Multiple Molecular Modalities of Tug1 locus

Locus, lncRNA and sORFs

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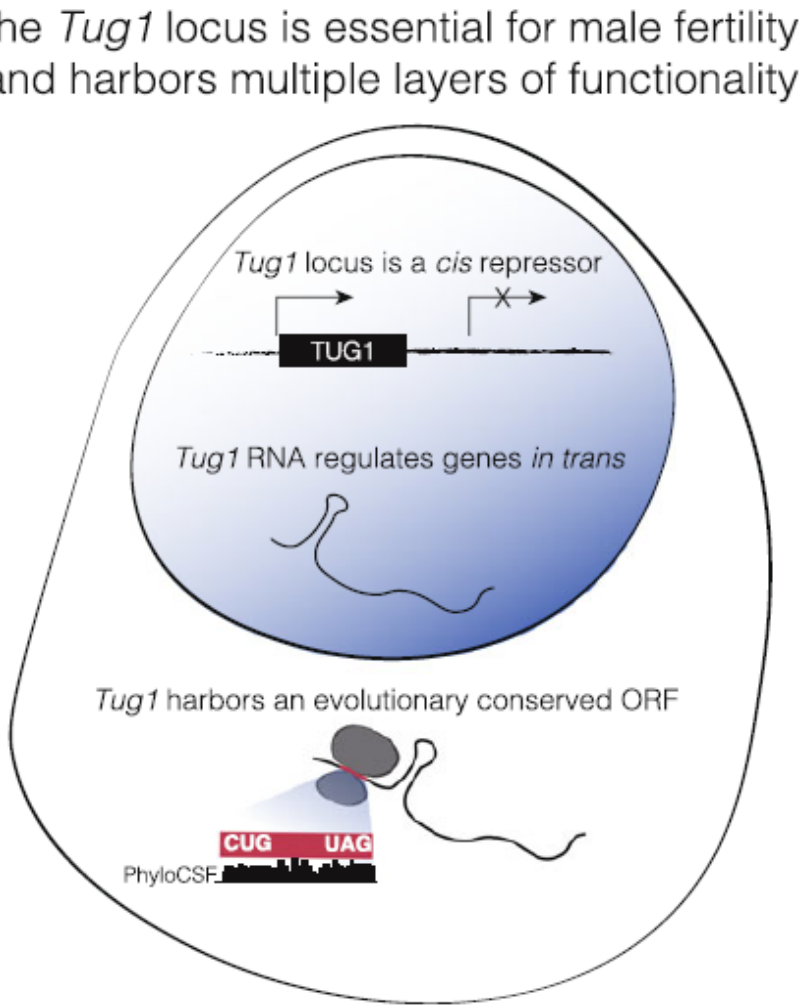
Organization

1. **Introduction**: lncRNA overview and main mechanisms of action
2. **Paper presentation**:
   a) The role of Tug1 in male fertility
   b) Cis-acting role of Tug1 locus
   c) Trans-acting role of Tug1 transcript
   d) Putative Tug1-BOAT protein



Features of lncRNAs

- LncRNAs are a group of transcripts that are longer than 200 nucleotides.
- LncRNAs are mostly polyadenylated and are transcribed by RNA Pol II.

New functions of lncRNAs:

They can possess coding ORFs that can encode for stable, functional small peptides.

Functions covered by lncRNA loci

Cis-action

The lncRNA loci can influence the expression and/or chromatin state of nearby genes.

Trans-action

The lncRNA transcript can operate far away from the transcription site.

Potential translation of micropeptides

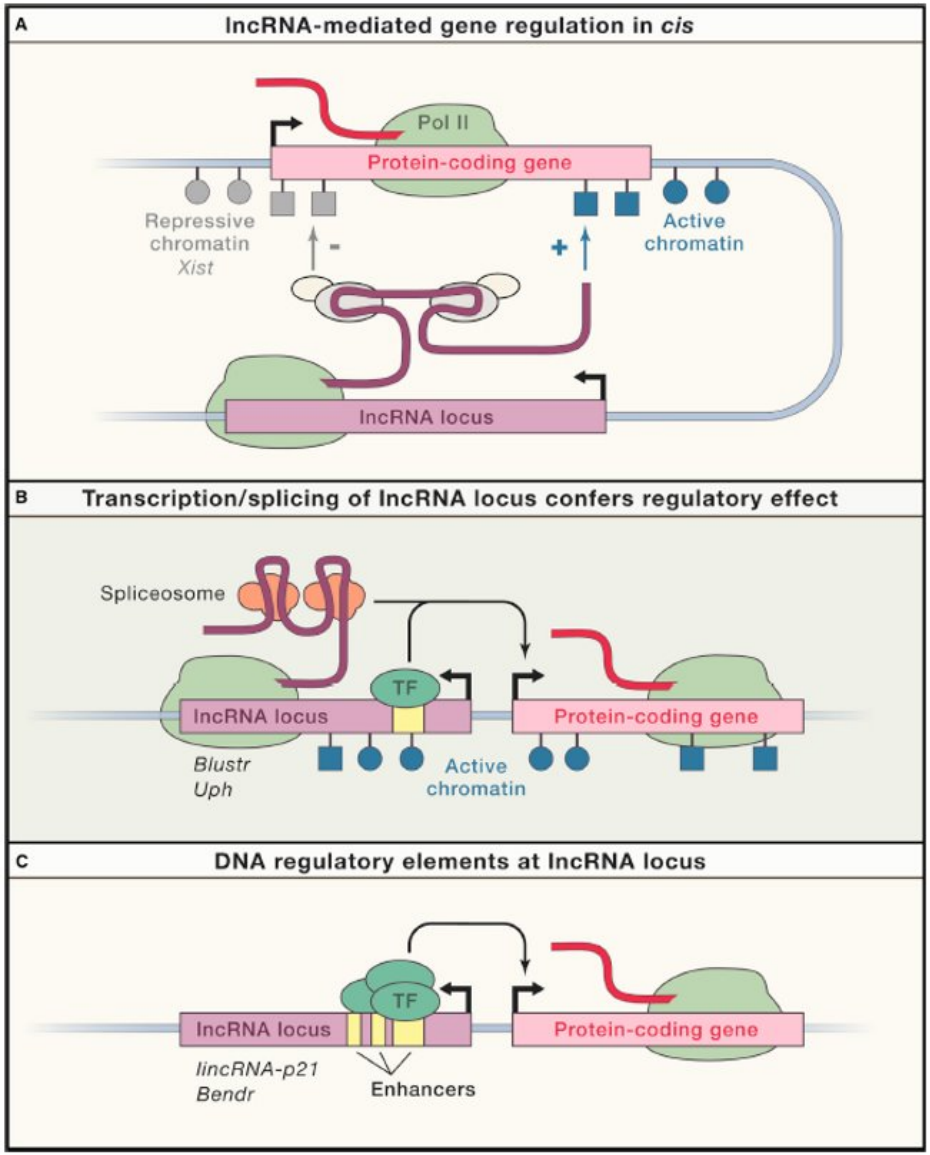
The lncRNA can harbor a sORF that could be translated into a micropeptide with a putative biological function.

Cis-acting lncRNA loci

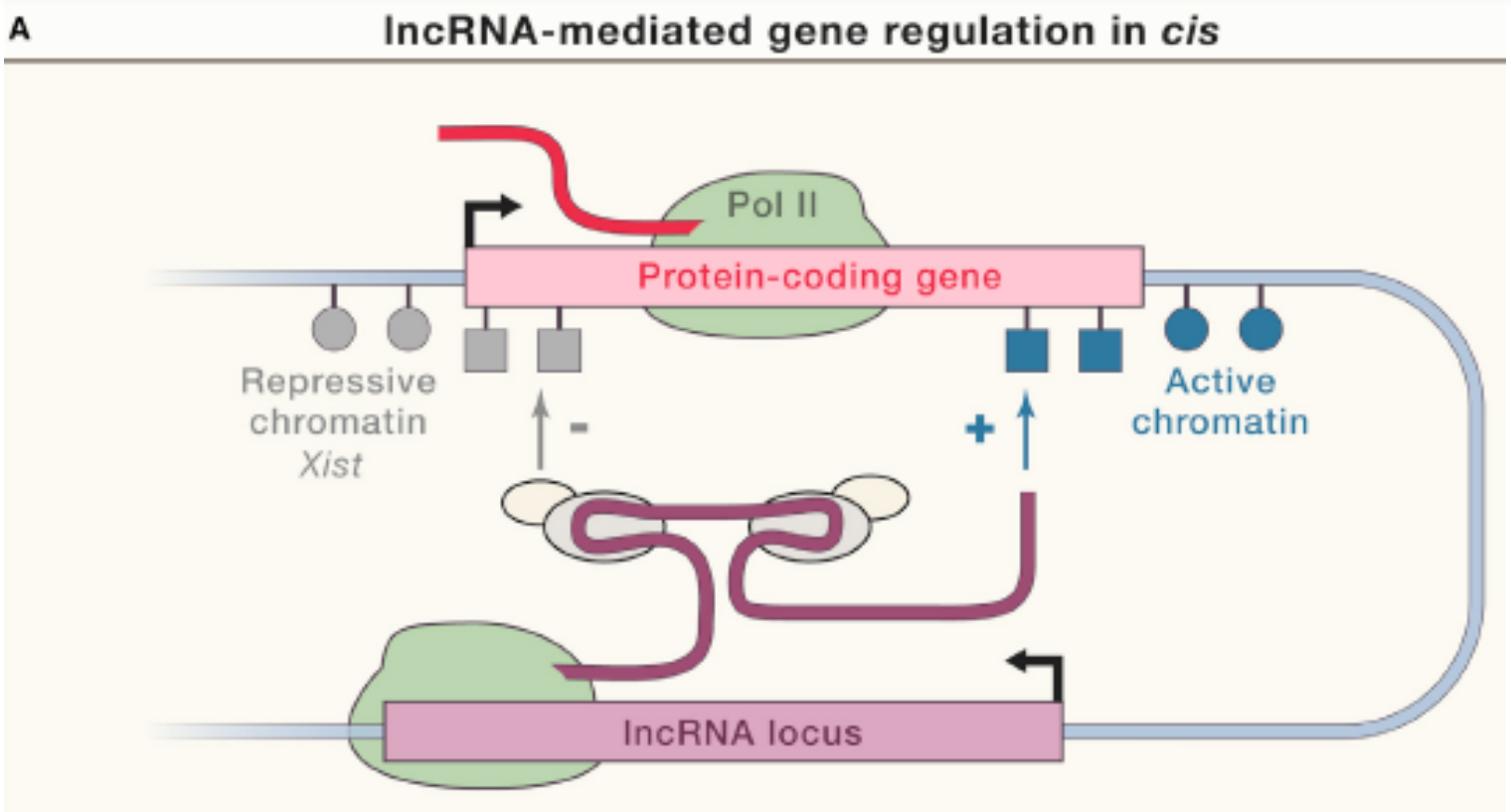
A) lncRNA-mediated gene regulation in cis

B) Transcription/splicing of lncRNA locus confers regulatory effect

C)DNA regulatory elements at lncRNA locus



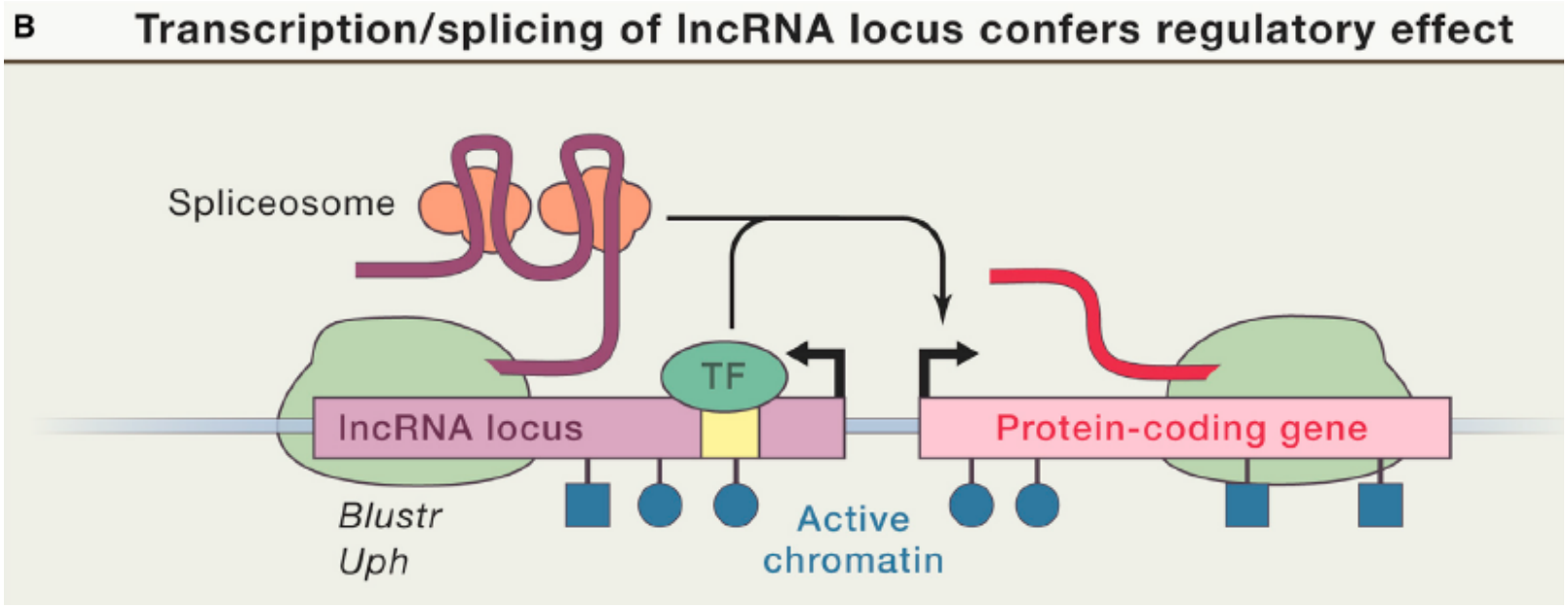
Cis-acting lncRNA loci



Positive and negative regulation

How to study :
-deletion of exons
-siRNA

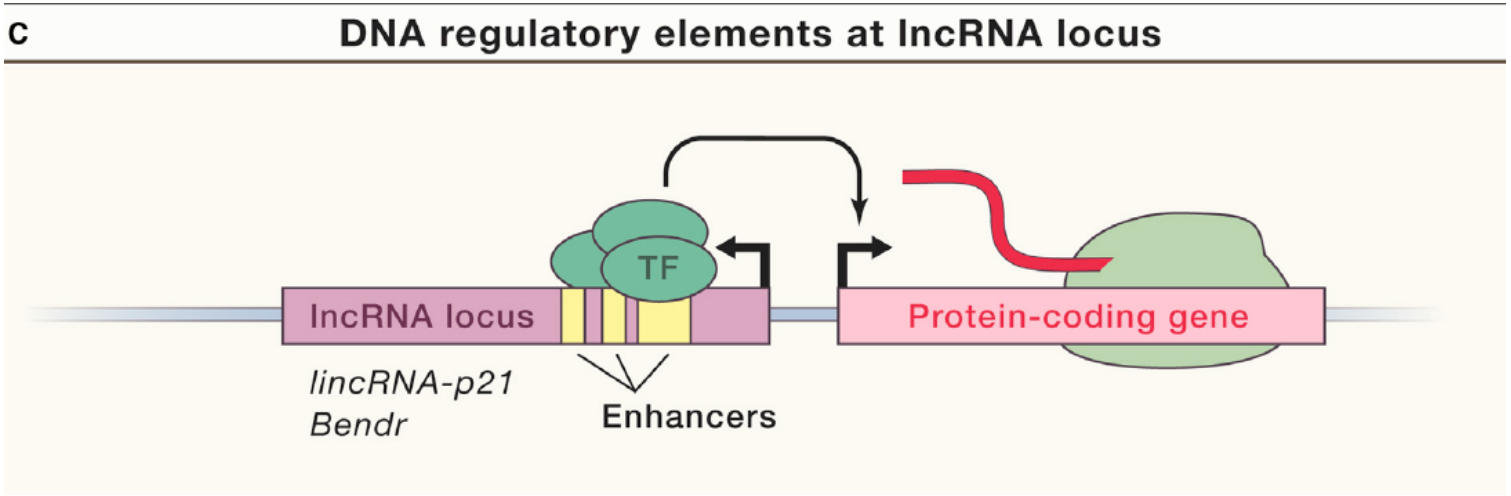
Cis-acting lncRNA loci



Positive and negative regulation

How to study :
-mutation in lncRNA splice sites
-poliA signal at lncRNA 5'end
-lncRNA promoter deletion

