Investigating the Predicted Enzymatic Activity of *Asteroid* in *Drosophila* Oogenesis and DNA Repair

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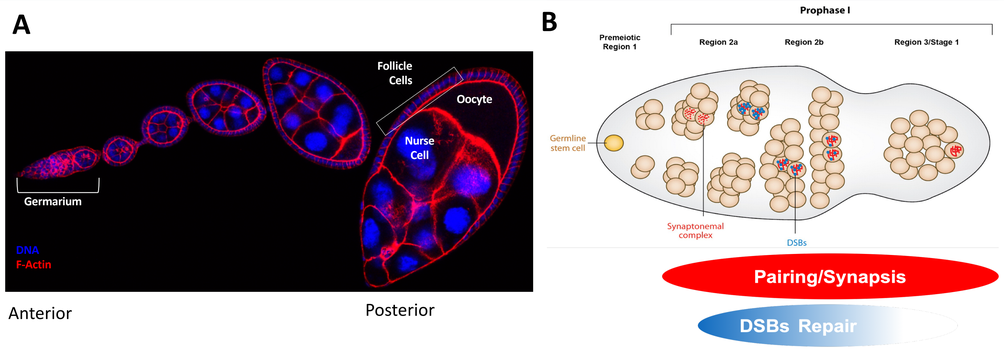
Abstract

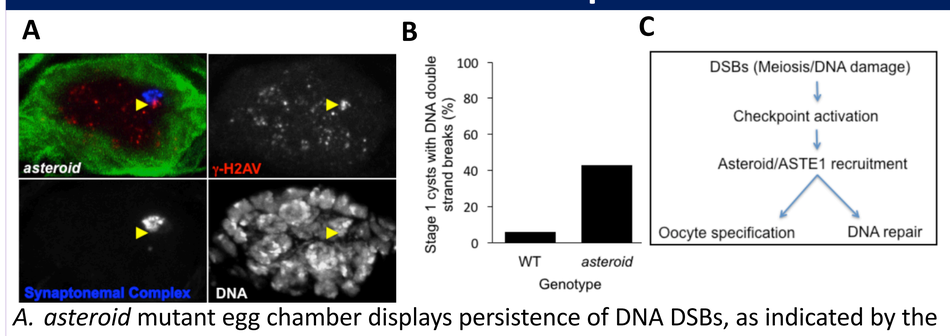
The intricate process by which gametes are formed from the germline stem cells is a fundamental question in biology. In *Drosophila*, oogenesis, begins by asymmetric division of the germline stem cells, and ultimately produces a cyst of 16 cells surrounded by a layer of somatic cells. One of these 16 cells is selected as the oocyte, the future egg, while the remaining become supporting cells. A genetic screen in *Drosophila* identified mutations in several evolutionarily conserved genes that result in a failure of oocyte fate determination, leading to loss of mature eggs and fertility. Strikingly, when the germline cells are mutant for *asteroid* (*ast*), one of the genes identified in this screen, the resulting cysts contain no oocyte. Further characterization of *asteroid* mutants revealed a persistence of double-stranded DNA breaks (DSBs) during meiosis. Interestingly, the protein encoded by *ast* and its human ortholog (ASTE1) both contain XPG domains, suggesting they are nucleases involved in DNA repair. Additionally, ASTE1 is mutated in a subset of patients with colorectal cancers, although its molecular function is unknown. To study the role of Asteroid *in vivo* and *in vitro*, biochemical and genetic experiments are currently underway. These studies aim to address the necessity of Ast's predicted nuclease domain during *Drosophila* oogenesis, as well as to examine the enzymatic activity of Asteroid and ASTE1 *in vitro*. Further investigation of asteroid and ASTE1 will shed much needed light on oocyte fate determination, as well as their roles in DNA repair, and possibly cancer.

