

ARTICLE

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[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

Video Enhanced DIC Microscopy
of Mitosis in Newt Lung Cells
(*Taricha granulosa*)

Victoria Skeen,
Robert Skibbens, and
E. D. Salmon

University of North Carolina at Chapel Hill
(see Skibbens et al., 1993, J. Cell Biol.
122:859-875)
Frame Time = HR:MIN:SEC

Kinetochores: microtubule attachment sites

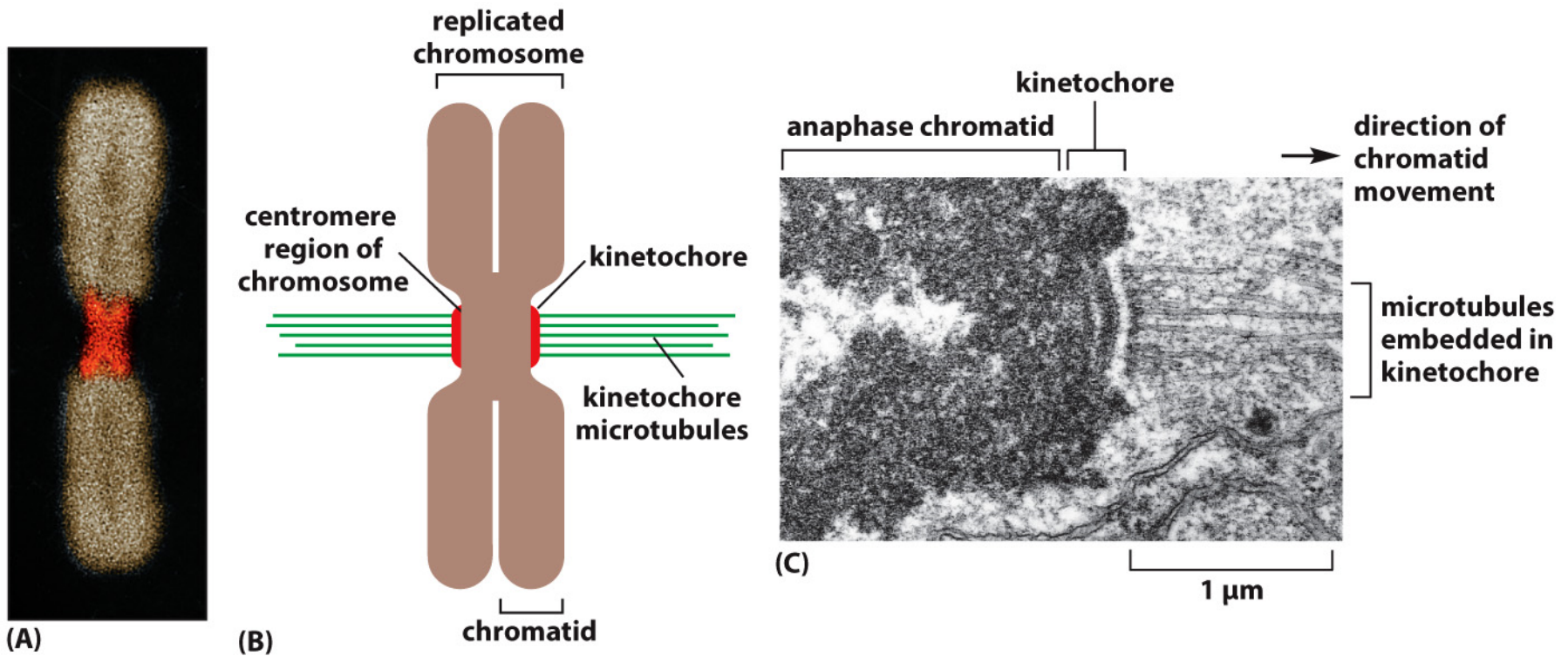
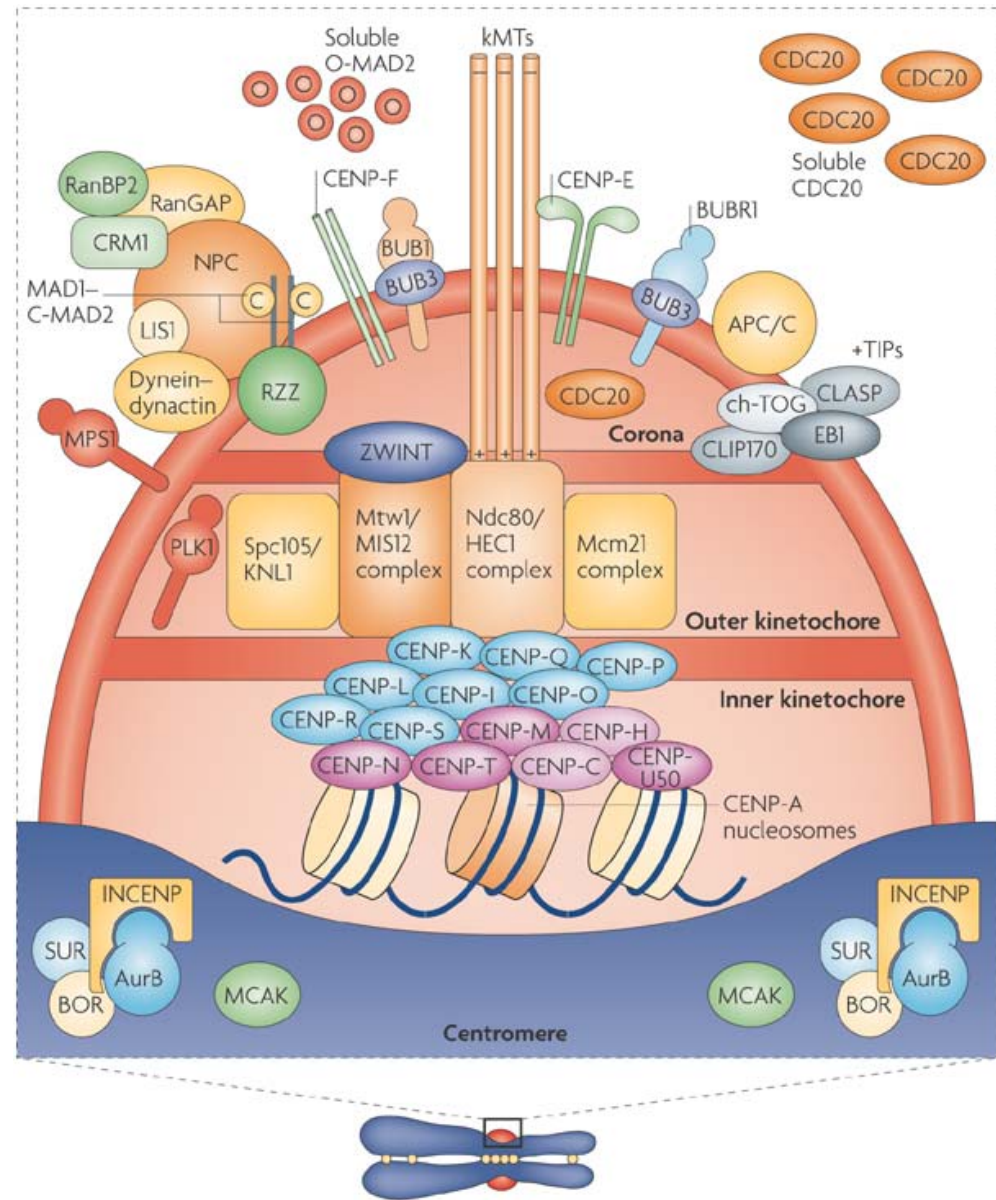
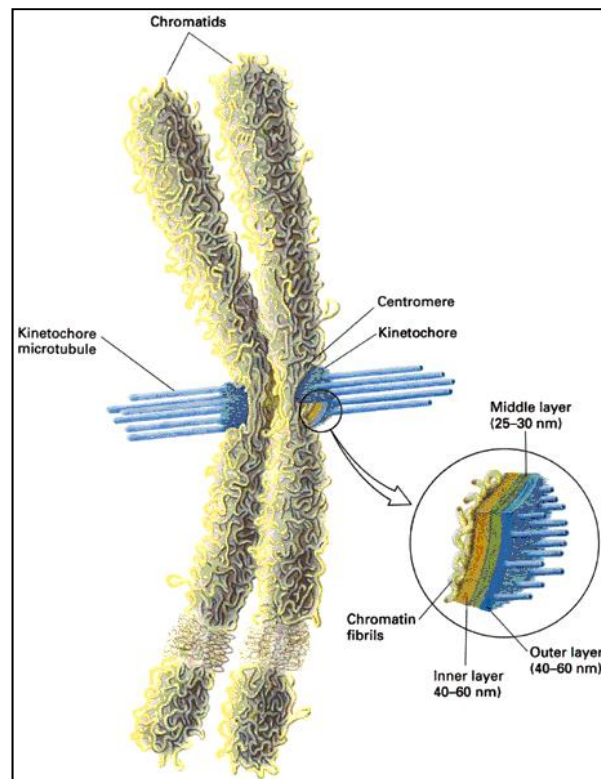


Figure 17-30 Molecular Biology of the Cell 6e (© Garland Science 2015)



stable end-on attachments

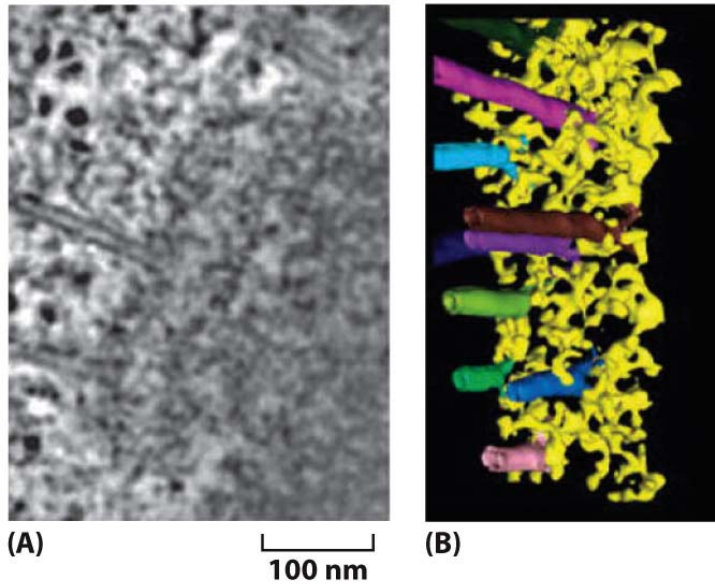
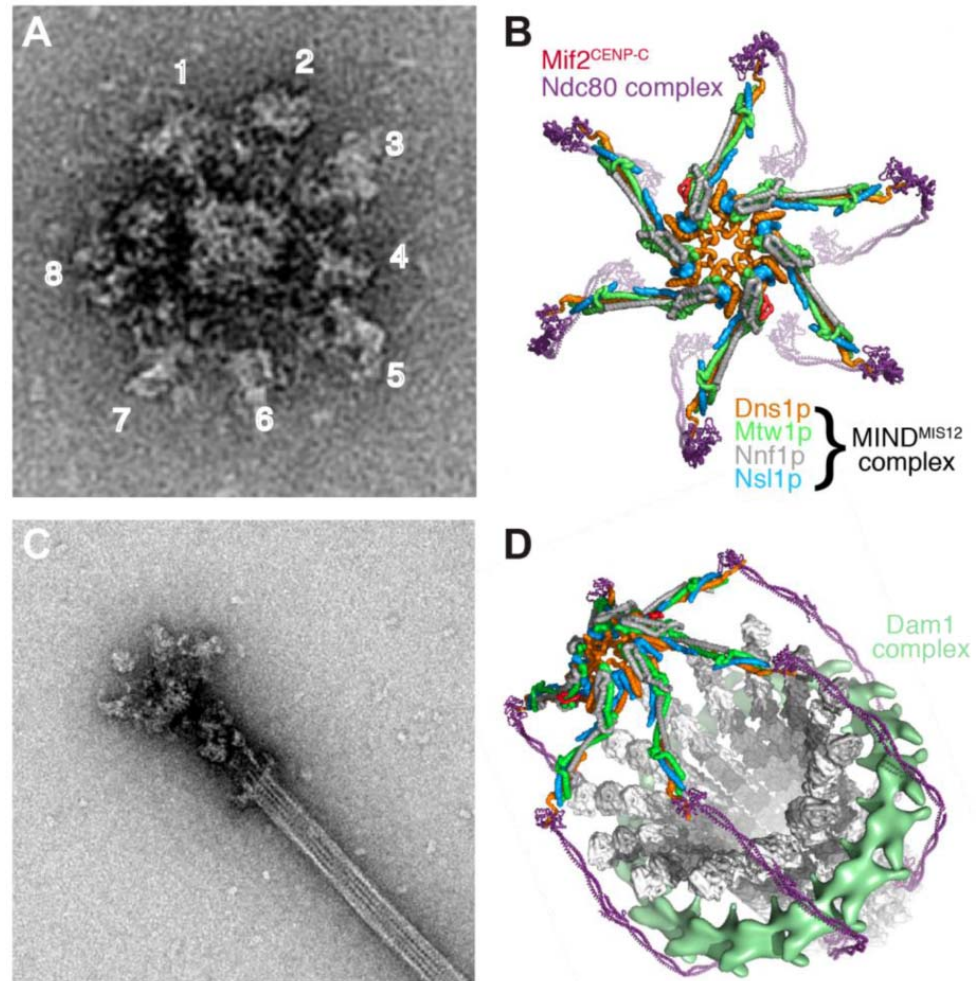
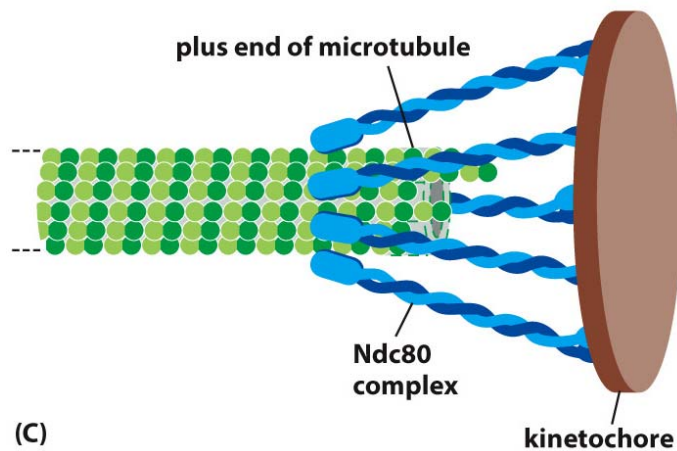


