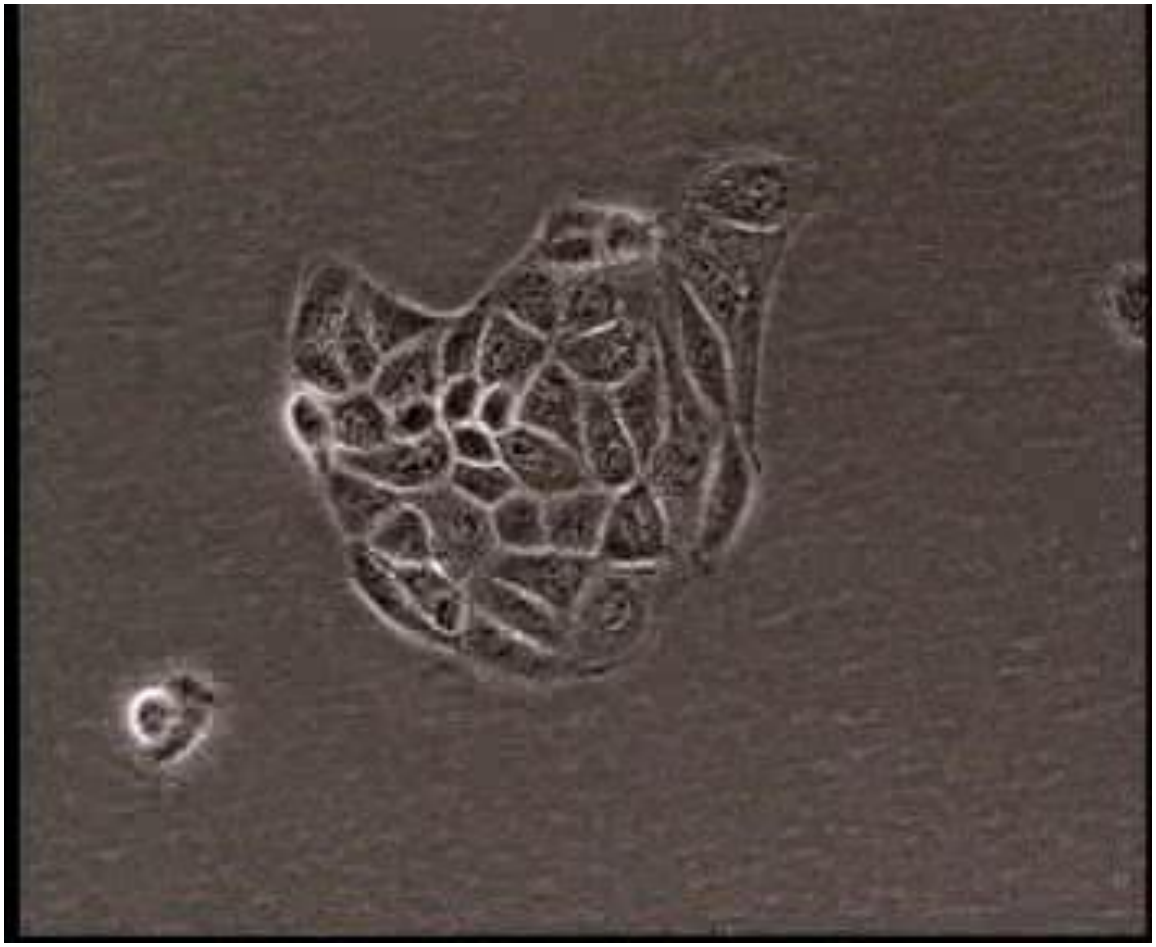
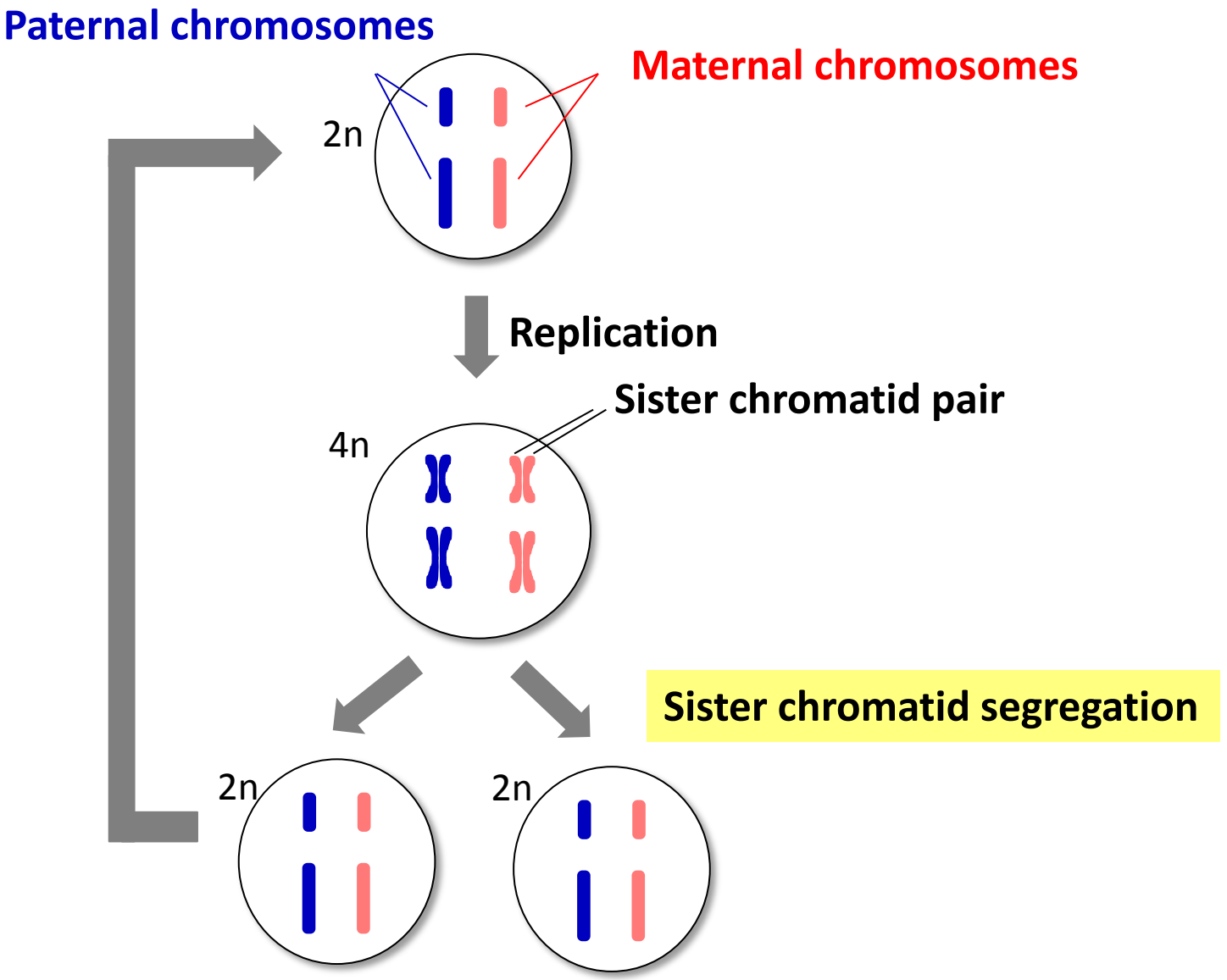
The mechanism of chromosome segregation during cell division

Cell division

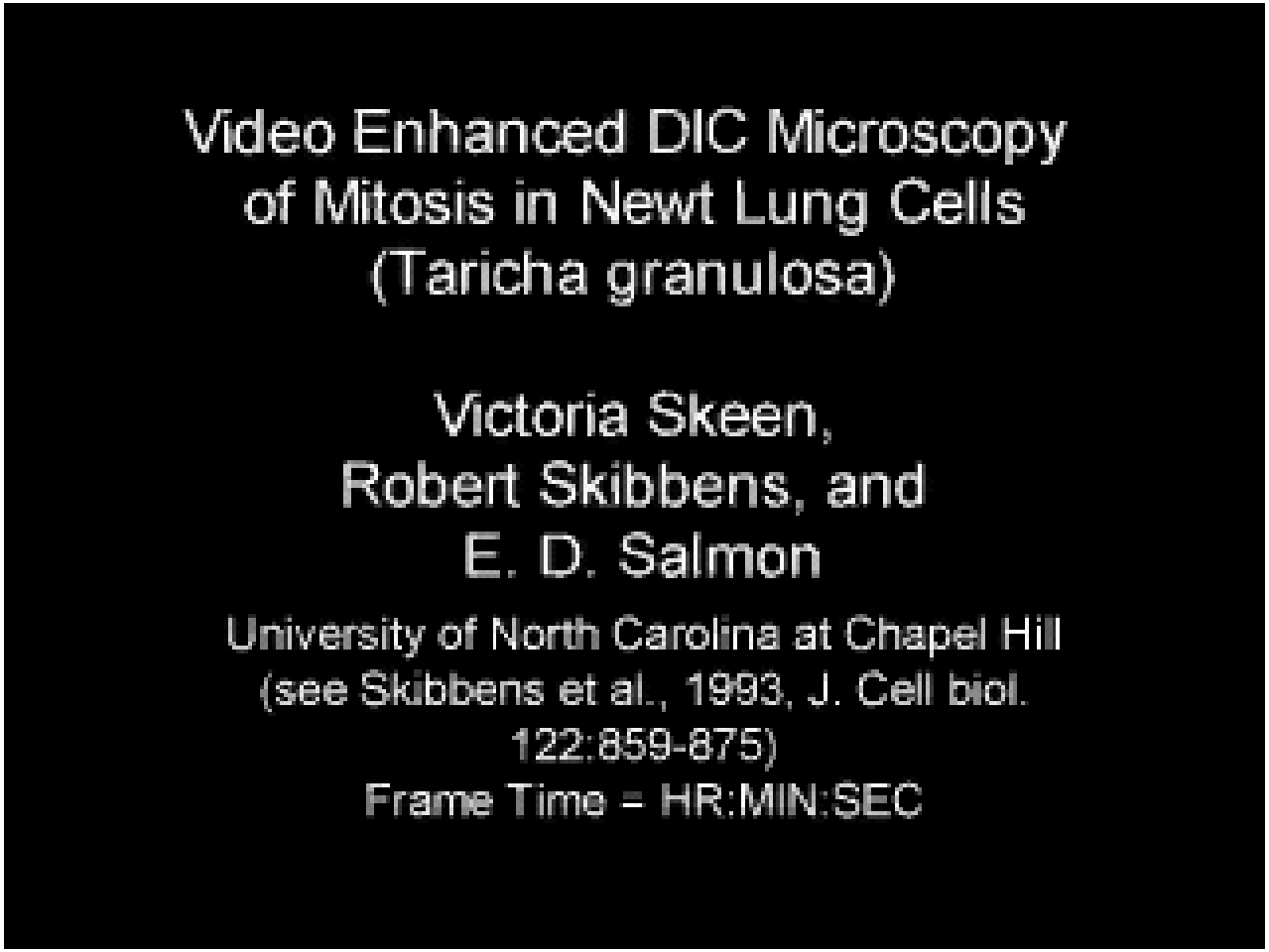


A most fundamental biological process for cells

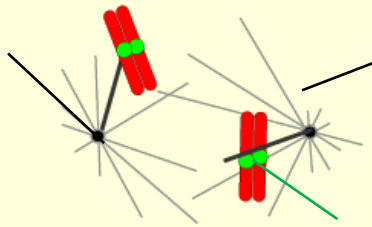
In each division, chromosomes have to be segregated **EQUALLY** into daughter cells



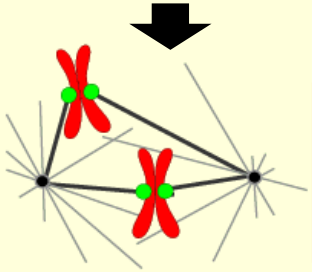
How are chromosomes segregated during cell division?



