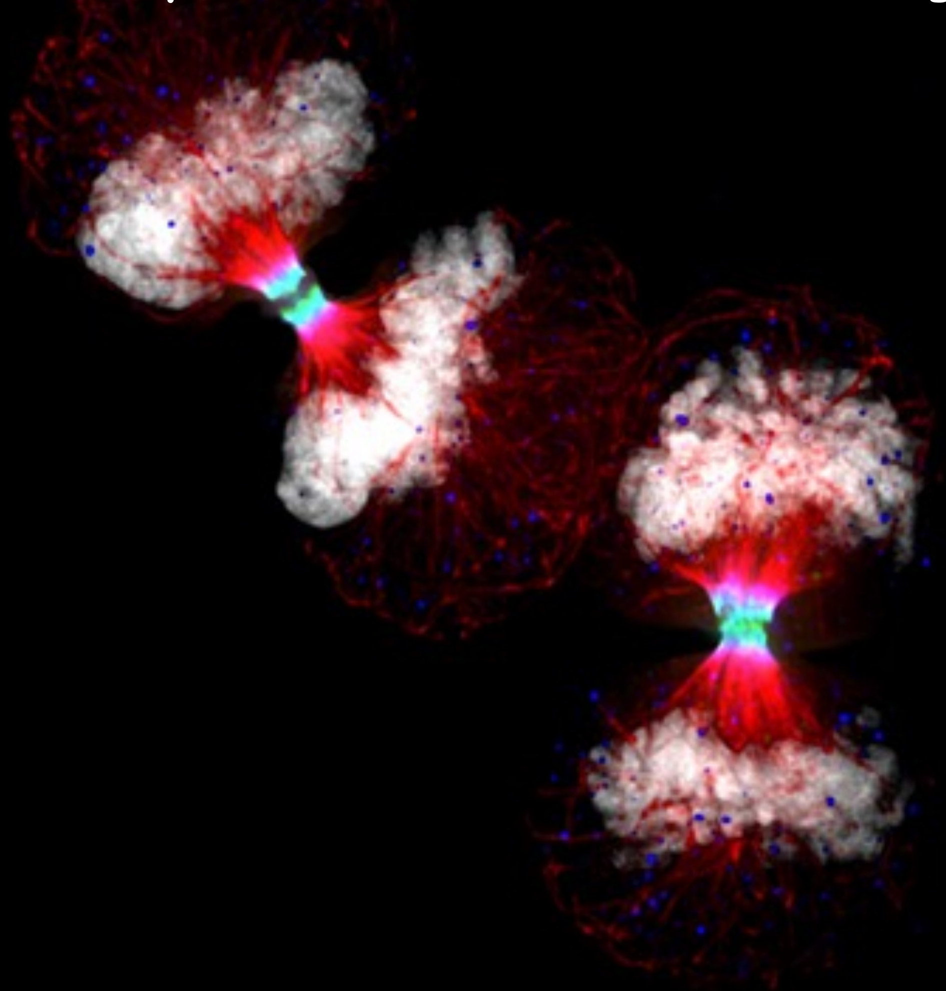
Cytokinesis in animal cells and fungi

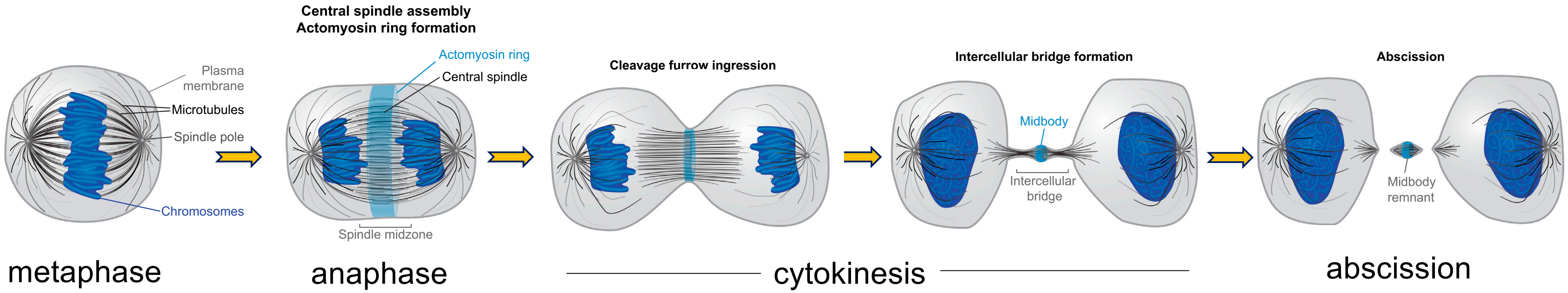


Simonetta Piatti, CRBM

Mitosis in PtK cells

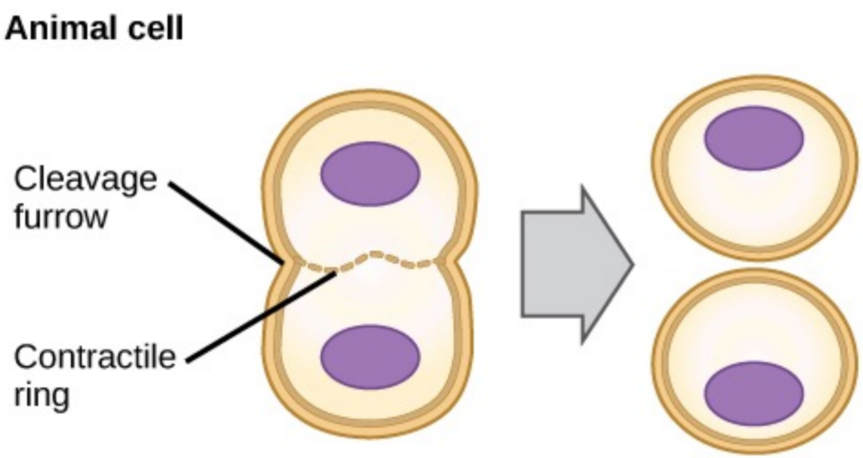
Courtesy of C. Rieder

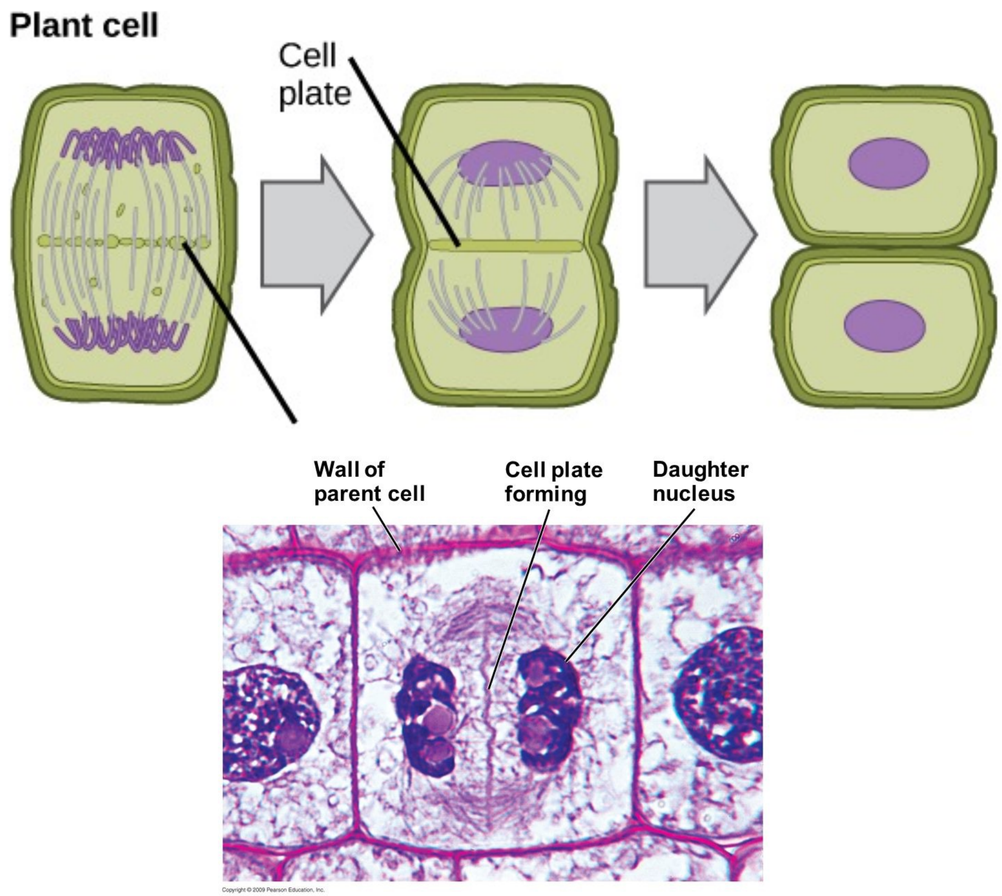
The main steps of cytokinesis in animal cells and fungi



From Mierzwa and Gerlich, 2014, Dev. Cell 31:525

In plants cytokinesis does not involve a contractile ring, but rather relies on local deposition of membrane and cell wall



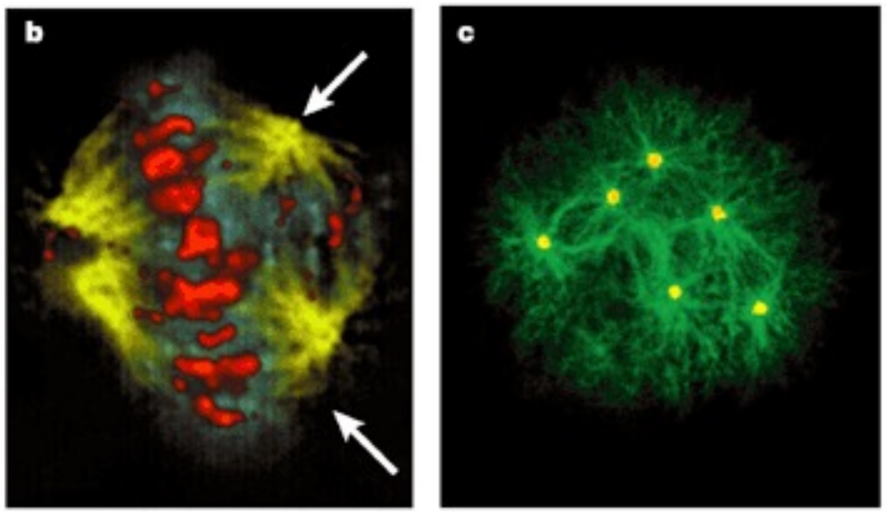


WHY STUDYING CYTOKINESIS IS IMPORTANT?

Polyploid cells frequently undergo segregation errors (Theodor Boveri, 1926)

Tumor cells are often polyploid and have multipolar spindles

Some pre-malignant cell types are polyploid; the polyploid condition precedes malignancy, which arises with p53 loss



Vol 437|13 October 2005|doi:10.1038/nature04217 nature

LETTERS

Cytokinesis failure generating tetraploids promotes tumorigenesis in *p53*-null cells

Takeshi Fujiwara[1*], Madhavi Bandi[1*], Masayuki Nitta[1], Elena V. Ivanova[2], Roderick T. Bronson[4] & David Pellman[1,3]

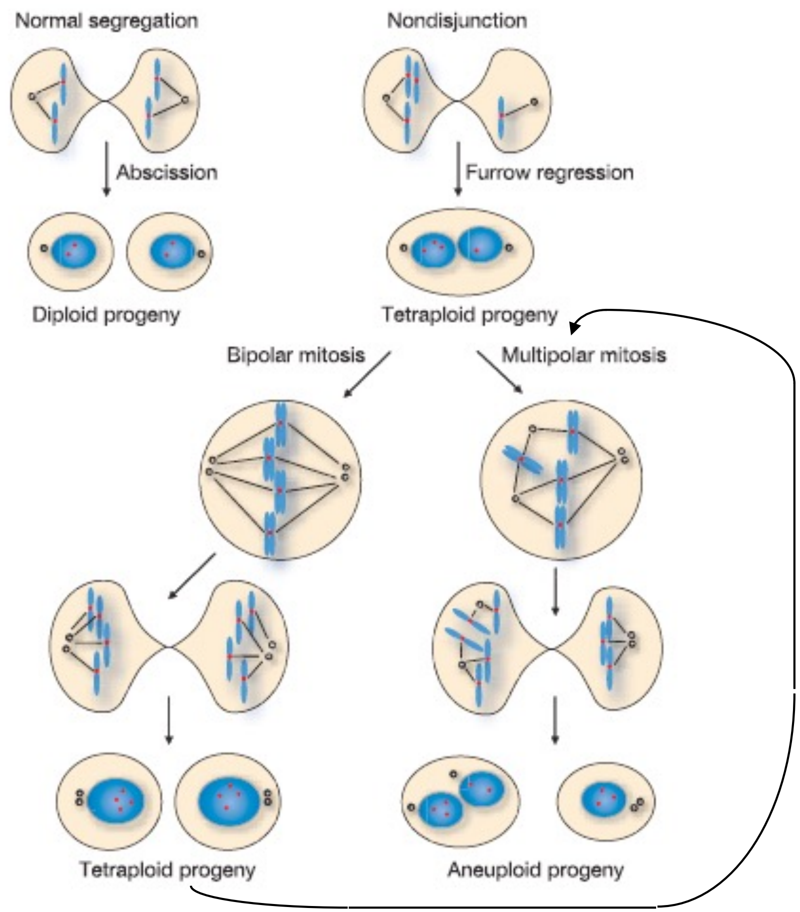
nature Vol 437|13 October 2005|doi:10.1038/nature03958

LETTERS

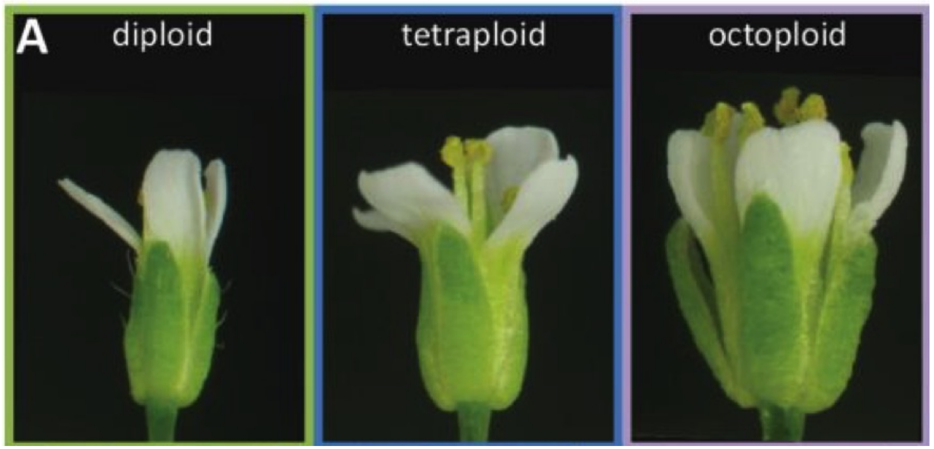
Chromosome nondisjunction yields tetraploid rather than aneuploid cells in human cell lines

Qinghua Shi[1]† & Randall W. King[1]

Chromosome non-disjunction (i.e. missegregation) often generates binucleate cells that are genetically unstable



Change in ploidy is also exploited to increase genetic diversification during evolution



Polyploidy

Examples of Polyploid Plants	
Name	**Number**
Common wheat	**6N** = 42
Tobacco	**4N** = 48
Potato	**4N** = 48
Banana	**3N** = 27
Boysenberry	**7N** = 49
Strawberry	**8N** = 56

Many **ferns** are polyploid with chromosome number up to 400N

Part I

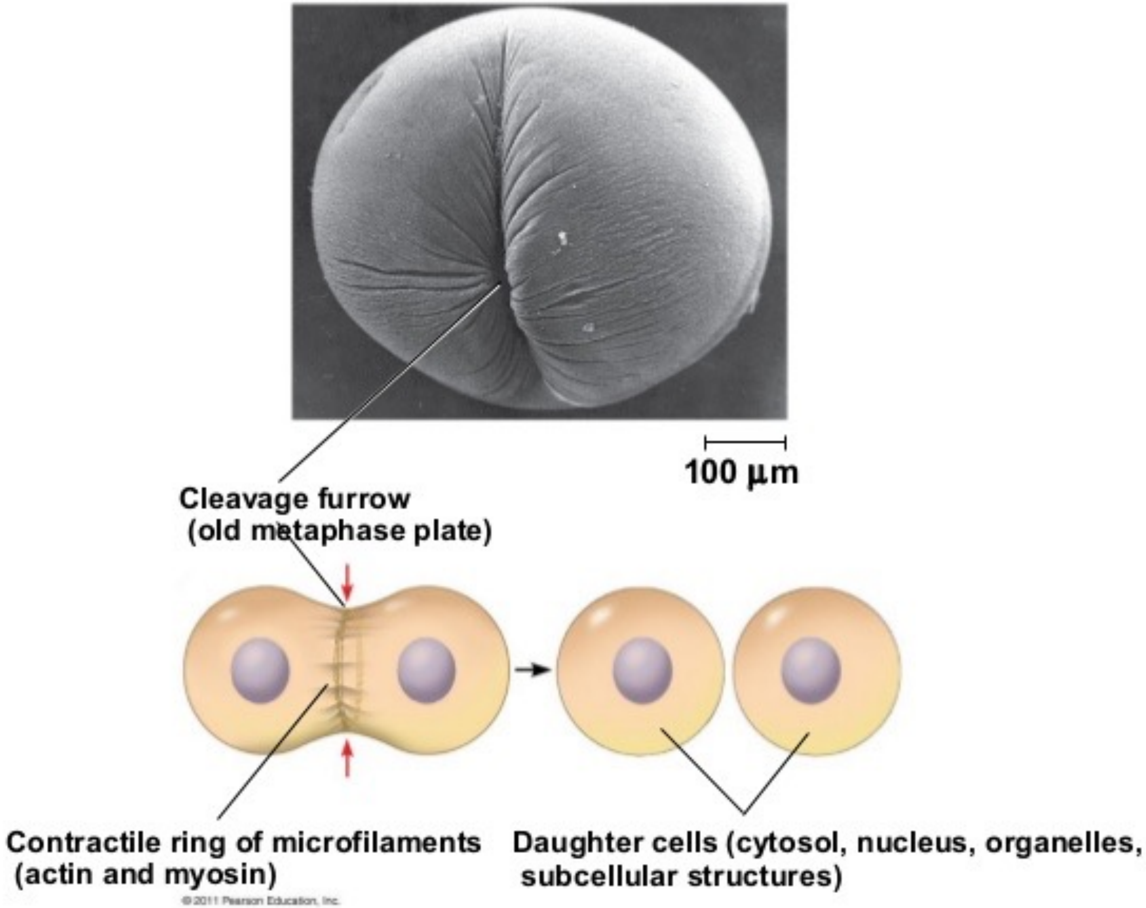
1. The mechanics of cytokinesis
2. How cells position the cleavage furrow in a precise place
3. How cytokinesis is coordinated with chromosome segregation

Part II

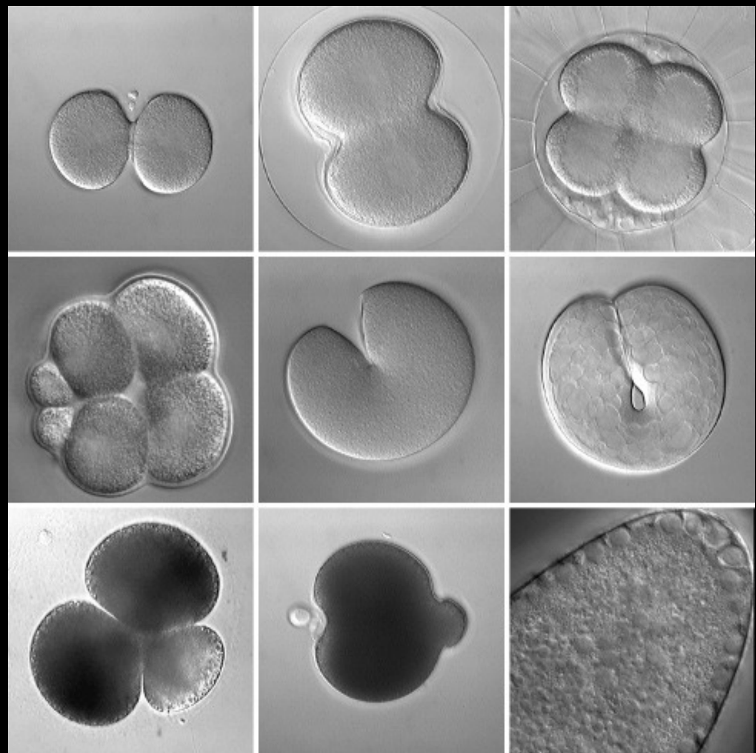
Septin ring dynamics for cytokinesis in budding yeast

