Notes

Pringle in CSHL yeast III, checkpoints vs clock, and defn of checkpoints vs substrate ie chechkpoints can be detected by mutations that kill the gene feed back loop, while, substrate dependency is immune to LOF mutations.

Growth vs division, are both processses influcen by nutrition status, but is there also direct mass feed back to division events?? I Rupe 2002. Could be feed backs from division to growth, but empirically growth seems autonomous, and cell cycle delays to wait for growth

Propaganda: cell cycle models should be based on single cell data, not averaged cell. In Cln3- we are testing if Cln3 reaches deeper into start module than just Cln12. Current models -> strong 'contraction' onto universal orbit contrary to fluctu, > 2variables needed for determinism, relax initial conditions. Cell-Cell variability common. Same talk to bio or physics audience

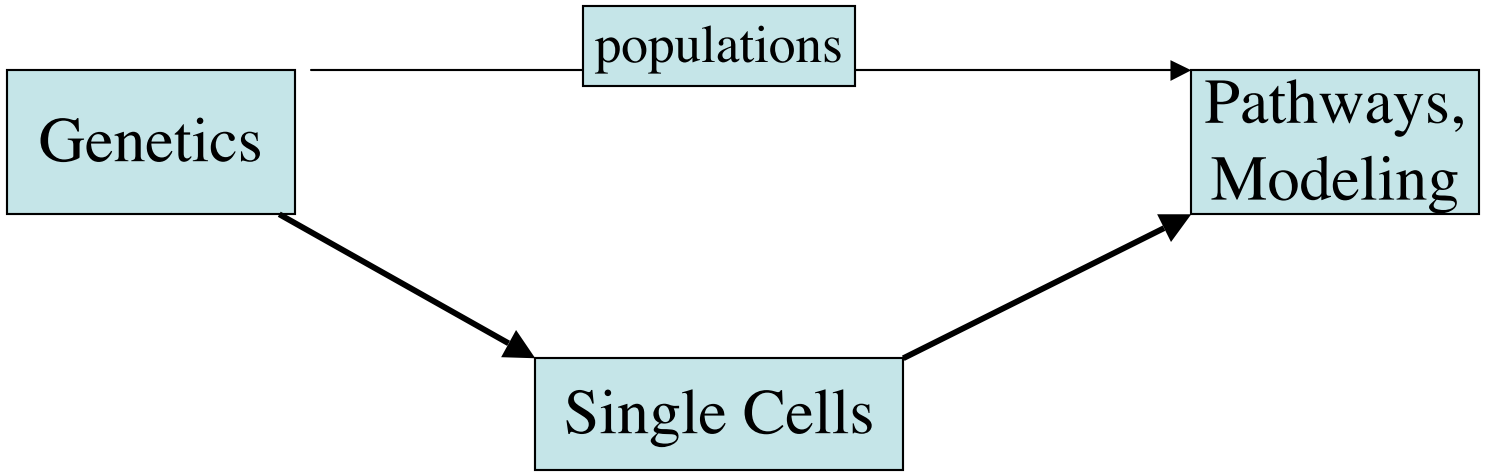
sizer and timer, use our current data,
jan poor media met-Cln2, cln2PR-GFP, compare Cln12- with wt, former
Jamie's data Cln3- strong signal. latest stefano mbud vs mass div

Founding Fathers

1854-1912

1866-1945

Problem



Cell-cell variability large and ubiquitous in biology.
Origins?

- History
- Small numbers of molecules
- Sensitivity to environment

Networks operate within a cell not on the 'average' cell,
Should one use deterministic ODE's, or markov model: 'clocks vs dominos'

Summary

Model: Cell cycle budding yeast

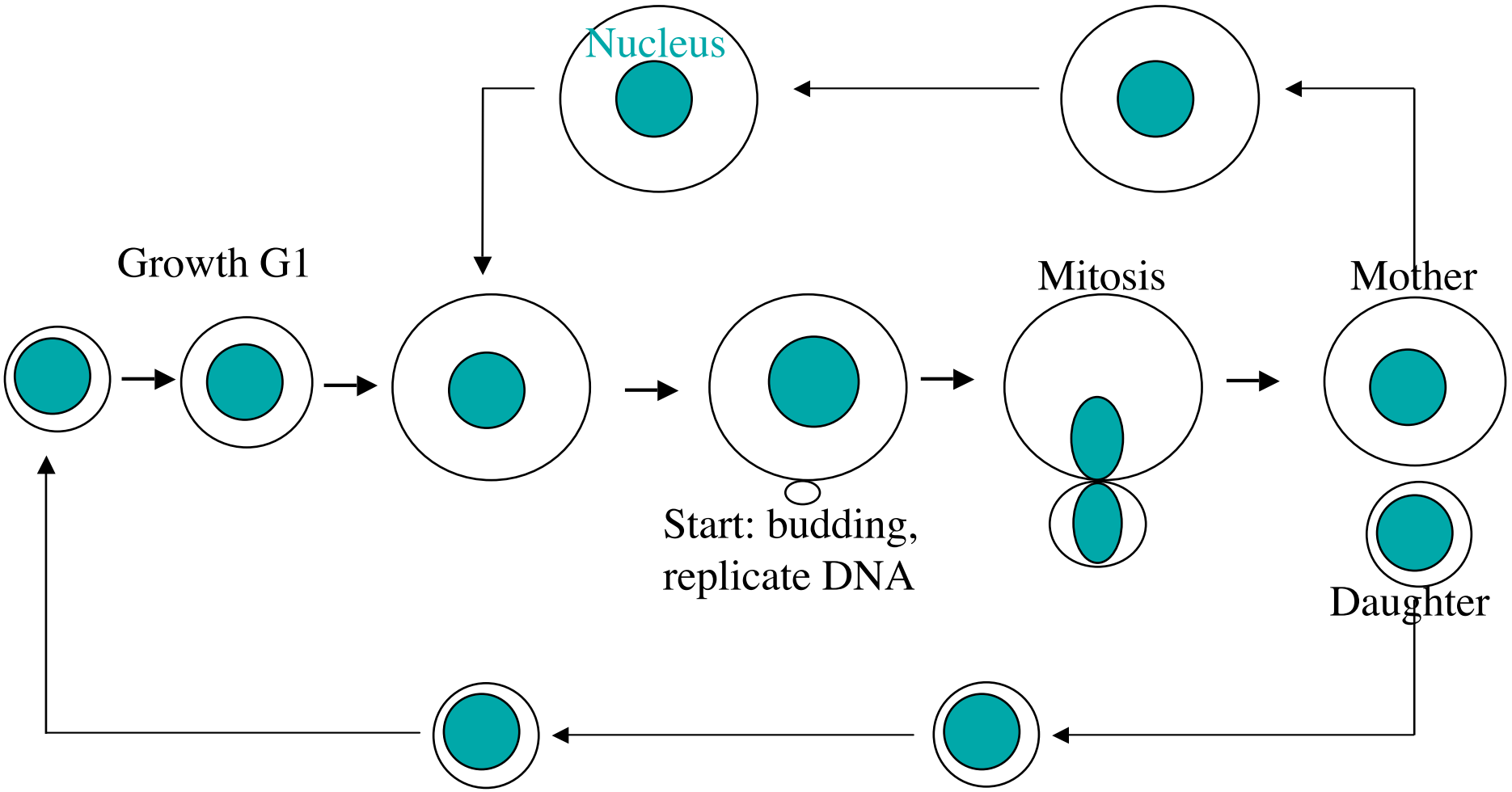
- Δ size cell (at ref points) > Δ pop medians almost all size mutants
- Cyclic process (hence know relevant past history)
- Grows variable ploidy (fluctuations scale with size)
- Single cell and grows on surface (measurements ~ natural state)

Outline:

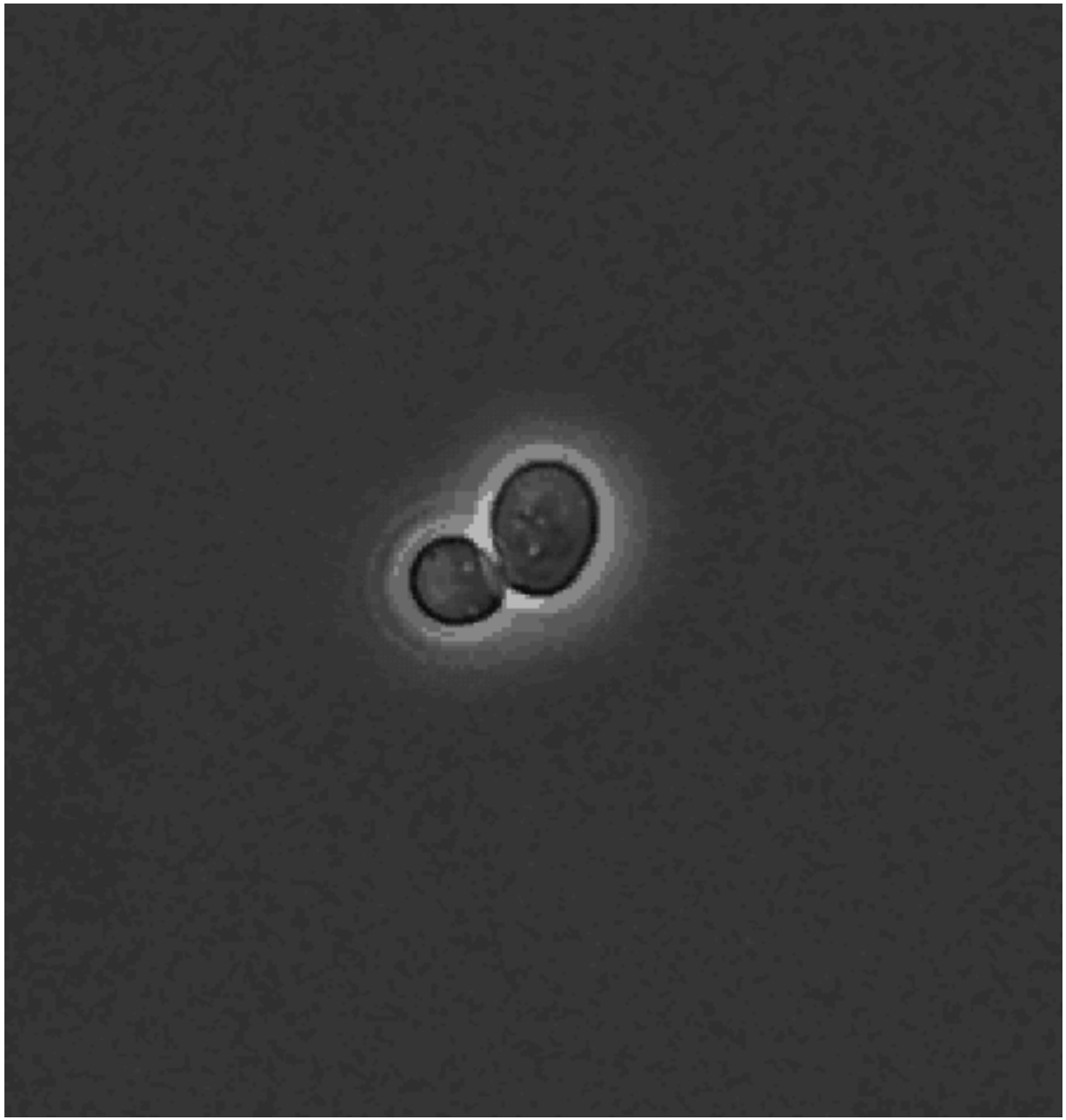
- Methods
- Genetics of Noise
- Noise phenotype of Start mutants, and deciphering pathways.

Cell cycle in budding yeast

(Unequal division, daughter < mother, $T_D > T_M$)



Phase contrast, wt-cells (~100min cycle)



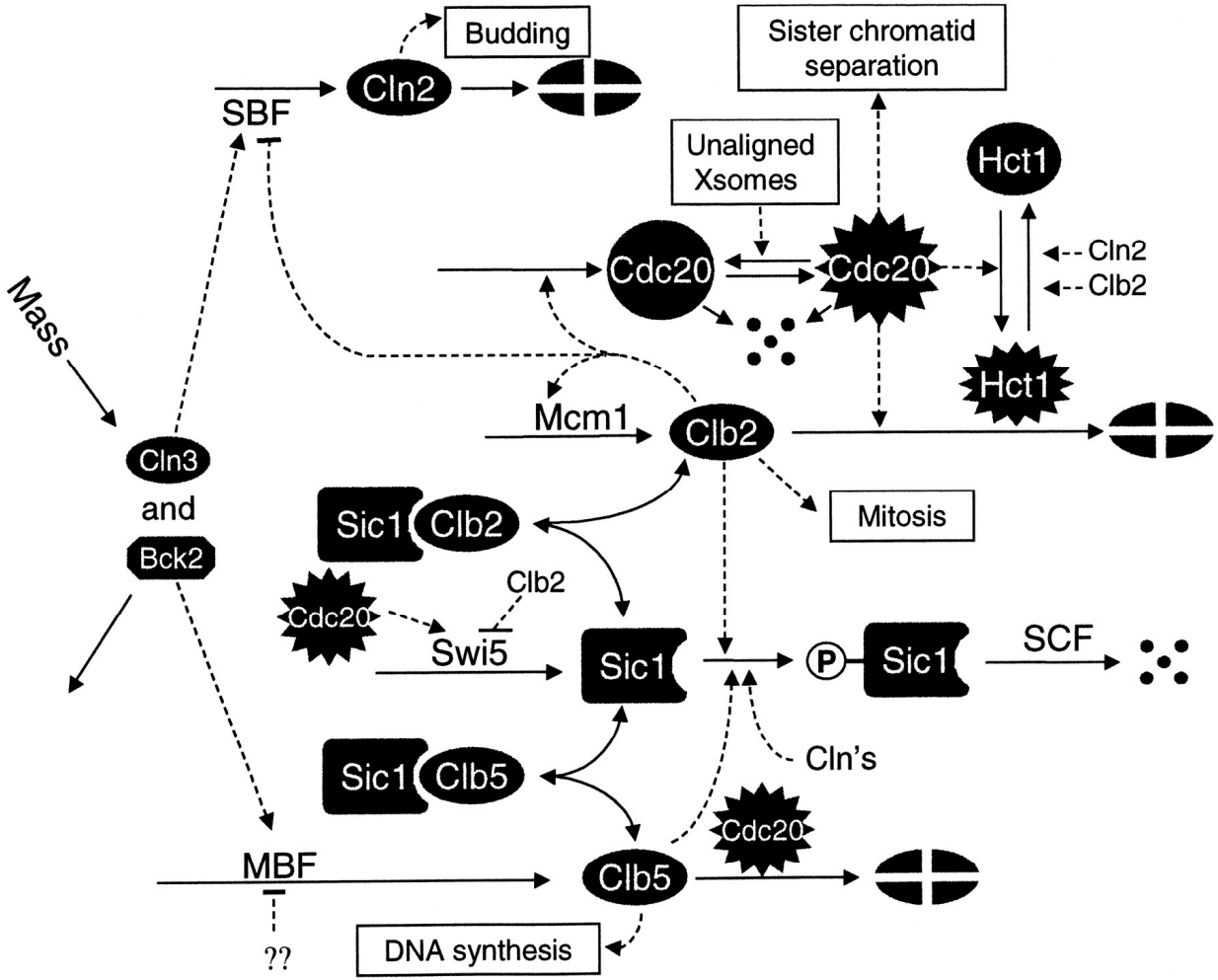
Molecular architecture of cell cycle

Problem: how to insure that a series of events happen once and in correct order, ie make 1 copy of genome, grow the bud, create mitotic spindle, separate chromosomes, partition cells and coordinate growth and division under multiple environments.

Solution: create central oscillator based on ‘cyclins’ (eg Clb2) that mark phases of cell cycle and act by targeting kinase, (metabolism +growth runs independently, --> period defined by growth rate, cell cycle slaved to growth)

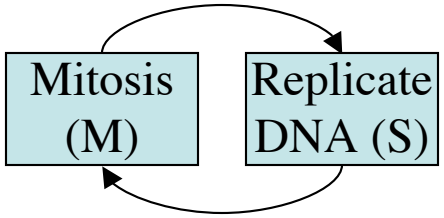
Cell cycle components conserved from yeast to man.
“Cancer and the cell cycle in yeast”

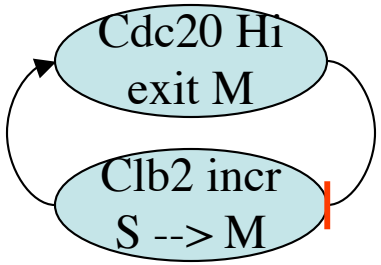
Chen-Tyson model (MBC 2000)



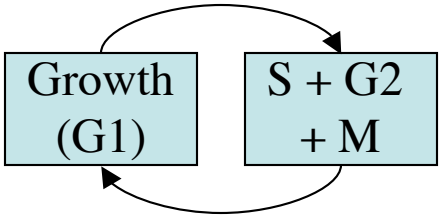
Cell cycle - (Poincare's version)

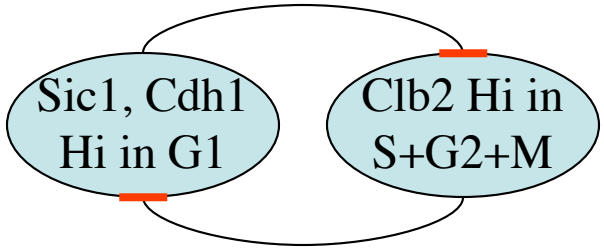
(Two coupled oscillators, driving common mitotic cyclin Clb2)





Negative feedback oscill, Clb2 activates its repressor, Cdc20/APC. Prominent: fly embryo, egg extracts.

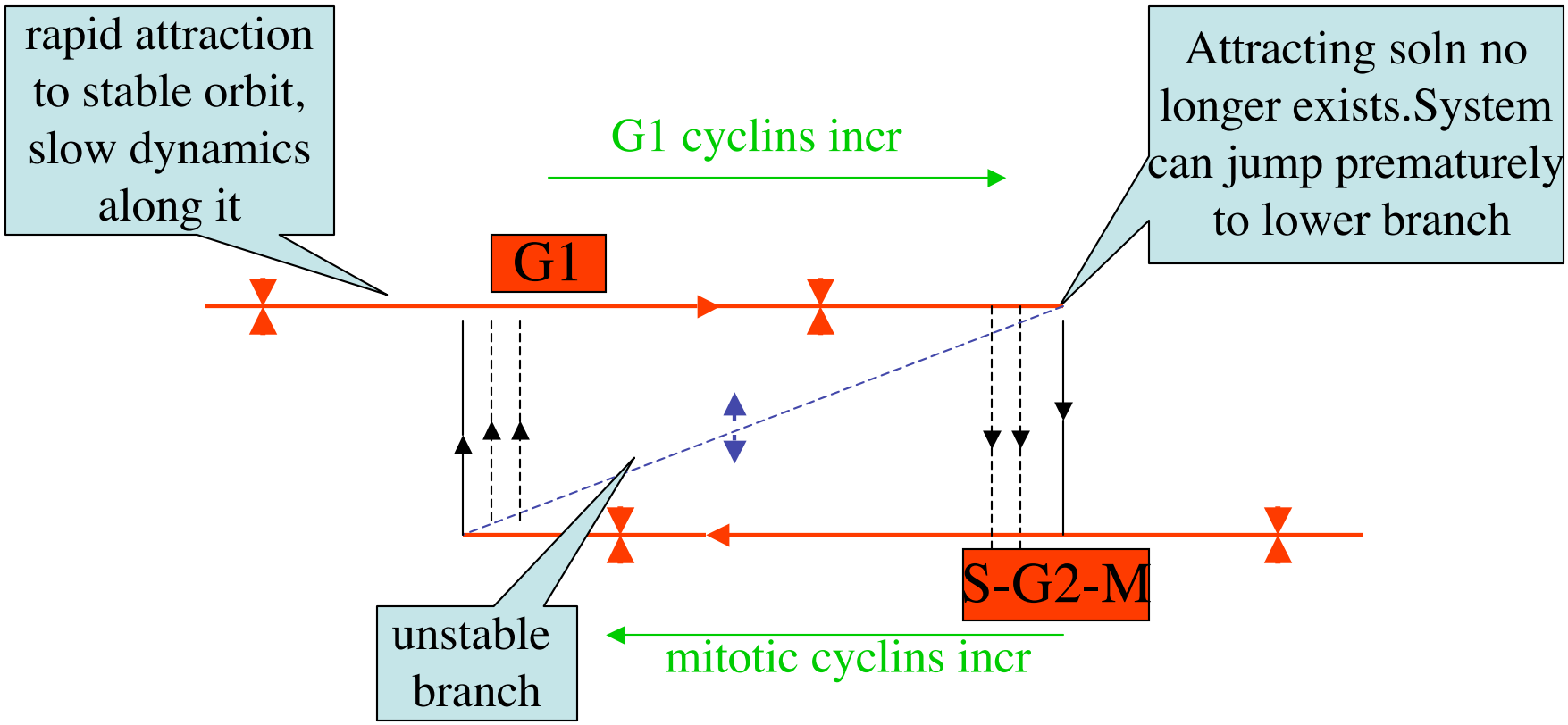




Relaxational oscillator. Sic1, Cdh1/APC inhibit Clb2, and visa versa. Prominent: yeast poor media, slow growth

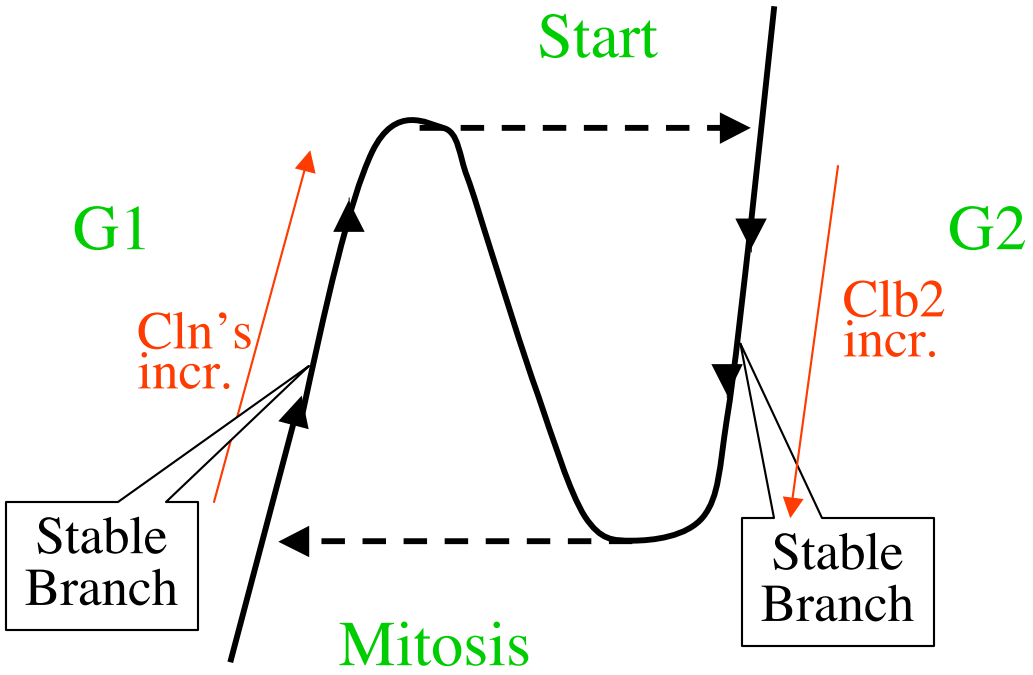
FR Cross, *Dev Cell* 2003

Hysteresis in relaxational oscill .