Spindle microtubules drive chromosomes to segregate

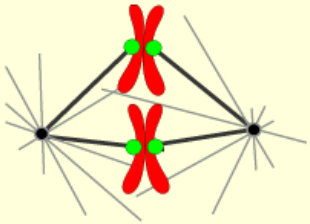


Microtubules pull kinetochores into opposite poles

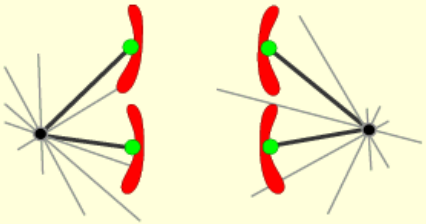


Kinetochore-microtubule attachment

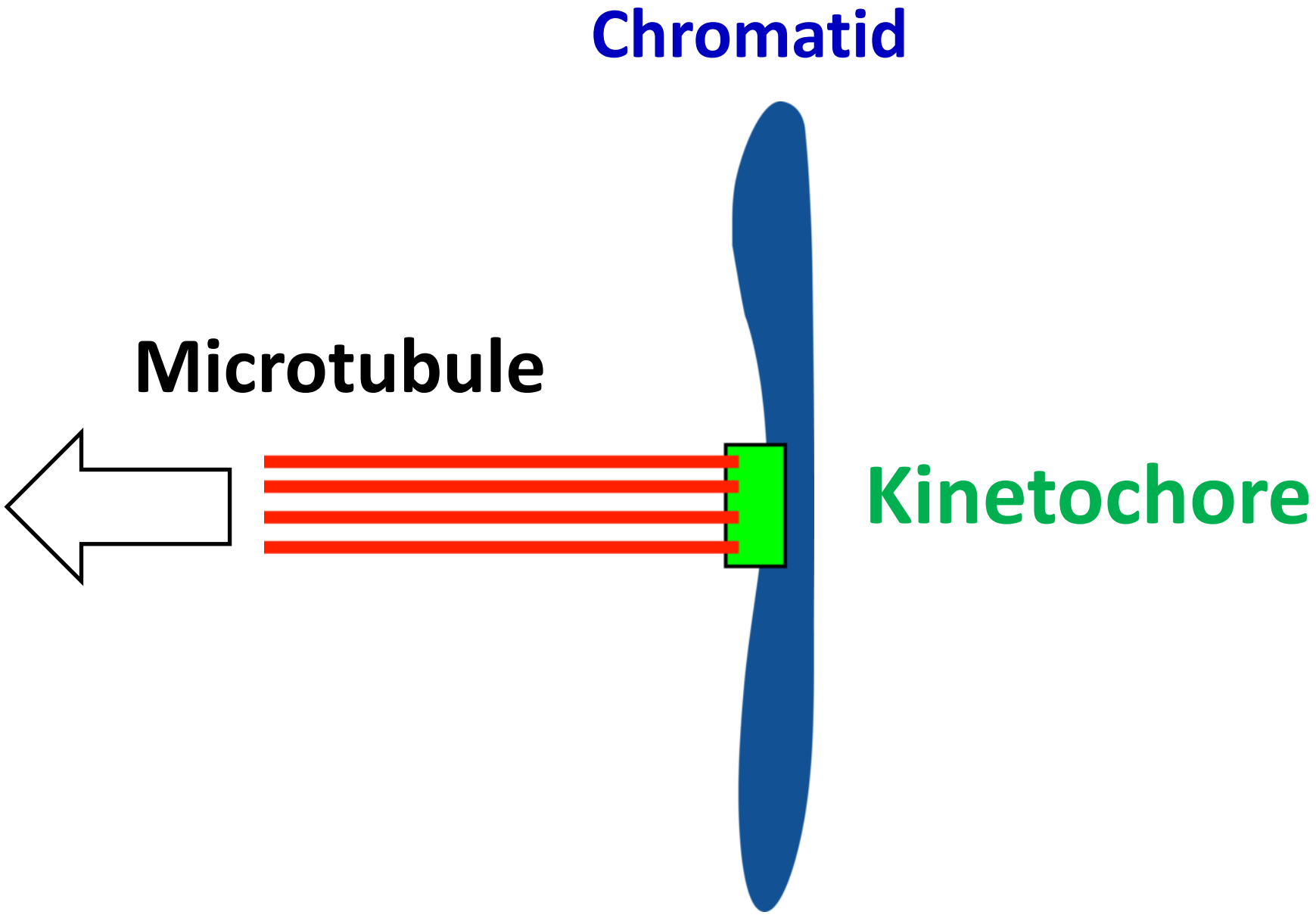
Chromosomes move to the spindle equator



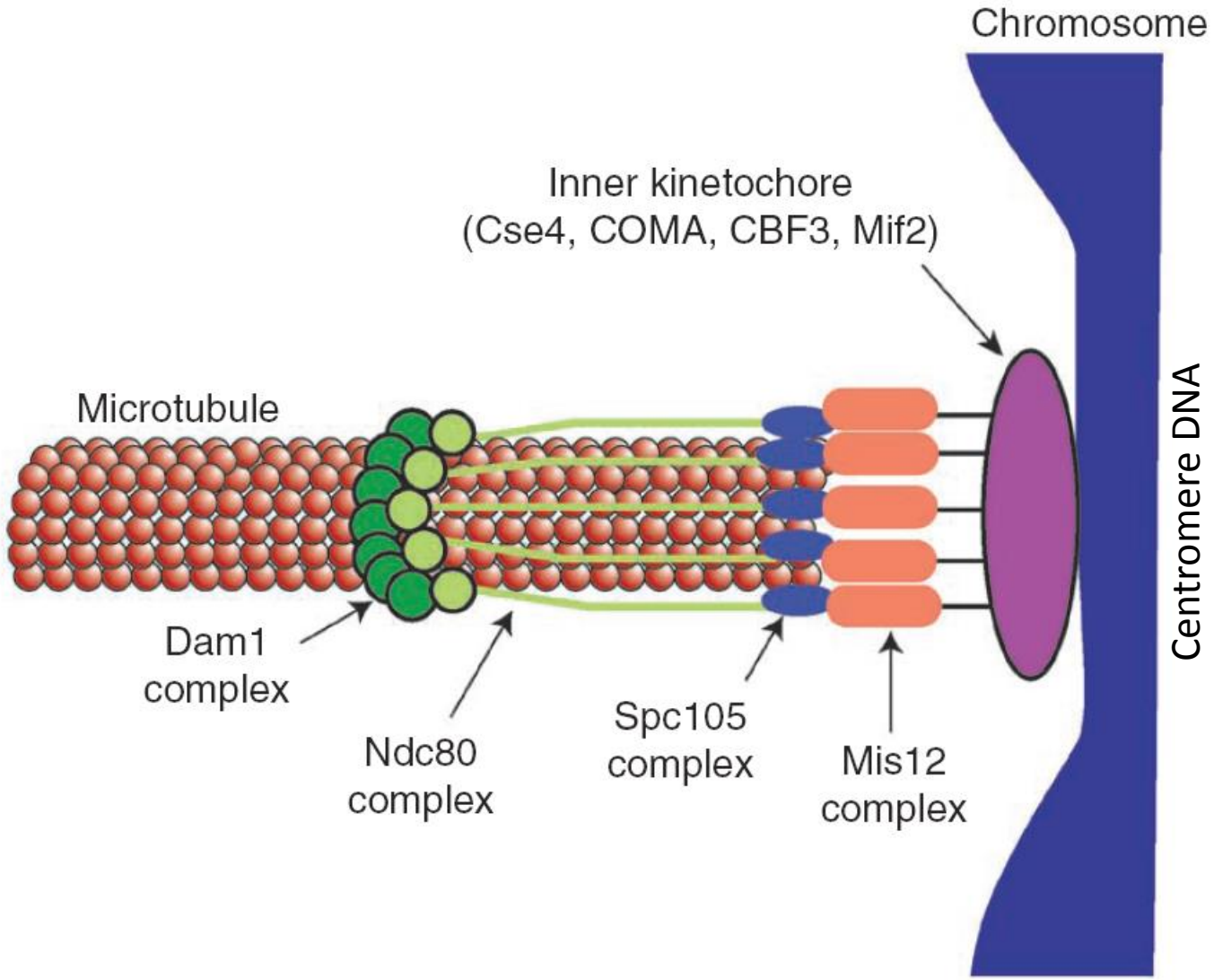
Microtubules segregate chromosomes into daughters



A minimum device that moves a chromosome

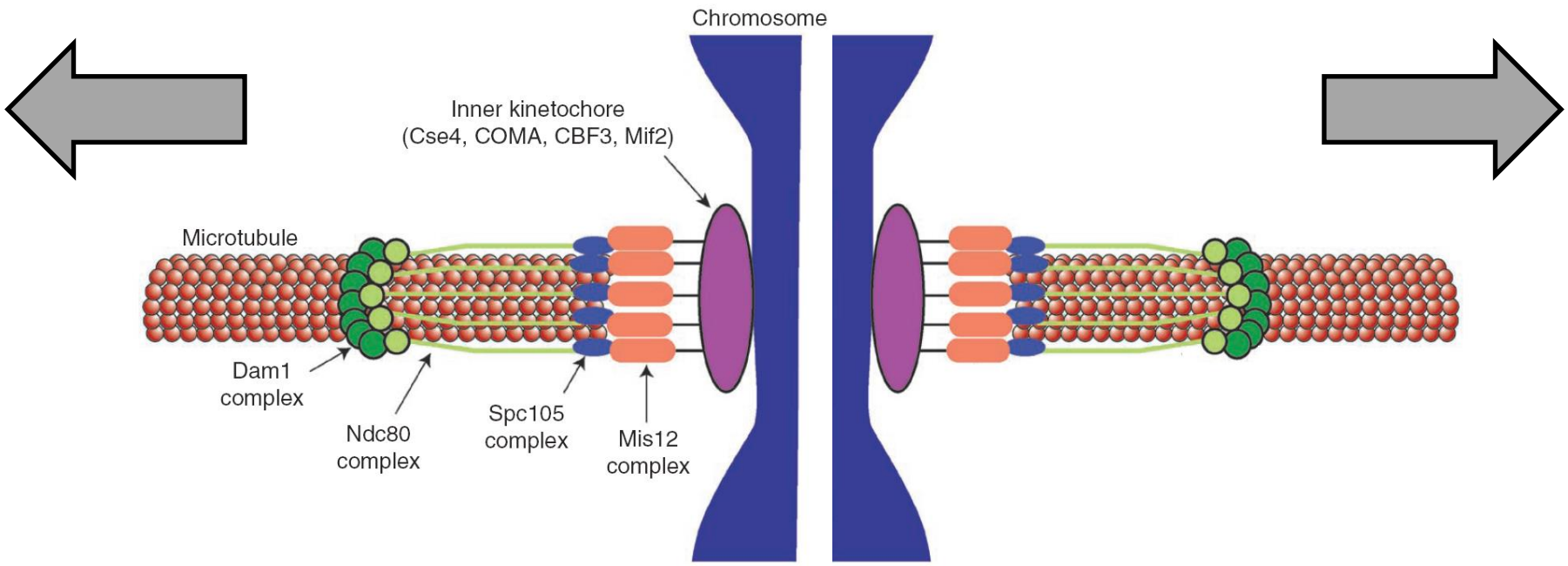


A current view of kinetochore-microtubule attachment



Gonen et al, Nat Str Mol Biol 2012

Pull into opposite sides!

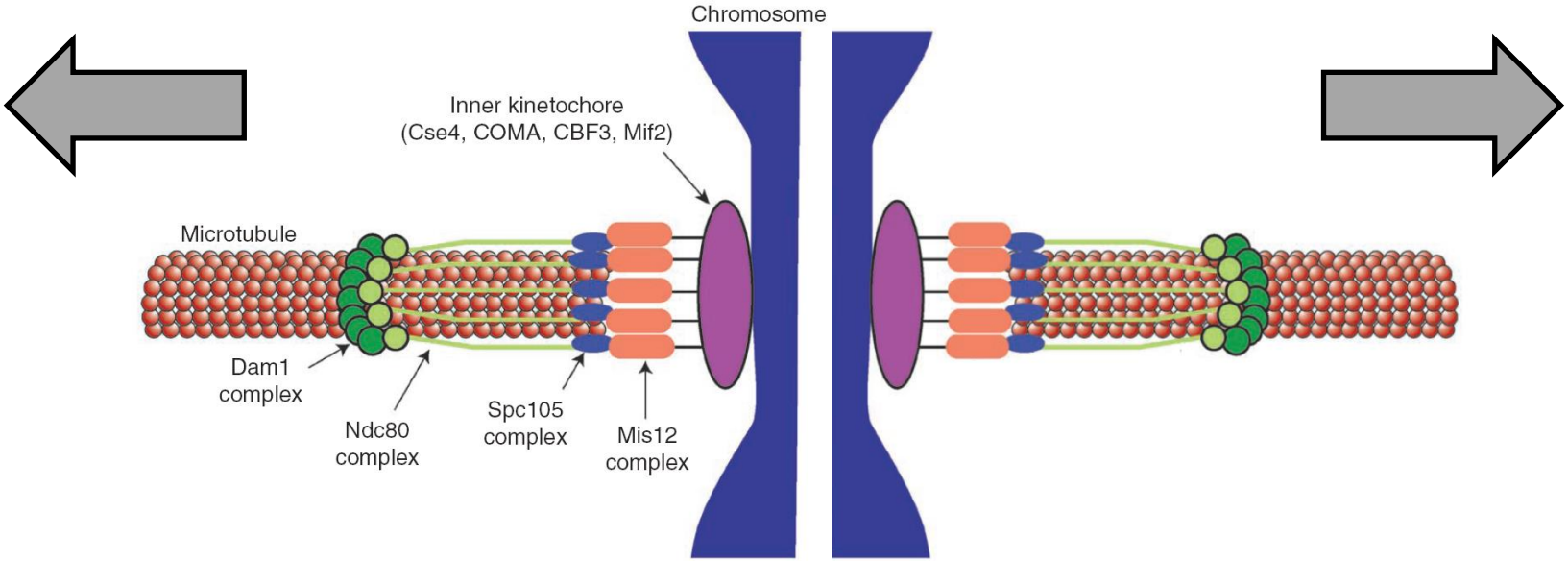


How are kinetochores attached by microtubules from opposite poles?

Today's Question

How are kinetochores attached by microtubules from opposite poles?

