DNA REPAIR EFFICIENCY IN GERM CELLS AND EARLY MOUSE EMBRYOS AND CONSEQUENCES FOR RADIATION-INDUCED TRANGENERATIONAL GENOMIC DAMAGE

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ABSTRACT

Exposure to ionizing radiation and other environmental agents can affect the genomic integrity of germ cells and induce adverse health effects in the progeny. Efficient DNA repair during gametogenesis and the early embryonic cycles after fertilization is critical for preventing transmission of DNA damage to the progeny and relies on maternal factors stored in the egg before fertilization. The ability of the maternal repair machinery to repair DNA damage in both parental genomes in the fertilizing egg is especially crucial for the fertilizing male genome that has not experienced a DNA repair-competent cellular environment for several weeks prior to fertilization. During the DNA repair-deficient period of spermatogenesis, DNA lesions may accumulate in sperm and be carried into the egg where, if not properly repaired, could result in the formation of heritable chromosomal aberrations or mutations and associated birth defects. Studies with female mice deficient in specific DNA repair genes have shown that: (i) cell cycle checkpoints are activated in the fertilized egg by DNA damage carried by the sperm; and (ii) the maternal genotype plays a major role in determining the efficiency of repairing genomic lesions in the fertilizing sperm and directly affect the risk for abnormal reproductive outcomes. There is also growing evidence that implicates DNA damage carried by the fertilizing gamete as a mediator of postfertilization processes that contribute to genomic instability in subsequent generations. Transgenerational genomic instability most likely involves epigenetic mechanisms or error-prone DNA repair processes in the early embryo. Maternal and embryonic DNA repair processes during the early phases of mammalian embryonic development can have far reaching consequences for the genomic integrity and health of subsequent generations.

INTRODUCTION

Living organisms have developed complex DNA repair mechanisms to maintain genomic integrity and limit the introduction of mutations into the genetic pool (Friedberg et al., 2005). The importance of efficient DNA repair is demonstrated by the many human diseases, such as Xeroderma Pigmentosum and Fanconi Anemia, that are associated with defects in specific DNA repair proteins and that confer exquisite sensitivity to carcinogenesis (Friedberg et al., 2005). Efficient DNA repair is particularly important during gametogenesis and the early phases of mammalian development to prevent the transmission of *de novo* mutations that can affect the health of the offspring. Genetic defects transmitted through male and female germ lines are associated with a variety of abnormal reproductive outcomes (McFadden & Friedman, 1997; Marchetti & Wyrobek, 2005; Wyrobek et al., 2006) and diseases in the offspring including cancer (Hassold et al., 1996; McFadden & Friedman, 1997; Hassold & Hunt, 2001). Although, we still lack conclusive proof of environmentally-induced heritable damage in humans, decades of work in rodents have shown that numerous environmental, occupational and medical agents, such as ionizing radiation, are capable of affecting the genomic integrity of germ cells and have detrimental effects on the health of the offspring (Marchetti & Wyrobek, 2005). Embryonic lethality (Ehling, 1971), heritable chromosomal tranlsocations (Searle et al., 1974), heritable gene mutations (Russell et al., 1998), genomic instability (Dubrova et al., 1993) and cancer in the offspring (Nomura, 1982; Nomura et al., 2004) are some of the abnormal reproductive outcomes that have been associated with parental exposure to ionizing radiation. There is also suggestive evidence for increased mutation rates in the offspring of parents exposed to the radioactive fallout following the Chernobyl accident (Dubrova et al., 1996).

The perifertilization period, or perigametic interval, encompassing the postmeiotic phase of spermatogenesis (spermiogenesis) and the early cycles of embryonic development, is a key period for the prevention of inherited DNA damage (Russell, 1999; Marchetti & Wyrobek, 2005). Two unique features of this period are that: (a) the latter part of spermiogenesis is DNA repair-deficient and genetic lesions induced during this period may accumulate unrepaired in the maturing sperm and persist until fertilization; and (b) fertilized eggs (zygotes) are unique among cells because all cellular functions between fertilization and transcriptional activation of the embryonic genome rely on stored maternal products. Both these features play important roles in determining how much DNA damage is transmitted by the sperm to the zygote and how much of the transmitted damage is converted into *de novo* mutations after fertilization (Figure 1).

