From Genes to Growth and Form
KITP
08/23/16

Time-keeping mechanisms of embryonic cell cycles

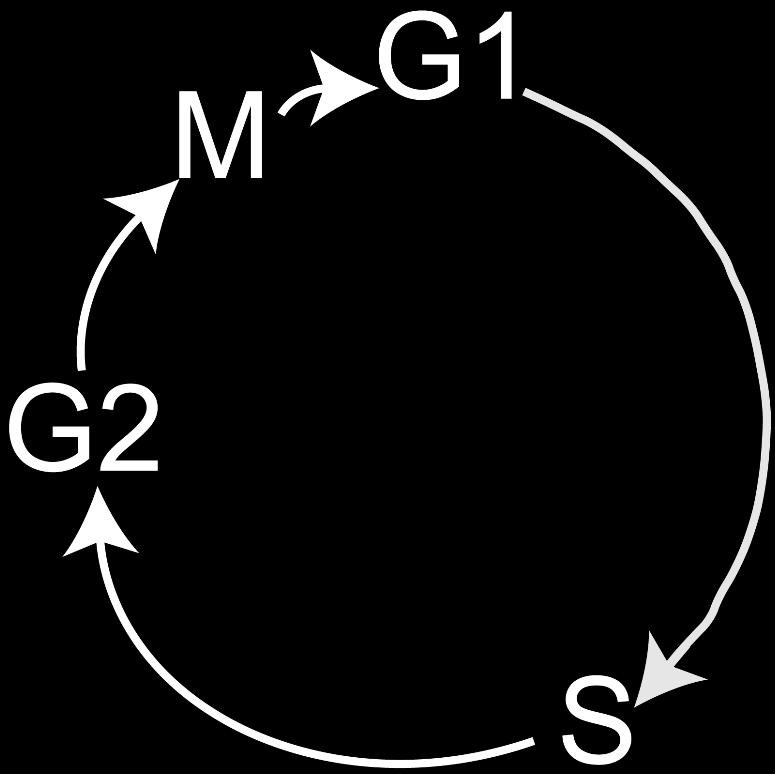
Stefano Di Talia
Department of Cell Biology
Duke University Medical Center

Biological systems are ‘noisy’. Yet, embryonic development is regulated so precisely!

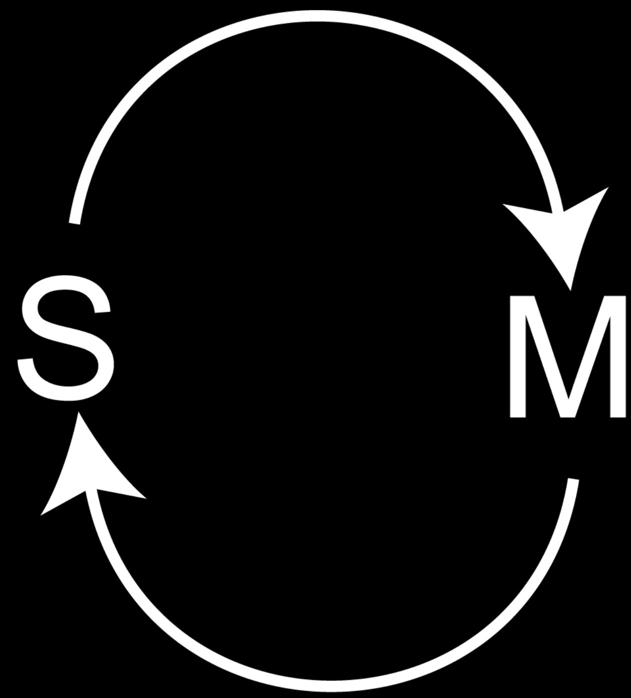
How are developmental transition accurately controlled in time?

The cell cycle as a model to study the precision of development

Somatic cell cycle



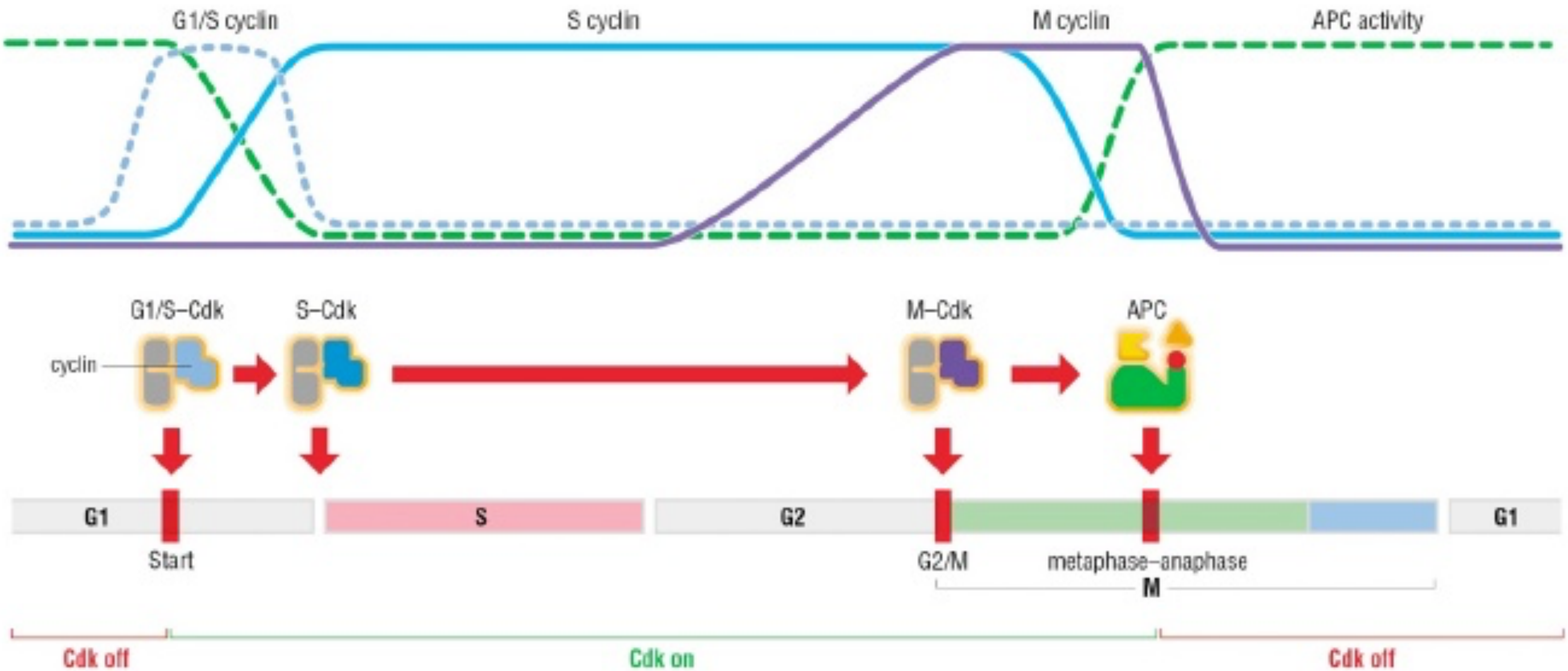
Early embryonic cell cycle



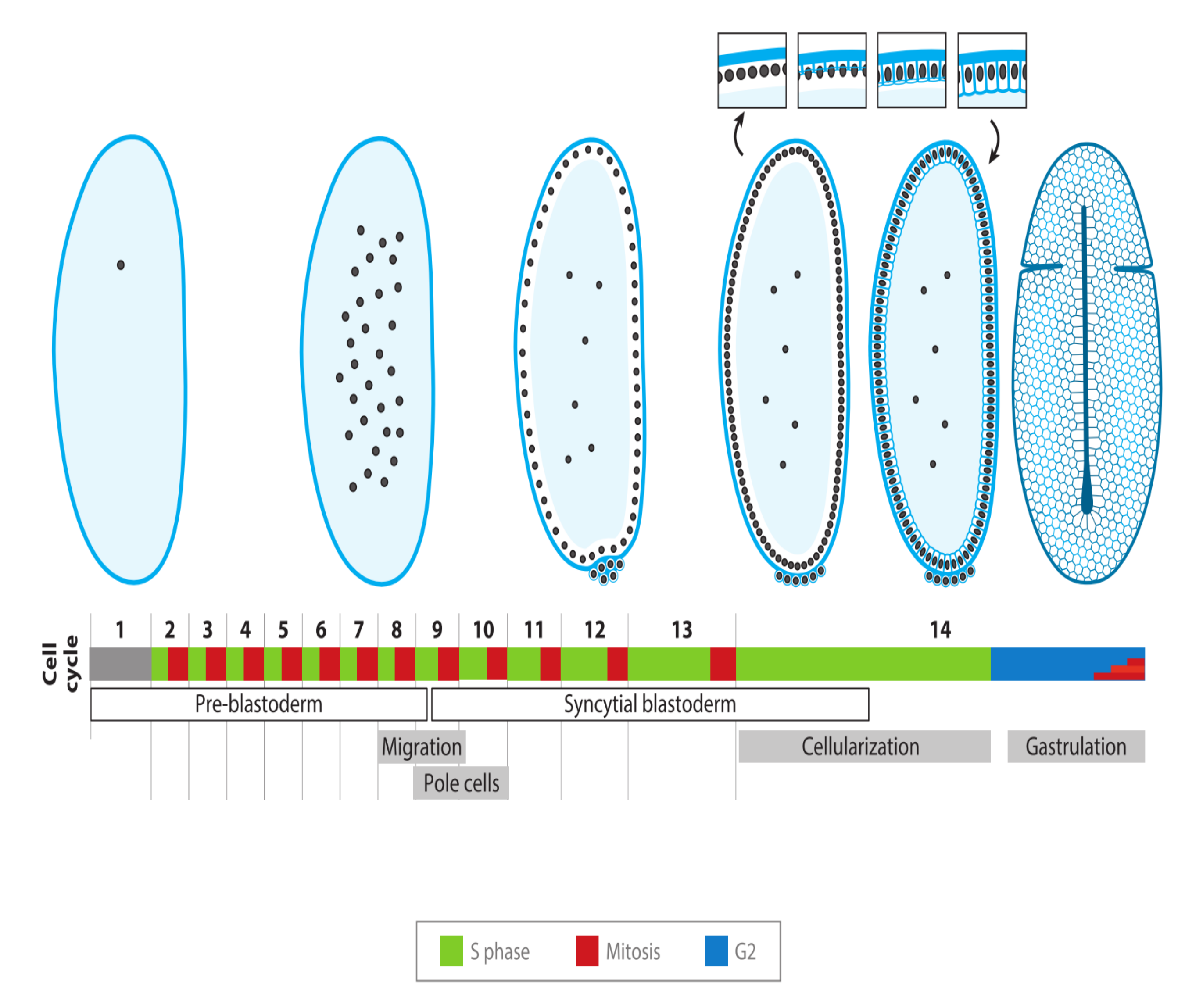
The major molecular pathways controlling the cell cycle have been identified

The cell cycle is controlled by oscillations in Cdk activity

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

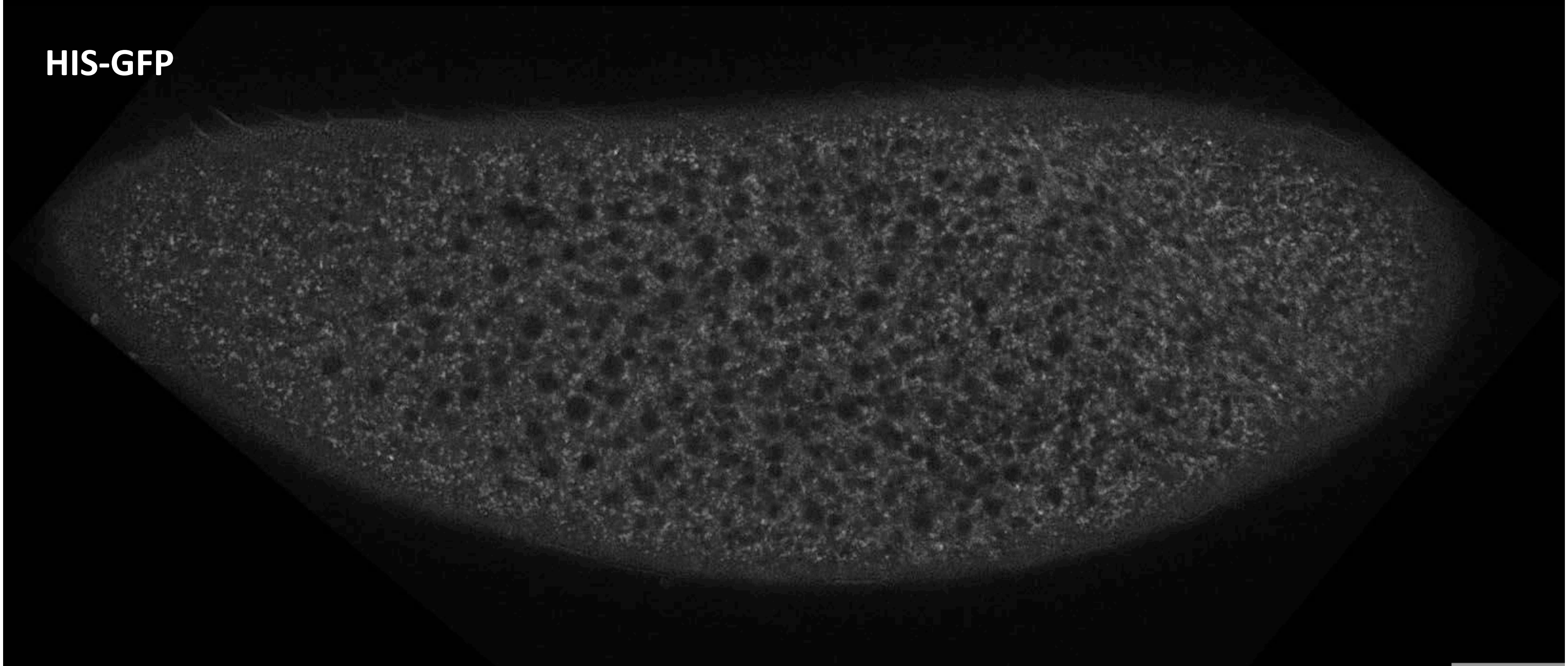


Early development in *Drosophila*



Farrell and O'Farrell 2014

Visualization of *Drosophila* early cell cycles



Three remarkable examples of regulation

1. Synchrony of the early mitosis
2. Cell cycle pause at the mid-blastula transition
3. Cell cycle re-entry in a spatiotemporal accurate pattern

Synchronization of mitosis across large scale (~0.5 mm)

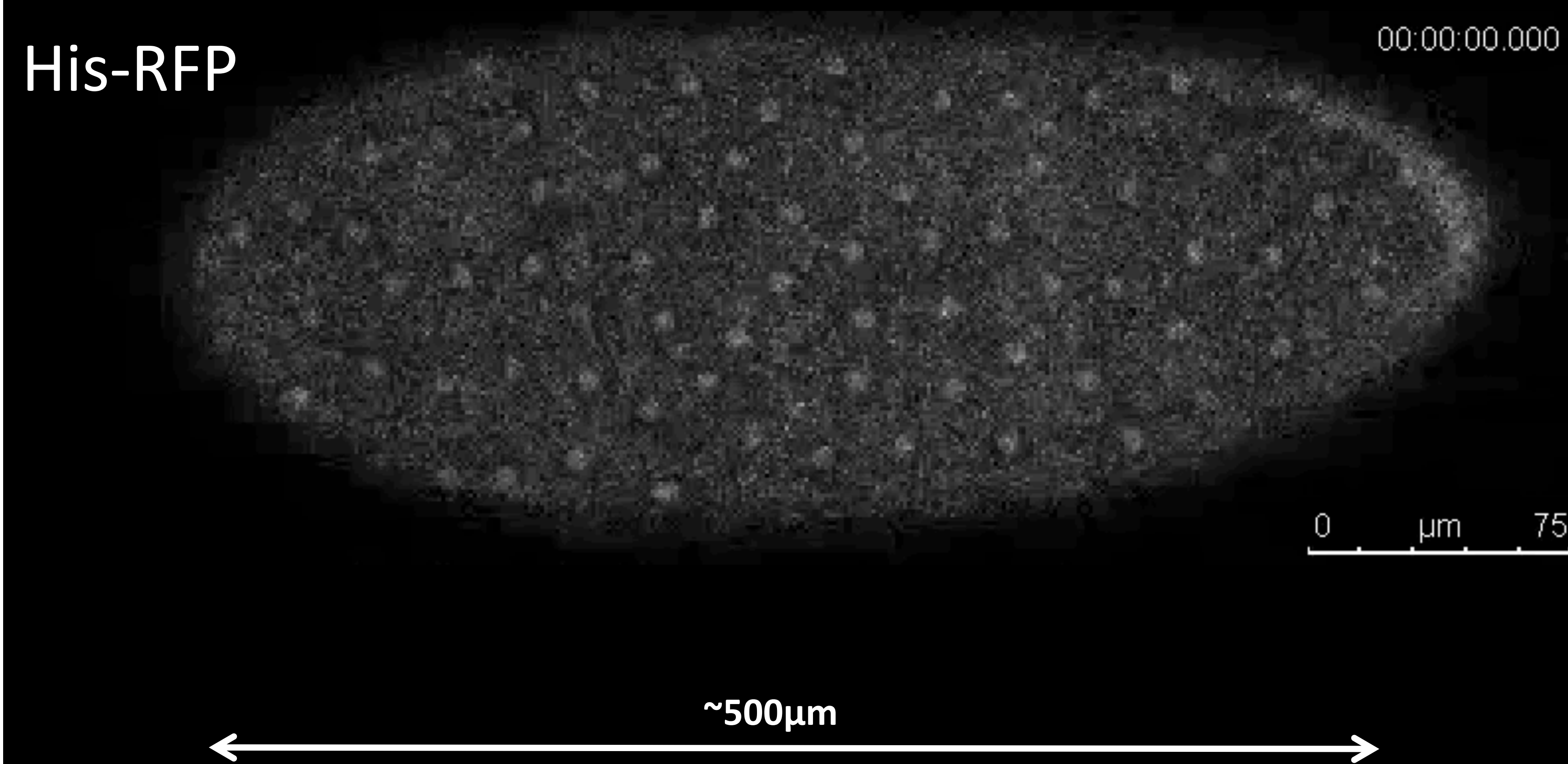
Victoria Deneke

Anna Melbinger

Massimo Vergassola

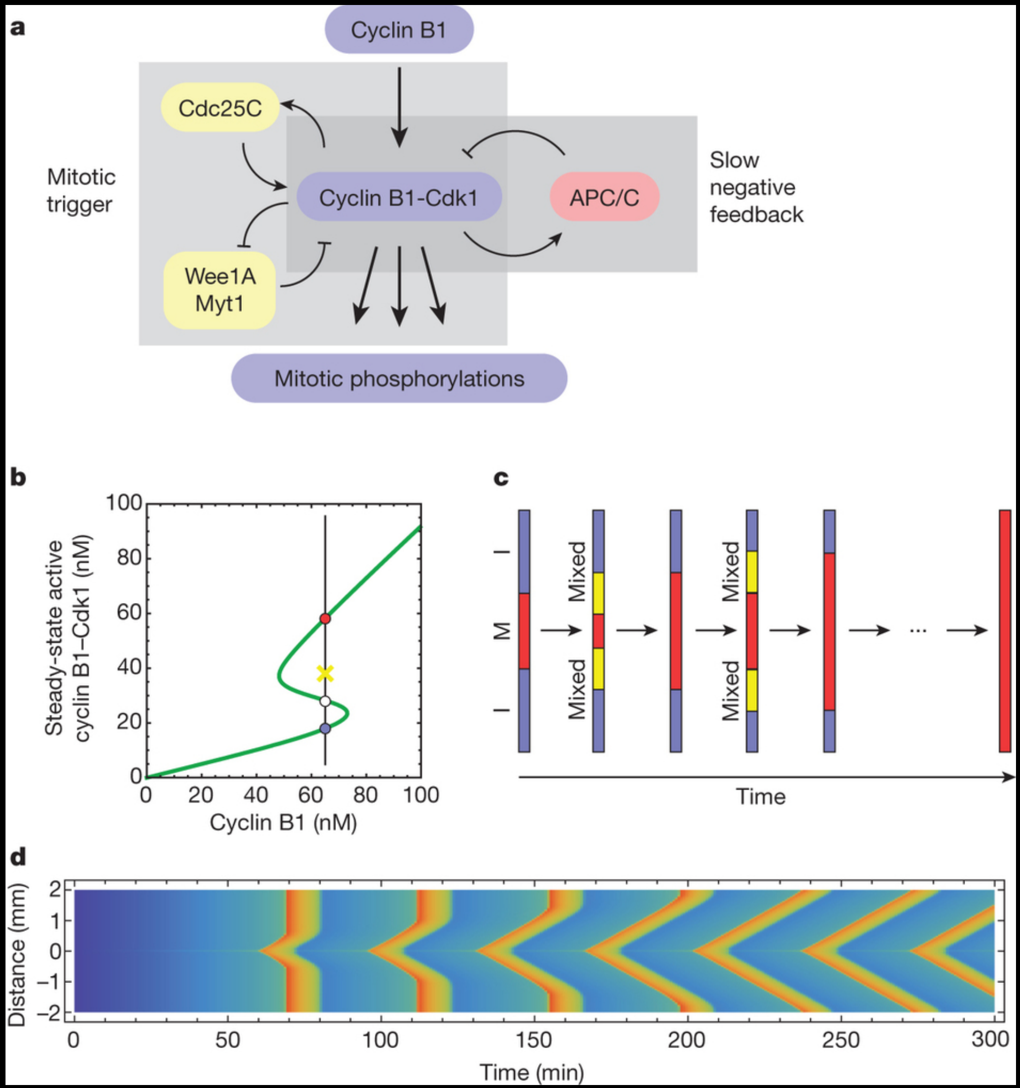
UCSD, Physics

How are mitotic events synchronized across large spatial scales?