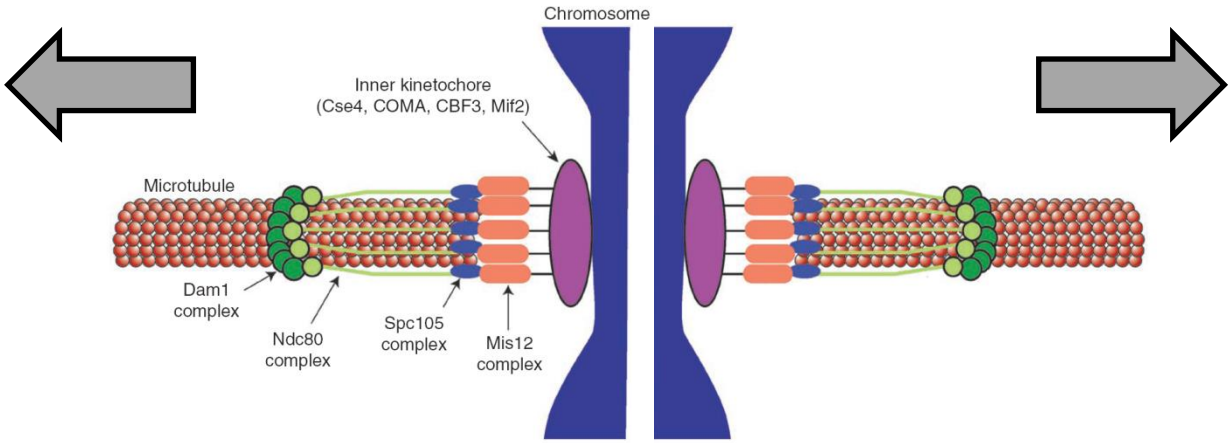
"Biorientation"



“Biorientation”

3 mechanisms so far proposed:

1. Kinetochore geometry
2. Kinetochore tension
3. Chromosome spatial arrangement



“Biorientation”

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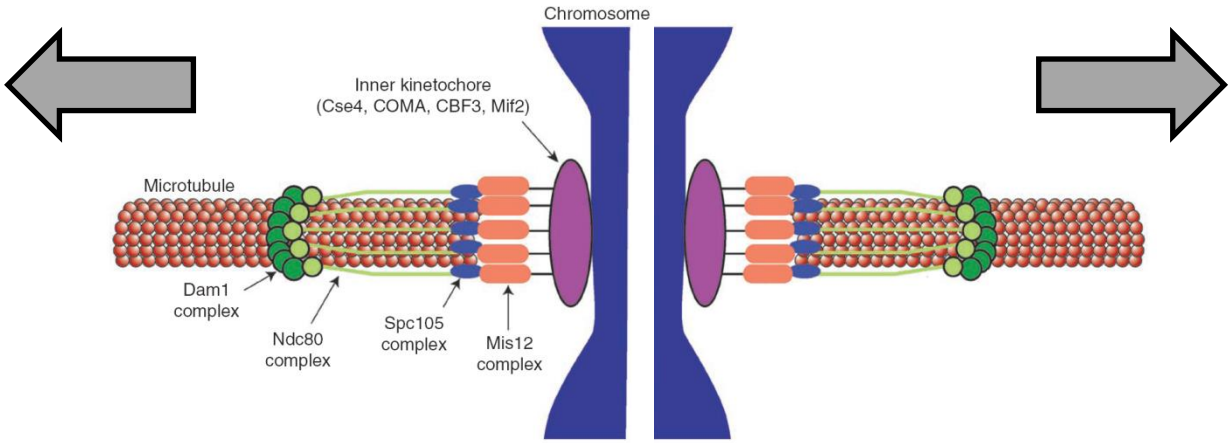
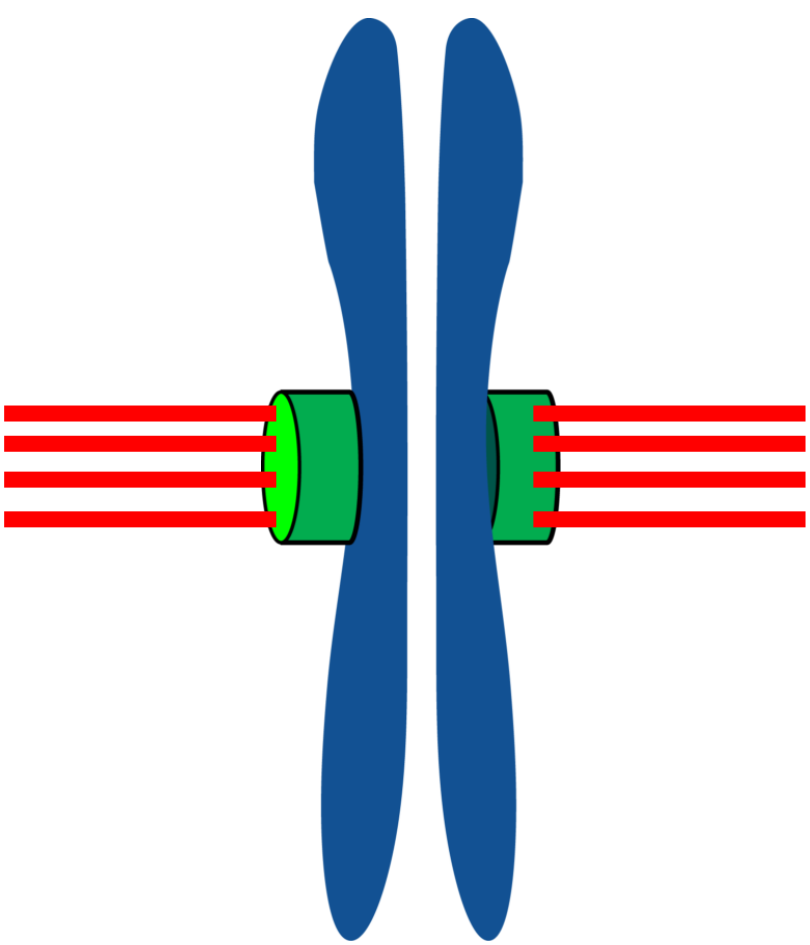
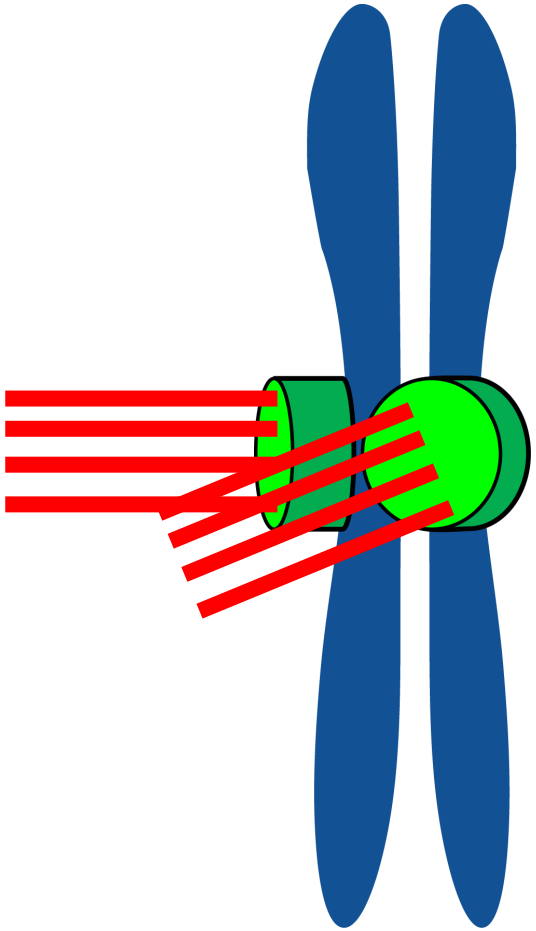


Kinetochore geometry

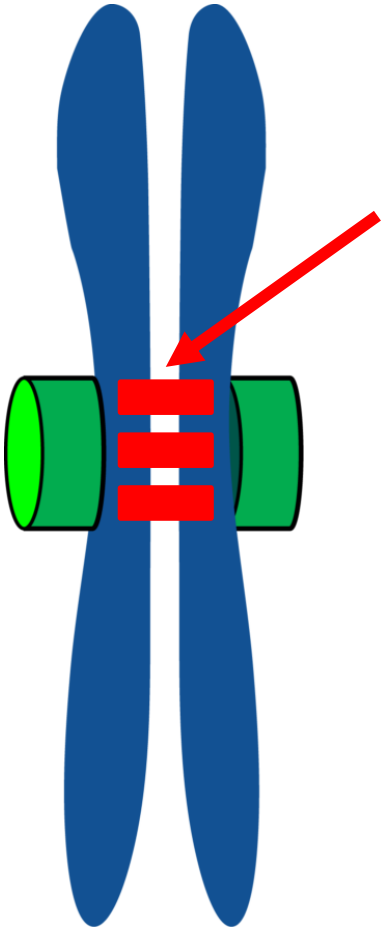


Back-to-back geometry



distorted

Kinetochore geometry



Molecules that fix the geometry?

Back-to-back geometry

nature Vol 458|16 April 2009|doi:10.1038/nature07876

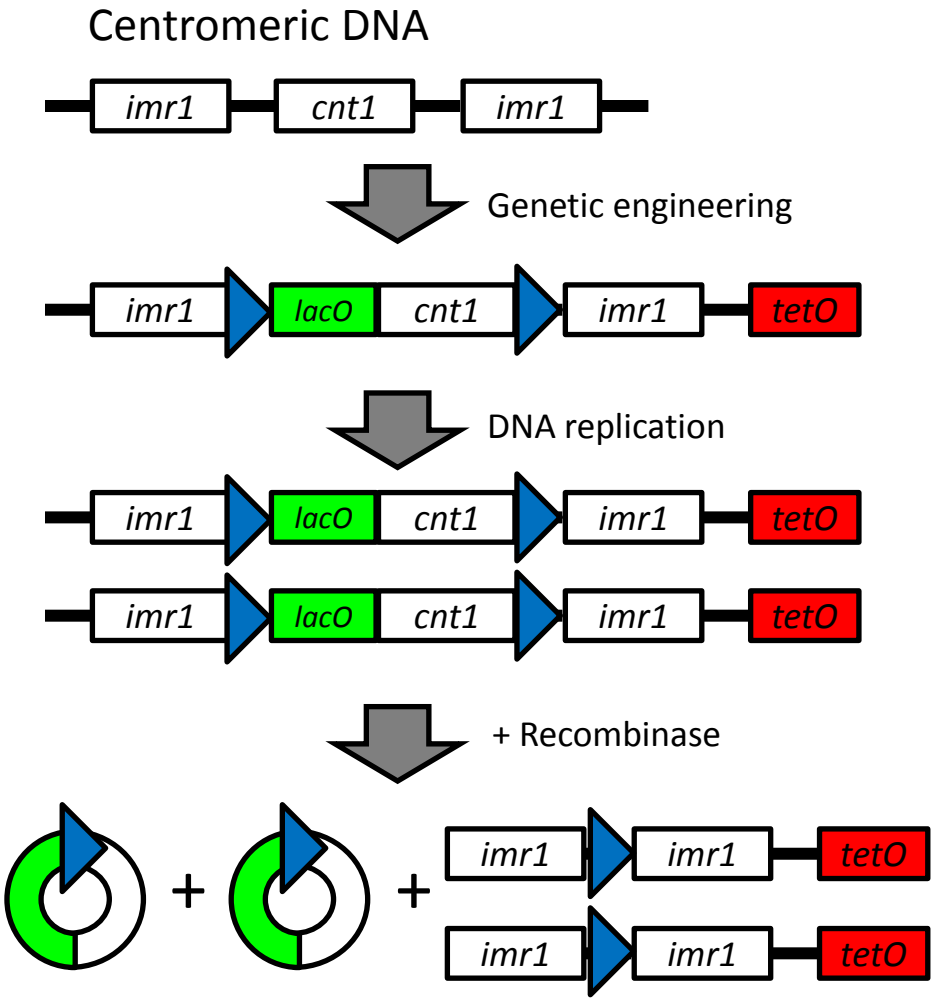
ARTICLES

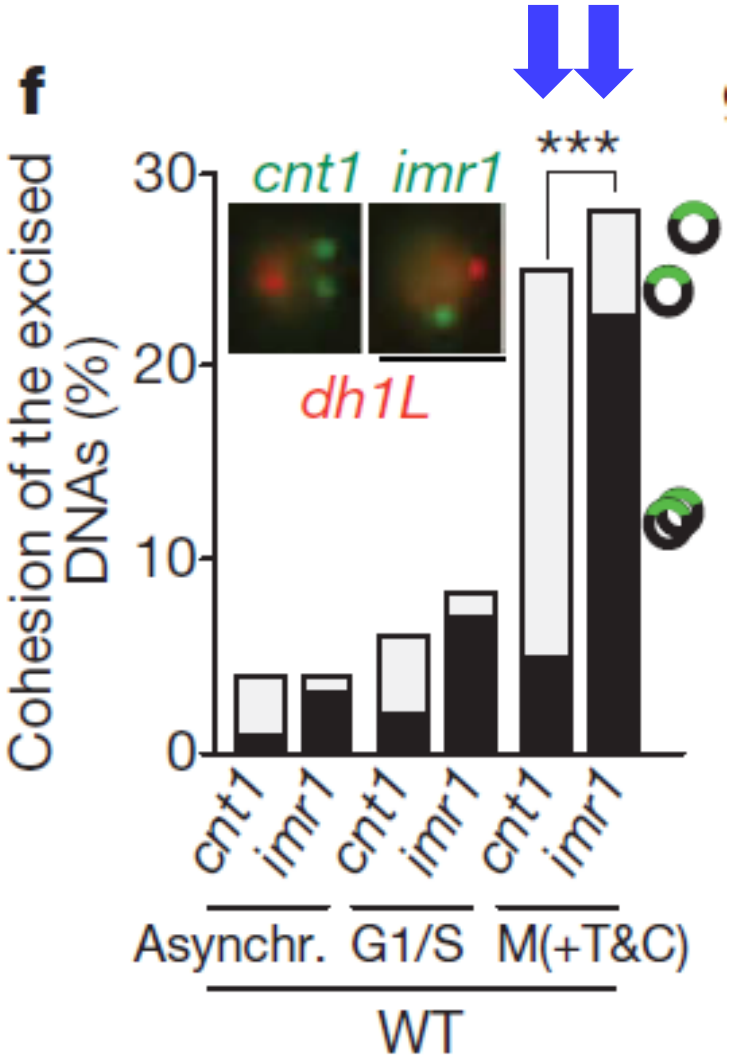
Kinetochore geometry defined by cohesion within the centromere

Takeshi Sakuno[1,2], Kenji Tada[1,3] & Yoshinori Watanabe[1,3]

