Use of CRISPR technology to investigate the mechanism of histone mRNA degradation

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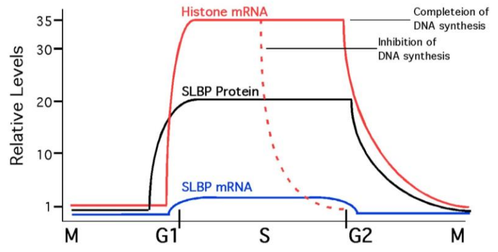
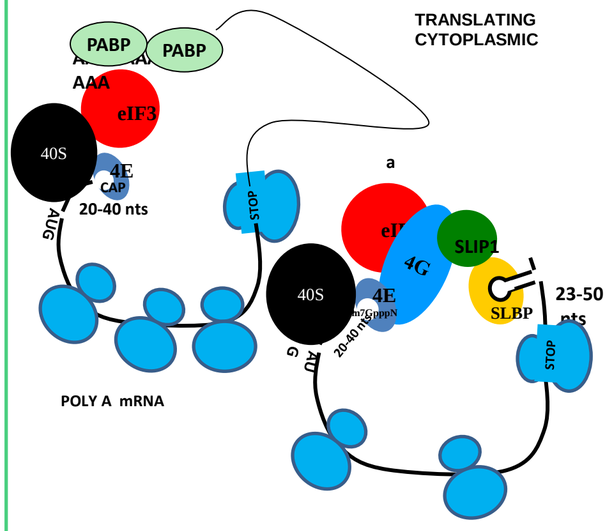
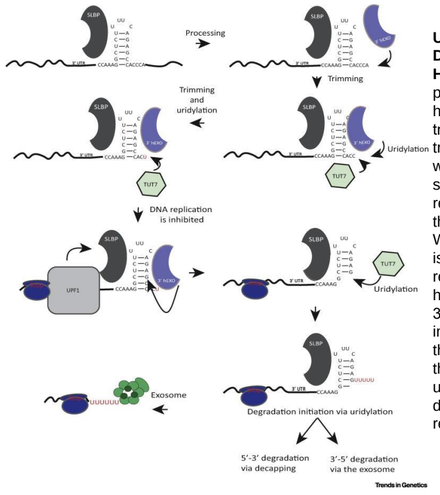
Histone mRNA degradation



histone mrna and slbp are cell-cycle regulated: histone mrna but not slbp are degraded when dna replication is inhibited



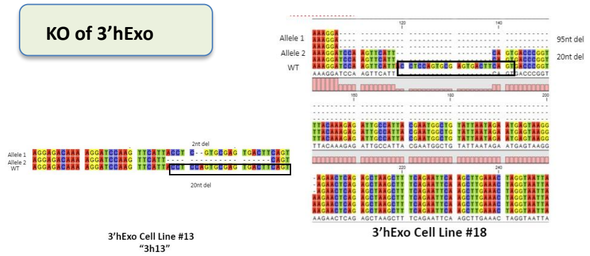
The stemloop at the 3' end is cis-element for regulated degradation of histone mRNA. Translation is required for histone mrna degradation



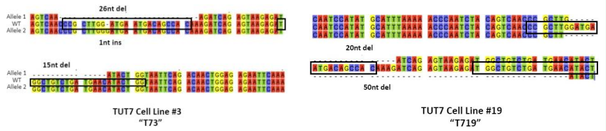
Uridylation and Degradation of Histone mRNA After processing the histone mRNA is trimmed by 3'hExo. If trimming proceeds to within 1–2 nt of the stem the mRNA is repaired by TUT7 to the proper length. When DNA replication is inhibited, Upf1 is recruited to the histone mRNA, and 3'hExo can degrade into the stem. When the nuclease stalls the mRNA is then uridylated and degradation can resume.

KO of 3'hExo&TUT7

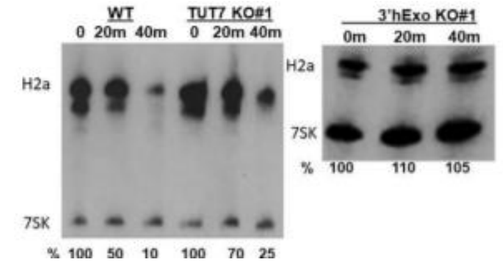
KO of 3'hExo



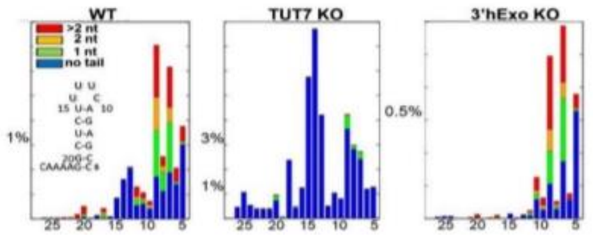
KO of TUT7



3'hExo&TUT7 KO Phenotype



TUT7 reduces 3'hExo KO abolish the degradation. TUT7 may reduces the degradation rate.