In this chapter, we review the available data on the DNA repair capacity during gametogenesis and early stages of mammalian development and the consequences that improper repair of DNA damage during this critical window of development may have for the genomic integrity and health of the offspring.

DNA REPAIR IN MALE GERM CELLS

The ability to repair DNA damage changes during spermatogenesis as male germ cells differentiates to form mature sperm (Olsen et al., 2005). DNA repair capacity declines dramatically during the postmeiotic phase of spermatogenesis (Sega, 1979;

Sotomayor & Sega, 2000) as spermatids undergo major nuclear chromatin reorganization (Meistrich, 1989; Wouters-Tyrou et al., 1998) and epigenetic reprogramming (Hazzouri et al., 2000). During chromatin reorganization, histones are replaced (~14 days before ejaculation in the mouse; ~21 days in human) first with basic transition proteins, and then with protamines (Meistrich, 1989), which are arginine-rich proteins that condense the chromatin and cause DNA to become transcriptionally inactive (Kierszenbaum & Tres, 1978). Sperm are then released into the lumen of the seminiferous tubules and pass through the rete testis and the epididymis where they acquire motility and the biochemical ability to fertilize the egg (Gatti et al., 2004). The reduced DNA repair capacity of late-step spermatids and sperm (Sega, 1974; Sega, 1979; Sotomayor & Sega 2000; Baarends et al., 2001; Olsen et al., 2005) is thought to be responsible for the high sensitivity of postmeiotic male germ cells to mutagenic exposures (Marchetti & Wyrobek ,2005).

DNA double strand breaks (DSBs) are one of the most cytotoxic lesions induced by ionizing radiation. An early step of the cellular response after exposure to ionizing radiation is the phosphorylation of γH2AX and its localization at sites of DSBs (Rogakou et al., 1998). In somatic cells, γH2AX foci have been extensively used to investigate the induction of DSBs after exposure to ionizing radiation (Burma et al., 2001; Ward & Chen, 2001; Fernandez-Capetillo et al., 2002; Kobayashi et al., 2002; Taneja et al., 2004). The number of γH2AX foci per nucleus increases linearly with dose (Rogakou et al., 1998; Burma et al., 2001; Ward & Chen, 2001; Stiff et al., 2004) and it is generally accepted that the appearance and disappearance of γH2AX foci are indicative of recognition and repair of DSBs (Redon et al., 2002). Irradiation of the testis rapidly elicits γH2AX foci formation in spermatogonia, spermatocytes and round spermatids (Hamer et al., 2003; Ahmed et al., 2007), but not in late spermatids and sperm. This pattern is shared by other DNA repair proteins, such as Rad51, 53Bp1 and Mdc1 (Ahmed et al., 2007) suggesting that the ability to detect and repair DSBs is progressively lost during the final stages of sperm maturation. The decline in DNA repair capacity in late spermatids and sperm is not restricted to DSB repair, and involves all other major DNA repair pathways (van Loon et al., 1993; Hamer et al., 2003; Olsen et al., 2005; Xu et al., 2005).

Genomic damage induced in late spermatids and sperm may accumulate and persist unrepaired in the fertilizing sperm and be transmitted to the embryo. Indeed, we recently showed that the different abilities of postmeiotic germ cells to repair DNA lesions has a significant impact on the amount of heritable genetic damage that is transmitted to the zygote (Marchetti & Wyrobek, 2008). This inability of sperm to repair DNA lesions as they occur may make them particularly susceptible to repeated exposures that take place because of occupational (radiation workers) or life style (e.g. tobacco smoking) reasons.

