nature COMMUNICATIONS

ARTICLE

Received 11 Mar 2016 | Accepted 13 Sep 2016 | Published 20 Oct 2016

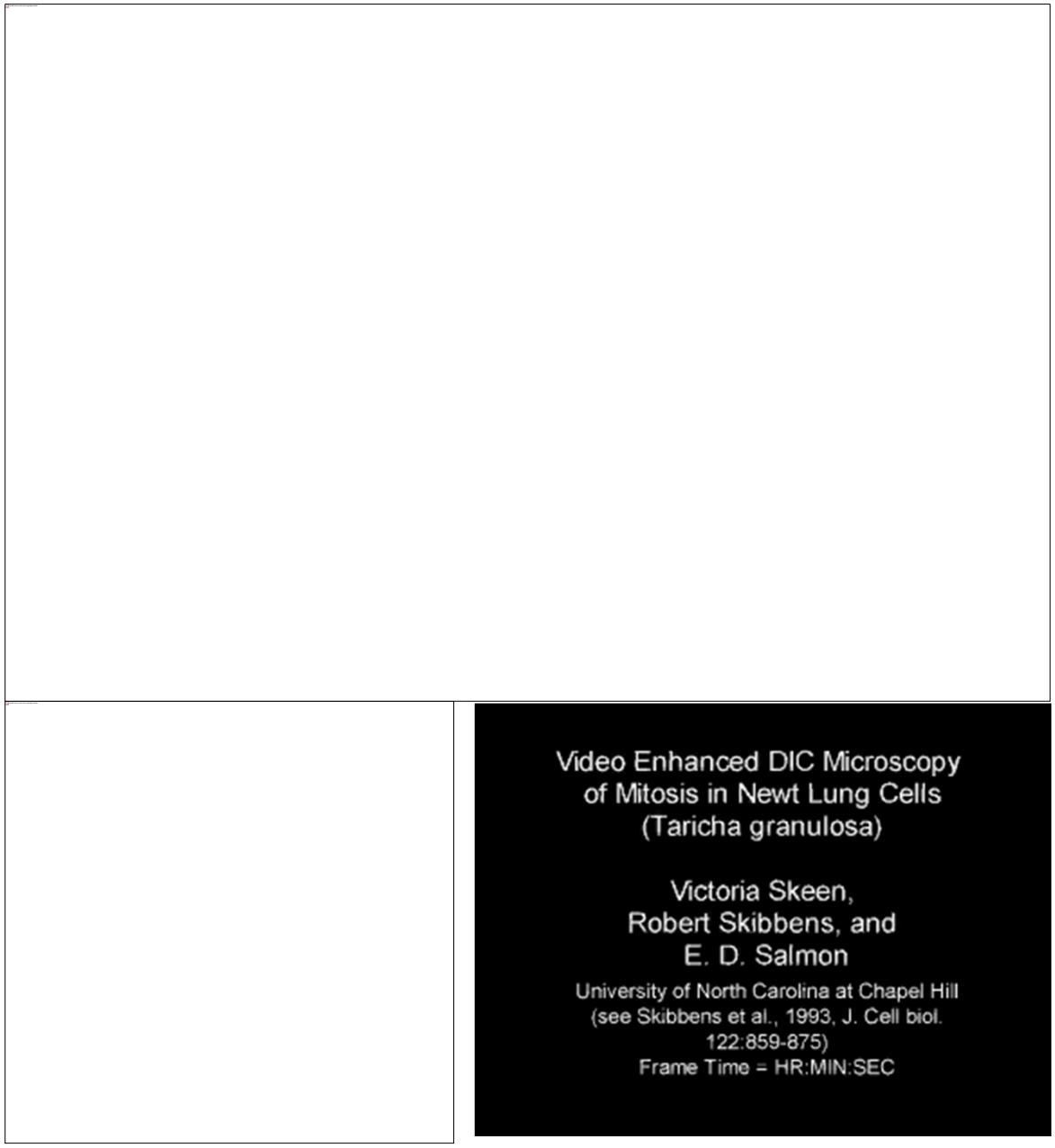
DOI: 10.1038/ncomms13221 OPEN

Chromosome biorientation produces hundreds of piconewtons at a metazoan kinetochore

Anna A. Ye[1,2], Stuart Cane[1,2] & Thomas J. Maresca[1,2]

Tension sensors:
centromere and kinetochore springs

How to measure
mechanical forces
within a cell?



Mechanical forces in mitosis

- Microtubule dynamics
- Motor proteins
- Required for chromosome movements
- Microtubule attachment
- SAC signalling

Kinetochore: microtubule attachment sites

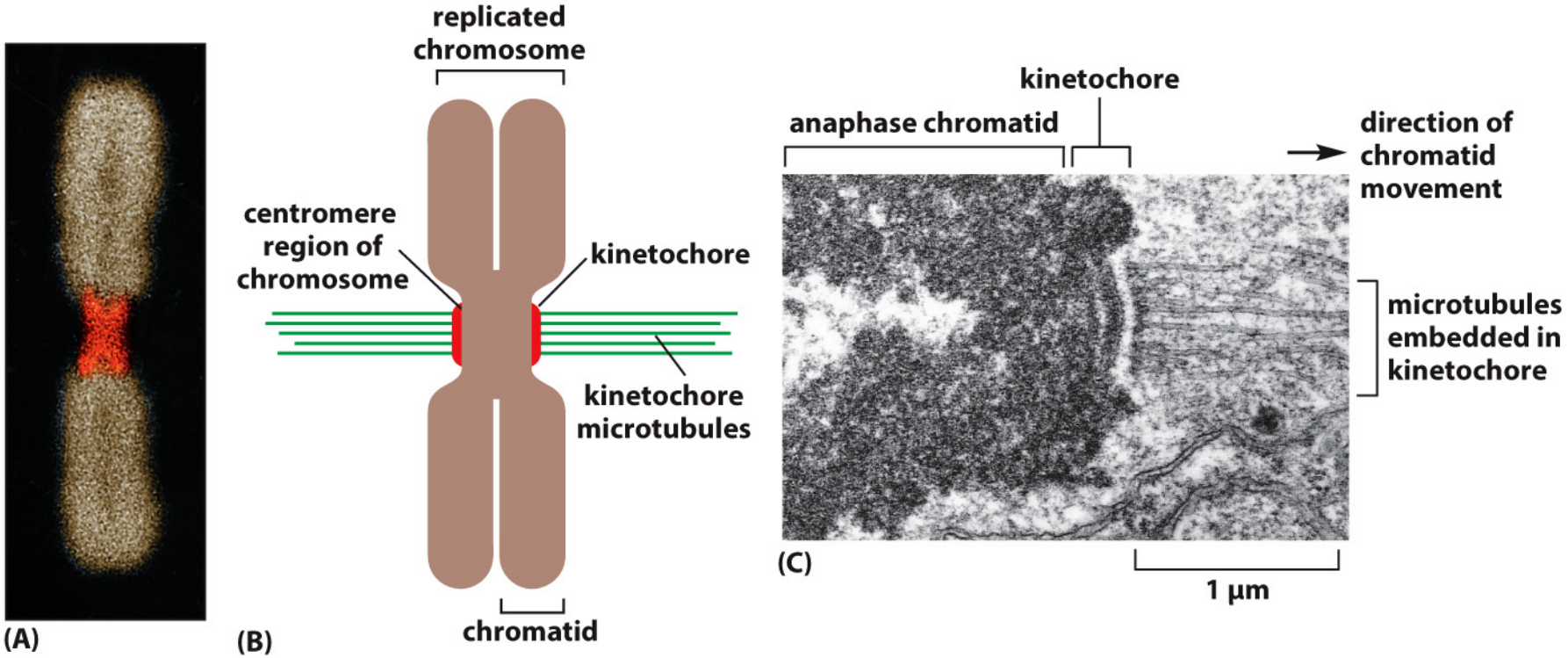
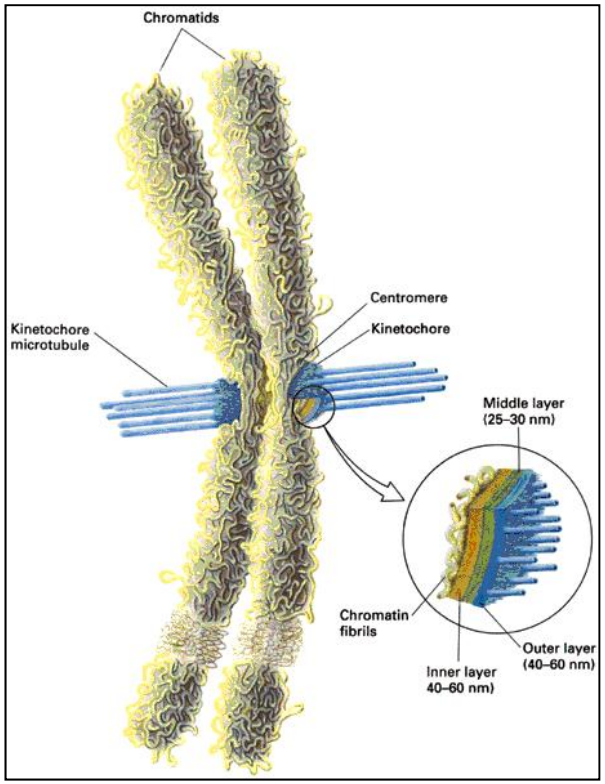
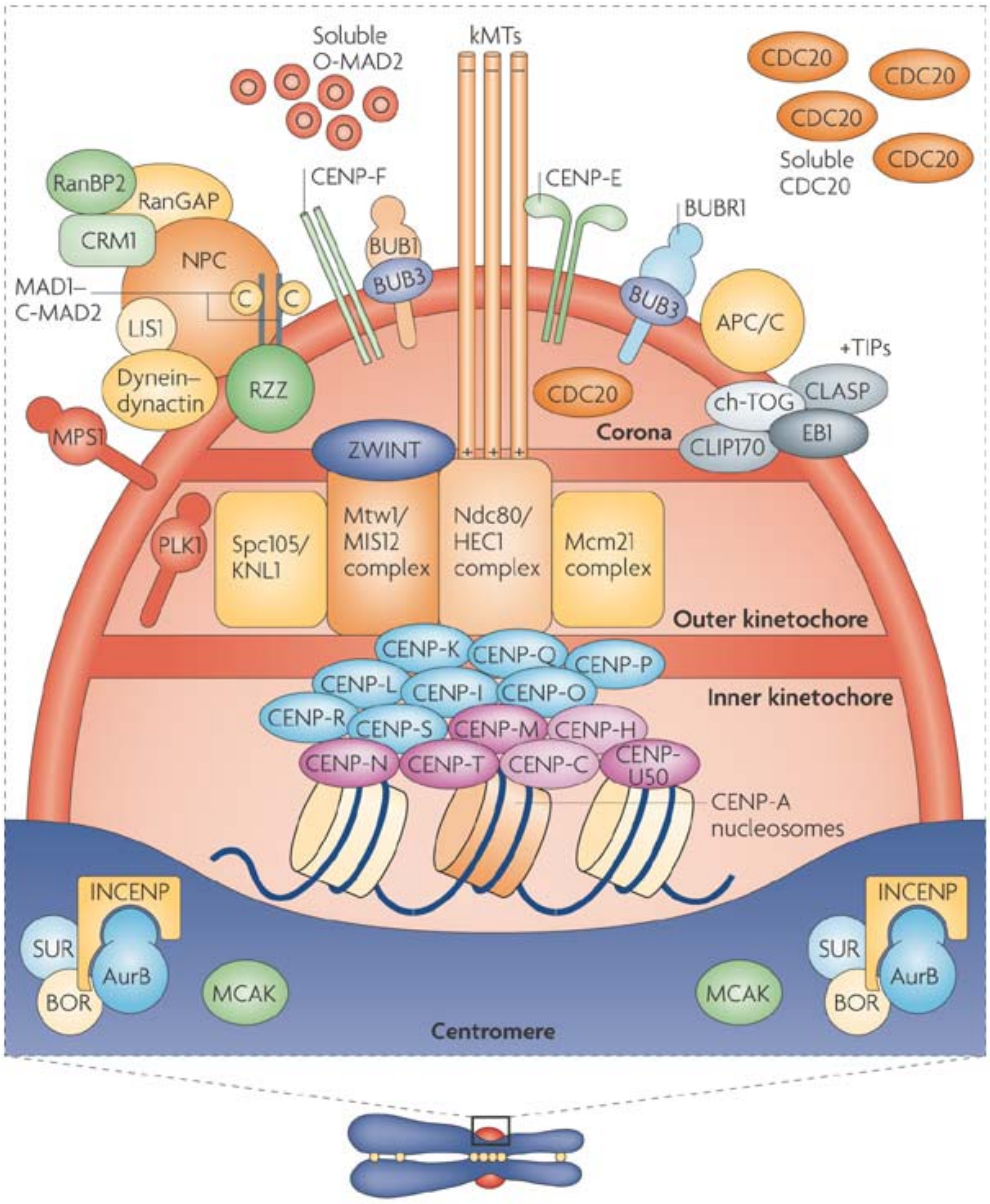


Figure 17-30 Molecular Biology of the Cell 6e (© Garland Science 2015)





