Topaz1 suppression disrupts the expression of testicular long non-coding RNAs during murine spermatogenesis

Manon Chadourne, Elodie Poumerol, Bruno Passet, Luc Jouneau, Eric Pailhoux, Béatrice Mandon-Pepin

Topaz1 suppression disrupts the expression of testicular long non-coding RNAs during murine spermatogenesis.

Chadourne Manon, Poumerol Elodie, Passet Bruno*, Jouneau Luc, Pailhoux Eric and Mandon-Pépin Béatrice
Université Paris-Saclay, UVSQ, INRAE, BREED, 78352, Jouy-en-Josas, France.
*Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France.
manon.chadourne@inrae.fr / beatrice.mandon-pepin@inrae.fr

Background of Topaz1: Topaz1, for Testis and Ovary specific PAZ domain gene 1, has been characterized in our lab as a new germ cell specific gene highly conserved in vertebrates with a role during gametogenesis of mammals[1]. To study the role of this gene, a line of Topaz1-depleted mice has been generated. Thus, whereas female mice are fertile, homozygous male mutants are infertile[2]. Abnormal meiosis occurs in testes Topaz1$^{-/-}$ mice causing cell death and an absence of spermatozoa. Previous transcriptional study of Topaz1$^{-/-}$ mice by microarray comparative analysis highlighted the significant proportion of deregulated long non-coding RNA (lncRNA) in mutant testis.

The aim of this work is to study (1) the whole transcriptome of Topaz1$^{-/-}$ mouse testes and (2) the involvement of lncRNA in murine spermatogenesis.

Methodologies :

- RNA sequencing (RNA Seq) to identify all deregulated genes in Topaz1$^{-/-}$ testis whose lncRNA.
- Localization by *In Situ* hybridization (ISH) of some deregulated lncRNA.
- Generation of a new line of knockout mice (CrispR/Cas9 technology) without one of these lncRNA → Studies of the phenotype.

1

Biotype of differential expressed genes between Topaz1$^{-/-}$ and WT testes (Adj-p<0,05)

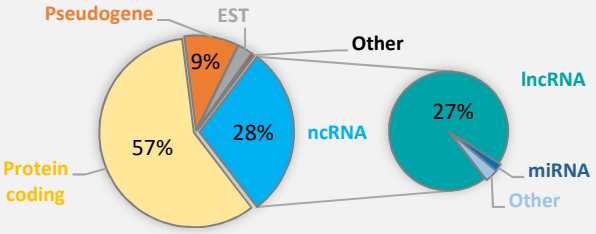


Fig.1. Overview of 2748 deregulated transcripts in P18.5 testes lacking Topaz1. 91% (2491) are down-regulated; 9% (257) are up-regulated. A large proportion (27%) of deregulated genes are lncRNAs.

2

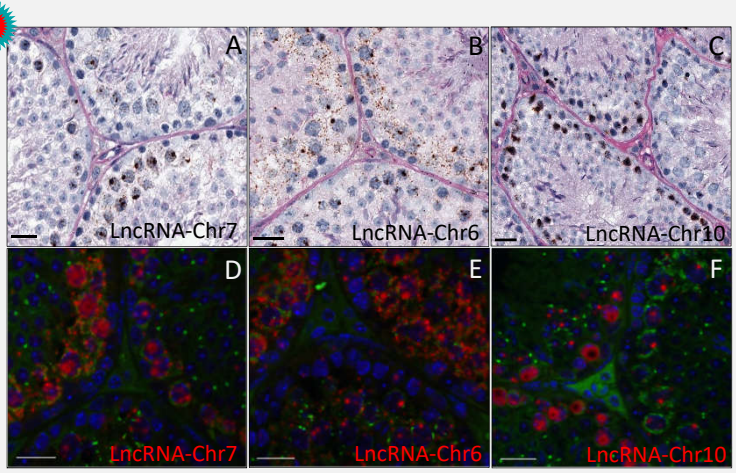


Fig.2. **A-C**: ISH *(RNAscope® Brown)* of different deregulated lncRNAs: LncRNA-Chr7(A), LncRNA-Chr6 (B) and LncRNA-Chr10 (C) in 2 months-old WT testis section. **D-F** : Merge images showing ISH with lncRNA (red) probes, immunodetection of VASA (DDX4) (green) and DAPI staining (blue).

