

# Project report

## Rotation in the Hauf Lab

Amogh Jalihal<sup>1</sup>

<sup>1</sup>Genetics, Bioinformatics and Computational Biology

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# Outline

## Background

The Biology

...and the Beast

## Two topics

Order in chromosome segregation

Temporal dynamics of mitotic regulators

## Summary

## Outlook

# The Biology

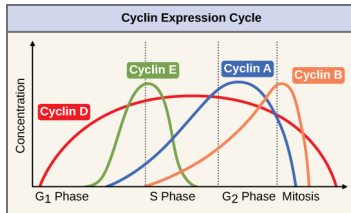
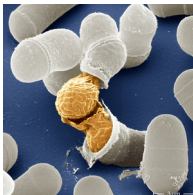


Figure : Cyclin dynamics through the cell cycle. Source

- The cell cycle
  - Driving the cell through the division cycle
  - Regulators and checkpoints
  - Dynamics of molecular players
- 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

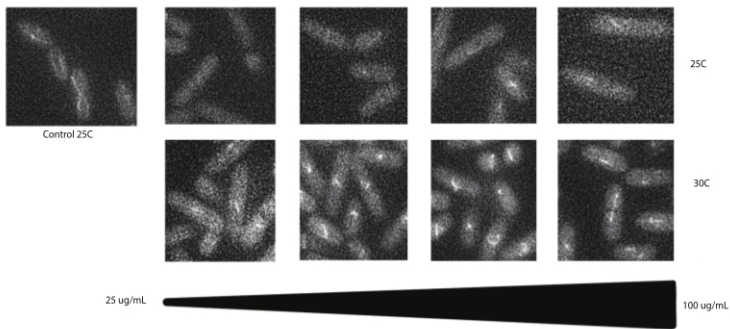
- Julia Kamenz's manuscript [1]
- Possible explanations of order?
- Castagnetti et al (2010) report nuclear division in absence of spindle microtubules [2]  
*... during nuclear fission SPBs and sister chromatids separate in absence of spindle microtubules, that **some level of chromosome segregation 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”

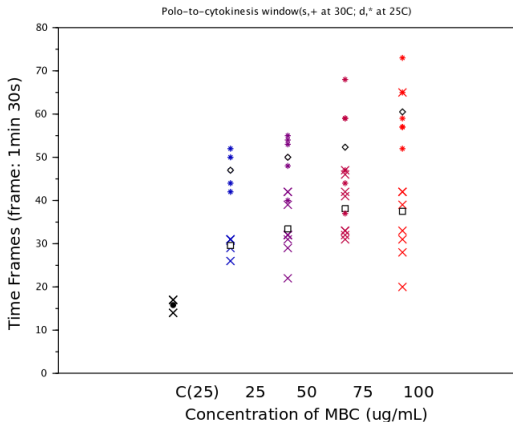
- Perils of overeager strain construction: *mad3Δ cen2-lacI-GFP*  
*cen3-tetR-tdTomato/dh1L-tetR-tdTomato*
- Controls
  - MBC effect on tubulin
  - Temperature
- Control 1: SK399: *atb2-GFP plo1-mCherry*
- Control 2: SU603/SU604: *mad3Δ plo1-mCherry cen2-GFP*

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



SK399: atb2-GFP plo1-mCherry

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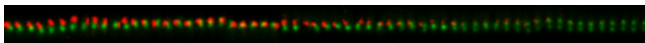
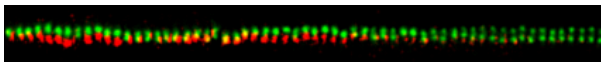


To verify observations of Castagnetti et al and to study chrososome segregation in  
“nuclear fission”

*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 chrososome segregation in  
“nuclear fission”



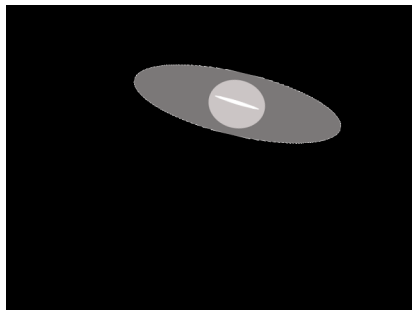
# Conclusions 1

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

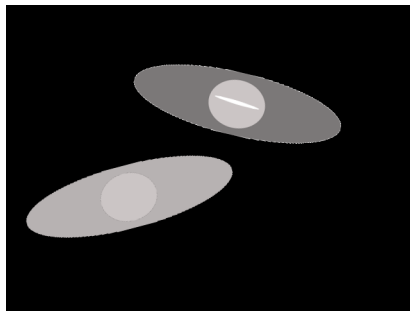
# Temporal dynamics of mitotic regulators

