DNA double-strand break repair of parental chromatin in ooplasm and origin of de novo mutations

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Dad's problems vs. mom's work:
Paternal DNA damage vs. DNA repair in the zygote

Part 1: Decondensation of sperm (protamine to nucleosome transition and survival of paternal nucleosomes) involves double strand DNA breaks

Part 2: Double strand DNA repair by the zygote

- Repair systems active in the zygote (Homologous recombination, HR and Non-Homologous End Joining, NHEJ)
- Consequences of repair problems for first cleavage and the induction of reciprocal translocations

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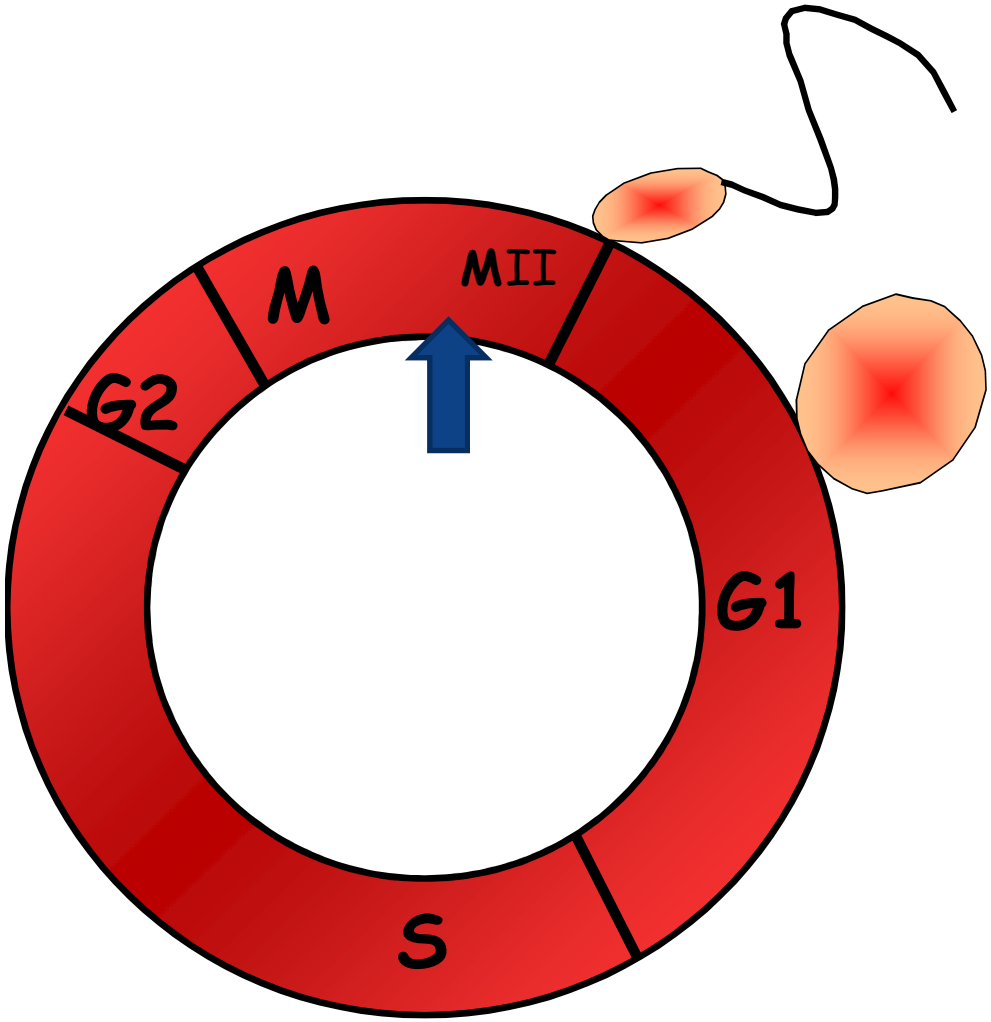
Meiosis II block, cell cycle resumption by membrane fusion



- As a result of IVF/ICSI more of the oocyte repair system will be asked ?
- When are DNA lesions detected and repaired?
- Is there a preference in stage of the zygotic cell cycle for repair to take place?
- Does a lower paternal DNA quality affect cell cycle progression?

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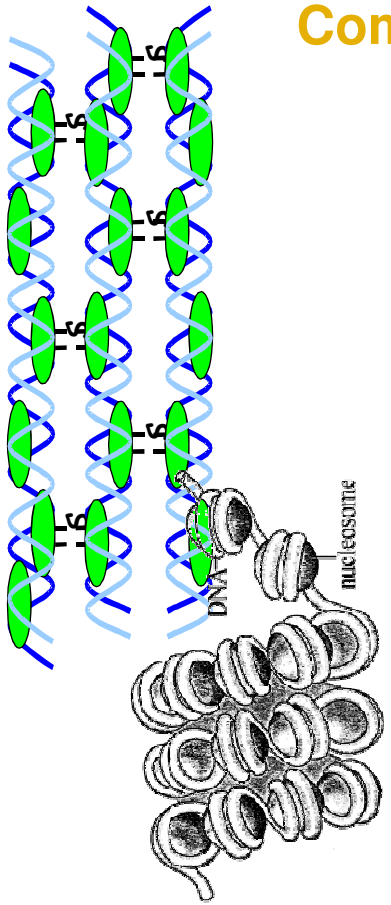
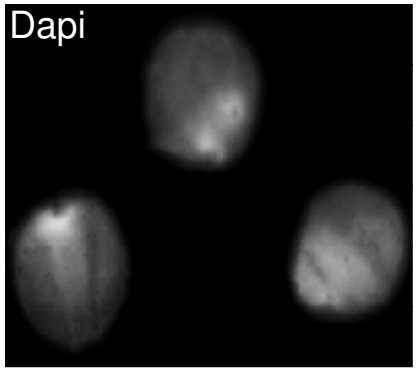
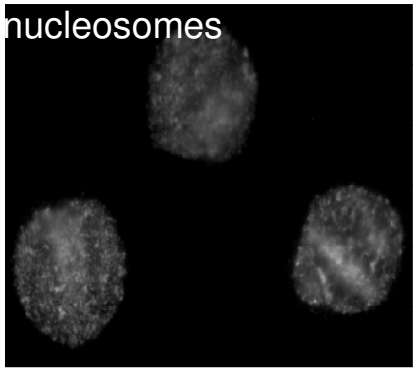
Sperm chromatin, special chromatin

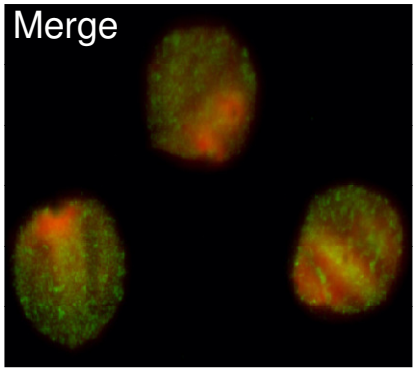


Compaction and radiation resistance is aided by protamines

- Mouse 1 -2 % of DNA is bound to Nucleosomes
- Human > 5% of DNA is bound to Nucleosomes*







Heparin decondensed human sperm stained with an antibody against nucleosomes

* More variable in OAT men

Ramos, vd Heijden et al.
Hum. Reprod. Vol.23, No.2 pp. 259–270, 2008

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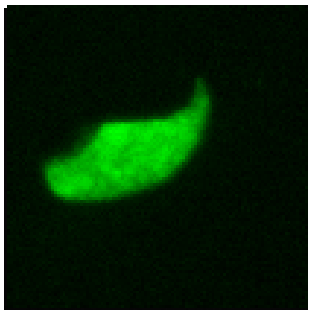
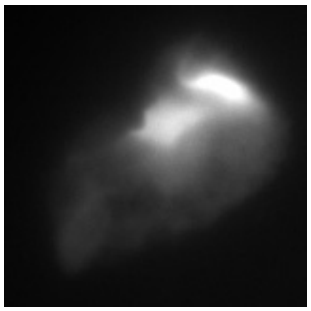
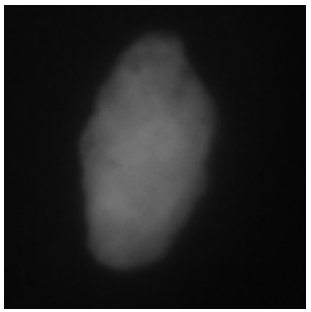
First cell cycle (mouse)