Nature Reviews | Molecular Cell Biology

Cheeseman/Desai 2008

stable end-on attachments

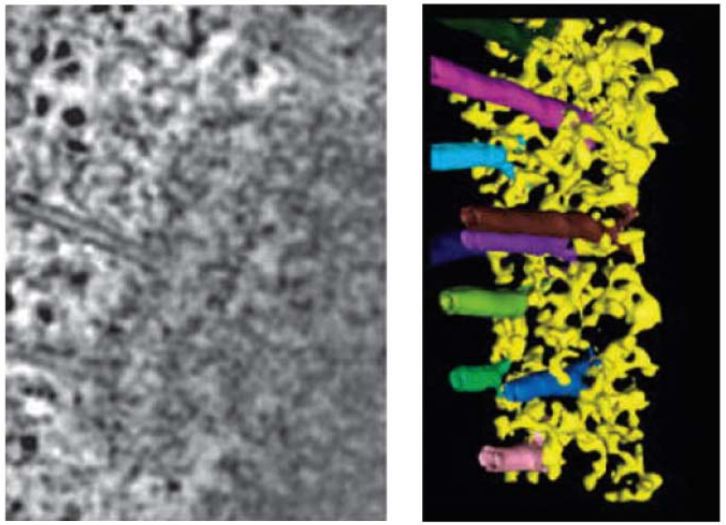
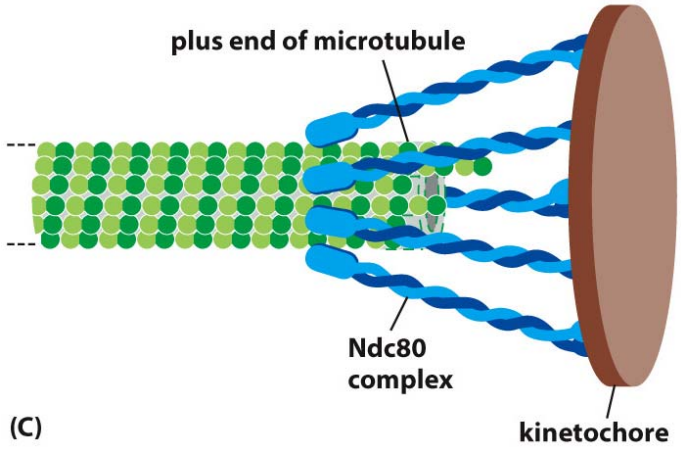
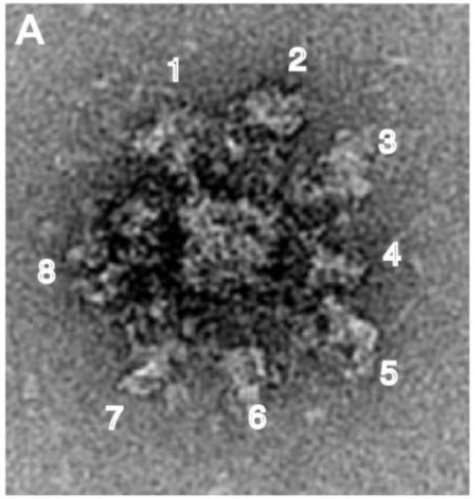
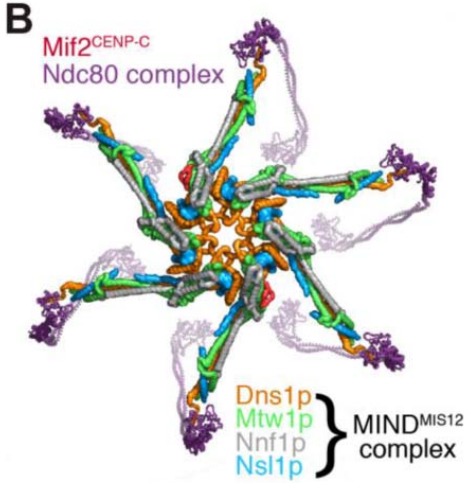
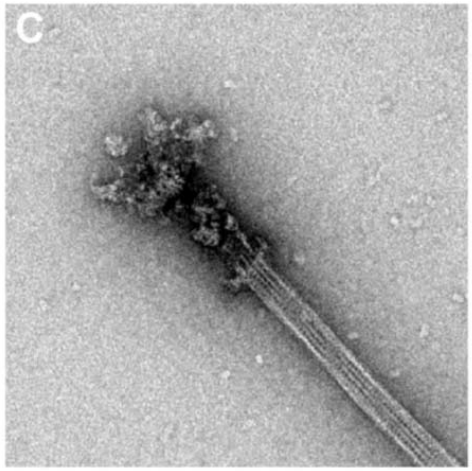


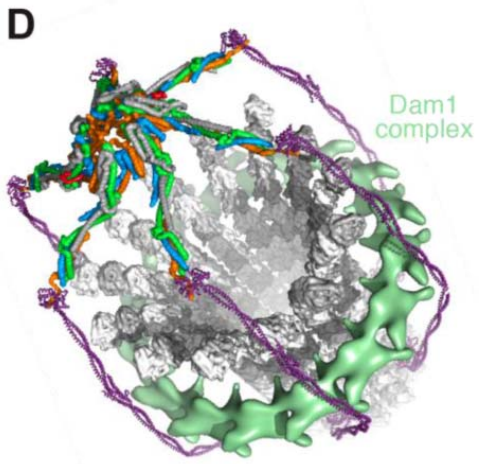
Figure 17-31 Molecular Biology of the Cell 6e (© Garland Science 2015)











Musacchio, A.; Desai, A. A Molecular View of Kinetochore Assembly and Function. *Biology* **2017**, *6*, 5.

chromosome bi-orientation

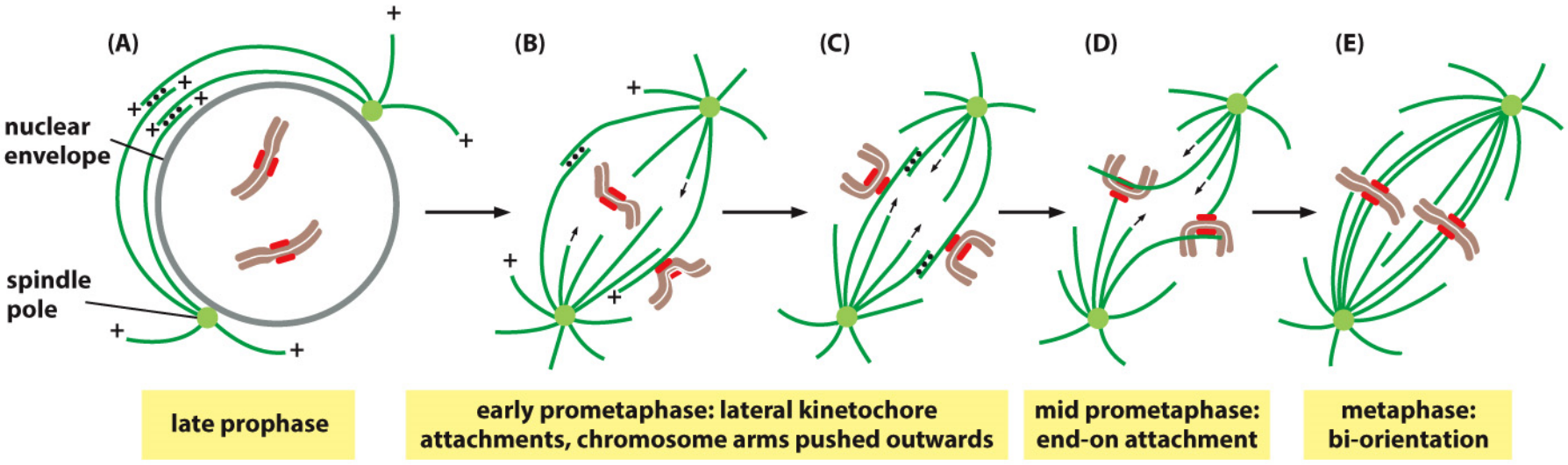
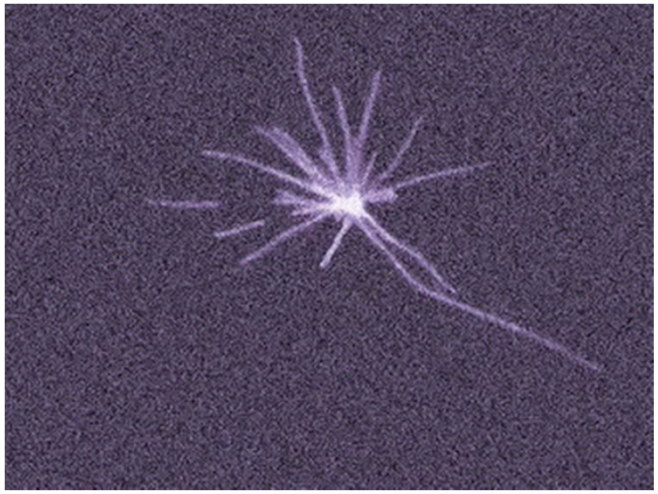
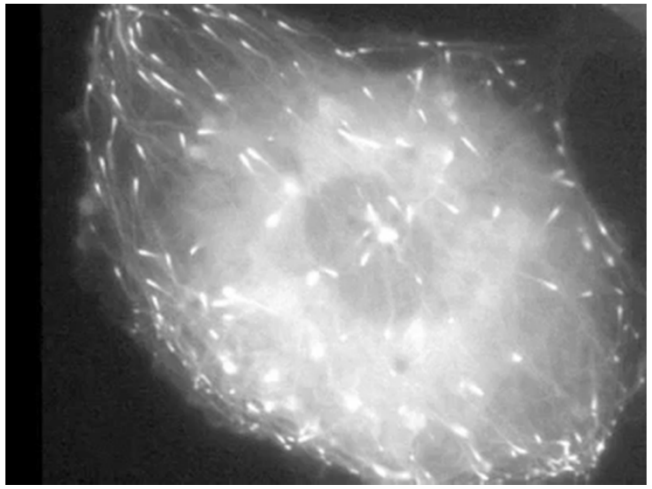
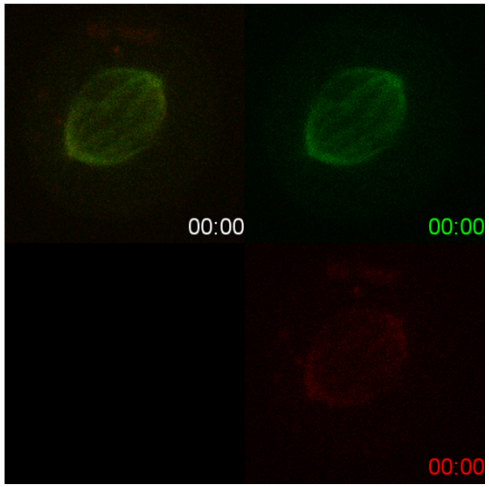


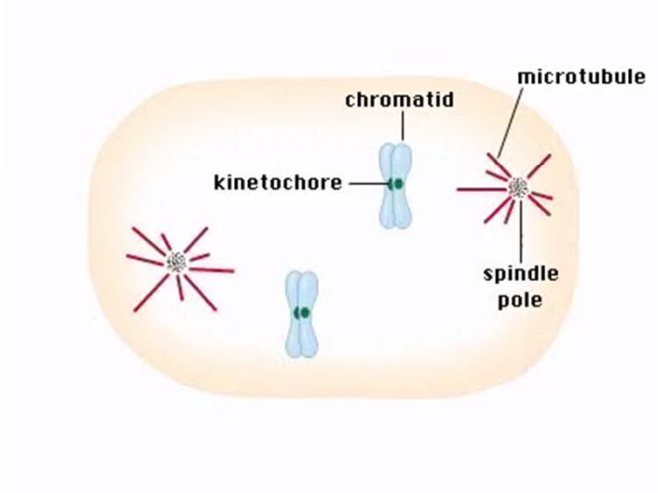
Figure 17-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

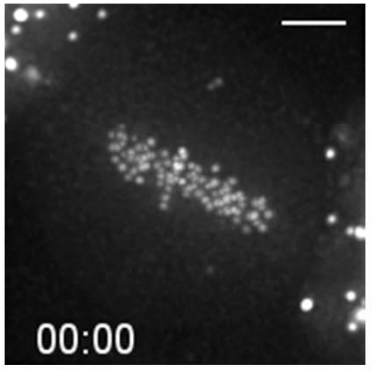
microtubule dynamics



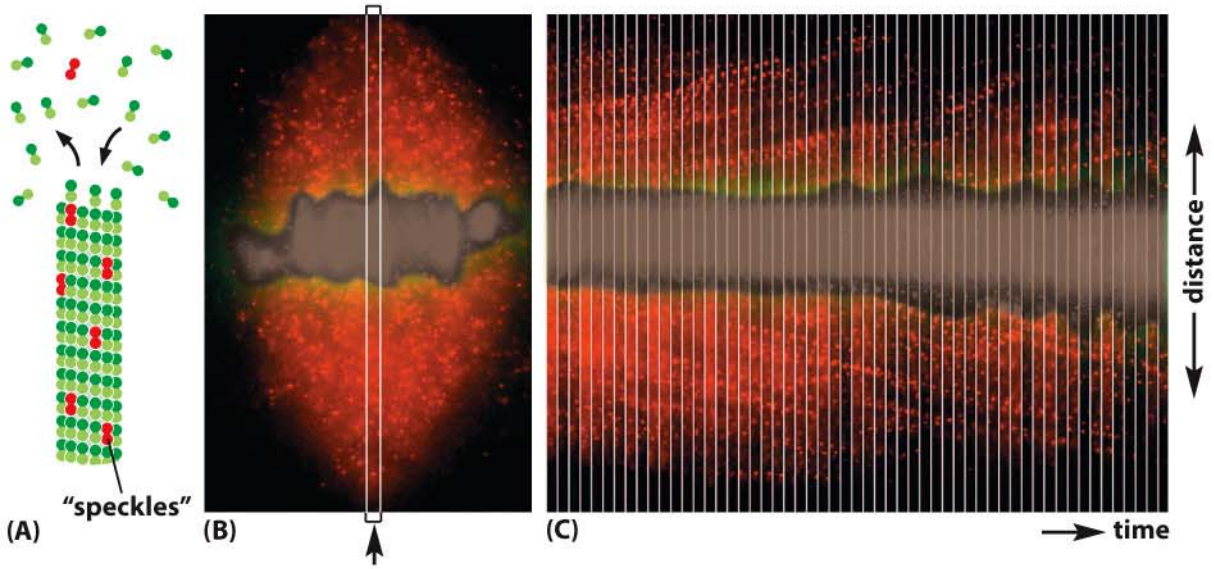








microtubule flux



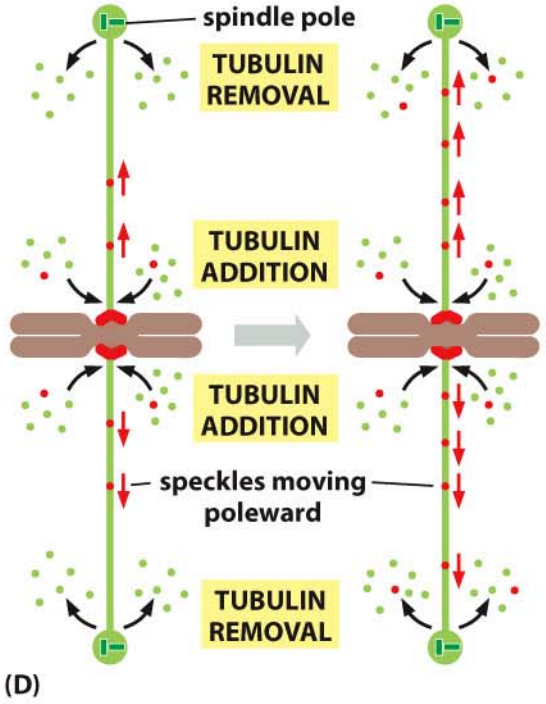
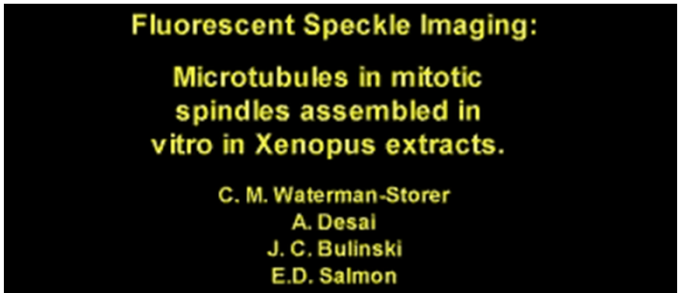
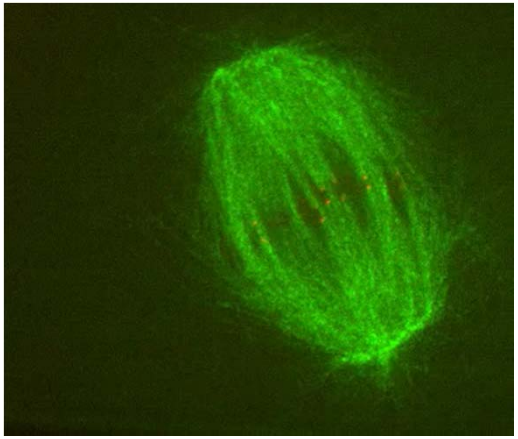


