# Radiation genetics, epigenetics and effects on clock genes

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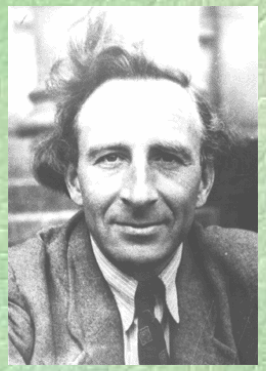
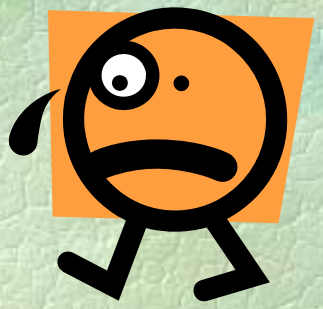


# Radiation genetics = target theory
# Independently developed in 1949
# by NV Timofeev-Ressovsky & DE Lea

Random targeting



DNA damage

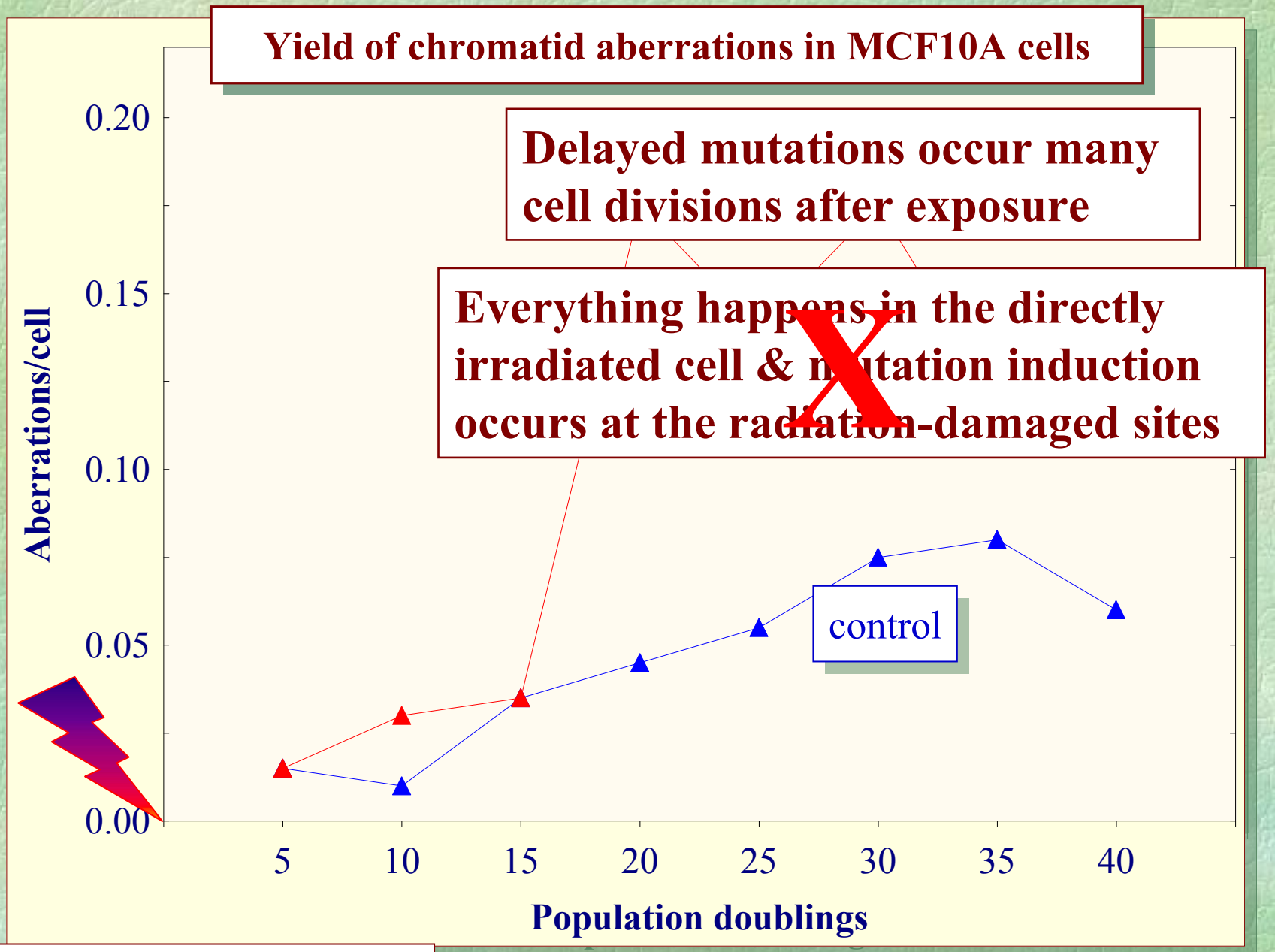
DNA repair

Chromosome aberrations

Gene mutations

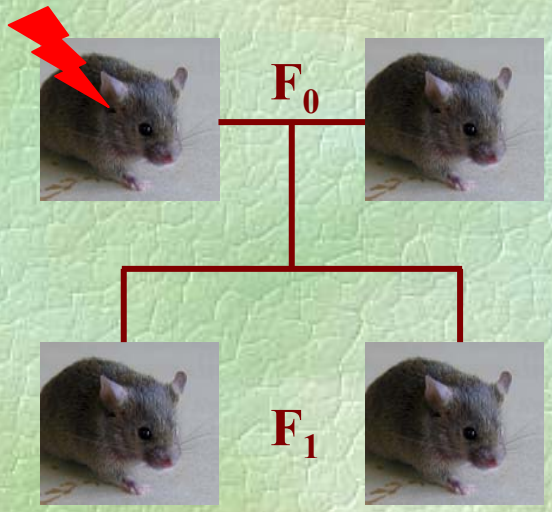
- Everything happens in the directly irradiated cell & mutation induction occurs at the radiation-damaged sites (targets)
- The yield of mutations is proportional to the amount of initial DNA damage & efficiency of its repair, *i.e.* depends on the dose, dose-rate & type of irradiation
- The risk of exposure to ionising radiation is described by the Linear No-Threshold Model

# Radiation-induced genomic instability in somatic cells



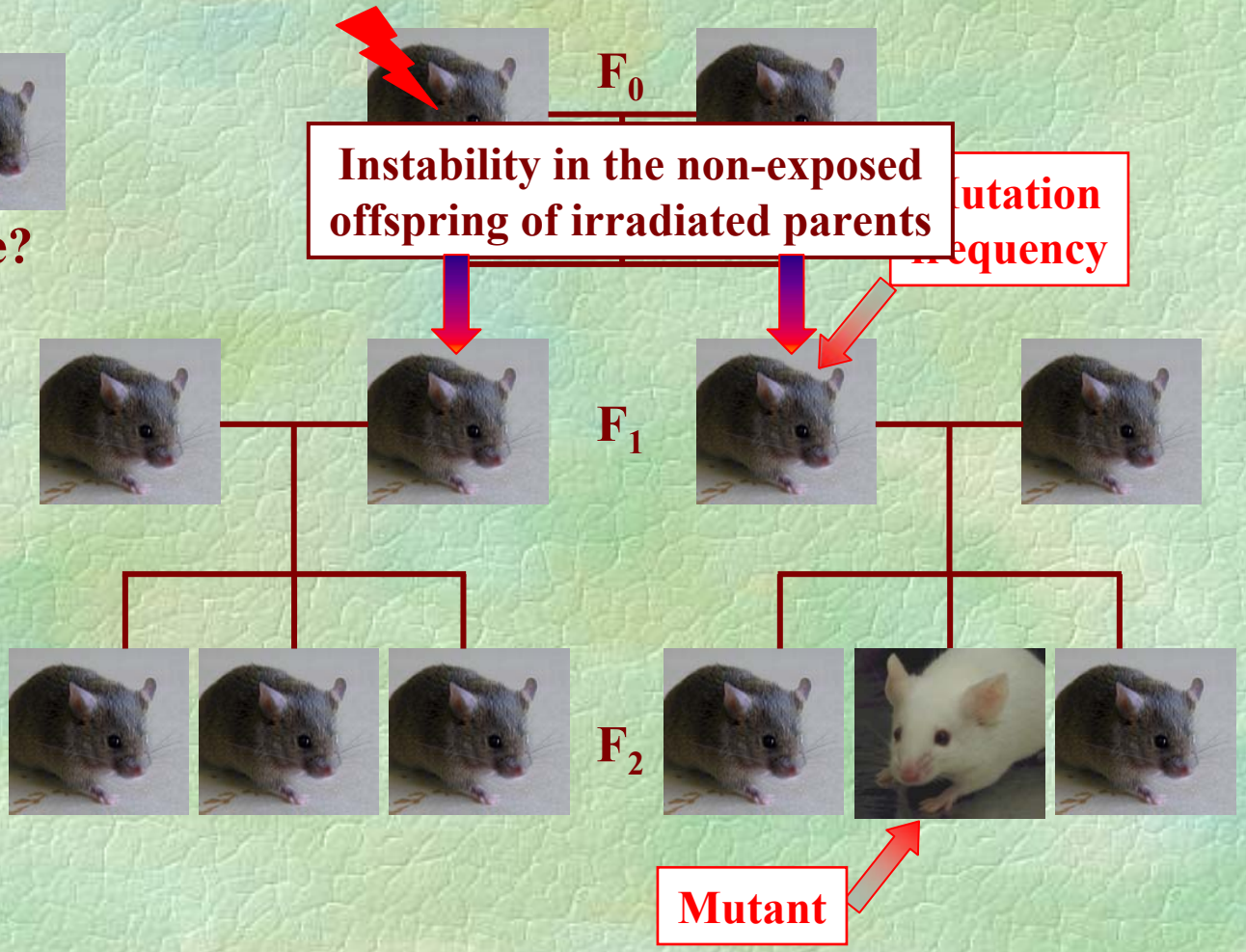

From: Ullrich & Ponnaiya, 1998, *Int J Radiat Biol* **74,** 747

# What about the germline?



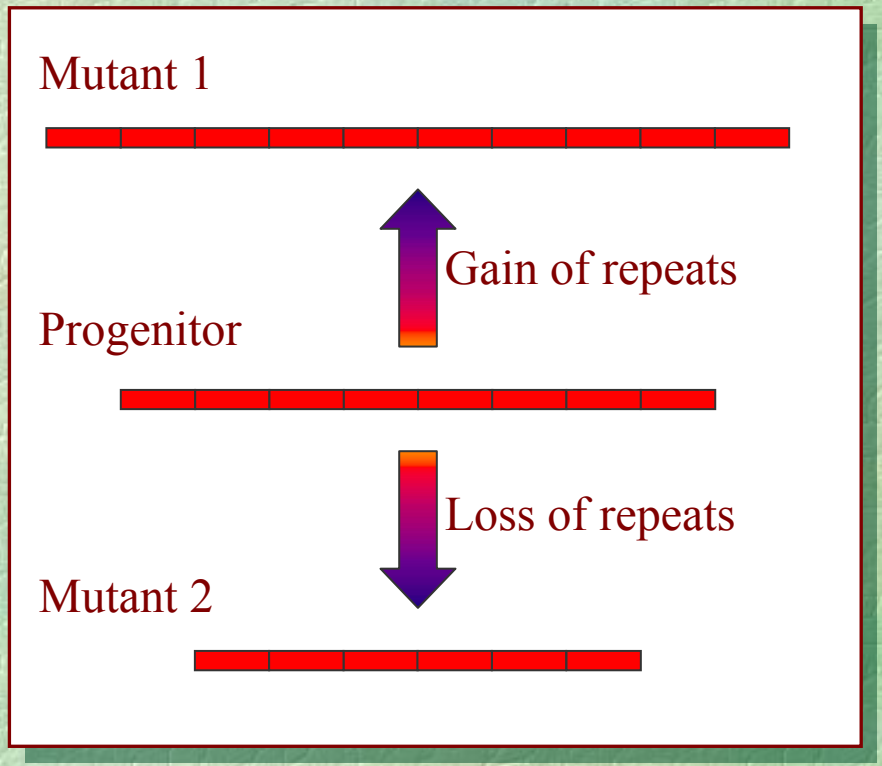

Are they unstable?

## How to analyse?

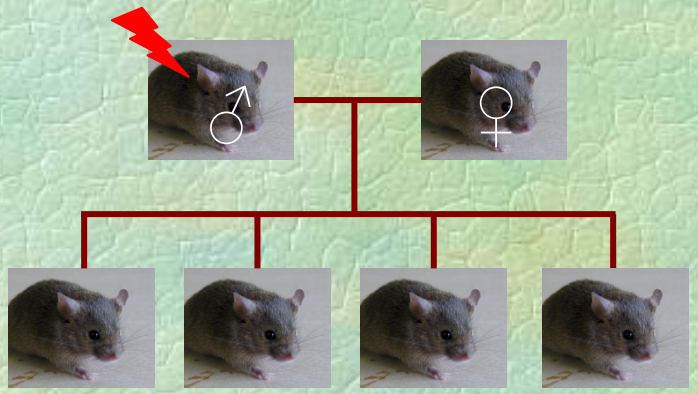


# Mouse Expanded Simple Tandem Repeat (ESTR) loci

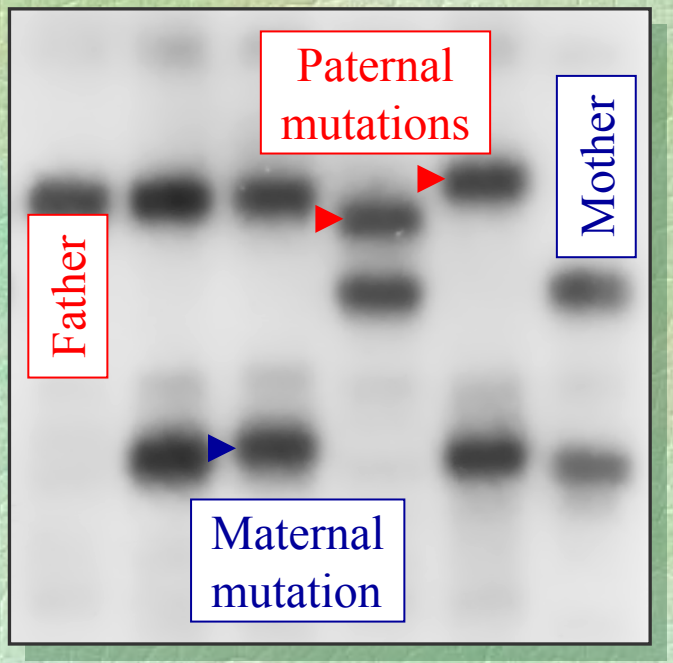
- 4-10 bp repeats, 100 bp - 20 kb arrays, non-coding
- Very spontaneous mutation rate (up to 15% per gamete)
- Mutations result in the loss/gain of repeats



