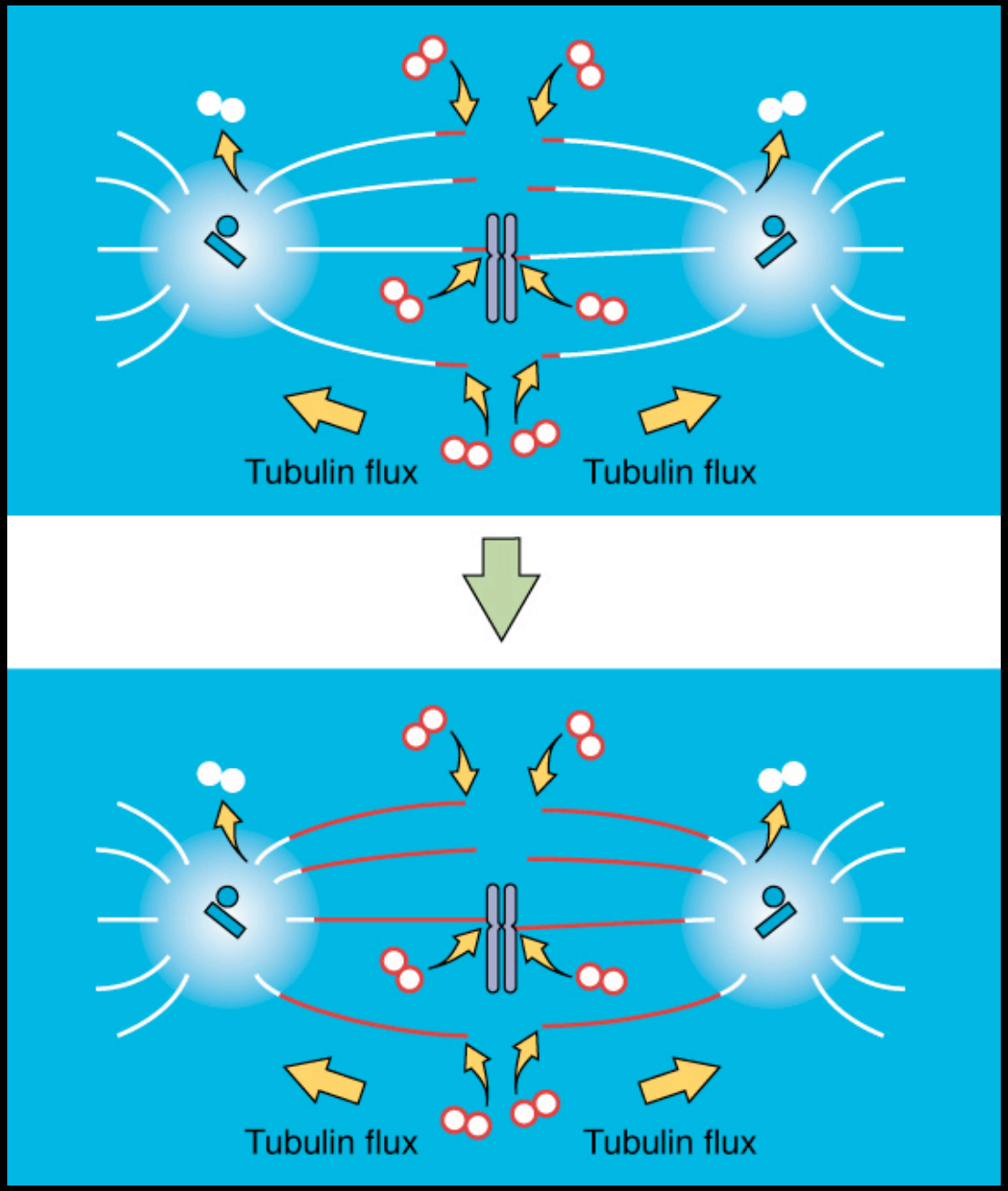


newly polymerized tubulin at (+) ends

depolymerize at poles

and kinetochores remain attached (Dam, linkers)

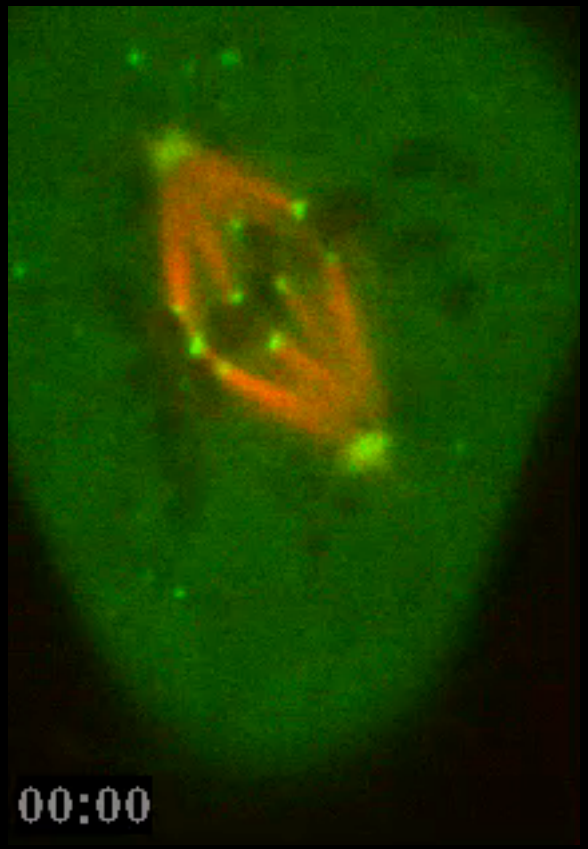
Implications of poleward flux



MT ends are free to exchange tubulin subunits in the spindle
dynamic attachments contributes to force balance

MT movement could be harnessed to move chromosomes poleward in anaphase

Stability of metaphase spindle : chromosomes still oscillate

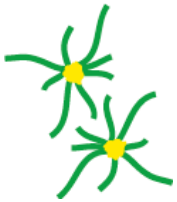


flux

kinetochore: motors and MT polymerization

global changes in microtubule dynamics: less catastrophe

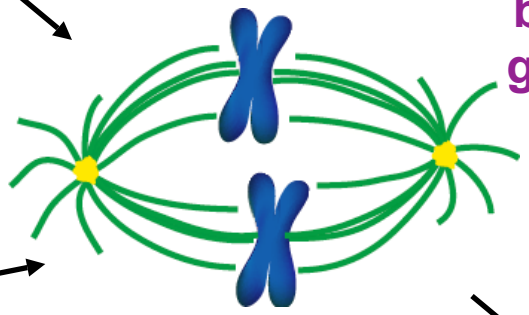
?



local regulation of microtubule dynamics
poleward flux of MTs

?

balance of dynamic forces
generates metaphase state



?

organization by microtubule-based motor proteins

at kinetochores

on chromosome arms

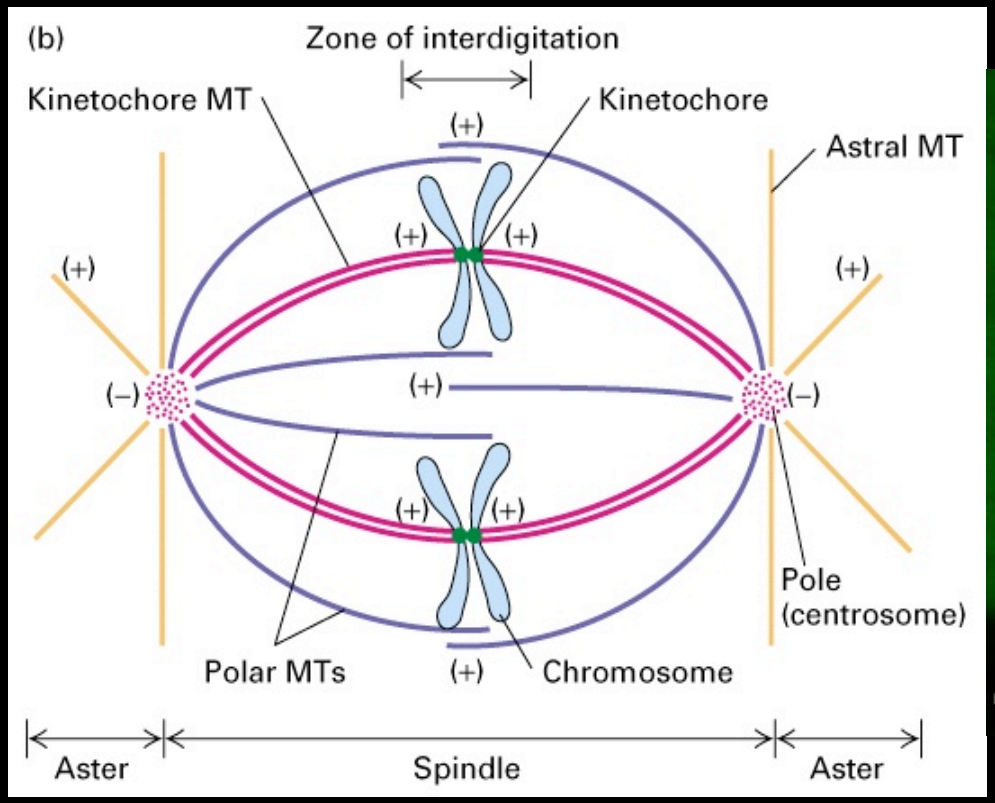
on spindle and astral microtubules

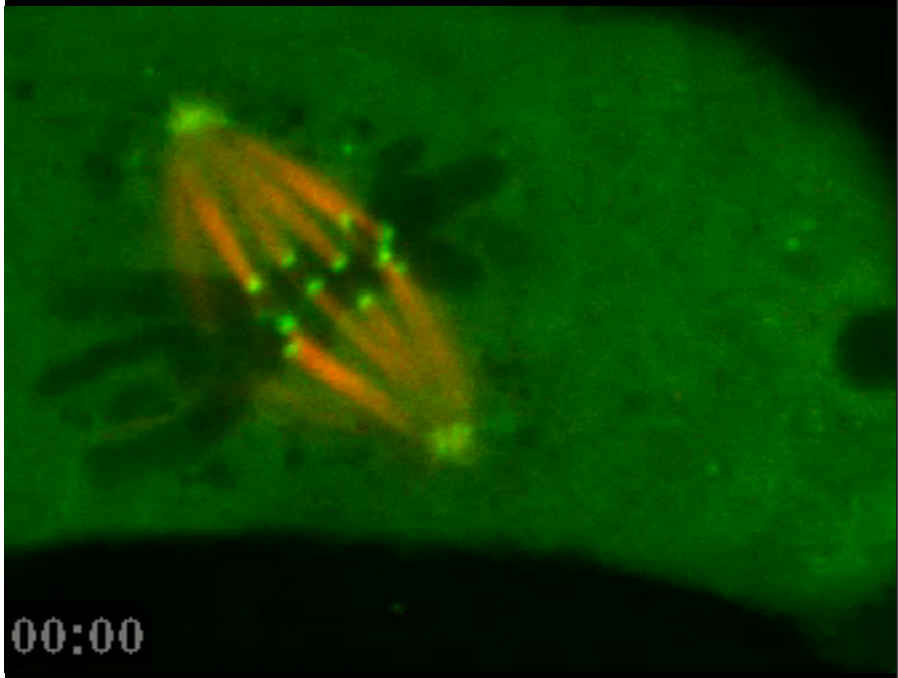
disrupt balance to enter
anaphase

The Onset of Anaphase

Balance of forces- MT dynamics, motors and chromosomes

- asters pushing on cortex (dynein)
- polar MTs and motors (kinesins) pushing poles together
- antipoleward forces on arms (MTs and chromokinesins)
- poleward flux of MTs (assembly at + end disassembly at - end)
- MT assembly and motors (kinesins) at kinetochores causes oscillations
- sister separation resisted by cohesion and bipolar orientation of sister kinetochores





What's needed to initiate Anaphase ?

