

Project report

Rotation in the Hauf Lab

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9th Dec, 2015

Outline

Background

The Biology
...and the Beast

Two topics

Order in chromosome segregation
Temporal dynamics of mitotic regulators

Summary

Learnings

The Biology

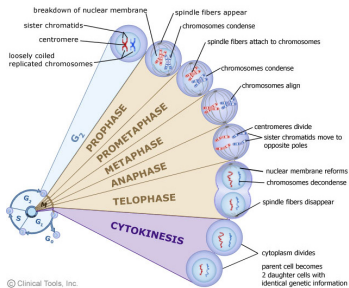
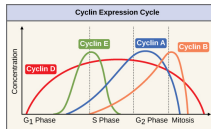


Figure : The cell cycle. Cyclin dynamics through the cell cycle. Source

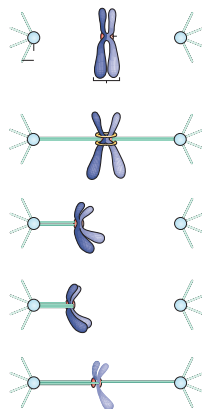


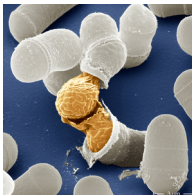
Figure : Source



- But why study it?
 - Basis of life
 - Cancer
 - Growth and development

...and the beast

- *Schizosaccharomyces pombe*



<http://www.hauflab.org/microscopy-images/>

- Why we use it..
 - Growth and maintenance
 - Strain construction
 - Live cell imaging
- ..for studying the cell cycle
 - A single Cdk-1
 - Only 3 chromosomes

Order in chromosome segregation

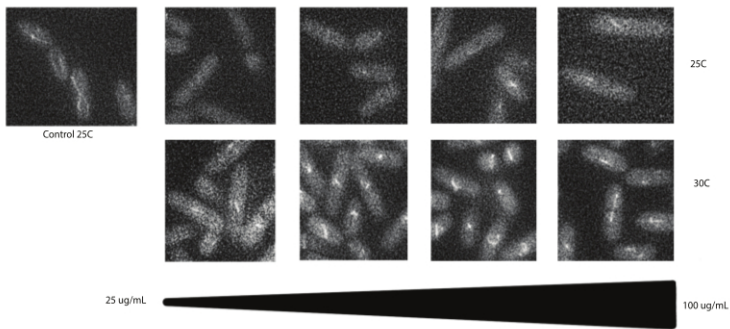
- Motivation

- Julia Kamenz's manuscript [1]
- Possible explanations of order?
- Castagnetti et al (2010) report nuclear division in absence of spindle microtubules [2]
*... during nuclear fission SPBs and sister chromatids separate in absence of spindle microtubules, that **some level of chromosome segregation can take place**,... for efficient nuclear fission functional SPBs and sister chromatid separation is required. [Emphasis mine]*

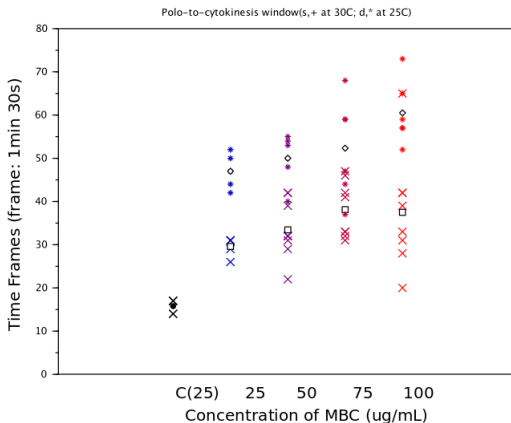
Story 1: MBC, mitosis, microscopy..

