Maternal effects of the *scid* mutation on radiation-induced transgenerational instability in mice

T Hatch[1,§], AAHA Derijck[2,§], PD Black[1], GW van der Heijden[2], P de Boer[2] and YE Dubrova[1,*]

[1]*Department of Genetics, University of Leicester, Leicester LE1 7RH, United Kingdom;*
[2]*Department of Obstetrics and Gynaecology, Radboud University Nijmegen Medical Centre P.O. Box 9101, 6500 HB Nijmegen, The Netherlands*

Running title: Maternal effects and genetic instability

Key words: Radiation; transgenerational instability; maternal effects; *scid*; NHEJ; mouse; germline

[*]Correspondence:
Professor Yuri E Dubrova
Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, United Kingdom
Tel: +44 (116) 252 5654
Fax: +44 (116) 252 3378
E-mail: yed2@le.ac.uk
[§] TH and AAHAD contributed equally to this work.

The results of a number of recent studies show that mutation rates in the offspring of irradiated parents are substantially elevated, however the effect of parental genotype on transgenerational instability remains poorly understood. Here we have analysed the mutation frequency at an expanded simple tandem repeat (ESTR) locus in the germline and bone marrow of the first generation male offspring of control and irradiated male mice. The frequency of ESTR mutation was studied in the offspring of two reciprocal matings ♂*scid* x ♀BALB/c and ♂BALB/c x ♀*scid,* which were compared with that in BALB/c mice. In the offspring of the BALB/c x BALB/c and ♂*scid* x ♀BALB/c matings, which were conceived after paternal sperm irradiation, the frequency of ESTR mutation was significantly elevated in both tissues. In contrast, ESTR mutation frequency was only slightly elevated in the offspring of ♂BALB/c x ♀*scid* mating conceived after paternal irradiation. The results of this study suggest that the oocytes of *scid* females are unable to fully support the repair of double-strand breaks induced in paternal sperm which may in turn result in the elimination of cells/embryos containing high levels of DNA damage, thus partially preventing the manifestation of genomic instability.

To analyse transgenerational changes in mutation rate, tissue samples were taken from the non-exposed 7-week-old male offspring of control and irradiated (2 Gy of acute X-rays) BALB/c and severe combined immunodeficient (*scid*) males. The frequency of mutations at an expanded simple tandem (ESTR) locus, *Ms6-hm,* was analysed in the offspring of two reciprocal matings ♂*scid* x ♀BALB/c and ♂BALB/c x ♀*scid* which were compared with those of BALB/c mice (Table 1). To ensure that all offspring included in this study were derived from irradiated sperm from the caudal epididymis and vas deferens, the exposed males were mated within 5 days after irradiation with non-exposed females (Searle, 1974). Homozygous *scid* mice used in this study are on the C.B17 genetic background. The C.B17 strain was derived from a multiple backcross of (BALB/c x C57BL/Ca) x BALB/c, its characteristics are essentially those of BALB/c (Festing, 1996) and this strain should therefore possess a similar genetic background with BALB/c mice. The progenitor allele size at the *Ms6-hm* locus in *scid*, C.B17 and BALB/c mice included in this study were all approximately 3 kb. Importantly, the same allelic variants of the genes encoding the proteins $p16^{INK4a}$ (Zhang *et al.*, 1998) and DNA-dependant protein kinase catalytic-subunit, $DNA\text{-}PK_{cs}$ (Yu *et al.*, 2001) are found in both strains (Barber *et al*., 2002, 2004). Given that the $p16^{INK4a}$ protein plays a vital role in cell-cycle control and $DNA\text{-}PK_{cs}$ is involved in the non homologous end-joining (NHEJ) pathway of DNA double-strand break (DSB) repair, it therefore appears that the presence of these allelic variants in both strains may have a similar effect on the genome stability. Indeed, our previous data show a remarkable similarity in ESTR mutation rates in the germline of non-exposed BALB/c and C.B17 males (Barber *et al*., 2004).