# ESTR mutation detection in the germline & somatic tissues



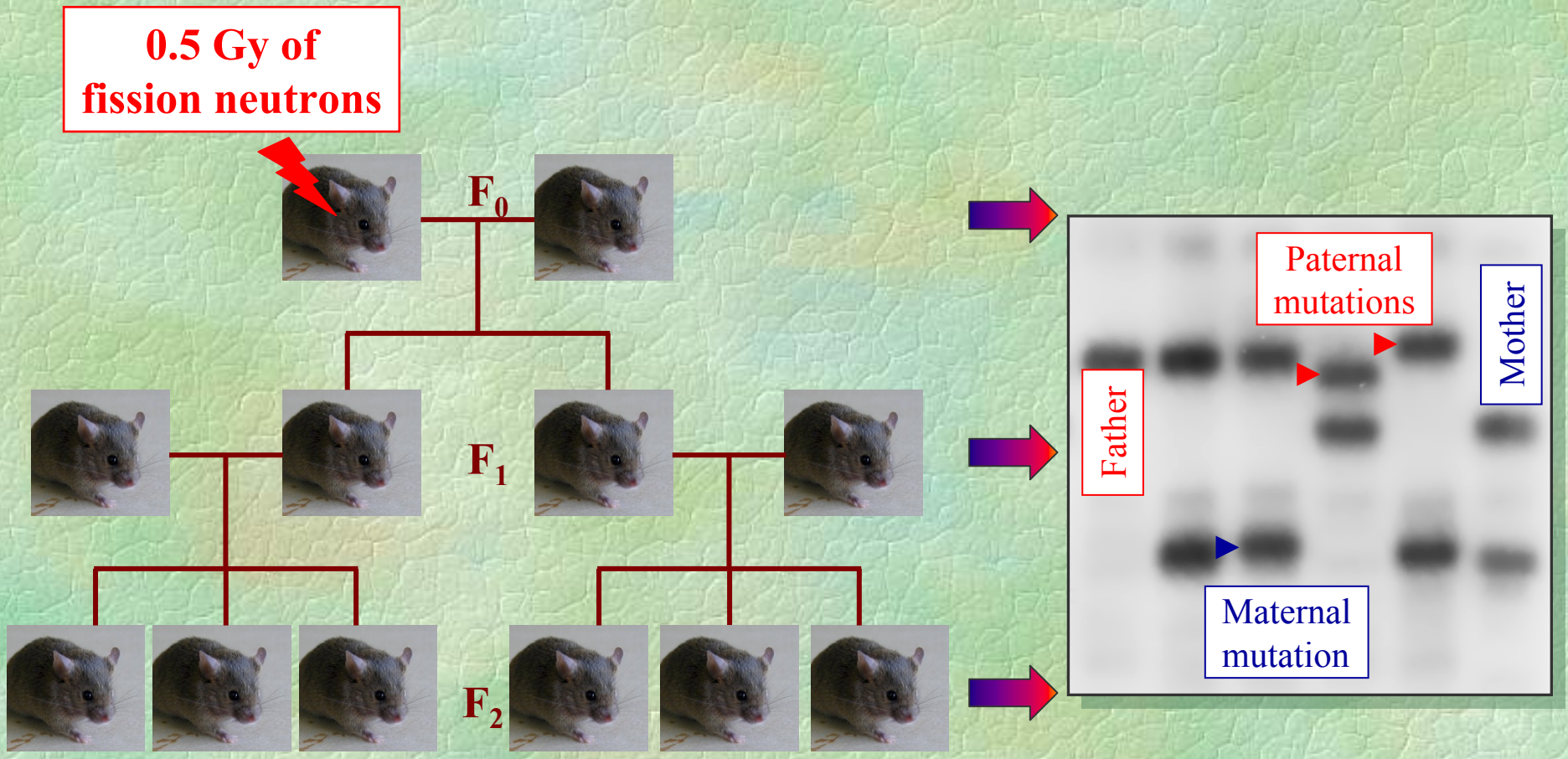

## Pedigree approach




**Mutants**

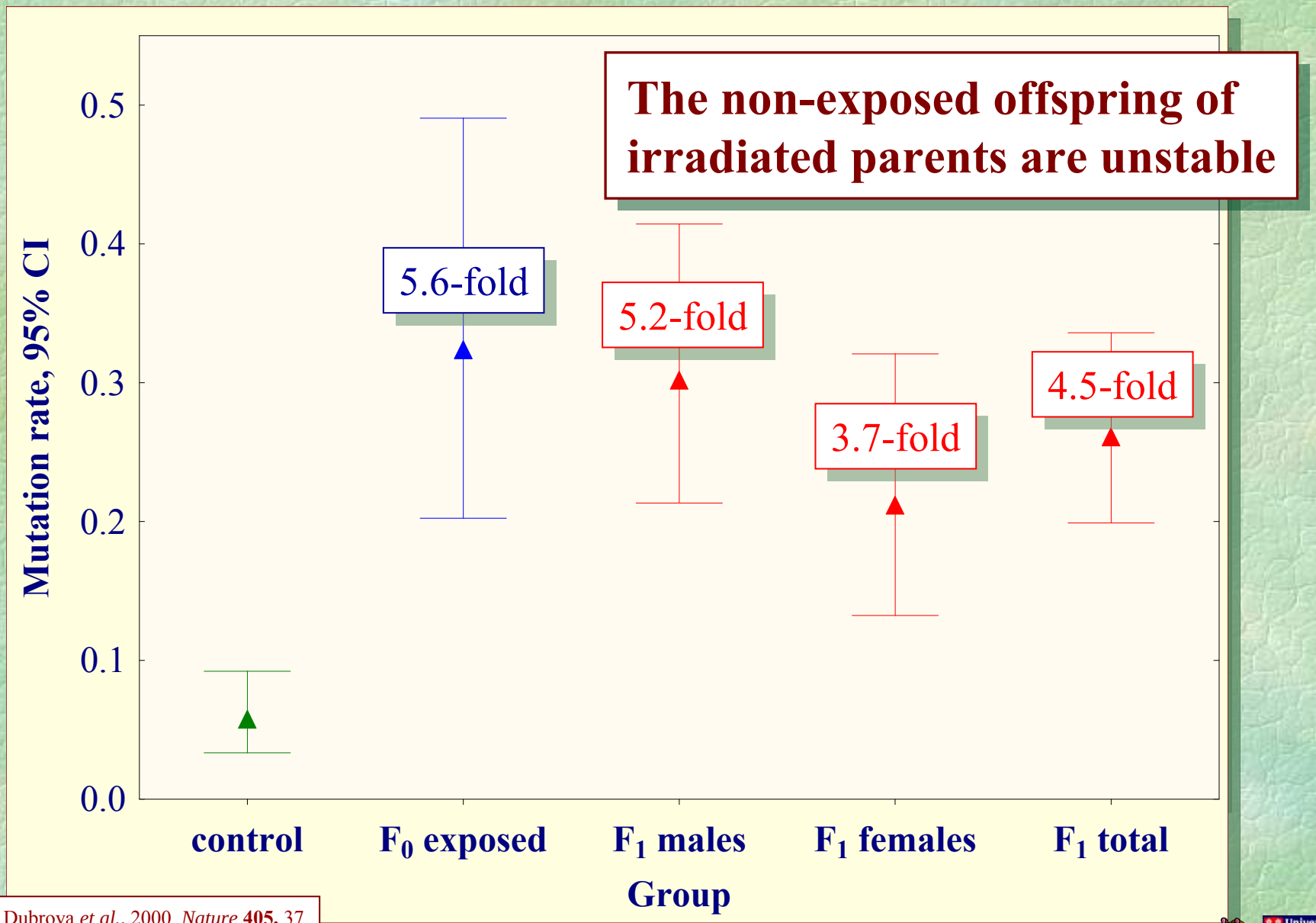
## Single-molecule PCR approach

# Let's go transgenerational…



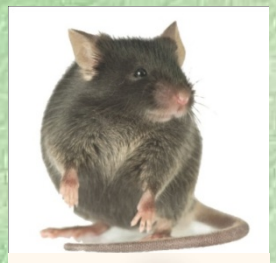

From: Dubrova *et al.*, 2000, *Nature* **405,** 37

# Transgenerational germline instability in the $F_1$ offspring of CBA/H male mice exposed to 0.5 Gy of fission neutrons




From: Dubrova *et al.*, 2000, *Nature* **405,** 37

# Is transgenerational instability strain-specific?

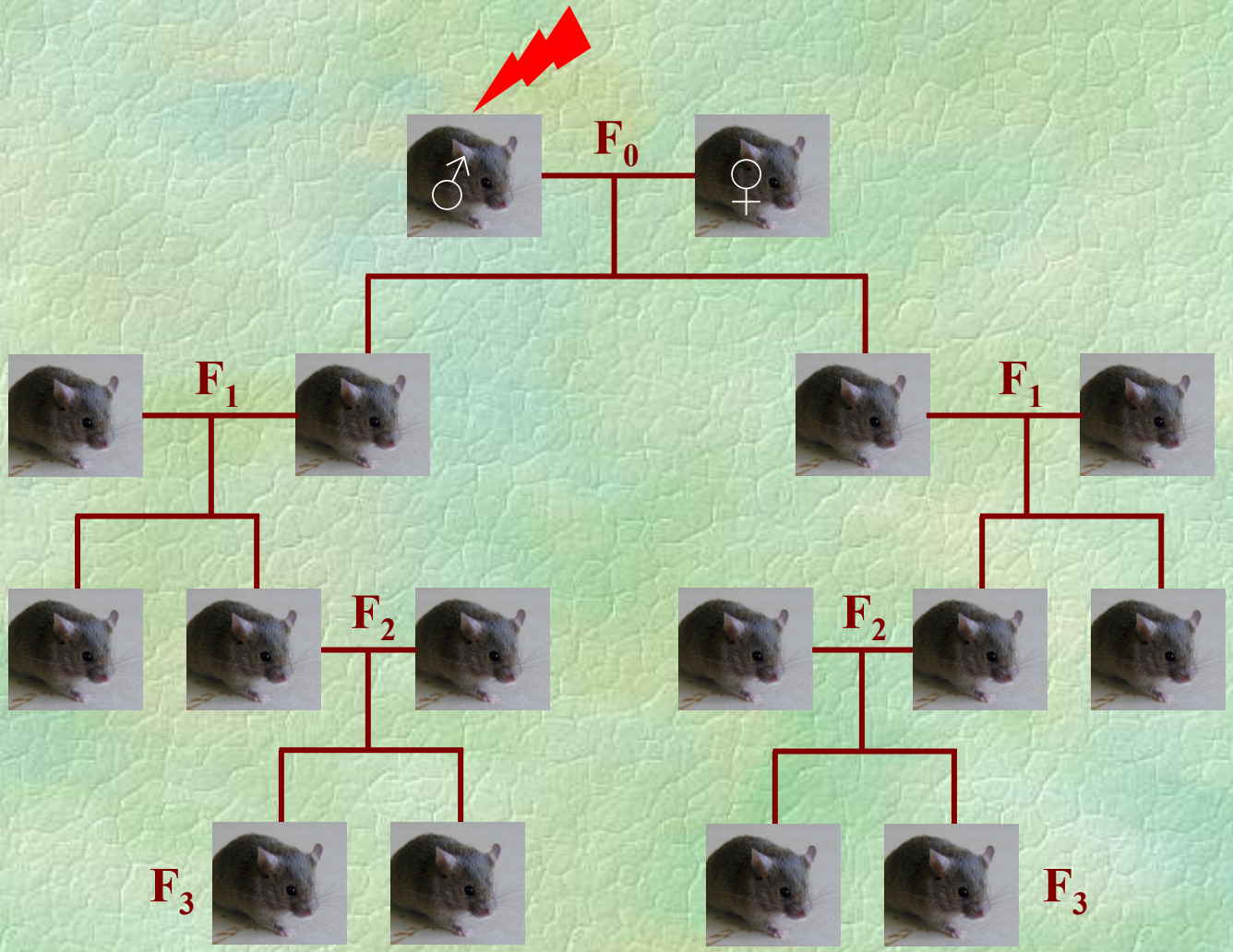


**CBA/H**

**BALB/c**

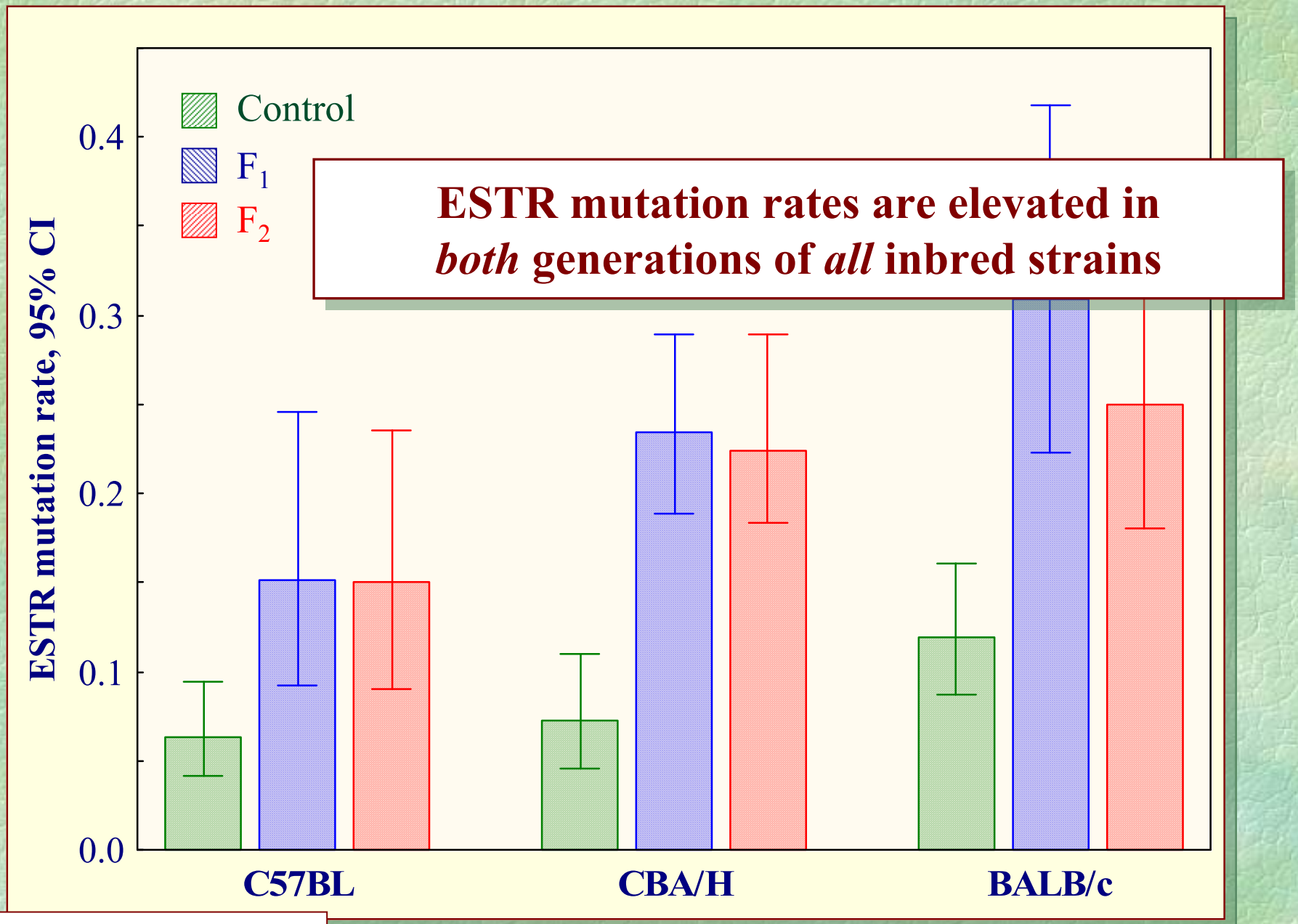
**C57BL/6J**

Fission neutrons, 0.4 Gy: CBA/H; C57BL/6
Acute X-rays, 2 Gy: CBA/H
Acute X-rays, 1 Gy: BALB/c