Introduction



A. Each ovariole is a chain of increasingly more mature egg chambers. An egg chamber contains 16 germline cells, 15 nurse cells and one oocyte, which are encapsulated by somatic epithelial cells.
B. To allow recombination during meiosis I, Synaptonemal Complexes (SCs) form between homologous chromosomes in the two pro-oocytes. This is followed by the formation of DNA double-strand breaks (DSBs), which are subsequently repaired. Oocyte selection occurs at or around the time of DSB repair. Adapted from Lake and Hawley, 2012.

asteroid mutant clones show persistent DNA DSBs



A. asteroid mutant egg chamber displays persistence of DNA DSBs, as indicated by the presence of DSB marker γ-H2AV (arrowhead). Synaptonemal Complex marks the oocyte.
B. Quantification of DSBs in *asteroid* mutant clones compared to wildtype (WT).
C. Proposed model for Asteroid function during oocyte determination and DNA repair.

Drosophila asteroid encodes ortholog of human ASTE1

B Comparison of Drosophila Asteroid and Human ASTE1

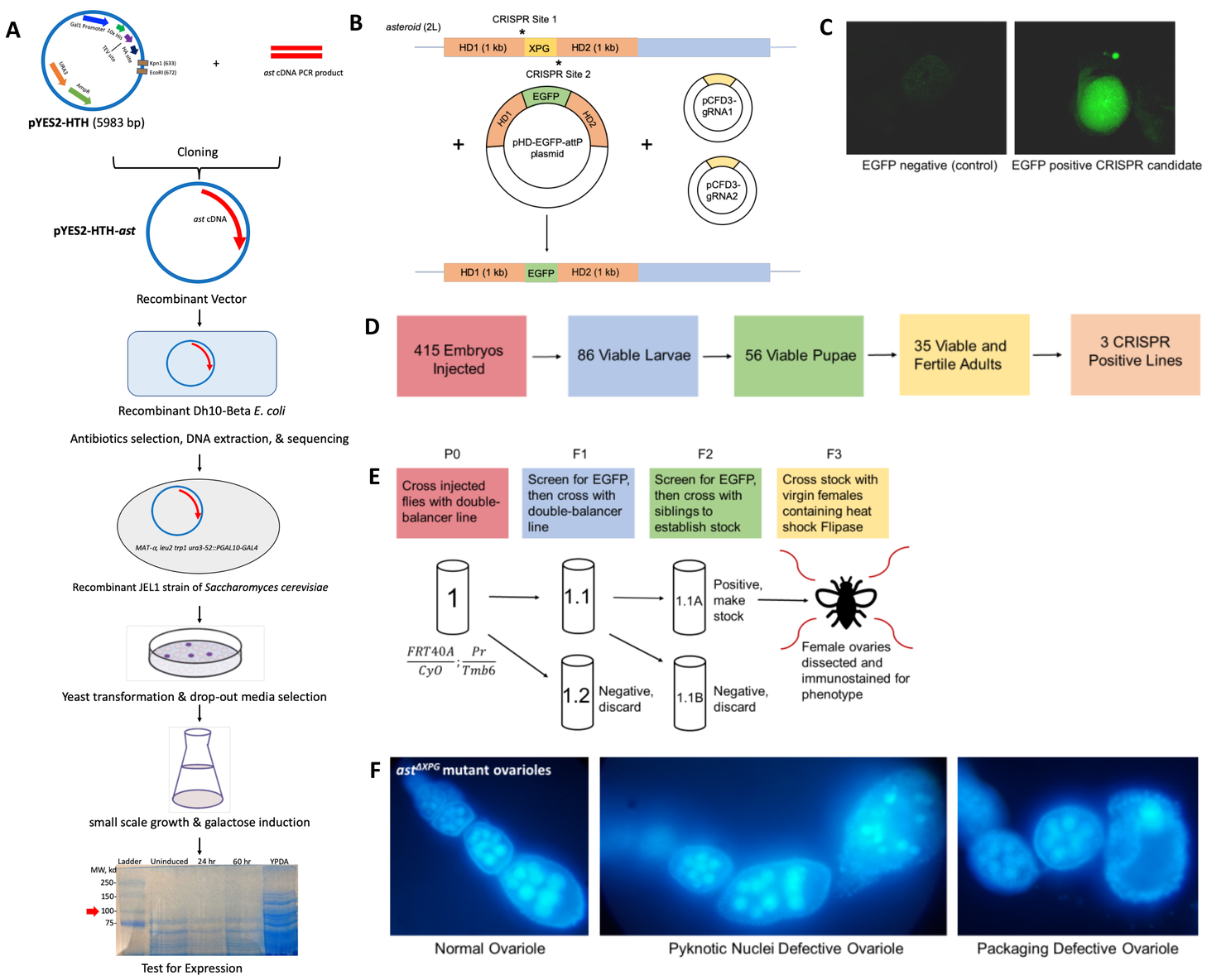
- 43% similar & 28% identical
- Contain XPG domains
- Predicted nuclease activity
- Role in DNA repair?
  - XPG domains associated with base excision repair (BER) & mismatch repair (MMR)

C Human ASTE1 is linked to cancer

- 80% of colorectal tumors with microsatellite instability had mutations in *ASTE1*.
- *ASTE1* may be linked to as many as 12% of colon cancers.