Victoria Deneke

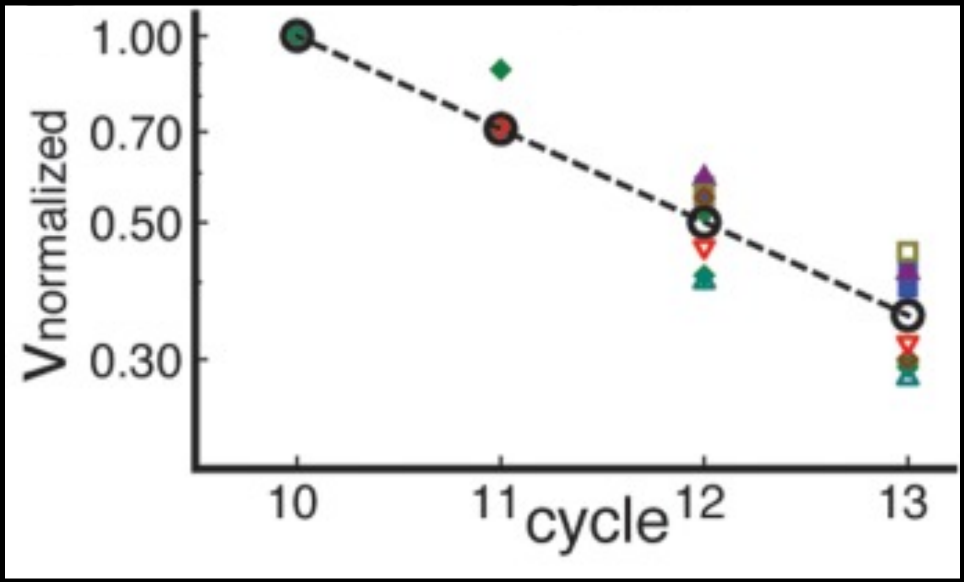
Cdk1 trigger waves

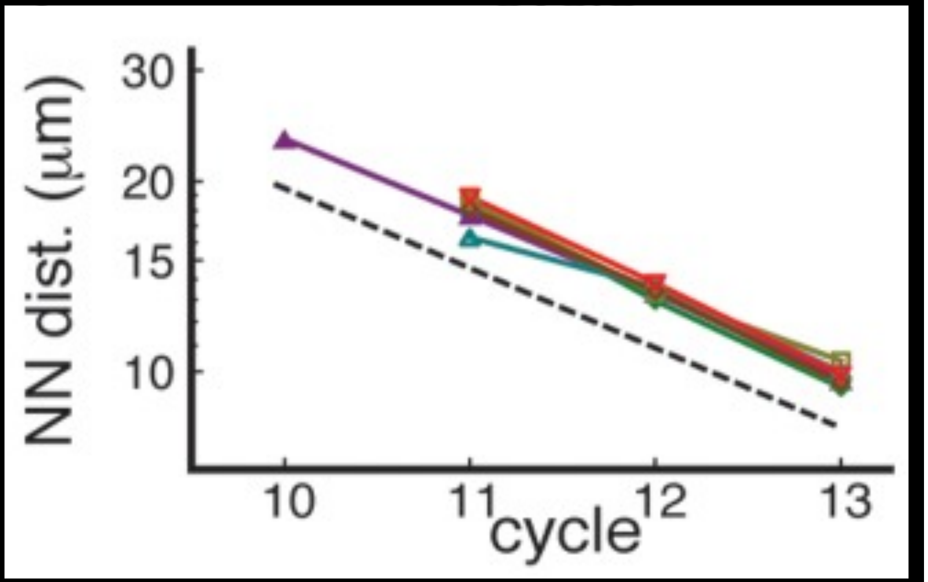


Novak and Tyson J Cell Sc and J Theor Biol 1993
Chang and Ferrell Nature 2013

Why do waves slow down during development?

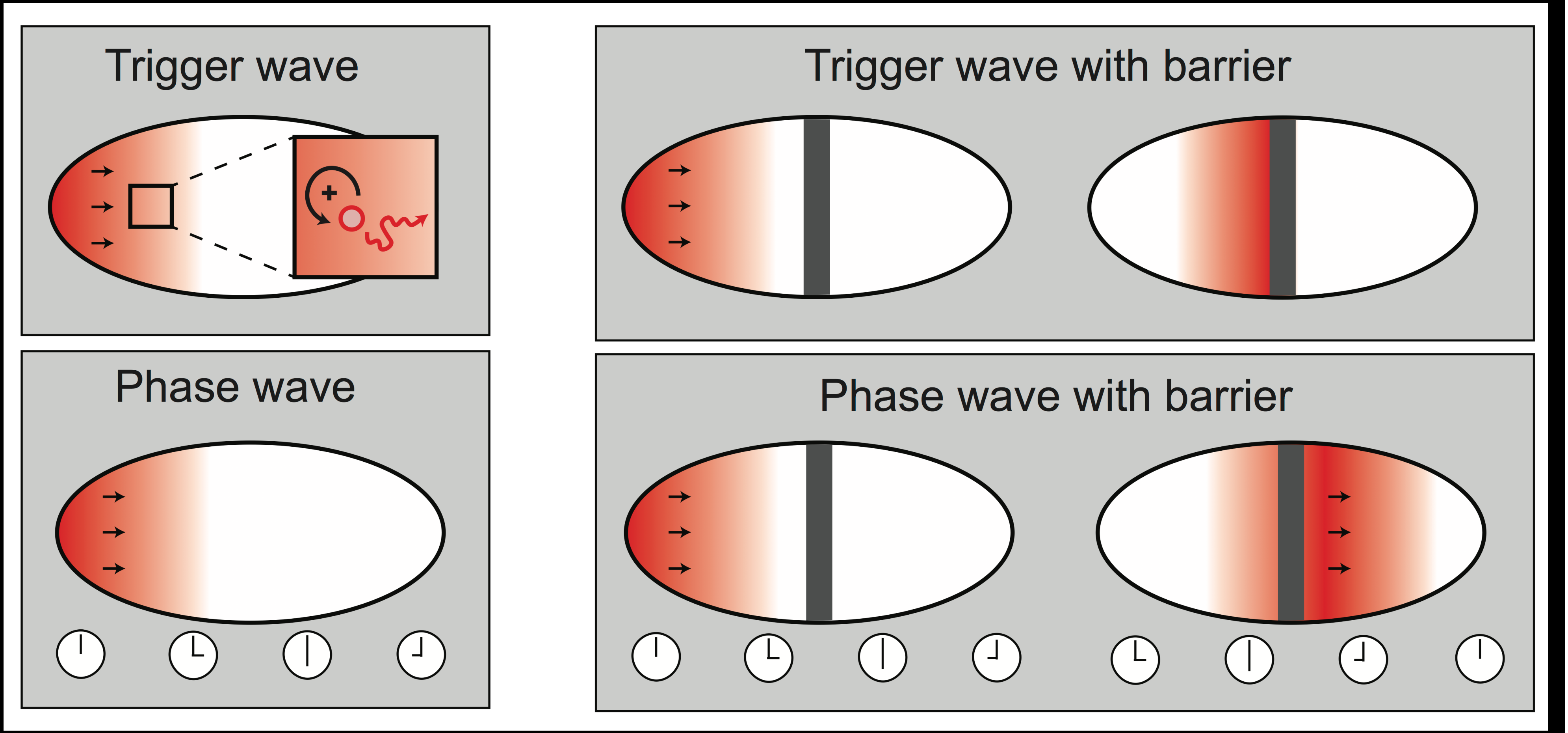
Scaling of the mitotic wave speed with nucleus-to-nucleus distance in *Drosophila* has been proposed as a signature of mechanical waves propagating through the embryo





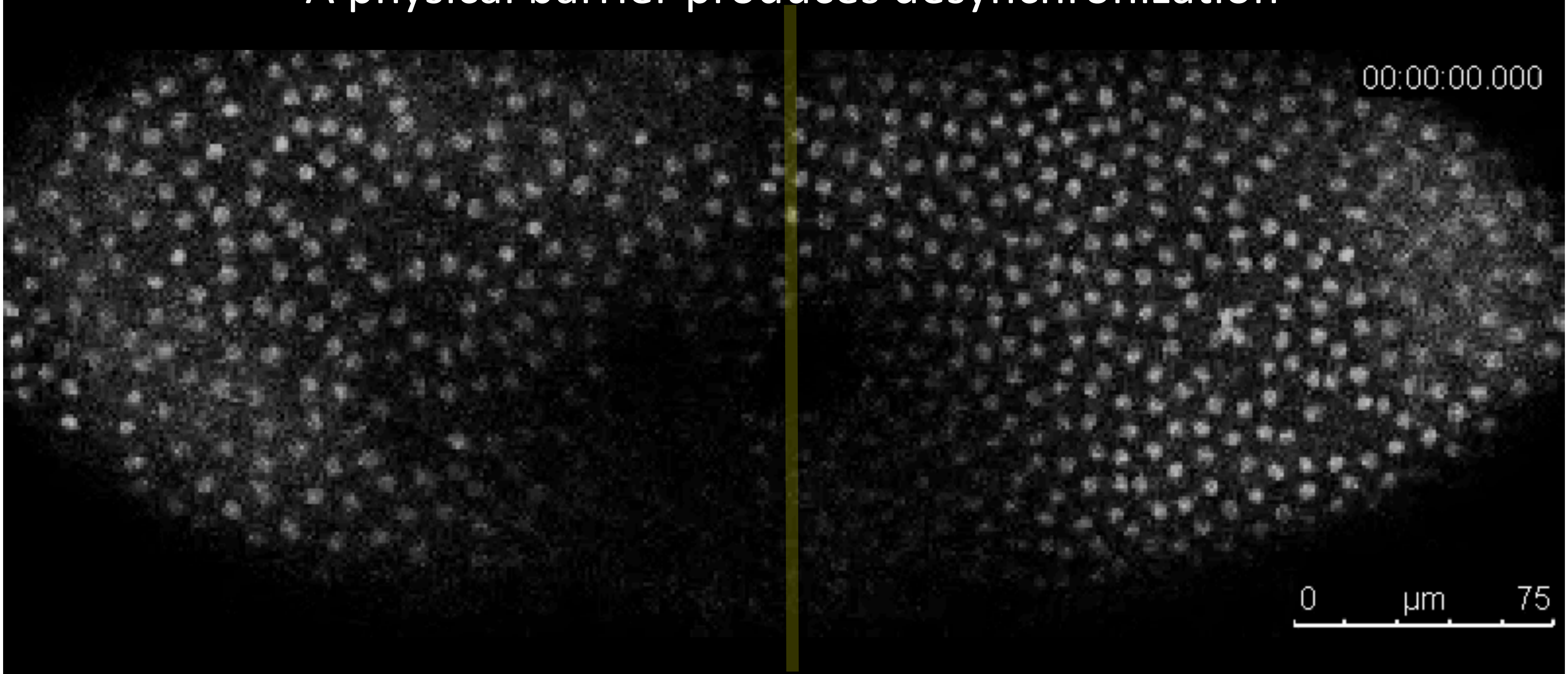
Idema et al Plos One 2013

Physical mechanisms of chemical waves



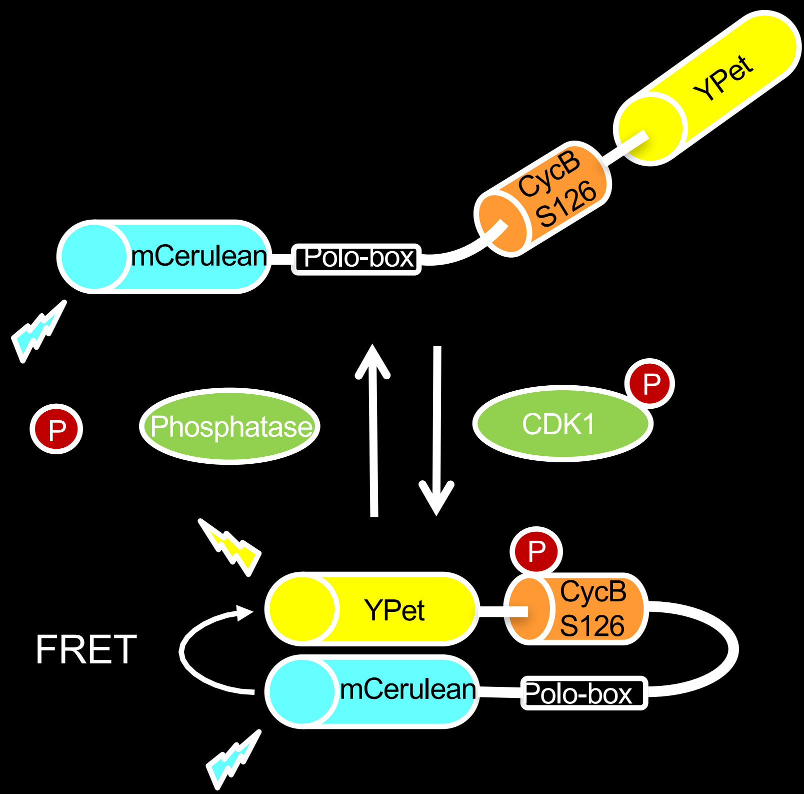
Is it really collective or just well synchronized clocks?

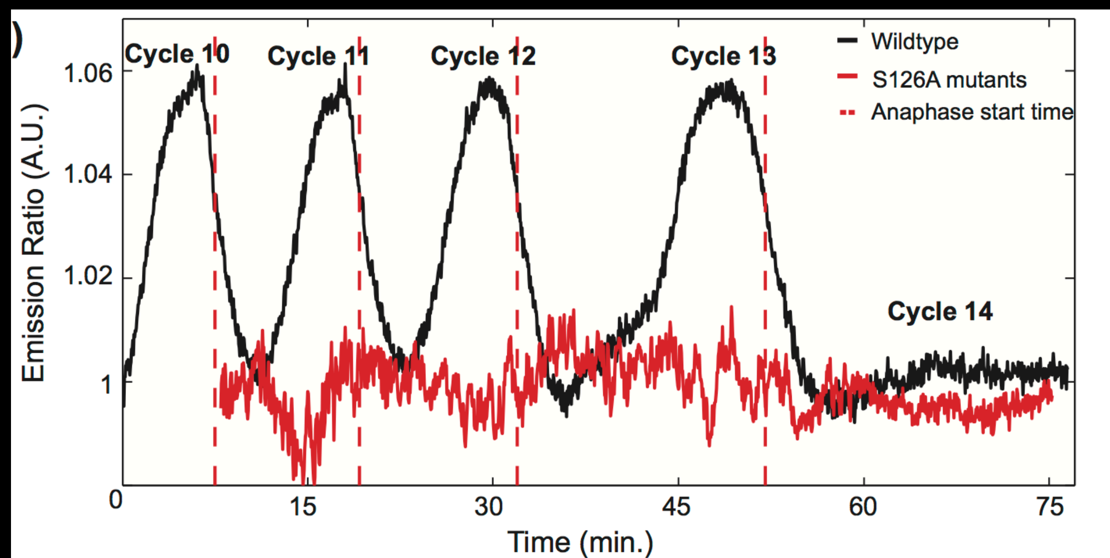
A physical barrier produces desynchronization



Can we understand the molecular and physical mechanisms controlling the wave?

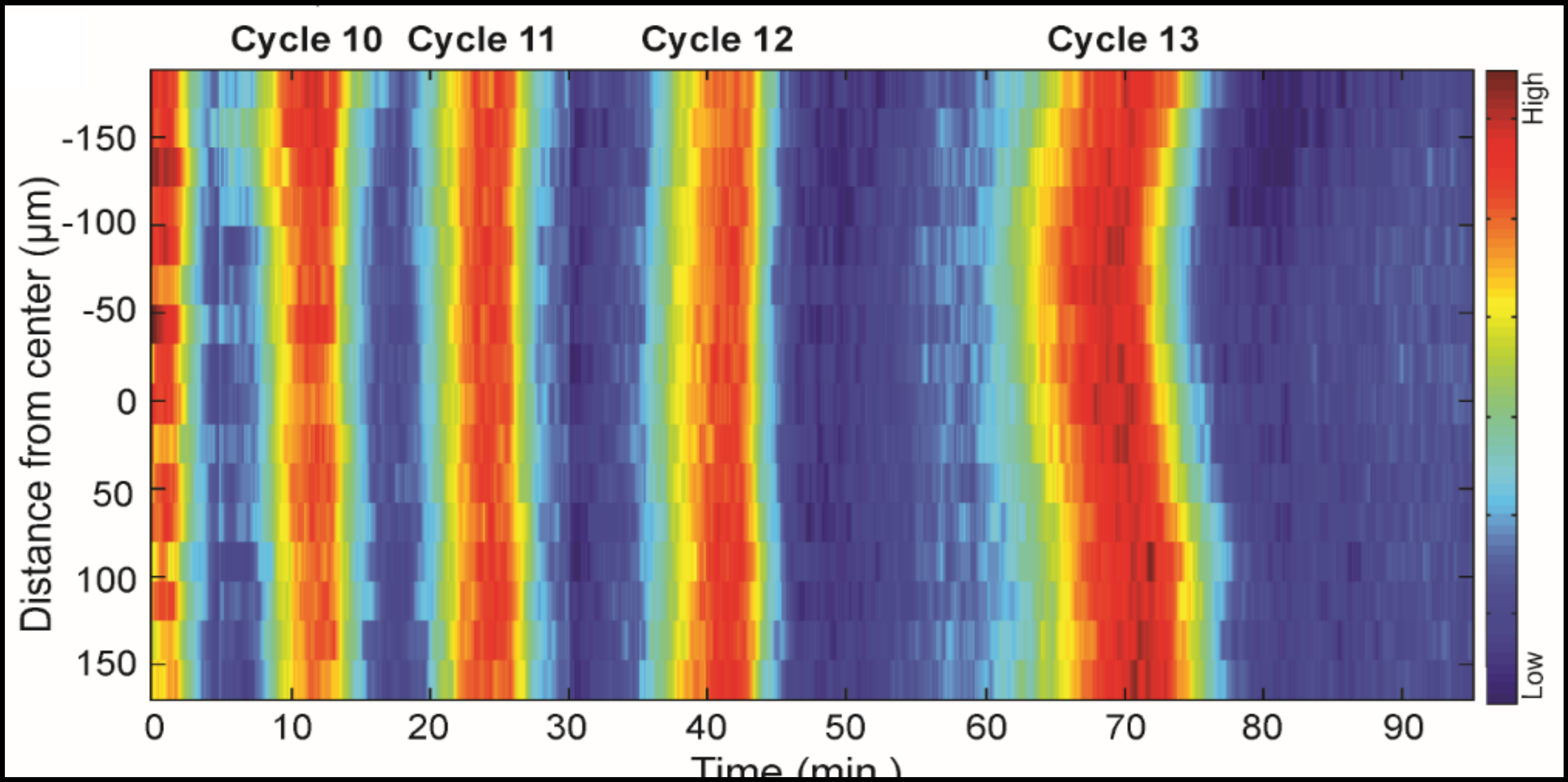
Cdk1 FRET biosensor specifically measures Cdk1 activity