From: Barber *et al.*, 2002, *PNAS* **99,** 6877-82

# Transgenerational instability in three inbred mouse strains

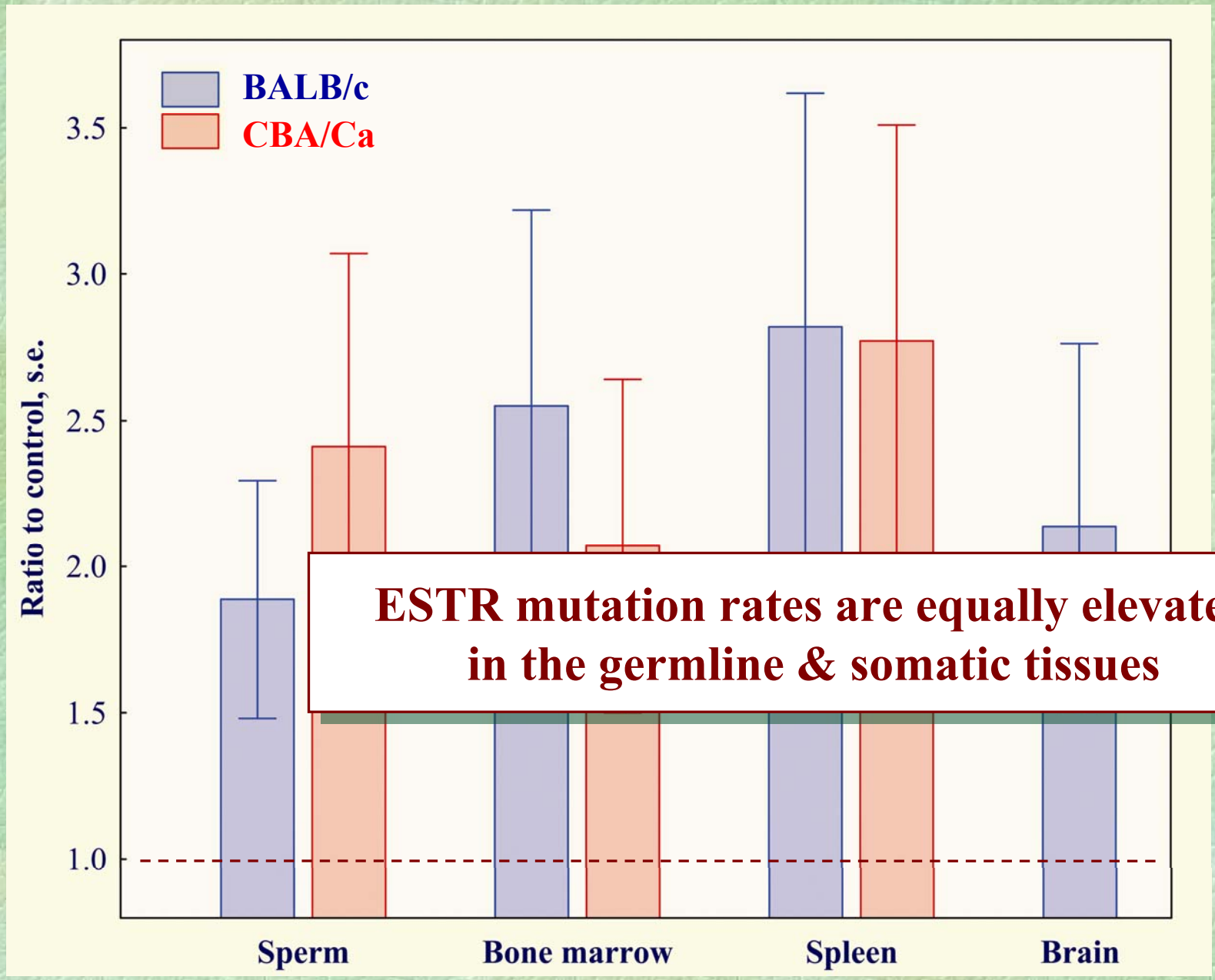



**ESTR mutation rates are elevated in *both* generations of *all* inbred strains**

From: Barber *et al.*, 2002, *PNAS* **99,** 6877-82

# Is transgenerational instability tissue-specific?

# Transgenerational instability in the germline & somatic tissues



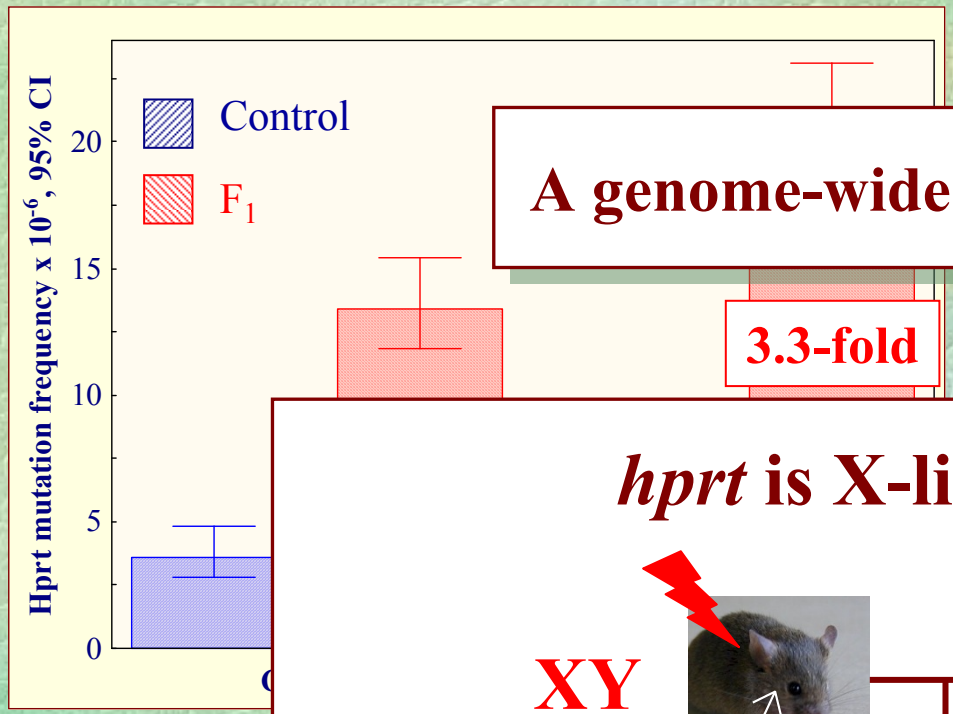

**ESTR mutation rates are equally elevated in the germline & somatic tissues**

From: Barber *et al*., 2006, *Oncogene* **25,** 7336-42; 2009, *Mutat Res* **664**, 6-12

# Is transgenerational instability specific for tandem repeat loci?

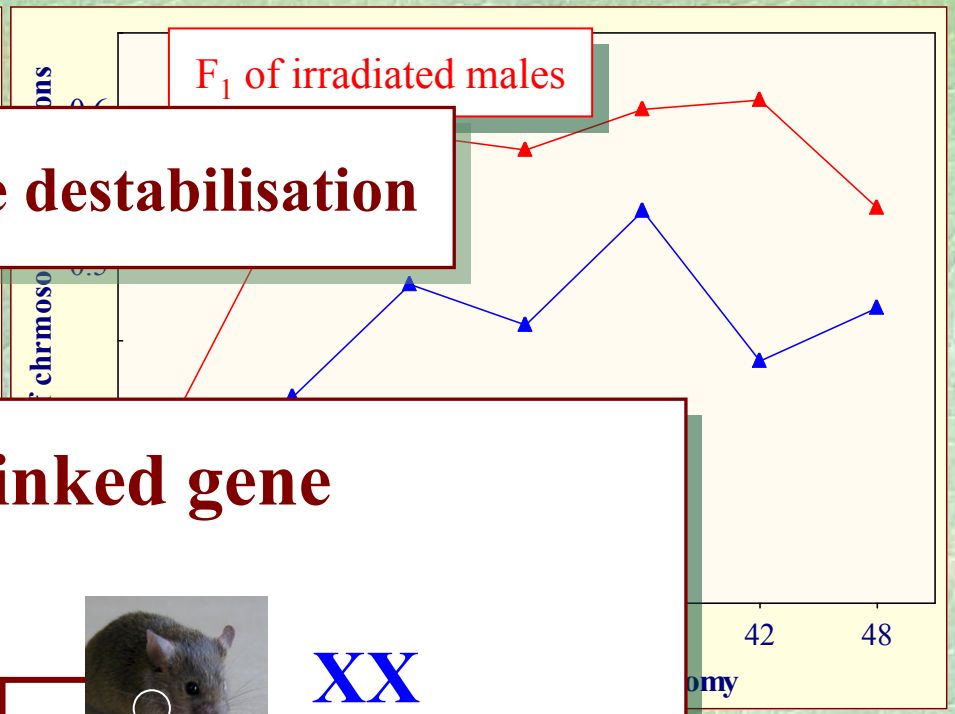
# Transgenerational instability at the mouse *hprt* locus

# Chromosome aberrations in the $F_1$ offspring of irradiated rats



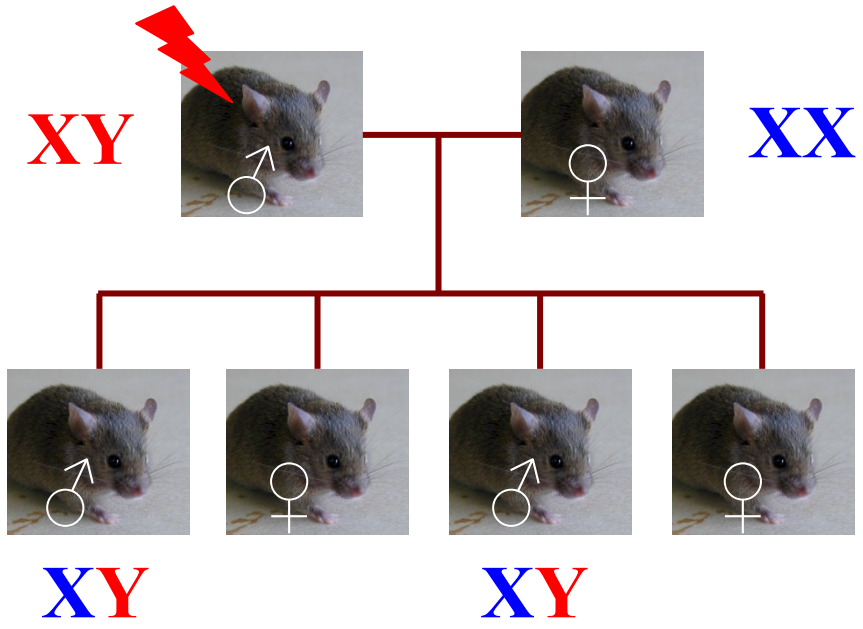

**A genome-wide destabilisation**

**3.3-fold**

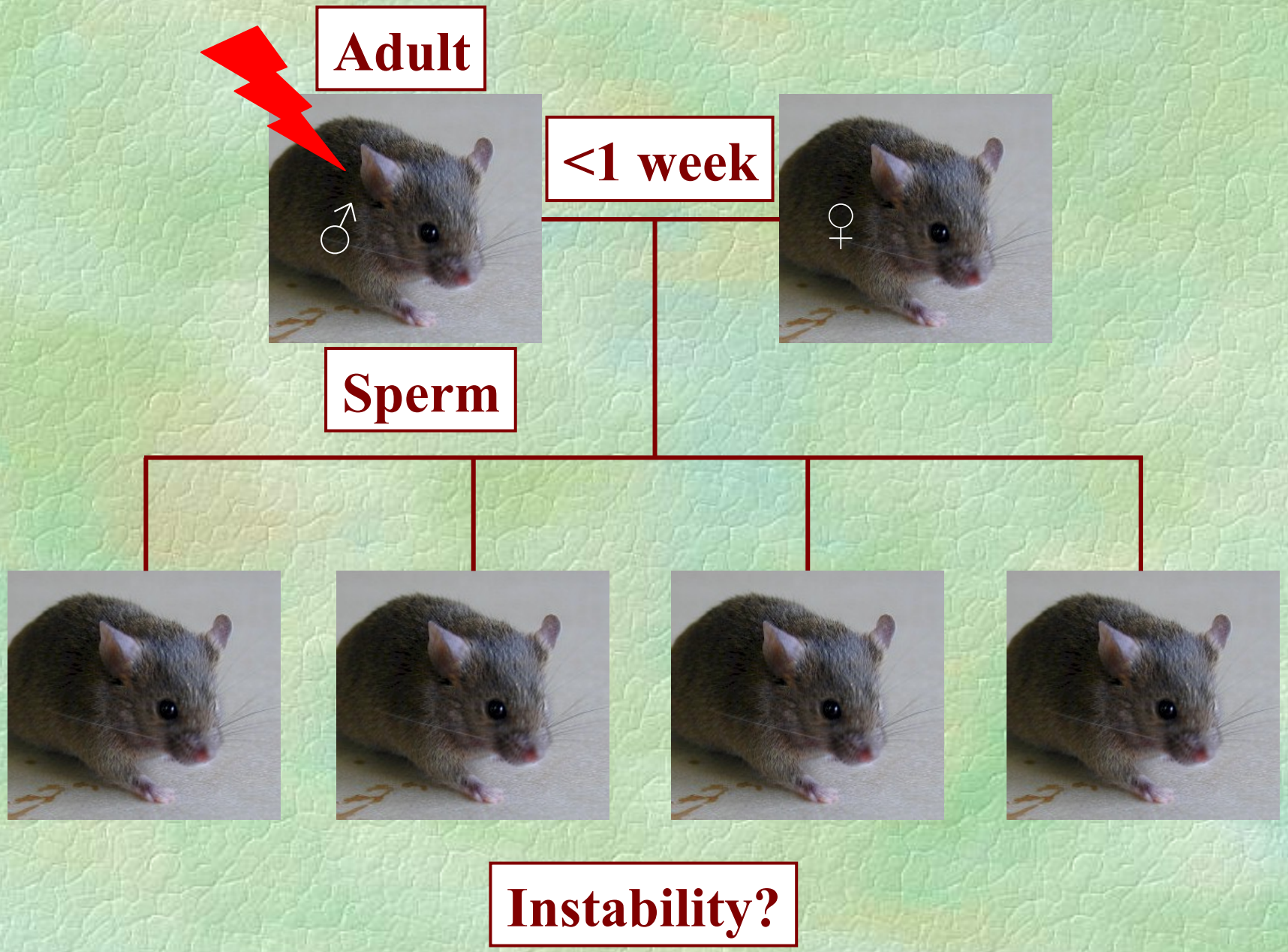



From: Barber *et al.*, 20

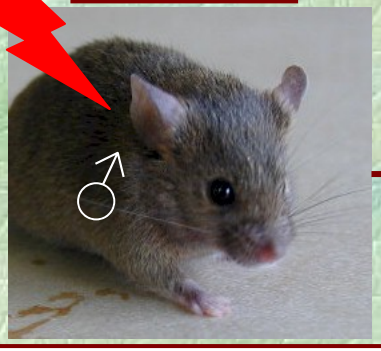
## *hprt* is X-linked gene



**For how long can a transgenerational signal survive in the irradiated males?**


Adult
<1 week
♂
♀
Sperm
Instability?

