Meeting with Terry - Wednesday, April 2

5 genes, RNAi treatment for several days
cell cycle = 24 hours

Analysis I

- A. no effect
- B. longer S (incomplete DNA replication) (actually do broad shoulder instead of 2 peaks)
- C. G2/M
- D. G1 arrest
- E. extreme aneuploidy

Analysis II - microscopy looking at DNA by DAPI

Analysis III
protein localization by IF
just look at Mad2 - localizes to kinetochore - possibly in G2, but maybe not until prophase - then comes off at metaphase
Mad2 senses MT attachment

- B -
- E - look like WT in terms of Mad2 (defect in kinetochore but not in microtubule attachment)

Analysis IV
Antibodies

- securin
- cyclin B
- cycin E
- cohesin
- chk1 phosphorylation

- C. M arrest because of separated sisters

would be cleaved cohesin, high cyclin B, no cyclin E, no phosphorylated chk1

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no microscopy, no western blotting

Ask Iain for DAPI and Mad2 images

DAPI & Mad2 - make sure to ask if he has images with separated sisters

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Search for Microscopy Images:

- Mad2 localization resources:

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  - Article 1

UCSF RNAi screen for mitotic spindle assembly in Drosophila S2 cells: http://rnai.ucsf.edu/mitospindlescreen/index.html

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Images needed:

DAPI

1. Some cells in interphase, some in normal mitosis
2. chromosome bridges in mitosis
3. mitotic arrest @ metaphase, other cells with premature sister separation
4. cells in interphase
5. cells in interphase, others with abnormal mitosis with unequal numbers of chromosomes moving to the 2 poles

Mad2 IF

1. Some cells with Mad2 on kinetochores (until metaphase), and some cells with Mad2 off
2. Cells in metaphase with Mad2 on some kinetochores and off others
3. Mad2 on separated sister chromatid kinetochores
4. No Mad2 signal
5. Mad2 off kinetochores

UCSF Database:
Chromosome bridges? - chromosome misalignment

- CG13329/cid

Chromosome misalignment

- CG31258 /CENP-C
- CG5148/Cal1
- CG7242/Ca2/Spc25
- CG9938/Ndc80

Premature sister chromatid separation? - Chromosome misalignment (sister chromatid cohesion proteins)

- CG8598/deco
- CG4008/DRad21 (cohesin)
- CG4029/Jumeau

Normal Metaphase

- Figure 1B?
- Figure 2D - "None" RNAi

- Figure 4A

Aneuploidy

- CG4029/Jumeau - multipolar
- look at cells with >2 spindle poles

Interphase

- Look at Supplemental Figure S3 D -obtain images from this?
- Supplemental Figure 7B CG7242/Cal2? (would alpha-tubulin appear throughout the cytoplasm?)

Questions for Terry

1. Actually 4- color staining in the paper
gamma tubulin (red), alpha tubulin (green), phosphor histone H3 (?), DNA (blue). Are you ok if we ignore the Histone H3 staining?

Other resources:

- FACS profiles of cell cycle phenotypes —> look at Figure 1 from this paper.
- Examples of mitotic phenotypes in this figure.
- Mad2 localization: this figure
- Mad2 not on chromosome during metaphase: here
- Potential Mad 2 and DNA pictures: here, here
- Time courses of Mad2 localization here
- Nice anaphase pictures in S2 cells here
- Premature sister chromatid separation (Identification of Drosophila Mitotic Genes by Combining Co-Expression Analysis and RNA Interference)? here (Figure C) here (Figure A) here (Figure B) here (Figure C)
- Mad2 RNAi in S2 cell here
- Premature sister chromatid separation:
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    - Figure 4 C & D from here
    - UCSF RNAi Screen of securing RNAi knockdown images
    - No securin in *Drosophila* embryo cells from this paper leads to metaphase arrest here.
- Colchicine and MG132 treated S2 cell images here
- APC15 (APC subunit) that mediates Mitotic Check Point coming off the chromosomes from here and here
- Mad2 western with and without nocodazone here
- Images of chromosome bridges here