A multiplex F1 RNAi screen for defects in Drosophila female meiosis

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1. Abstract:

Traditional genetic screens to identify genes required for meiosis induce mutations, make mutated chromosomes homozygous, and then examine progeny of mutant females for evidence of chromosome segregation errors. This requires that mutant females be viable and fertile, and will therefore miss any meiotic genes that are required to produce viable offspring. Our lab is currently screening the germline-specific VALIUM22 collection that was produced by the Harvard TRiP Project, which contains RNAi constructs targeting genes known to be expressed in the germline. By driving RNAi only in the germline, we can test genes that would be lethal if knocked down in all cells, and by examining unfertilized metaphase-arrested mature oocytes by confocal microscopy, we can identify genes even if they would result in sterility.

We are screening this collection to identify genes that disrupt either of two phenotypes: the ability of meiotic chromosomes to undergo congression to a single mass at the end of prometaphase, and the structure of cytological filaments that become heavily decorated with Mps1-GFP during acute hypoxia. As of the time of this meeting, we have tested ~1400 out of the ~1500 lines in the VALIUM22 collection, and have obtained multiple hits for both phenotypes, including several genes that were not known to play a role in meiosis as well as finding the first phenotypes to be associated with multiple previously uncharacterized genes.

2. Experimental Approach:

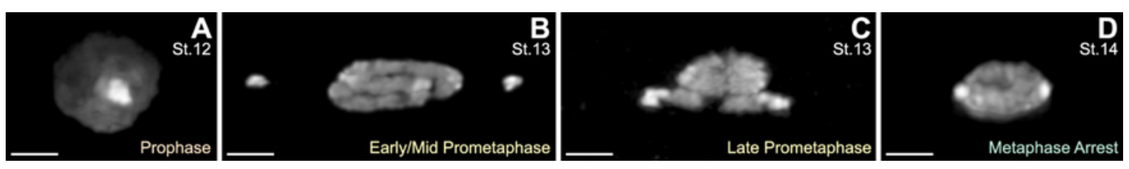
Leverage collection of RNAi construct lines from TRiP
- Cross males from each line to tester stock virgins
  - (Genotype FM7; P{mps1-GFP}; P{nos::Gal4}; pol)
- Brings in Gal4 driver to induce RNAi
- Other items (FM7, mps1-GFP) needed to test phenotypes
- Dissect, fix and examine ovaries of F1 females

Advantages:
- Experimental females are F1s -- minimize effort
- RNAi only driven in germline — can test lethals
- Scoring in mature oocytes — can test steriles
- Identity of any hits known immediately

Disadvantages:
- RNAi is only knockdown, not knockout
- RNAi can have off-target effects

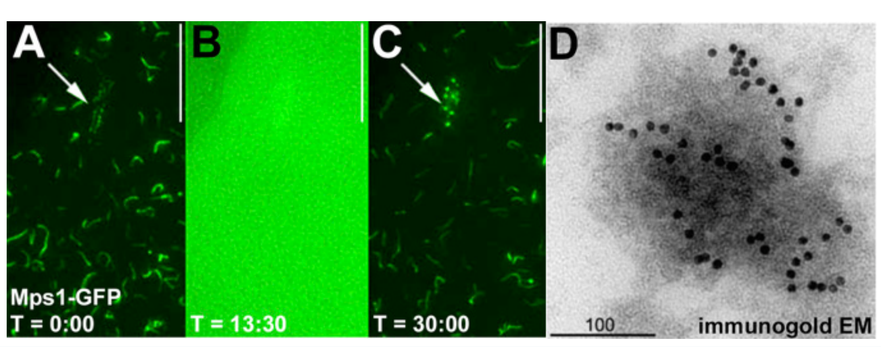
3. Phenotypes to Test For:

Congression:
- At end of prometaphase, meiotic chromosomes move to meta phase plate and form a single mass



- Congression failure results in multiple masses at metaphase arrest

MPS1-binding filaments:
- Mps1-GFP is sequestered to filaments during hypoxia. What proteins build the scaffold?



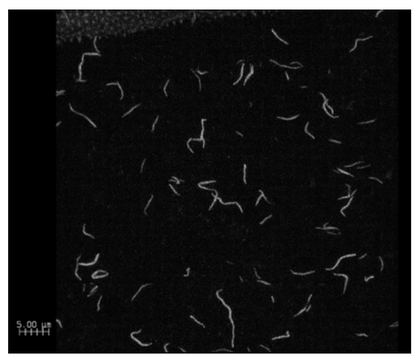
4. Results: Lines Tested

We have been delayed because of the coronavirus shutdown, but as of poster submission, of the ~1500 VALIUM22 lines, we have
- 1183 lines tested and found not interesting
- ~140 lines that still need to be ordered
- ~100 lines that have been dissected and await scoring
- 18 lines excluded as untestable (no mature ovaries)
- 47 lines excluded as duplicates (2nd constructs, only 1 tested)

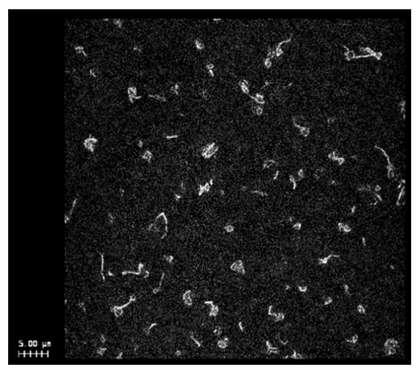
We have identified around 40-50 hits, including known meiotic genes, CG genes, and genes not previously known to be required in meiosis. We'd tell you what they all were but I have to work from home because of the plague and I don't have them all memorized. Sorry.

5. Results: Some Interesting Hits

• RNAi vs bin1 (Bicoid Interacting Protein 1) causes shorter, straighter filaments than in normal females
• Bicoid establishes A-P axis during oogenesis
•Filaments formation triggered by wave from posterior to anterior at GVBD (PLoS One 2009)



• RNAi vs nuf2 forms twisty "balls" instead of longer filaments
•Nuf2 homolog found in similar filaments in in C. elegans oocytes (Monen et al 2005)



• RNAi vs mustard (mtd) causes congression failure by reducing recombiantion rates by 95% (confirmed by 2nd RNAi construct)
• Essential gene not previously known to be in meiosis
• mtd is involved in IMD pathway to protect against gram-negative bacteria, and infection is known to increase recombination rates

6. Conclusions

If we ever get a chance to get back in the lab, and finish this screen, it will have worked quite well. We had around an 8% dud rate, a 2% hit rate, we'll get to name several new CG genes that have never been characterized, and the idea would have been finished within the 3-year schedule except that the world is ending and this all feels rather pointless.

So instead have a nice picture of a puppy. His name is Jupiter, he is a Swedish Vallhund, and he is four months old. We got him about 3 days before the governor ordered us to shelter in place, and so while I have been stuck at home and unable to finish this project, or go to a conference I really wanted to attend, at least I get to play with a puppy every day, so it's not completely bad.

But I wish he was housebroken.