Functional characterization of *atlas*, a putative *de novo* evolved gene essential for *Drosophila* male fertility

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Background

***De novo* genes**

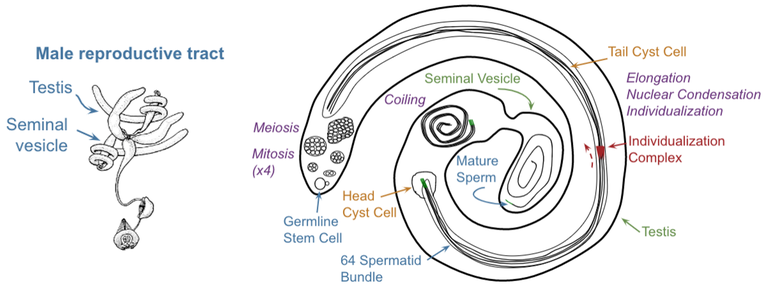
De novo genes evolve from previously non-coding DNA through mutations that produce an open reading frame and a functional promoter [1]. Previous studies have shown that *de novo* genes can quickly become integral to an organism's fitness [2]. Many of these *de novo* genes exhibit testis-biased expression, suggesting that they may play roles in male fertility. However, the functions of most of these genes remain largely unknown.

Using testis-specific RNAi knockdown (KD) of putative *de novo* genes, we have identified several testis-expressed *de novo* genes that contribute to male fertility [3]. Males knocked down for one such gene, *atlas*, were nearly completely sterile. We have sought to characterize the function *atlas* plays in *Drosophila melanogaster* reproduction and spermatogenesis.

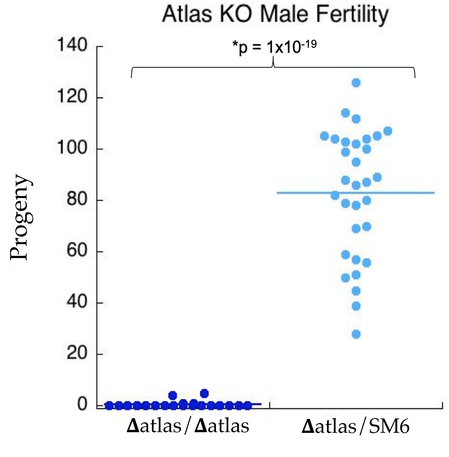
CRISPR/Cas9-mediated *atlas* knockout (KO)

Because low levels of *atlas* transcript remained detectable in KD males, we used CRISPR/Cas9 to create a null allele. Using guide RNAs that targeted either side of *atlas*, we deleted the gene so that there would be no protein expression in flies that were homozygous for the mutation.

***Drosophila melanogaster* spermatogenesis**

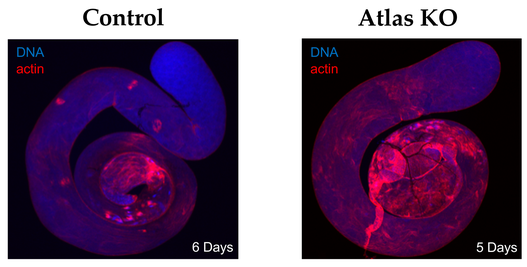


Atlas is essential for male fertility



Atlas functions in late spermatogenesis

We used phase microscopy to visualize *atlas* KO male reproductive tracts. We observed that few sperm in KO males make it to the seminal vesicle. Instead, we saw the progressive accumulation of sperm tails in the basal end of the testis (arrows), suggesting that *atlas* plays a role in post-meiotic spermatogenesis.

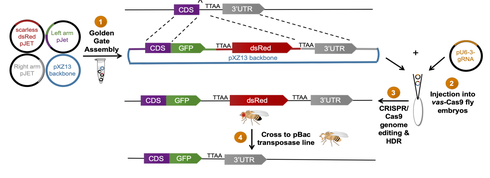


We stained testes for actin and imaged them using confocal microscopy to study the investment complexes (ICs) that individualize the spermatid bundles. In *atlas* KO males, we observed a lack of ICs progressing along the length of the testis.

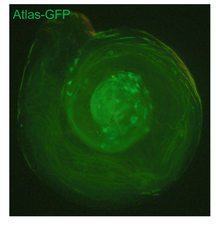
Visualization of Atlas protein with scarless CRISPR knock-in

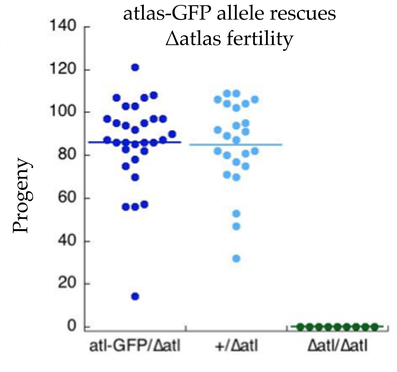
Endogenous Expression

We used CRISPR/Cas9-mediated scarless genome editing to tag atlas C-terminally with GFP at its endogenous locus [4]. We removed the dsRed marker from flies that expressed both the atlas-GFP and dsRed and then sequenced the *atlas* locus to verify the expected final insertion at the correct genomic location.

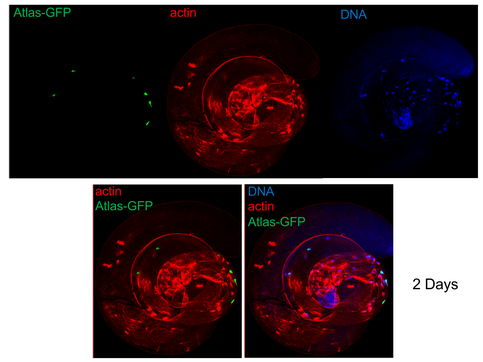


Visualization of Atlas-GFP in whole-mount testes.



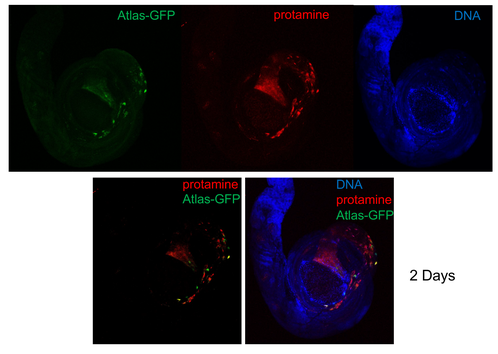


Atlas does not localize with investment complexes



We stained homozygous atlas-GFP males with Phalloidin to observe the localization of the Atlas protein relative to the ICs to investigate the phenotype previously observed. Atlas does not appear to localize with the ICs in the basal end of the testis.

Atlas co-localizes only partially with protamines



We crossed our *atlas*-GFP line with flies containing a protamine-dsRed marker [5] to study the temporal and spatial localization of atlas relative to protamine proteins used in the final stages of spermatid nuclear condensation. The observed Atlas-GFP pattern resembled condensing nuclear bundles, but Atlas-GFP overlapped only partially with protamine-dsRed. These data suggest that Atlas may act as a transition protein in nuclear condensation that facilitates the replacement of histones with protamines [6].

Acknowledgments

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