DNA REPAIR IN FEMALE GERM CELLS

In contrast to the differentiation of male germ cells, the mammalian oocyte is capable of repairing DNA damage throughout oogenesis (Brandriff & Pedersen, 1981; Ashwood & Edwards, 1996). Evidence from a number of in vivo and in vitro systems indicates that the mammalian oocyte is capable of repairing a variety of DNA damage (Ashwood & Edwards, 1996). Indirect evidence that DNA repair pathways are

functional in the egg come from microarray studies of oocytes and early embryonic stages which showed that oocytes and zygotes have significantly higher levels of mRNAs for DNA repair genes than later embryonic stages and that transcripts for genes of all major DNA repair pathways are present in the egg (Hamatani et al., 2004; Zeng et al., 2004). Zeng et al. (2004) suggested that the overrepresentation of DNA damage and DNA repair genes in oocyte and zygotes may reflect the oocyte's response to selective pressure to insure genomic integrity. This is because the egg provides the gene products that are responsible for repairing DNA damage carried by both parental genomes at fertilization. These maternal gene products persist to sustain the zygote until its genome is fully activated, which occurs at the 2-cell stage in the mouse (Schultz & Worrad, 1995; Schultz, 2002; Hamatani et al., 2004; Zeng et al., 2004) and even later in the human embryo (Braude et al., 1988). As discussed in the next section, this has important implications for the prevention of inherited DNA damage.

DNA REPAIR DURING THE FIRST CELL CYCLE AFTER FERTILIZATION

The mammalian first cell cycle after fertilization is dramatically different from any other somatic cell. Specifically: (i) the fertilizing sperm nucleus undergoes major chromatin alterations as it decondenses and protamines are removed and replaced by histones (Garagna & Redi, 1988; Nonchev & Tsanev, 1990; Perreault ,1992); (ii) all cellular events that take place during the first cell cycles of development, including DNA repair and synthesis, occurs in the absence of transcription (Schultz, 2002); and (iii) male and female pronuclei proceed through G1, S-phase and G2 to mitosis as separate pronuclei (McGaughey & Chang, 1969). Progression of the two parental pronuclei through the first cell cycle occurs in a coordinated fashion and if the timing of these critical events is disrupted in one pronucleus, the development of the other pronucleus will also be affected (Perreault, 1992). The apparent necessity for the parental pronuclei to undergo development in a coordinated fashion is thought to be one mechanism to prevent zygotes containing one pronucleus with unrepaired DNA damage from reaching the metaphase stage and progressing further into development.

The G1 phase of the first cell cycle also represents the first opportunity for the repair of DNA lesions that were induced in the sperm during the DNA-repair deficient window of spermatogenesis. In order to carry out the repair of these lesions the egg must: (i) be capable of recognizing the presence of DNA damage in the fertilizing sperm; (ii) transmit the information to the female pronucleus to maintain the coordinated development of the two pronuclei, and (iii) activate the necessary DNA repair pathways. The following sections describe the available data showing that the egg is capable of carrying out these three functions.

Detection of DNA damage in the fertilized egg

Recent research is showing that the egg is very efficient in sensing the presence of damaged DNA in the sperm and that its repair occurs immediately following fertilization. In a careful and detailed study, Derijck et al. (2006) showed a rapid and efficient γH2AX signaling during sperm chromatin remodeling in G1 after fertilization. Exposure of sperm to ionizing radiation prior to fertilization induced γH2AX foci that were detected during the first couple of hours after fertilization as the sperm head underwent transformation into the male pronucleus. The detected foci could be divided

in two groups based on appearance: small foci that did not show a dose-response following irradiation of sperm and large foci that showed dose-related increases. No γH2AX foci were detected over the unirradiated maternal pronucleus. Treatment of zygotes with etoposide, an efficient inducer of DSB breaks (Marchetti et al., 2001), induced strong γH2AX signaling in both parental pronuclei. These results show that the egg's response to DNA damage is rapid and occurs during the first few hours after fertilization. This may help to keep the broken DNA ends in close proximity and allowing their repair before they are spatially separated during the significant nuclear enlargement that is associated with pronuclear formation and before DNA synthesis takes place to reduce the risk of misrejoning and the generation of chromosomal rearrangements, and/or the formation of acentric fragments.

