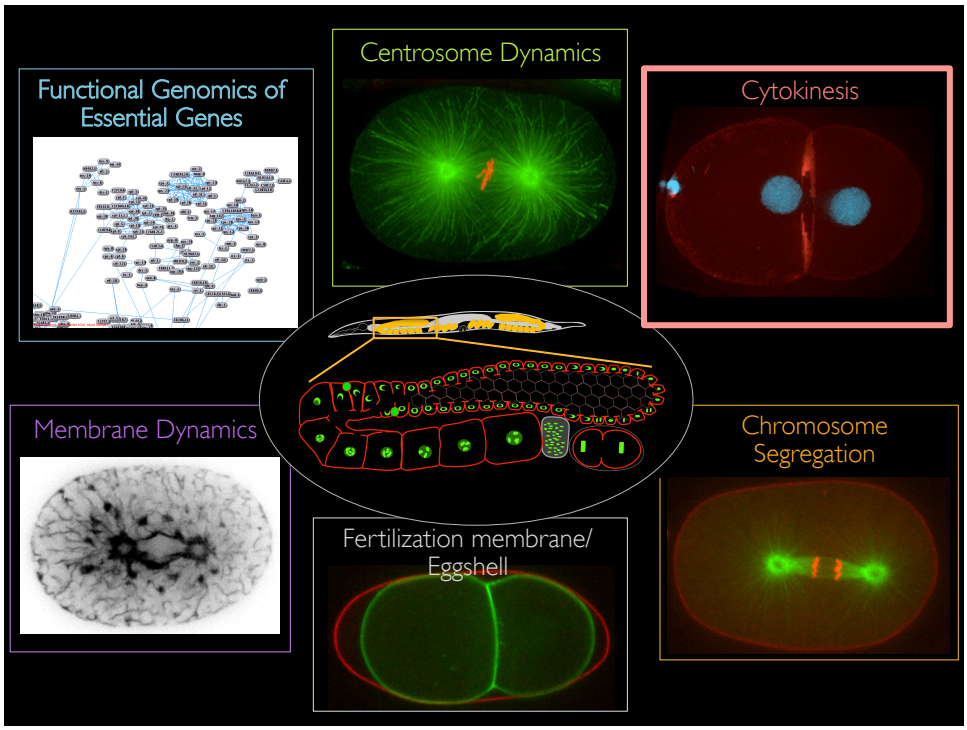
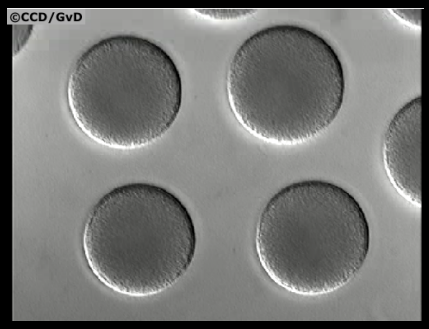
Using *C. elegans* to dissect cell division mechanisms

Karen Oegema
Laboratory of Mitotic Mechanisms
Ludwig Institute for Cancer Research, San Diego Branch
Cellular and Molecular Medicine, UCSD



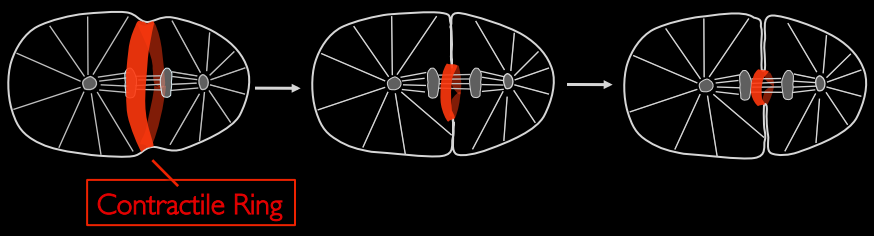
Cytokinesis partitions the contents of the mother cell to the two daughter cells



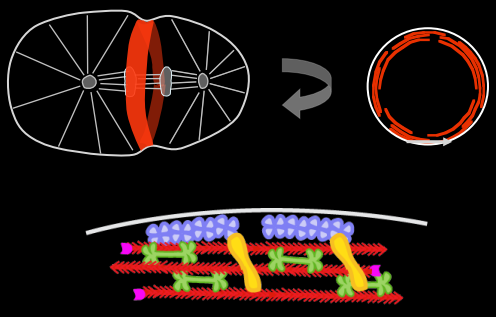
Purple sea urchin embryos
George von Dassow

DNA/ Plasma Membrane/ Microtubules
C. elegans embryo

Cytokinesis is accomplished via constriction of a contractile ring

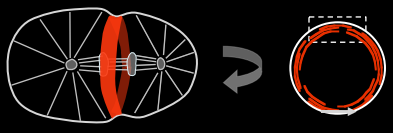


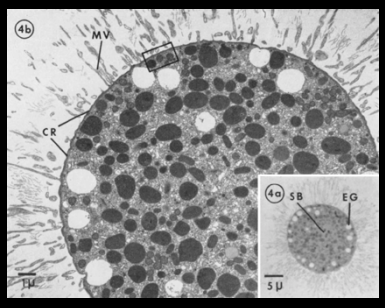
The contractile ring is a thin layer of cross-linked protein filaments

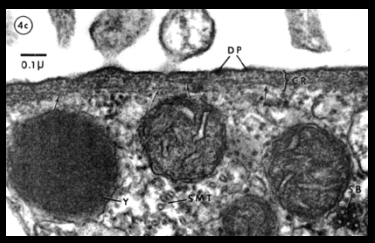


F-actin Formin Myosin II Septins Anillin

The contractile ring is a thin layer that lies beneath the plasma membrane

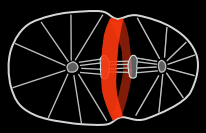


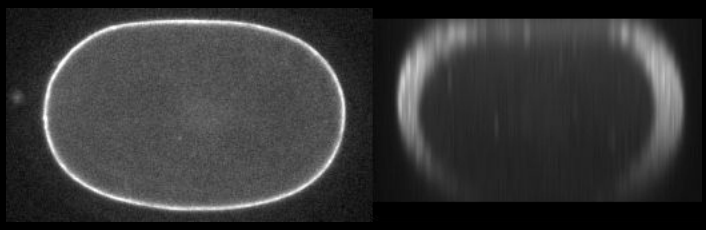




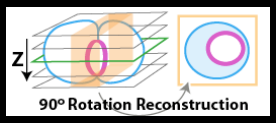
Images from:
TE Schroeder JCB, 1972

The contractile ring closes asymmetrically during the first division of the *C. elegans* embryo