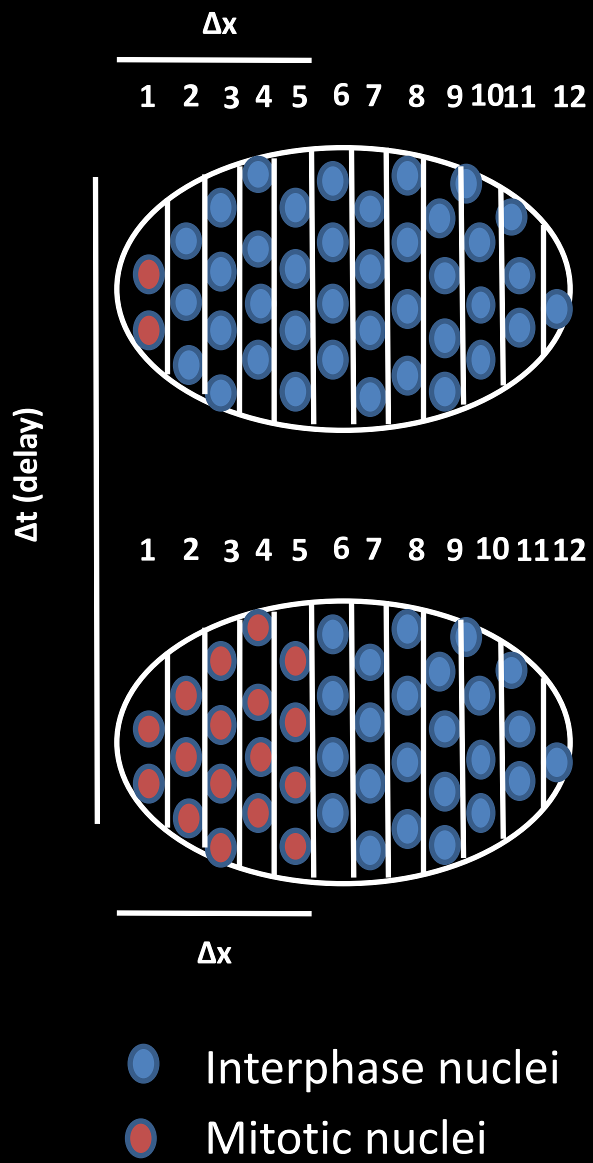


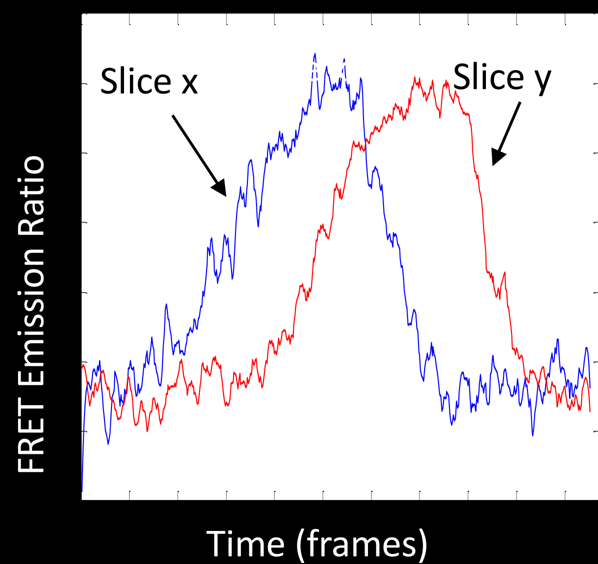
Gavet and Pines Dev Cell and JCB 2010

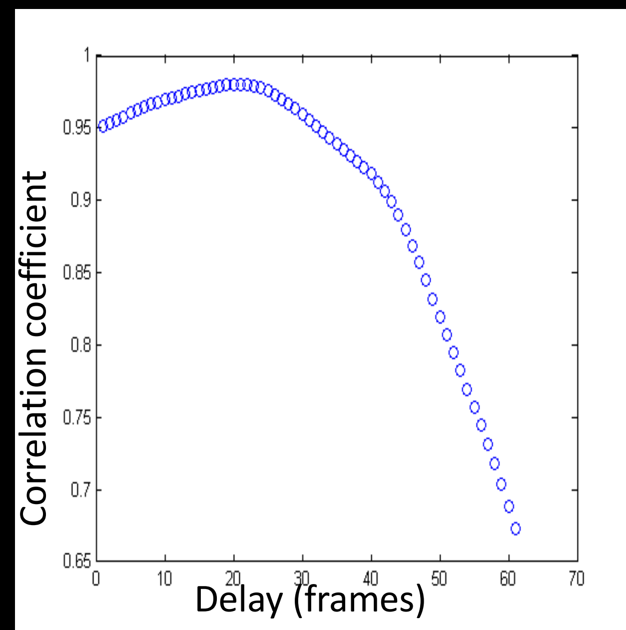
Measuring Cdk1 activity over multiple cell cycles with seconds resolution



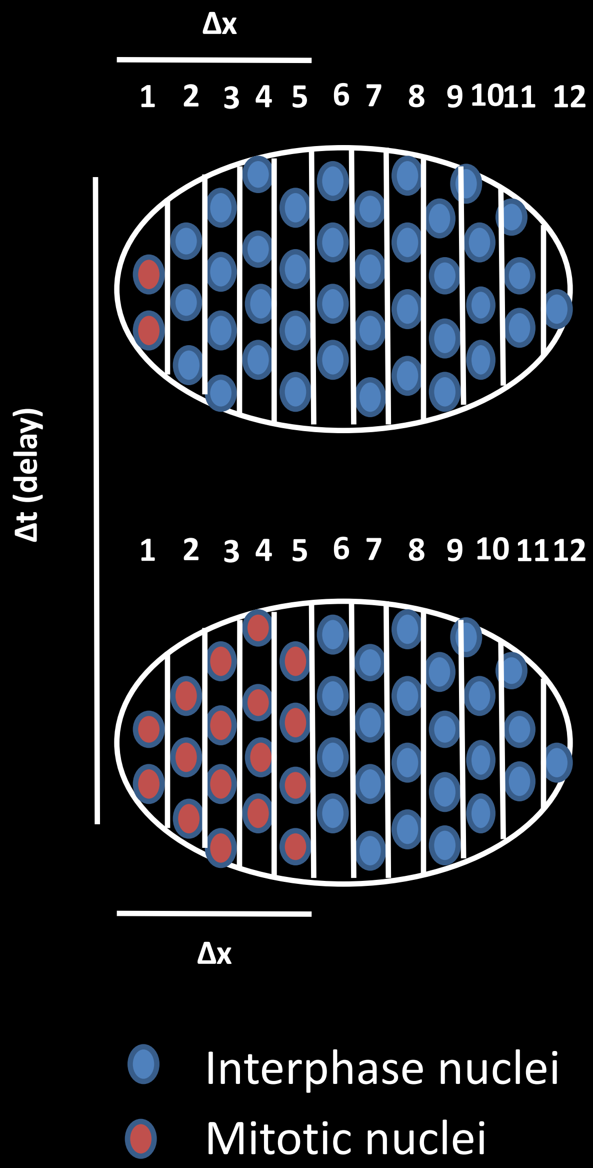
Wave speed can be determined from time delays in vertical slices

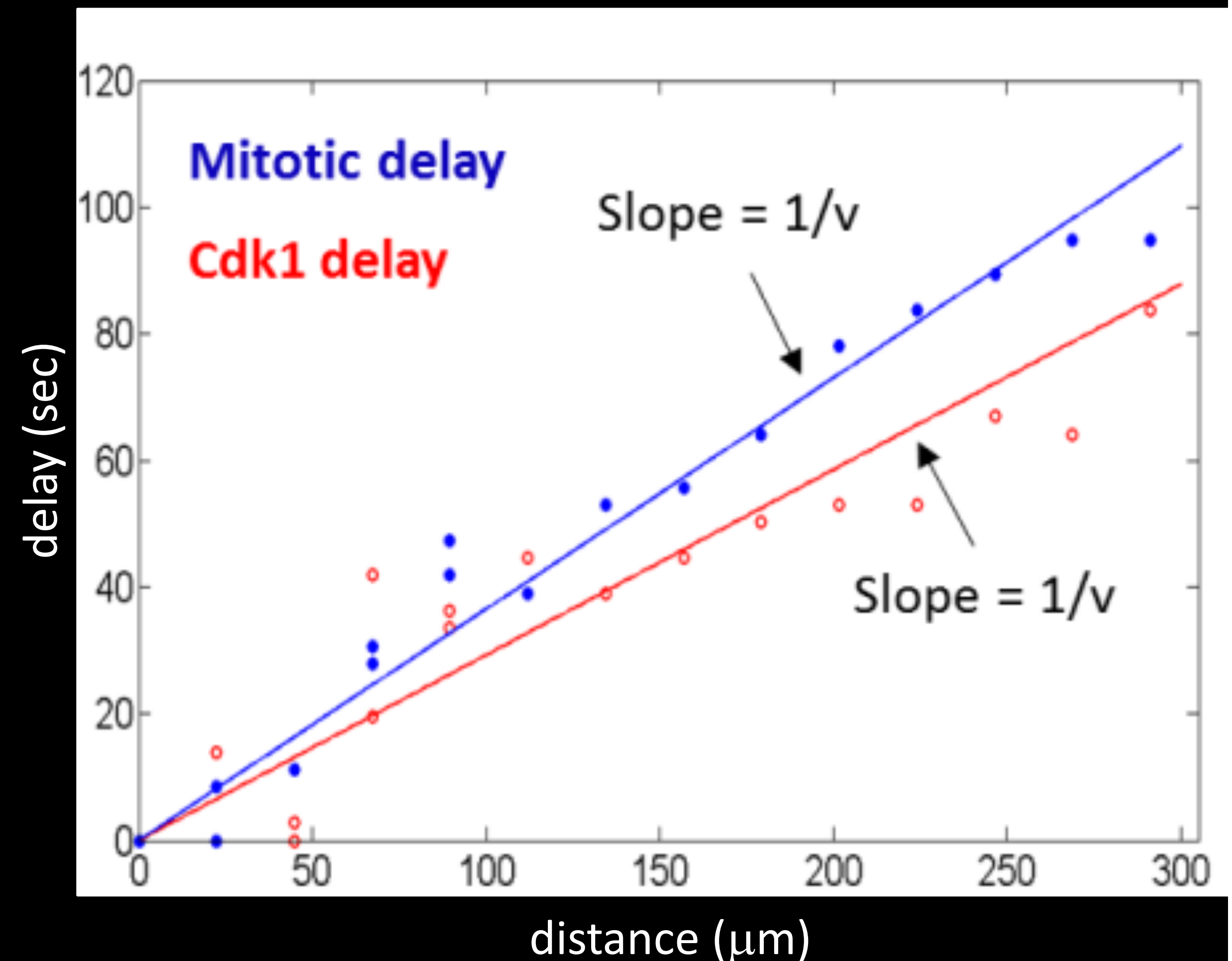




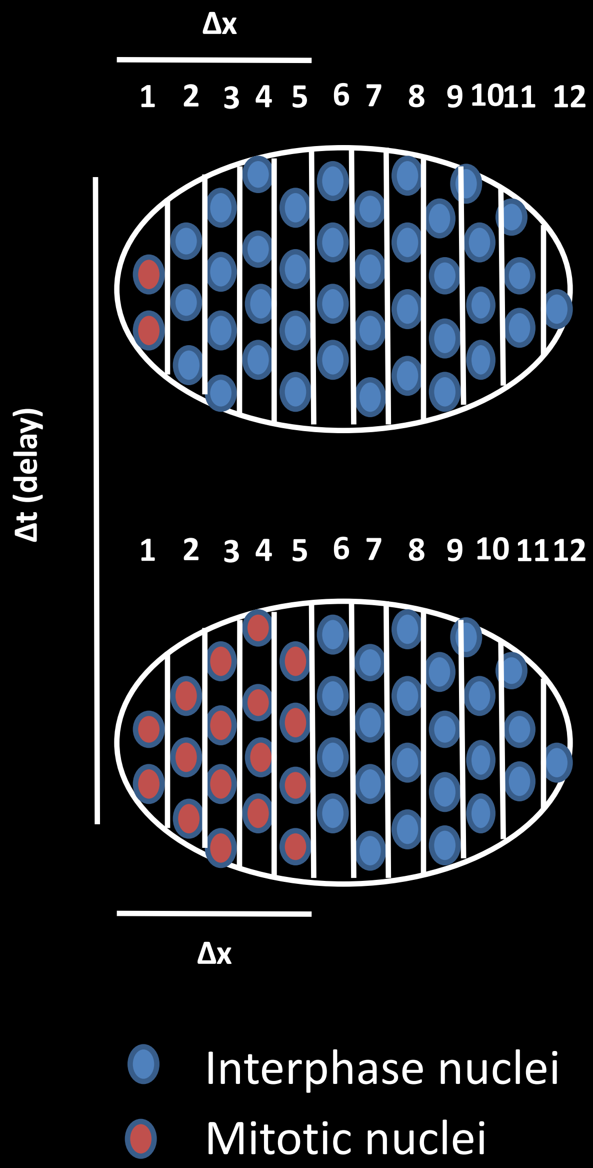


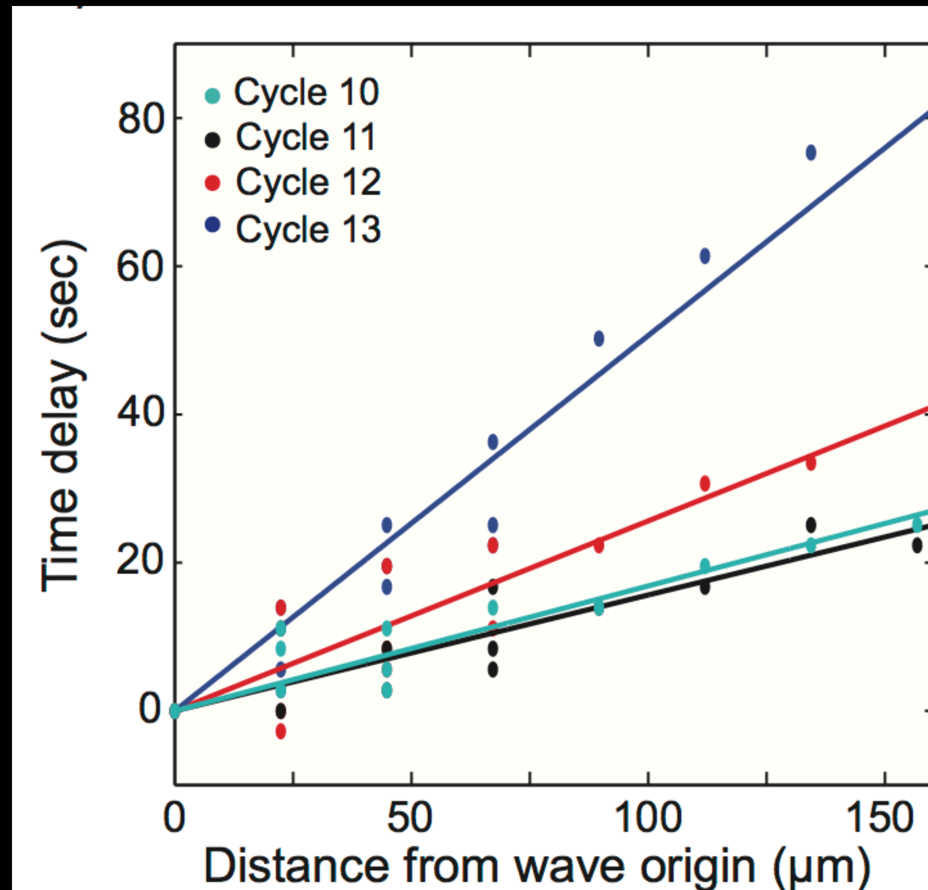
Wave speed can be determined from time delays in vertical slices



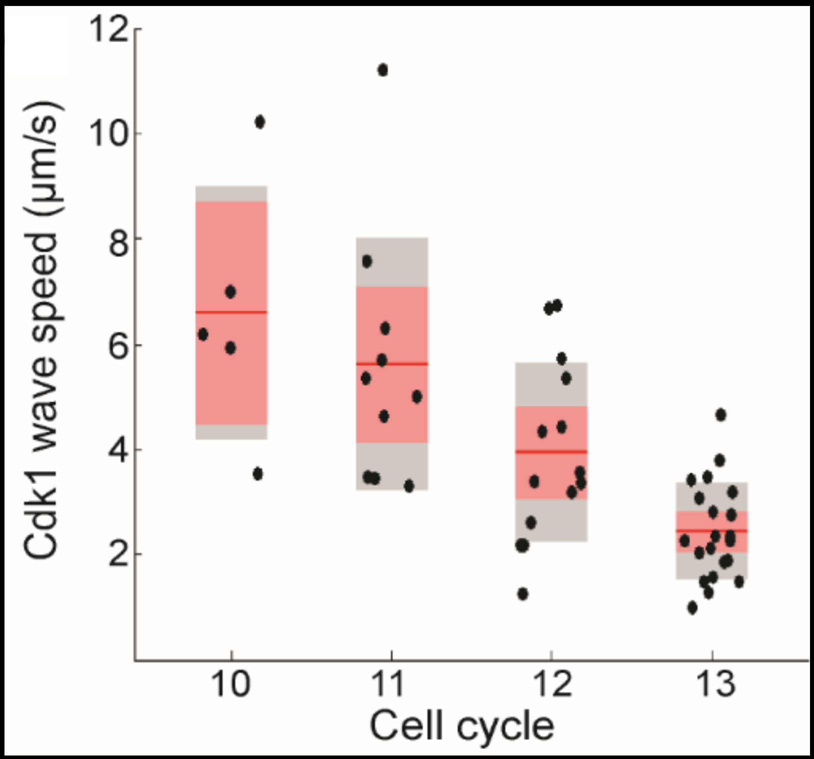


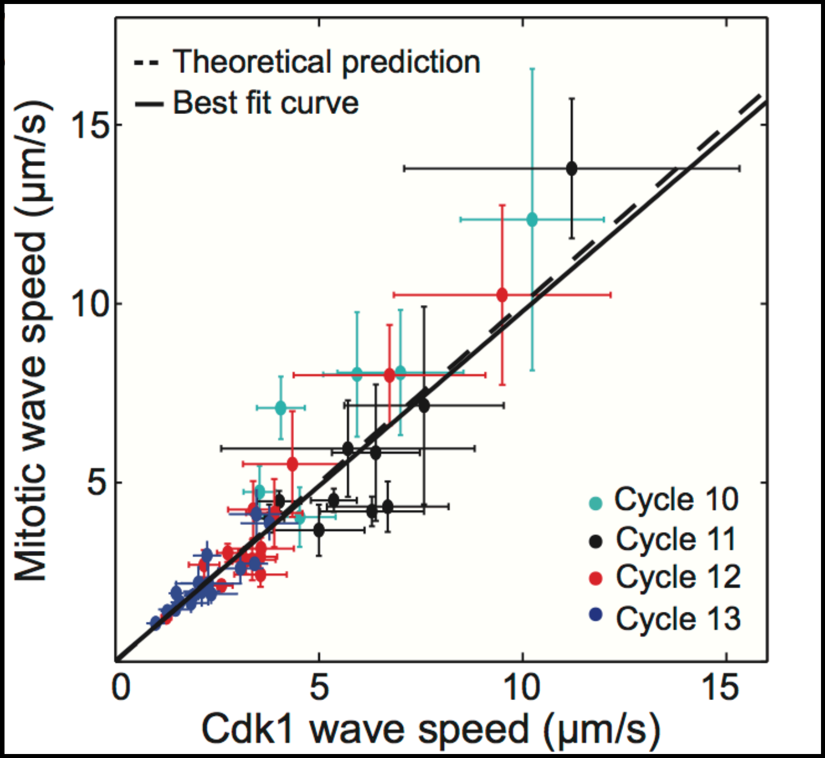
Wave speed can be determined from time delays in vertical slices





Cdk1 waves control mitotic waves





Can we understand what controls the physical properties of the waves?

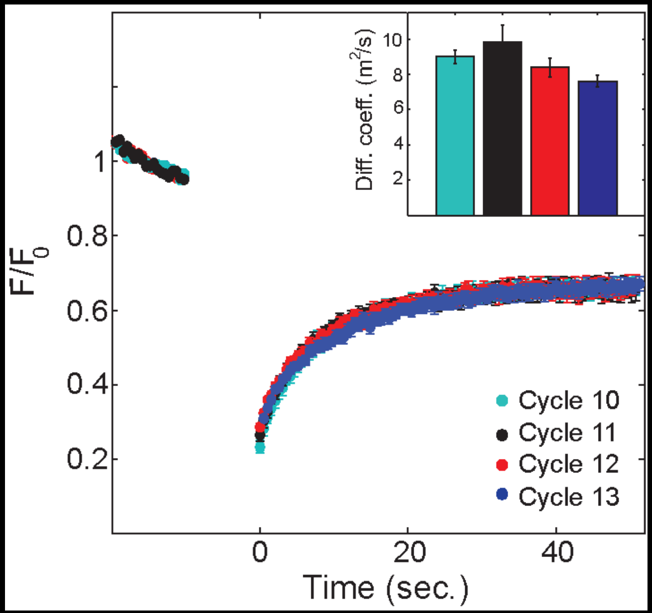
Back of the envelope

Dimensional analysis suggests that the speed should scale as:

$$v \approx \sqrt{\frac{D}{\tau}}$$

where D is the diffusion coefficient and τ is the relevant timescale.

