Paul Nurse
KITP Evo Cell, Feb 25, 2010

SCIENTIA · PRO · BONO · HUMANI · GENERIS

The Rockefeller University

1901

Overview: The Cell-Cycle Control System 3-0

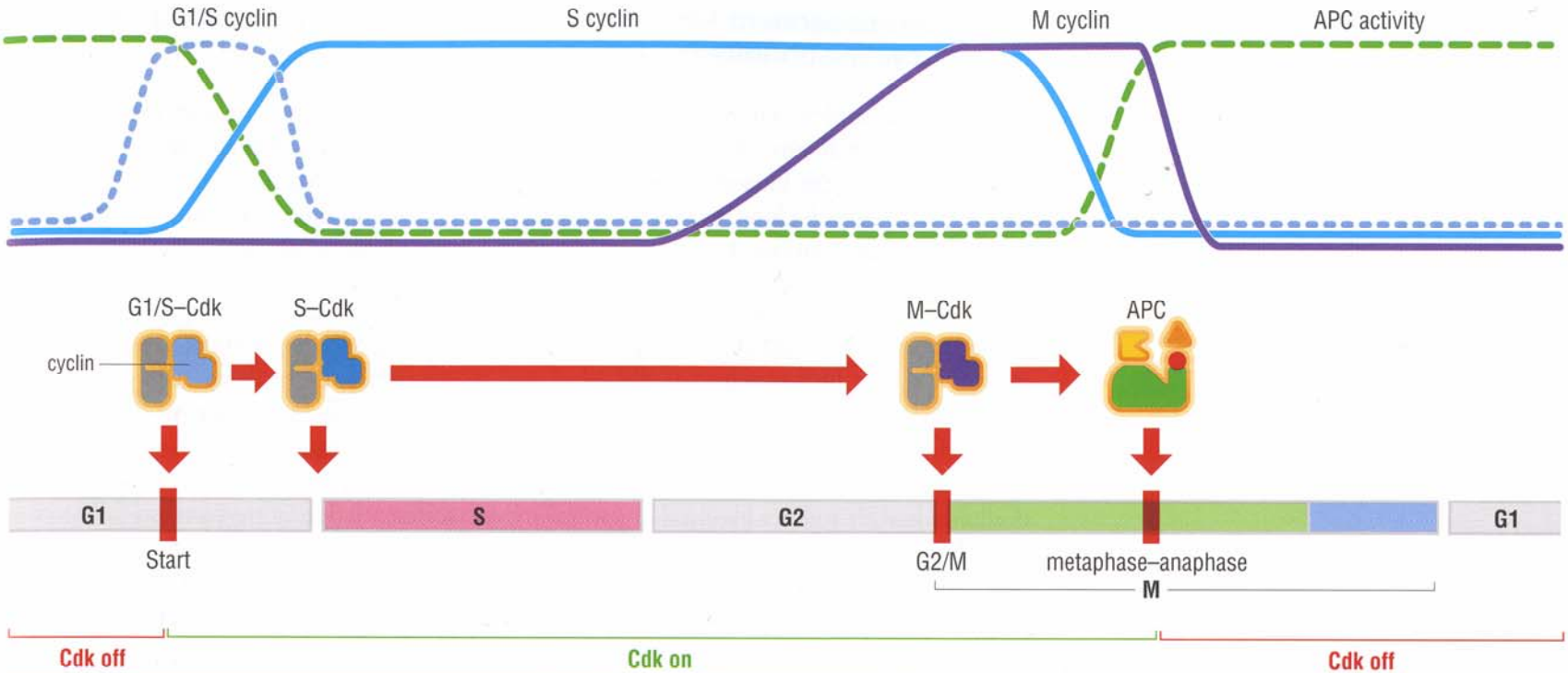
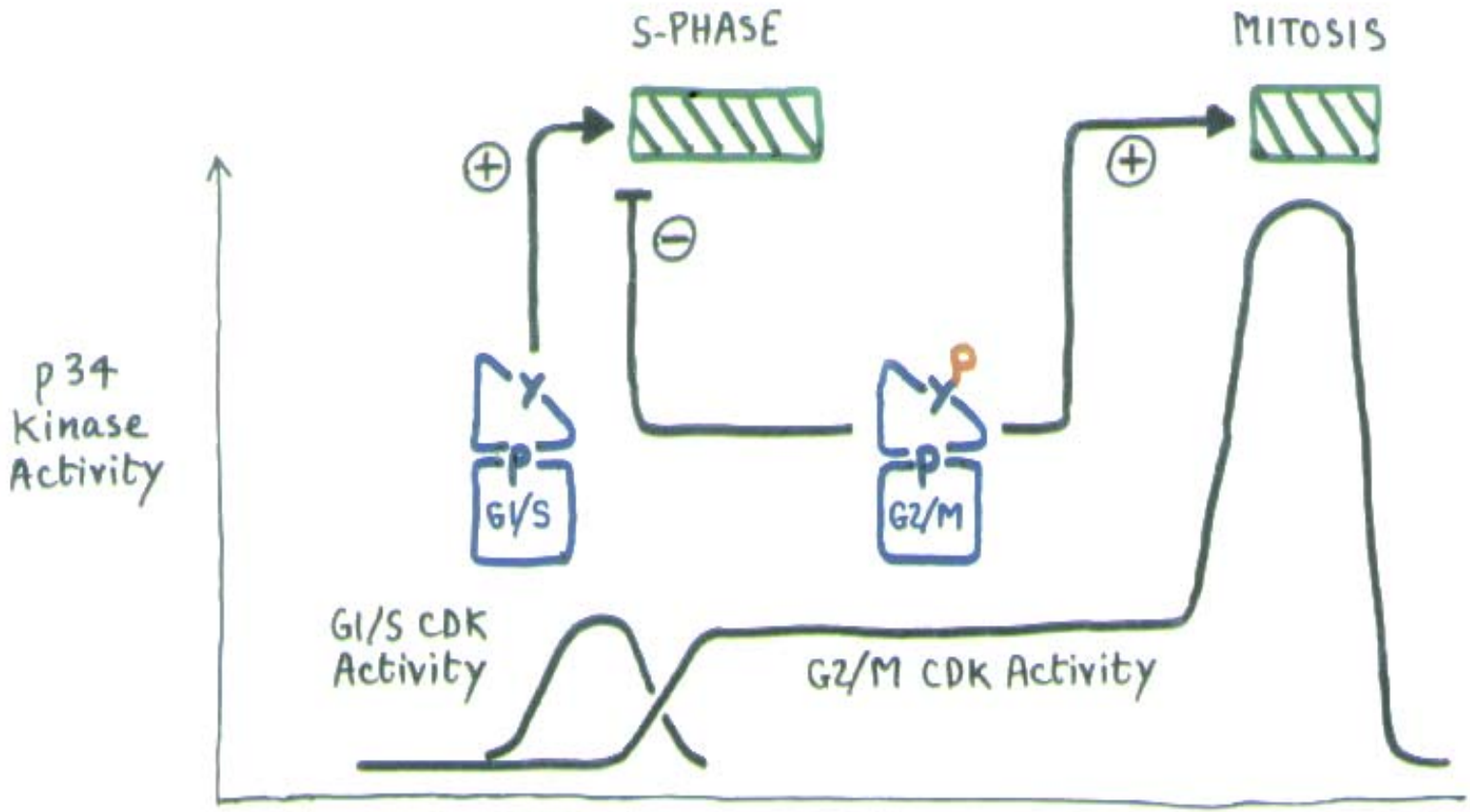
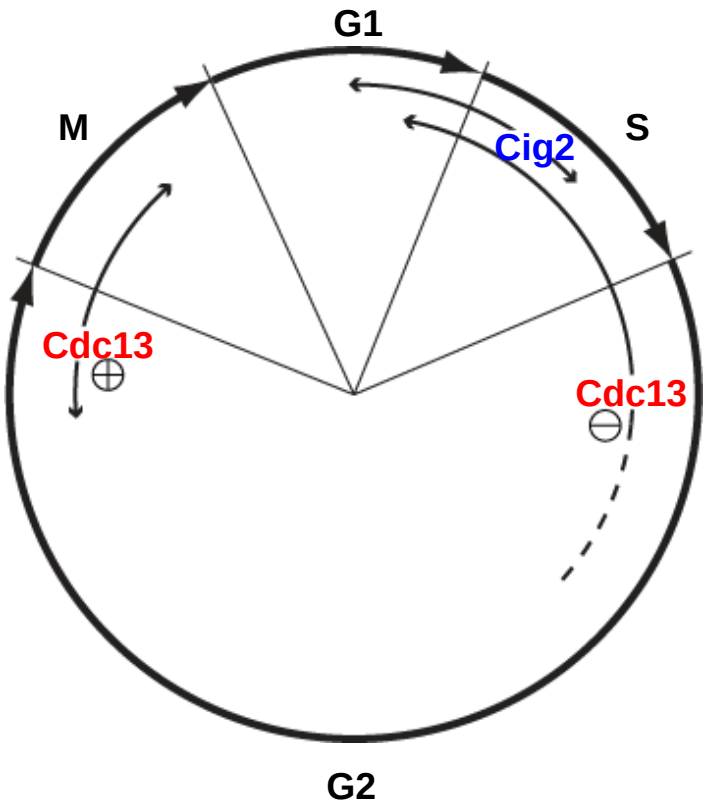


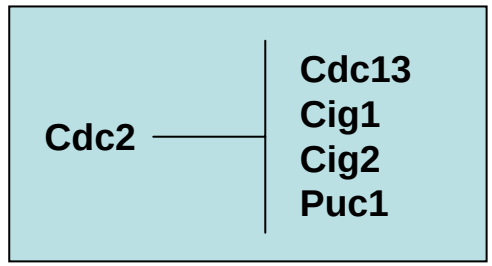
Figure 3-1 A simplified view of the cell-cycle control system Levels of the three major cyclin types oscillate during the cell cycle (top), providing the basis for oscillations in the cyclin–Cdk complexes that drive cell-cycle events (bottom). In general, Cdk levels are constant and in large excess over cyclin levels; thus, cyclin–Cdk complexes form in parallel with cyclin levels. The enzymatic activities of cyclin–Cdk complexes also tend to rise and fall in parallel with cyclin levels, although in some cases Cdk inhibitor proteins or phosphorylation introduce a delay between the formation and activation of cyclin–Cdk complexes. Formation of active G1/S–Cdk complexes commits the cell to a new division cycle at the Start checkpoint in late G1. G1/S–Cdks then activate the S–Cdk complexes that initiate DNA replication at the beginning of S phase. M–Cdk activation occurs after the completion of S phase, resulting in progression through the G2/M checkpoint and assembly of the mitotic spindle. APC activation then triggers sister-chromatid separation at the metaphase-to-anaphase transition. APC activity also causes the destruction of S and M cyclins and thus the inactivation of Cdks, which promotes the completion of mitosis and cytokinesis. APC activity is maintained in G1 until G1/S–Cdk activity rises again and commits the cell to the next cycle. This scheme serves only as a general guide and does not apply to all cell types.

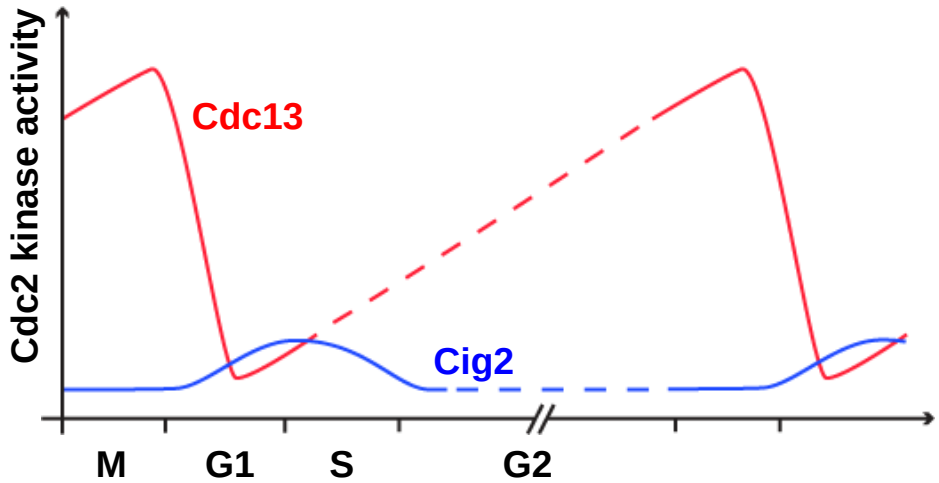
"The Cell Cycle" David Morgan


S-PHASE
MITOSIS
p34
Kinase
Activity
G1/S
G2/M
G1/S CDK
Activity
G2/M CDK Activity

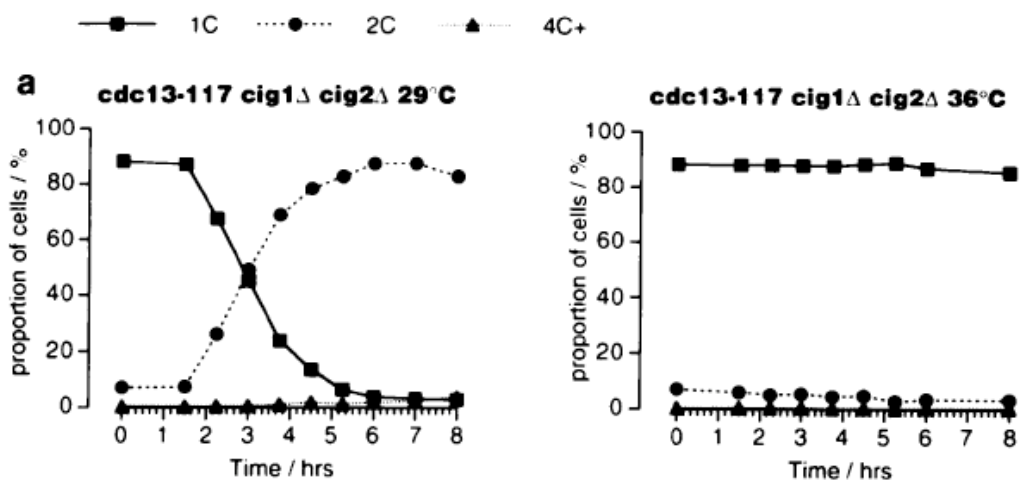
Different cyclins control different phases of the cell cycle

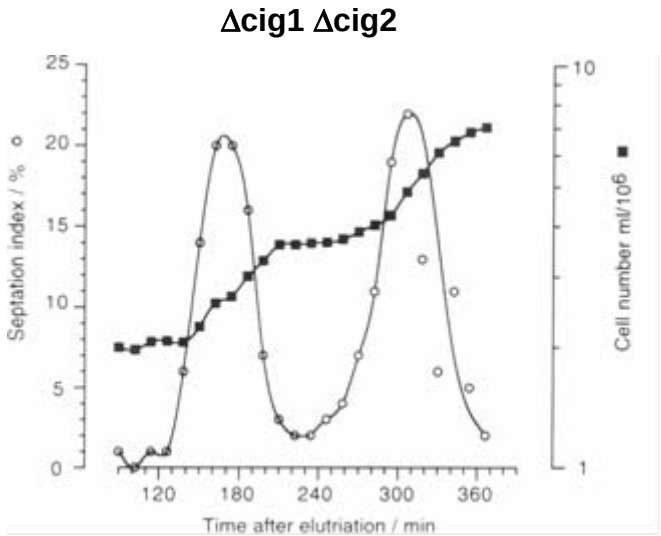






Questioning the dogma of cyclin mediated cdk specificity





(Fisher and Nurse, 1996)

Cdk1 is sufficient to drive the mammalian cell cycle

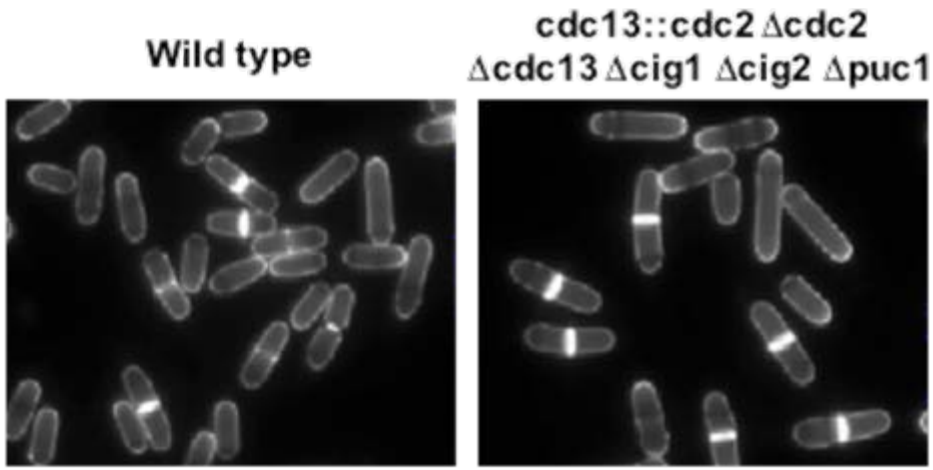
David Santamaría[1]*, Cédric Barrière[1,2]*†, Antonio Cerqueira[1], Sarah Hunt[1]†, Claudine Tardy[1], Kathryn Newton[3], Javier F. Cáceres[3], Pierre Dubus[2], Marcos Malumbres[1] & Mariano Barbacid[1]

Nature 2007

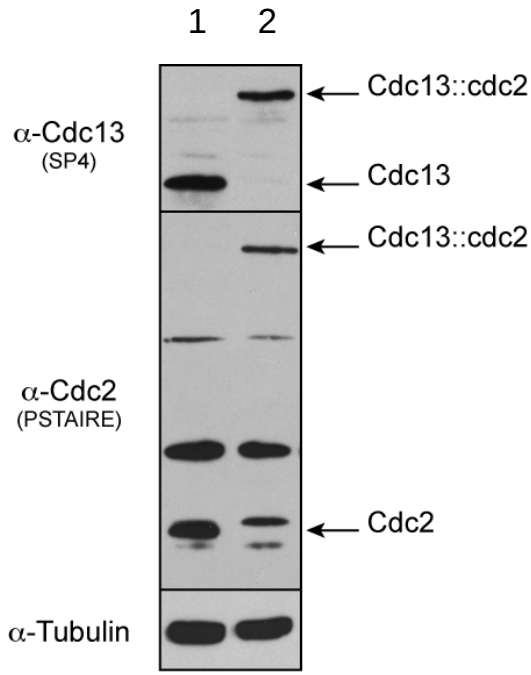
- Is a single cdk-cyclin oscillator sufficient and flexible enough to drive dramatically different events?
- What constitutes the minimal machinery that supports the cell cycle?

Damien Coudreuse

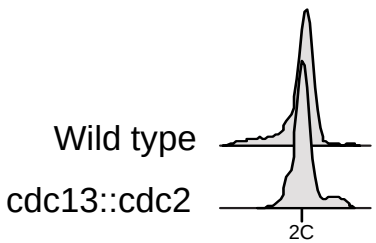
Reduction of the fission yeast Cdk oscillator to a single fusion protein



	Wild type	cdc13::cdc2 Δcdc2 Δcdc13 Δcig1 Δcig2 Δpuc1
Size at division (μm)	13.7 ± 0.2	15.9 ± 0.2
Generation time (min)	163 ± 2.3	162 ± 5.5
Septation index (%)	11.7 ± 0.7	13.4 ± 0.1

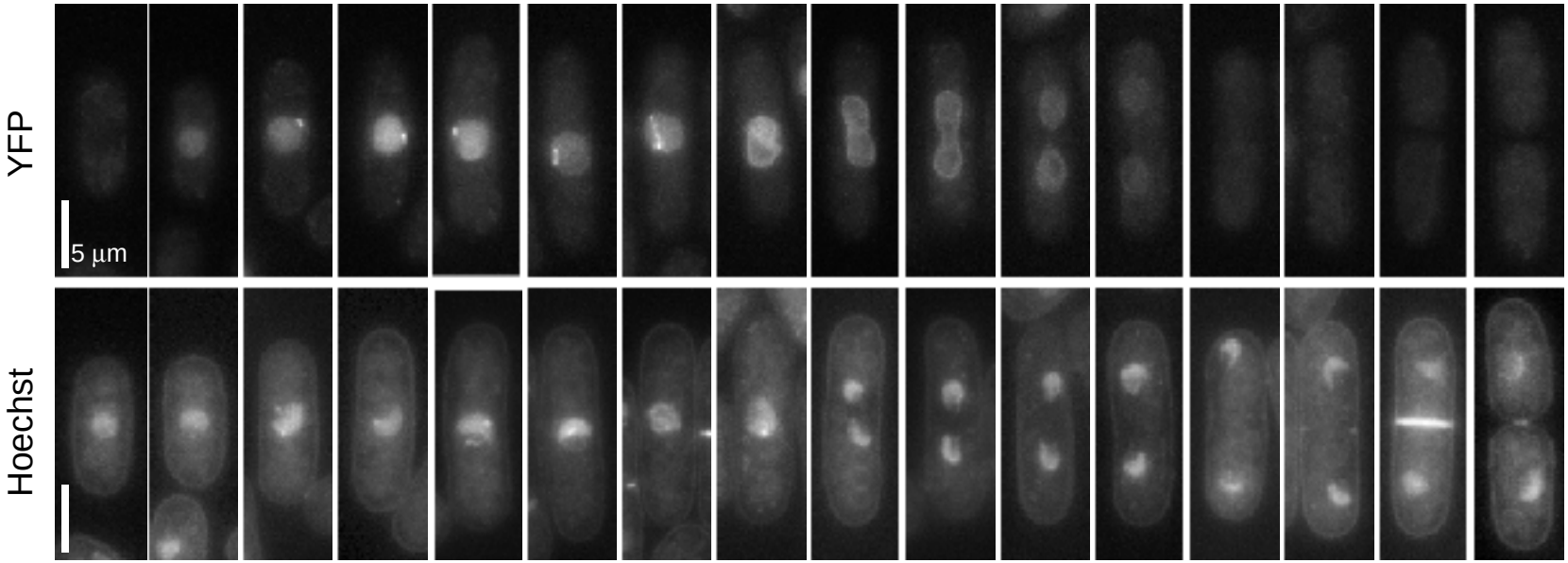


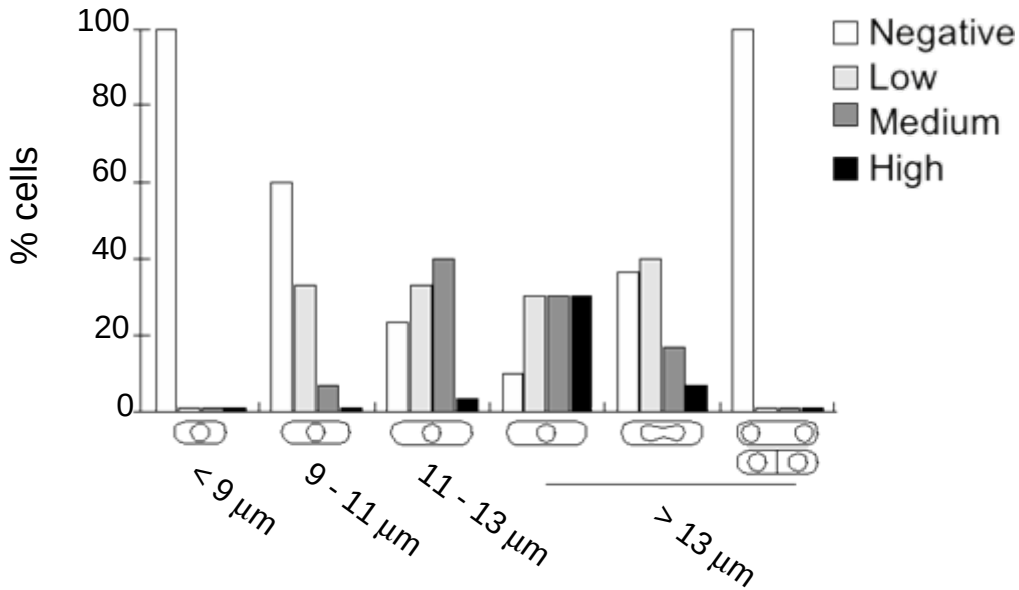
DNA content



Reduction of the fission yeast Cdk oscillator to a single fusion protein

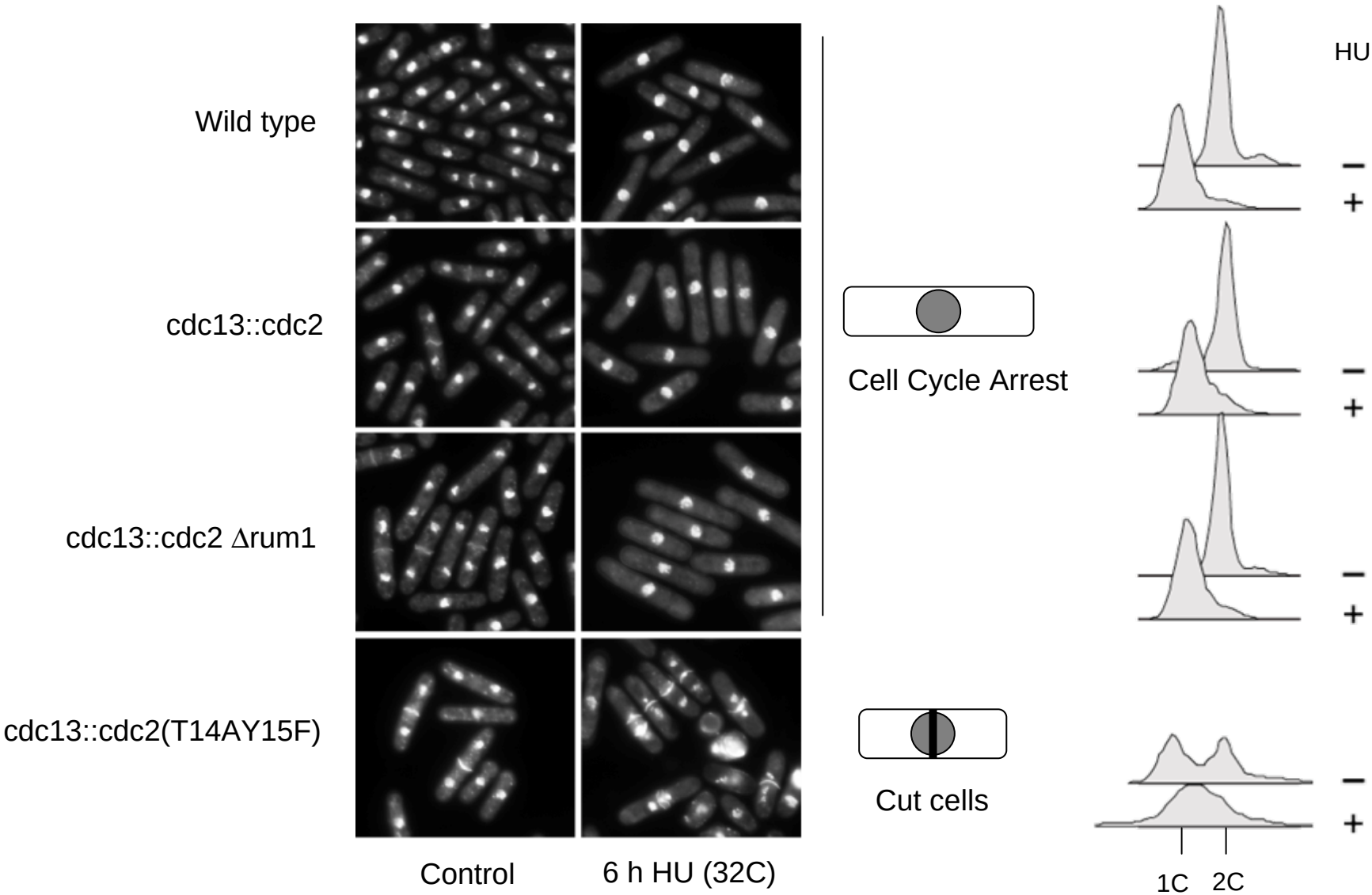
cdc13::cdc2::YFP Δcdc2 Δcdc13Δcig1 Δcig2 Δpuc1