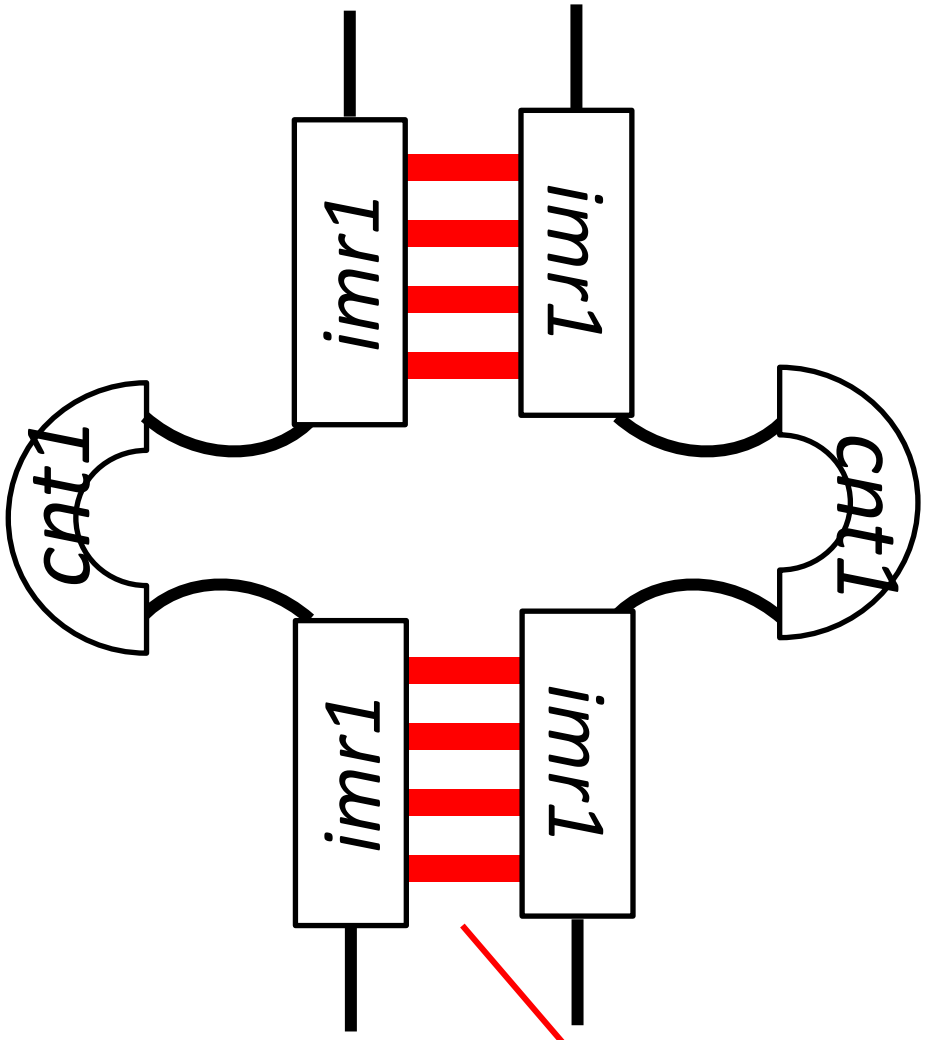
During cell division microtubules capture chromosomes by binding to the kinetochore assembled in the centromeric region of chromosomes. In mitosis sister chromatids are captured by microtubules emanating from both spindle poles, a process called bipolar attachment, whereas in meiosis I sisters are attached to microtubules originating from one spindle pole, called monopolar attachment. For determining chromosome orientation, kinetochore geometry or structure might be an important target of regulation. However, the molecular basis of this regulation has remained elusive. Here we show the link between kinetochore orientation and cohesion within the centromere in fission yeast *Schizosaccharomyces pombe* by strategies developed to visualize the concealed cohesion within the centromere, and to introduce artificial tethers that can influence kinetochore geometry. Our data imply that cohesion at the core centromere induces the mono-orientation of kinetochores whereas cohesion at the peri-centromeric region promotes bi-orientation. Our study may reveal a general mechanism for the geometric regulation of kinetochores, which collaborates with previously defined tension-dependent reorientation machinery.

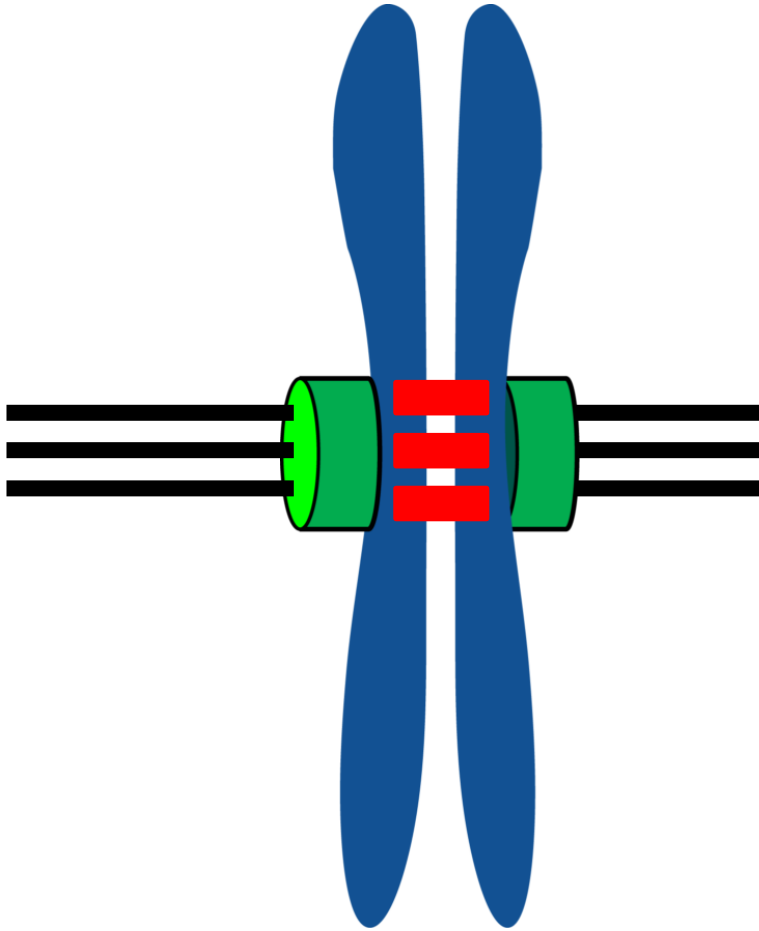
Centromeric cohesion visualized by excision





Cohesion-mediated kinetochore geometry?





Back-to-back geometry

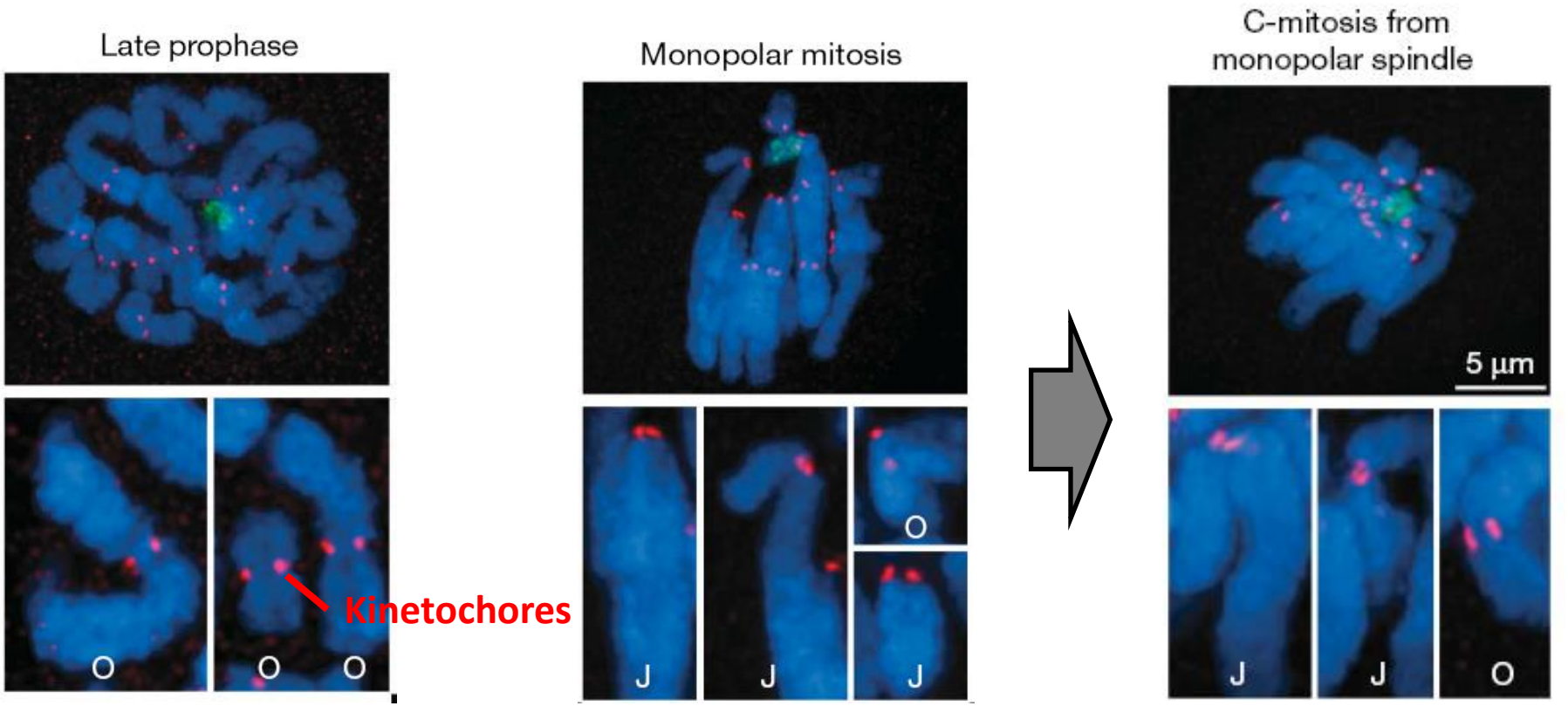
Importance of kinetochore geometry

Vol 450 | 29 November 2007 | doi:10.1038/nature06344 nature

LETTERS

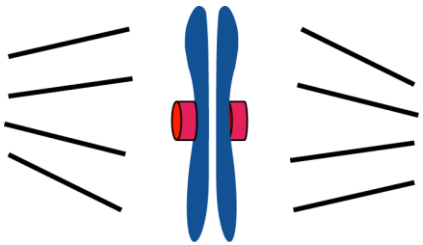
The centromere geometry essential for keeping mitosis error free is controlled by spindle forces

Jadranka Lončarek[1]*, Olga Kisurina-Evgenieva[1]*†, Tatiana Vinogradova[1], Polla Hergert[1], Sabrina La Terra[1,2], Tarun M. Kapoor[3] & Alexey Khodjakov[1,2,3]



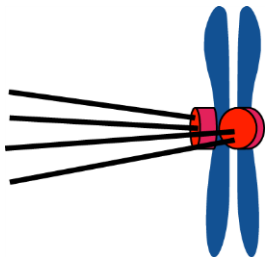
Before kinetochore-microtubule attachment

Back-to-back kinetochore geometry



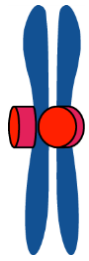
After kinetochore-microtubule in Monastrol

Distorted kinetochore geometry

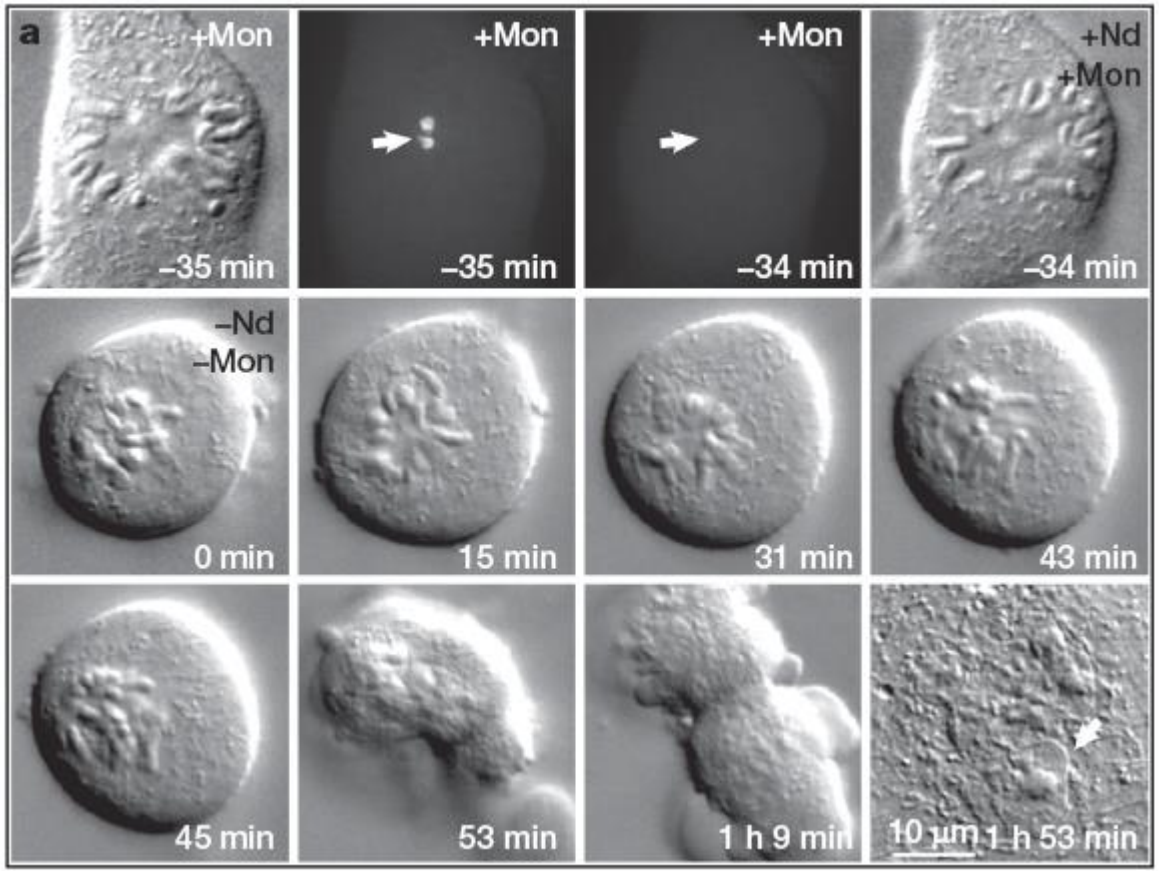


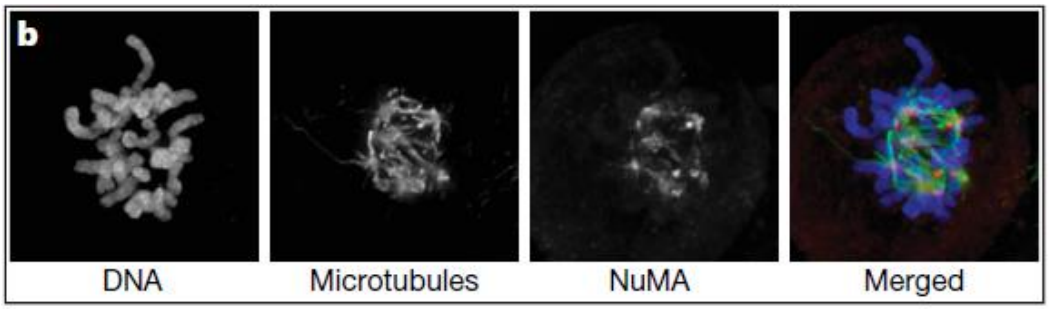
Remove kinetochore-microtubule by Nocodazole