Figure 17-31 Molecular Biology of the Cell 6e (© Garland Science 2015)



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

chromosome bi-orientation

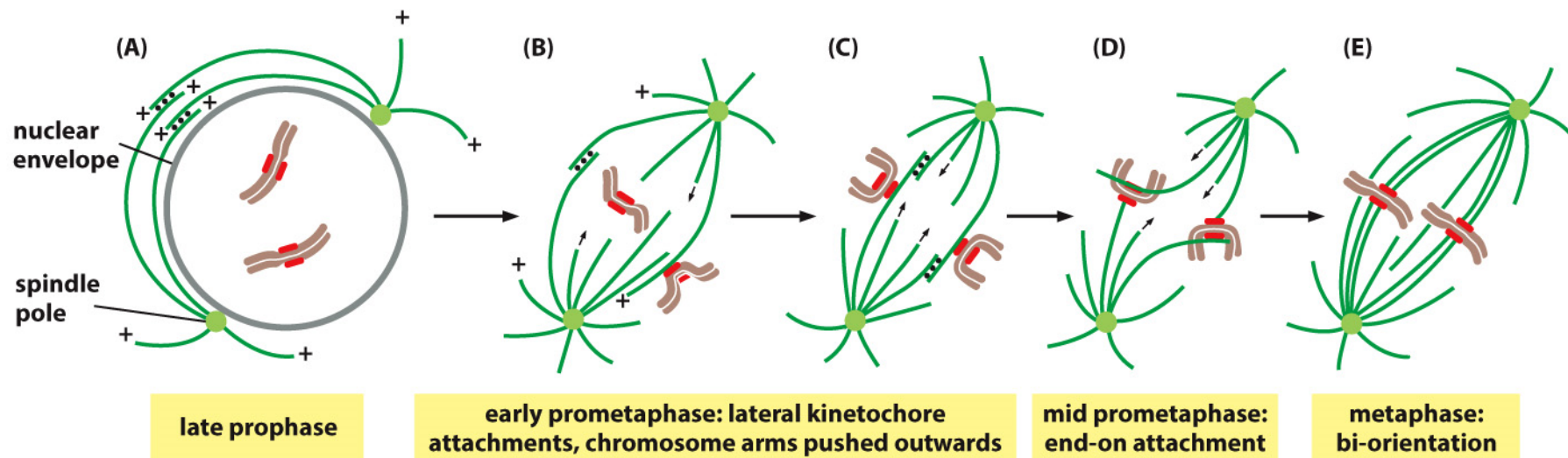
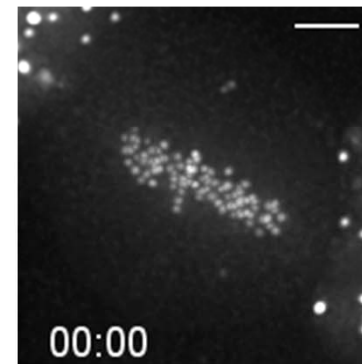
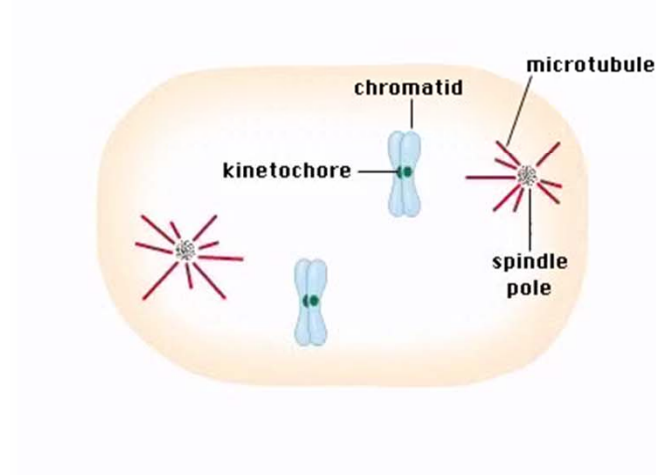
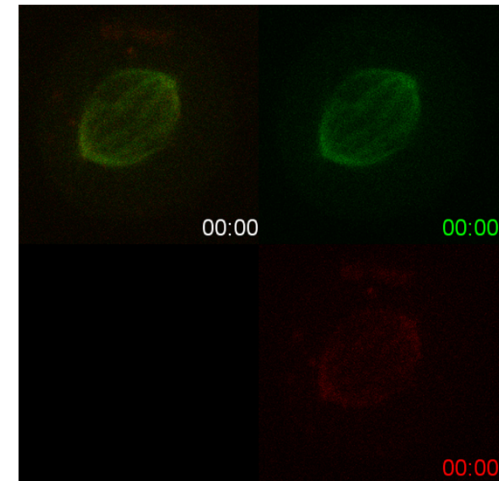
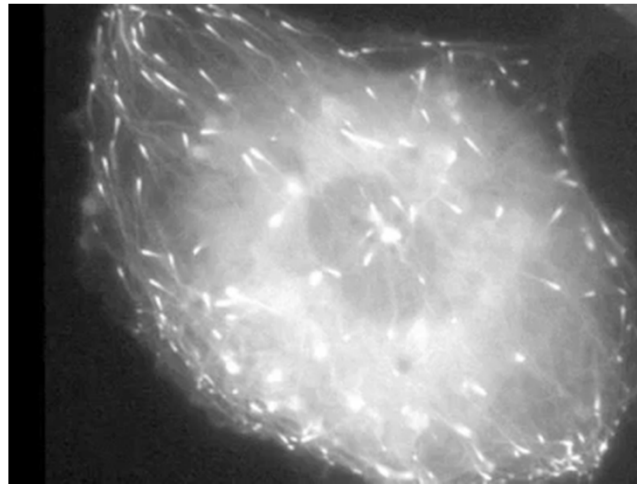
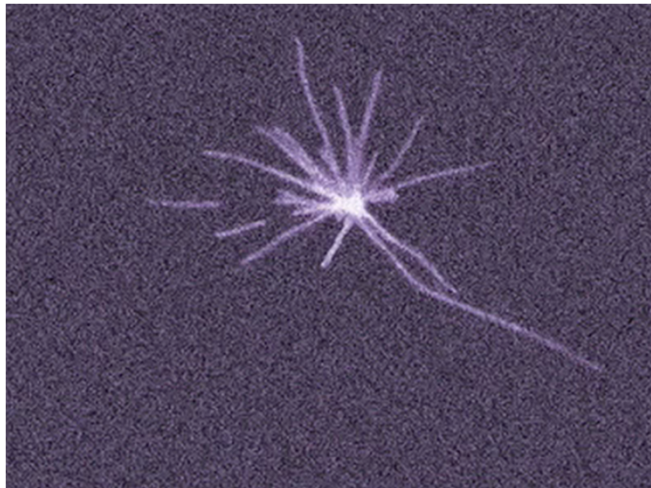


Figure 17-32 Molecular Biology of the Cell 6e (© Garland Science 2015)

microtubule dynamics



microtubule flux

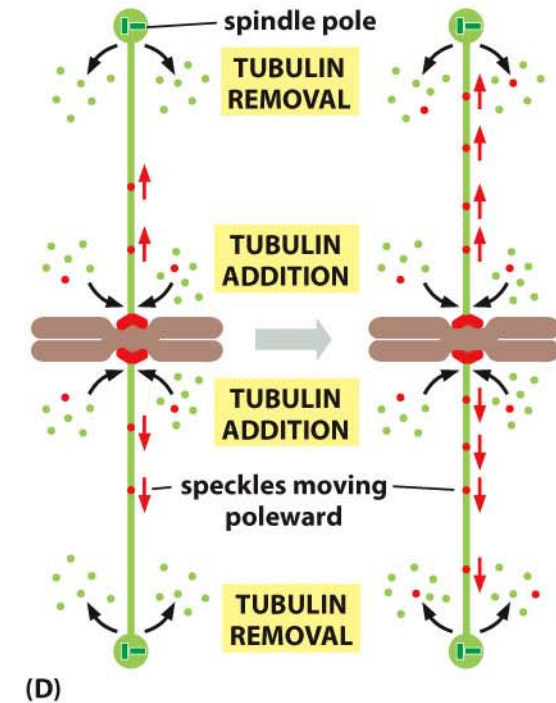
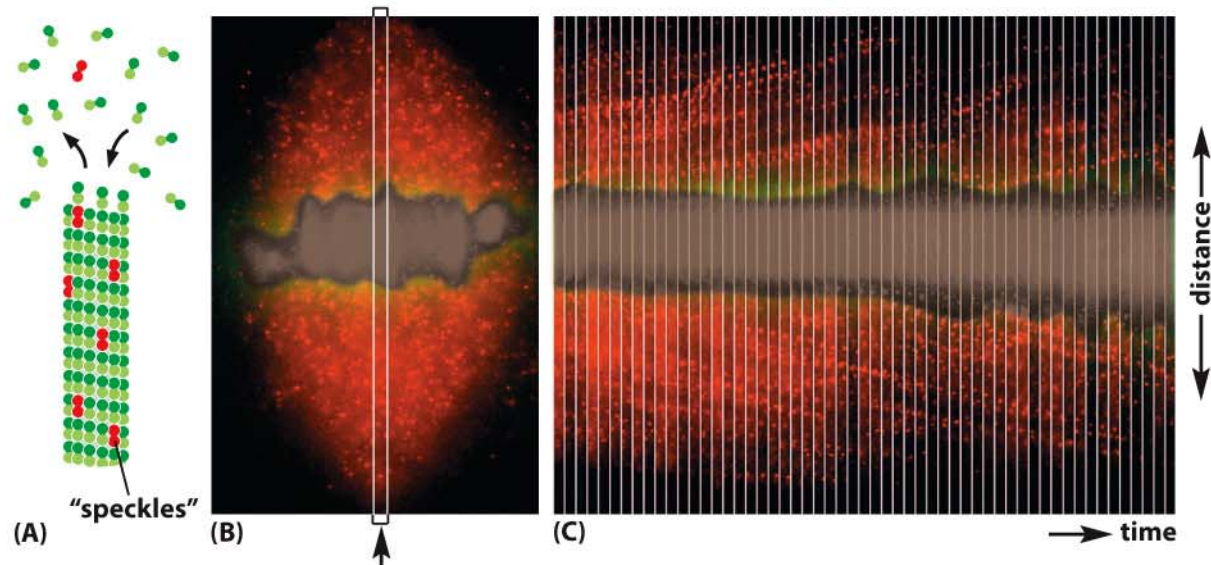
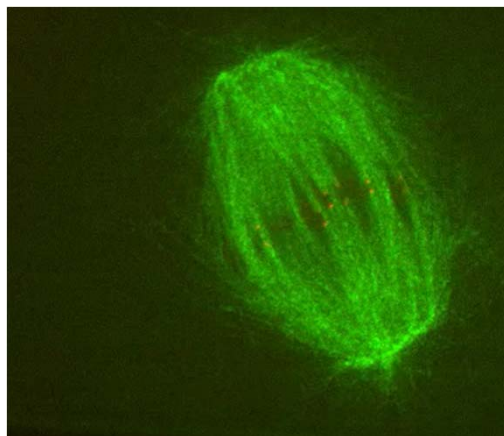


Figure 17-35 Molecular Biology of the Cell 6e (© Garland Science 2015)

Fluorescent Speckle Imaging:

Microtubules in mitotic
spindles assembled in
vitro in *Xenopus* extracts.