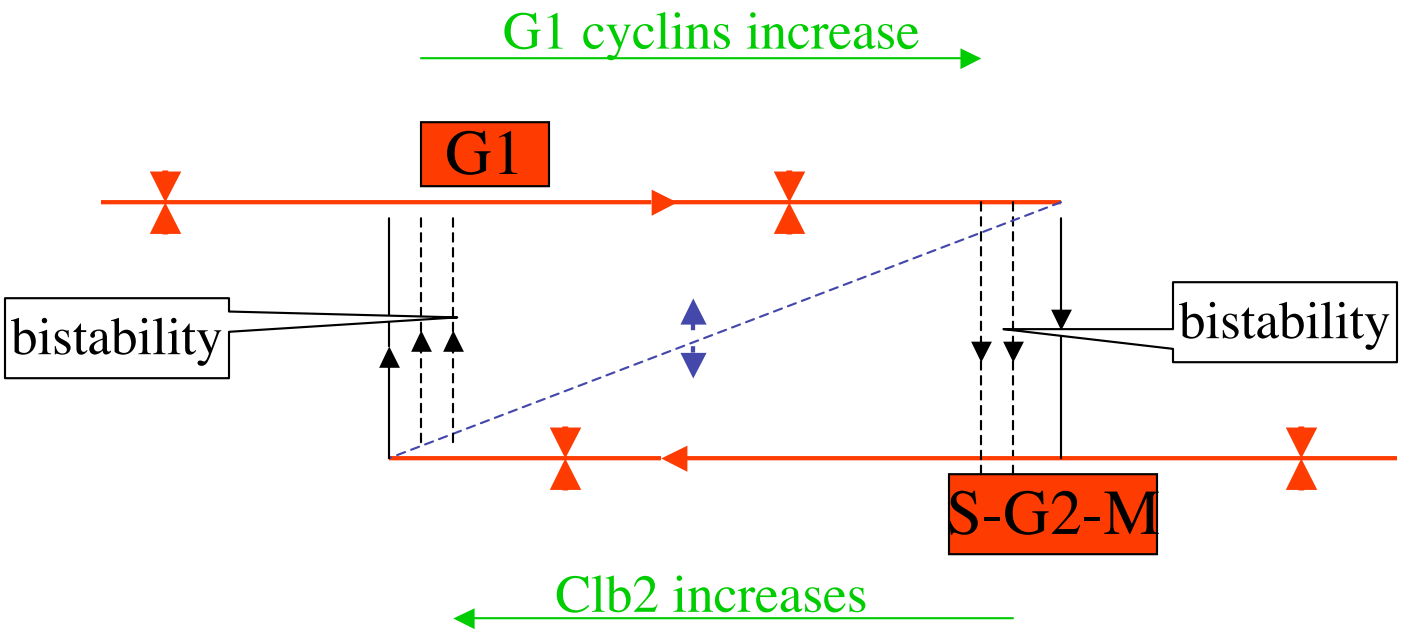


Consequences: Motion 1 dimensional except near jump where susceptible to noise, natural way to adapt period to environ.
Experiments: cell extracts and yeast have seen bistability

Geometry of Relaxational Oscillator



Hysteresis in relaxational oscill.

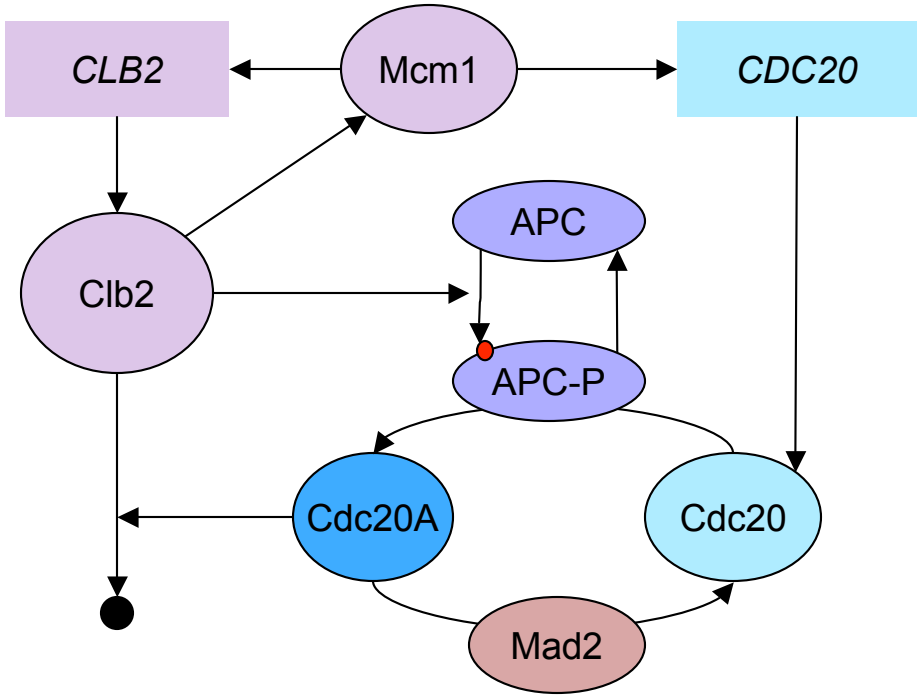


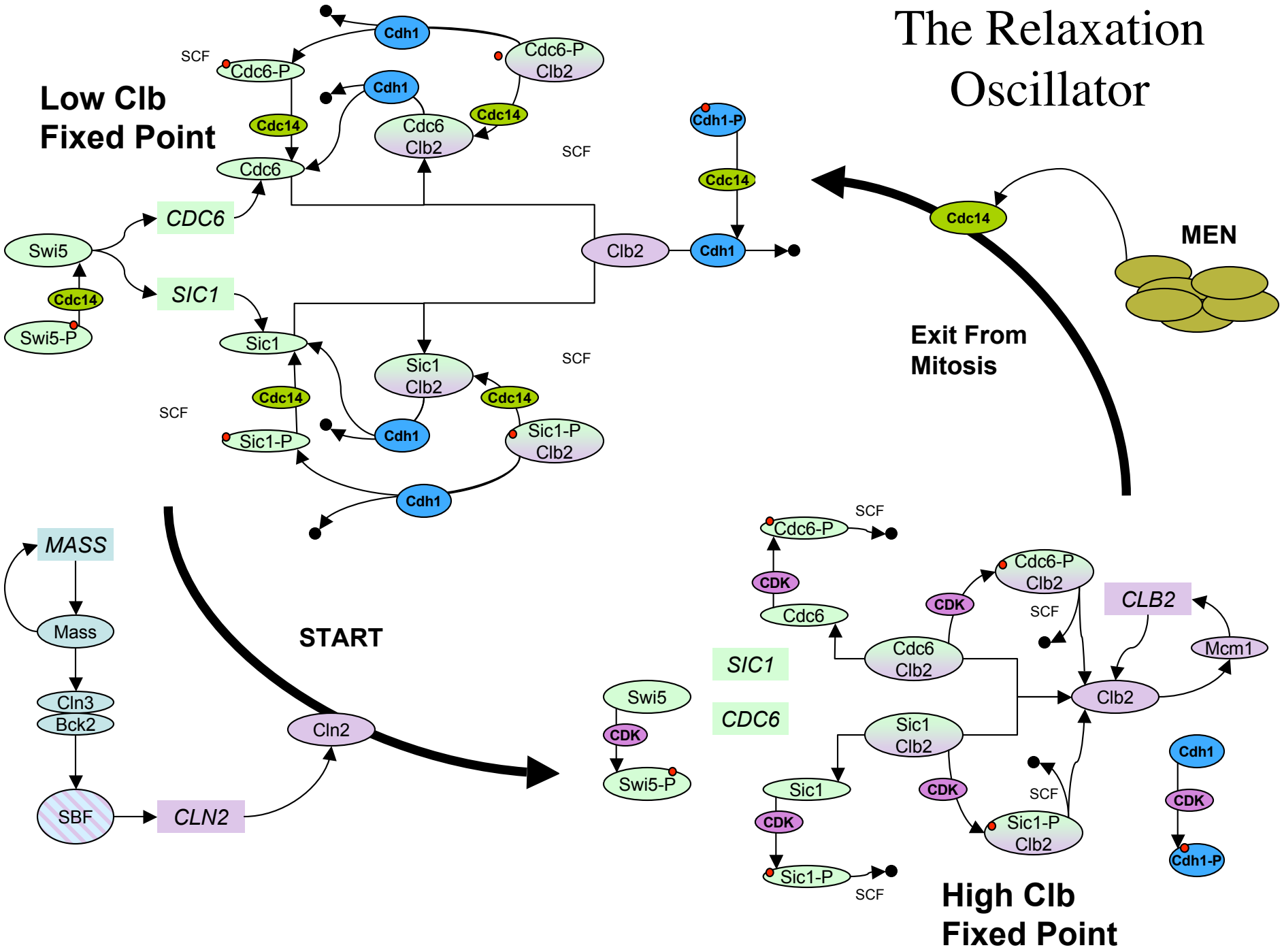
Consequences: Motion 1 dimensional except near jump where susceptible to noise.

Experiments: cell extracts and yeast have seen bistability

The Negative Feedback Oscillator

(Chen-Tyson 2004)




The Relaxation Oscillator
Low Clb Fixed Point
SCF
Cdh1
Cdc6-P
Cdc6-P Clb2
Cdc14
Cdc6
Cdc6 Clb2
CDC6
Swi5
Swi5-P
SIC1
Sic1
Sic1 Clb2
Sic1-P
Sic1-P Clb2
Clb2
Cdh1-P
MEN
Exit From Mitosis
START
MASS
Mass
Cln3
Bck2
SBF
CLN2
Cln2
CDK
CLB2
Mcm1
High Clb Fixed Point

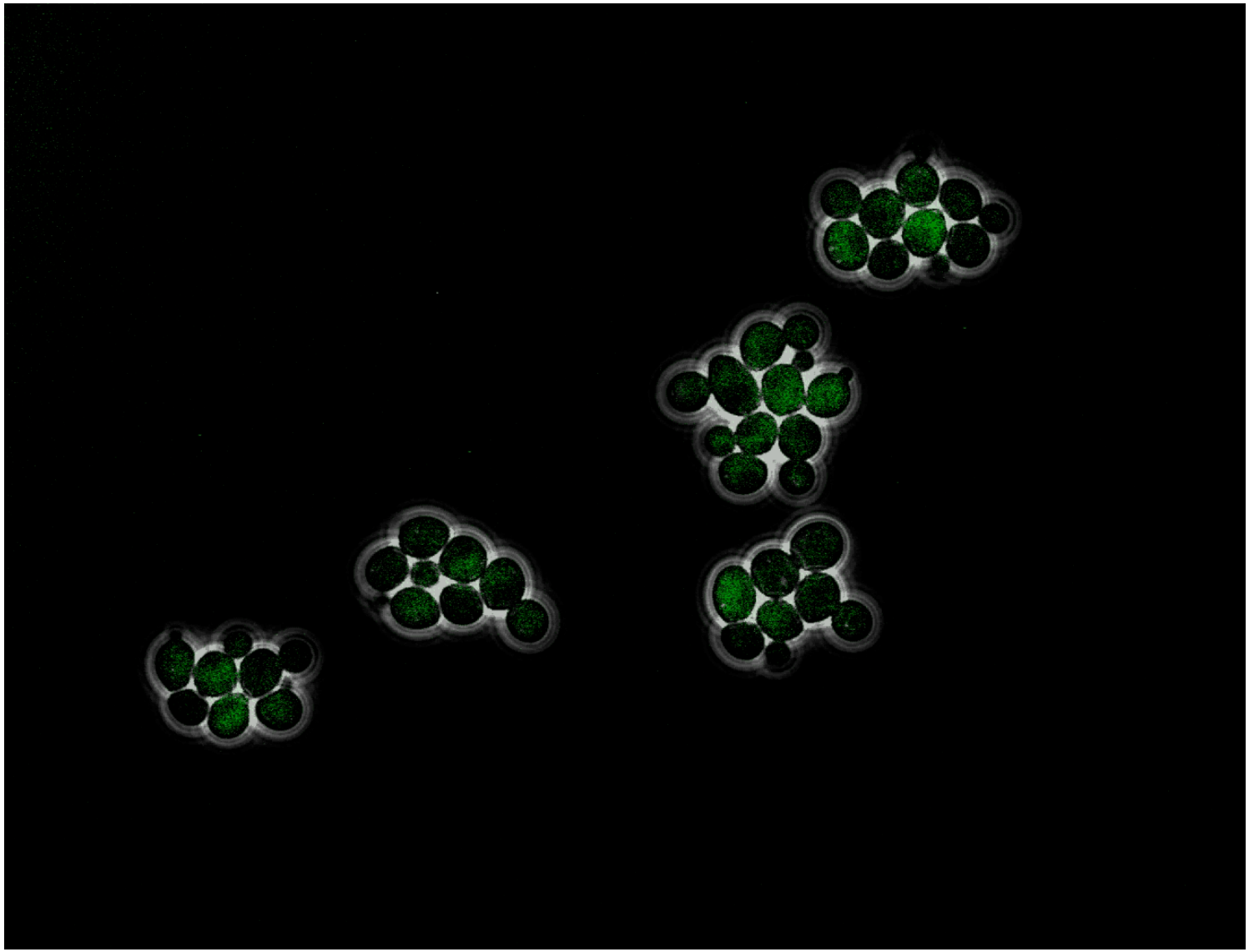
Time lapse imaging

- Sample between gel slab and coverslip, temp humidity control, computer controlled stage.
- Phase contrast + 2 fluorescent channels image every 3 min, for 6-8 hrs(4-5 divisions) cells remain in layer. Multiple fields followed in parallel.
- Software to segment phase image, follow cells from image to image, integrate fluorescence over cells, manually annotate bud/division times, remember parentage
- Markers: bud emergence (morphology); bud+division(Myo1-GFP); cell 'mass' (ACT1pr-DsRed); gene activation at start (CLN2pr-GFP-PEST); 'G1' phosphorylation state (nuclear local Whi5-GFP)…

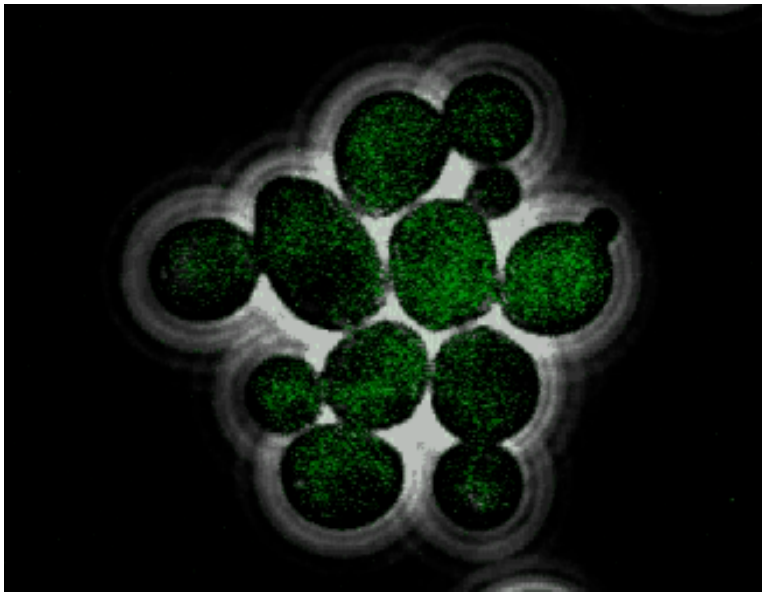
Technology

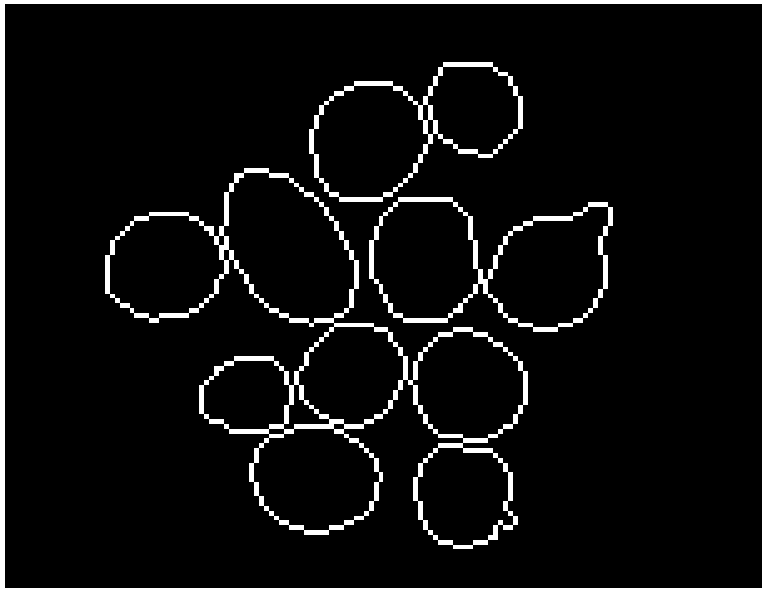
- Sample between gel slab and coverslip.
- Phase contrast + 2 fluorescent channels image every 3 min, for 6-8 hrs(~5 divisions) cells remain in layer (controls)
- Markers:
    - bud ring (time bud,division by relocaliz): Myo1, Cdc10
    - phosphorylation G1/S gene transcription: Whi5, Sic1
    - cyclin transcription: CLN2pr-GFP-PEST
    - mass marker: Act1pr-dsRed
- Software