Adult

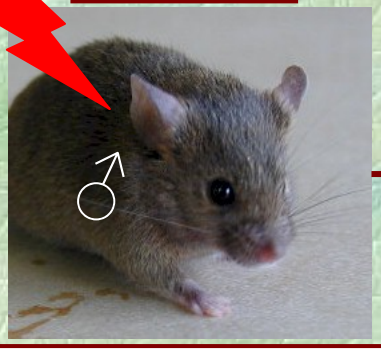



3 weeks


Spermatids

Instability?

Adult

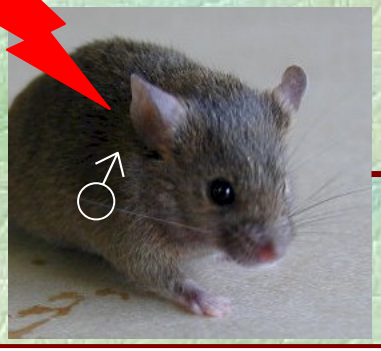



6 weeks


Spermatogonia

Instability?

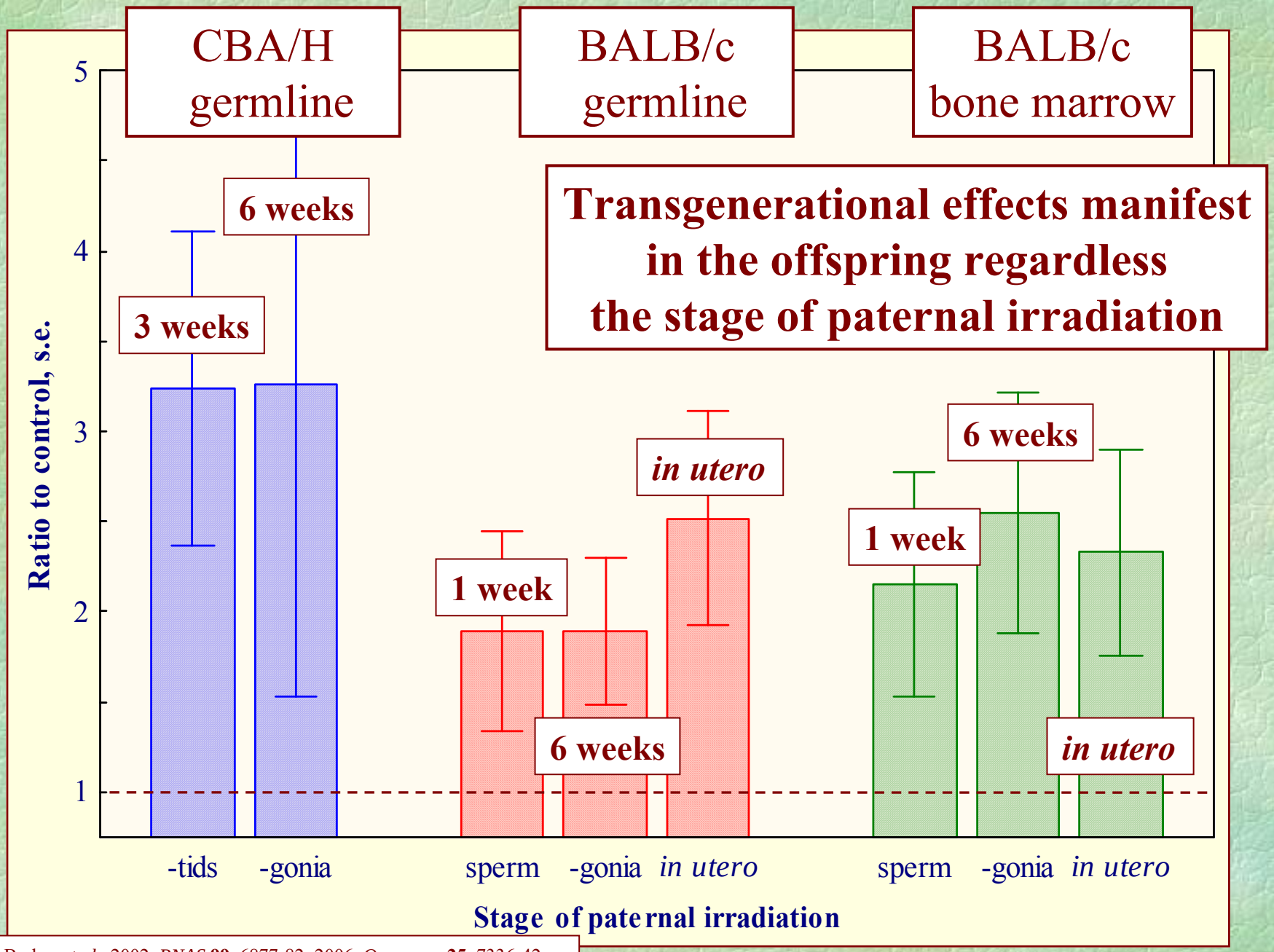
*in utero*





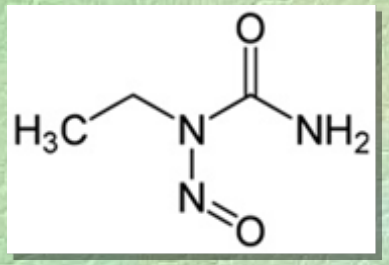
**Primordial stem cells**

**Instability?**

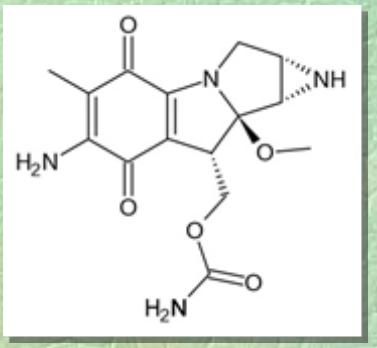



From: Barber *et al.*, 2002, *PNAS* **99,** 6877-82; 2006, *Oncogene* **25,** 7336-42; 2009, *Mutat Res* **664**, 6-12; Hatch *et al.*, 2007, *Oncogene*, **26,** 4720-4

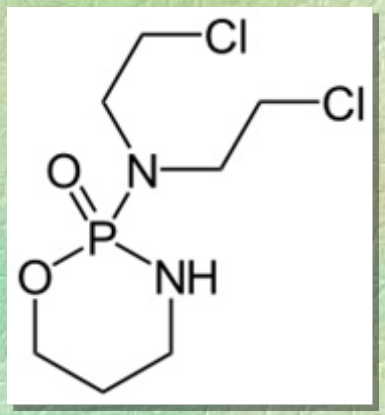
# Can paternal exposure to chemical mutagens destabilise the $F_1$ genomes?



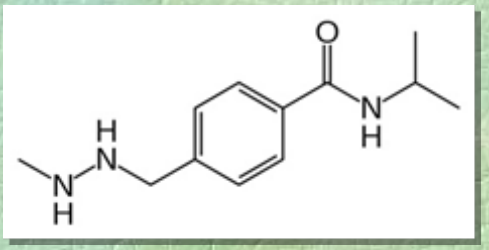

Alkylating agent ethynitoesurea, ENU
- mostly base damage
- results in base substitutions
- ~ no ENU-induced DSBs




Anticancer drug mitomycin C, MMC
- alkylated monoadducts & crosslinks
- base substitutions
- crosslinks can result in DSBs

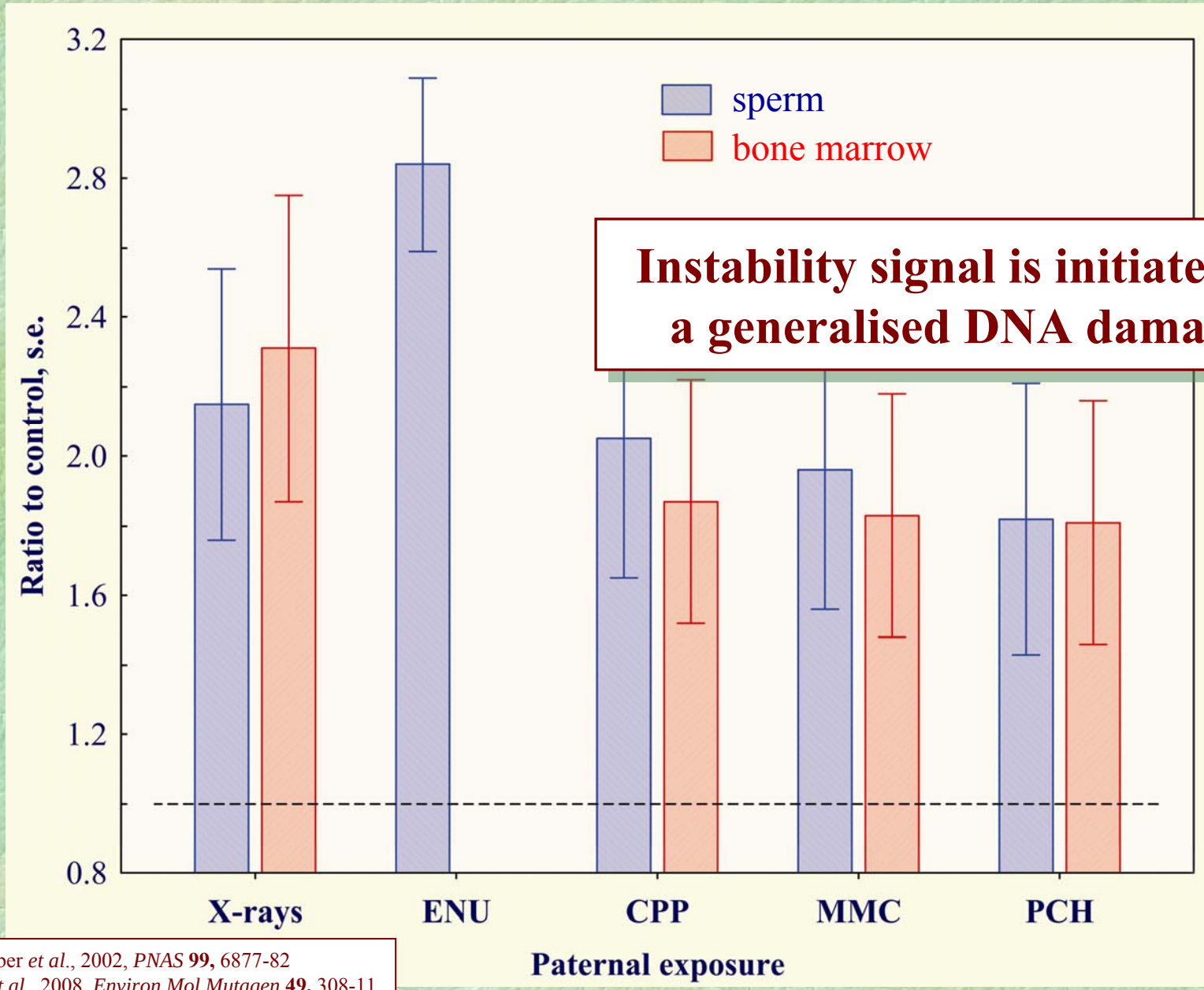



Anticancer drug cyclophosphamide, CPP
- alkylated monoadducts & crosslinks
- results in base substitutions
- crosslinks can result in DSBs after replication/repair




Anticancer drug procarbazine, PCH
- alkylated monoadducts
- free radical species
- base substitutions & SSBs