The mechanics of cytokinesis

Cytokinesis depends on a cleavage furrow where the membrane invaginates

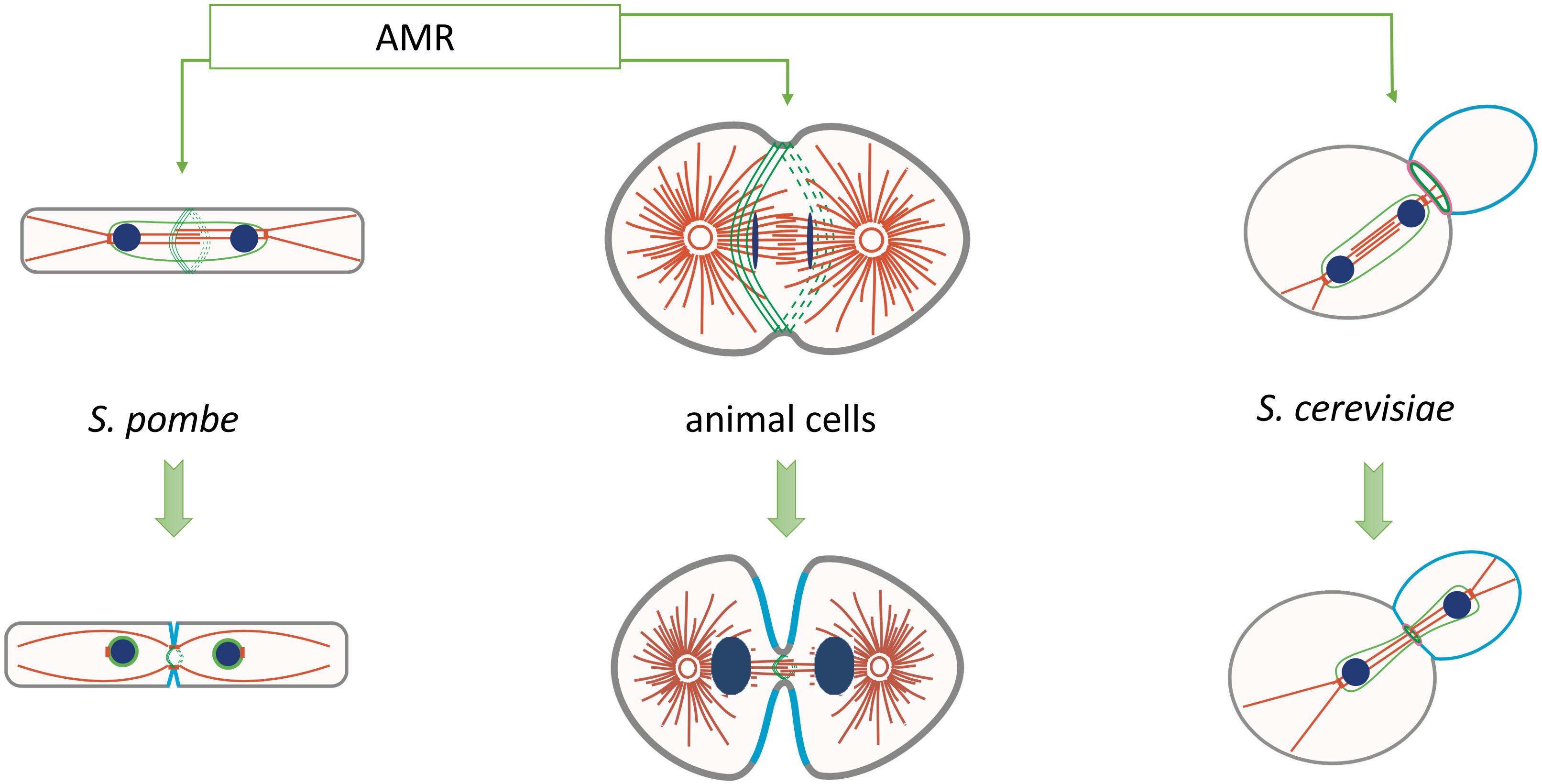


The extraordinary variability in cytokinesis modes

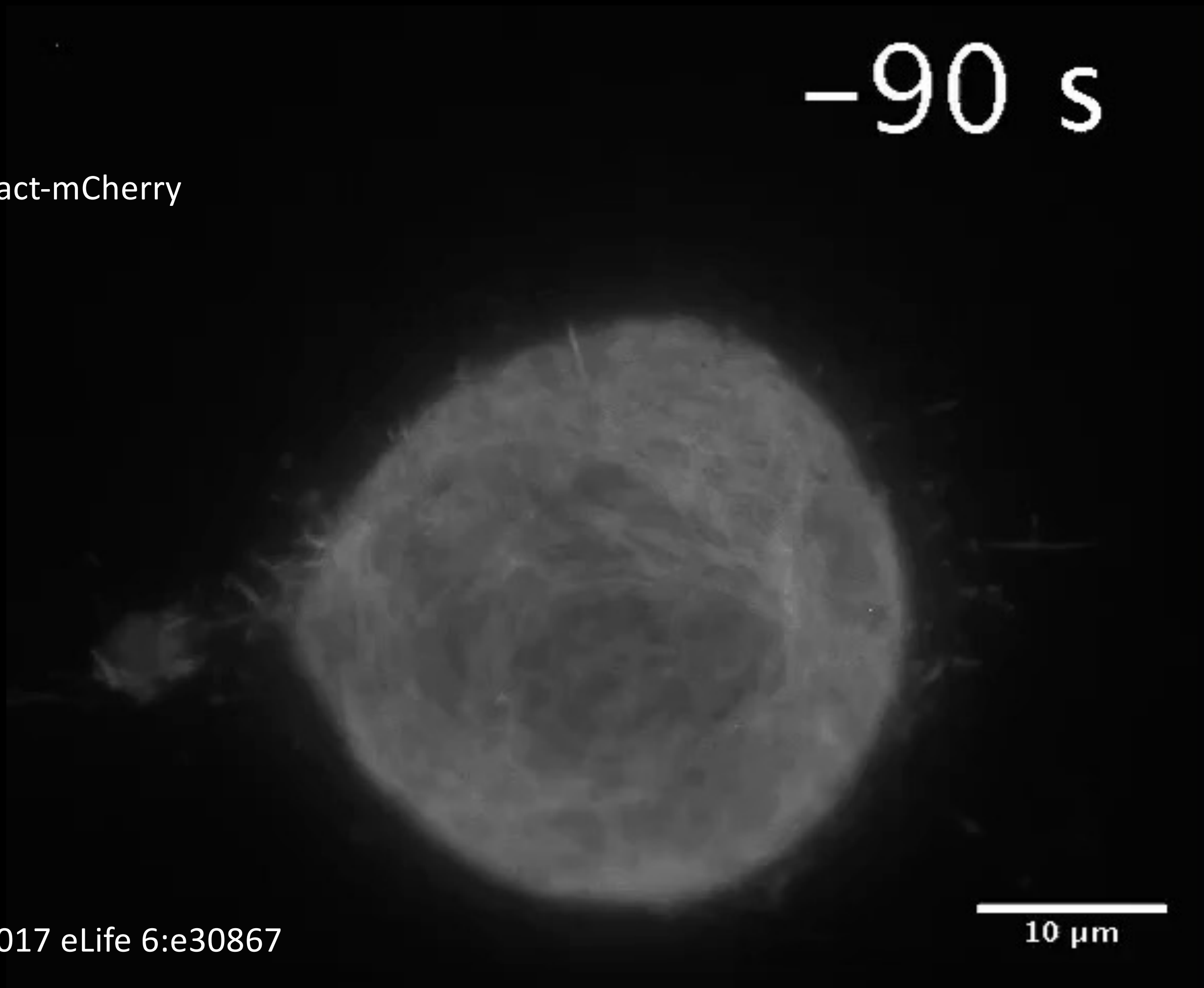


Various embryos during early cleavage. Top row: normal cytokinesis in the nemertean *Cerebratulus* (left), the urchin *S. droebachiensis* (middle), and the ascidian *Corella* (right). Middle row: variations on cytokinesis include unequal cleavage in urchin embryos (left; the urchin *S. purpuratus*), and "unipolar" cytokinesis in the embryonic cells of cnidarians (middle; the jellyfish *Aequorea*) and ctenophores (right; *Pleurobrachia*). Bottom row: cytokinesis-like processes include polar lobe formation in diverse spiralians including scaphopods (left; *Pulsellum*) and bivalves (middle; the clam *Acila*), and somatic budding (right; the wasp *Nasonia*). All panels are DIC images taken from time-lapse movies.

The contractile actomyosin ring (AMR) drives cleavage furrow ingression in many eukaryotes



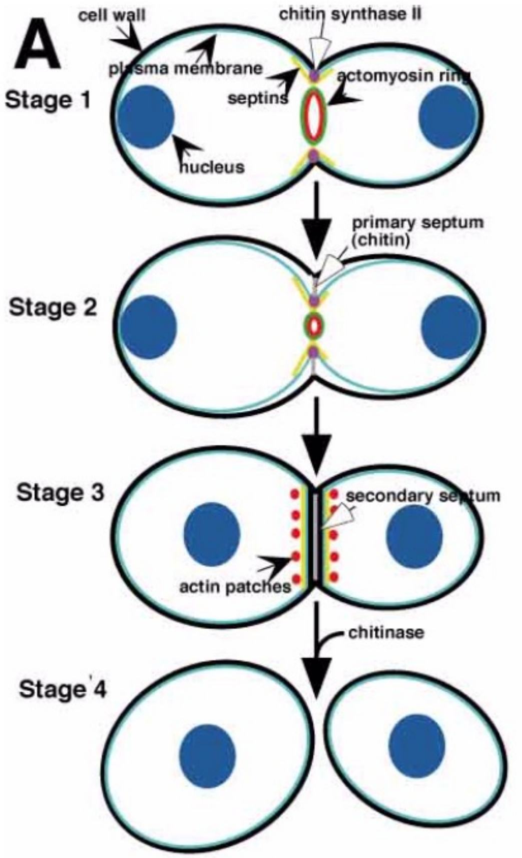
Actomyosin ring contraction in human cells



Lifeact-mCherry

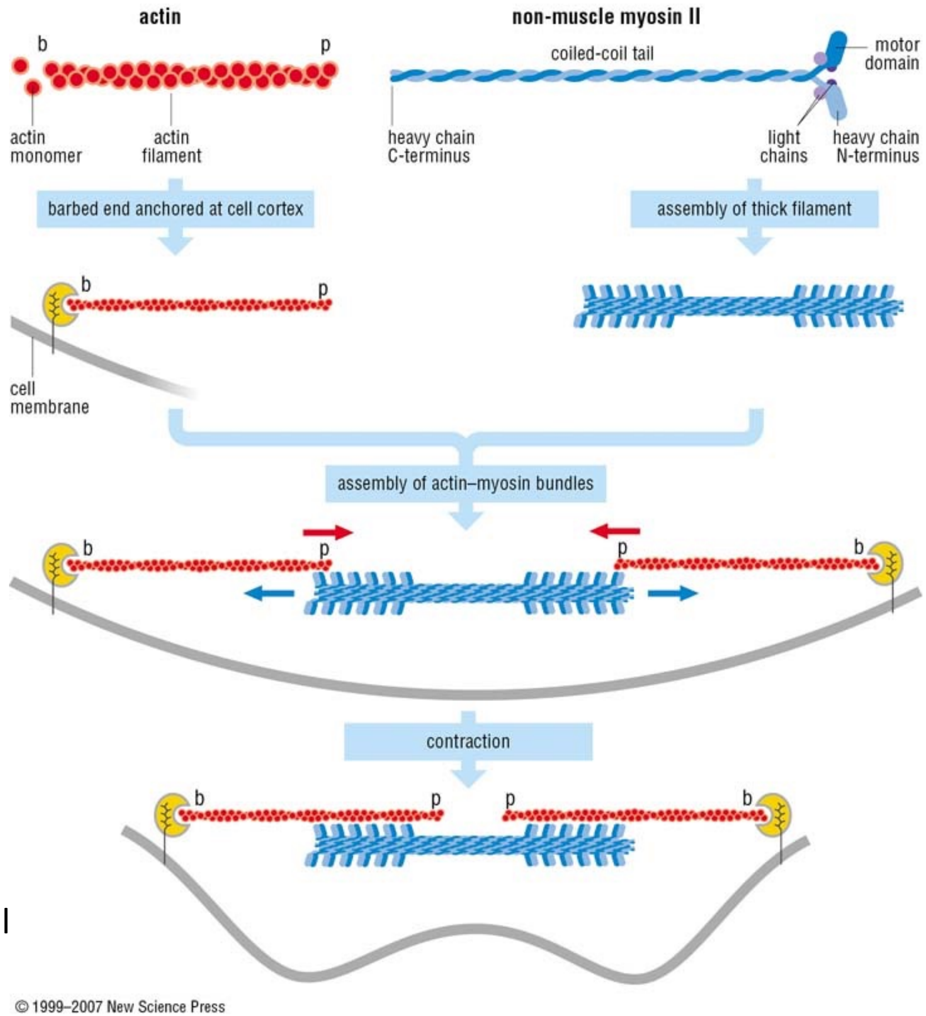
From Spira et al., 2017 eLife 6:e30867

In yeasts AMR contraction is coupled to septum (i.e. cell wall) deposition



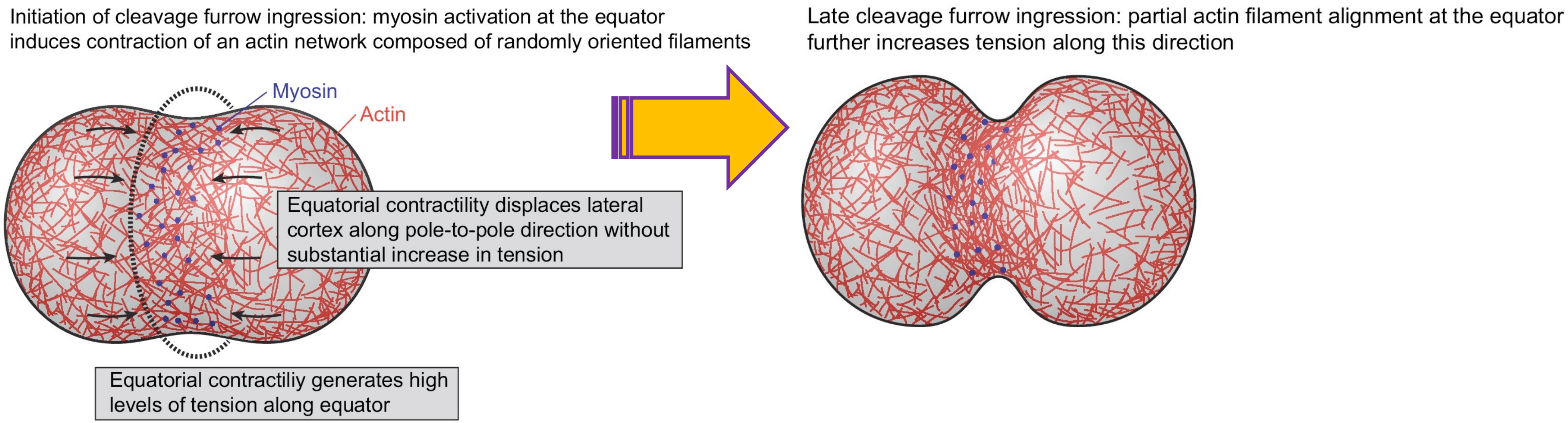
How does the actomyosin ring constricts?

b: barbed end (fast dynamics)
P: pointed end (slow dynamics)



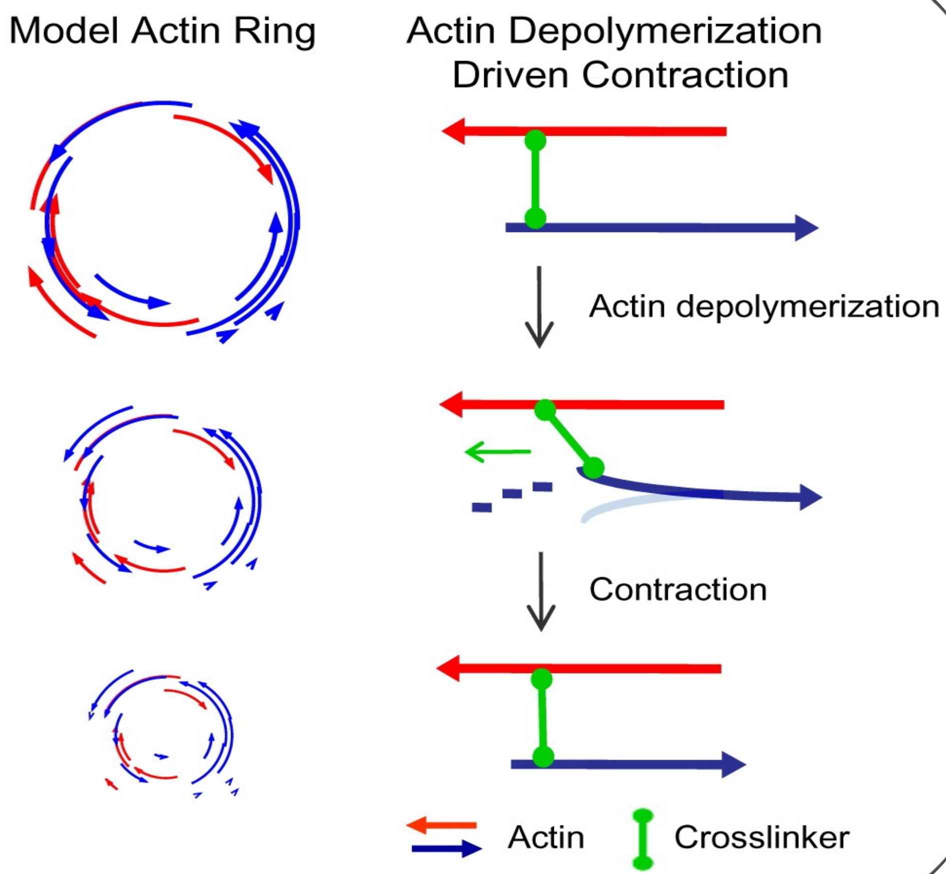
From The Cell Cycle: Principles of Control (D. Morgan)

The actomyosing ring has randomly oriented actin filaments at the beginning of furrow ingression



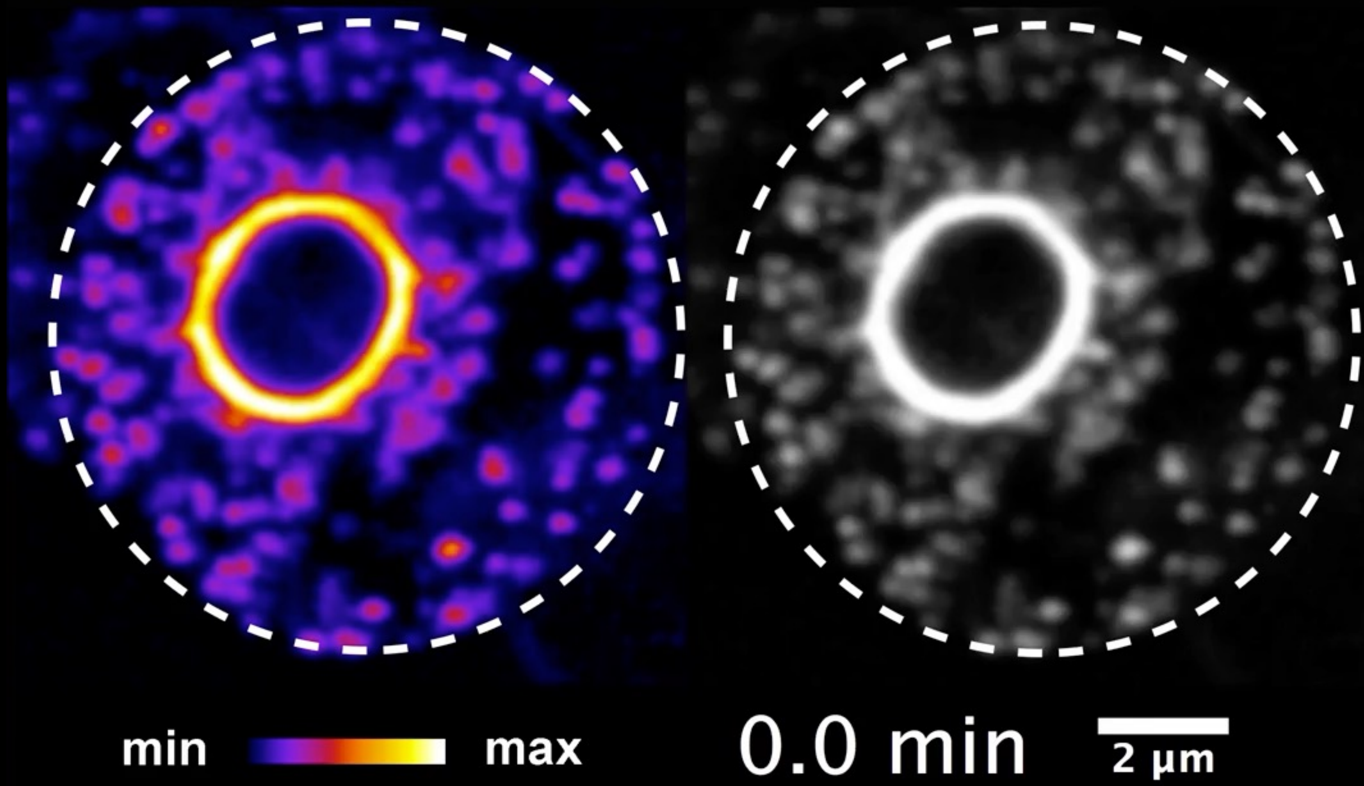
From Spira et al., 2017 eLife 6:e30867

Actin depolymerization contributes to AMR constriction



From Mendes Pinto et al., 2012

In fission yeast AMR contraction is accompanied by ejection of actomyosin bundles containing cytokinesis proteins



From Huang et al., 2016 eLife 5:e21383

Most of the proteins involved in cytokinesis are involved in either organizing or regulating the acto-myosin ring