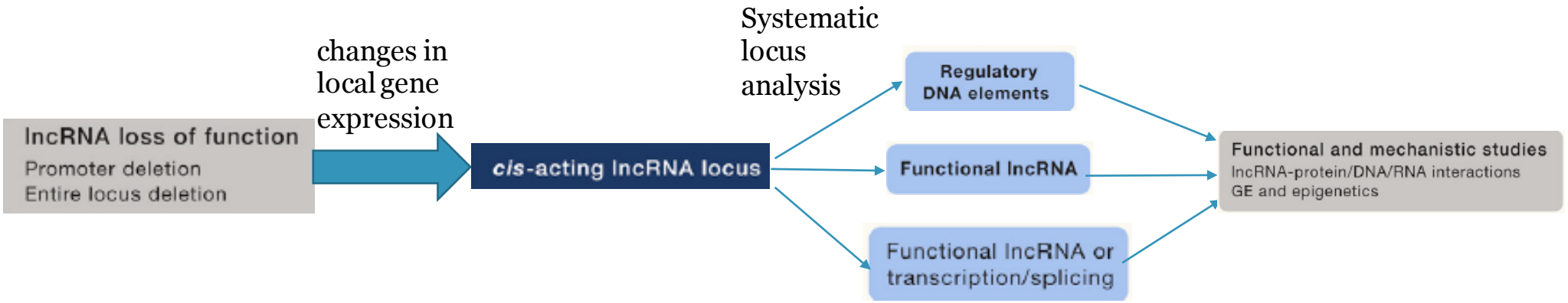
Cis-acting lncRNA loci



Positive and negative regulation

How to study :
-deletion of lncRNA promoter
-allele specific expression analysis

Experimental dissection of lncRNA loci in cis

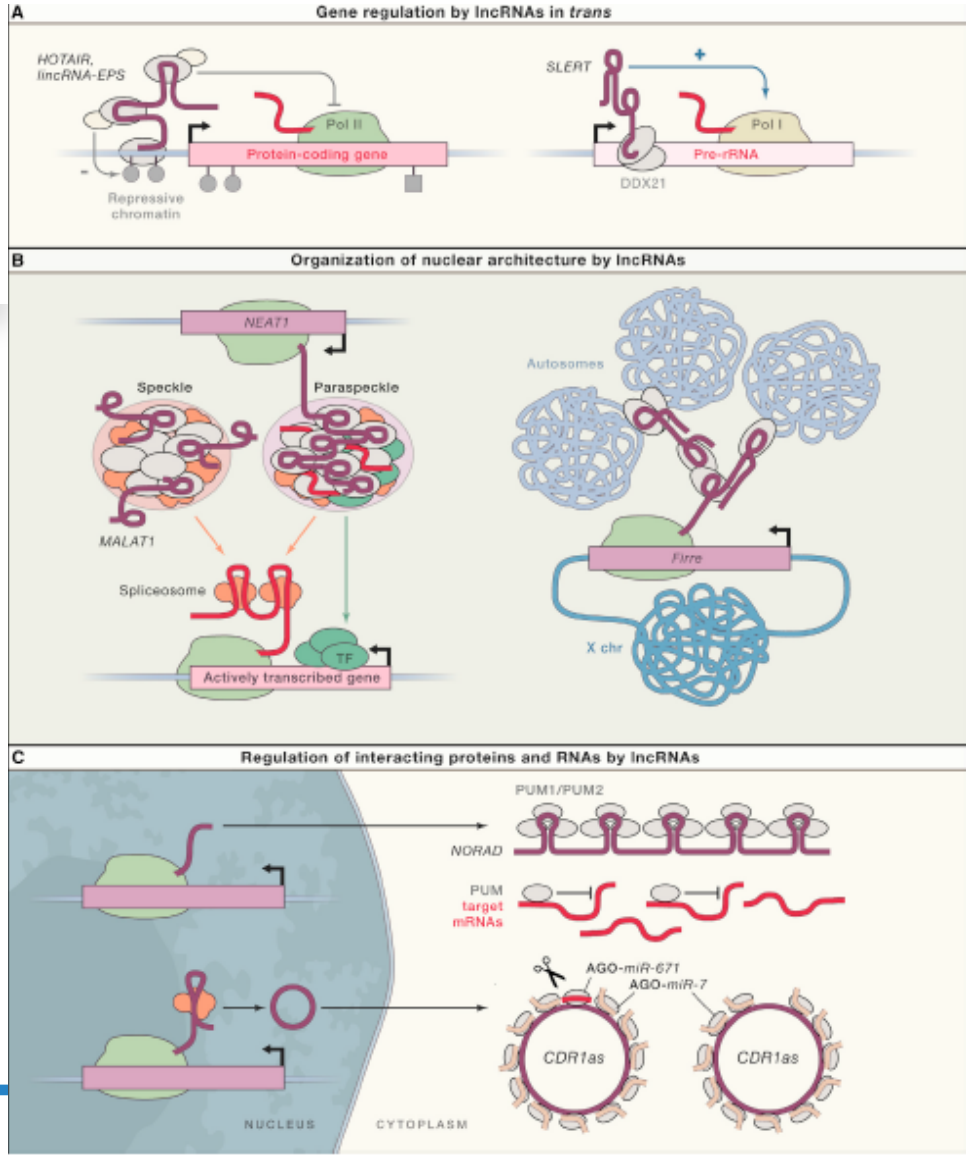


Trans-acting lncRNAs

A) Gene regulation by lncRNAs in trans

B) Organization of nuclear architecture by lncRNAs

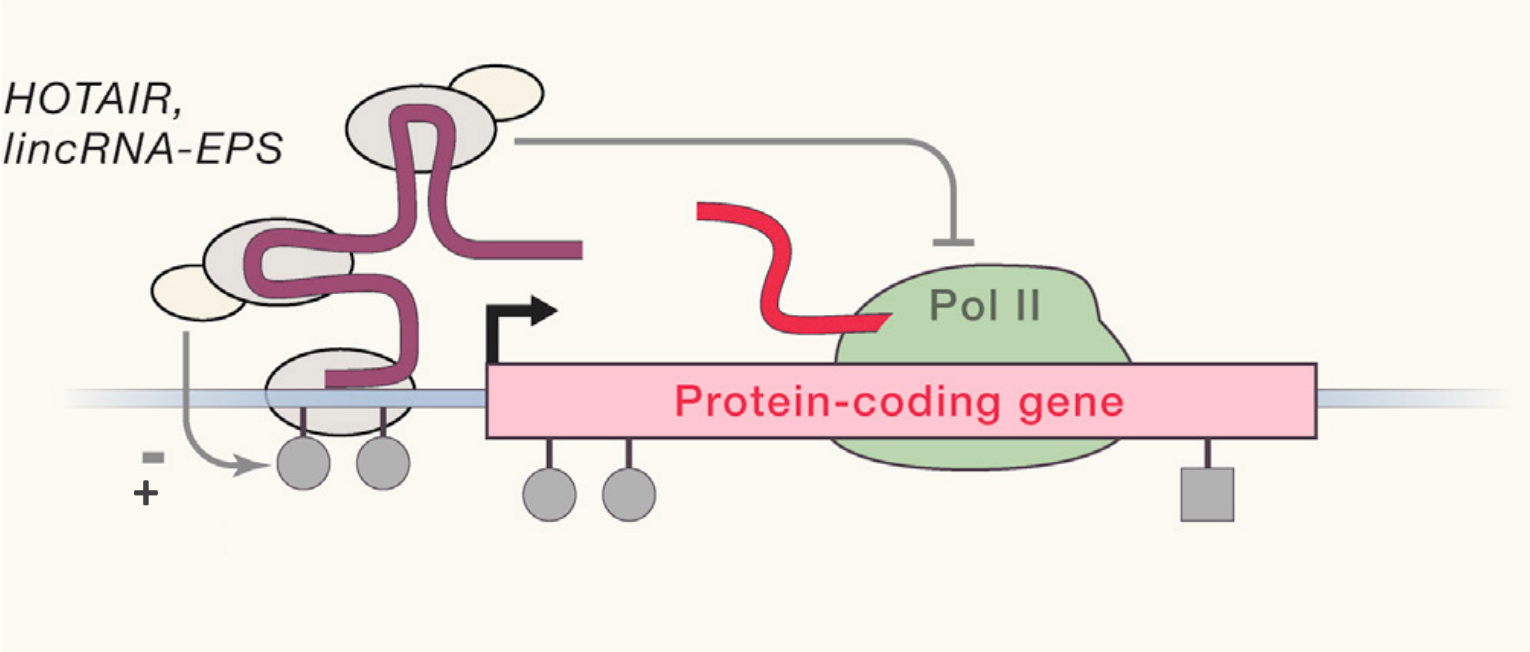
C) Regulation of interacting proteins and RNAs by lncRNAs



Trans-acting lncRNAs

LncRNA transcripts binding to distant loci is important to the addition and maintenance of activating/repressive chromatin marks.

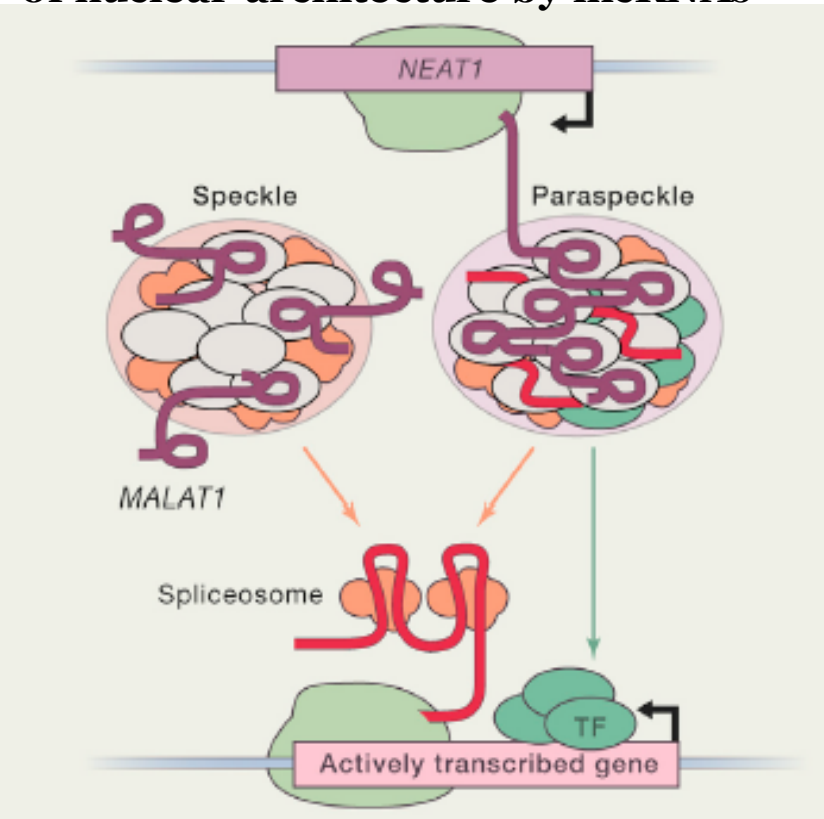
Gene regulation by lncRNAs in trans



Trans-acting lncRNAs

Some lncRNAs appear to influence nuclear architecture to orchestrate transcription, RNA processing, and other steps in gene expression.

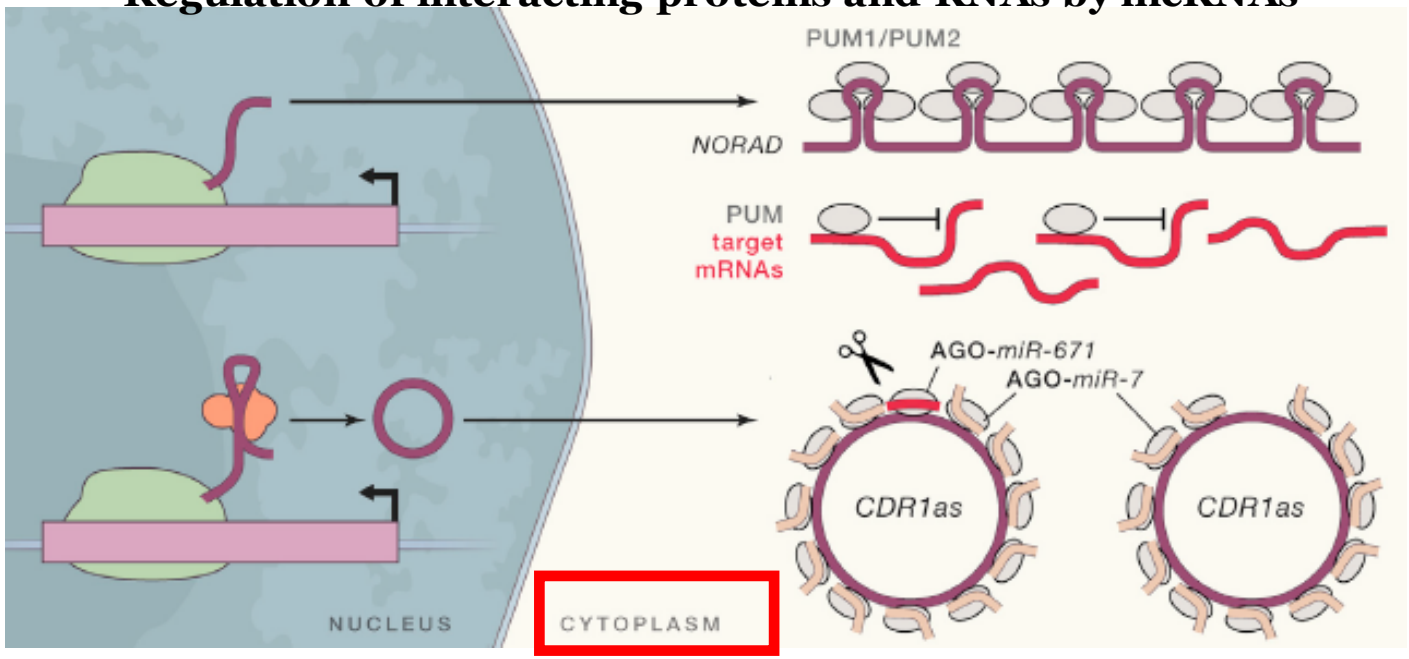
Organization of nuclear architecture by lncRNAs