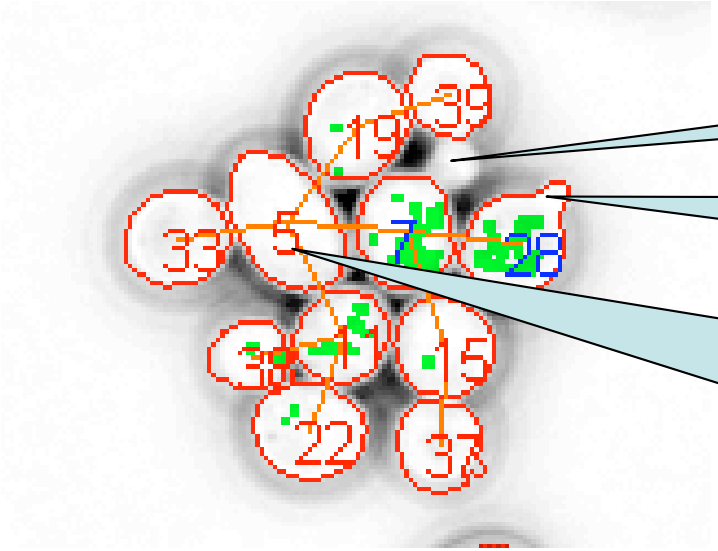
Phase + CLN2pr-GFP



Segmentation and annotate with genealogy





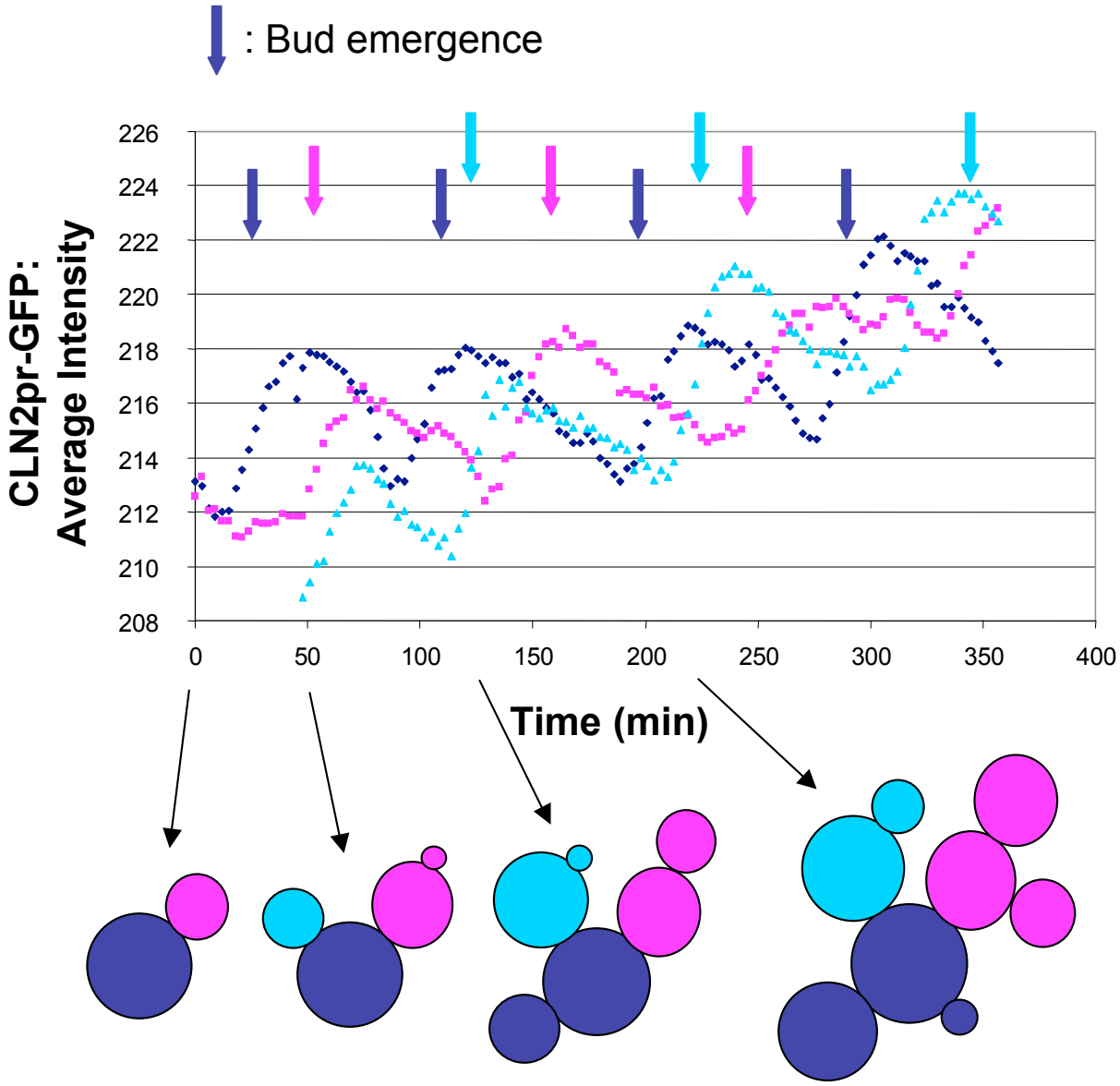


bud segmented and dropped as too small

bud not segmented

annotation; red lines
founder 5 ->{7, 11, 19, 33};
7->{15, 28}; 11->{22, 38};
15->33; 19->39;

Plot cell integrated intensities vs time



Growth and size control (history)

- Yeast maintain cytoplasmic/nuclear vol ~ cst, haploid->tetraploid, mutants disrupt ratio ~2x, large cells long G1, mechanism unclear.
- Major proteins increase exponentially, d_tP/P ~ cst independent of cell size (Elliott.. PNAS **75**, 4384 1978)
- Poor media size daughter/mom decrease, G1 increases for D's (Hartwell et al 70's)
- Cell cycle variable ~50% as measured by times or volume at bud (Hartwell, Lord-Wheals '79-82)
- Variability under genetic control, pseudo-hyphal strains (Kron..Fink '94), much more regular than isolated (no cytoplasmic connections), Thus affects fitness and evolution.
- Size/growth and control of cell cycle common to all eukaryotes.

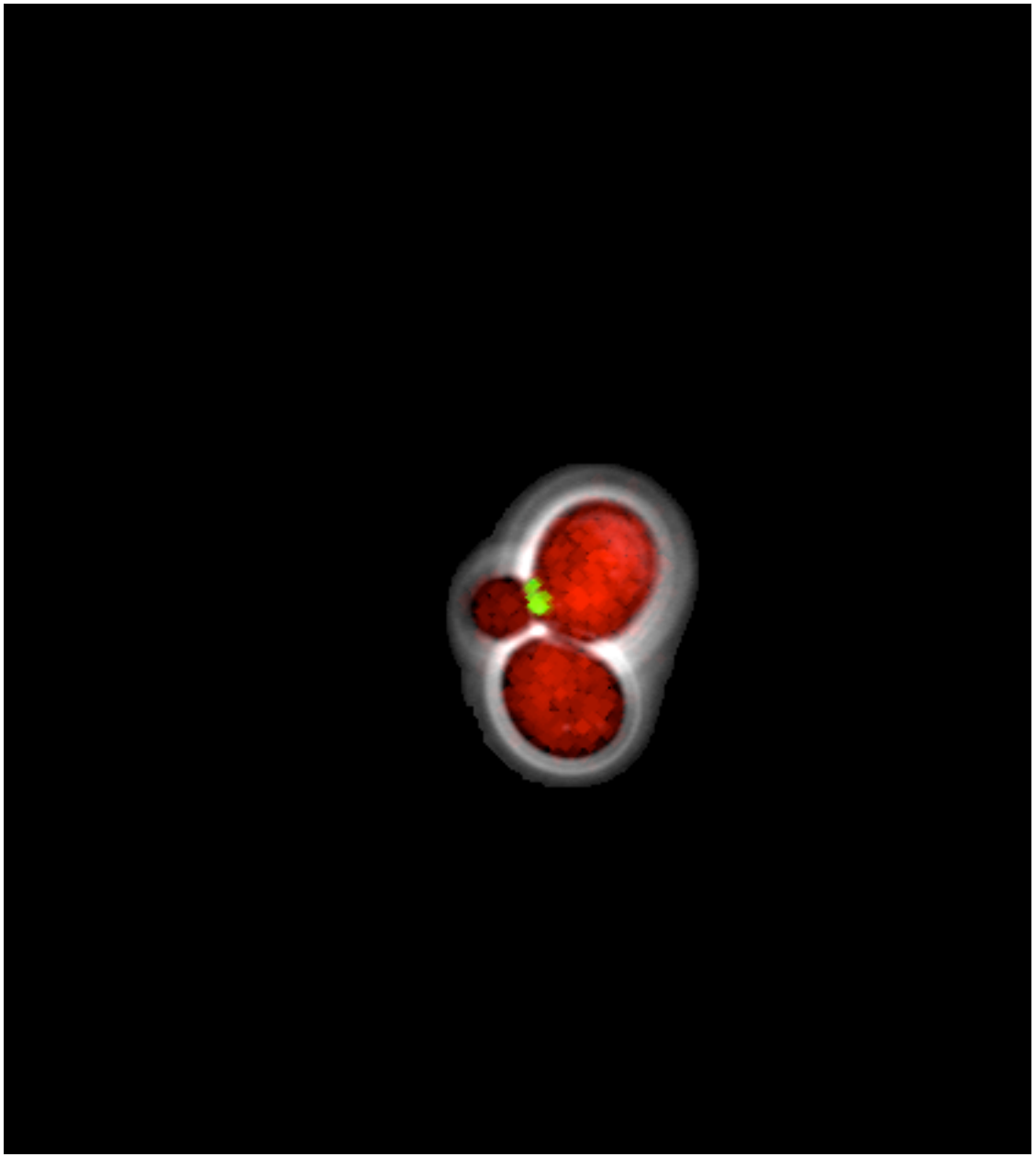
Growth and size control (questions)

- Are cells less variable (eg size at bud, time division to bud etc) when quantified with mass rather than volume (nb yeast have vacuole)? Dimension of phase space?
- Molecular origins of variability, correlations, what's least variable?
- How is mass partitioned between mother and daughter?
- How do mutants affect single cell variability? Genetics of noise

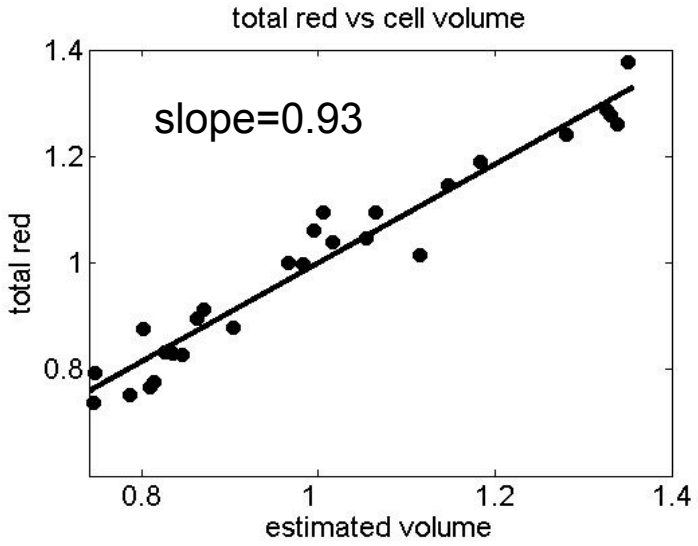
Growth and size control- issues

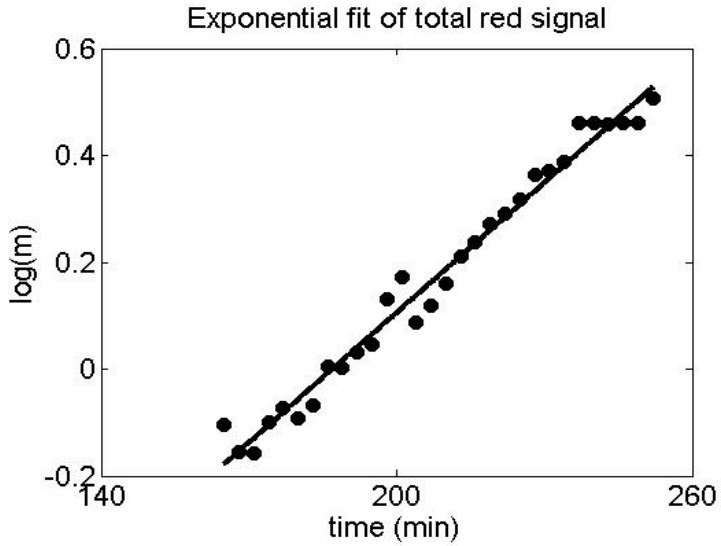
•Cells grow exponentially, population doubling time set by metabolism
•Poor media: daughters born small, long growth phase (G1) prior to ‘Start’ (bud emergence, DNA repl)
•Size control at G1/S, classic size mutants leave doubling time fixed, advance (smaller cells), retard (larger cells) ‘Start’. biophysical mechanism unclear
.
•Population screens can not examine for one cell, Size(Start) vs Size(Division), strict defn of size control.
•Budding yeast more variable than E.coli or fission yeast which divide symmetrically.
•Molecular origins of variability (genetics of noise)

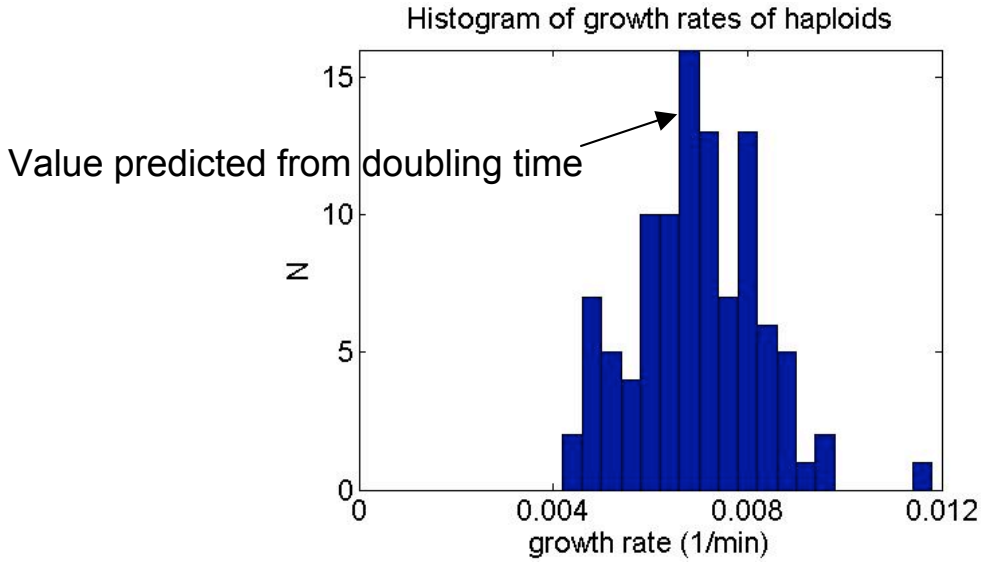
Movie: protein, budring, phase



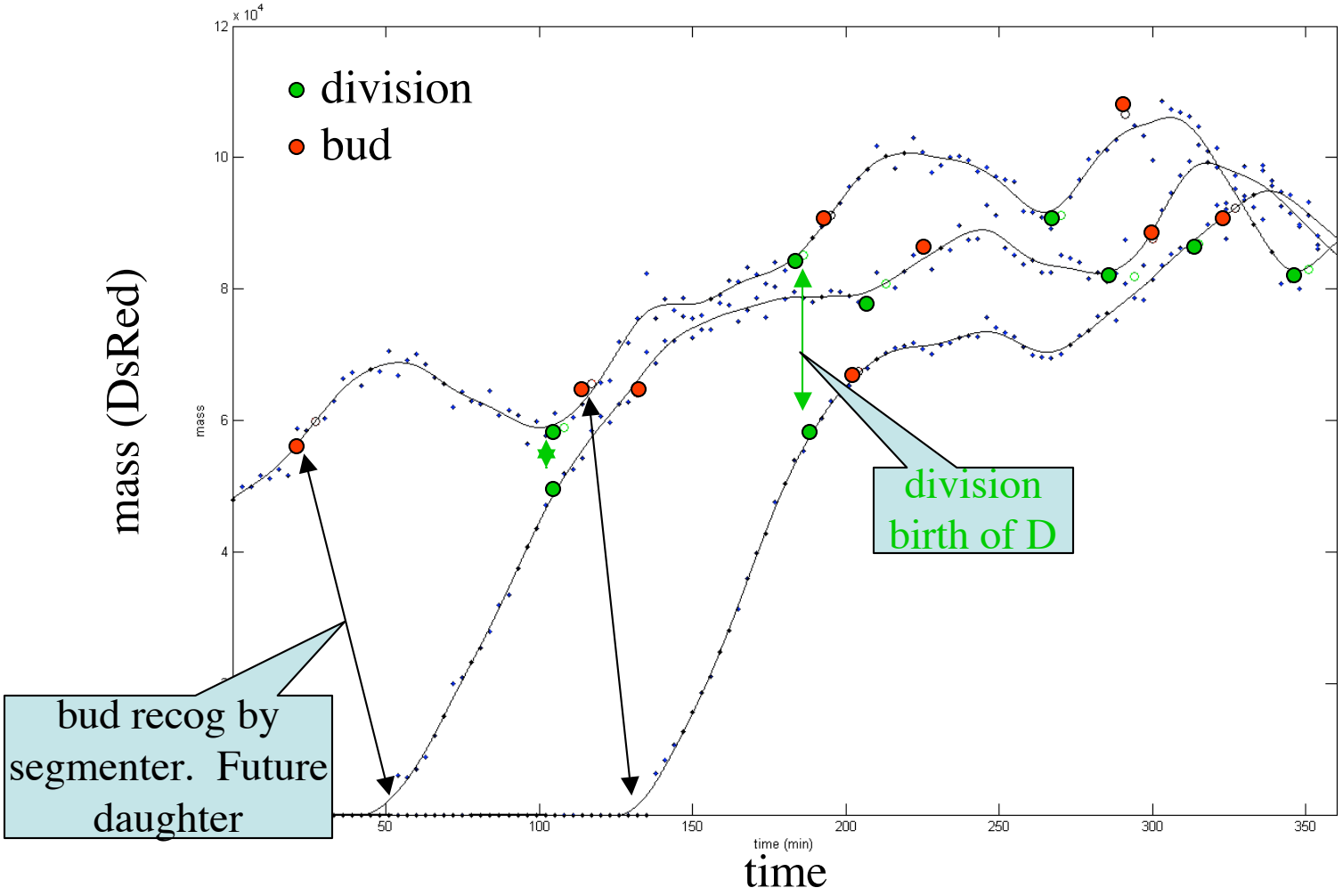
Measuring mass with ACT1pr-dsRed







mass vs time


division
bud
mass (DsRed)
mass
x 10^4
12
10
8
6
4
50
100
150
200
250
300
350
time (min)
time
division
birth of D
bud recog by
segmenter. Future
daughter

Noise in yeast gene expression

Control of Stochasticity in Eukaryotic Gene Expression

Jonathan M. Raser and Erin K. O'Shea*

Noise, or random fluctuations, in gene expression may produce variability in cellular behavior. To measure the noise intrinsic to eukaryotic gene expression, we quantified the differences in expression of two alleles in a diploid cell. We found that such noise is gene-specific and not dependent on the regulatory pathway or absolute rate of expression. We propose a model in which the balance between promoter activation and transcription influences the variability in messenger RNA levels. To confirm the predictions of our model, we identified both *cis*- and *trans*-acting mutations that alter the noise of gene expression. These mutations suggest that noise is an evolvable trait that can be optimized to balance fidelity and diversity in eukaryotic gene expression.

Heterogeneity in a number of factors that affect gene expression may underlie extrinsic noise, including heterogeneity in cell size and shape, cell cycle stage, or gene-specific signaling. We tested if extrinsic noise factors could be eliminated by flow cytometry to isolate subpopulations of cells that are homogeneous in size and shape. We found that, although diminished by this process, extrinsic noise predominated relative to intrinsic noise in these subpopulations (fig. S2A). Similarly, neither correction for individual cellular volume nor segregation by cell cycle stage (6) resulted in more than a ~25% decrease in extrinsic noise (fig. S2B). To distinguish

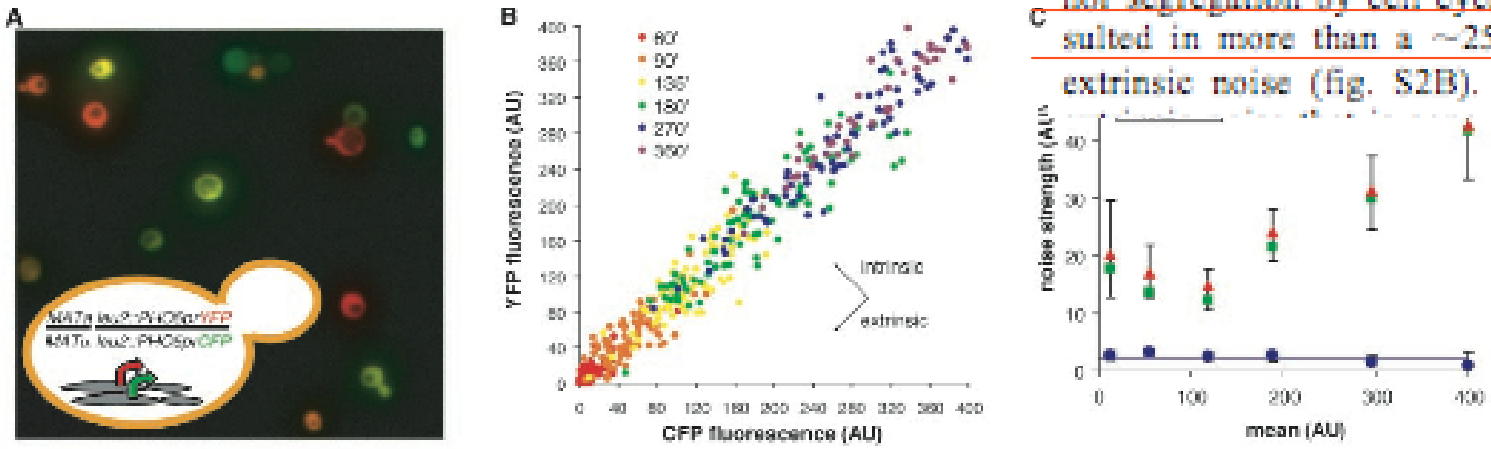


Fig. 1. Separation of intrinsic and extrinsic noise for the *PHO5* promoter. (**A**) A false-color overlay of YFP (red) and CFP (green) fluorescence micrographs from a diploid yeast strain that expresses YFP and CFP from identical promoters at homologous loci, as diagrammed in the inset. (**B**) Scatter plots showing CFP and YFP values for each cell (solid circles) during a time course of *PHO5* induction by phosphate starvation. Populations from different time points (in minutes) are indicated with different colors. Extrinsic noise is manifested as scatter along the diagonal and intrinsic noise as scatter perpendicular to the diagonal. AU, arbitrary units of fluorescence. (**C**) Total, extrinsic, and intrinsic noise strength as functions of population mean for (**B**). The solid line represents expectations for a single stochastic process, and error bars represent bootstrap values (*6*).