FRAP measurements of the diffusion coefficient of Cdk1-YFP



Diffusion cannot explain why the waves slow down

Back of the envelope

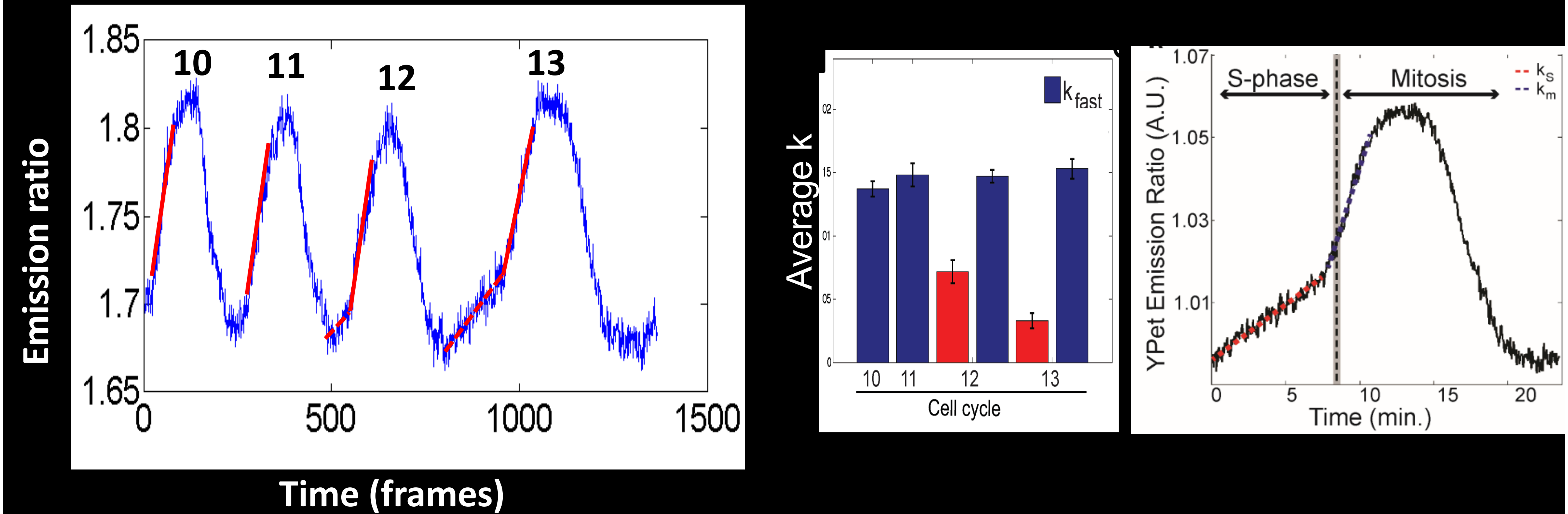
Dimensional analysis suggests that the speed should scale as:

$$v \approx \sqrt{\frac{D}{\tau}}$$

where D is the diffusion coefficient and τ is the relevant timescale.

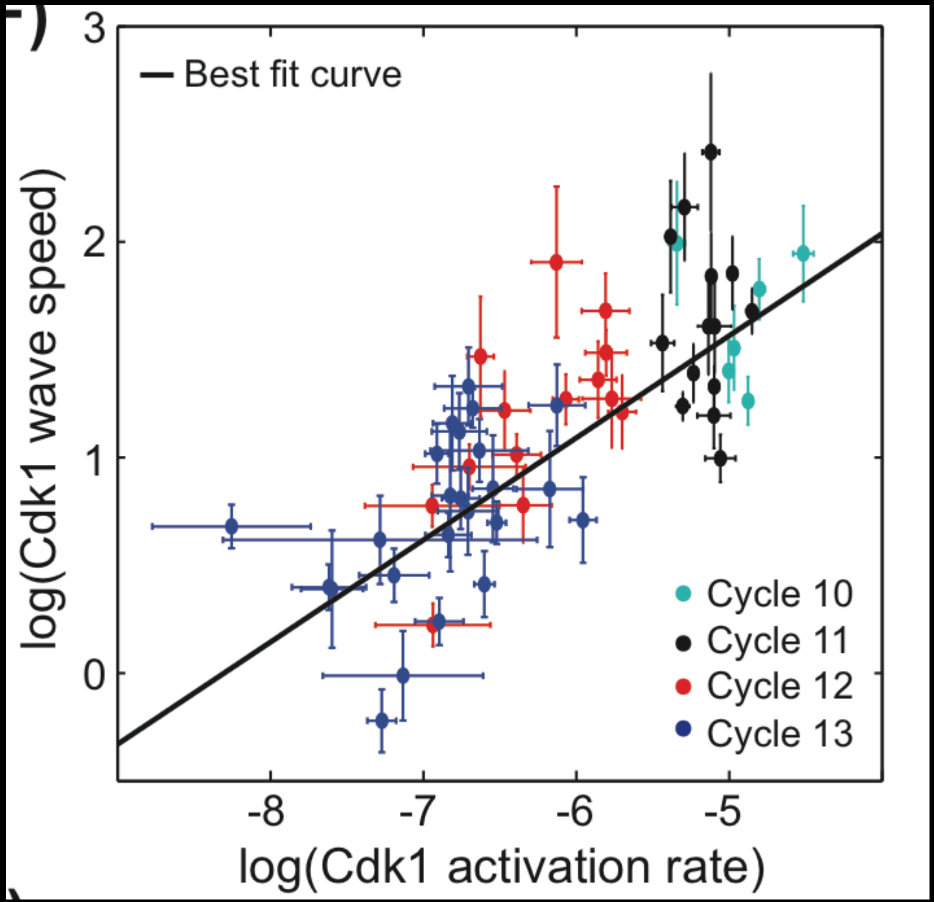
Which changes in the Cdk1 reaction dynamics can explain the change in speed?

The activity of Cdk1 during mitosis does not regulate the speed of Cdk1 waves

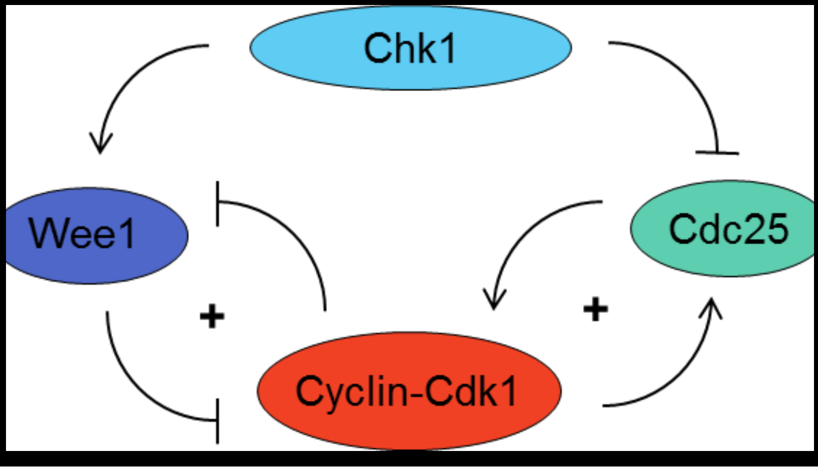


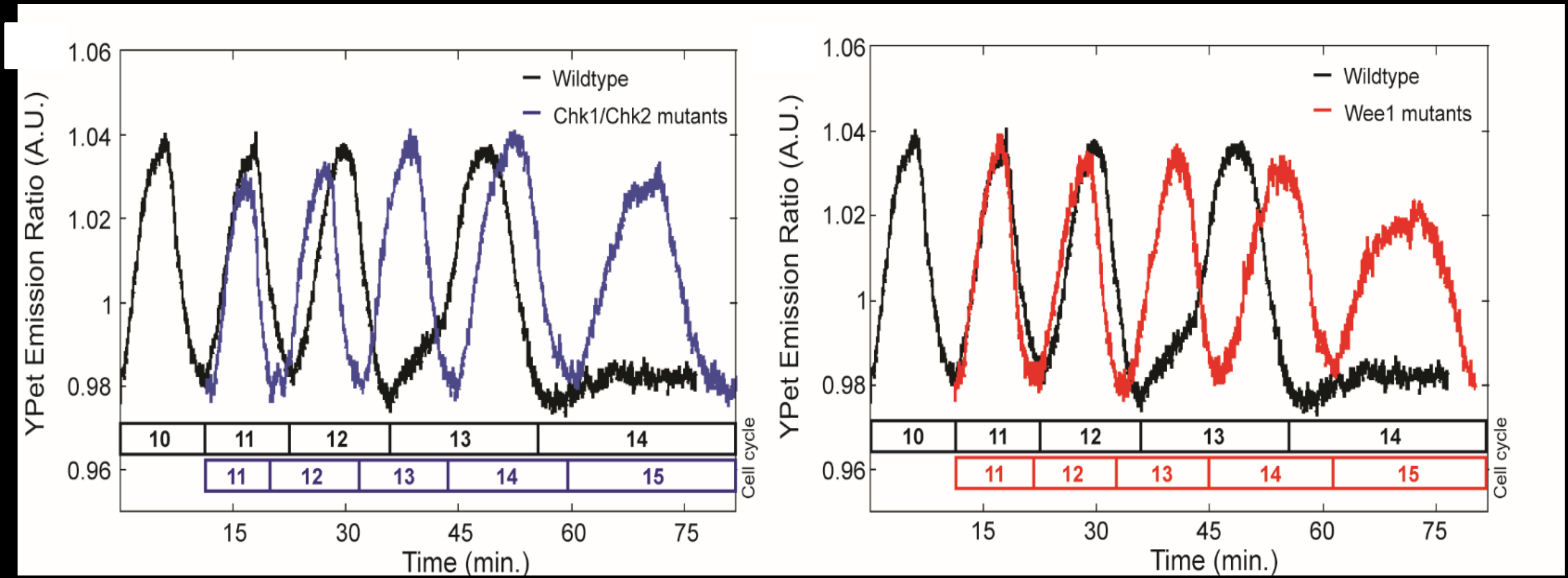
Cdk1 wave speed can be predicted by the rate of activation of Cdk1 in S-phase

$v \sim (Dk_S)^{1/2}$; $\log(v) = 0.5 * \log(k_S) + C$

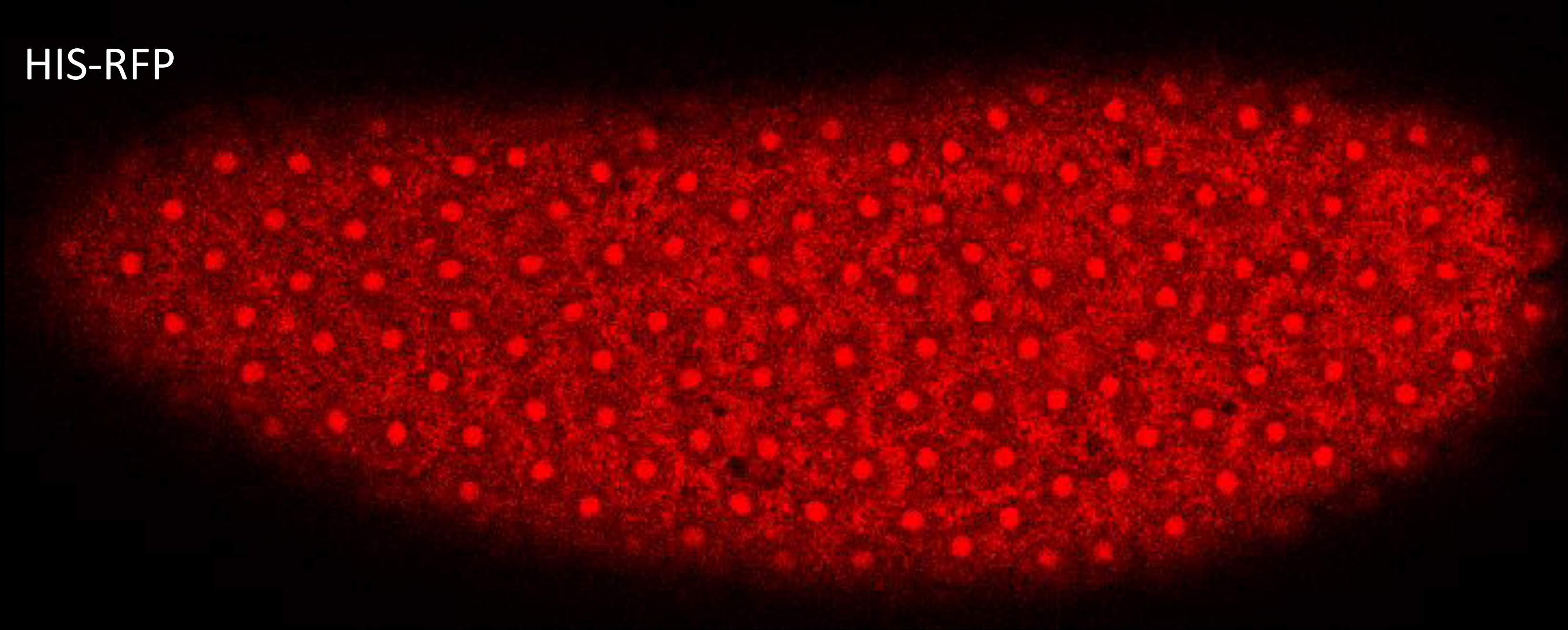


The dynamics of Cdk1 is regulated by the DNA replication checkpoint via Chk1/Wee1

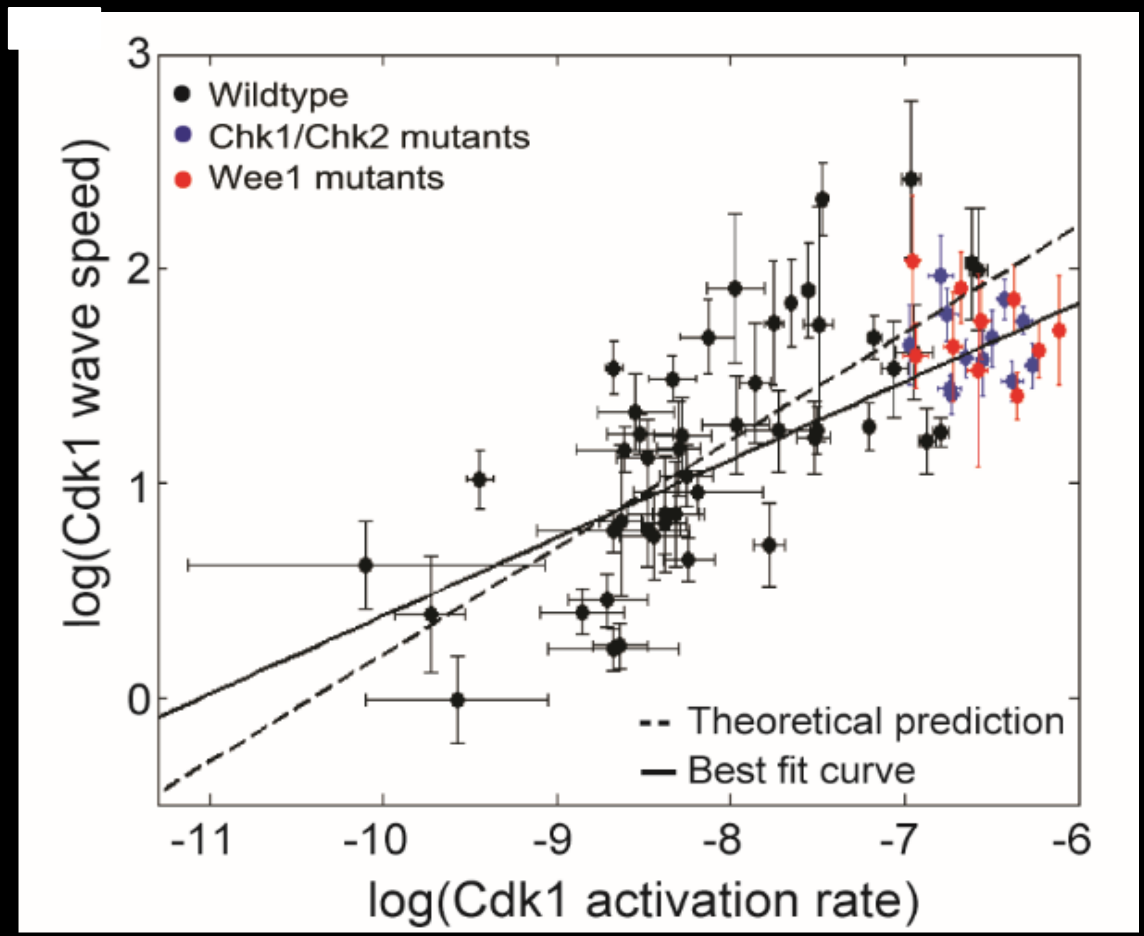




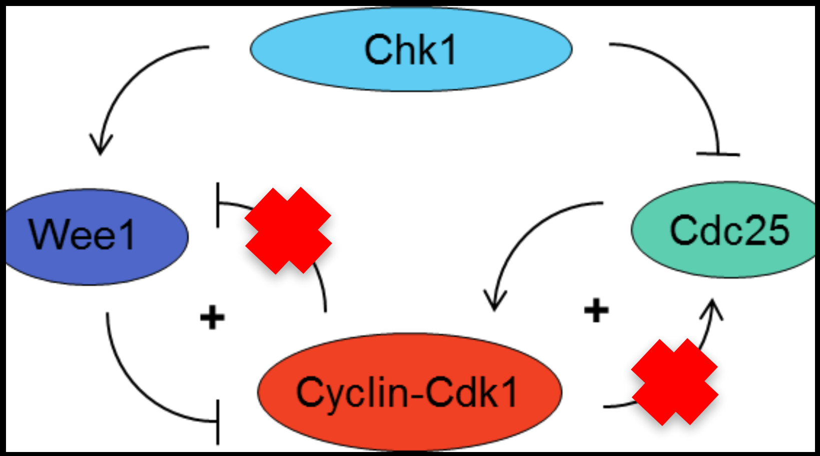
The mitotic waves do not slow down in *chk1 chk*2 mutants

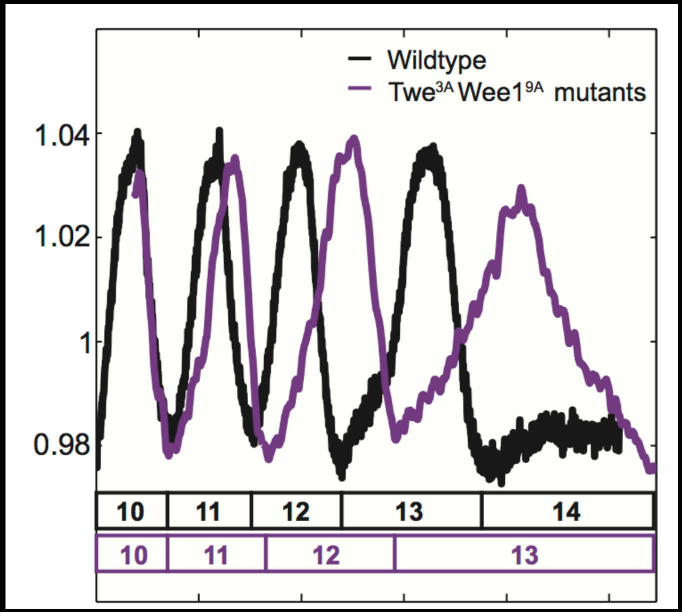


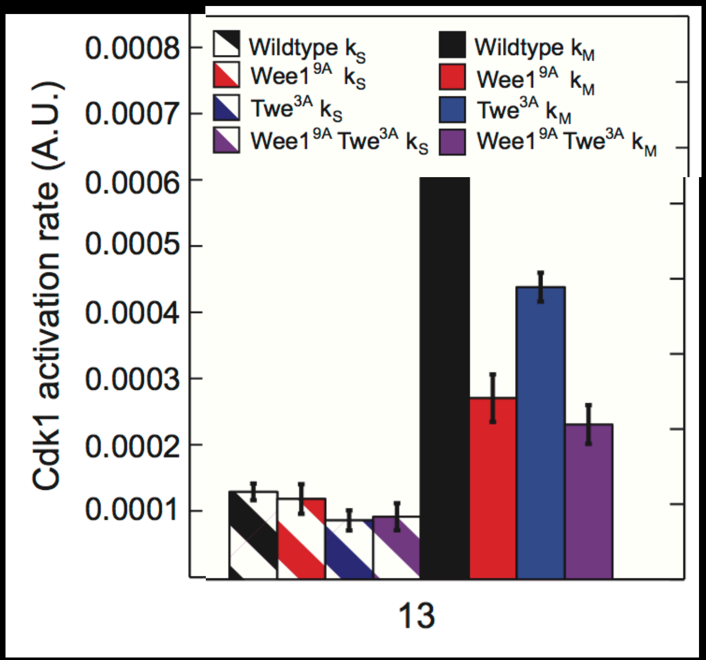
The slowdown of the Cdk1 waves requires the Chk1/Wee1 pathway