# ESTR instability in the $F_1$ offspring of mutagen-treated male mice

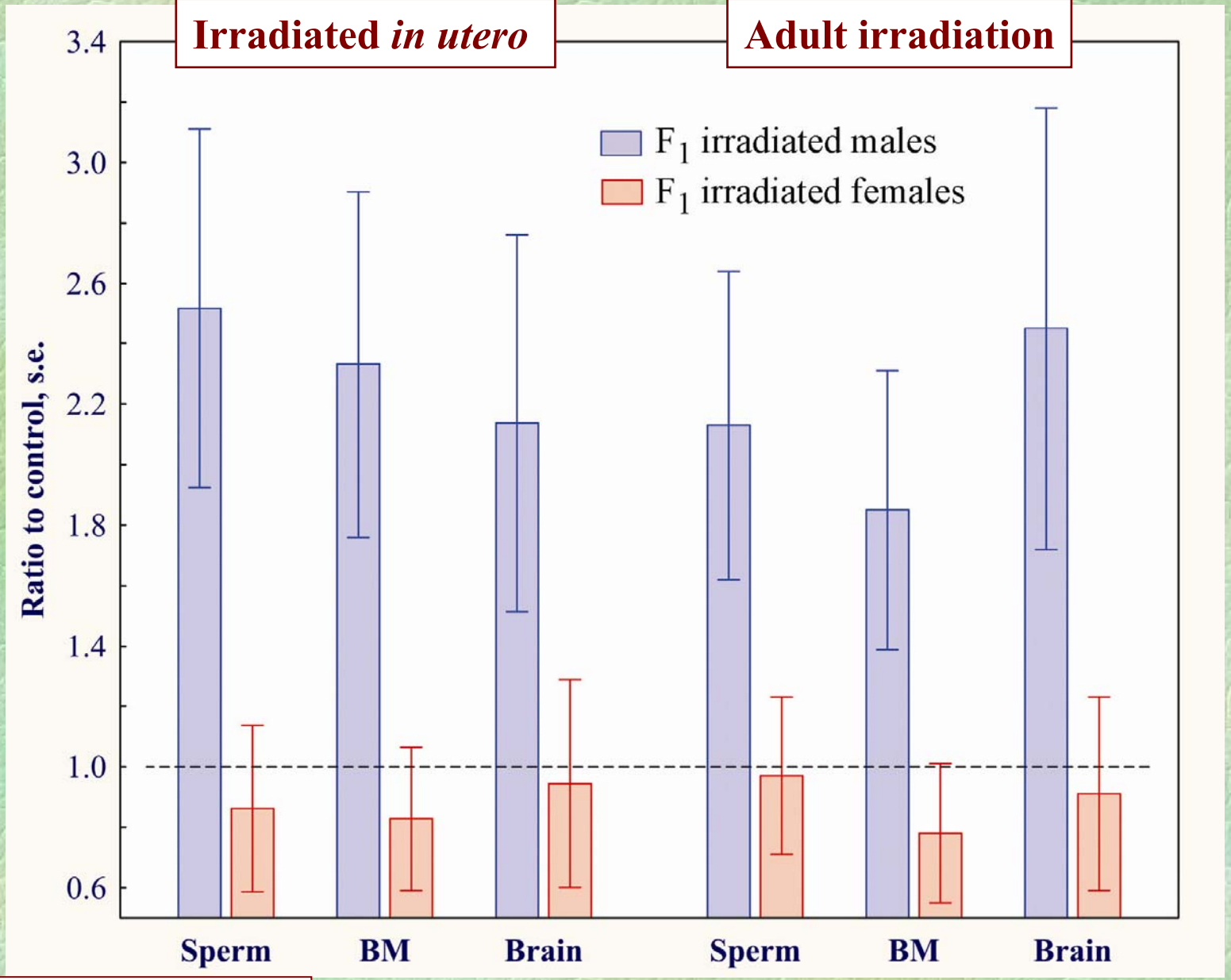



**Instability signal is initiated by a generalised DNA damage**

From: Barber *et al.*, 2002, *PNAS* **99,** 6877-82
Dubrova *et al.*, 2008, *Environ Mol Mutagen* **49,** 308-11
Glen, Dubrova 2012, *PNAS* **109,** 2984

# Is transgenerational instability sex-specific?

# The offspring of irradiated females are stable



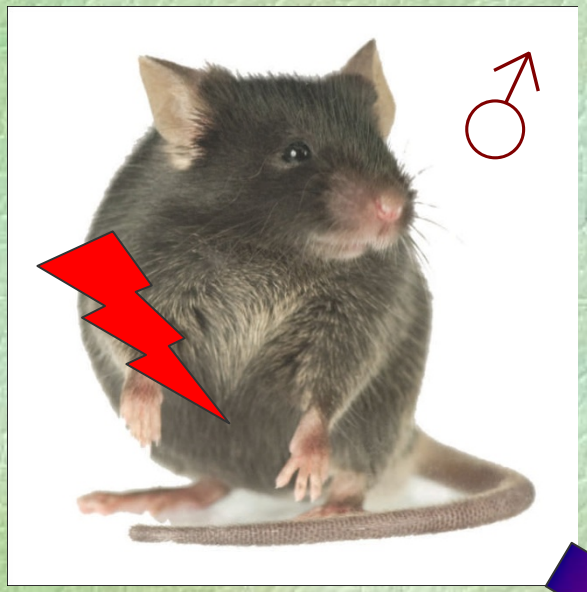

From: Barber *et al*., 2009, *Mutat Res* **664**, 6-12;
Abouzeid Ali et al., 2012, *Mutat Res* **732**, 21-5

# Mechanisms

# Some back of the envelope exercises…

- ~ 1,000 genes are involved in maintain genome stability in mammals (DNA repair, apoptosi e arrest etc)
- max spontaneous mutation r
- exposure to 1 Gy of X- old increase in mutation rate
- if ANY radiati at ANY of 1,000 genes is DOMINA tially compromise the genome s
  $1000 \times 3 \times 10^{-6} =$ e $F_1$ offspring should be unstable
- according to our data ~100% of the $F_1$ offspring of of irradiated males are unstable