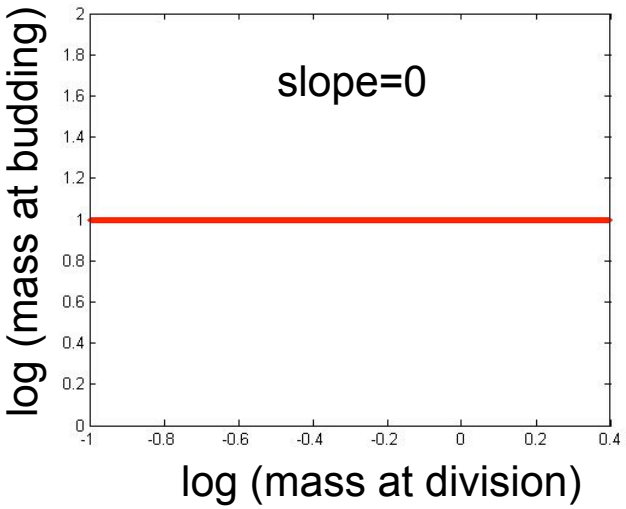
Sizer vs Timer

Sizer: Network that halts cell cycle progression until critical size is reached

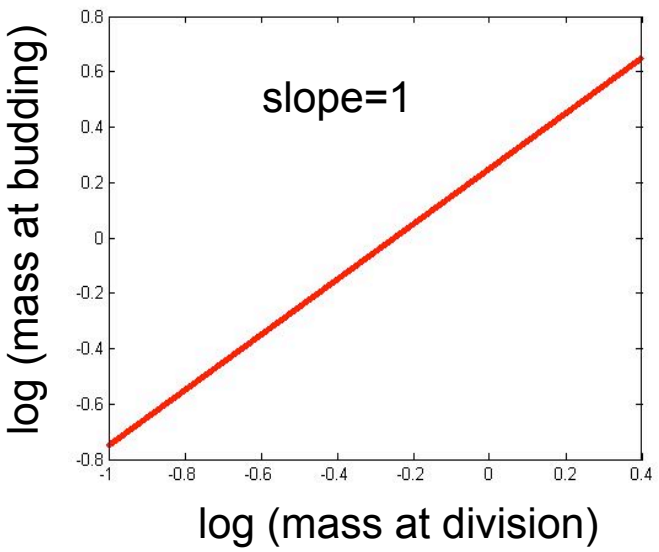
Timer: Imposes fixed time delay irrespective of mass --> (via exponential growth) $mass_{bud} = cst\ mass_{div}$

therefore in log-log plot:

ideal sizer:

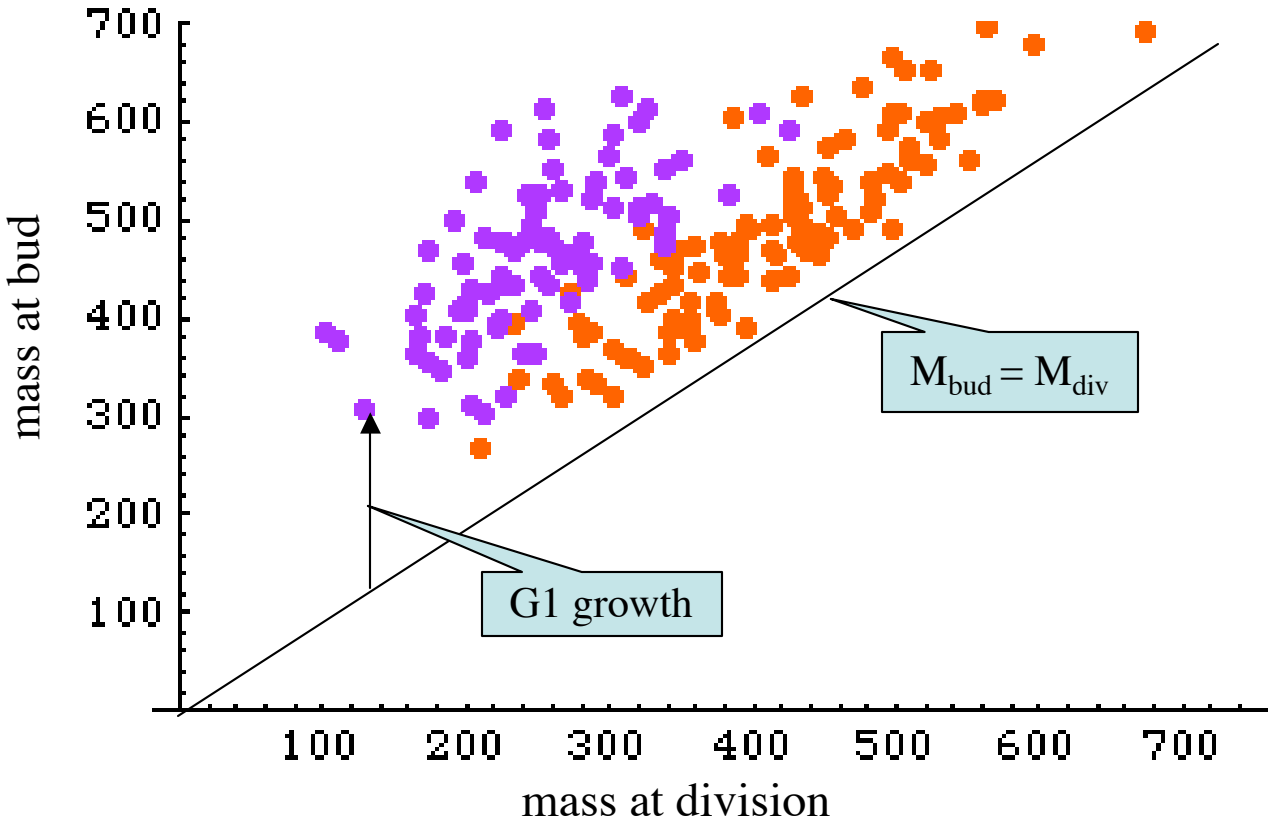


ideal timer:



Sizer vs Timer: Data gly/eth media

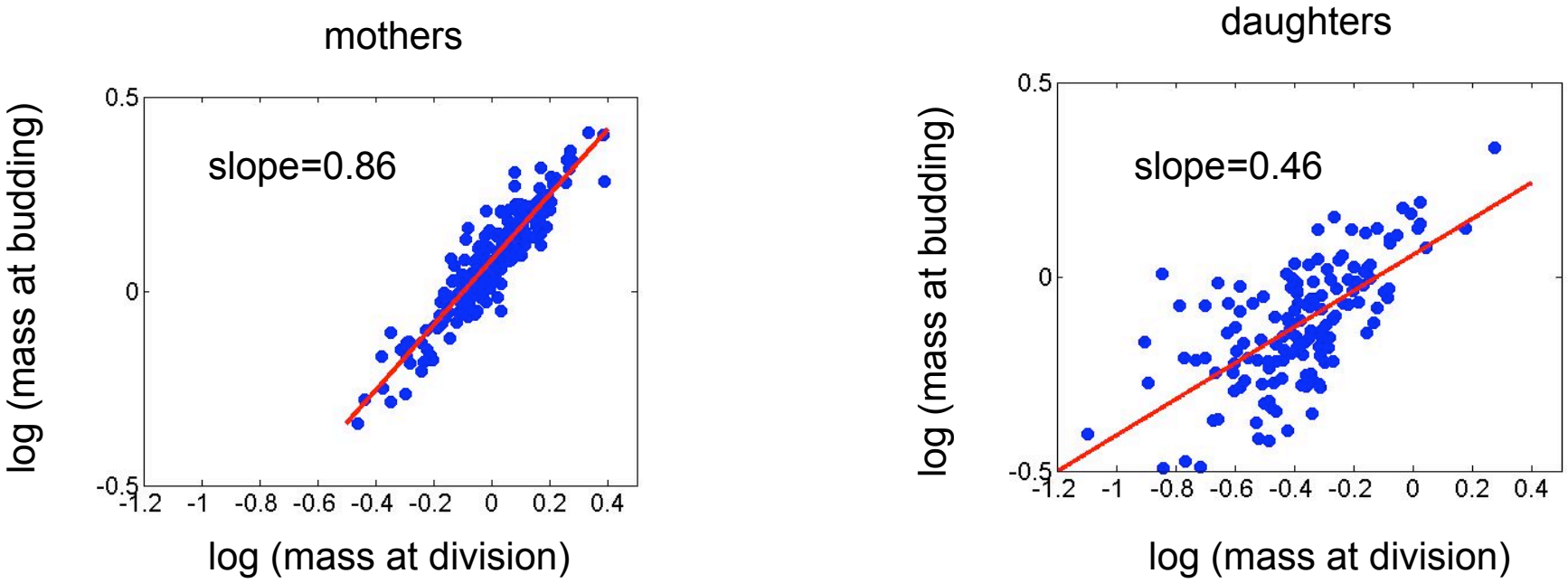
Mass bud vs division (D, M)



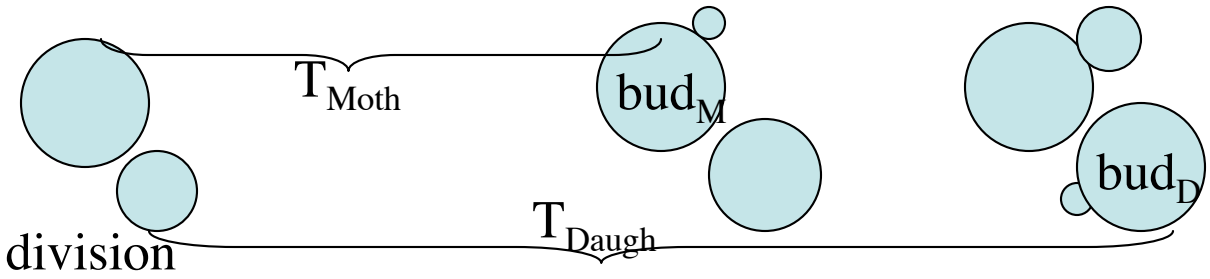
Mothers on timer; G1 daughter delay; small daughers on sizer

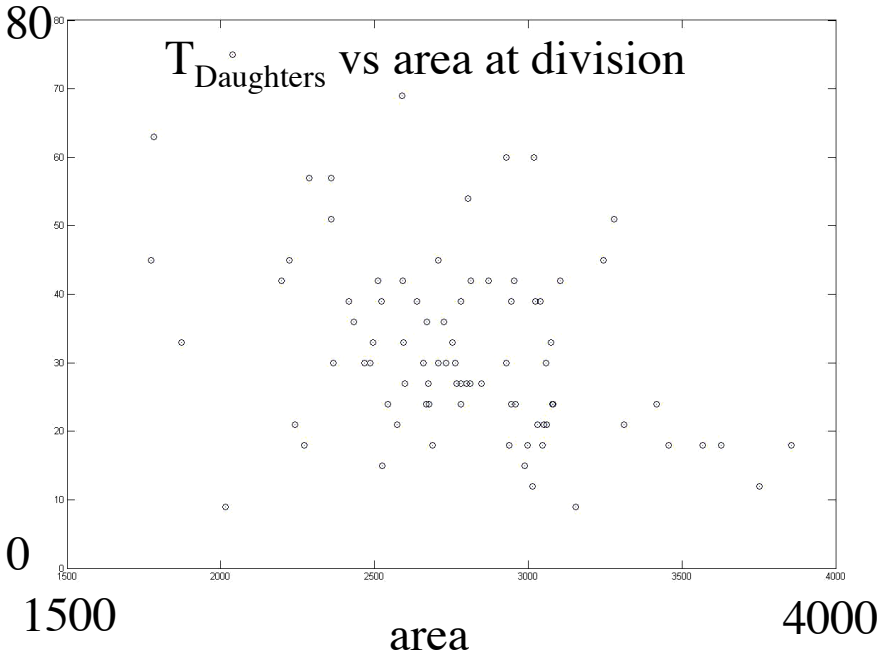
Sizer vs timer: Data good media

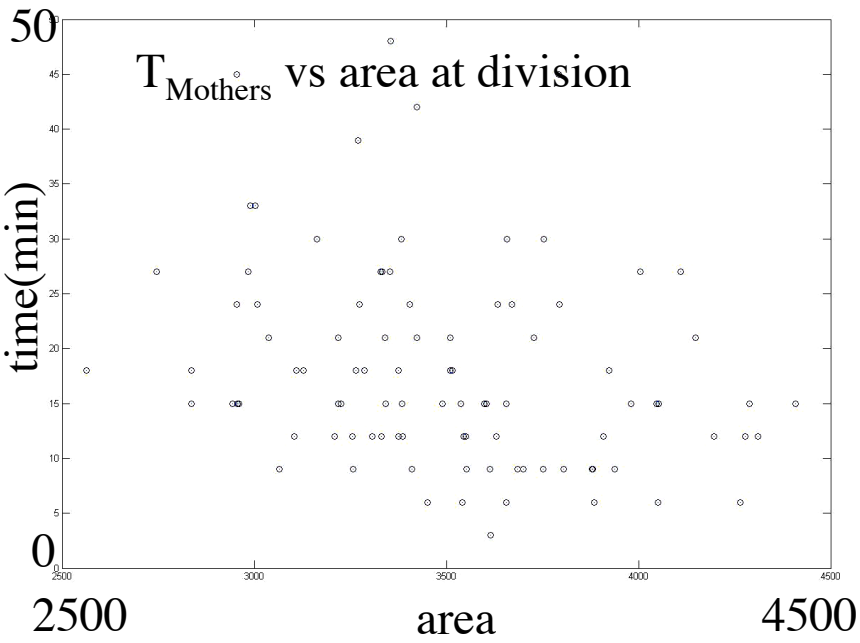
Correlation between mass at budding and mass at division



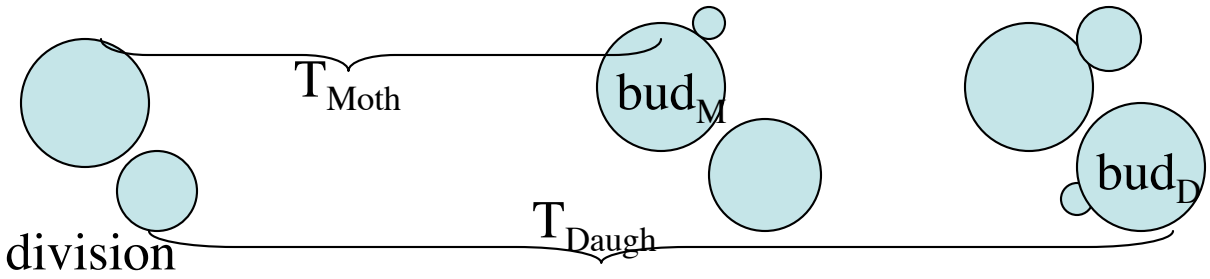
Time [division to bud] vs area [at division]



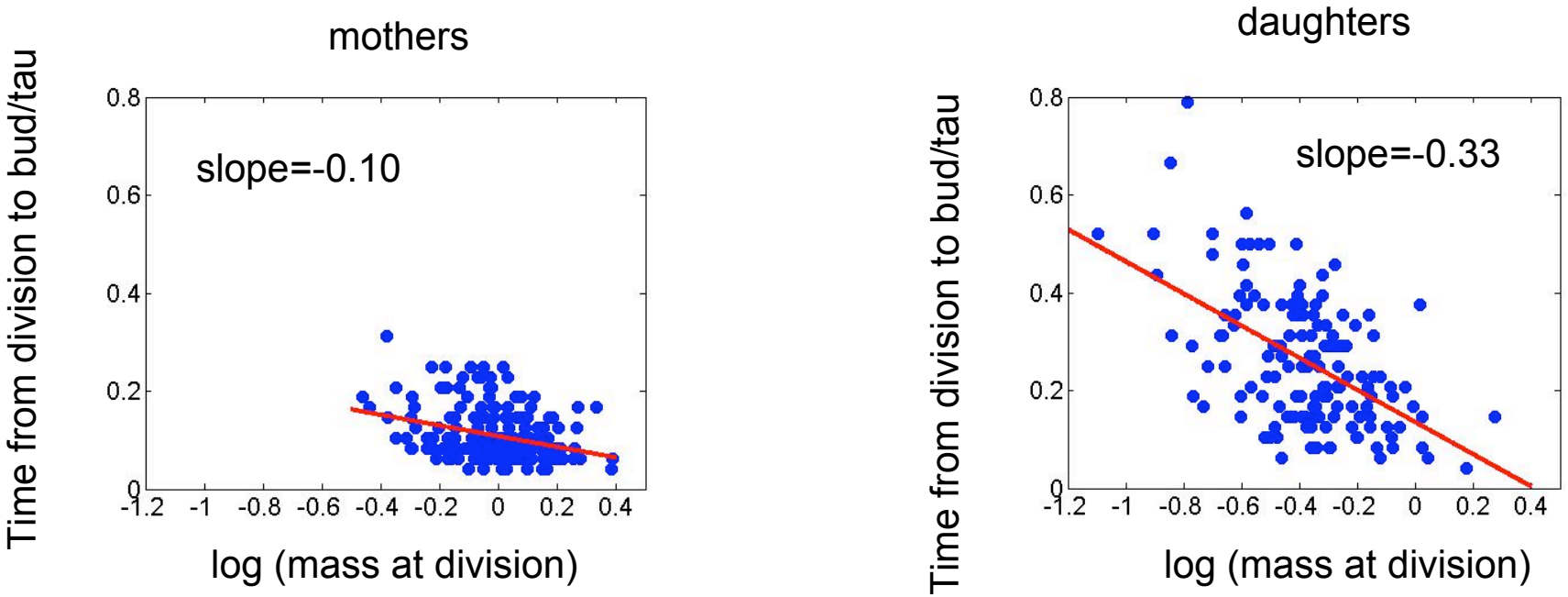




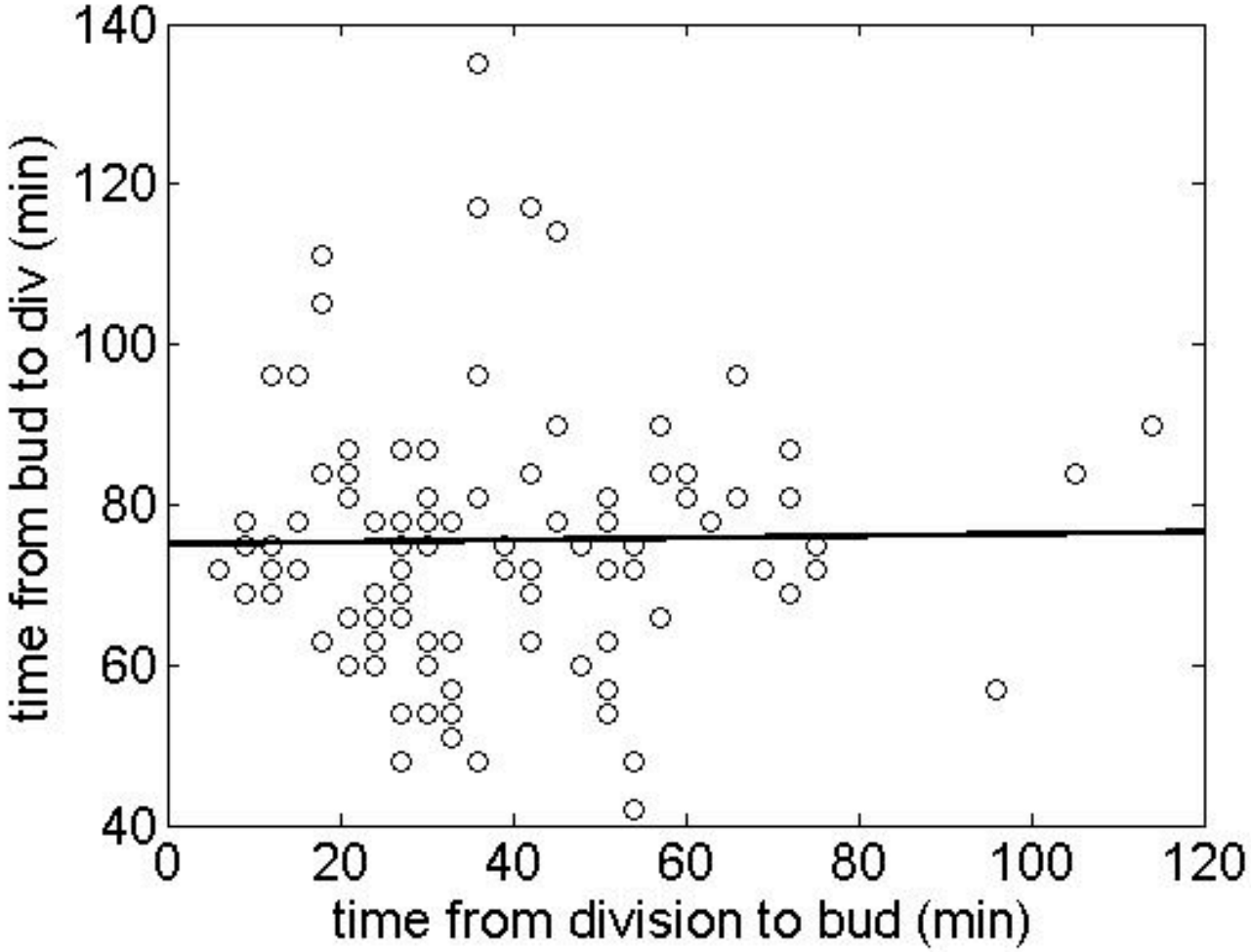
Time [division to bud] vs mass [at division]