Using a single-molecule (SM-PCR) approach (Yauk *et al.*, 2002; Barber *et al.*, 2006), the frequency of ESTR mutation at the *Ms6-hm* locus was evaluated in bone marrow (BM) and sperm DNA samples taken from 18 offspring (3 animals per experimental group). We first compared the frequency of ESTR mutation in the offspring of non-irradiated parents. Given that the frequency of mutation in the offspring of the reciprocal matings (♂*scid* x ♀BALB/c and ♂BALB/c x ♀*scid*) did not significantly differ (sperm, t=0.09; P=0.9283 and BM, t=0.75; P=0.4335, Table 1), these data were therefore combined for further analysis. Overall, a significant 1.8-fold increase in the mean frequency of ESTR mutation was found in the sperm of BALB/c x *scid* F_1 hybrid animals compared to that in the BALB/c strain (t=2.35; P=0.0190); a similar 1.5-fold increase was also detected in BM (t=1.88; P=0.0603). *scid* mice carrying a nonsense mutation in the $DNA\text{-}PK_{cs}$ are deficient in the repair of DSBs by the non-homologous end joining (NHEJ) pathway (Biedermann *et al*., 1991; Blunt *et al*., 1996). Given that the activity of $DNA\text{-}PK_{cs}$ in BALB/c mice is also substantially compromised (Okayashi check spelling *et al*., 2000), the effects of a nonsense *scid* mutation can therefore manifest in BALB/c x *scid* F_1 hybrid animals, thus resulting in elevated spontaneous mutation rate.

We next analysed transgenerational changes in the first generation (F_1) offspring of irradiated wild-type BALB/c mice. For both tissues, a statistically significant ~2-fold increase in the mean mutation frequency was found in the offspring of irradiated males. Most importantly, the frequency of ESTR mutation was elevated in the germline and somatic tissue of all the offspring of irradiated males (Figure 1). These data confirm our earlier results obtained in the F_1 offspring of irradiated males (Dubrova *et al.*, 2000; Barber *et al.*, 2002; 2006).

In our previous studies ESTR mutation rates were analysed in the offspring conceived 3 and 6 weeks after paternal exposure irradiation (Dubrova *et al.*, 2000; Barber *et al.*, 2002; 2006). Given that these stages of the mouse spermatogenesis are transcriptionally active, their exposure to radiation could result in an accumulation of certain classes of RNA in the paternal germ cells which, being transmitted to the fertilised egg, may affect gene expression and stability in the developing embryo. If transgenerational instability is attributed to the zygotic transfer of RNA (Rassoulzadegan *et al*., 2006), then the offspring conceived just few days

after paternal irradiation, from transcriptionally inert sperm cells (Rousseaux *et al*., 2005), should be genetically stable. However, a similar magnitude of transgenerational increases in ESTR mutation frequency was found in the F_1 offspring of exposed BALB/c males conceived from either transcriptionally active or inactive stages of spermatogenesis (Figure 2).

Given that pre-mutational radiation-induced lesions in sperm DNA are effectively recognised and repaired within a few hours of fertilisation (Matsuda and Tobari, 1989; Derijck *et al.*, 2006), it would therefore appear that radiation-induced damage to sperm DNA could trigger a cascade of events in the zygote, including profound changes in the expression of DNA repair genes in the pre-implantation embryo (Harrouk *et al*., 2000, Shimura *et al.*, 2002) and alterations in DNA methylation and histone acetylation (Barton *et al.*, 2005). The presence of such dramatic changes at fertilization could also result in delayed effects, which may influence the stability of the developing embryo.

To evaluate the effect of the *scid* mutation, the frequency of ESTR mutation was compared in the offspring of two reciprocal crosses, where irradiated BALB/c and *scid* males were mated to non-exposed *scid* and BALB/c females, respectively. In the offspring of ♂*scid* x ♀BALB/c mating, the frequency of ESTR mutation was significantly elevated in both tissues (Table 1). The magnitude of this increase was very similar to that observed in the offspring of irradiated ♂BALB/c x ♀BALB/c. In contrast, ESTR mutation frequency was only slightly elevated in the offspring of reciprocal cross. We therefore conclude that in the maternal *scid* background the manifestation of radiation-induced transgenerational instability is partially suppressed.

