# RNA-mediated heredity of an acquired pathology

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# RNA-mediated heritable controls of transcriptional activity

After the first experimental models of epigenetic inheritance, starting with **the plant paramutation** (Brink, 1956), and now characterized as 'heritable gene silencing',

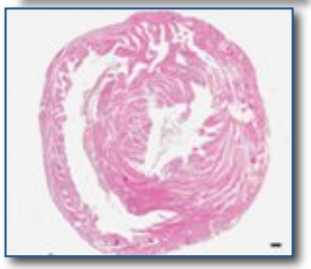
our laboratory reported in the recent period three examples of **RNA-mediated epigenetic heritable variations** corresponding to **modulation in transcriptional activity**

Their analysis led us to conclude that non-coding RNA molecules carried by the gametes, **sperm RNA in the case of paternal transmission**, may act as transgenerational determinants of epigenetic states

# The mouse paramutations: epigenetic variations inherited in a non-Mendelian manner

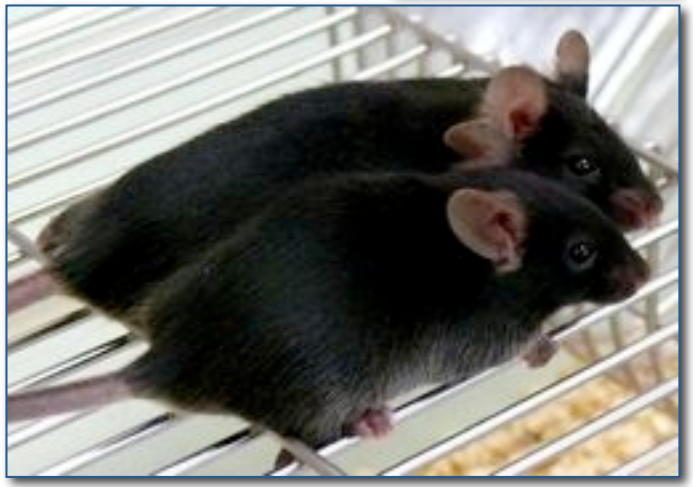
⇒ Kit locus : fur color, tumoral developments, diabetes