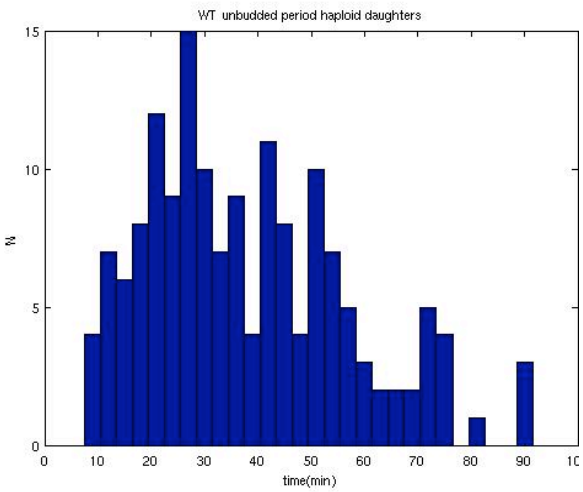
Correlation between mass at division and time from division to bud



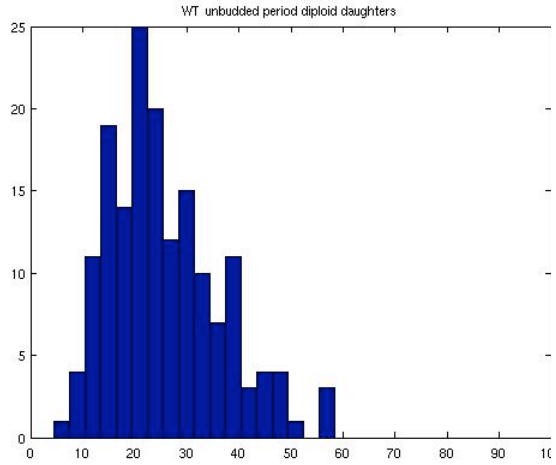
Time [bud->div] vs [div->bud] daughters (diploids)



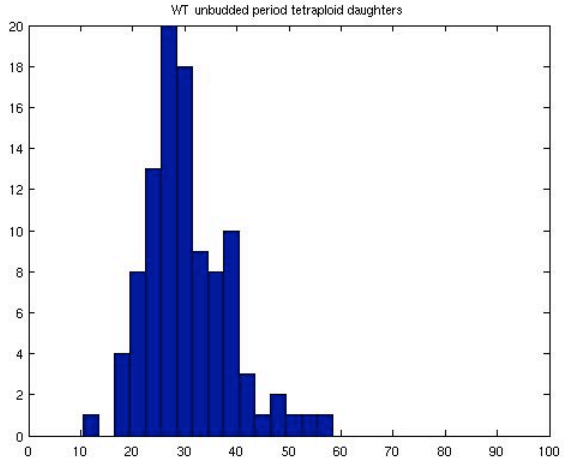
Time (division->bud) vs ploidy



haploid



diploid



tetraploid

Cell size accurately scales with ploidy

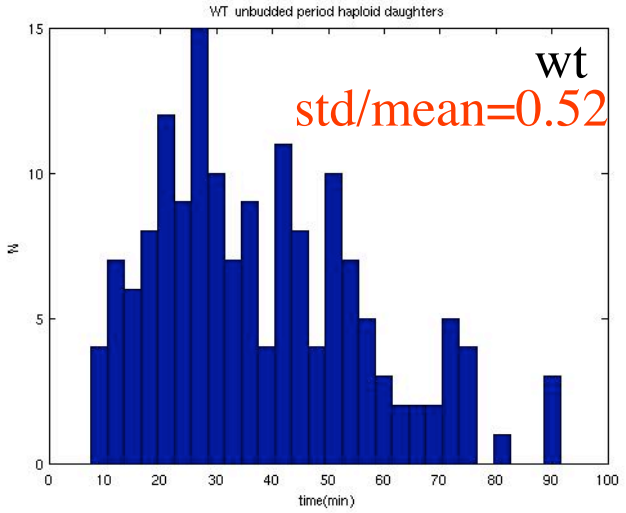
For daughters; std/mean 0.52, 0.41, 0.26 (averages: 38, 26, 30 min). -> expected scaling if molecular noise, sqrt(size). (Budded period std/mean = 0.21, 0.19, 0.19 vs ploidy)

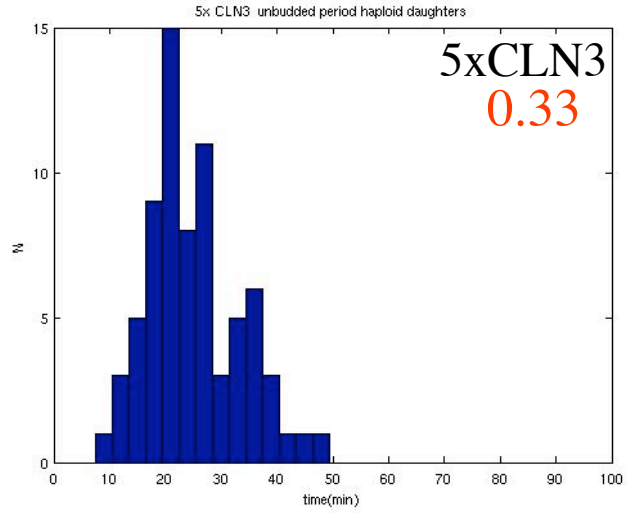
Similar scaling mothers. (unbudded: 0.50, 0.42, 0.28 ±0.04).

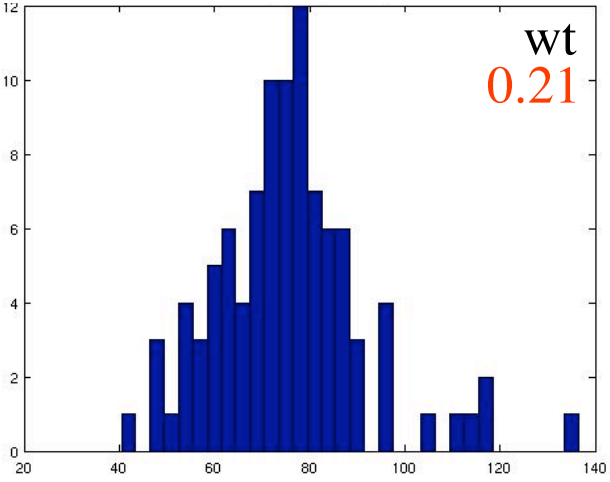
Timing vs CLN3 gene dosage(daughters)

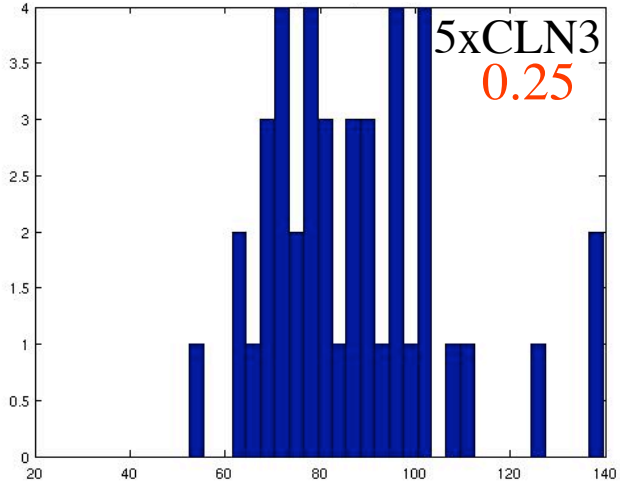
unbudded period

budded period







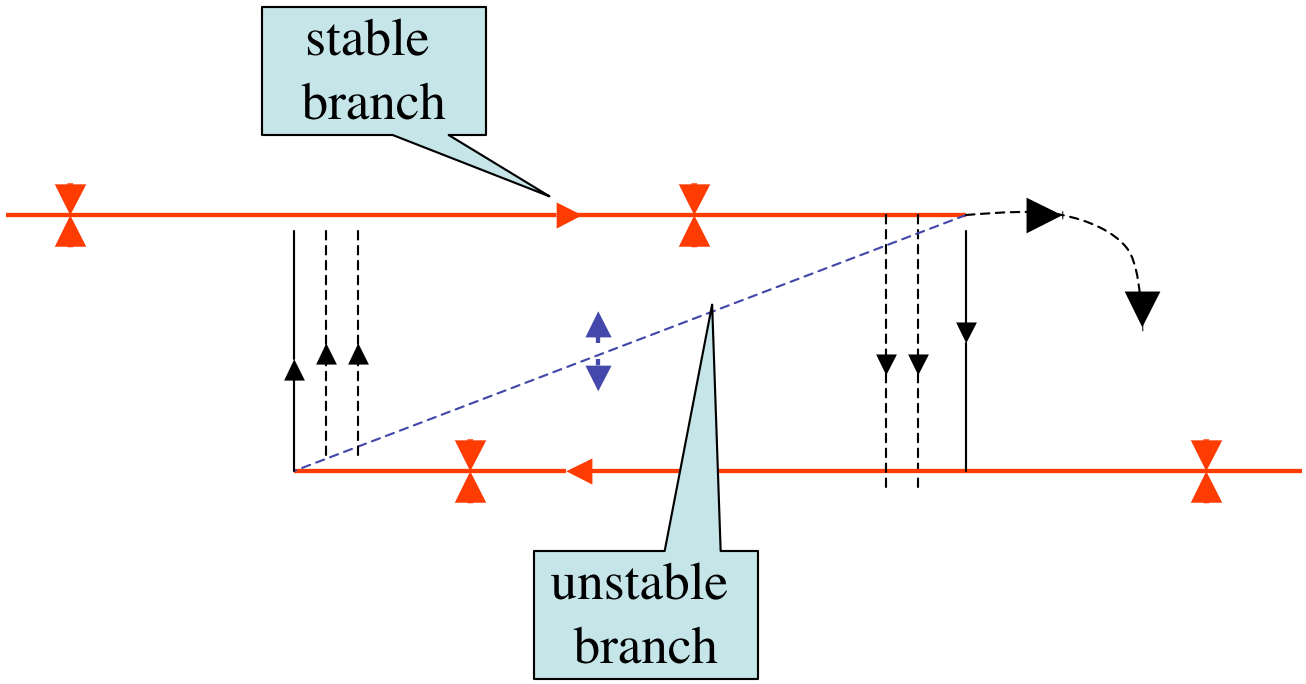


Total daughter cycle time 113(WT) vs 114 min. Std/mean, unbudded, decr by < 1/sqrt(6).

Conclusions from mass-timing measurements

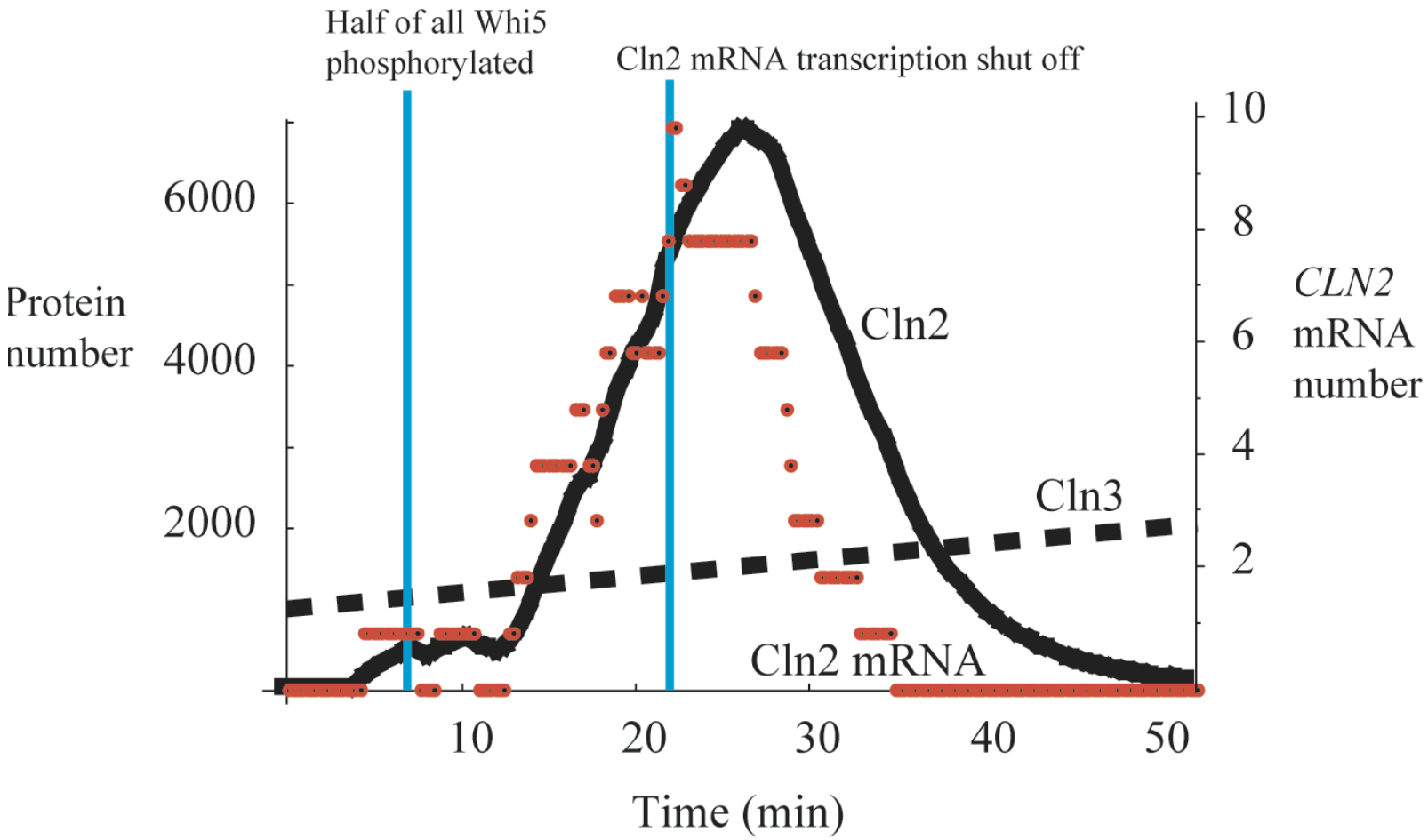
- Order 1 variability in mass at division and bud times
- Div->Bud, daughters monitor size if small, mothers on timer.
- Bud->Div, ~timer, not mass regulated, daughters~mothers
- Timing of certain sub-intervals scales with ploidy--> molecular origins of noise (vs 'morphological' noise of bud site selection)
- Trace noise to genes upstream of budding, vary dosage.
- Stochastic models of 'Start' (commitment to division) suggest transition happens after loose bistability.
- Prior (population) studies looked at change in size histogram, we look control of mass from div to bud cell by cell -> very different take on what genes matter.

Transition in relaxational oscill.



Due to delays in DNA->RNA->protein, initiation of ‘Start’ happens after stable branch disappears.

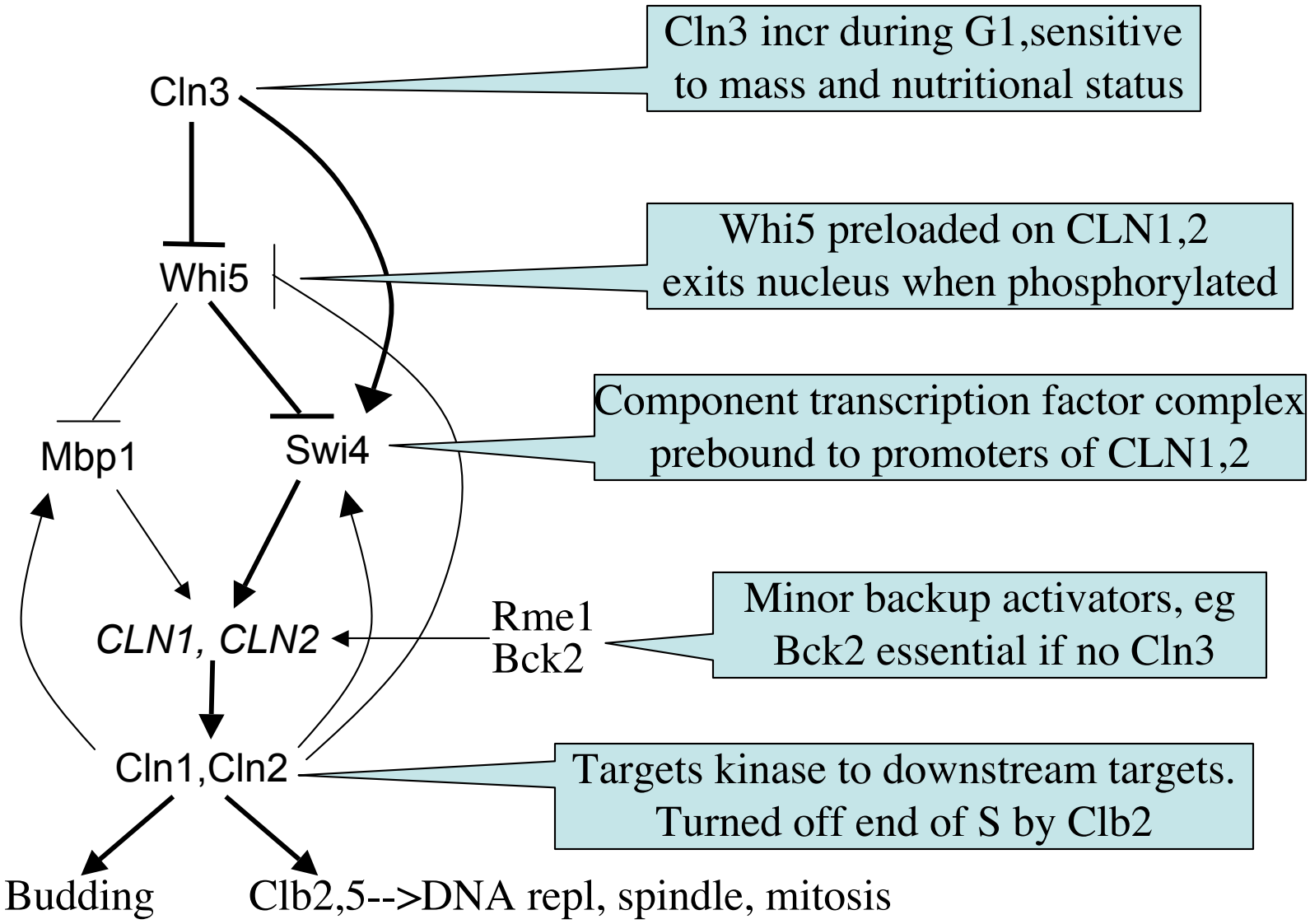
Stochastic Simulations



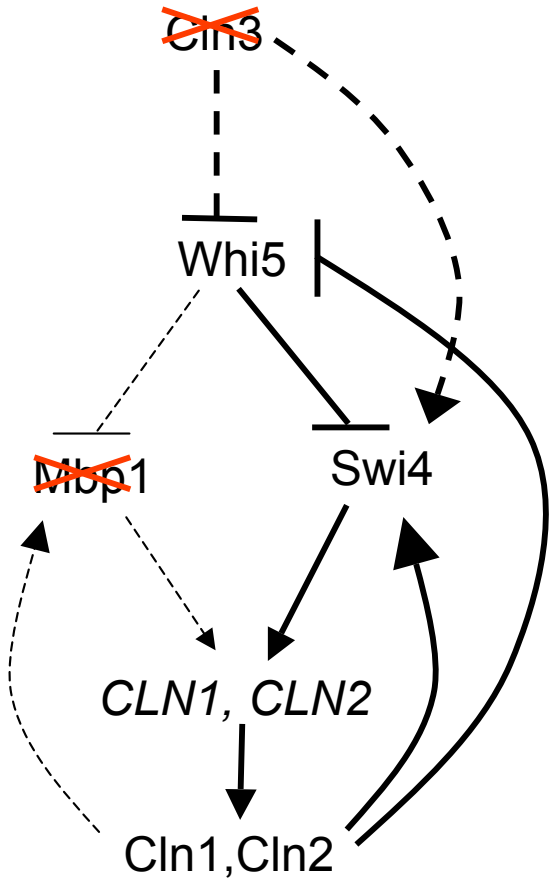
Extrinsic and intrinsic noise in the Start ‘module’

- Does Start (commitment to next round of division) function dynamically as unit?
- Contrast deletion of ostensible upstream activator, Cln3, vs removal of internal component, Swi4.
- Former increases the variability in time division->start, (‘extrinsic’ noise) but does not increase the relative variability for markers internal to pathway (‘intrinsic’ noise).
- Latter destroys coherence of Start eg budding but no reporter signal.
- Conclusions require single cell imaging.