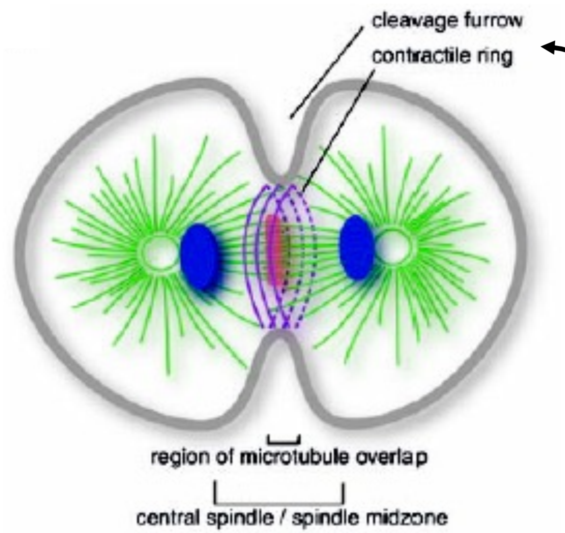
Components of the Contractile Ring

Protein family	Mammals	*Drosophila*	*C. elegans*	*S. cerevisiae*	*S. pombe*
actin	actin	actin	ACT-5	Act1	Act1
myosin II heavy chain	myosin II	Zipper	NMY-2	Myo1	Myo2, Myp2
myosin essential light chain	EMLC	Mlc-c	?	Mlc1	Cdc4
myosin regulatory light chain	RMLC	Spaghetti squash	MLC-4	Mlc2	Rlc1
formin	Dia1	Diaphanous (Dia)	CYK-1	Bni1, Bnr1	Cdc12
profilin	profilin	Chickadee	PFN-1	Pfy1	Cdc3
cofilin	ADF/cofilin	Twinstar	UNC-60A	Cof1	Cof1

Proteins that Organize and Regulate the Contractile Ring

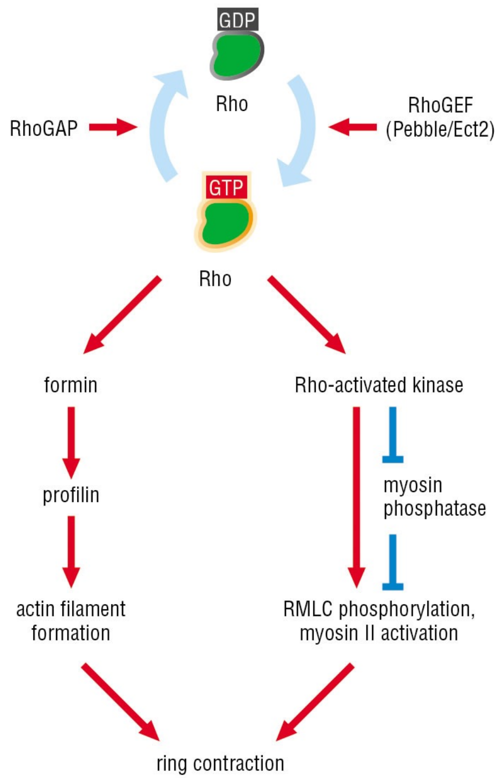
Protein family	Mammals	*Drosophila*	*C. elegans*	*S. cerevisiae*	*S. pombe*
septins	Sept1-10	Peanut, Sep1, 2, 4, 5	UNC-59, UNC-61	Cdc3, 10, 11, 12, Sep7	Spn1-7
anillin	anillin	Scraps	anillin	?	Mid1, Mid2
IQGAP	–	–	–	Iqg1/Cyk1	Rng2
Rho	RhoA	Rho1	RhoA	Rho1	Rho1
RhoGEF	Ect2	Pebble	LET-21	Rom1, Rom2	Gef1, Scd1
Rho-activated kinase	ROCK	dROK	LET-502	–	–
Citron kinase	Citron-K	Citron kinase	Citron kinase	–	–
myosin light-chain kinase	MLCK	MLCK	MLCK	–	–
myosin phosphatase	MYPT	dMYPT	MEL-11	–	–

Assembly and constriction of the actomyosin ring requires the RhoA GTPase

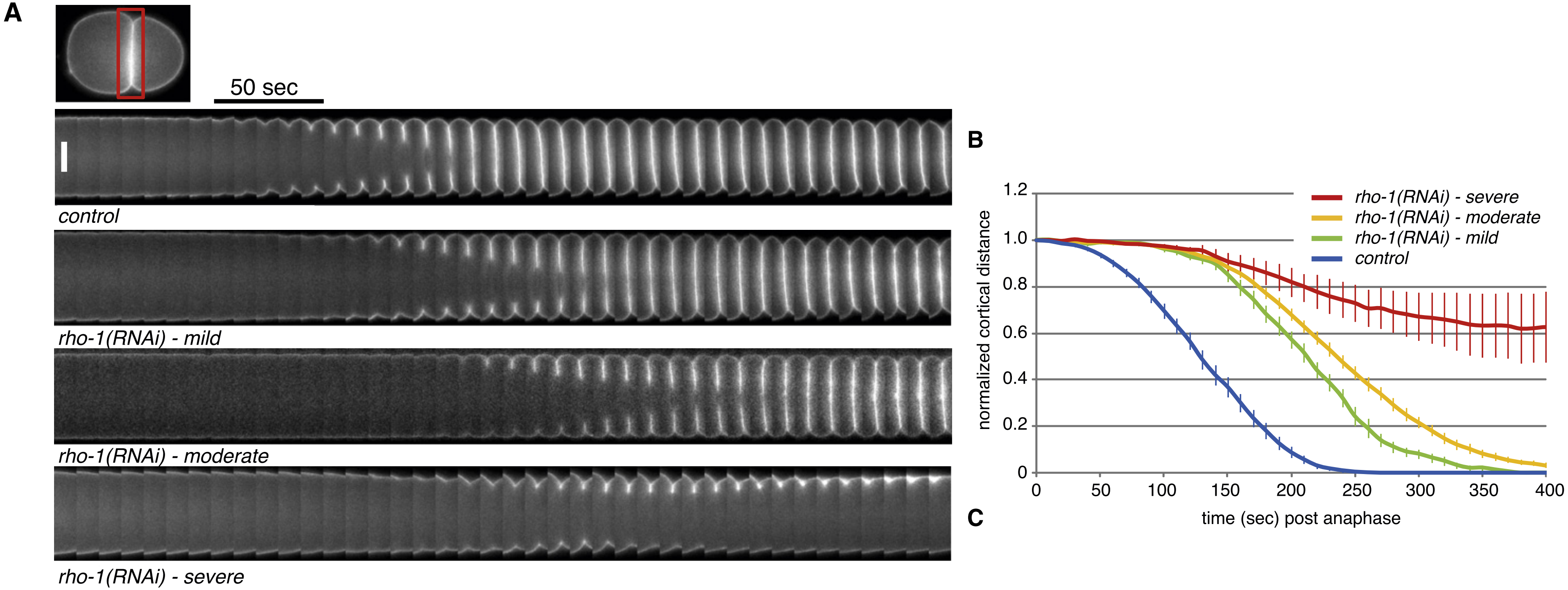


Rho GTPase

From **The Cell Cycle: Principles of Control**
by David O Morgan



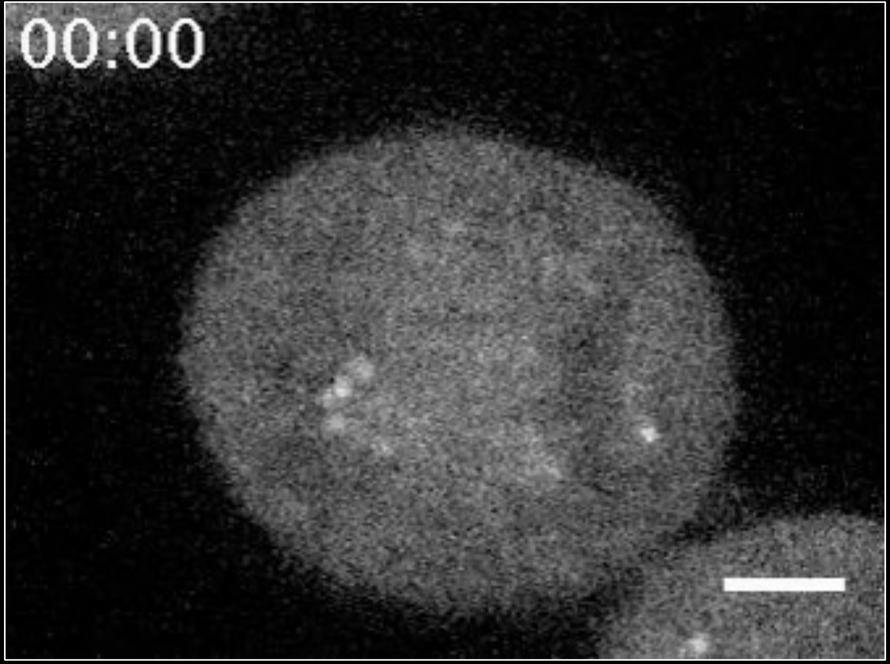
The RhoA GTPase is a dosage-sensitive regulator of cytokinesis



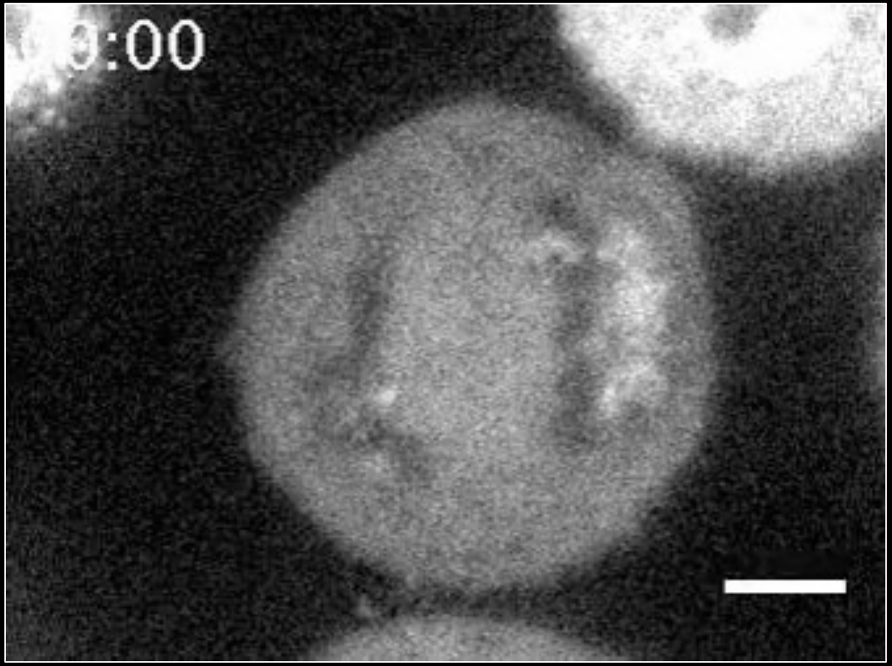
From Loria et al., 2012, Curr. Biol. 22:213

Local activation of RhoA by optogenetics is sufficient to drive cleavage furrow ingression

Activation of RhoA perpendicular to the spindle axis

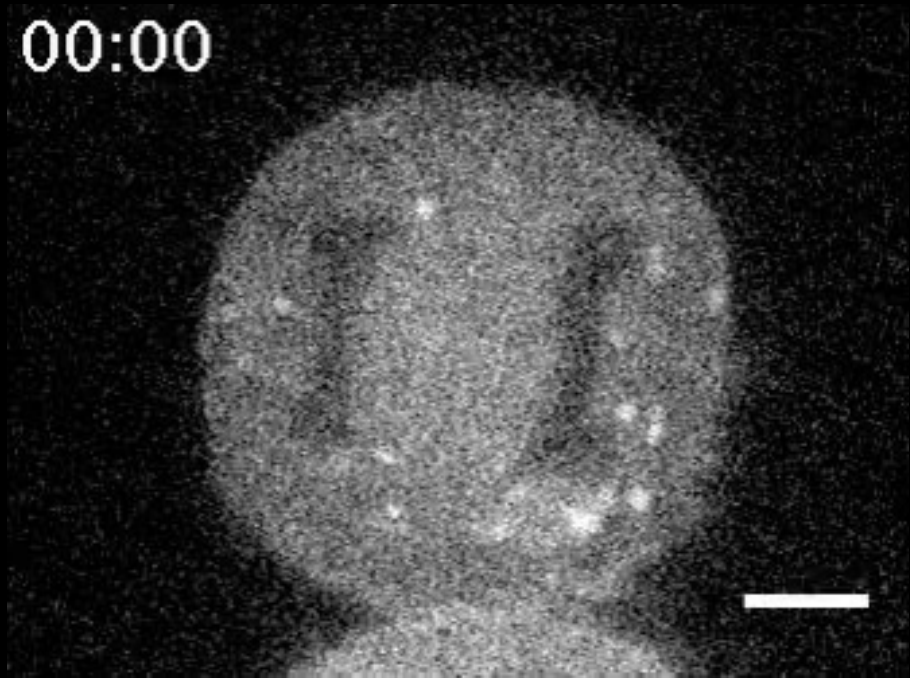


Activation of RhoA along the spindle axis



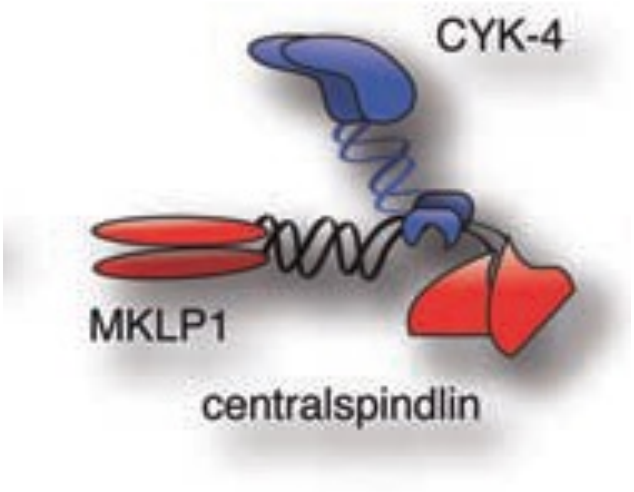
The plasma membrane is homogeneously responsive to RhoA activation!

Artificial activation RhoA in different regions of the membrane can drive ingression of multiple furrows

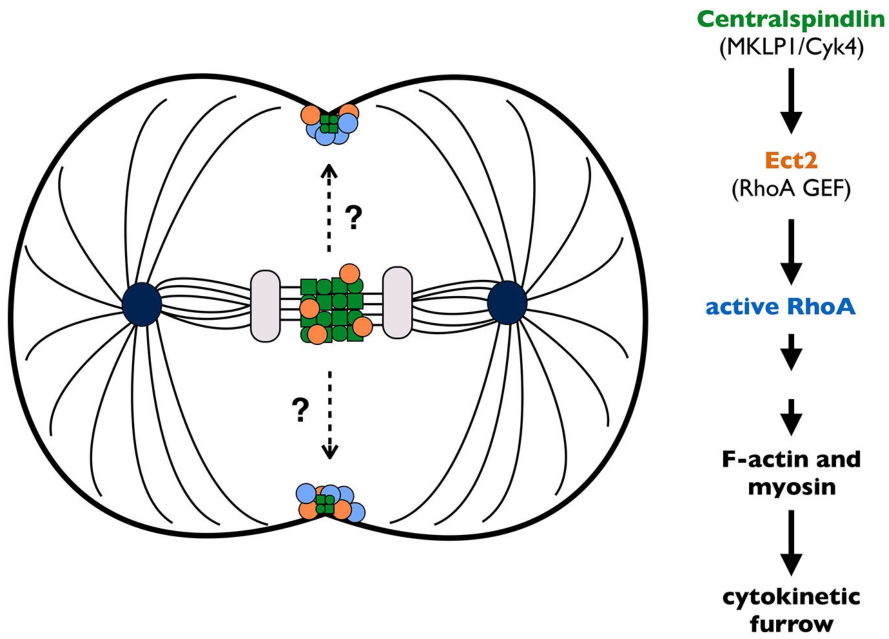


From Wagner and Glotzer, 2016, J. Cell Biol. 213:641

The centralspindlin complex promotes RhoA accumulation and accumulation at the cleavage furrow in metazoans



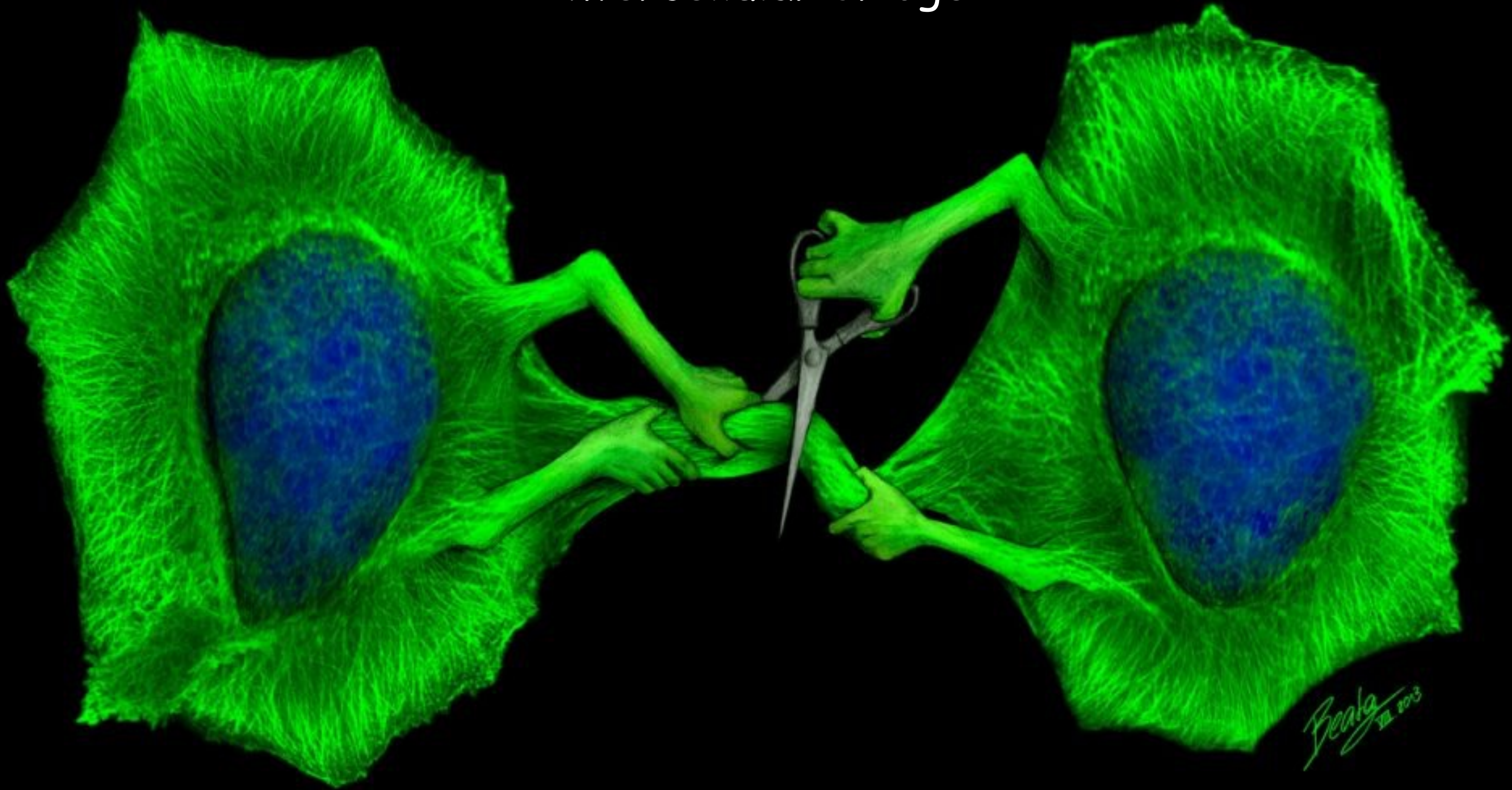
Cyk-4: GAP for Rho-like G-proteins
Mklp1: kinesin



From Glotzer, 2005, Science 307:1735

From Basant and Glotzer, 2005, Curr. Biol. 28:R570

Abscission is the final step of cytokinesis and involves the cut of the intercellular bridge



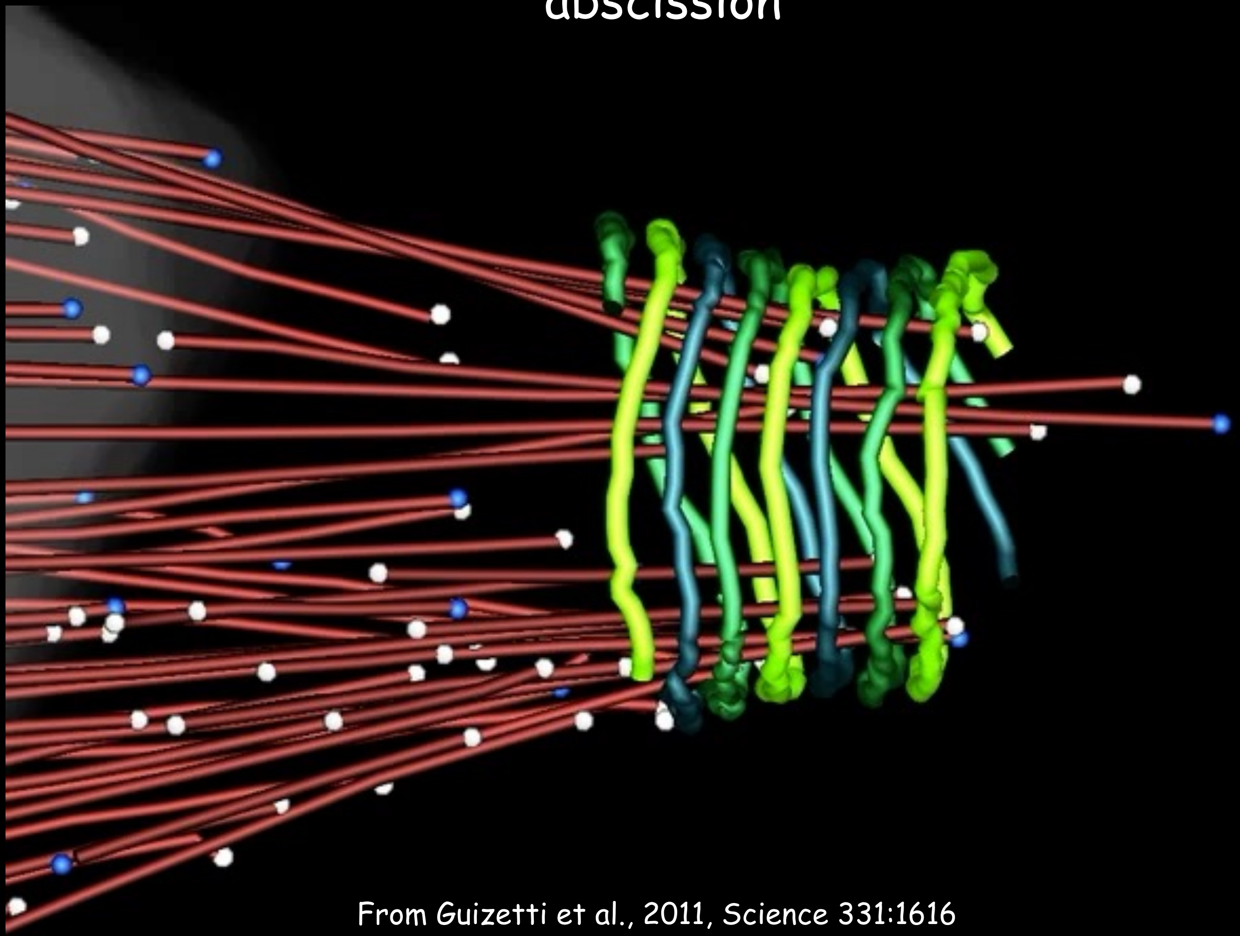
Drawing from Beata Mierzwa

Hela Kyoto cells

Histone2B-mCherry   a-tubulin-EGFP

3 different modes of abscission

The ESCRT-III complex forms spirals that mediate membrane fission during abscission