Activation of cell cycle checkpoints in the fertilized egg

Tp53 is a central player in the response to DNA damage in somatic cells (Kastan et al., 1991; Enoch & Norbury ,1995; Siliciano et al., 1997; MacCallum et al., 2001; Wahl & Carr, 2001). Tp53 phosphorylation in one of the earliest events after the induction of DNA damage and triggers a series of cellular responses that activate cell cycle checkpoints to allow cells to repair DNA damage (Siliciano et al., 1997; Banin et al., 1998; Canman et al., 1998; Saito et al., 2002). Tp53 is also a key player in the zygotic response to DNA damage. Fertilization with irradiated mouse sperm activated a Tp53-dependent damage S-phase checkpoint that delayed DNA synthesis in both pronuclei in the zygote (Shimura et al., 2002). Two unique features of this checkpoint were that: (i) it was not dependent on p21, as the checkpoint was activated even when p21 null females were used; and (ii) it also suppressed DNA synthesis in the maternal pronucleus, which was never exposed to ionizing radiation indicating that DNA damage carried by the sperm activated pronuclear cross talk between the parental pronuclei. Although the identity of the signaling protein(s) is yet to the discovered, pronuclear cross-talking has also been demonstrated after paternal exposure to cyclophosphamide in the rat (Barton et al., 2005). Continuous communication between the two parental pronuclei appears to be a mechanism for the coordinated progression through the first cell cycle.

Repair of DNA damage in the fertilized egg

Because DNA repair in the zygote is carried out by maternal factors stored in the egg before fertilization, differences in DNA repair capacity among eggs may affect the efficiency by which DNA lesions are repaired in the two parental pronuclei. The importance of the maternal genotype in modulating the repair of DNA damage in the zygote was first demonstrated by Generoso et al. (1979) who reported large variations in the yields of chromosomal aberrations at zygotic metaphase and embryonic lethality after implantation following mating of mutagen-exposed males with females of various genetic strains. These results indicated that strains of females differed in their ability to repair the DNA lesions induced by the paternal mutagenic exposure.

Subsequent studies have suggested that several types of DNA lesions could be repaired in the zygote. Brandriff & Pedersen (1981) demonstrated that DNA damage was being repaired in the fertilized egg by measuring radionucleotide grain counts over pronuclei. UV irradiation of mouse zygotes induced unscheduled DNA synthesis (UDS)

in both parental pronuclei, however, when the sperm only was irradiated before fertilization, UDS was detected just in the paternal pronucleus. The authors interpreted these results as the egg's attempt to repair the DNA damage induced by UV radiation to the sperm DNA. In the late 80's, Matsuda and colleagues performed a series of experiments with DNA repair inhibitors during the first cycle of development in mouse zygotes and showed that interference with DNA repair markedly altered the amounts and types of chromosomal abnormalities detected at metaphase after paternal exposure to X-rays and chemical agents (Matsuda et al., 1989a; Matsuda et al., 1989b; Matsuda & Tobari, 1989; Matsuda & Tobari, 1995). These findings provided compelling evidence that chromosomal aberrations were formed after fertilization rather than before. Also, the observation that both chromosome- and chromatid-type aberrations were affected by the use of DNA repair inhibitors indicated that both pre- and post-replication repair mechanisms were operating in the zygote. Similar findings were obtained using the hamster egg method to investigate the repair of DNA damage in human sperm (Genesca et al., 1992).