Start pathway activity of G1 cyclins



Start ‘module’ remains intact: cln3Δ mbp1Δ



Isolate putative feed back loop
Cln1,2 self activate (with Bck2)

1. Monitor Whi5 exits nucleus vs bud emergence
2. Peak in CLN2pr-GFP vs bud emergence

Increased variability mitosis->S.
no change to intra Start markers

Whi5-GFP reporter defines 3 intervals

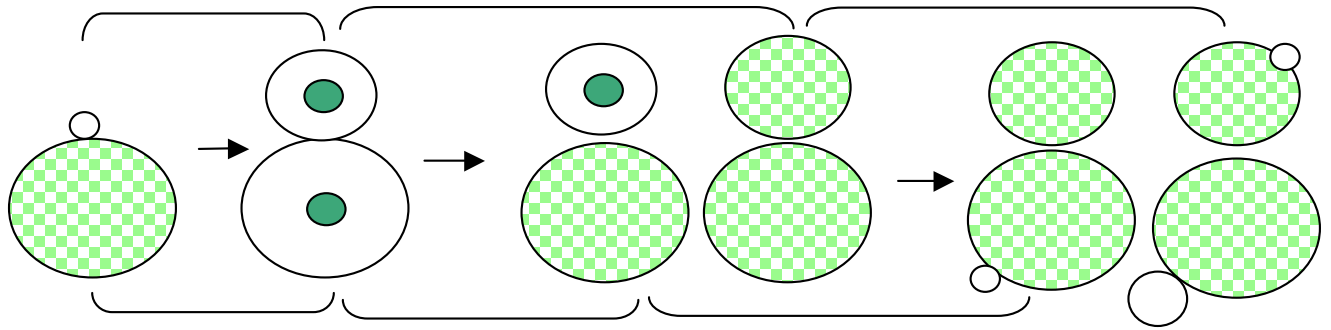
BE -> Whi5 IN
(D and M sychron)

Whi5 IN -> Whi5 OUT
(D lags M)

Whi5 OUT -> BE
Two events in Start module

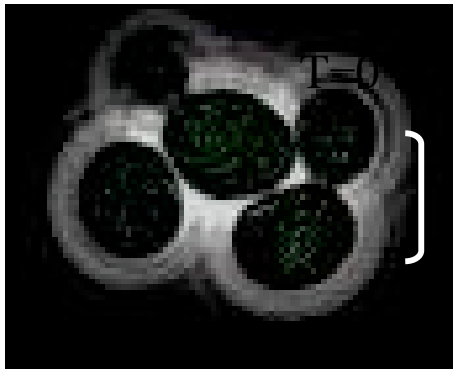
Daughter intervals

Mother intervals

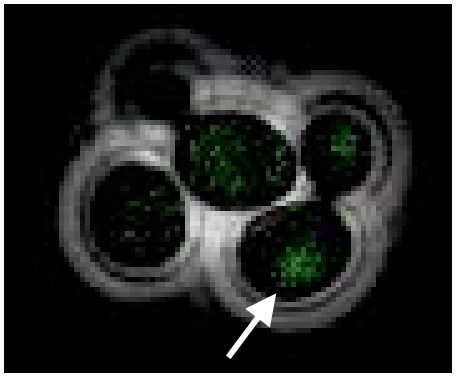


Whi5 cycles into nucleus post mitosis, exits prebud

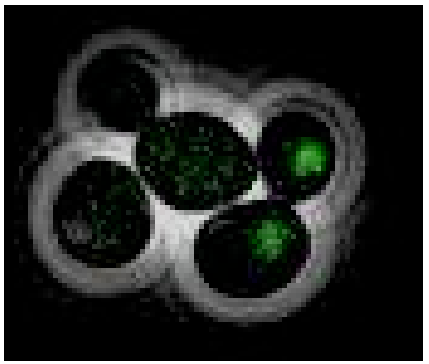
T=0 mitosis



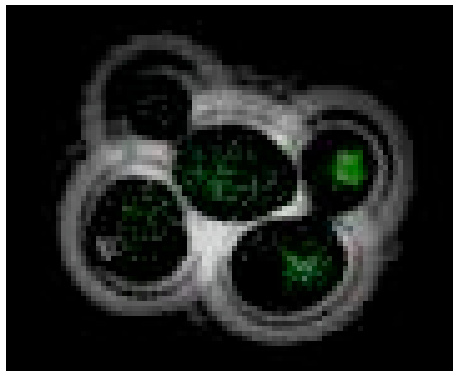
T=3 Whi5 In



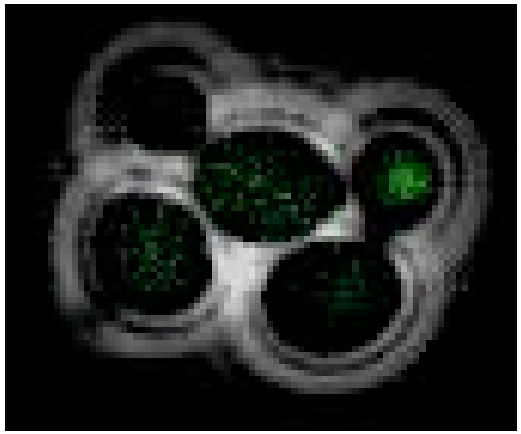
T=9 Whi5 In



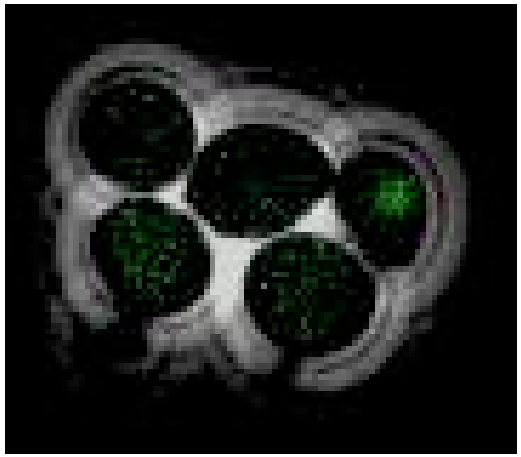
T=12



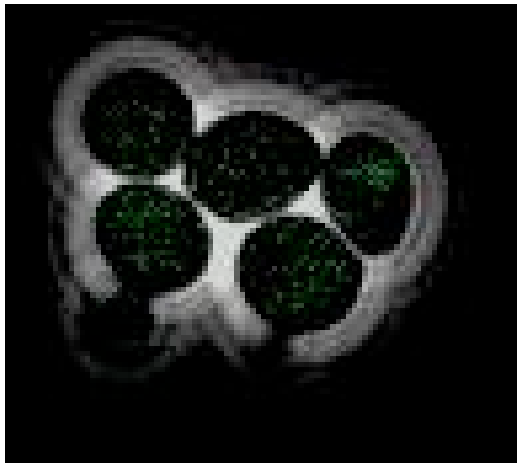
T=15 Out M



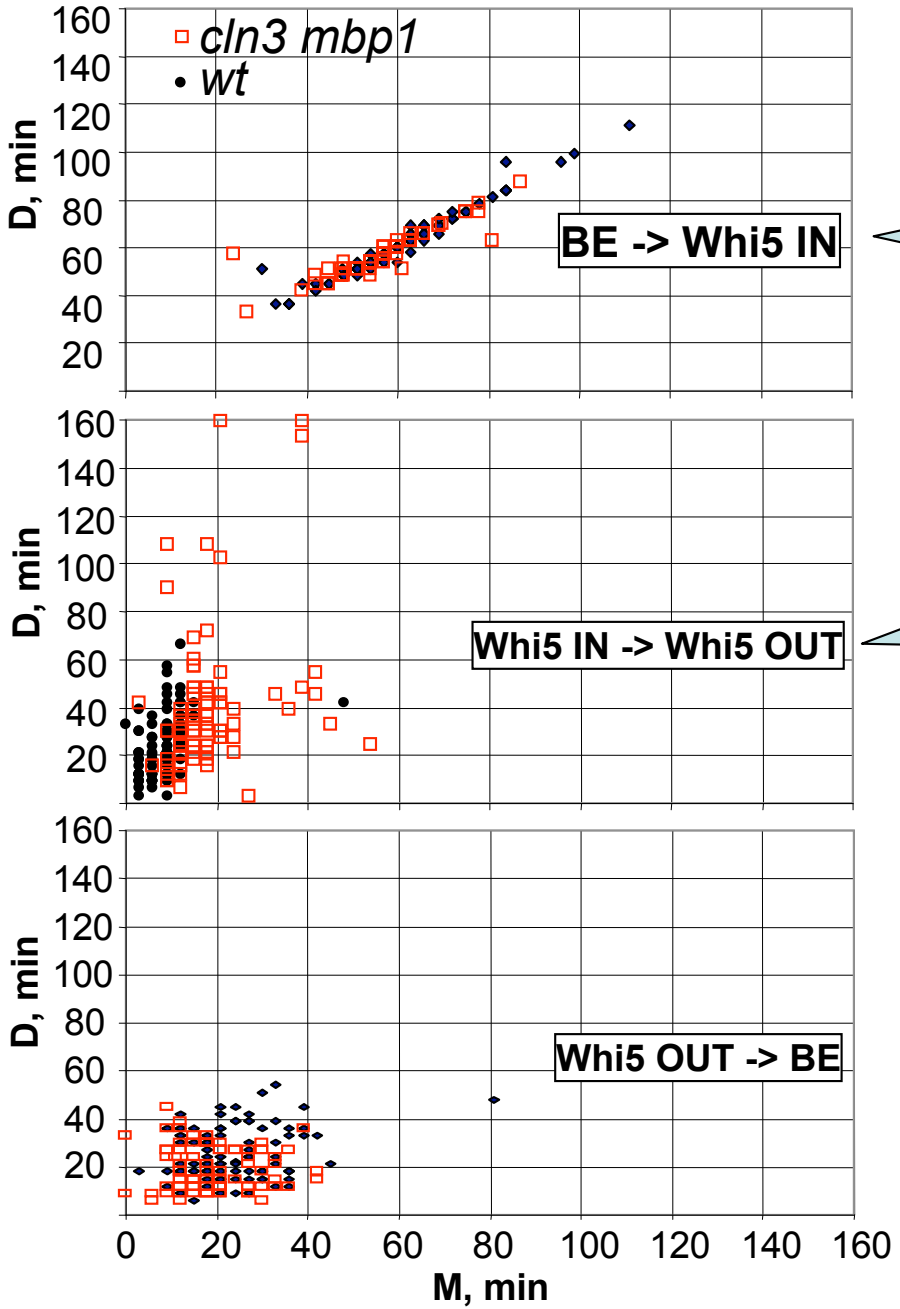
T=42



T=45 Out D



Mother vs Daughter timing of Whi5 in nucleus



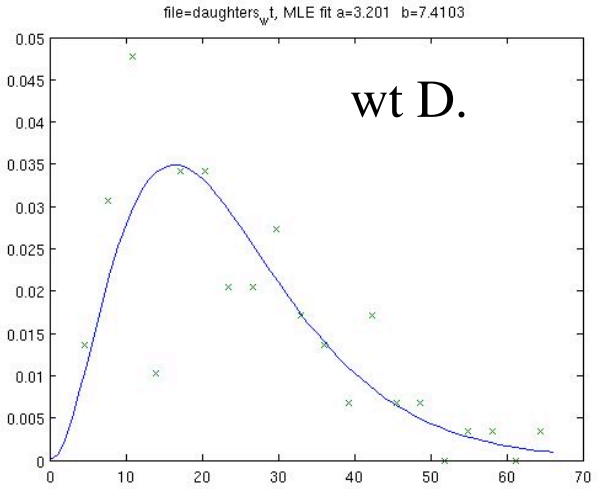
Bud emergence (BE) common to D,M
Whi5 enters nucleus end of mitosis prior to separation -> times correl.

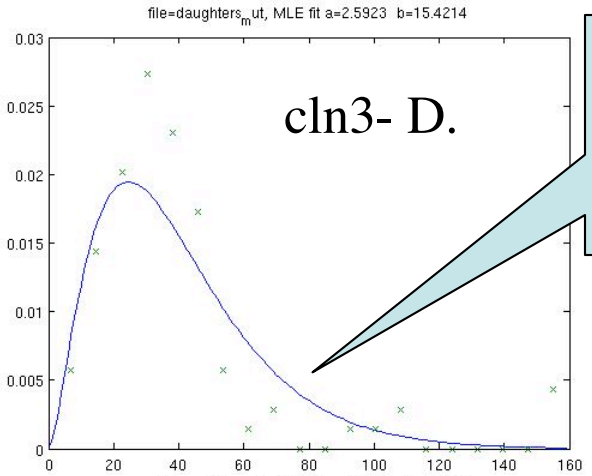
Length G1 (M->S) longer in D vs M due to size. Times increase and get more variable in mutant.

Intra-start time; M vs D similar
Mutant a bit less variable

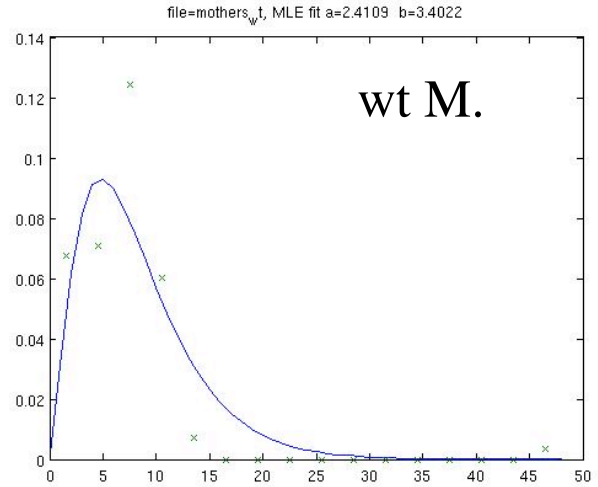
Histograms: time-Whi5 nuclear (~G1)

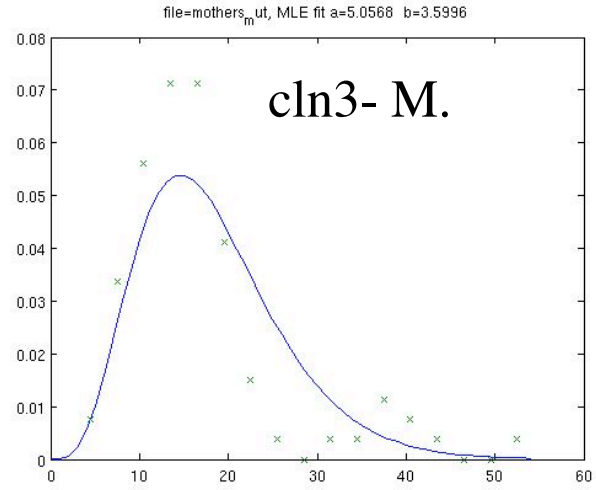
(fit to ~ $t^{(a-1)}$ exp(-t/b))



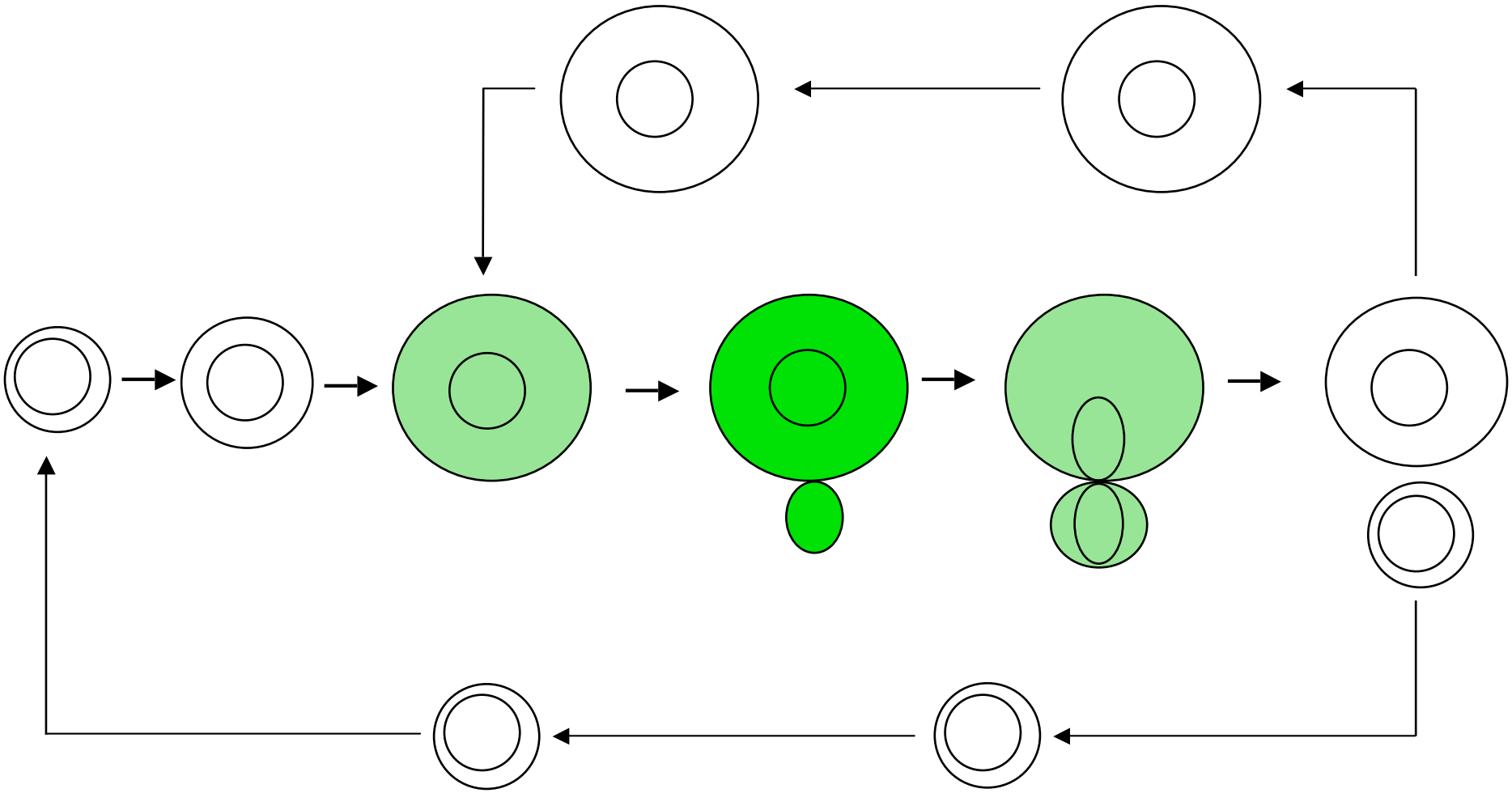


mutants: narrow peak + tail to longer times. Similar in D & M.