Tension as a regulator:

signals anaphase onset

influences microtubule-kinetochore attachments and movement

Evidence:

laser ablation of unattached kinetochore induces anaphase

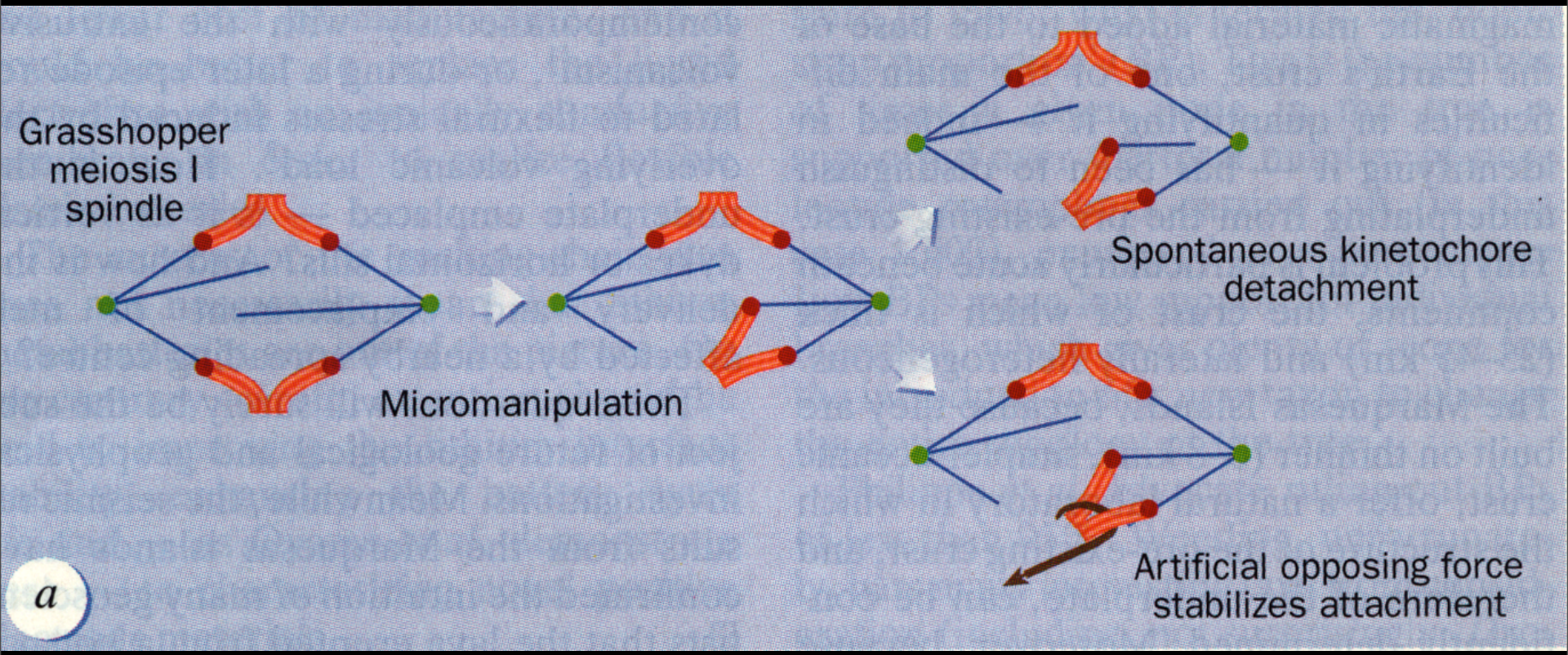
Checkpoint:
unattached kinetochore sends WAIT! signal

signal depends on having all kinetochores attached to the spindle (Spindle Assembly Checkpoint (SAC) or mitotic checkpoint

responds to tension or attachment, or both

Nicklas lab - insect spermatocyte micromanipulation

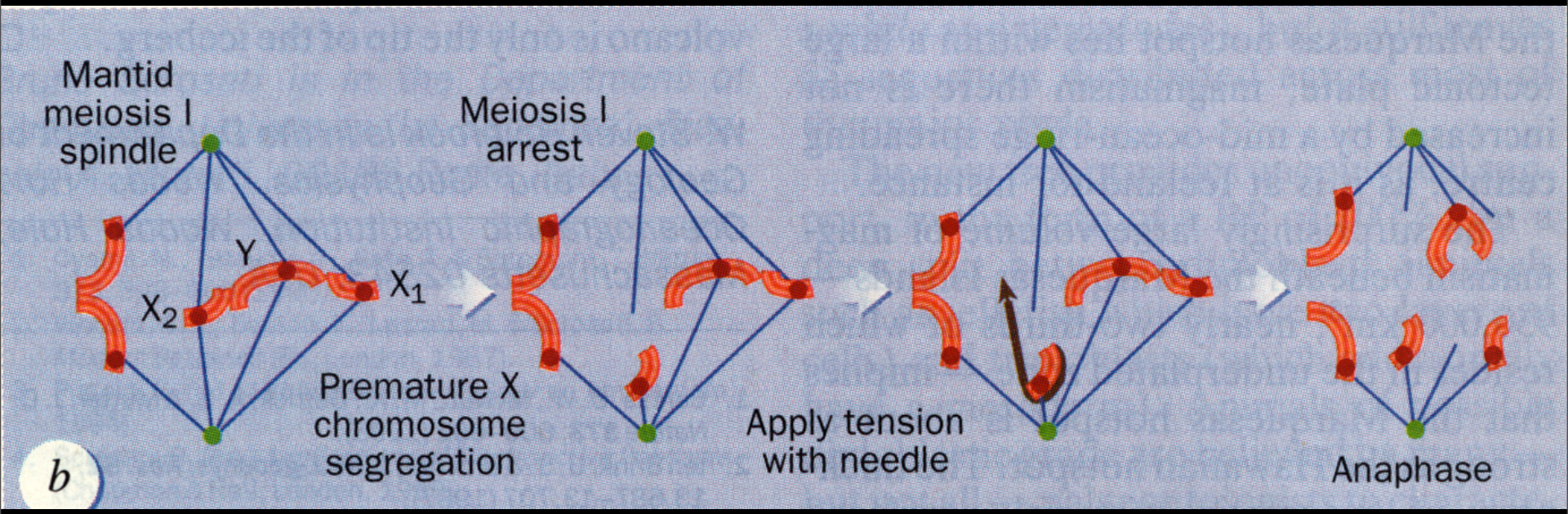
1) only bipolar attachments are stable



1969!

2) tension regulates anaphase onset

sex chromosome segregation:
2 X chromosomes pair with Y, unstable



1995

What's needed to initiate Anaphase ?

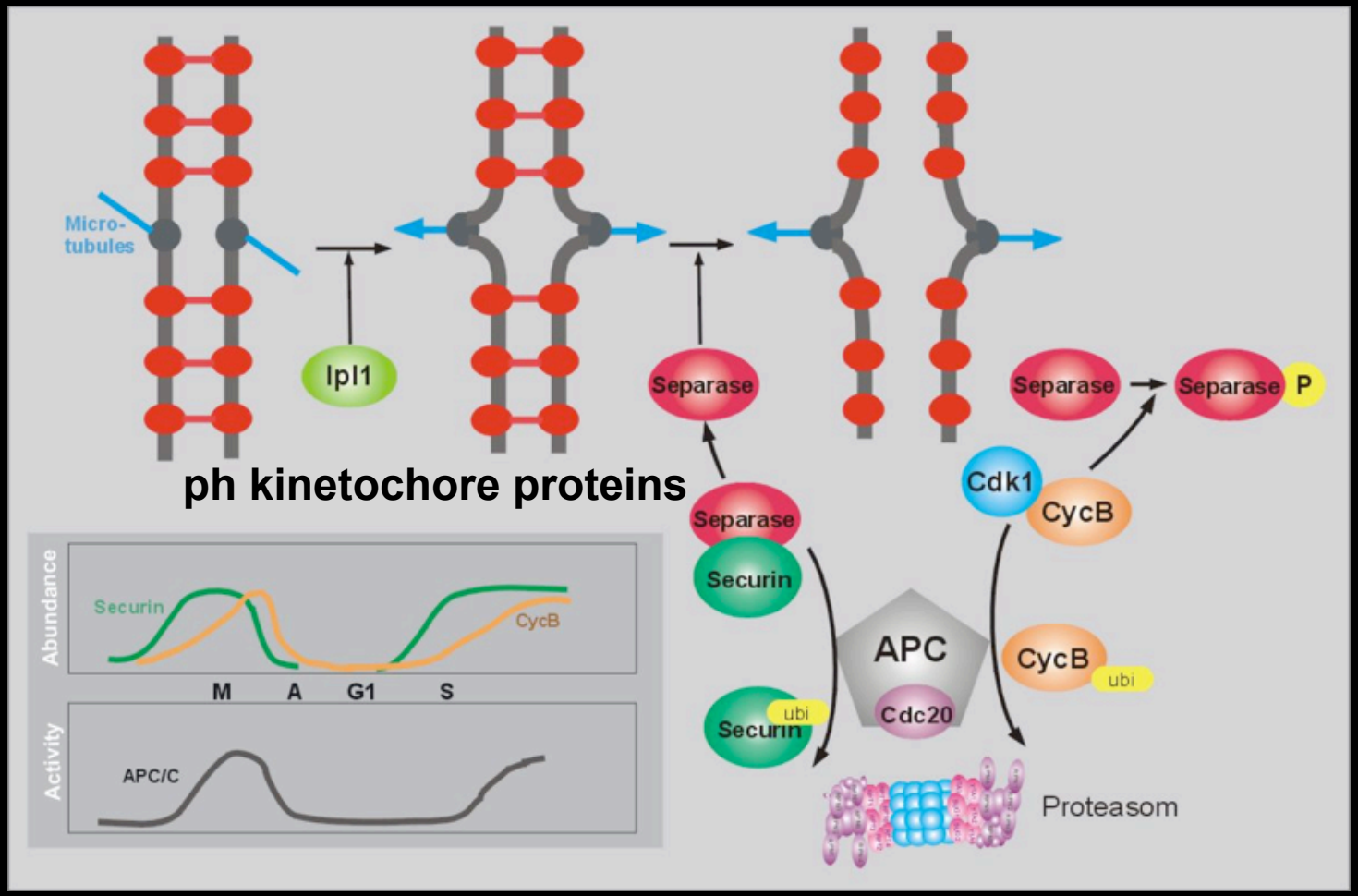
disrupt balance of forces

- remove sister cohesion
  - allows separated sister kinetochores to move poleward
- maintain polar MTs pushing poles toward the poles
- maintain poleward flux of MTs, BUT depolymerize MTs at kinetochore
- maintain antipoleward forces on arms

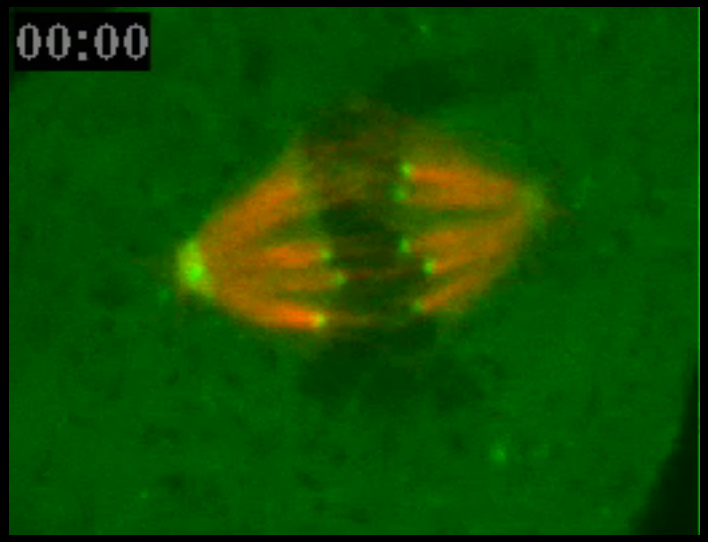
Anaphase initiated by release of cohesion

triggered by activation of the APC (Anaphase Promoting Complex)