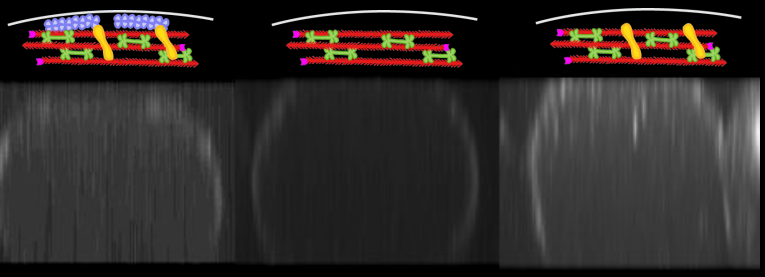


GFP Plasma membrane probe 90x real time



Anillin and the Septins are required for asymmetric constriction

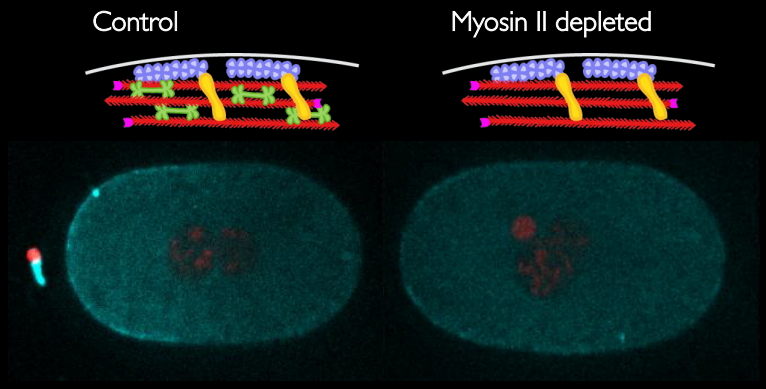
Control | Anillin depleted | Septin depleted



GFP Myosin II 90x real time

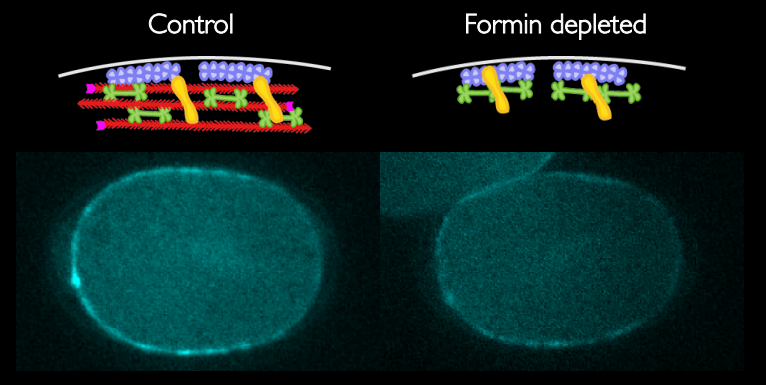
Amy Maddox (IRIC/University of Montreal)

Contractile ring constriction requires the motor myosin II



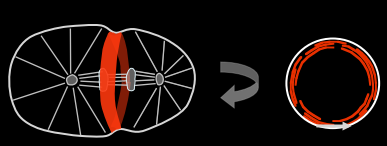
Anillin/ Chromosomes
Playback 80x realtime

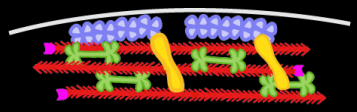
Contractile ring constriction requires the cytokinesis formin CYK-1



GFP-Septin
Playback 90x realtime

Key questions





How are the filaments in the contractile ring organized?

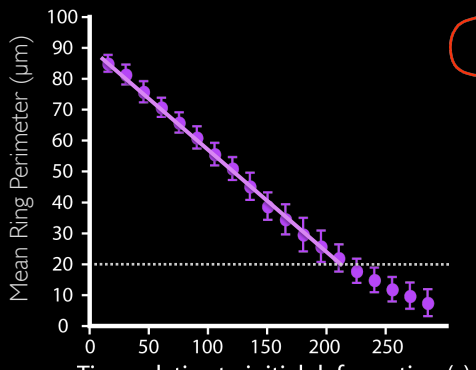
How is contractile stress generated?

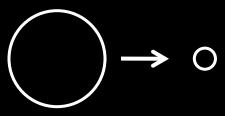
What determines the constriction rate?

What happens to ring structure/components during constriction?

How dynamic are the ring components?

During the first division, the contractile ring closes at a constant rate

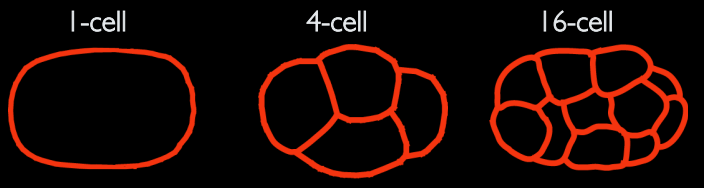




Zumdieck et al., PLoS one, 2007

Analyze scaling of cytokinesis with cell size

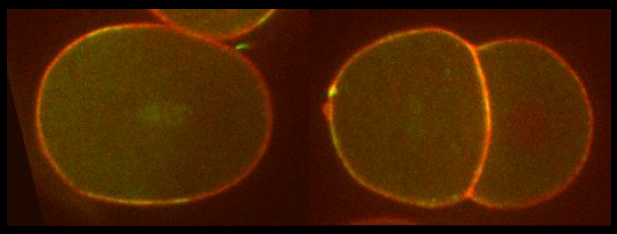
Ana Carvalho

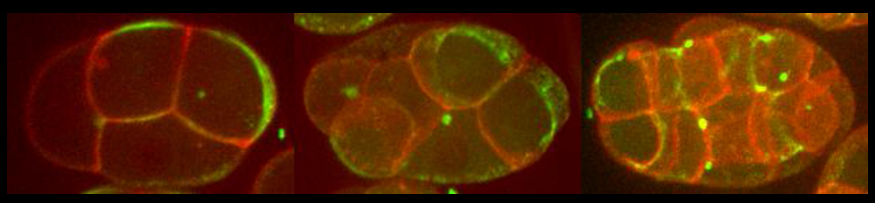


	1-cell	4-cell	16-cell
Volume (embryo)	V	V	V
Volume (cell)	V	V/4	V/16
Perimeter (cell)	P	0.63 P	0.40 P

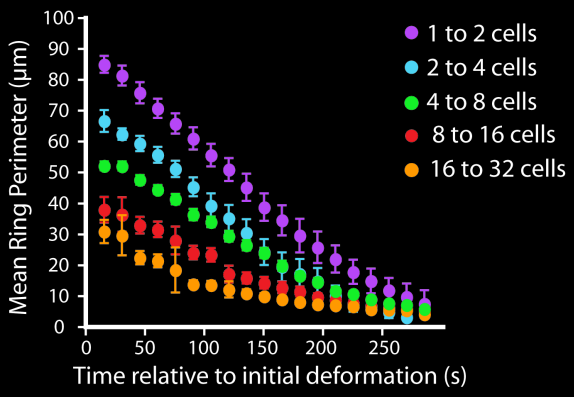
Measuring the kinetics of contractile ring constriction during the first 5 rounds of cell division