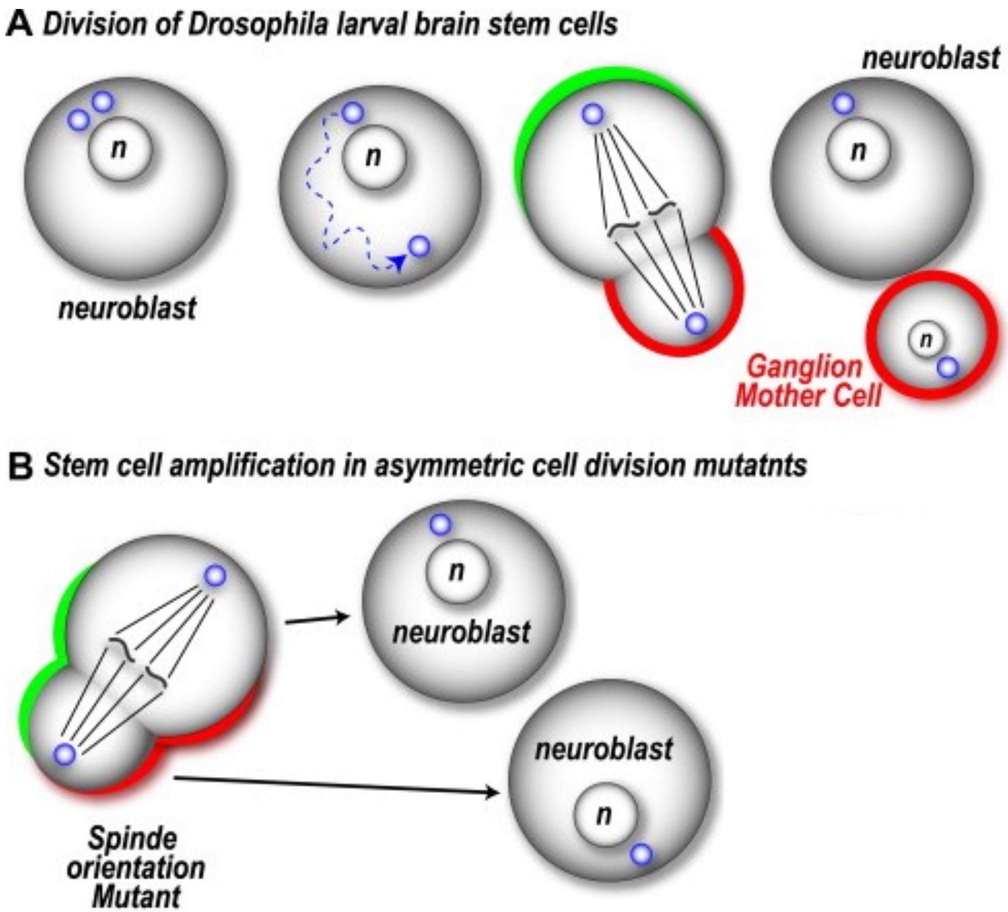


From Guizetti et al., 2011, Science 331:1616

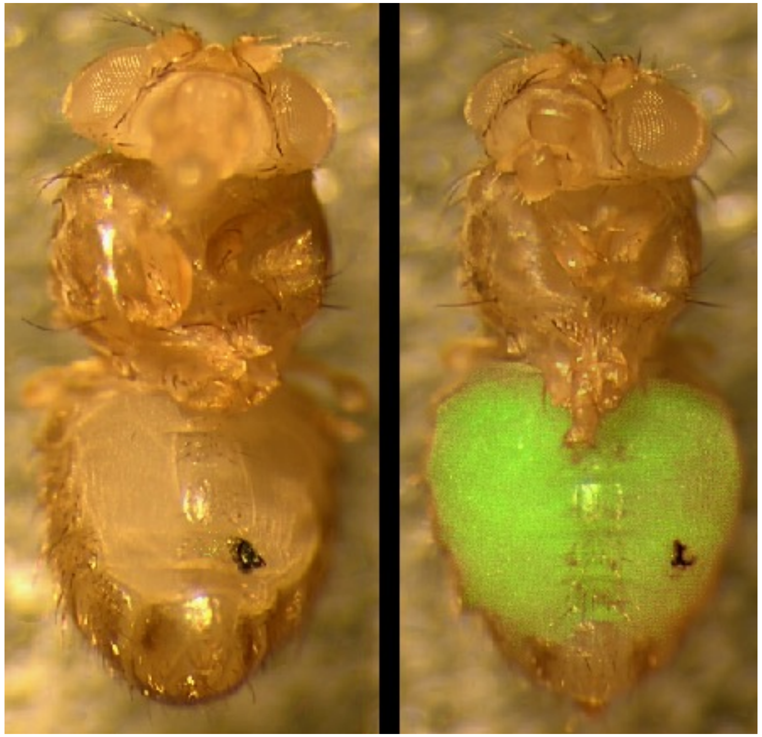
Positioning of the division site

Asymmetric distribution of cell fate determinants in *C. elegans* embryos

Cytokinesis following spindle misorientation leads to aberrant distribution of polarity factors, thereby affecting cell fate



Perturbing asymmetric cell division causes tumor growth in *Drosophila*



Caussinus and Gonzalez, 2005, Nature Genetics 37:1125

What signals determine the position of the cleavage furrow?

Nature 382, 466 - 468 (01 August 1996); doi:10.1038/382466a0

'Anaphase' and cytokinesis in the absence of chromosomes

Dahong Zhang & R. Bruce Nicklas

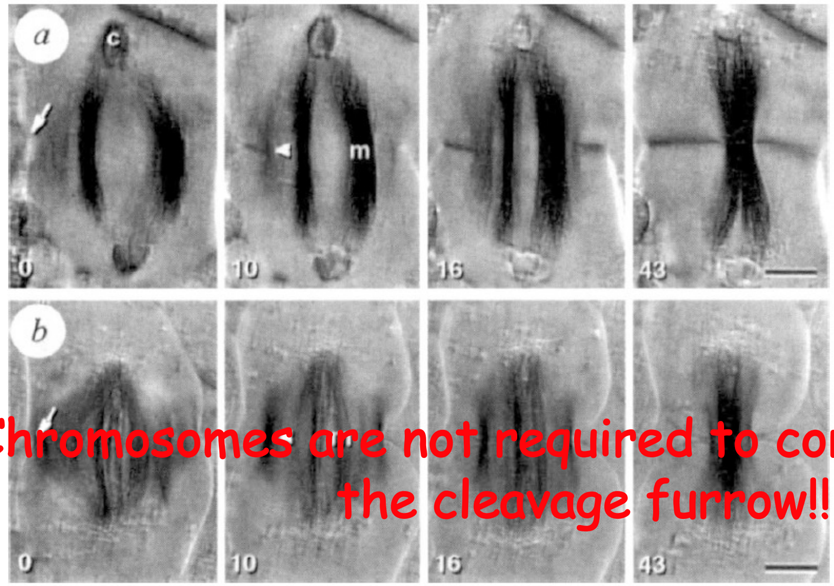


FIG. 3 Cytokinesis in the presence (*a*) and the absence (*b*) of chromosomes. A cleavage furrow (arrows) appeared midway between spindle poles regardless of the presence (*a*) or the absence (*b*) of chromosomes. Interzone microtubules (arrow heads) and mitochondria (m) were bundled together while the furrow gradually moved inward (10 min onwards). In the absence of chromosomes, the nuclear envelope did not reform. The final separation of daughter cells is not shown. Scale bars, 10 μm.

Grasshopper spermatocytes

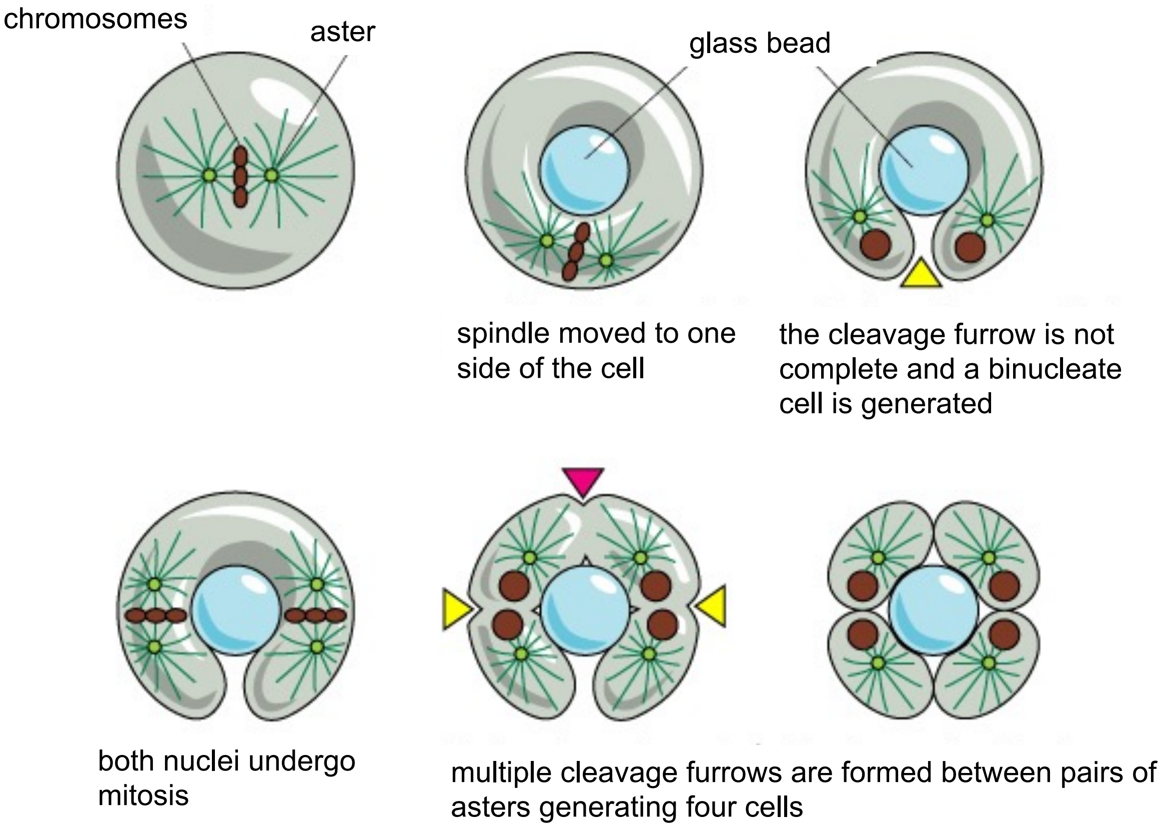
Chromosomes are not required to correctly position the cleavage furrow!!

The mitotic spindle is required to define the correct cleavage plane

S. pombe cell after microtubule depolymerisation (Rlc1-GFP)

From Pardo and Nurse, 2003

Cleavage furrow positioning depends on spindle position

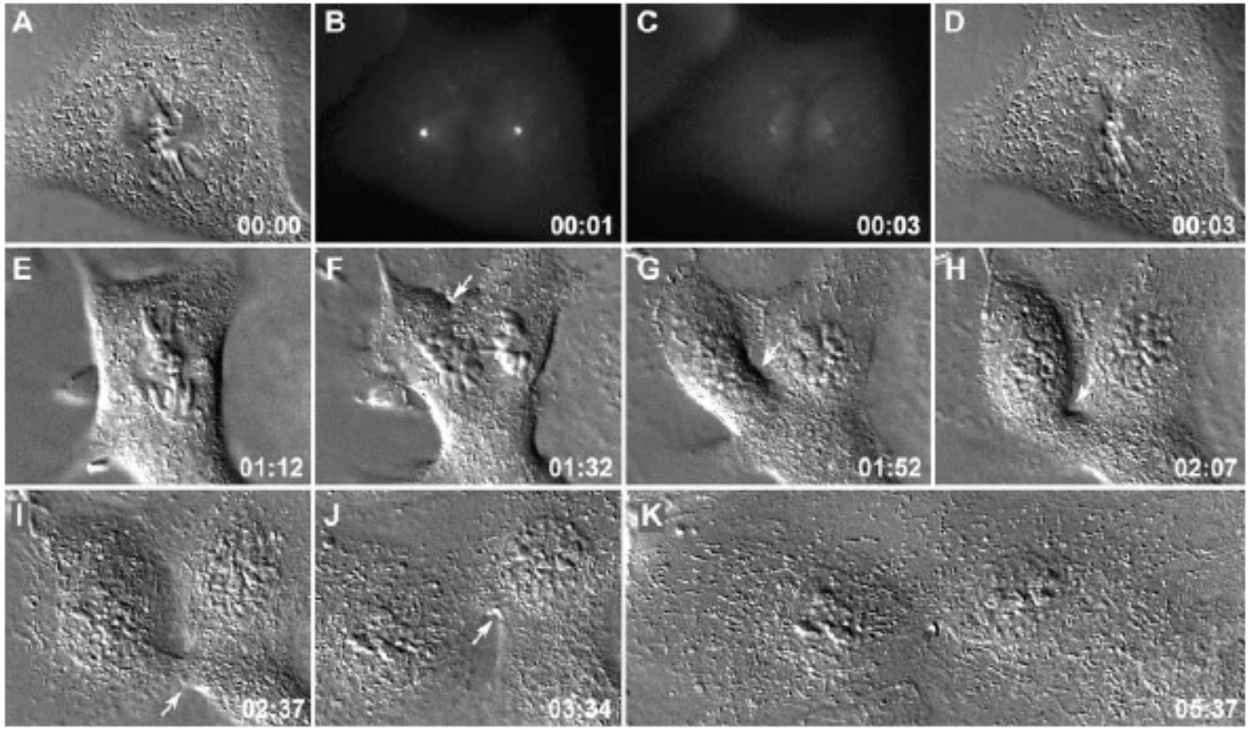


Ray Rappaport
(marine invertebrate embryos)

Centrosomes Enhance the Fidelity of Cytokinesis in Vertebrates and Are Required for Cell Cycle Progression✪

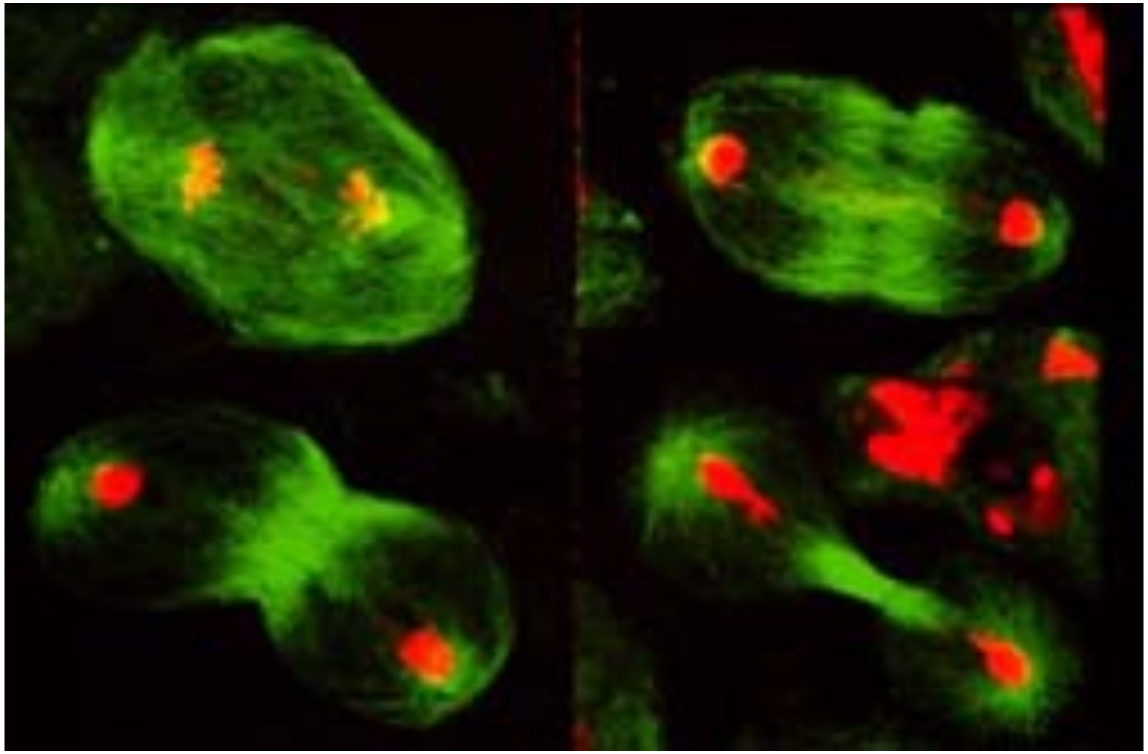
Alexey Khodjakov and Conly L. Rieder

Laboratory of Cell Regulation, Division of Molecular Medicine, Wadsworth Center, New York State Department of Health, Albany, New York 12201; and Department of Biomedical Sciences, State University of New York, Albany, New York 12222
J. Cell Biol. 2001, 153:237



Laser ablation of centrosomes in Ptk1 cells

In *Drosophila* cytokinesis is strictly dependent on the central spindle and the midbody

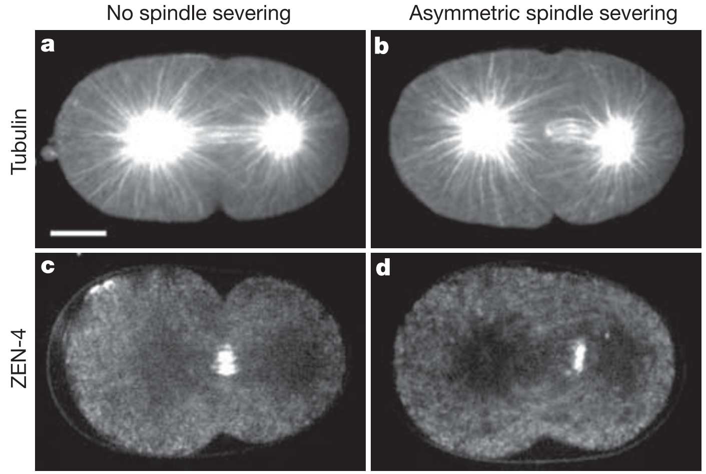


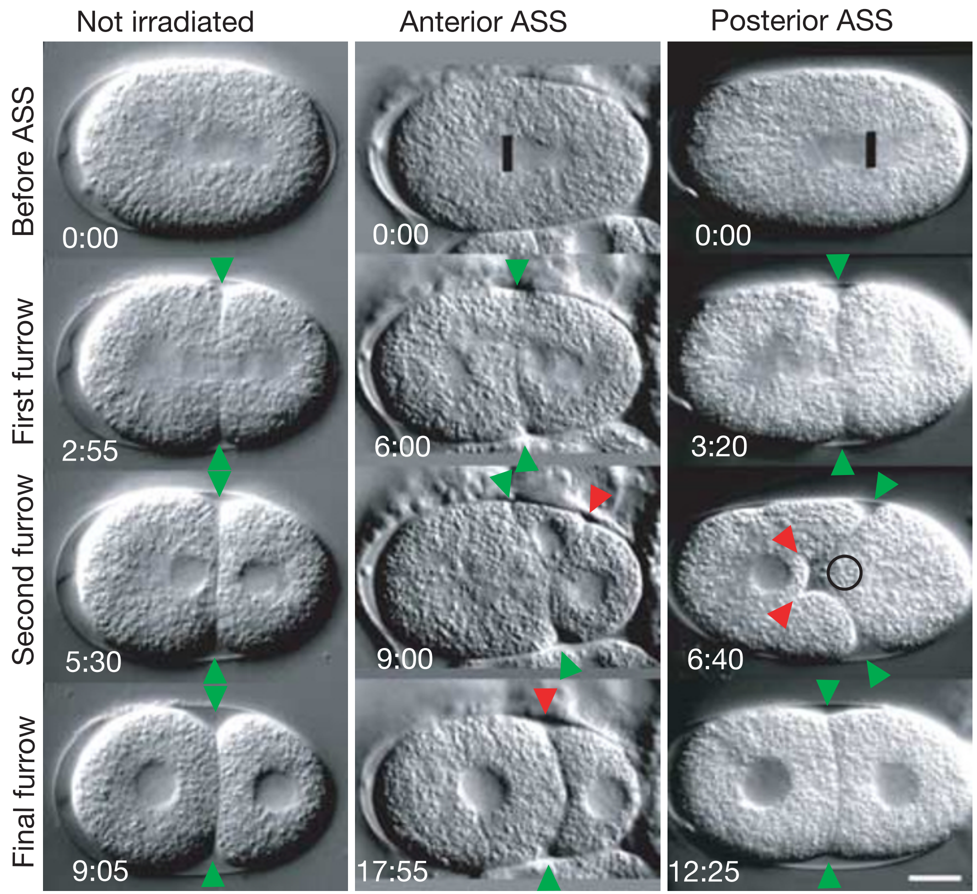
Both the spindle poles and the spindle midzone contribute to cleavage furrow positioning