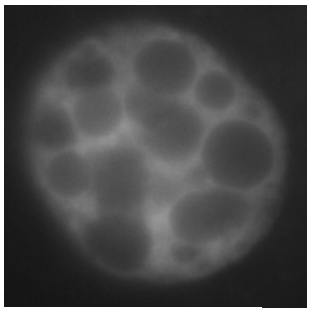
5-15 min



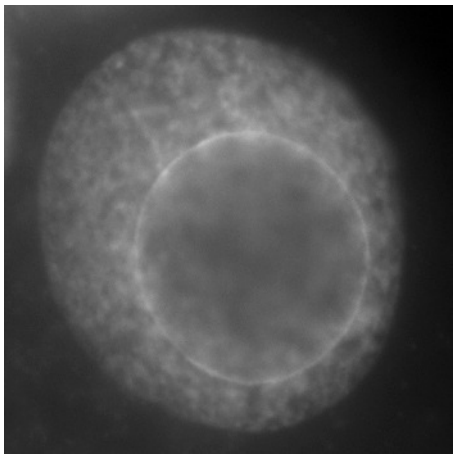
50 min



1 h 20 min



3 h

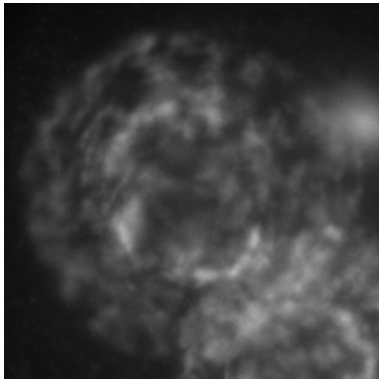


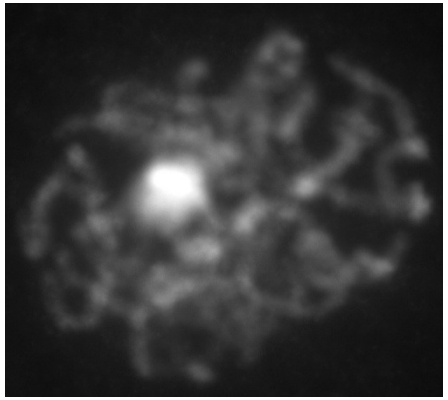
7 h

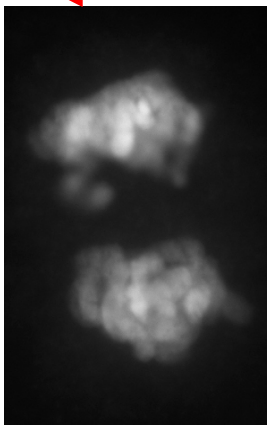
14 h

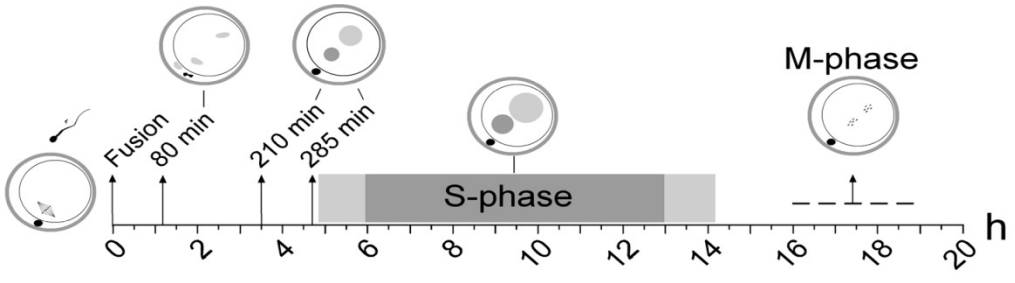
15 h

17 h









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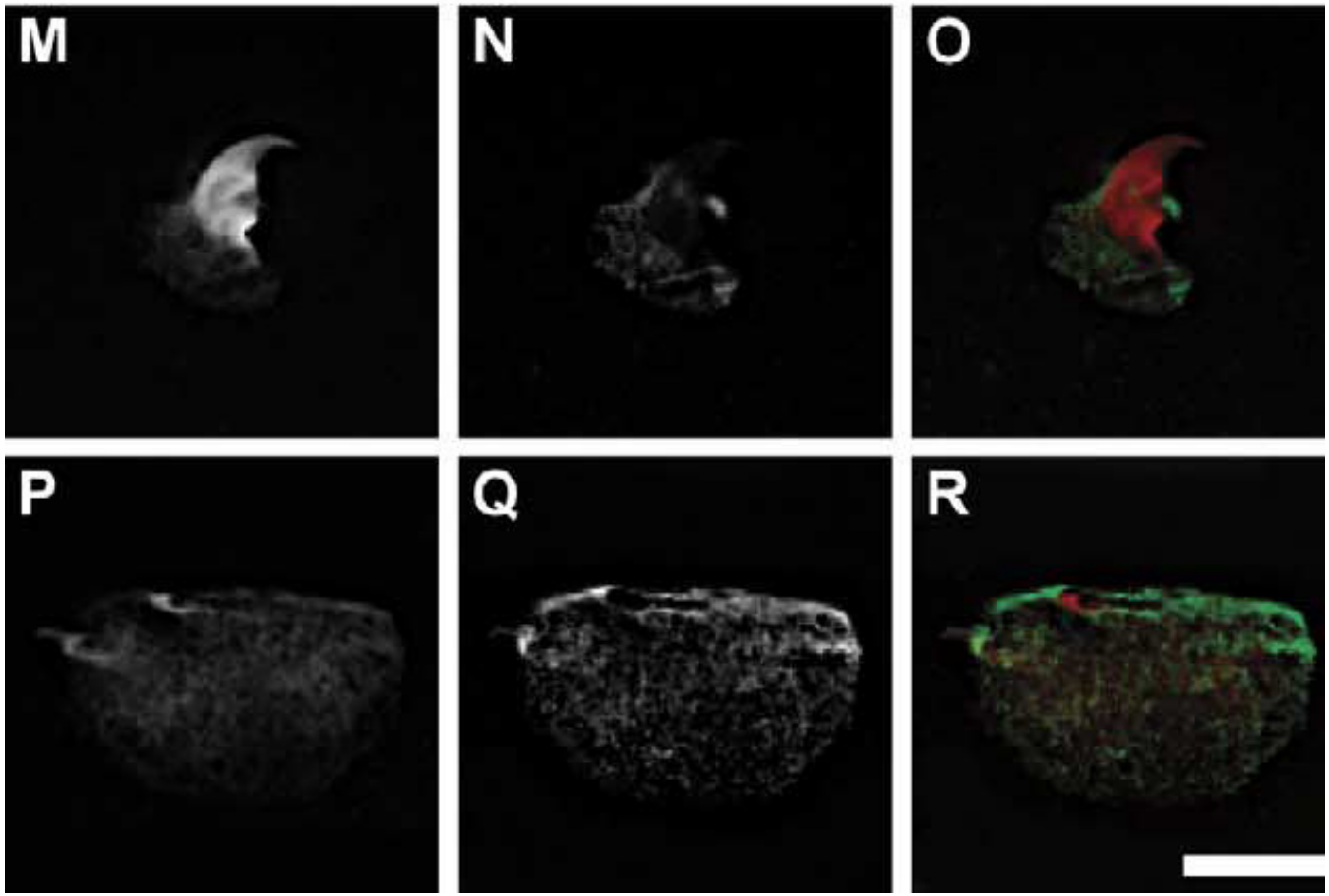
Upon gamete fusion protamines are rapidly removed and replaced by nucleosomes (n,q)



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Tools

- Mouse mutants defective for double strand DNA repair

- Knowledge of the zygotic cell cycle as to male and female pronucleus formation, male and female S-phase, and mitotic division

- Use of histone modifications for:

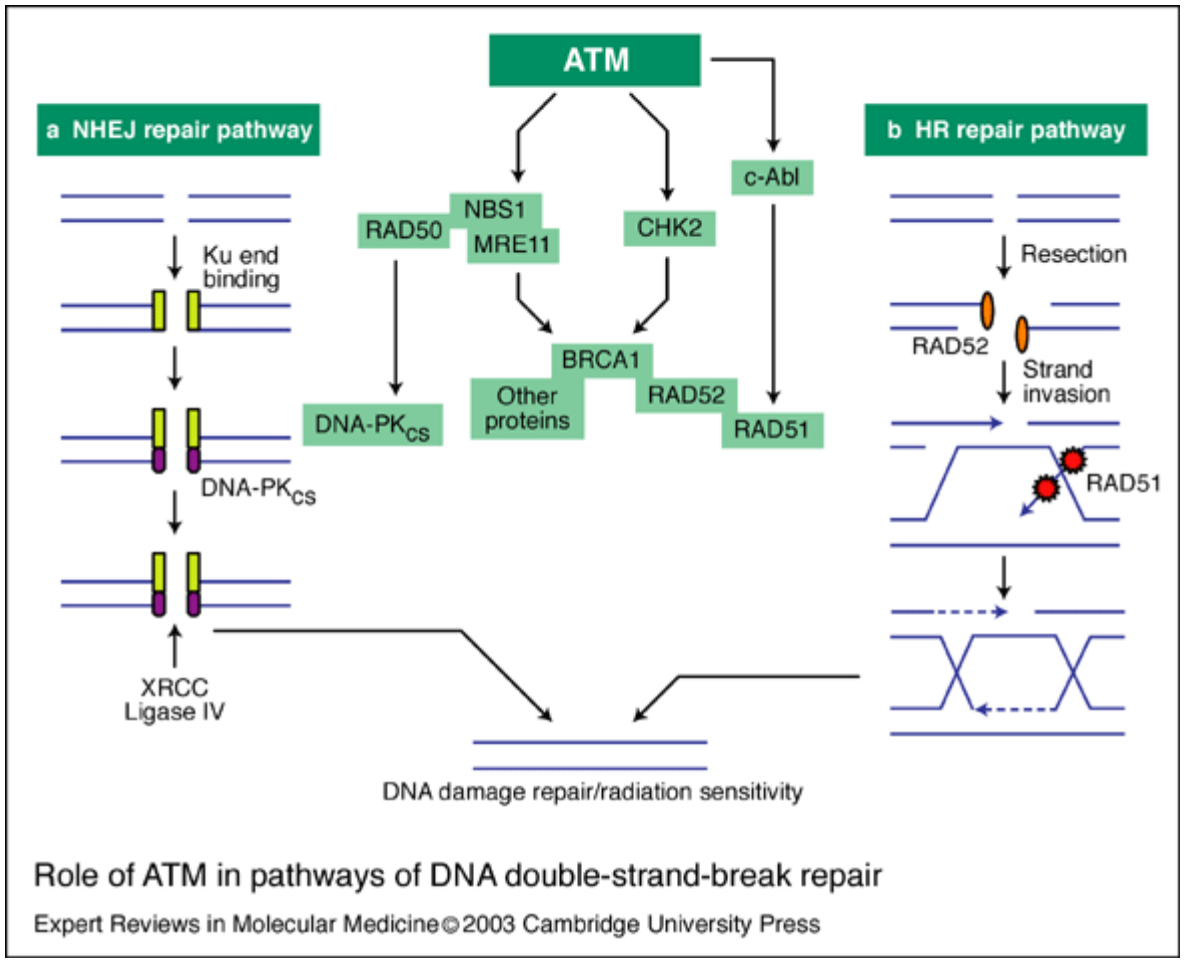
a) Male chromatin remodeling after gamete fusion
b) The detection of double strand DNA breaks
c) Identification of male vs. female chromatin (epigenetic asymmetry)