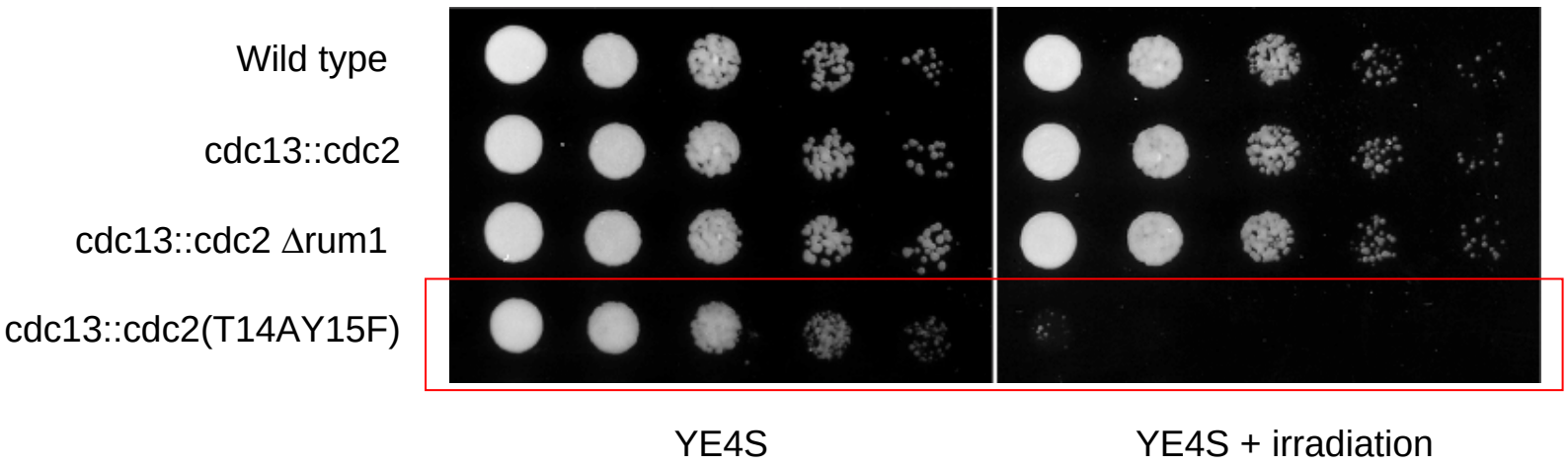
The Wee1 and Cdc25 network is essential for checkpoint activation

S phase checkpoint

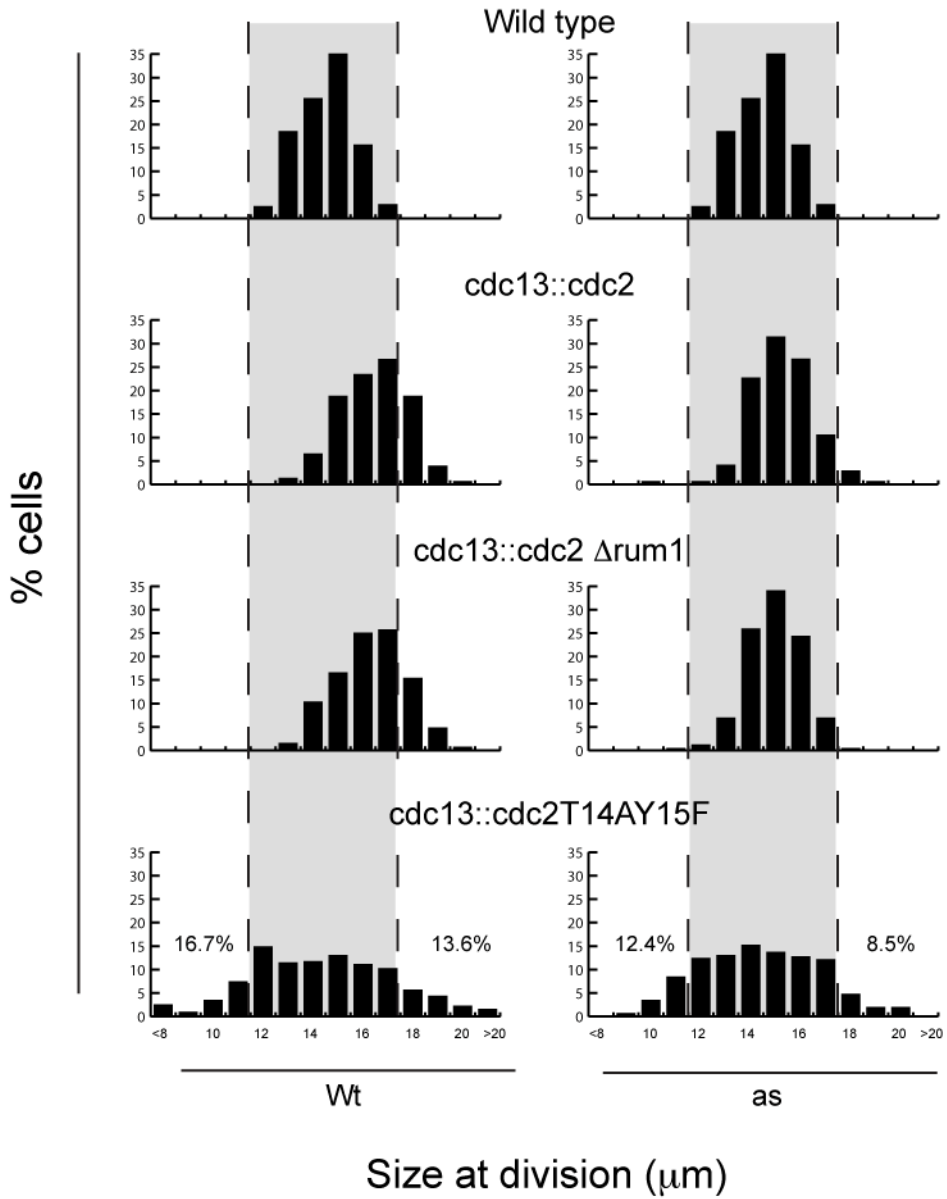


The Wee1 and Cdc25 network is essential for checkpoint activation

DNA damage checkpoint



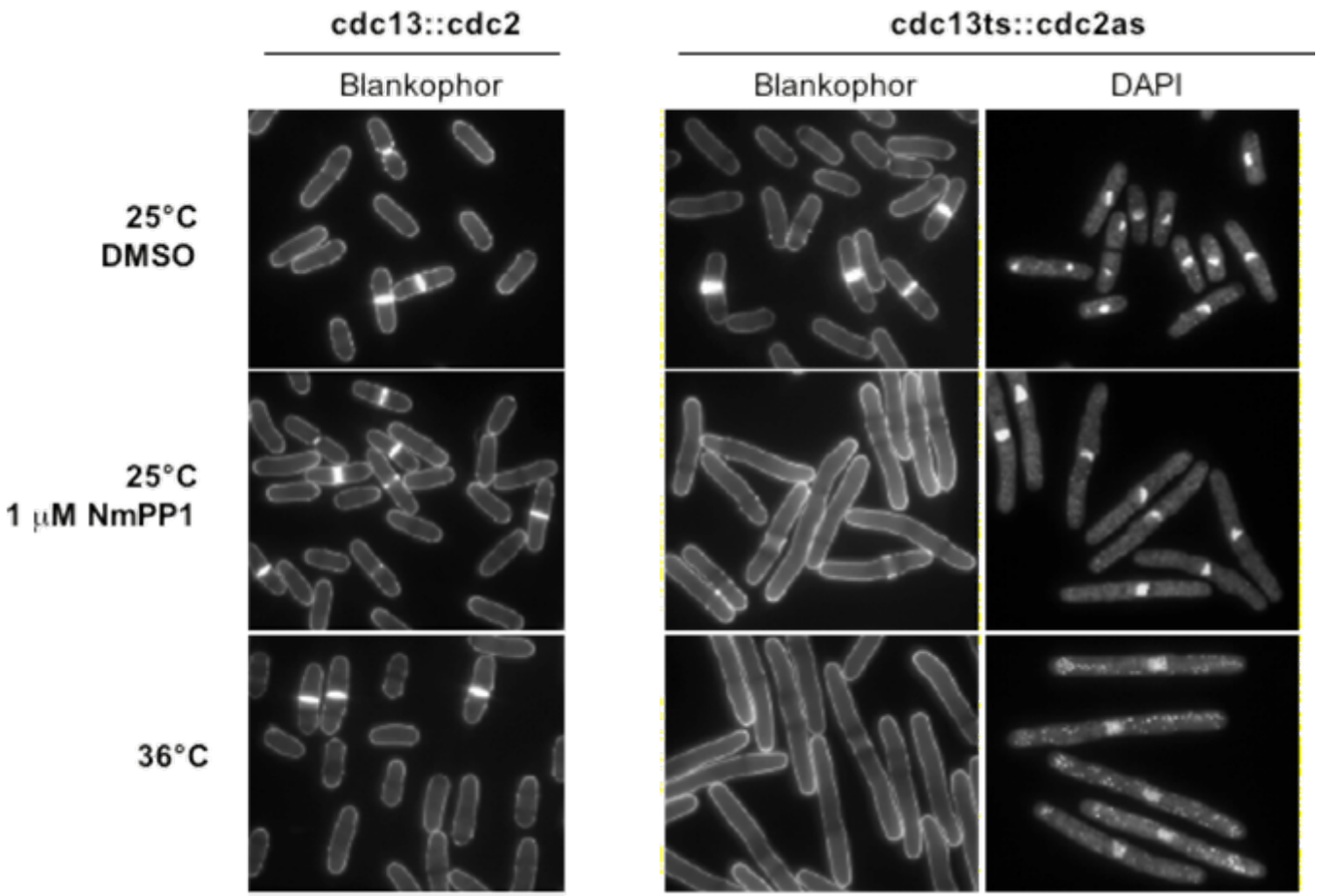
The Wee1/Cdc25 loop is essential for the homogeneity of the population



The Cdc13::cdc2 fusion protein drives the cell cycle autonomously

cdc13C379Y::cdc2as Δcdc2 Δcdc13

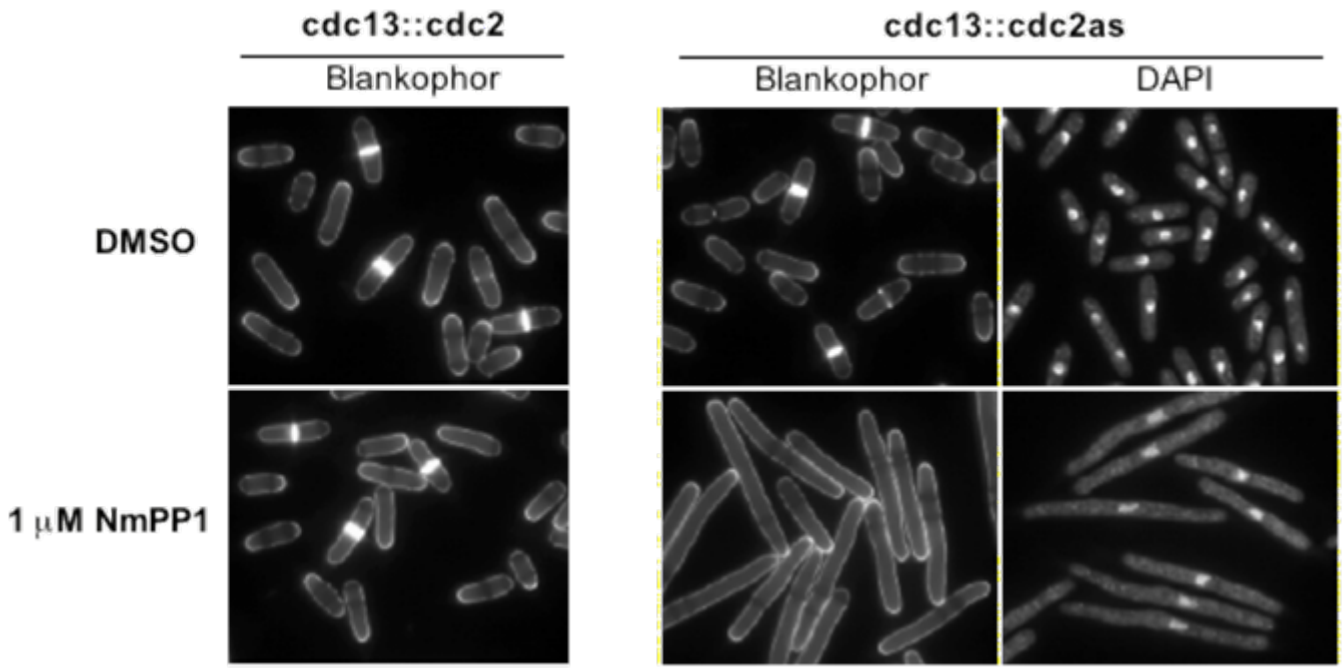
(ts mutation) (sensitivity to NmPP1)



YE4S

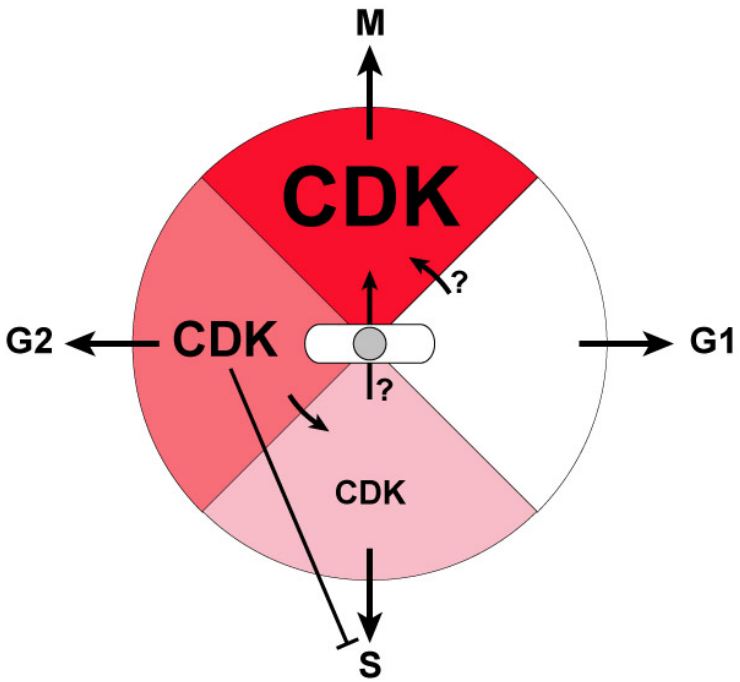
A tunable and minimal cell cycle

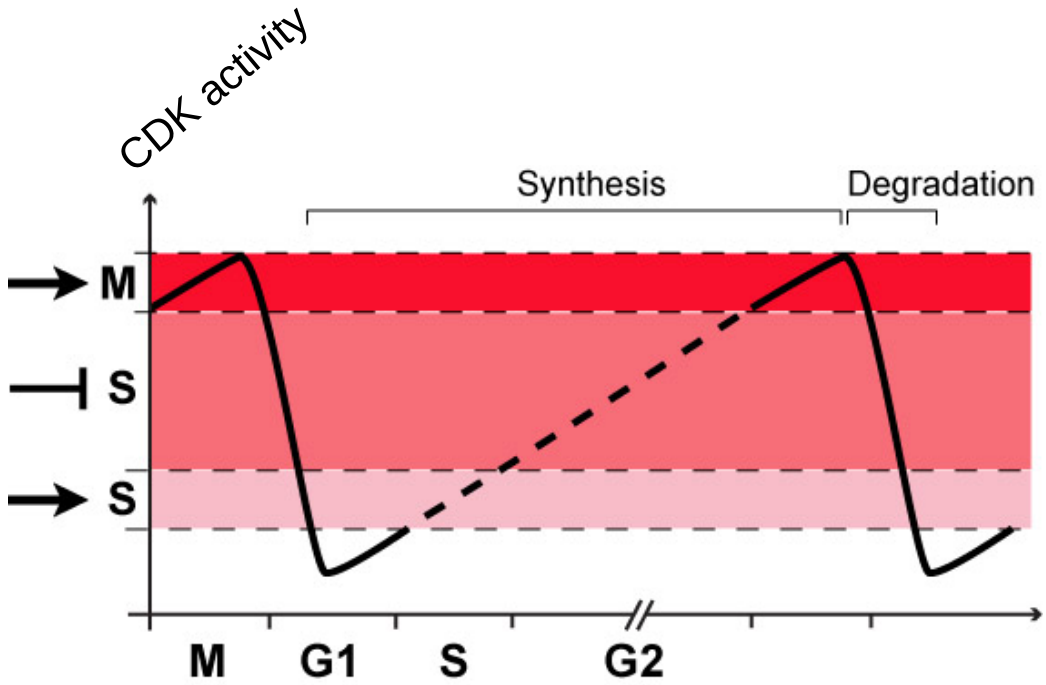
cdc13::cdc2as Δcdc2 Δcdc13 Δcig1 Δcig2 Δpuc1



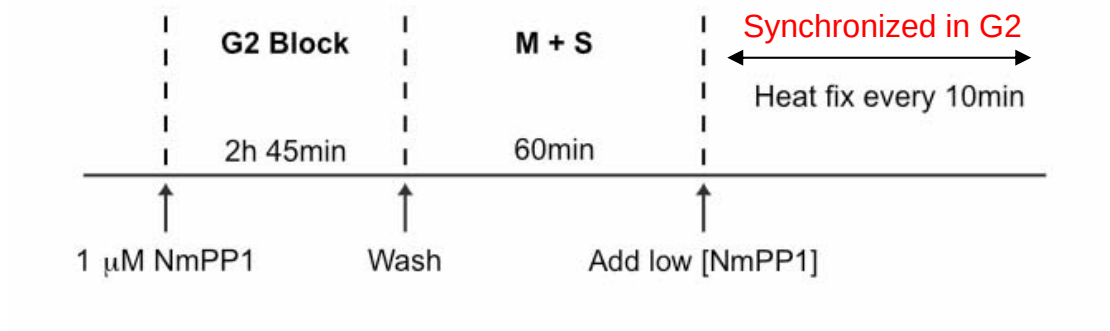
	Wt	as
Size at division (μm)	15.9 ± 0.2	14.7 ± 0.3
Generation time (min)	162 ± 5.5	158 ± 5.5
Septation index (%)	13.4 ± 0.1	13.7 ± 0.6

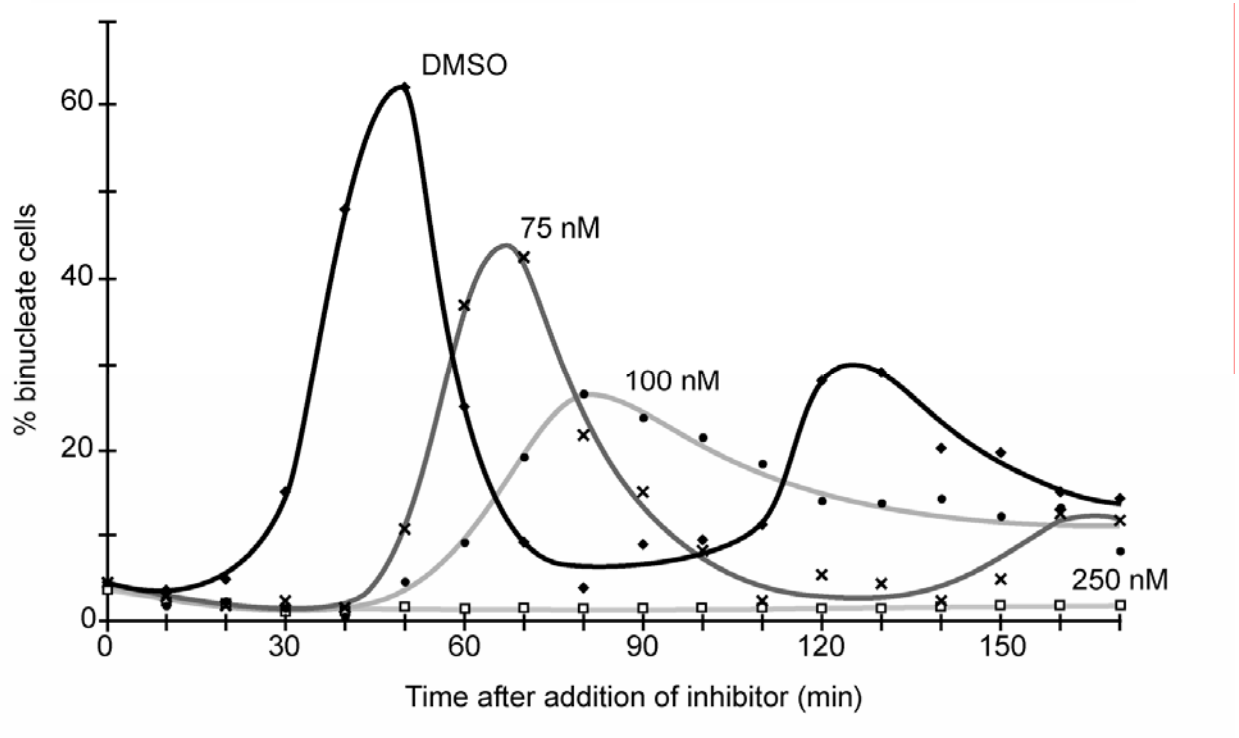
The minimal cell cycle is dependant upon changes in a single CDK activity