Figure 17-35 Molecular Biology of the Cell 6e (© Garland Science 2015)





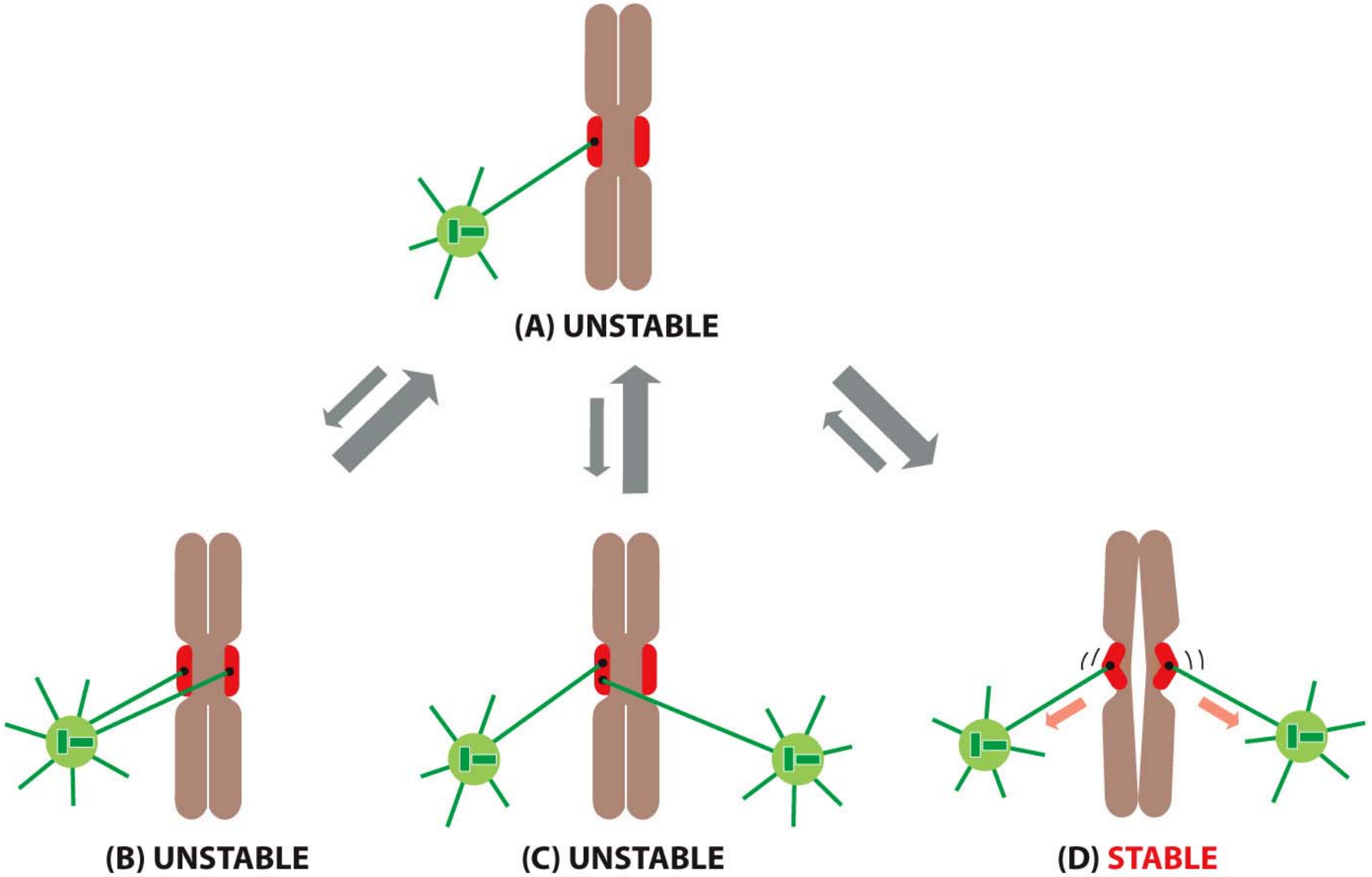
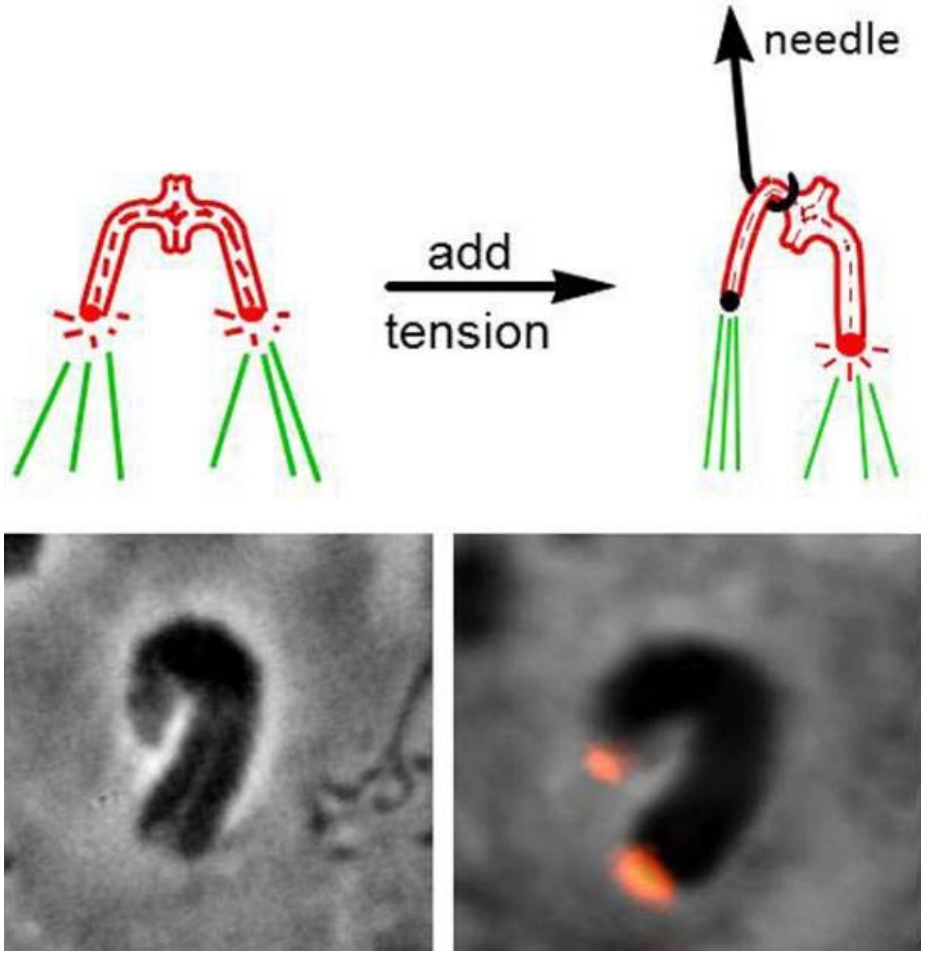


Figure 17-33 Molecular Biology of the Cell 6e (© Garland Science 2015)