A cytokinesis furrow is positioned by two consecutive signals

Henrik Bringmann[1] & Anthony A Hyman[1]

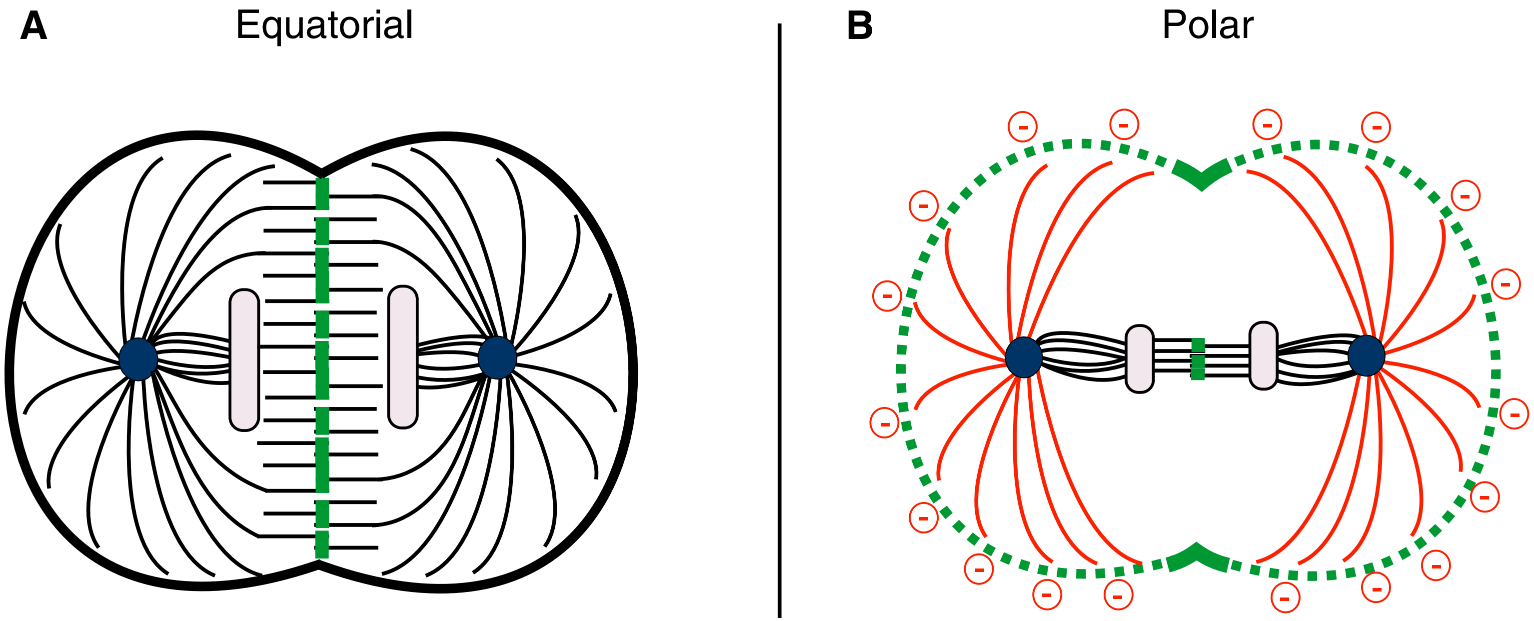
Nature (2005) 436:731





ASS: asymmetric spindle severing

The spindle has a crucial function in furrow positioning: the central spindle stimulates RhoA accumulation at the medial cortex, while astral microtubules inhibit RhoA accumulation at the cell poles

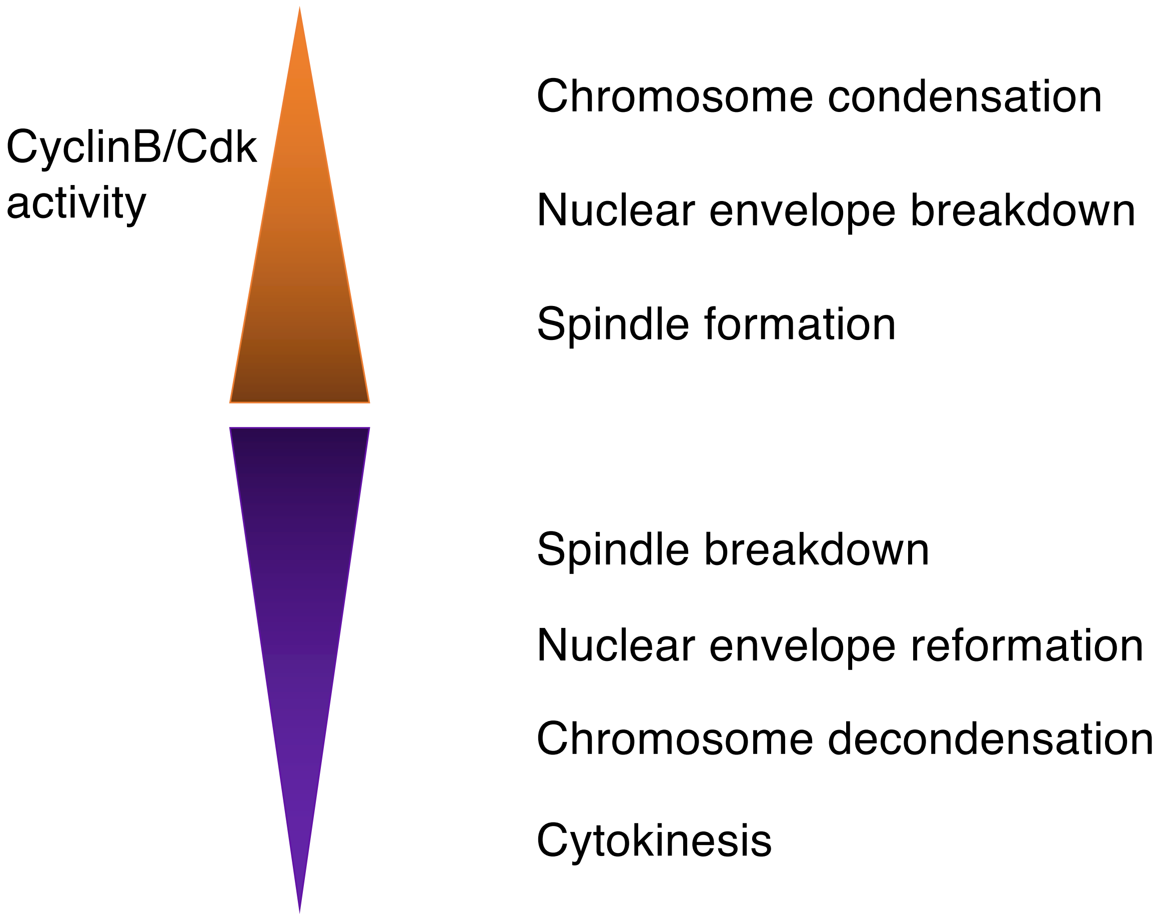


From Basant and Glotzer, 2005, Curr. Biol. 28:R570

Coupling cytokinesis to chromosome segregation

How do eukaryotic cells avoid that cytokinesis occurs before chromosome segregation?

Mitotic events controlled by CyclinB/Cdk activity



The presence of chromatin in the intercellular bridge prevents abscission through a checkpoint that depends on the Aurora B kinase

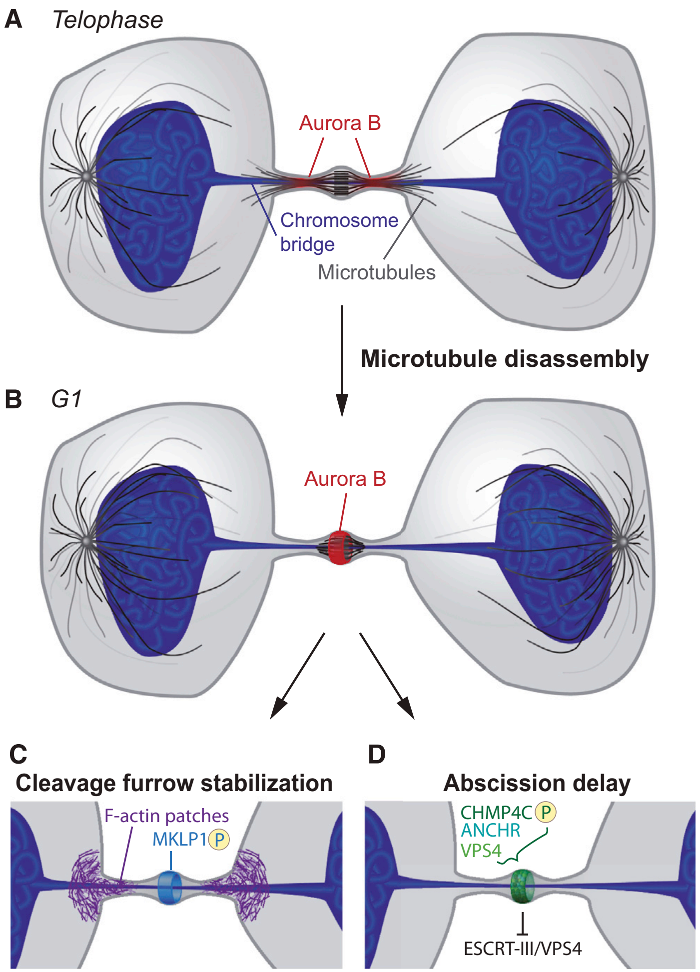


Figure 6. Aurora B-Mediated Abscission Delay

The presence of a chromosome bridge in the intercellular bridge sustains Aurora B activity to stabilize the ingressed cleavage furrow and to delay abscission.

(A) During telophase, active Aurora B localizes adjacent to the midbody, as in normally segregating cells.

(B) Midbody-associated microtubules disassemble at a time similar to normally segregating cells, resulting in Aurora B relocalization to the central midbody (Steigemann et al., 2009).

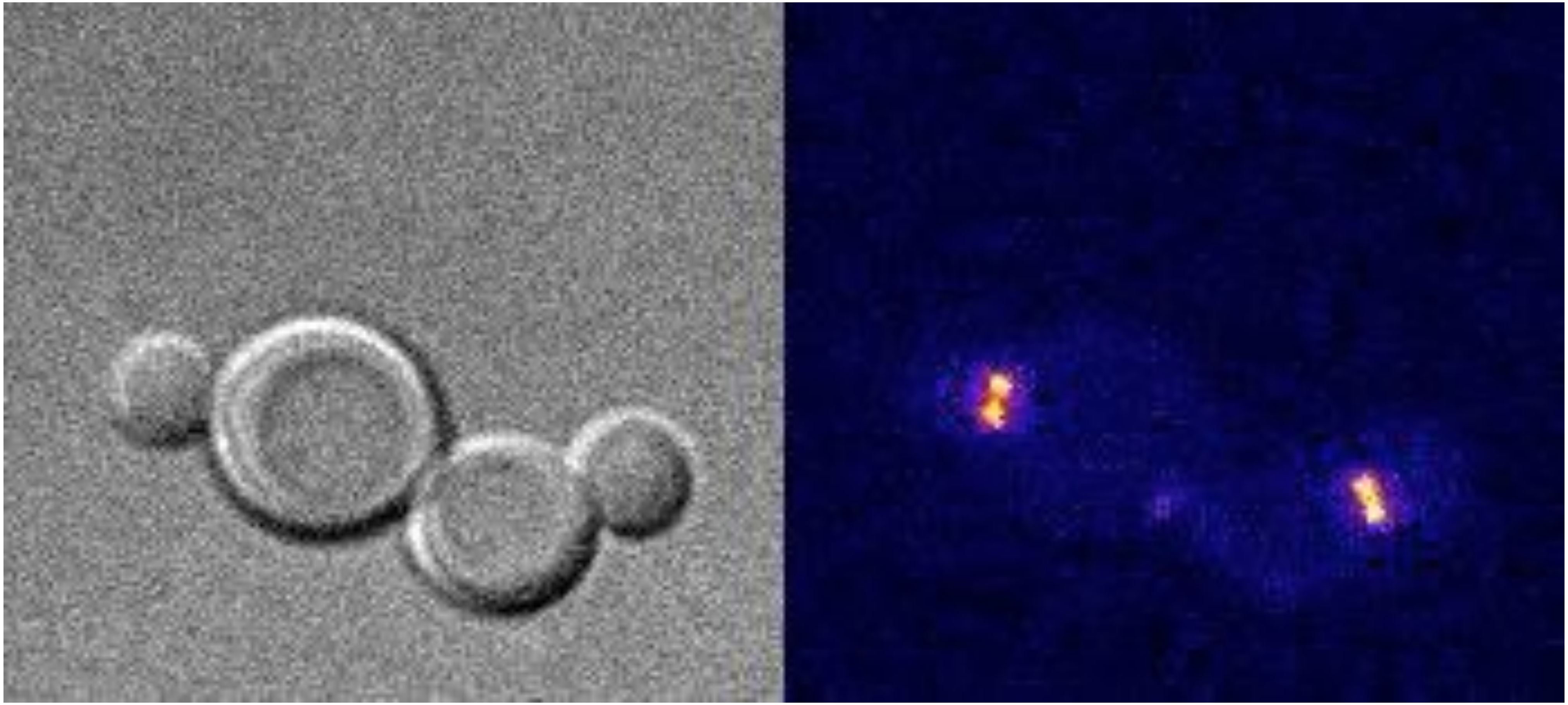
(C) Accumulation of large F-actin patches on both sides of the intercellular bridge and phosphorylation of MKLP1 may contribute to the maintenance of an ingressed cleavage furrow in cells with persistent chromosome bridges (Steigemann et al., 2009).

(D) Aurora B regulates the abscission machinery by phosphorylating ESCRT-III subunit CHMP4C (Capalbo et al., 2012; Carlton et al., 2012). A protein complex formed between ANCHR, CHMP4C, and VPS4 at the central midbody inhibits ESCRT-III and VPS4 (Thoresen et al., 2014).

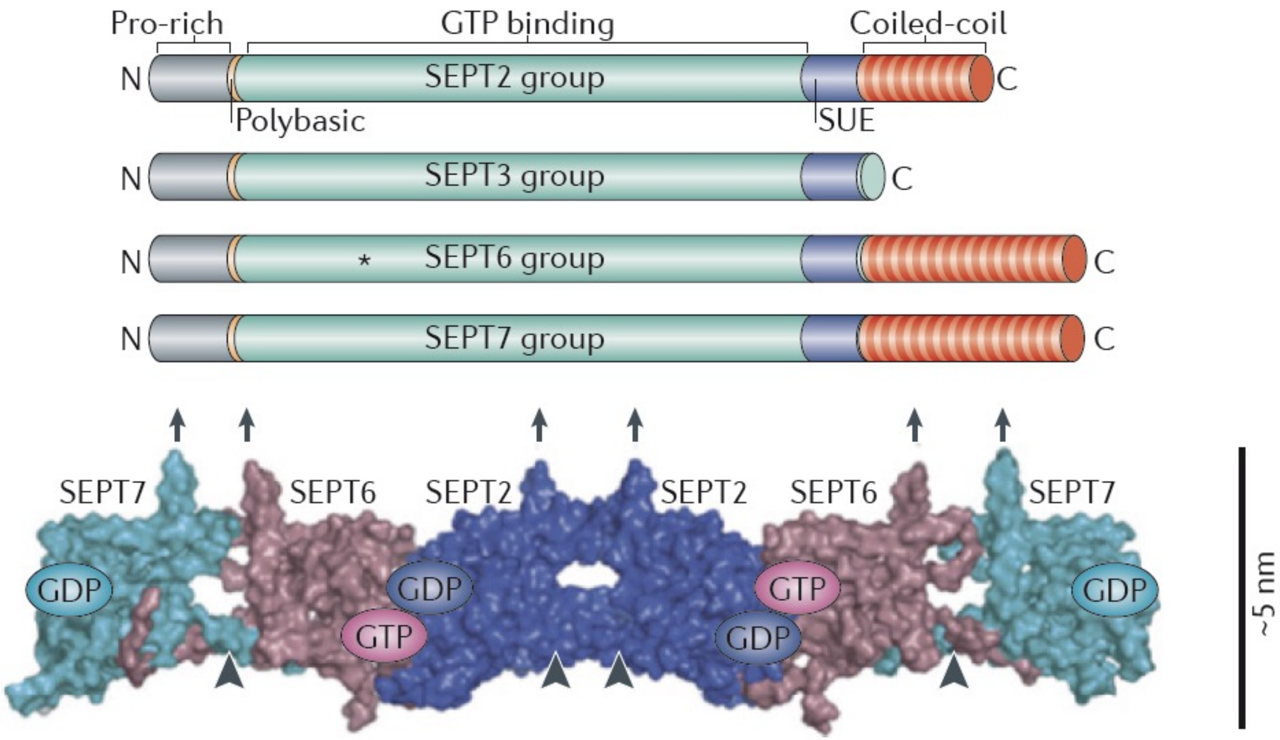
From Mierzwa and Gerlich, 2014, Dev. Cell 31:525