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

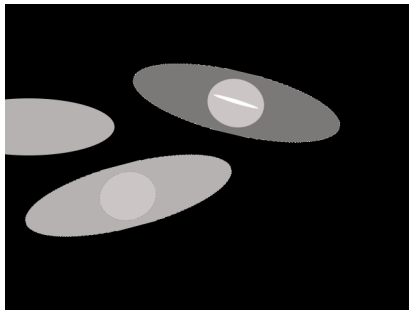
To attempt to create an ImageJ based workflow to automate image analysis to obtain temporal dynamics



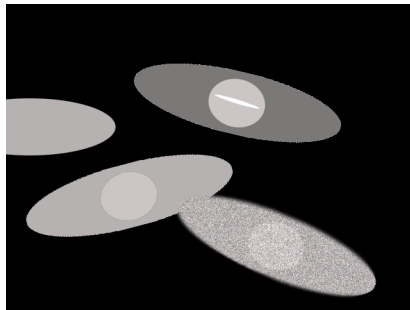
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To attempt to create an ImageJ based workflow to automate image analysis to obtain temporal dynamics

- 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.

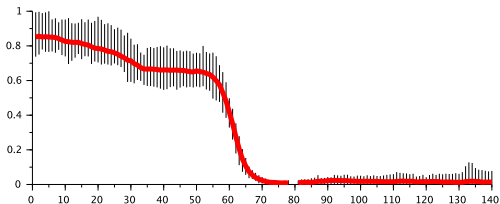
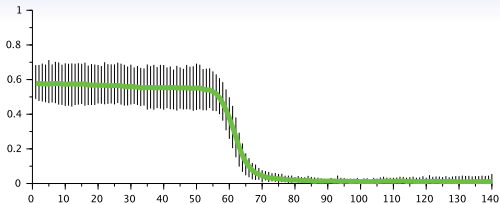


Figure : ST957: *cdc13-sfGFP dh1L-tetR-tdTomato*

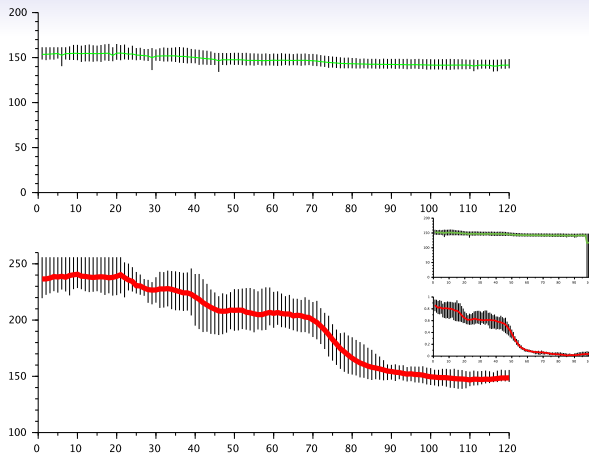


Figure : ST650: *cdc2-GFP dh1L-tetR-tdTomato*

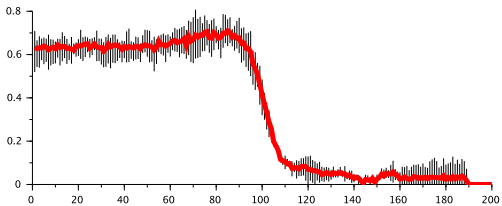
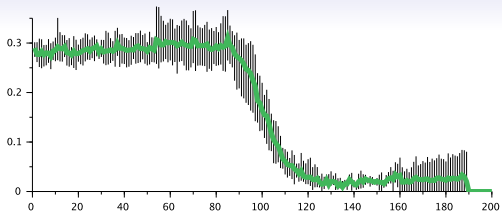