C. M. Waterman-Storer
A. Desai
J. C. Bulinski
E.D. Salmon



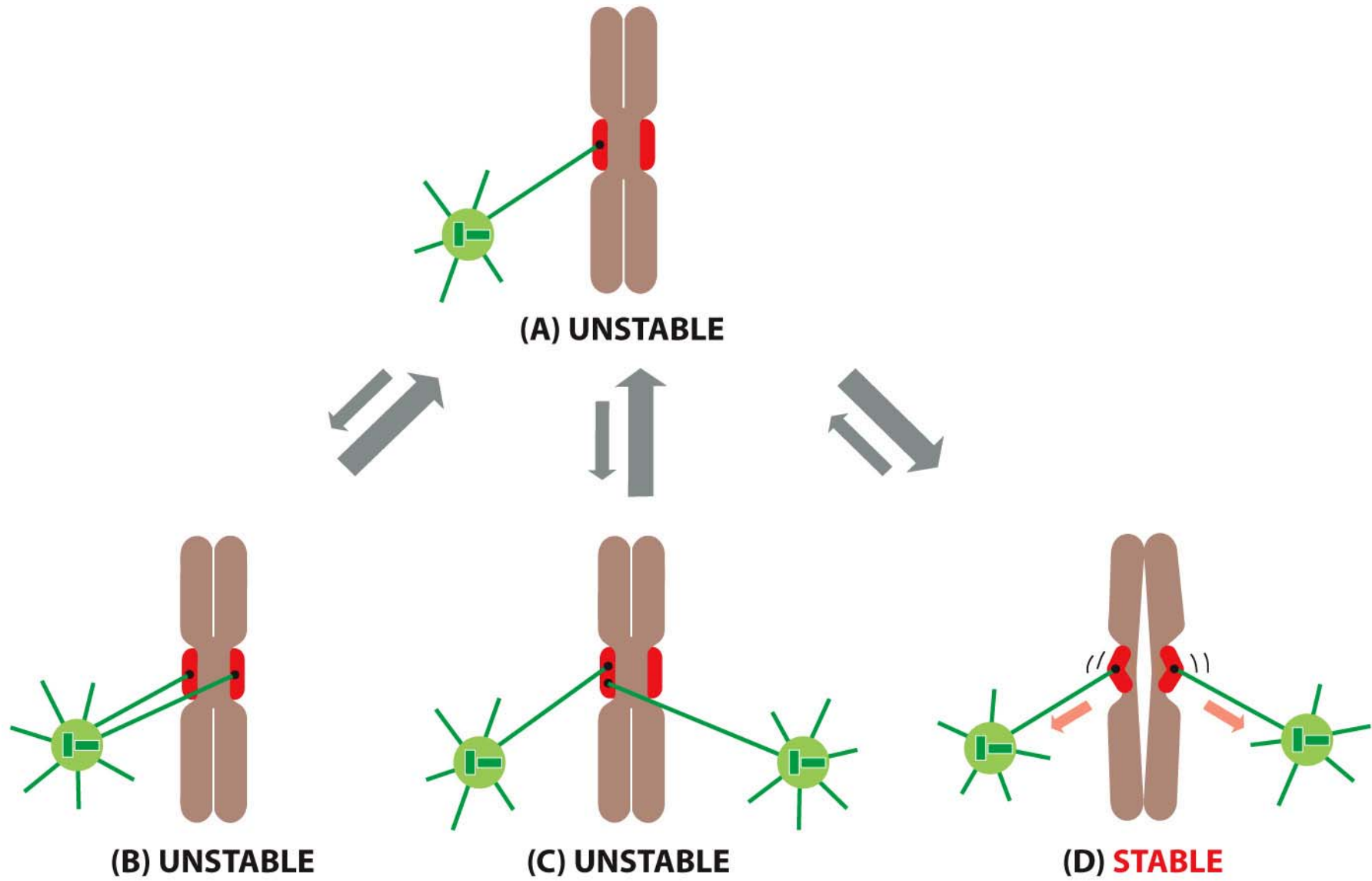
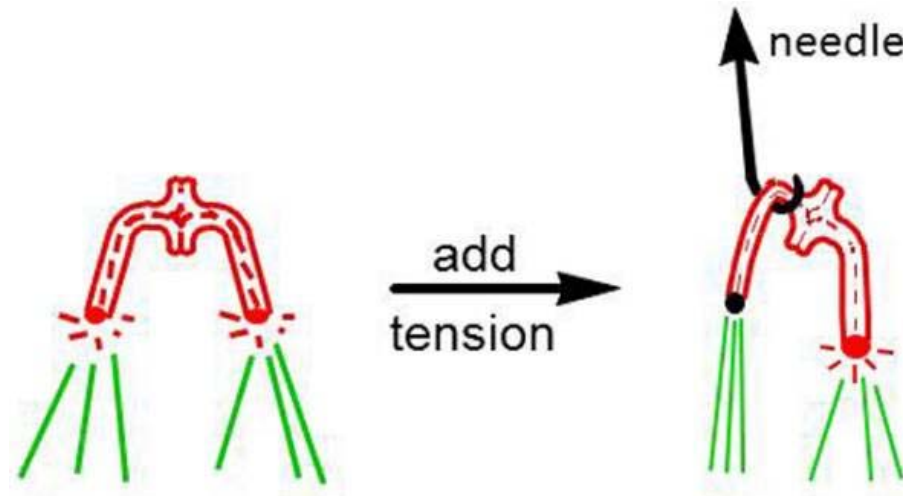
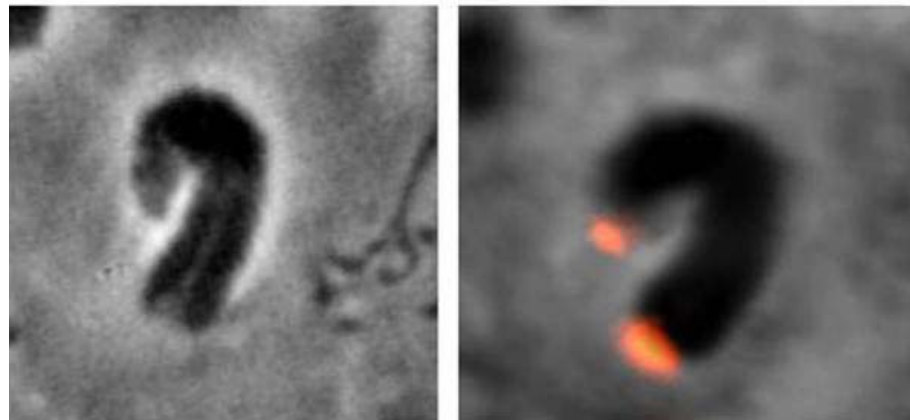


Figure 17-33 Molecular Biology of the Cell 6e (© Garland Science 2015)

tension affects kinetochore signalling



3F3 antibody



Nicklas et al. JCB 1995, 130: 929

tension stabilises attachment

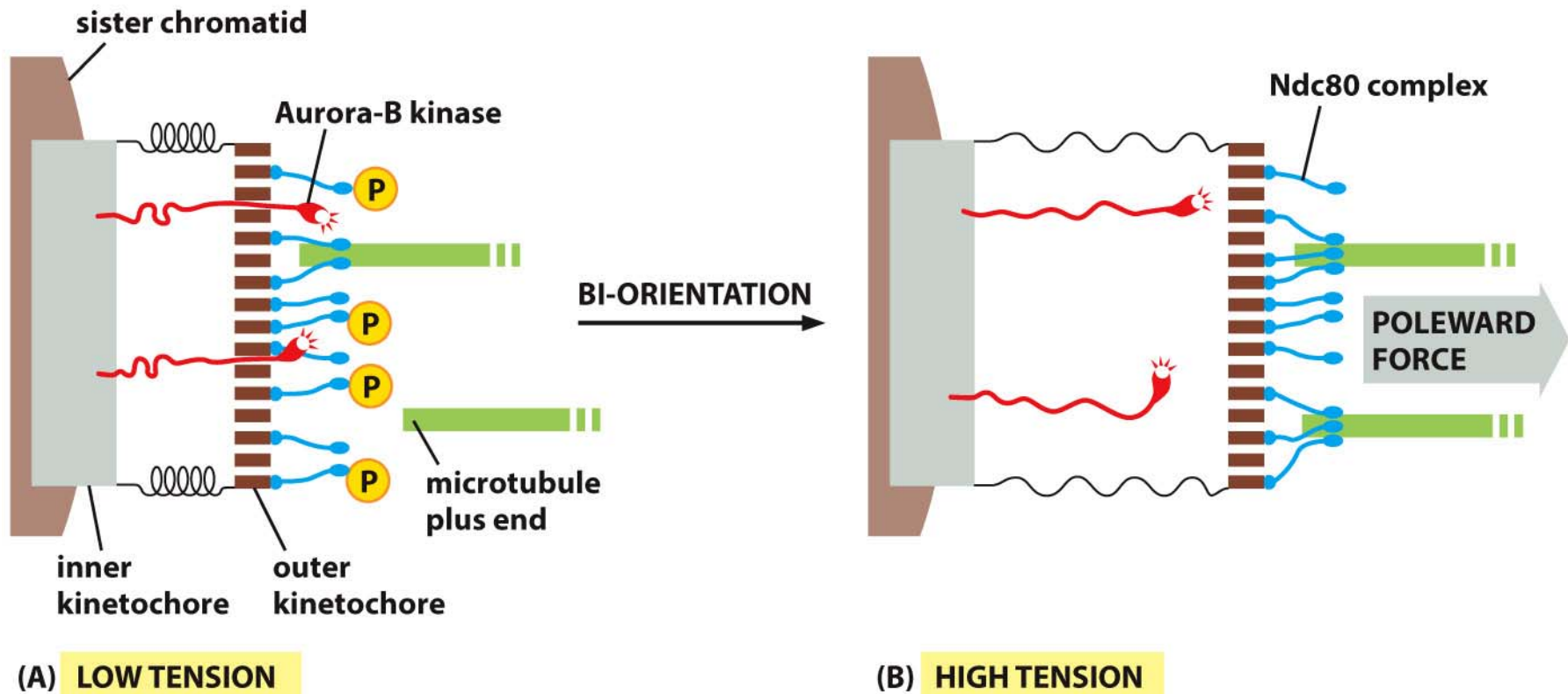


Figure 17-34 Molecular Biology of the Cell 6e (© Garland Science 2015)

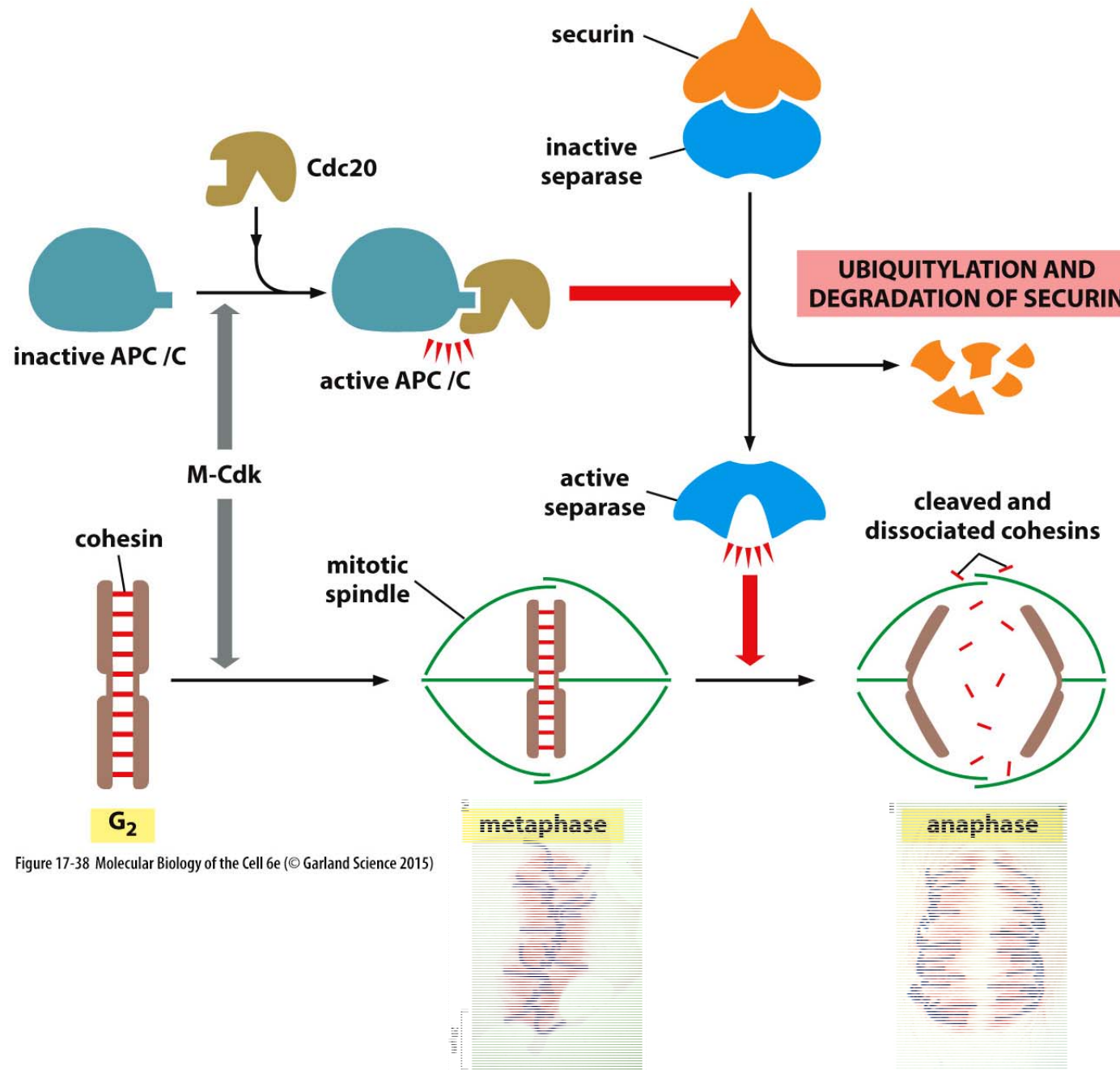
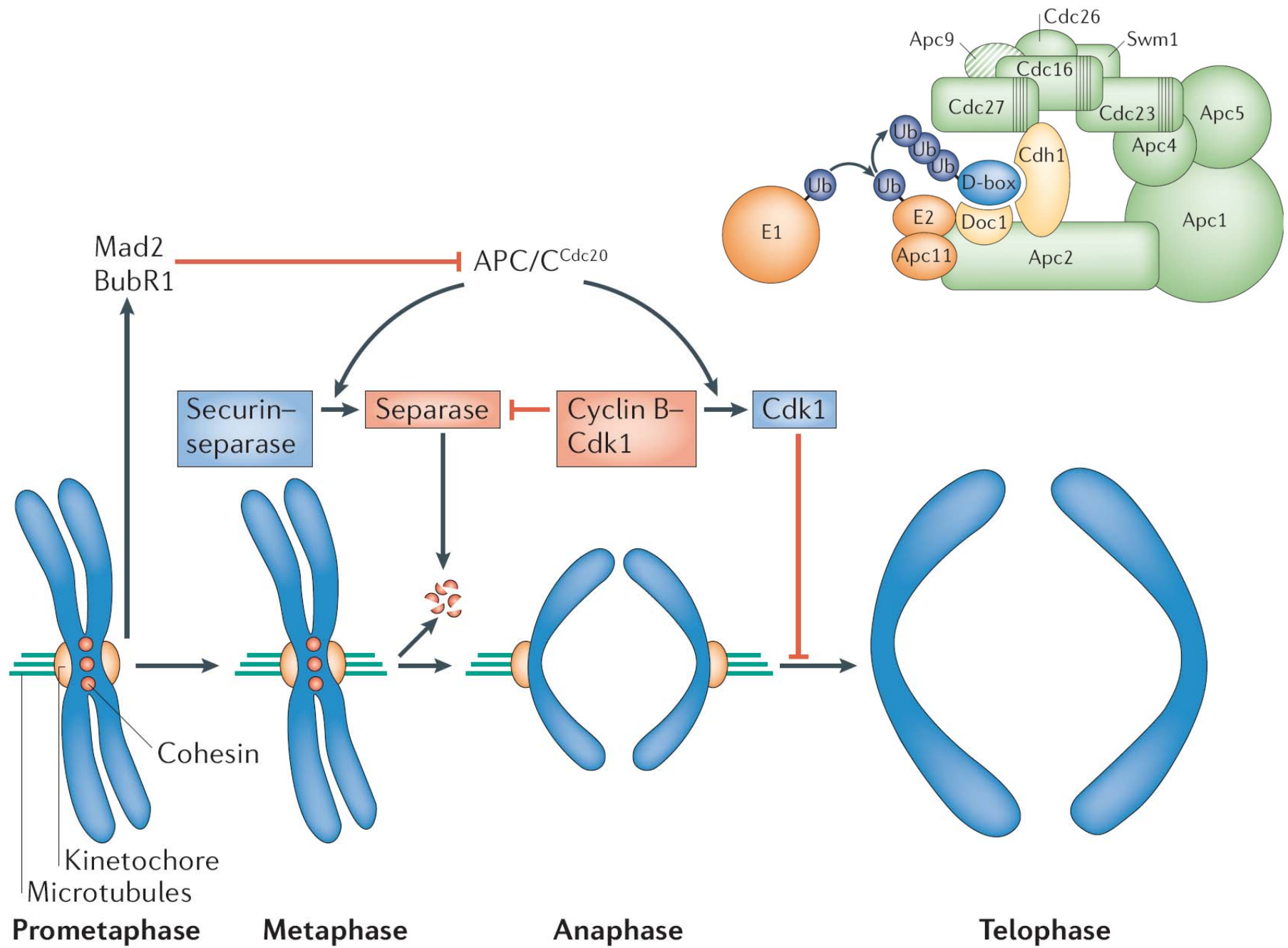
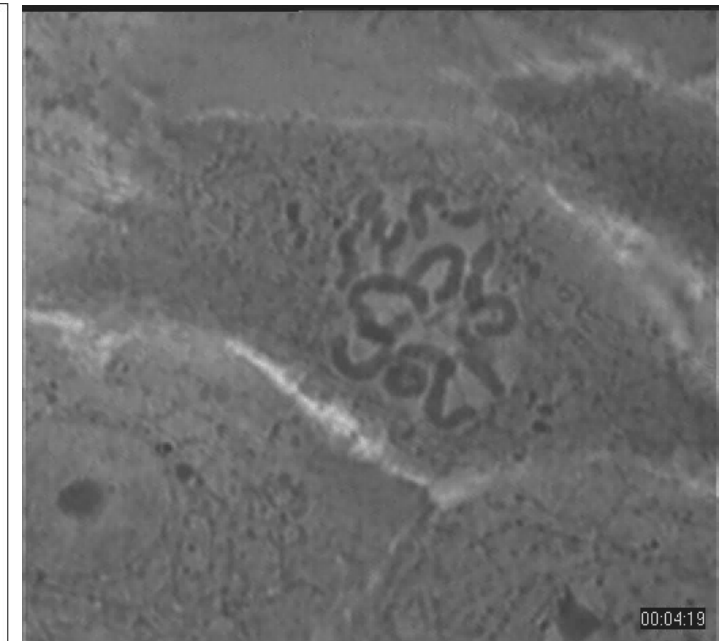
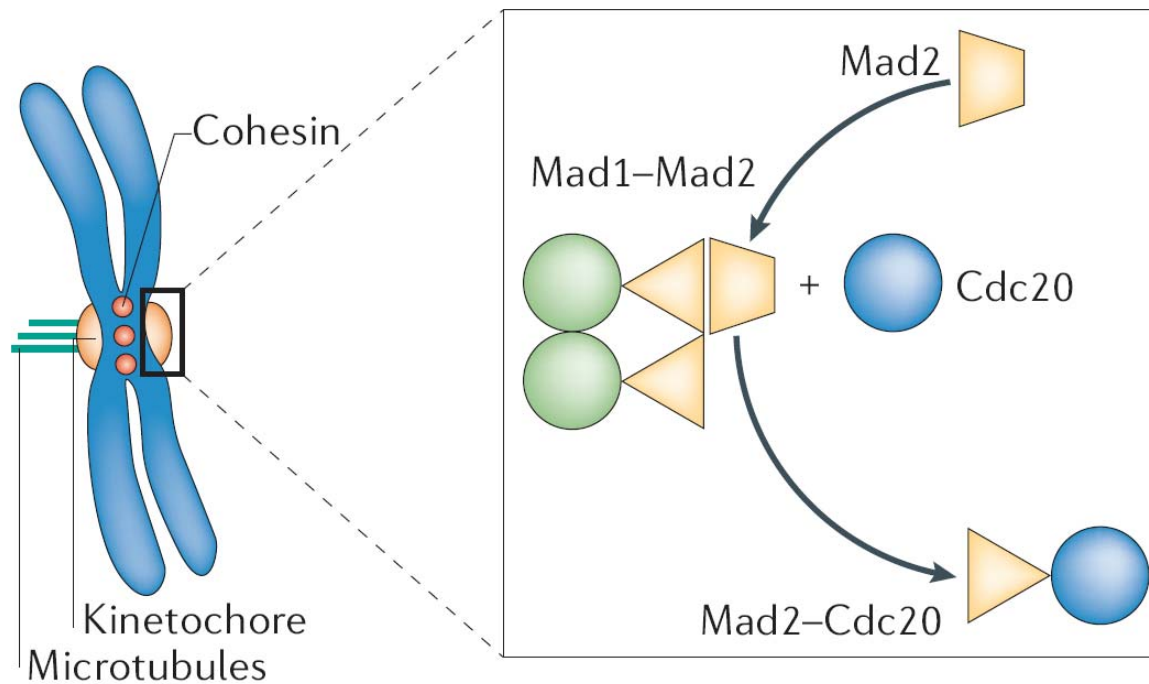