- Mutagenesis:

a) Sperm irradiation in vivo
b) Zygote irradiation in vitro
c) Chemical mutagenesis by 4NQO in vitro

Readouts: gammaH2AX foci, Rad51 foci, zygote mitotic index and chromosome abnormalities at first cleavage

Why focus on double strand break repair? dsDNA breaks cause chromosome abnormalities, balanced (reciprocal translocations) and unbalanced (lethal, dicentrics)



Role of ATM in pathways of DNA double-strand-break repair

Double strand breaks arise spontaneously at chromatin remodeling and during DNA replication at S-phase

Genetic dissection:

NHEJ
DNA-PKcs = scid mouse

HR
Rad54/Rad54B double ko

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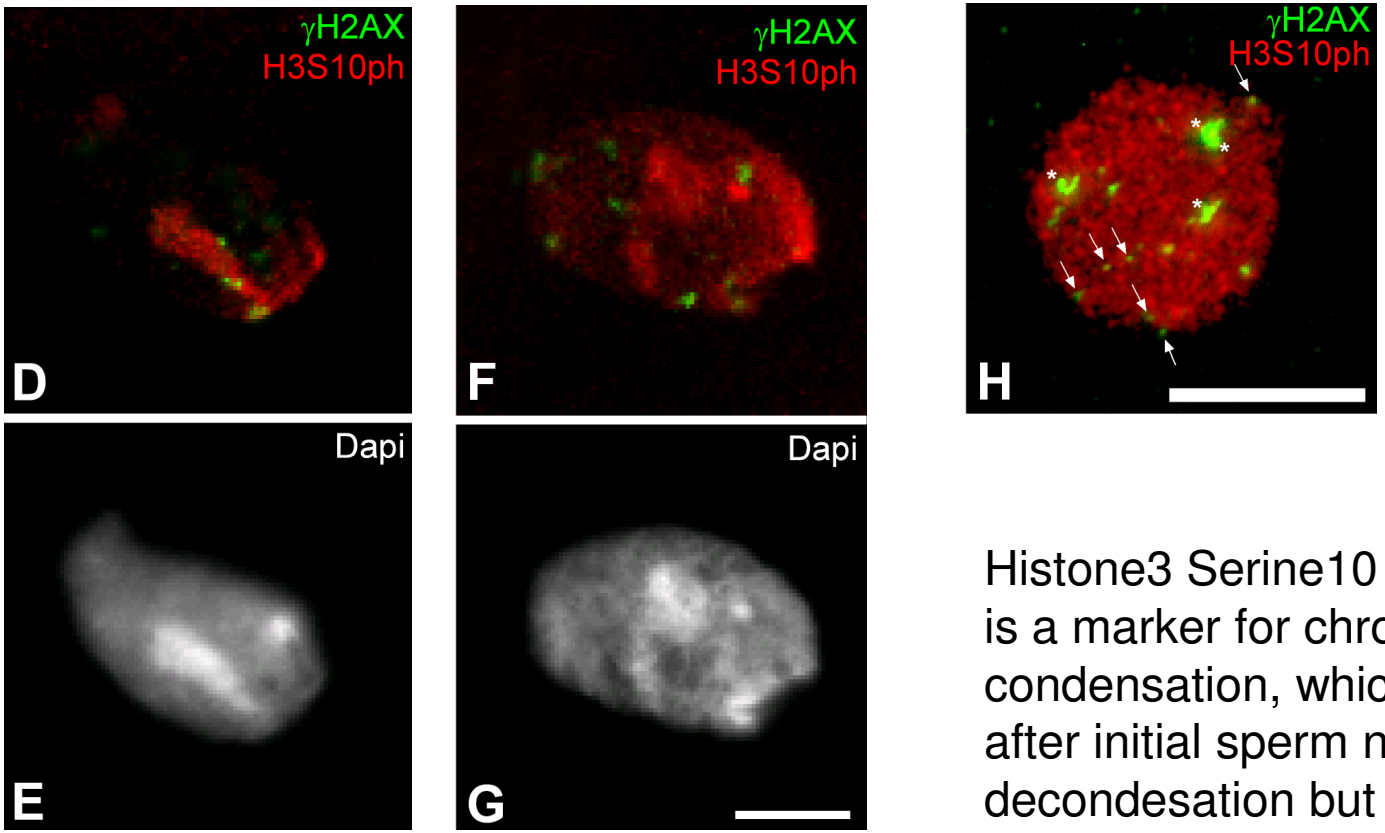
γH2AX signalling is active during sperm chromatin remodelling after gamete fusion and reacts on sperm irradiation



Histone3 Serine10 phosphorylated is a marker for chromosome condensation, which takes place after initial sperm nucleus decondesation but before pronucleus formation

Bar 10 um

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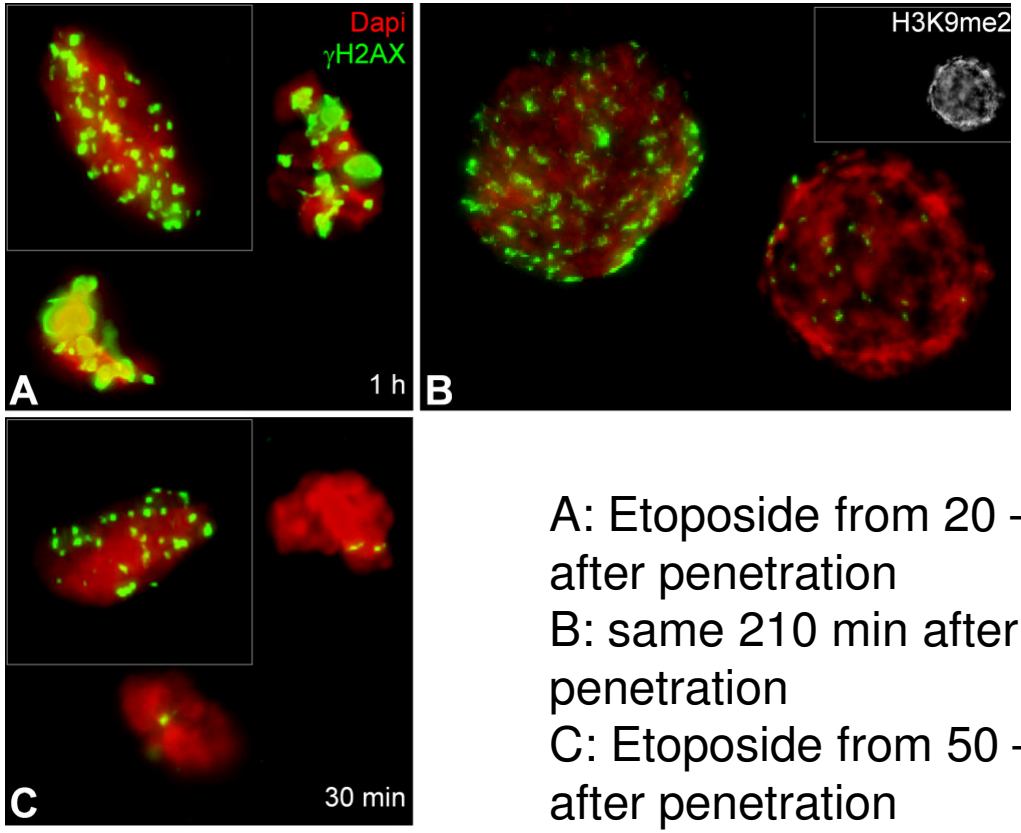
Topoisomerase II is involved in sperm chromatin remodeling as the specific inhibitor etoposide leads to ds DNA breaks, that persist in G1



A: Etoposide from 20 – 80 min after penetration
B: same 210 min after penetration
C: Etoposide from 50 – 80 min after penetration

A.Derijck, G. vd Heijden et al.
DNA Repair, 2006, 5(8): 959 - 71

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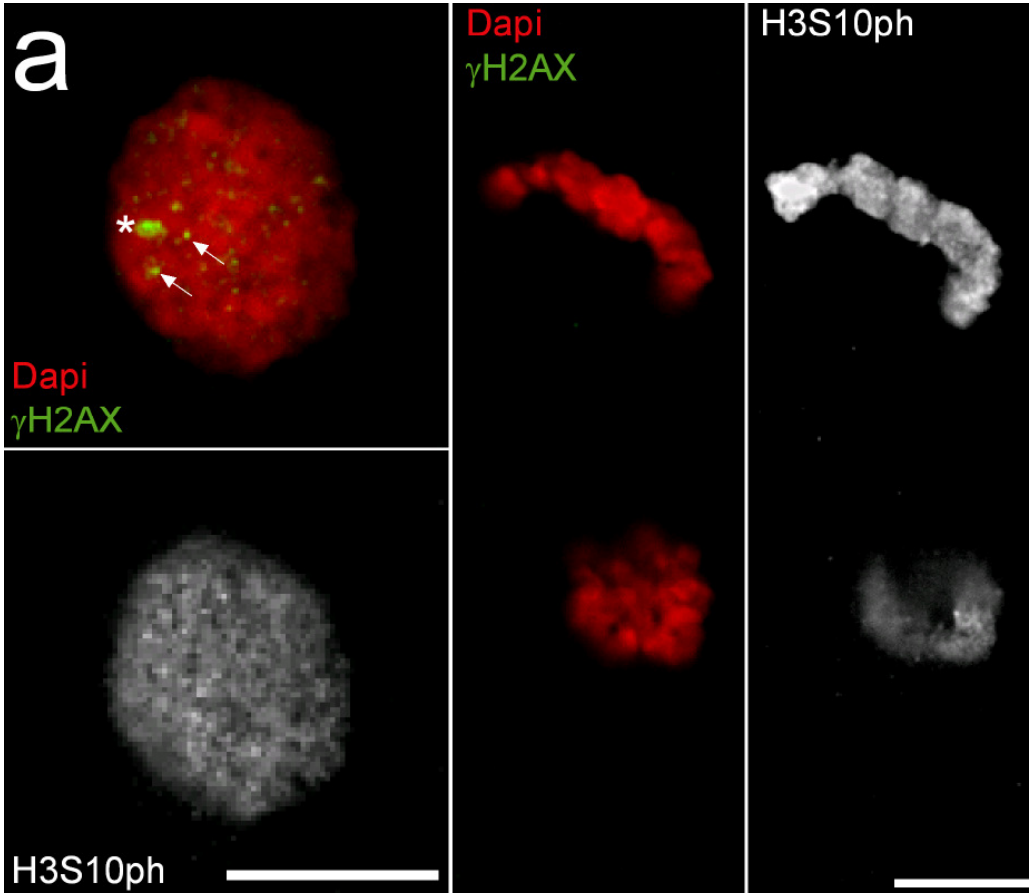
ϒH2AX signalling in heterologous ICSI with human sperm