Rassoulzadegan et al. Nature, 2006



⇒ Cdk9 locus: cardiac hypertrophy

Wagner et al. Dev Cell, 2008

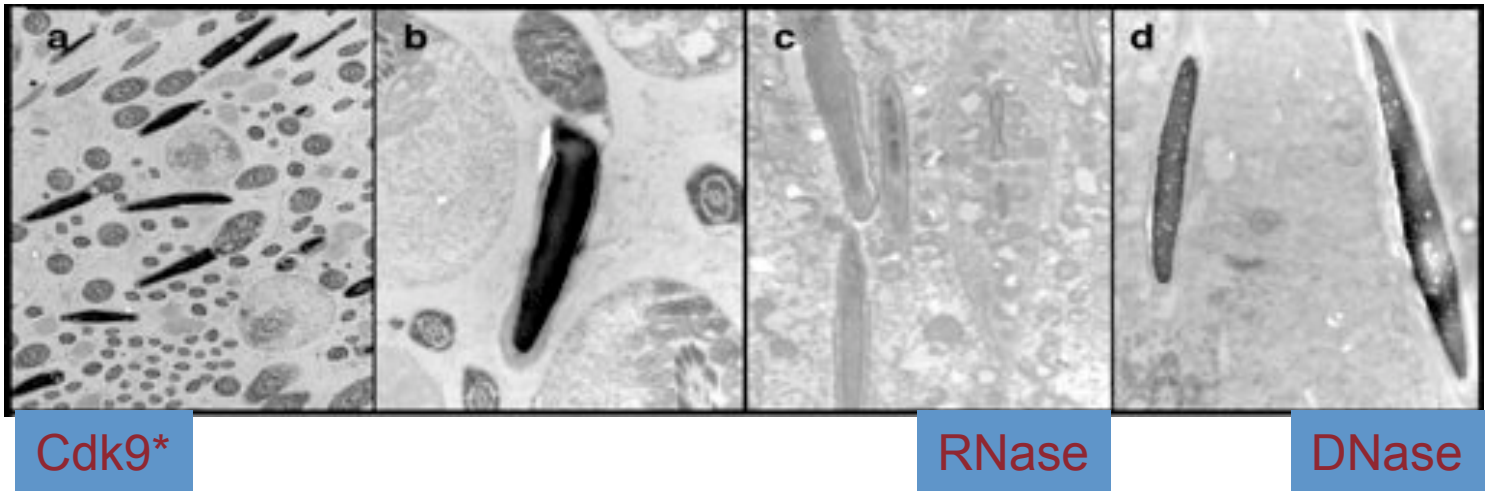


⇒ Sox9 locus: gigantism and gemellity

Grandjean et al. Development, 2009

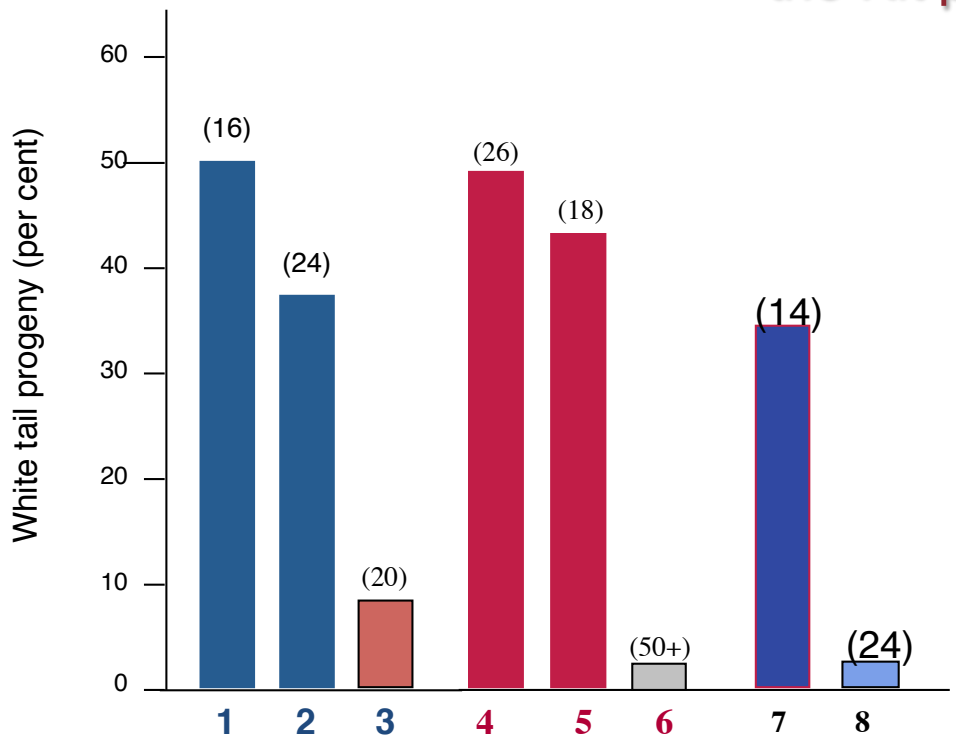
# Sperm RNA, transgenerational signal of the epigenetic variation

Since its discovery by S.Krawetz and colleagues (Ostermeier et al., Nature, 2004), the possible functions of sperm RNA had remained essentially a matter of speculation.




Reverse EDTA staining of epididymis sections (courtesy of P. Gounon)

# RNA microinjection in fertilized eggs: the Kit paramutation




1: heterozygote sperm
2: heterozygote brain
3: wild type brain

4: miR221
5: miR222
6: miR-1, miR-124, …

7: Kit oligonucleotide
8: unrelated (Cdk9) oligonuclleotide

⇒ Microinjection in fertilized normal eggs of RNA prepared from sperm and somatic cells of heterozygotes induces the heritable white-spotted phenotype

⇒ MicroRNAs 221 and 222, which target *Kit* RNA, are also efficient inducers of the paramutated phenotype

⇒ Oligoribonucleotides with randomly chosen sequences from the Kit coding sequences induce as well

# Common features to the three known instances of mouse paramutation

1. RNA induction

The epigenetic change was initiated

- In heterozygous with one allele inactivated by a Lac or GFP insert (Kit)
- In one-cell embryos after microinjection of fragments of the transcript (20-30nt oligonucleotides) (all three instances)
- In one-cell embryos after microinjection of the cognate microRNAs

=> presence of abnormal RNAs with sequence homology to the transcript

=> presence of abnormal RNAs in spermatozoon ?

# Part I

# A role of RNA methylation in epigenetic heredity

## A requirement for the methyltransferase Dnmt2

Kiani, J., Grandjean, V., Liebers, R., Tuorto, F., Ghanbarian, H., Lyko, F., Cuzin, F., Rassoulzadegan, M., RNA-mediated epigenetic heredity requires the cytosine methyltransferase Dnmt2, *PLoS genetics* 9, e1003498 (2013).