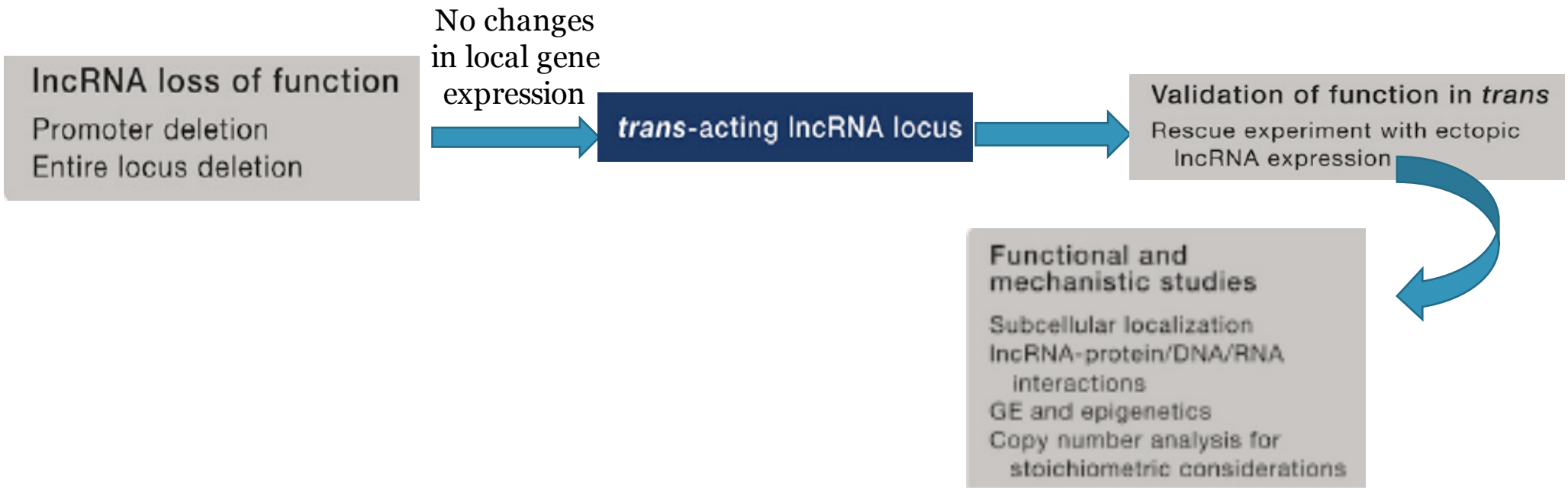
Regulation of Interacting Proteins and RNAs by lncRNAs

Trans-acting lncRNAs may also function by modulating the activity or abundance of proteins or RNAs to which they directly bind.

Regulation of interacting proteins and RNAs by lncRNAs



Experimental dissection of lncRNA loci in trans



INTRODUCTION

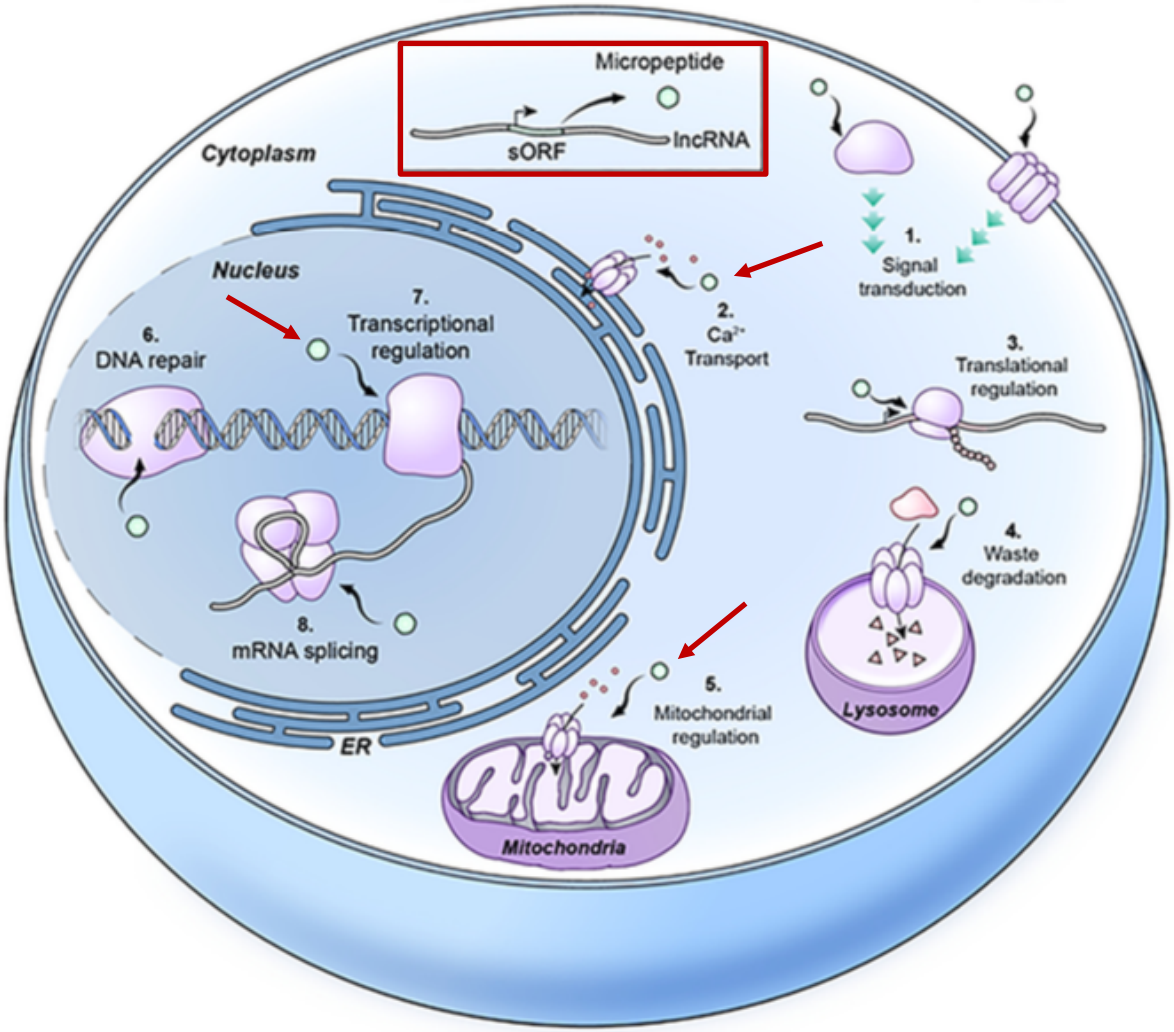
Short ORFs in lncRNA

- Short open reading frames (sORFs), of ~100-200 codons in lenght.
- sORFs conisist of a string in frame sense codons beginning with a canonical or not canonical starting codon and end with a stop codon.

INTRODUCTION

Micropeptides Encoded by sORF in lncRNA

Micropeptides have diverse regulatory roles, although the mechanisms that underlies their roles are yet to be fully characterized.



Corrine Corrina, et. Al Molecular and Cellular Biology (2020)

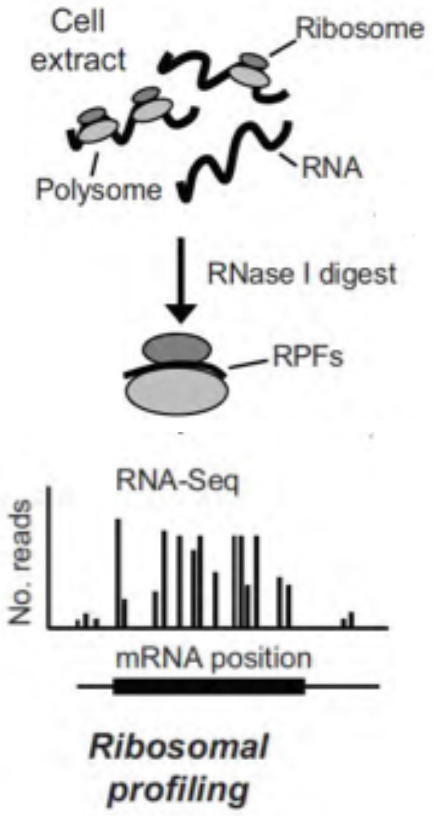
The Identification of Coding ORFs

- **Cross-species comparisons** of sORF sequences
- **Examination of the codon content** through bioinformatics tools. It is used to differentiate coding sORF from non-coding ones.
- Analysis of **transcriptional** and **translational** experimental data to identify sORFs expression.

The Identification of Coding ORFs

- **Ribosome Profiling** results in a "Ribosome Footprint" that highlights potentially translated lncRNAs.

- **Proteomics approach**, using MS spectrometry.



Validation of sORF Translation

A common way to determine if a sORF is translated into a micropeptide is by *in vitro* translation.

Lewandowski *et al. Genome Biology* (2020) 21:237
https://doi.org/10.1186/s13059-020-02081-5

Genome Biology

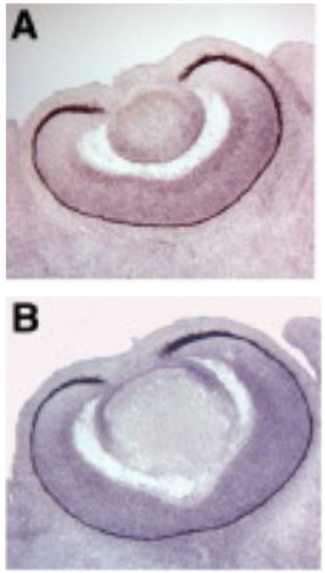
RESEARCH Open Access

The *Tug1* lncRNA locus is essential for male fertility

Jordan P. Lewandowski[1†], Gabrijela Dumbović[2†], Audrey R. Watson[2,3†], Taeyoung Hwang[2†], Emily Jacobs-Palmer[4], Nydia Chang[1], Christian Much[2], Kyle M. Turner[5], Christopher Kirby[5], Nimrod D. Rubinstein[5], Abigail F. Groff[1,6], Steve C. Liapis[1], Chiara Gerhardinger[1], Assaf Bester[7,8], Pier Paolo Pandolfi[7,8], John G. Clohessy[7,8], Hopi E. Hoekstra[4,5,9*], Martin Sauvageau[10,11*] and John L. Rinn[1,2,3*]

Tug1 lncRNA

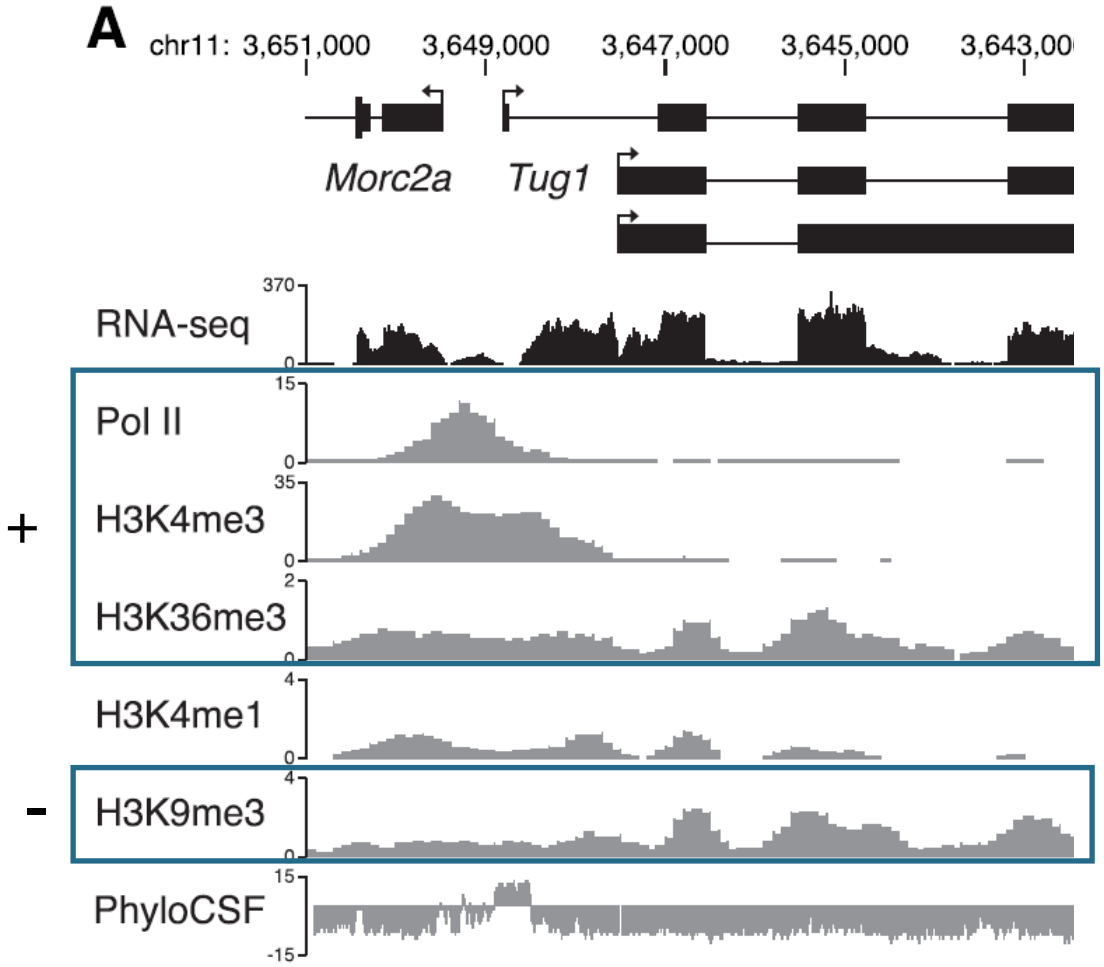
- **Tug1** *(taurine-upregulated gene 1)* is a highly conserved lncRNA locus, of 7.1 kb, located on chromosome 11.
- Tug1 was first identified in screenings for genes upregulated in response to taurine in murine retinal cells.
- In fact Young et. Al. Showed the RNA-based roles of Tug1 during the development of photoreceptors.
- The Tug1 locus is expressed in multiple tissues and its lncRNA is spliced.



Tug1 has other roles:

- **Gene expression**: the lncRNA interacts with PRC2.
- **Malignacy**: e.g. it acts as a miRNA sponge in cancer cell lines; acts as a suppressor in human gliomas.
- **Male fertility**: studied by Lewandowski et al.