Remains distorted



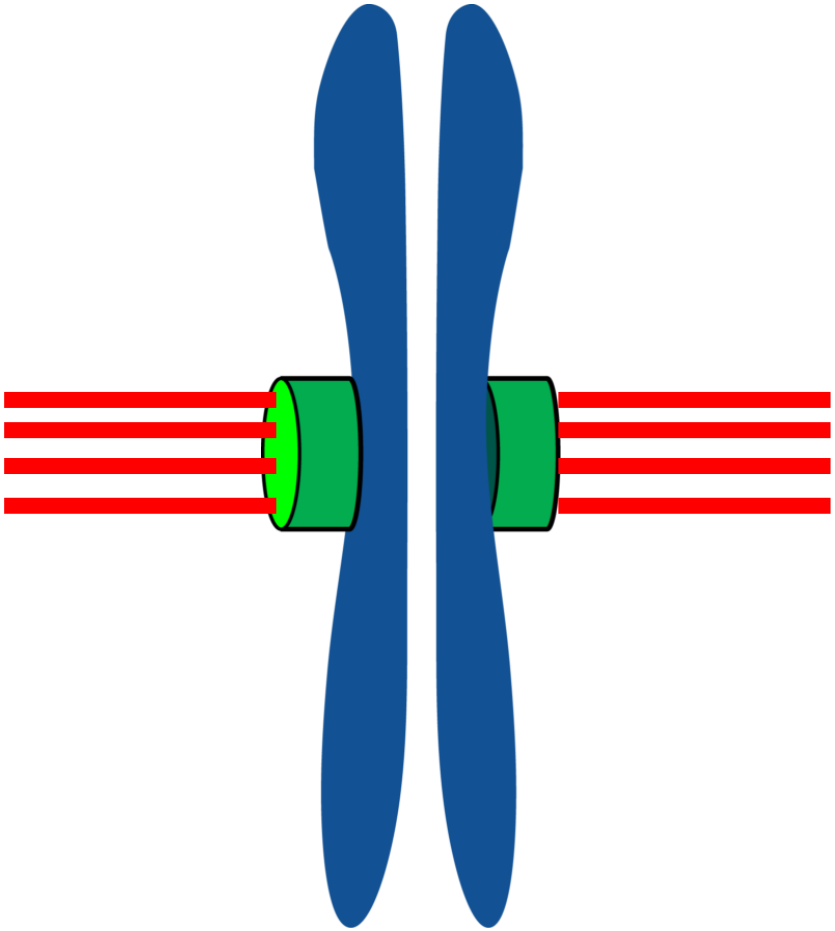
Errors in chromosome segregation with distorted kinetochore geometry



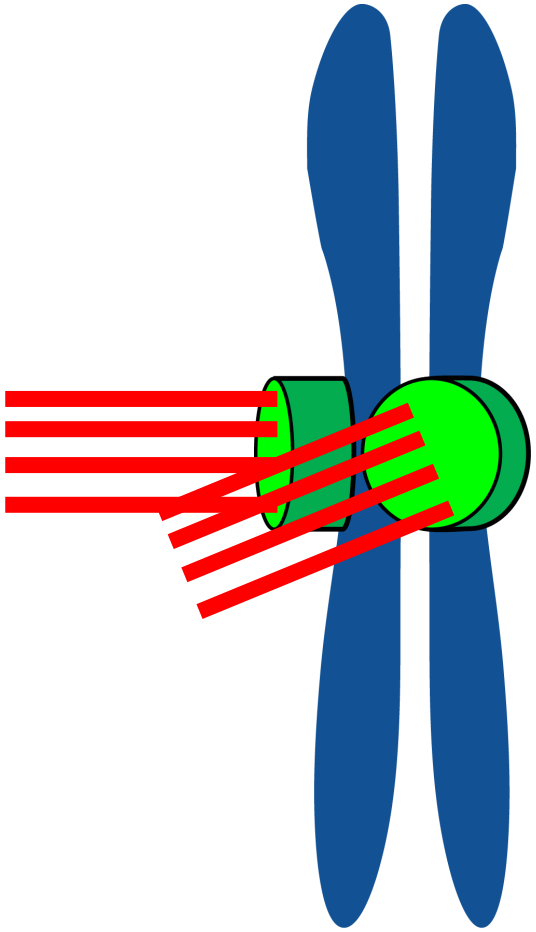


1. Create distorted kinetochore geometry by Monastrol (-35 min)
2. Remove existing kinetochore-microtubule attachments by Nocodazol (-34 min)
3. Restart kinetochore-microtubule attachments by washout (0 min)

Kinetochore geometry



Back-to-back geometry



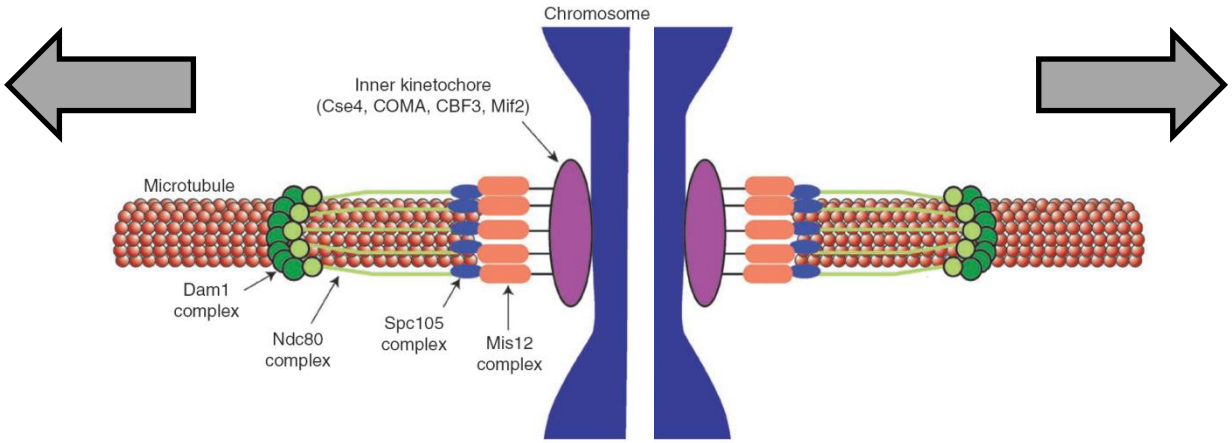
distorted

Increases the likelihood of initial attachment from opposite poles

“Biorientation”

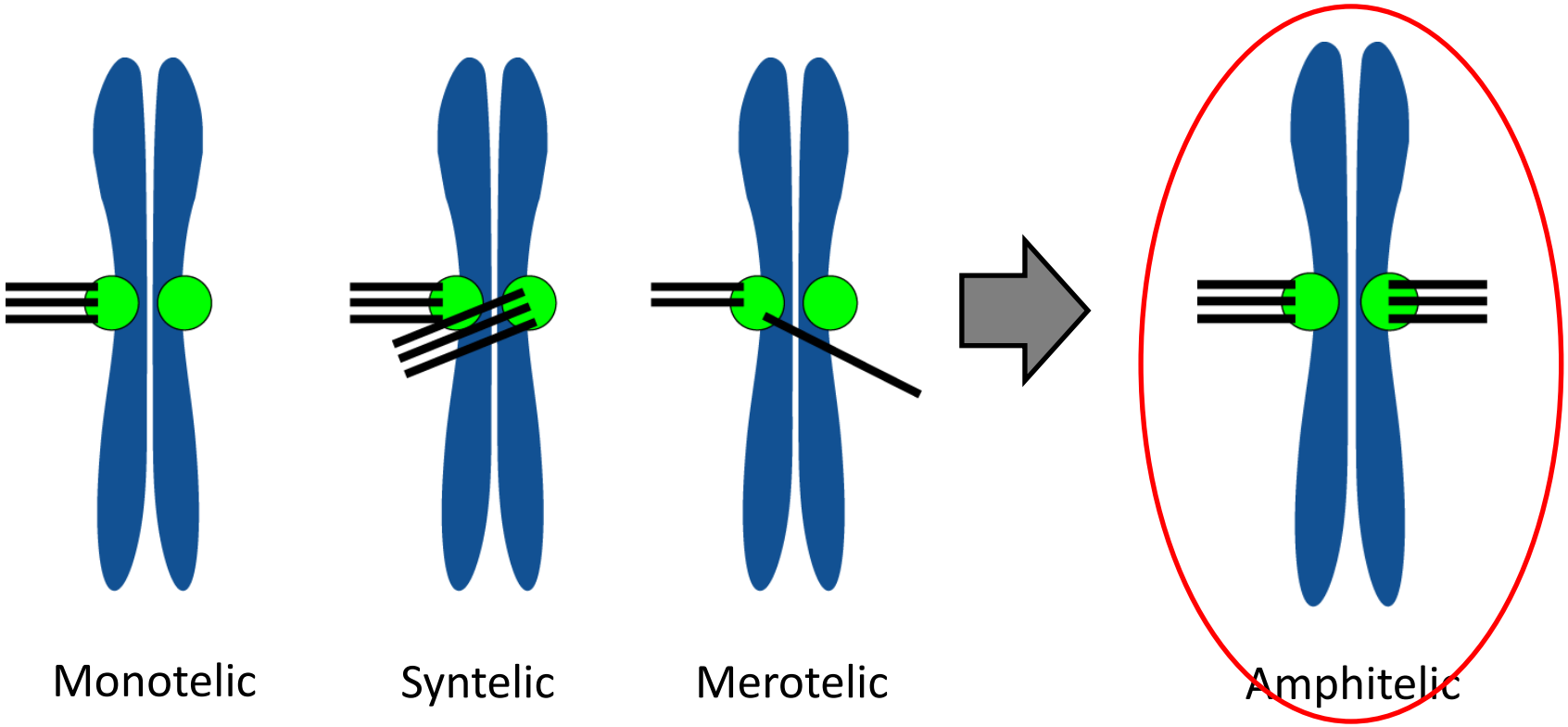
3 mechanisms so far proposed:

1. Kinetochore geometry
2. Kinetochore tension
3. Chromosome spatial arrangement

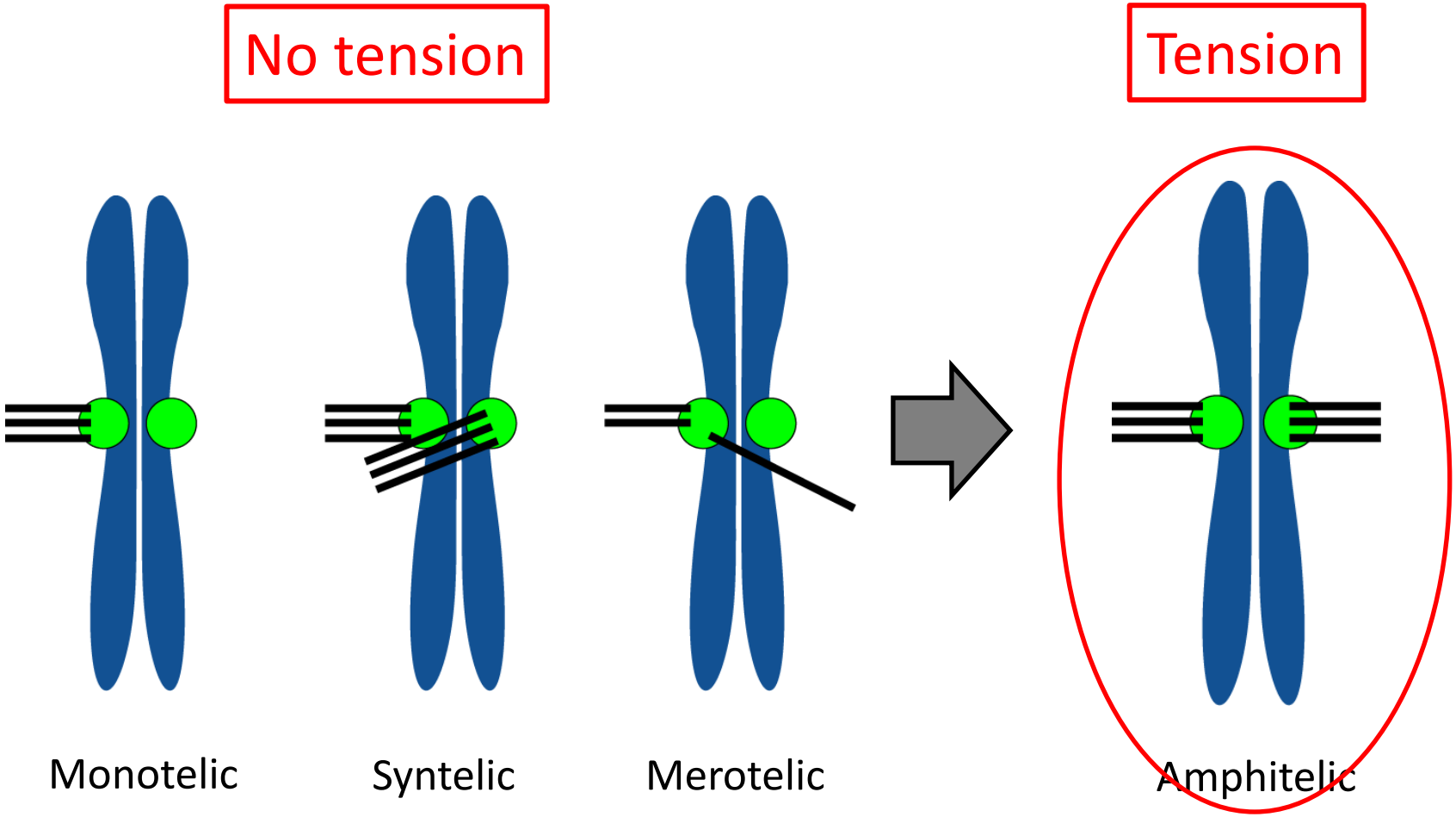


What happens if initial attachment is wrong?

