IRSN
INSTITUT
DE RADIOPROTECTION
ET DE SÛRETÉ NUCLÉAIRE

Faire avancer la sûreté nucléaire

« Multi- and trans-generational effects : importance of epigenetic processes»

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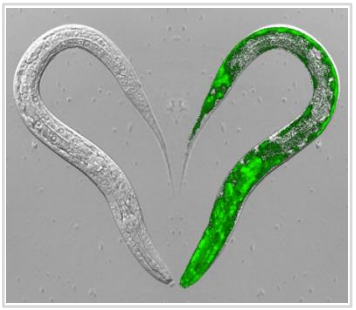


How to predict consequences of long term exposure ?

- Distinguish **multigenerational** exposure…chronic exposure over several generations. Toxicity observed for the F1, F2, F3… = or <> the one observed for F0 (adaptation or increased sensitivity ?)

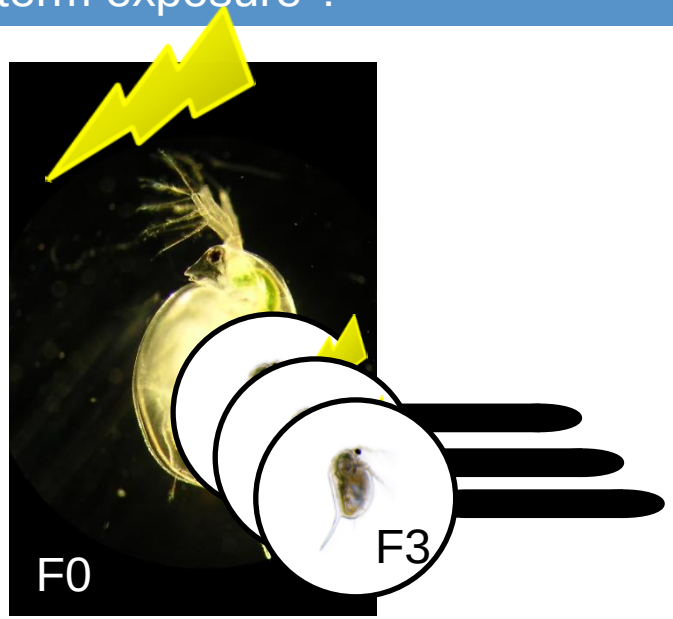
- Some examples :

Chronic exposure of microcrustaceans to Am-241 (15 mGy/h max). Altered survival and fecundidty in F1 and F2 but not in F0 (Alonzo et al., 2008). Effects higher in F2 than in F1 (5-fold)

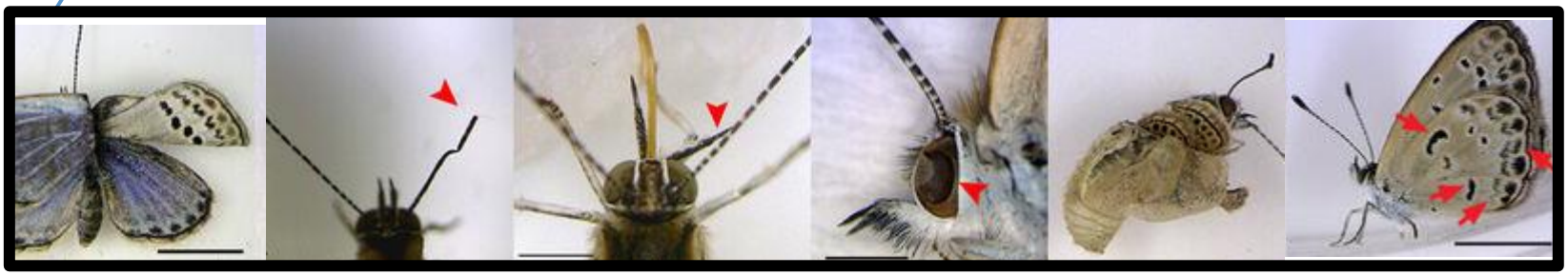
Chronic exposure of nematodes to uranium. Drastic decrease of fertility until F3, then, from F3 to F21, selection of individuals being the more fertile and having the fastest growth (Dutilleul et al., 2014)

