# Regulation of Spermatogenesis by Notch Signaling

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## Abstract:

Spermatogenesis is essential for production of sperm for sexual reproduction. Defects in this process can lead to infertility. In order to understand the basis of infertility it is necessary to identify and characterize the genetic mechanisms that promote sperm development. Previous studies have demonstrated that Notch signaling is required in the somatic cells of the gonad to promote sperm development. We have shown that increased levels of Notch signaling in somatic cells results in the arrest of somatic cyst cell development in the testes and a subsequent arrest in spermatogenesis. In a parallel study we also observed that increased levels of the transcription factor Ribbon in somatic cells also arrests spermatogenesis. Given that testes with increased Notch and Ribbon exhibit similar phenotypes, we wanted to examine expression of potential target genes in the testes. Previous studies have demonstrated that the Drosophila homologue of mammalian Ras Responsive Element Binding Protein 1 (RREB1), known as *hindsight* (*hnt*), is a target of Notch signaling in other tissues, suggesting it may be a target in the testes as well. We find that Hnt is expressed in the somatic cells of the testes during the transition from early to late somatic cyst cells, when Notch signaling is active. When Notch is overexpressed, somatic cells persist in this transition state and Hnt expression is expanded. We also observed that Hnt expression is present in the early germline cells of the controls and expression of Hnt appears to be expanded in the germ line cells when Notch is overexpressed. Interestingly, when Ribbon is overexpressed, we observe less Hnt in somatic cells. These experiments suggest that Notch and Ribbon may not cooperate to promote spermatogenesis, but rather may act antagonistically. We plan to further explore the relationship between Notch, Ribbon and Hnt to better understand how these genes, and their mammalian homologues promote spermatogenesis.

## Previous Work:

- Overexpression of *Notch* causes spermatogenesis defects.
- Overexpression of *ribbon* causes spermatogenesis defects.
- Similar phenotypes were observed when *Notch* and *ribbon* were overexpressed.
- This led the lab to believe that Notch and Ribbon may be interacting together to regulate spermatogenesis. In other contexts, Notch and Ribbon may regulate the expression of the same genes which led us to think that there may be a broader interaction.

## Overexpression of activated Notch causes prolonged Traffic jam expression

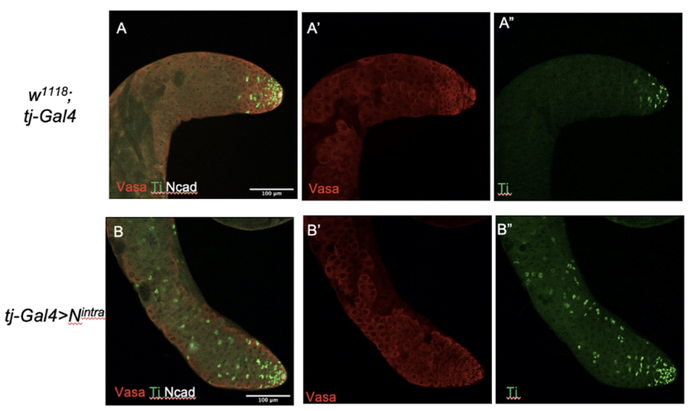



**Figure 2. Overexpression of the activated Notch causes the transcription factor Traffic jam (Tj) to have prolonged expression. Tj is a known marker for early somatic cells in the testes. It appears that Notch overexpression causes cells to stay in the early somatic cell stage and not transition to late-stage somatic cells.**