Entry into M depends upon a high activity threshold

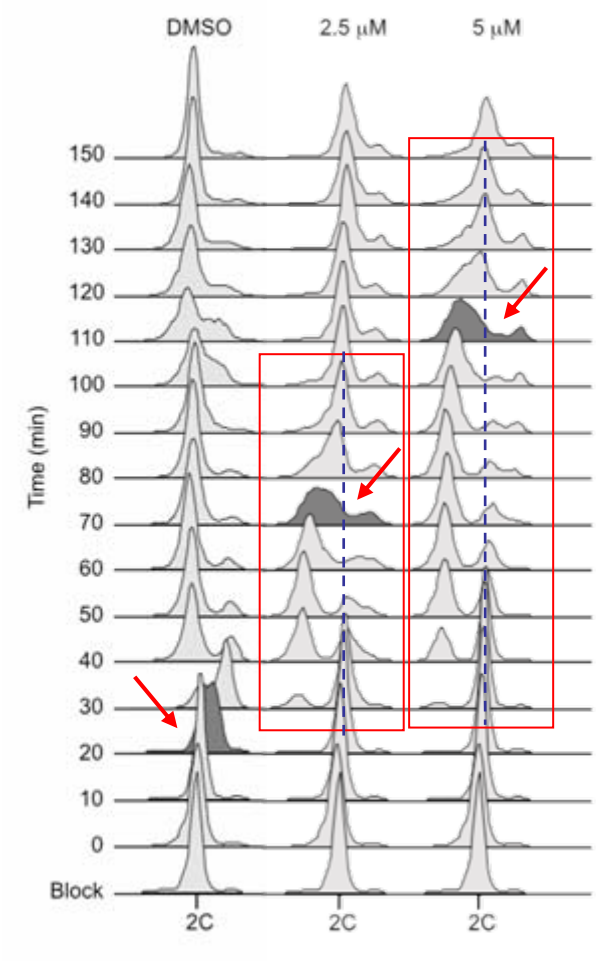


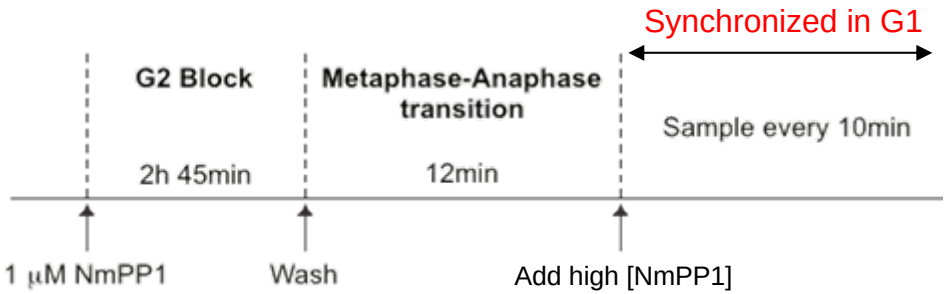


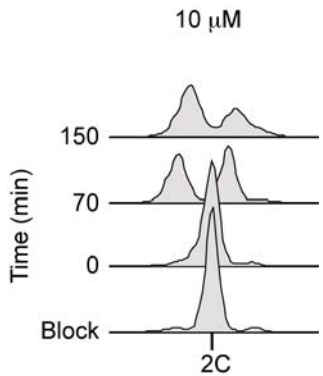
0.25 μM NmPP1 prevents mitotic entry

Entry into S depends upon a low activity threshold

cdc13::cdc2as Δcdc2 Δcdc13Δcig1 Δcig2 Δpuc1



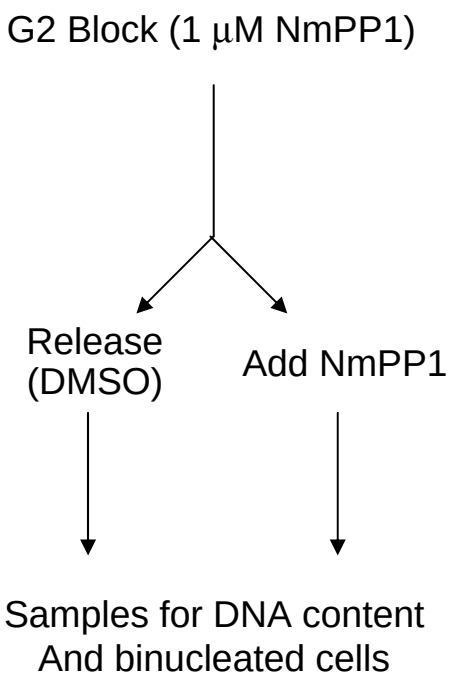


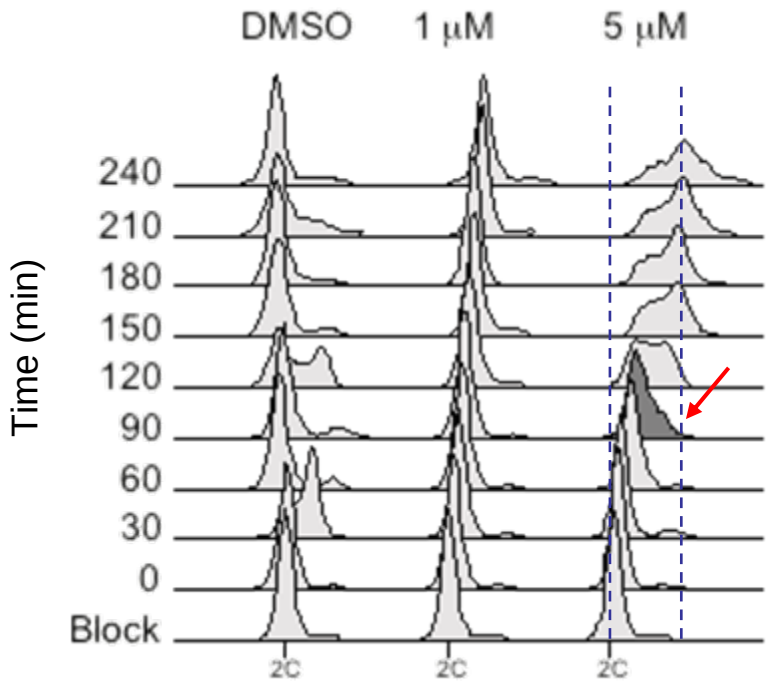


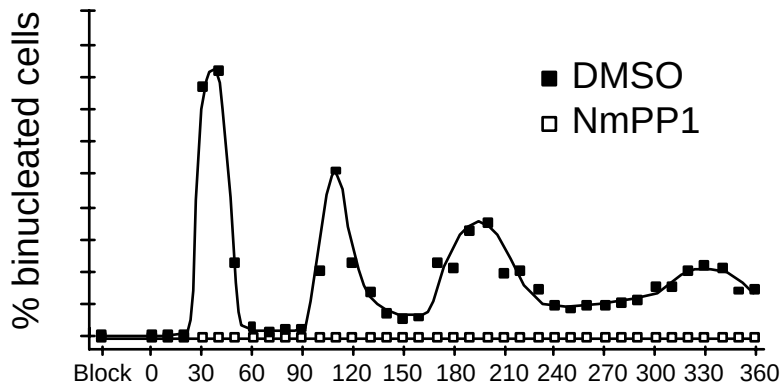
10 μM NmPP1 prevents S phase for 2.5 hours

The M or S decision depends on CDK activity level

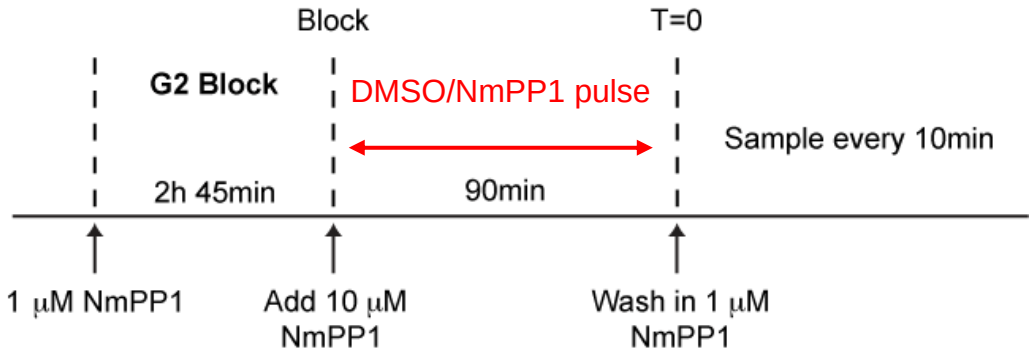
cdc13::cdc2as Δcdc2 Δcdc13Δcig1 Δcig2 Δpuc1

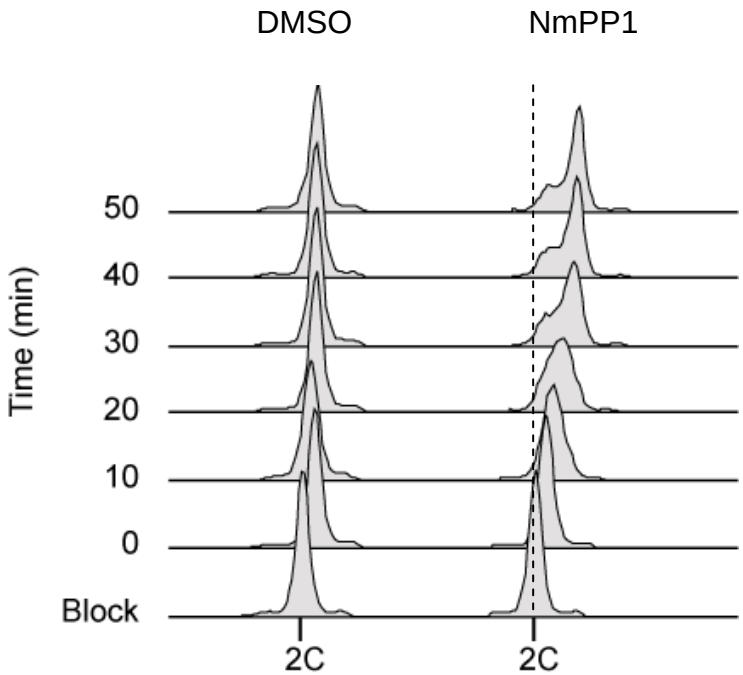


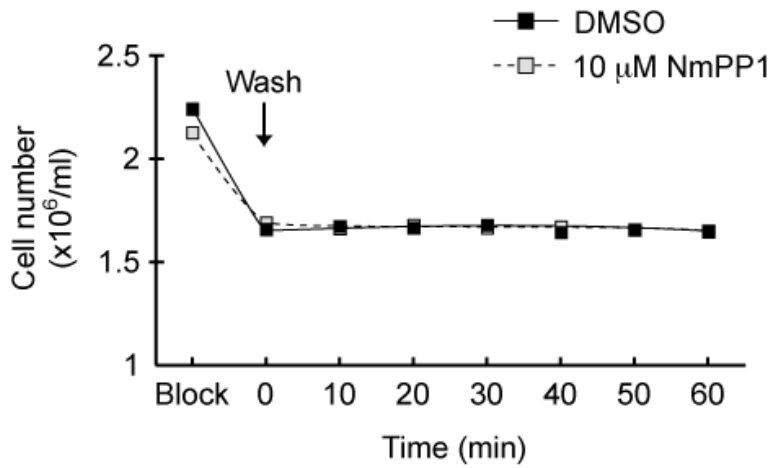




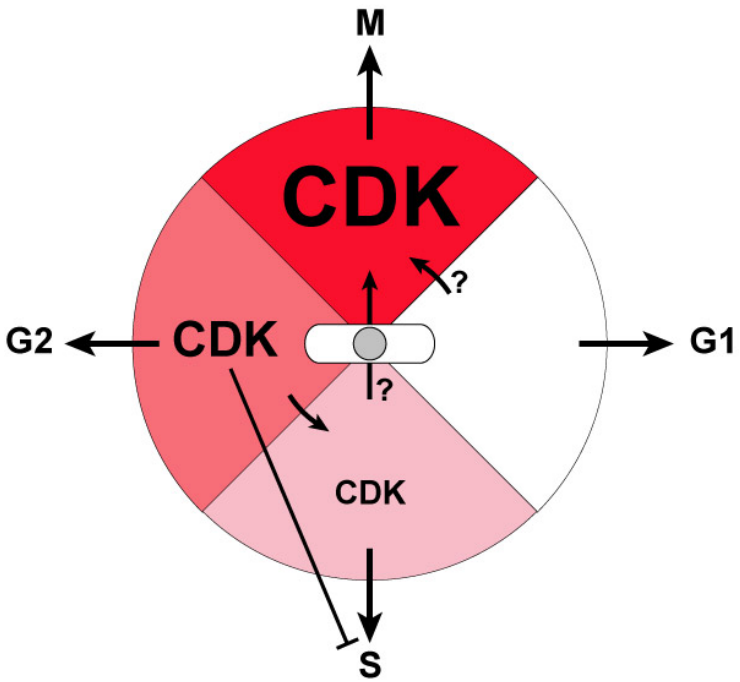
Transient inhibition of CDK activity in G2 allows resetting in G1

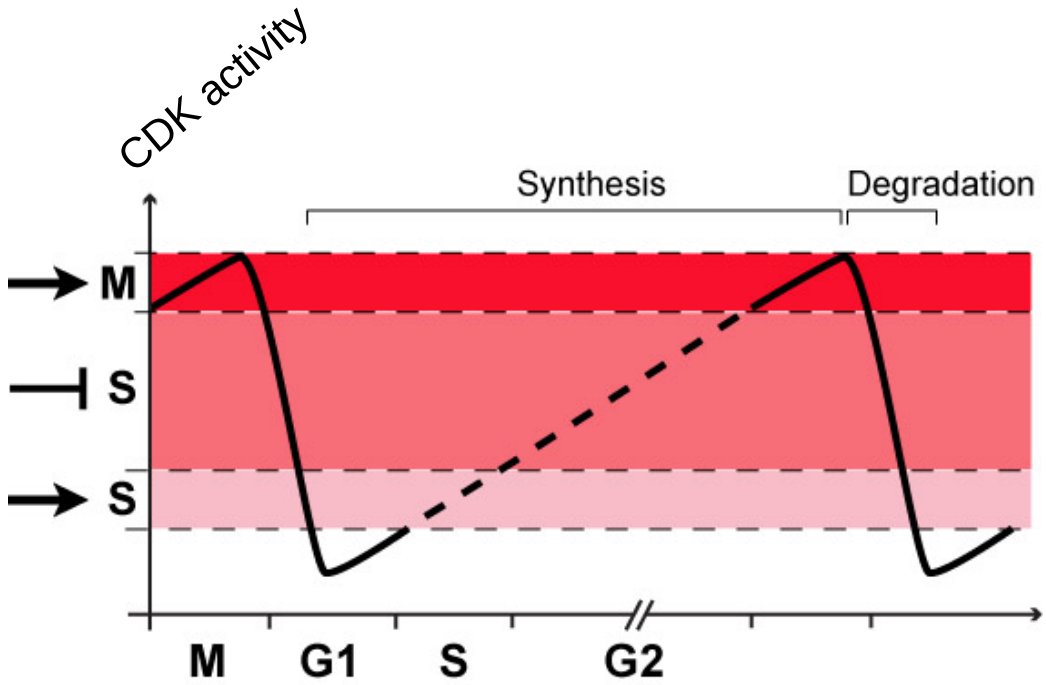




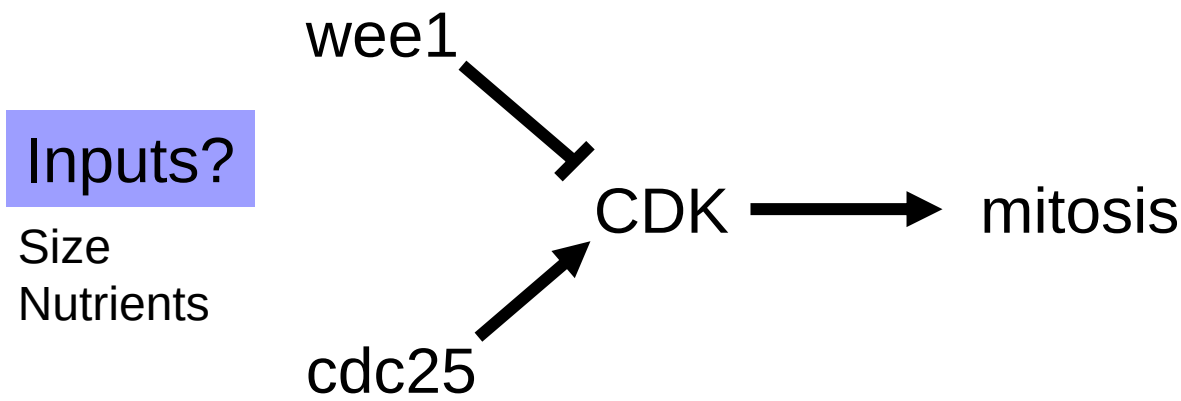


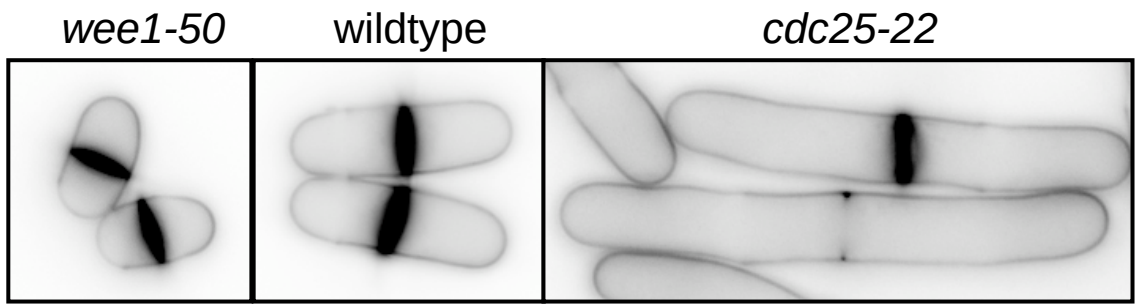
The minimal cell cycle is dependant upon changes in a single CDK activity





Regulation of the G2/M transition



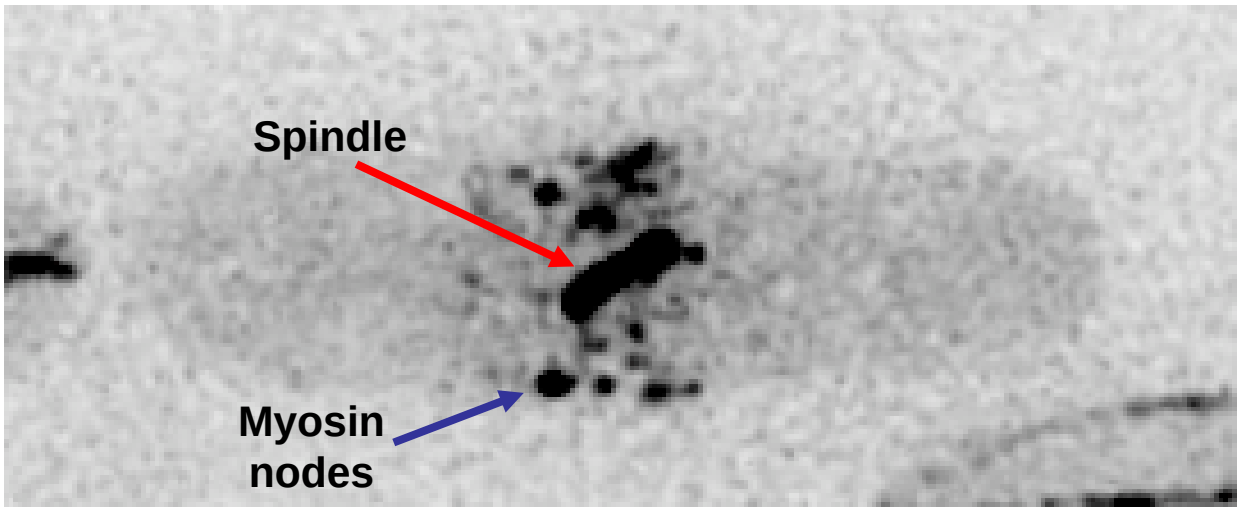


Jamie Moseley

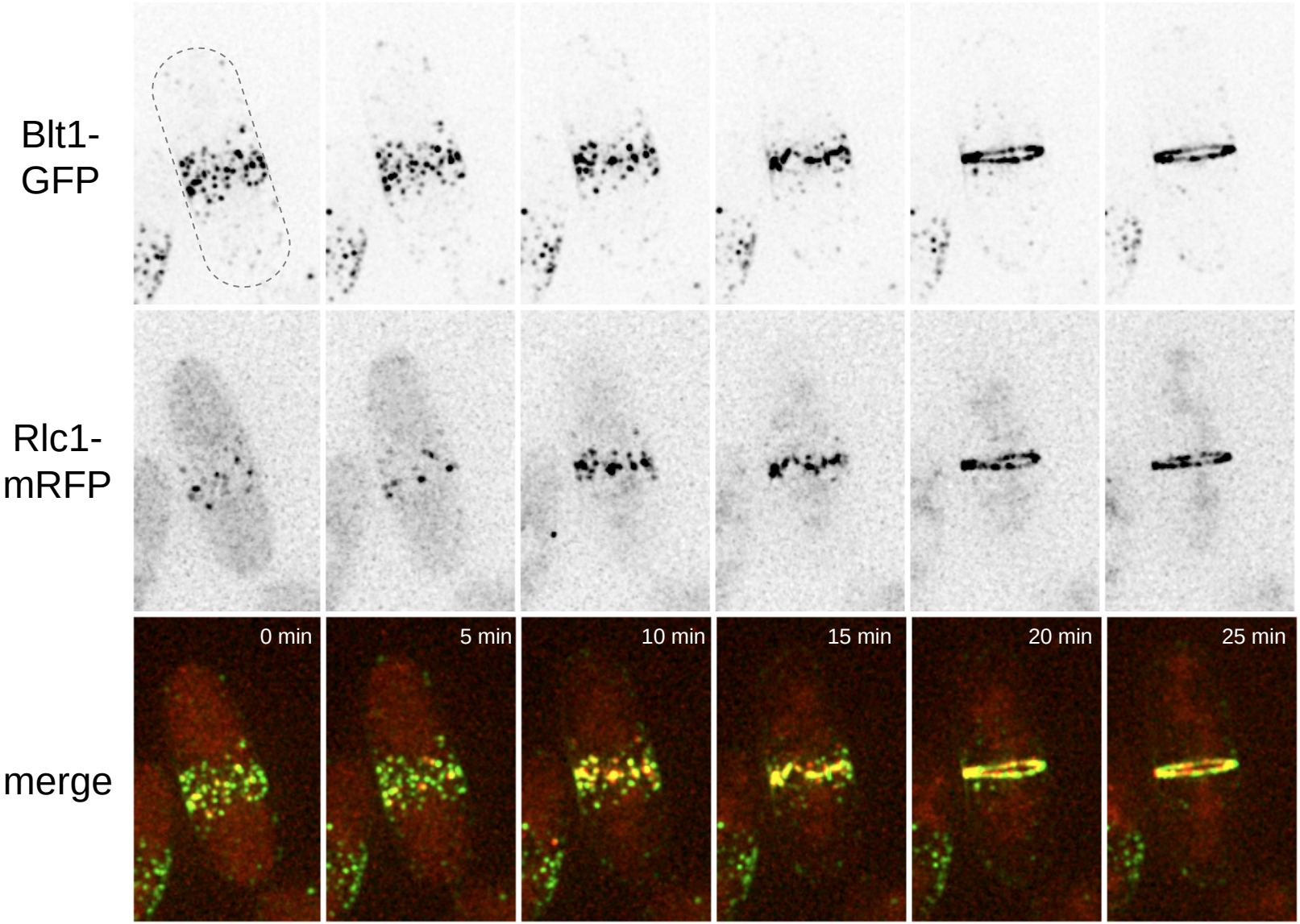
in collaboration with Anne Paoletti’s group
at Institut Curie