Although these studies clearly indicated that DNA repair was occurring in the zygote, the exact DNA repair pathways and proteins that were affected were unknown. In recent years, the development of knockout mice for specific DNA repair proteins (Friedberg & Meira, 2006) provides a valuable tool for investigating how DNA lesions are recognized by the egg, and how the DNA repair capacity of the fertilized egg affects the amount of DNA damage that is converted into chromosomal aberrations in the zygote. We recently used this approach to investigate how disruption of specific DNA repair pathways in the zygote affected the repair of DNA lesions in the fertilizing sperm. We focused on DSB repair pathways because the majority of chromosomal aberrations that are detected at zygotic metaphase are of the chromosome-type, i.e., affecting both sister chromatids, regardless of the paternal mutagen used and its mechanism of action (Marchetti & Wyrobek, 2005). This strongly suggests that DSBs are an obligatory step in the processing of sperm lesions into chromosomal aberrations.

DSBs can be repaired via two mechanistically distinct pathways: non-homologous end joining (NHEJ), in which broken ends are rejoined directly with minimal requirement for homology (Lieber et al., 2003; Valerie & Povirk, 2003), and homologous recombination (HR), in which a sister duplex provides information to repair the damaged duplex (West, 2003; Wyman & Kanaar, 2006). The NHEJ pathway involves the DNA-PK (DNA protein kinase) complex which is composed of the Ku70/Ku86 DNA end-binding heterodimer and the catalytic subunit DNA-Pkcs (Smith & Jackson, 1999). The HR pathway involves the RAD52 epistasis group including RAD54 (Valerie & Povirk ,2003), which interacts with RAD51 and is required for DSB repair (Essers et al., 1997; Dronkert et al., 2000). Disruption of either DSB repair pathway in somatic cells confers increased radiation sensitivity (van Gent et al., 2001). We found that disruption of NHEJ through abrogation of DNA-PK catalytic subunit, resulted in a doubling of the frequencies of zygotes with chromosomal aberrations after in vivo irradiation of male mice with respect to controls. Disruption of HR through abrogation of Rad54, resulted in a 5-fold increase in the frequencies of zygotes with chromatid-type aberrations with respect to controls (Marchetti et al., 2007). These findings were later confirmed in another laboratory (Derijck et al., 2008). These authors also showed: (i) a direct correlation between γH2AX foci in the forming male

pronucleus and the levels of chromosomal aberrations at zygotic metaphase; and (ii) increased levels of chromosomal aberrations in both paternal and maternal chromosomes when zygotes deficient in HR were treated in S-phase with 4-nitroquinoline 1-oxide, an agent that partially mimics damage induced by UV.

These findings unequivocally show that DNA DSB repair in the zygote is occurring throughout the entire first cell cycle after fertilization and that inefficient zygotic DNA repair increases the risk of inherited chromosomal aberrations. As chromosomal aberrations at first metaphase are directly associated with the risk of spontaneous abortion or offspring with chromosomal abnormalities (Marchetti and Wyrobek, 2005; Marchetti et al., 2007), these results show that inefficient DNA repair during the first few hours after fertilization can have profound effects on the health of the offspring.

GERM CELL-INDUCED GENOMIC INSTABILITY

In the previous section, we described the immediate effects induced during the first cell cycle of development after exposure to ionizing radiation. Evidence is accumulating that exposure to ionizing radiation, and other germ cell mutagens, can induce delayed effects (Figure 1). In recent years, a series of studies have suggested that DNA damage in germ cells can mediate postfertilization processes that lead to an increased risk for genomic instability in the progeny (see below). A key feature of genomic instability is the delayed induction of genomic changes in the descendants of exposed cells (Niwa, 2003). Genomic methods, such as the expanded simple tandem repeats (ESTR) assay (Dubrova et al., 2002; Yauk et al., 2002; Dubrova, 2005; Bouffler et al., 2006), have shown that paternal exposures to chemical mutagens (Vilarino-Guell et al., 2003; Glen et al., 2008), ionizing radiation (Niwa & Kominami, 2001; Dubrova, 2005) and particulate air pollution (Somers et al., 2002; Yauk et al., 2008) not only increase mutation frequencies in sperm, but more importantly, induced persistent genomic instability in the F1 and F2 offspring of exposed mice (Dubrova et al., 1998; Dubrova et al., 2000; Barber et al., 2002; Hatch et al., 2007; Dubrova et al., 2008). In addition, the introduction of DNA damage by irradiated sperm triggers genomic instability that can induce mutations in the unirradiated maternal genome (Niwa & Kominami, 2001). These results suggest that the mechanisms contributing to transgenerational genomic instability most likely involve epigenetic mechanisms or error-prone DNA repair processes in the embryo.