# Dnmt2, the enigmatic methyltransferase

- Dnmt2 is a member of the family of the DNA methyltransferases
- DNA methylation activity is either weak or absent
- RNA methylation was established by several groups with tRNAs identified as substrates
- Its functions are still a matter of discussion

# Pedigree analysis in the progeny of Kit heterozygotes

*Kit* $^{tmlAlf1/+}$ *Kit* $^{+/+}$

(4 crosses)

*Kit* $^{tmlAlf1/+}$ (16)

*Kit* $^{+/+}$ * (24)

*Kit* $^{+/+}$ (4)

*Dnmt2*$^{+/+}$ mouse

*Kit* $^{tmlAlf1/+}$ *Kit* $^{+/+}$

(8 crosses)

*Kit* $^{tmlAlf1/+}$ (47)

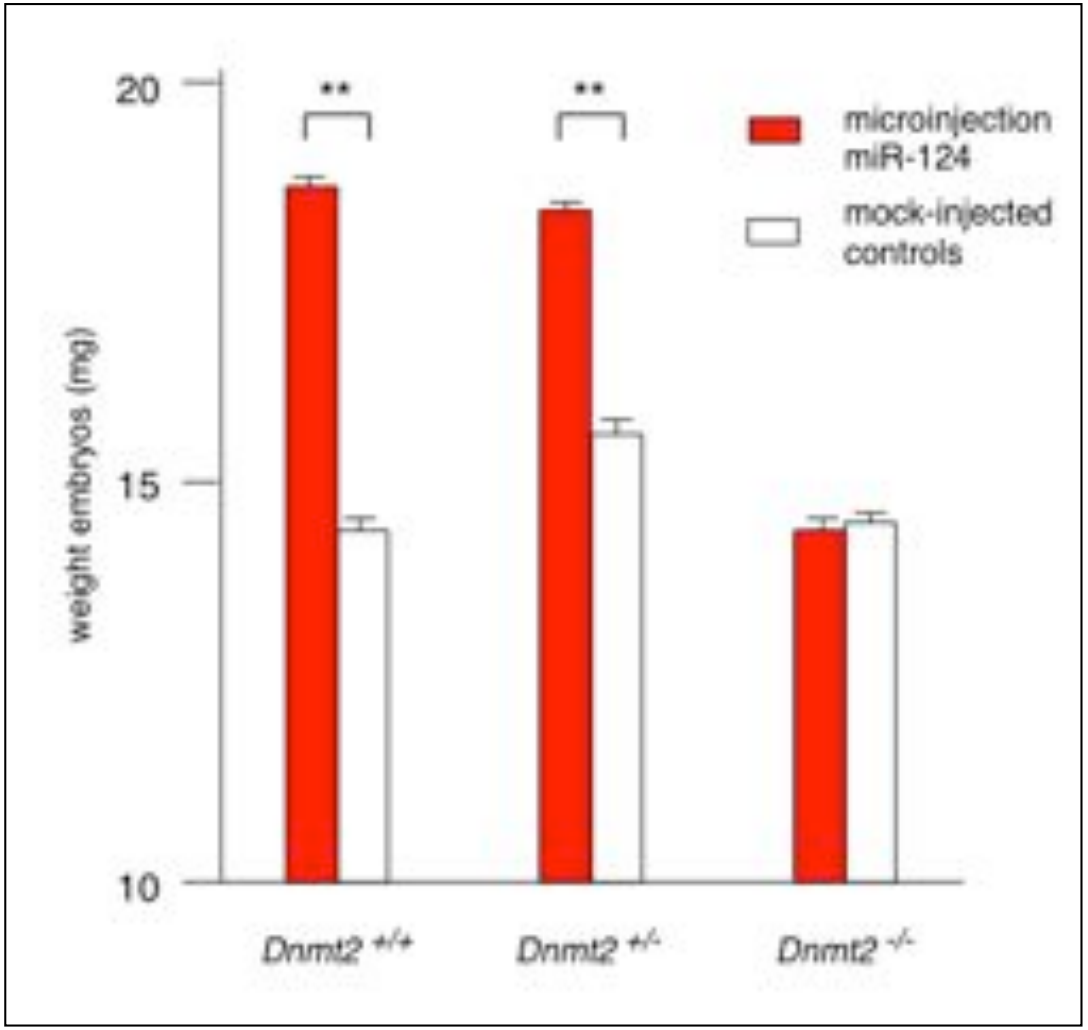
*Kit* $^{+/+}$ * (0)