Given that the maternally-derived NHEJ pathway is fully active in zygote (Fiorenza *et al.*, 2001), in the offspring of ♂BALB/c x ♀*scid* mating it should be severely compromised during the early stages of their development. The inability of the *scid* oocytes to fully support the repair of radiation-induced DSBs in sperm may therefore lead to the elimination of embryos containing high level of DNA damage. Previously reported lack of mutation induction in irradiated *scid* mice can also be explained by the high cell killing effects of irradiation on their germline (Barber *et al.*, 2004).

As the mouse embryo undergoes early zygotic activation of transcription during the 2-cell stage where over 800 genes, including the gene encoding DNA-PK_{cs}, are re-expressed (Zeng et al., 2004), the level of NHEJ in the offspring of ♂BALB/c x ♀scid mating should be restored very early by transcription of the paternal BALB/c allele. The alleged elimination of some highly unstable embryonic cells should therefore occur at the very early stages of development, perhaps before the 4-cell stage, where the ability to repair DSBs by NHEJ still remains under the *scid* maternal control.

The suppression of mutation induction and radiation-induced genomic instability in homozygous *scid* cells can be explained by the DNA-PK-independent activation of p53 and p21, resulting in a high level of apoptosis and cell-cycle arrest in the irradiated DNA-PK_{cs} deficient cells (Jimenez *et al.*, 1999). The inter-strain variation in the responses to ionising radiation, including the manifestation of radiation-induced genomic instability, has previously been explained by differences in the intensity of apoptosis (Wallace *et al.*, 2001). According to the results of this study, cells from the radiation-resistant C57BL/6 mice undergo rapid apoptosis after irradiation, which could in turn suppress radiation-induced genomic instability in this strain.

Our recent data show that radiation-induced DSBs in the sperm head are repaired in the wild-type zygote during pre S-phase (Derijck *et al.*, 2006), where they should be almost exclusively repaired by the NHEJ pathway (Rothkamm *et al.*, 2003). Given that unrepaired DSBs are highly mutagenic and result in chromosome type aberrations in early zygote (Matsuda and Tobari, 1989), their early repair in the offspring of ♂BALB/c x ♀*scid* mating should be compensated, most likely by homologous recombination (Allen *et al*., 2002). Given

the high fidelity of HR and its well-established role in the repair of DSBs during replication, the activation of this pathway may suppress mutation process at ESTR loci, the mechanisms of which are most probably attributed to replication slippage (Yauk *et al.*, 2002; Barber *et al.*, 2004). However, as the period of time when in the offspring of ♂BALB/c x ♀*scid* mating remains under the *scid* maternal control is very brief, it appears unlikely that such a short burst of HR activity may substantially affect ESTR stability, unless the long-term epigenetic up-regulation of HR in these animals is suspected.

In conclusion, the data presented here show that radiation-induced transgenerational instability in the offspring of ♂BALB/c x ♀*scid* mating is suppressed and therefore suggest a long lasting effect of maternal genotype and DNA repair proficiency on ESTR mutation rate in the germline and somatic tissues. Together with the results of our previous study showing strain-specific differences in the extent of transgenerational instability in mice (Barber *et al.*, 2002), these data highlight the importance of genetic factors in the manifestation of this phenomenon. Moreover, they provide further insights on the mechanisms underlying the phenomenon of maternal effect for DNA repair in mammals. The results of early studies show that the inter-strain differences in the efficiency of DNA repair in oocyte can significantly affect the yield of dominant-lethal mutations induced in male germ cells (Generoso *et al.*, 1979; Marchetti and Wyrobek, 2005). According to these data, the frequency of mutations detected in the offspring of exposed male mice depends on the ability of maternal strain to repair DNA lesions induced in sperm. However, these maternal effects were detected in the wild-type strains of mice where the efficiency of DNA repair is not substantially compromised. In contrast, the *scid* mutation almost completely abolishes the activity of DNA-PK_{cs}, thus dramatically affecting the early responses to DNA damage in zygote. Presumably on this maternal background, a considerable fraction of DNA lesions induced in sperm cannot be properly repaired, resulting in the elimination of most unstable cells/embryos. As in the surviving embryos, the manifestation of transgenerational instability is suppressed after paternal sperm irradiation, maternal effects of the *scid* mutation may thus be regarded as protective. Finally, given the wide range of inherited variation in DNA repair capacity in humans (Mohrenweiser *et al.*, 2003), there is potential that the same phenomenon may also exist in humans.