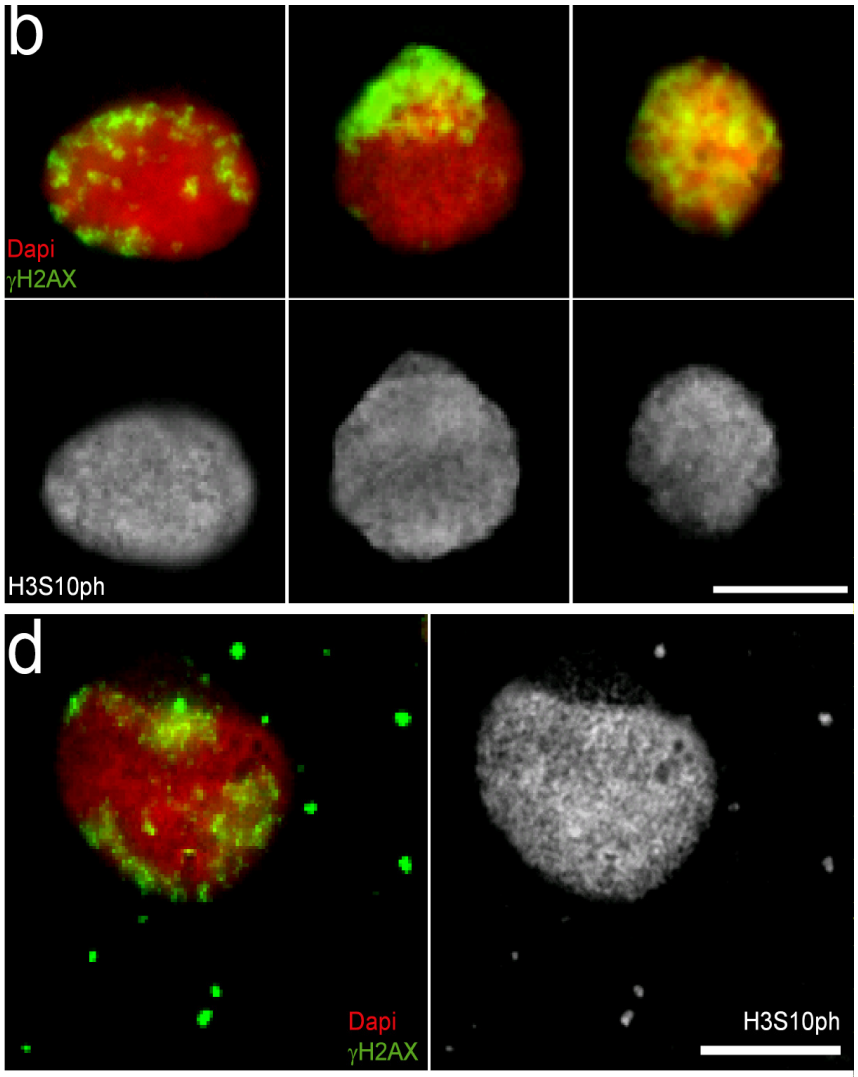


male female

Breaks in human sperm heads and mouse sperm heads occur at about the same frequency

Were these breaks present in sperm DNA, or do they originate from an interaction with the oocyte at chromatin remodeling ?

A. Derijck, G.vd Heijden, et al.
Hum. Reprod. 2007 Sep;22(9):2368-76



Injection of non-motile dead spermatozoa with normal morphology mostly yields abnormal yH2AX staining patterns (b) up to fragmenting male chromatin (d)

A.Derijck, G.vd Heijden, et al.
Hum. Reprod. 2007 Sep;22(9):2368-76

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Mouse models for the two major dsDNA break repair pathways (mouse IVF)

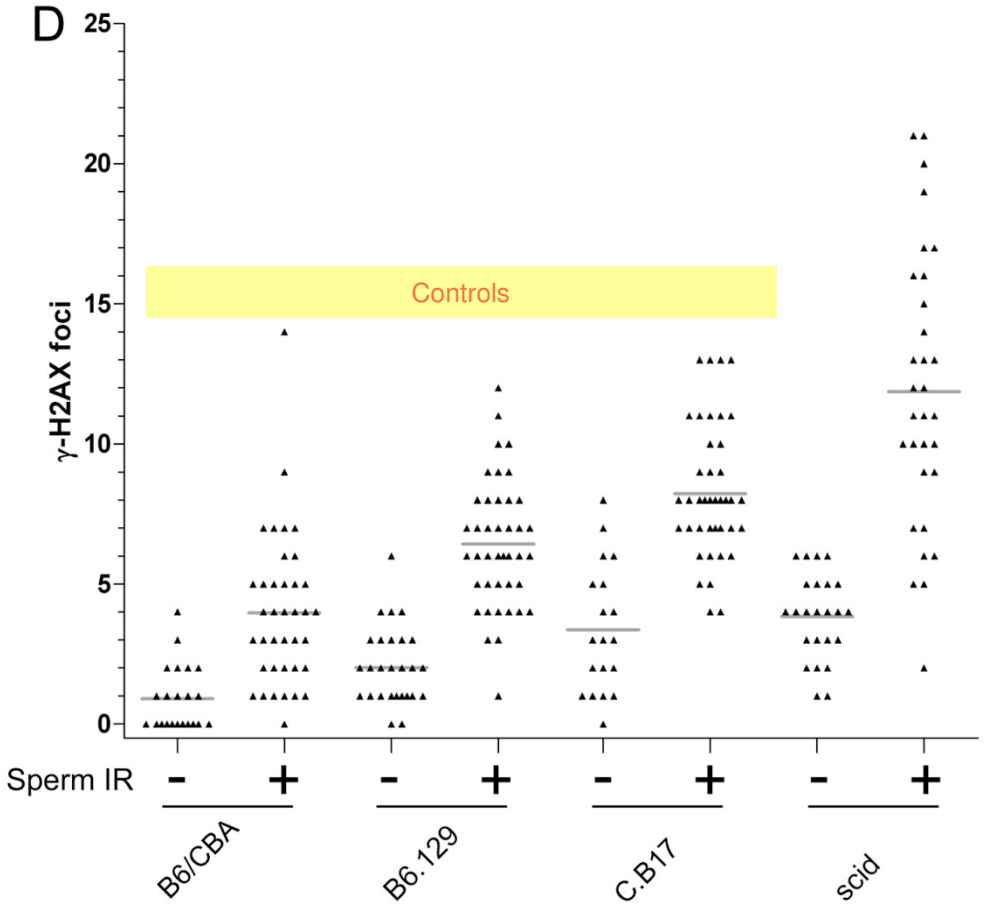
Oocyte sources

Rad54-/- Rad54B-/- (HR hypomorphic)

Scid (NHEJ deficient due to DNA.PKcs strongly hypomorphic allele)

Controls: for Rad54 is the B6/129 synthenic
for Scid is the C.B17, carrying a less severy DNA.PK hypomorphic allele.

A. Derijck, G. vd Heijden et al.
DNA Repair 2006, august; 5(8):959-71



Spontaneous and sperm (B6 CBA) induced breaks are, 80 min post penetration, dependent on oocyte genotype

NHEJ is involved in chromatin remodeling and in the repair of sperm irradiation damage

Also, we do not see every paternal break in a normal zygote

	C.B17	Scid (NHEJ)	B6.129	*mRad54/54* $B^{-/-}$ (HR)
% of zygotes with male chr.abnorm. (3 Gy)	29.3 (17/58)	69.0[a] (40/58)	31.3 (15/48)	35.9 (38/106)

Derijck A, van der Heijden G, et al.
Hum Mol Genet. 2008 Jul 1;17(13):1922-37

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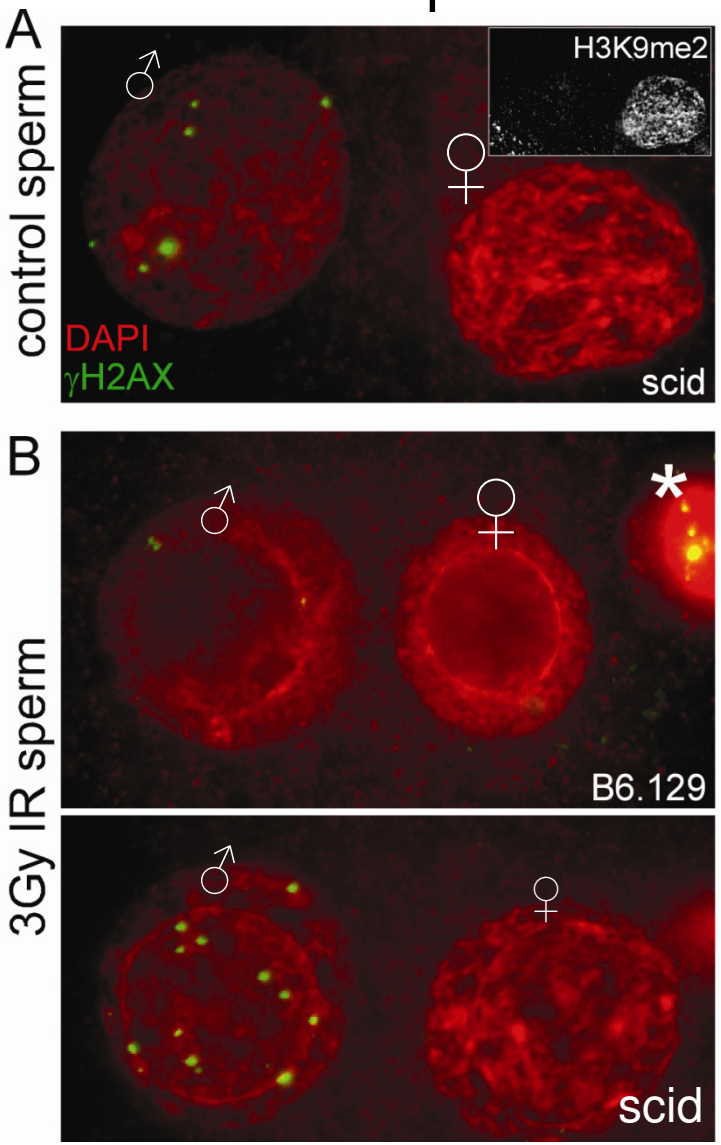
How is sperm irradiation damage removed?