Part II
Septin dynamics for cytokinesis in budding yeast

Septin dynamics during budding yeast cell division

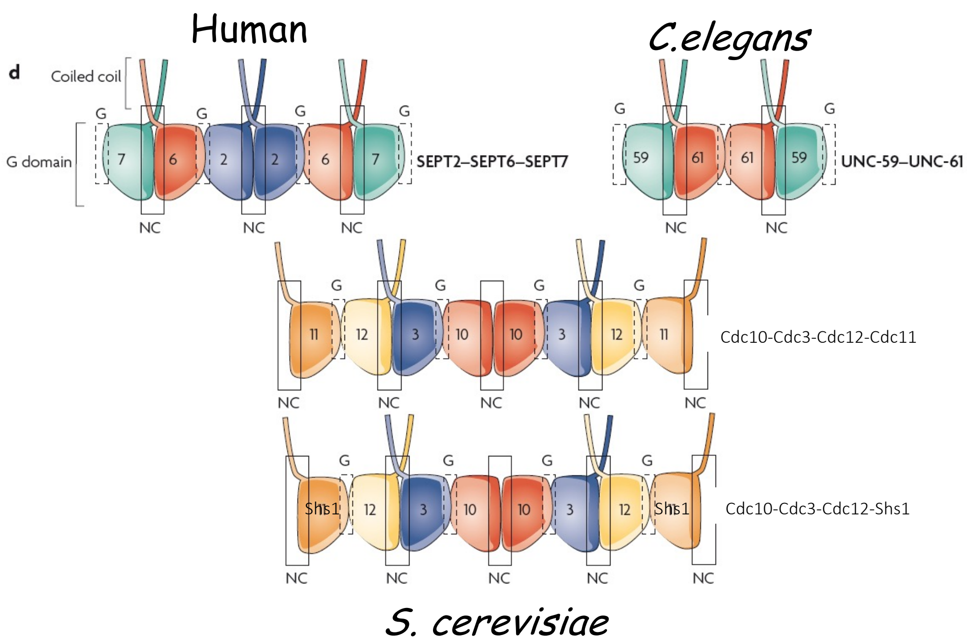


Septin domain organisation

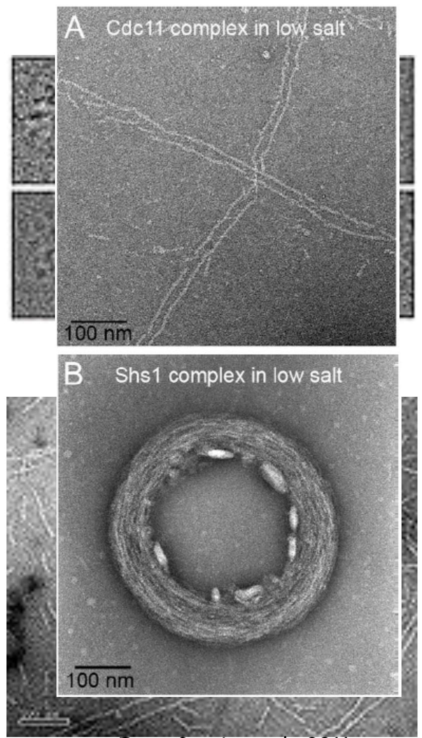


From Mostowy and Cossart, 2012

Structure of septin complexes

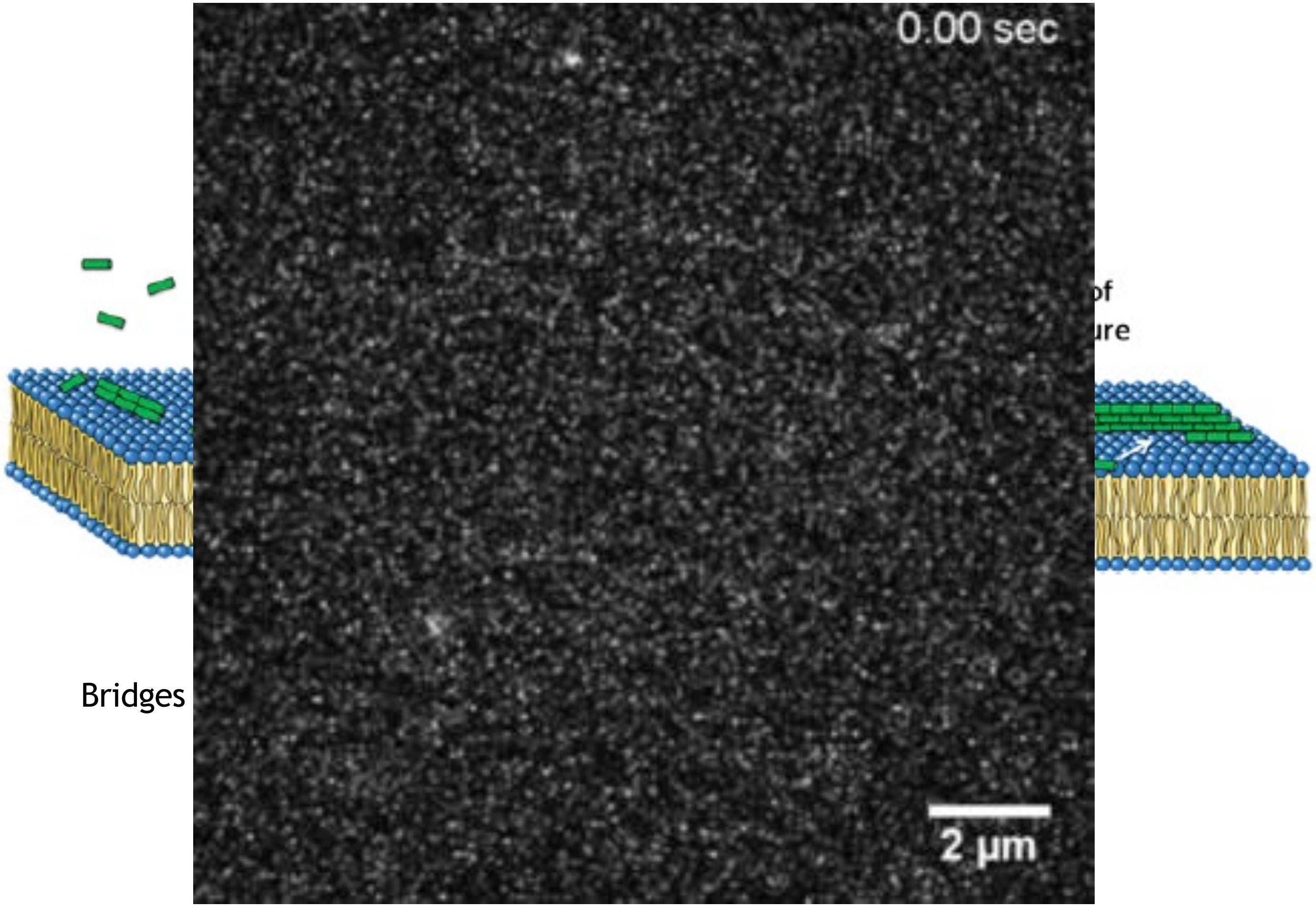


Adapted from Weireich et al., 2008

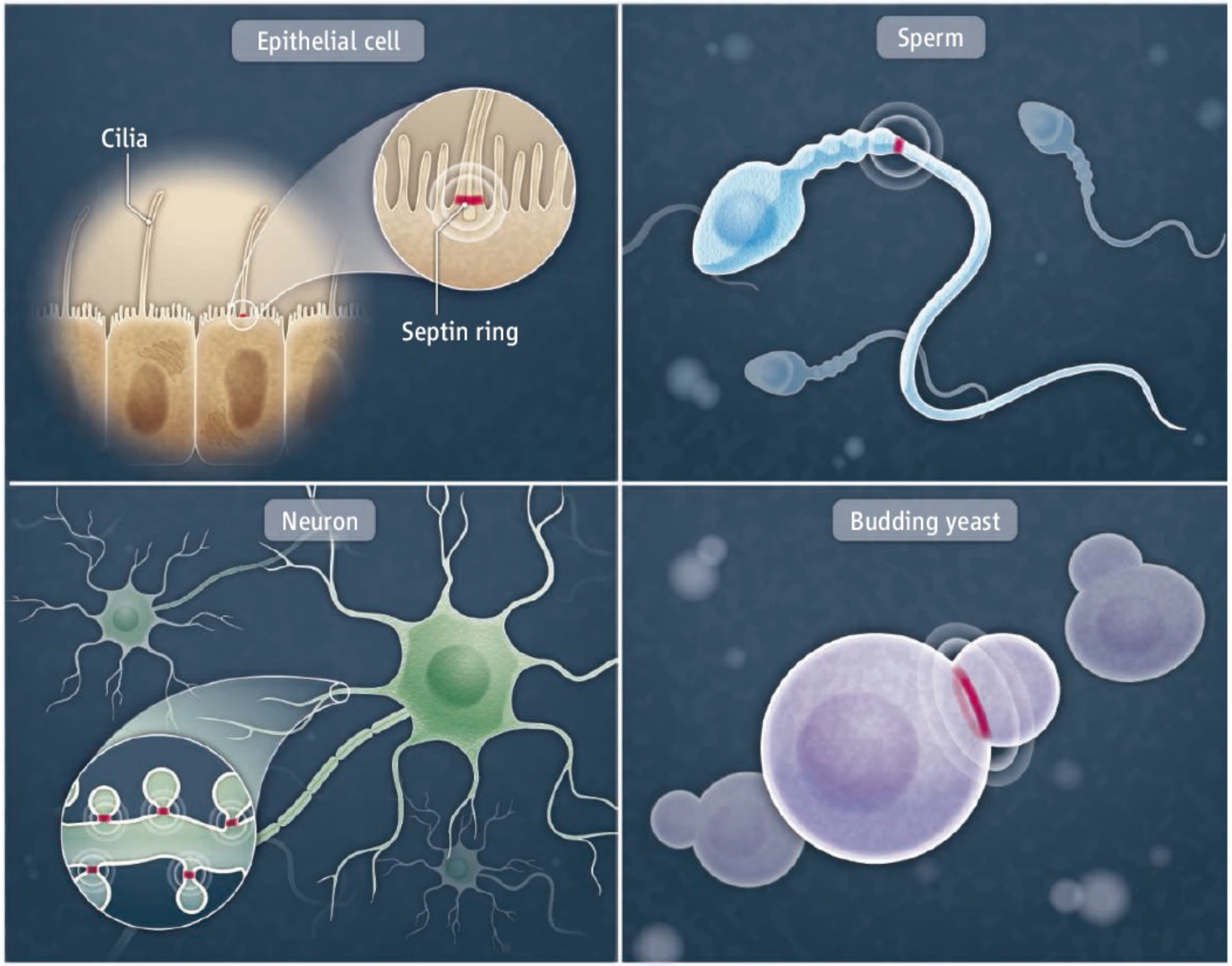


From Garcia et al., 2011
From McMurray and Thorner., 2008

Septin filaments assemble by self-annealing of septin rods

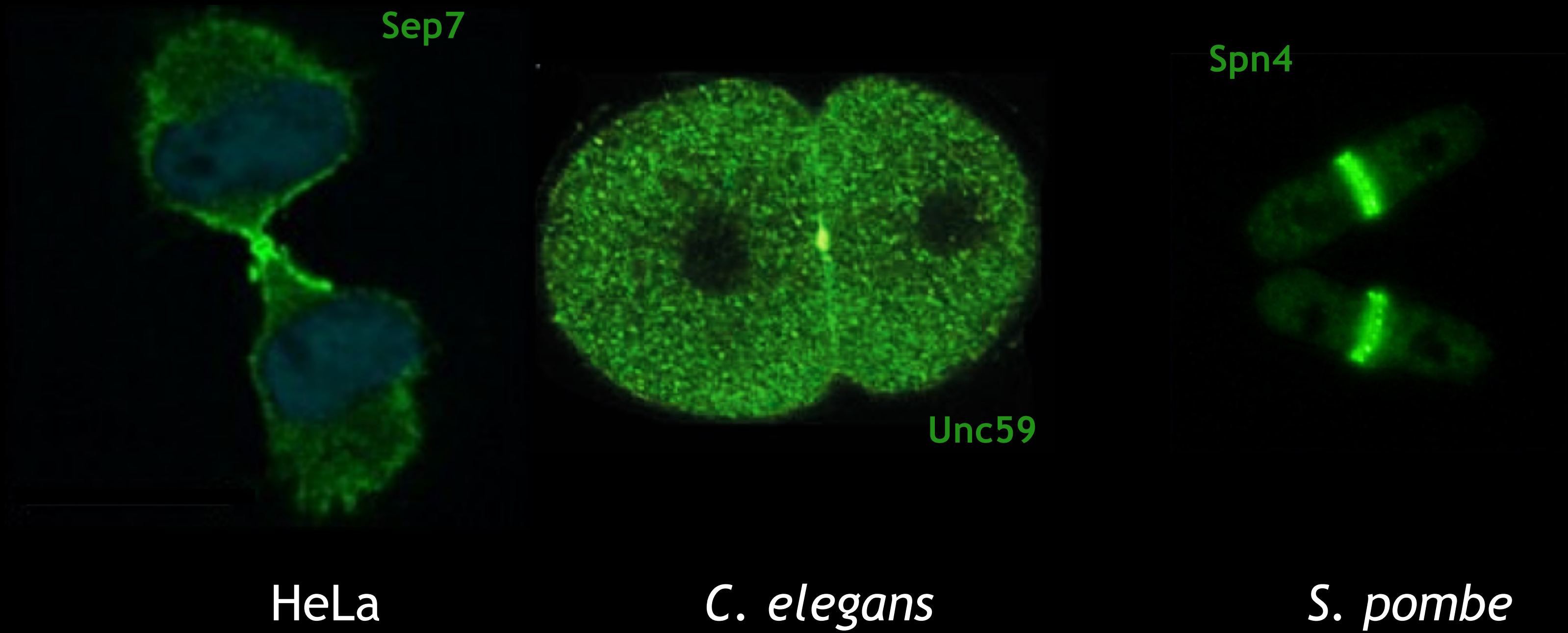


Distribution of septin rings

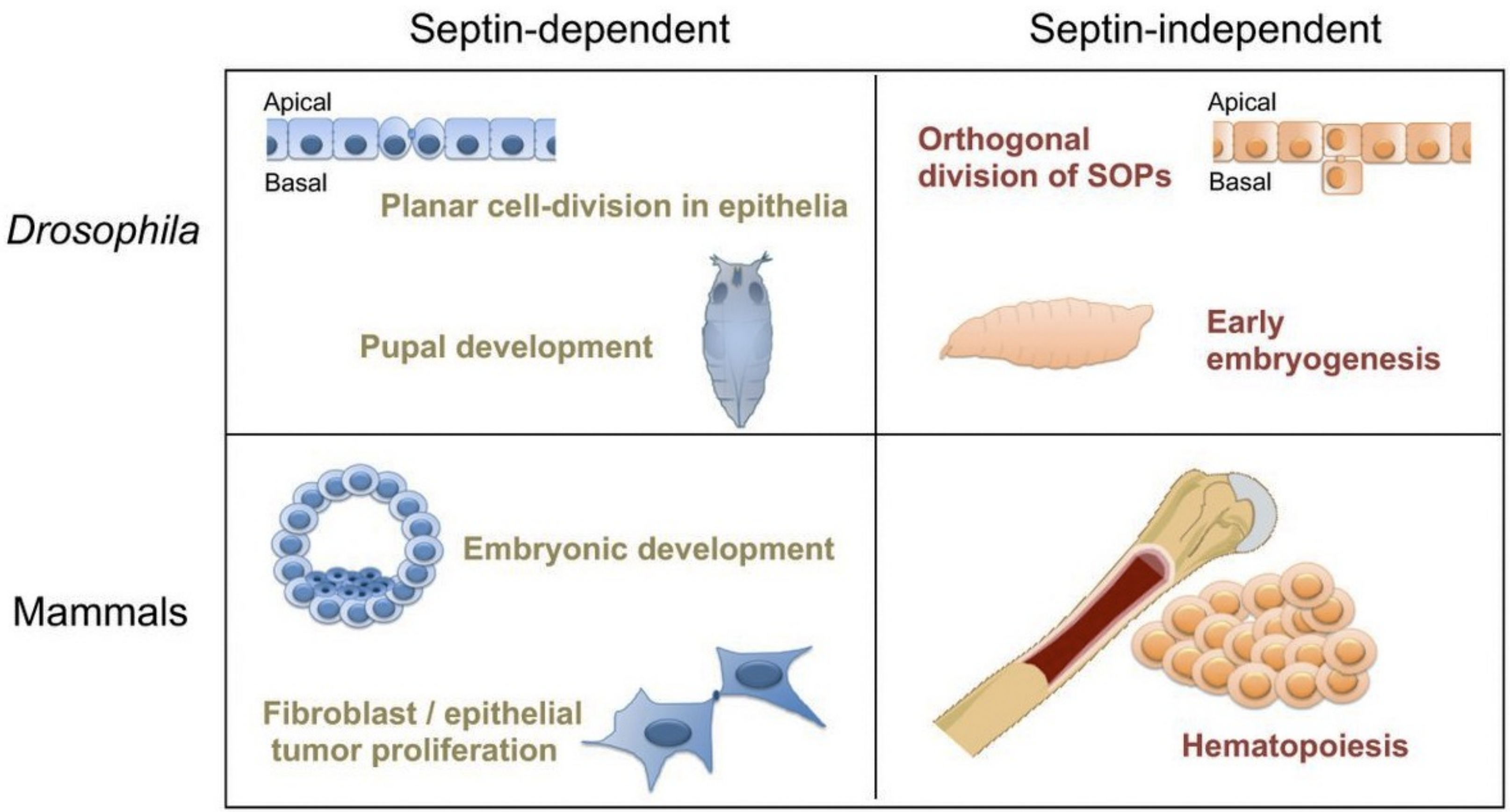


From Barral, 2010

Septins often localize at the cell division site



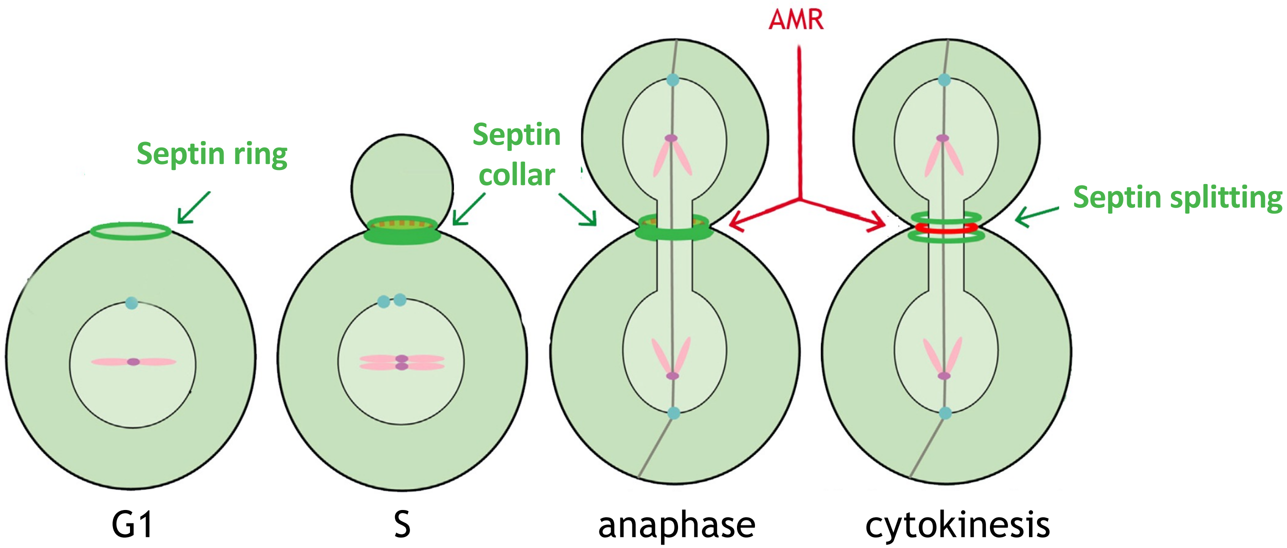
The need for septins during cytokinesis is variable



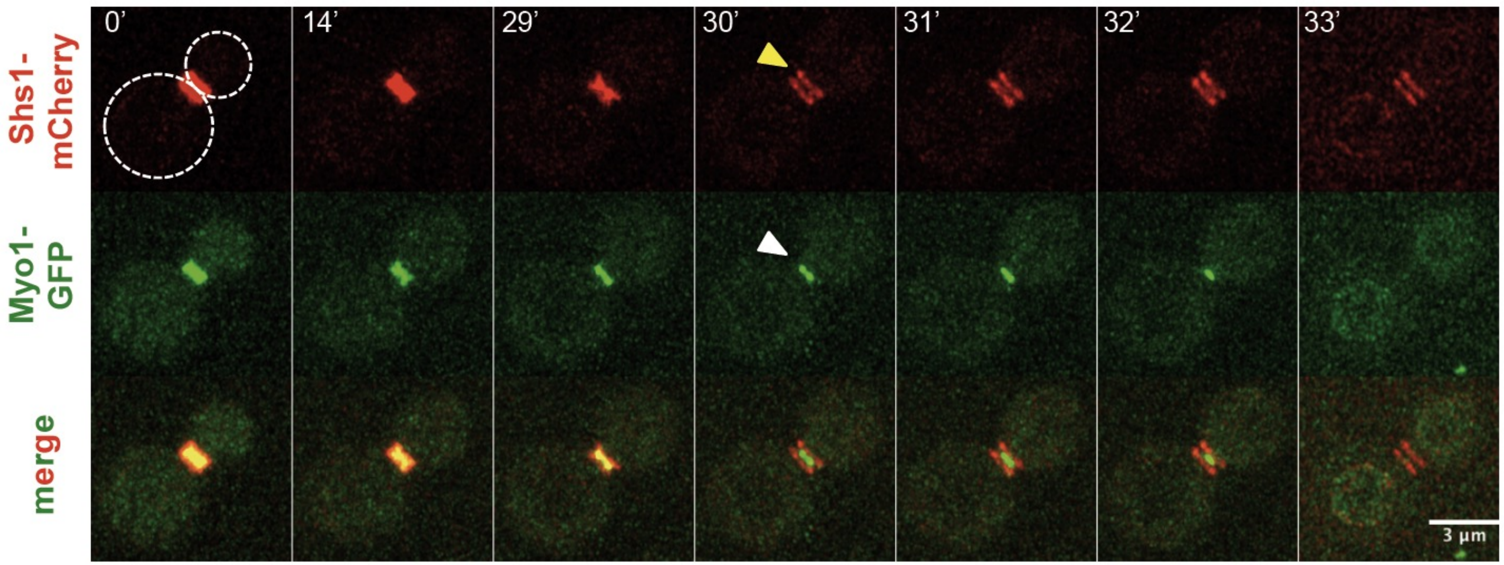
SOP: sensory organ precursor

From Menon and Gaestel, 2015, J. Cell Sci. 128:1877

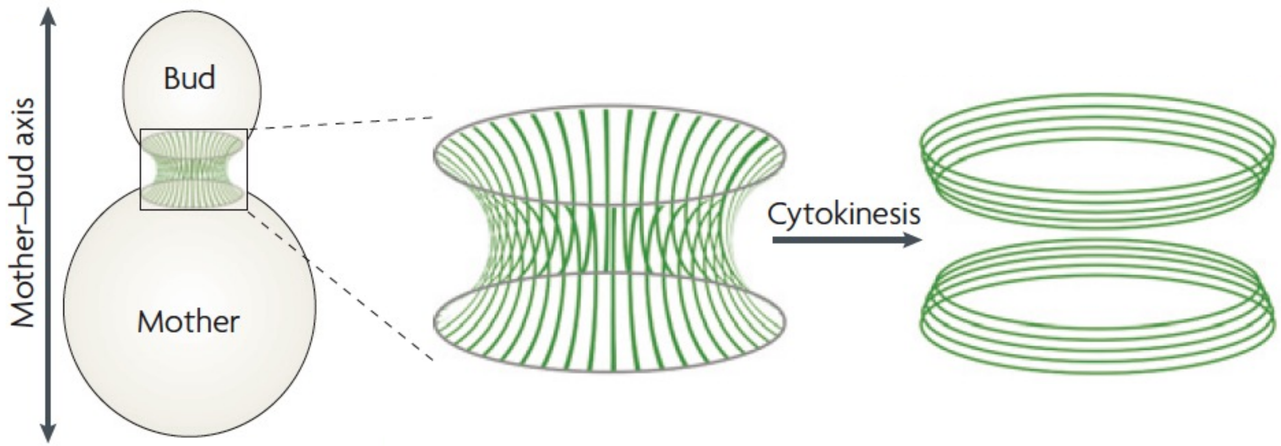
A septin ring at the bud neck recruits AMR components and is essential for cytokinesis in budding yeast