Kinetochore geometry increases
the likelihood of initial correct attachment,
but we still see many wrong attachments in cells!



A mechanism to sense the tension across kinetochores?



Cells are capable of sensing tension between kinetochores

Mitotic forces control a cell-cycle checkpoint

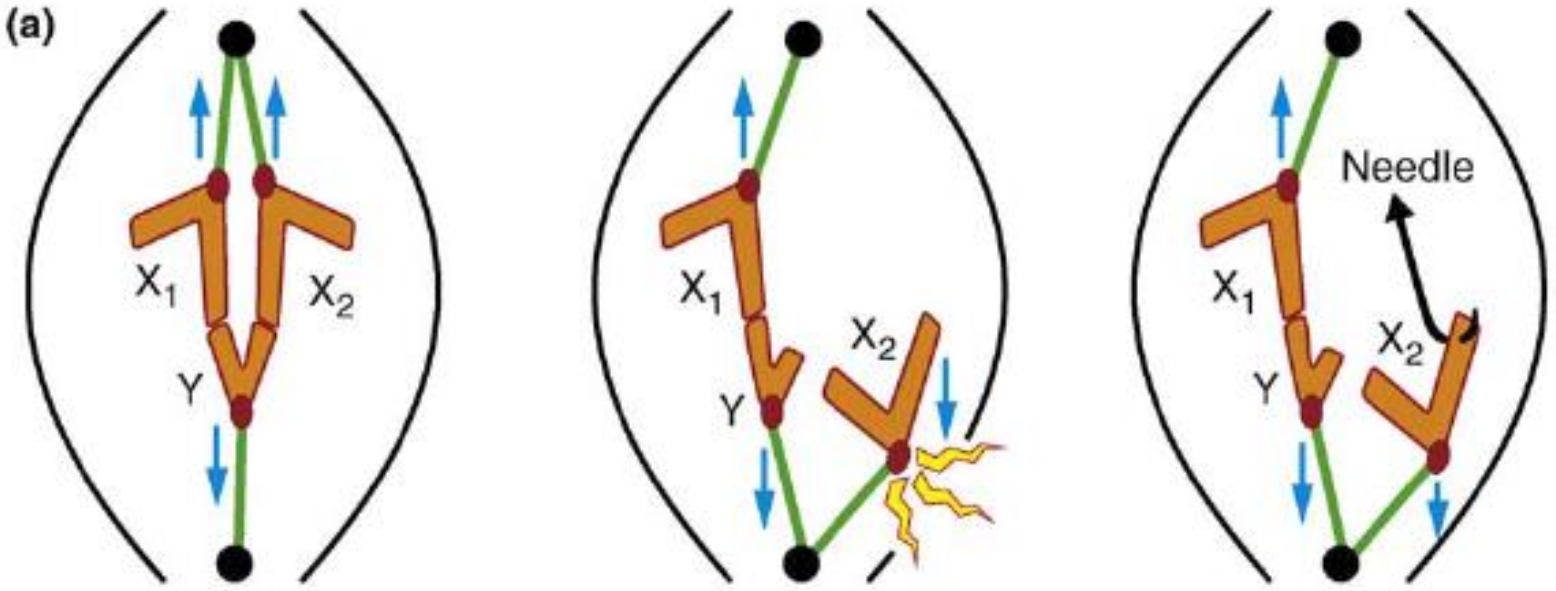
Xiaotong Li & R. Bruce Nicklas

Department of Zoology, Duke University, Box 91000, Durham, North Carolina 27708-1000, USA

EVERY time a cell divides, the chromosomes must be distributed accurately to the daughter cells. Errors in distribution arise if chromosomes are improperly attached to the mitotic spindle. Improper attachment is detected by a cell-cycle checkpoint in many cells[1,2] and the completion of cell division is delayed, allowing time for error correction. How is an improperly attached chromosome detected? An absence of tension from mitotic forces is one possibility[3]. Here we test this possibility directly by applying tension to an improperly attached chromosome with a micromanipulation needle. In the absence of tension, the entry into anaphase and the completion of mitosis was delayed by 5–6 hours. When the misattached chromosome was placed under tension, however, the cell entered anaphase in 56 minutes, on average. Tension from mitotic forces or from a micromanipulator's needle evidently signals to the checkpoint that all is in order and that cell division can proceed.

NATURE · VOL 373 · 16 FEBRUARY 1995

Nicklas' experiment



(reviewed in Nezi & Musacchio, 2009 Curr Opi Cell Biol)

The cell somehow senses
the tension across the kinetochore pair

Tension between kinetochores is sufficient to achieve correct attachment

letters to nature

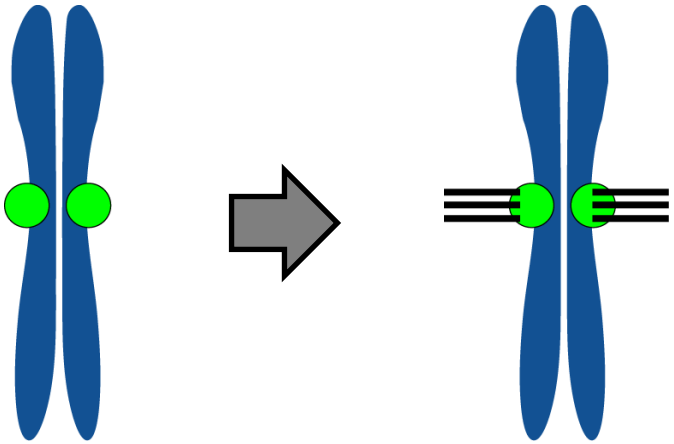
Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle

Hilary Dewar[1], Kozo Tanaka[1], Kim Nasmyth[2] & Tomoyuki U. Tanaka[1]

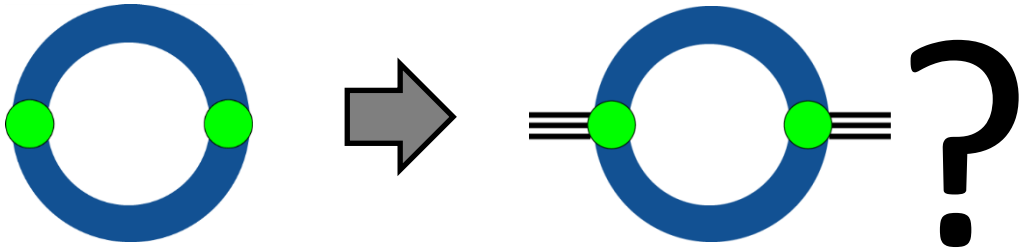
[1]*School of Life Sciences, University of Dundee, Wellcome Trust Biocentre, Dundee DD1 5EH, UK*
[2]*Research Institute of Molecular Pathology, Dr Bohr-Gasse 7, A-1030 Vienna, Austria*

The movement of sister chromatids to opposite spindle poles during anaphase depends on the prior capture of sister kinetochores by microtubules with opposing orientations (amphitelic attachment or bi-orientation)[1]. In addition to proteins necessary for the kinetochore–microtubule attachment, bi-orientation requires the Ipl1 (Aurora B in animal cells) protein kinase[2–7] and tethering of sister chromatids by cohesin[8,9]. Syntelic attachments, in which sister kinetochores attach to microtubules with the same orientation, must be either 'avoided' or 'corrected'. Avoidance might be facilitated by the juxtaposition of sister kinetochores such that they face in opposite directions; kinetochore geometry is therefore deemed important. Error correction, by contrast, is thought to stem from the stabilization of kinetochore–spindle pole connections by tension in microtubules, kinetochores, or the surrounding chromatin arising from amphitelic but not syntelic attachment[10,11]. The tension model predicts that any type of connection between two kinetochores suffices for efficient bi-orientation. Here we show that the two kinetochores of engineered, unreplicated dicentric chromosomes in *Saccharomyces cerevisiae* bi-orient efficiently, implying that sister kinetochore geometry is dispensable for bi-orientation. We also show that Ipl1 facilitates bi-orientation by promoting the turnover of kinetochore–spindle pole connections in a tension-dependent manner.

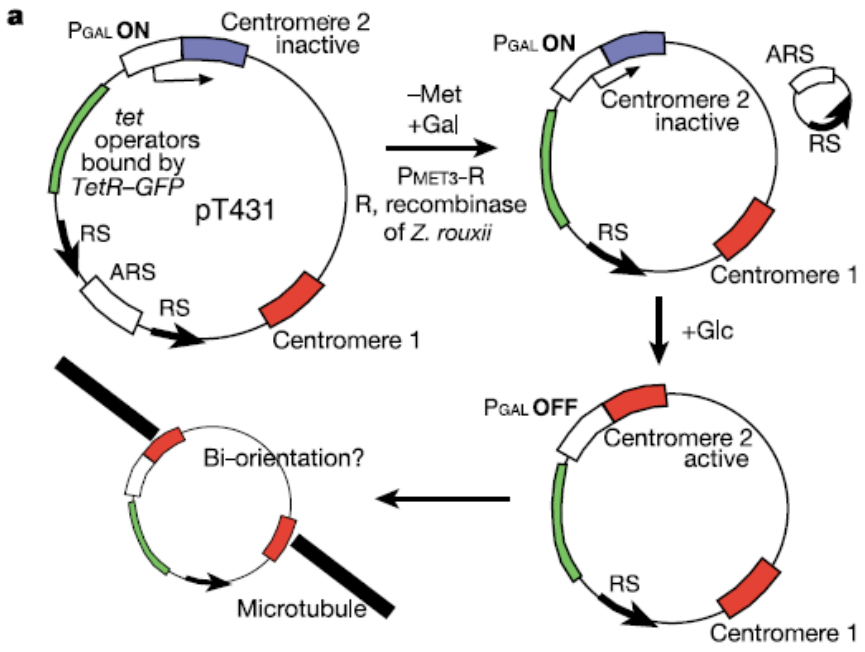
NATURE | VOL 428 | 4 MARCH 2004 | www.nature.com/nature



Chromosomes in normal cells achieve biorientation

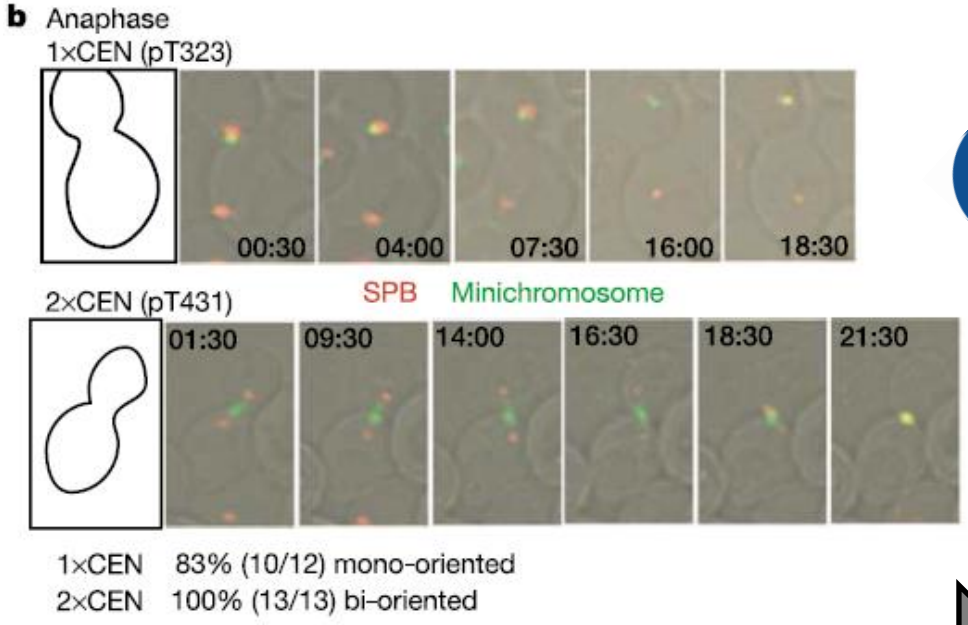


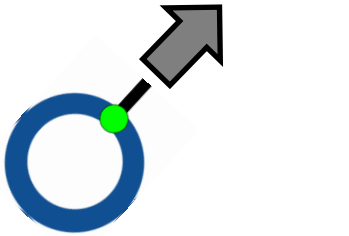
How about this engineered chromosome?