Figure 17-38 Molecular Biology of the Cell 6e (© Garland Science 2015)

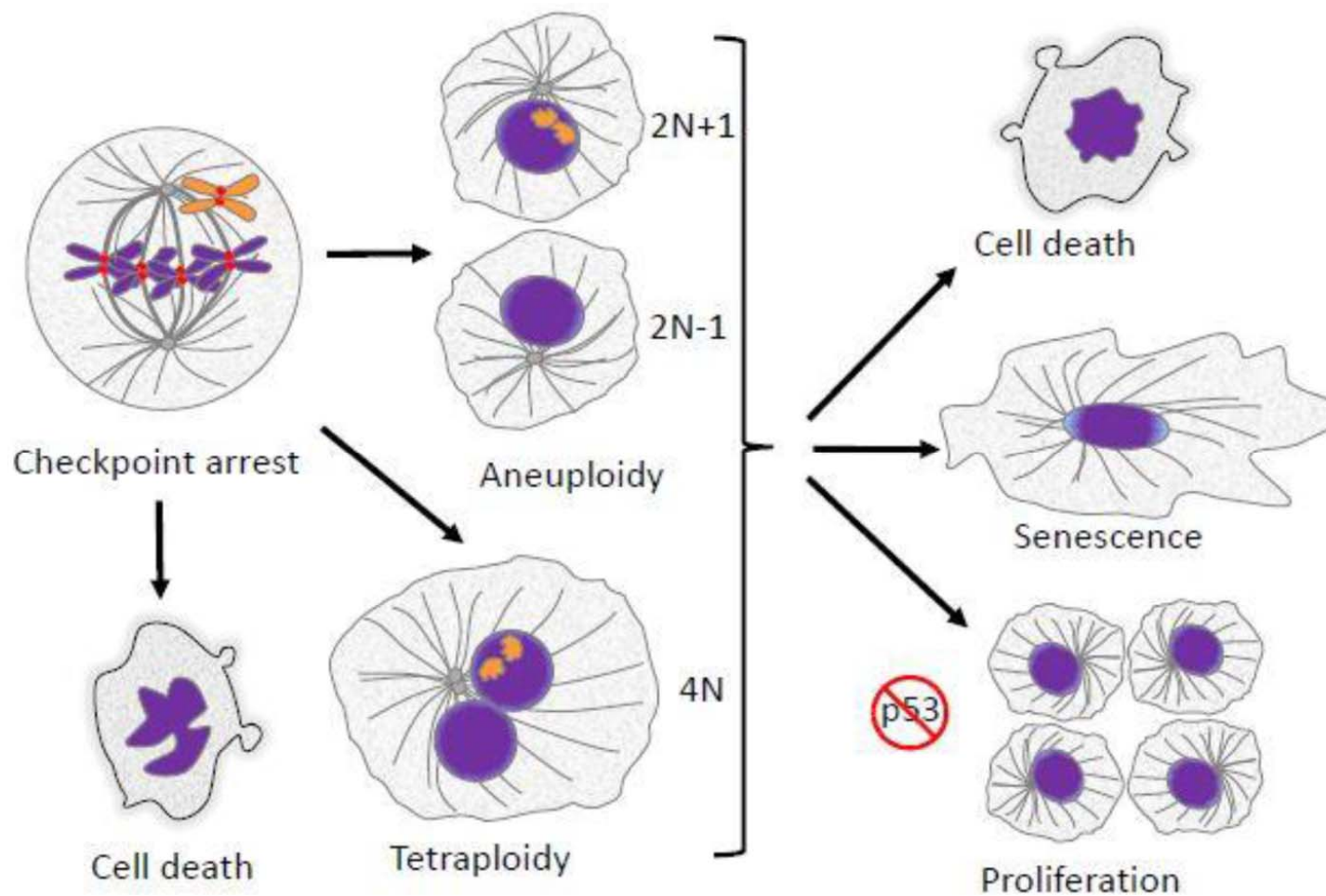


The anaphase-wait signal (the spindle checkpoint) is generated at the kinetochore



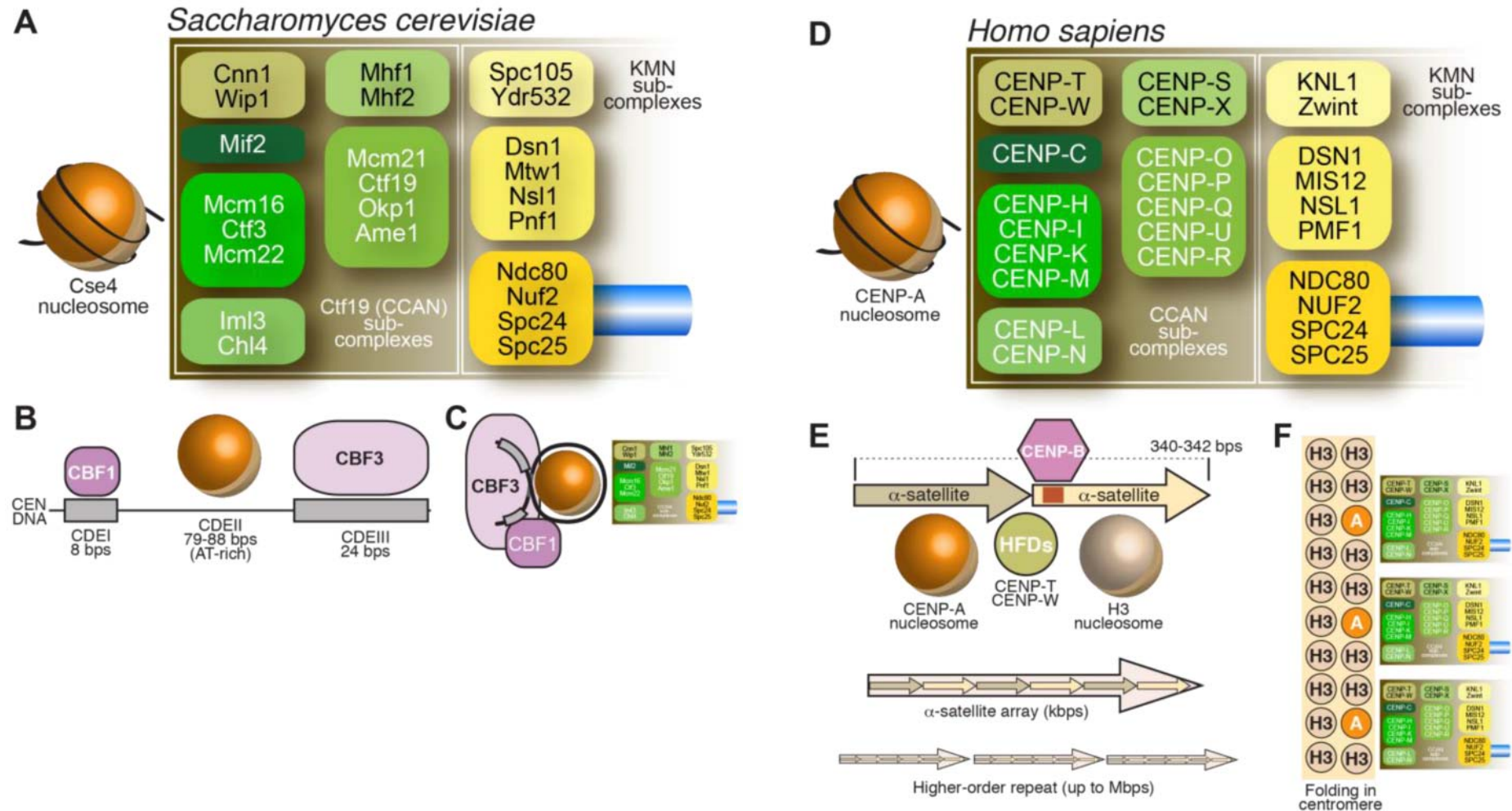
exit from mitosis without
a spindle and without
SAC signalling