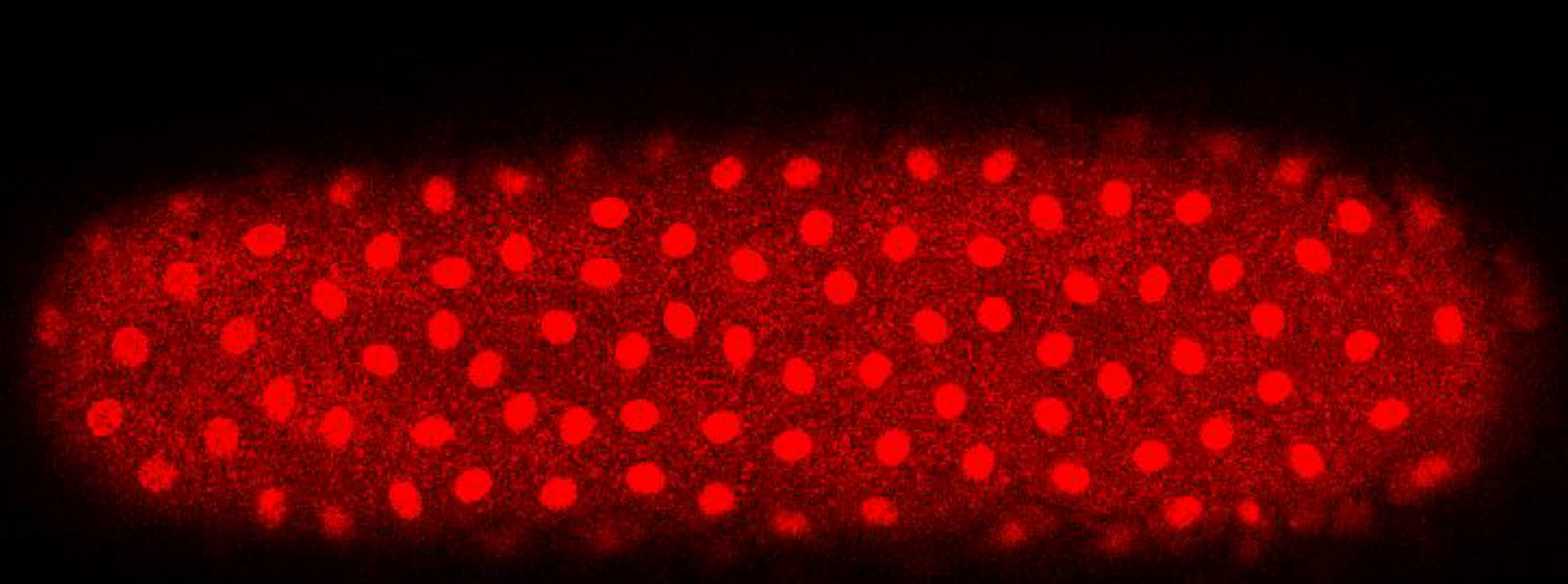
Disabling the Cdk1/Wee1/Cdc25 feedbacks alters the rapid phase of Cdk1 activation



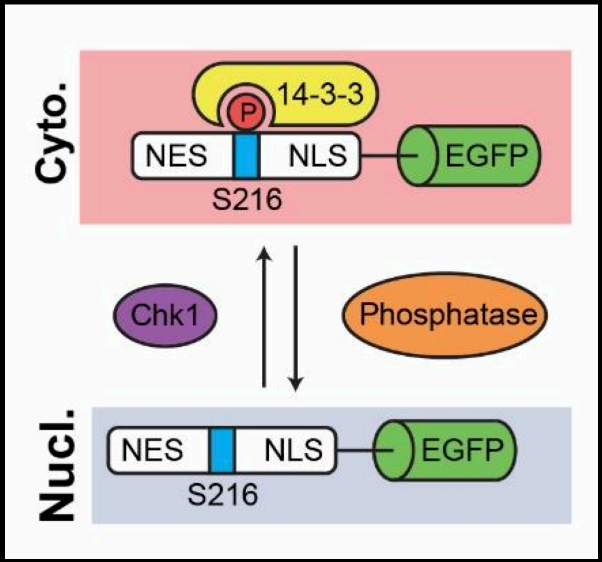


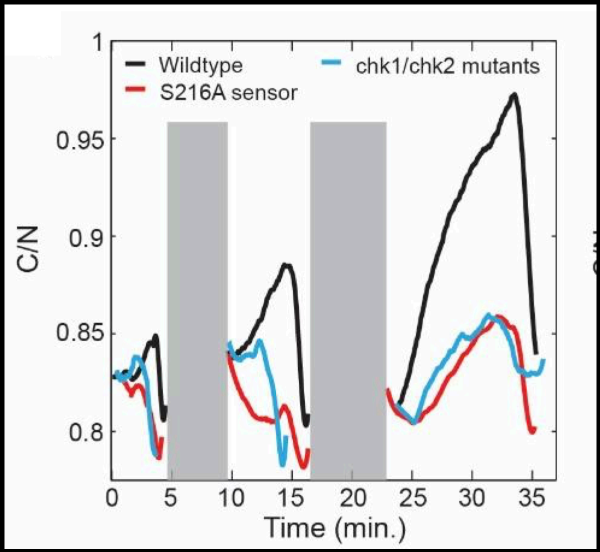


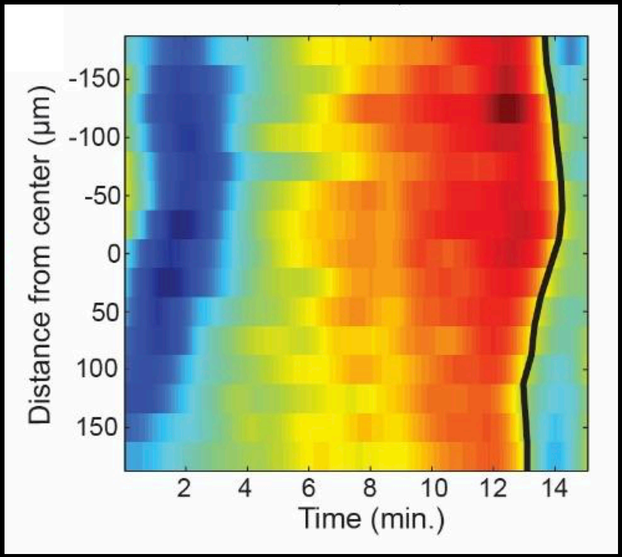
Disabling the Cdk1/Wee1/Cdc25 feedbacks does not prevent the propagation of mitotic waves



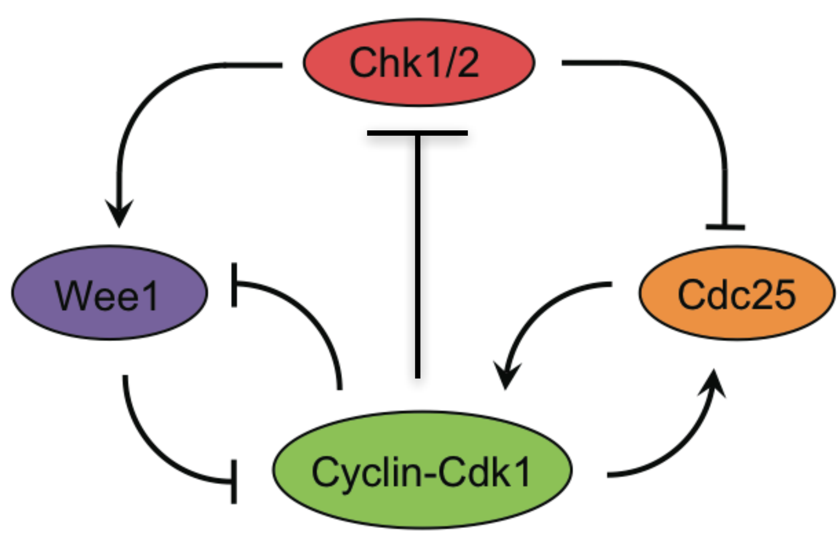
Chk1 activity is turned off in a wave-like pattern

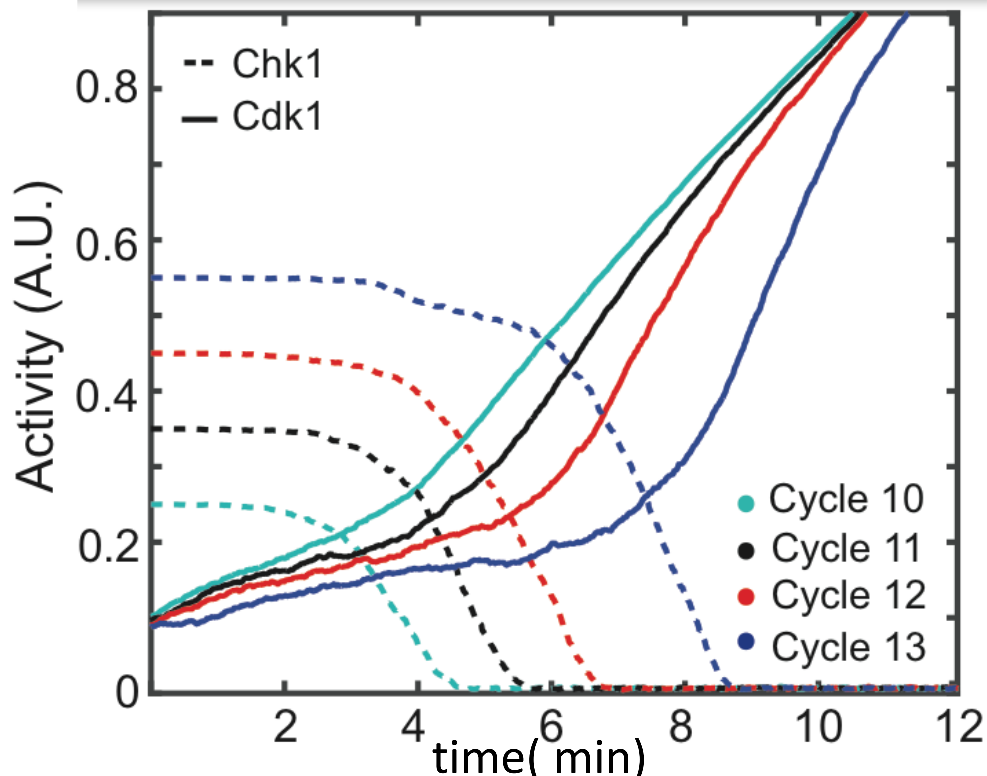




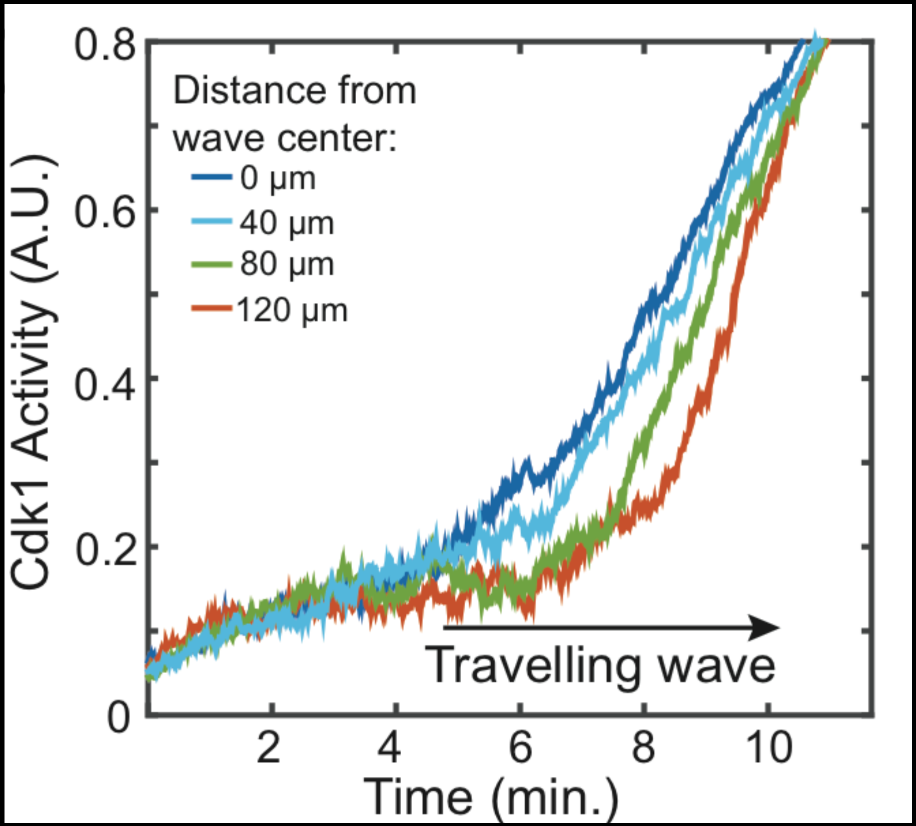


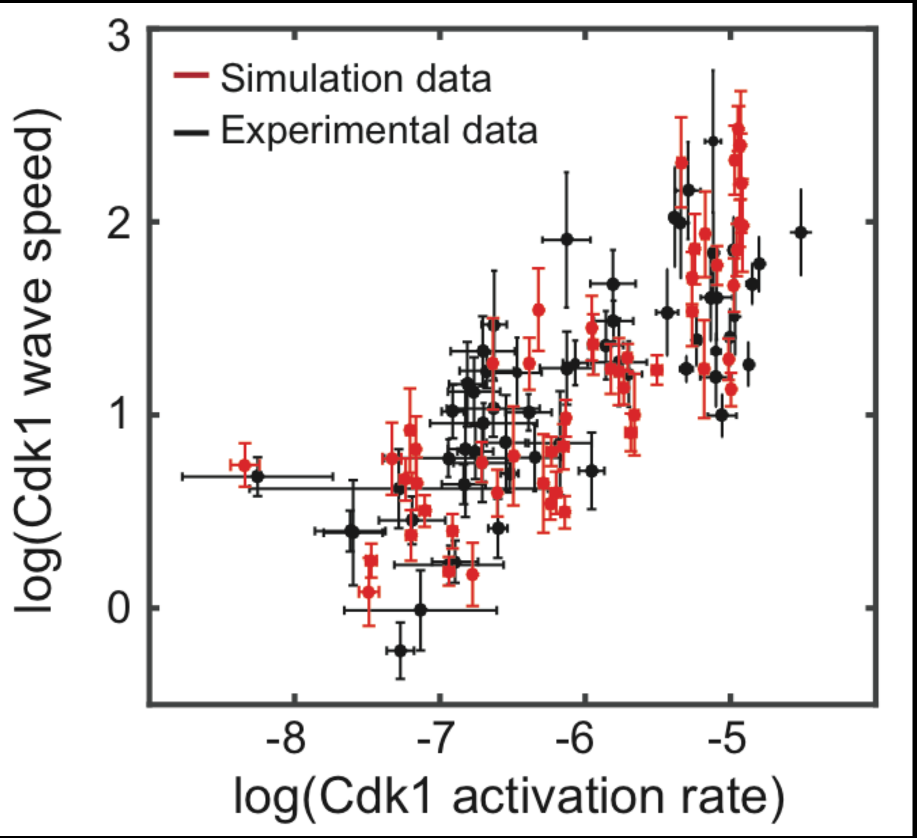
A mathematical model of Cdk1 waves



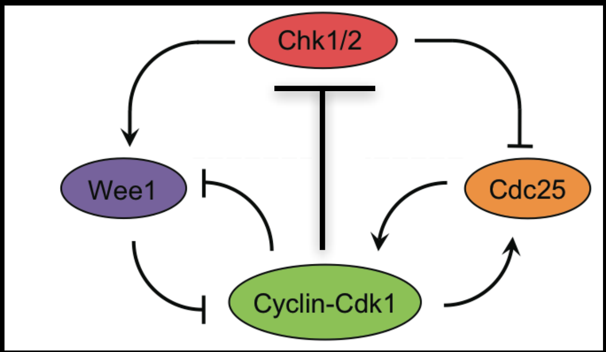


A simple model can reproduce the scaling of the speed of Cdk1 waves





Reduction to an effective one-species dynamics



$$\frac{\partial f}{\partial t} = D_{Chk1}\frac{\partial^2 f}{\partial x^2} - \frac{a^\sigma}{K^\sigma_{Chk1}+a^\sigma} r_0 f + \xi_f(x,t)$$

$$\frac{\partial a}{\partial t} = D_{Cdk1}\frac{\partial^2 a}{\partial x^2} + \alpha + r_+(a,f)(c(x,t)-a) - r_-(a,f)a + \xi_c(x,t) + \xi_r(x,t)$$

$$\frac{\partial c}{\partial t} = D_{Cdk1}\frac{\partial^2 c}{\partial x^2} + \alpha + \xi_c(x,t)$$

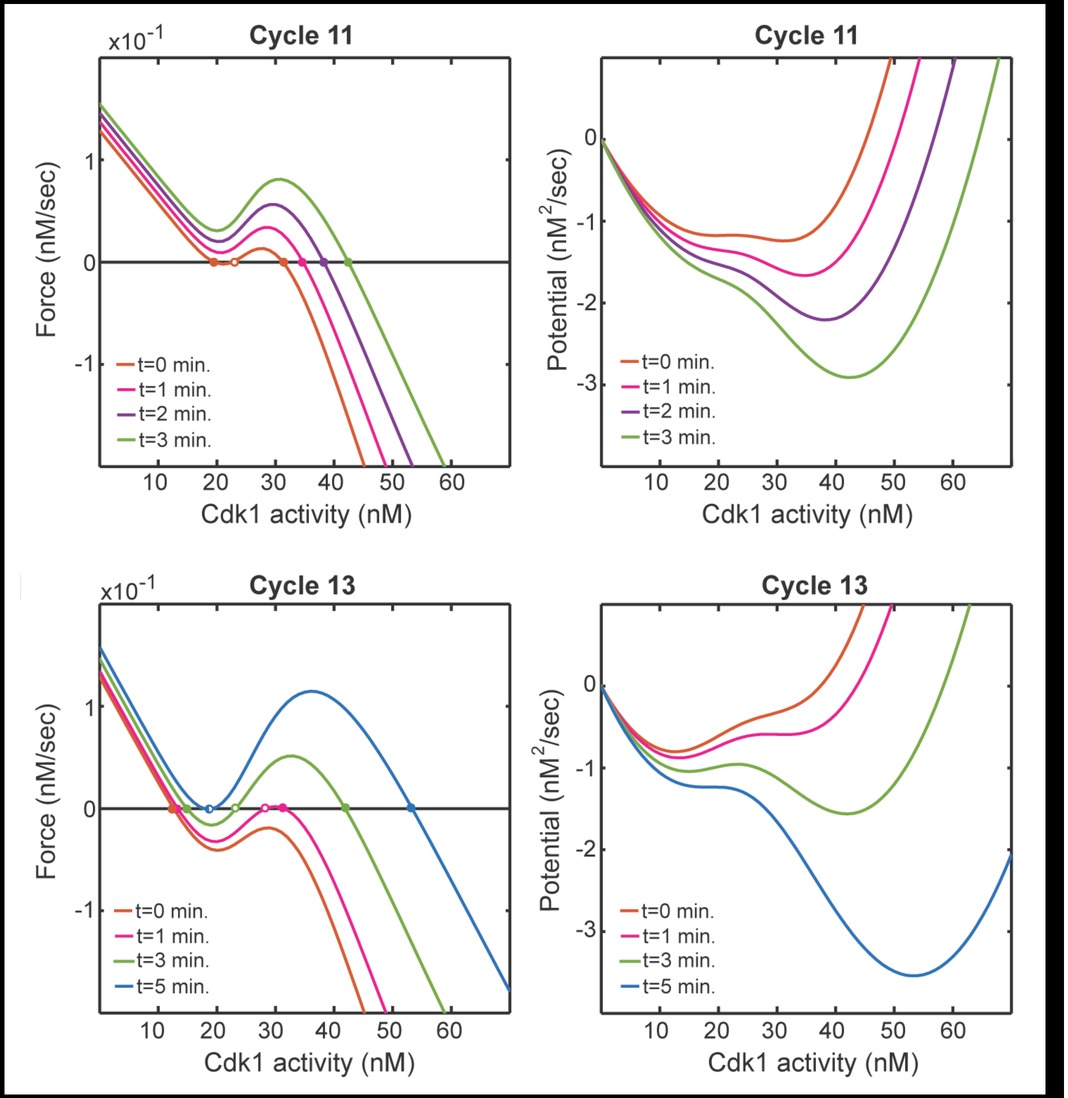
1. The dynamics of f is fast and effectively slaved to the dynamics of Cdk1
2. The dynamics of c (total Cdk1) is correlated over scales $(4Dt)^{1/2} \approx 70\ \mu m$ large compared to $D/v \approx 1\ \mu m$ so safely $c(x,t) \approx c(t)$
3. Curvature of the embryo safely ignored by same reasoning