Time: 285 minutes after gamete fusion, close to S=phase

When NHEJ is deficient, γH2AX foci persist, also in control sperm

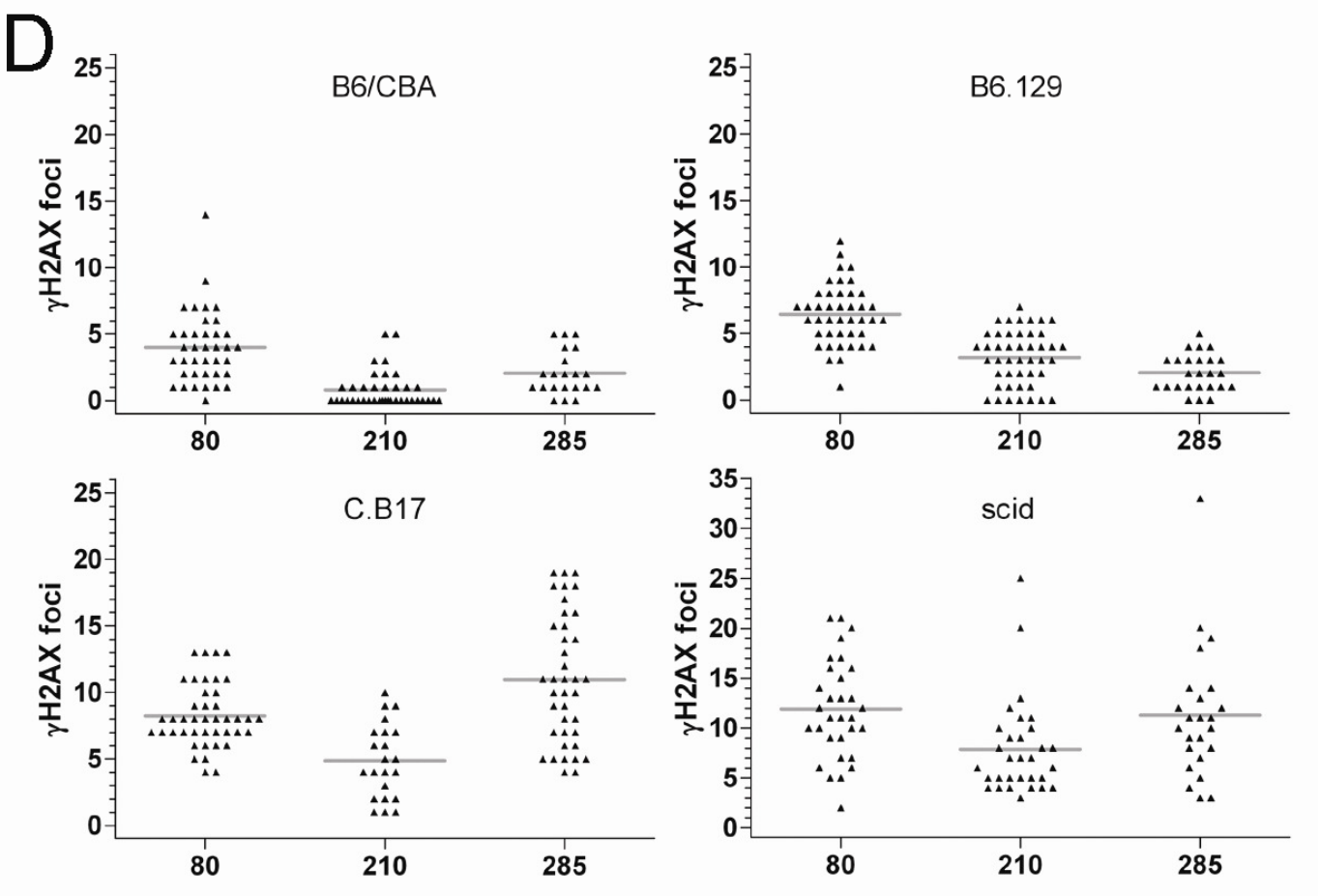
When scid is compared with the Rad54 control with a normal DNA.PK allele, this can be demonstrated

PS H3K9me2 is female chromatin marker

Graphical representation

Also in C.B17, G1 repair is deficient, not leading to increased CA

Why do close to S-phase foci increase?



A. Derijck, G. vd Heijden et al.
DNA Repair 2006, august; 5(8):959-71

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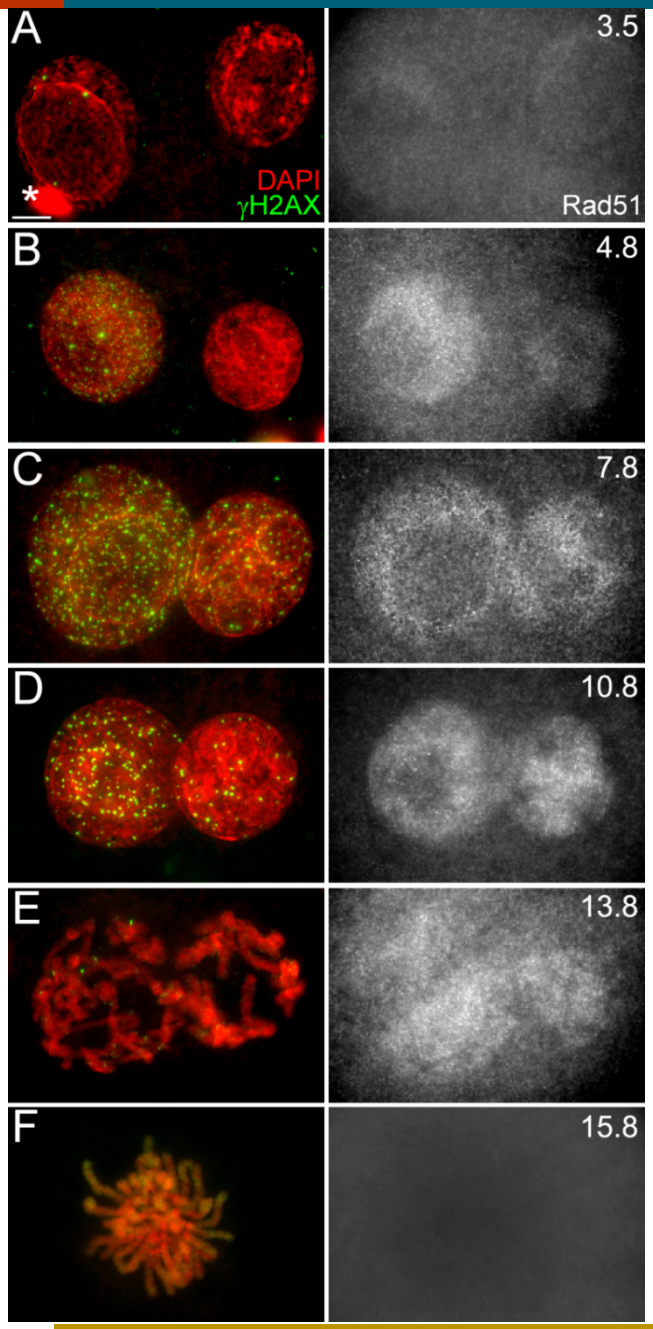
Observations, more questions

•In early G1 γH2A.X foci disappear. Not in SCID (DNA.PK deficient) oocytes, leading to chromosome abnormalities and in C.B17, not leading to chromosome abnormalities

Do G1 paternal lesions interfere with the progression of the cell cycle thereafter?

A G1 S phase checkpoint does not exist in the zygote

Events in S-phase

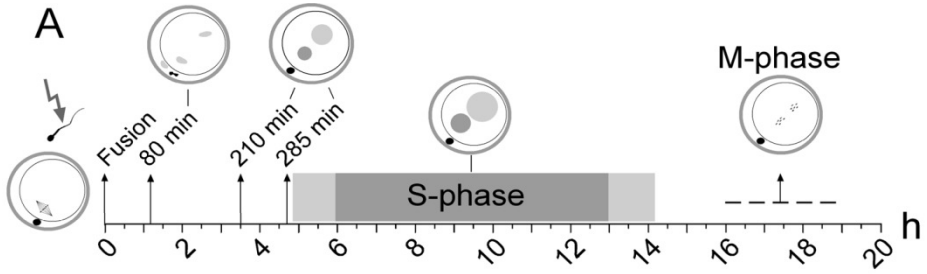


A. Derijck, G. vd Heijden et al.
DNA Repair 2006, august; 5(8):959-71

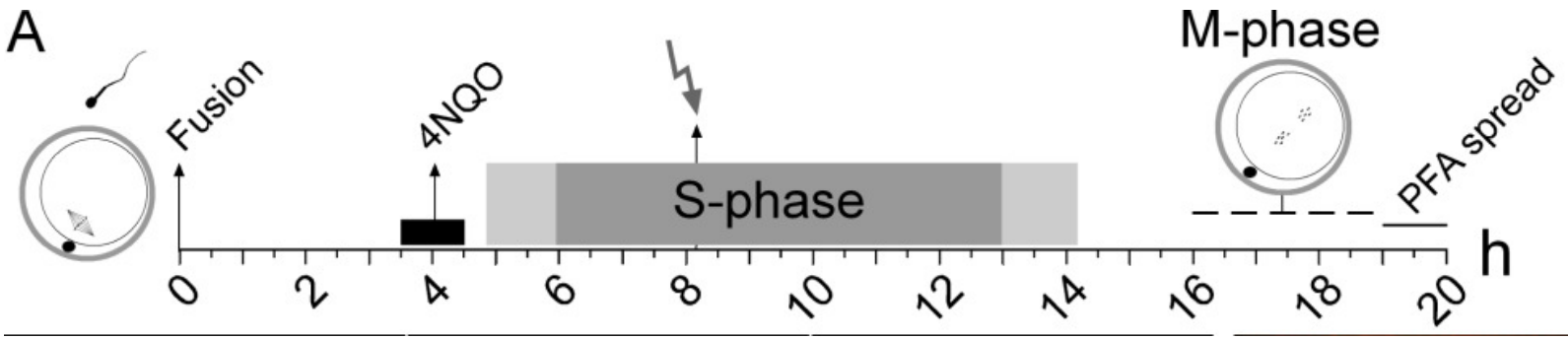
S-phase, earlier in the male PN, has a specific γH2AX pattern that in time coincides with import of the HR key protein Rad51

