Dynamics of recruitment of transcription elongation factor SPT6 to the Histone Locus Body during the activation of replication-dependent histone genes in early embryogenesis

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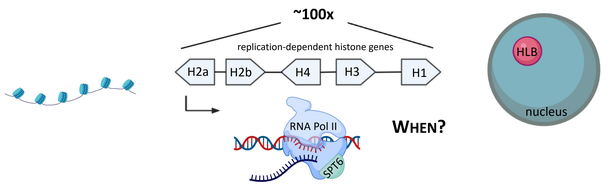
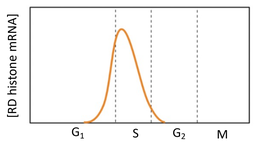
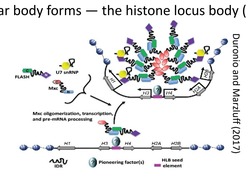
Background



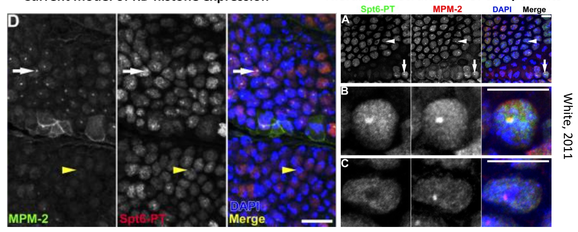
Histones are essential for packaging DNA, and their expression is tightly controlled and coupled to DNA replication. In *Drosophila*, replication-dependent (RD) histone mRNAs are transcribed from a single locus at which an evolutionarily conserved, phase-separated nuclear body forms — the histone locus body (HLB).



Current model of RD histone expression



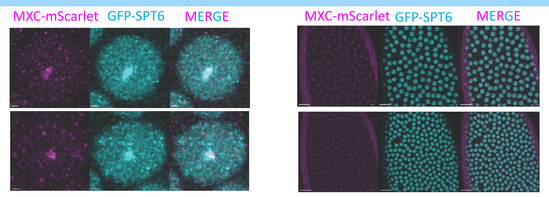
Hierarchal recruitment of components



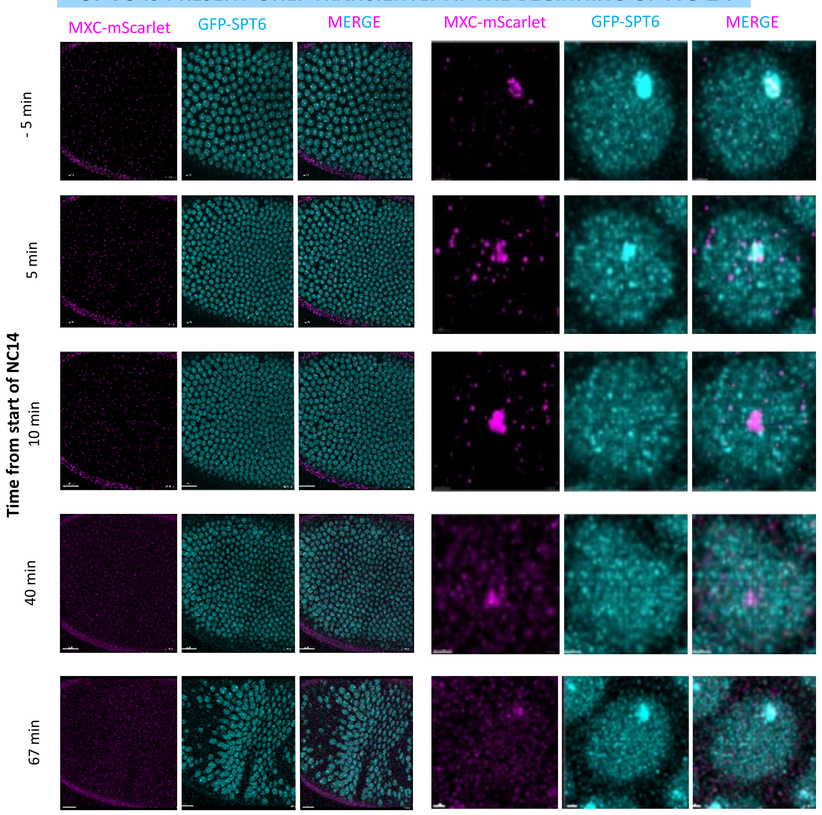
Previous understanding: SPT6 (visualized via α-SPT6) is a cell-cycle dependent component of the HLB, present when histone genes are being transcribed

Here, using antibodies and fluorescently tagged proteins we investigate the spatiotemporal localization of the transcription elongation factor SPT6 and RNA Pol II at the HLB in developing *Drosophila* embryos. By combining this approach with a sensitive FISH probe that can detect nascent RD histone transcripts, we examined the relationship between the localization of these factors and actively transcribing RD histone genes.

