Translational regulation of *cycB* in the *Drosophila* male germline

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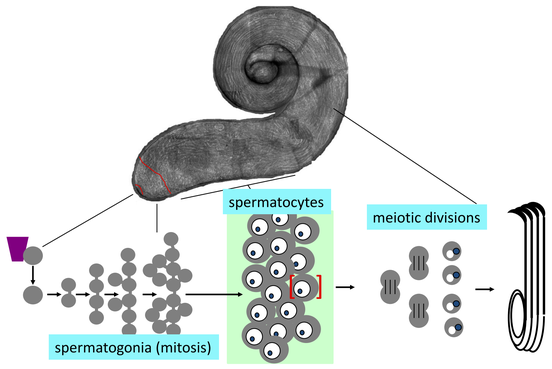
Abstract

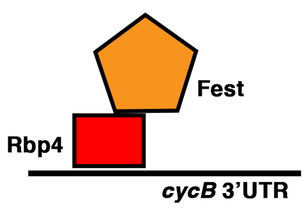
Tissue-specific regulation of the cell cycle is critical for proper development and homeostasis. Such regulation can be mediated by the function of cell-type-specific proteins to control the expression and/or activity of the core cell cycle machinery. The *Drosophila* male germline contains both mitotic cells (spermatogonia) and meiotic cells (spermatocytes), and the regulation of cell division in these two cell types is dramatically different. Spermatogonia divide regularly and efficiently; spermatocytes, in contrast, undergo a meiotic G2 prophase that lasts 3.5 days, and the concurrent delay of the meiotic divisions is mediated by fine-tuned control of the temporal expression of core cell cycle components. One such cell cycle factor is Cyclin B (CycB). CycB protein expression is high in mitotic spermatogonia, and then low in immature spermatocytes. CycB protein levels spike again just before spermatocytes enter the meiotic divisions. Published work from our lab has shown that the RNA-binding protein Rbp4 and its co-factor Fest repress *cycB* translation, mediated by sequences in the 130nt *cycB* spermatocyte 3'UTR (Baker, Gim, & Fuller 2015). Fest has no recognizable protein domains but is conserved in protostomes. Subsequent work has revealed that a novel protein, Lutin (Lut, formerly CG1690), is also required for *cycB* repression in early spermatocytes. Lut binds Fest independent of RNA, and co-precipitates with Rbp4 in the presence of Fest. In addition, we have found that testis-specific isoforms (the product of spermatocyte-specific transcription and splicing) of the RNA regulator Syp are required for activation of *cycB* translation in mature spermatocytes. Loss of function of *syp* (by double-CRISPR of the unique N-terminal coding sequence from promoters 1 and 4) in the testis causes germ cells to advance to the late spermatocyte stage, but these germ cells fail to translate *cycB* RNA and arrest prior to meiotic division. Syp, like Rbp4, binds the 130nt *cycB* spermatocyte 3'UTR in biotin pulldown experiments. Curiously, Syp binds to Fest independent of RNA (and can co-precipitate with Rbp4 in the presence of Fest). Further experiments should reveal whether Lut and Syp can co-precipitate in the presence of Fest. Experiments exploiting a synchronized differentiation time-course technique (Kim et al 2017) are underway to determine whether any of the major interactions (Fest-Rbp4, Fest-Lut, and Fest-Syp) change as spermatocytes mature to allow *cycB* translation to switch from off to on.

An overview of fly spermatogenesis



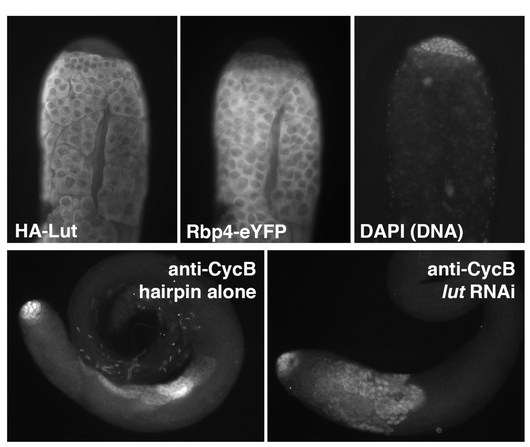
Rbp4 represses *cycB* translation in immature spermatocytes via the *cycB* 3'UTR

- Rbp4-eYFP associates with the *cycB* 3'UTR
- 3'UTR sequences required for Rbp4-eYFP binding are also required for translational repression of an *in vivo* CycB-eYFP reporter
- Fest, an Rbp4 co-factor, is also required for repressing *cycB* translation early.