Septin ring splitting in live cells

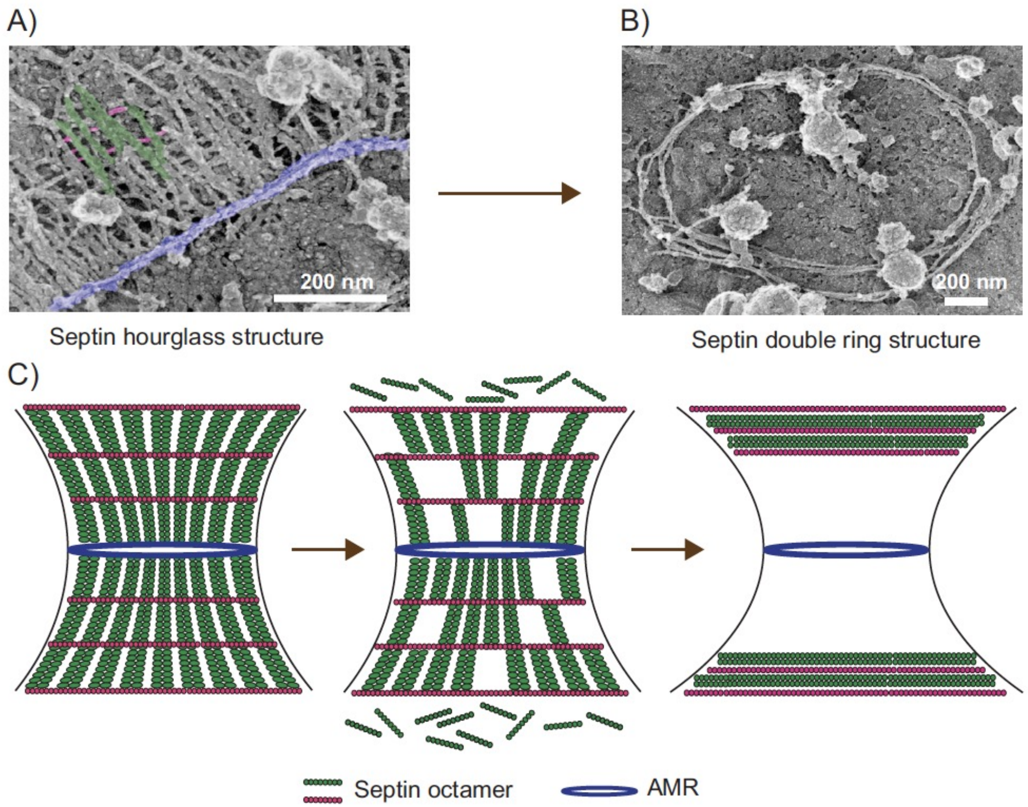


The hourglass to double ring transition involves a drastic remodelling of septins at the division site



From Weirich et al., 2008

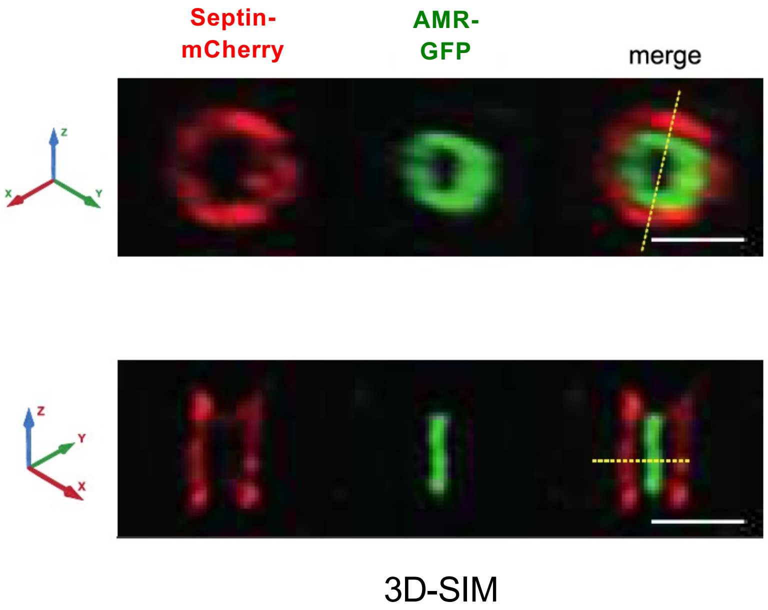
The collar to split-rings transition likely implicates partial disassembly and re-assembly of septin filaments

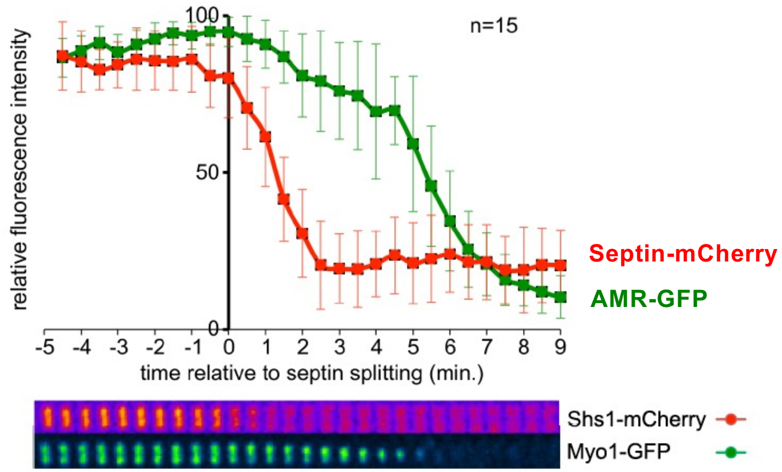


From Bhavsar-Jog and Bi, 2017

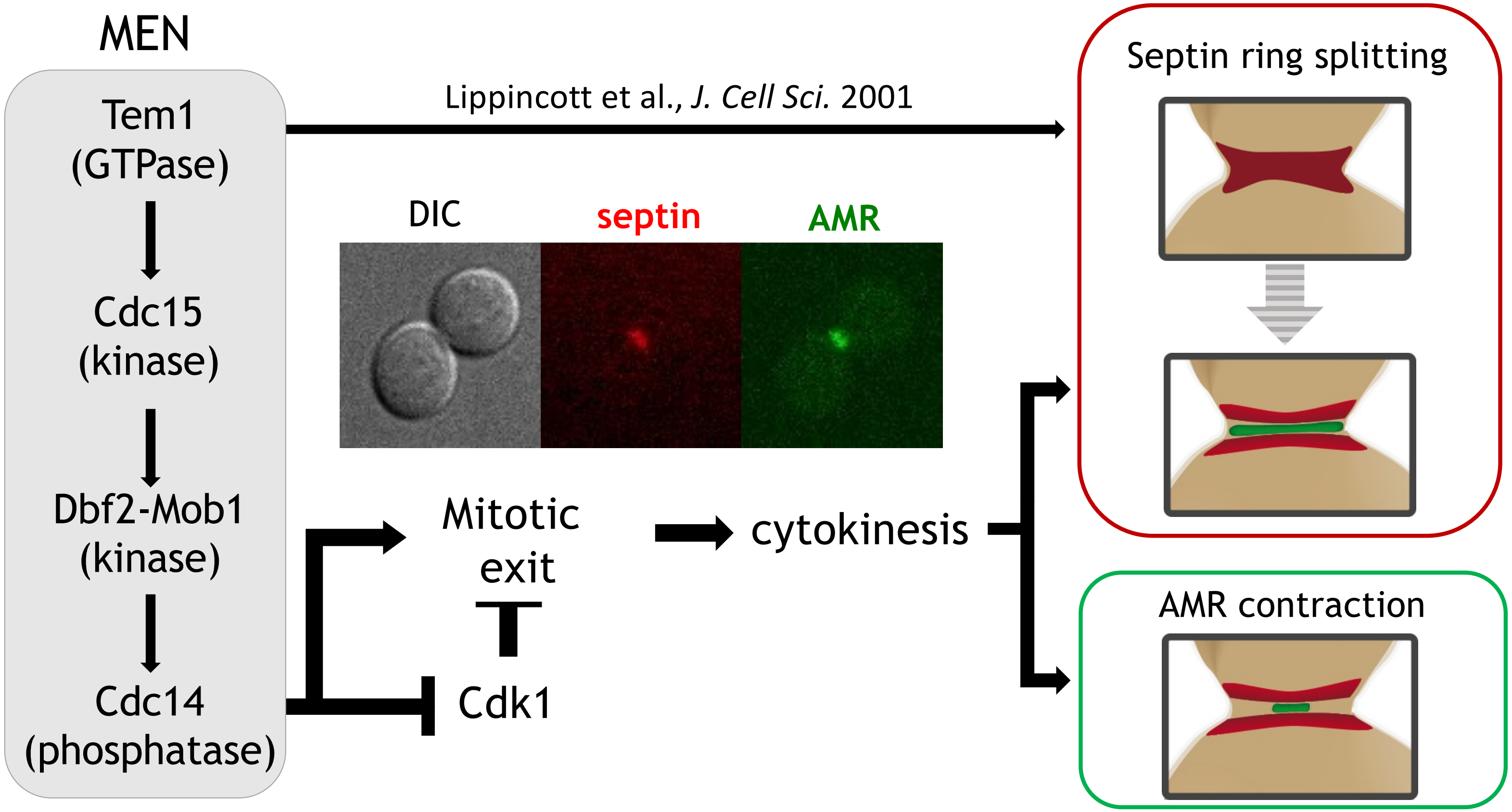
What is the functional relevance of septin ring splitting?

Septin ring splitting and AMR contraction are spatially and temporally distinct processes





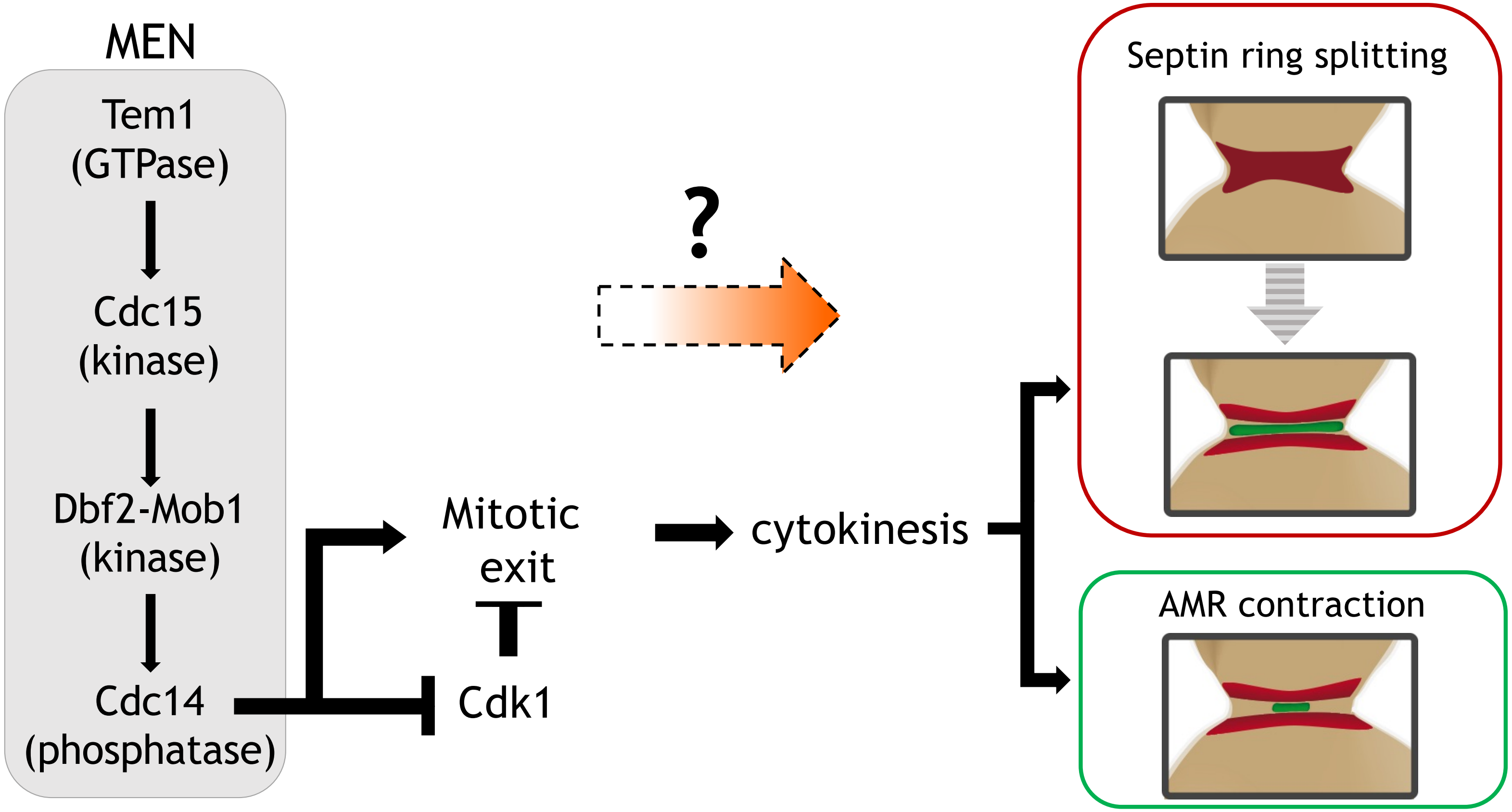
The Mitotic Exit Network promotes mitotic exit and cytokinesis



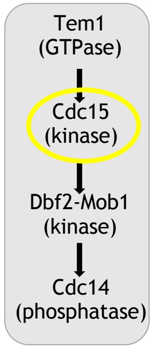
Is septin ring splitting important for cytokinesis?

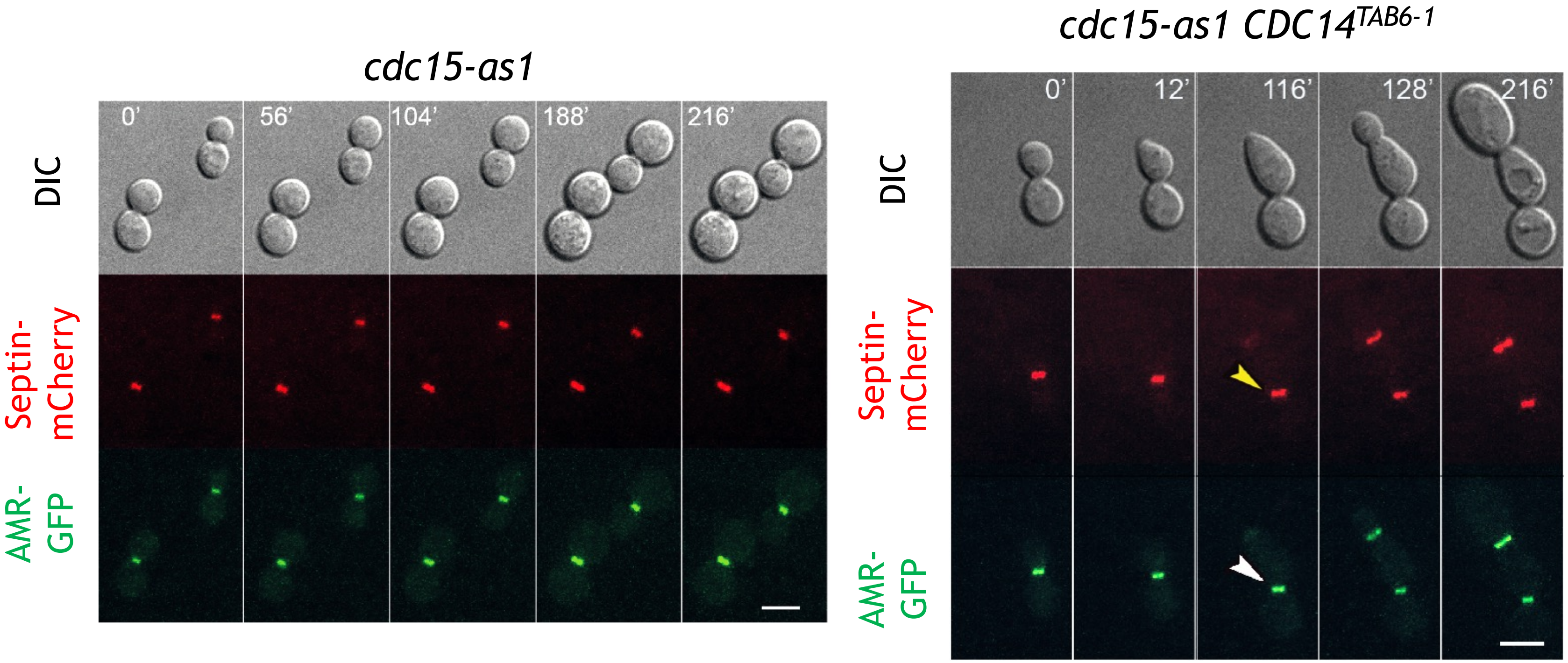
Davide
Tamborrini

Is MEN involved in septin ring splitting independently of mitotic exit?

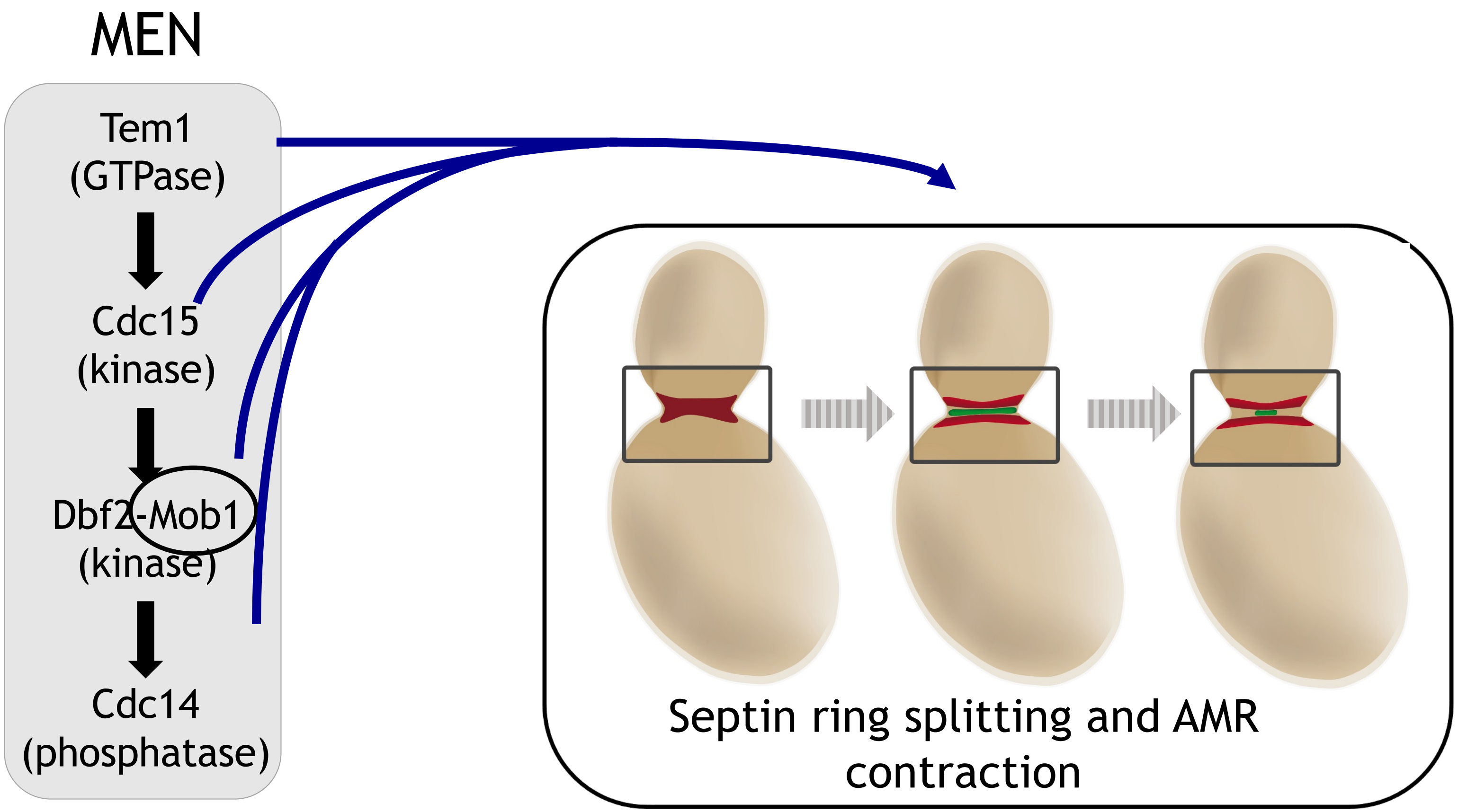


The hyperactive *CDC14^{TAB6-1}* allele allows mitotic exit upon MEN inactivation





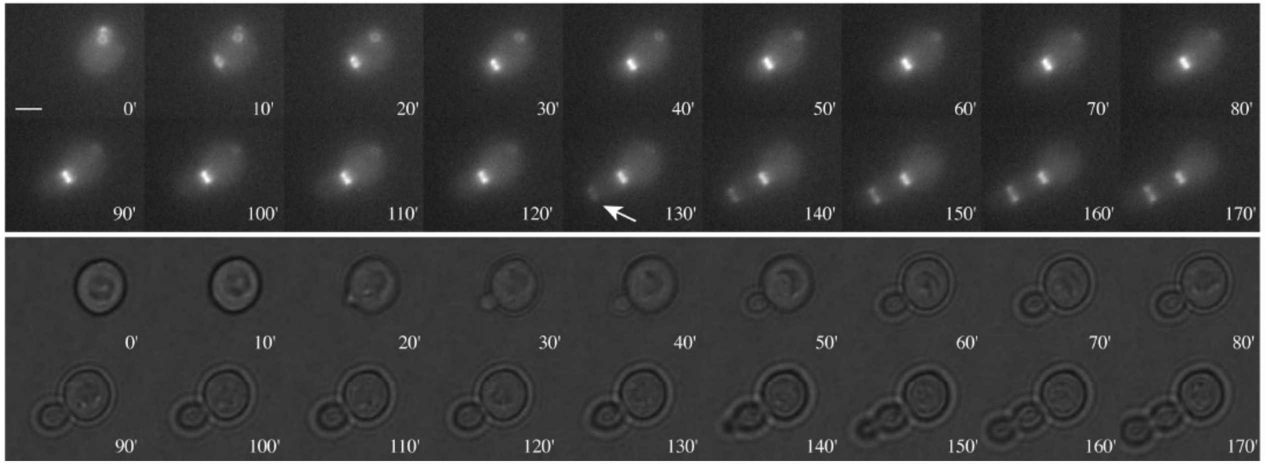
MEN promotes septin ring splitting and AMR contraction independently of mitotic exit



Overexpression of the E3 ligase *DMA2* delays septin ring splitting and inhibits cytokinesis