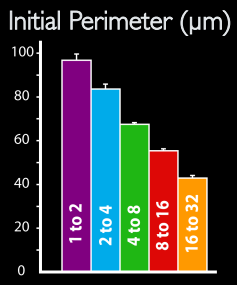
Contractile ring
Plasma Membrane

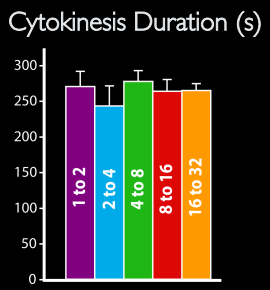




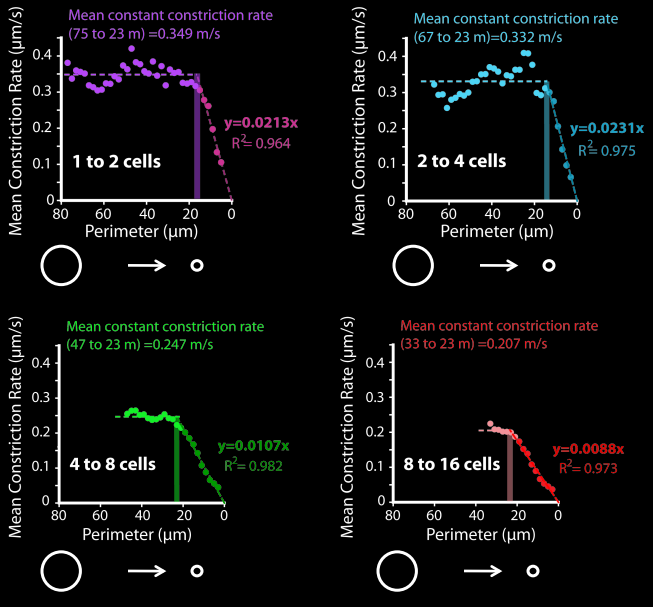
Cytokinesis duration is the same despite the decreasing initial size of the ring



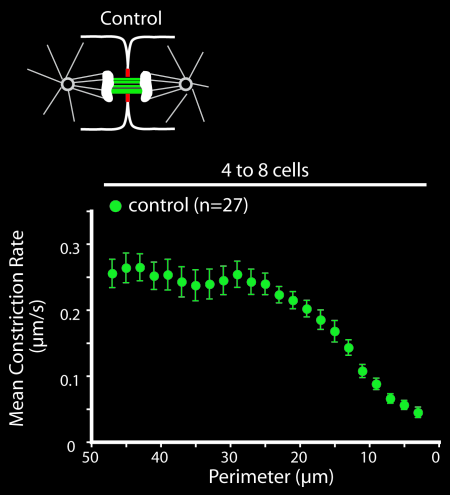




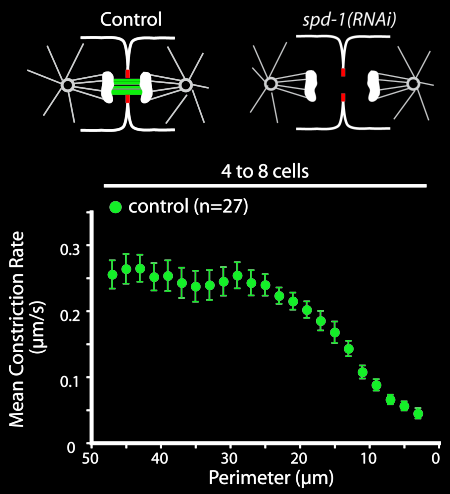
Contractile rings transition from constricting at a constant rate to a decreasing rate at a fixed perimeter



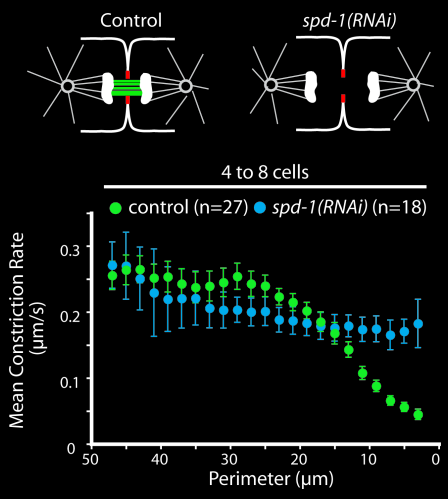
The transition from constant to decreasing constriction rate is due to contact with microtubule bundles in the spindle midzone



The transition from constant to decreasing constriction rate is due to contact with microtubule bundles in the spindle midzone



The transition from constant to decreasing constriction rate is due to contact with microtubule bundles in the spindle midzone

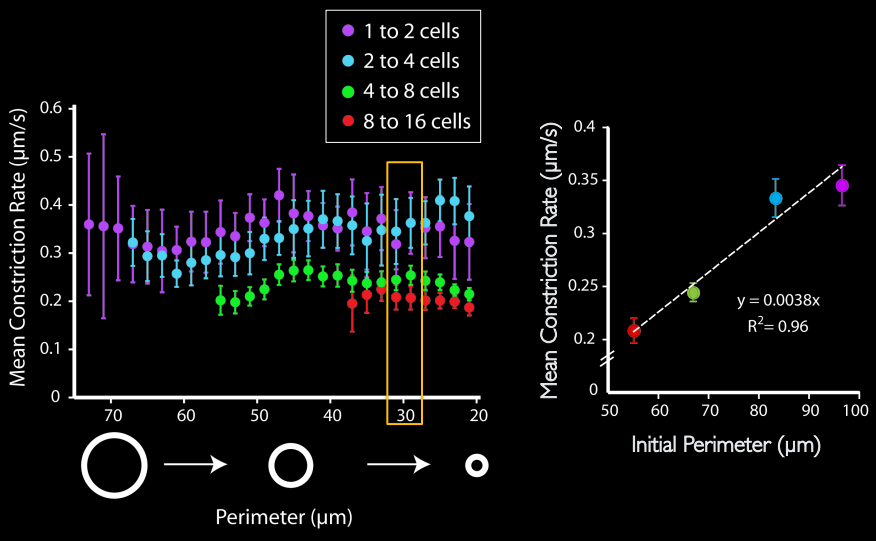


Conclusions:

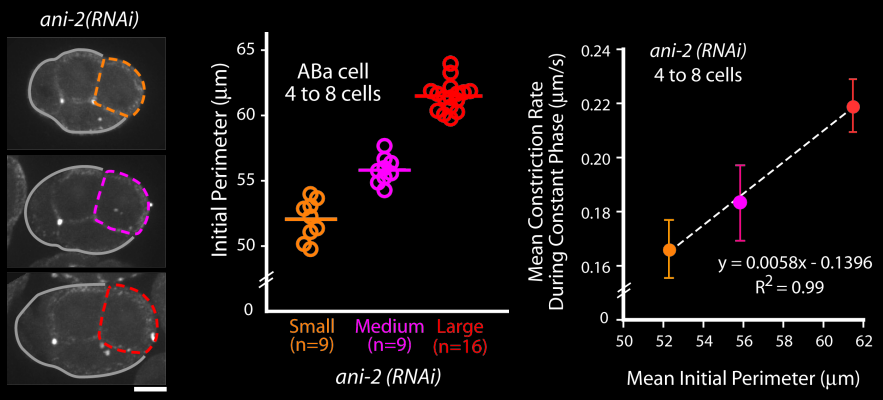
- Contractile rings have an intrinsic capacity to close at a constant rate
- Contractile rings transition from constricting at a constant rate to constricting at a decreasing rate when they come into contact with the microtubules in the spindle midzone

Why is the duration of cytokinesis INVARIANT?