SPT6 Localization in Syncytial Nuclear Cycles



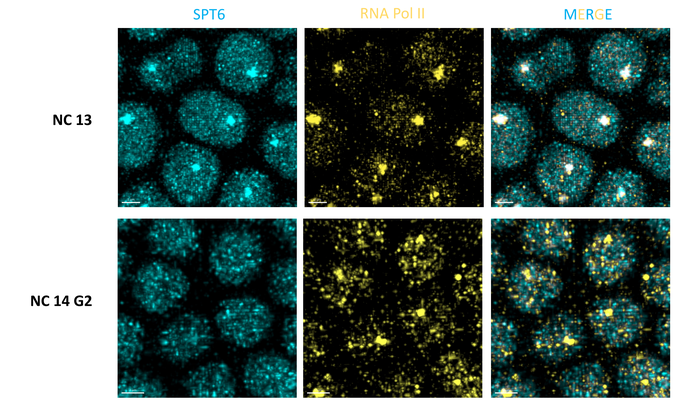
SPT6 is present only transiently at the beginning of NC 14



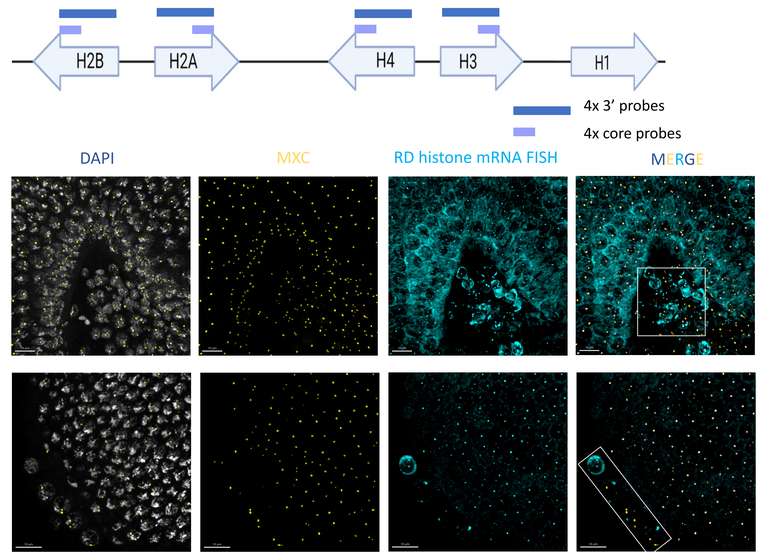
Representative stills from live imaging of developing embryo

Nascent RD Histone mRNA accumulates in the HLB independent of detectable SPT6 Enrichment during G2 of Nc 14

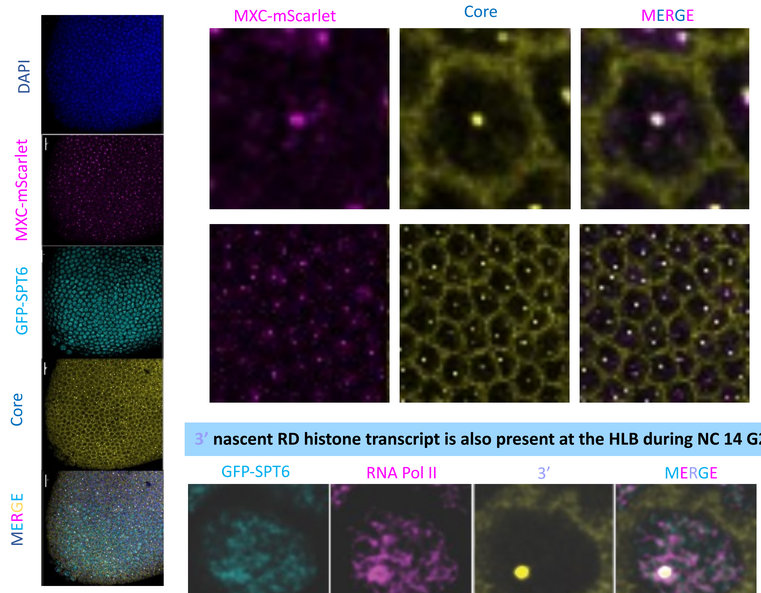
Decoupling of SPT6 and RNA Pol II in NC 14 G2