American Cancer Society

Cytokinesis myosin cortical nodes

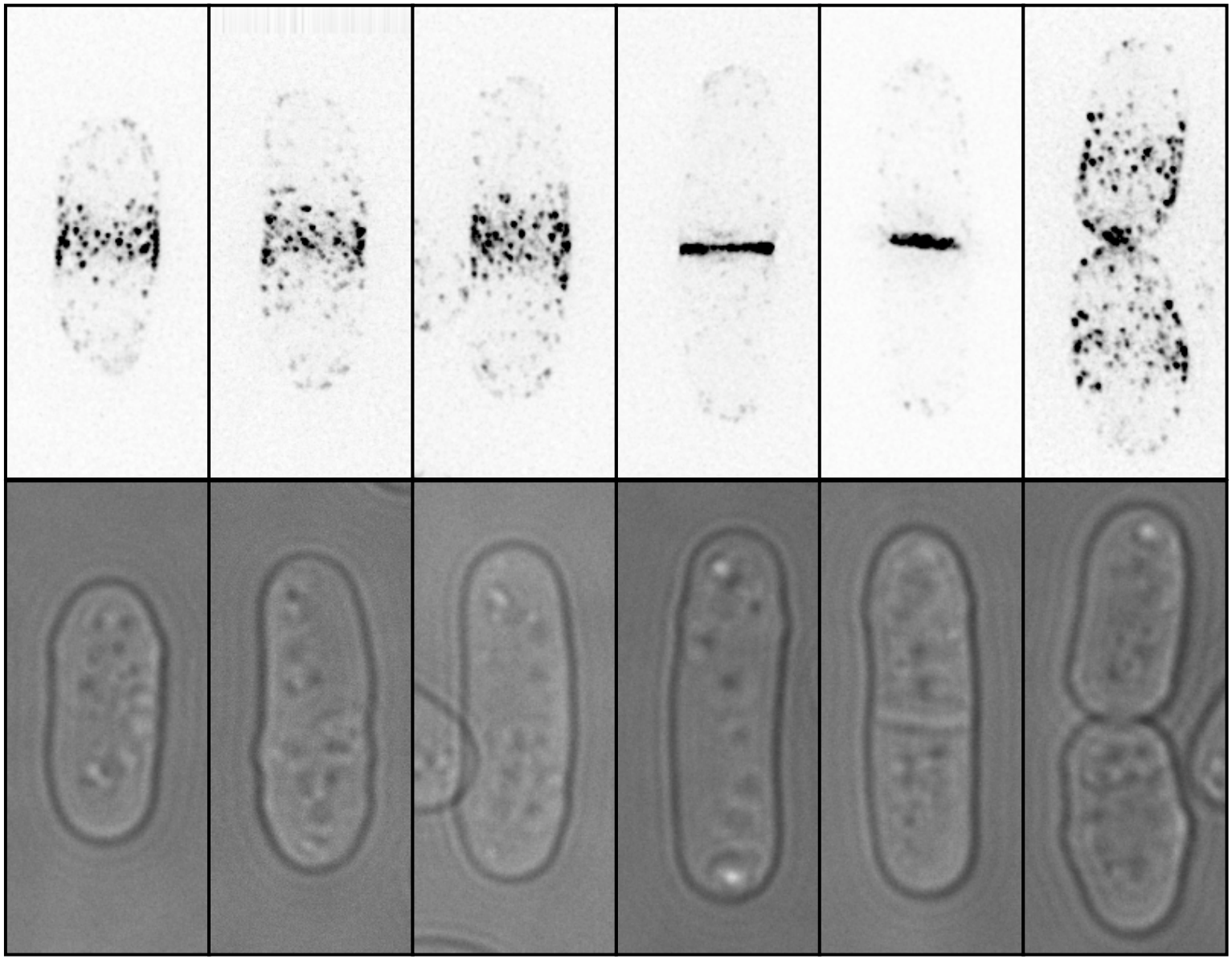


Blt1 is at myosin cortical nodes and actomyosin ring

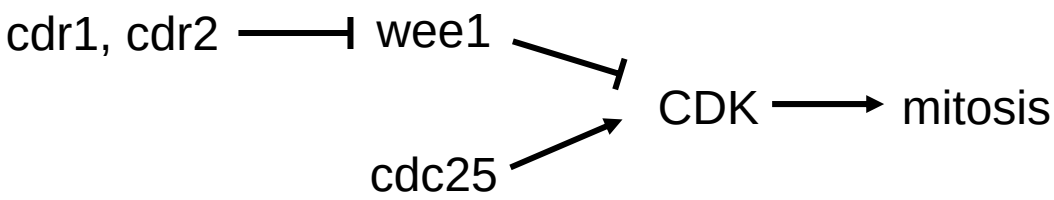


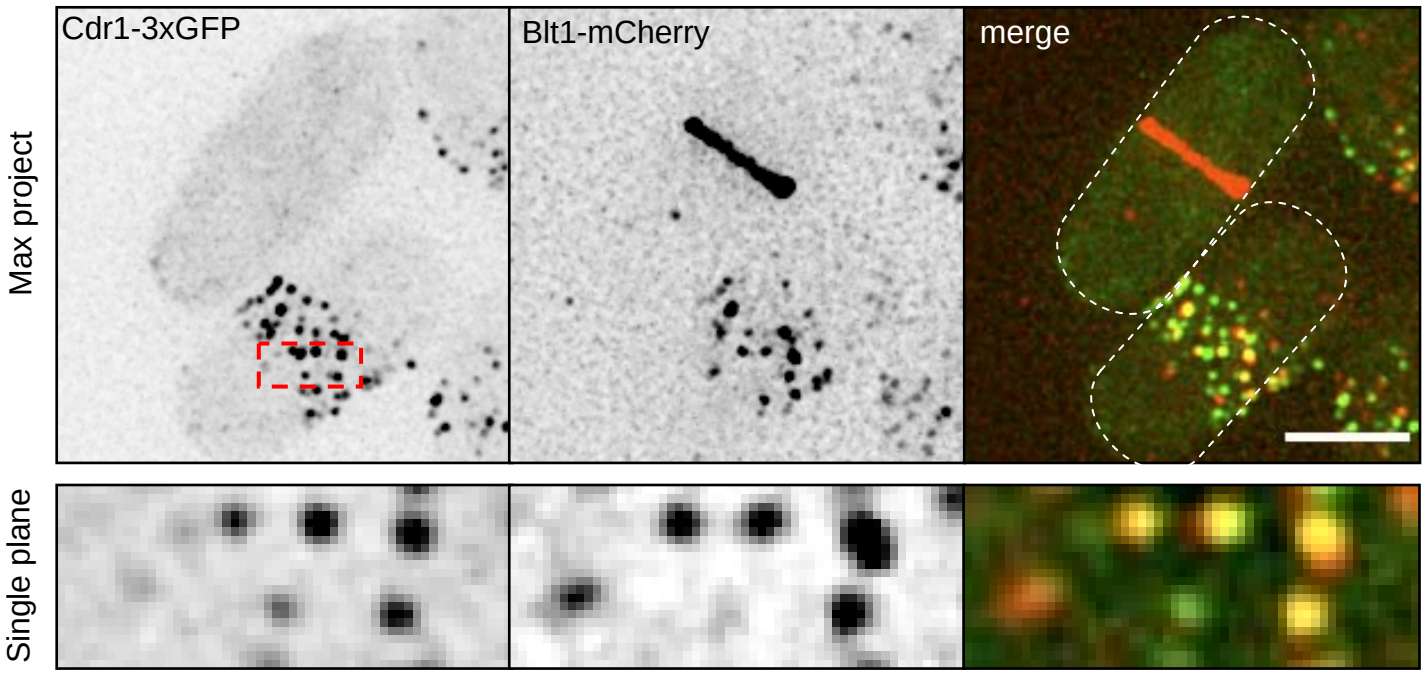
Unlike actomyosin, Blt1 localizes to cortical nodes throughout G2

Blt1-mEGFP

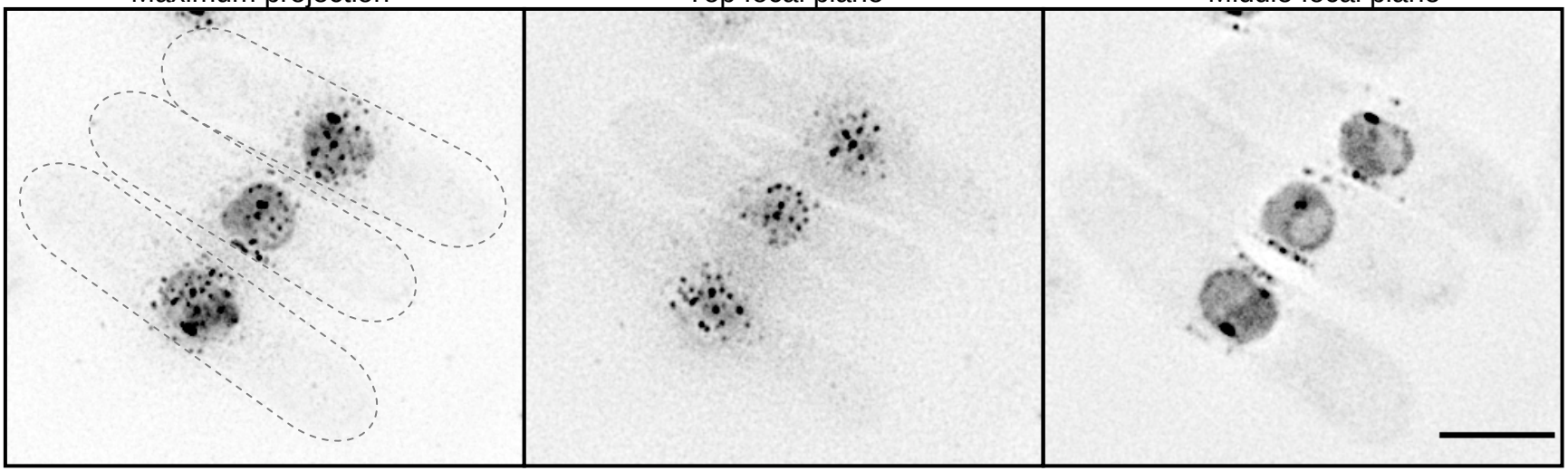


Both Wee1 inhibitory kinases associate with interphase nodes

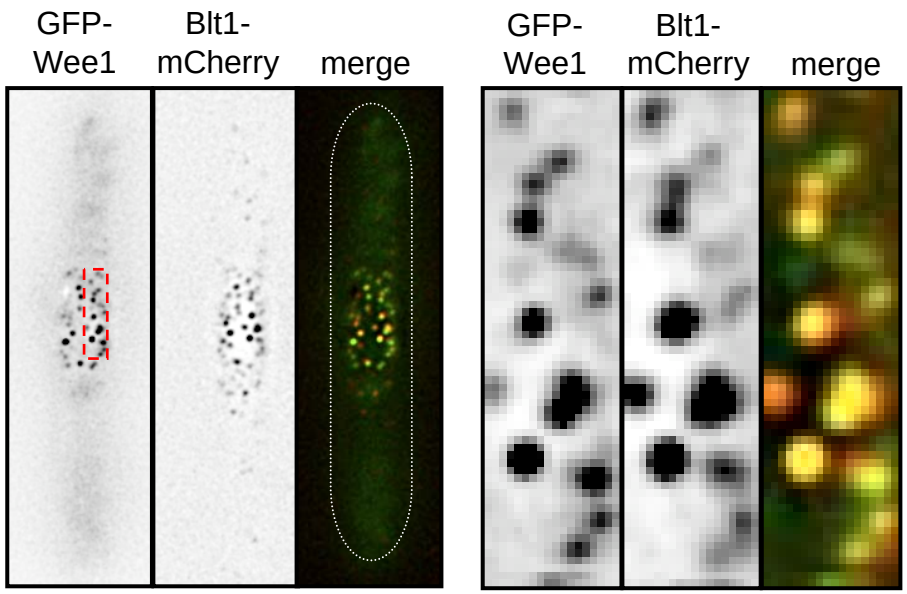




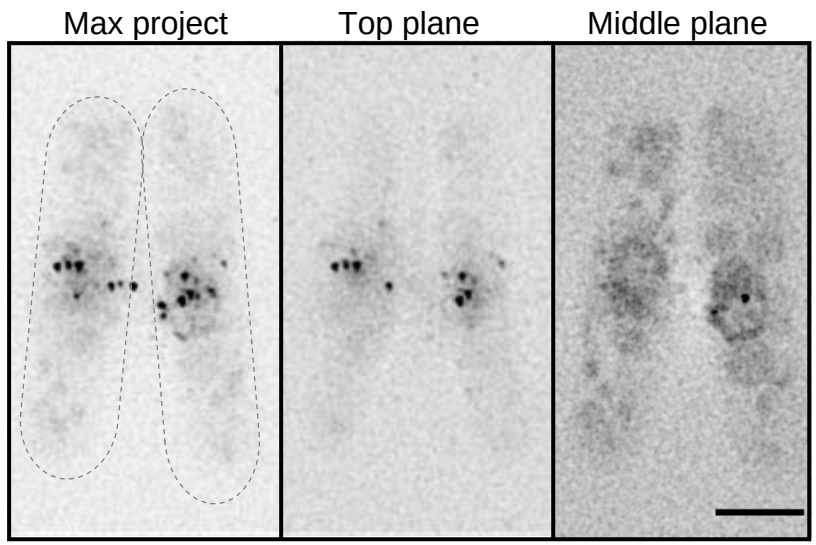
Wee1 localizes to nodes



P81nmt1-GFP-wee1

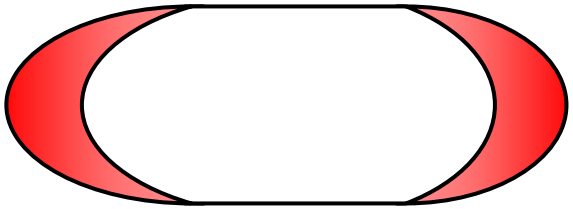


wee1-3xGFP::kanMX6

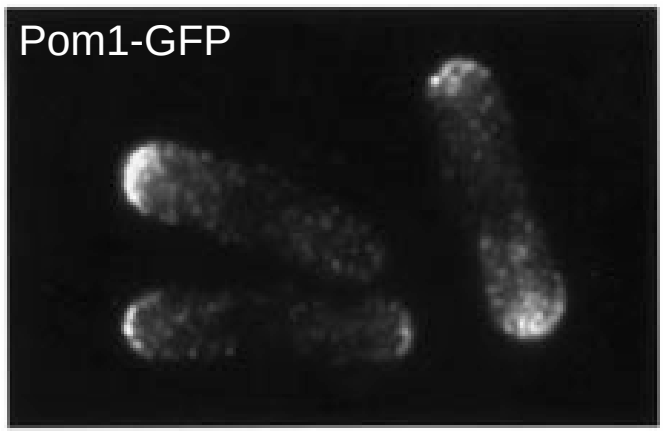


A

Negative cell tip signals

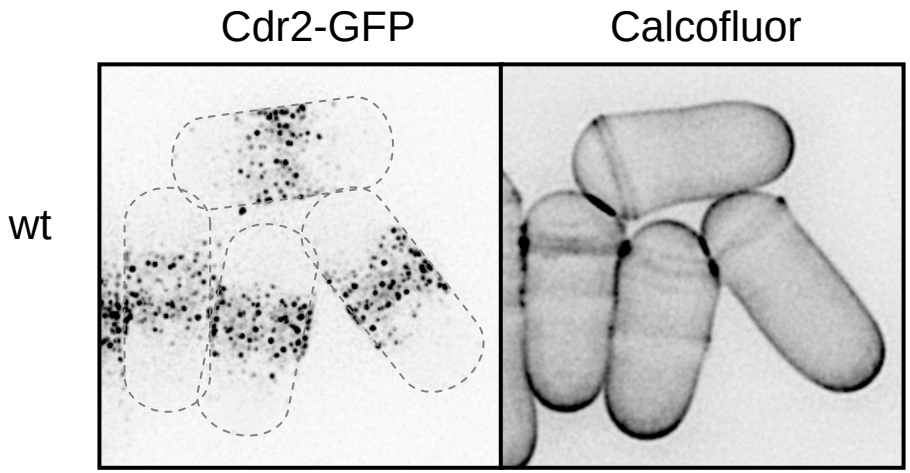


B



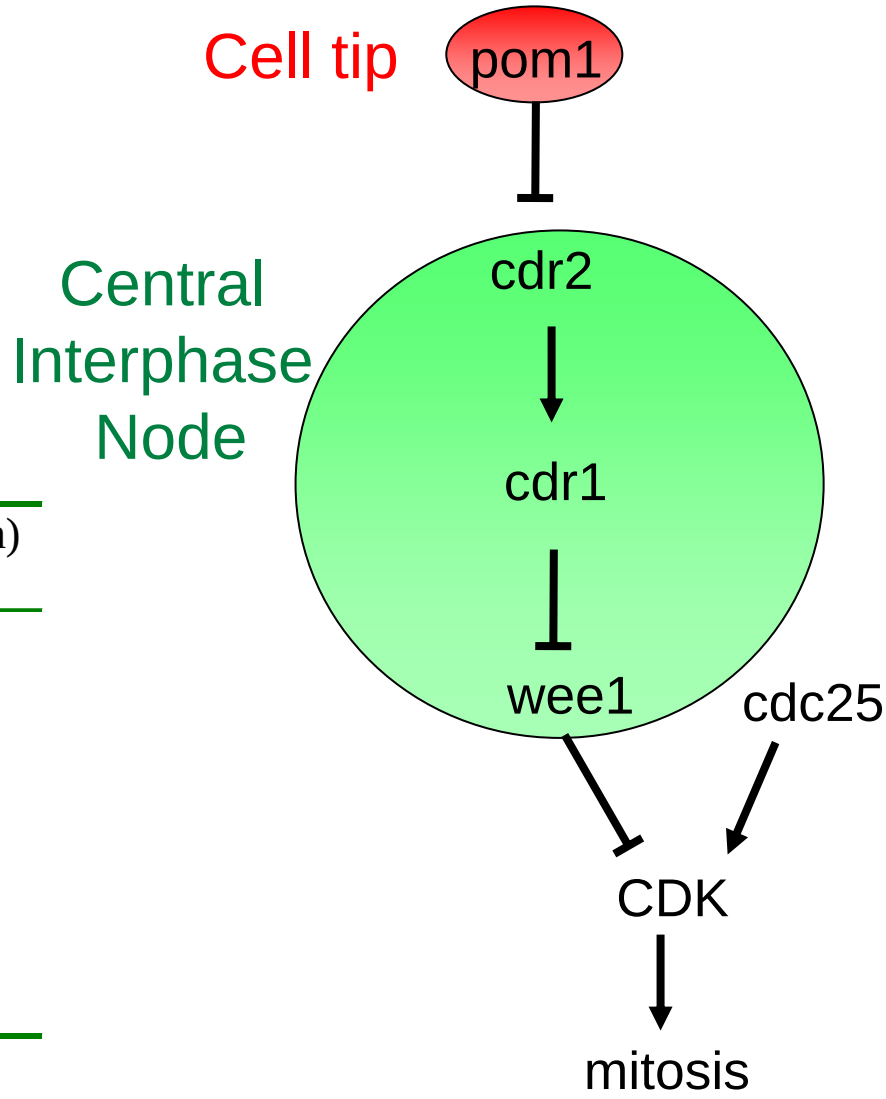
Bähler and Pringle, 1998
Bähler and Nurse, 2001

C

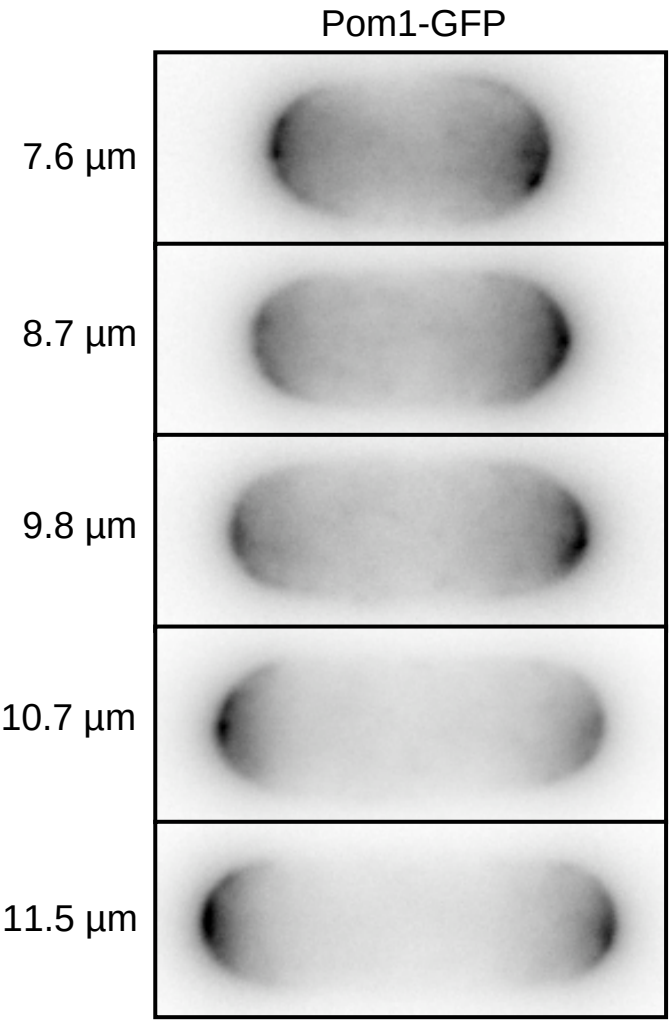


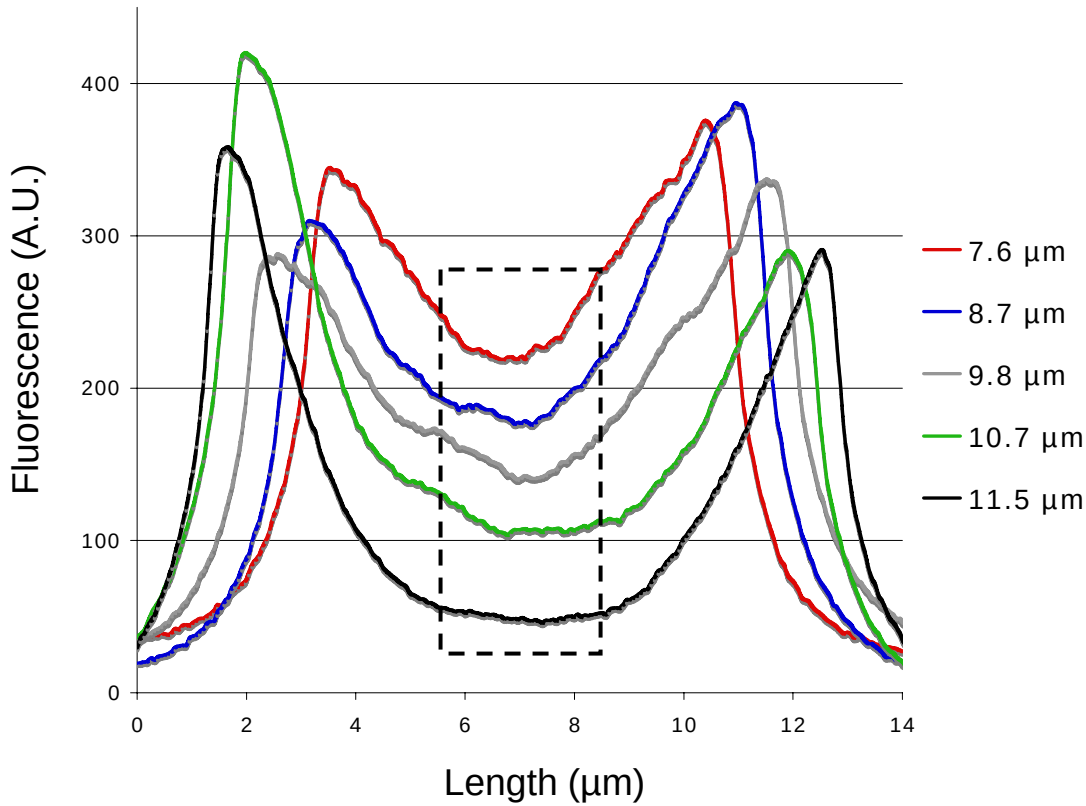
pom1 inhibits mitosis through cdr2 and wee1

Strain	Length at division (µm)
wildtype	13.7 ± 1.0
pom1Δ	11.7 ± 1.8
cdr1Δ	17.8 ± 1.4
pom1Δ cdr1Δ	17.2 ± 1.4