tension affects kinetochore signalling



3F3 antibody

Nicklas et al. JCB 1995, 130: 929

tension stabilises attachment

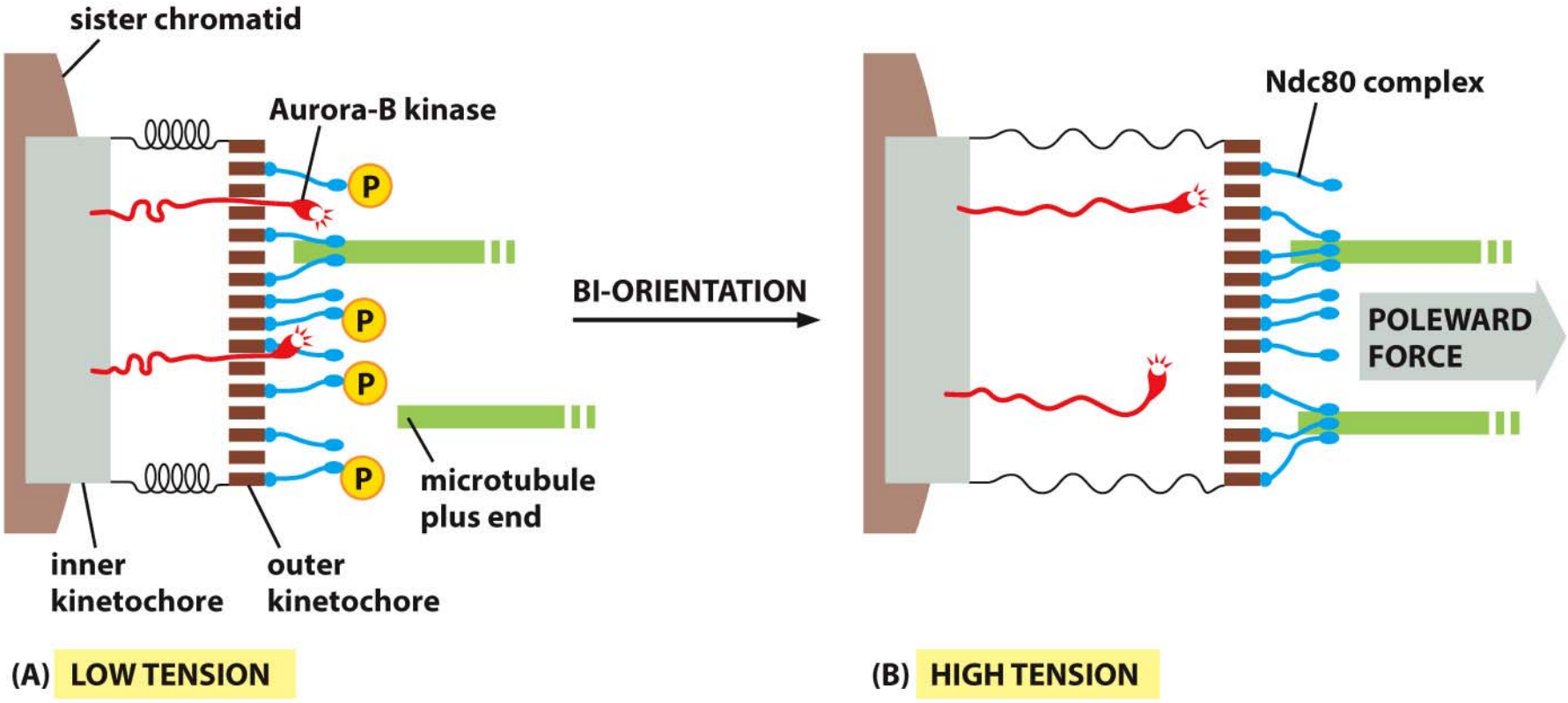


Figure 17-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

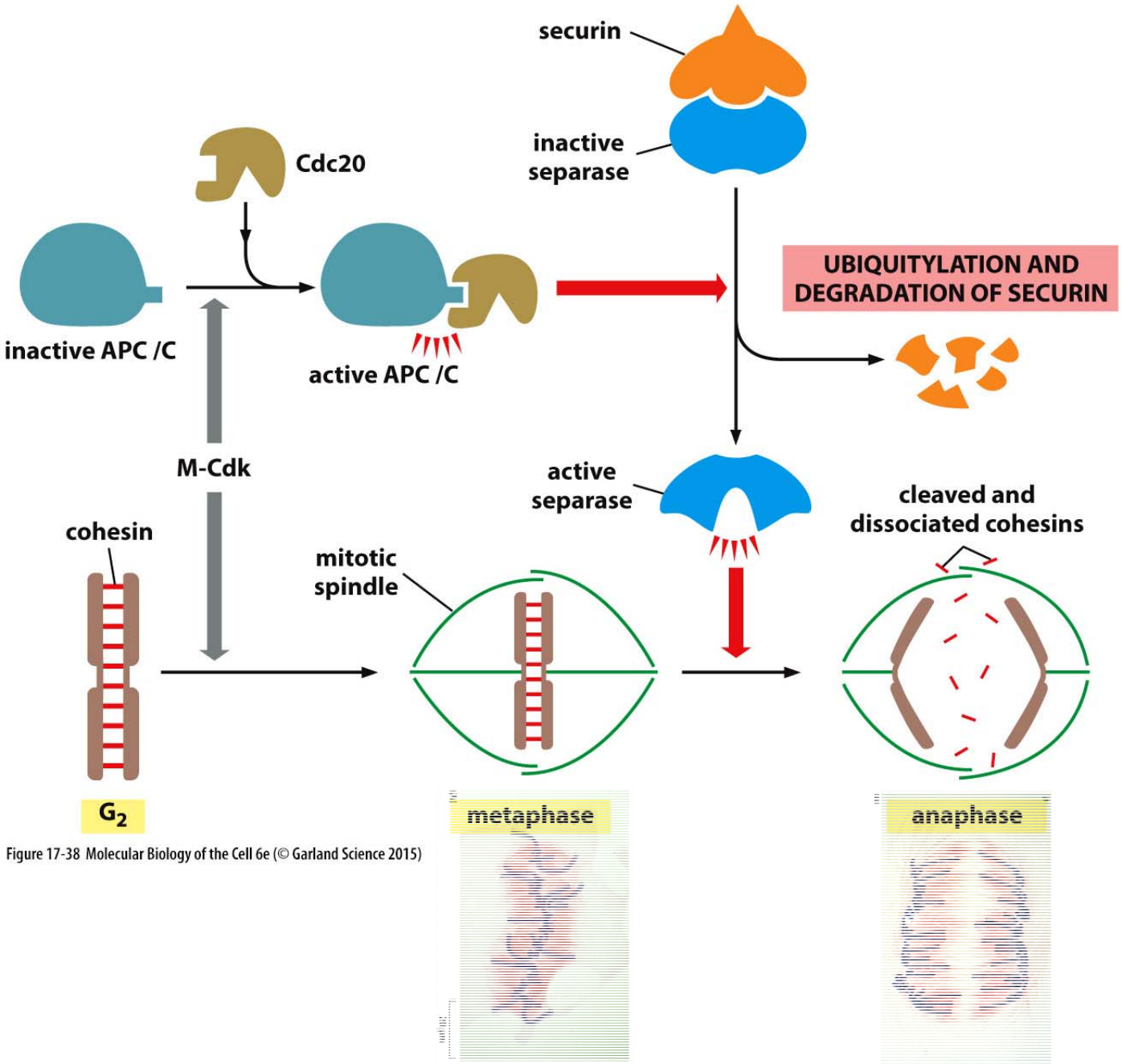
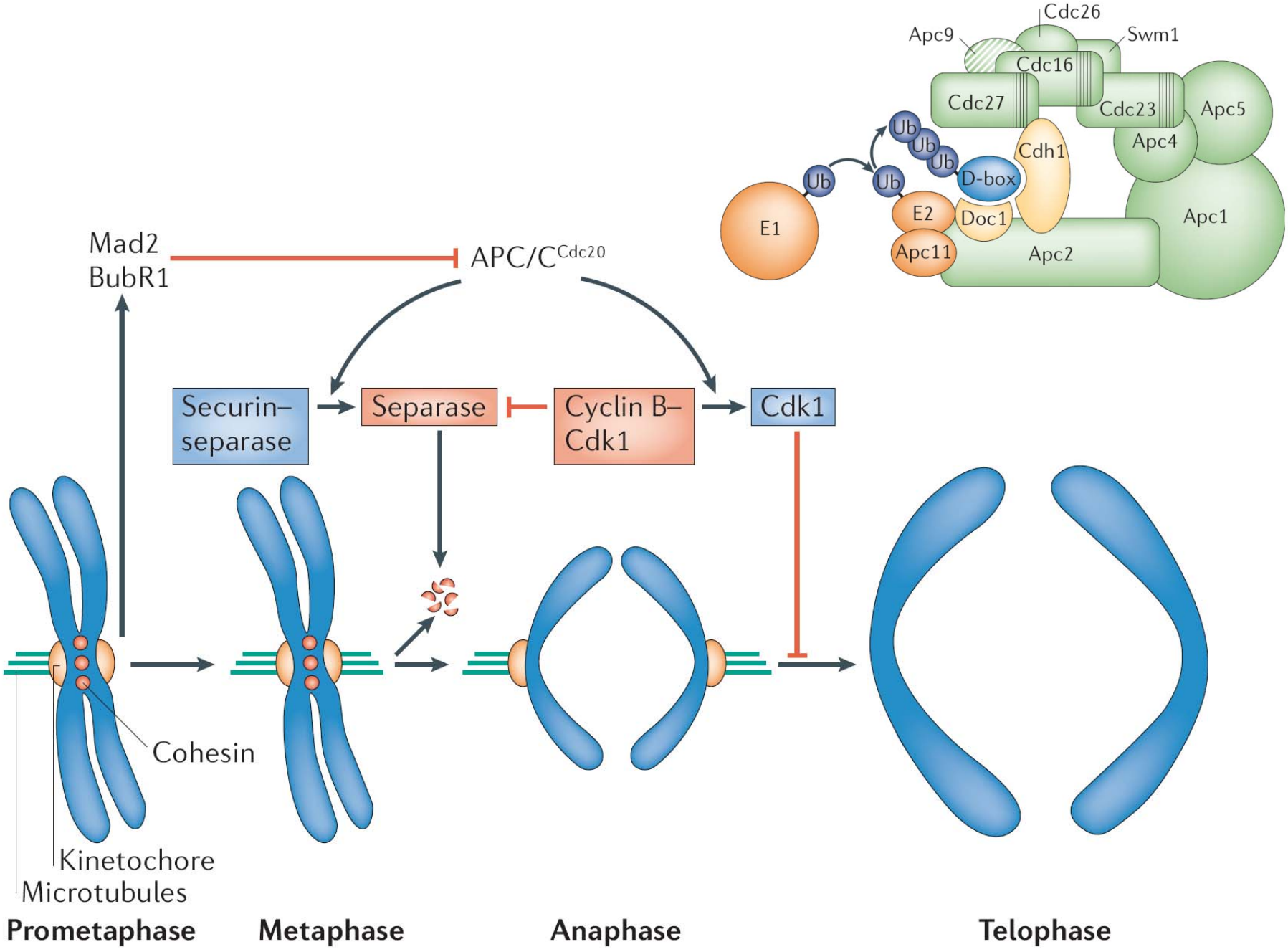
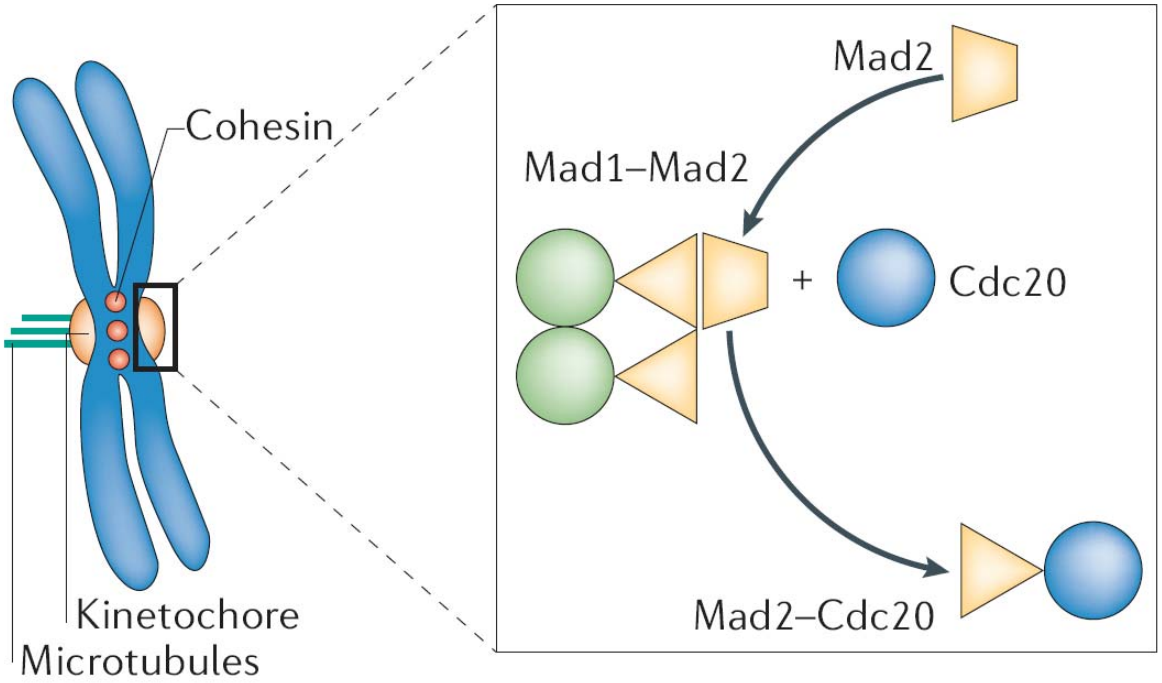
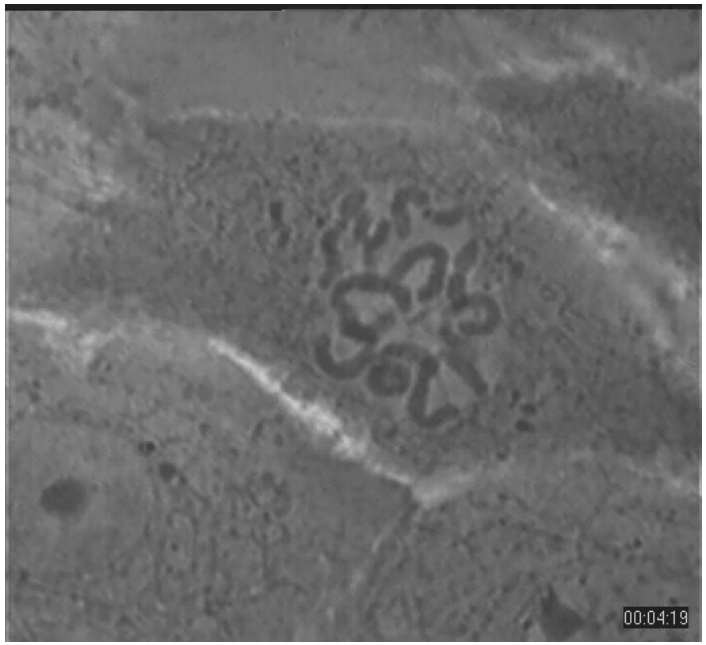


Figure 17-38 Molecular Biology of the Cell 6e (© Garland Science 2015)


Cdc26
Apc9
Swm1
Cdc16
Cdc27
Cdc23
Apc5
Apc4
Ub
Ub
Ub
Ub
Ub
D-box
Cdh1
E1
E2
Doc1
Apc1
Apc11
Apc2
Mad2
BubR1
APC/C^Cdc20
Securin–separase
Separase
Cyclin B–Cdk1
Cdk1
Cohesin
Kinetochore
Microtubules
Prometaphase
Metaphase
Anaphase
Telophase

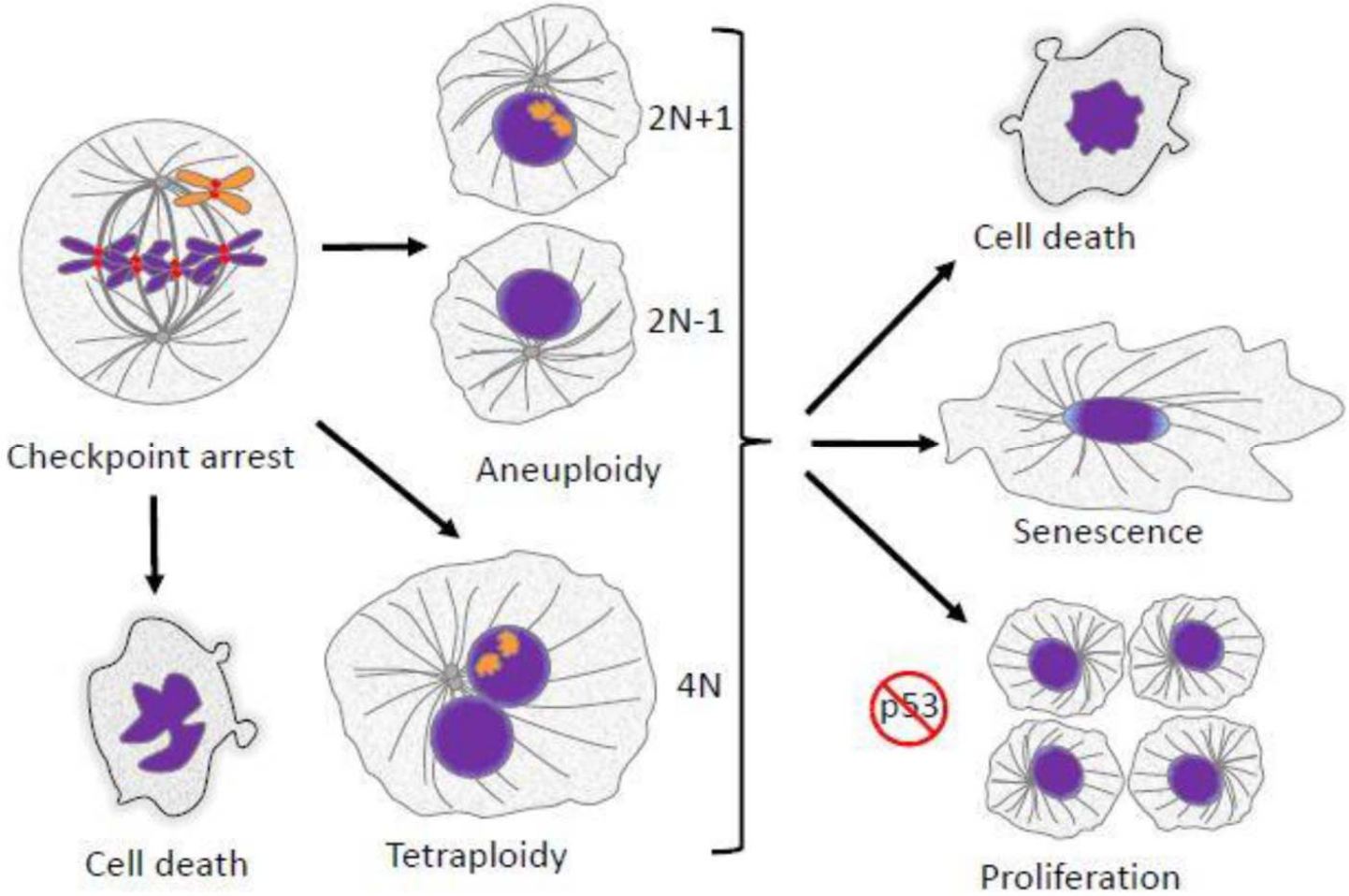
The anaphase-wait signal (the spindle checkpoint) is generated at the kinetochore