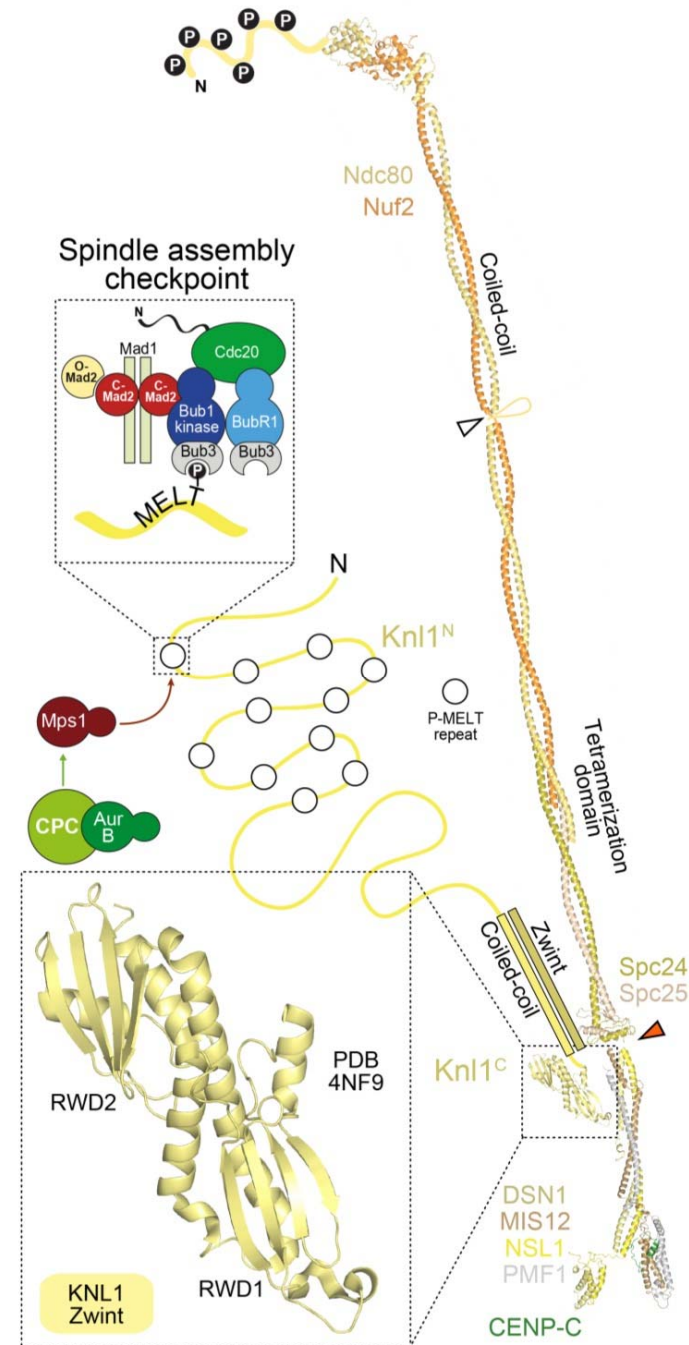
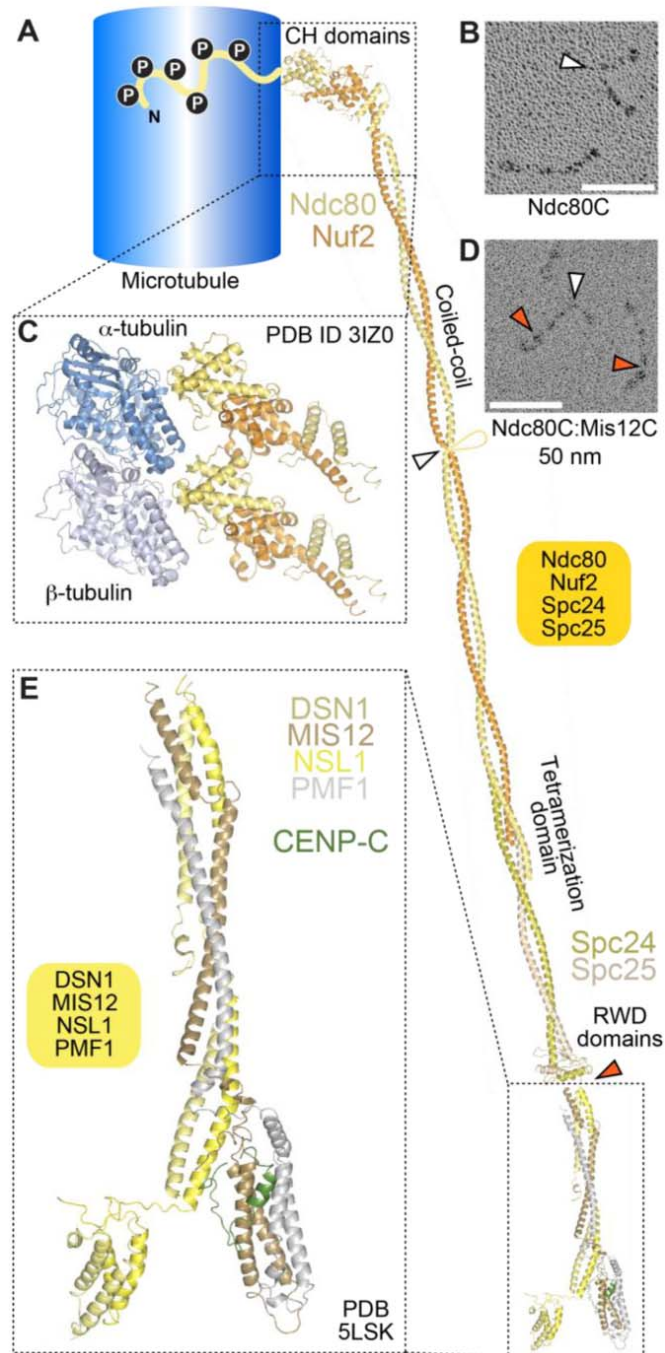
chromosome segregation errors result in aneuploidy



point kinetochores

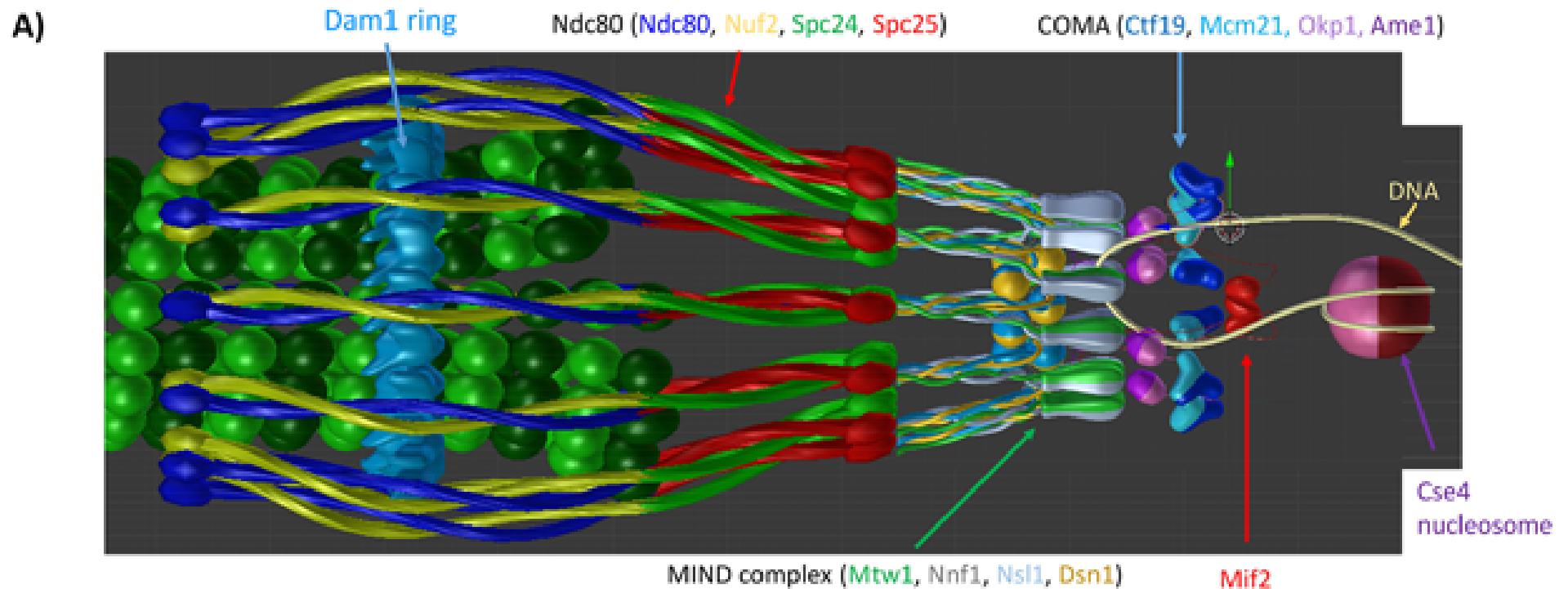
regional kinetochores



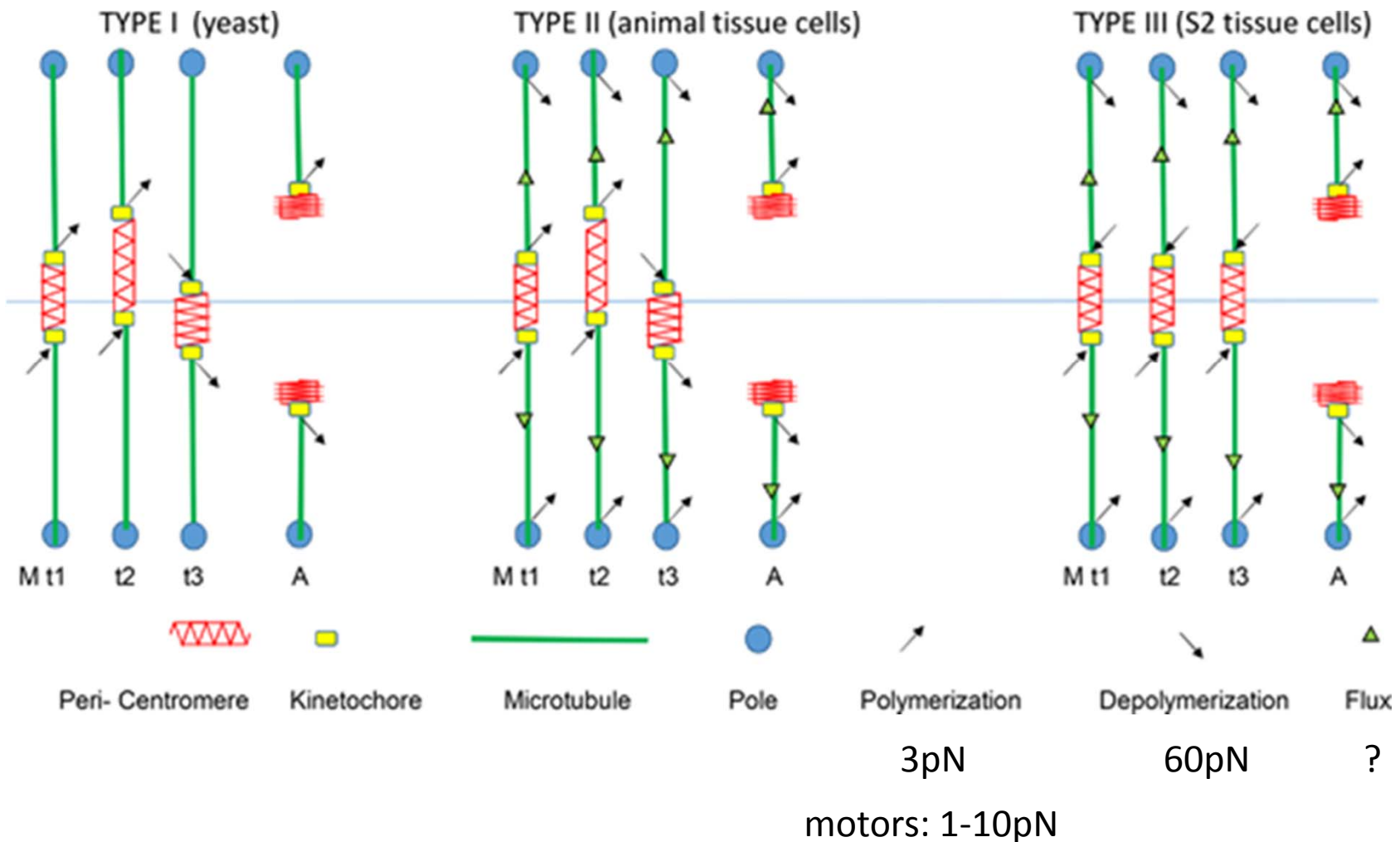


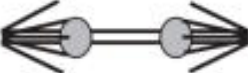
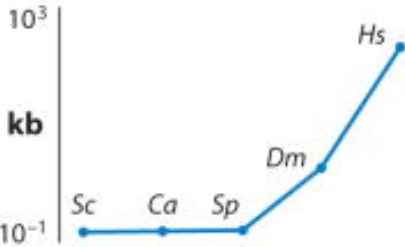
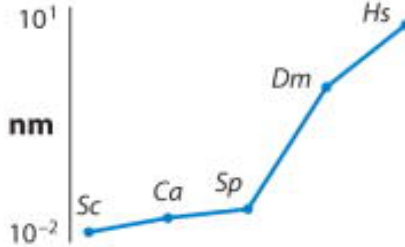
Musacchio, A.; Desai, A. A Molecular View of Kinetochore Assembly and Function. *Biology* **2017**, *6*, 5.

model of a yeast point kinetochore

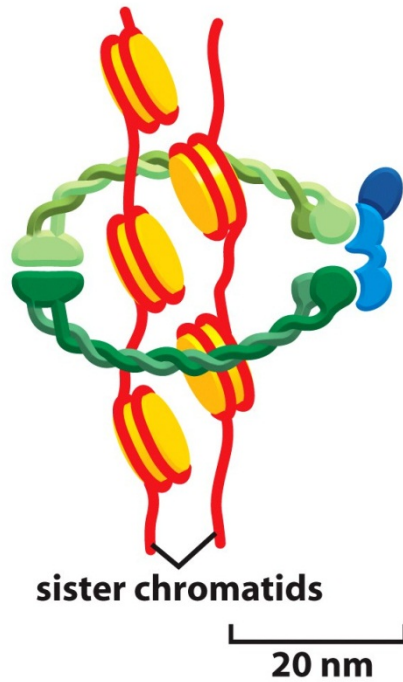


Mechanisms controlling chromosome movements

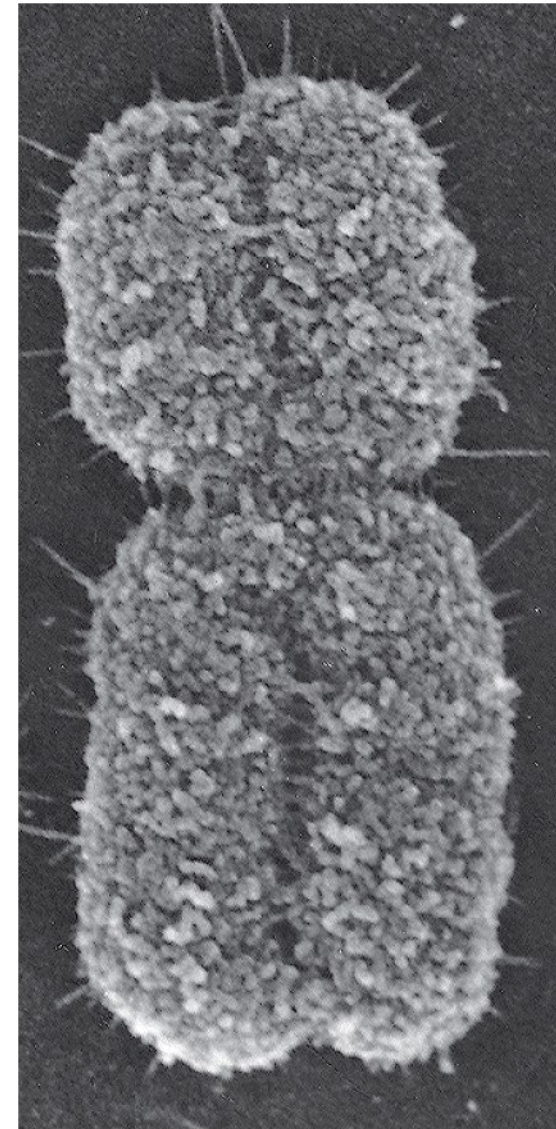
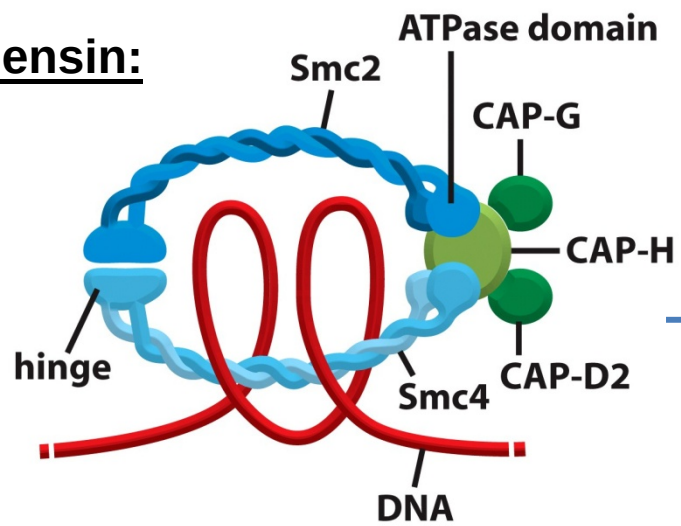