Septin-GFP

DIC

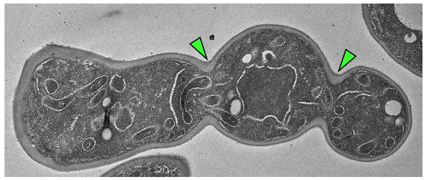


GAL1-DMA2

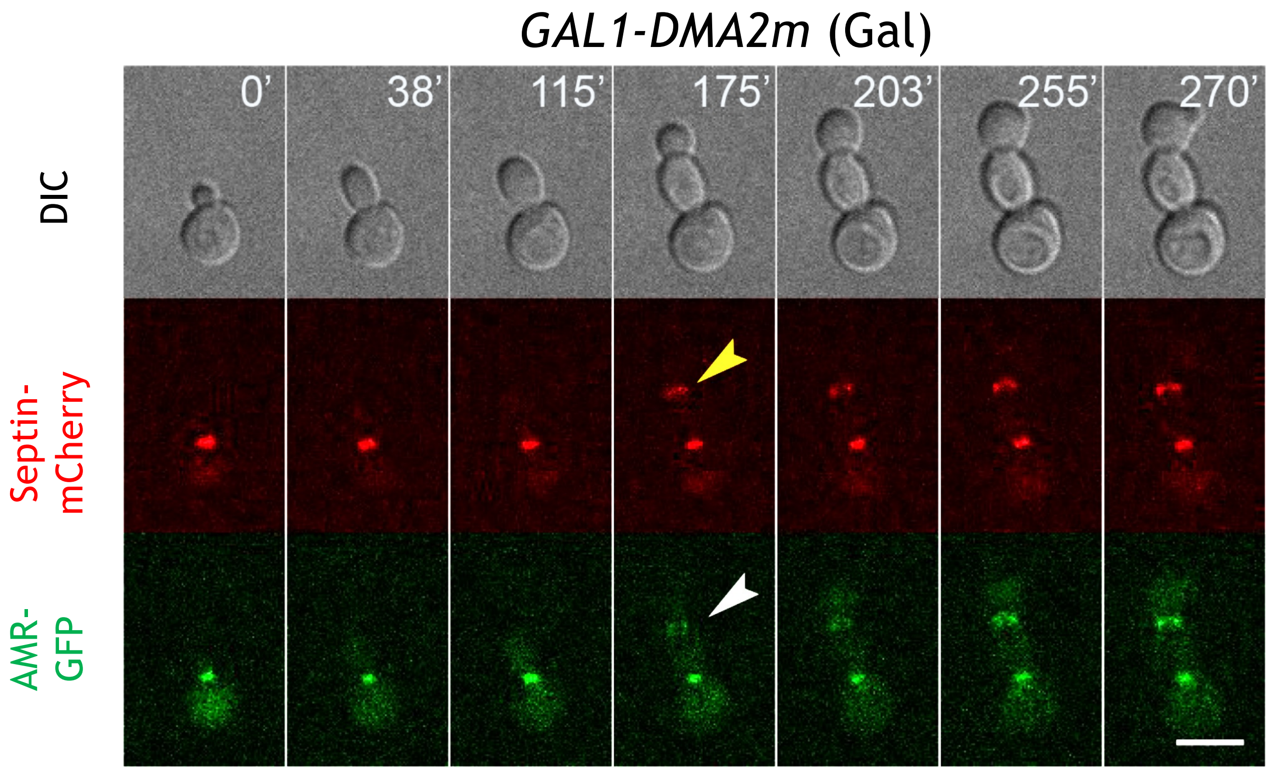
Merlini et al., 2012 *PloS Gen.*

wild type

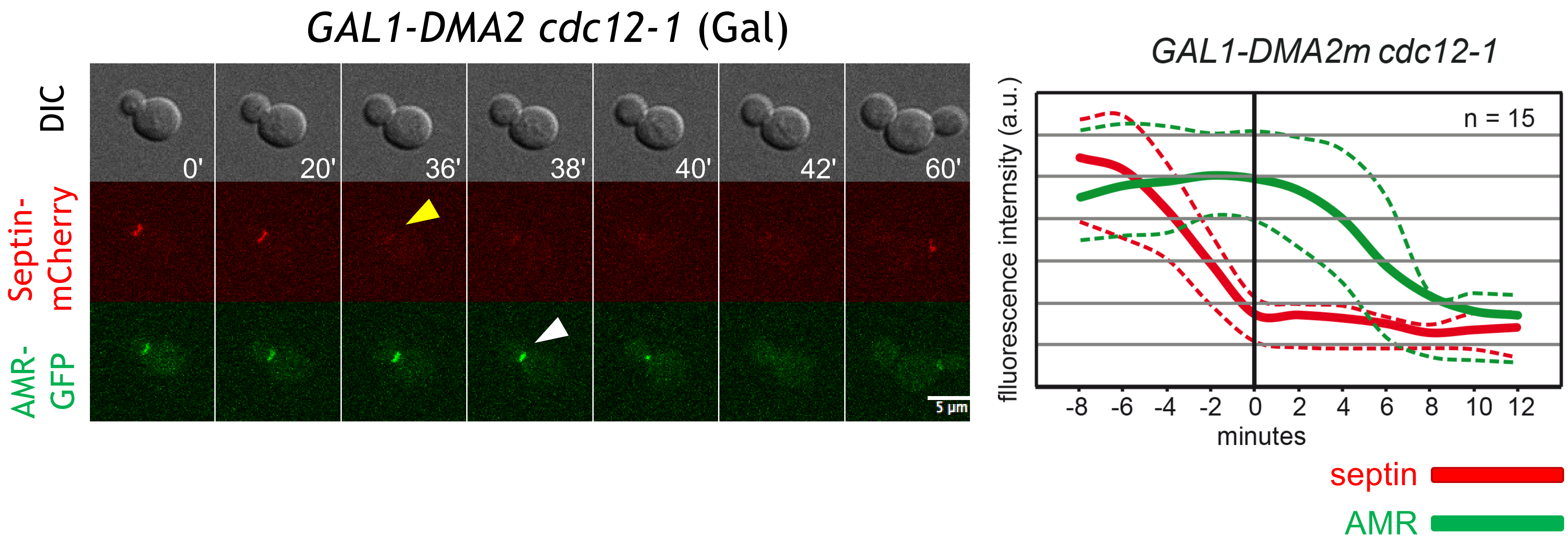
GAL1-DMA2



DMA2 overexpression inhibits septin ring splitting and AMR contraction



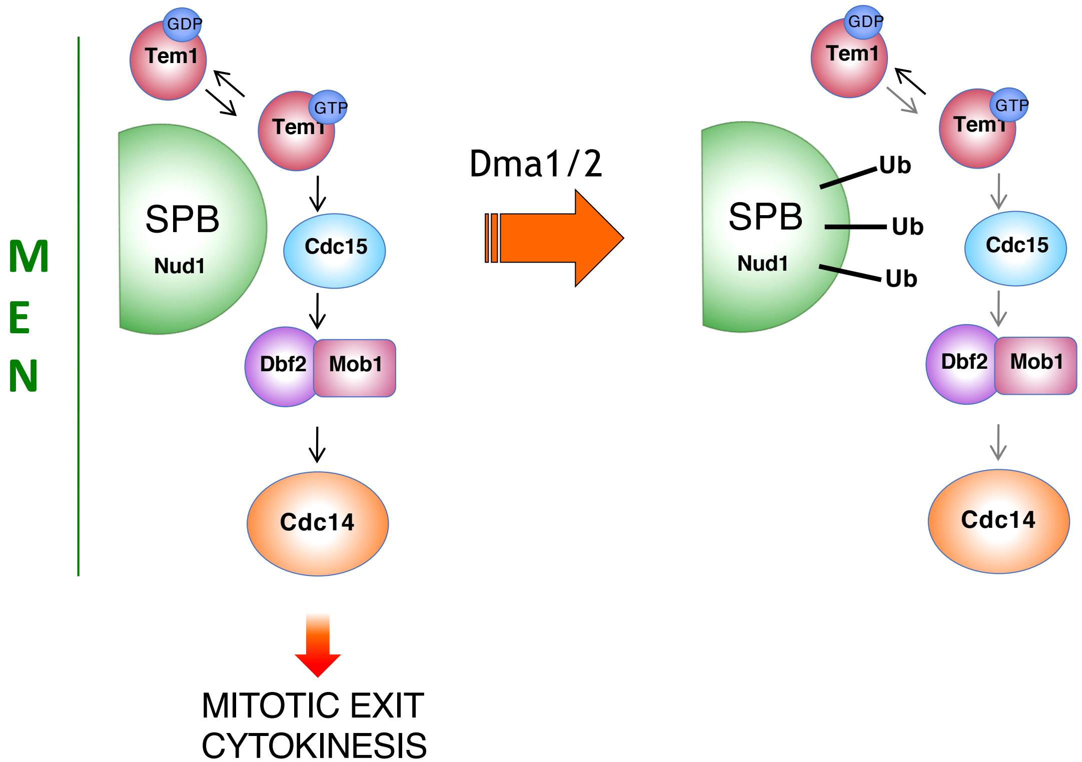
Septin destabilization with the *cdc12-1* allele is sufficient to allow AMR contraction in *DMA2*-overexpressing cells



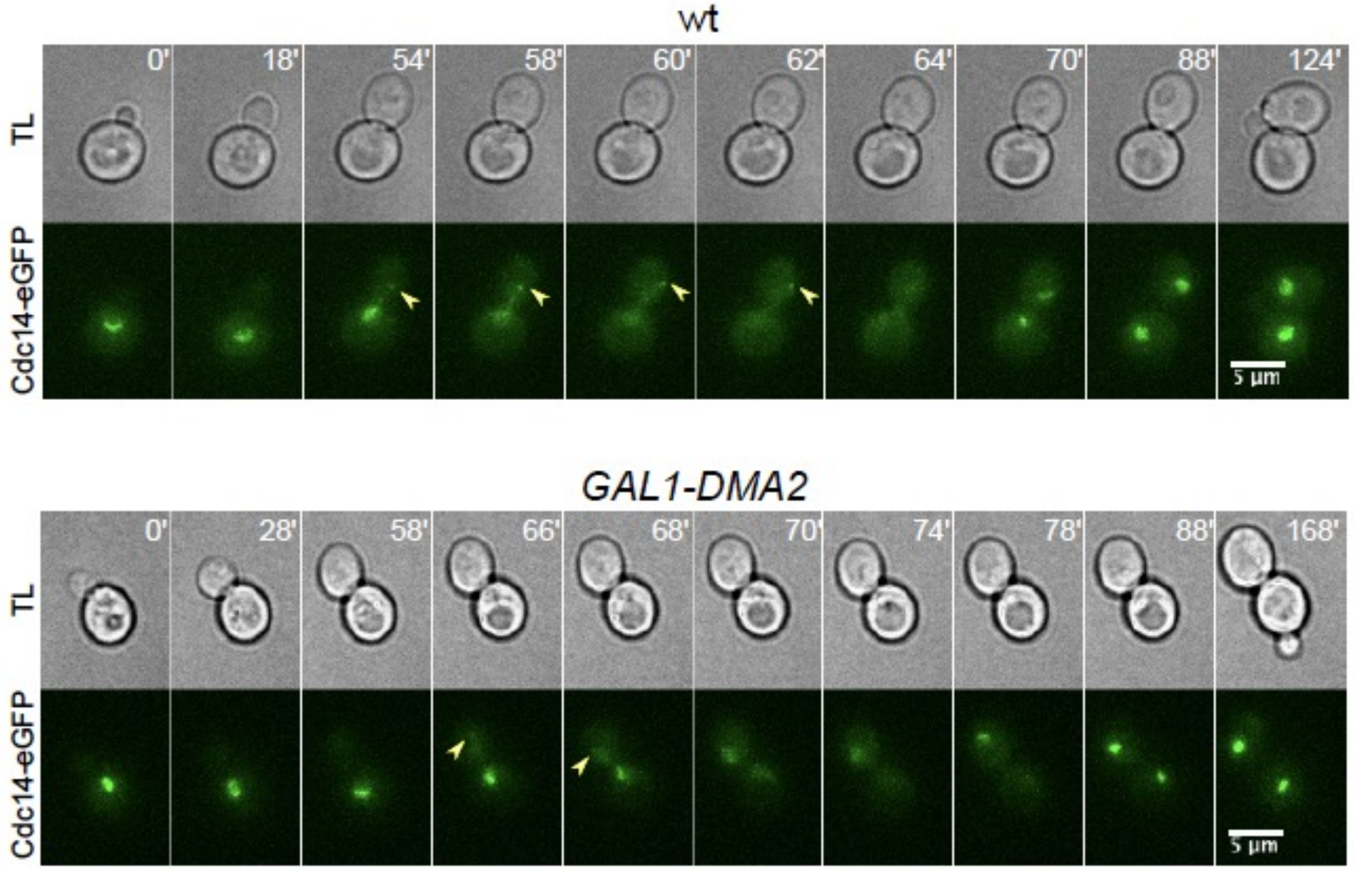
Septin displacement/clearance from the division site is a prerequisite for AMR contraction!

How does Dma2 prevent septin ring splitting?

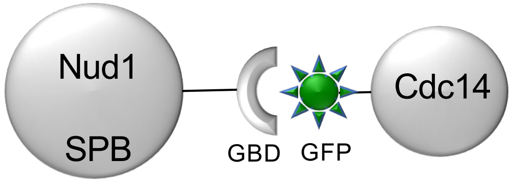
Dma2 promotes the ubiquitination of the MEN scaffold at SPBs Nud1



Recruitment of Cdc14 to SPB is impaired upon Dma2 overexpression

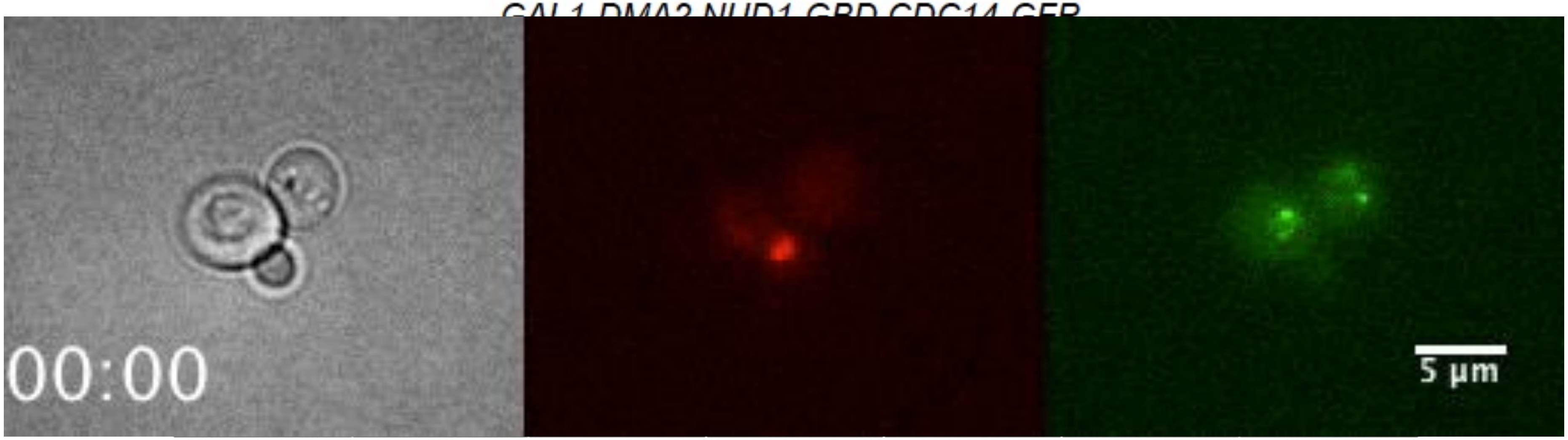


Artificial recruitment of Cdc14 to SPB restores septin displacement and cytokinesis in *DMA2*-overexpressing cells



Septin-mCherry

Cdc14-GFP



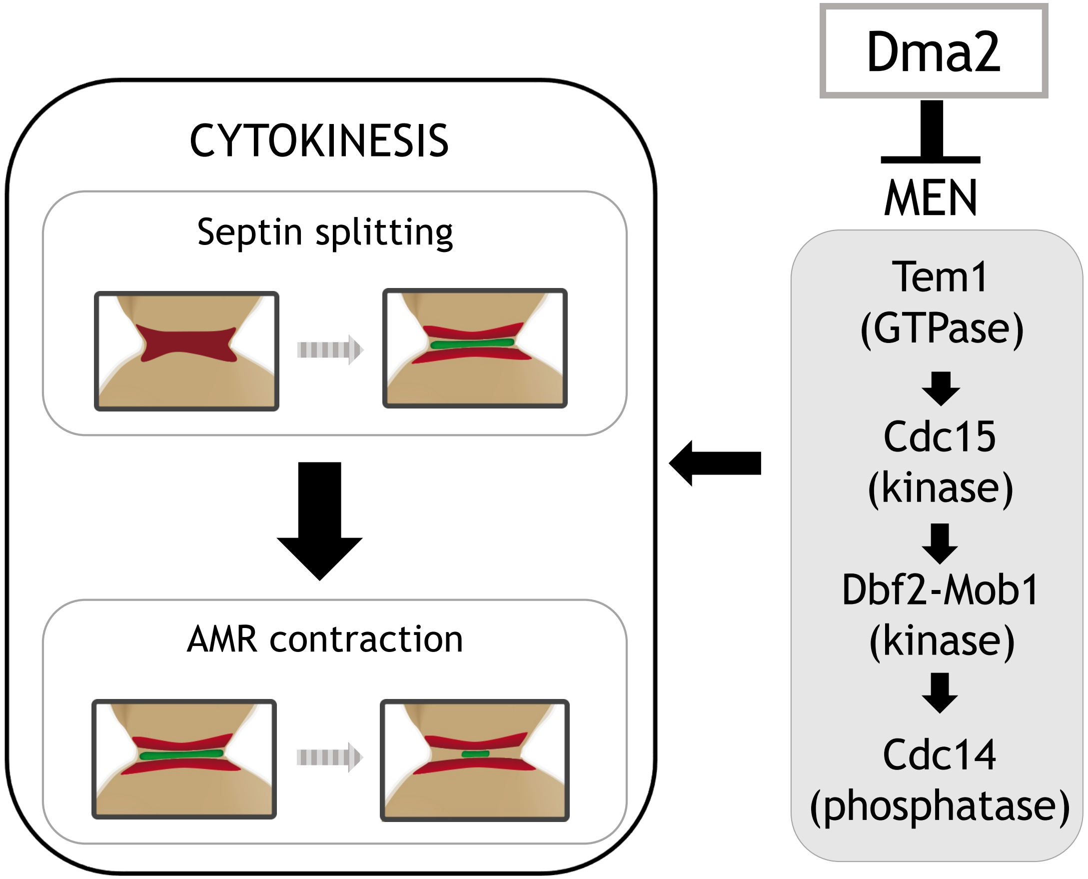
Conclusions

1. Septin reorganisation in yeast is necessary for AMR contraction

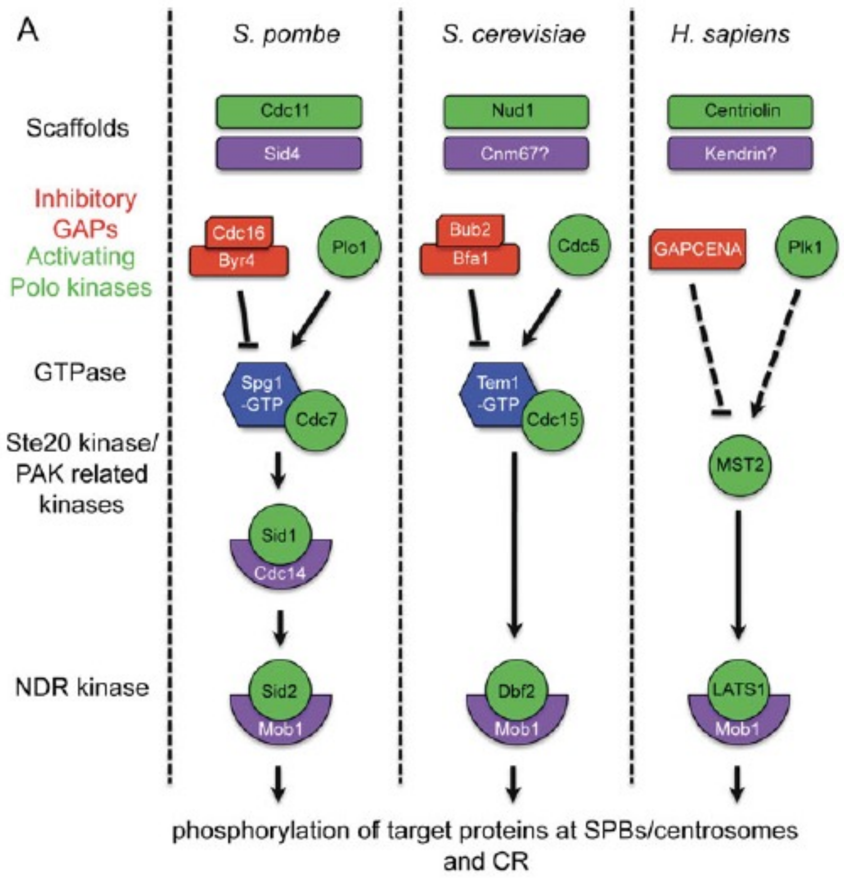
2. A subset of MEN factors promote both events independently of mitotic exit

3. Dma2 dampens MEN signalling at SPBs through Nud1 ubiquitination

Tamborrini et al., 2018, Nature Comm. 9:4308



Regulation of cytokinesis by a scaffold protein at centrosomes/SPBs is conserved



From Johnson et al., *Cytoskeleton* 2012

CRBM

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