- cleaves Mitotic Cyclin
- cleaves inhibitor (securin) of cohesion protease (separase)



Anaphase is really two stages



Anaphase A

initial poleward chromosome movement after cohesin is degraded

motor activity (dynein) and shortening of K-MTs at kinetochores,

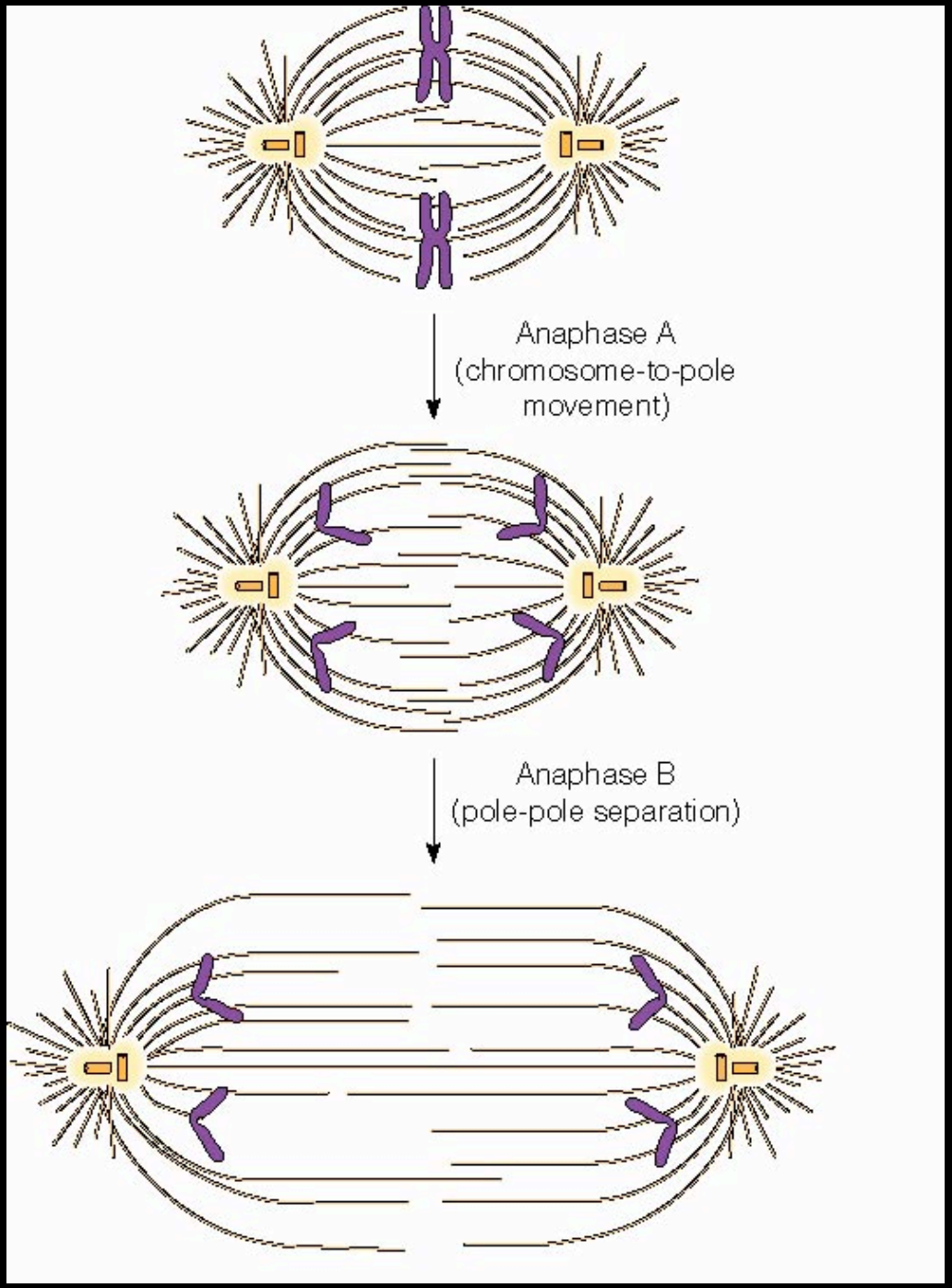
plus some depolymerization at poles (flux)

Anaphase B

poles separate, after sisters separate, moves sisters further apart

motor activity (dynein) pulls poles to cortex

motor activity (kinesins) and MT assembly at overlap between polar MTs


Anaphase A
(chromosome-to-pole
movement)
Anaphase B
(pole-pole separation)

Anaphase A

kinetochore MTs shorten as chromosomes are transported poleward

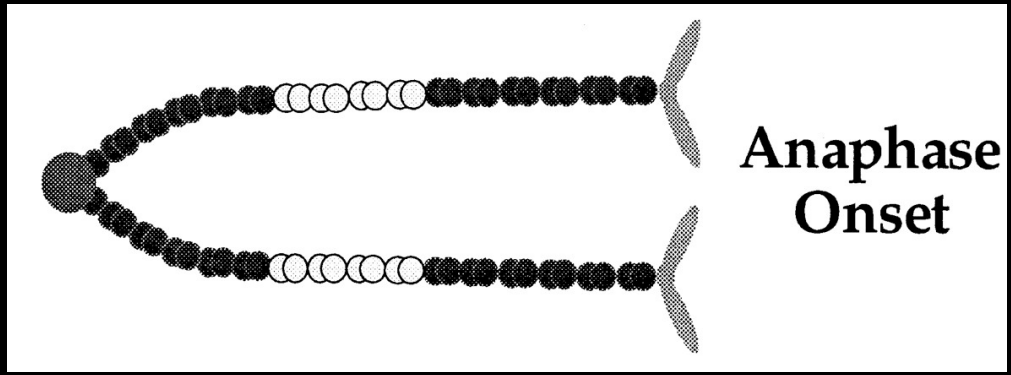
2 models (not mutually exclusive)

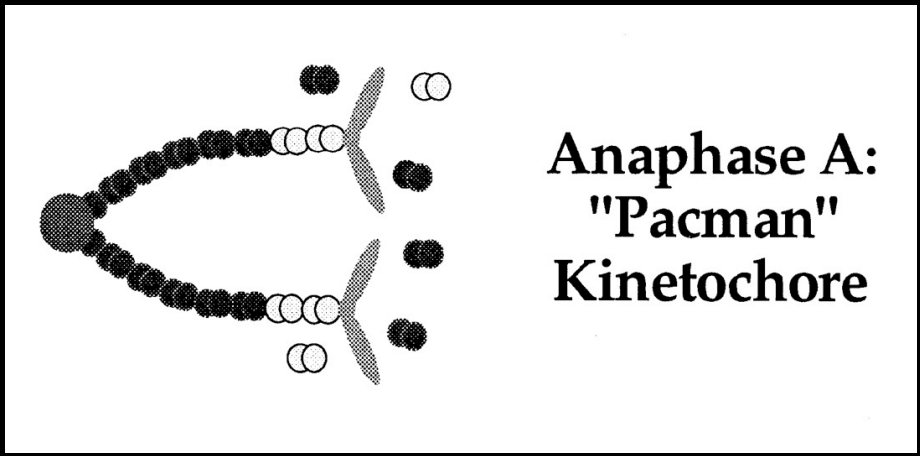
1) pacman:
kinetochore MTs disassemble at (+) ends

2) traction fiber:
kinetochore MTs disassemble at (-) ends
= poleward flux

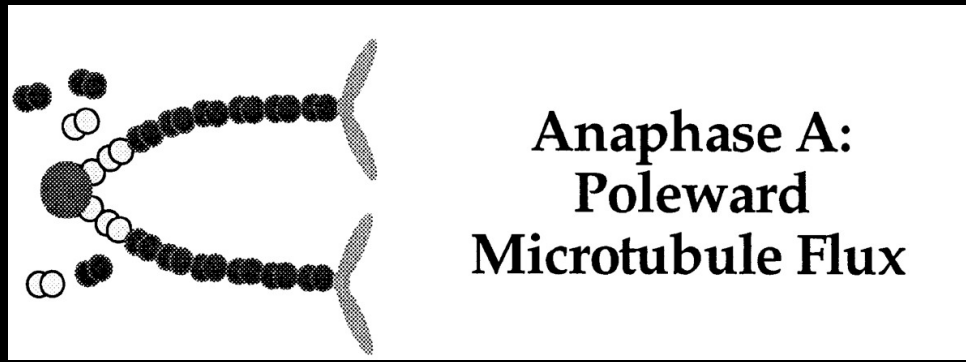
Experiments:
photoactivation, in vitro reconstitution

predicted fate of photoactivated tubulin subunits

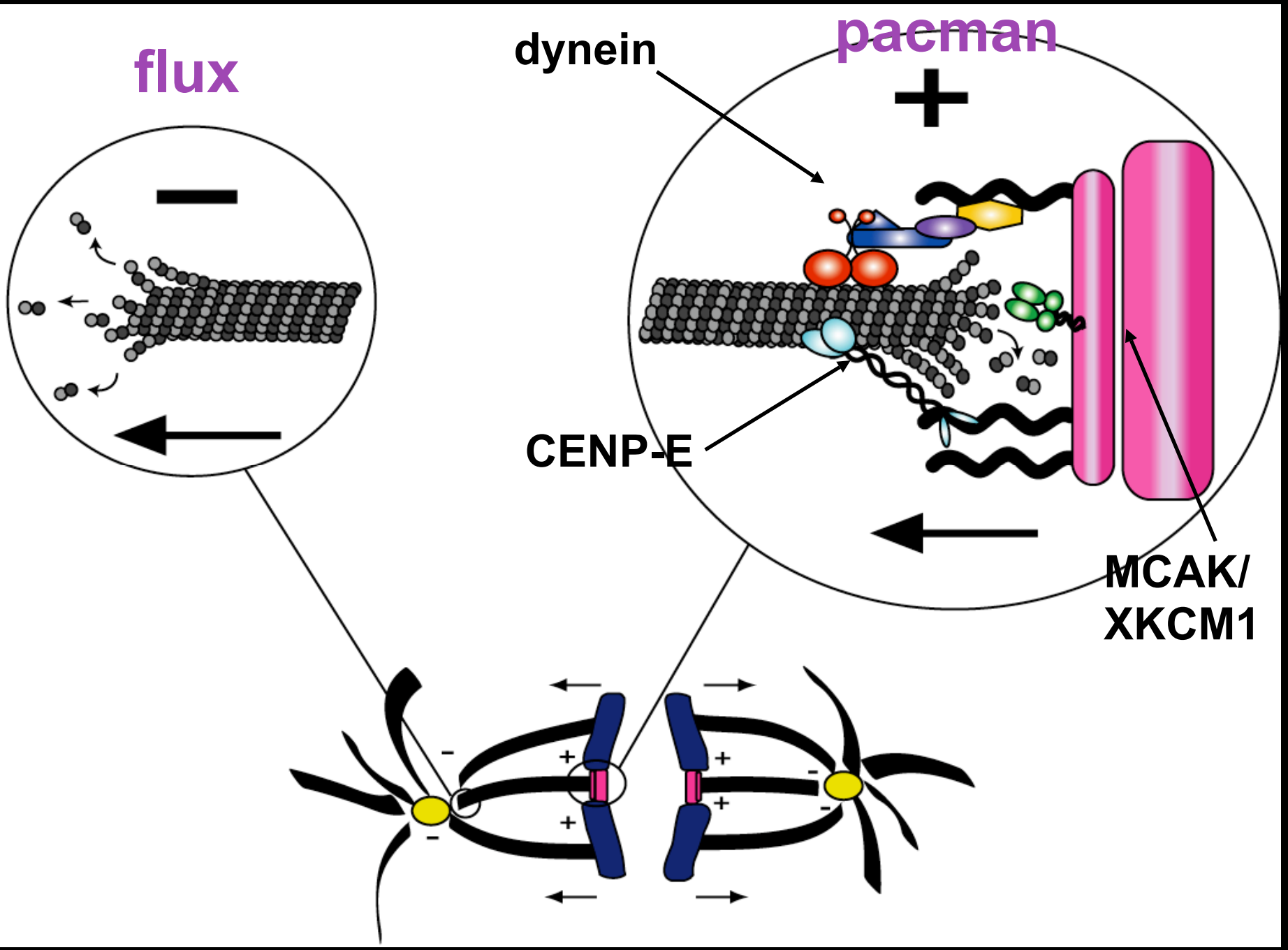




depolymerization at (+) end



depolymerization at (-) end


flux
dynein
pacman
CENP-E
MCAK/
XKCM1

Evidence for Pacman model

tissue culture cells:

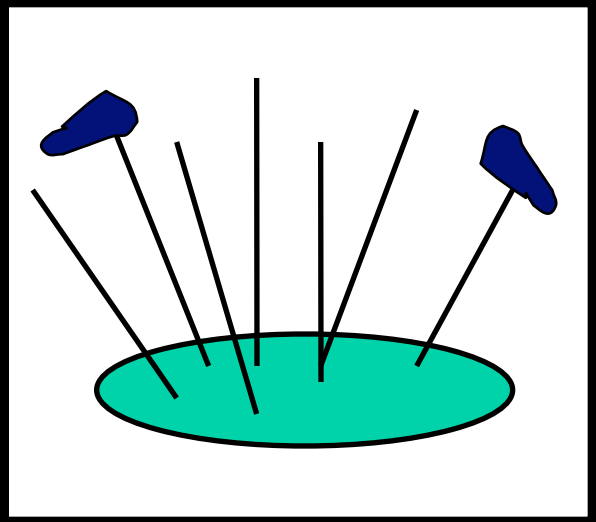
chromosome movement 2 μm/min
flux 0.5 μm/min
kinetochores move past photoactivated region

in vitro:

chromosomes can maintain attachment to shrinking MTs

Tetrahymena pellicle experiments

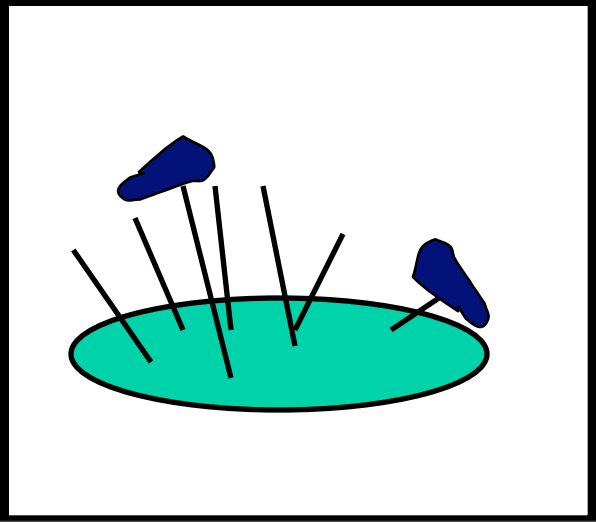
grow MTs from
pellicle in flow chamber

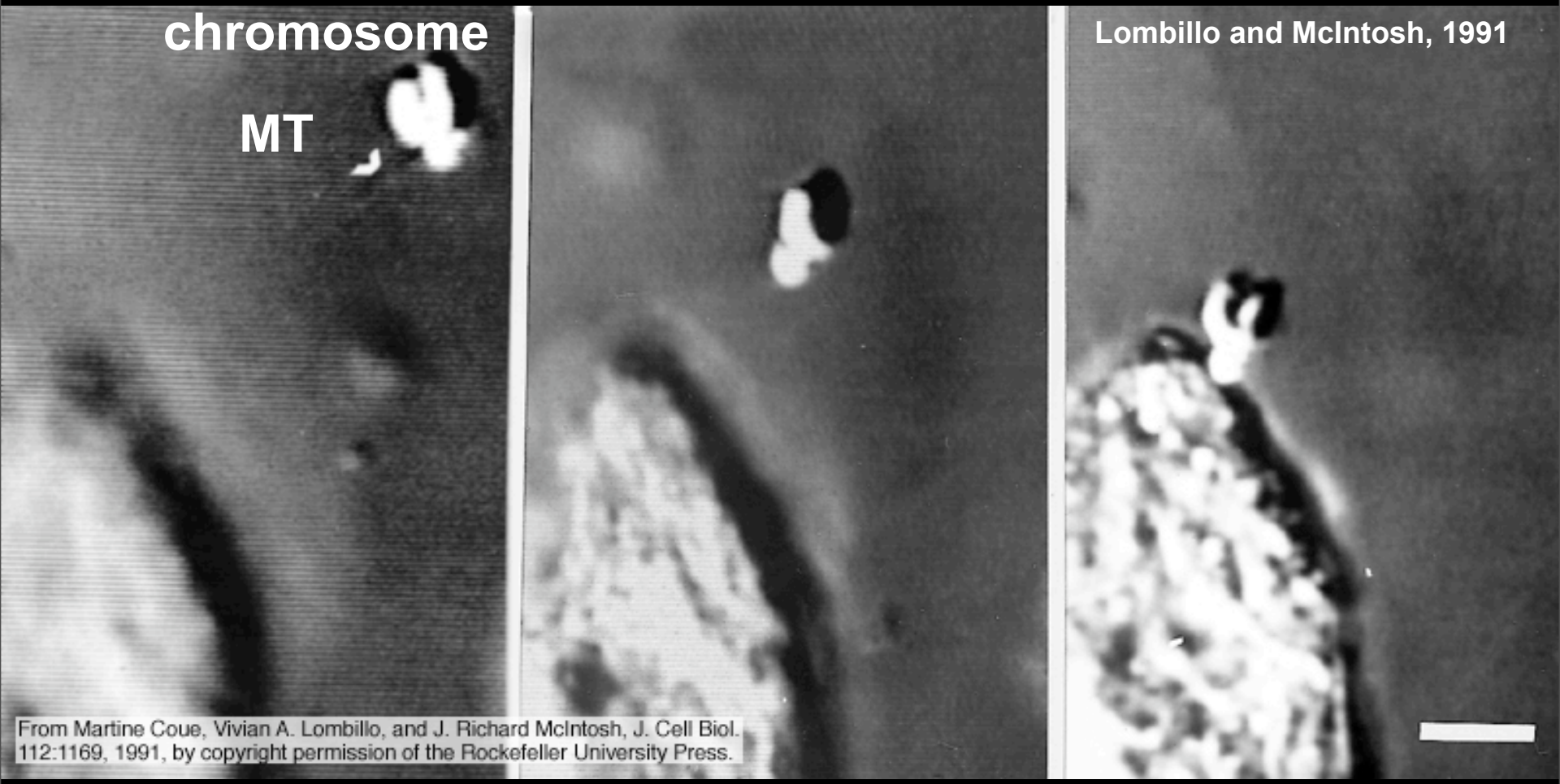


flow in
chromosomes

depolymerize MTs by diluting tubulin

chromosomes stay attached
and move toward pellicle





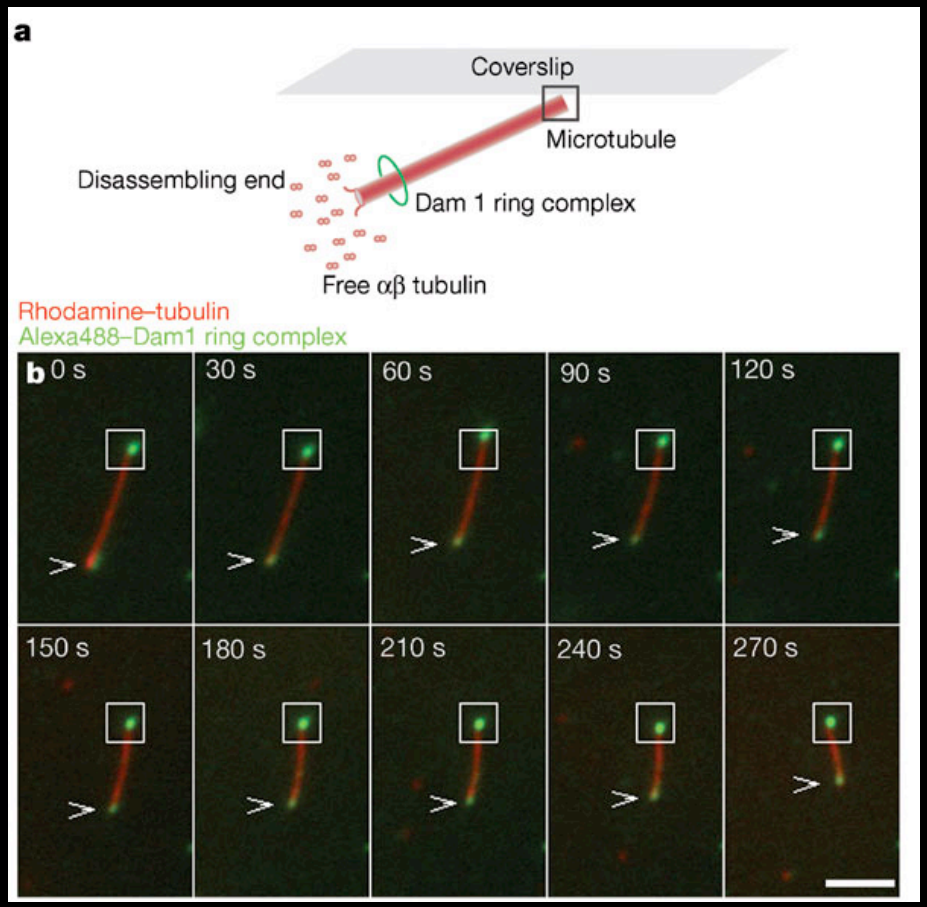
ATP not required

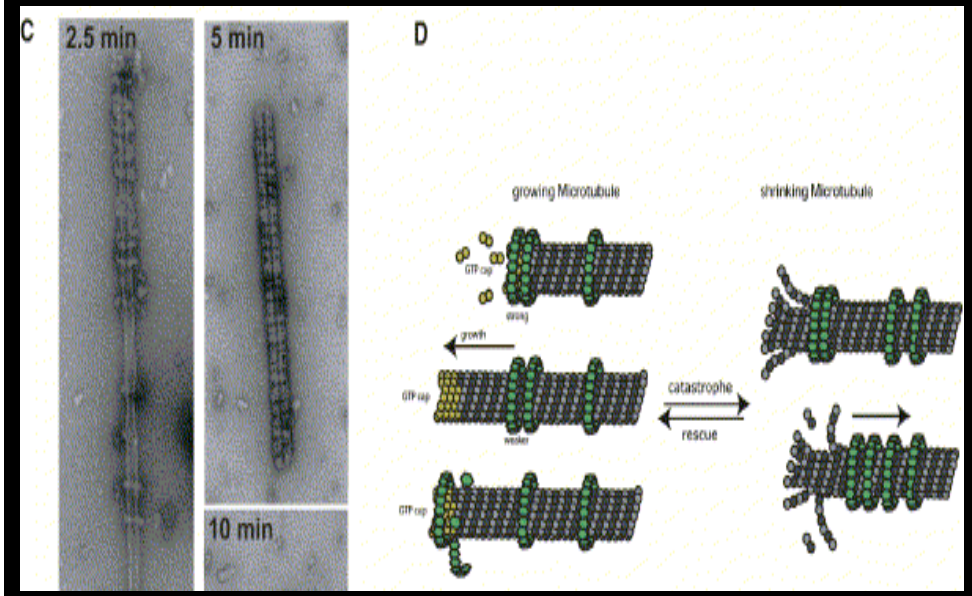
blocked by antibodies to CENP-E

beads coated with (+) end-directed kinesin also work, but not dynein

Dam1 complex provides another linking mechanism

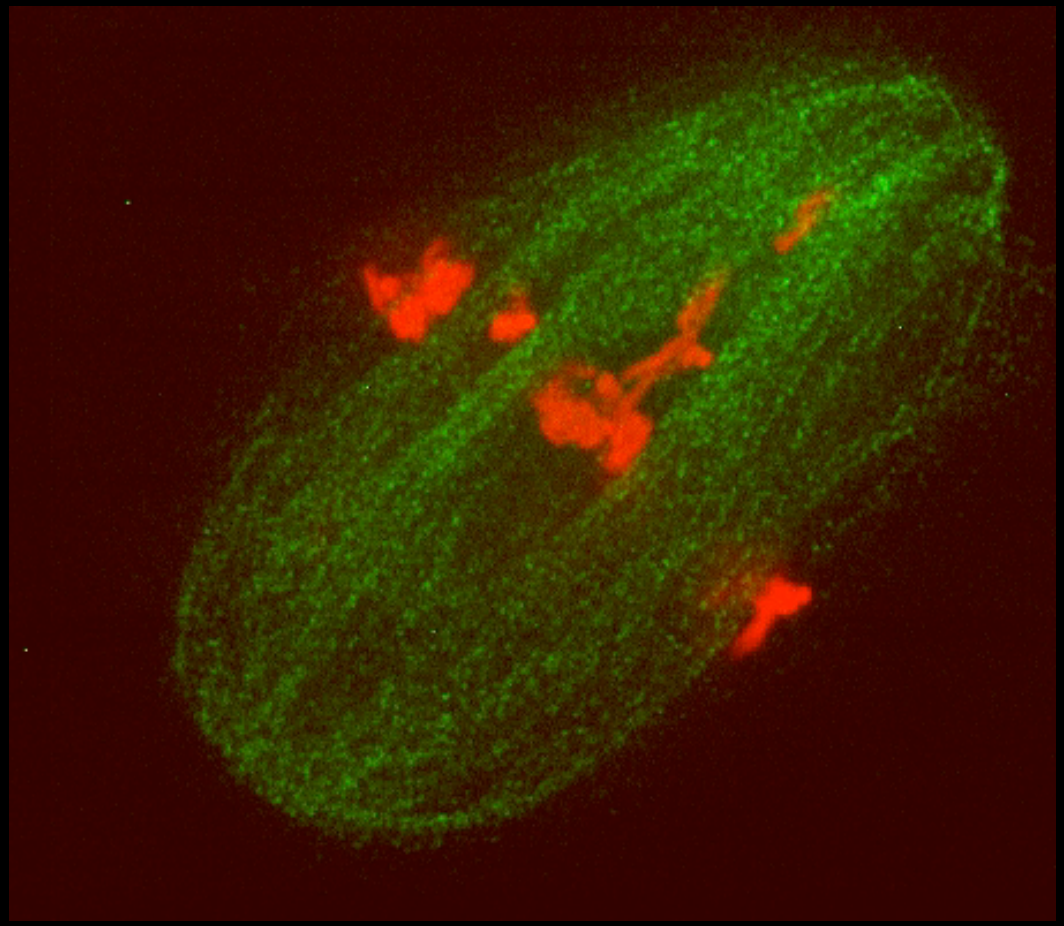