	Centromere DNA size 		Kinetochore separation in mitosis
<i>S. cerevisiae</i>	0.125 kb	0.04 μM 	800 nm 
<i>C. albicans</i>	3–4 kb	0.15 μM 	~800 nm
<i>S. pombe</i>	10 kb	0.23 μM 	~1,000 nm
<i>D. melanogaster</i>	200–500 kb	1–1.65 μM 	~1,000 nm
<i>H. sapiens</i>	500–1,500 kb	2.8 μM 	~1,000 nm 
Scaling			

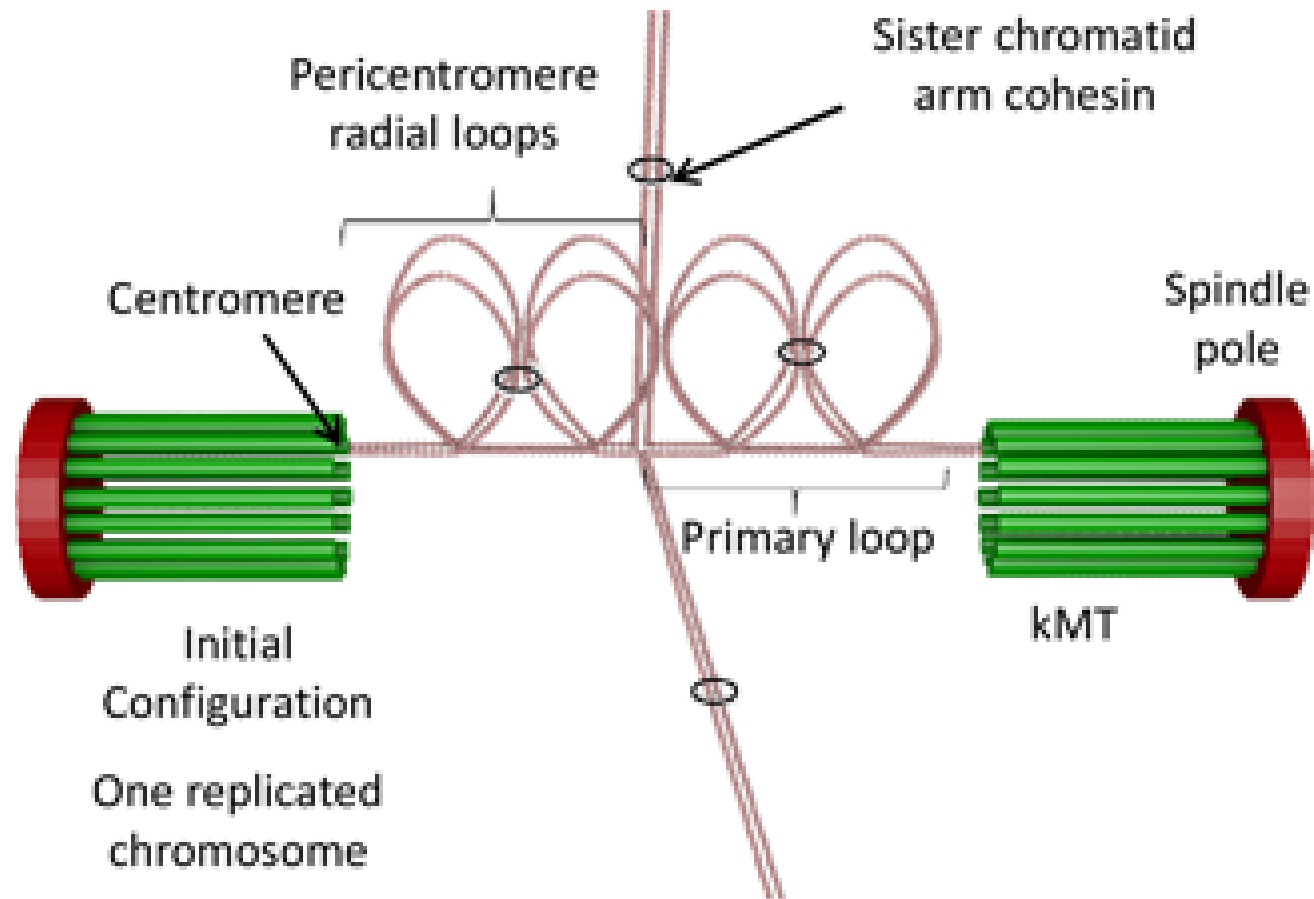
Cohesin:



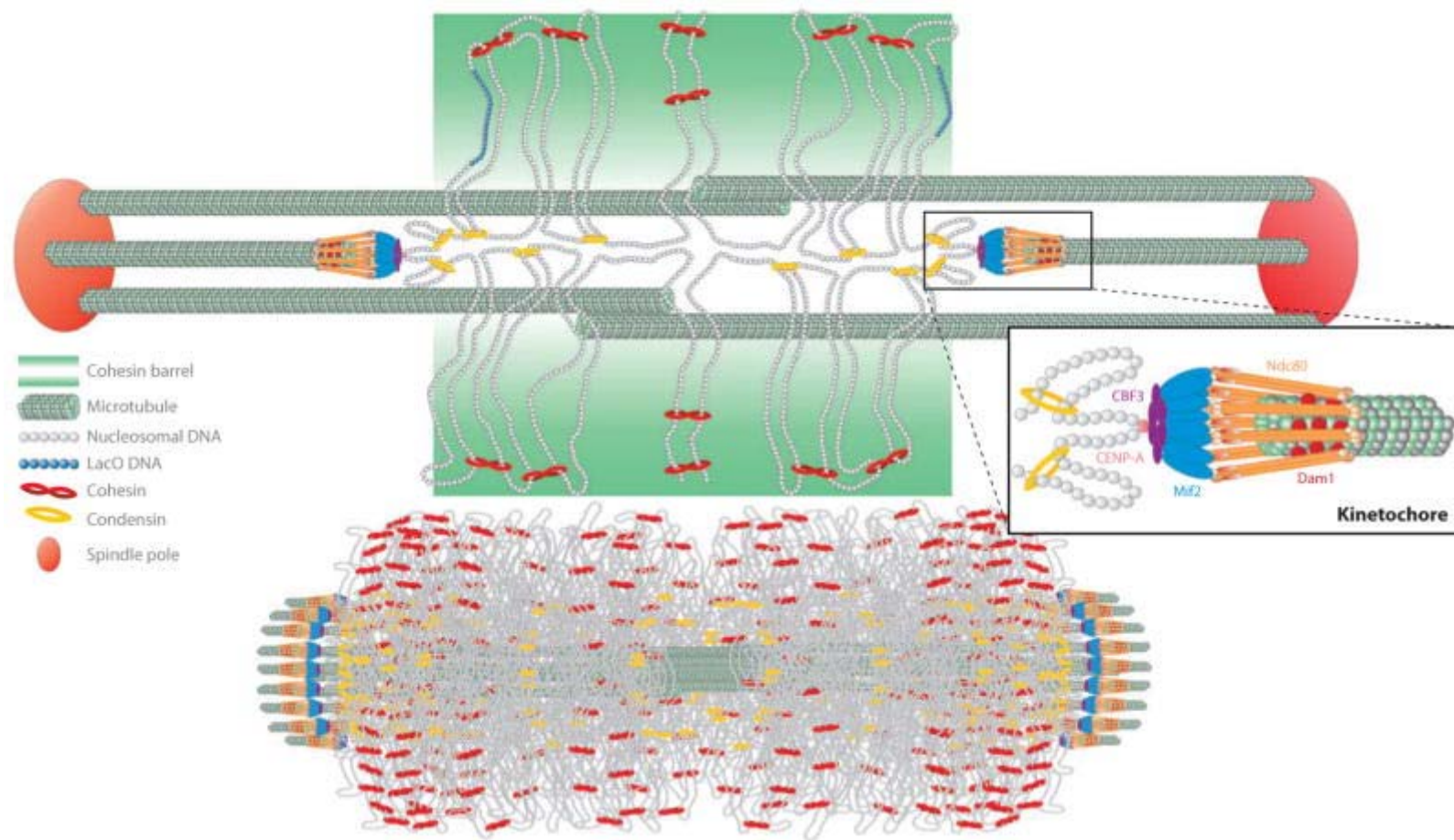
Condensin:



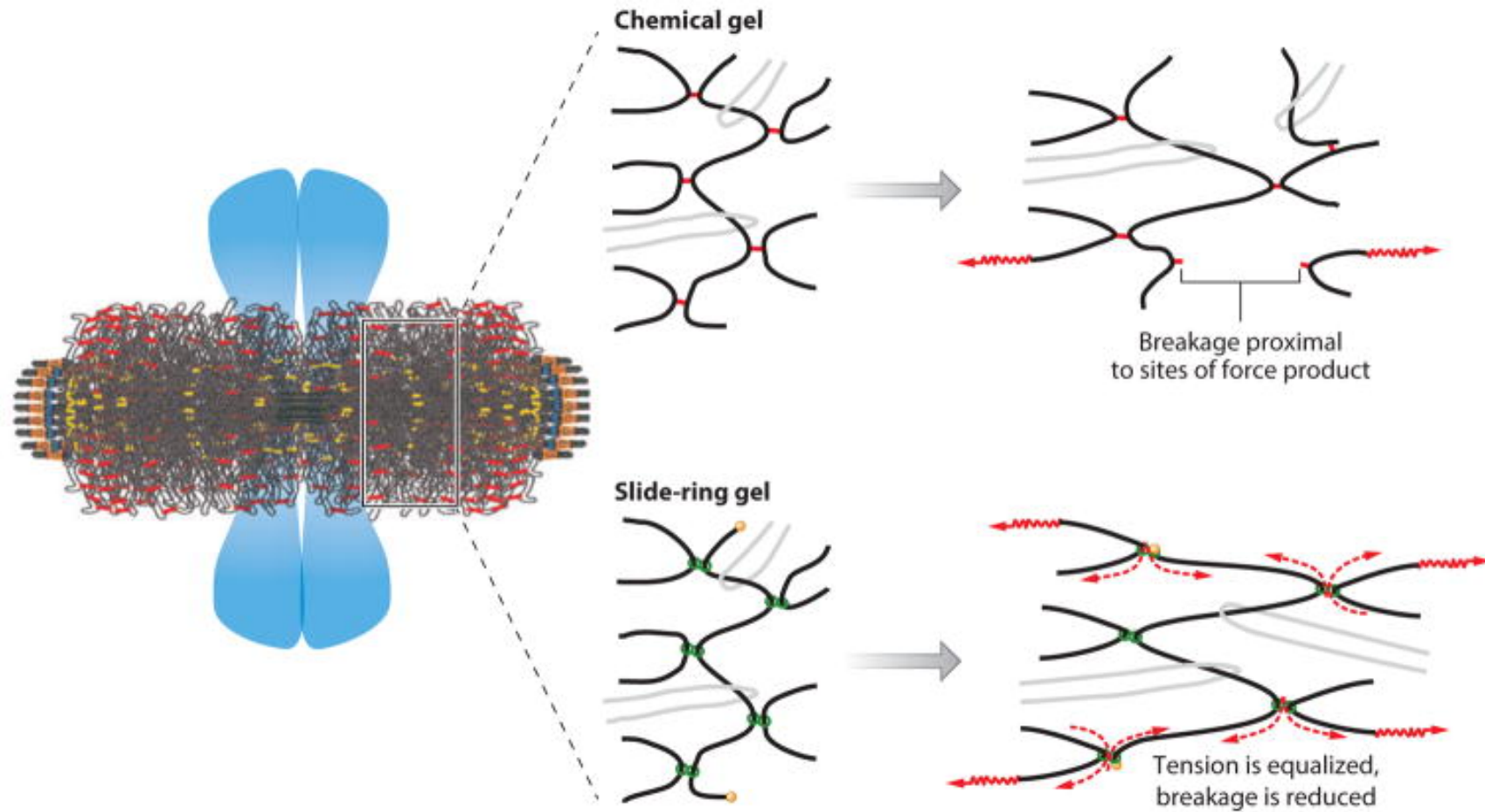
centromere scheme



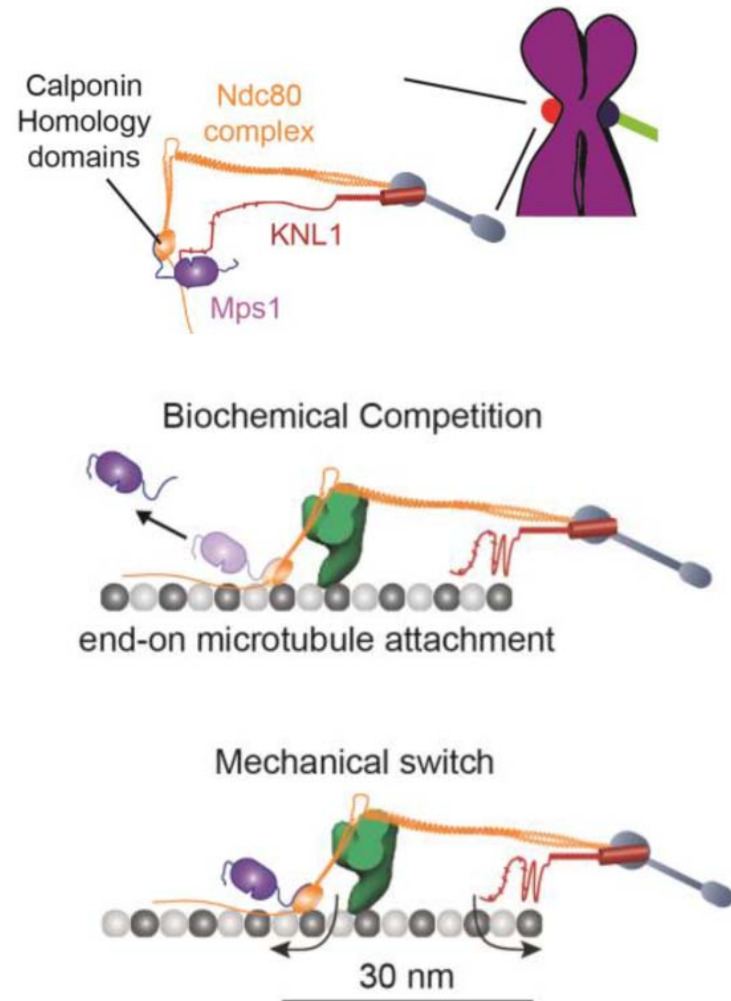
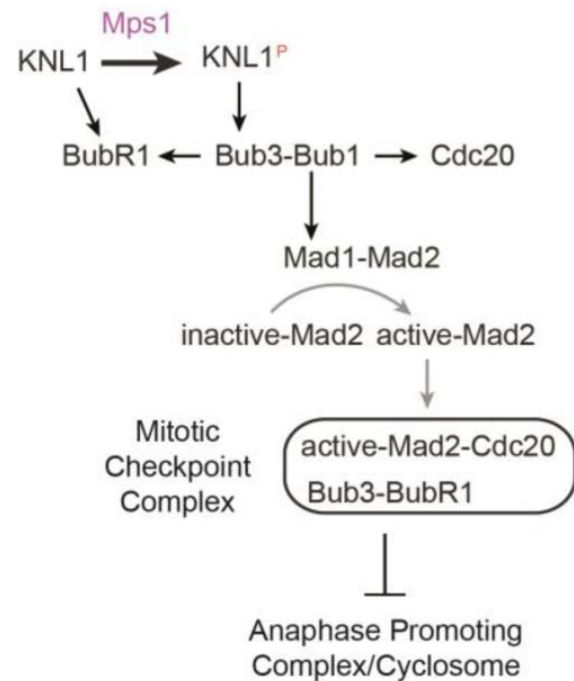
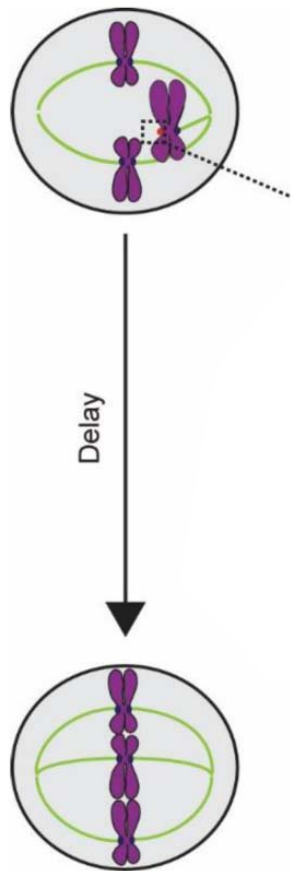
organisation of centromeric DNA



rings avoid breakage



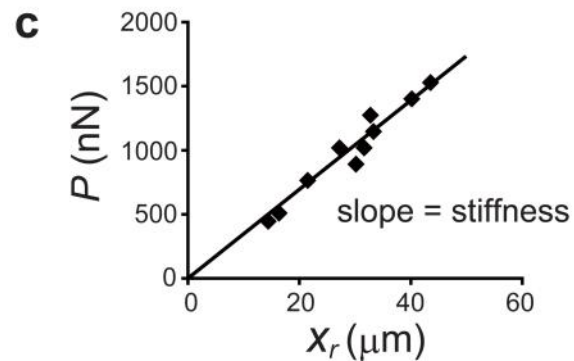
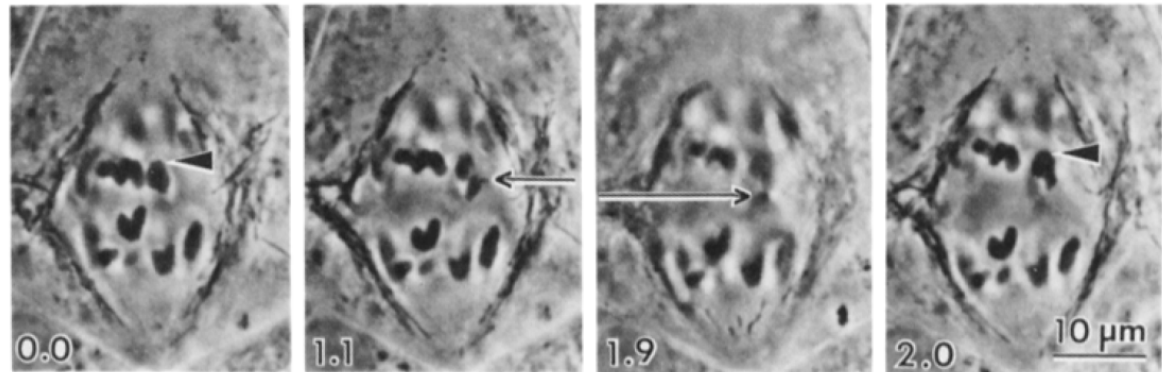
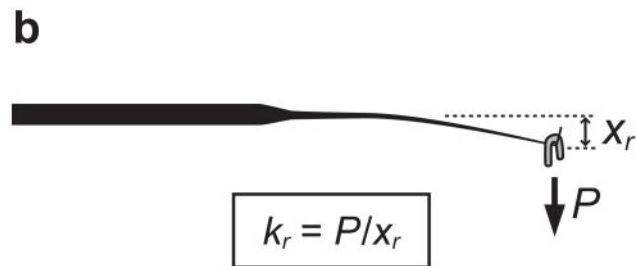
SAC inactivation by attachment and tension



What is the function of the pulling forces that cause interkintochores 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

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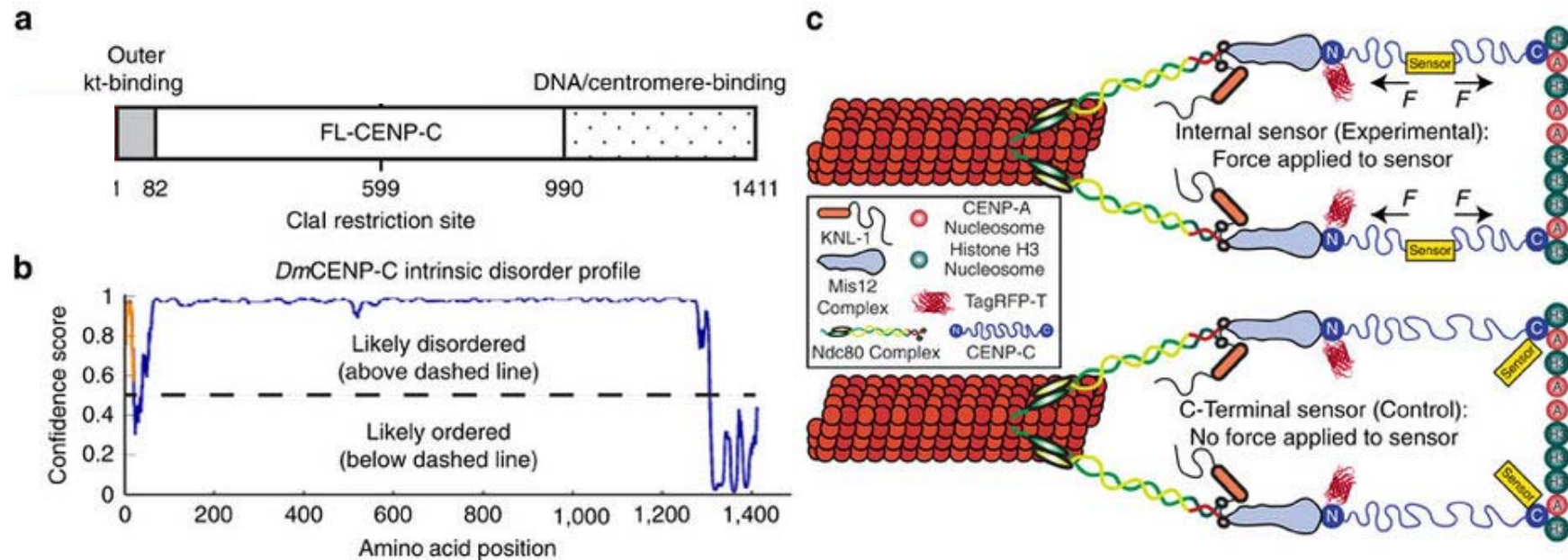
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Chromosome biorientation produces hundreds of piconewtons at a metazoan kinetochore

Anna A. Ye^{1,2}, Stuart Cane^{1,2} & Thomas J. Maresca^{1,2}

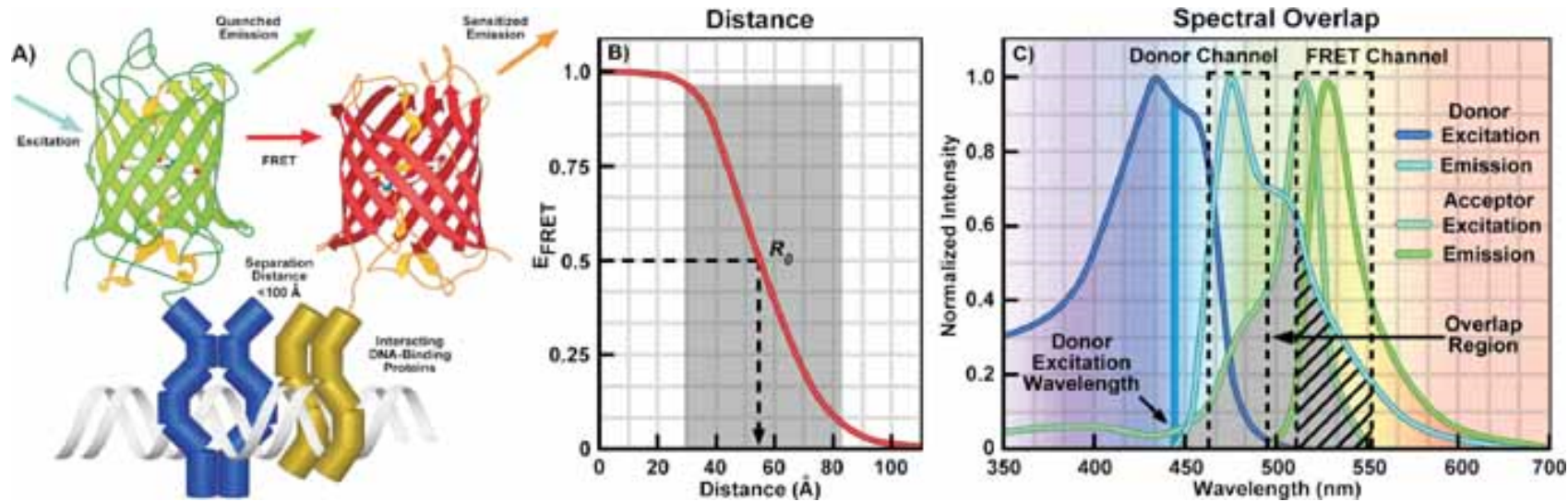
Intramolecular stretch sensor



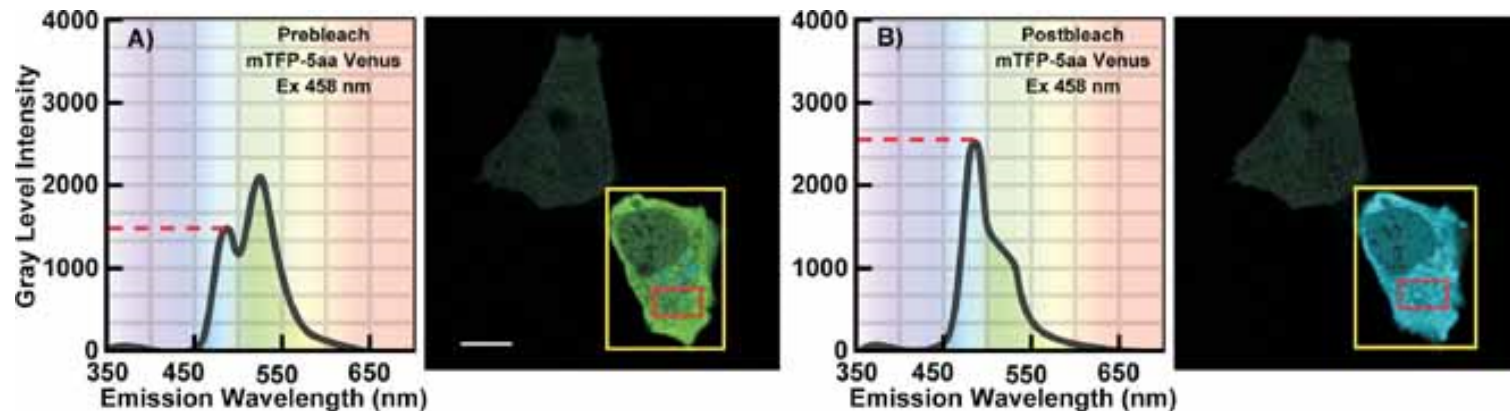
- **sensor (FRET):** TSMoD (mTurquoise2-spider silk protein-mVenus)
- **model system:** drosophila S2 cells (no metaphase oscillation)
- **target molecule:** CENP-C (single linker between chromatin and MT attachment site)