exit from mitosis without
a spindle and without
SAC signalling

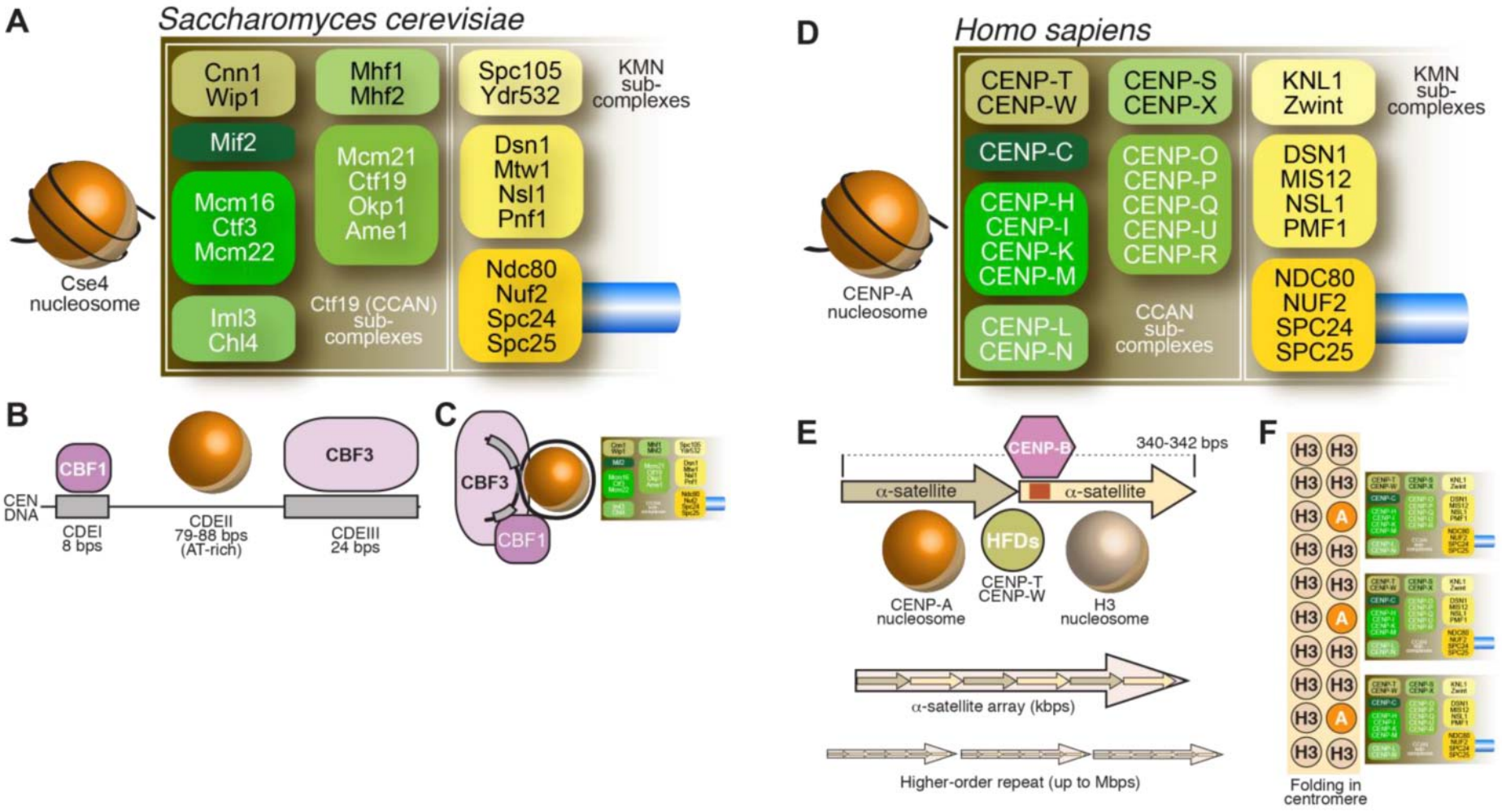
chromosome segregation errors result in aneuploidy



Potapova, T.; Gorbsky, G.J. *Biology* **2017**, *6*, 12.

point kinetochores

regional kinetochores



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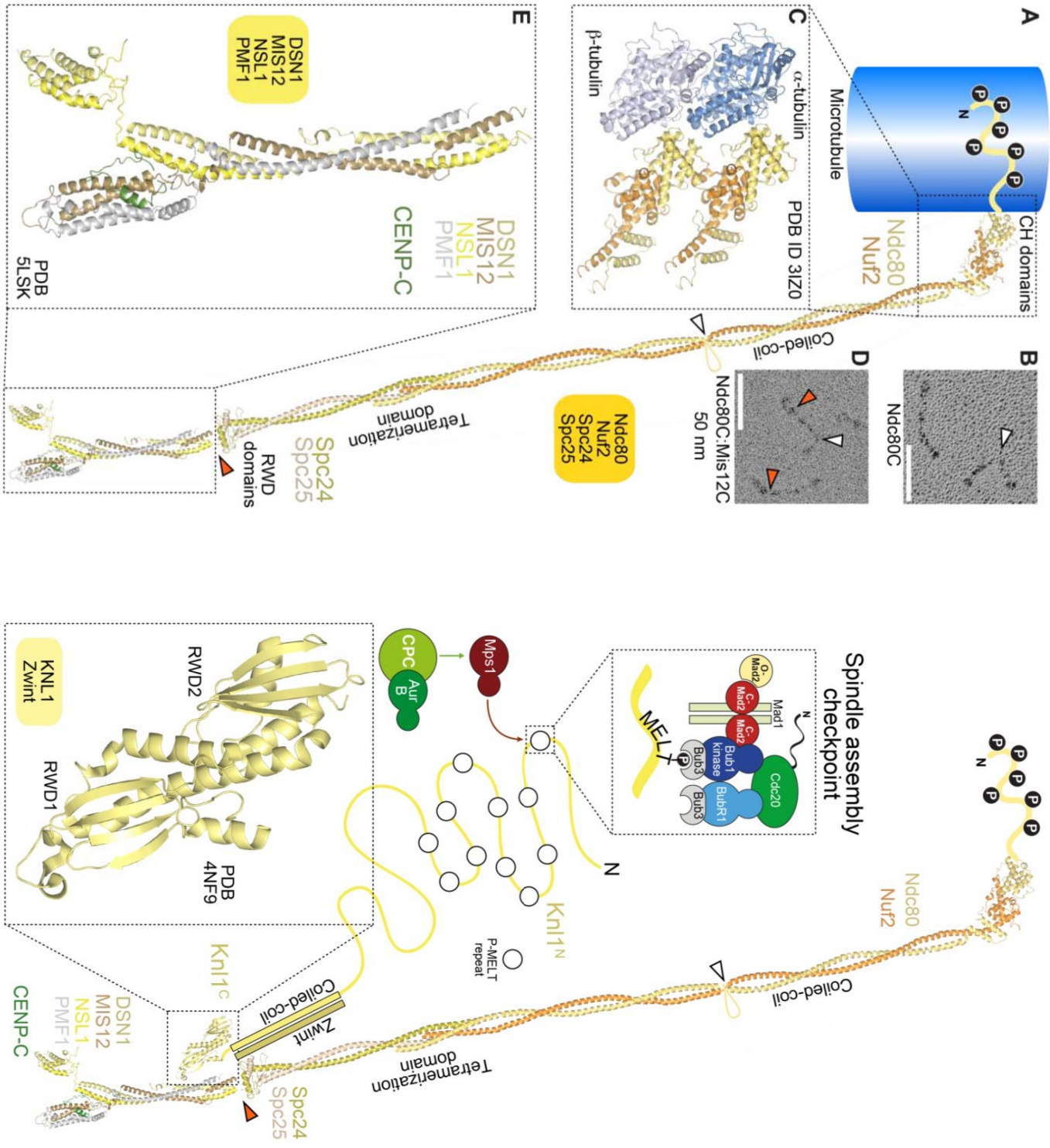
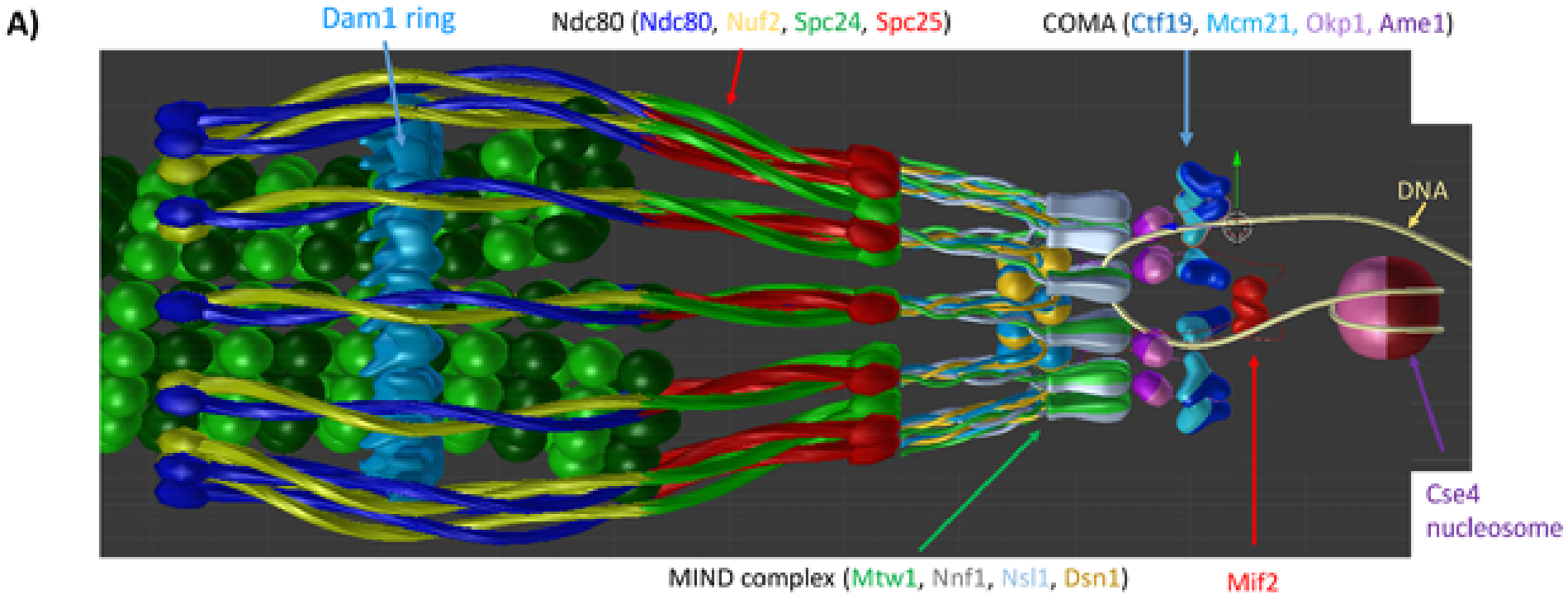


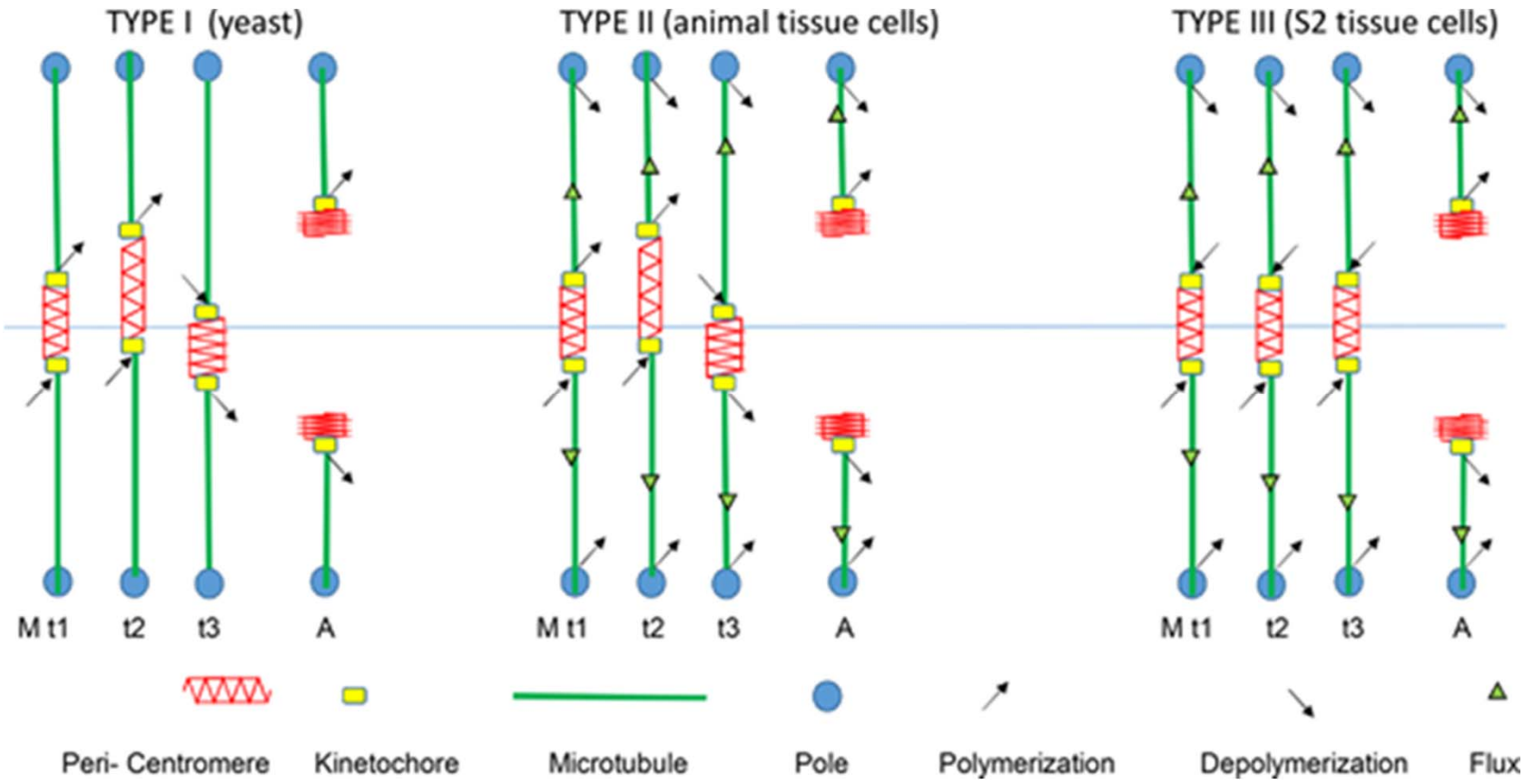
Musacchio, A.; Desai, A. A Molecular View of Kinetochore Assembly and Function. *Biology* **2017**, *6*, 5.