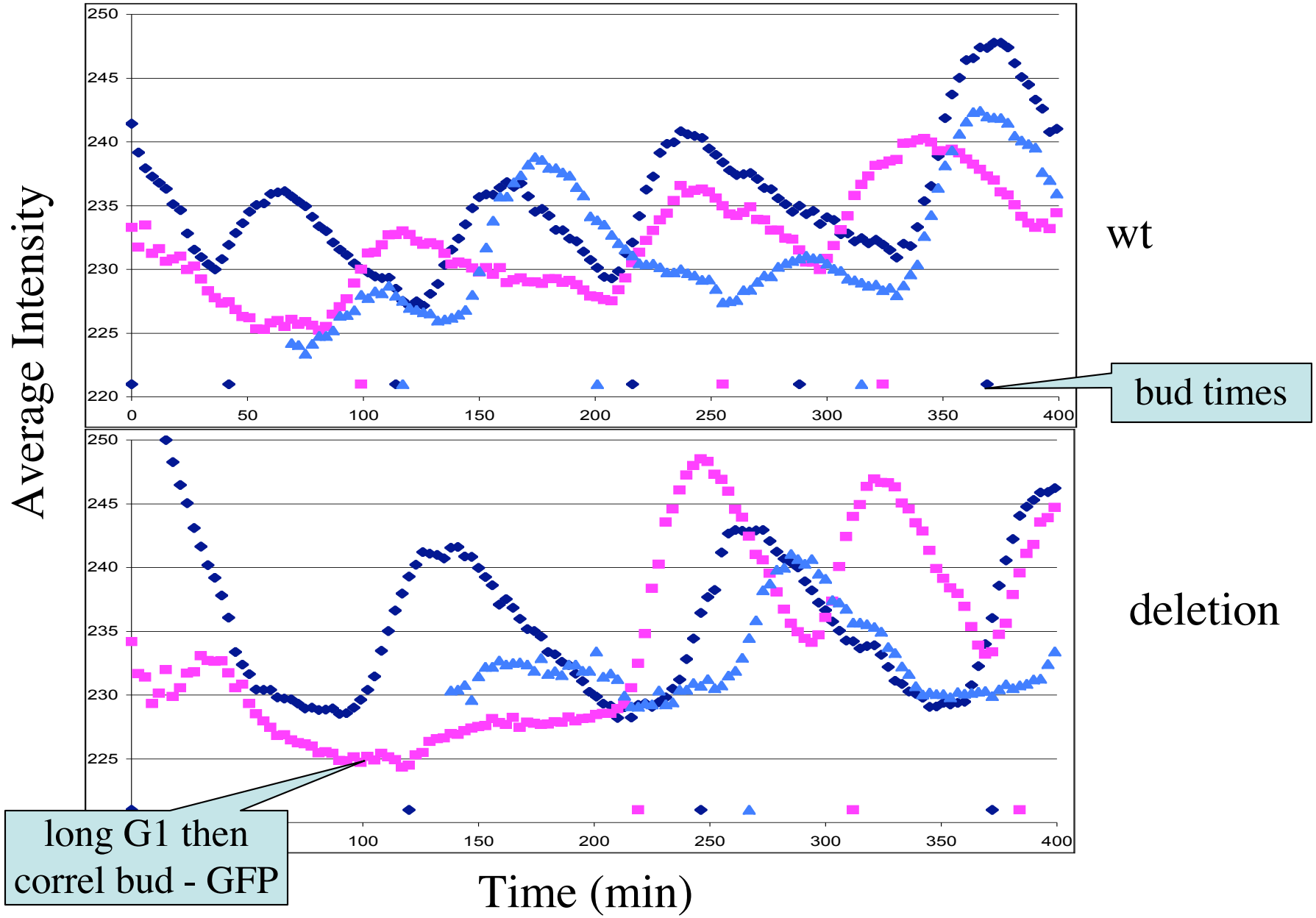




CLN2pr-GFP reporter for Start



CLN2pr-GFP cln3 mbp1 rme1

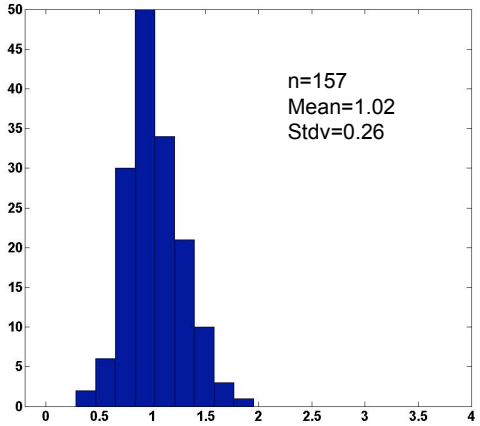


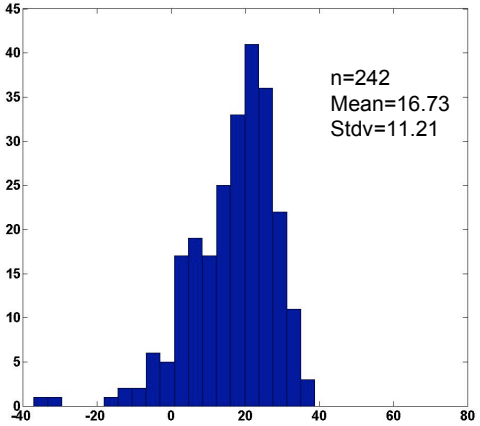
Cln2pr-GFP peak and timing histograms

Peak Amplitude

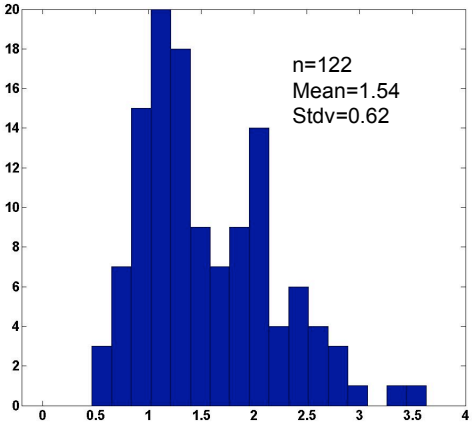
Bud-to-Peak Time (min)

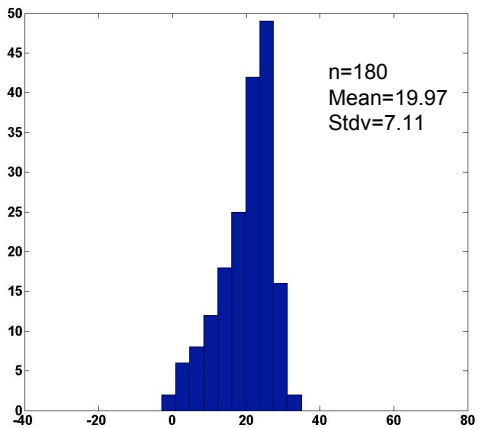
wt



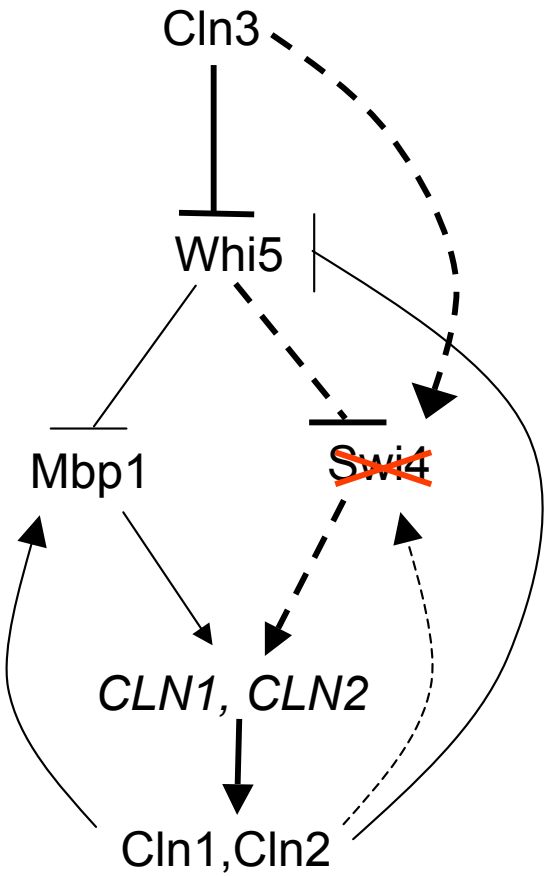


cln3 mbp1 rme1

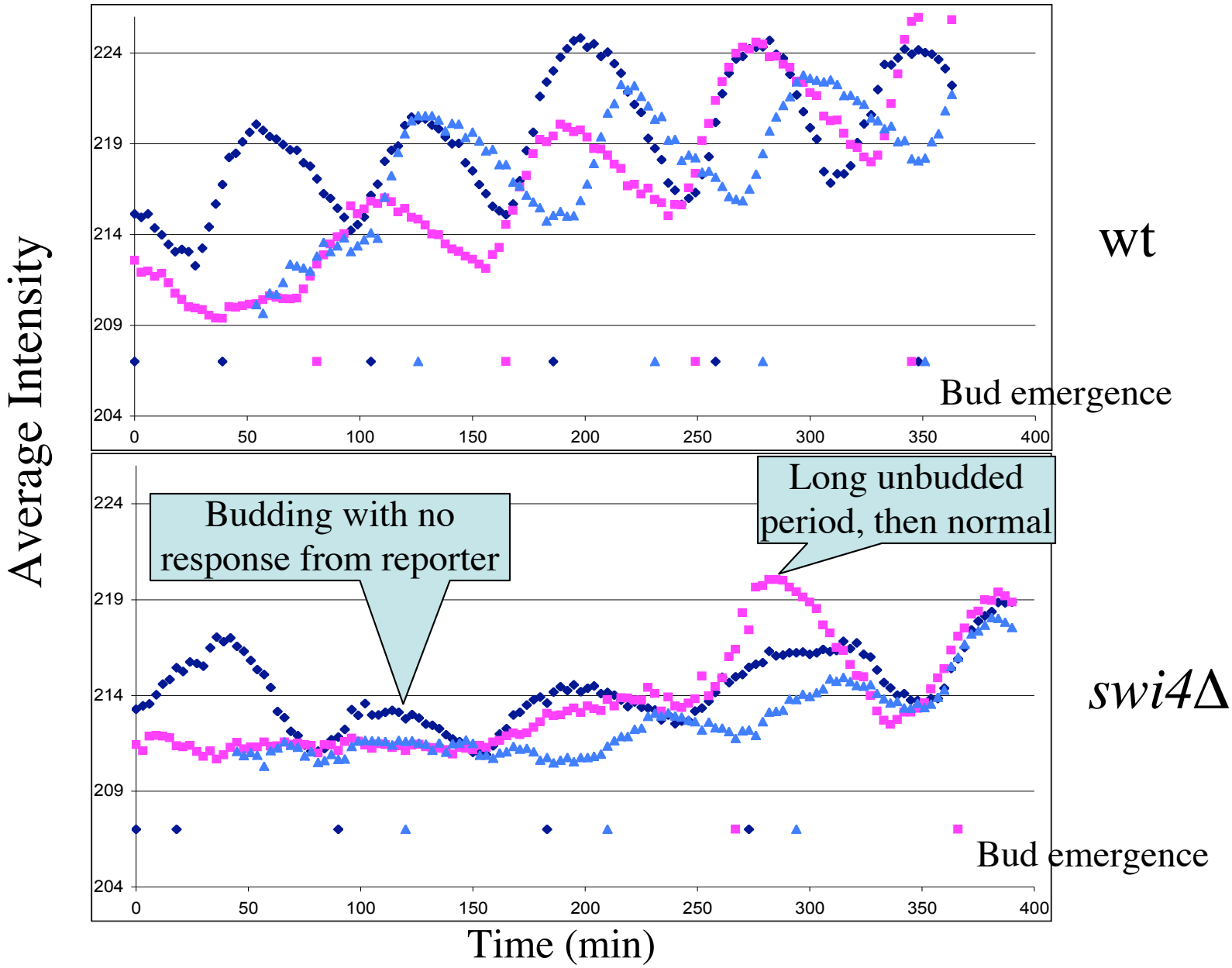




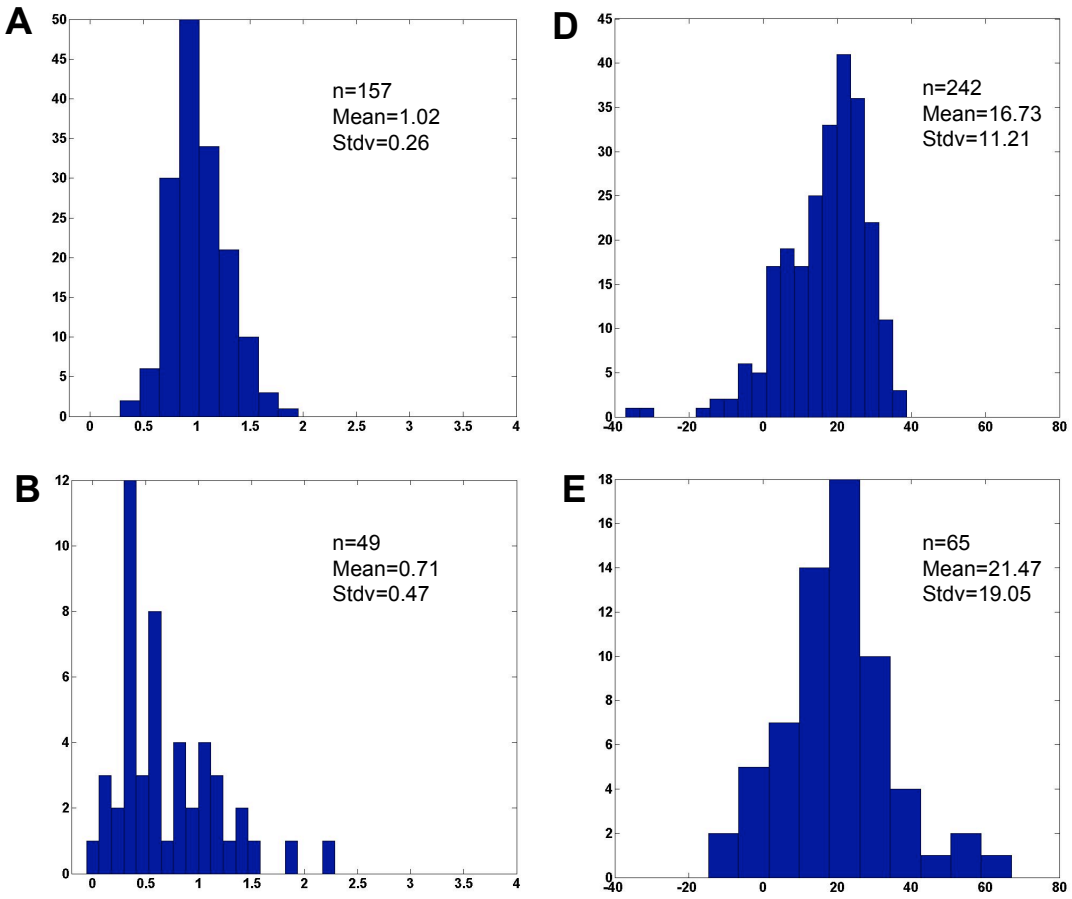
Start module disrupted by swi4Δ



CLN2pr-GFP swi4Δ



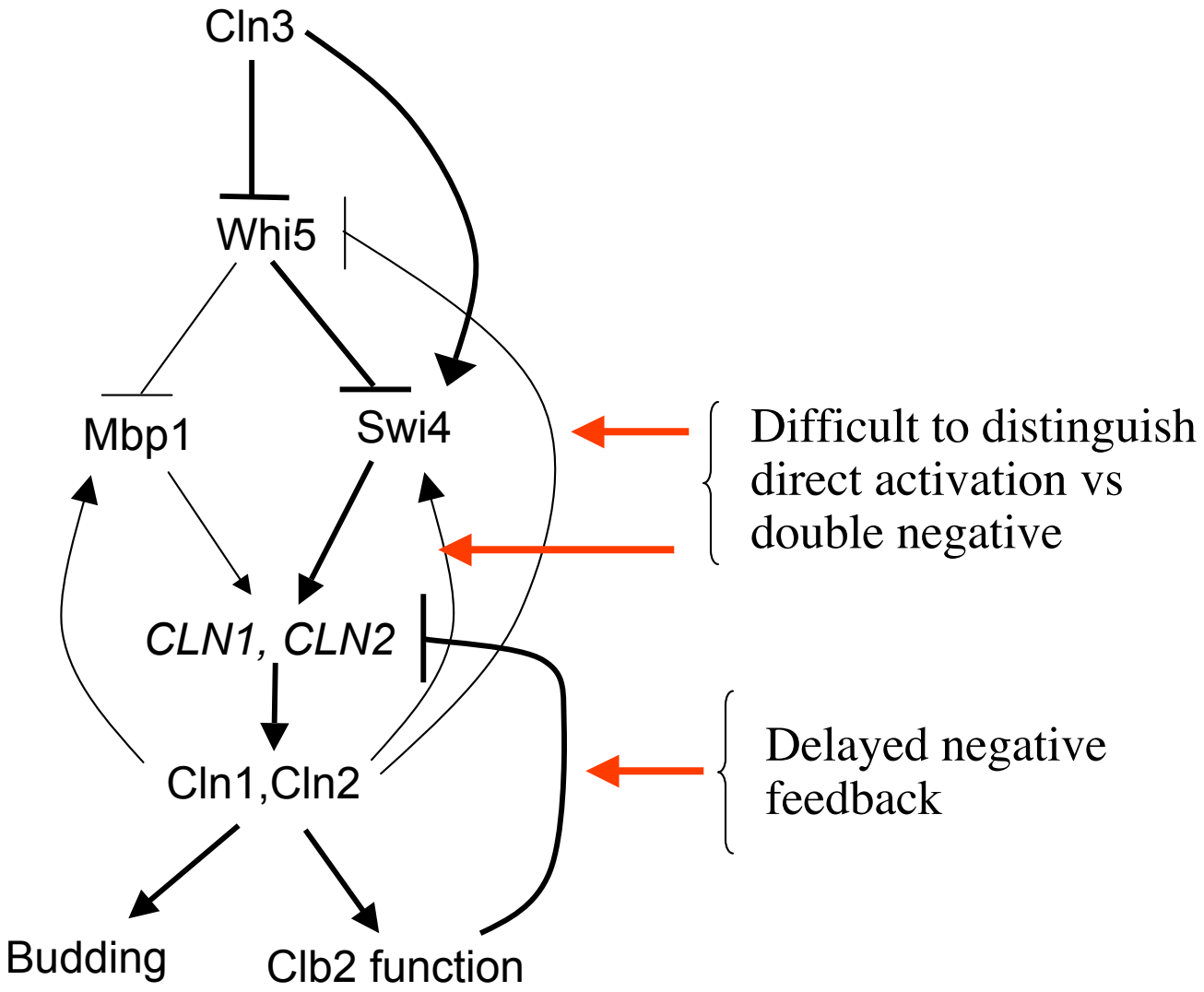
Cln2pr-GFP peak and timing swi4Δ



Conclusions from Start mutants

- cln3Δ causes variablity in initiation of start, swi4Δ interferes with coherence of intra module markers.
- Variability in swi4Δ , with Cdc10-GFP bud ring marker see either normal ring+bud, or incipient ring that ‘tries’ several positions before leading to bud.
- ‘Morphological noise’ associated with either placement of bud ring, spindle check points etc, independent of molecular fluctuations, relevant to wt also.

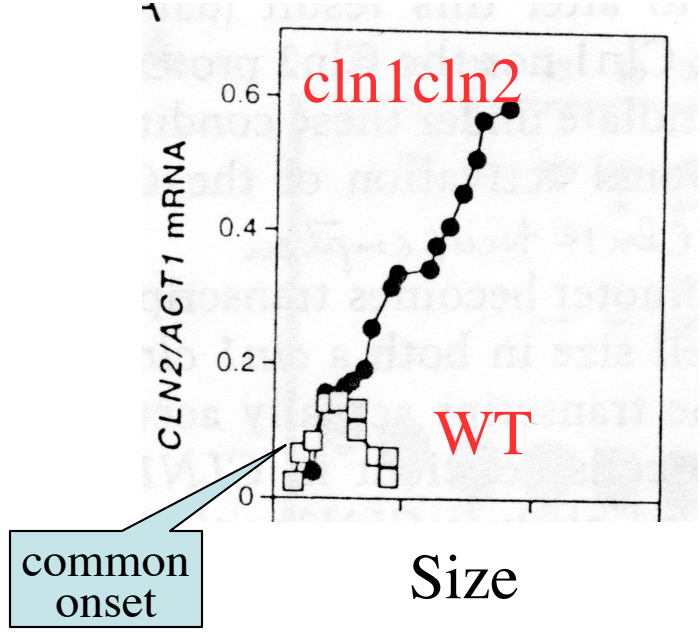
What's the evidence for this arrow?



Are CLN1,2 controled by positive feedback in wt?

Cross&Tinkelberg (Cell '91) show that in cln3- cell CLN2 is enough to excite CLN2 burst.
Problem: could CLN3 backup pathway (eg BCK2) be responsible, and does this happen in wt.

Counter experiment., Stuart&Wittenberg (G&D '95). Cells GALprCLN3 + elutriation+shift to Glu -> get uniformily small daughter cells just divided, with endogenous CLN3 only compare CLN2 message levels, wt vs cln1$^-$ cln2mutant.
Problem: CLN1,2 involved in neg feedback via CLB2, population measurement



Our strategy (Skotheim)

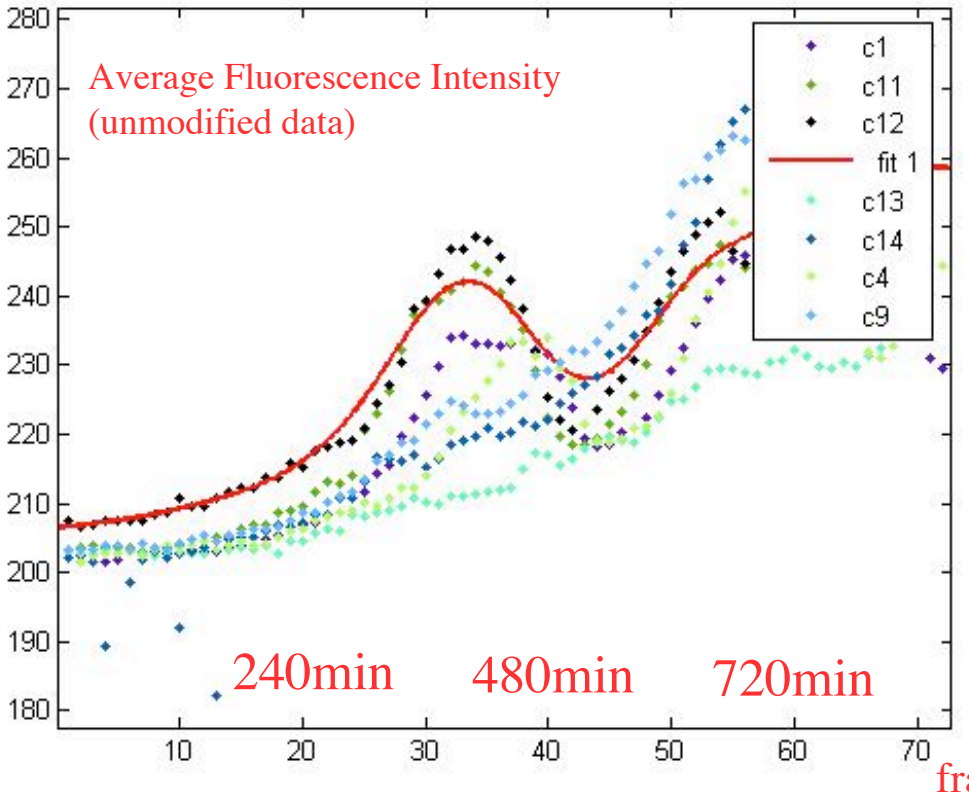
Cells:
•METprCLN2, MYO1-GFP (score division&bud times), CLN2prGFP (reporter)
•compare WT vs cln1,2-.