Figure : SL249: *cut2-GFP dh1L-tetR-tdTomato*

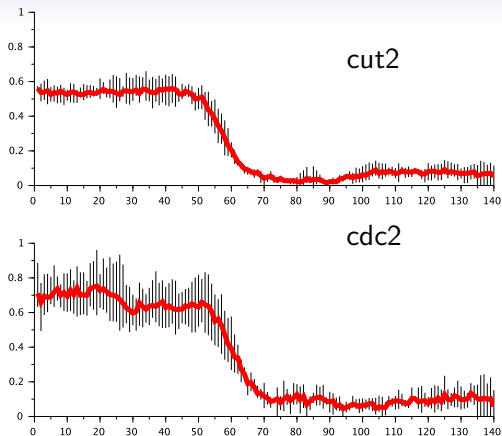


Figure : SU076: *cdc2-mCherry cut2-GFP*

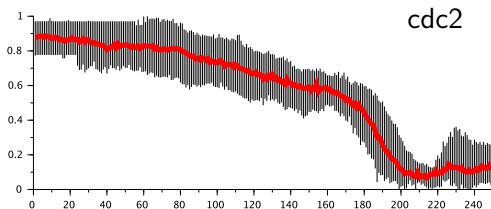
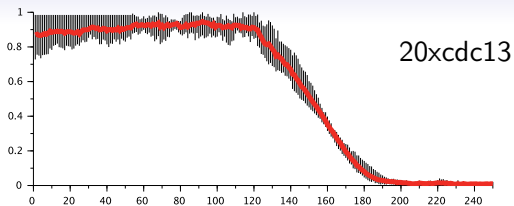


Figure : ST998: *20X-cdc13-sfGFP cdc2-mCherry*

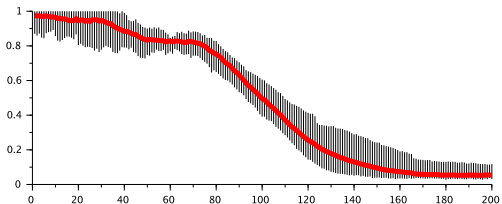
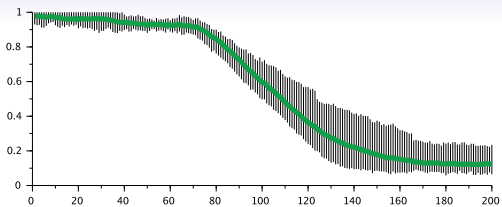


Figure : SU417: *plo1-mCherry 20Xcdc13-sfGFP*

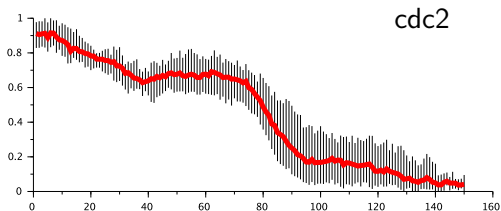
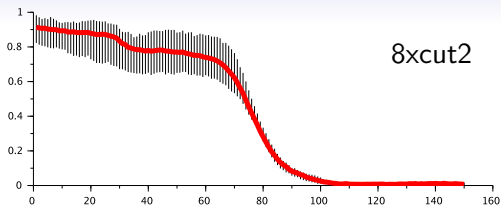


Figure : SU302: *8Xcut2-GFP cdc2-mCherry*



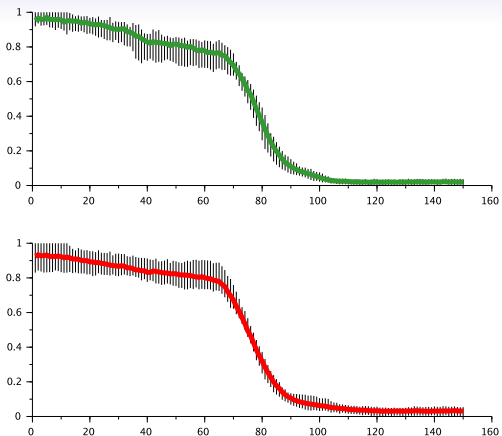


Figure : SU303: *8Xcut2-GFP cdc2-mCherry*

## 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

- MBC treatment experiment inconclusive
- Cdc13, cdc2, cut2 dynamics studied

# Outlook

- Other approaches to study centromere separation
- 3 color imaging?
- Cdc13-GFP cdc2-mCherry analysis/ refilming?
- Single comparison of all dynamics?



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