As for chromosomal aberrations, maternal DNA repair efficiency can affect genomic instability and mutation frequencies in the offspring. Radiation-induced transgenerational instability varies among mouse strains (Barber et al., 2002), and Hatch et al. (2007) demonstrated that ESTR mutation frequencies in the offspring of irradiated males that were mated with Scid females were lower than those in the offspring of irradiated males mated to normal females. However, as the studies with knockout females demonstrated that Scid females are deficient in the ability to repair DSBs in the paternal genome (Marchetti et al., 2007; Derijck et al., 2008), this is most likely an indirect effect due to the elimination of embryos with unrepaired or misrepaired DNA damage that prevents the manifestation of genonic instability in the offspring. Nevertheless, it is clear that DNA repair in the zygote is important for both the repair of

the initial radiation-induced DNA lesions and the prevention of delayed genomic instability.

Induced genomic instability can contribute to oncogenic mutations in somatic cells and malignant transformation (Little, 2000). Therefore, radiation-induced delayed transgenerational instability may have important health consequences that may become apparent in later generations after the original exposure. Clearly, understanding the DNA repair capacity of the zygote and the mechanisms that contribute to transgenerational genomic instability are areas that will require significant attention in the future.

CONCLUSIONS

There has been significant progress toward understanding the role of DNA repair during the perifertilization period in modulating the amount of transmitted damage and the consequences for the genetic integrity and health of the offspring. The available evidence shows that:

(1) decline in DNA repair during spermiogenesis results in the accumuation of DNA lesions that are transmitted to the egg at fertilization;
(2) fertilization with sperm with damaged DNA activates the egg's damage response that occurs immediately after fertilization;
(3) maternal and paternal pronuclei "talk" to each other as they move in a coordinated fashion through the first cell cycle of development;
(4) disruption of maternal DNA repair significantly increases sperm-derived chromosomal damage;
(5) germ cell DNA damage induces genomic instability during embryonic development and untargeted effects in the progeny.

The results presented in this review suggest that DNA repair processes during the early phases of mammalian embryonic development can have far reaching consequences for genomic stability and the health of future generations.

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REFERENCES

Ahmed, E. A., van der Vaart, A., Barten, A., Kal, H. B., Chen, J., Lou, Z., Minter-Dykhouse, K., Bartkova, J., Bartek, J., de Boer, P., de Rooij, D. G. (2007). Differences in DNA double strand breaks repair in male germ cell types: Lessons learned from a differential expression of Mdc1 and 53BP1. *DNA Repair* **6**, 1243-54.

Ashwood, M. J., Edwards, R. (1996). DNA repair by oocytes. *Mol Hum Reprod* **2**, 46-51.

Baarends, W. M., van der Laan, R., Grootegoed, J. A. (2001). DNA repair mechanisms and gametogenesis. *Reproduction* **121**, 31-9.

Banin, S., Moyal, L., Shieh, S., Taya, Y., Anderson, C. W., Chessa, L., Smorodinsky, N. I., Prives, C., Reiss, Y., Shiloh, Y., Ziv, Y. (1998). Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* **281**, 1674-7.

Barber, R., Plumb, M. A., Boulton, E., Roux, I., Dubrova, Y. E. (2002). Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. *Proc Natl Acad Sci U S A* **99**, 6877-82.

Barton, T. S., Robaire, B., Hales, B. F. (2005). Epigenetic programming in the preimplantation rat embryo is disrupted by chronic paternal cyclophosphamide exposure. *Proc Natl Acad Sci U S A* **102**, 7865-70.