Contractile rings constrict at a constant rate proportional to their initial size

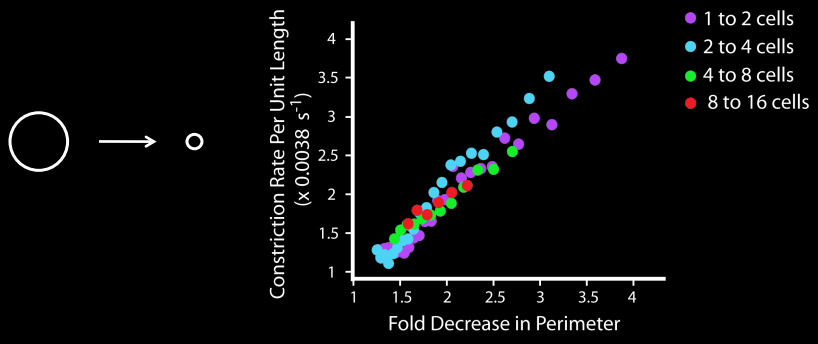


Constant constriction rate is proportional to initial cell size at a single developmental stage



Initial size of the ring determines the constriction rate throughout closure → Ring retains a MEMORY of its initial size

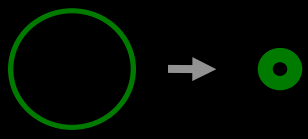
CONSTANT CONSTRICTION RATE = Rate PER UNIT LENGTH
increases in proportion to decrease in perimeter



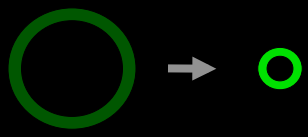
Is this because the amount of myosin per unit length (concentration) INCREASES during constriction?

What happens to contractile ring structure/components during constriction?

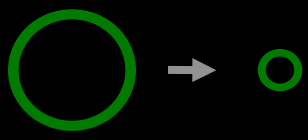
A) Increase in width

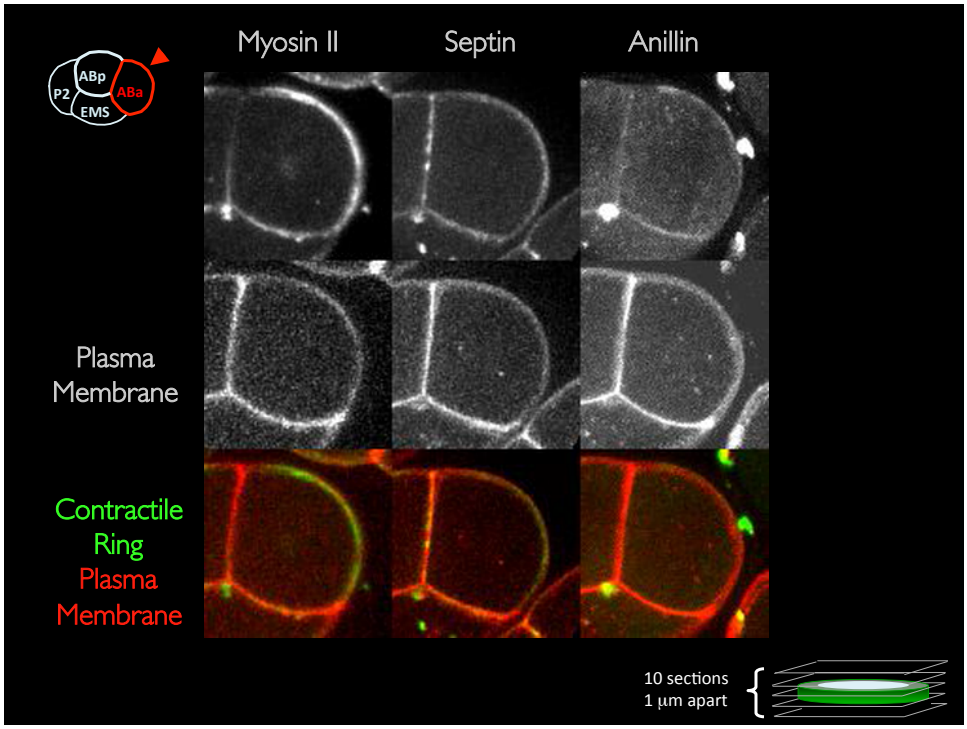


B) Increase in concentration or thickness

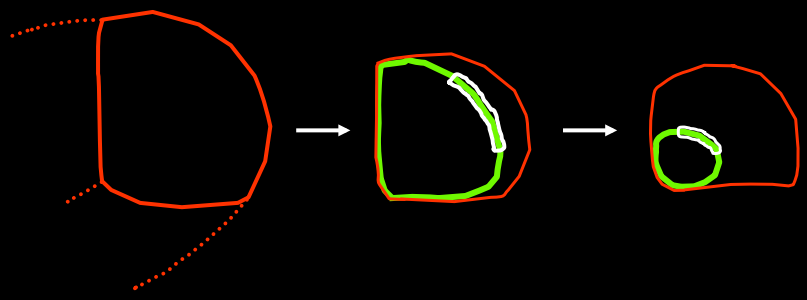


C) Constant concentration and width/thickness



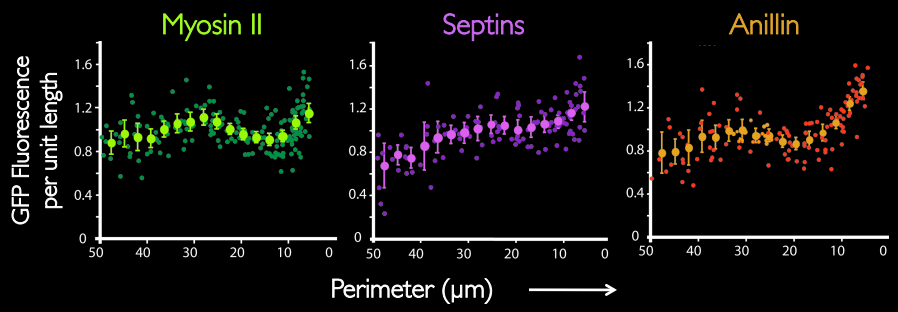


Analyzing the distribution of contractile ring components: GFP fluorescence per unit length