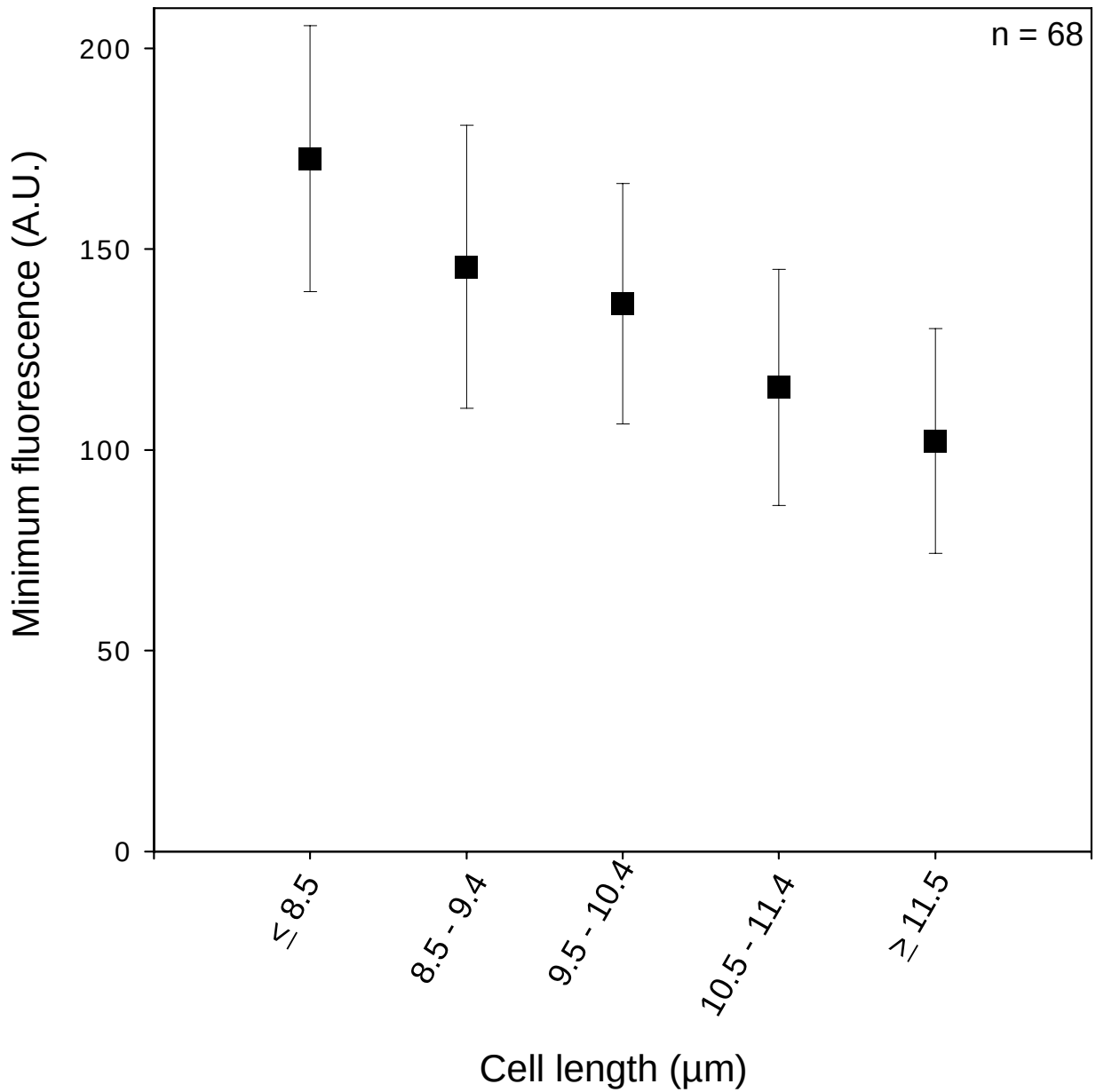


Pom1 polar gradient through the cell cycle



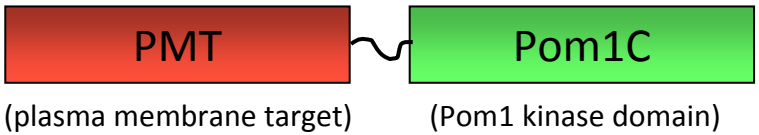


Pom1 levels at cell center decrease during G2



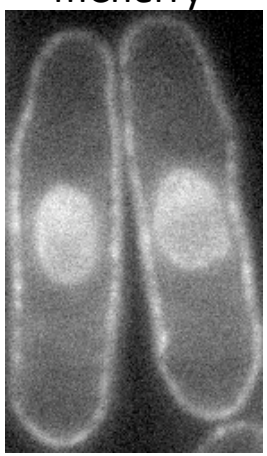
Ectopic targeting of Pom1 to cell middle disrupts Cdr2 localization and cell cycle

Chimera

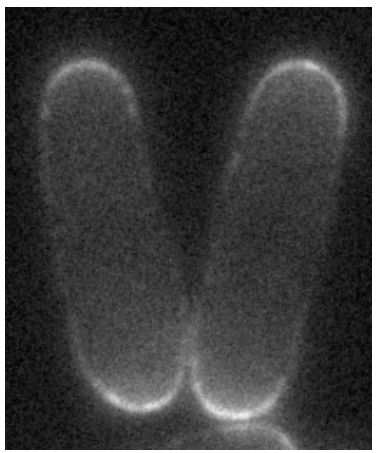


Strain	Length at division (μm)
WT	14.3 ± 0.9
chimera	19.7 ± 1.9
cdr2Δ	19.9 ± 1.8
cdr2Δ + chimera	19.1 ± 1.9
cdc25-22	23.8 ± 2.6
cdc25-22 + chimera	39.3 ± 7.9

Chimera-mCherry

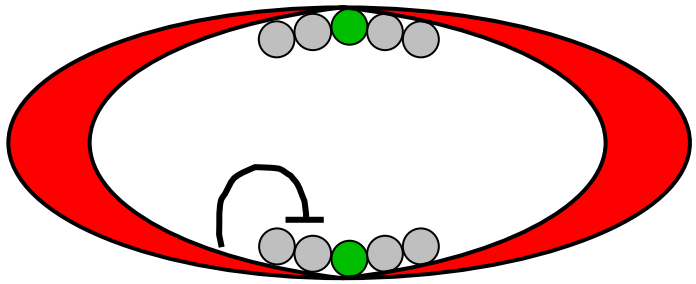


Pom1-GFP

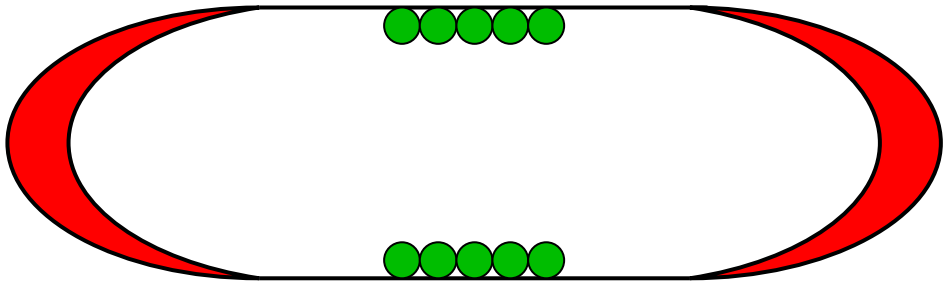


Model for coordinating polarized growth with mitosis

Early G2



Late G2



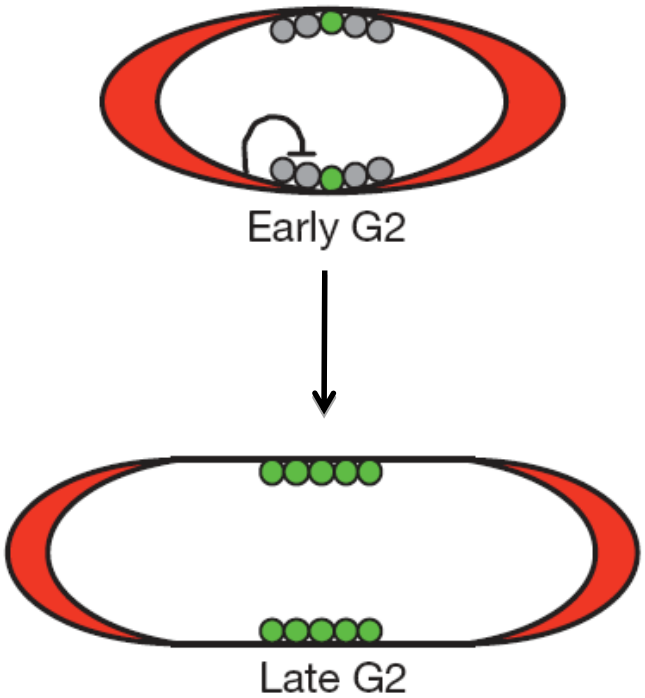
Pom1

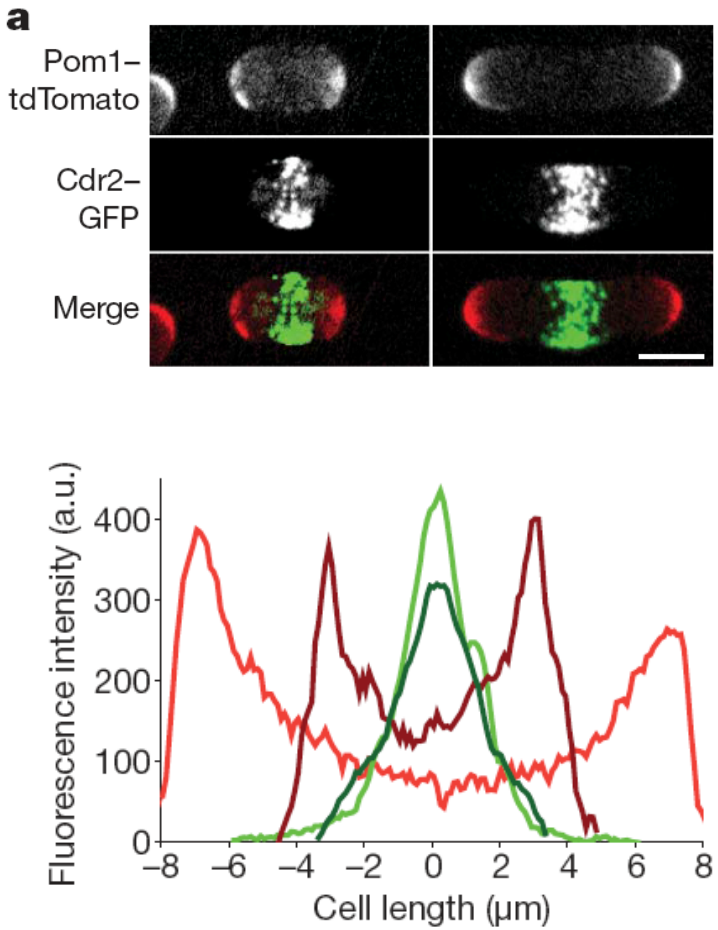
Cdr2 (ON)

Cdr2 (OFF)

	Cdr2	Wee1	CDK
Early G2	OFF	ON	Low
Late G2	ON	OFF	High

Combined picture of pathway linking cell size and mitotic entry



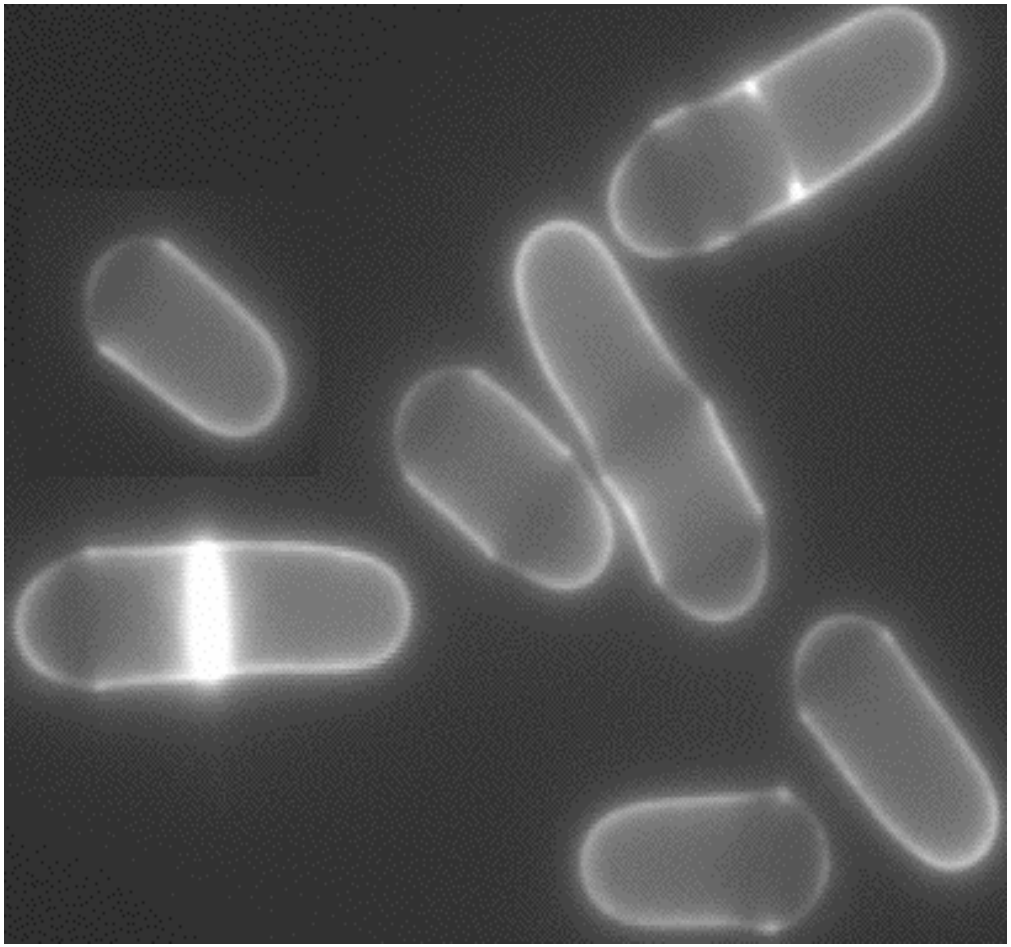


Pom1 (small cell) Cdr2 (small cell)

Pom1 (large cell) Cdr2 (large cell)

Martin and Berthelot-Grosjean 2009

Schizosaccharomyces pombe cells



- Genome 12. 4Mb (exc rDNA)
- 3 chromosomes
- 4914 protein coding genes
- 46% have introns

Jacky Hayles

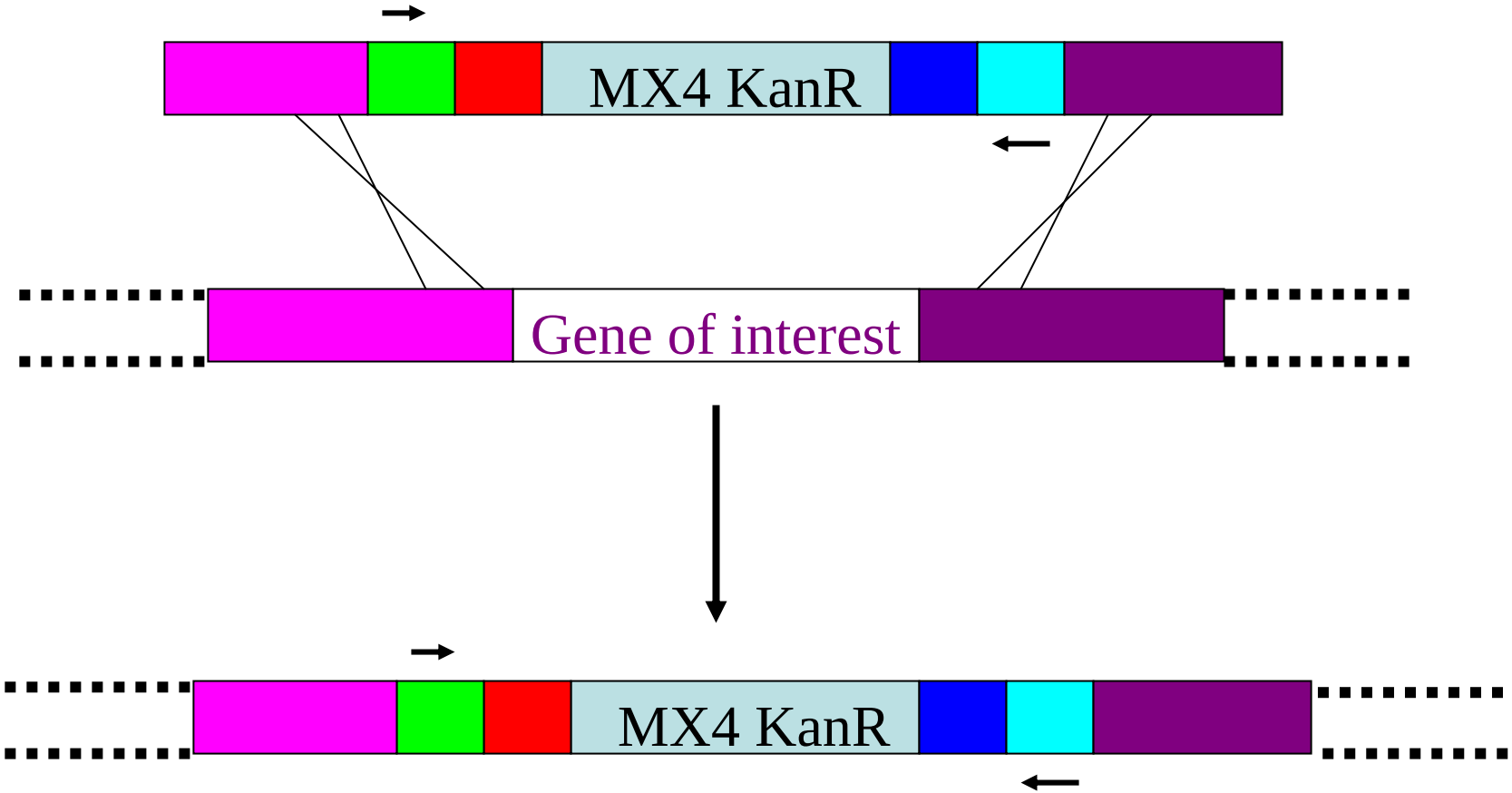
Fission Yeast
Genome Wide Deletions

KRIBB	Kwang-Lae Hoe, Dong-Uk Kim
Bioneer	Han-Oh Park
Sanger	Valerie Wood
CRUK	Jacky Hayles

\W=528,457

Genome wide systematic deletions

Constructed in the diploid h+/h+
ade6-M210/ade6-M216, leu1-32/leu1-32, ura4-D18/ura4-D18



Universal priming sequences

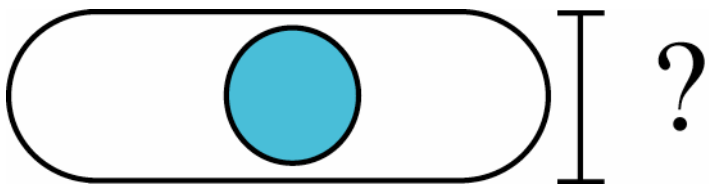
Unique up & down tags

Progress to date

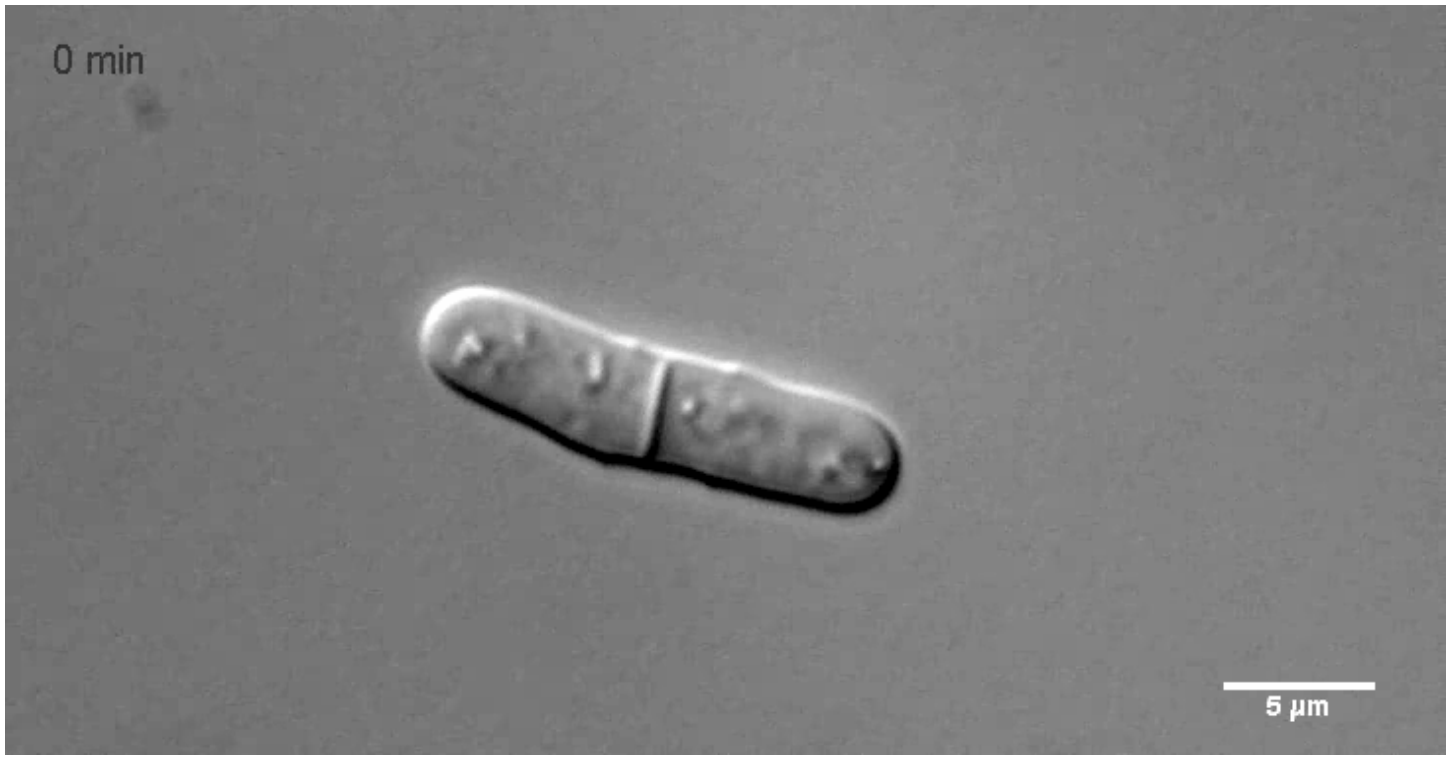
- **Total data-set of genes:** **4914 (100%)**
- **Deletions constructed to date:** **4836 (98.5%)**
- **Essential genes:** **1260 (26%)**

Encoding a Cellular Ruler

Felice Kelly

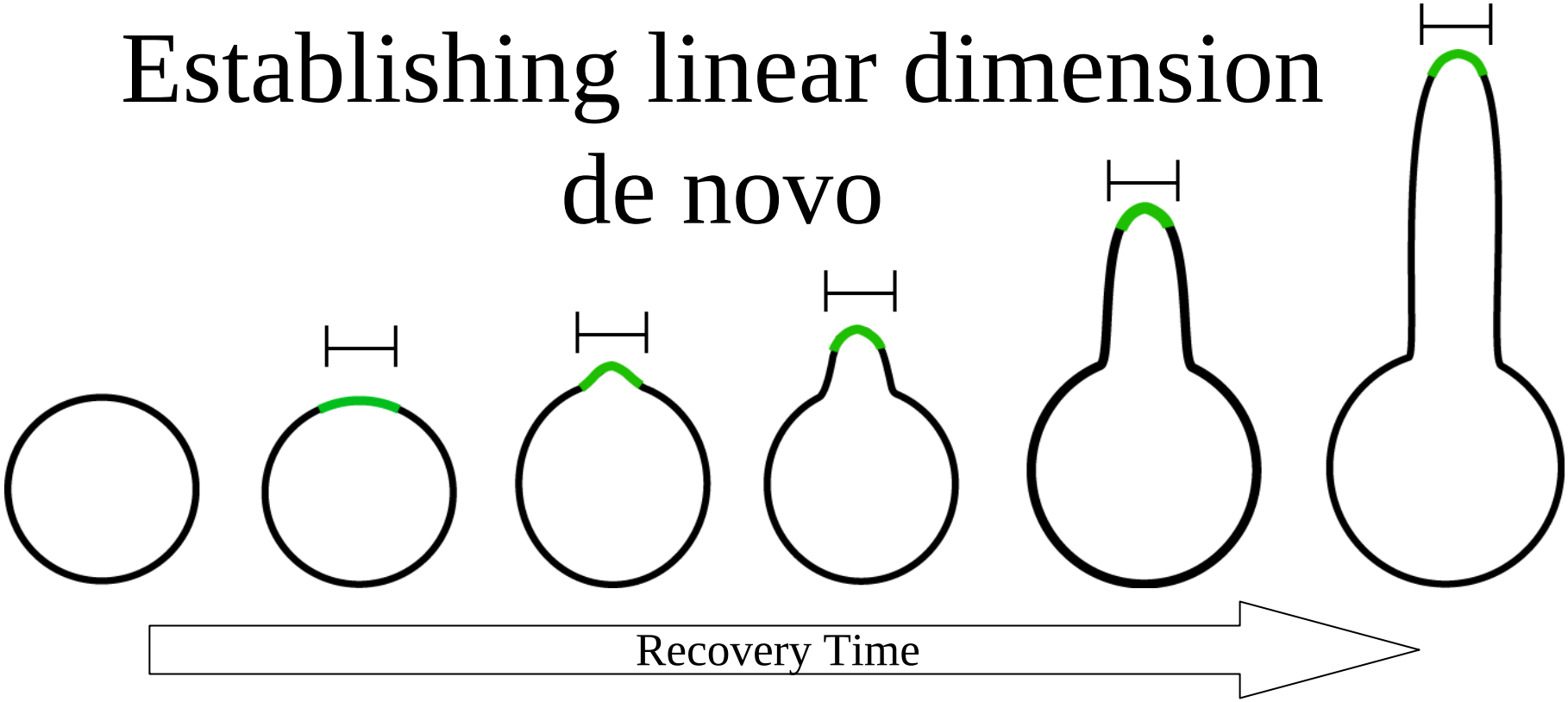


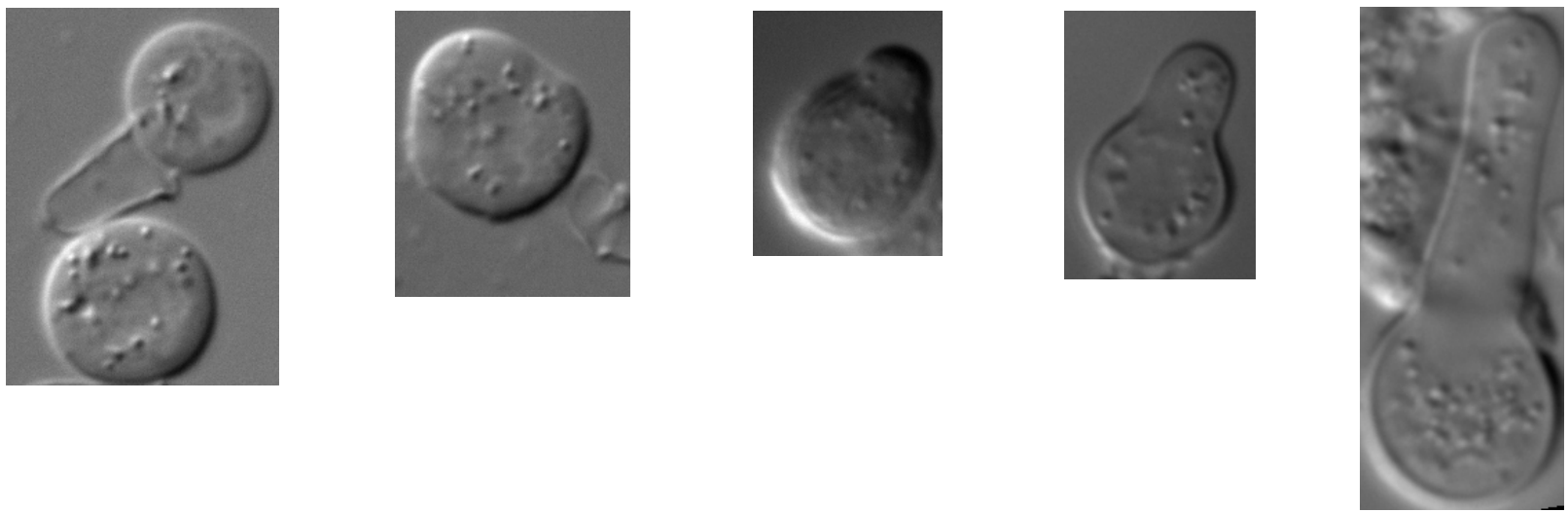
Fission Yeast grow with a constant width