Acknowledgments

We thank Bianca Lemmers and Iris Lamers of the Central Animal Facility of Radboud University for expert animal care. This work was supported by a grant from the European Commission (YED and PDB). YED work was also supported in part by grants from the Wellcome Trust, the Department of Energy and the Medical Research Council.

References

Allen C, Kurimasa A, Brenneman MA, Chen DJ, Nickoloff JA. (2002). *Proc Natl Acad Sci USA* **99:** 3758–3763.

Barber RC, Miccoli L, van Buul PPW, Burr KL-A, van Duyn-Goedhart A, Angulo JF *et al.* (2004). *Mutat Res* **554:** 287-295.

Barber R, Plumb MA, Boulton E, Roux I, Dubrova YE. (2002). *Proc Natl Acad Sci USA* **99:** 6877-6882.

Barber RC, Hickenbotham P, Hatch T, Kelly D, Topchiy N, Almeida G *et al.* (2006). *Oncogene* Jun 5 [Epub ahead of print].

Barton TS, Robaire B, Hales BF. (2005). *Proc Natl Acad Sci USA* **102:** 7865-7870.

Biedermann KA, Sun JR, Giaccia AJ, Tosto LM, Brown JM. (1991). *Proc Nat Acad Sci USA* **88:** 1394-1397.

Blunt T, Gell D, Fox M, Taccioli GE, Lehmann AR, Jackson SP *et al.* (1996). *Proc Natl Acad Sci USA* **93:** 10285-10290.

Derijck AA, van der Heijden GW, Giele M, Philippens ME, van Bavel CC, de Boer P. (2006). *DNA Repair* **5**: 959-971.

Dubrova YE, Plumb M, Brown J, Fennelly J, Bois P, Goodhead D *et al.* (1998). *Proc Natl Acad Sci USA* **95:** 6251-6255.

Dubrova YE, Plumb M, Gutierrez B, Boulton E, Jeffreys AJ. (2000). *Nature,* **405:** 37.

Festing M. (1996). *Genetic Variants and Strains of the Laboratory Mouse*. Lyon MF, Rastan S, Brown SDM (eds). Oxford University Press: Oxford, **2:** pp. 1537-1576.

Fiorenza MT, Bevilacqua A, Bevilacqua S, Mangia F (2001) *Dev Biol*, **233**: 214-224.

Generoso WM, Cain KT, Krishna M, Huff SW. (1979). *Proc Natl Acad Sci USA* **76:** 235-437.

Harrouk W, Codrington A, Vinson R, Robaire B, Hales BF (2000) *Mutat Res* **461**: 229-241.

Jimenez GS, Bryntesson F, Torres-Arzayus MI, Priestley A, Beeche M, Saito S *et al.* (1999). *Nature* **400:** 81-83.

Marchetti F, Wyrobek AJ. (2005). *Birth Defects Res* C **75:** 112-129.

Matsuda Y and Tobari I. (1989). *Mutat Res* **210**: 35-47.

Mohrenweiser HW, Wilson DM, Jones IM. (2003). *Mutat Res* **526:** 93-125.

Okayashu R, Suetomi K, Yu Y, Silver A, Bedford JS, Cox R *et al.* (2000). *Cancer Res* **60:** 4342-4345.

Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F. (2006). *Nature* **241:** 469-474.

Rothkamm K, Kruger I, Thompson LH, Lobrich M (2003) *Mol Cell Biol* **23**: 5706-5715.

Rousseaux S, Caron C, Govin J, Lestrat C, Faure AK, Khochbin S. (2005). *Gene* **345:** 139-153.

Searle AG. (1974). *Adv Radiat Biol* **4:** 131-207.

Shimura T, Inoue M, Taga M, Shiraishi K, Uematsu N, Takei N *et al.* (2002). *Mol Cell Biol* **22:** 2220-2228.

Wallace M, Coates PJ, Wright EG, Ball, KL. (2001). *Oncogene* **20:** 3597-3608.