Effective one-species dynamics

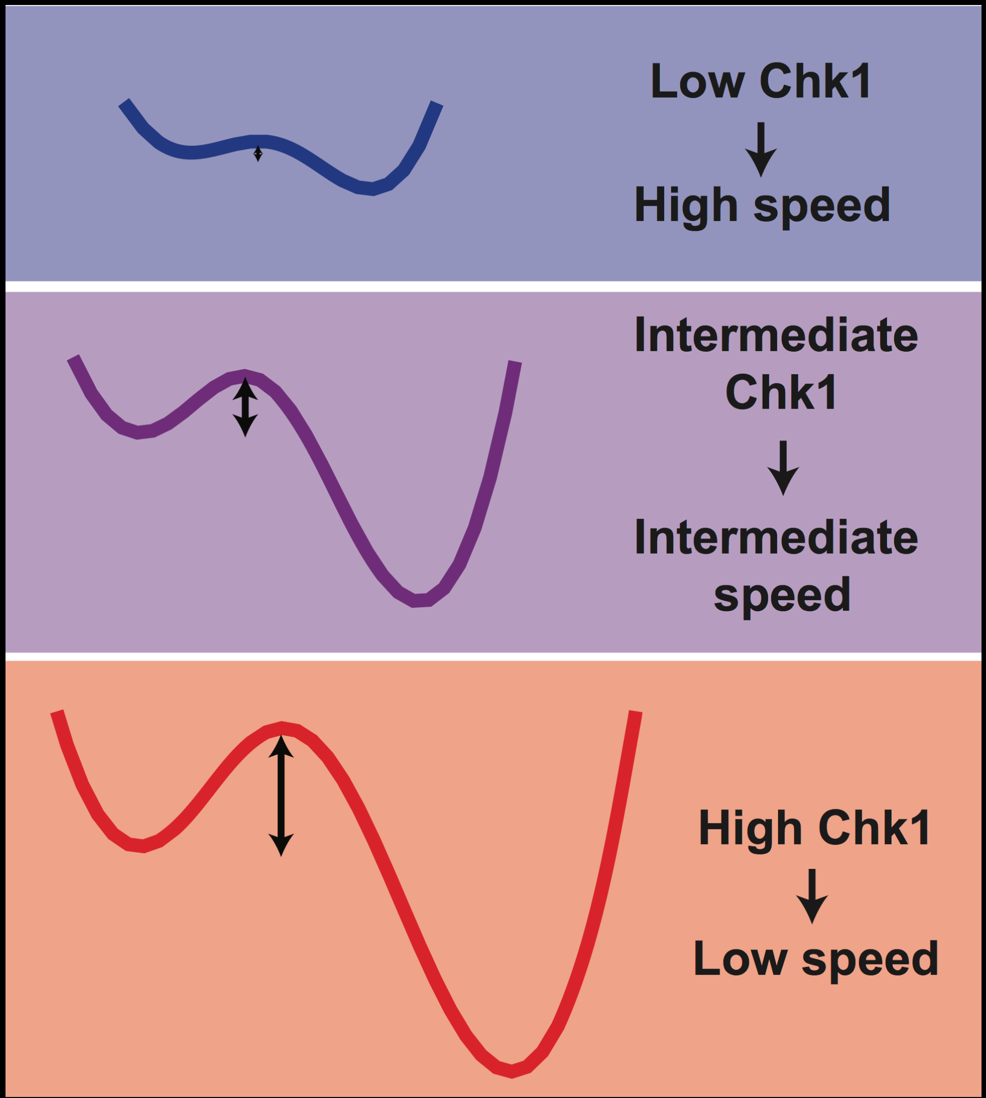
$$\frac{\partial a}{\partial t} = D_{Cdk1}\nabla^2 a - \frac{\partial V(a)}{\partial a} + \xi_{eff}(x,t)$$

The waves are noise triggered: noise comparable to barrier

1. **Noise controls the time of the jump and as a consequence the speed of the wave as potential depends on time**
2. **The spreading is not a perfect wave**



An intuitive explanation for the wave slowdown

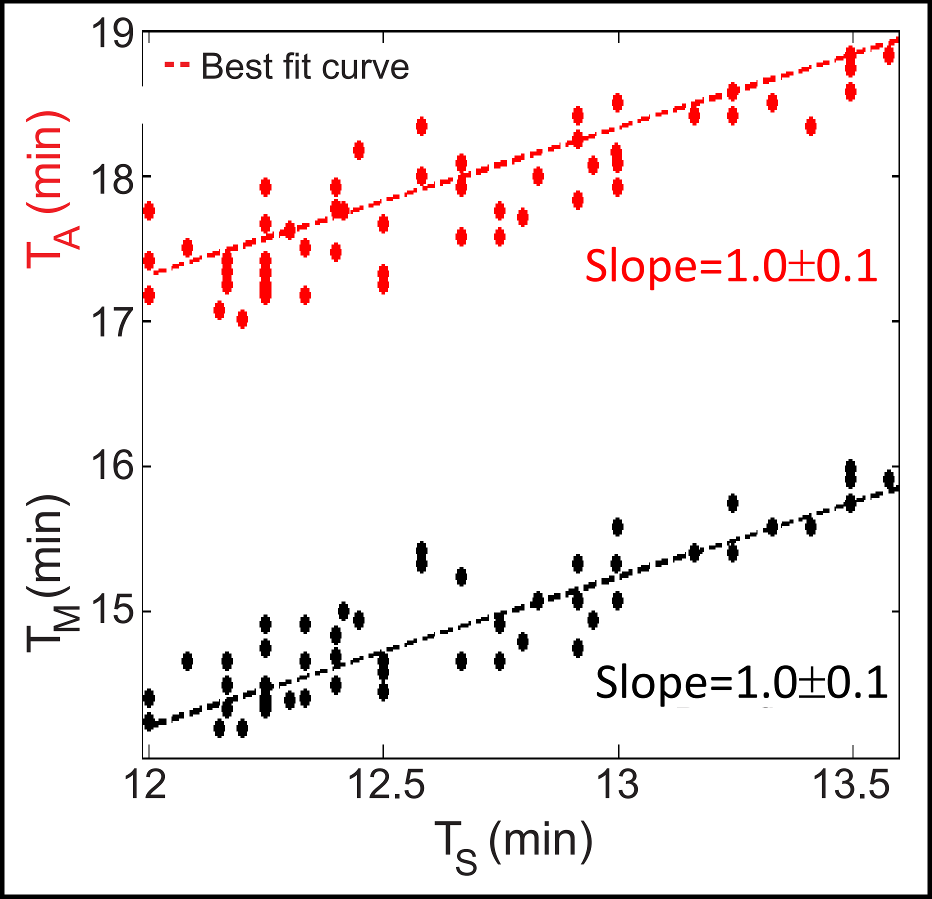


Important prediction:

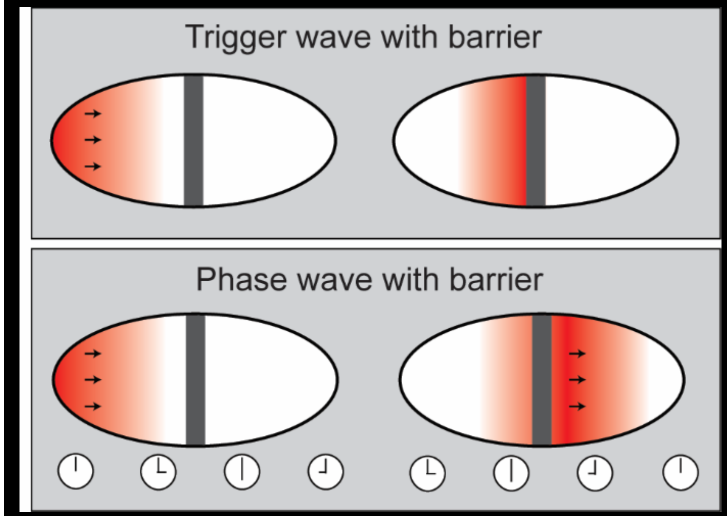
Once the wave has passed and M-phase begins, diffusion is slow and cannot alter the delays set by the S-phase Cdk1 trigger wave: the system is effectively on a clock

S-phase waves predict the mitotic and anaphase waves

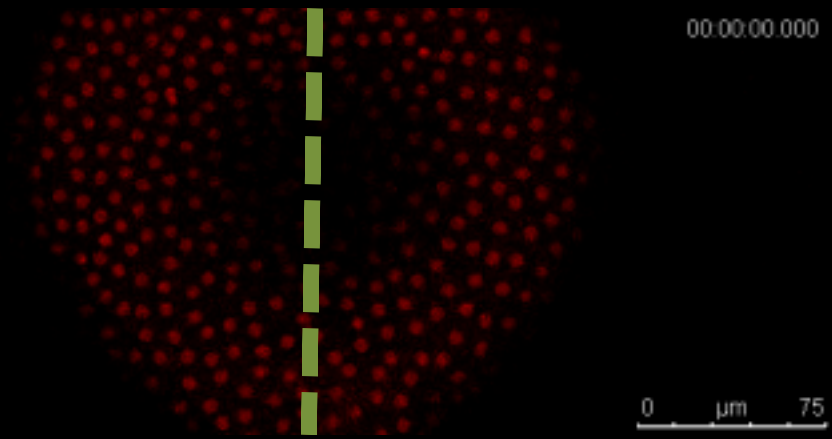
Phase wave prediction: $T_M = T_S + \Delta_M$; $T_A = T_S + \Delta_A$



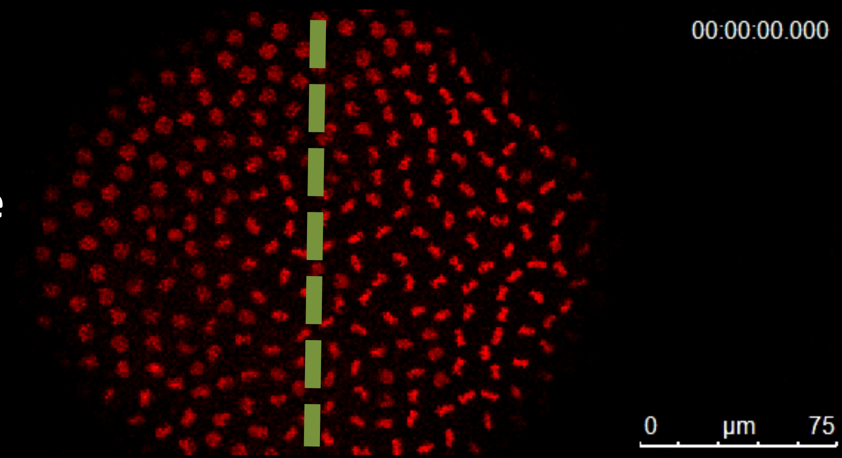
Causality: timed ligation experiments



S-phase ligation

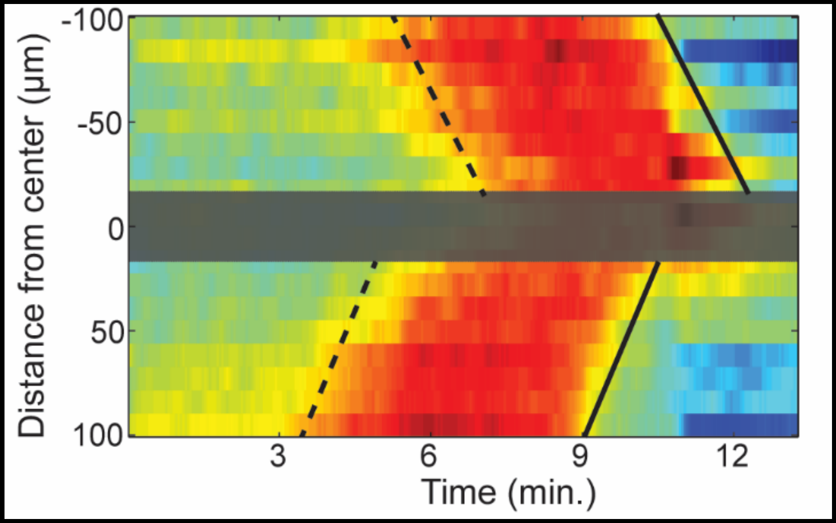


M-phase ligation

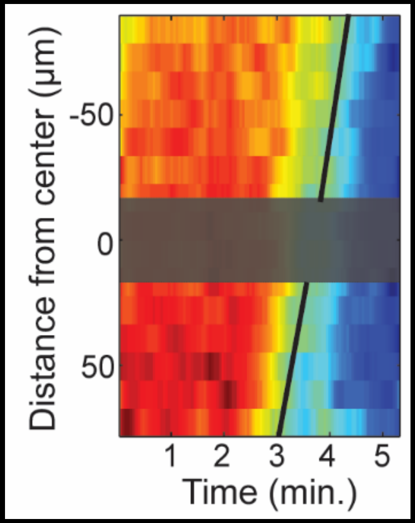


The wave of anaphase is a phase wave, which reflects the delays set by the trigger wave during S-phase

S-phase ligation



M-phase ligation



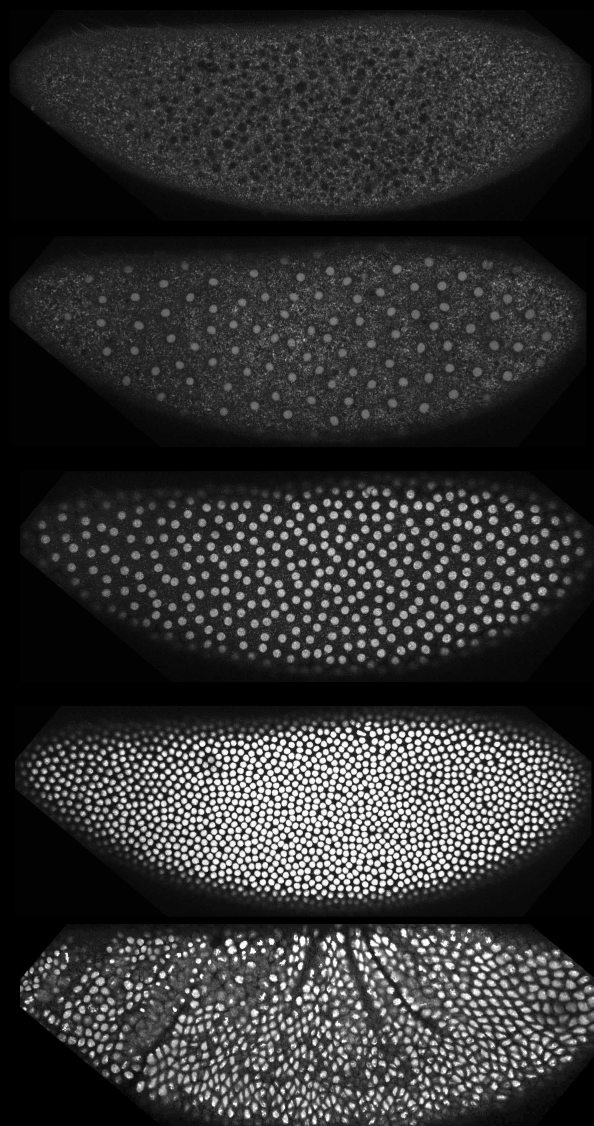
Deneke, Melbinger, Vergassola, Di Talia *Dev. Cell* (2016)

Summary

- The speed of Cdk1 waves depends on the S-phase DNA replication checkpoint
- Cdk1 positive feedback serves primarily to ensure the rapid onset of mitosis and is dispensable for wave propagation in the *Drosophila* early embryo
- The Cdk1 wave during S-phase is a trigger wave and the mitotic wave is a phase wave that follows the delays set by the trigger wave

Part II

Cell cycle control during gastrulation

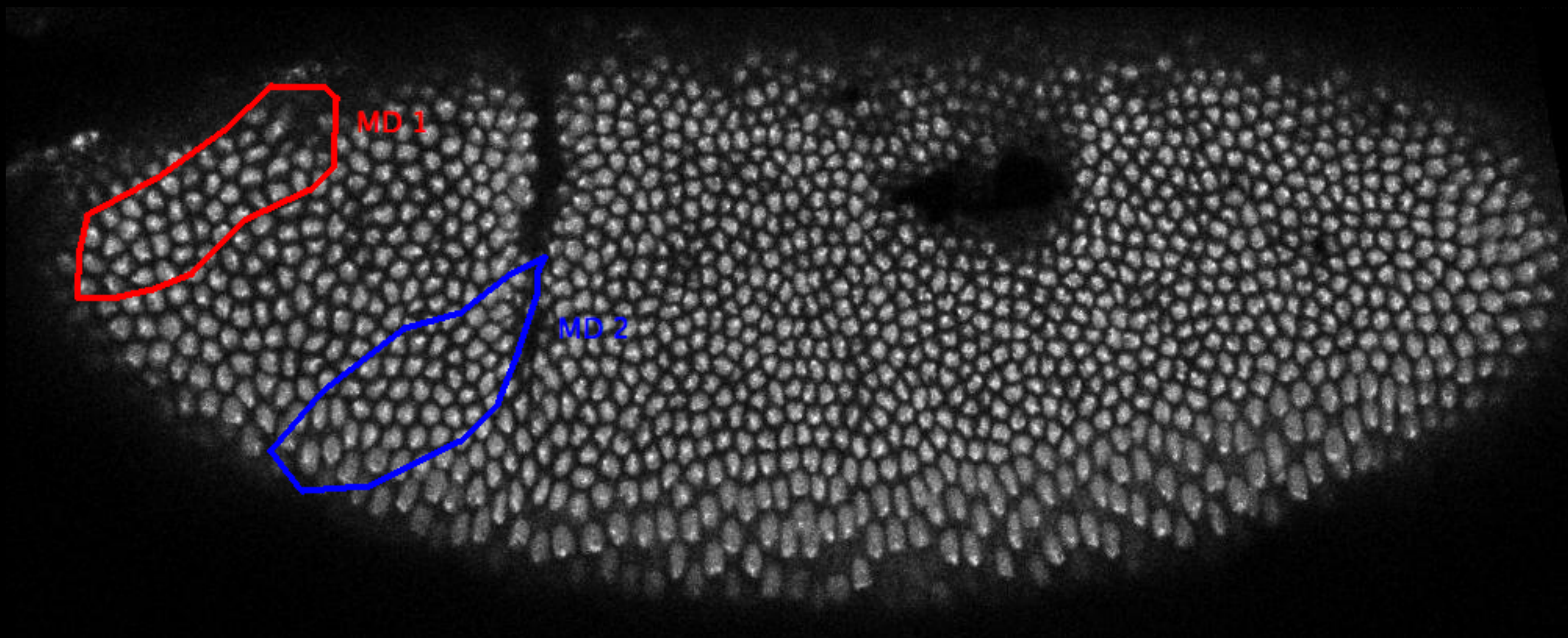


Synchronous Nuclear Replication

Cell Cycle Pause
maternal gradients establish cell fate

Gastrulation
cells in different regions behave differently

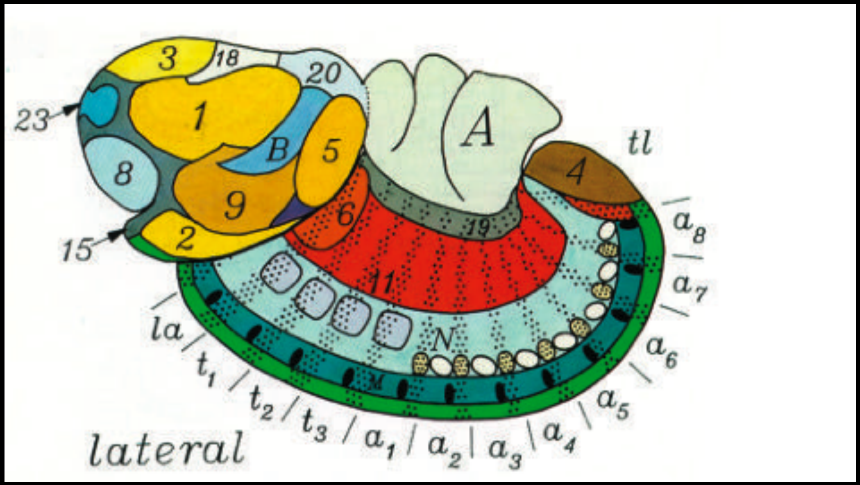
Drosophila Embryo Shows a Distinct Temporal Patterning



Pattern of cell division correlates with the activation of transcription of *string*

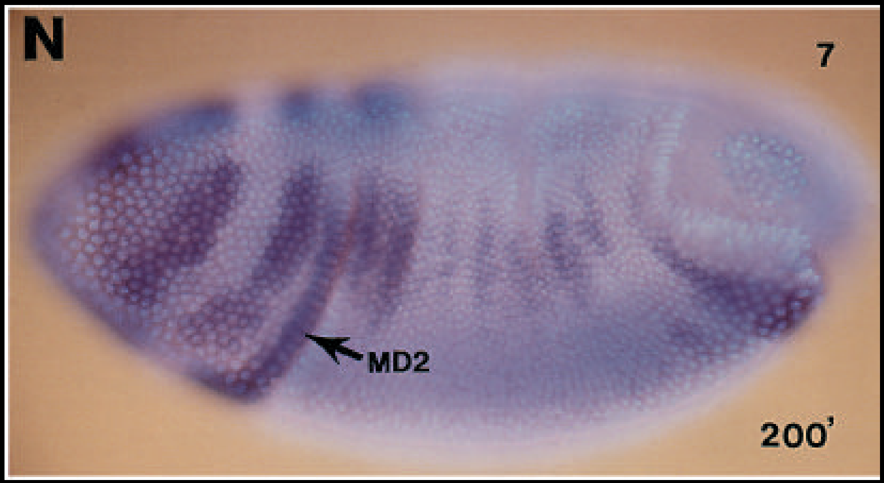
Following depletion of String and Twine and the MBT, maternal gradients establish cell fates. Cell cycle re-entry correlates with transcription of *string* in specific mitotic domains.