Chronic exposure of rats to uranium. Modification of urinary and blood metabolome of F0 and F1(Grison et al., 2014,2013 and PhD S. Grison)

…and **transgenerational exposure** : only F0 is exposed, the following generations are not exposed. Toxicity observed for F0>F1>F2>**F3**… = or not. Transgenerational effects are generally proven from **F3**



Field studies: observation of morphologic abnormalities in butterflies exposed at Fukushima at 3 µSv/h. Worse morphologic abnormalities observed in the following generations obtained in laboratory controlled conditions ; return to normal phenotypes after 2 years (Hiyama et al., 2012, 2015)



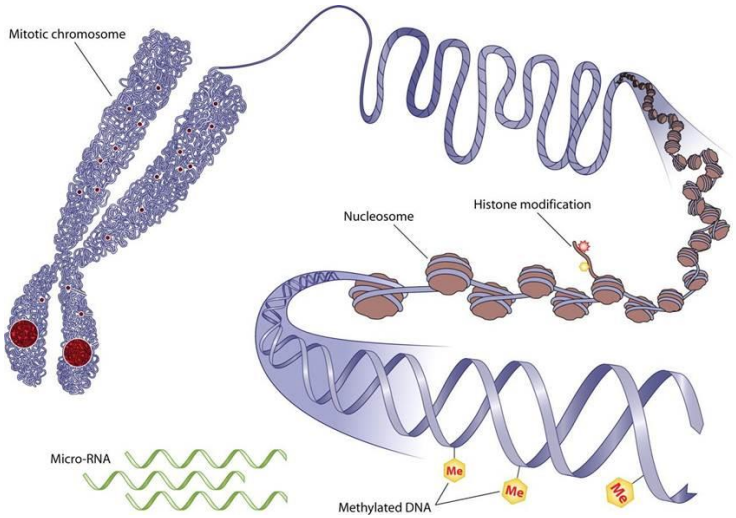
- Morphological abnormalities are observed in F1 of irradiated mice (X rays). Epidemiological data on offspring of patients treated for cancer : no transgenerational effect (review Goodhead et al., 2013). Too short exposure ? Exposed stage/window of exposure ? Exposed organ, somatic vs germinal?


- What are the mechanisms underlying these multi- and transgenerational effects ?

Genetic…Decrease of fertility and increase of mutation rates, DNA damages or to chromosomes in sparrows (Ellegren et al., 1997 ; Bonisoli-Alquati et al., 2010) and mice in Chernobyl (Pomerantseva et al., 1997) or in the lab (>1 Gy) (Goodhead et al., 2013).

…Or not? Adaptation of pines to high doses in Chernobyl despite deformities and DNA damages (Kovalchuck et al., 2003). These adaptive mechanism cannot be only genetic (10^{-5}-10^{-6} mutation per germ cell)…increase of DNA methylation : **epigenetic mechanism**

Epigenetics : **nuclear inheritance which is not based on changes in DNA sequence** (Wu and Morris, 2001)



Trygve and Tollefsbol (2012)
Burggren (2016)

Results from interactions between **genome** and **environment leading to phenotypic changes (adaptation, adverse effects, evolution…)**

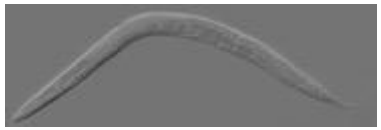
Involved in several **biological processes (development, specialisation ex : bees), and diseases** (prostate and colorectal cancers, diabete, cardiovascular diseases, obesity…)

Most studied epigenetic mechanisms :

1. **DNA methylation**
2. micro-RNA
3. Histone modifications

Use of biological models of complementary characteristics (radiosensitivity, sexual or clonal reproduction, short or long life cycle), in laboratory controled conditions…..