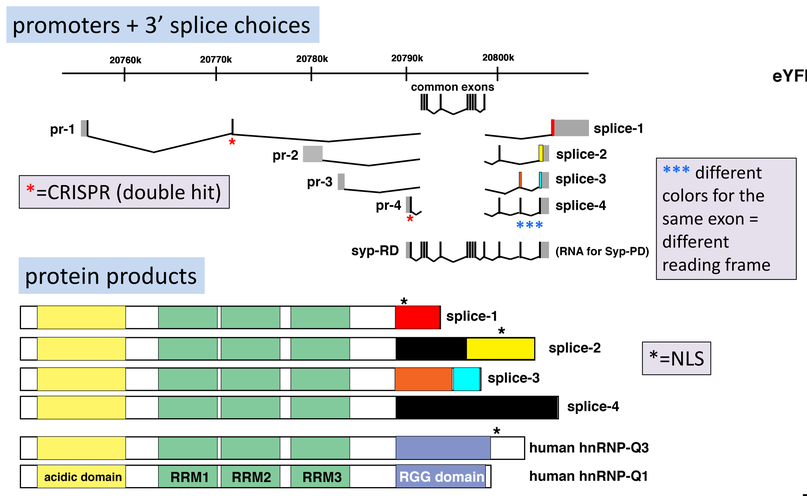
Baker, Gim, & Fuller, 2015

Lutin (formerly CG1690), like Rbp4, is expressed starting in early spermatocytes and is needed to repress *cycB* translation early

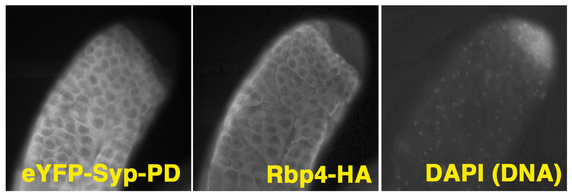


Not shown: Lut binds Fest (and through it, Rbp4)

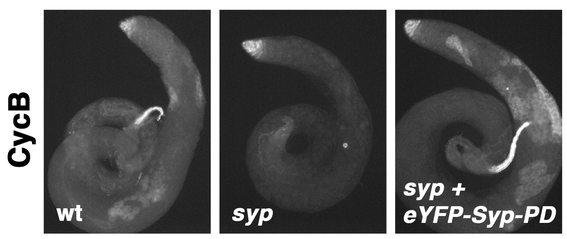
Syp is the *Drosophila* homolog of mammalian hnRNP Q



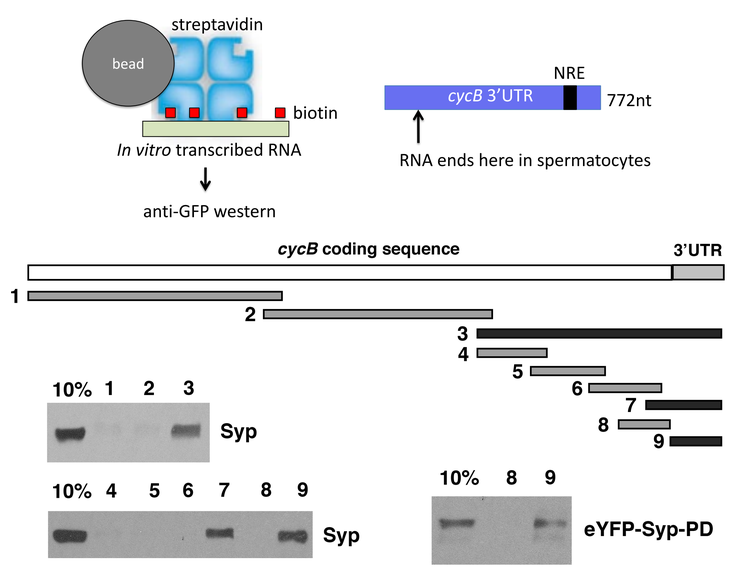
An eYFP-Syp-PD reporter is expressed in similar pattern to Rbp4 - starting in early spermatocytes, and entirely cytoplasmic