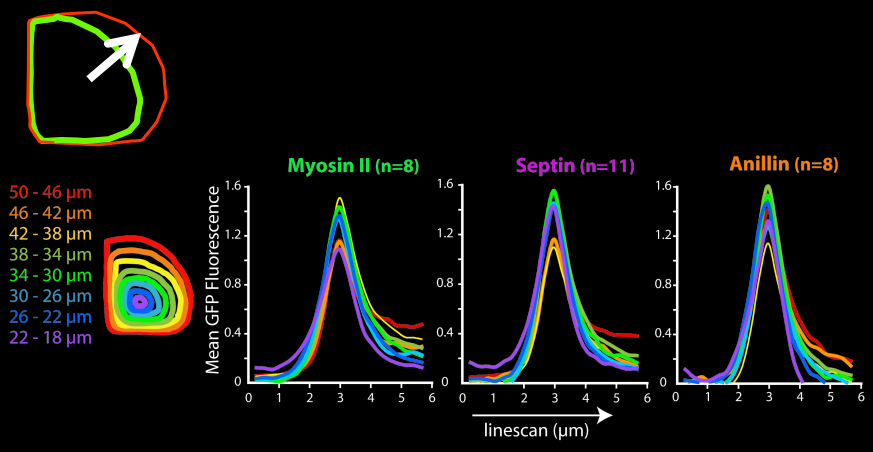


Draw box of constant width over the arc at each time point
Measure GFP intensity per unit length

The amount per unit length of three contractile ring components remains constant during constriction

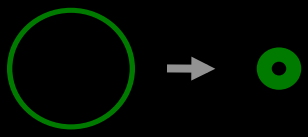


Contractile ring width does not change during ring constriction

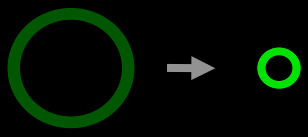


What happens to contractile ring structure/components during constriction?

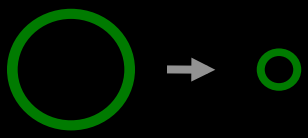
A) Increase in width



B) Increase in concentration or thickness



C) Constant concentration and width/thickness



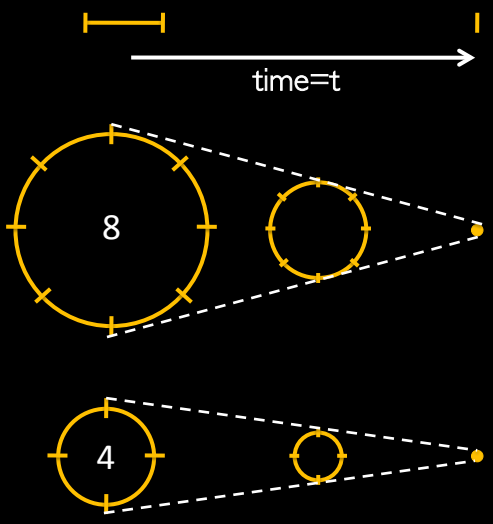
IN AGREEMENT WITH CLASSIC EM Studies of Sea Urchin Embryos by Schroeder *JCB* 1972

Properties of the Contractile Ring:

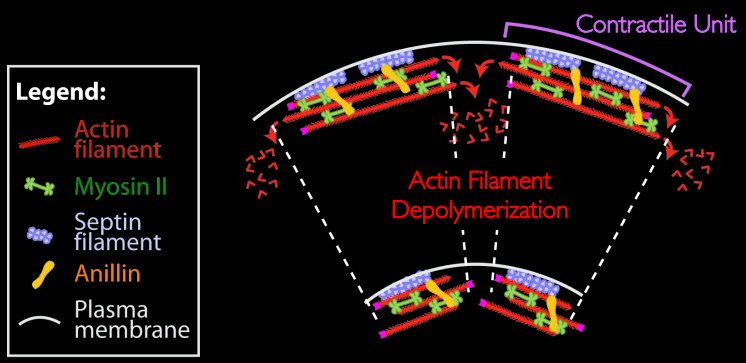
1) Inherent ability to constrict at a CONSTANT RATE
2) Constant constriction rate proportional to initial ring size
3) Constriction-coupled loss of ring components
4) No/slow component turnover

Contractile Unit Model

Contractile rings are built from UNITS of FIXED SIZE
that SHORTEN at a CONSTANT RATE = 1 unit/t



Model for contractile ring constriction

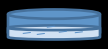


Constriction rate may be governed by actin filament depolymerization

Analyze partial depletion phenotypes of myosin heavy chain (NMY-2) and the cytokinesis formin CYK-1

1. Inject L4s

2. Leave at 16C for increasing amounts of time to vary the extent of depletion

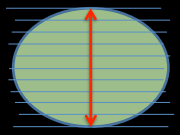


22 hours → 37

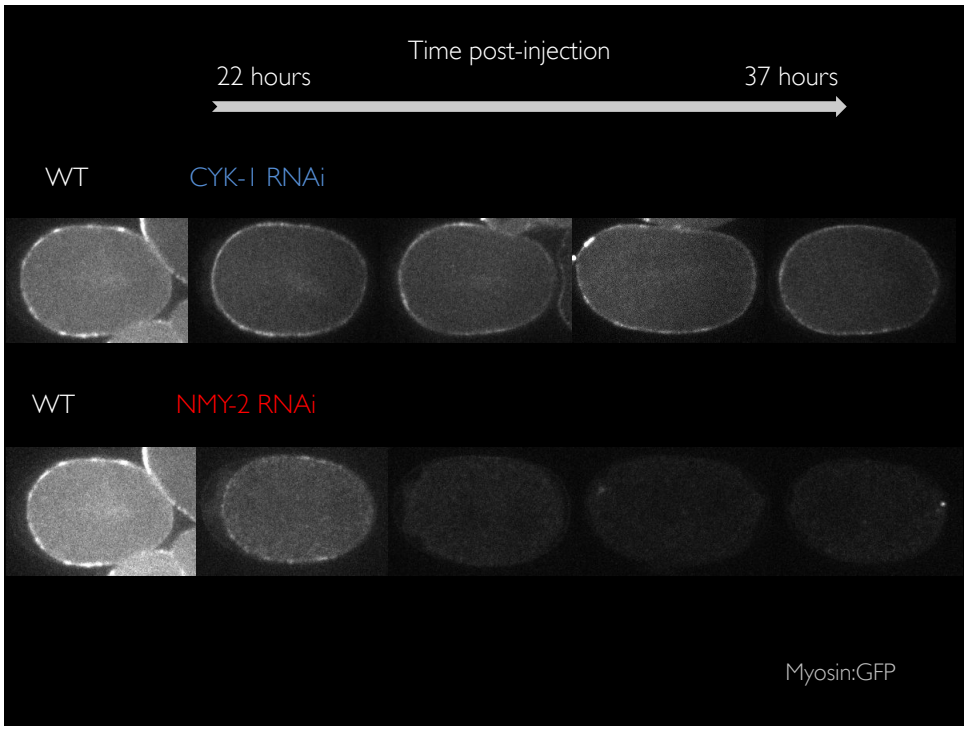
22 → 26
24 → 28
26 → 30
28 → 32
30 → 34
32 → 36
34 → 37

