Drosophila Phosducin-like Protein 3 Regulates Spermatogenesis

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Phosducin-like Protein 3 (Phlp3)

- CG4511 encodes *Drosophila melanogaster Phlp3*
- Thioredoxin-domain containing protein
- Hypothesized to function as a member of the Chaperonin-Containing T (CCT) Complex, which promotes folding of actin and tubulin
- *C. elegans* homolog required for tubulin acetylation and microtubule formation
- Highly expressed in *Drosophila* ovaries and testis
- Mainly expressed in germ cells throughout the testis

Fertility Crosses with *Phlp3*$^{-/-}$ Males

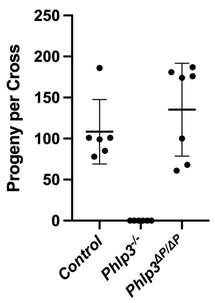


Figure 2: *Phlp3*$^{-/-}$ males are sterile. Homozygous *Phlp3*$^{-/-}$ males crossed to control females did not produce any progeny, indicating *Phlp3*$^{-/-}$ males are sterile. Excision of the P element restores fertility.

Phlp3$^{-/-}$ Mutants

Figure 3: Gene structure of *Phlp3*. The gene structure of *Phlp3* is shown. The orientation is 5' (left) to 3' (right). Gray boxes indicate the 5' and 3' untranslated regions. Black boxes indicated the coding parts of the exons. The arrowhead indicates the location of the P element insertion ($E\{Pgy2\}^{EY13373}$).

Phlp3 P-Element Insertion Results in *Phlp3* Downregulation

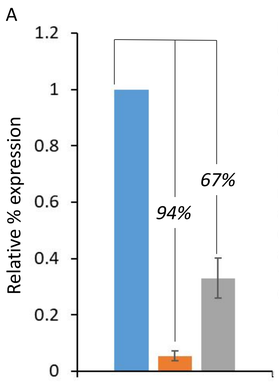
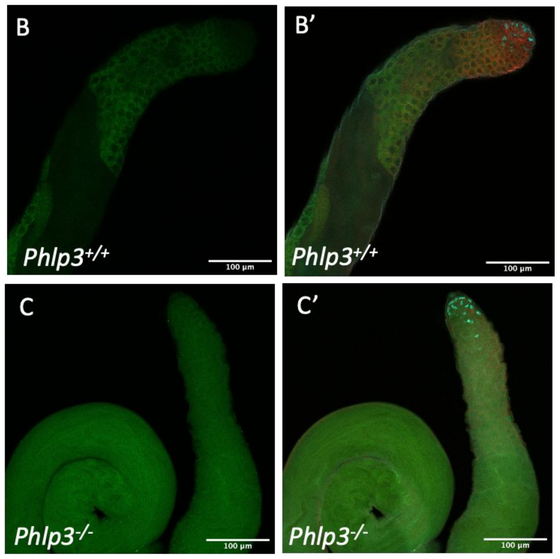


Figure 4: Expression of *Phlp3*. A) qRT-PCR shows downregulation of *Phlp3* expression in testes from males homozygous for the P-element insertion in *Phlp3 (-/-)*, compared to wild-type (+/+) levels. Excision of the P-element (ΔP/ΔP) partially-rescues *Phlp3* expression. (Data from previous undergraduates Michaela Marra and Elizabeth Kojak, Kanzok lab). B-C) Fluorescent *in situ* hybridization for *Phlp3 (green)* reveals expression in germ cells *in Phlp3*$^{+/+}$ testes (B) and loss of expression in *Phlp3*$^{-/-}$ testes (C). Testes co-stained with anti-Vasa (red) to label germ cells and anti-Tj (cyan) to label somatic cyst cells. Scale bar is 100 µm. (Data from previous undergraduate Claire Chaikin, Mierisch Lab).