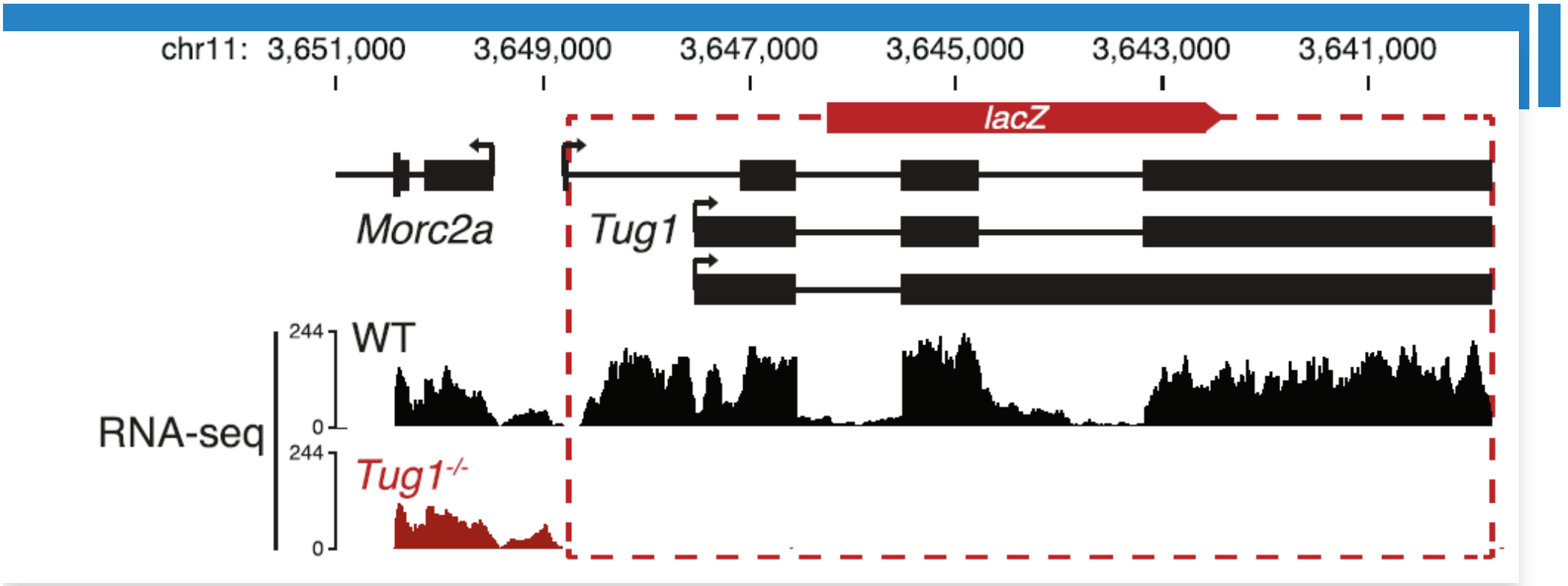
Tug1 locus analysis



3 annotated transcripts

This atypical combination of H3K9me3 and H3K36me3 is conserved in human cells.

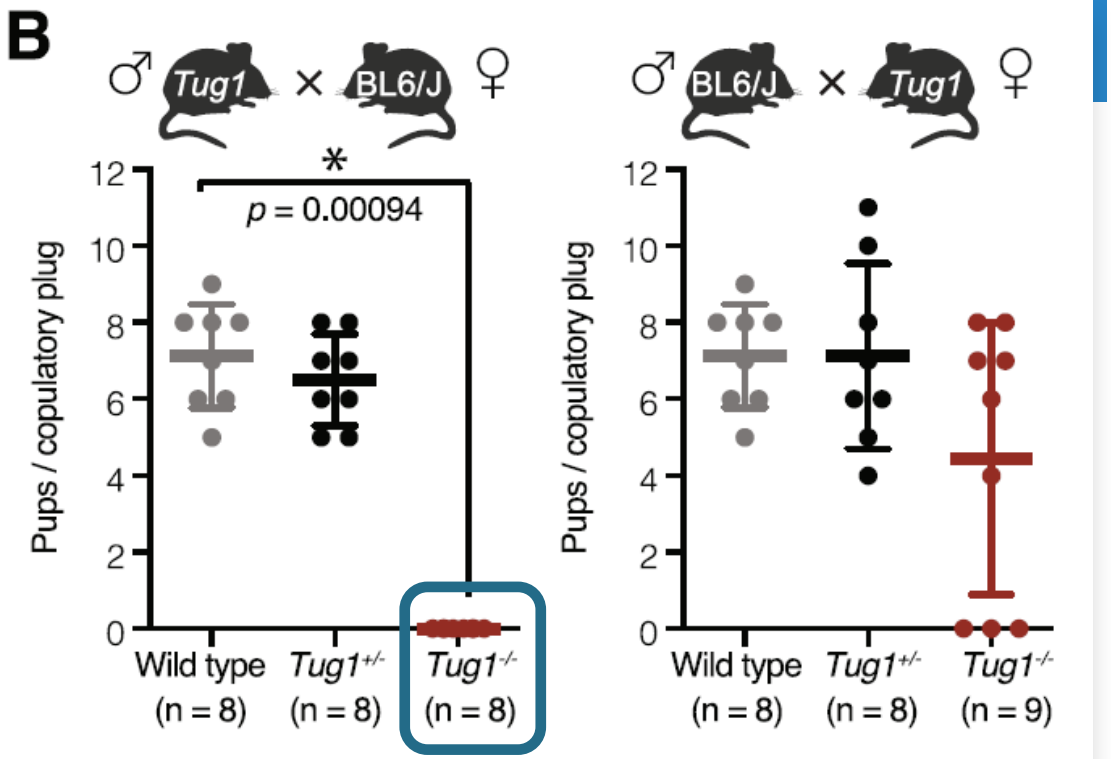
Model system:
Tug1-knockout (Tug1–/–) mouse



Main Phenotype

- Tug1-/- x Wild type mice
- Tug1-/- x Tug1-/-mice
- Absence of offspring from Tug1-/- males

Conclusion: Tug1-/- mice are sterile.



Mice Spermatogenesis

During spermatogenesis mice tubules cycle through 12 epithelial stages.

Spermatogenesis ends with **spermiation:** a 16-step process by which mature spermatids are released into the seminiferous tubule lumen, prior to their passage to the **epididymis**.

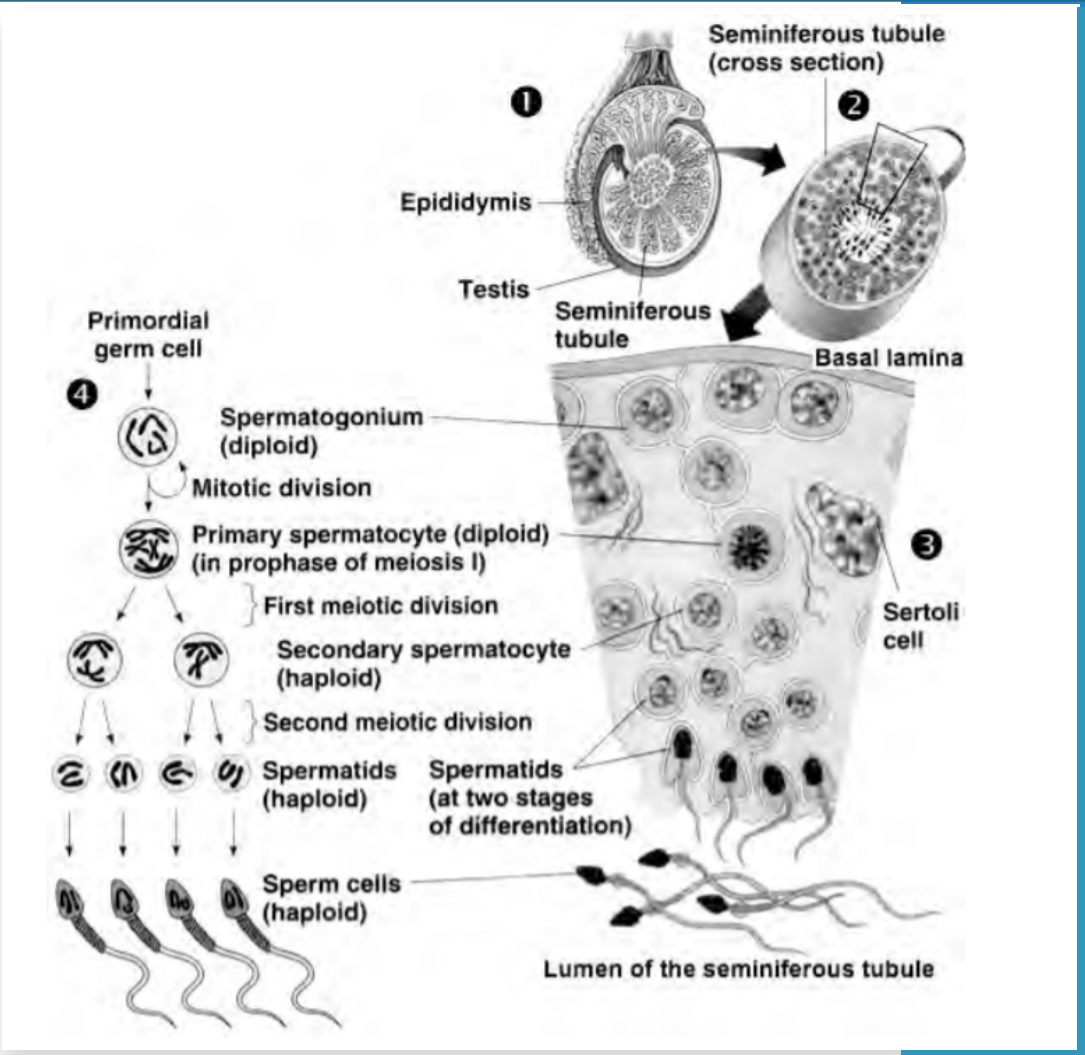
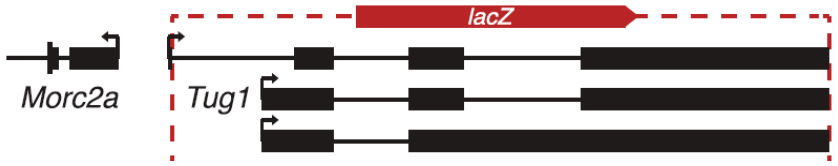
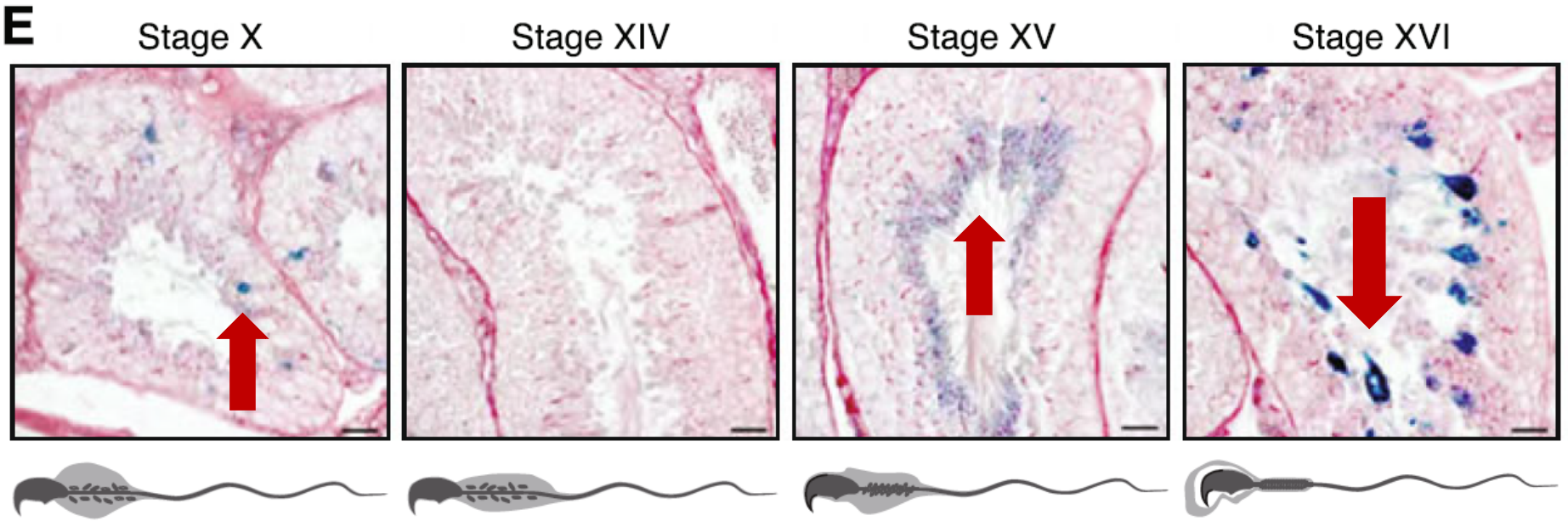


Image: Guan, Jikui. (2009). Mammalian sperm flagella and cilia.

TUG1 & MALE FERTILITY

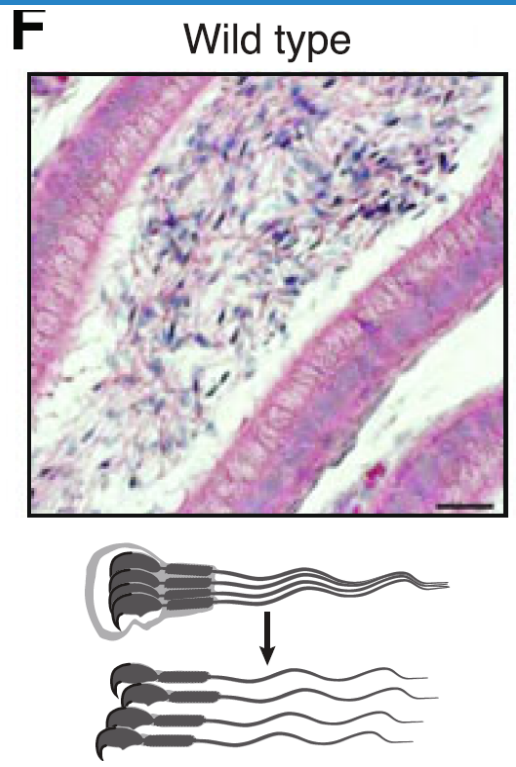
Is Tug1's promoter active during spermatogenesis?





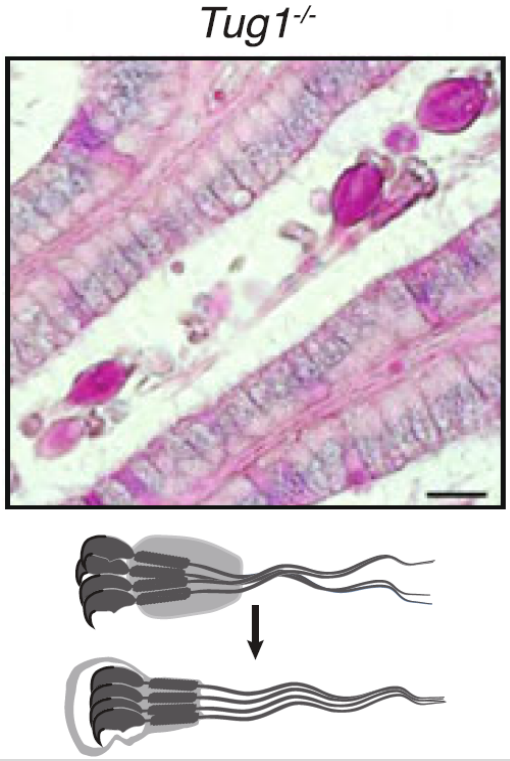
Conclusion: LacZ is expressed, so the promoter is active during spermatogenesis.