Frank Neumann

Establishing linear dimension de novo



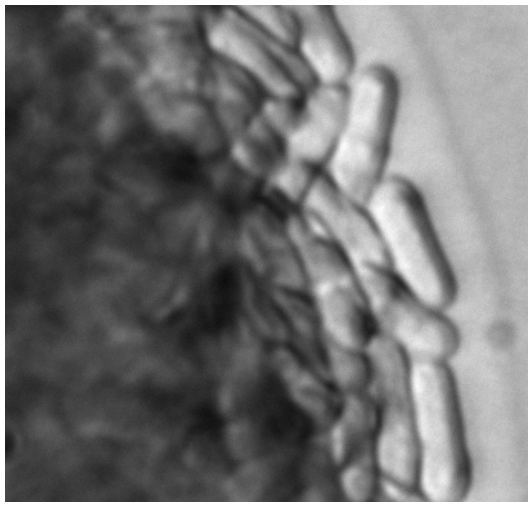


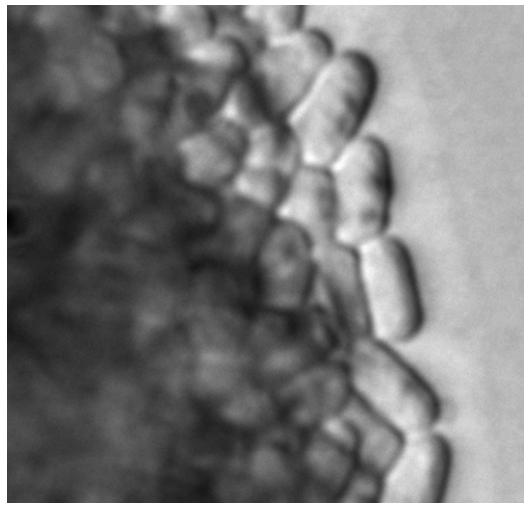
Screening for a subtle phenotype

Wild-type cells

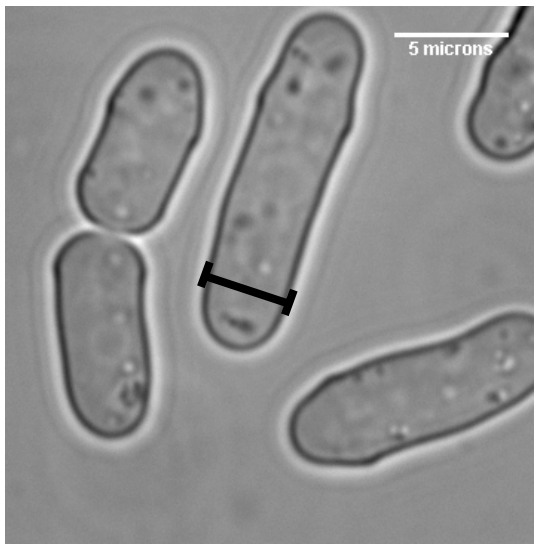
Wide mutant cells

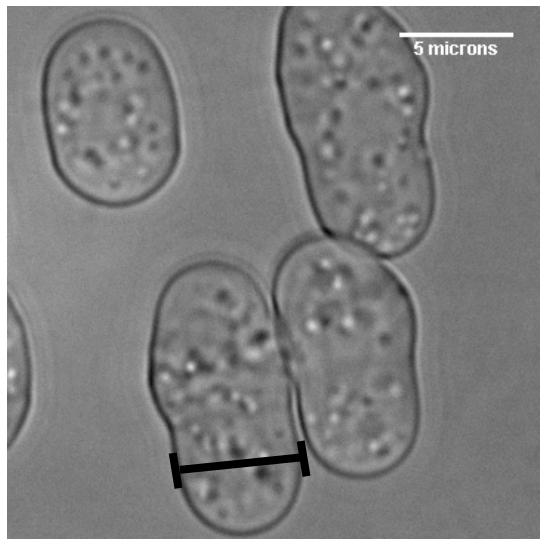
Initial Categorization
by Jacky Hayles





Directed Screen
of Exponentially
Growing Cells





Genomic screen for width mutants

3488 viable
deletions

↓

142 putative
width mutants

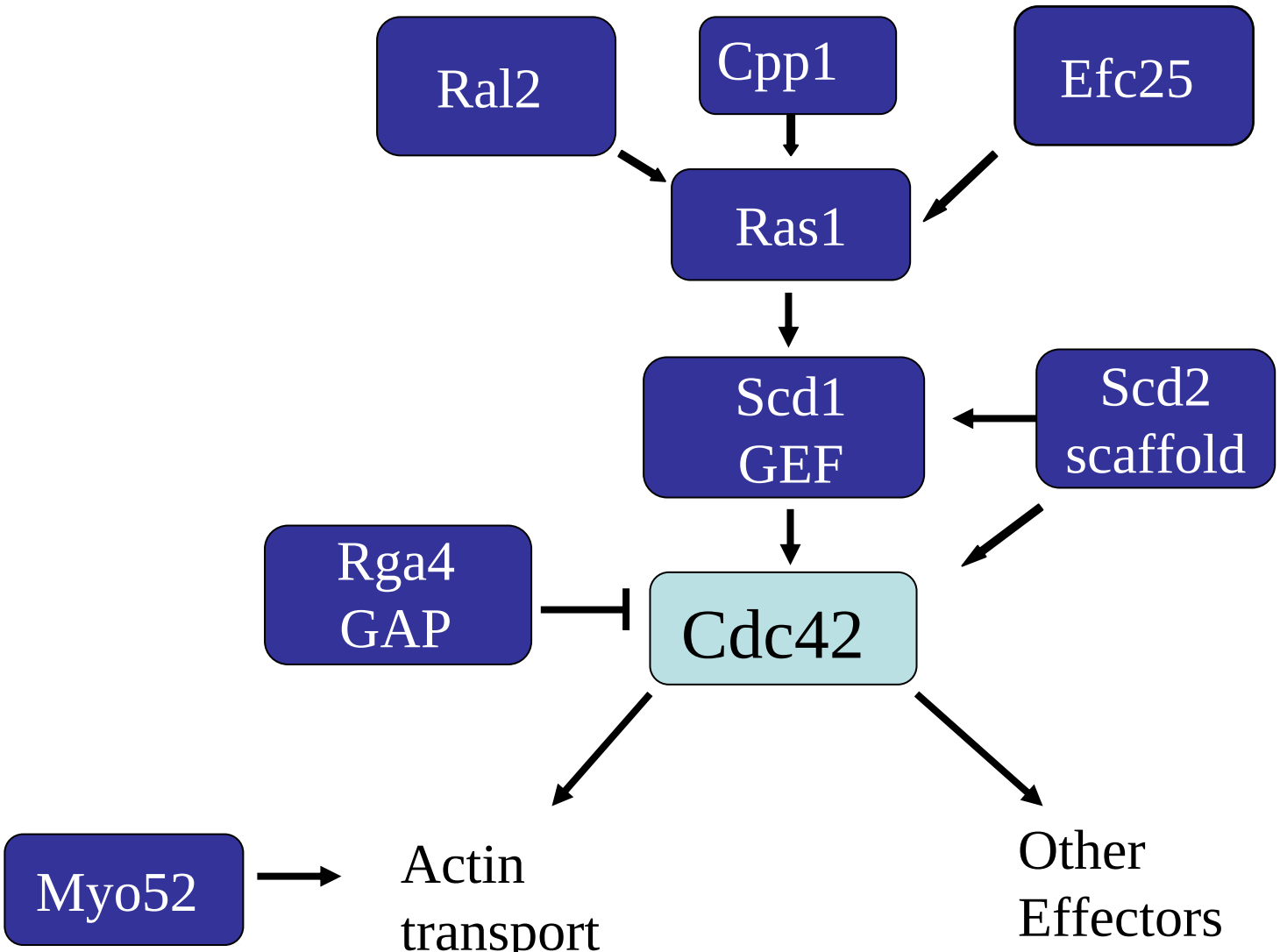
↓

12 confirmed
width mutants

↓

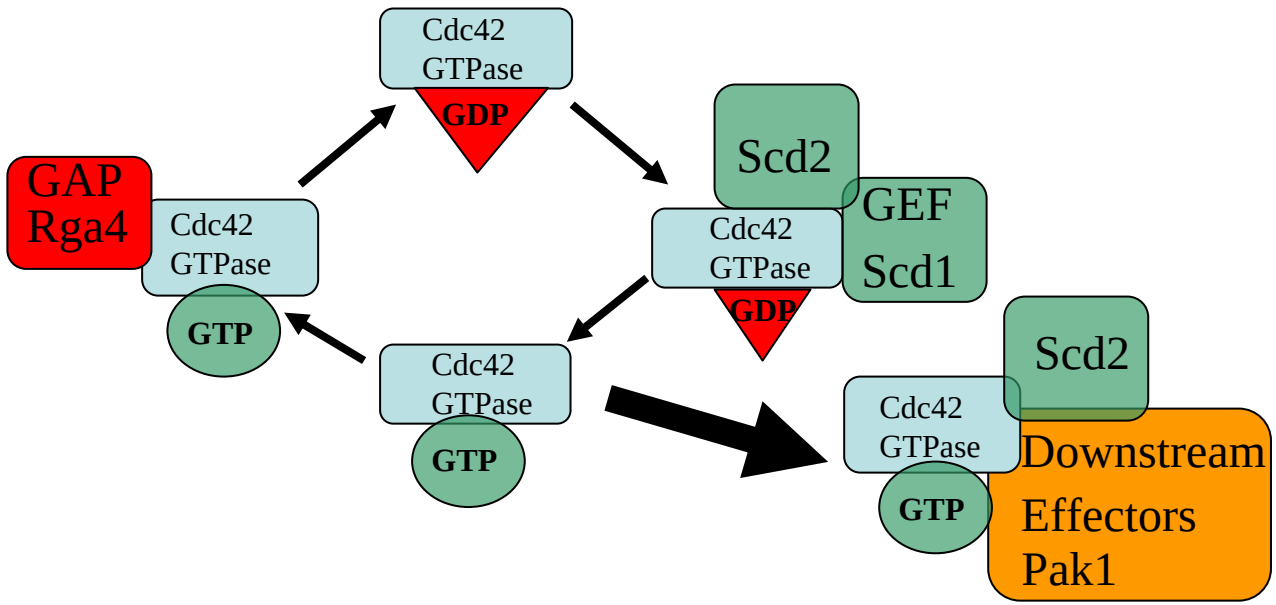
8 genes in a
single pathway

Genomic screen revealed a conserved pathway controlling cell width



Cdc42 is a conserved polarity regulator in all eukaryotes

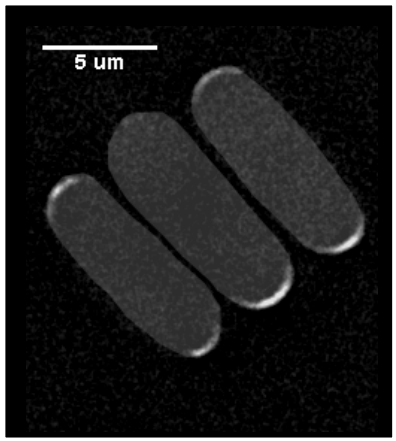
- Cdc42 binds GTP (active form) or GDP (inactive form).
- Wide ranging activities include filapodia formation and polarization in mammalian cells.
- Many functions involve actin cytoskeleton polarization.



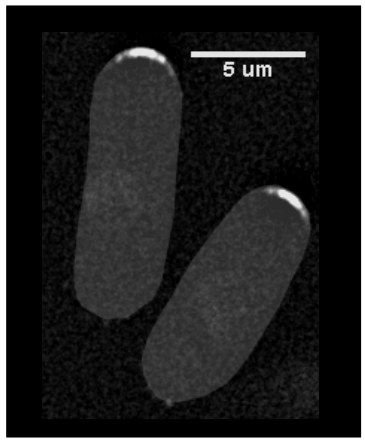
Cdc42 activators localize to the growing cell tip

GFP signal
and
cell shape

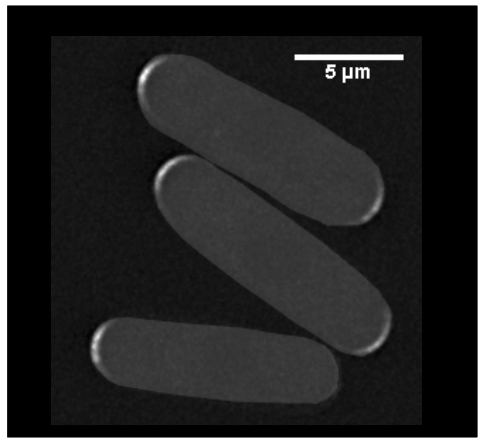
Scd1-GFP
(Cdc42 GEF)

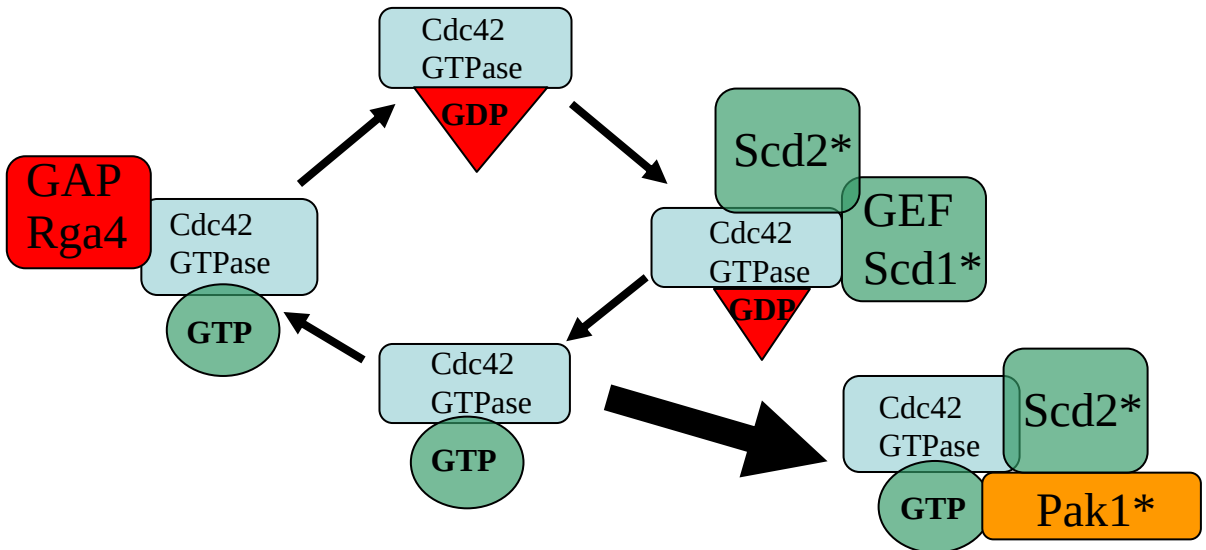


Scd2-GFP
(scaffold)



Pak1-GFP
(effector kinase)





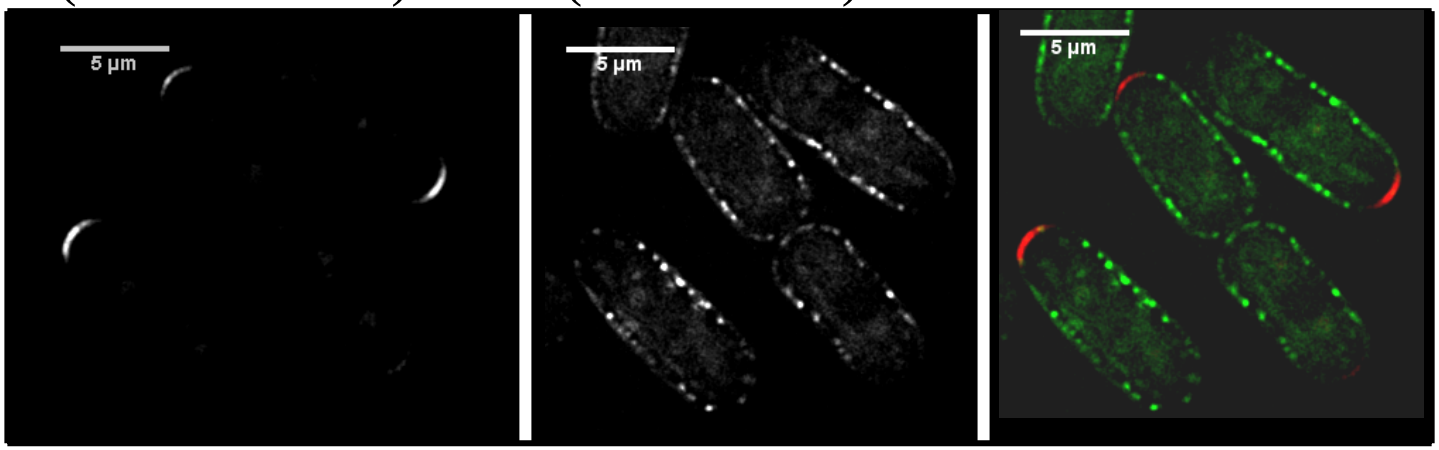
Negative Regulator of Cdc42 is excluded from cell tips

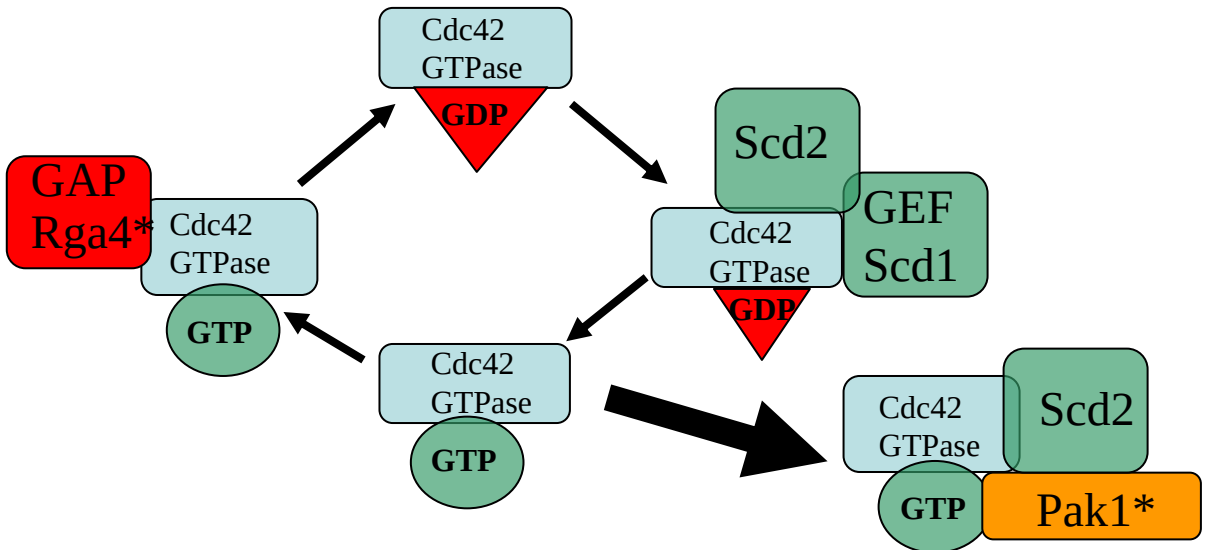
Pak1-mCherry (effector kinase)

Rga4-GFP (Cdc42 GAP)

merge

Fluorescent protein signal



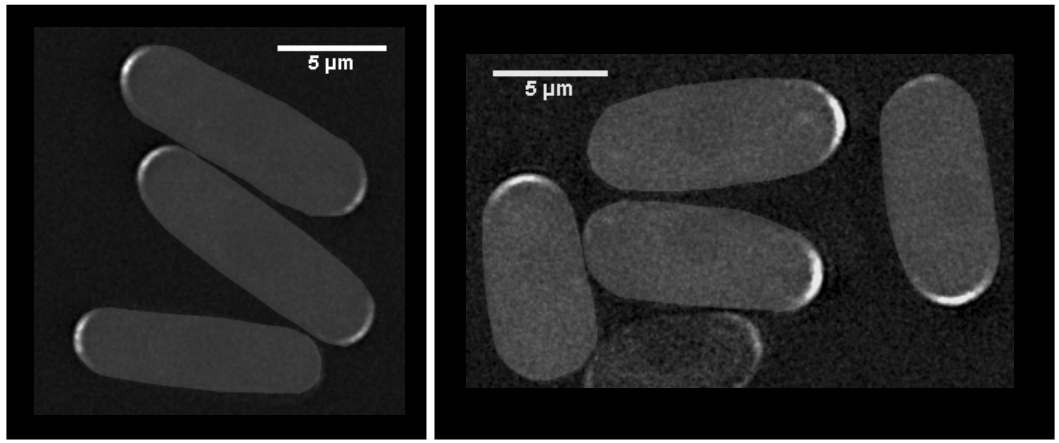


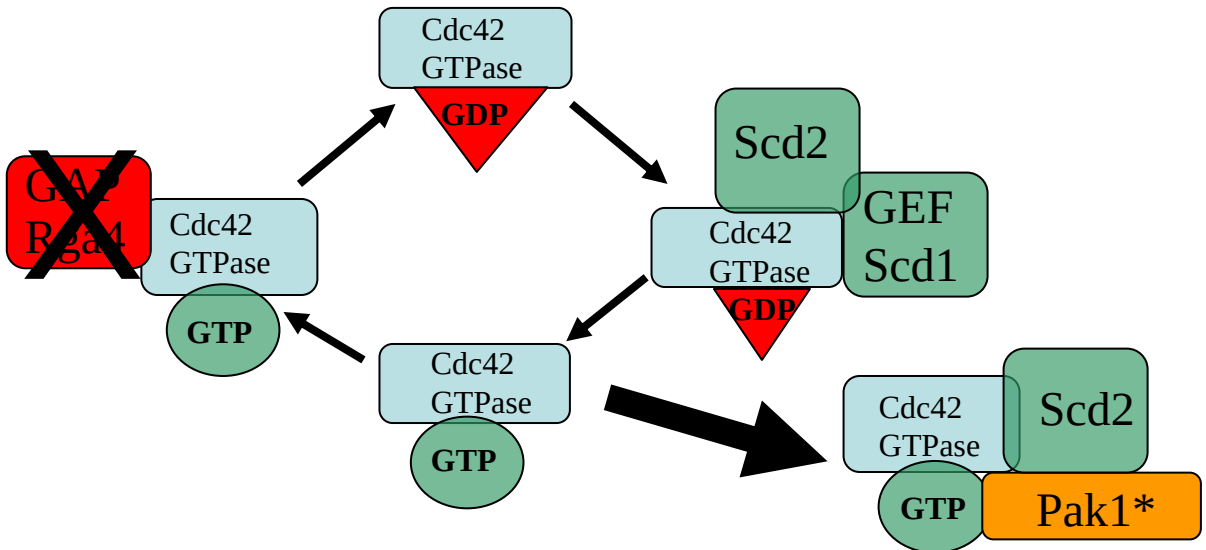
Rga4 is required for restriction of Pak1