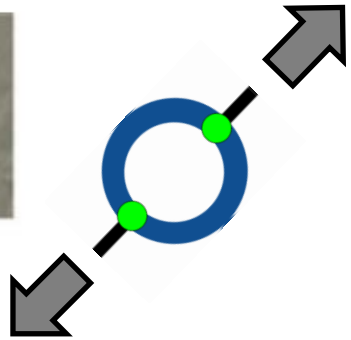


A plasmid-based minichromosome

- 2 Centromeres
- No Replication origin
- Labeled by GFP

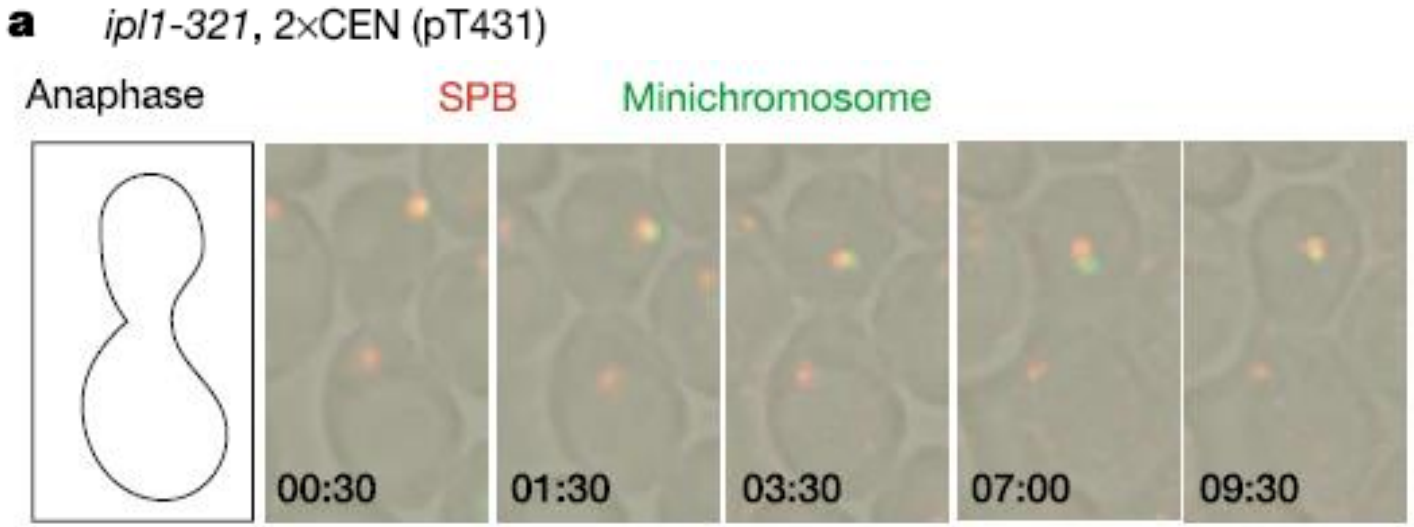


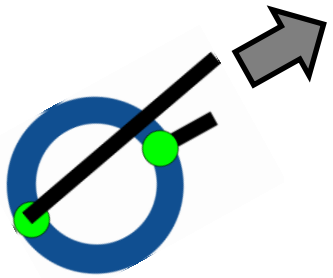




Tension suffices for chromosome biorientation !

Aurora kinase (Ipl1 in yeast) is required for sensing tension across kinetochores





Cell, Vol. 108, 317–329, February 8, 2002, Copyright ©2002 by Cell Press

Evidence that the Ipl1-Sli15 (Aurora Kinase-INCENP) Complex Promotes Chromosome Bi-orientation by Altering Kinetochore-Spindle Pole Connections

Tomoyuki U. Tanaka,[1,2,4] Najma Rachidi,[1]
Carsten Janke,[3] Gislene Pereira,[3] Marta Galova,[2]
Elmar Schiebel,[3] Michael J.R. Stark,[1]
and Kim Nasmyth[2]
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United Kingdom
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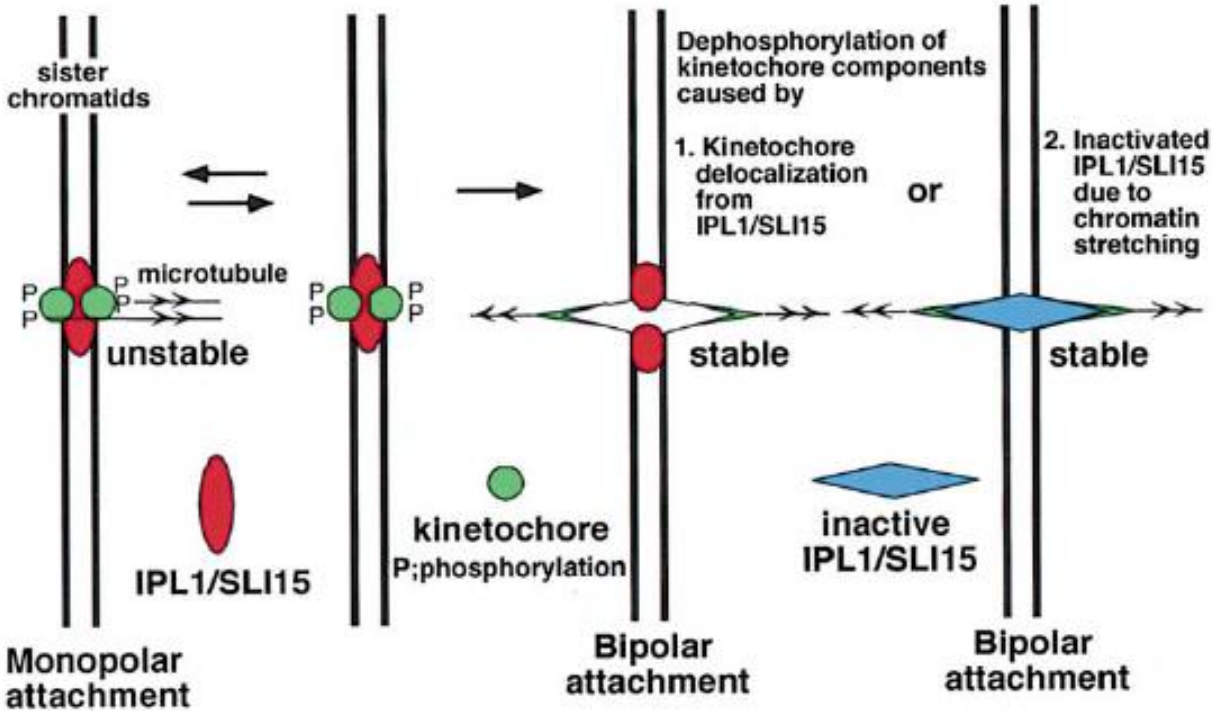
Vienna
Austria
[3]The Beatson Institute for Cancer Research
CRC Beatson Laboratories
Glasgow G61 1BD
United Kingdom

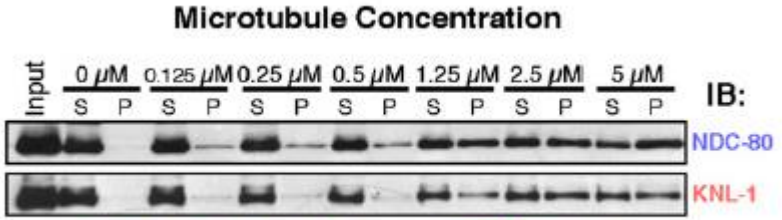


What is the target of Aurora B?

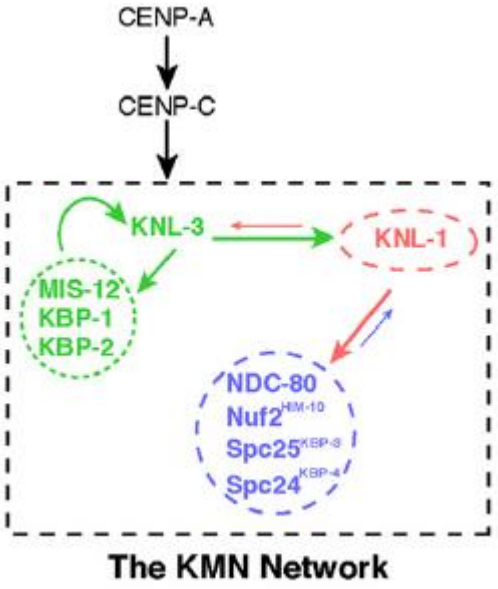
Biochemical approach to assay kinetochore-microtubule attachment

The Conserved KMN Network Constitutes the Core Microtubule-Binding Site of the Kinetochore

Iain M. Cheeseman,[1,*] Joshua S. Chappie,[2] Elizabeth M. Wilson-Kubalek,[2] and Arshad Desai[1,*]
[1] Ludwig Institute for Cancer Research, Department of Cellular and Molecular Medicine (UCSD), CMM-East, Room 3052, La Jolla, CA 92093, USA
[2] Center for Integrative Molecular Biosciences, Department of Cell Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA
*Contact: icheeseman@ucsd.edu (I.M.C.), abdesai@ucsd.edu (A.D.)
DOI 10.1016/j.cell.2006.09.039

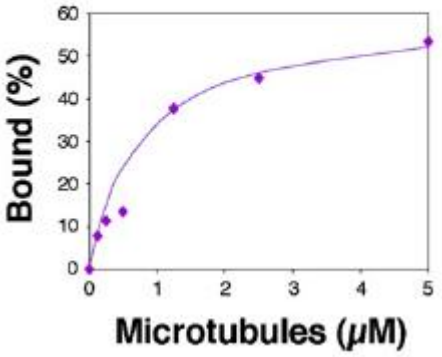


Partially Purified *C. elegans* KMN Network

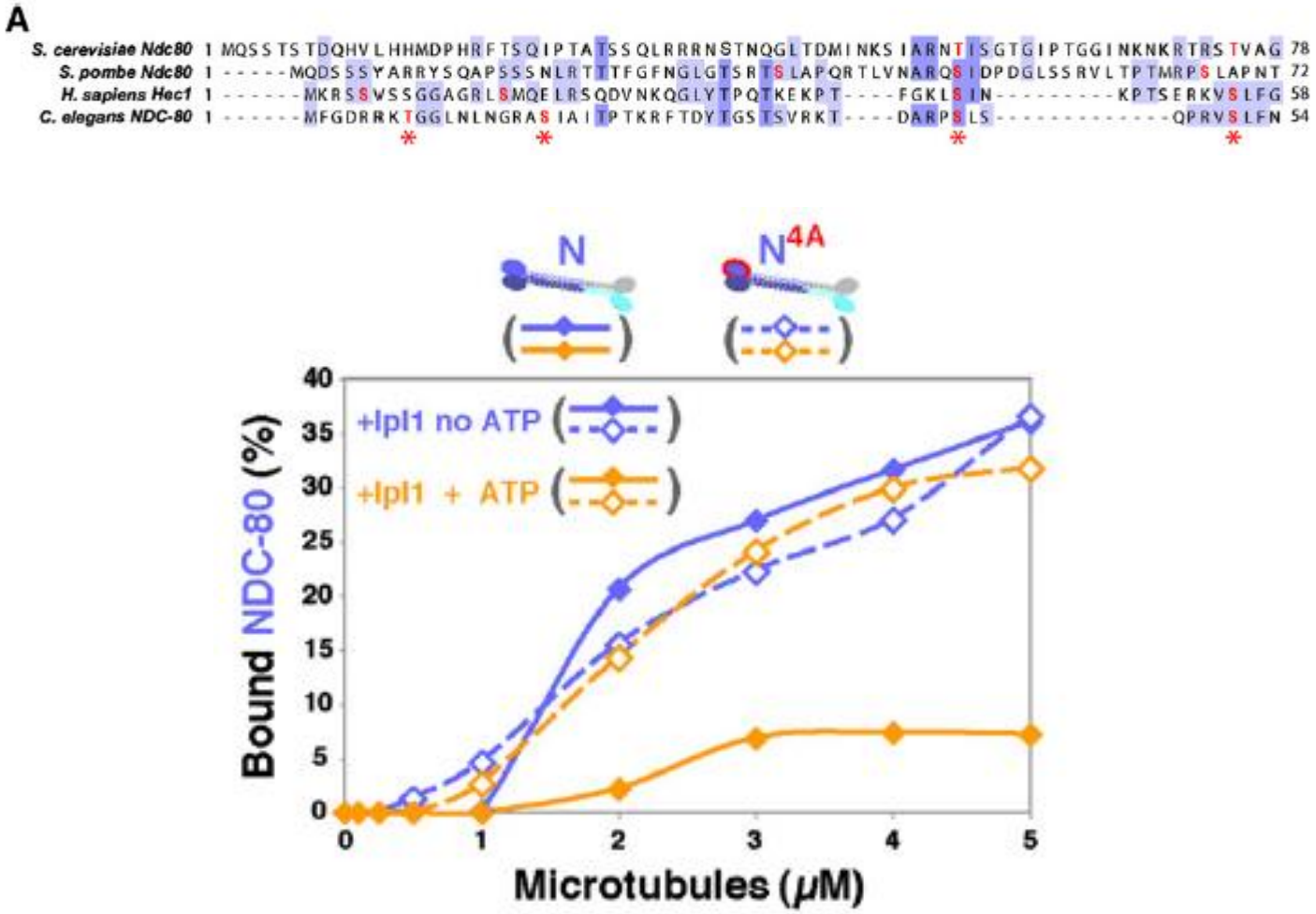


The KMN Network

Partially Purified
C. elegans KMN Network



Phosphorylation of kinetochore components by Aurora B weakens microtubule binding affinity



How does Aurora B "sense" tension between kinetochores?

Sensing Chromosome Bi-Orientation by Spatial Separation of Aurora B Kinase from Kinetochore Substrates

Dan Liu,[1] Gerben Vader,[2]* Martijn J. M. Vromans,[2] Michael A. Lampson,[1]†‡ Susanne M. A. Lens[2]†

Successful cell division requires that chromosomes attach to opposite poles of the mitotic spindle (bi-orientation). Aurora B kinase regulates chromosome-spindle attachments by phosphorylating kinetochore substrates that bind microtubules. Centromere tension stabilizes bi-oriented attachments, but how physical forces are translated into signaling at individual centromeres is unknown. Using fluorescence resonance energy transfer–based biosensors to measure localized phosphorylation dynamics in living cells, we found that phosphorylation of an Aurora B substrate at the kinetochore depended on its distance from the kinase at the inner centromere. Furthermore, repositioning Aurora B closer to the kinetochore prevented stabilization of bi-oriented attachments and activated the spindle checkpoint. Thus, centromere tension can be sensed by increased spatial separation of Aurora B from kinetochore substrates, which reduces phosphorylation and stabilizes kinetochore microtubules.