A. Protein alignment of *Drosophila* Asteroid (Ast) and its human ortholog ASTE1. The point mutation for *asteroid* allele identified in our screen is indicated (*, A670T). Ast/ASTE1 contain predicted XPG domains (light blue).
B. Comparison of *Drosophila* Asteroid and Human ASTE1 proteins.
C. Human ASTE1 is mutated in 80% of colorectal tumors with microsatellite instability. Tourgeron, et al., *Modern Pathology*, 2009.

Results



A. Heterologous expression of *Drosophila* Ast in *S. cerevisiae*. HA-10xHis-Asteroid (~100 kDa; red asterisk) expression detected by SDS-PAGE, followed by Coomassie blue staining. Asteroid protein expression is not detected.
B. Generation of $ast^{\Delta XPG}$ mutant allele. CRISPR sites were identified on either side of the XPG-encoding region and pCFD3 plasmids encoding gRNAs targeting these sites were generated (CRISPR sites, *). A pHD-EGFP plasmid was designed with 1 kb homology domains (HD) flanking EGFP and recognized the *asteroid* region for homology-directed repair. CRISPR and homology domain plasmids were introduced by embryo injection by Rainbow Transgenics.
C. A control fly eye negative for EGFP (left) and a fly eye positive for EGFP (right). EGFP+ flies were used to generate stocks of candidate $ast^{\Delta XPG}$ alleles.
D. Process of obtaining EGFP+ CRISPR candidate lines from injected embryos. In total, 415 embryos were injected with the CRISPR gRNA plasmids and HDR donor plasmid by Rainbow Transgenics, Inc. Of those, only 86 larvae survived. Of the surviving larvae, 35 adults were viable and fertile and used to established independent lines. Many of these lines were not EGFP positive and only 3 EGFP+ CRISPR candidate lines were produced and were confirmed by DNA sequencing.
E. Progeny of the injected flies were screened for EGFP. Positive flies were crossed with a double-balancer line (P0). EGFP eyes were screened in the F1 and F2 crosses to establish stocks. Progeny of the F2 cross were crossed with virgin females containing heat shock-activated flipase to produce mutant clones. Ovaries were then dissected and immunostained and are currently being examined for mutant mosaic phenotypes.
F. DNA staining of ovarioles from the EGFP+ $ast^{\Delta XPG}$ candidate lines. Most ovarioles are phenotypically normal (left), while others exhibit a variety of defects, including pyknotic nuclear, likely indicative of cell death (middle), and packaging defects (right).

Predicted Results

Asteroid cleaves single- or double-stranded DNA.

- Asteroid is predicted to cleave DNA on the 3' site of a damaged site. In XPG domain-containing proteins, this cleavage excises an oligonucleotide consisting of approximately 30 bases.

XPG domain is responsible for DNA repair and oocyte selection

- $ast^{\Delta XPG}$ mutant clones are predicted to exhibit loss of oocyte identity and persistent DNA damage.

Aims & Future Directions

Aim 1. Choose an appropriate host for heterologous Asteroid protein expression and purification.

Aim 2. Examination of Asteroid's role in DNA repair during *Drosophila* oogenesis

A. Determine if Asteroid has a role in the DNA damage checkpoint during *Drosophila* oogenesis.
B. Test for genetic interactions between *asteroid* and other DSB formation/repair genes.

Aim 3. Elucidate the role(s) of human ASTE1 during cancer progression

A. Investigation of ASTE1's predicted nuclease function in DNA repair
B. Use UAS-hASTE1 transgenic line in *Drosophila* to confirm that human ASTE1 is the ortholog of Asteroid.

Acknowledgements

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