Epididymis Tubule Sections

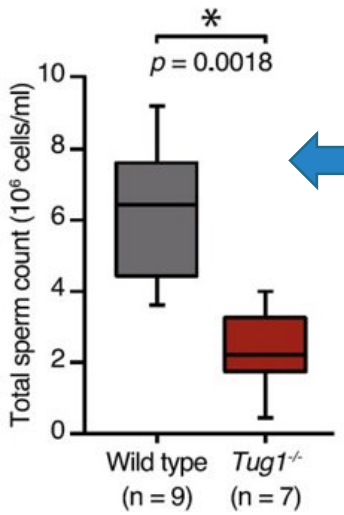


Morphological differences between wild type and KO Tug1 mice tissues.

Conclusion:
The morphological defects of the KO contribute to the sterility phenotype.



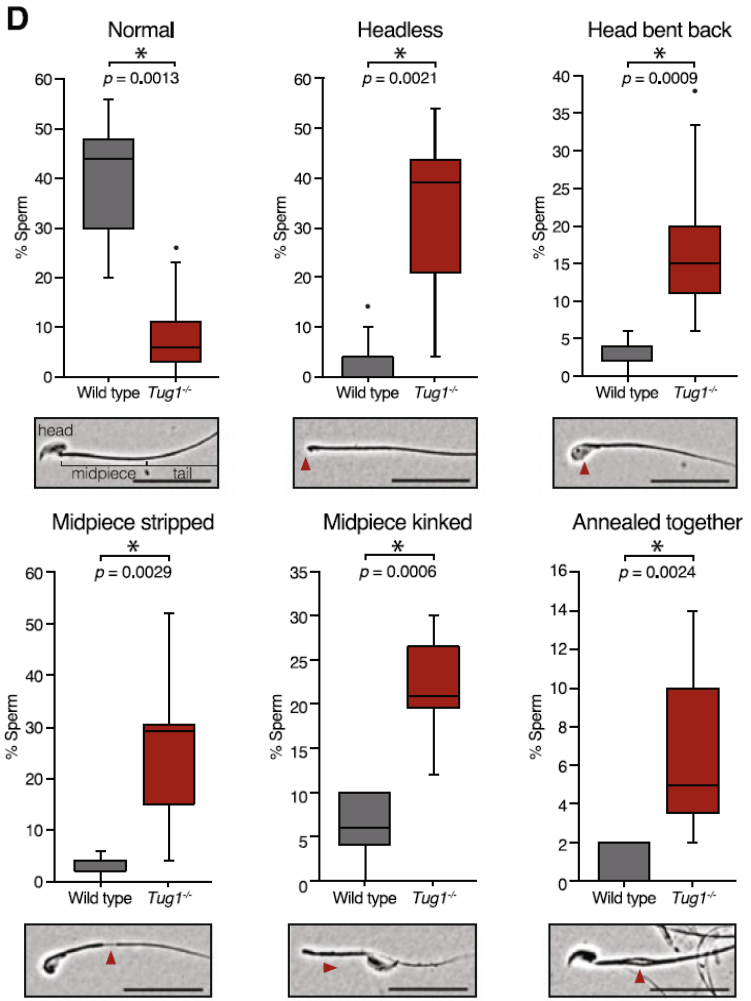
Sperm count & morphology of Tug1–/– males



Oligozoospermia

No Azoospermia

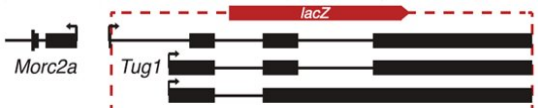
Morphological defects in sperm are called **teratozoospermia**

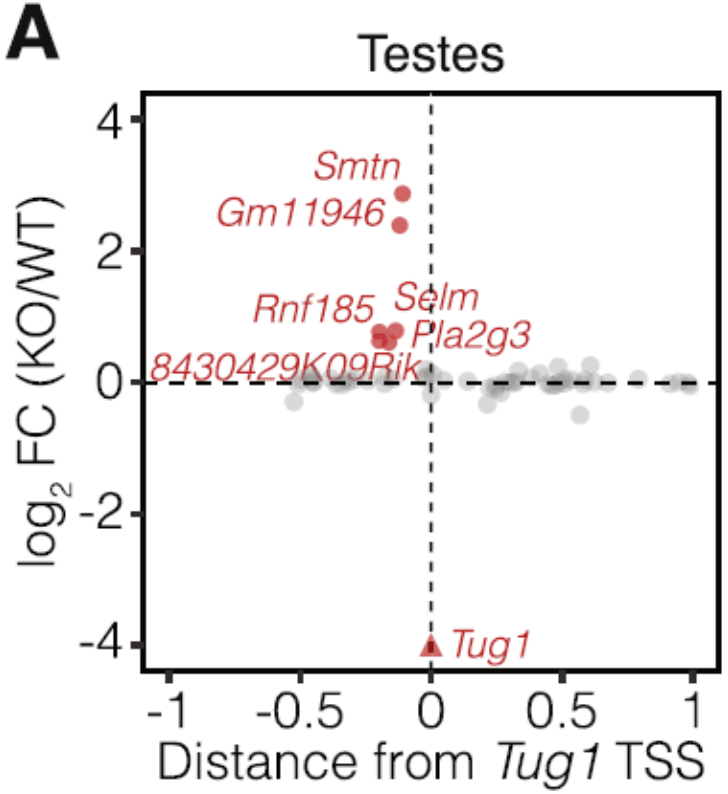


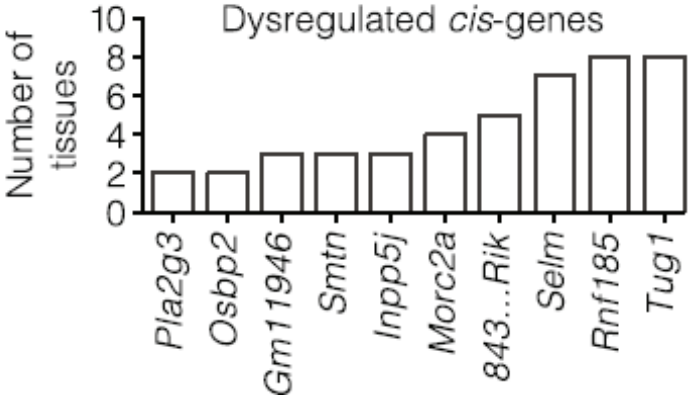
Conclusion: The sterility phenotype arises from a combination of oligozoospermia and teratozoospermia.

CIS-ACTING ROLE OF TUG1

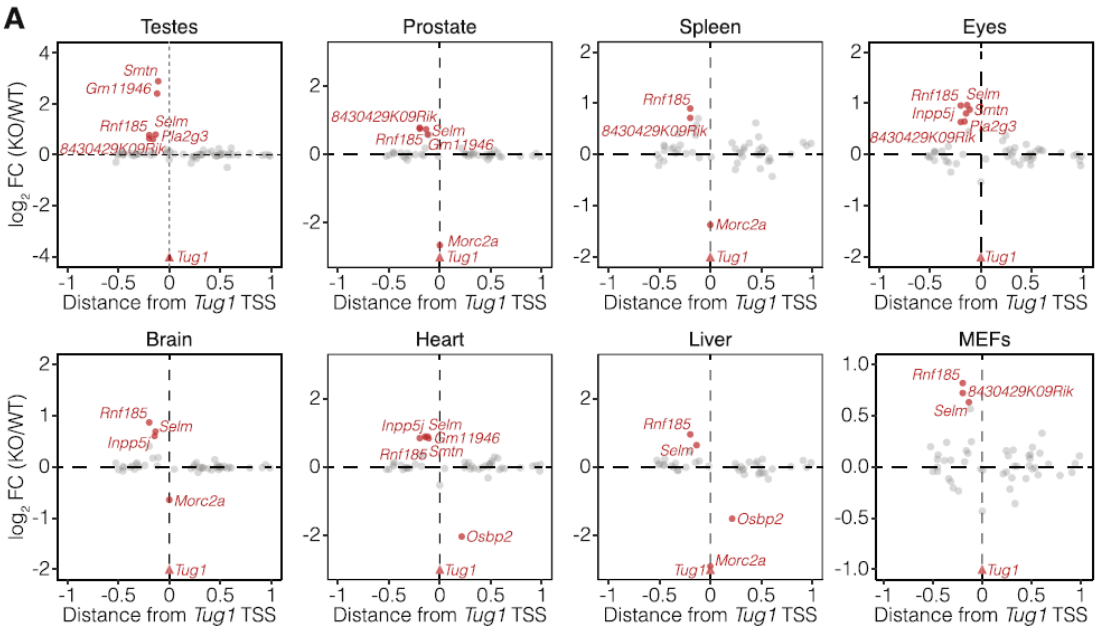
1. Does Tug1 locus affect local genes?





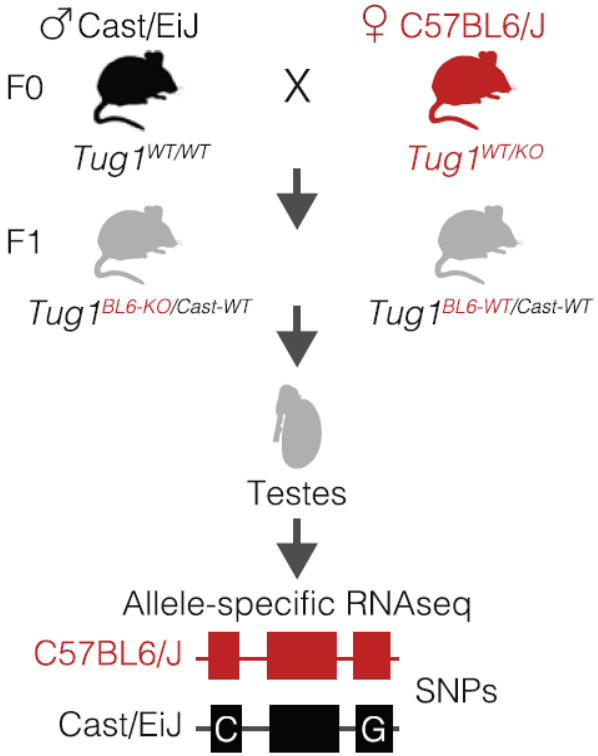


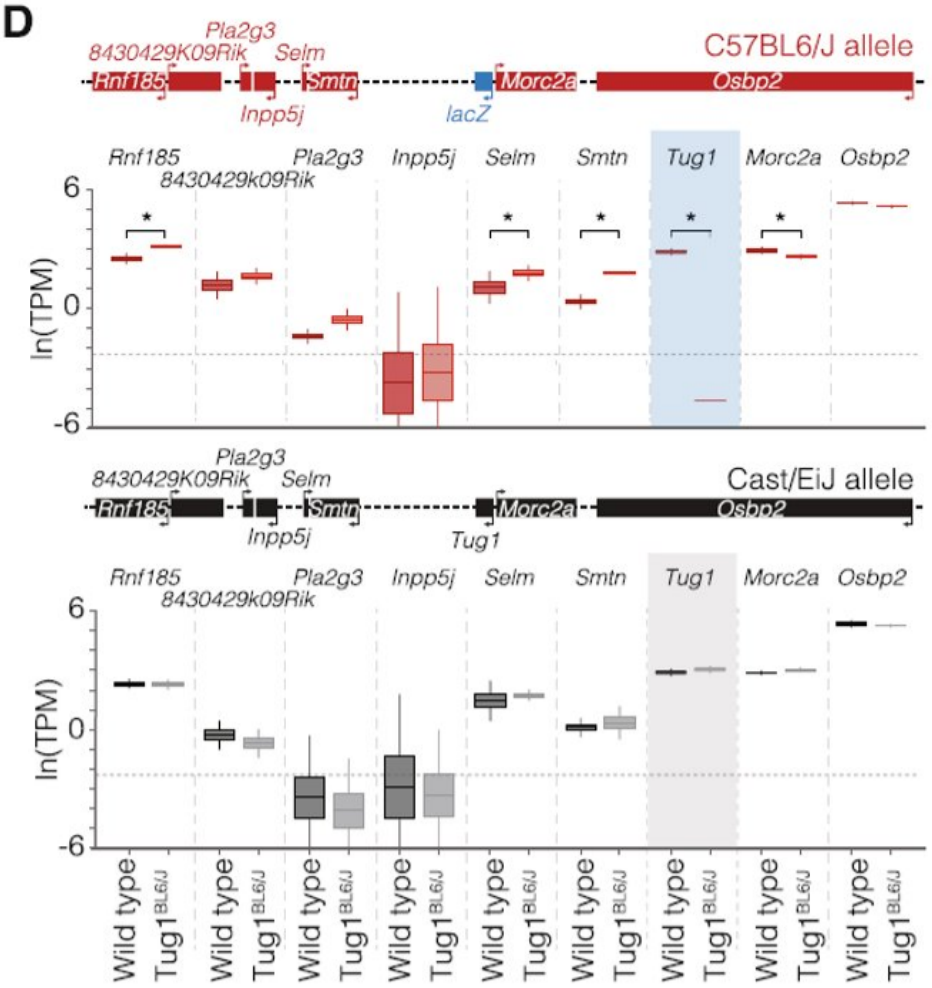
Conclusion: Tug1 loci regulates neighboring genes in many tissues.



CIS-ACTING ROLE OF TUG1

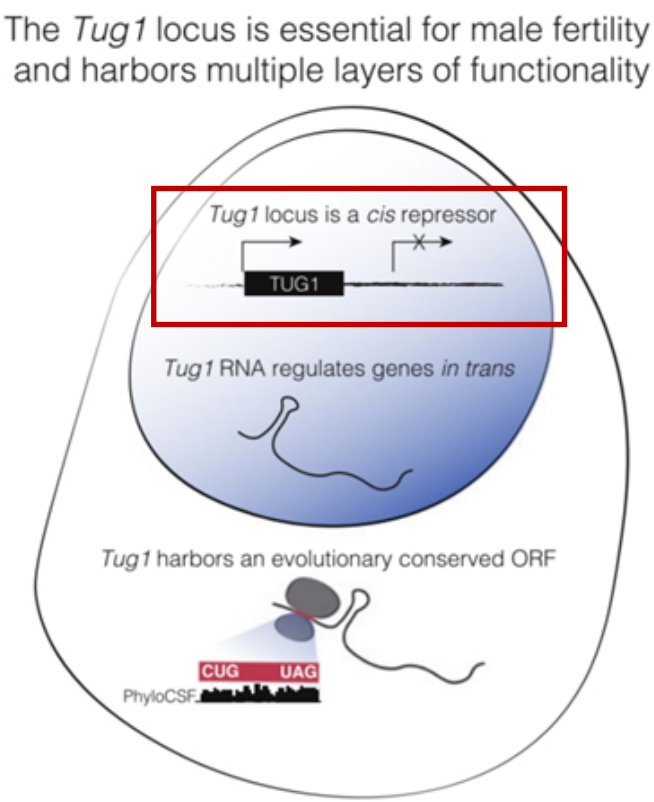
2. Does the repressive effect happens on the same allele?