Drosophila Spermatogenesis

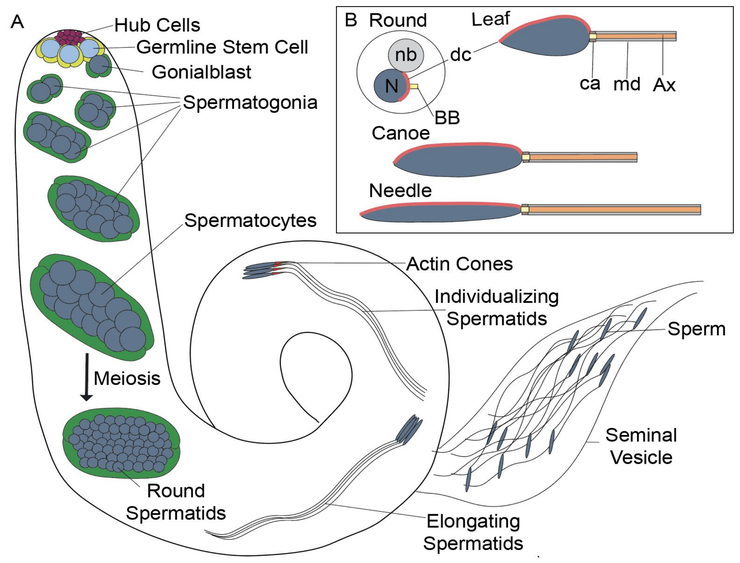


Figure 1: *Drosophila* spermatogenesis. A) Stages of *Drosophila* spermatogenesis. Cyst stem cells (light green), somatic cyst cells (dark green). B) Stages of spermatid maturation: Round stage to leaf stage to canoe stage to needle stage. N: nucleus, nb: nebenkern; BB: basal body; ca: centriolar adjunct, dc: dense complex, mitochondrial derivative (md) and Ax: axoneme.

Arrest and Rescue of Spermiogenesis

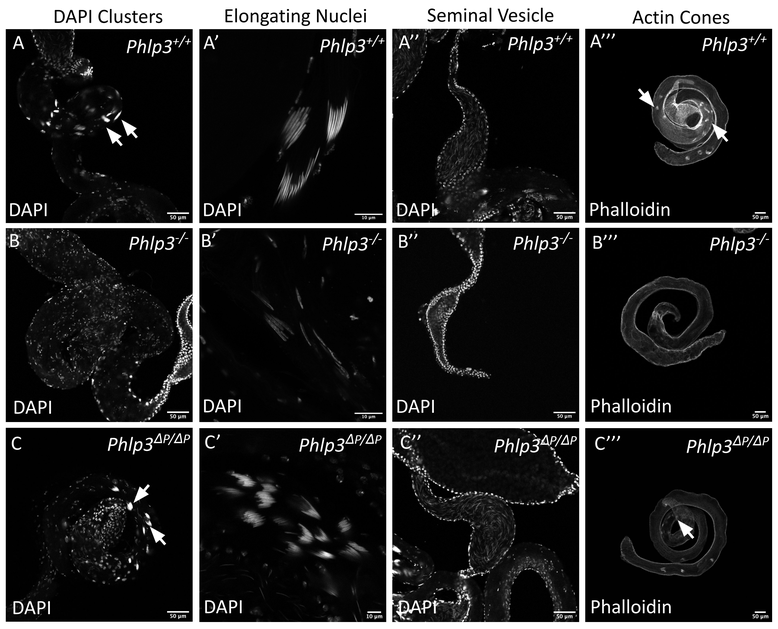


Figure 5: Mutation of *Phlp3* causes arrest of spermatogenesis. A) *Phlp3*$^{+/+}$ testes have DAPI clusters in the distal testes, elongating nuclei, large seminal vesicles with mature sperm, and actin cones. B) *Phlp3*$^{-/-}$ testes have dispersed DAPI clusters, less elongated nuclei, smaller seminal vesicles, and no actin cones and smaller seminal vesicles devoid of mature sperm. C) Excision of the P element in *Phlp3*$^{\Delta P/\Delta P}$ rescues the mutant phenotype.