Nematode



Daphnid

Zebra fish

Shepherds purse

Arabidopsis

Arabidopsis

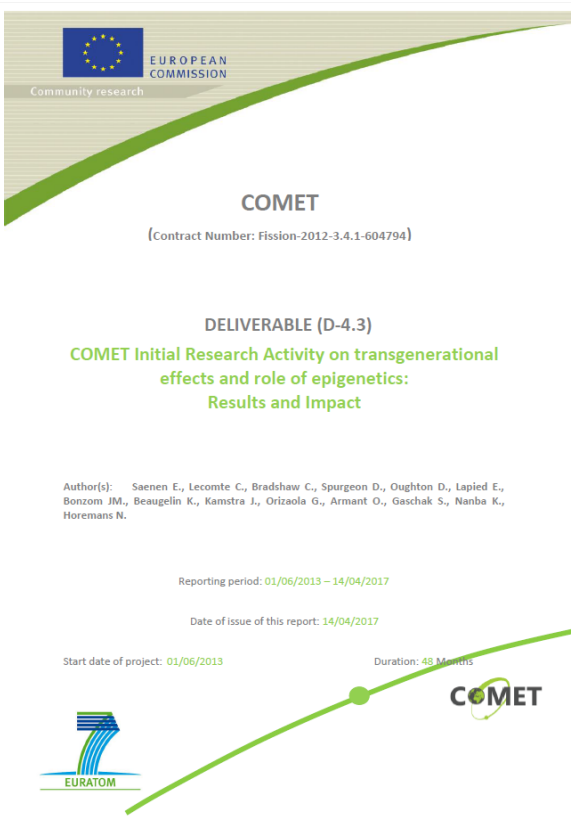
Earth worm

Tree frog

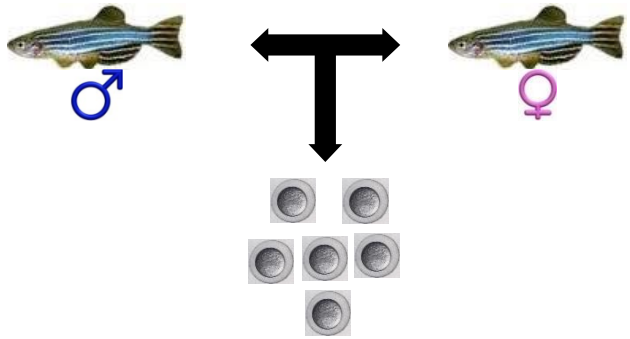
…or in field studies (national or international projects)

DNA methylation : potential biomarker of IR

- EU-project COMET gave the first indications that methylation might be affected over several generations in different organisms both lab and field sampled but that further experiments are needed



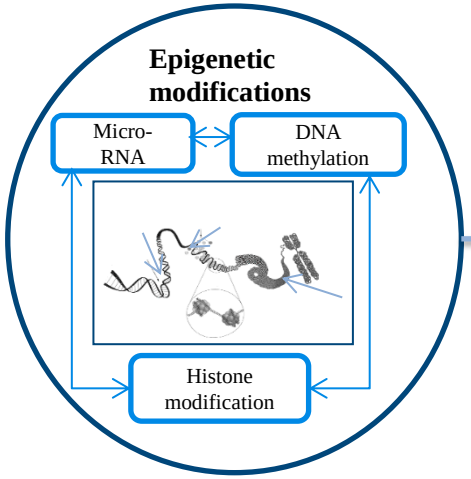
Exposure to 20 µg **uranium**/L and monitoring of global DNA methylation in parents and offspring

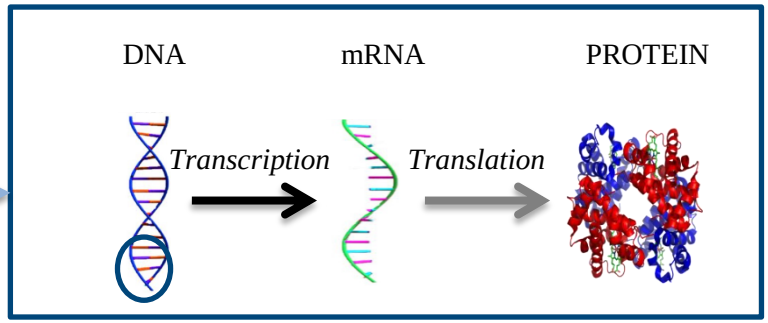


Gombeau et al. (2016)