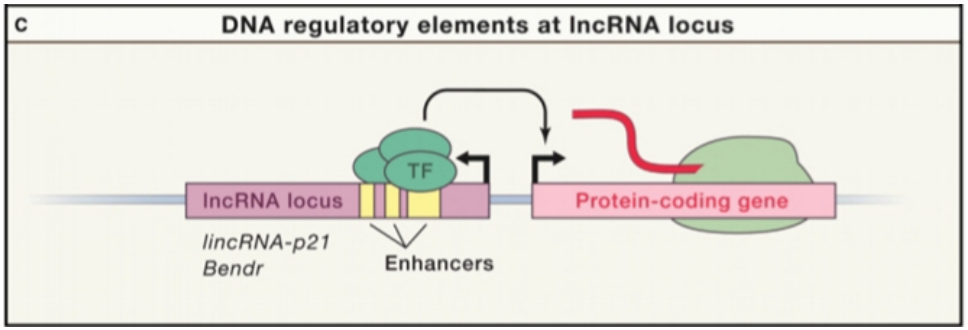


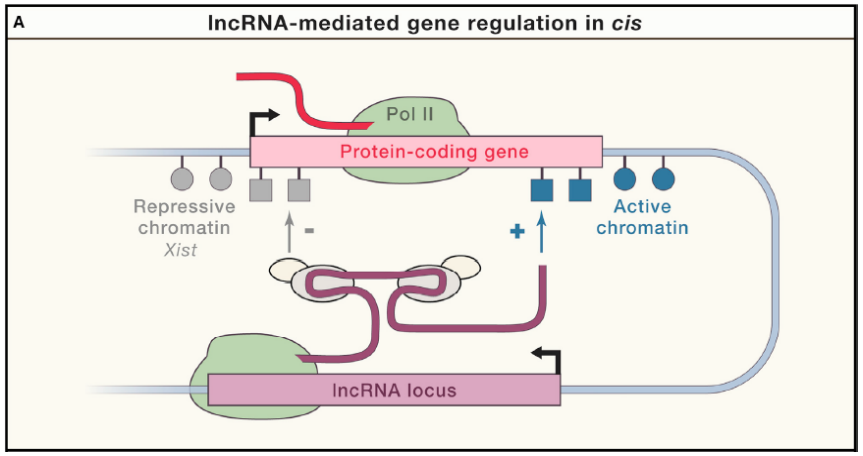


Conclusion: There is an haplotype-specific regulation

Tug1 locus is a cis repressor







But....They cannot exclude a functional transcript!

TRANS-ACTING ROLE of TUG1

3. Does the deletion of Tug1 locus affect distant genes?

☐

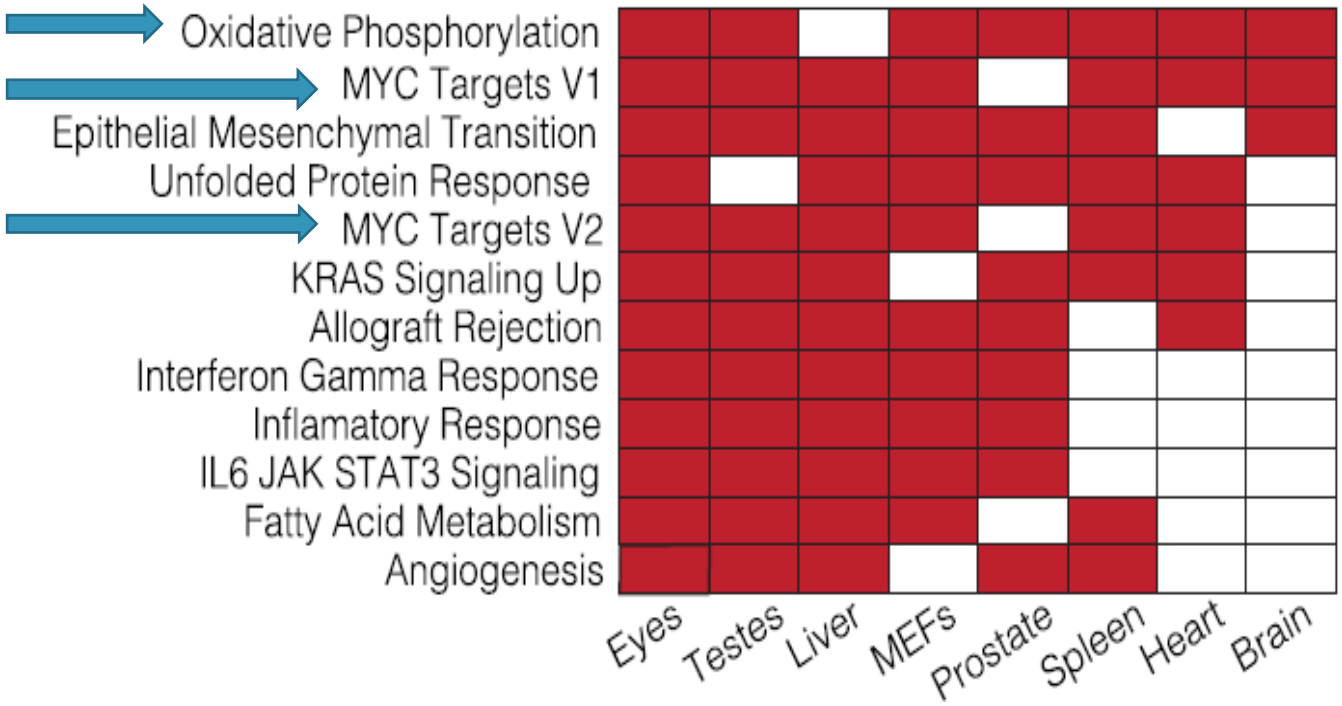
RNA-seq data from WT and *Tug1-/-*

Tug1 deletion causes a general dysregulation of gene expression in many tissues

→ **Hypothesis**: Tug1 can have a *trans-acting role* in gene regulation

TRANS-ACTING ROLE of TUG1

Several pathways are dysregulated in Tug1-/- tissues



TRANS-ACTING ROLE of TUG1

Rescue experiment

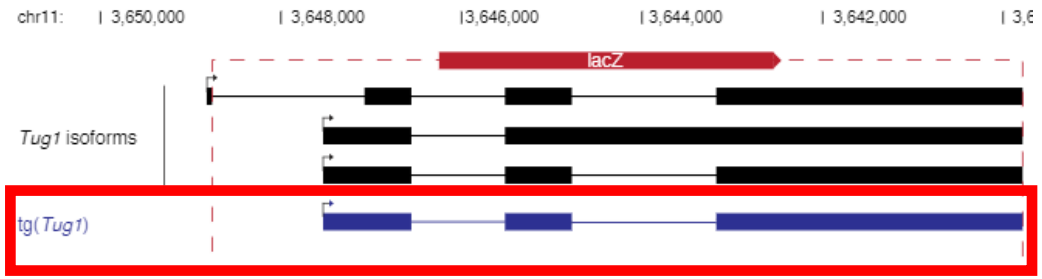
□

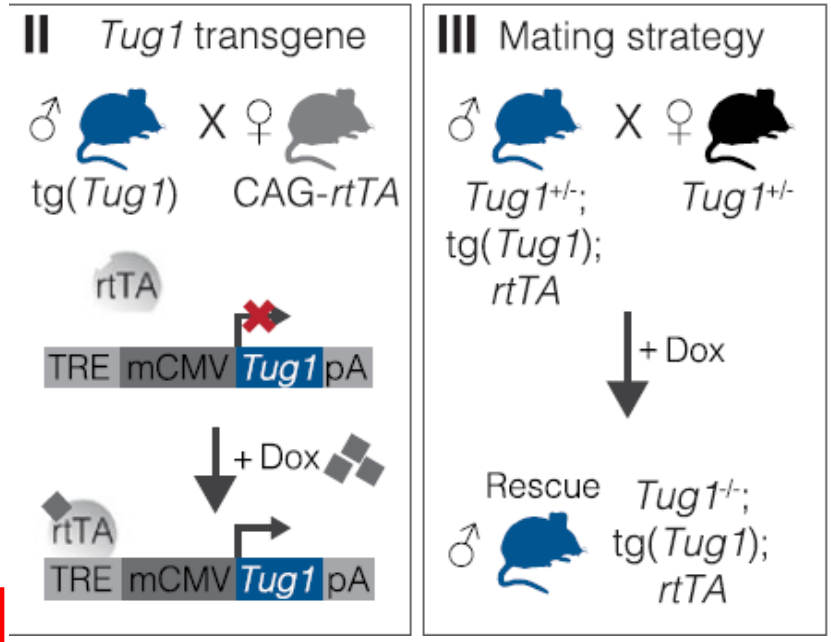
Tet-On system: □

•5'region absent Tug1 isoform□ [tg(Tug1)]

•doxycicline-controlled Tug1 induction in Tug1-/- mice □

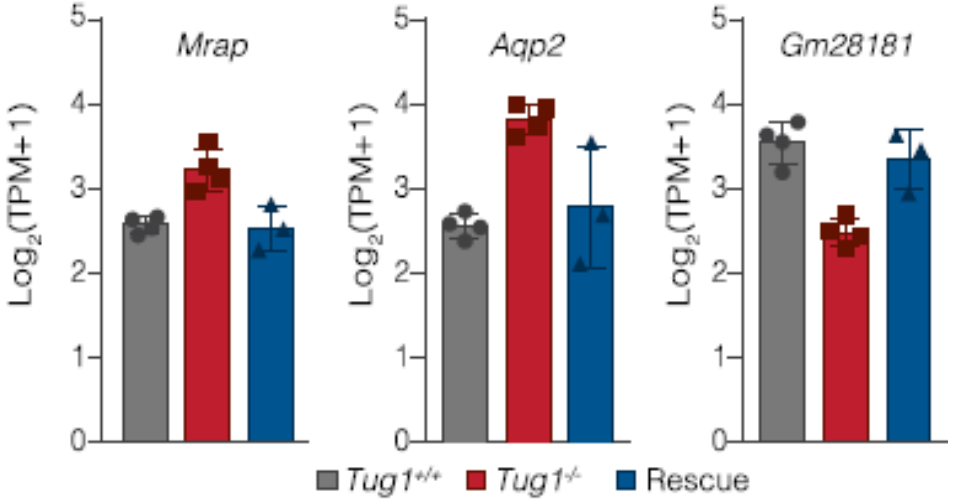
□

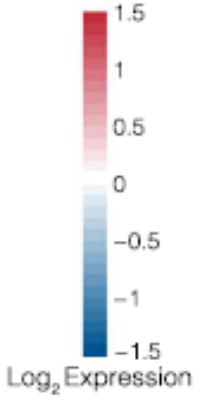




TRANS-ACTING ROLE of TUG1

4. Does the ectopic expression of Tug1 recover the dysregulation occuring in Tug1-/-?





Conclusion: Tug1 rescue recapitulates wild-type expression in many distant genes

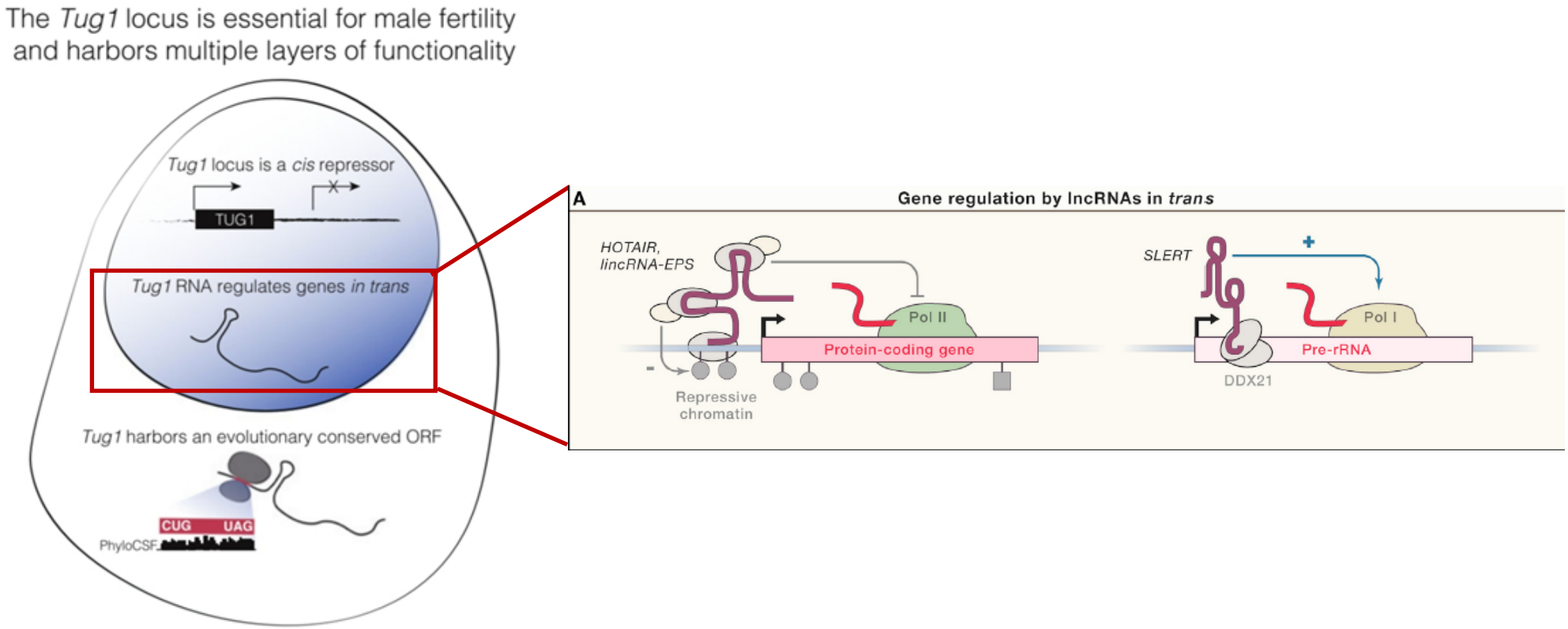
5. Is Infertility phenotype recovered in $Tug1^{rescue}$?

$Tug1^{rescue}$ male mice are still sterile.