*Kit* $^{+/+}$ (35)

*Dnmt2*$^{-/-}$ mouse

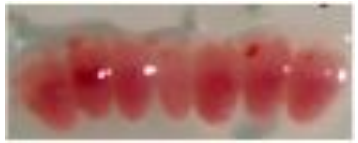
# Sox9* induction by microinjection of miR-124

E7.5 embryos after miR-124 microinjection




***Dnmt2 +/+***

***Dnmt2 -/-***



***Dnmt2 -/-***

***12-day mice*** ***Dnmt2 +/+***

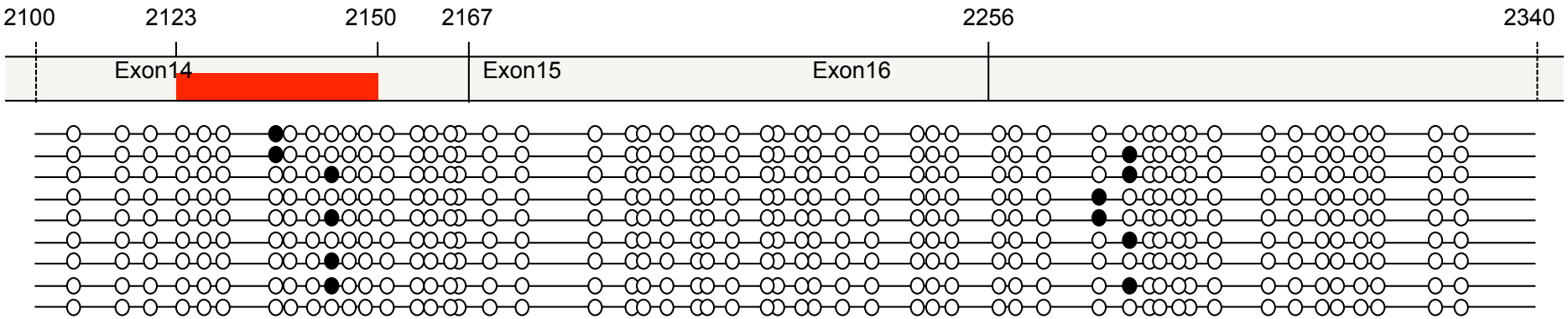
# Cytosine 5' methylation of Kit RNA following microinjection of a Kit oligoribonucleotide

Induction of the Kit* paramutation by microinjection of the Kit $_{2123\text{-}2150}$ oligonucleotide (underlined)

RNA extraction at day E11.5 and bisulfite assay (Schaefer et al., NAR, 2009)

```
2120        2130        2140            2150        2160
 .           .           .met            .met        .
…gaagcggcactttataagaaC  cttctgcaC  tcaacggagcctt……

            2260        2270        2280        2290
             .           .met        .met        .
……aatagactcgtacatagaaagagaC  gtgaC  tcctgccatc…
```



# Induction of paramutation by a fragment of the *Kit* mRNA requires *both* methylation *and* Dnmt2 expression

| *Dnmt2* genotype | Oligoribonucleotide | Injected embryos | Total births (litters) | White-tailed mice |
|---|---|---|---|---|
| +/+ | Kit 2123-2150 | 80 | 28 (7) | 4 |
| +/+ | MetKit2 | 80 | 38 (8) | 13 |
| -/- | MetKit2 | 100 | 45 (9) | 0 |

# Part II

# RNA-mediated inheritance of an acquired pathology: the diet-induced metabolic syndrom

# The metabolic syndrom (obesity and type 2 diabetes)

induced by high fat diets, currently a worldwide epidemic

The New York Times

Most Popular | Video |

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### Top News

**Obesity Rate for Young Children Plummets 43% in a Decade**

By SABRINA TAVERNISE

The data, reported in a major federal health survey, offered the first clear evidence that America's children have turned a corner in the obesity epidemic, and came as a surprise to researchers.

**The metabolic syndrom (obesity and type 2 diabetes)**

induced by high fat diet in human and experimental organisms
currently a worldwide epidemics.

Both human epidemiology and experimental work in the mouse have established efficient paternal heredity.