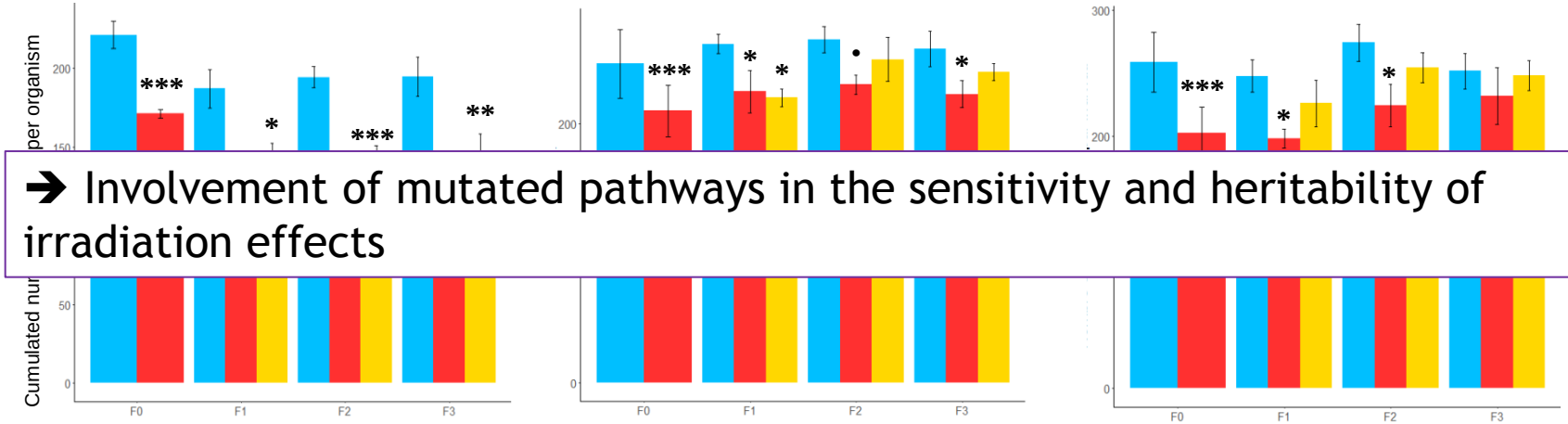
- **Increase of global DNA methylation during embryonic development** of offspring coming from parents exposed to U
- RNA sequencing shows the alteration of mechanisms linked to DNA methylation, chromatine remodelling and DNA repair : **role of epigenetic mechanisms in DNA stabilization** (decrease of genomic instability)
- **Decrease of global DNA methylation is higher in males exposed to U**
- **Same tendency in gonads, brain and kidney of rats exposed to natural U (Elmhiri et al., 2017)**

Long term and transgenerational effects in nematodes





C. elegans: Methylations of histone H3K4 and adenines jointly control the epigenetic inheritance of phenotypes



➔ Involvement of mutated pathways in the sensitivity and heritability of irradiation effects

Wild type N2

Strain *DAMT-1* : DNA 6mA methyltransferase depleted

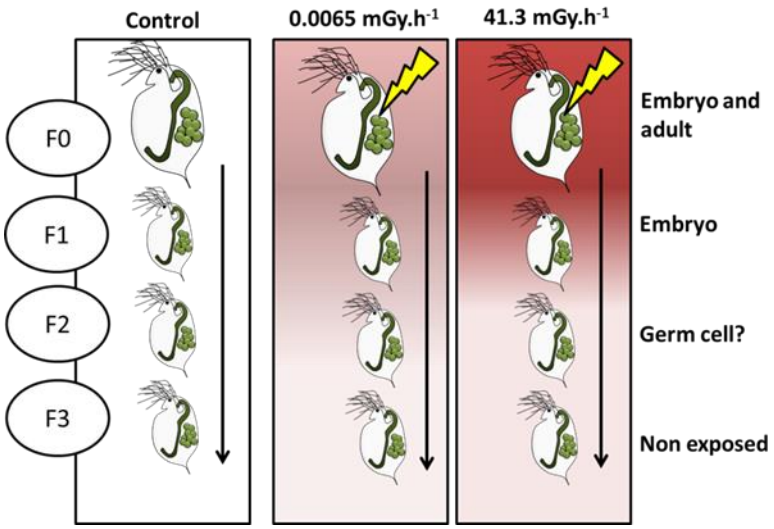
Strain *SET-30* : H3K4me methytransferase depleted

Control | Recovery | Exposed 50 mGy/h

*P-value : *** < 0,001 ; **<0,01 ; *<0,05 ; 0,05<.<0,1*

Long term and transgenerational effects in daphnids

Specific methylation of genome (bisulfite sequencing)