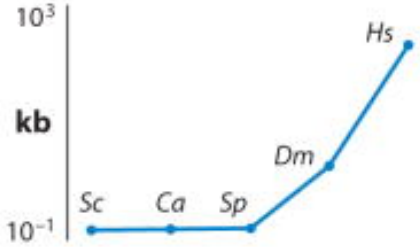
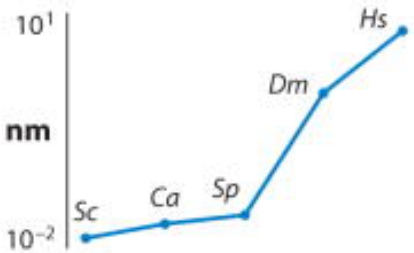
model of a yeast point kinetochore



Salmon ED, Bloom K. Bioassays 2017, 1600216

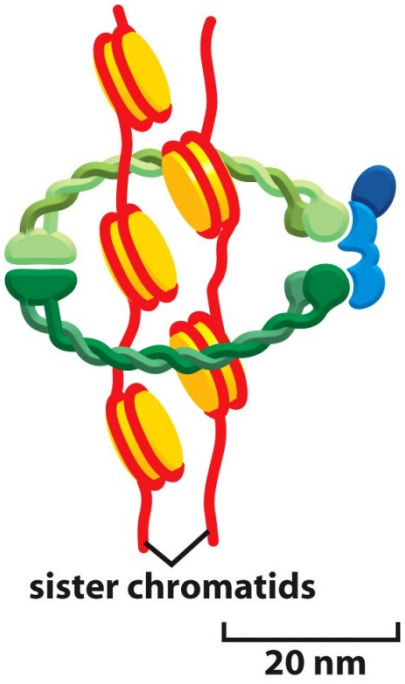
Mechanisms controlling chromosome movements



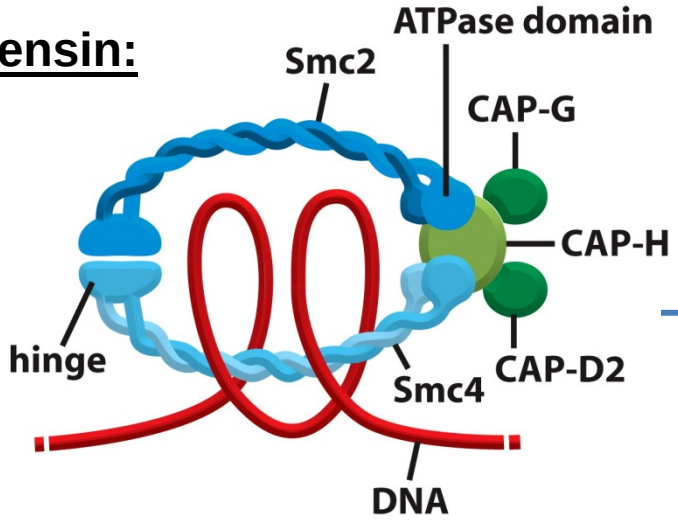
	Centromere DNA size	R_g	Kinetochore separation in mitosis
S. cerevisiae	0.125 kb	0.04 μM	800 nm
C. albicans	3–4 kb	0.15 μM	~800 nm
S. pombe	10 kb	0.23 μM	~1,000 nm
D. melanogaster	200–500 kb	1–1.65 μM	~1,000 nm
H. sapiens	500–1,500 kb	2.8 μM	~1,000 nm
Scaling			

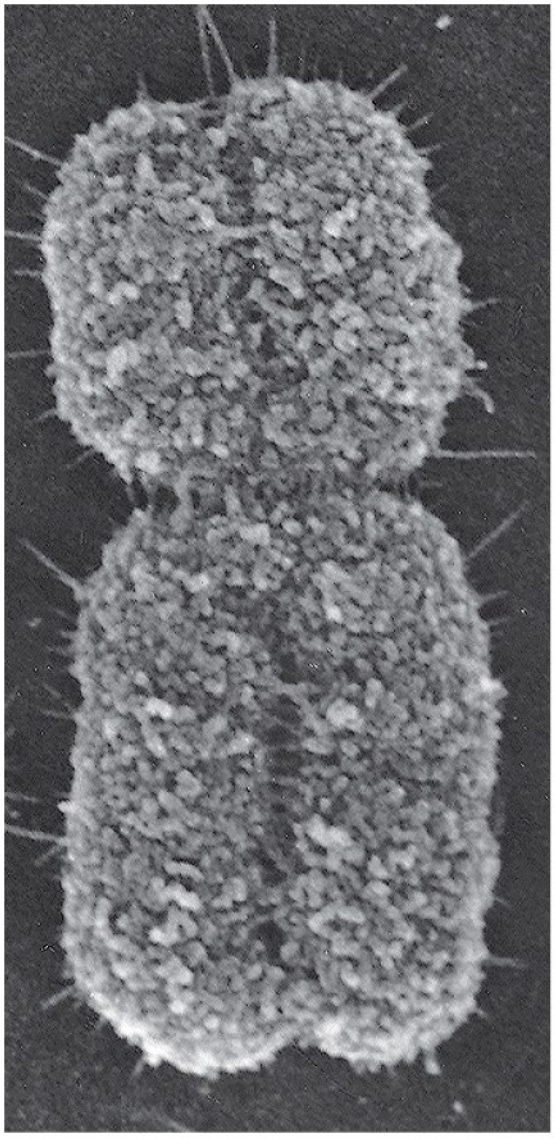
Bloom K., Annu. Rev. Genetics 2014, 48:457-584

Cohesin:

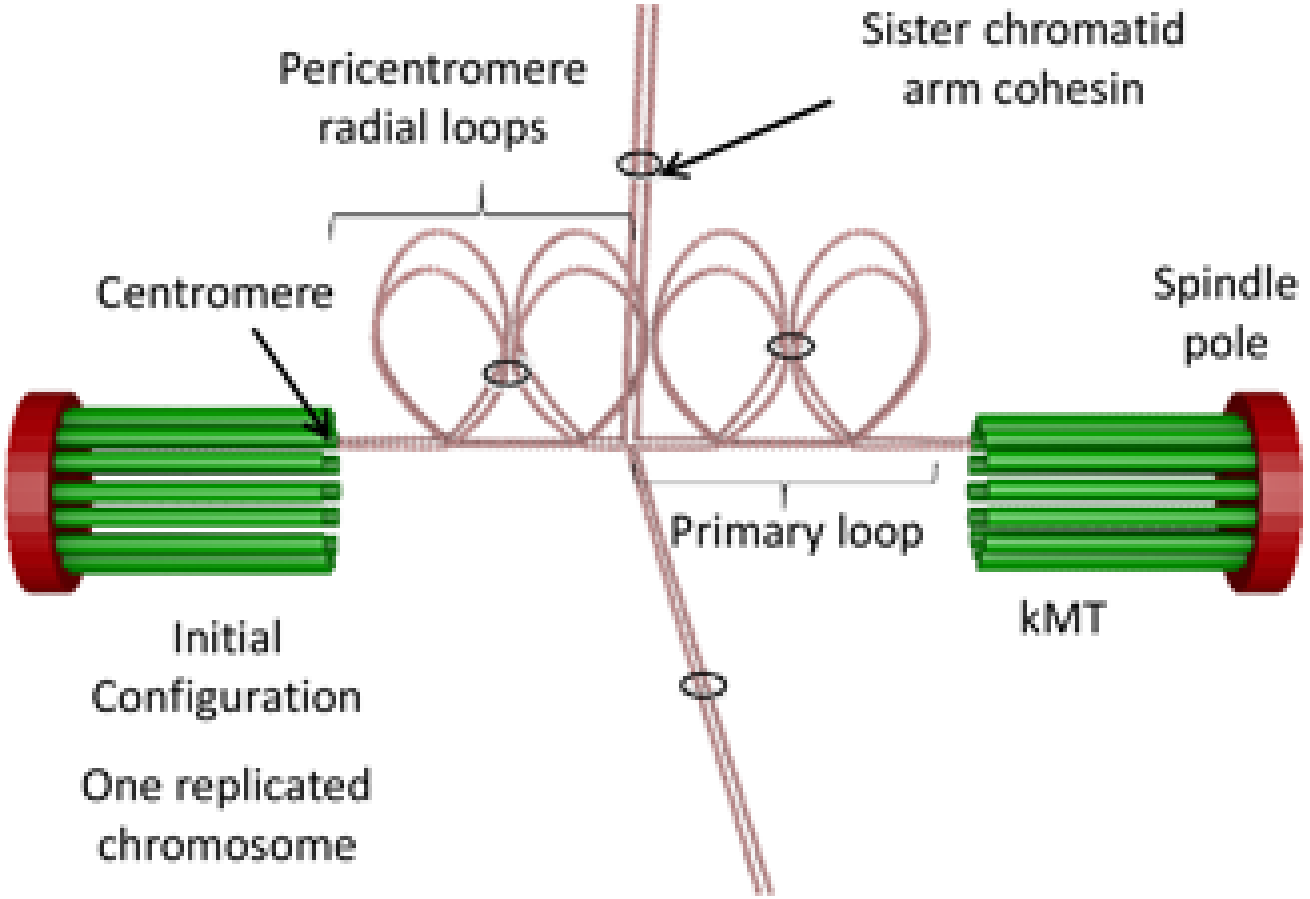


Condensin:

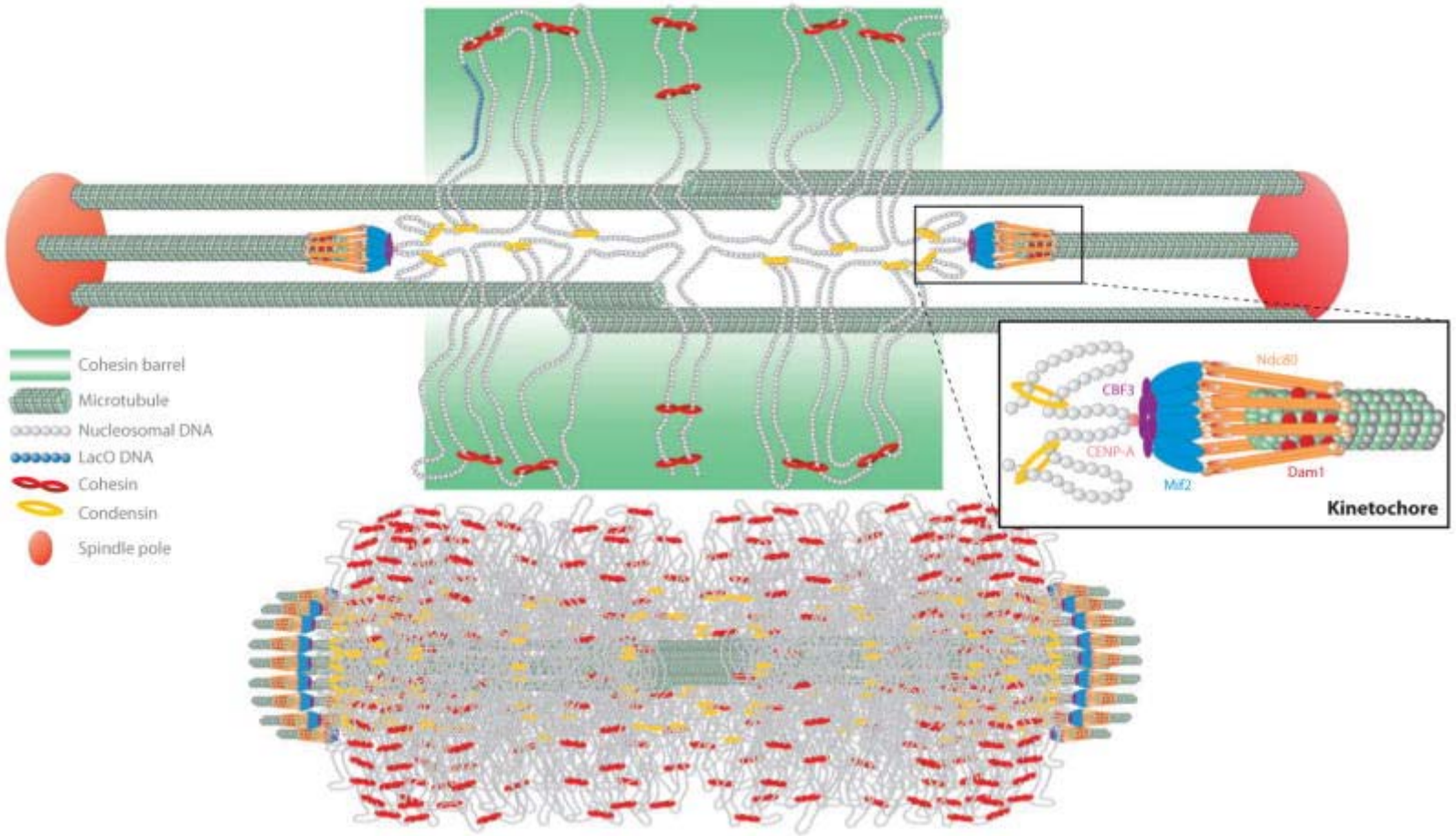




centromere scheme

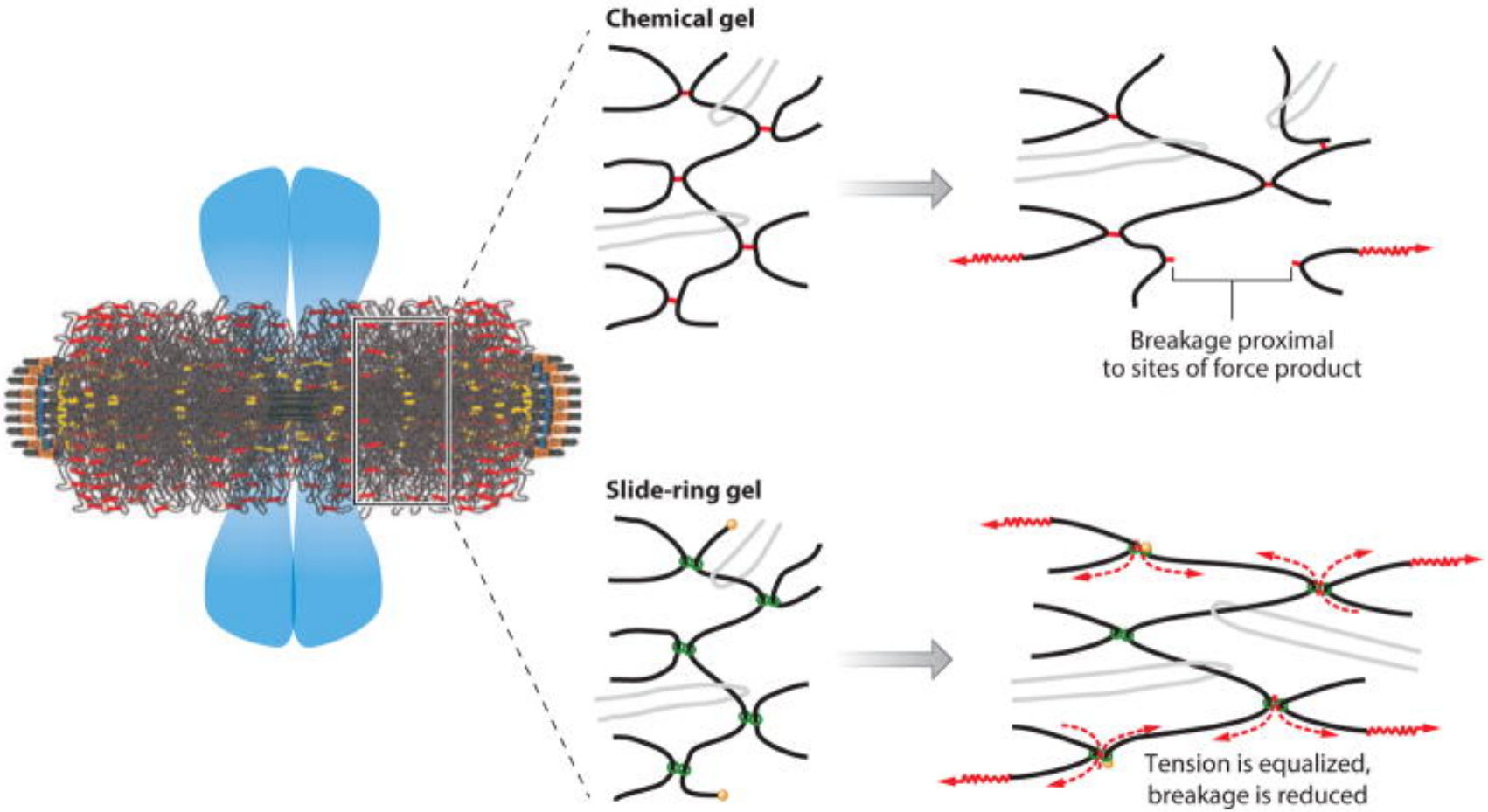


organisation of centromeric DNA



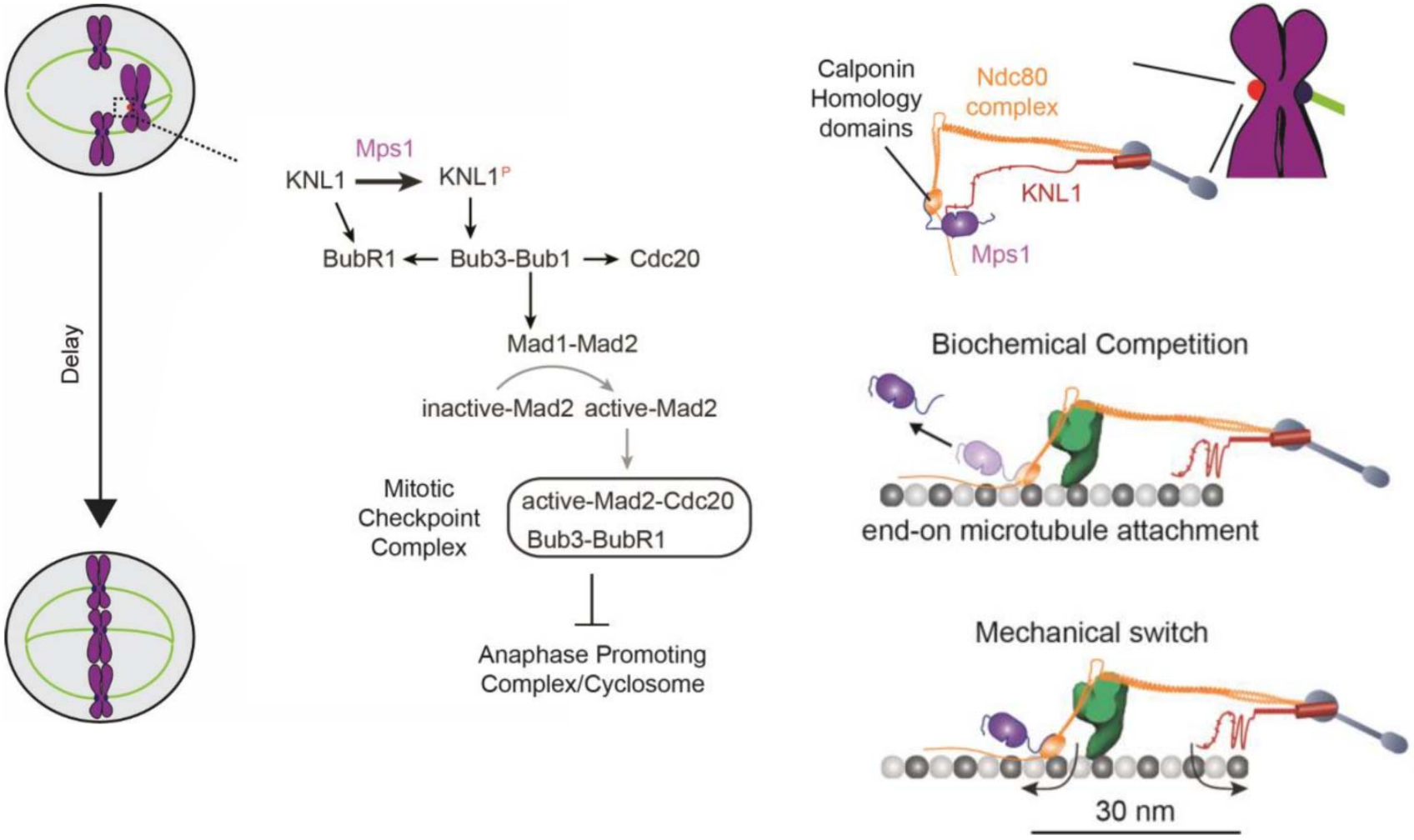
Bloom K., Annu. Rev. Genetics 2014, 48:457-584

rings avoid breakage



Bloom K., Annu. Rev. Genetics 2014, 48:457-584

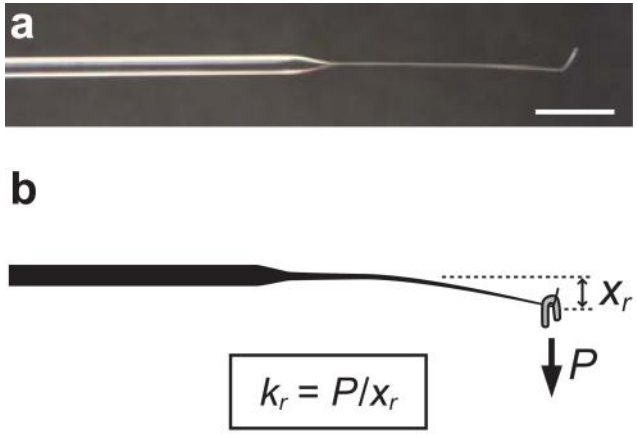
SAC inactivation by attachment and tension

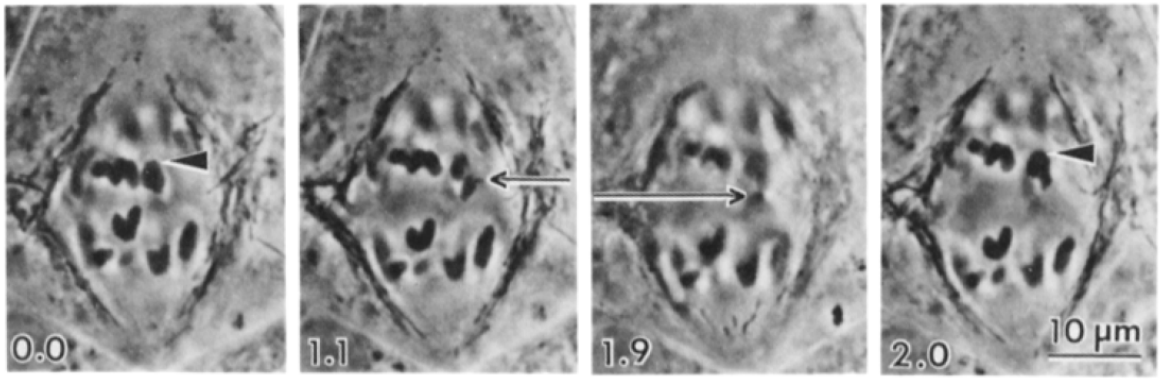


What is the function of the pulling forces that cause interkintochore tension?

- Required for:
  - chromosome movement (congression and oscillation)
  - stabilisation of microtubule attachment (catch bond)
  - SAC silencing
- Magnitude:
  - estimates range from 1 to 700 pN (!) depending on cell types, experimental set ups…..
  - more experiments needed!

How big is the force that acts on kinetochores?





stall force = 700pN

Nicklas B, JCB, 1983 97: 542

nature COMMUNICATIONS

ARTICLE

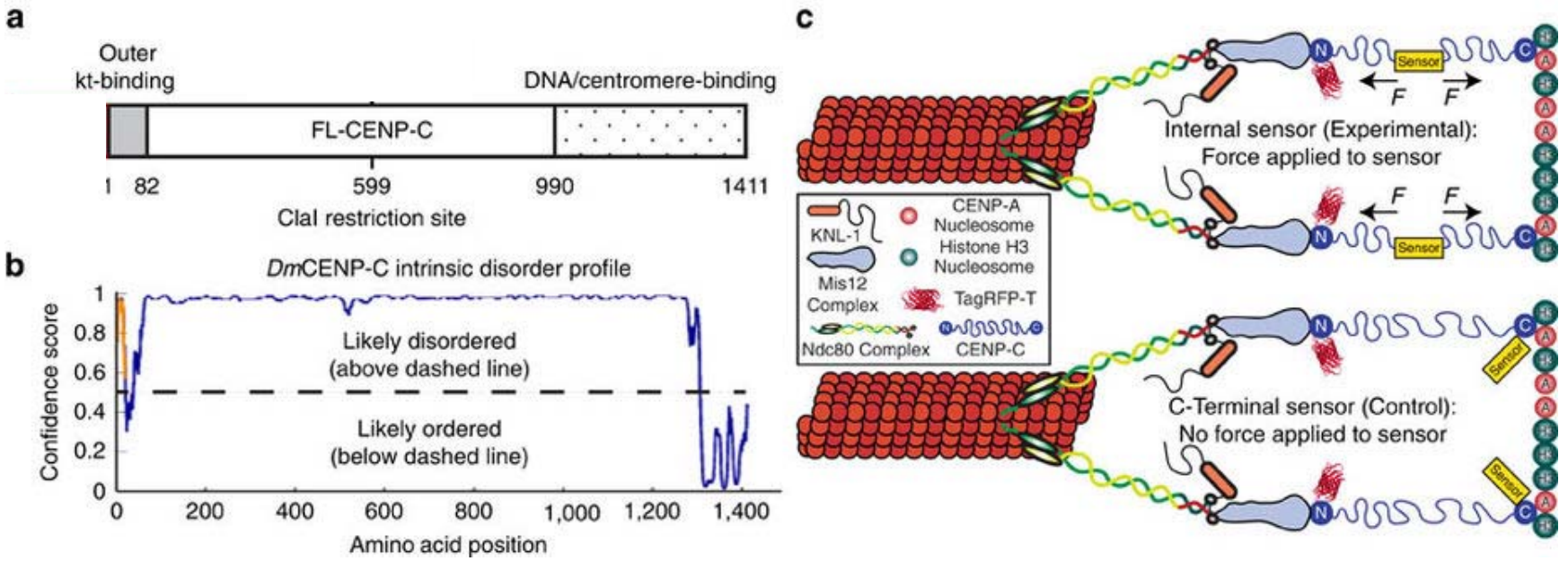
Received 11 Mar 2016 | Accepted 13 Sep 2016 | Published 20 Oct 2016

DOI: 10.1038/ncomms13221 OPEN

Chromosome biorientation produces hundreds of piconewtons at a metazoan kinetochore

Anna A. Ye[1,2], Stuart Cane[1,2] & Thomas J. Maresca[1,2]

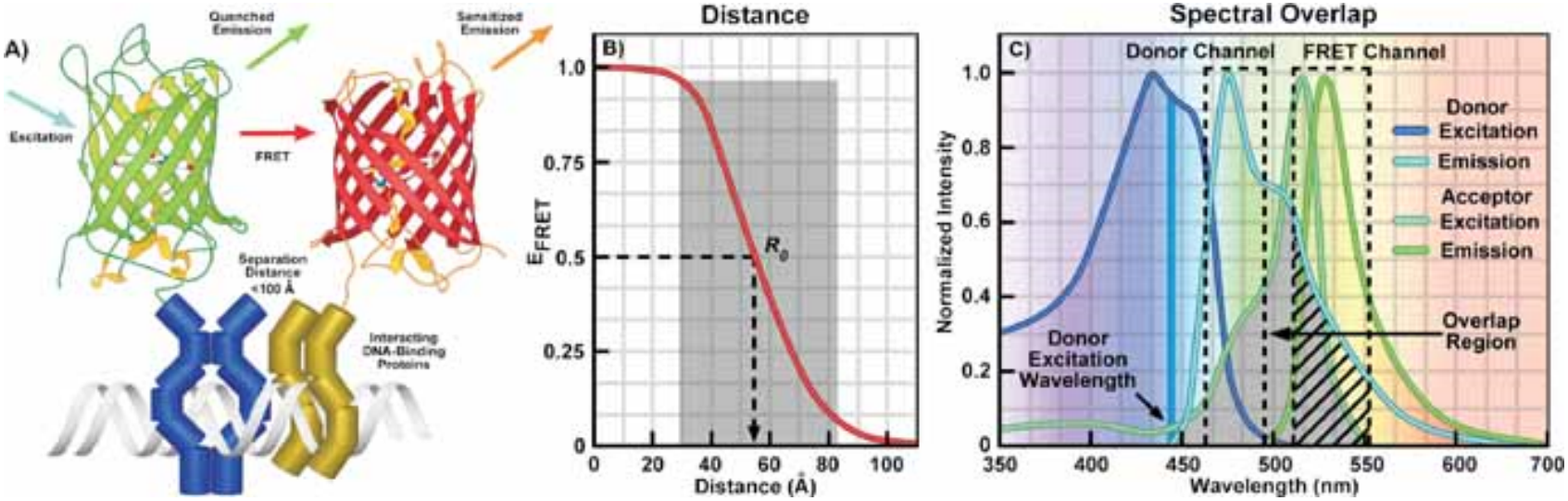
Intramolecular stretch sensor



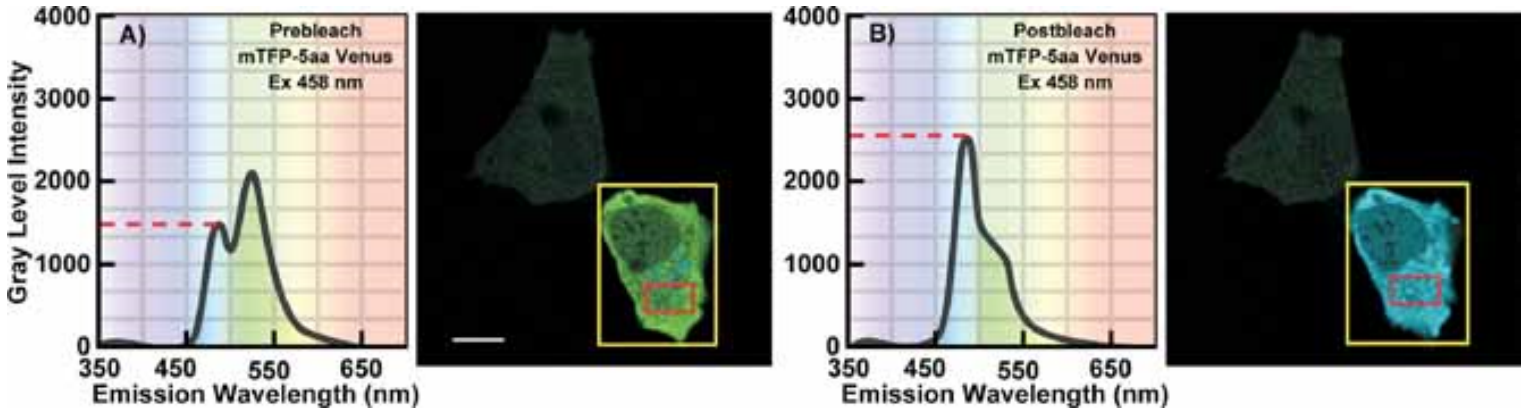
- **sensor (FRET):** TSMod (mTurquoise2-spider silk protein-mVenus)
- **model system:** drosophila S2 cells (no metaphase oscillation)
- **target molecule:** CENP-C (single linker between chromatin and MT attachment site)