**The mechanisms must be epigenetic**



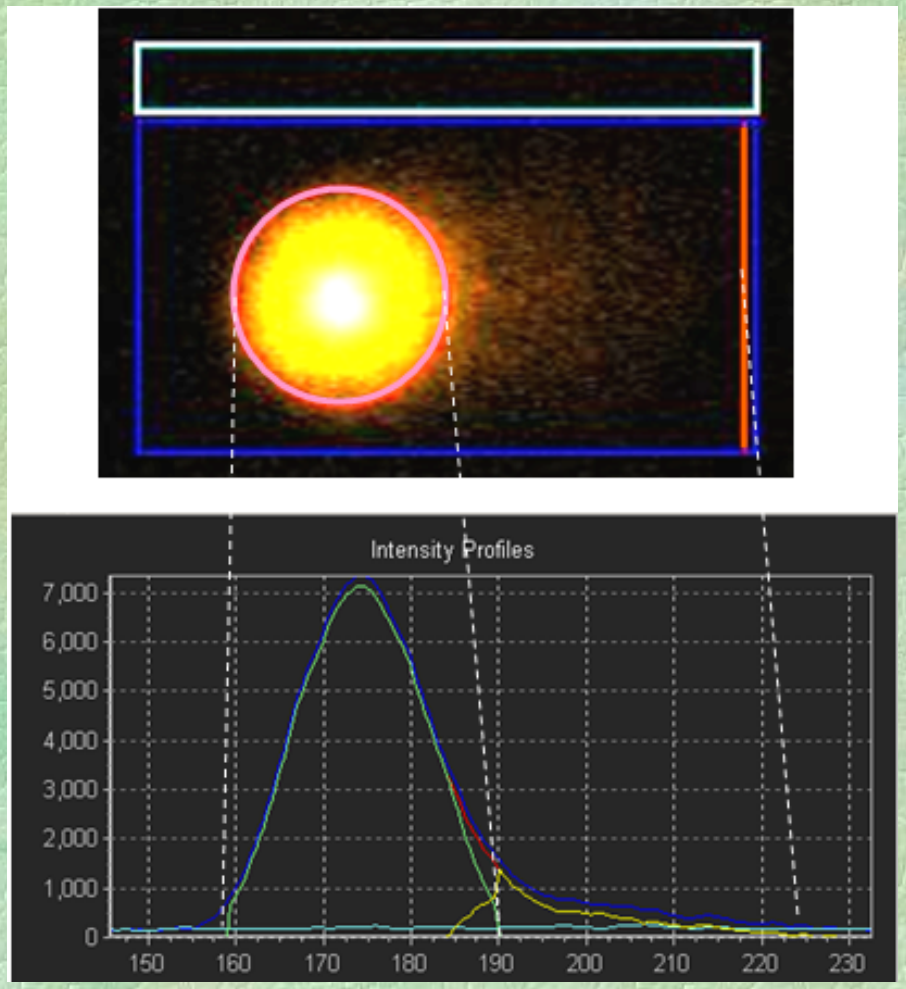


**Initiation of an epigenetic instability signal in the directly exposed male germ cells**

**Transmission of an epigenetic instability signal to the offspring & its manifestation**

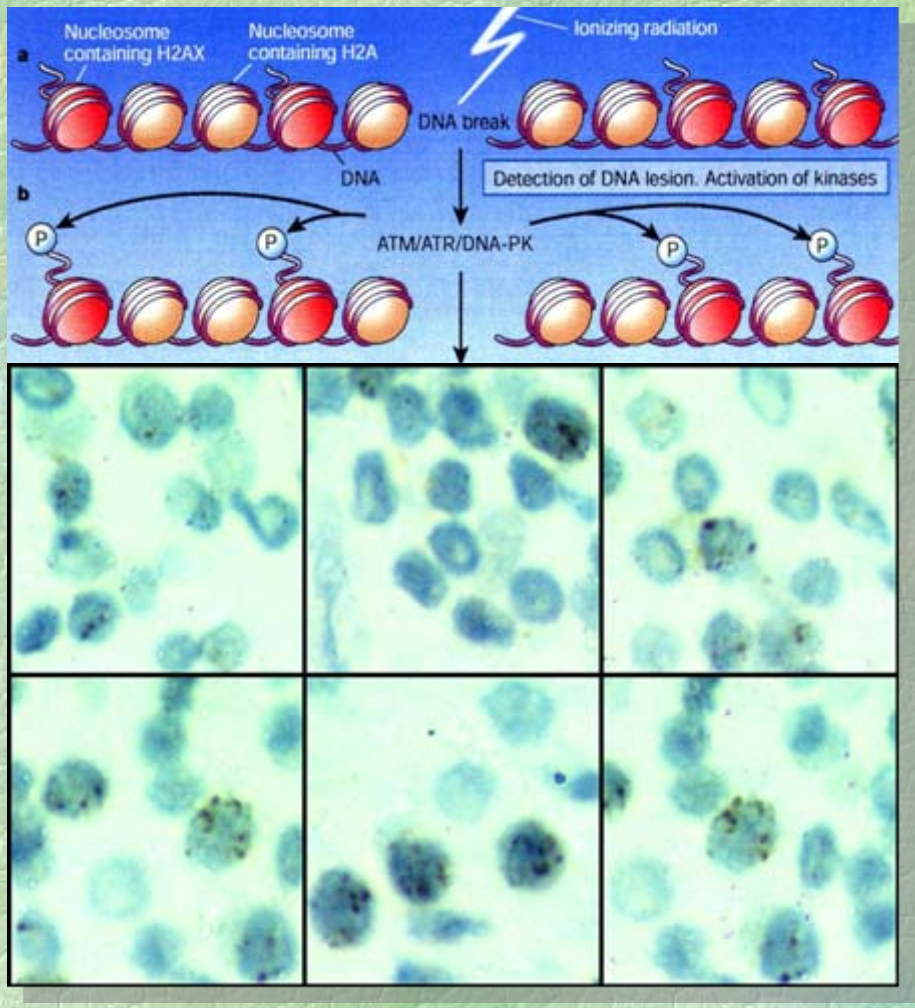
# Measuring DNA damage *in vivo*

## The alkaline Comet assay




**Mostly single-strand DNA breaks**
**+ some DNA adducts**

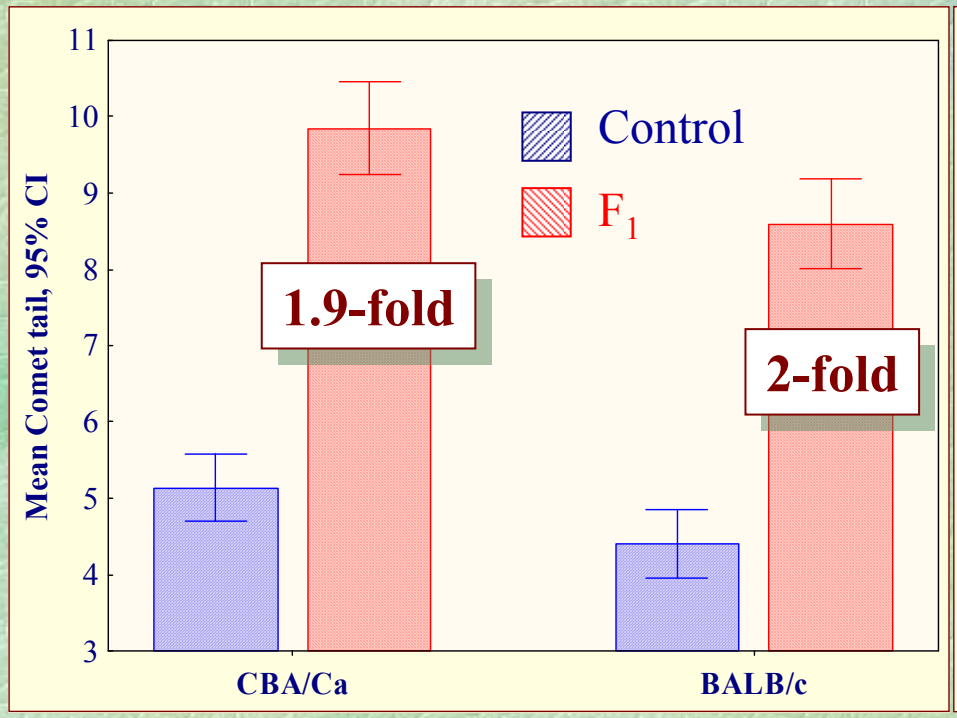
## The *γH2AX* assay



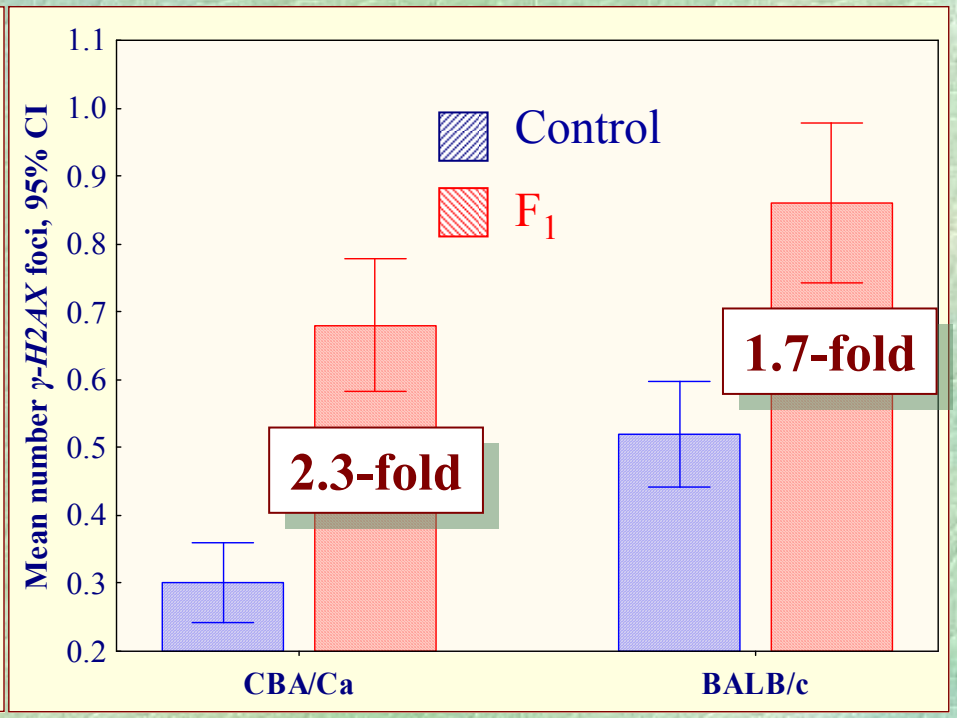

**Double-strand DNA breaks only**

# Endogenous DNA damage in controls & the $F_1$ offspring of irradiated males

## Single-strand DNA breaks
## Comet assay, bone marrow

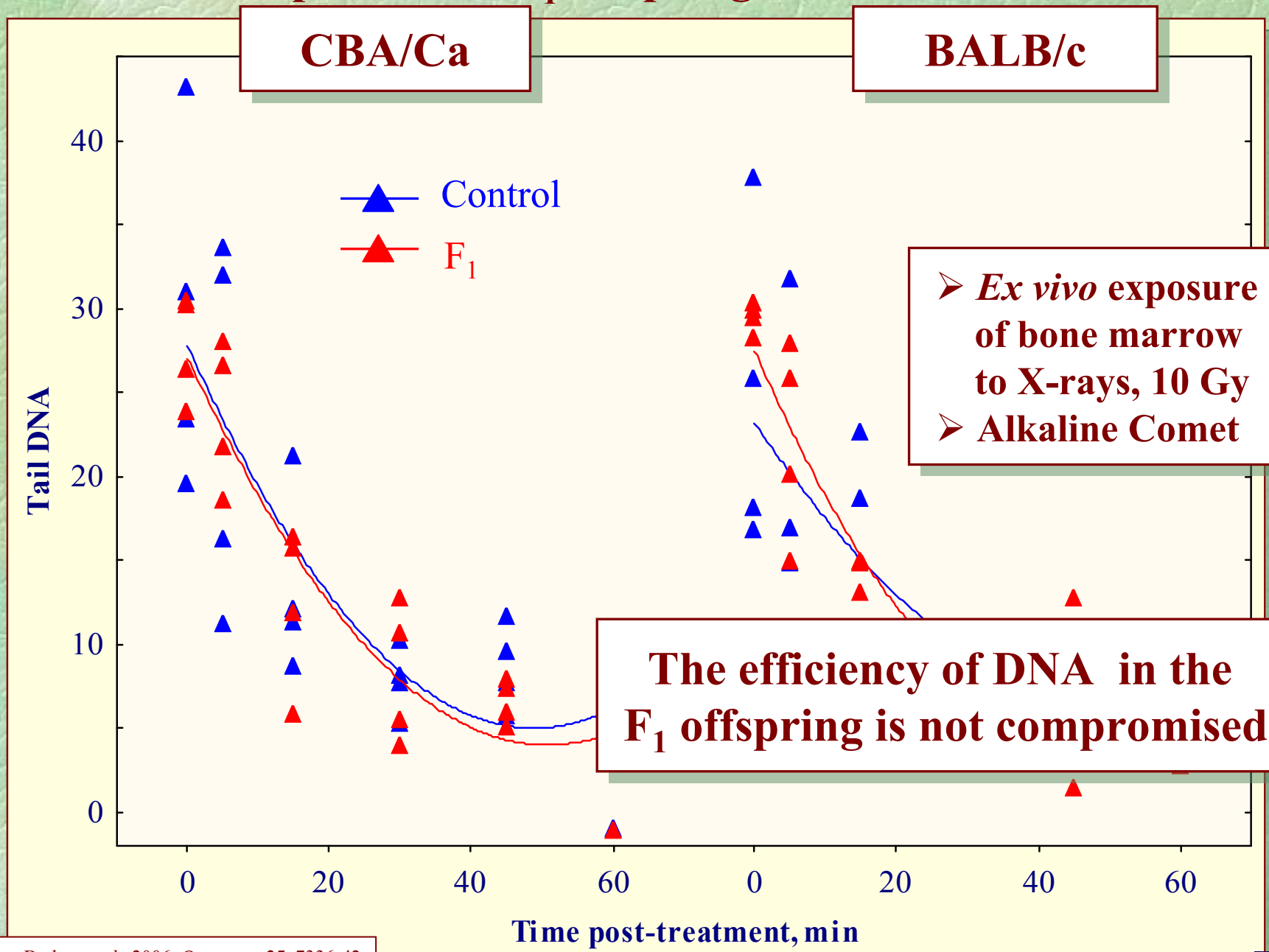



## Double-strand DNA breaks
## *γ-H2AX* assay, spleen




From: Barber *et al.*, 2006, *Oncogene* **25,** 7336-42

# DNA repair in the $F_1$ offspring of irradiated males




- *Ex vivo* exposure of bone marrow to X-rays, 10 Gy
- Alkaline Comet

**The efficiency of DNA in the $F_1$ offspring is not compromised**

From: Barber *et al.*, 2006, *Oncogene* **25,** 7336-42

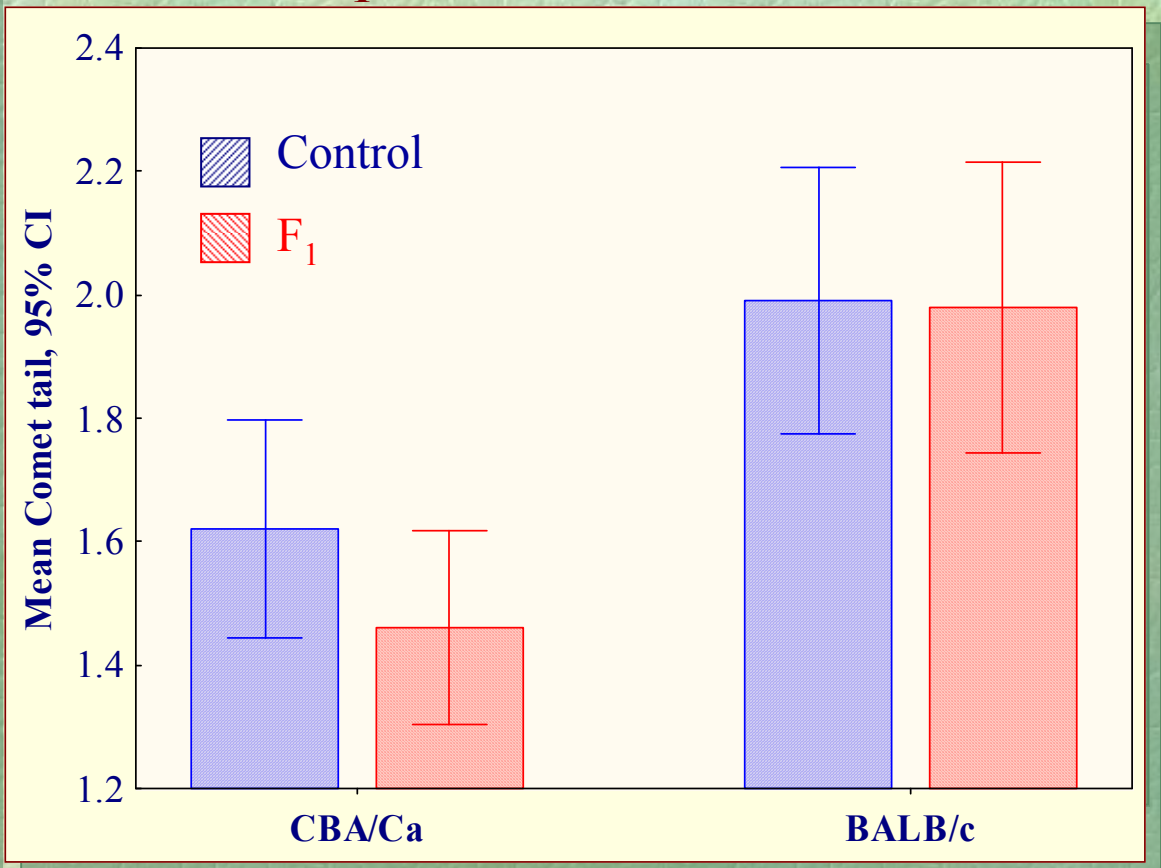
# Oxidative DNA damage in the $F_1$ offspring (FPG Comet)

## Oxidative stress

**DNA damage:**

- modified bases
- single-strand breaks
- double-strand breaks

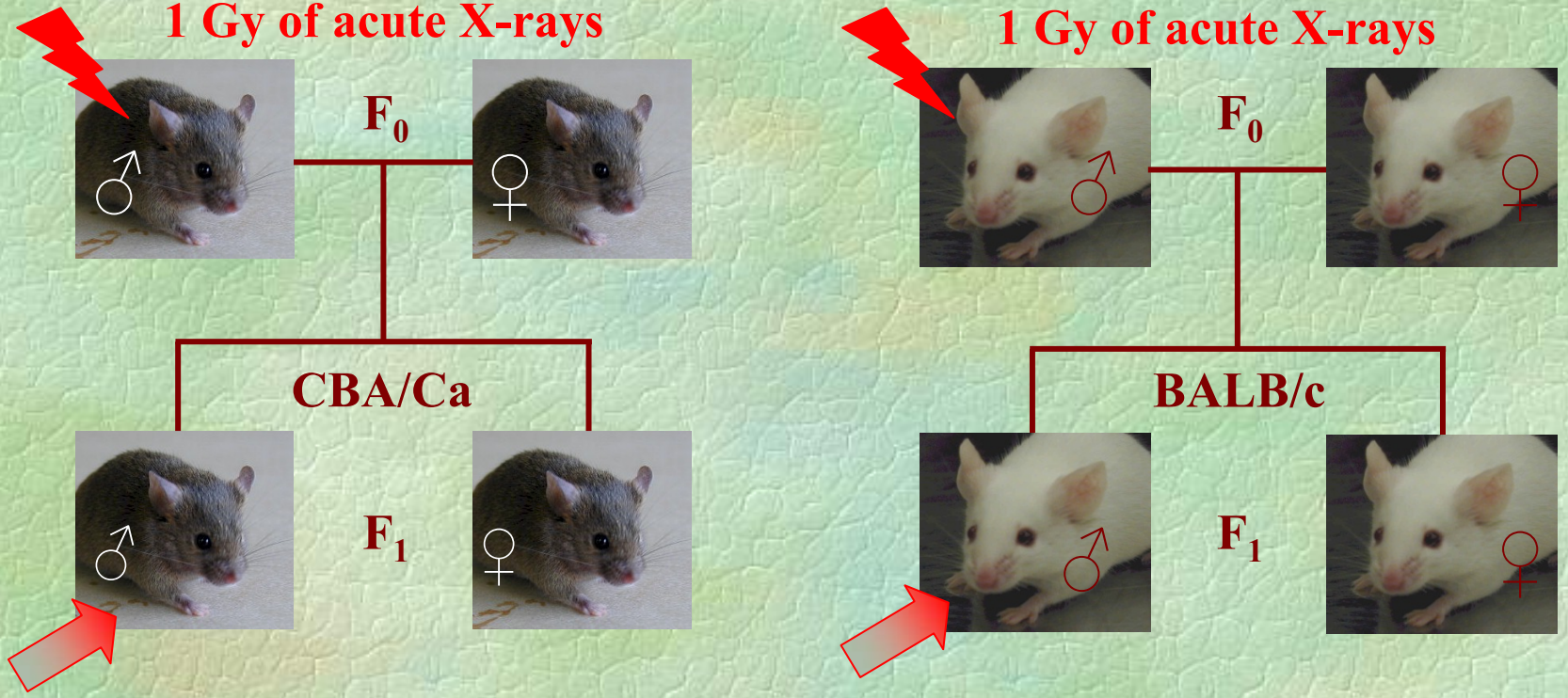
**Hallmark:**
**Accumulation of oxidatively damaged nucleotides in DNA**




- The efficiency of DNA in the $F_1$ offspring is OK
- No sign of oxidative stress in the $F_1$ offspring
- What else?

From: Barber *et al.*, 2006, *Oncogene* **25,** 7336-42

# Transcriptome analysis of transgenerational effects




RNA extraction


NimbleGen 12x135K expression arrays:

- 135,000 probes per array; 45-60mer long
- Complete coverage of the mouse transcriptome (42,576 transcripts)
- 3-4 probes per transcript
- 12 arrays per slide