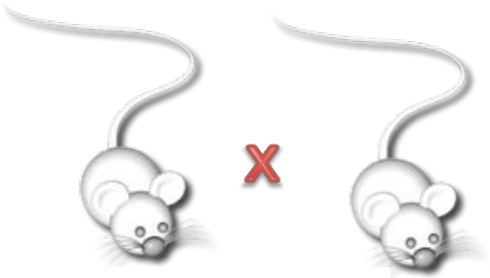
**However …**

The nature of the transgenerational signal has remained a matter of speculation, covalent modifications of histones and DNA methylation being the ‘usual suspects’…

But experimental evidence was lacking.

**Transfer by paternal RNA of an epigenetic information?**

# A straightforward experiment



Normal diet

High-fat diet (20 percent butter)

Sperm or Testis RNA preparation and microinjection

One-cell embryo

*Acquired obesity & type 2 diabetes*

Normal diet

*Epigenetic heredity*

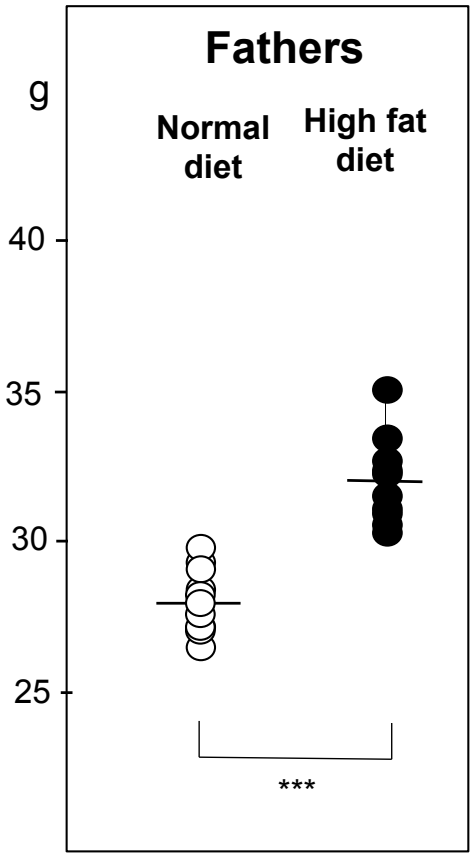
Normal diet

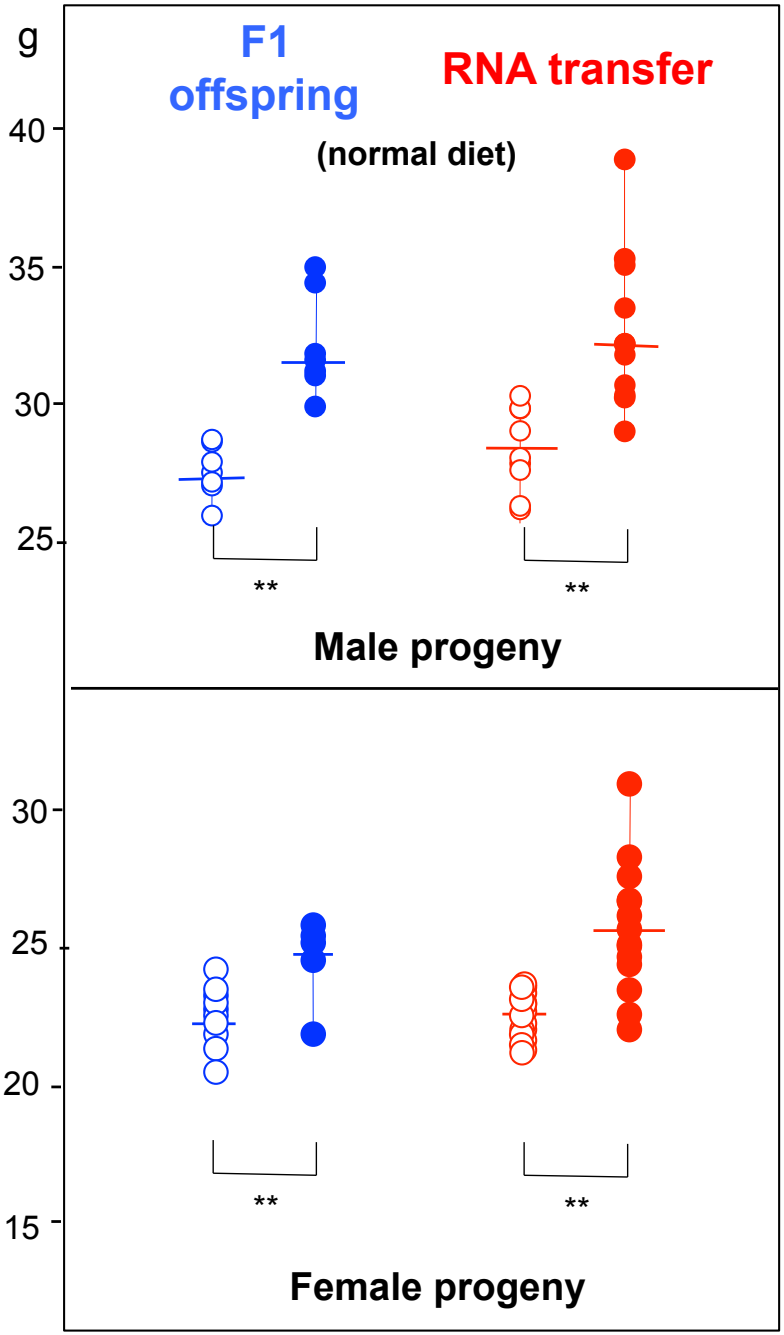
*RNA transfer of epigenetic determination?*