Ring complex moves processively for several micrometres at the ends of depolymerizing microtubules without detaching from the lattice





Westermann et al., Nature (2006)

Evidence for flux model in Anaphase A



Xenopus in vitro spindles:

flux and anaphase same rate, ~2 μm/min

same pharmacology

requires ATP, taxol insensitive

What is depolymerizing the MTs?

Drosophila embryo experiments:

antibody injection and movies

2 Kinl kinesins required for chromosome alignment and segregation

KLP59C: at kinetochore
inhibition blocks pac-man

KLP10A: at spindle poles
inhibition blocks flux

Rogers et al. (2004) Nature 247, 364

Anaphase B

poles separate

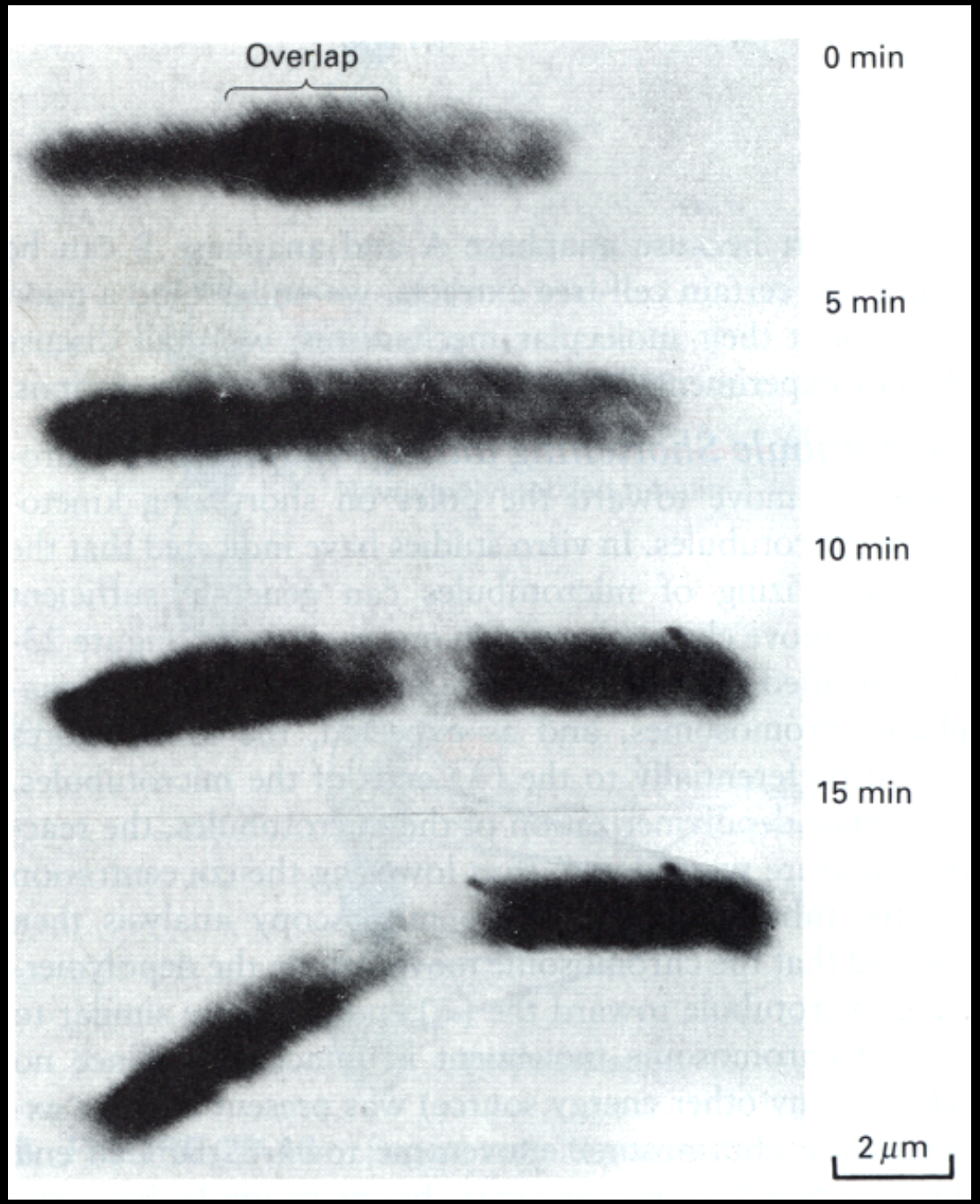
2 mechanisms:

sliding of antiparallel microtubules

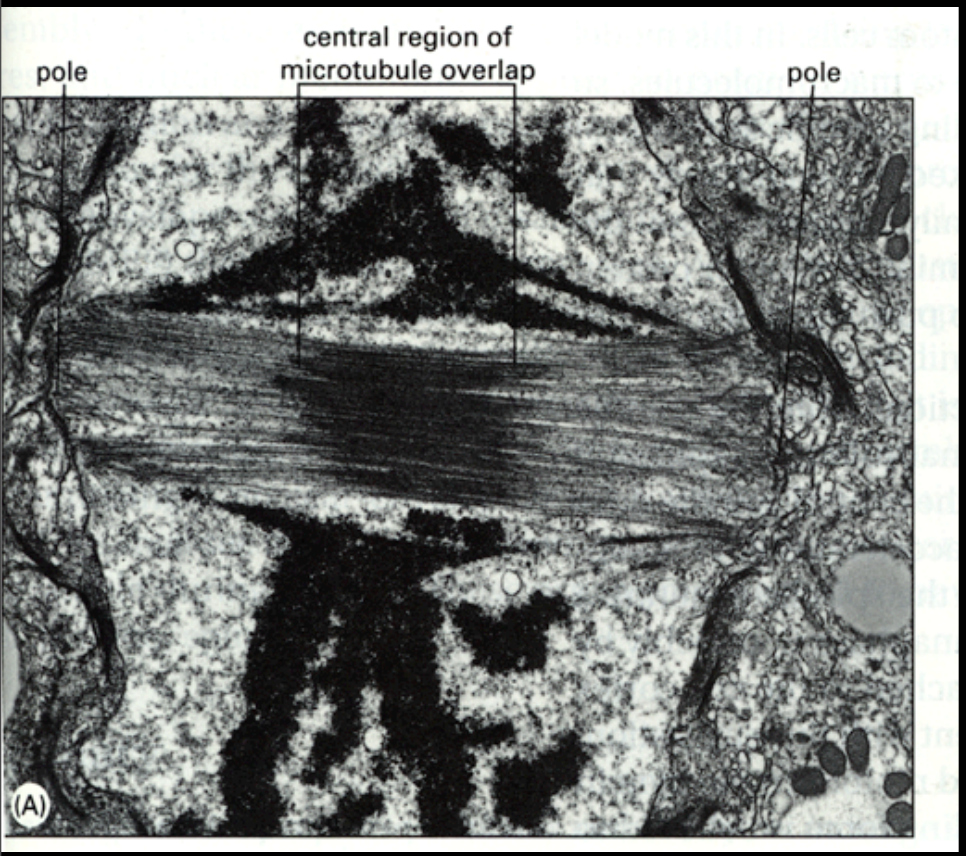
pulling of astral microtubules by cortex