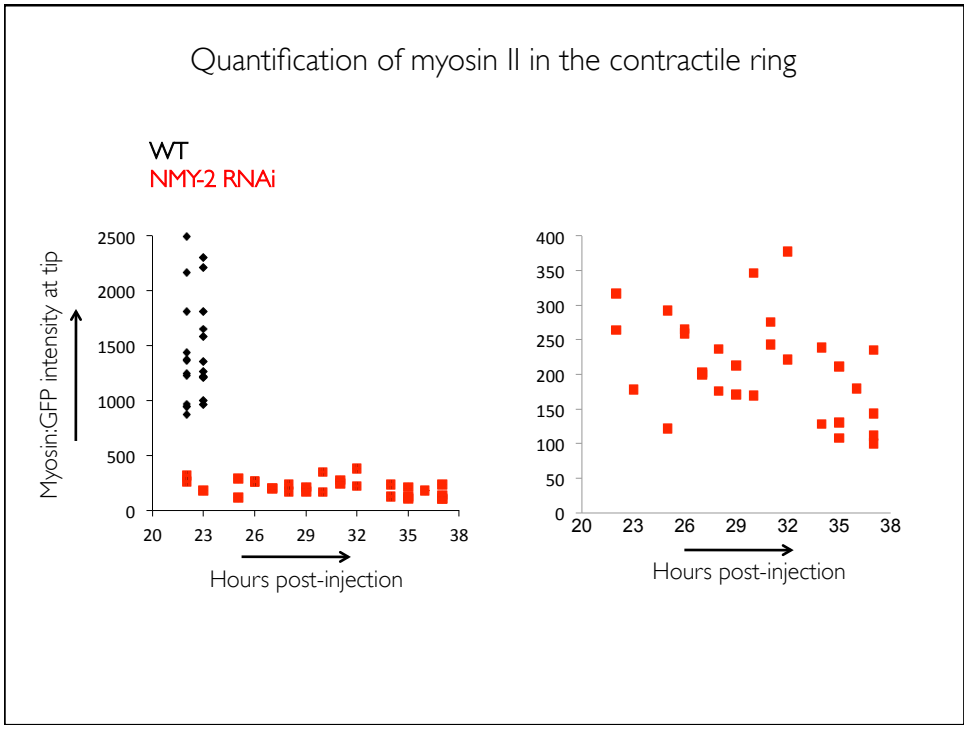
3. Image the first cytokinesis

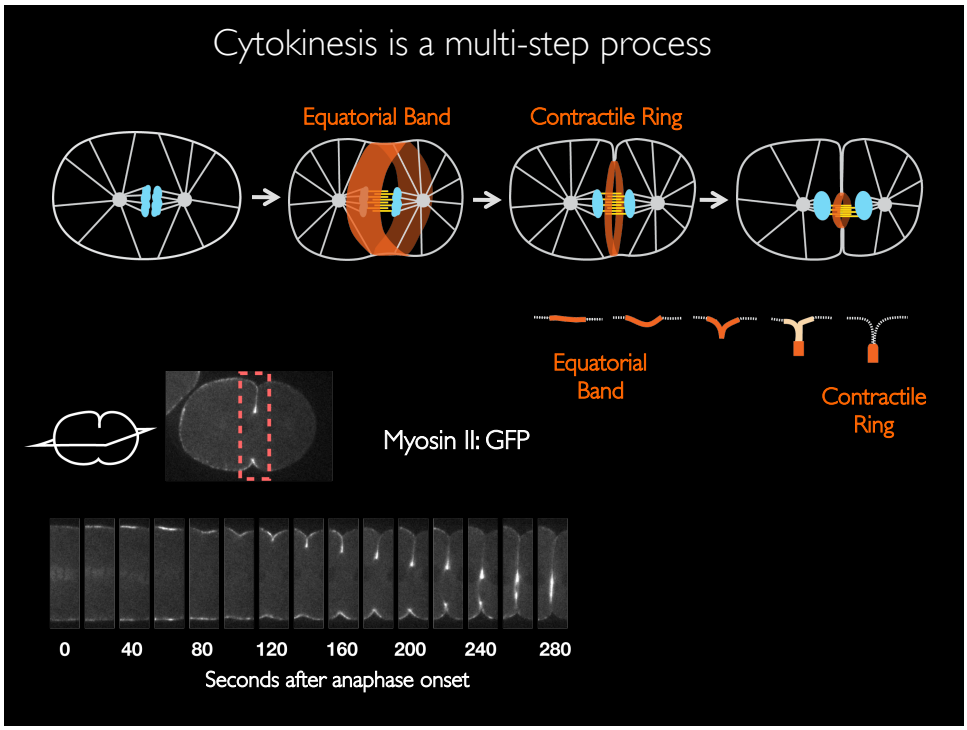
Image 11 z-sections, 2.5um apart



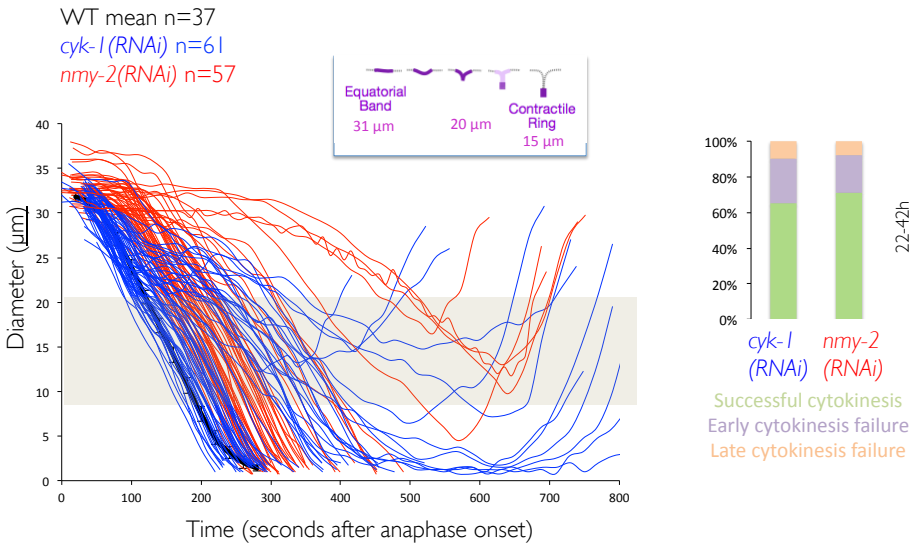
Myosin-GFP
mCherry- histone

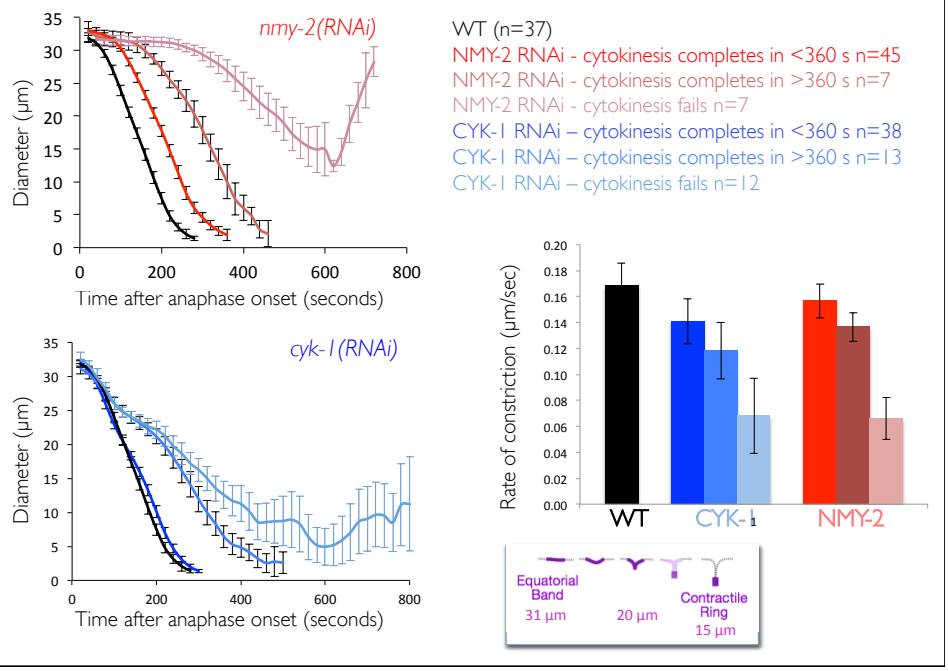


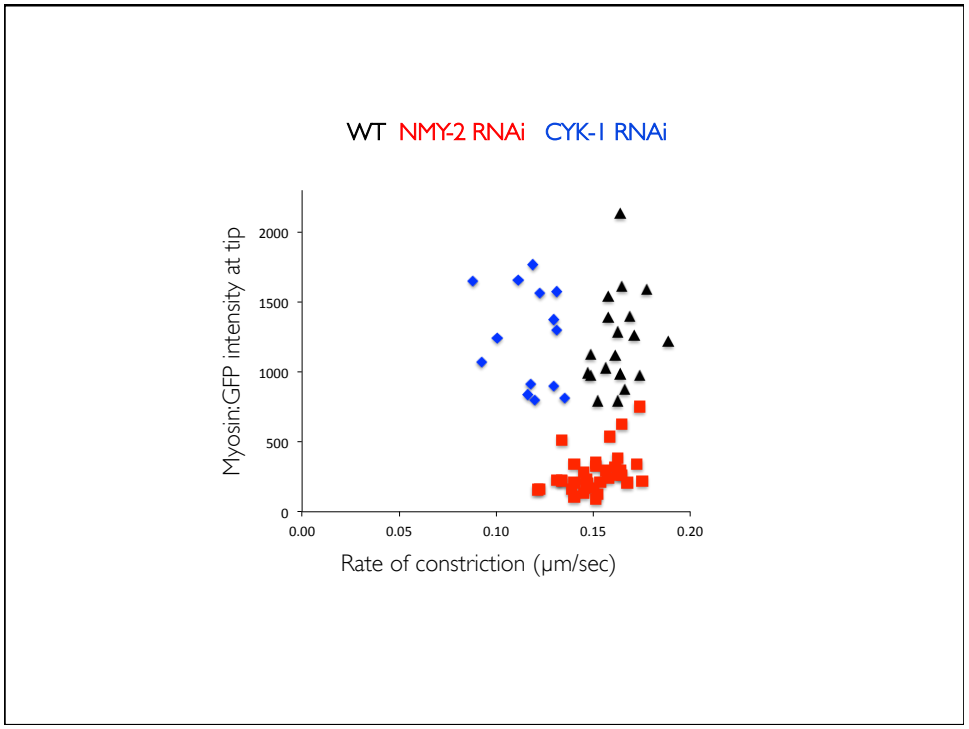

Quantification of myosin II in the contractile ring
WT
NMY-2 RNAi