Rad51 is always lost from chromatin at late prophase

Male S-phase is more heavily labelled with γH2AX foci, that persist longer

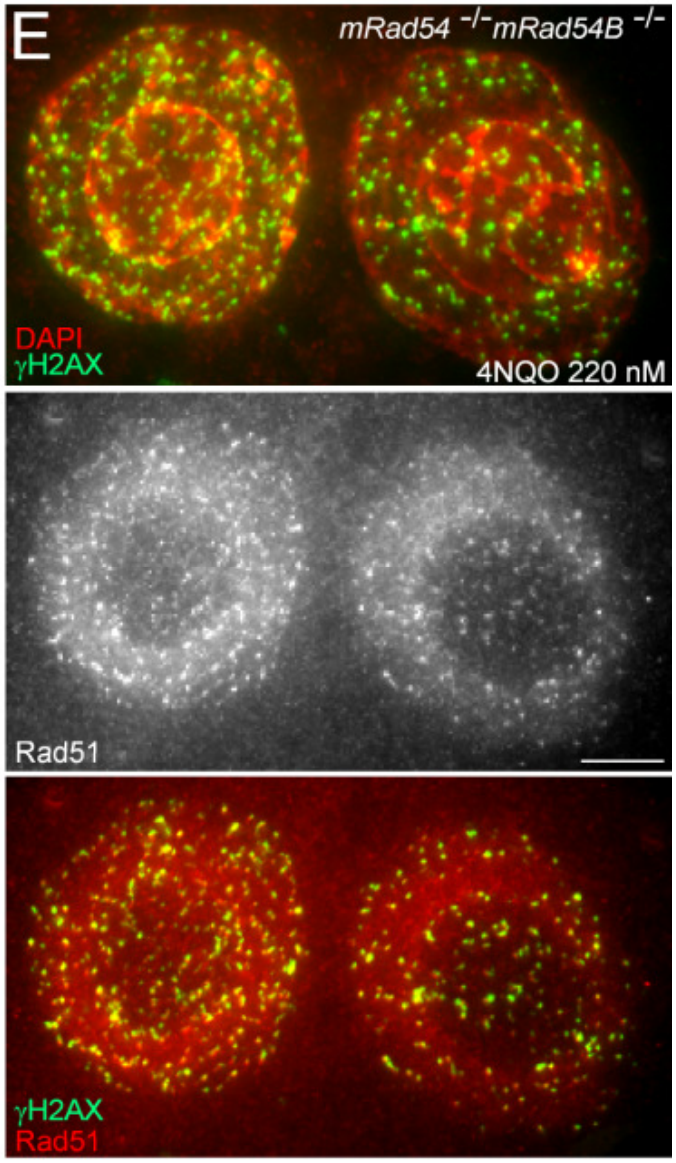


Analysing DNA repair from S-phase on



4NQO: 4-Nitroquinolione 1-oxide, ao mimics UV light

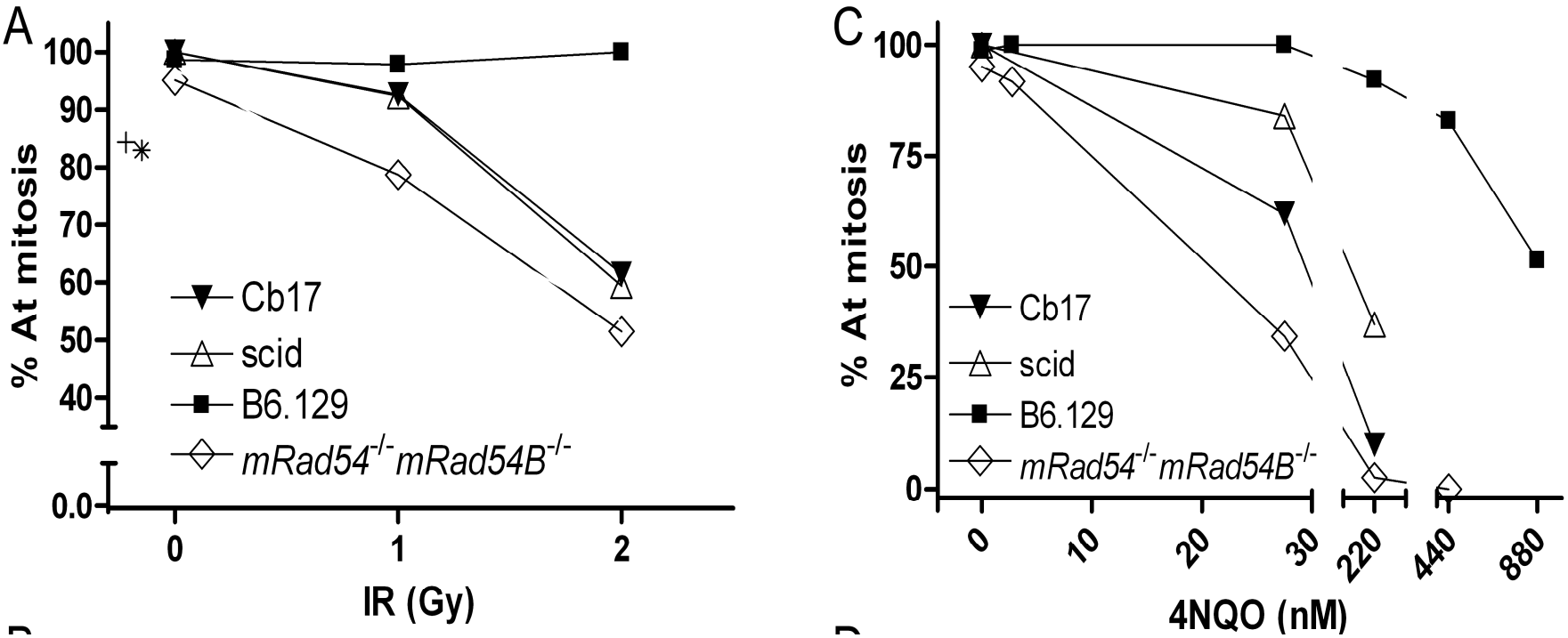
After 4NQO, Rad51 and γH2AX foci can be detected, single strand lesions interfere with replication and are repaired in the context of replication



Rad54 ko *(HR deficient)* zygotes are very sensitive to 4NQO, leading to many Rad51 foci that coincide with γH2AX foci in especially the blocked male PN

Paternal pronucleus on left side (more foci)

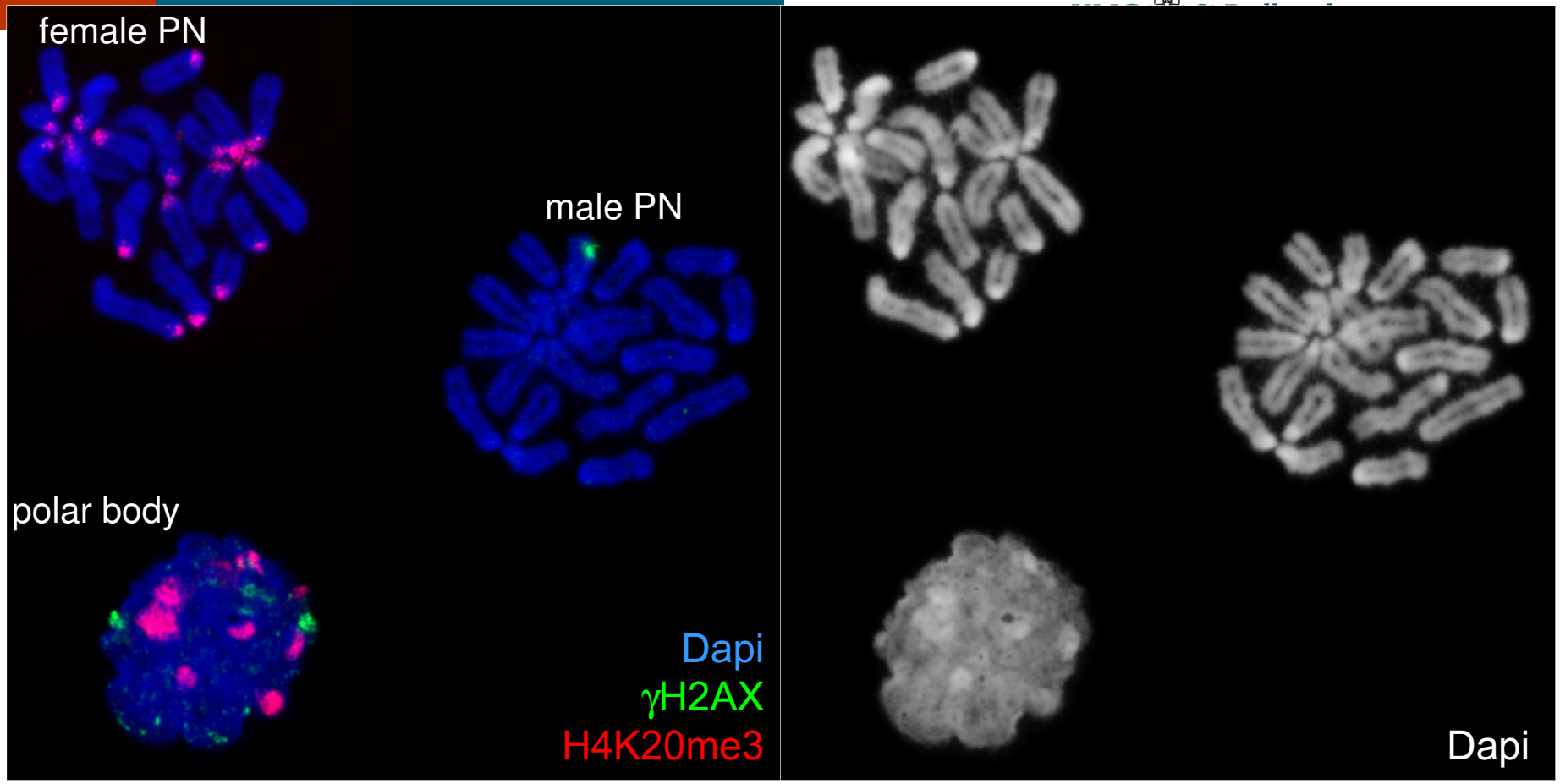
A. Derijck, G. vd Heijden et al.
DNA Repair 2006, august; 5(8):959-71