Isolated Diatom spindle in vitro

Cande Lab

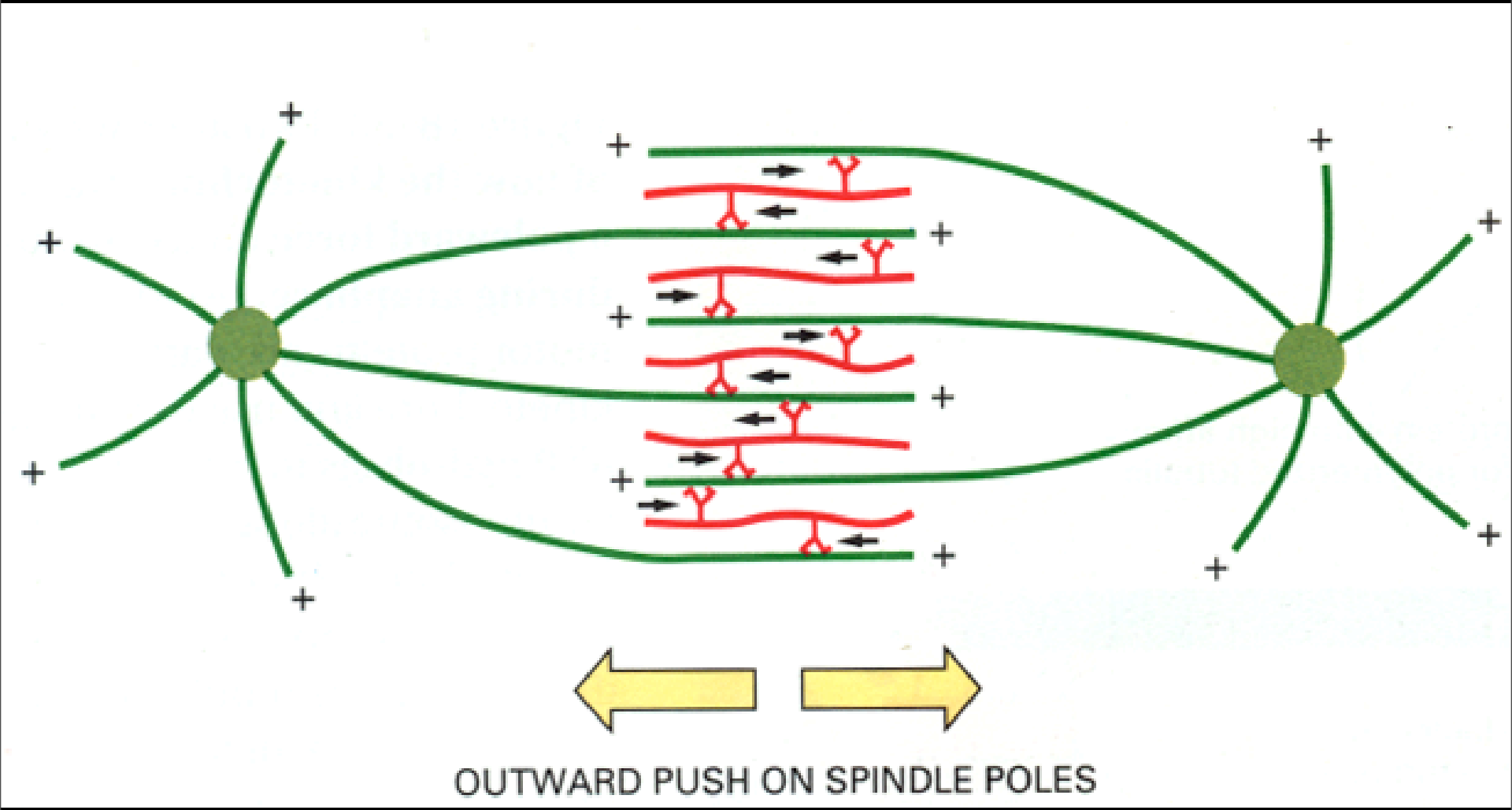


Diatom spindle



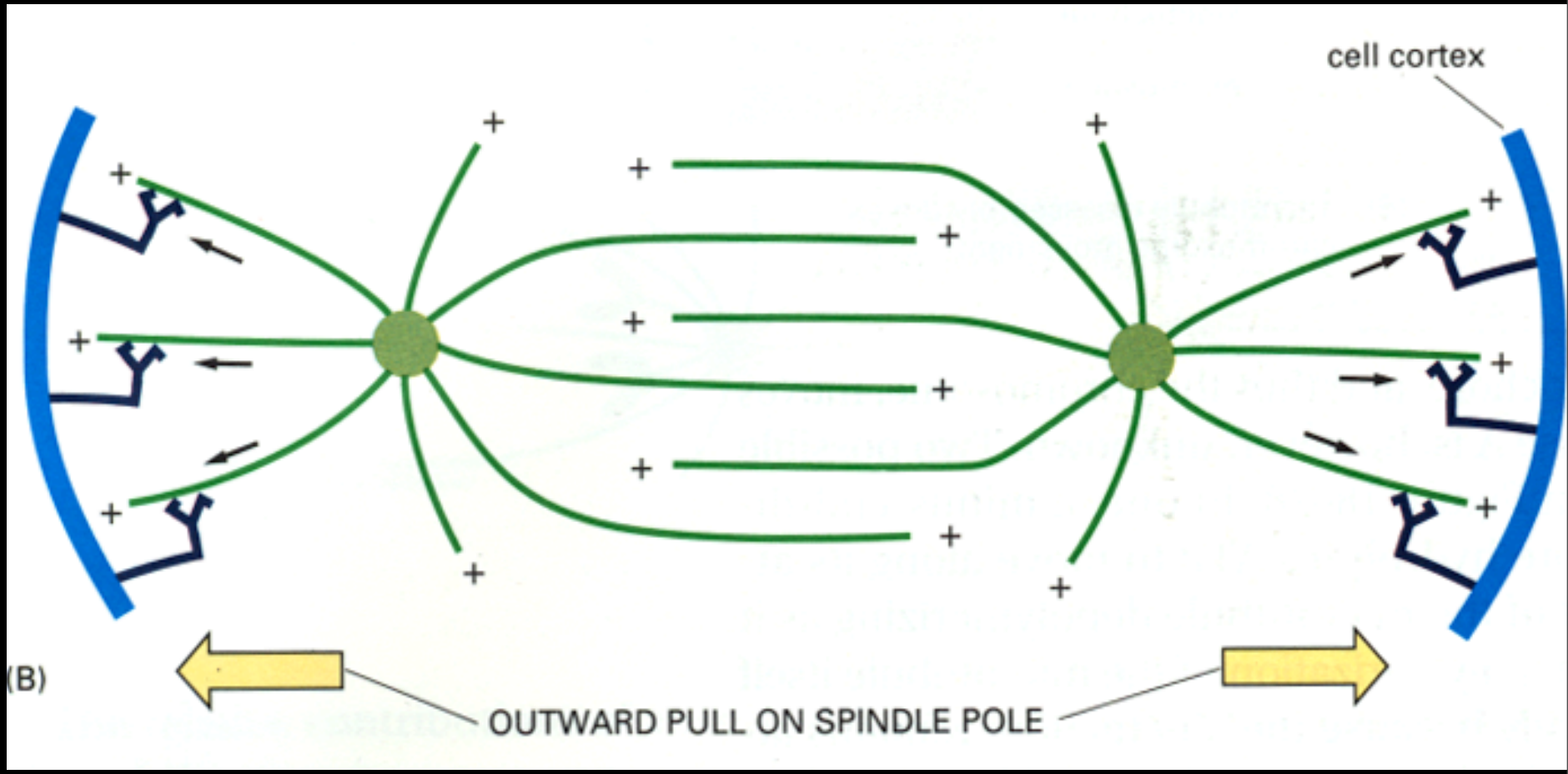
reduced overlap of polar MTs in Anaphase B

sliding of antiparallel microtubules



motor candidate: kinesin-like-protein MKLP1/CHO1

pulling of astral microtubules by cortex



motor candidate: cytoplasmic dynein

Cytokinesis

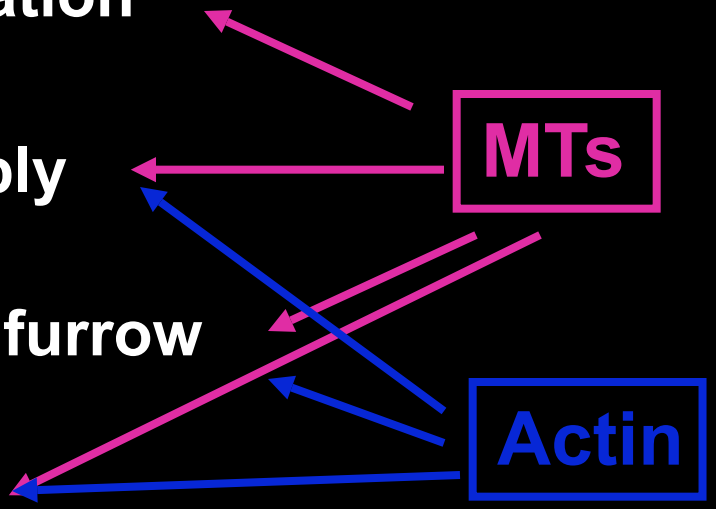
coordination between microtubules and actin