Yauk CL, Dubrova YE, Grant GR, Jeffreys AJ. (2002). *Mutat Res* **500:** 147-156.

Yu Y, Okayashi R, Weil MM, Silver A, McCarthy M, Zabriskie R *et al.* (2001). *Cancer Res* **61:** 1820-1824.

Zeng F, Baldwin DA, Schultz RM. (2004). *Dev Biol* **272:** 483-496.

Zhang S, Ramsay ES, Mock BA. (1998). *Proc Natl Acad Sci USA* **95:** 2429-2434.

Zheng N, Monckton DG, Wilson G, Hagemeister F, Chakraborty R, Connor TH *et al.* (2000). *Environ Mol Mutagen* **36:** 134-145.

Titles and legends to figures

Figure 1 Frequency of ESTR mutations in controls males (open bars) and the first-generation offspring of irradiated males (hatched bars). Each bar represents the frequency of mutations measured in an individual male. (**a**) Frequency of ESTR mutations in sperm. (**b**) Frequency of ESTR mutations in the bone marrow tissue. The 95% confidence intervals (CI) are shown on all graphs. DNA samples were prepared as previously described (Barber *et al.*, 2006). Approximately 5 μg of each DNA sample was digested with 20 U *Mse*I (New England Biolabs). The frequency of ESTR mutation was be evaluated using a single-molecule PCR (SM-PCR) approach (Yauk *et al*., 2002; Barber *et al.*, 2006). DNA was amplified on an MJ DNA engine PTC 220 in 10 μl reactions using 0.6 μM flanking primers, 1 U enzyme mix (Expanded High Fidelity PCR system, Roche), 1 M Betaine and 200 μM dNTPs. After denaturing at 96ºC for 3 min, PCRs were cycled at 96ºC for 20 sec, 58ºC for 30 sec, and 68^{o}C for 3 min for 30 cycles, ending with 10-min incubation at 68ºC. PCR products were resolved on a 40 cm long agarose gel and detected by Southern blot hybridisation (Dubrova *et al*., 1998). To increase the robustness of the estimates of individual ESTR mutation frequencies, on average 139 amplifiable molecules were analyzed for each tissue for each male mouse. The frequencies of ESTR mutation, 95% CI's and standard errors were estimated using modified approach proposed by Chakraborty (Zheng *et al*., 2000).

Figure 2 Frequency of ESTR mutations in the F_1 offspring of BALB/c males conceived 5 days (sperm) and 6 weeks (spermatogonia) after acute exposure to X-rays. Data for the spermatogonia irradiation are taken from Barber *et al.* (2006).

Figure 1

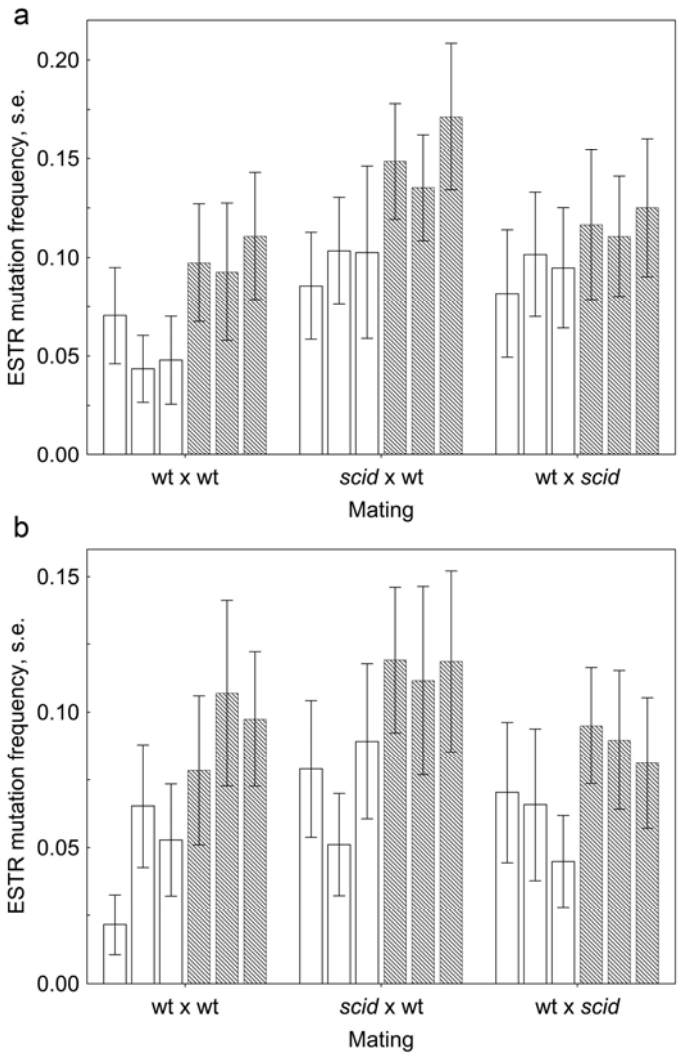
a
ESTR mutation frequency, s.e.
0.00
0.05
0.10
0.15
0.20
wt x wt
scid x wt
wt x scid
Mating
b
ESTR mutation frequency, s.e.
0.00
0.05
0.10
0.15
wt x wt
scid x wt
wt x scid
Mating