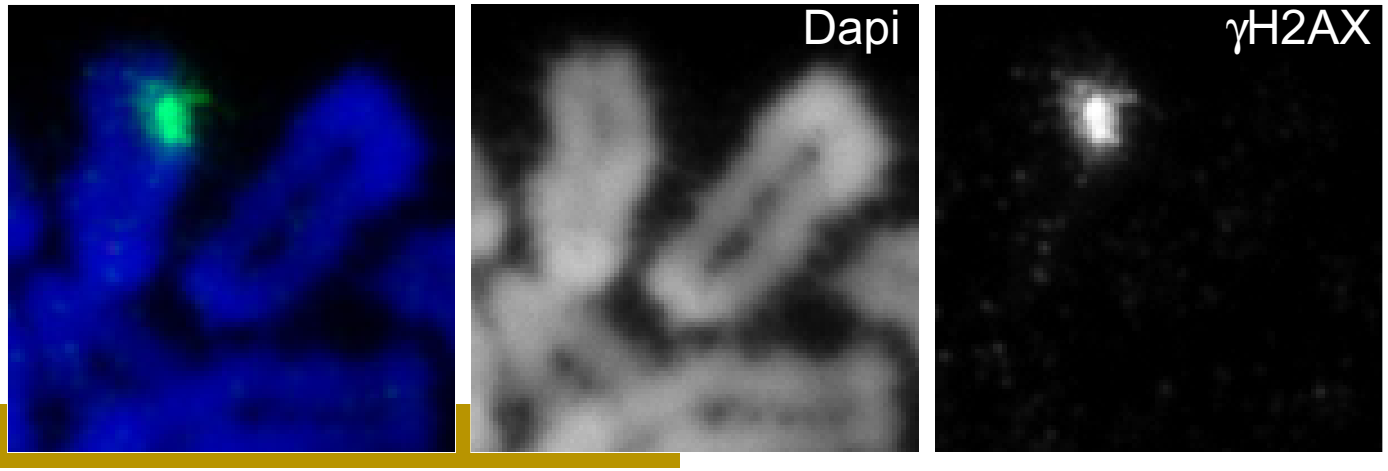


Mitotic efficiency of the various maternal genotypes *(S-phase irradiation and preS-phase chemical mutagenesis)*.
Note the order of sensitivities, both HR and NHEJ are involved

After sperm irradiation, some zygotes hypomorphic for DNA.PK (+,*) block at the pronuclear stage

Derijck A, van der Heijden G, et al.
Hum Mol Genet. 2008 Jul 1;17(13):1922-37


female PN
male PN
polar body
Dapi
γH2AX
H4K20me3
Dapi


Dapi
γH2AX

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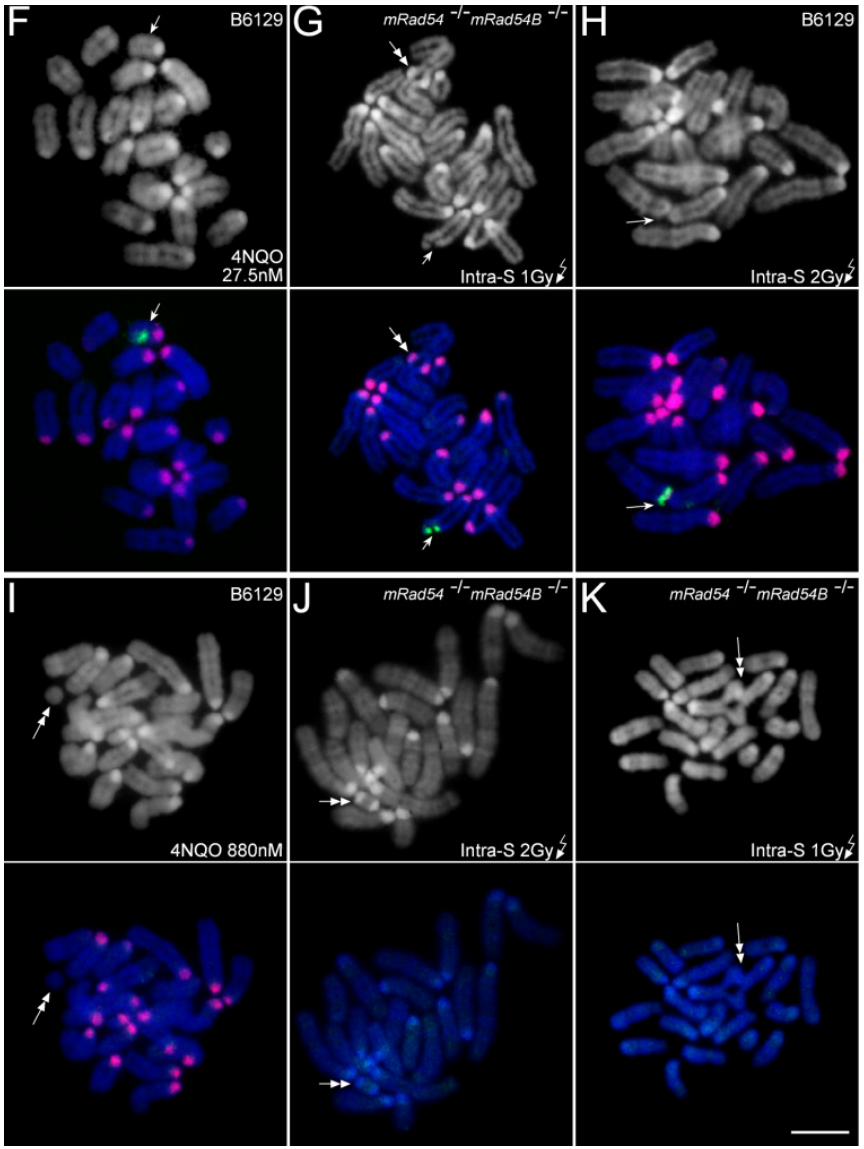
Chromosomes of zygotes that divide



Red is fluorescent mark (Histone4 Lysine20 trimethyl) on female centric heterochromatin

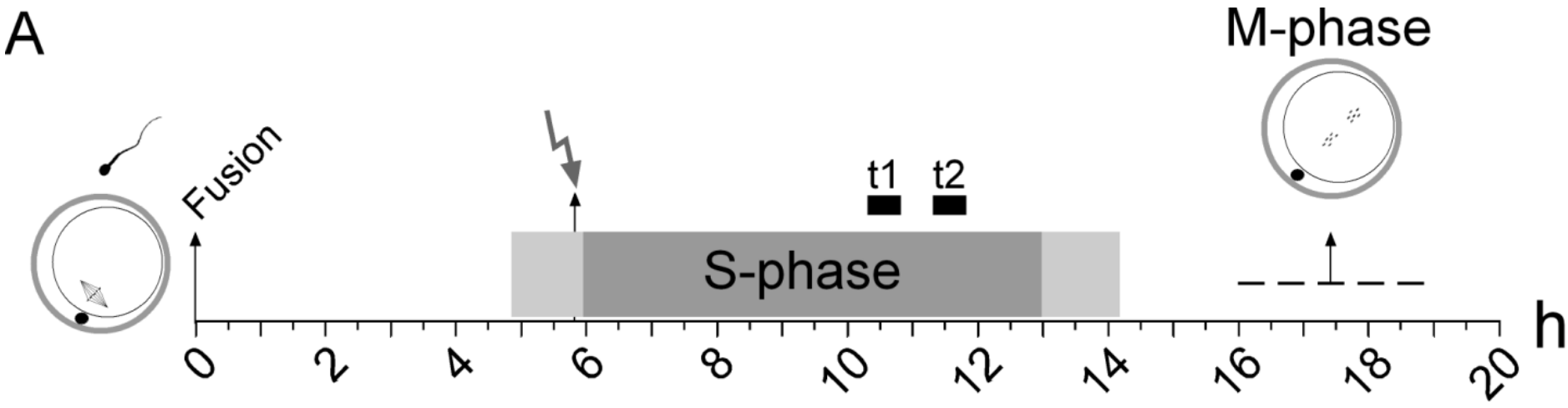
Green is γH2AX foci on 1 or 2 (iso)chromatids, usually not connected with a chromosome abnormality (single arrow)

Double arrow is chromosome abnormality

In G and K quadriradials are visible produced by inadequate action of the HR pathway