Trijau et al., in prep

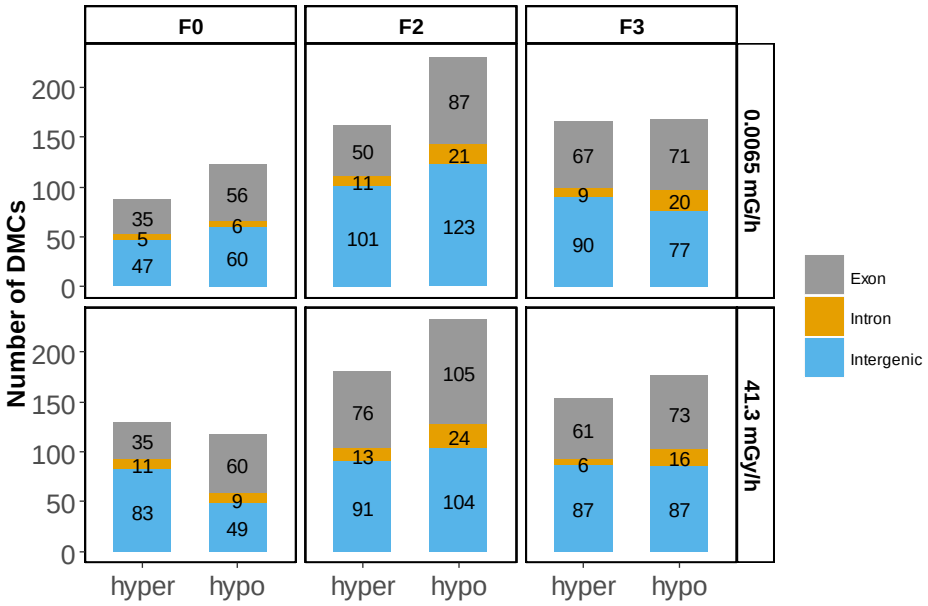
Decrease of F0 daphnid fecundity (exposed to IR)

	F0		F1		F2		F3	
mGy/h	0.007	40	0.007	40	0.007	40	0.007	40
Survival	ø	ø	ø	ø	ø	ø	ø	ø
Growth	ø	ø	ø	ø	ø	ø	ø	ø
Reproduction	ø	+	ø	ø	ø	ø	ø	ø

Long term and transgenerational effects in daphnids

- **No significant effect on average global methylation levels was observed in generations F0, F2 and F3, at 0.007 and 40 mGy/h**

- **Differentially (hypo- or hyper-) methylated cytosines (DMC) were detected in various genomic locations in generations F0, F2 and F3 after F0 exposure to 0.0065 and 41.3 mGy/h**



- **Hypomethylation of DNA** in generations F2 and F3 coming from mother exposed to IR and **heritability of this methylation pattern between F2 and F3 generations : β-mpp** (biogenesis of mitochondrial proteins), **Hsp 70** (protein repair, **IR**), **rpl28** (protein synthesis, **IR**)

- → Epigenetic biomarkers for IR exposure and effect

	D. magna	*C. elegans*
Multigenerational effects on reproduction	✓ 35 mGy/h in F0 ✓ 70 µGy/h in F2	✓ 26 mGy/h in F0 ✓ 7 mGy/h in F2
Transgenerational effects on reproduction	✗ F1, F2, F3 (41mGy/h)	✓ F1, F2, F3 (50 mGy/h)
Epigenetic changes	DMCs: ↗ F0, F2, F3	Increase in radioresistance of mutants depleted on 6mA and H3K4me2

(Parisot et al., 2015 ; Buisset-Goussen et al., 2014 ; Lecomte-Pradines et al., 2017 ; Trijau et al., in prep.)

→ Chronic effects has to be considered in a multigenerational context
→ Epigenetic mechanisms play a major role in these effects
→ Need to understand the link between molecular process and phenotype changes (Adverse Outcome Pathway)

Perspectives:

→ High throughput analyses to identify finderprints of ionising radiation effects and early and sensitive biomarkers

→ Most realistic conditions of exposure (lower dose rates, more generations, use of complex systems)

Thank you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