FRET: protein proximity assay

sensitized emission FRET

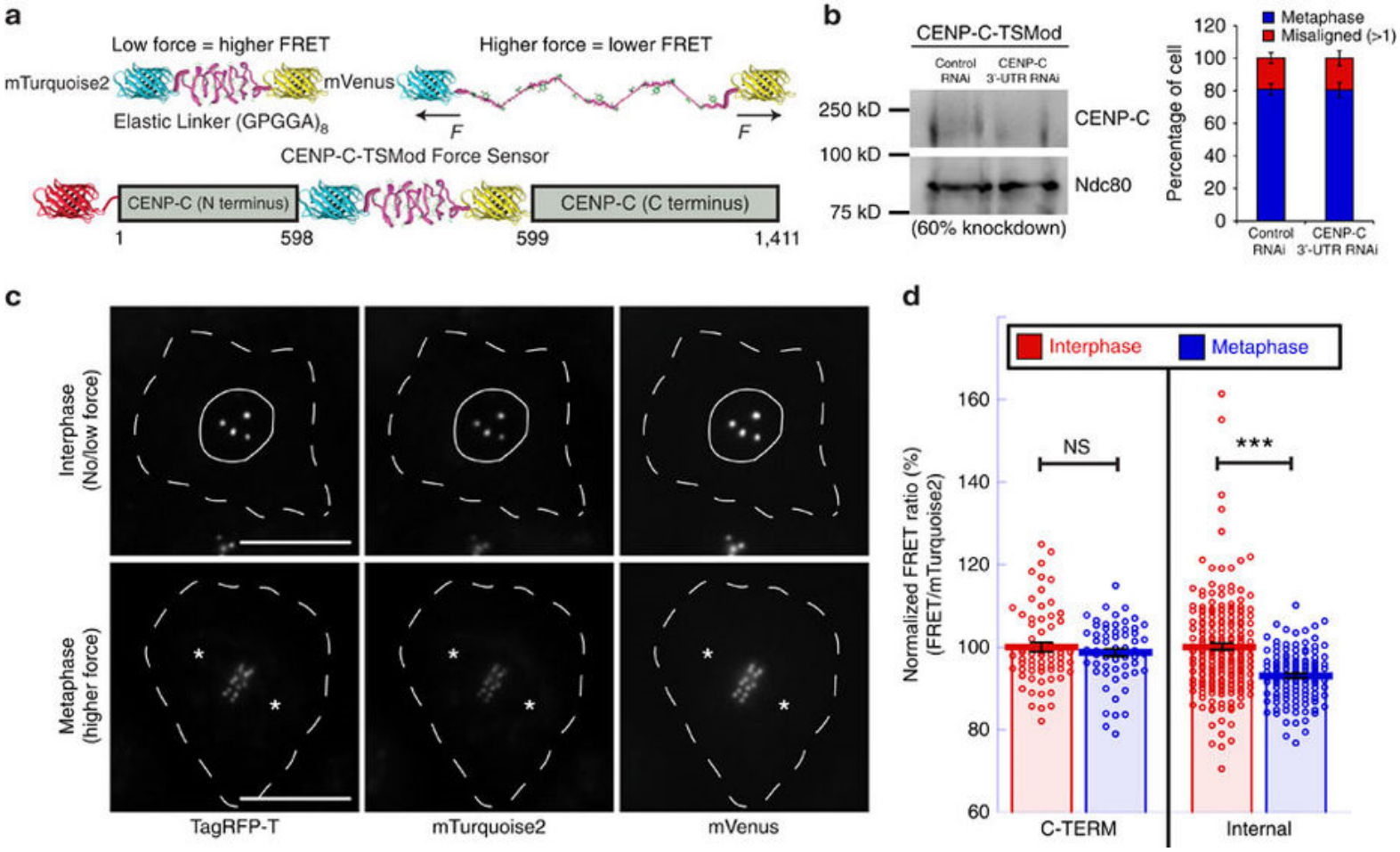


acceptor photobleaching FRET

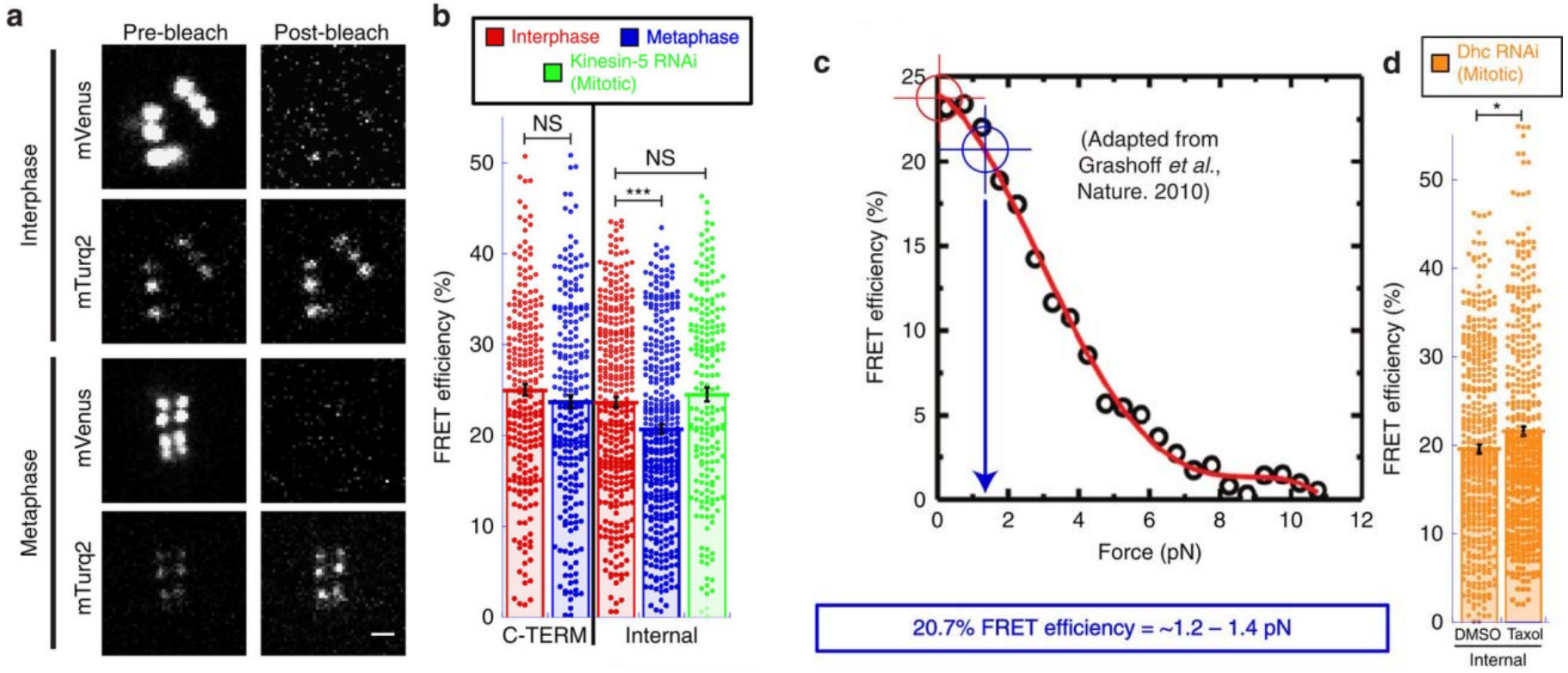


BioEssays
Volume 34, Issue 5, pages 341-350, 7 MAR 2012 DOI: 10.1002/bies.201100098
http://onlinelibrary.wiley.com/doi/10.1002/bies.201100098/full#fig1

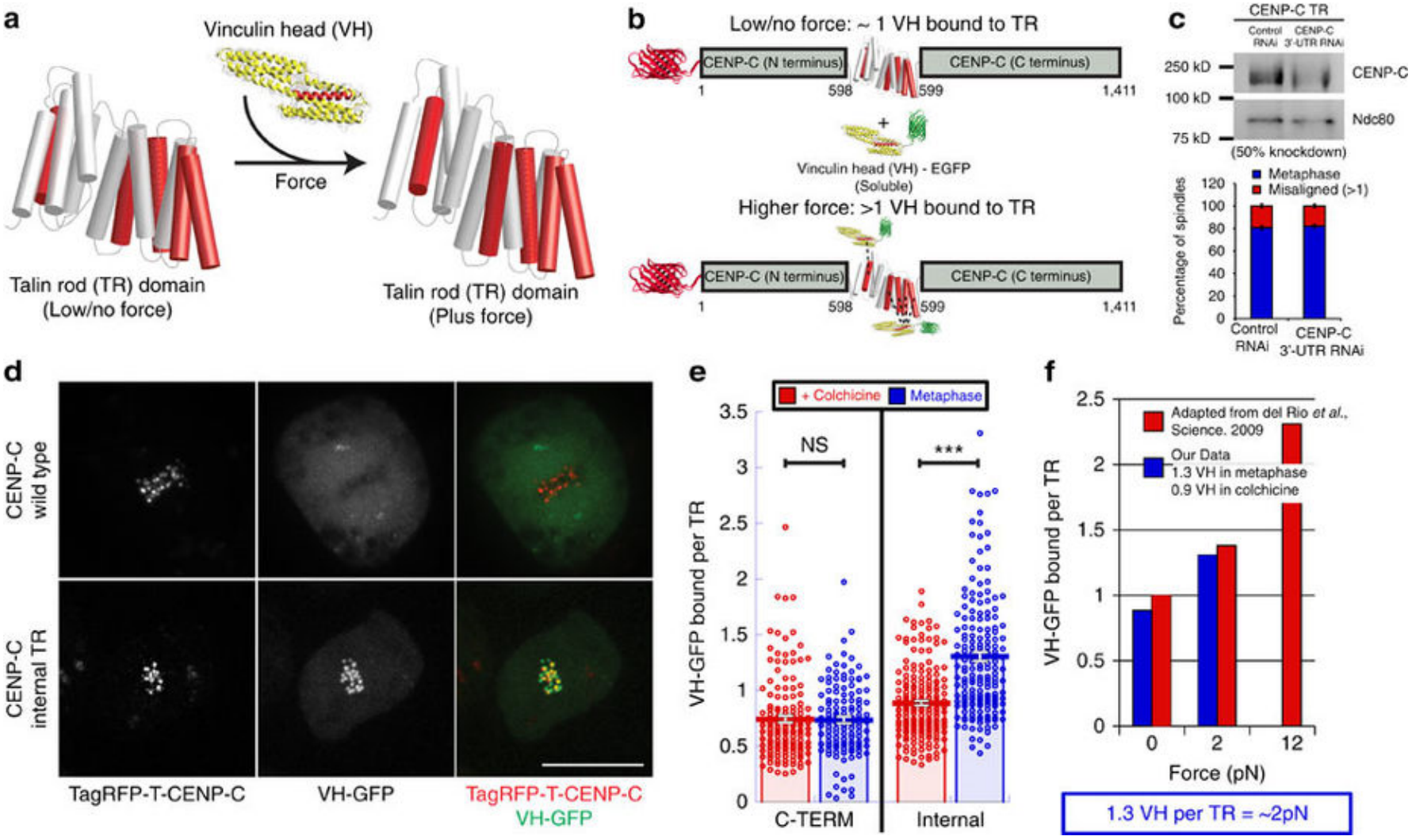
TSMod FRET changes with tension



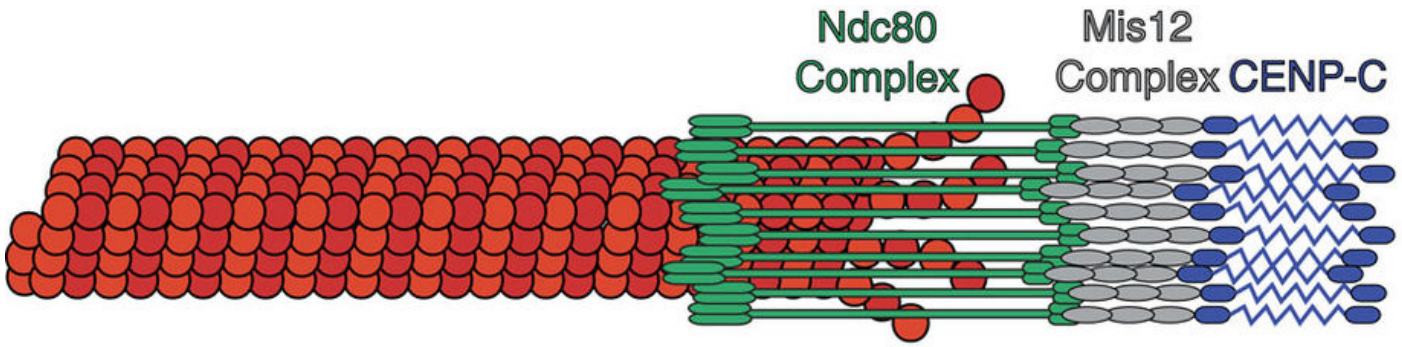
Force estimation using acceptor photobleaching



TR force sensor increases signals with tension



How much force acts on a kinetochore?



A simple *Drosophila* kinetochore model: Linkages arranged as a set of parallel springs

$F_{MT} = F_{CENP\text{-}C}$ X # CENP-C molecules per MT

$F_{kt} = F_{MT}$ X # kinetochore-microtubules

	$F_{CENP\text{-}C}$	# CENP-C per MT	F_{MT}	# kt-MTs^{+}	Max F_{kt}
Low	1 pN	12.3*	12.3 pN	11	135 pN
High	2 pN	30.8**	61.5 pN	11	677 pN

Conclusions

- From the paper:
  - each CENP-C linker on average bears 1-2pN measured by two tension sensors
  - total of several 100s pN per kinetochore (although probably < 10pN are required for chromosome movement)
  - mechanisms not elaborated (motors, flux, etc.)
- My conclusions:
  - elegant and rather easy to understand measurement of forces acting on kinetochores
  - clever use of existing reporters to address an old question
  - drawback: doesn‘t fully replicate mammalian spindles
  - would be nice to develop a force sensor for human cells