Mitotic Domains



Foe

string mRNA expression



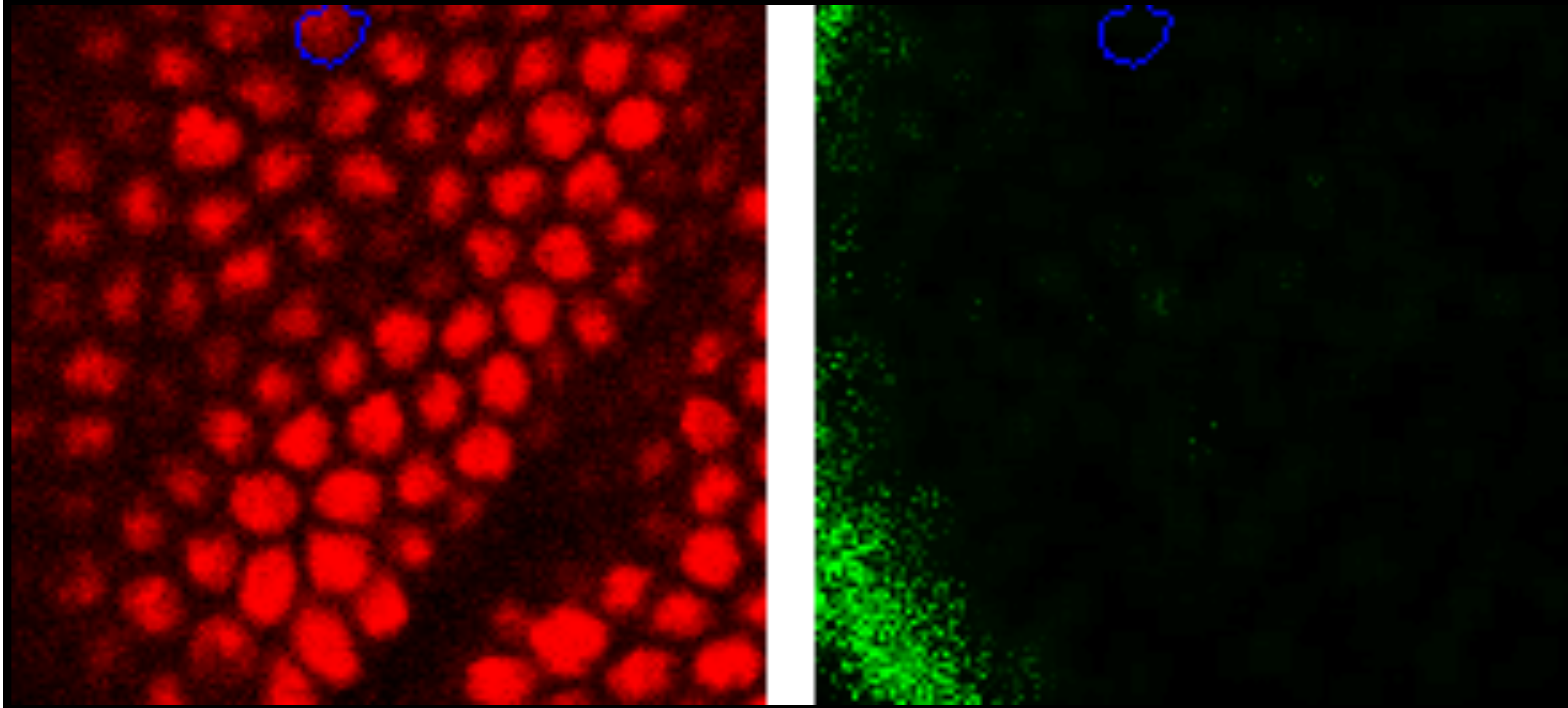
Edgar, O'Farrell

Is the time of mitosis controlled solely by *string* transcription?

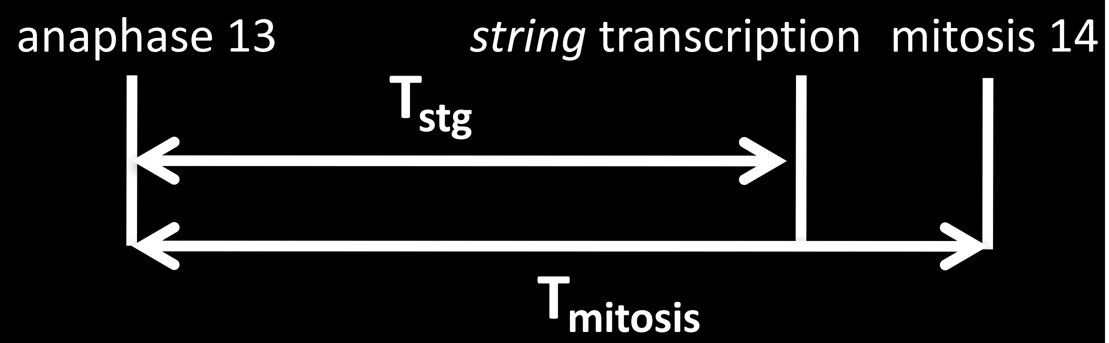
Or is there some other process that controls entry into mitosis once adequate levels of String are expressed?

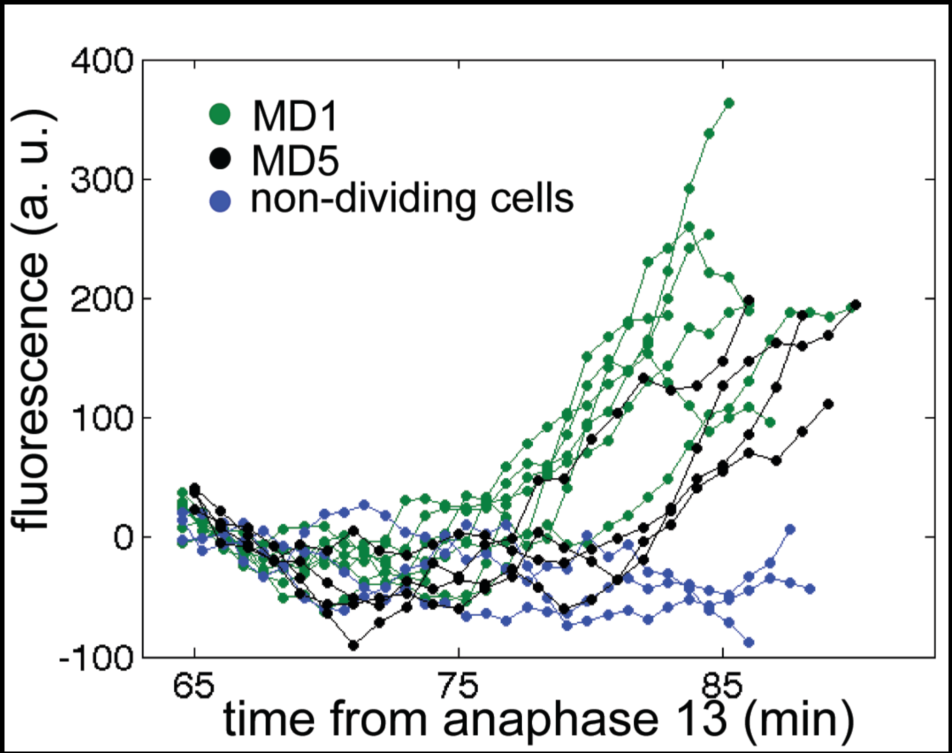
Dynamical analysis of *string* transcription

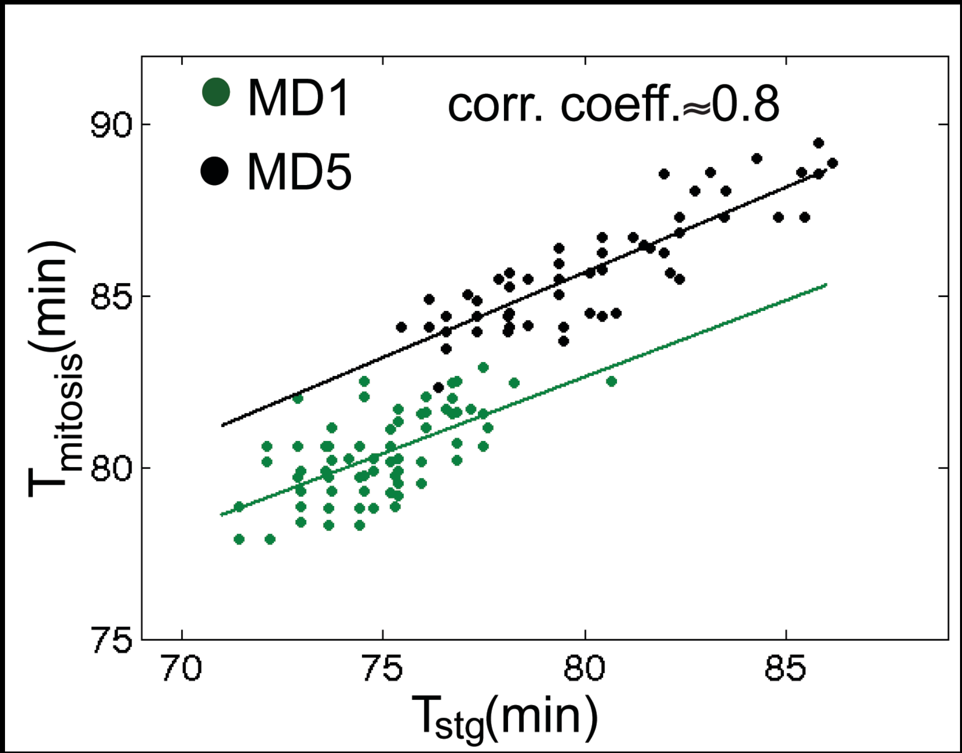
HIS-RFP | *string* enhancer-GFPNLS



Activation of *string* transcription controls the timing of mitosis





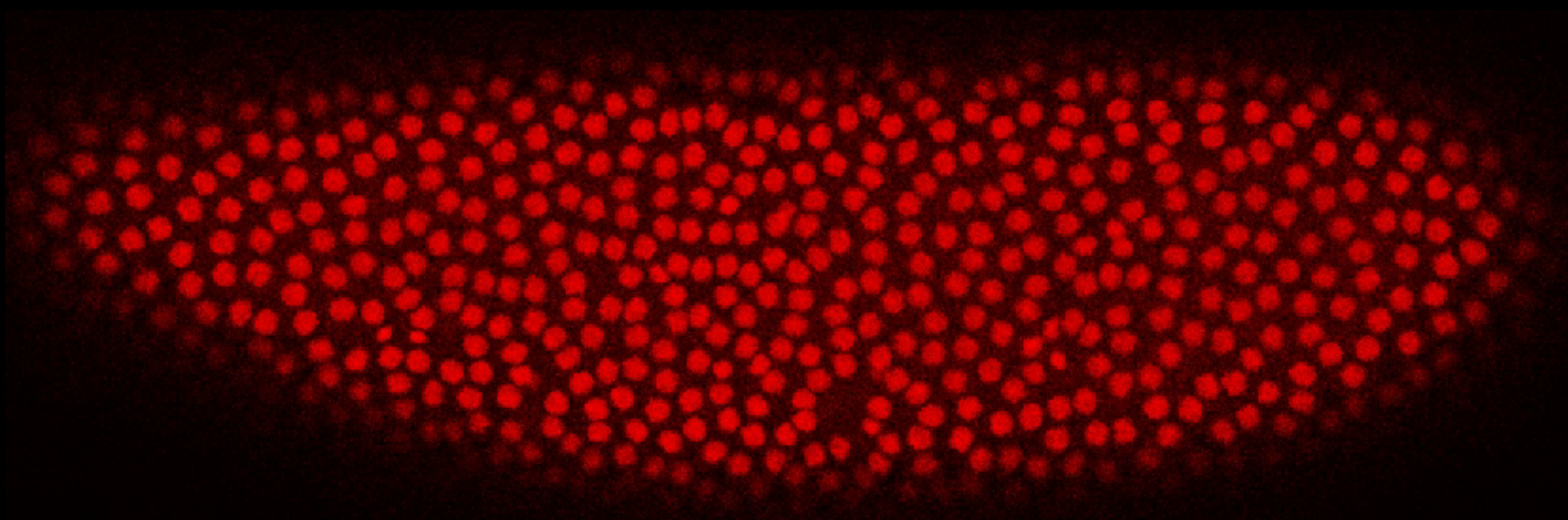


How is the expression of *string* translated into reliable control of mitosis?

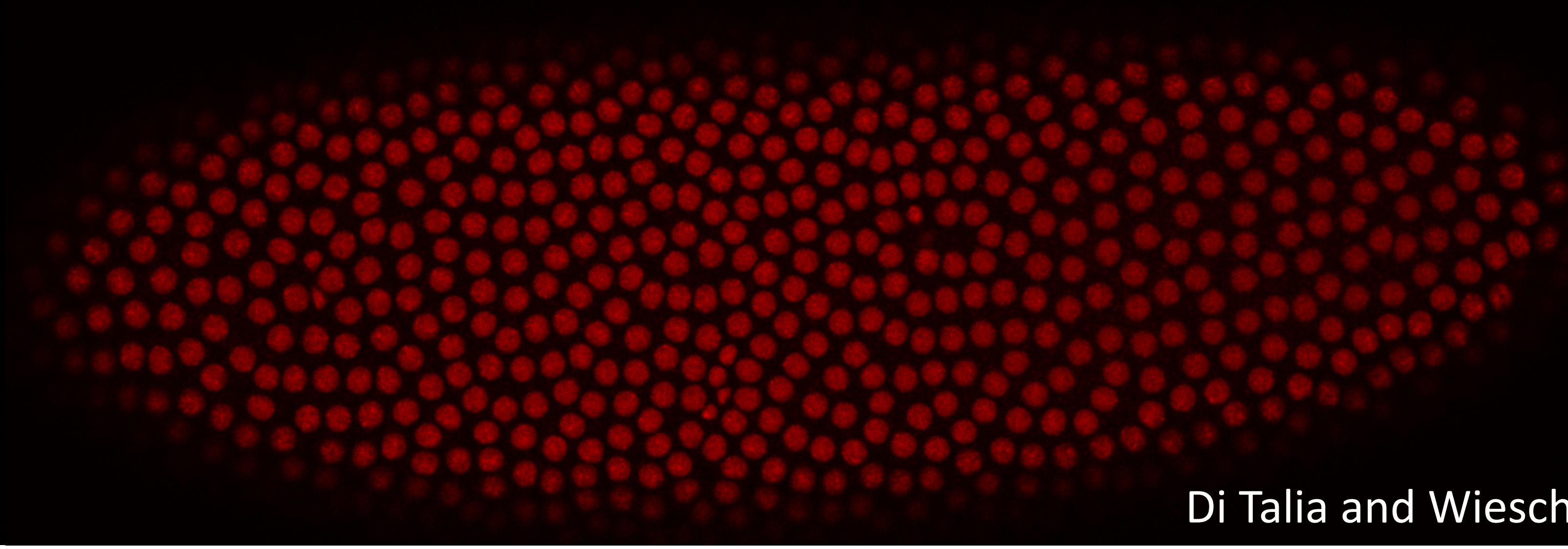
Is String the only input in the decision of entering mitosis? Eliminate transcriptional variability (in space and time) to study how the levels of String control mitosis

Uniform expression of String induces a synchronous mitosis

wild type



uniform String expression



Di Talia and Wieschaus 2012

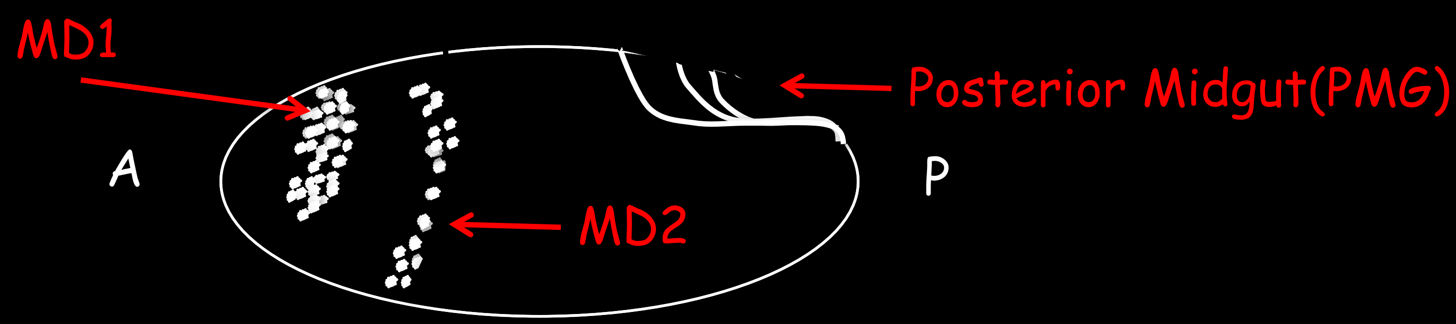
Identifying Dosage-Dependent Regulators of Mitosis

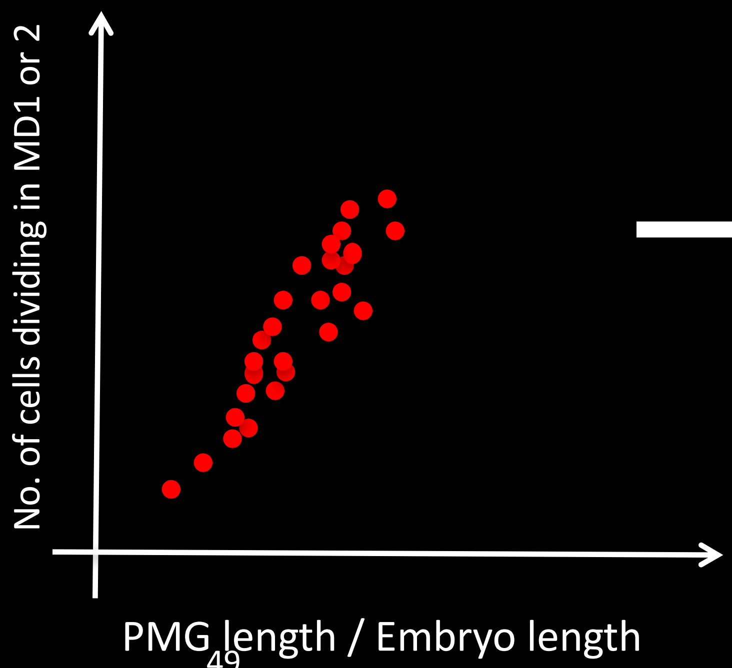
A genetic screen for trans-activating factors

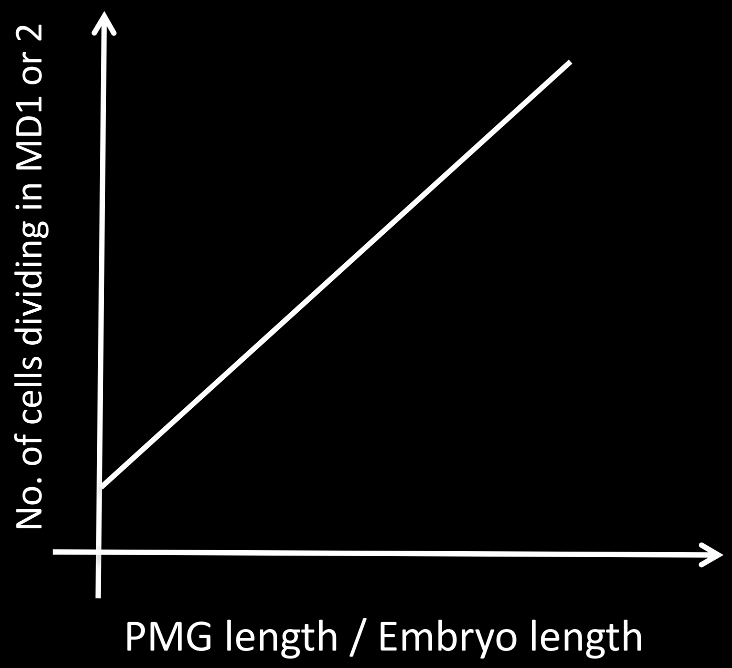
Amir Momen Roknabadi
Princeton

Eric Wieschaus

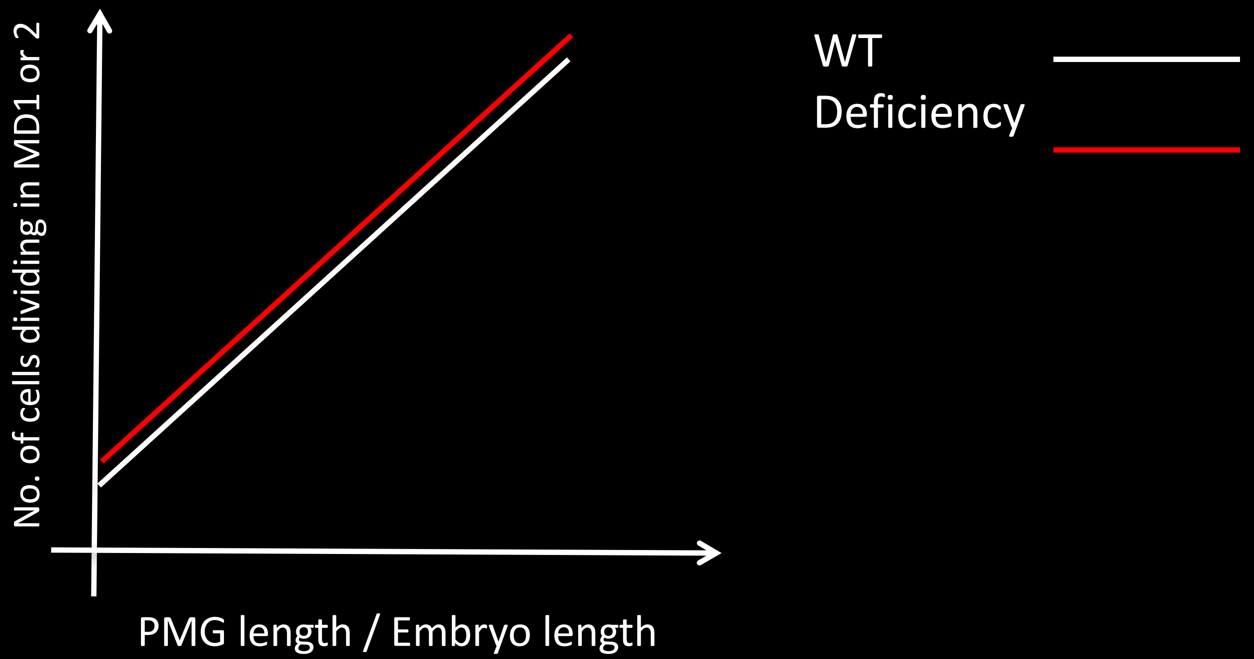
Identifying Dosage-Dependent Regulators of Mitosis