Control embryos: RNA from male raised on normal diet

Valérie Grandjean, manuscript in prep

# RNA-mediated inheritance of body weight



# The mounting evidence for RNA-mediated heredity
# (“the transgenerational gap”)

starting with

**the plant paramutation** (Brink, 1956)

in **C. elegans**

Lee et al. (2012); Ashe et al. (2012); Shirayama et al. (2012)

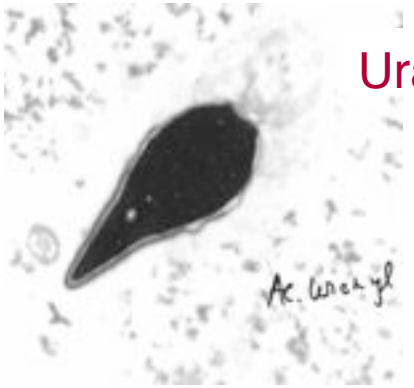
(reviewed by Lim & Brunet, 2013, Trends Genetics, 29:176-186)

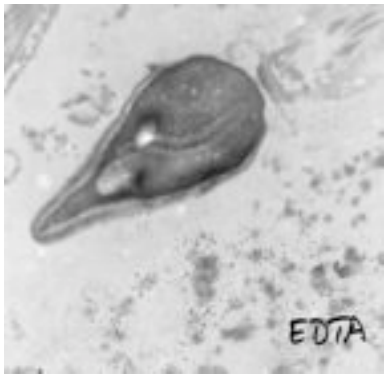
in **mice**

Paramutations (2006, 008, 2009, 2013), metabolic syndrom, …

Psychiatric disorders (MSUS depression): Gapp et al., Nature Neuro, 2014

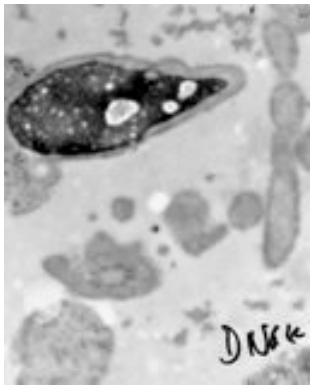
Uranyl acetate



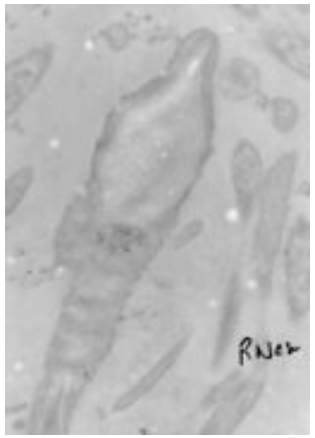



EDTA reverse

Human sperm RNA?



DNase control



RNase control

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- Sandra Lacas
  -Gervais

Plate-forme
transcriptome

- Sandra Fourré
- Pascal Barbry

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UMR 636

# Epigenetics : the first report

Genesis 30:37-39

**Figure 1.** Then Jacob took fresh sticks of poplar and almond and plane trees, and peeled white streaks in them, exposing the white of the sticks. **38** He set the sticks that he had peeled in front of the flocks in the troughs, that is, the watering places, where the flocks came to drink. And since they bred when they came to drink, **39** the flocks bred in front of the sticks and so the flocks brought forth striped, speckled, and spotted.