Design:
• Grow cells with METprCLN2 ON: WT and cln12- uniformly small.
•Move to slide METprCLN2 OFF, follow only budded, G2 cells
•post-bud -> normal Start, CLN2 negative feedback has finished;
•pre-division -> subsequent cell cycle in wt normal, control for transfer.
•Measure time CLN2prGFP first turns on -vs- time of division for each cell, for WT and cln12-
•Check various media

Raw Data

One field of cells on glycerol/ethanol

cln1cln2

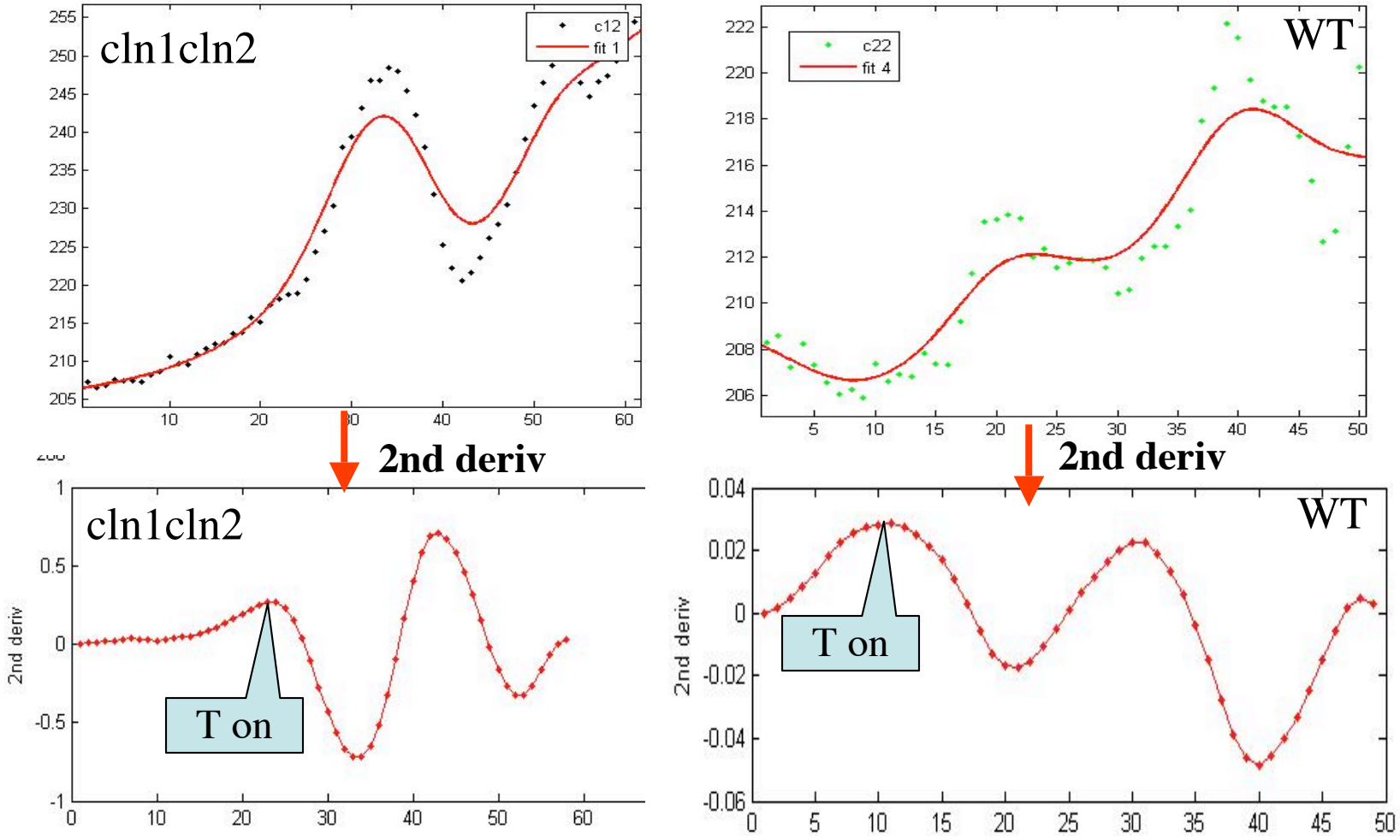


Average Fluorescence Intensity (unmodified data)

One frame = 12 minutes

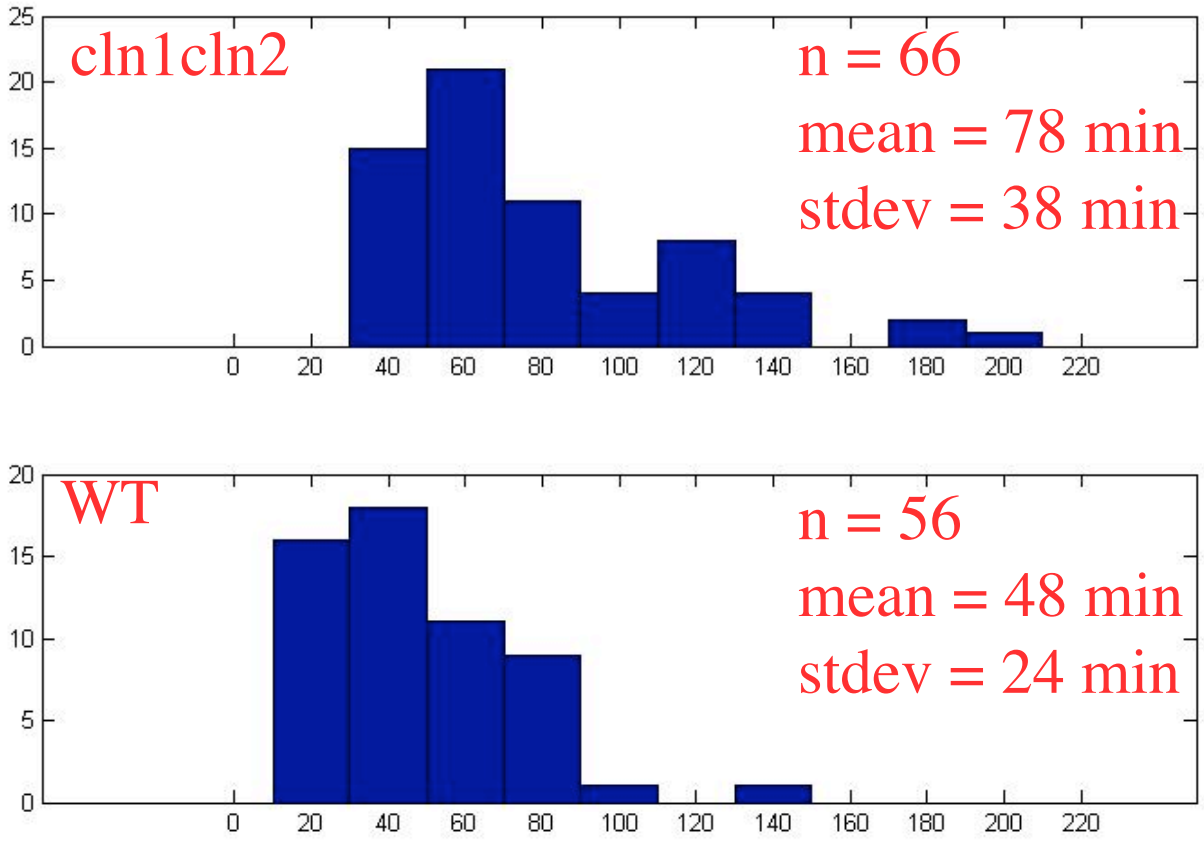
Data processing: smoothing spline + max 2nd deriv

Average cell fluor vs time (12 min pts) in gly-eth



Positive feedback emerges

Glucose histograms T_{on} - T_{div}



Turn-on timing
is not the same
for both distributions

We can conclude
that SBF regulated
genes use positive
feedback

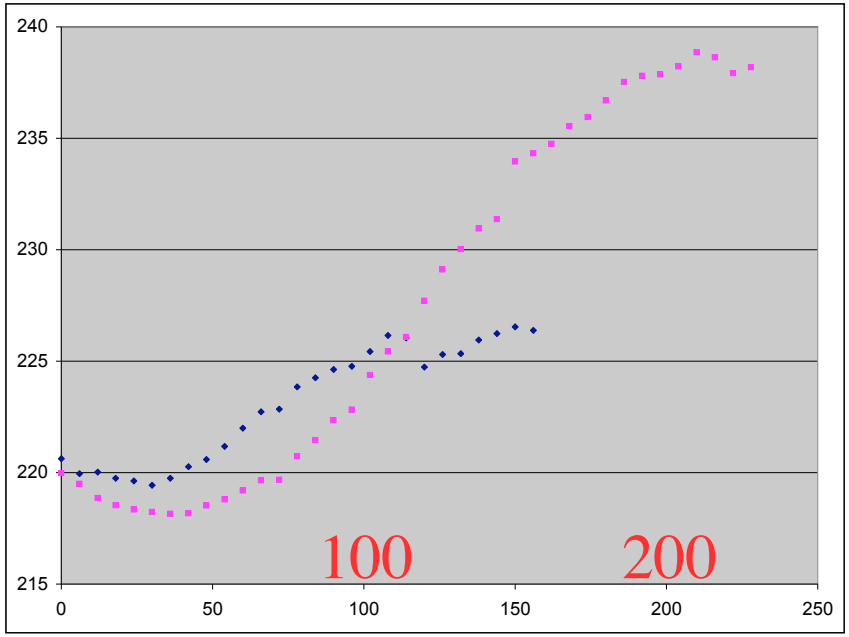
$P < 0.001$ that these points are sampled from the same distribution

Glycerol/Ethanol
slightly larger effect

Hindsight

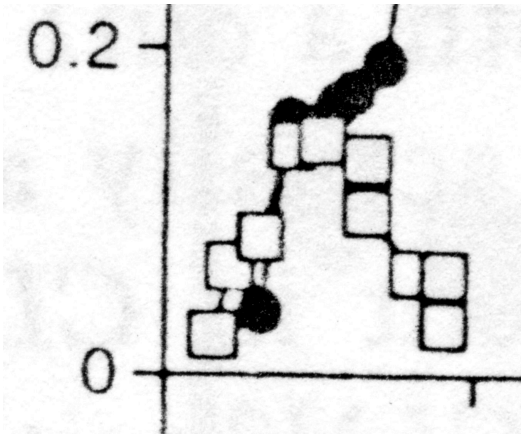
Time series averaged over ~10cells

Avg Fluor. Int.



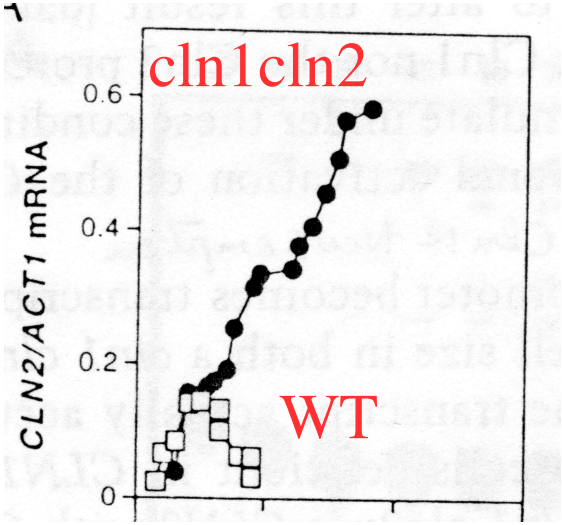
Time (min)

Closeup



Stuart and Wittenberg data revisited - Wt first off the mark

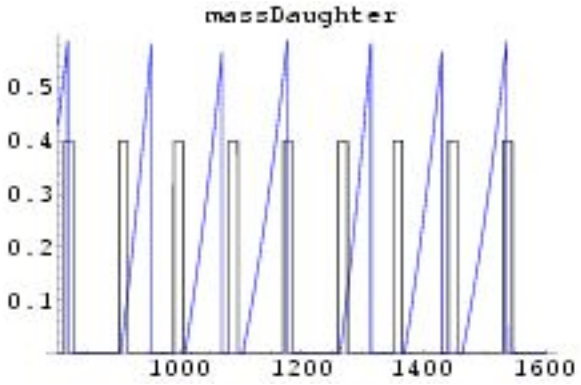
GALprCLN3 and elutriation to start with small cells
Both functional and nonfunctional CLN12 transcript level was measured



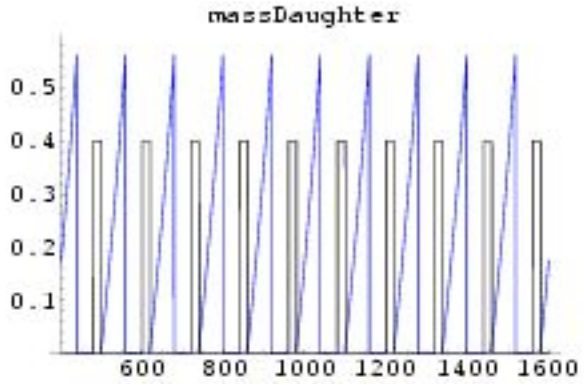
Size

Fantasy: Phase Lock the Cell cycle

Simulate Chen-Tyson 2000 model. Add external pulses of gene (eg Cln3 -black) monitor phase of cell cycle (eg mass-Daughter(time)), for various pulse periods. Cell will **phase-lock** for a range of periods depending on force amplitude.

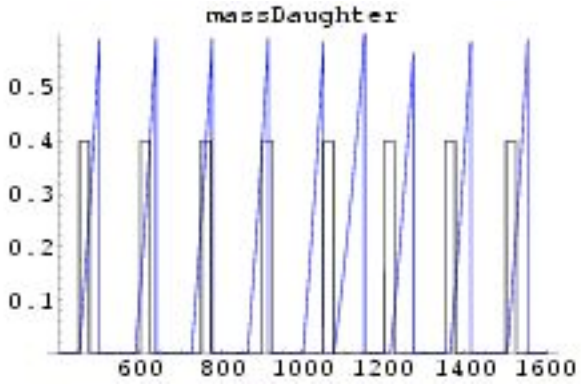


$T_{pulse} << T_{cell}$



$T_{pulse} \sim T^{wt}_{cell}$

$T_{cell} == T_{pulse}$



$T_{pulse} >> T_{cell}$

Results (simulations):

1. Pulsing in Cln3, Cln2 (G1 cyclins) phase locking will advance Start but not delay it (shorten but not lengthen cell period).
2. Pulses of Cdc20 will not phase lock cell cycle (even though this is standard laboratory method to synchronize cells via block-release)
3. Pulses of Clb2 lengthen daughter period (premature division & small size).

Real?? 1&3 plausible, 2 perhaps artifact.
External pulse acts as restoring ‘force’ against noise.

Perspective (ie what's next)

- Single cell measurements give richer phenotype (eg Start mutants)
- Are continuous time systems best description, given level of fluctuations?
- Does the cell 'read' the phase of the cyclin oscillator (eg mitotic exit <-> level of Clb2) or is the 'oscillator' a series of discrete steps (clock vs dominos). Analogies with morphogens and patterning.
- Noise under genetic control, some events limited by few molecules,
- Noise not inherent in size, eg E.coli and S.pombe have more regular cell cycles than S.cer.
- Speculate: asymmetric division allows population level control of size but sloppy cells, symmetric division does not.
- Fitness effects of cell cycle noise (growth in steady environment)

Credits:

Fred Cross (Yeast Genetics)

Jamie Bean

Stefano Ditalia

Gilles Charvin

Jan Skotheim

Ref: Bean-Siggia-Cross *Molecular Cell* Jan '06

Background (History Genetics)

"George Beadle", P. Berg, M. Singer

"History of Genetics", A. Sturtevant

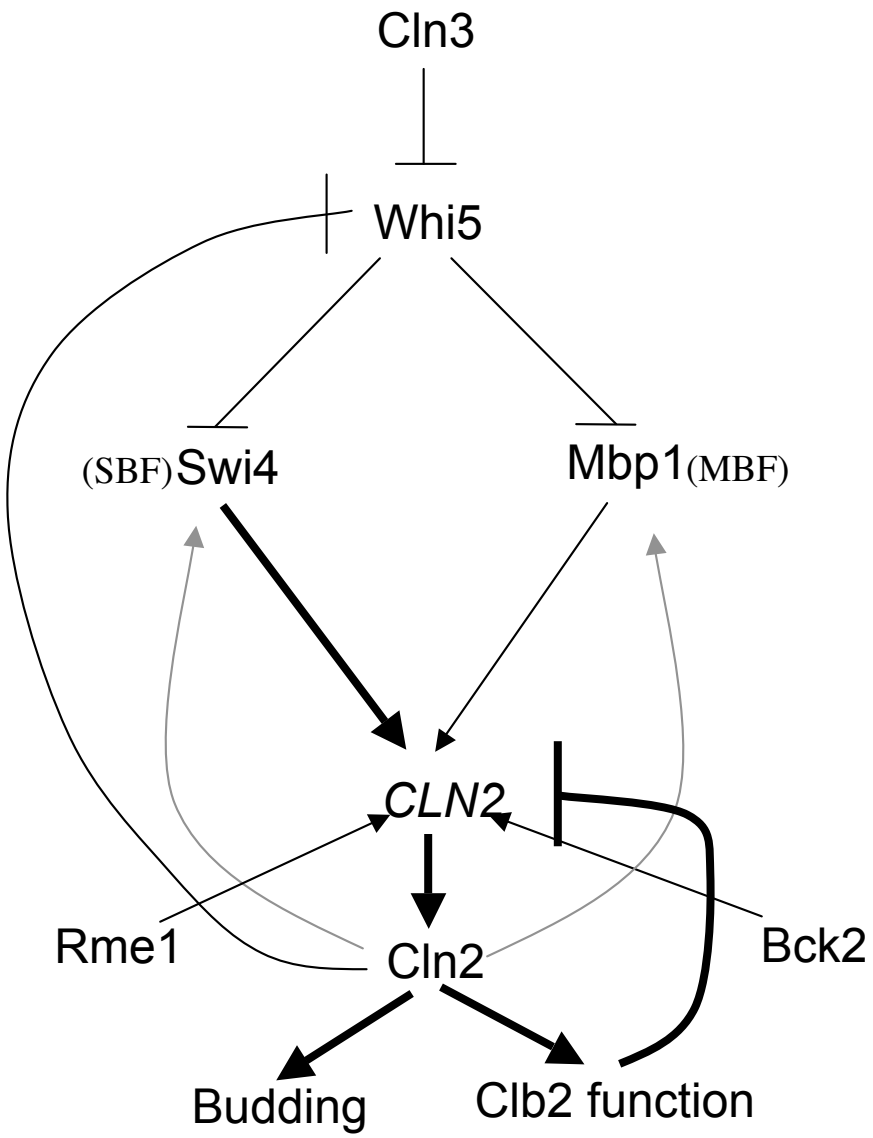
"Phage & Origins of Mol. Bio", Cairns, Stent, Watson..

Biology:

"Cell Cycle..", Dave Morgan

"Cell Growth,.. CSHL press", M. Hall, M.Raff

The Start pathway (complete)



Perspective (old)

- Single cell measurements give richer phenotype (eg Start mutants)
- What does the cell measure at Start, cyclin protein levels, translation rates (eg ribosomes), cumulative phosphorylation…
- Are continuous time systems best description, given level of fluctuations?
- Does the cell 'read' the phase of the cyclin oscillator (eg mitotic exit <-> level of Clb2) or is the 'oscillator' a series of discrete steps (epicycles). Analogies with morphogens and patterning.
- Noise under genetic control, and not inherent in size, eg E.coli and S.pombe have more regular cell cycles than S.cer. Map via scaling of $T_{div\text{-}bud}$ with ploidy.