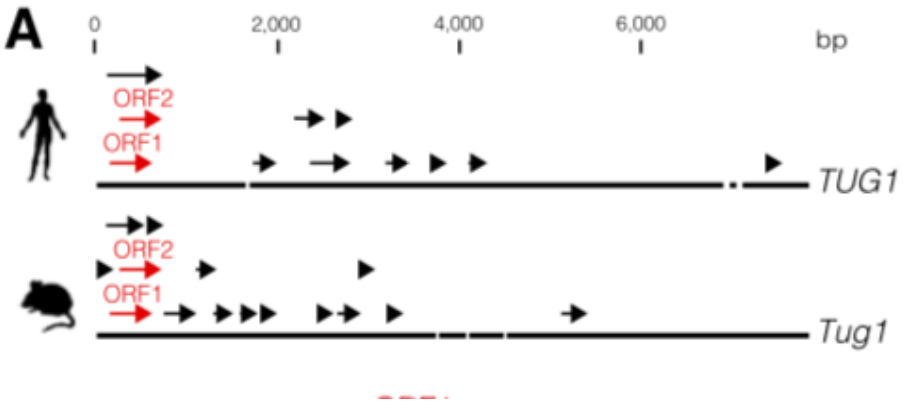
Hypothesis:

- Used Tug1 RNA isoform
- low level of Tug1 expression from the transgene in the testes
- LncRNA Tug1 transcript doesn't impair reproduction capabilities

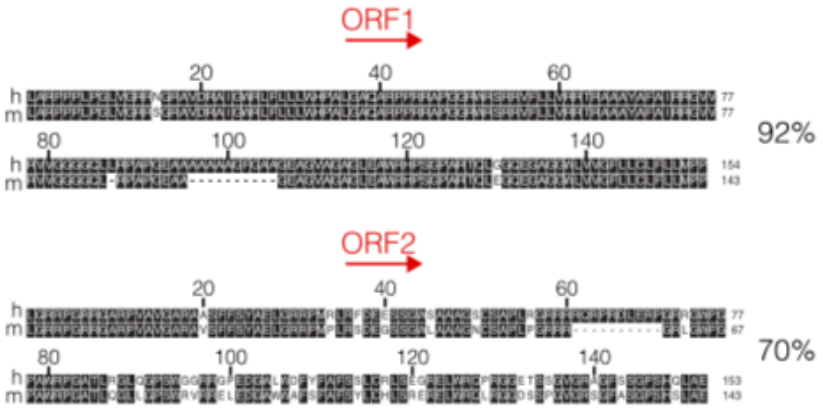
TRANS-ACTING ROLE of TUG1



Tug1 locus Contains a Conserved ORF in Human and Mice

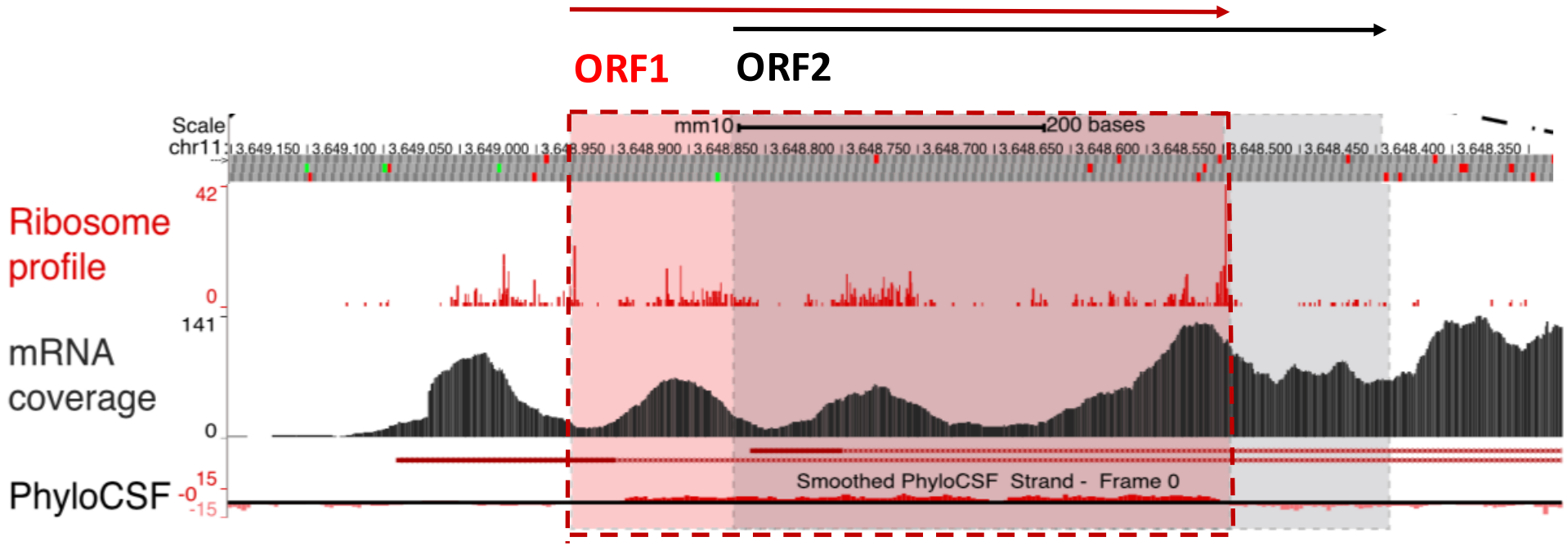


Multiple short ORFs in the human and mouse TUG1/*Tug1*.



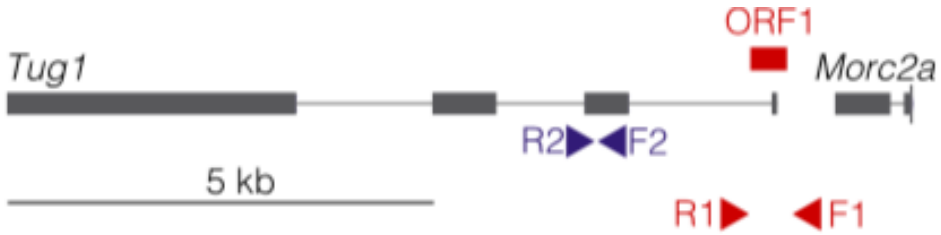
ORF1 and ORF2 share 92% and 70% cross species-identity respectively.

Ribosme Occupancy, RNA-seq and PhyloCSF across the Tug1 Locus in MEFs

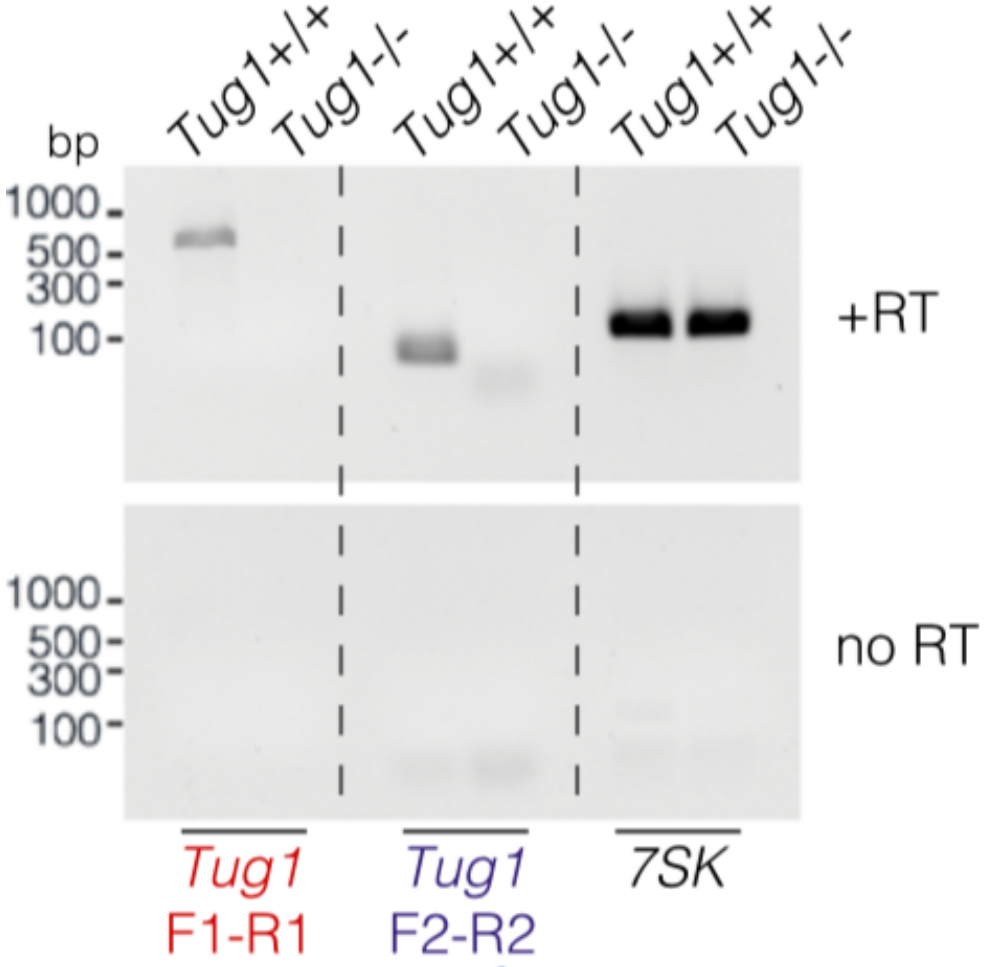


sORF in Tug1 lncRNA

6. Is the transcript containing ORF1 present in the Testes ?

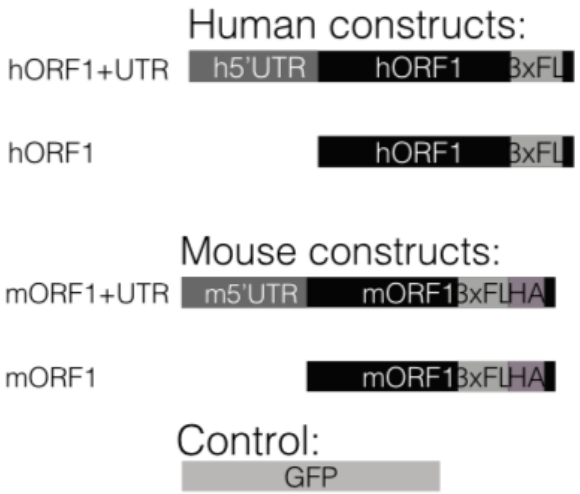


RT-PCR on wilde type and *Tug1* -/- testes.

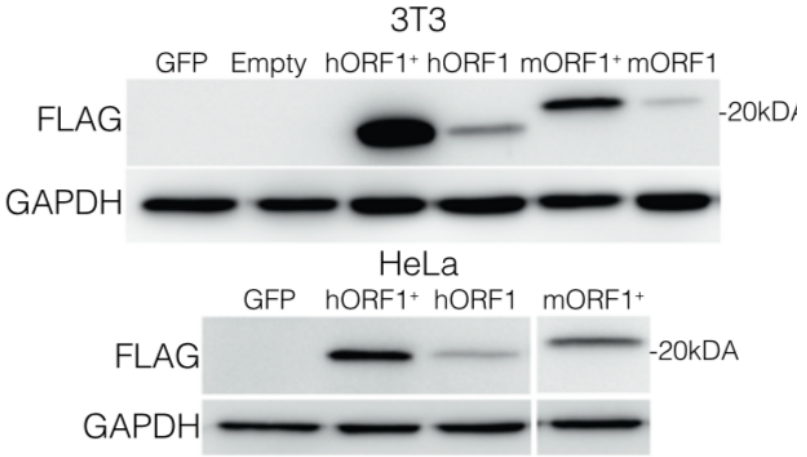


Conclusion: the RT-PCR confirm the presence of ORF1's transcript in the testes in WT samples.

7. Can TUG1-BOAT produce a stable protein?



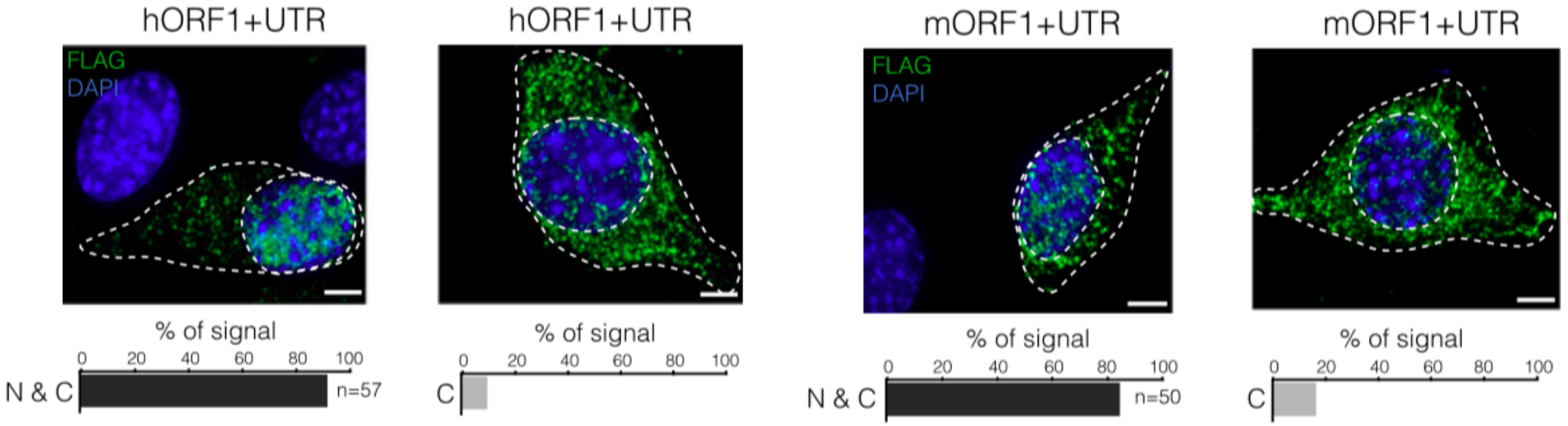
Human and mouse ORF1 construct design.



Western-Blot analysis to test TUG1-BOAT translation in 3T3 and HeLa cells.

Conclusion: thanks to WB there is evidence of the presence of a stable protein.