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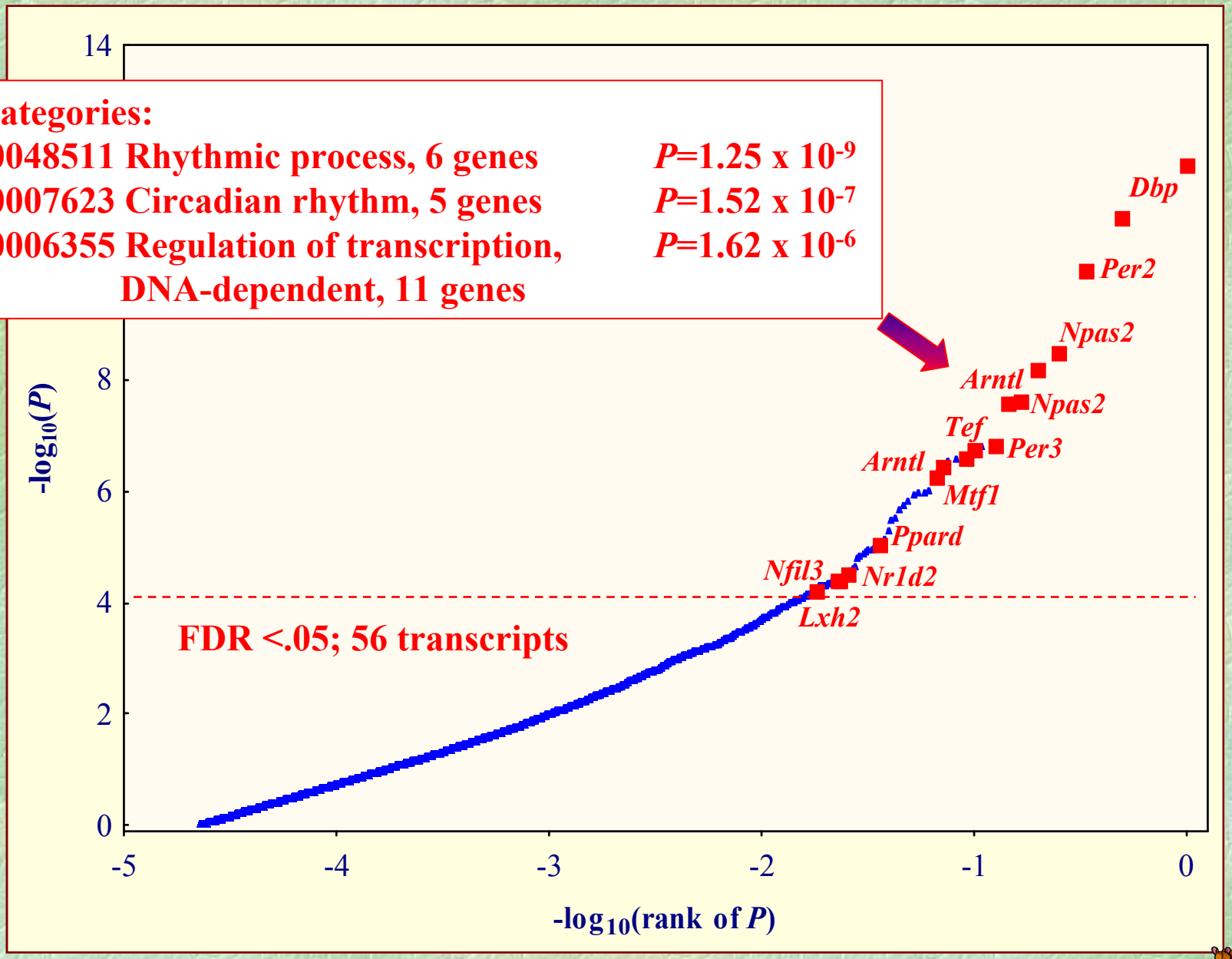
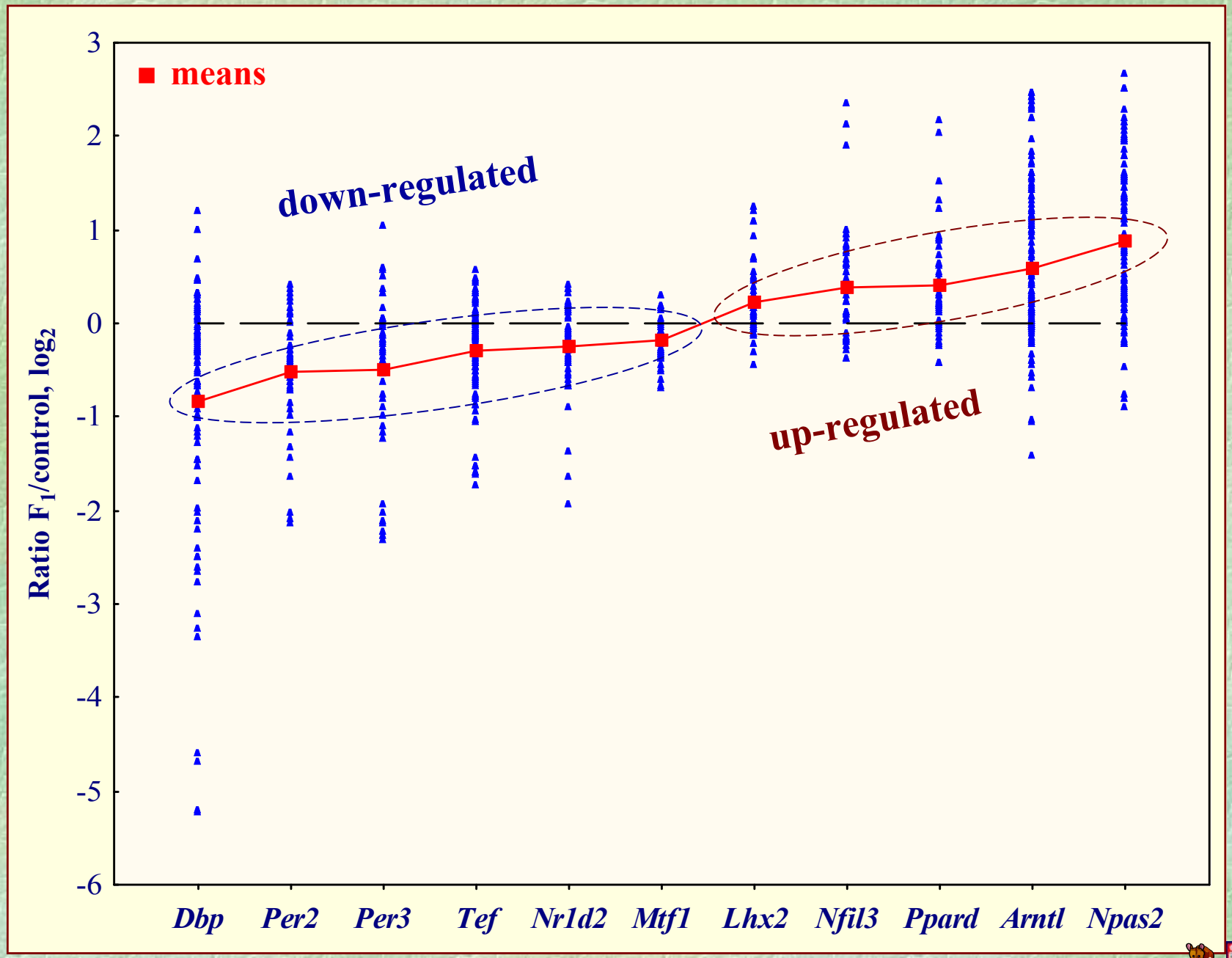
# Probabilities for the effects of paternal irradiation on $F_1$ gene expression

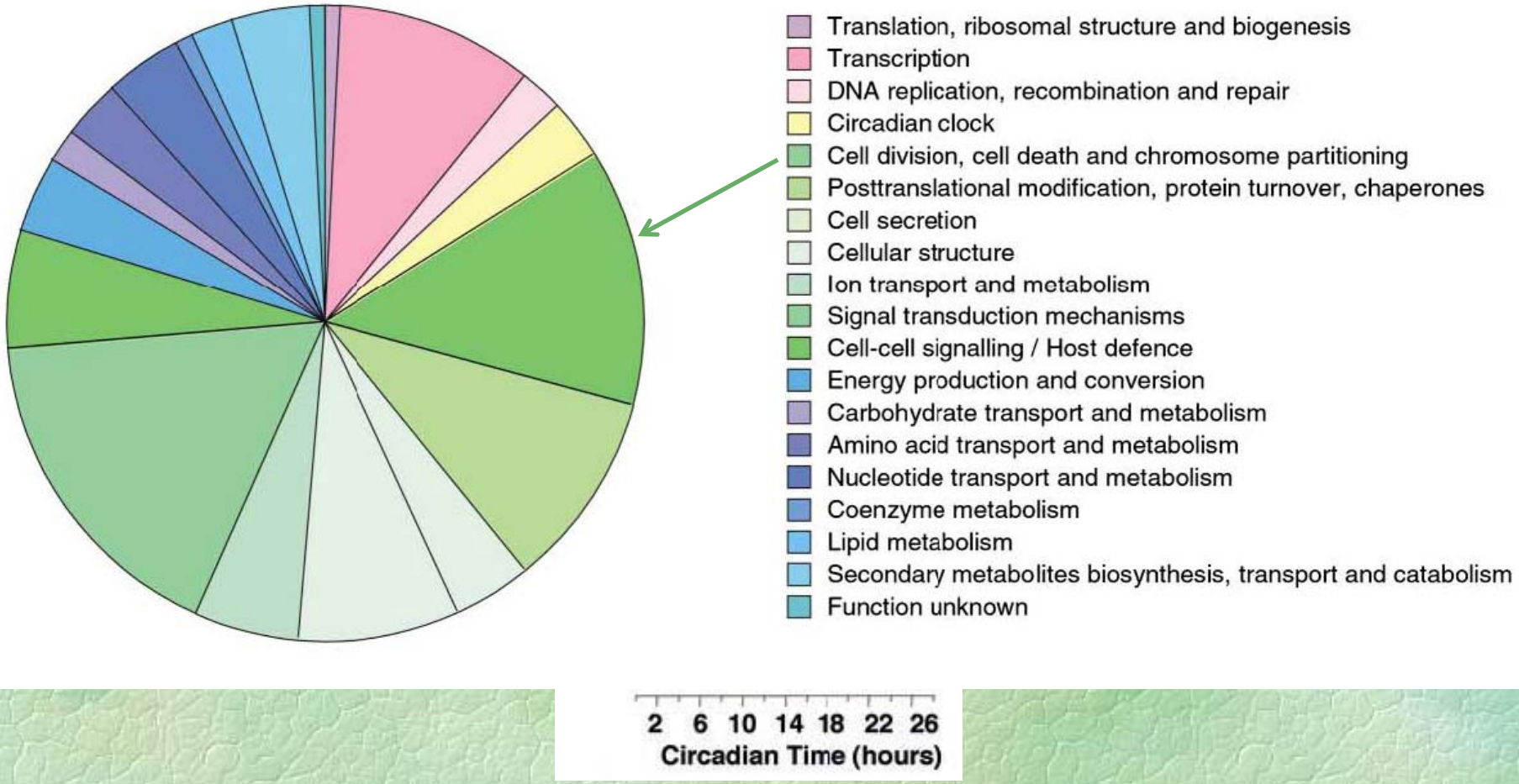


# Compromised gene expression in the $F_1$ offspring



# Circadian trascriptome & circadian metabolism in mice

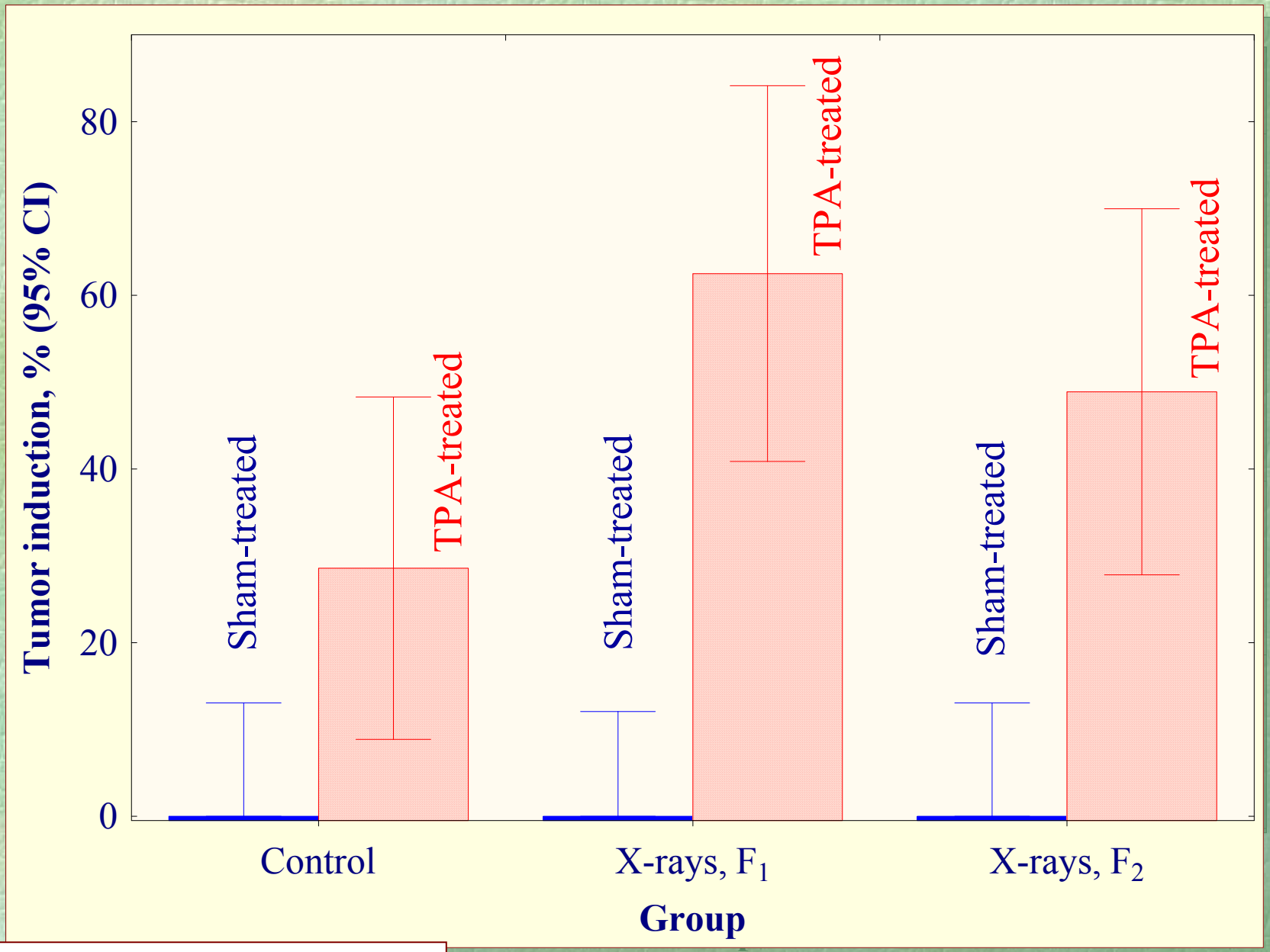
## Circadian transcripts in mouse liver




From: Maywood *et al*., 2007, *Cold Spring Harb Symp Quant Biol* **72,** 85
Akhtar *et al*., 2002, *Curr Biol* **12,** 540

# And so what?

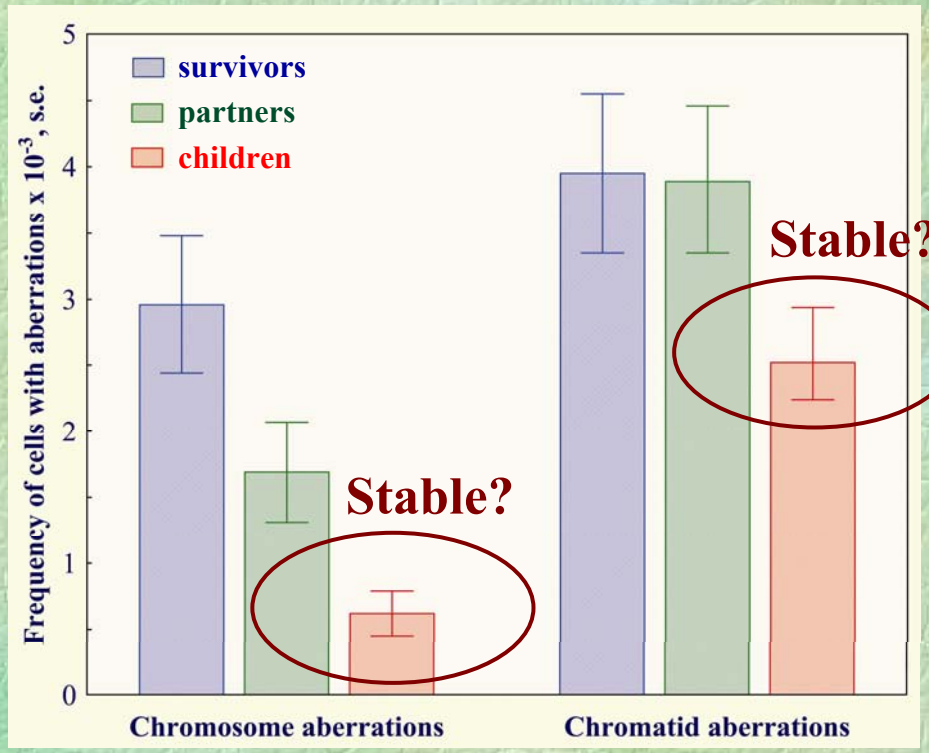
# Incidence of skin tumour in the offspring of irradiated male mice