Bouffler, S. D., Bridges, B. A., Cooper, D. N., Dubrova, Y., McMillan, T. J., Thacker, J., Wright, E. G., Waters, R. (2006). Assessing radiation-associated mutational risk to the germline: repetitive DNA sequences as mutational targets and biomarkers. *Radiat Res* **165**, 249-68.

Brandriff, B., Pedersen, R. A. (1981). Repair of the ultraviolet-irradiated male genome in fertilized mouse eggs. *Science* **211**, 1431-3.

Braude, P., Bolton, V., Moore, S. (1988). Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. *Nature* **332**, 459-61.

Burma, S., Chen, B. P., Murphy, M., Kurimasa, A., Chen, D. J. (2001). ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *J Biol Chem* **276**, 42462-7.

Canman, C. E., Lim, D. S., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M. B., Siliciano, J. D. (1998). Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* **281**, 1677-9.

Derijck, A., van der Heijden, G., Giele, M., Philippens, M., de Boer, P. (2008). DNA double-strand break repair in parental chromatin of mouse zygotes, the first cell cycle as an origin of de novo mutation. *Hum Mol Genet* **17**, 1922-37.

Derijck, A. A., van der Heijden, G. W., Giele, M., Philippens, M. E., van Bavel, C. C., de Boer, P. (2006). gammaH2AX signalling during sperm chromatin remodelling in the mouse zygote. *DNA Repair* **5**, 959-71.

Dronkert, M. L. G., Beverloo, H. B., Johnson, R. D., Hoeijmakers, J. H. J., Jasin, M., Kanaar, R. (2000). Mouse RAD54 Affects DNA Double-Strand Break Repair and Sister Chromatid Exchange. *Mol Cell Biol* **20**, 3147-56.

Dubrova, Y. E. (2005). Radiation-induced mutation at tandem repeat DNA loci in the mouse germline: spectra and doubling dose. *Radiat Res* **163**, 200-7.

Dubrova, Y. E., Grant, G., Chumak, A. A., Stezhka, V. A., Karakasian, A. N. (2002). Elevated minisatellite mutation rate in the post-chernobyl families from ukraine. *Am J Hum Genet* **71**, 801-9.

Dubrova, Y. E., Hickenbotham, P., Glen, C. D., Monger, K., Wong, H. P., Barber, R. C. (2008). Paternal exposure to ethylnitrosourea results in transgenerational genomic instability in mice. *Environ Mol Mutagen* **49**, 308-11.

Dubrova, Y. E., Jeffreys, A. J., Malashenko, A. M. (1993). Mouse minisatellite mutations induced by ionizing radiation. *Nat Genet* **5**, 92-4.

Dubrova, Y. E., Nesterov, V. N., Krouchinsky, N. G., Ostapenko, V. A., Neumann, R., Neil, D. L., Jeffreys, A. J. (1996). Human minisatellite mutation rate after the Chernobyl accident. *Nature* **380**, 683-6.

Dubrova, Y. E., Plumb, M., Brown, J., Fennelly, J., Bois, P., Goodhead, D., Jeffreys, A. J. (1998). Stage specificity, dose response, and doubling dose for mouse minisatellite germ-line mutation induced by acute radiation. *Proc Natl Acad Sci U S A* **95**, 6251-5.

Dubrova, Y. E., Plumb, M., Gutierrez, B., Boulton, E , Jeffreys, A. J. (2000). Transgenerational mutation by radiation. *Nature* **405**, 37.

Ehling, U. H. (1971). Comparison of radiation- and chemically-induced dominant lethal mutations in male mice. *Mutat Res* **26**, 285-93.

Enoch, T., Norbury, C. (1995). Cellular responses to DNA damage: cell-cycle checkpoints, apoptosis and the roles of p53 and ATM. *Trends Biochem Sci* **20**, 426-30.