1) cleavage plane specification

2) cleavage furrow assembly

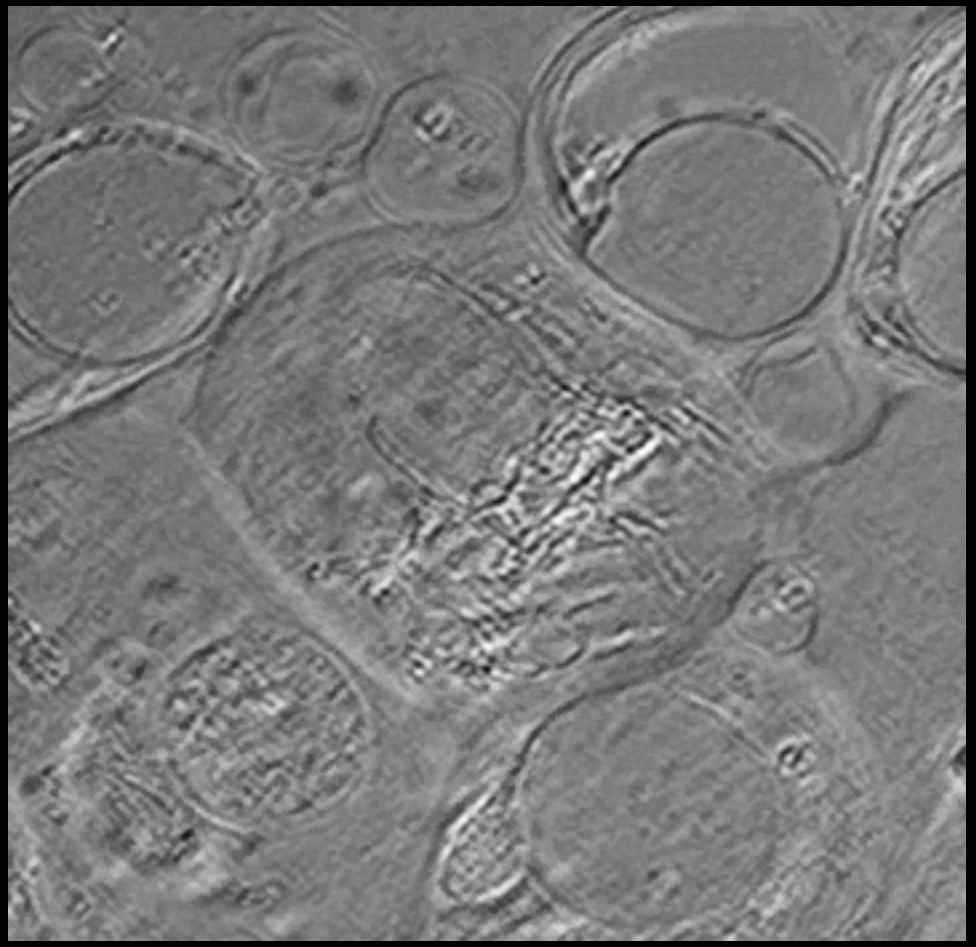
3) contraction of cleavage furrow

4) cleavage furrow seals



membrane insertion

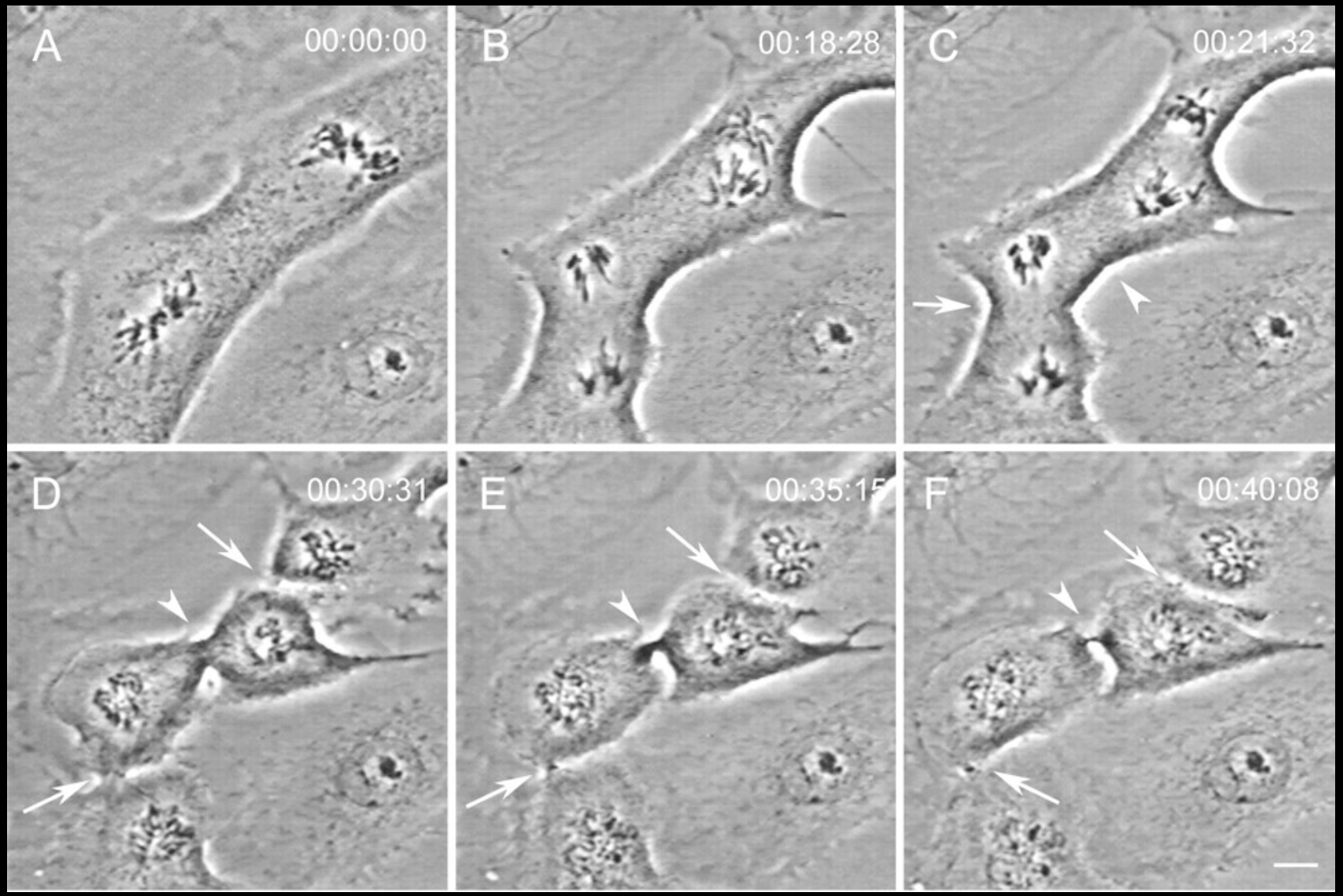
Cytokinesis occurs at Midbody



region of overlap between polar MTs at end of mitosis associated with contractile ring

Evidence that sites of cytokinesis are determined by poles

Cell with two spindles: Savoian et al. MBC 10, 297 (1999)



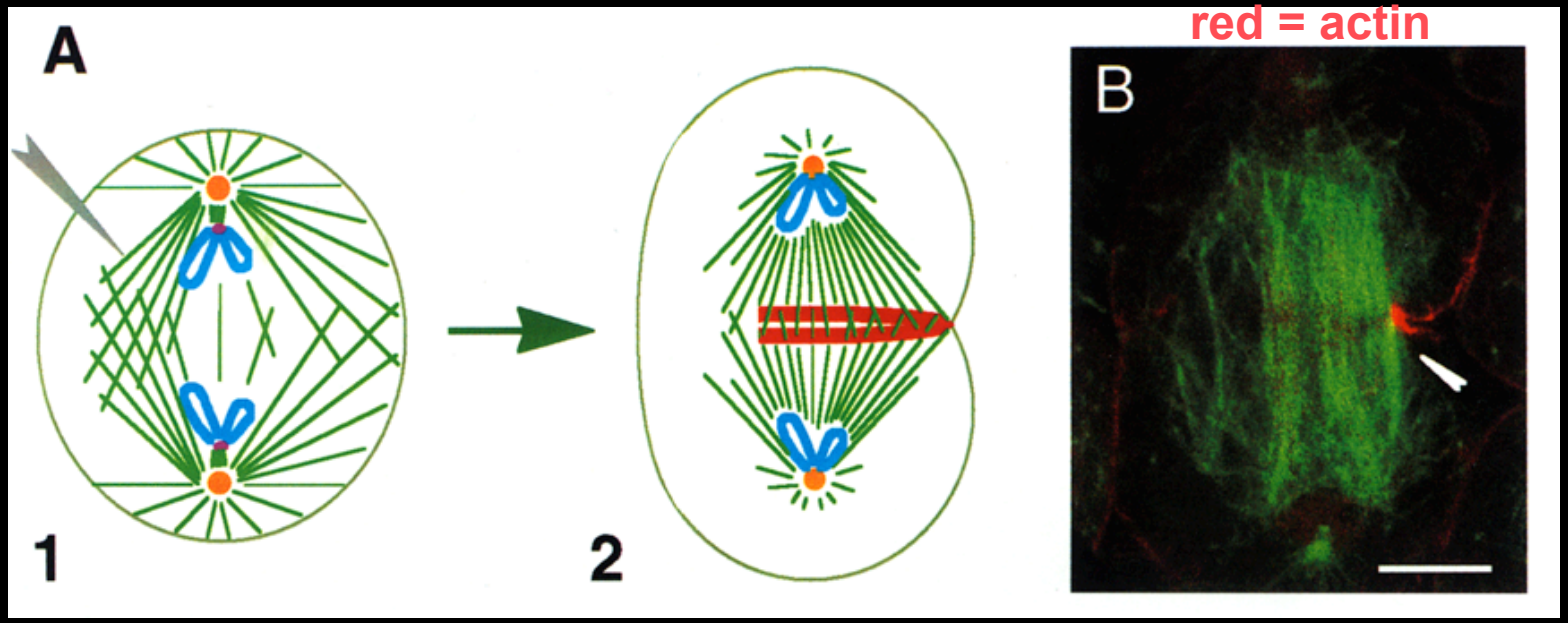
1) Astral relaxation model:

signals from asters cause relaxation of the cortex
cleavage occurs where signal is weakest = spindle midzone

CONCEPTS: Astral MTs important
Contractile activity motile

2) Astral stimulation model:

signal from where MTs from opposite poles interact at cortex



push astral MTs away from cortex at midzone
no furrowing on that side

CONCEPT: MTs required for furrowing

3) Equatorial stimulation from spindle mid-zone

Evidence:

physical barrier between spindle midzone and cortex blocks cytokinesis

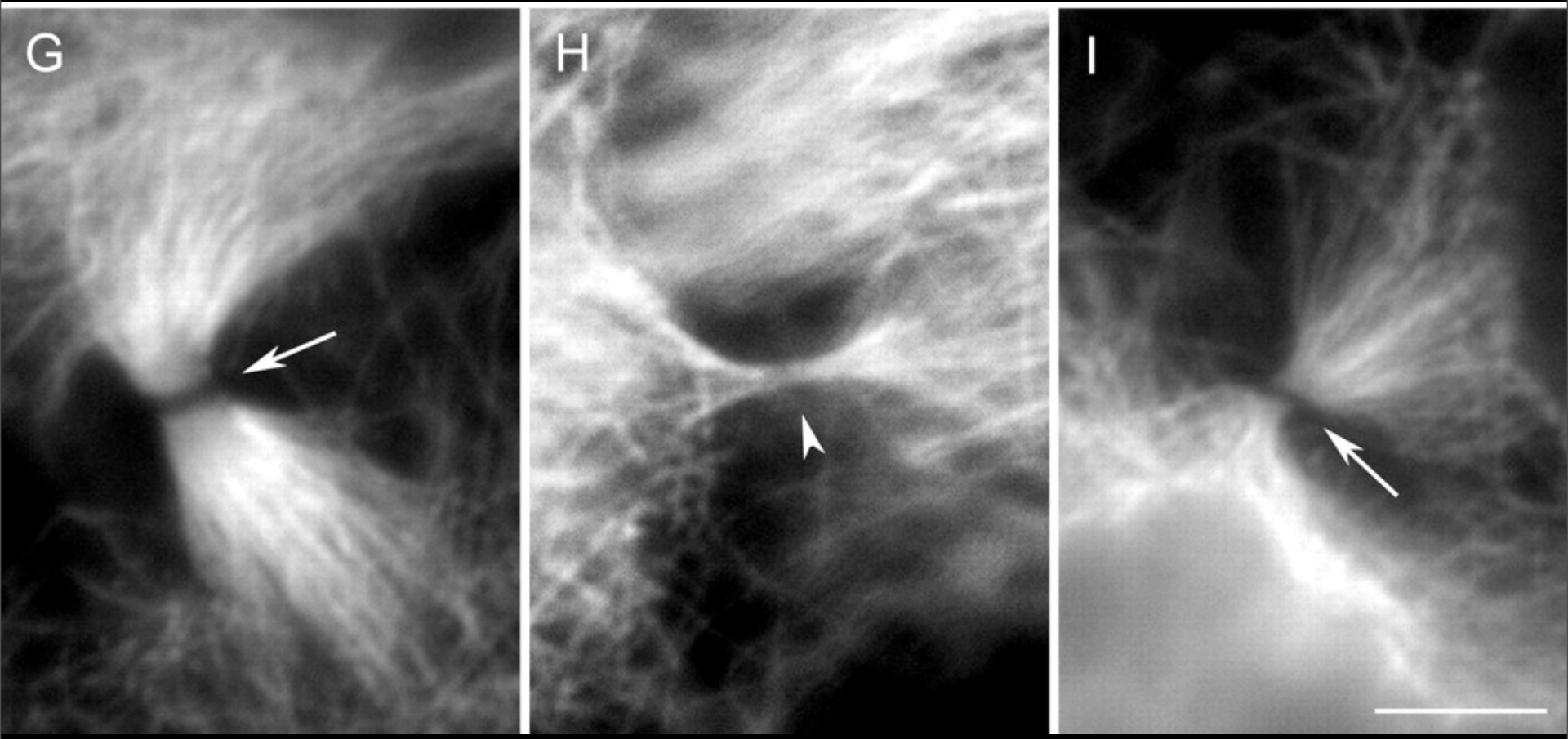
if midzone forms, then cytokinesis proceeds