Myosin:GFP intensity at tip
2500
2000
1500
1000
500
0
20 23 26 29 32 35 38
Hours post-injection
400
350
300
250
200
150
100
50
0
20 23 26 29 32 35 38
Hours post-injection

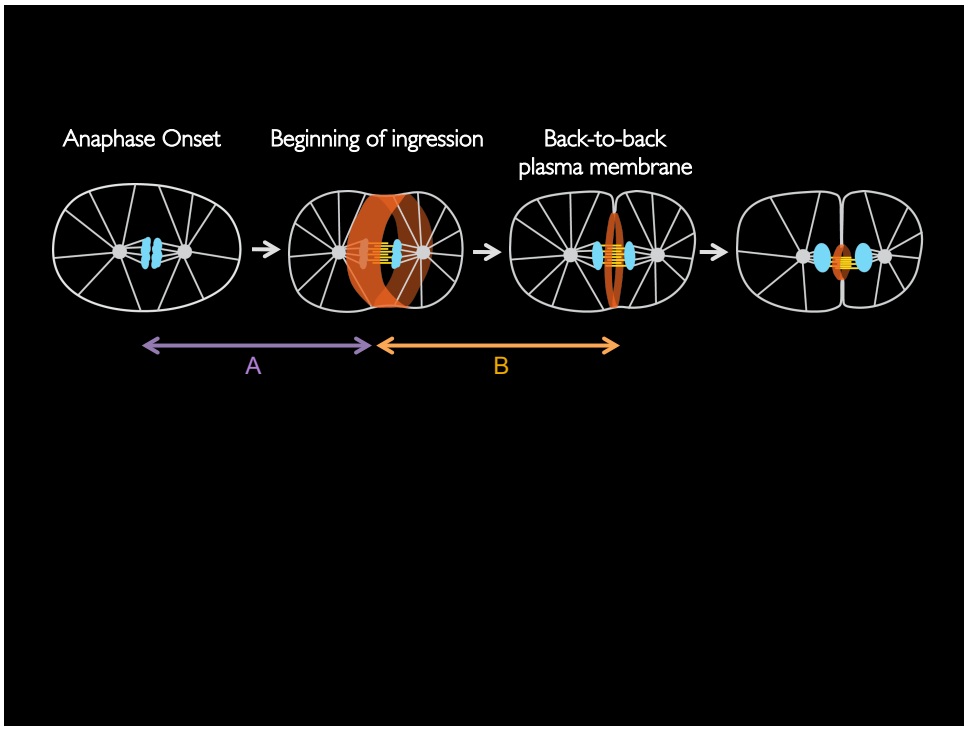

Cytokinesis is a multi-step process
Equatorial Band
Contractile Ring
Equatorial Band
Contractile Ring
Myosin II: GFP
0 40 80 120 160 200 240 280
Seconds after anaphase onset