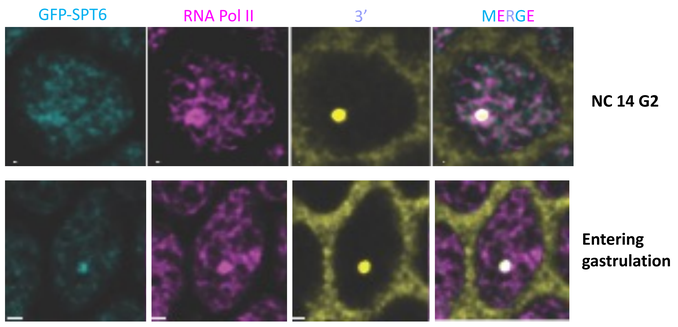
Different probes reveal paused polymerase at RD histone genes



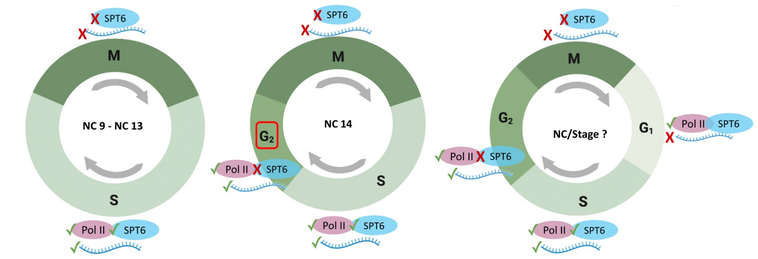
Core nascent RD histone transcript is present at the HLB during NC 14 G2



3' nascent RD histone transcript is also present at the HLB during NC 14 G2



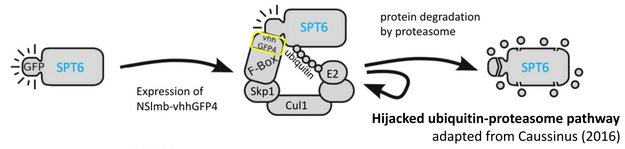
RD Histone Transcription & The Cell Cycle



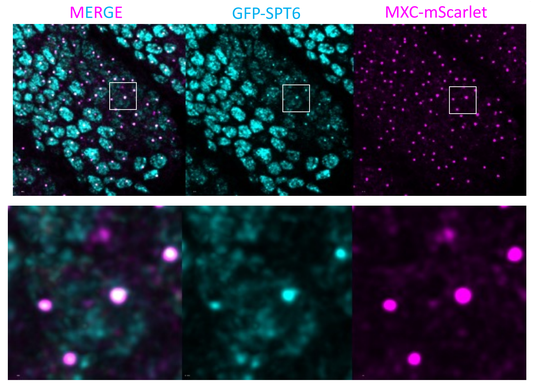
Result: Uncoupling of SPT6 association with RNA Pol II and active transcription of RD histone mRNA
RNA Pol II is not paused during transcription of RD histone mRNA in NC 14 G2

Is SPT6 necessary for HLB formation or Activation?

Attempted depletion of SPT6 from nucleus via **DeGradFP system**



Hijacked ubiquitin-proteasome pathway
adapted from Caussinus (2016)

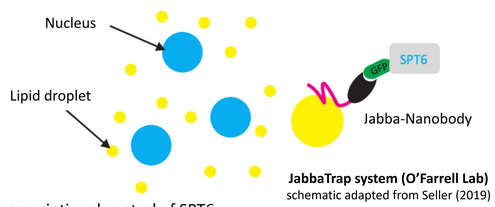


Stage 14 post-gastrulation embryo

♀ $\frac{yw GFP\text{-}SPT6, MXC\text{-}mScarlet, En2.4Gal4\ DegradFP}{yw}$

♂ $yw GFP\text{-}SPT6, MXC\text{-}mScarlet; \frac{En2.4Gal4\ DegradFP}{}$

Future Directions



JabbaTrap system (O'Farrell Lab)
schematic adapted from Seller (2019)

- Interrogate transcriptional control of SPT6
- Is SPT6 under String/cdk1 control at NC 14?
  - string mutants and heat-shock string

Acknowledgements

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