To verify observations of Castagnetti et al and to study chromosome segregation in
“nuclear fission”

- Perils of overeager strain construction: *mad3Δ cen2-lacI-GFP*
cen3-tetR-tdTomato/dh1L-tetR-tdTomato
- Controls
 - MBC effect on tubulin
 - Temperature
- Control 1: SK399: *atb2-GFP plo1-mCherry*
- Control 2: SU603/SU604: *mad3Δ plo1-mCherry cen2-GFP*



SK399: atb2-GFP plo1-mCherry



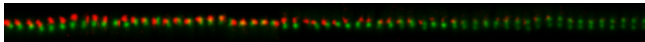
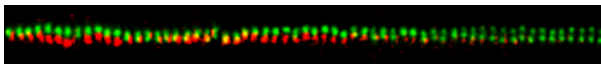
SK399: atb2-GFP plo1-mCherry

To verify observations of Castagnetti et al and to study chromosome segregation in
“nuclear fission”

mad3Δ plo1-mCherry cen2-GFP

cen2 split after observable plo1 split	10
cen2 split during plo1 window (plo1 split not observed)	23
cen2 split after plo1 window	9
non-splitting cen2 in plo1 window	8
cen2 split in absence of plo1	3
Total number observed	53

To verify observations of Castagnetti et al and to study chromosome segregation in “nuclear fission”



Conclusions 1

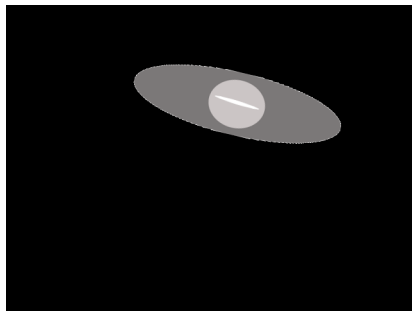
- Observations coincide with that of Castagnetti et al.
- Practical considerations: 3 color filming?
- General take-home: What effect are is really being descibed?

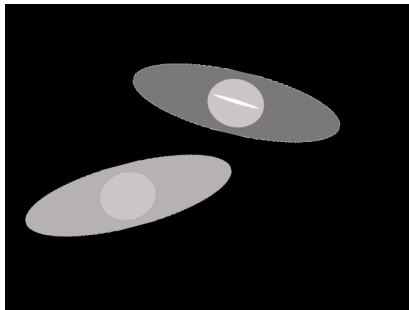
Temporal dynamics of mitotic regulators

- Motivation
 - Cdc13 and the apparent stabilization
 - Automating image analysis
 - Tool to study temporal dynamics from microscopy

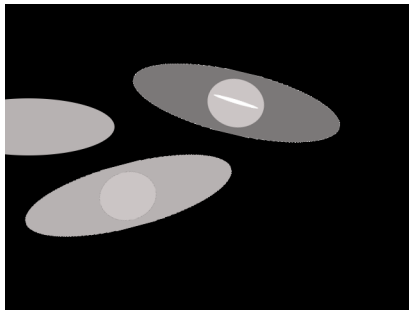
Story 2: ImageJ, automation, insight

To attempt to create an ImageJ based workflow to automate image analysis to obtain temporal dynamics

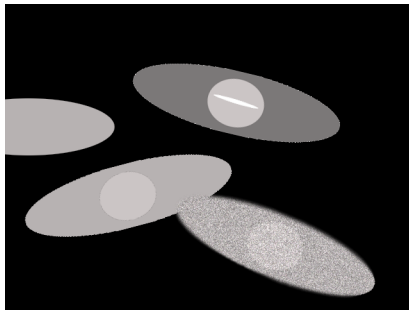




To attempt to create an ImageJ based workflow to automate image analysis to obtain temporal dynamics



To attempt to create an ImageJ based workflow to automate image analysis to obtain temporal dynamics



To attempt to create an ImageJ based workflow to automate image analysis to obtain temporal dynamics

- Workflow

1. IF single cell AND fixed AND no cells enter
2. Transform cells → STEP 1.
3. Threshold ROI OR create Mask
4. "Analyze Particles"
5. "Clean" output
6. Scilab script plots, assembles plot lines.
7. Registration? Segmentation → GOTO STEP 1.