FRET: protein proximity assay

sensitized emission FRET



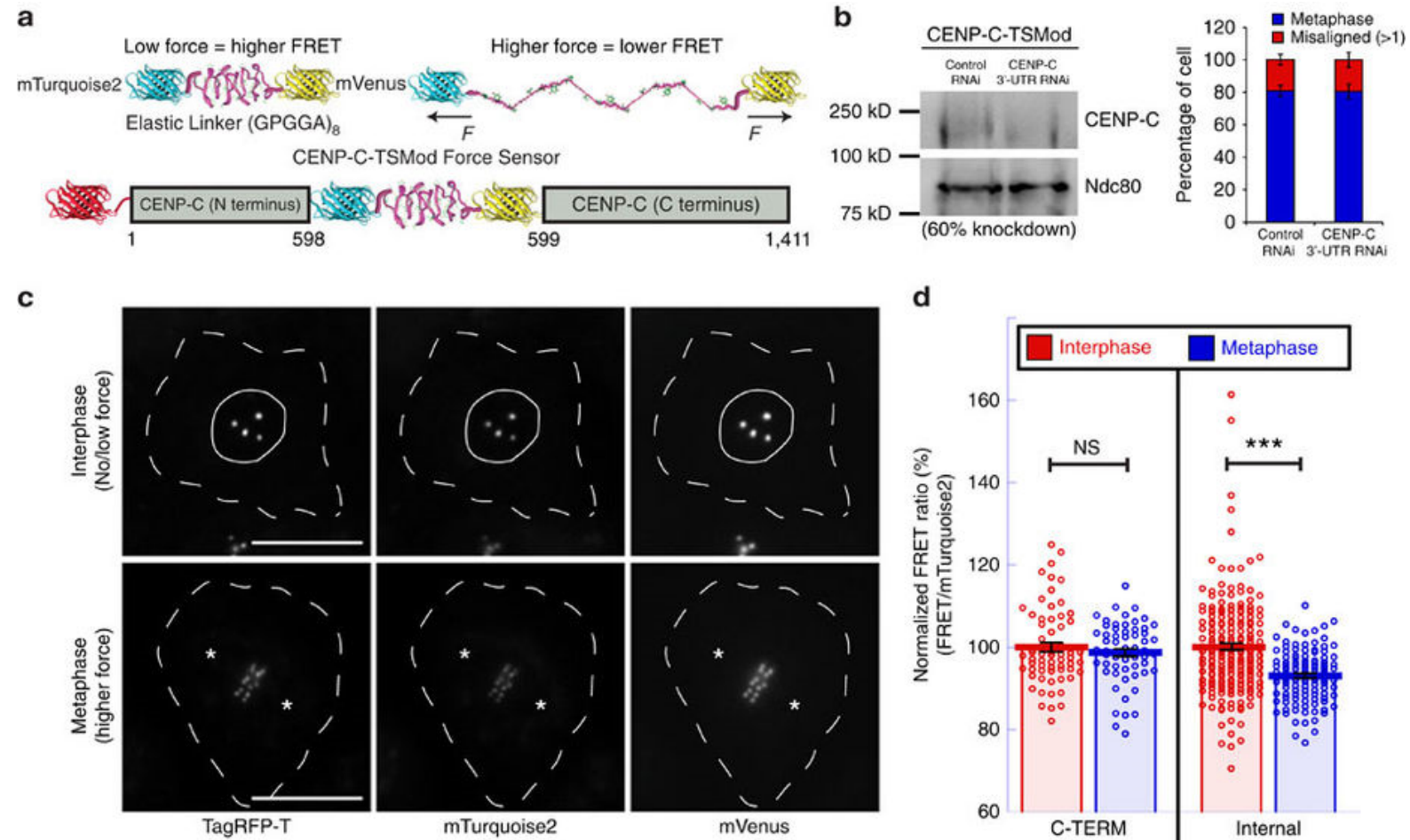
acceptor photobleaching FRET



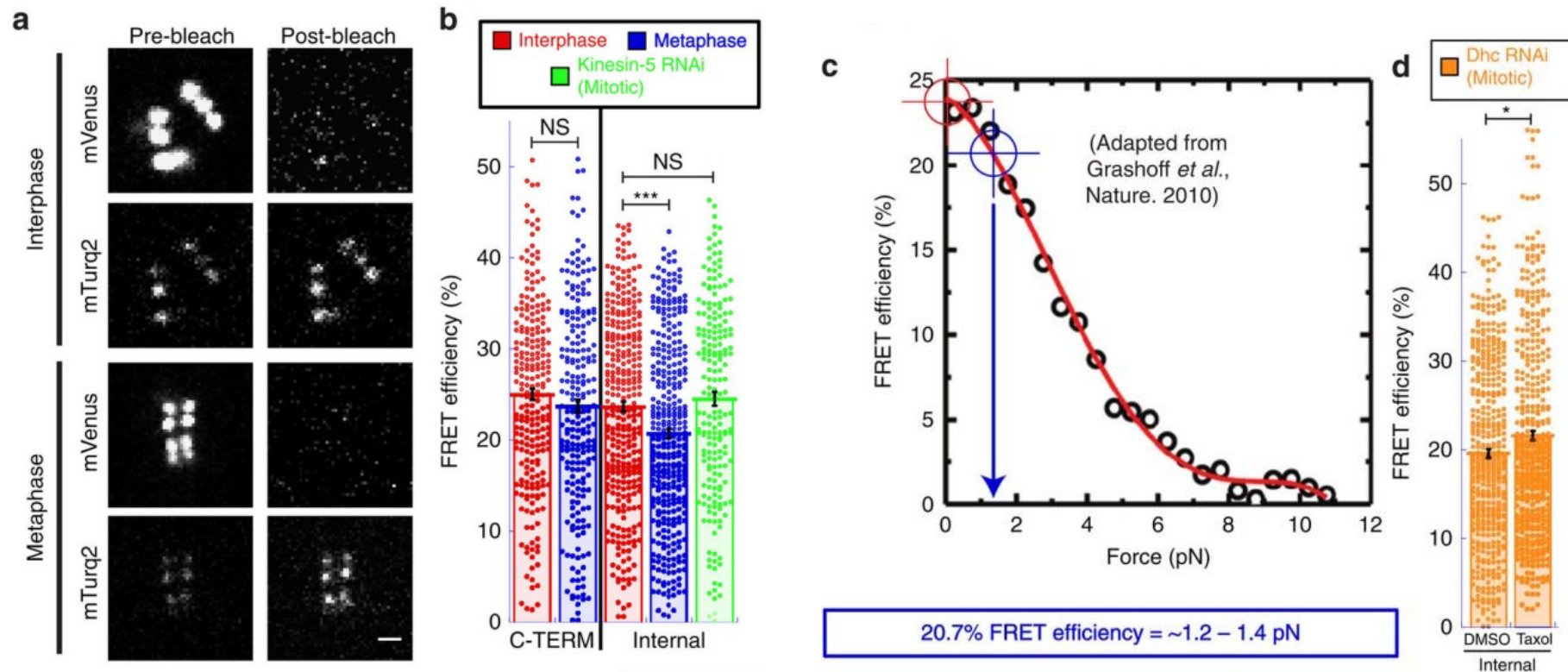
BioEssays

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>

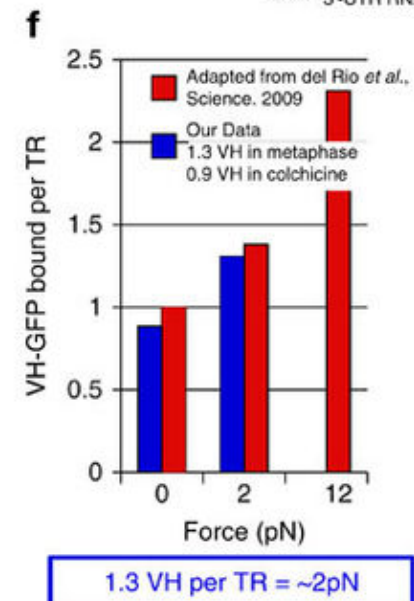
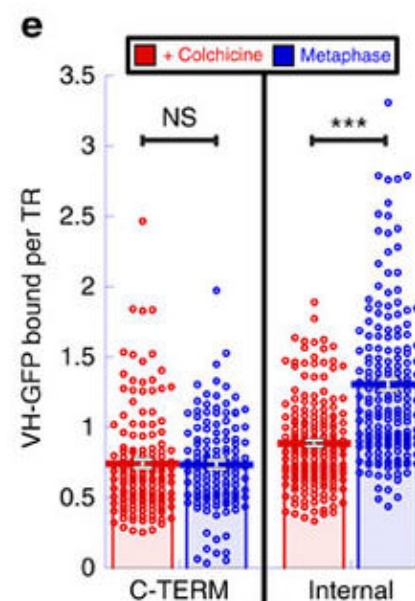
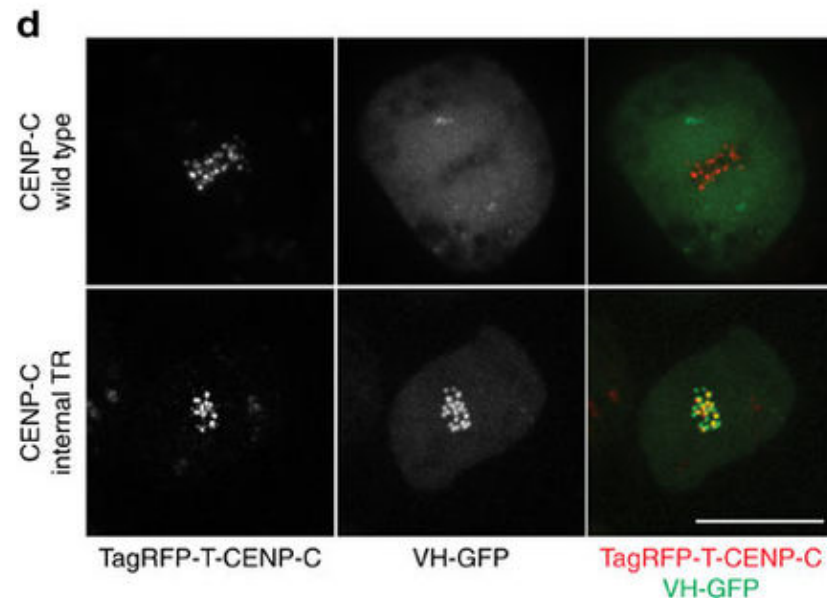
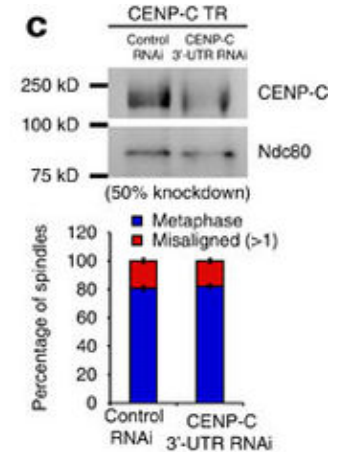
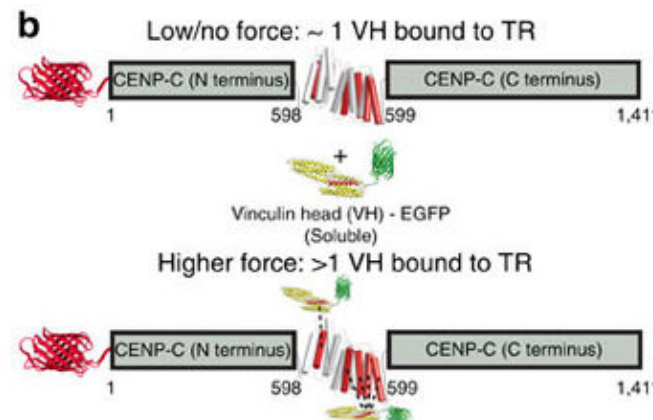
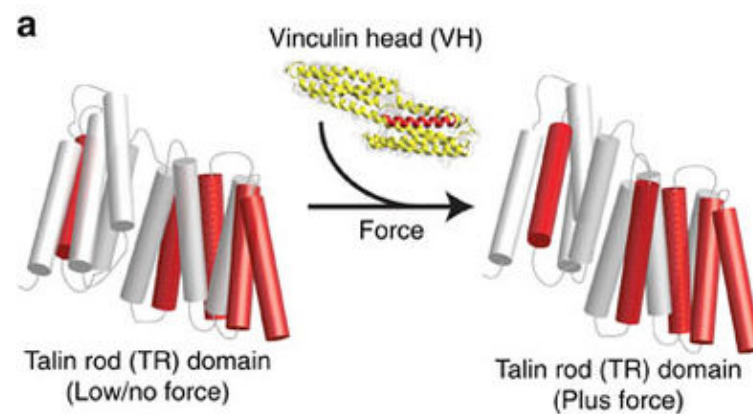
TSMoD FRET changes with tension



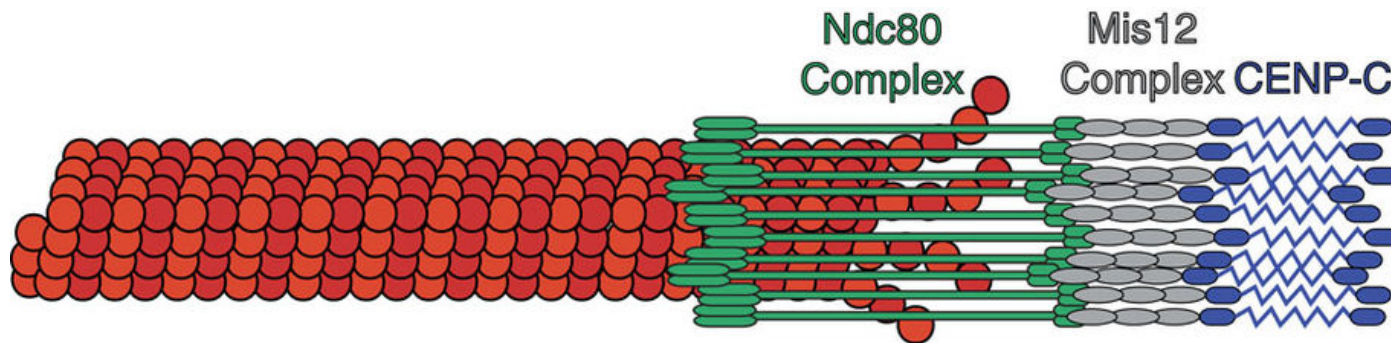
Force estimation using acceptor photobleaching



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

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

$$F_{kt} = F_{MT} \times \# \text{ kinetochore-microtubules}$$

	F_{CENP-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 < 10 pN 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