## Overexpression of activated Notch results in prolonged Tj/Eya co-localization

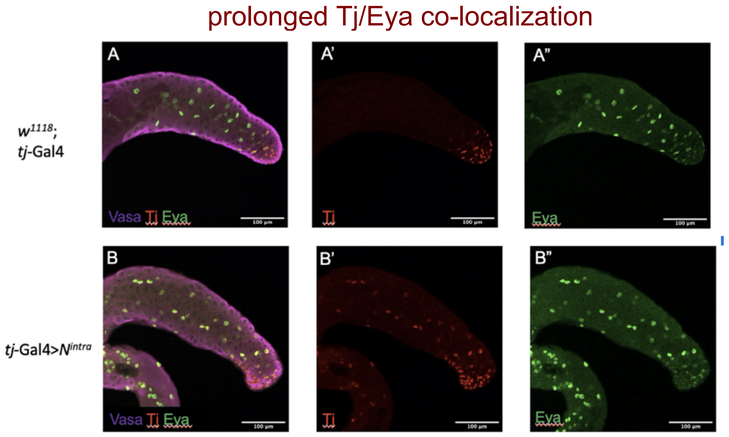



**Figure 3. Overexpression of activated Notch also results in prolonged Eya expression. Eya is a late-stage somatic cell marker. It appears that when Notch is overexpressed, this increases the number of transition-stage somatic cells, and they don't progress to the late stage. Somatic cells are not transitioning which suggests that the germ cells also fail to progress in spermatogenesis because their development is coordinated.**

## Structure of *Drosophila* Testes

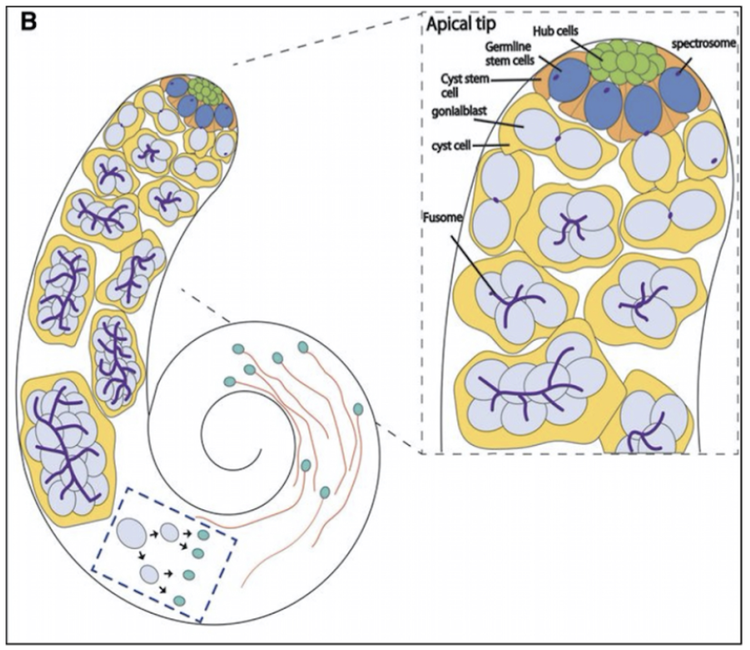



**Figure 1. Schematic of the structure of the testes. Hub cells are non-dividing cells that maintain stem cells. Stem cell divides to give rise to gonial blast which undergo 4 rounds of division to form 16 spermatogonia that will undergo meiosis to form 64 spermatids which will then undergo further processes such as elongation, actin cone addition and individualization until they become sperm. In regard to our research, Notch signaling known to be active in transition stage somatic cells so we are going to be looking at the effect of too much and too little Notch signaling in this stage of development. Gleason et al., 2018.**