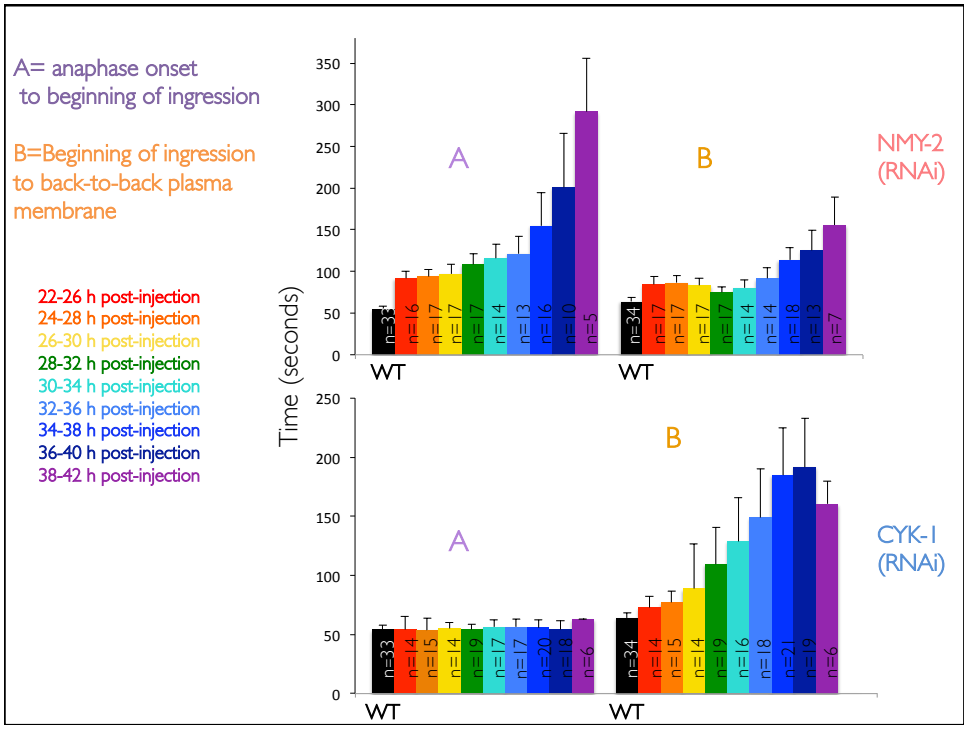
Most partially depleted embryos either fail early or complete cytokinesis successfully

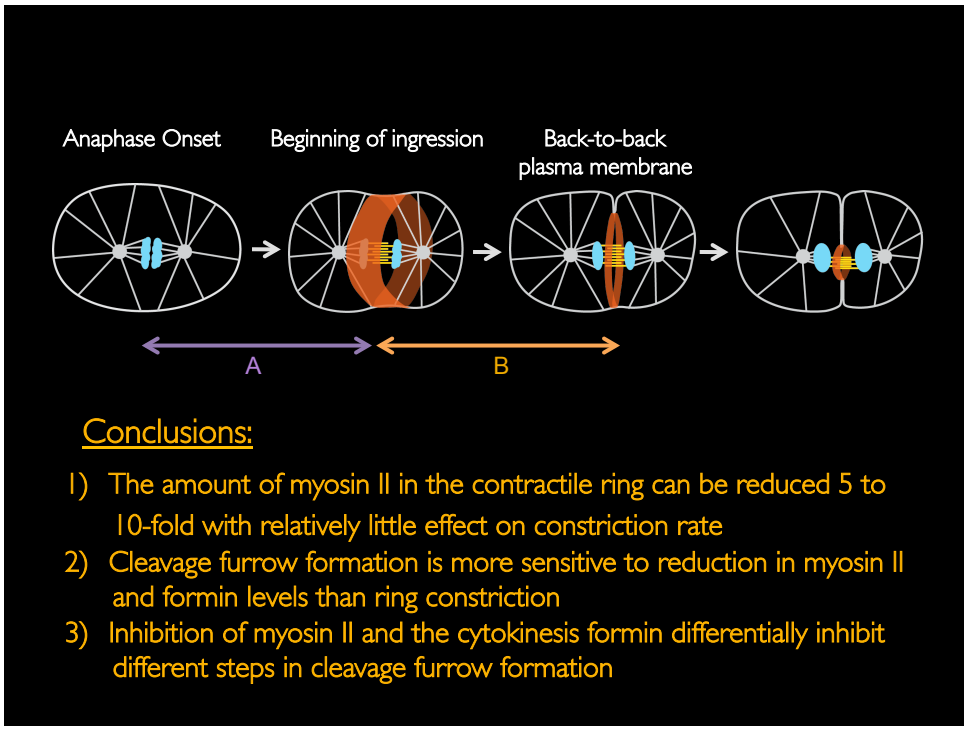





WT NMY-2 RNAi CYK-1 RNAi
Myosin:GFP intensity at tip
2000
1500
1000
500
0
0.00
0.05
0.10
0.15
0.20
Rate of constriction (µm/sec)

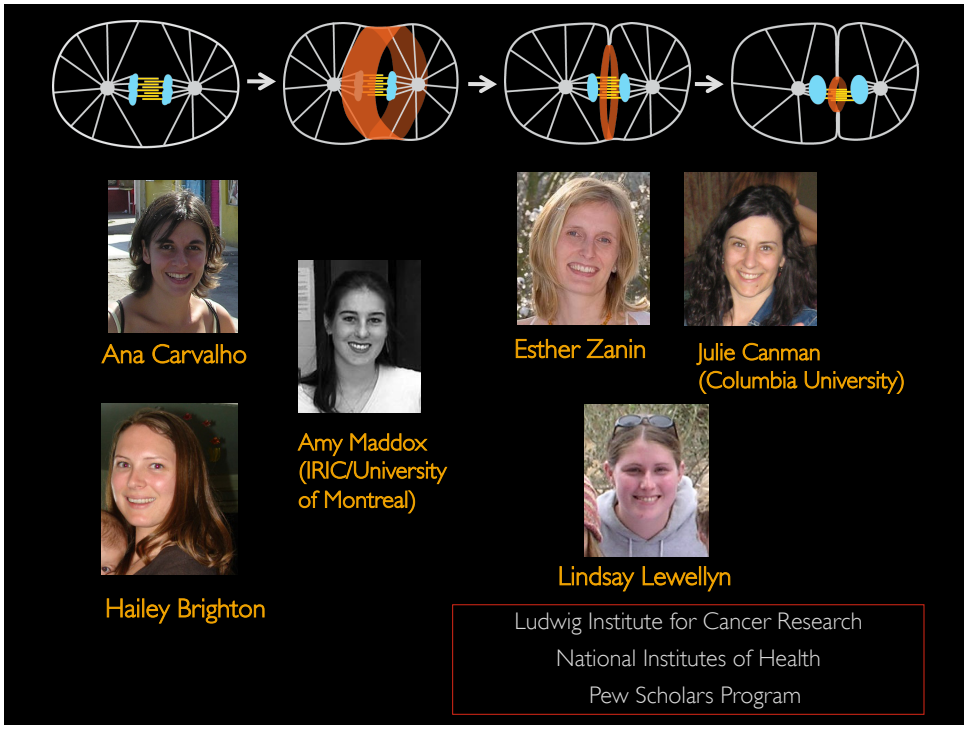

Anaphase Onset
Beginning of ingression
Back-to-back plasma membrane
A
B





Conclusions:

1) The amount of myosin II in the contractile ring can be reduced 5 to 10-fold with relatively little effect on constriction rate
2) Cleavage furrow formation is more sensitive to reduction in myosin II and formin levels than ring constriction
3) Inhibition of myosin II and the cytokinesis formin differentially inhibit different steps in cleavage furrow formation


Ana Carvalho
Amy Maddox
(IRIC/University
of Montreal)
Esther Zanin
Julie Canman
(Columbia University)
Hailey Brighton
Lindsay Lewellyn
Ludwig Institute for Cancer Research
National Institutes of Health
Pew Scholars Program