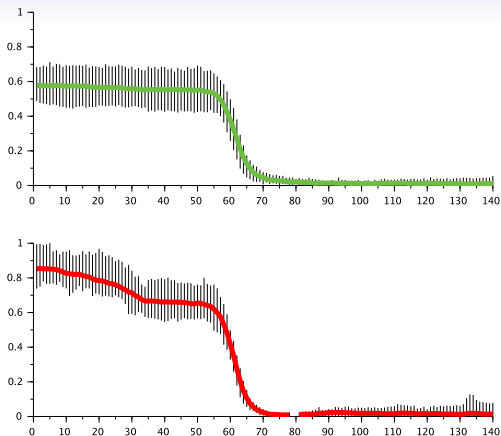


Figure : ST957: *cdc13-sfGFP dh1L-tetR-tdTomato* $T = 15s$

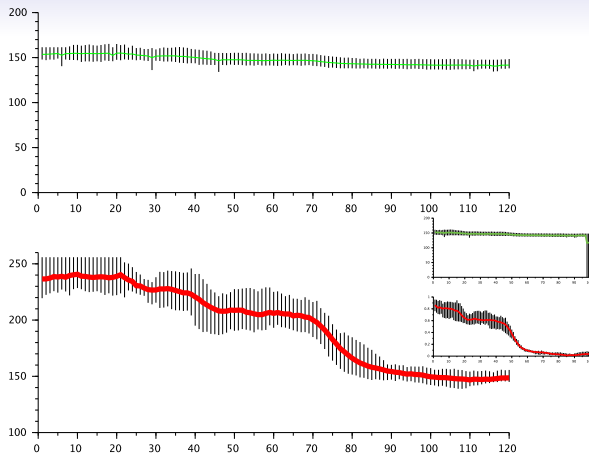


Figure : ST650: *cdc2-GFP dh1L-tetR-tdTomato* $T = 15s$

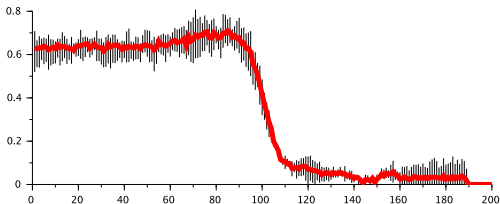
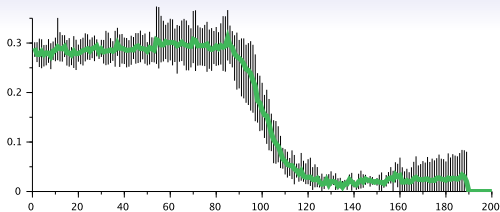