## Mutation of Notch and Ribbon does not cause spermatogenesis defects

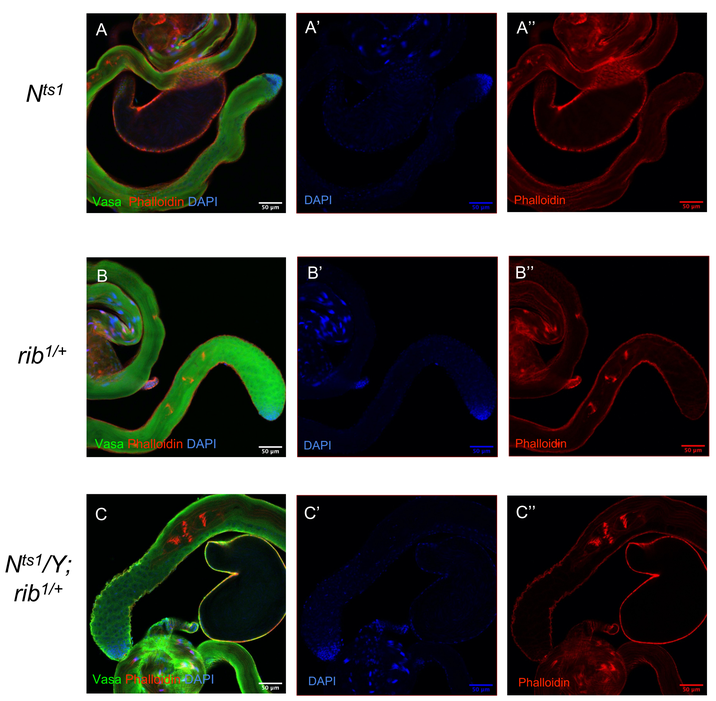



**Figure 4. (A) *Notch$^{ts1}$* mutant testis. (B) *rib* heterozygous mutant. (C) *Notch$^{ts1}$* mutant heterozygous for *rib*. Hypothesis was that the *N$^{ts1}$/Y; rib$^{1/+}$* mutants would have an enhanced phenotype when compared to the weaker phenotypes of the *Notch* and *Ribbon* controls. The results indicate that there was no enhancement of a phenotype. Spermatogenesis and spermiogenesis are still functioning given the mature sperm formed. This may indicate that there is not a strong genetic interaction between *Notch* and *Ribbon*. Vasa = somatic cells, Phalloidin = actin, DAPI = nuclei.**

## Hnt Expression in the Testes

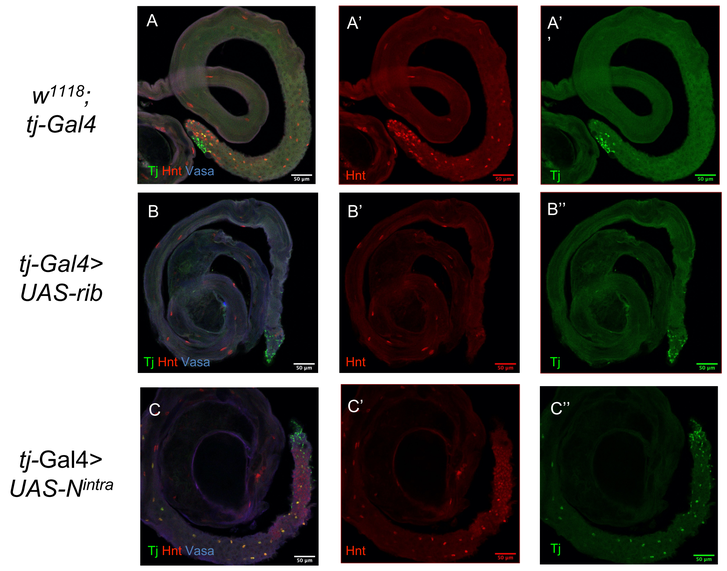


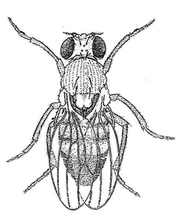

**Figure 5. Overexpression of *rib* (B) and *Notch* (C) in somatic cells of the testes. Expression in somatic cells using *traffic jam (tj)*-Gal4 throughout development. (A) *w$^{1118}$; tj-Gal4* controls. Vasa = somatic cells, Tj = early somatic cells, Hnt = Hindsight.**

## Conclusions and Further Analysis:

- We can use Hnt as a marker for transition stage and late-stage somatic cells and spermatogonia.
- Notch may regulate Hnt expression in the somatic cells of the testes, as observed in the ovarian follicle cells.
- Overexpression of *rib* in early somatic cells leads to an arrest in somatic cell and germline development.
- Overexpression of activated *Notch* in early somatic cells leads to arrest in the transition stage of somatic cell development and an arrest in germline development.
- Rib and Notch do not cooperate to promote spermatogenesis and may act antagonistically to regulate Hnt expression.

## Broader Impacts:

- Notch signaling has been observed to disrupt spermatogenesis across species. Our work suggests Hnt may be a target through which Notch signaling functions.
- Hnt is the Drosophila homologue of Ras Responsive Element Binding Protein 1 (RREB1), which are for growth factor signaling.
- What we learn about the relationship of Notch, Hnt, and Rib (mammalian homologue is BTBD18) in *Drosophila melanogaster* may provide insight into how these proteins functions across species to promote spermatogenesis.



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