Acknowledgements

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Transmission Electron Microscopy

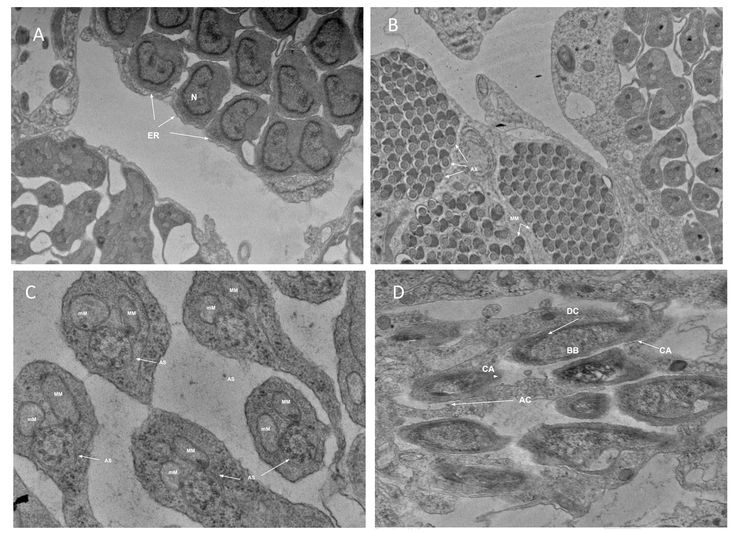

Figure 7: Control *Drosophila* Testis. A) Nuclei during the process of nuclear elongation at magnification of 23200x. **B)** Cross section through a cyst of individualized spermatids, each with a nebenkern and an axoneme (AX) and major mitochondrion (MM) shown and cluster of individualized spermatids at magnification of 15500x. **C)** Cross section of sample 5. The major mitochondrial derivative (MM) and minor mitochondrial derivative (mM) are both associated with the axoneme (AX). The axoneme is surrounded by the axonemal sheath (AS). Sample n = 5. Magnification 8,000X. **D)** Longitudinal sections of early elongation nuclei. Centriole adjunct (CA). Basal body (BB). Acrosome (AC). Dense Complex (DC). Taken of sample n = 1 at magnification 3000x..

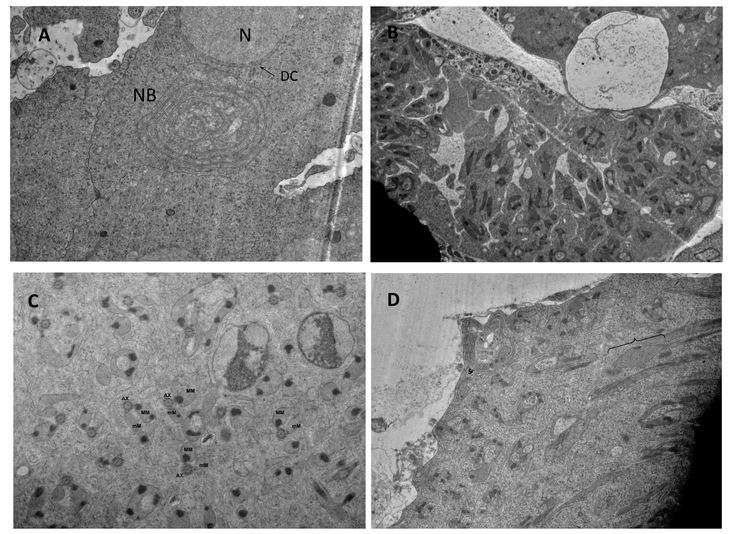

Figure 8: *Phlp3*$^{-/-}$ *Drosophila* Testis. A) Nuclei during the nebenkern stage taken at a magnification of 11600x. **B)** Cross section through a cyst showing major mitochondrion (MM) and minor mitochondria (mM). They appeared to be scattered and not attached to an axoneme (AX). Taken at a magnification of 7760x. **C)** Cross section of a post individualized spermatid, featuring the major mitochondrial derivative (MM) and minor mitochondrial derivative (mM) are both associated with the axoneme (AX), but in an unorganized manner. Magnification 19400X. **D)** Longitudinal sections of elongating nuclei. Bracket indicates an elongating nuclei. Centriole adjunct (CA), Basal body (BB), Acrosome (AC), and Dense Complex (DC) are not clearly depicted. Taken at magnification 9310x.

Conclusion and Further Analysis

- Investigating the dense complex and microtubule alignment during nuclear elongation using TEM.
- Generating additional mutants using CRISPR
  - Determining redox activity function in spermiogenesis by mutating cysteine in active site
  - Generating Myc-tagged Phlp3 at N and C terminus to determine if Phlp3 colocalizes with microtubules in testis.
- Test the ability of human Phlp3 (Txndc9) to functionally substitute for *Drosophila* Phlp3.