Quadriradials segregate into normal and reciprocal translocation

In all, γH2AX foci are more numerous on male chromosomes



Evaluation of repair during S-phase (NHEJ and HR), involving the four maternal genotypes

Dose of irradiation is 2 Gy
At t1 and t2, the numbers of yH2AX and Rad51 foci are counted

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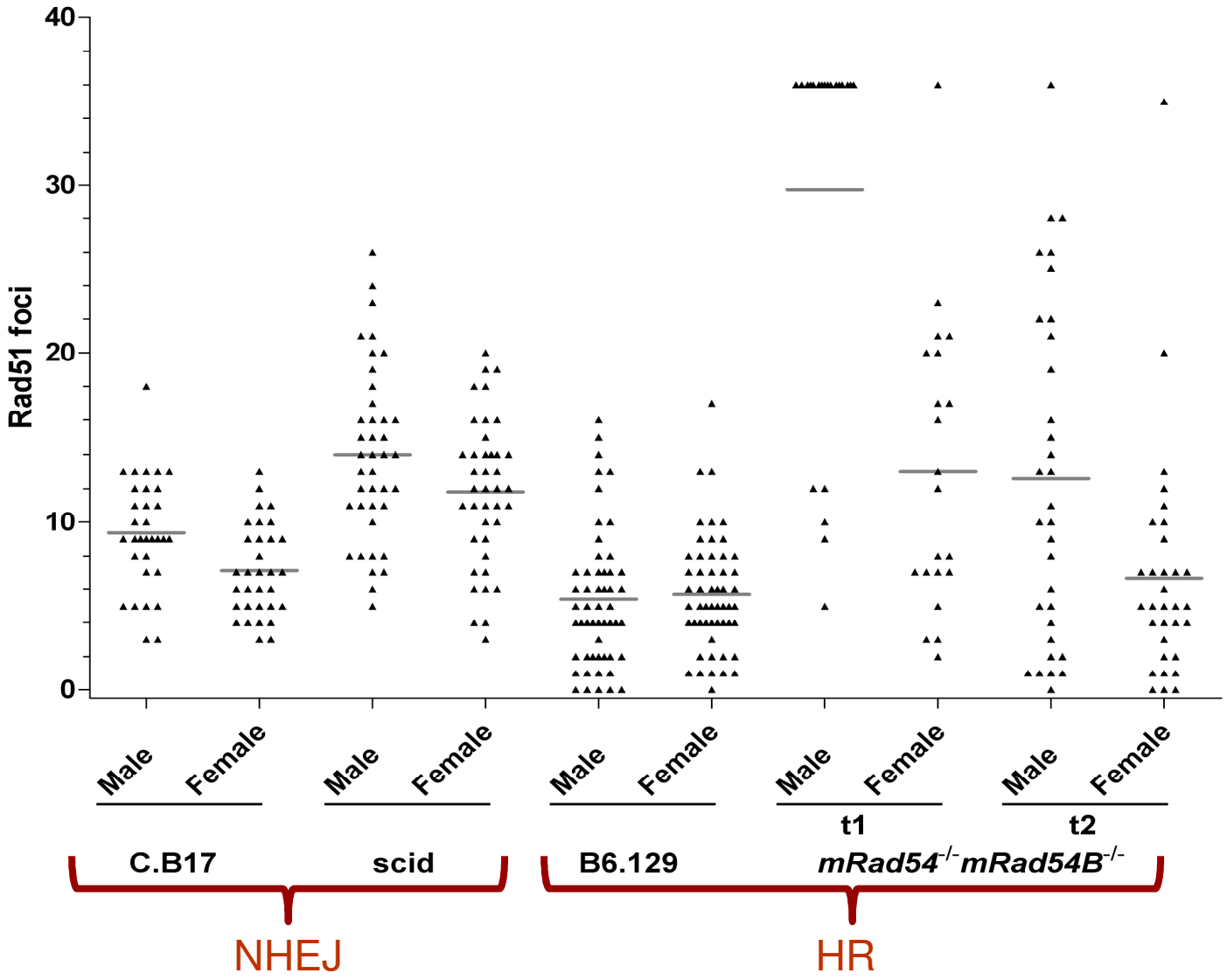
Rad51 foci in late S-phase for male and female pronuclei



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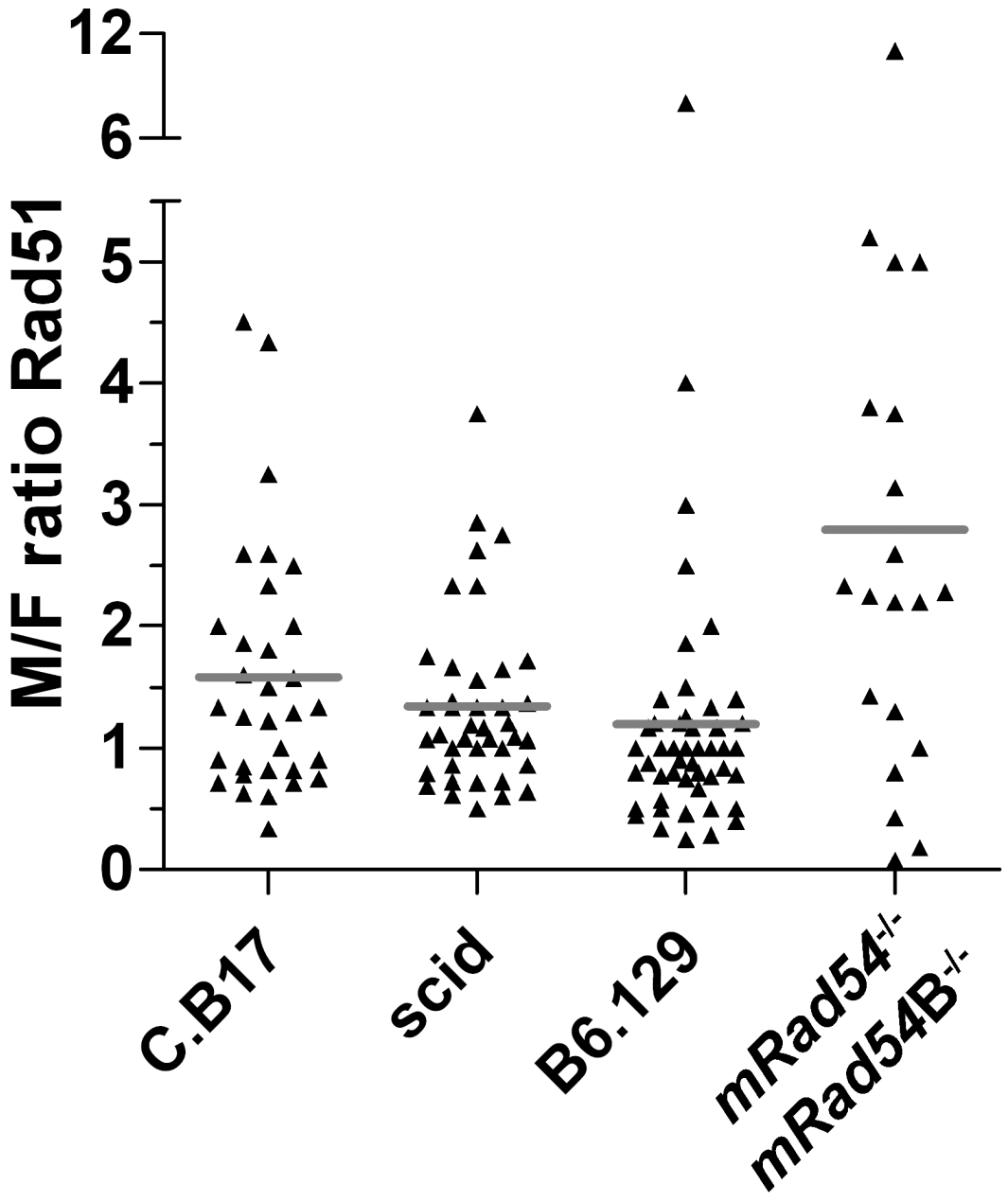

Maternal HR defects do lead more to paternal DNA repair problems than to maternal DNA repair problems

In oocytes from superovulation, there is a subclass with more paternal repair problems

Derijck A, van der Heijden G, et al.
Hum Mol Genet. 2008 Jul 1;17(13):1922-37

Male and female chromosome complements are differently sensitive to DNA damage indications

• At G1, γH2AX foci are only visible in the male chromatin (spontaneous + induced)

• At S-phase, these foci are more numerous in the male PN

• Mutagenesis at the onset of and during S-phase challenges the male PN more than the female PN

a. Higher numbers of residual γH2AX foci on male mitotic chromosomes

b. Higher numbers of Rad51 foci on male PN, especially when HR dsDNA break repair is compromised

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Conclusions part 2

- Both NHEJ and HR are active during the first cell cycle
- NHEJ is involved in sperm chromatin remodeling
- Before S-phase, NHEJ is involved in preventing male chr. abnorm.
- At S-phase, both NHEJ and HR are active, HR more so than NHEJ
- When NHEJ is compromised, HR takes over
- When HR is low, especially male PN are very sensitive for chromosome abnormalities .

Zygote S-phase as a readout of sperm quality

Origin of stalled replication forks
-DNA single strand breaks
-DNA adducts
-DNA helix crosslinks, DNA protein crosslinks

-Which repair pathways are active

-ATR signaling
-PARP1,2, Mre11 involvement

RecQ family involvement, ao Bloom and Werner syndrome proteins

Fanconi Anemia protein family

ERCC1/XPF involvement

Translesional synthesis

Checkpoint status

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Part of a double PhD project
2002 – 2007
Dr. Godfried van der Heijden
and Dr. Alwin Derijck

Acknowledgments:

Maud Giele
Johan van der Vlag
Marielle Philippens
Liliana Ramos
Jan Kremer
Radboud University MC

Roland Kanaar, Jeroen Essers
Dept Celbiology and Genetics Erasmus MC Rotterdam for Rad54,54B ko mice and Rad51 ab

Thomas Jenuwein, Max Planck, Freiburg for Histone mod abs