Figure 2

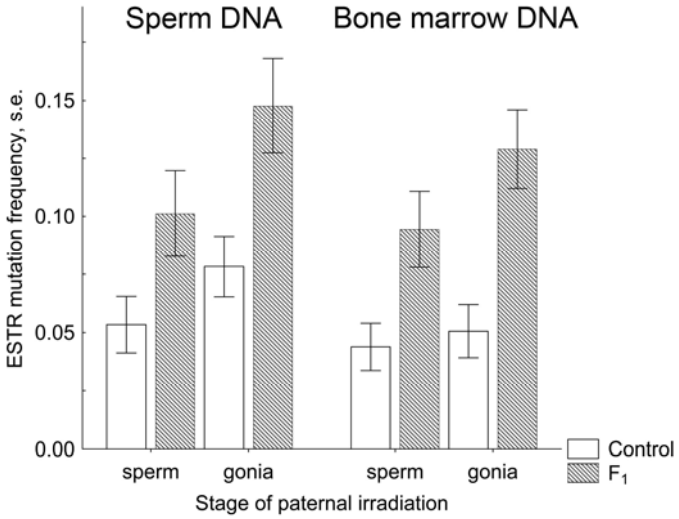
Sperm DNA
Bone marrow DNA
ESTR mutation frequency, s.e.
0.15
0.10
0.05
0.00
sperm
gonia
sperm
gonia
Stage of paternal irradiation
Control
F1

Table 1 ESTR mutation frequencies in controls and the offspring of irradiated males

Mating, Tissue	Control		F_1 of irradiated males				
	No mutations[*]	Mutation frequency[†]	No mutations[*]	Mutation frequency[†]	Ratio[‡]	*t*[§]	Prob.[§]
♂BALB/c x ♀BALB/c							
Sperm	21 (393)	0.053±0.012	34 (336)	0.101±0.018	1.89	2.16	0.0311
BM	20 (456)	0.044±0.010	38 (402)	0.094±0.016	2.15	2.62	0.0089
♂*scid* x ♀BALB/c							
Sperm	60 (643)[¶]	0.095±0.013	92 (616)	0.149±0.018	1.57	2.49	0.0129
BM	52 (802)[¶]	0.065±0.010	51 (435)	0.117±0.018	1.81	2.59	0.0097
♂BALB/c x ♀*scid*							
Sperm	60 (643)[¶]	0.095±0.013	41 (350)	0.117±0.020	1.24	0.95	0.3423
BM	52 (802)[¶]	0.065±0.010	51 (569)	0.090±0.014	1.38	1.50	0.1338

[*] Number of amplifiable molecules is given in brackets.

[†] ± standard error of mean.

[‡] Ratio to the frequency in the offspring of non-exposed males.

[§] Student's test and probability for difference between the offspring of irradiated males and controls.

[¶] Aggregated data for the offspring of non-exposed mating ♂*scid* x ♀BALB/c and ♂BALB/c x ♀*scid*.