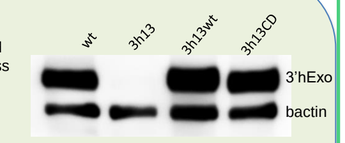


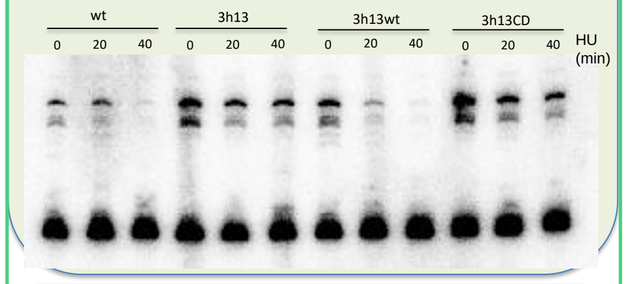
uridylation was reduced about 95% at the 3' end of histone mRNAs in the TUT7 KO Sphase cells

3'hExo&TUT7 KO Rescue

Rescue of 3'hExo KO

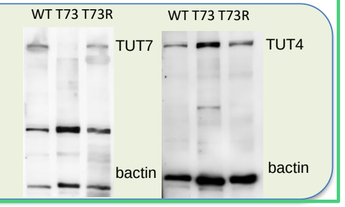
A lentivirus (made an active(wt) and catalytically dead(cd) virus - express 3'hexo from doxycycline inducible promoter – puromycin resistant



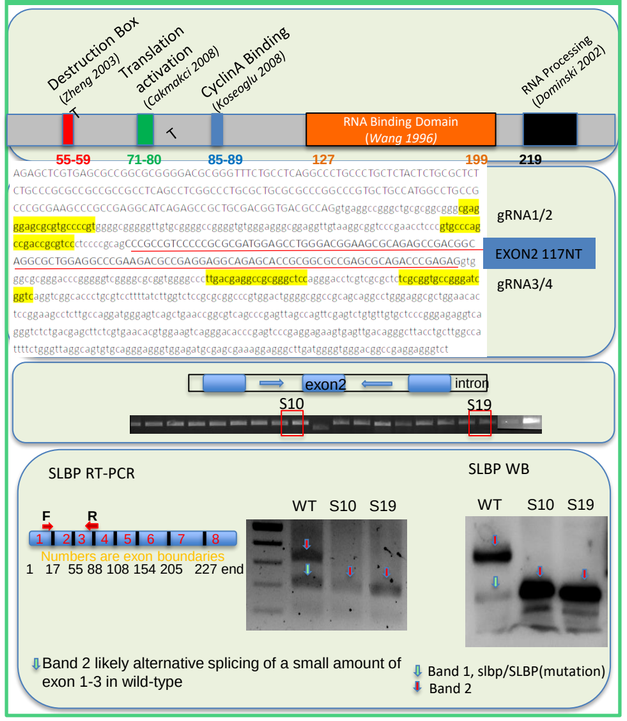


Rescue of TUT7

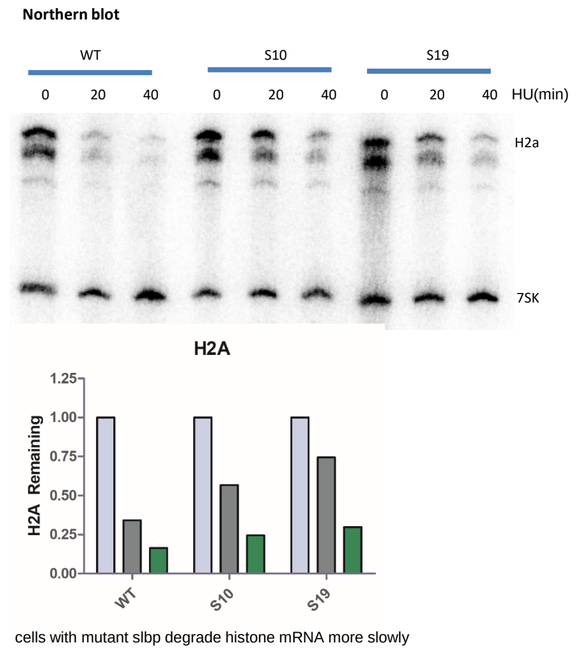
A lentivirus plasmid- express TUT7 had been infected to the TUT KO cells. TUT7 KO express higher TUT4, but TUT7R(rescue) express less TUT4.



Deletion of SLBP exon2



Phenotype of SLBP mutation



cells with mutant slbp degrade histone mRNA more slowly

Abstract&Conclusion

Histone mRNAs differ from all other cellular mRNAs in that they do not end in a polyA tail, but end instead in a stemloop. Histone mRNAs are tightly cell-cycle regulated and most of the regulation in mammalian cells is postranscriptional. An important regulatory step is rapid changes in the rate of histone mRNA degradation to balance histone mRNA levels with the rate of DNA replication. The stem-loop at the 3' end of histone mRNA binds SLBP and is the critical cis-element that controls histone mRNA half-life. The SLBP-stem-loop RNA complex is involved in all steps of histone mRNA metabolism. SLBP-mediated replication-dependent histone mRNAs decay depends on translation and UPF1 recruitment to the 3' UTR of histone mRNA when DNA replication is inhibited. UPF1 recruitment results in activation of 3'hExo and TUTase 7 to initiate histone mRNA degradation. Using crispr/cas9, we deleted exon2 (aa 14-50) of SLBP, on the N-terminal and found the rate of degradation of histone mRNA became slower. Dr.Sutapa Chakrabarti's lab (Free University of Berlin), have found that this region of SLBP directly binds Upf1, providing a mechanism to recruit Upf1 to histone mRNA. We postulate that Upf1 binds to the terminating ribosome and also to the N-terminal region of SLBP. This results in activation of 3'hExo and TUT7 to initiate histone mRNA degradation. We have knocked out both TUT7 and 3'hExo and will also determine the regions in these proteins required for histone mRNA degradation.

Acknowledgements

Marzluff Lab

UNC

Sutapa Lab

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