midzone proteins are required

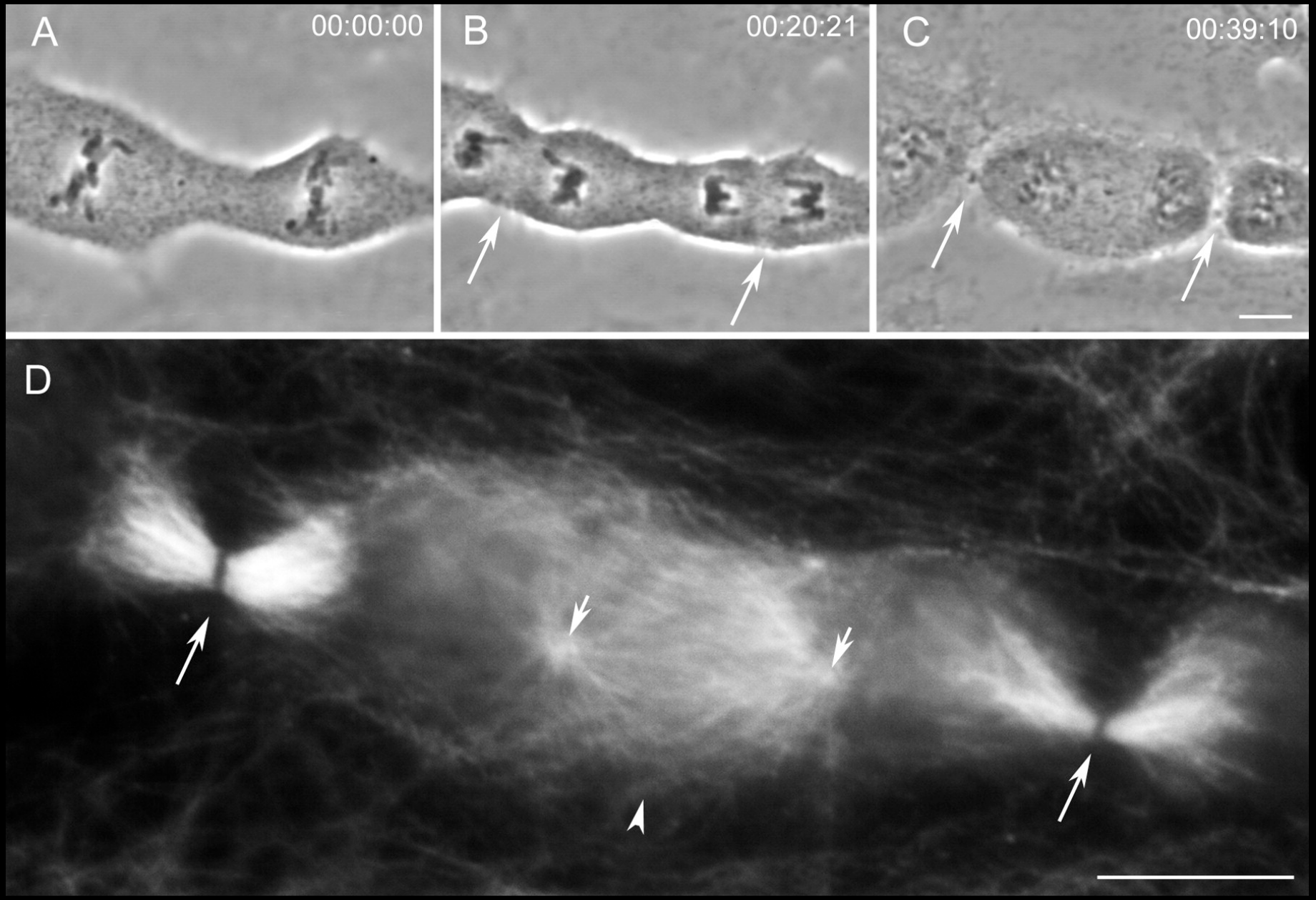
Central spindle/midzone proteins required for cytokinesis

proteins:	roles:
motor proteins	
chromokinesin KLP3A CHO1/MKLP1	stabilize MT bundles transport furrow components?
chromosome passengers	
INCENP BIR-1	relocate from chromosomes to midzone at anaphase targets kinases
kinases	
polo aurora-type	localization of motors
GTPases	
rho	MT and actin regulation

And many more…

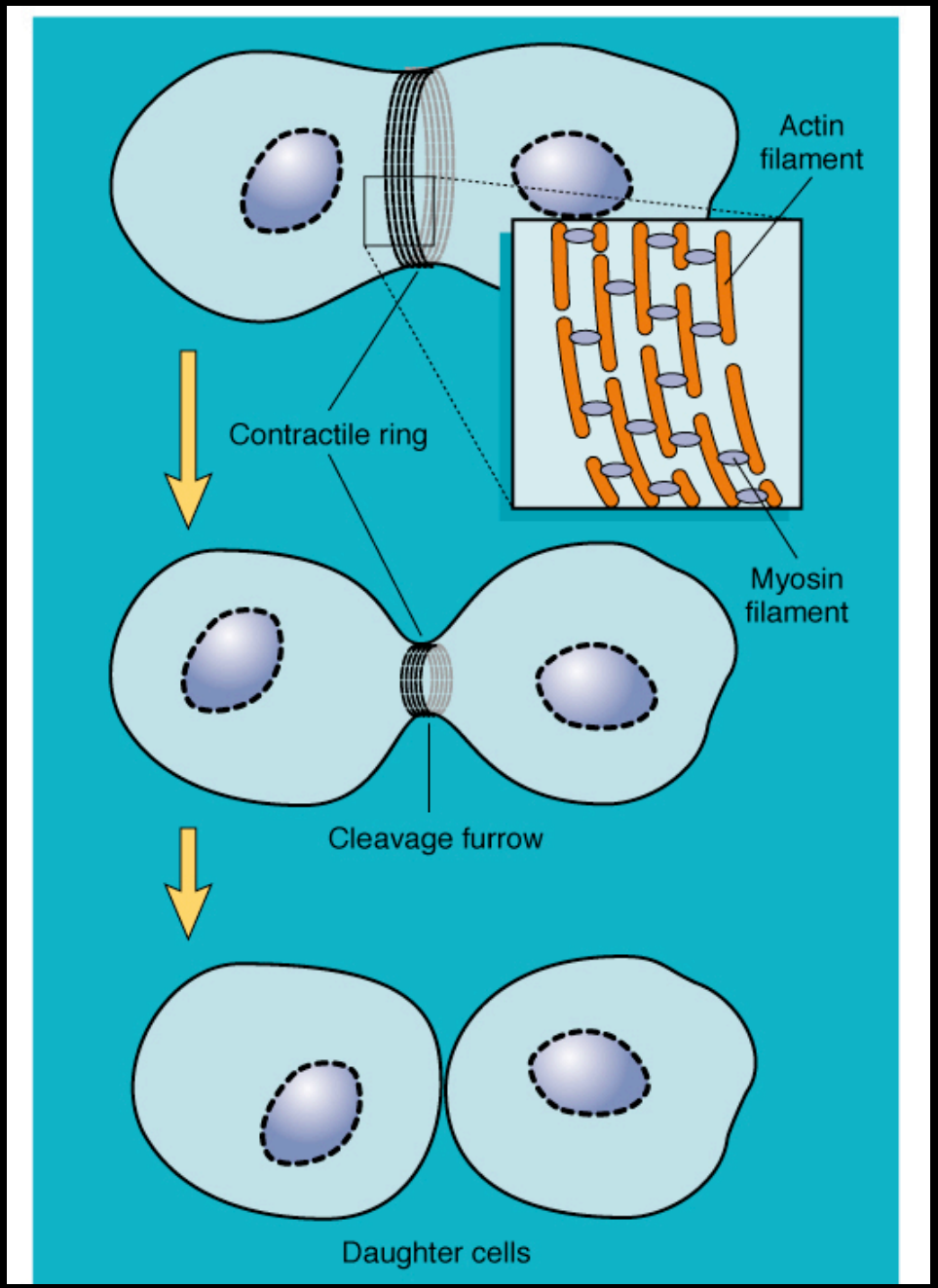


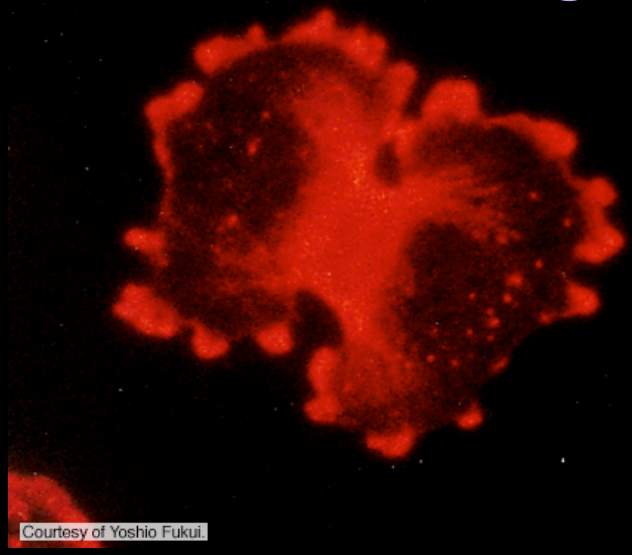
midbody forms between the two spindles:
MKLP1/CHO1 and INCENP recruited



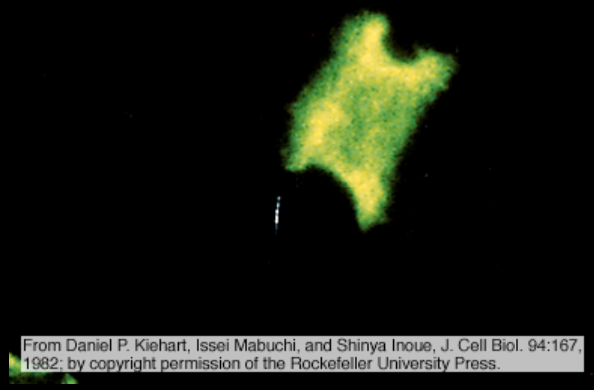
If ectopic midbody doesn't form - no furrowing

Actin and Myosin: formation of the contractile ring



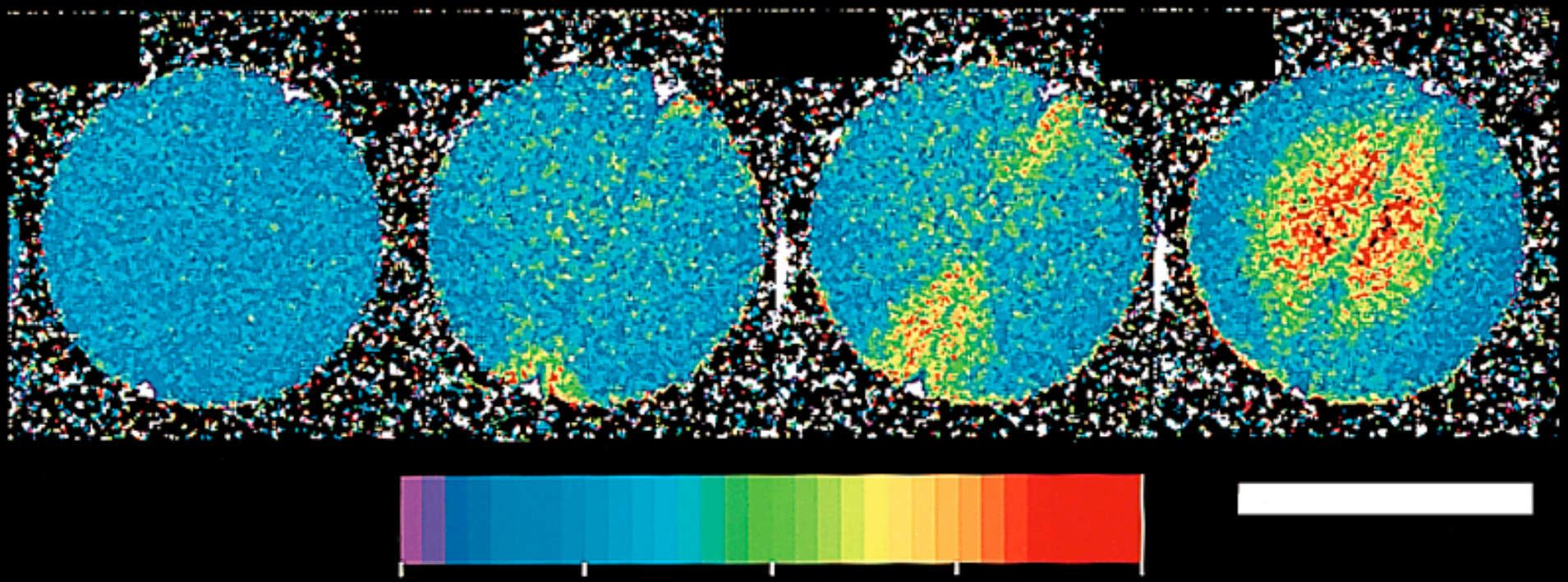


actin ↑ myosin II ↓



Local rise in calcium concentration associated with contraction

amphibian egg injected with calcium indicator dye



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what is the impact of increasing calcium concentration ?

Evidence for membrane insertion step to fuse membranes at end of cytokinesis

syntaxins required

target vesicles for fusion

Brefeldin A inhibits closure of intercellular bridge

inhibits GTPase required for membrane targeting

Golgi membrane delivery is involved

major source of membrane

model:

terminal step is analogous to secretion, requires vesicle trafficking