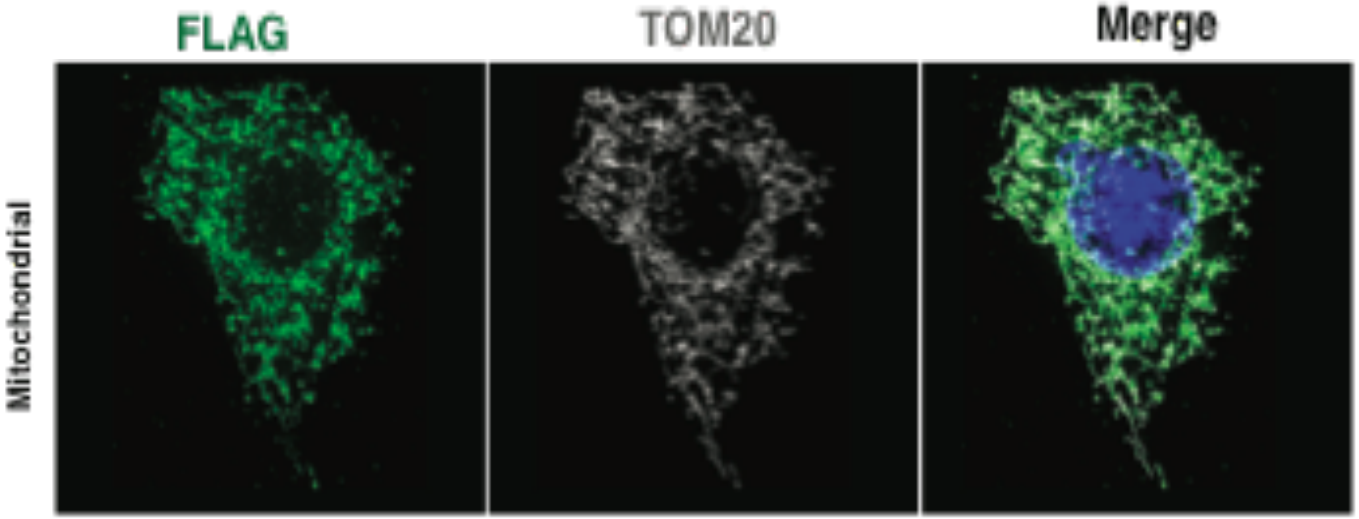
Immunostainig of 3T3 cells Express Human and Mouse Construct



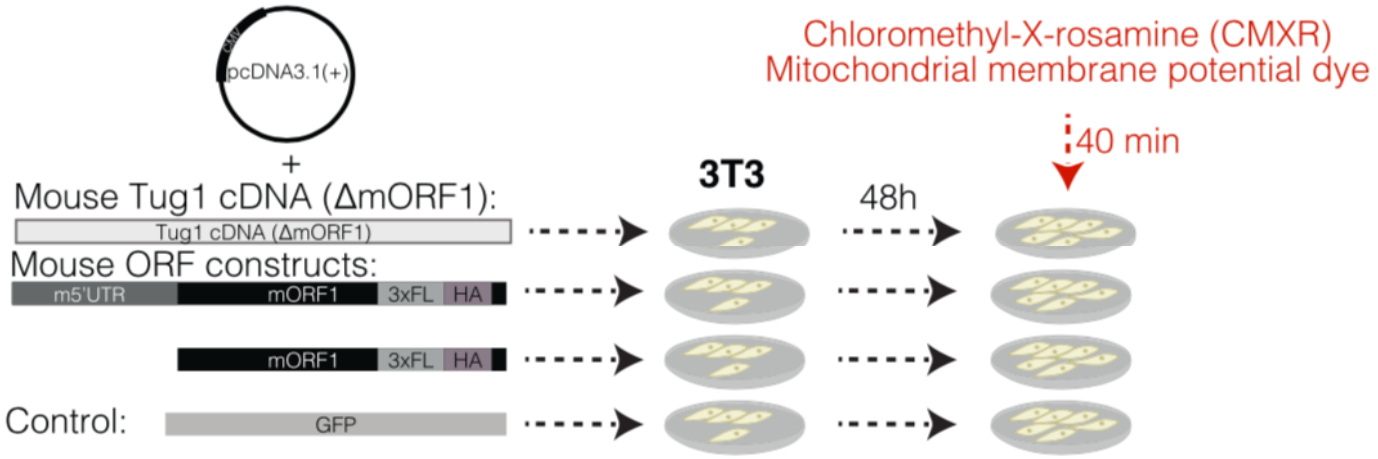
Nuclear and citoplasmatic localization of TUG1-BOAT.

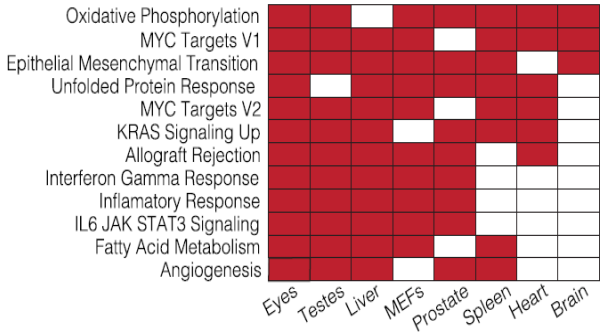
TUG1-BOAT co-localizes with the mitochondria

TUG1-BOAT FLAG-tagged (green) co-localized with mitochondrial membrane translocase TOM20 (gray).



8. Has Tug1-BOAT a role in the Mitochondria?





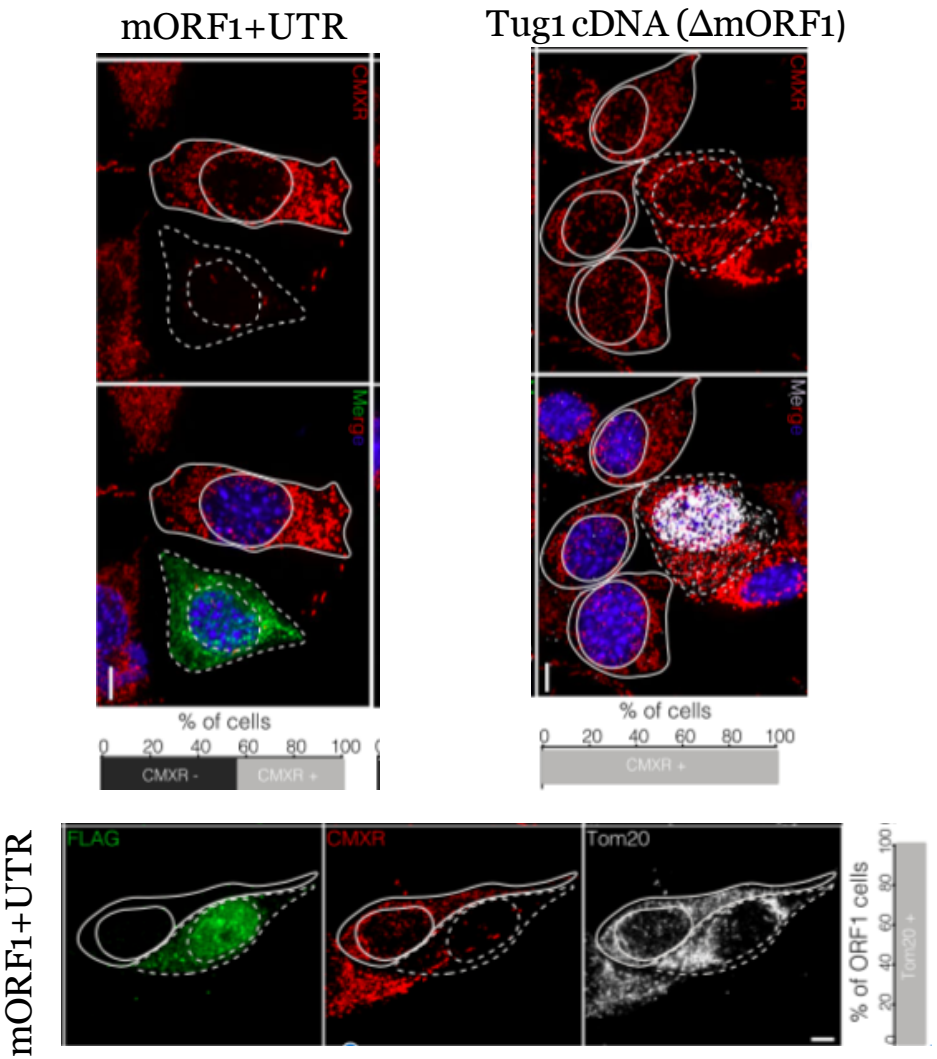
Construct of human and mouse Orf1, mouse Tug1 cDNA (ΔmORF1) inserted into pcDNA3.1.

sORF in Tug1 lncRNA

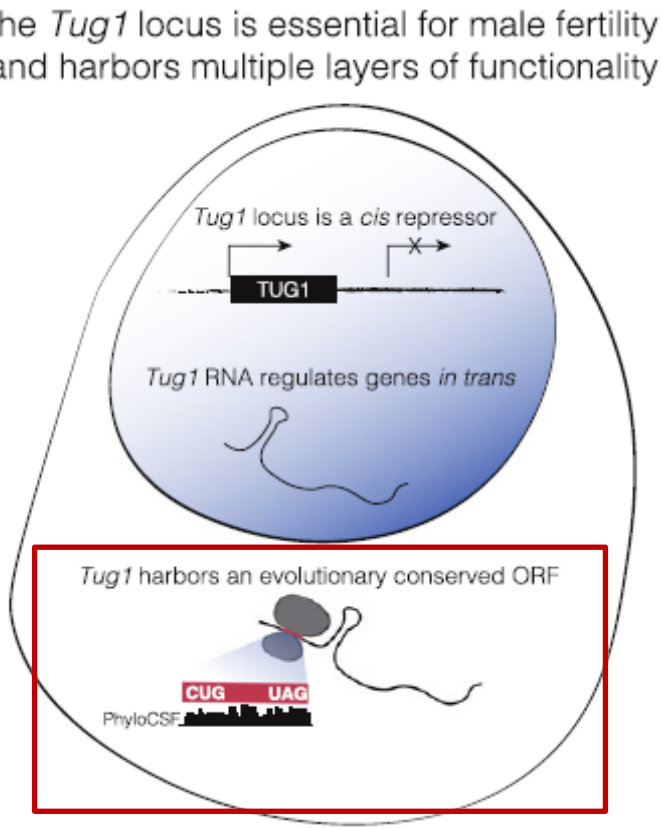
48h post-transfection
3T3 images analysis

DAPI
Flagged TUG1-BOAT
Tug1 RNA FISH
CMXR, mito-membrane potential stainig
TOM 20, mitochondrial translocase

Conclusion: the overexpression of TUG1-BOAT show a reduction in mitochondria staining by CMXR



Tug1 locus harbors a conserved ORF that could be translated into a stable protein



Our Hypothesis

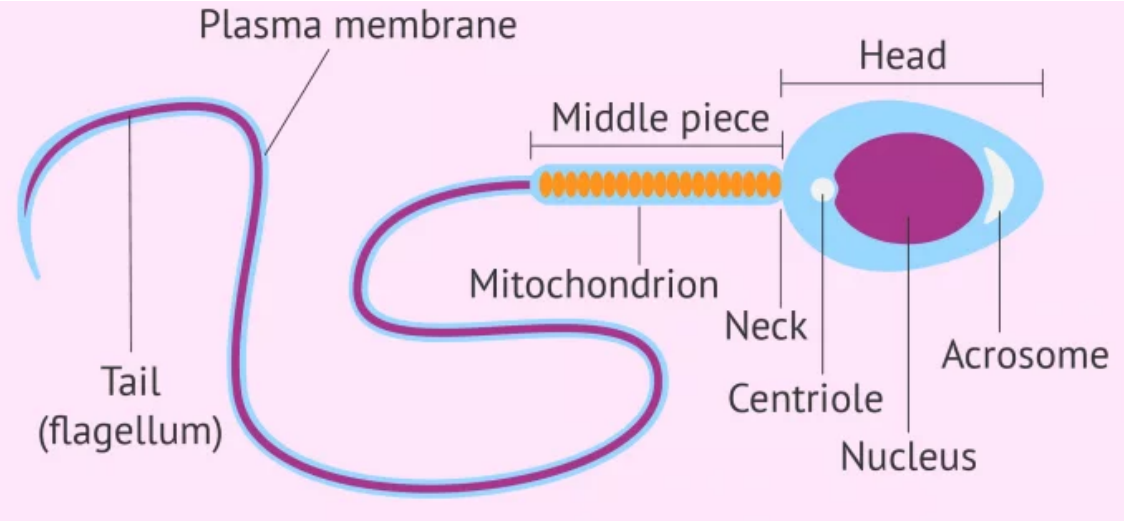


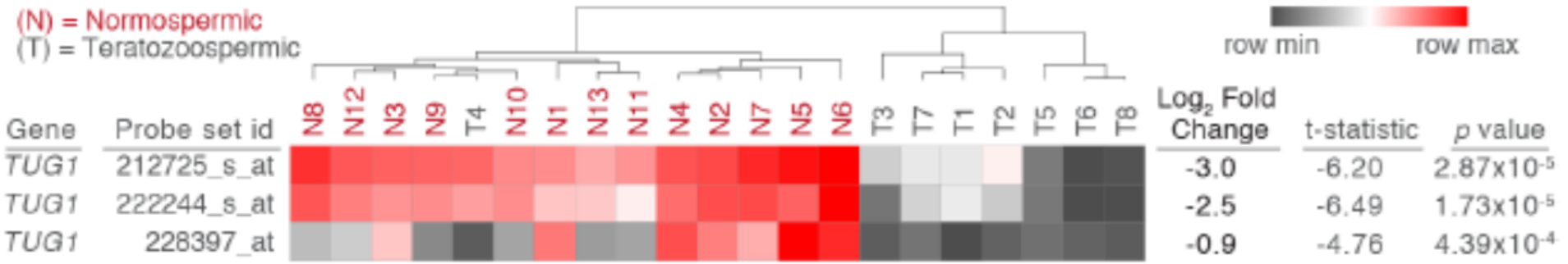
Image: sperm structure. Retrieved from https://www.invitra.com/en/sperm-cell/

- As we have seen so far Tug1 locus also has an effect at the mitochondrion level.
- We know that mitochondria are the major organelle in sperm cells.

Could there be a connection?

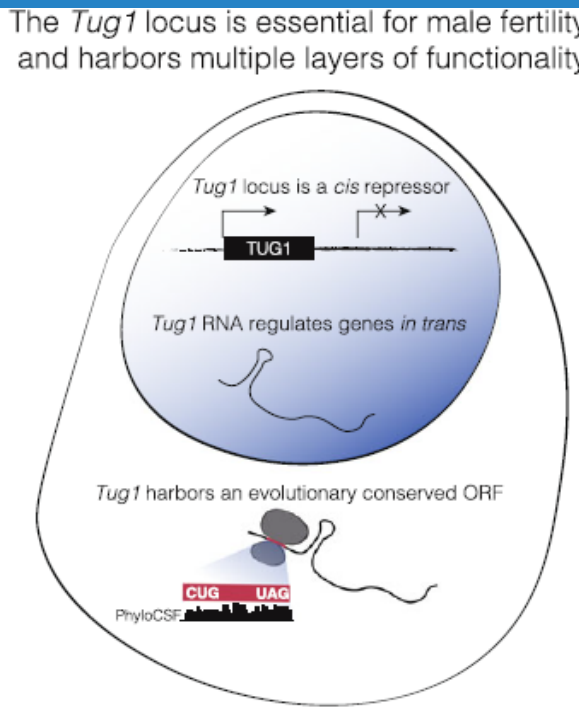
- Maybe the loss of Tug1 locus might also have an impact on sperm's mitochondria contributing to sterility phenotype. Further studies are needed.

Further studies



Heatmap of microarray data showing decreased expression of TUG1 in sperm from infertile teratozoospermic men.

Conclusions



Tug1 locus has an essential role in male fertility.

Tug1 locus harbors three molecular activities:

- cis DNA repressor
- trans-acting lncRNA
- potential coding capacity

Thank you for the attention

References

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Summary

- The deletion of the Tug1 locus in mice leads to male sterility.
- The Tug1 locus harbors two distinct non-coding activities:
  1. cis DNA repressor.
  2. trans-regulatory role of lncRNA.
- The dysregulated gene expression program in Tug1-knockout testes can be partially rescued by ectopic expression of Tug1 RNA in vivo, but not the fertility phenotype.
- The Tug1 locus contains an evolutionarily conserved ORF, which can be translated into a stable protein (TUG1-BOAT) that may impacts mitochondrial membrane potential upon overexpression.