Pak1-GFP

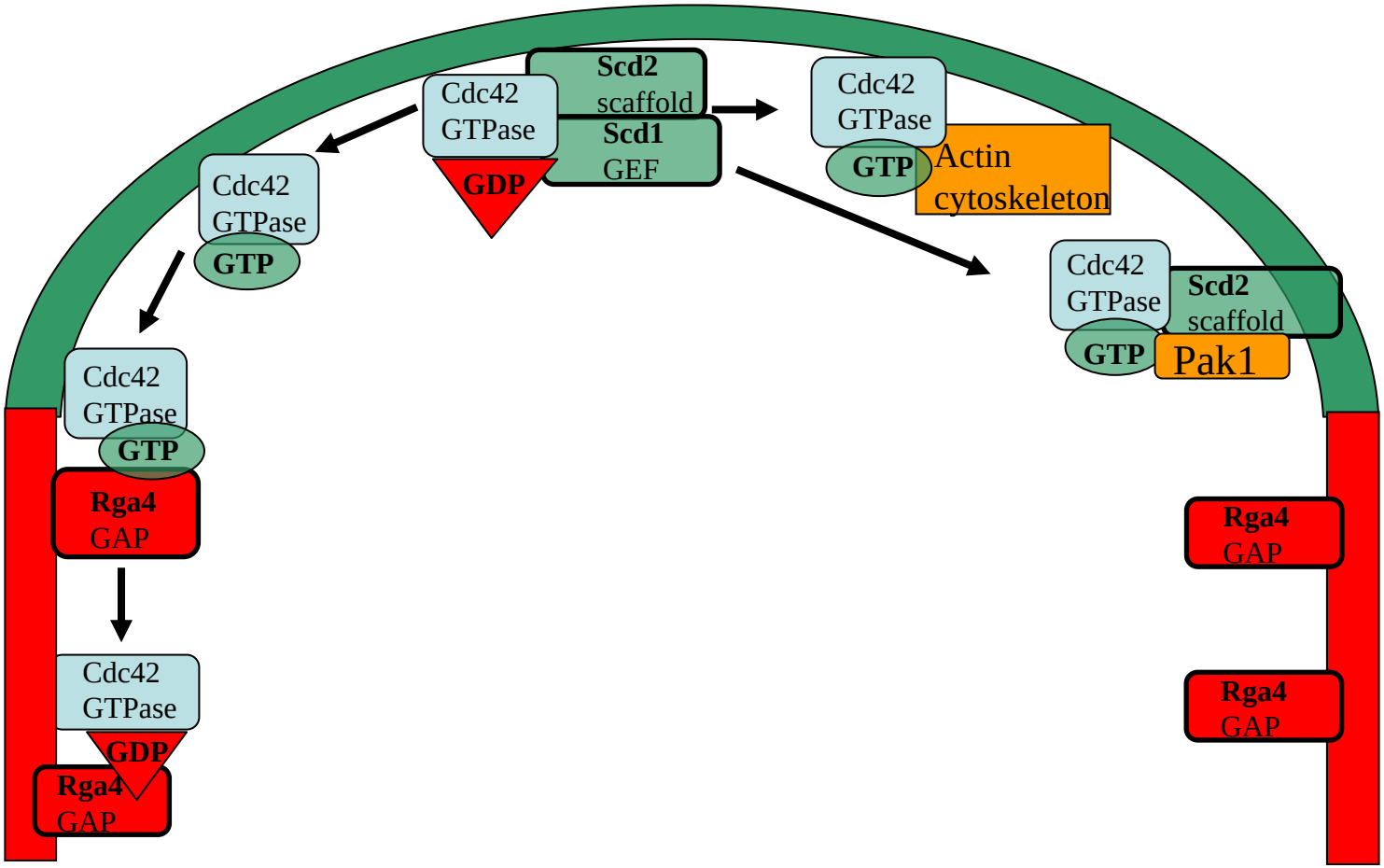
rga4Δ Pak1-GFP

GFP signal



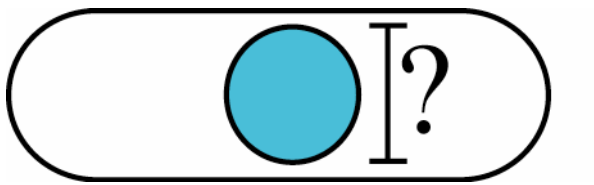


Rga4 forms a negative regulatory zone which limits cell width

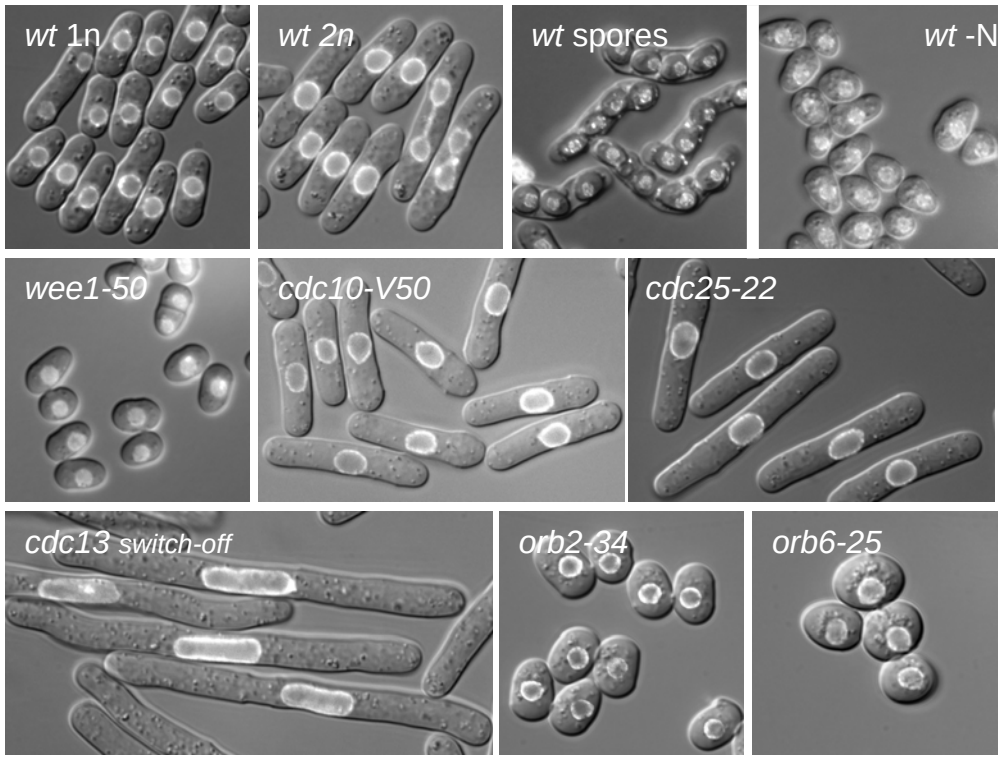


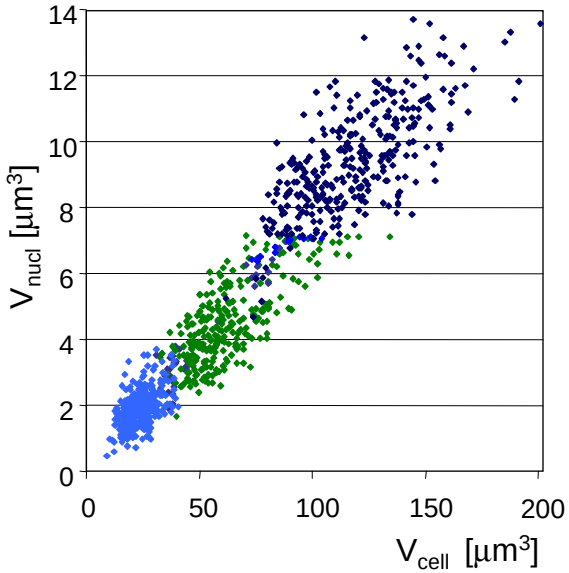
Controlling Nuclear Size

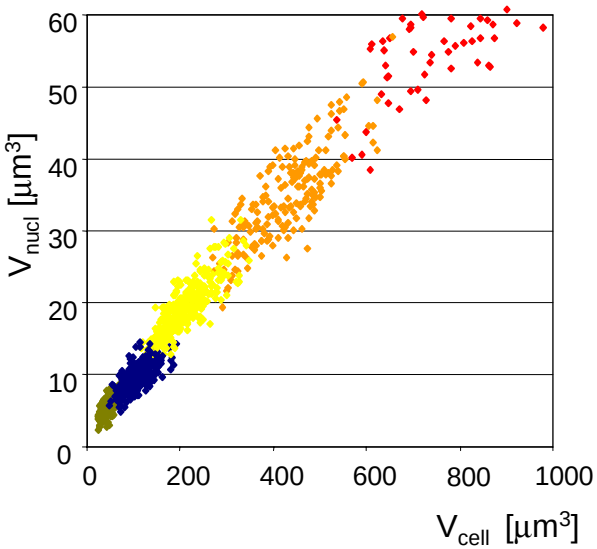
Frank Neumann



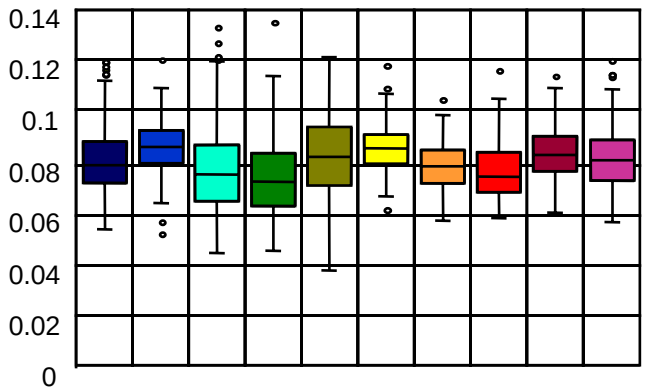
Cell size and shape



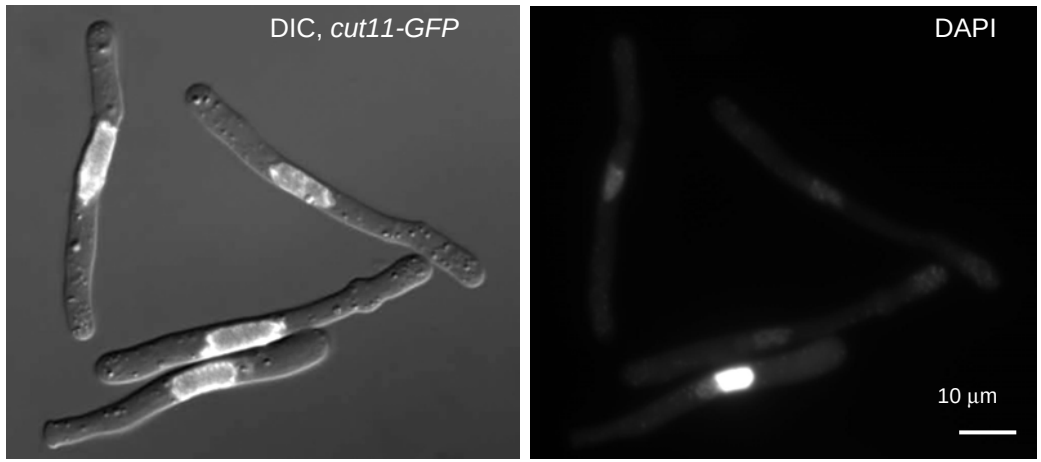


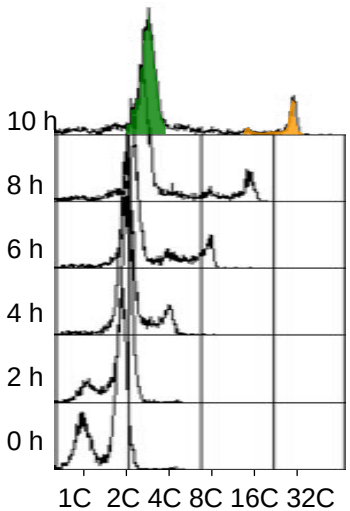


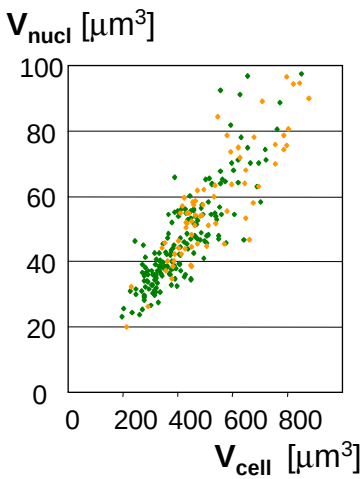
- *wt* haploid
- *wt* diploid
- *wt* spores
- *wt* –N starvation
- *wee1-50* 5h 36.5ºC
- *cdc10-V50* 4.5h 36.5ºC
- *cdc25-22* 3h 36.5ºC
- *cdc13* switch-off 10h
- *orb2-34* 5h 36.5ºC
- *orb6-25* 5h 36.5ºC

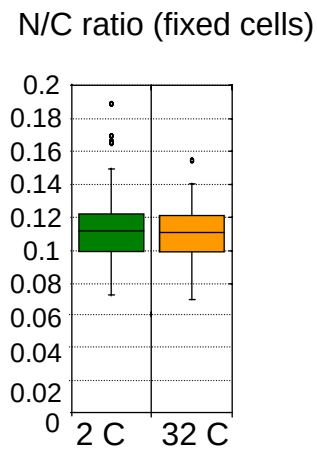


DNA content

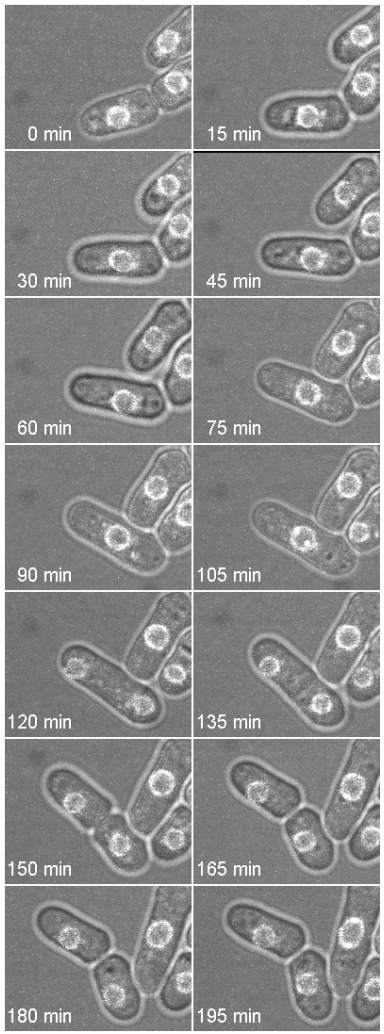

DIC, *cut11-GFP*
DAPI
10 µm

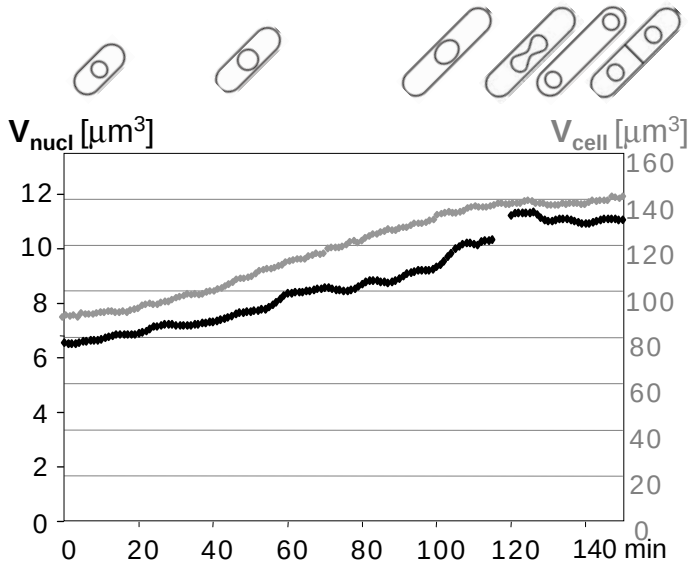

10 h
8 h
6 h
4 h
2 h
0 h
1C 2C 4C 8C 16C 32C

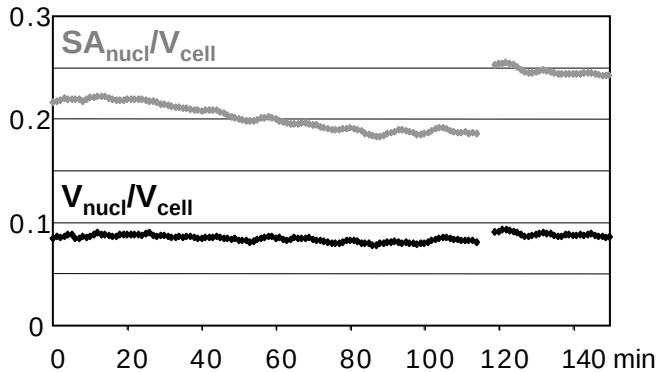

V_{nucl} [µm^3]
100
80
60
40
20
0
0 200 400 600 800
V_{cell} [µm^3]


N/C ratio (fixed cells)
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
2 C
32 C