→ LncRNAs are expressed in different spermatocytes depending on the stage of the seminiferous epithelium: lncRNA-Chr10 seems nuclear whereas lncRNA-Ch6 cytoplasmic. LncRNA-Chr7 looks as if located in both cytoplasm and nuclei. *Scale bar = 20µm*

3

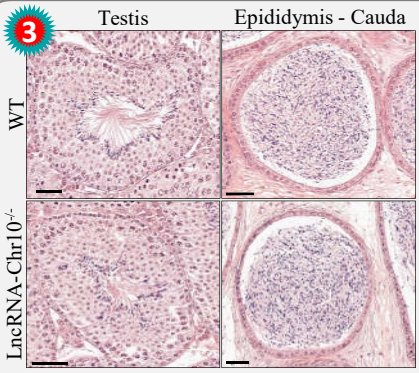
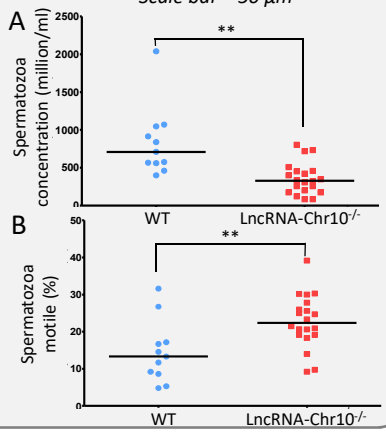
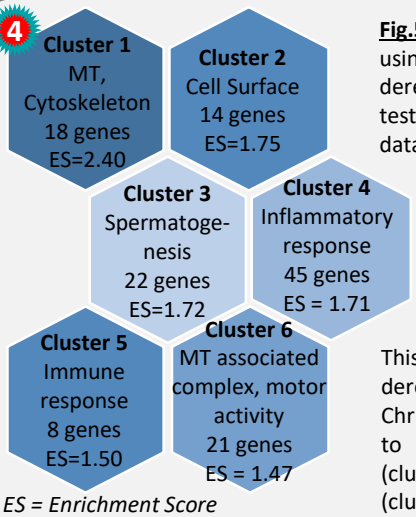


Fig.3. Histological analysis of 2 month-old testis showed the presence of spermatozoids in the lumen of the seminiferous tube and in the cauda of epididymis in both WT and lncRNA-Chr10$^{-/-}$ mice.
→ LncRNA-Chr10$^{-/-}$ mice are fertile with a normal progression of spermatogenesis.
Scale bar = 50 µm

Fig.4. Evaluation of epididymal spermatozoa concentration (A) and motility (B) show significantly differences between WT and lncRNA-Chr10$^{-/-}$ mice.
→ LncRNA-Chr10$^{-/-}$ mice have a 57% decrease of spermatozoa concentration and motility was significantly higher.
** : pvalue<0,01



4



ES = Enrichment Score

Fig.5. Functional annotation clustering using DAVID analysis with 206/258 deregulated genes in lncRNA-Chr10$^{-/-}$ testes compared to WT (from RNAseq data of P18.5 testes; data not show).

→ Despite the absence of noticeable phenotype in lncRNA$^{-/-}$ mouse line, transcriptomic analysis of P18.5 testes indicated that 6 gene clusters are significantly deregulated (ES >1,3).

This higlighted the biological process deregulated in absence of the lncRNA-Chr10 with the most interesting referred to microtubule (MT) base process (cluster 1 and 6) and spermatogenesis (cluster 3).

Conclusion :

Number of LncRNA expressed in the testes is significant. In Topaz1 null mice, with male meiotic arrest, a large number of testicular lncRNA are significantly deregulated (Fig1). Three of them were observed by ISH showing different localisations into spermatocyte cells, suggesting distinct roles during spermatogenesis (Fig2). To determine a potential spermatogenetic role, a new mouse line deleted of one of them (LncRNA-Chr10, expressed in nuclear germ cells) has been created. These mice are fertile in both sexes (Table 1) and spermatogenesis of mutant takes place normally (Fig3). However, they have a decrease of epididymal spermatozoa concentration and an increase of spermatozoa motility (Fig5). Moreover, transcriptomic analysis of P18.5 lncRNA-Chr10$^{-/-}$ testes (by RNAseq) shows more than 250 deregulated genes involved particularly in cytoskeleton and spermatogenesis (Fig5).
→ Thus, although LncRNA-Chr10, expressed exclusively in the testes with an expression linked with Topaz1 expression, its depletion does not appear crucial for fertility in mice. The lncRNA-Chr10 seems to play role in spermatozoa motility and concentration. This lncRNA may regulate protein coding gene expression especially genes in relation to the cytoskeleton.

References *: (1) Baillet A, et al. PLoS One. 2011;6(11):e26950; (2) Luangpraseuth-Prosper A, et al. Dev Biol 2015;406:158-171.*