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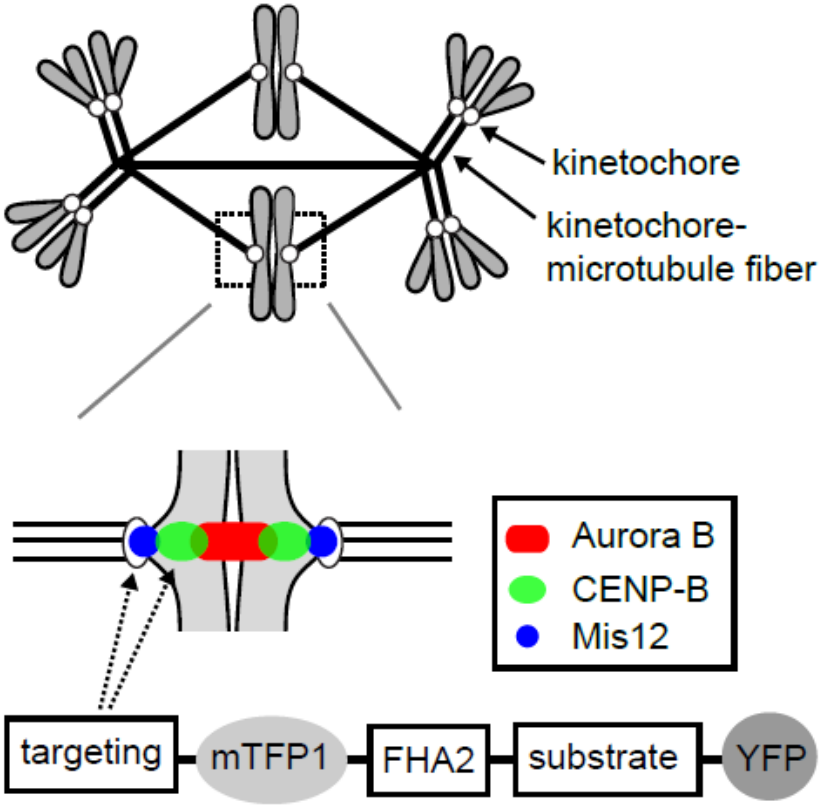
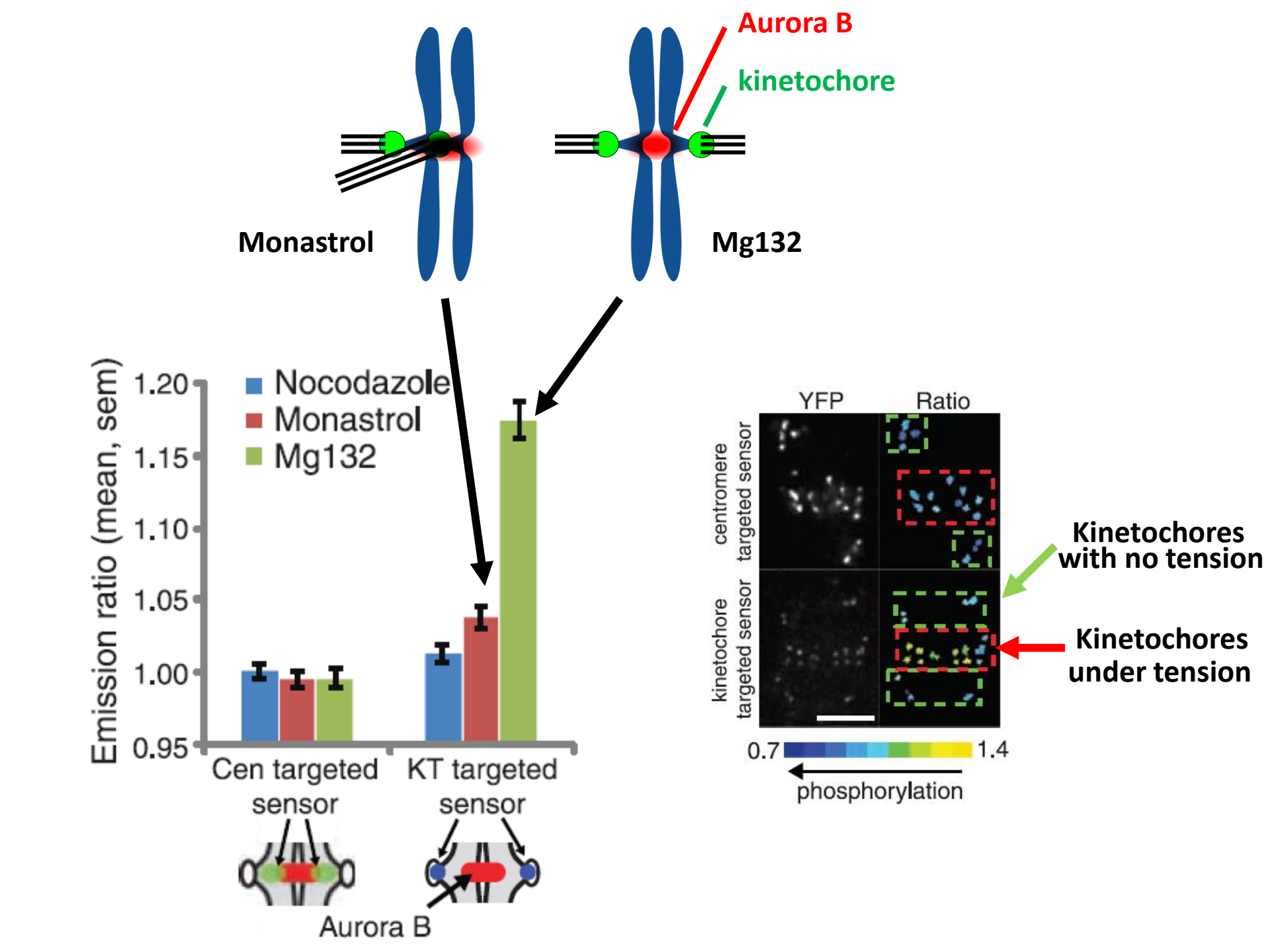
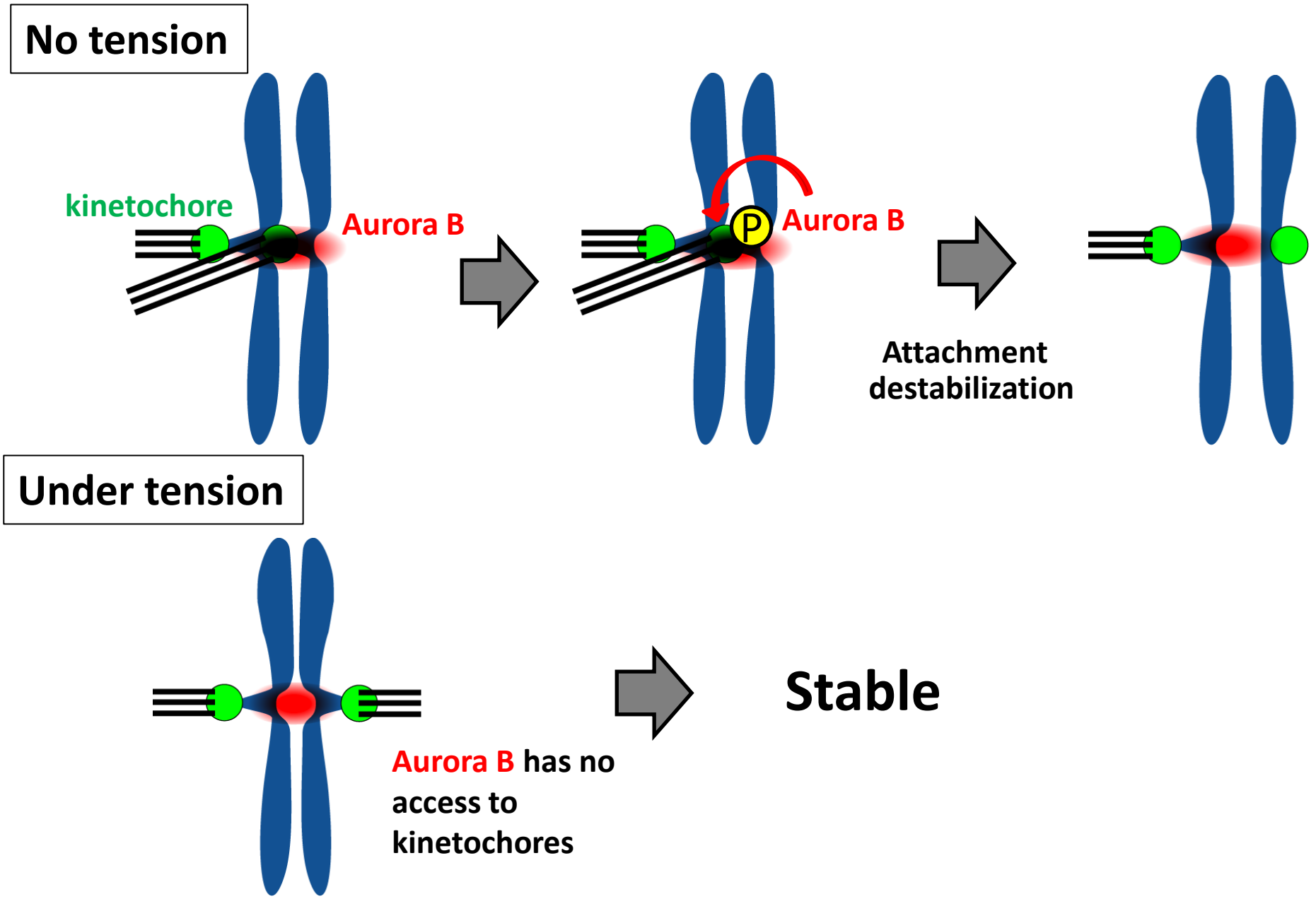


Aurora B-specific FRET sensor


Aurora B
kinetochore
Monastrol
Mg132
Nocodazole
Monastrol
Mg132
Emission ratio (mean, sem)
1.20
1.15
1.10
1.05
1.00
0.95
Cen targeted sensor
KT targeted sensor
Aurora B
YFP
Ratio
centromere targeted sensor
kinetochore targeted sensor
Kinetochores with no tension
Kinetochores under tension
0.7
1.4
phosphorylation

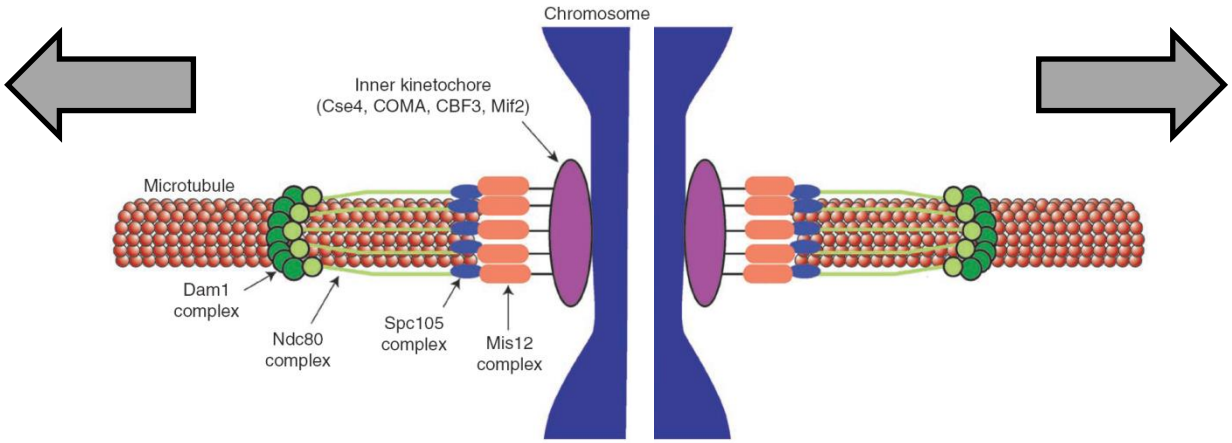
Aurora B senses changes in kinetochore geometry



“Biorientation”

3 mechanisms so far proposed:

1. Kinetochore geometry
2. Kinetochore tension
3. Chromosome spatial arrangement



“Biorientation”

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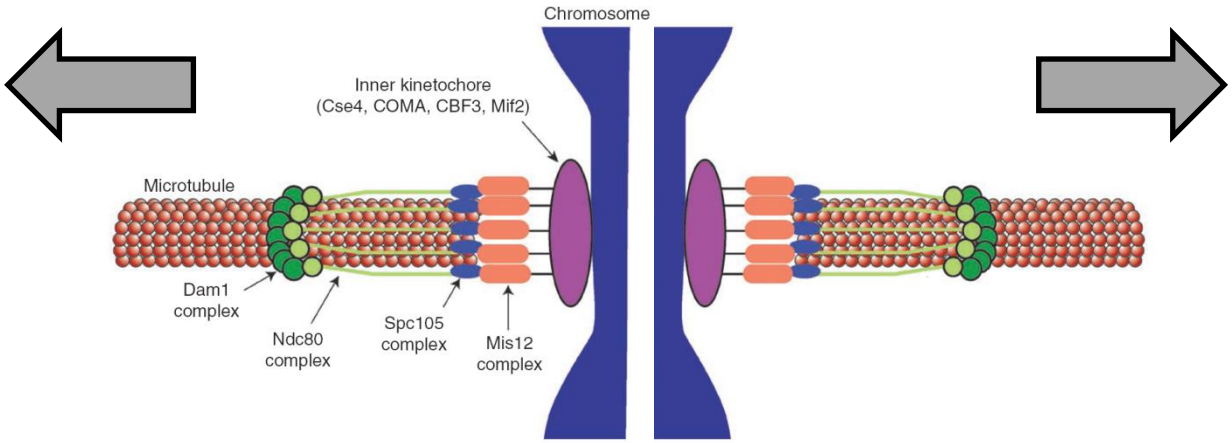

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