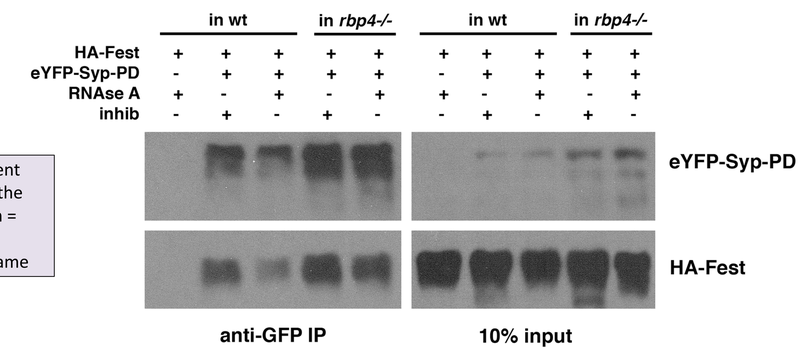
Syp is required for activation of *cycB* translation in late spermatocytes (and one copy of eYFP-Syp-PD rescues)



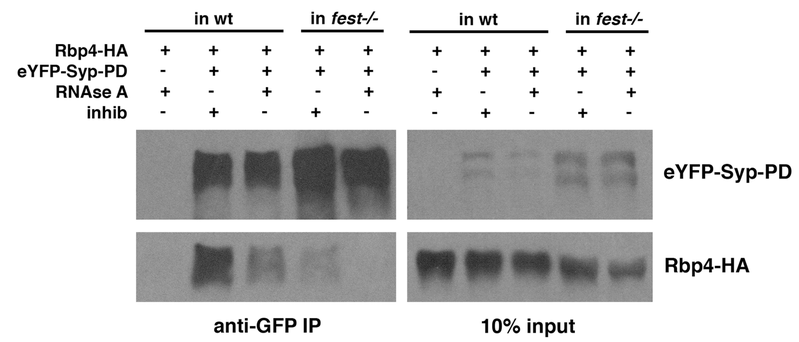
Syp binds the 130nt spermatocyte 3'UTR of the *cycB* RNA (via biotin pulldowns from testis extract)



eYFP-Syp-PD physically interacts with Fest

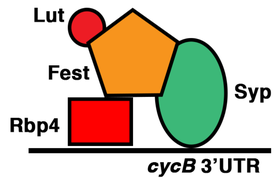


The Rbp4-Syp interaction requires Fest

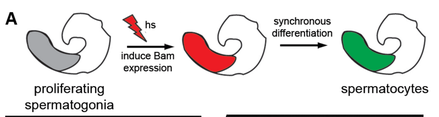


Updated model

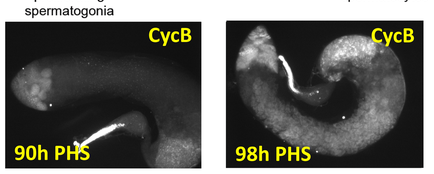
It assumes Syp and Lut interact in the presence but not absence of Fest. I meant to have this data for TAGC but COVID happened.



The synchronized differentiation time-course allows for enrichment of specific cell types



Kim, Lu, Srinivasan, Awe, Brehm, Fuller. *Science* 2017



strategy: repeat co-IPs early vs. late (72h vs. 98h PHS)

initial findings: no change in Lut-Fest or Fest-Rbp4 interactions

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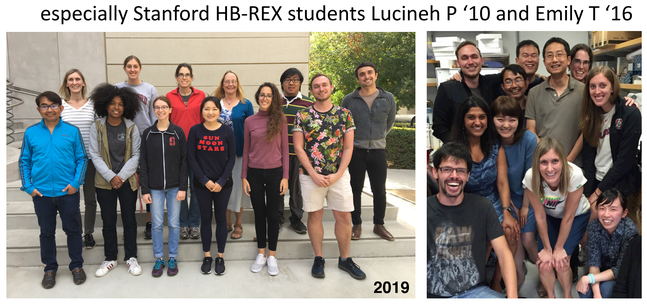
especially Stanford HB-REX students Lucineh P '10 and Emily T '16



e-mail me at ccb at stanford dot edu to chat about this project and/or the lab ☺