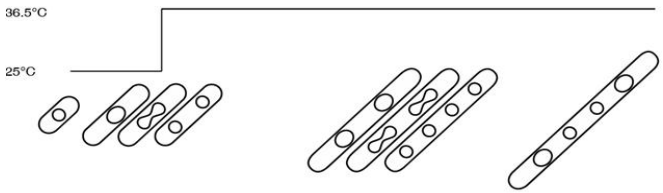
N/C ratio and cell cycle

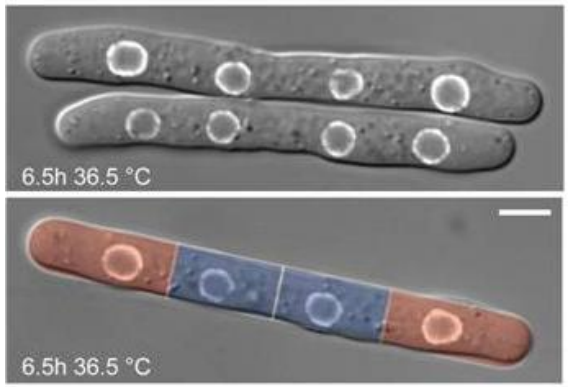


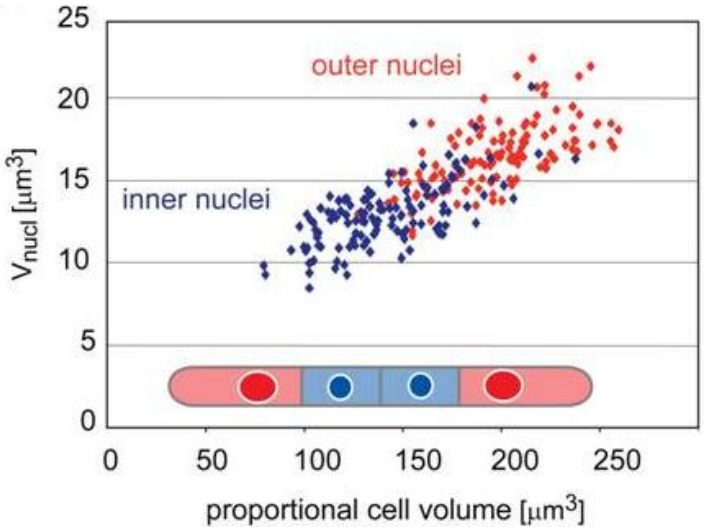


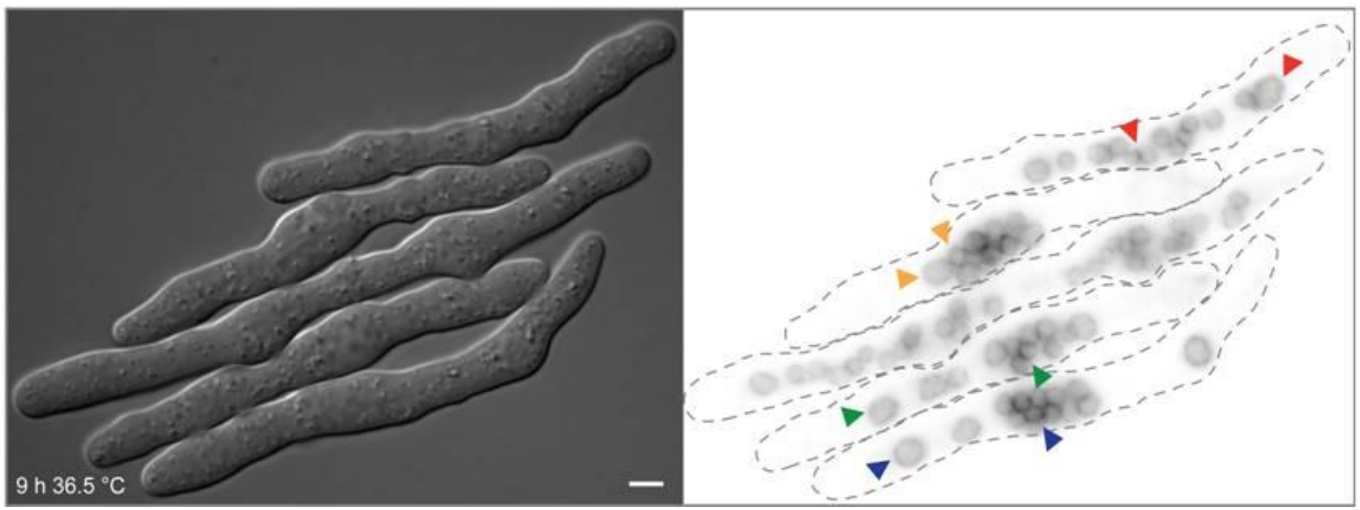


Subcellular environment

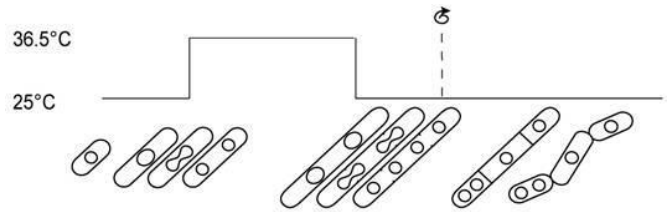


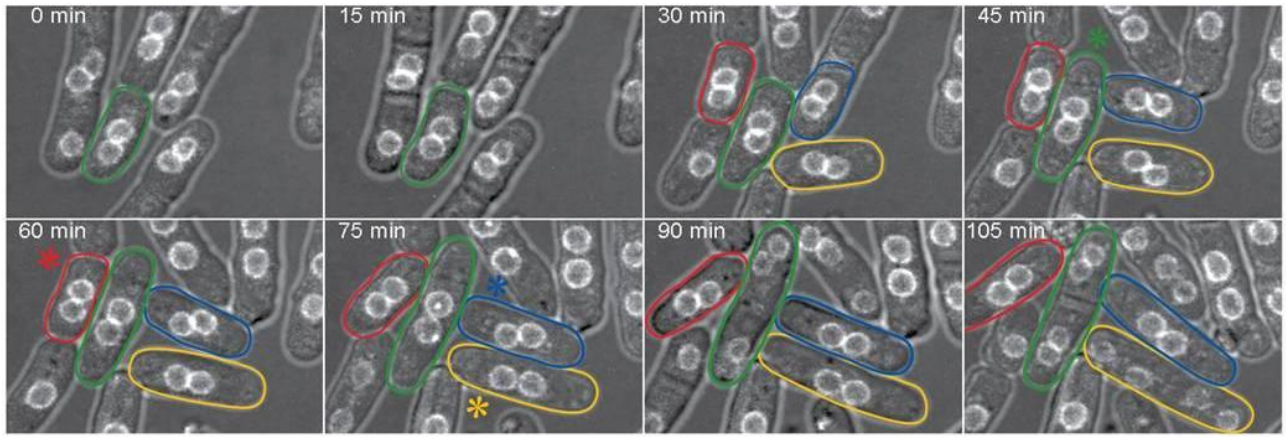


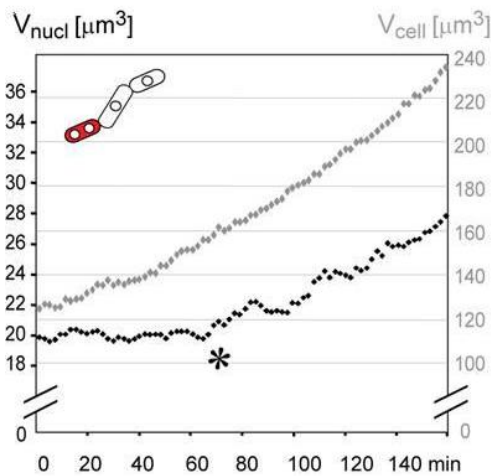


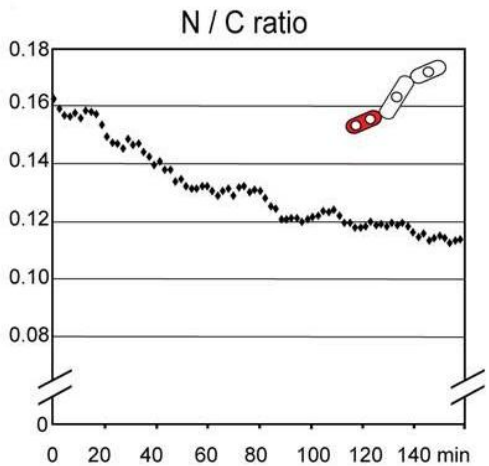


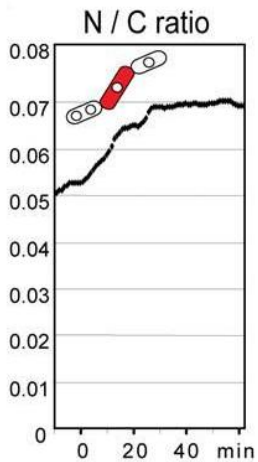
N/C ratio perturbation



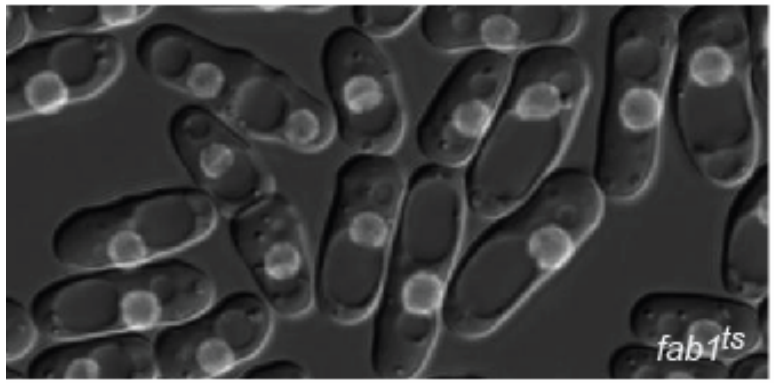


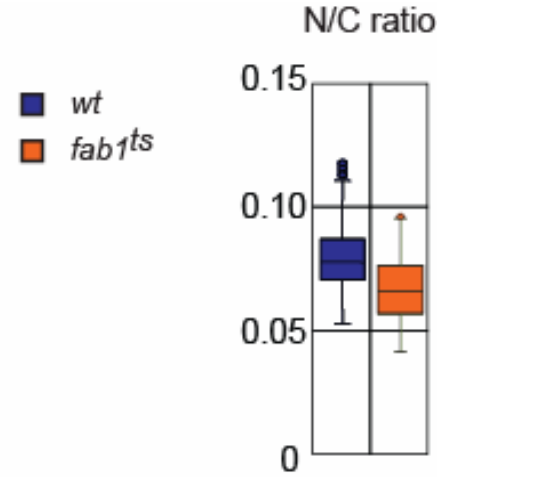


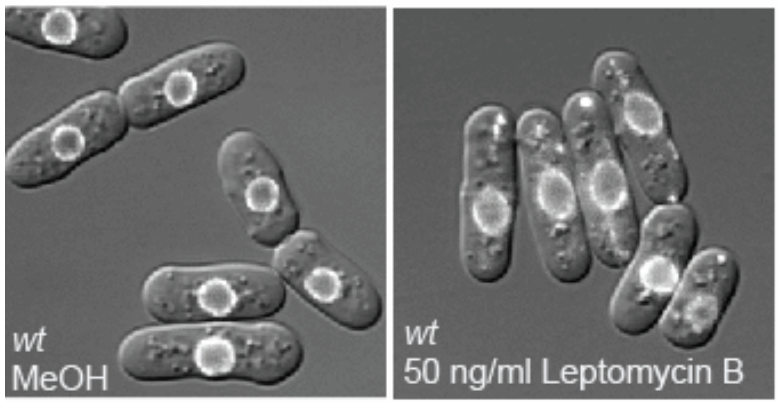


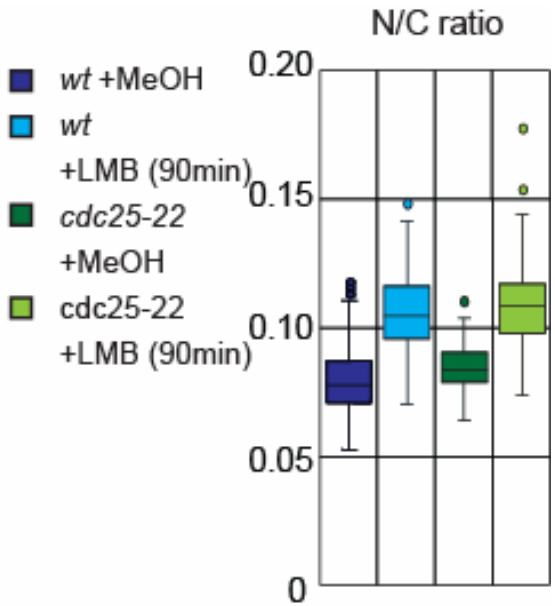


Changed N/C ratios









Systems-level study of cell size control in fission yeast

Francisco Navarro

Genome-wide screen of small size mutants

A

Primary screens
(on solid media)

↓

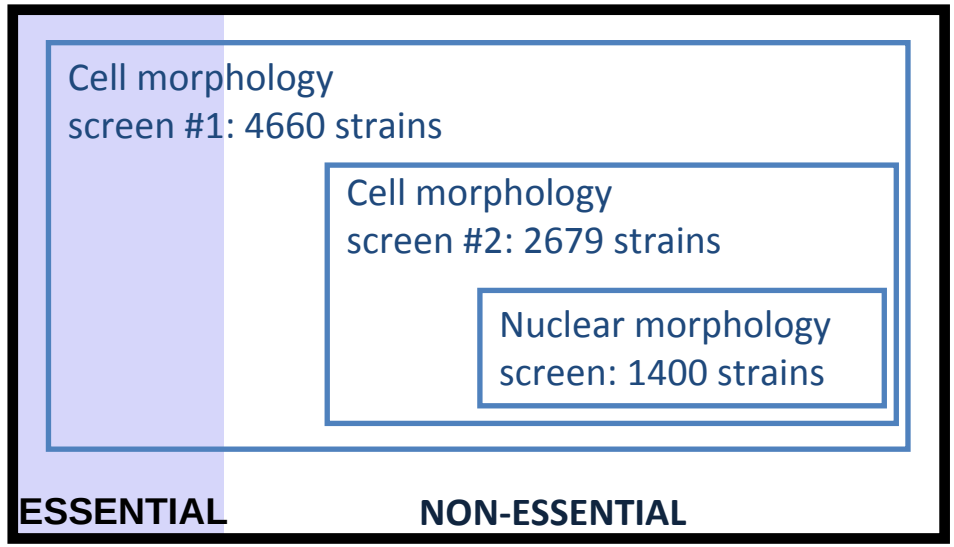
Secondary screen
(exponentially growing cultures)

↓

Verification of mutants
(Backcross with wt; PCR)

B

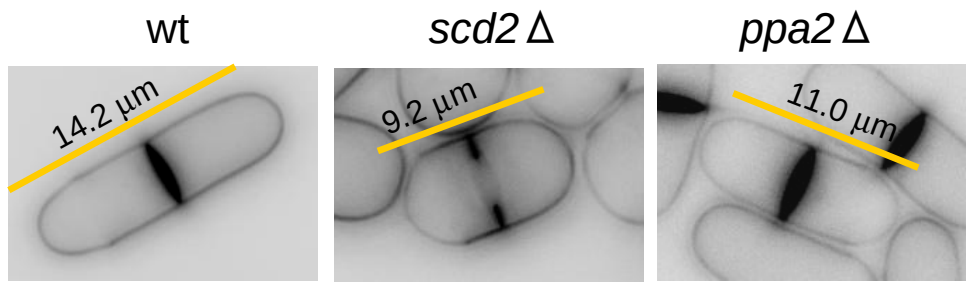
Coverage of the pombe genome by the primary screens



Pombe gene number: 5027

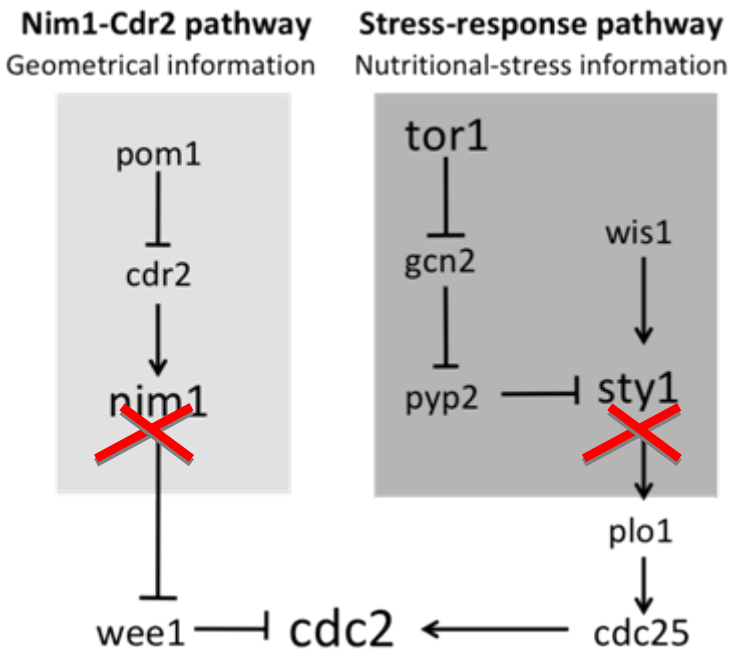
C

D

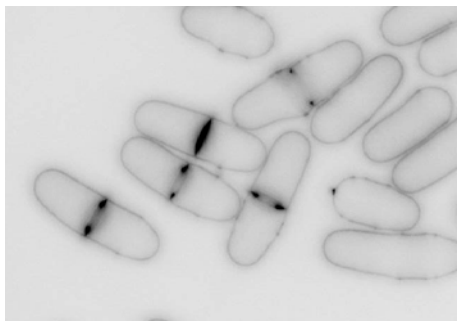


Small size mutants identified in the screen

Sys ID	Cell length (μm)	Doubling time (min)	GO_molecular function	GO_Biological Process
SPCC18B5.03	7.4 ± 0.7	156	Protein Ser/Thr/Tyr kinase	Regulation of G2/M transition of mitotic cell cycle
SPBC106.10	10.5 ± 0.7	157	cAMP-dependent protein kinase	cAMP-mediated signaling; negative regulation of transcription by glucose; response to osmotic stress;
SPAC23H3.13c	10.9 ± 0.9	152	GTPase, α-subunit	
SPCC1753.02c	12.7 ± 0.8	148	G-protein coupled receptor	
SPBC32H8.07	12.9 ± 0.9	142	GTPase activity	
SPBC1718.07c	12.3 ± 0.7	138	Protein binding	Negative regulation of mitosis; septum formation; mitosis exit; Cytokinesis checkpoint;
SPAC1782.09c	11.9 ± 0.9	138	Protein Ser/Thr/Tyr phosphatase	
SPAC2F7.03c	12.3 ± 1.6	144	Protein Ser/Thr kinase	Activation of bipolar cell growth; regulation of cytokinesis;
SPBC23G7.04c	12.5 ± 0.7	126	Protein kinase inhibitor	Negative regulation of mitotic cell cycle
SPAC2F7.08c	12.6 ± 0.7	137	General RNA polymerase II transcription factor activity	Chromatin remodeling; regulation of transcription from RNA polymerase II promoter
SPBC30B4.04c	12.9 ± 0.9	131		
SPCC126.04c	12.7 ± 0.8	163	unknown	Chromatin modification; histone acetylation
SPBC16E9.12c	13.1 ± 0.8	143	Poly(A) RNA binding	mRNA poly(A) tail shortening
SPCC1919.05	12.4 ± 0.6	136	Protein binding	mRNA catabolic process; protection against dsRNA virus; 3'-5' directed mRNA degradation.
SPBC19F8.02	12.6 ± 0.6	149	Protein binding	Cytoplasm organization
SPAC27E2.03	12.3 ± 0.7	154	GTP binding	unknown
SPBC16H5.07c	11.2 ± 1.0	136	Phosphoprotein phosphatase activity	Negative regulation of mitotic cell cycle; signal transduction
SPAC26F1.10c	11.3 ± 0.6	138	Protein tyrosine phosphatase	Negative regulation of stress-activated MAPK cascade
wt	14.1 ± 0.8	130		



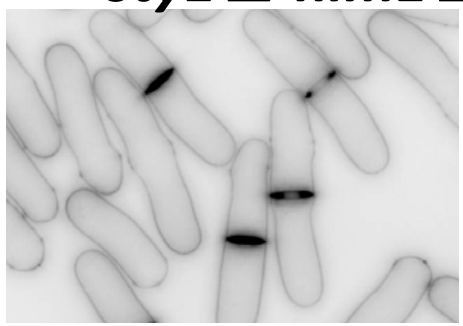
wt



Mean length=14.2m

CV=6.3%

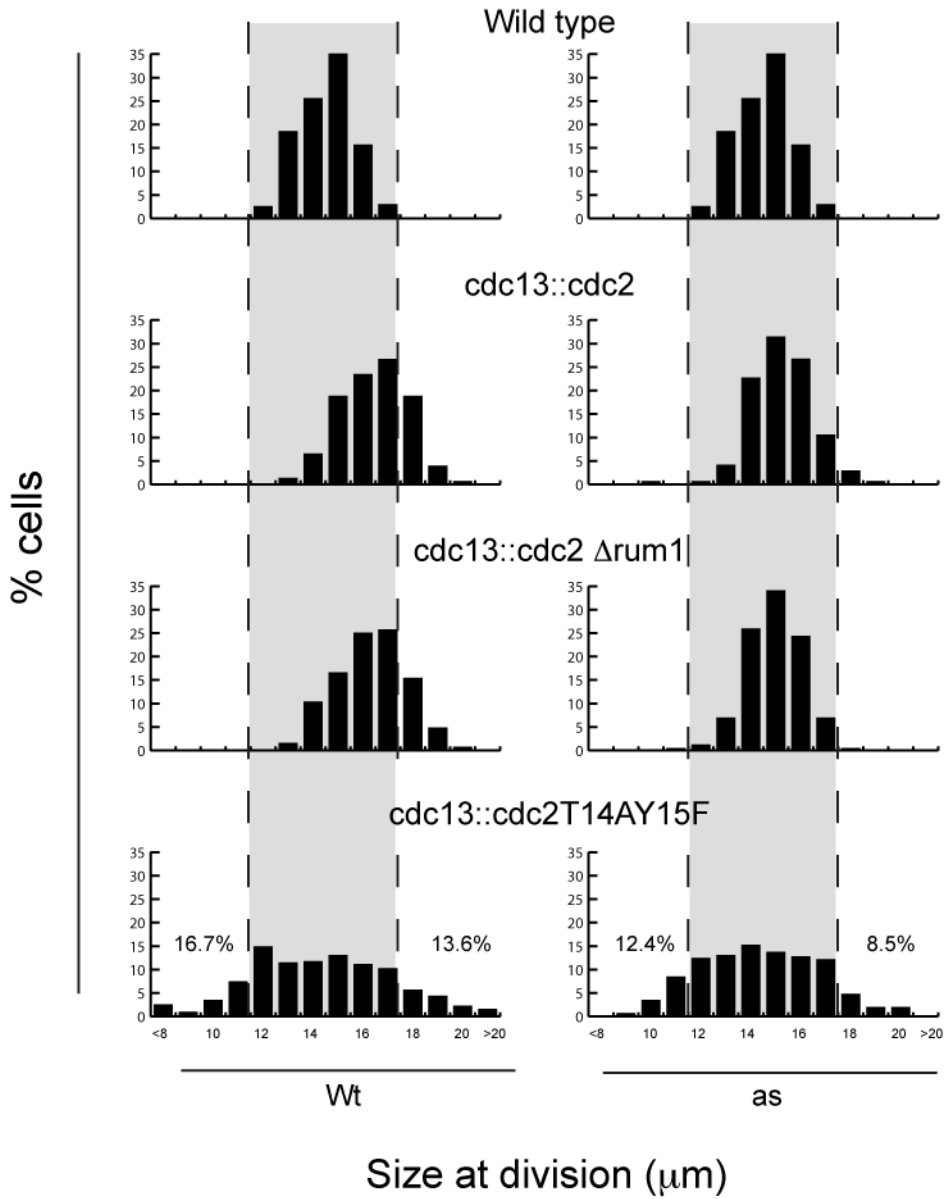
sty1 Δ nim1 Δ

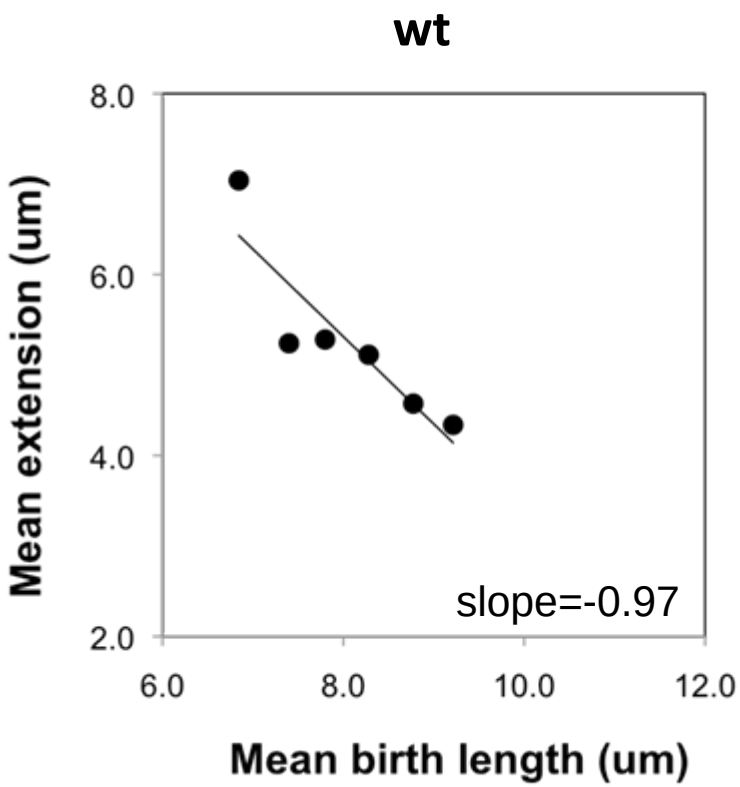


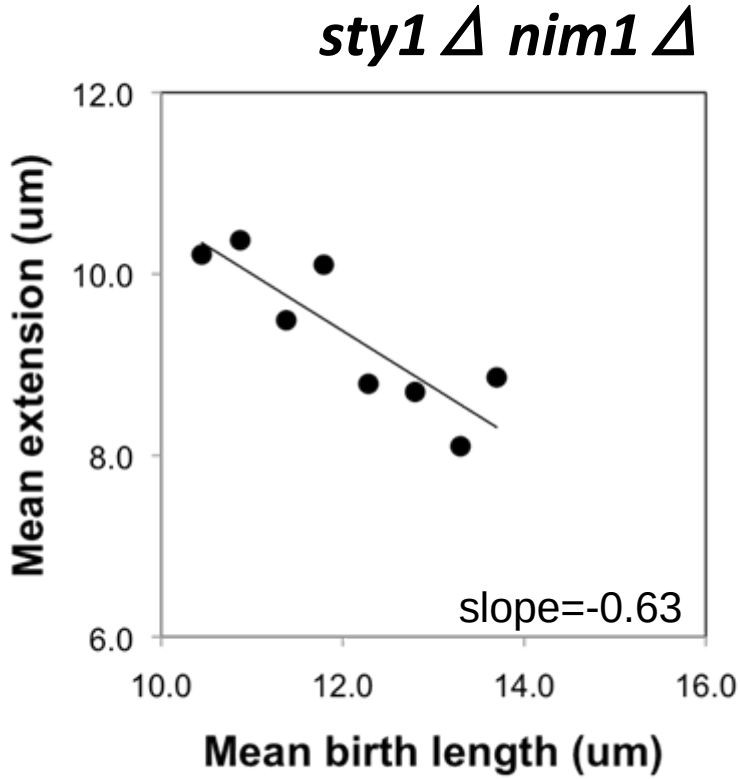
Mean length=22.6 μ m

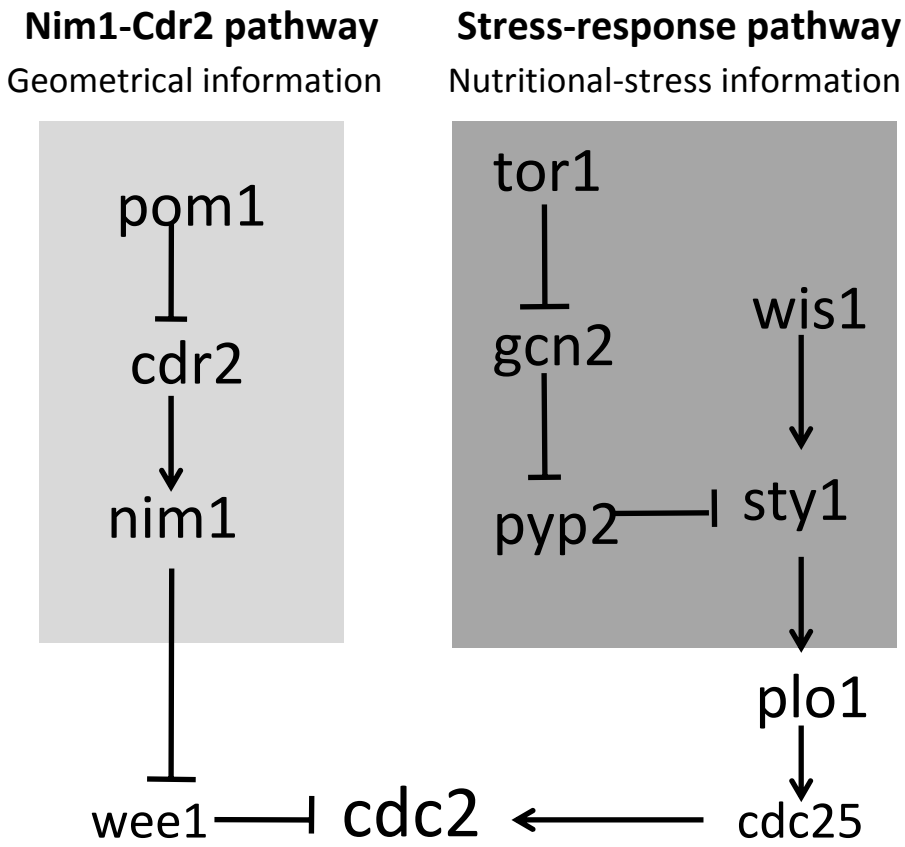
CV=7.5%

The Wee1/Cdc25 loop is essential for the homogeneity of the population




wt
8.0
6.0
4.0
2.0
Mean extension (um)
slope=-0.97
6.0
8.0
10.0
12.0
Mean birth length (um)


sty1 Δ nim1 Δ
12.0
10.0
8.0
6.0
Mean extension (um)
slope=-0.63
10.0
12.0
14.0
16.0
Mean birth length (um)


Nim1-Cdr2 pathway
Geometrical information
Stress-response pathway
Nutritional-stress information
pom1
cdr2
nim1
tor1
gcn2
pyp2
wis1
sty1
plo1
wee1
cdc2
cdc25


Breakfast
Lunch
Ice Cream
Keep
Governors Island
Clean


Raid