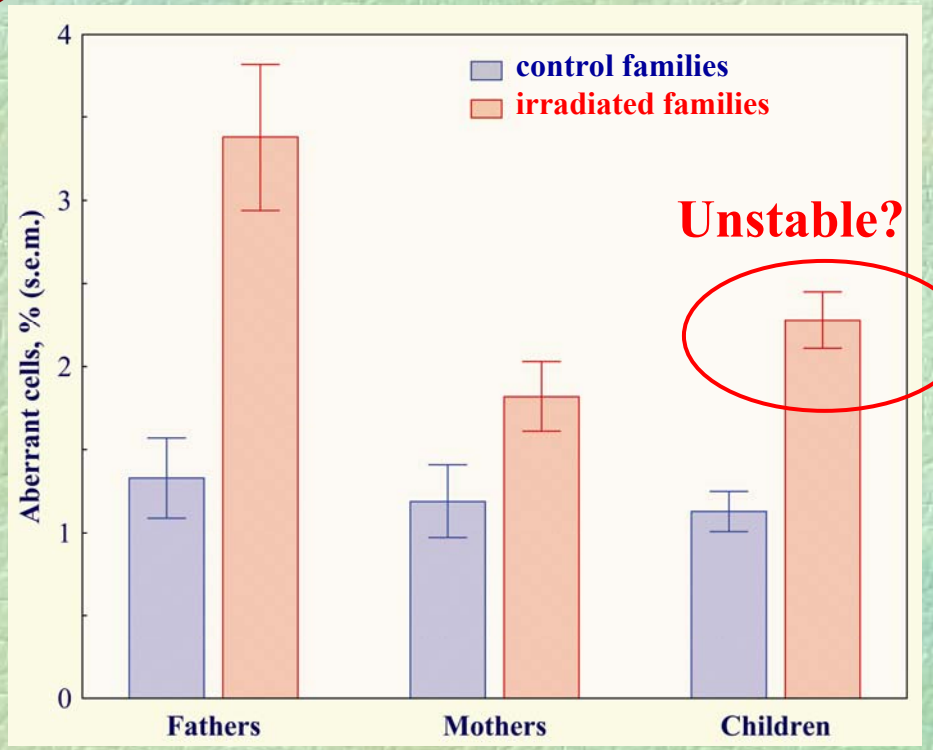

From: Vorobtsova *et al.*, 1993, *Mutat. Res.* **287,** 207-216

# Transgenerational effects in the children of irradiated parents

## Childhood cancer survivors




## Chernobyl clean-up workers




From: Tawn *et al*., 2005, *Mutat Res* **523,** 198-206; Aghajanyan & Suskov, 2009, *Mutat Res* **523,** 52-7

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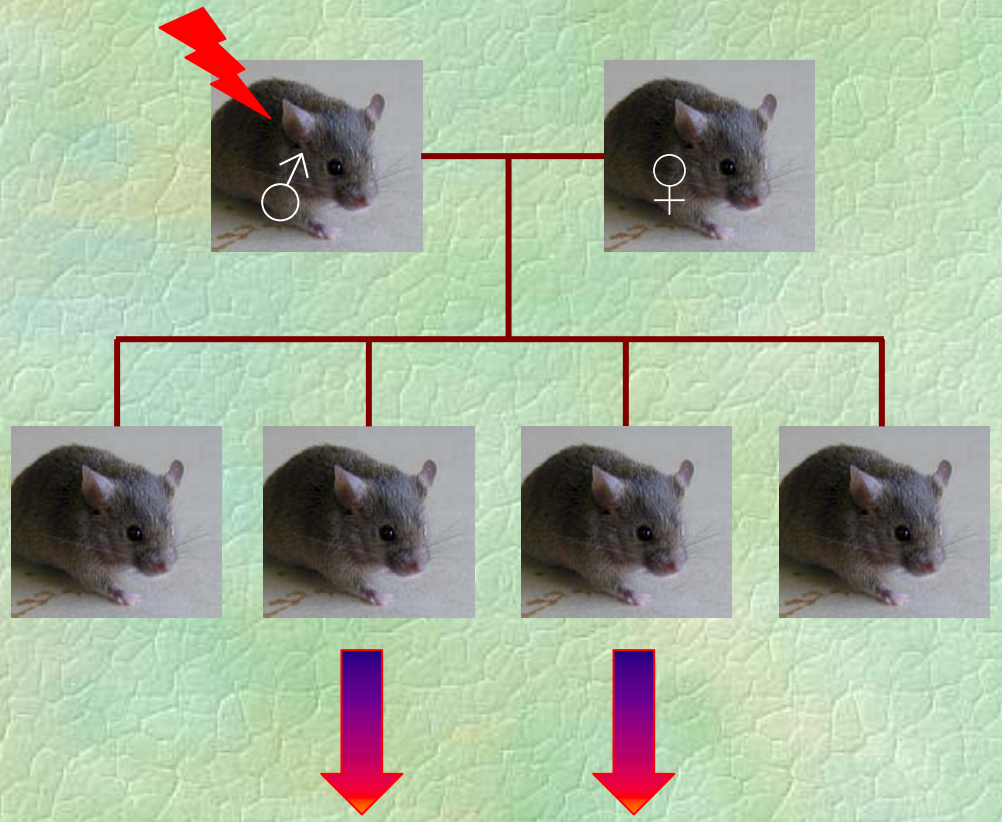
# From mice to humans....

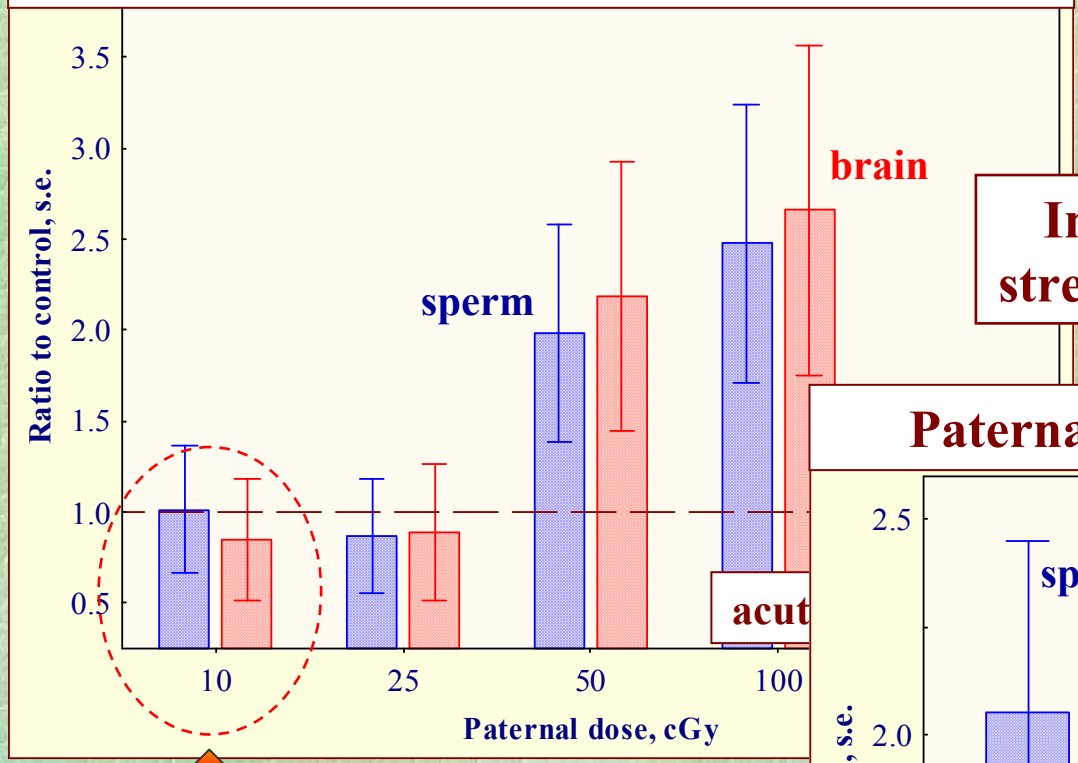
**Experiment one:**
**Male mice exposed to**
**10 – 100 cGy acute γ-rays**
**or 100 cGy chronic γ-rays**

**Experiment two:**
**Male mice exposed to**
**clinically-relevant doses**
**of 3 anticancer drugs:**
**Cyclophosphamide**
**Mitomycin C**
**Procarbazine**



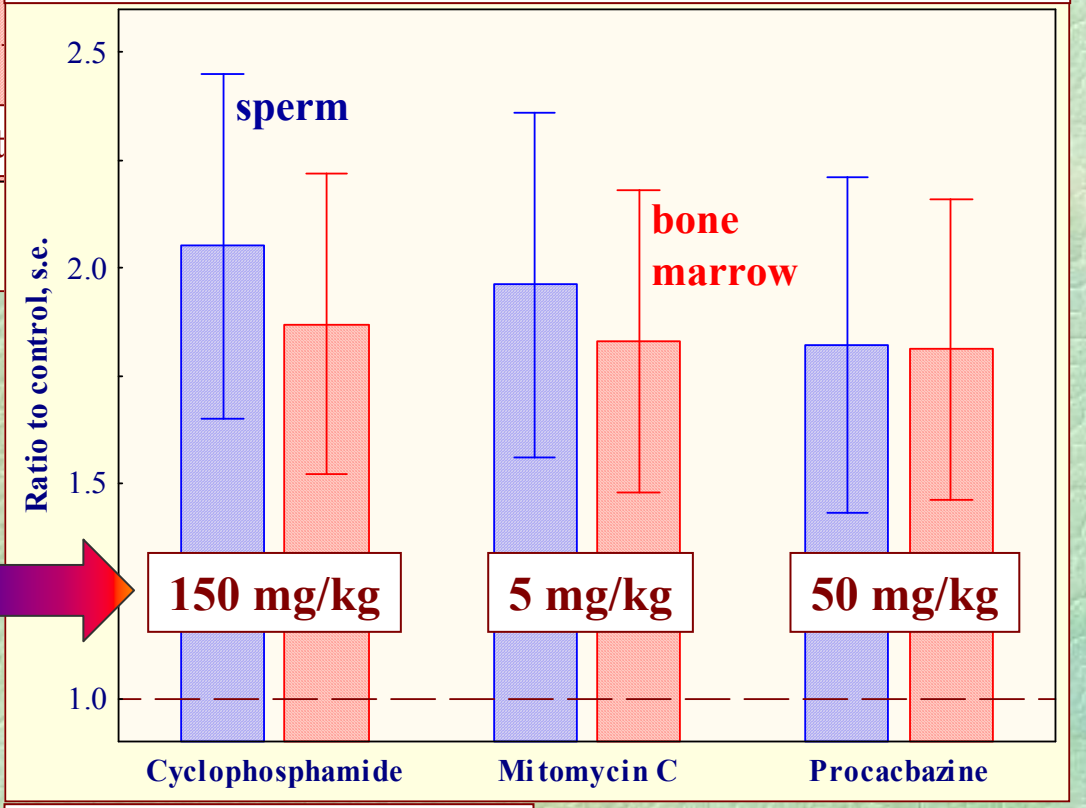

**Sperm, brain, bone marrow**

## Paternal exposure to acute & chronic γ-rays




**Instability signal is triggered by a stress-like response in irradiated cells**

## Paternal exposure to anticancer drugs




From: Glen, Dubrova 2012, *PNAS* **109,** 2984

**Dose per single radiotherapy procedure**

**Doses per single chemotherapy procedure**

# Conclusions

- High-dose acute paternal exposure to a number of mutagens can significantly destabilise the genomes of their offspring
- Transgenerational instability is a genome-wide phenomenon which affects the frequency of chromosome aberrations and gene mutations
- Transgenerational instability is triggered in the directly exposed germ cells by a stress-like response to a generalised DNA damage
- Transgenerational instability is attributed to the presence of a persistent subset of endogenous DNA lesions
- Transgenerational instability is attributed to the epigenetic changes affecting the expression of a subset of genes, involved in rhythmic process & regulation of transcription
- Transgenerational instability may represent an important component of the long-term genetic risk of human exposure to mutagens, but we need HUMAN data to prove it!

# Acknowledgements


- **Dubrova's lab**

  Ruth Barber, Colin Glen, Safeer Mughal, Andre Gomes, Robert Hardwick, Carole Yauk, Mariel Voutounou, Tim Hatch, Dominic Kelly, Peter Hickenbotham, Morag Shanks, Carles Vilarino-Guell, Karen Monger, Bruno Gutierrez, Karen Burr, Julia Brown, Natalya Topchiy, Isabelle Roux, Peter Black, Demetria Pavlou, Hamdy Ali Abouzeid
- **MRC Radiation and Genome Stability Unit, Harwell, UK**

  Mark Plumb, Emma Boulton, Jan Fennelly, Dudley Goodhead
- **Department of Cancer Studies and Molecular Medicine, University of Leicester, UK**

  George "Don" Jones, Gabriela Almeida — Comet Assay
- **NI Vavilov Institute of General Genetics, Moscow, Russia** — Chronic irradiation

  Alexander Rubanovich, Andrey Myazin
- **Medical Radiological Research Centre, Obninsk, Russia** — Chronic irradiation

  Leonid Zhavoronkov, Yuri Semin, Albert Brovin, Valentina Glushakova, Valentina Posadskaya, Olga Izmet'eva
- **MRC Toxicology Unit, Leicester, UK** — Anticancer drugs

  Andy Smith
- **Centre for Molecular Genetics and Toxicology, University of Wales, Swansea, UK**

  George Johnson, James Parry — *Hprt* assay
- **Catholic University of Nijmegen, The Netherlands**

  Peter de Boer, Alwin Derijck, Godfried van der Heijden — Sperm irradiation
- **Gray Cancer Institute, Northwood, UK** — *γH2AX* assay

  Kai Rothkamm



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