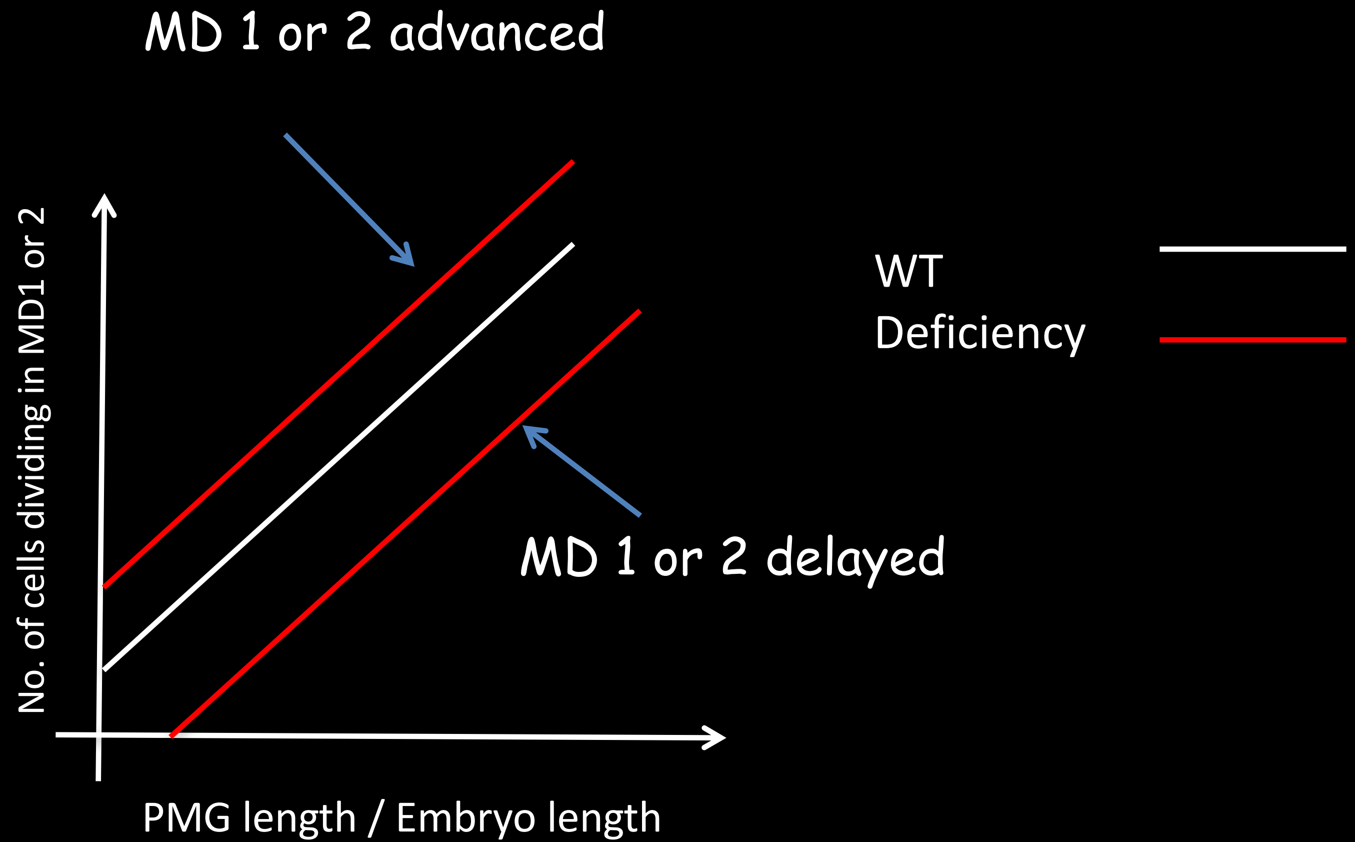




Deficiency Does Not Affect MD1 or MD2 Timing



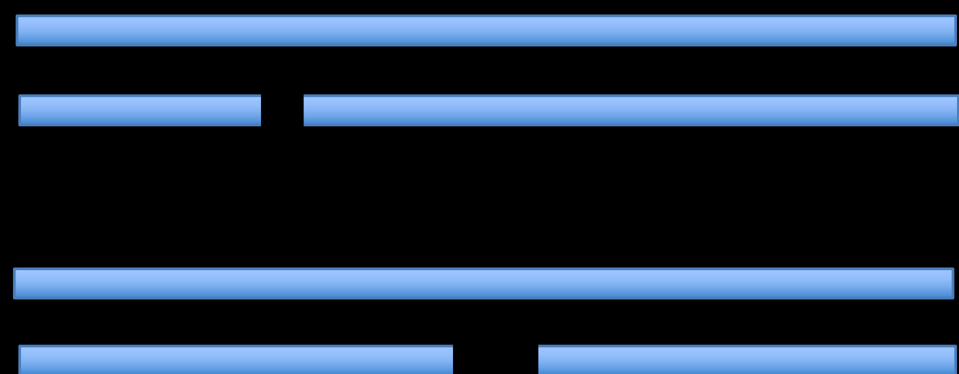
Deficiency Affects MD1 or MD2 Timing: Change in the Intercept



Temporal control by a two-step process

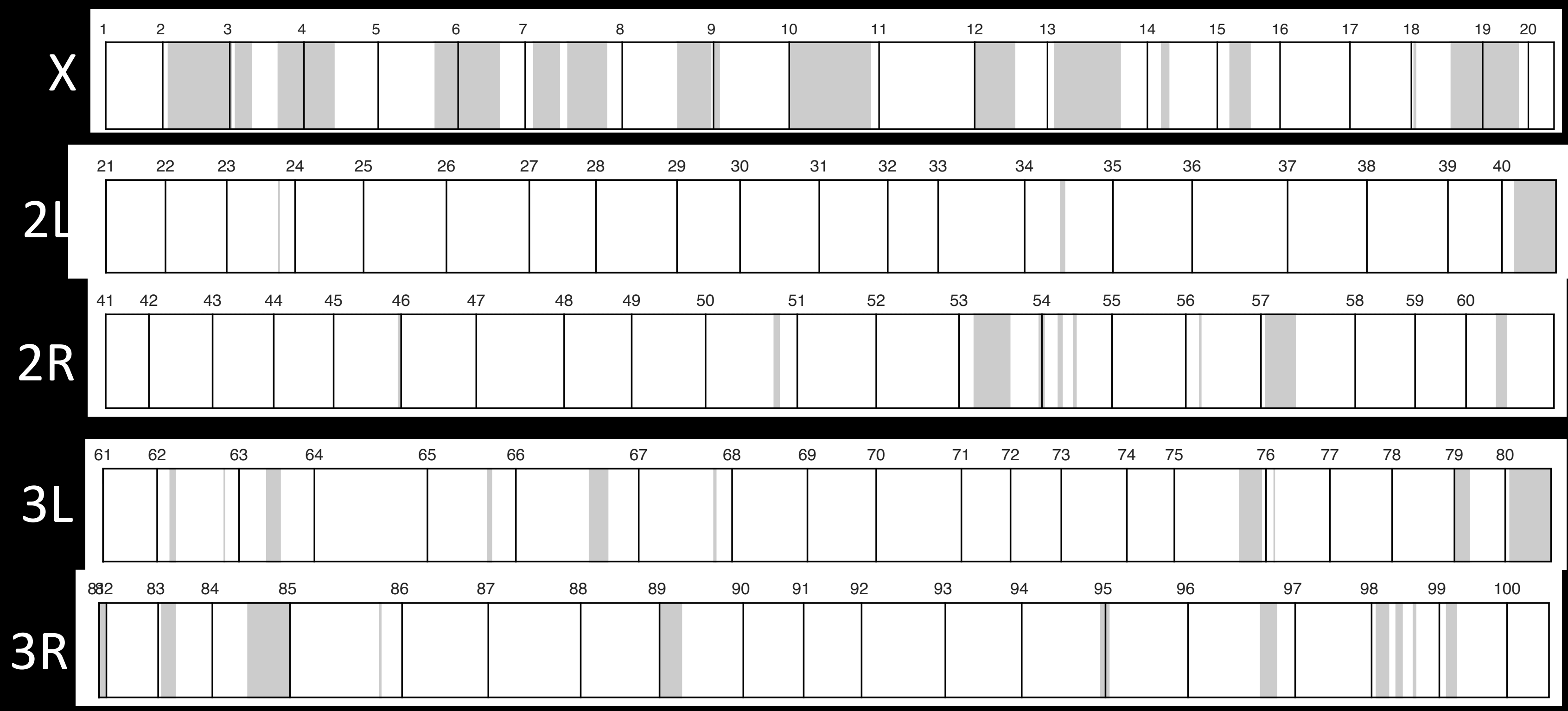
A genetic screen for trans-activating factors

Chromosomal deficiencies:
Flies can survive carrying large genomic deletion on one chromosome



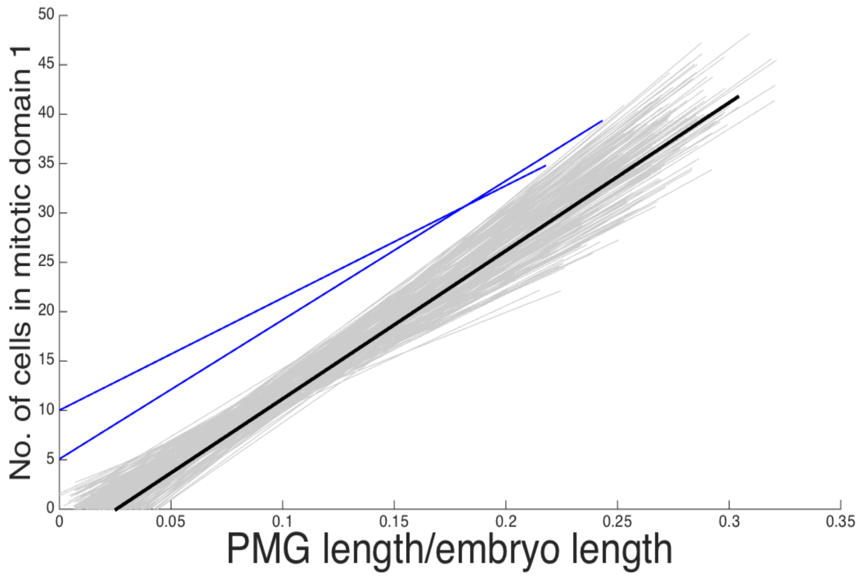
Identifying Dosage-Dependent Regulators of Mitosis

Genome-wide screen using heterozygote deficiencies, coverage of 85% of the genome

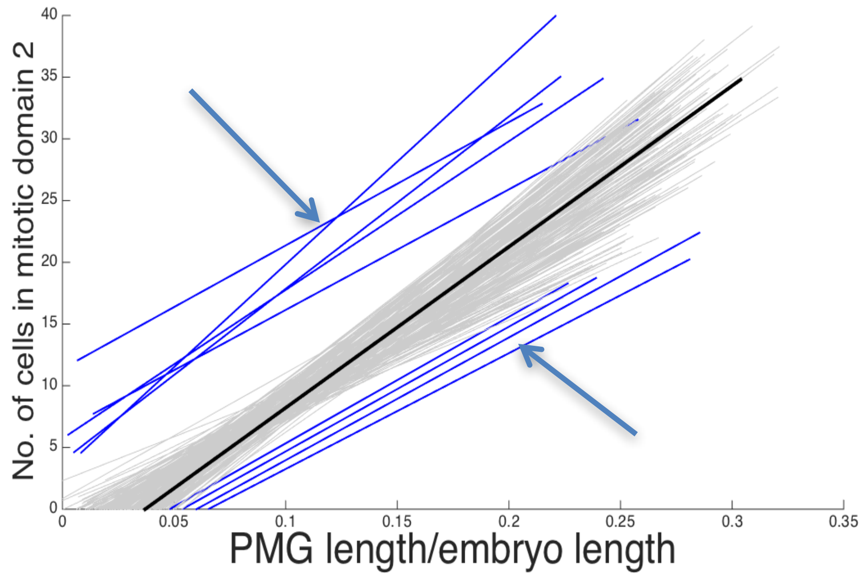


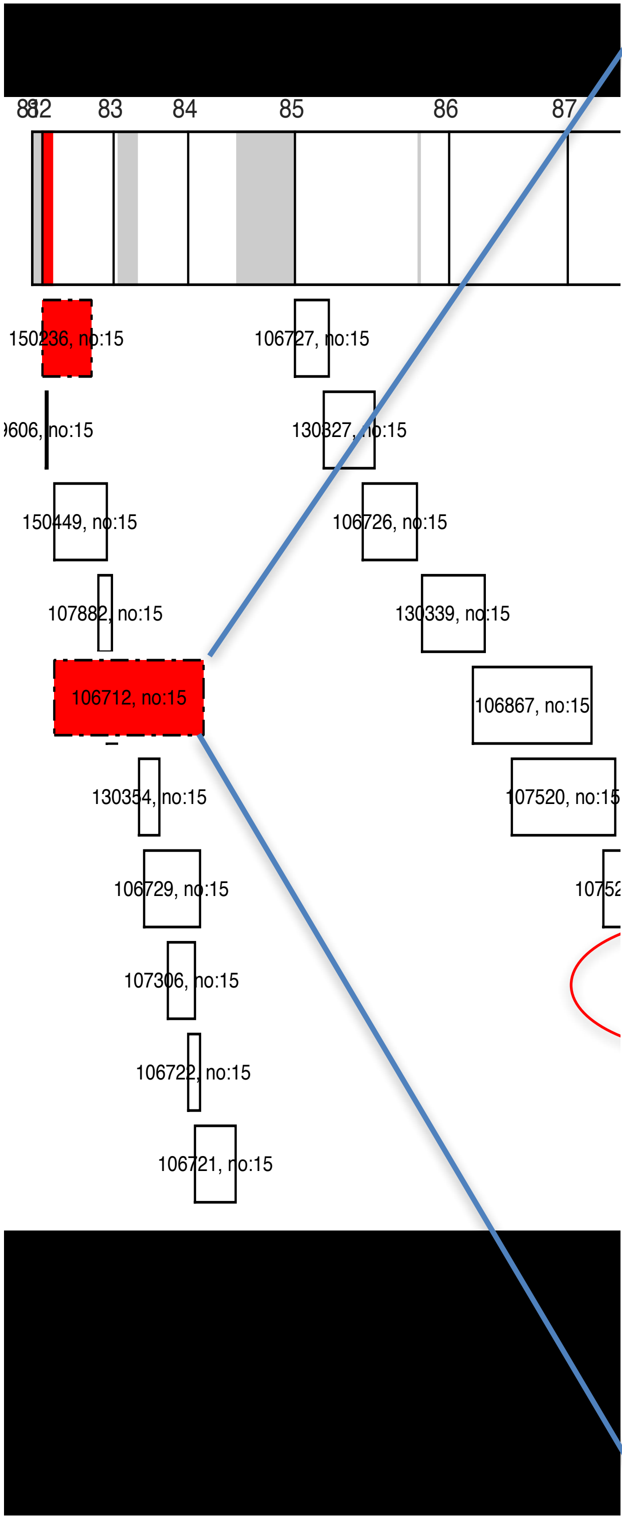
11 Regions Affect Either MD1 or MD2

Two deficiencies advance MD1



Four deficiencies delay MD2
Five deficiencies advance MD2





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87F11-87F11	3R:13703084..13705402	Adgf-D	5 stocks
87F12-87F12	3R:13706757..13709129	Adgf-C	2 stocks
87F12-87F12	3R:13709159..13709939	CG31469	5 stocks
87F12-87F12	3R:13710017..13711835	primo-2	4 stocks
87F12-87F12	3R:13710017..13711835	primo-1	5 stocks
87F12-87F12	3R:13712469..13714204	CR34044	
87F13-87F13	3R:13715207..13764667	CG34383	13 stocks
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87F13-87F13	3R:13765756..13767103	CG9312	3 stocks
87F13-87F13	3R:13767549..13770732	Cht5	7 stocks
87F13-87F13	3R:13771495..13779317	CG9297	3 stocks
87F13-87F13	3R:13779624..13780795	CG42375	3 stocks
87F13-87F13	3R:13779624..13780795	CG9288	4 stocks
87F13-87F13	3R:13780964..13782082	CG9286	4 stocks
87F13-87F13	3R:13782288..13785676	Dip-B	5 stocks
87F13-87F13	3R:13785806..13786583	CR45590	
87F14-87F14	3R:13797953..13798025	tRNA:CR31588	
87F14-87F14	3R:13798198..13798270	tRNA:CR31331	
87F14-87F14	3R:13813109..13814648	tal-2A	
87F14-87F14	3R:13813109..13814648	tal-3A	
87F14-87F14	3R:13813109..13814648	tal-1A	
87F14-87F14	3R:13813109..13814648	tal-AA	
87F14-87F14	3R:13815279..13815778	CR43641	
87F14-87F14	3R:13816231..13817127	CR43642	FBst0059486
87F14-87F14	3R:13827151..13828008	Mst87F	3 stocks
87F14-87F14	3R:13827968..13828427	CR43300	
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88A1-88A1	3R:13854599..13855454	CG14362	2 stocks
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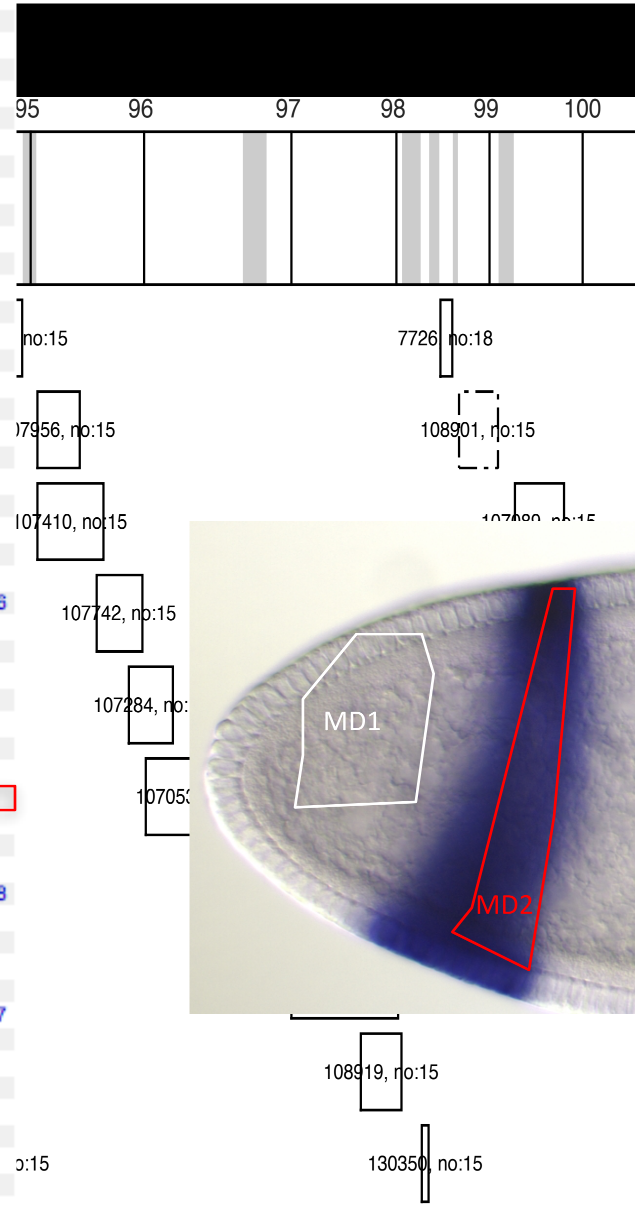

Momen-Roknabadi, Di Talia* and Wieschaus* to appear in Cell Reports

Conclusions

- The same transcription factors that control the spatial pattern of mitosis encode the temporal pattern

- The precise timing of mitosis is encoded by a combination of transcriptional activators and repressors.

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Di Talia lab
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Ben Cox
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Eric Wieschaus

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