Figure : SL249: *cut2-GFP dh1L-tetR-tdTomato* T = 15s

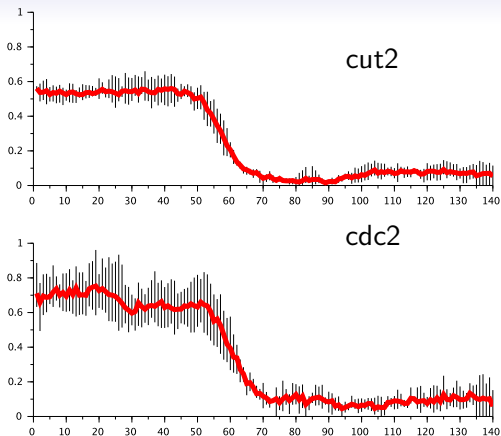


Figure : SU076: *cdc2-mCherry cut2-GFP* T= 15s

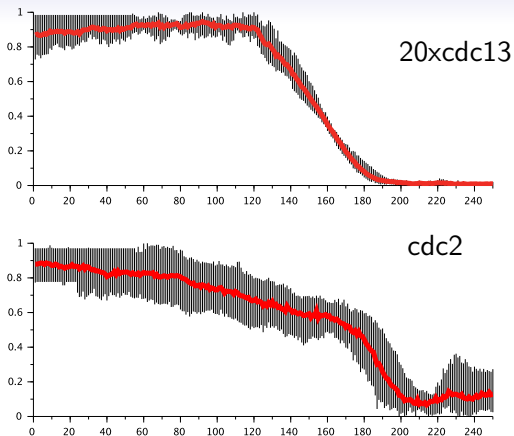


Figure : ST998: *20X-cdc13-sfGFP cdc2-mCherry* T= 15s

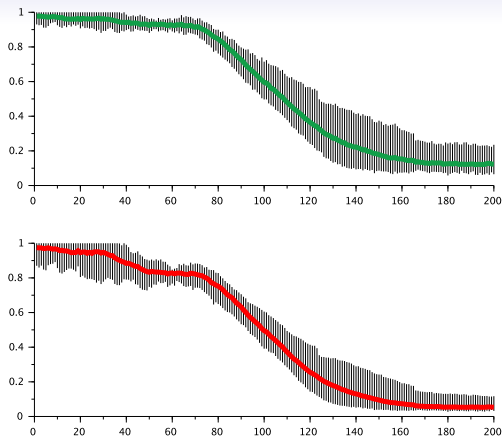


Figure : SU417: *plo1-mCherry 20Xcdc13-sfGFP* $T = 15s$

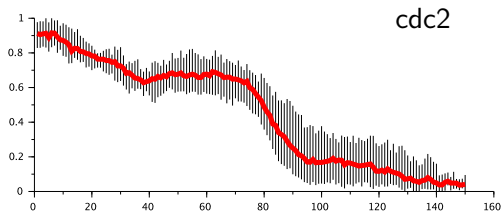
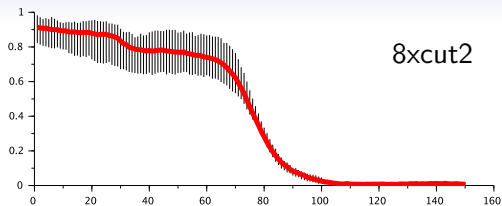


Figure : SU302: *8Xcut2-GFP cdc2-mCherry* T= 15s

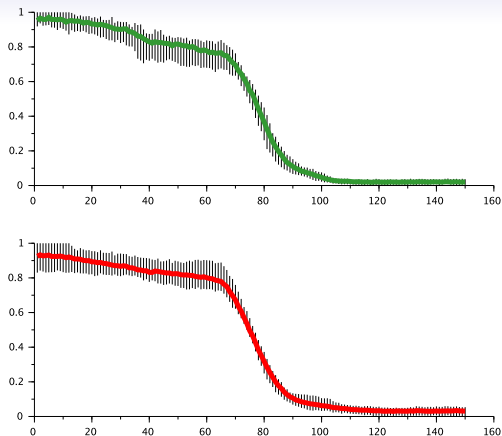


Figure : SU303: *8Xcut2-GFP cdc2-mCherry* T= 15s

Conclusions 2

- Cdc13 and Cdc2 seem to exhibit preferential export from the nucleus on entry into mitosis
- Overexpressing cut2 leads to whole cell degradation?

Summary and Outlook

- MBC treatment experiment inconclusive
 - Other approaches to study centromere separation
 - 3 color imaging?
- Cdc13, cdc2, cut2 dynamics studied
 - Cdc13-GFP cdc2-mCherry analysis/ refilming?
 - Single comparison of all dynamics?

Learnings

- Strain construction
- Live-cell fluorescence microscopy
- Rigorous documentation and data storage
- Shell scripting
- ImageJ Macro language
- Version control for scripts



Kamenz et al.

Synchronous sister chromatid splitting in anaphase occurs without obligatory positive feedback



Castagnetti et al.

Fission yeast cells undergo nuclear division in the absence of spindle microtubules

PLOS Biology, 2010.



Thank you