Essers, J., Hendriks, R. W., Swagemakers, S. M., Troelstra, C., de Wit, J., Bootsma, D., Hoeijmakers, J. H., Kanaar, R. (1997). Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell* **89**, 195-204.

Fernandez-Capetillo, O., Chen, H. T., Celeste, A., Ward, I., Romanienko, P. J., Morales, J. C., Naka, K., Xia, Z., Camerini-Otero, R. D., Motoyama, N., Carpenter, P. B., Bonner, W. M., Chen, J., Nussenzweig, A. (2002). DNA damage-induced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat Cell Biol* **4**, 993-7

Friedberg, E. C., Meira, L. B. (2006). Database of mouse strains carrying targeted mutations in genes affecting biological responses to DNA damage Version 7. *DNA Repair* **5**, 189-209.

Friedberg, E. C., Walker, G. C., Siede, W. (2005). DNA Repair and Mutagenesis. American Society of Microbiology Washington, D.C.

Garagna, S., Redi, C. (1988). Chromatin topology during the transformation of the mouse sperm nucleus into pronucleus in vivo. *J. Exp. Zool.* **246**, 187-93.

Gatti, J. L., Castella, S., Dacheux, F., Ecroyd, H., Metayer, S., Thimon, V., Dacheux, J. L. (2004). Post-testicular sperm environment and fertility. *Anim Reprod Sci* **82-83**, 321-39.

Generoso, W. M., Cain, K. T., Krishna, M., Huff, S. W. (1979). Genetic lesions induced by chemicals in spermatozoa and spermatids of mice are repaired in the egg. *Proc Natl Acad Sci U S A* **76**, 435-7.

Genesca, A., Caballin, M. R., Miro, R., Benet, J., Germa, J. R., Egozcue, J. (1992). Repair of human sperm chromosome aberrations in the hamster egg. *Hum Genet* **89**, 181-6.

Glen, C. D., Smith, A. G., Dubrova, Y. E. (2008). Single-molecule PCR analysis of germ line mutation induction by anticancer drugs in mice. *Cancer Res* **68**, 3630-6.

Hamatani, T., Carter, M. G., Sharov, A. A., Ko, M. S. (2004). Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell* **6**, 117-31.

Hamer, G., Roepers-Gajadien, H. L., van Duyn-Goedhart, A., Gademan, I. S., Kal, H. B., van Buul, P. P , de Rooij, D. G. (2003). DNA double-strand breaks and gamma-H2AX signaling in the testis. *Biol Reprod* **68**, 628-34.

Hassold, T., Abruzzo, M., Adkins, K., Griffin, D., Merrill, M., Millie, E., Saker, D., Shen, J., Zaragoza, M. (1996). Human aneuploidy: incidence, origin, and etiology. *Environ Mol Mutagen* **28**, 167-75.

Hassold, T., Hunt, P. (2001). To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* **2**, 280-91.

Hatch, T., Derijck, A. A., Black, P. D., van der Heijden, G. W., de Boer, P., Dubrova, Y. E. (2007). Maternal effects of the scid mutation on radiation-induced transgenerational instability in mice. *Oncogene* **26**, 4720-4.

Hazzouri, M., Pivot-Pajot, C., Faure, A. K., Usson, Y., Pelletier, R., Sele, B., Khochbin, S., Rousseaux, S. (2000). Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone deacetylases. *Eur J Cell Biol* **79**, 950-60.

Kastan, M. B., Onyekwere, O., Sidransky, D., Wogelstein, B., Craig, R. W. (1991). Participation of p53 protein in the cellular response to DNA damage. *Cancer Research* **51**, 6304-11.

Kierszenbaum, A. L., Tres, L. L. (1978). RNA transcription and chromatin structure during meiotic and postmeiotic stages of spermatogenesis. *Fed Proc* **37**, 2512-6.

Kobayashi, J., Tauchi, H., Sakamoto, S., Nakamura, A., Morishima, K., Matsuura, S., Kobayashi, T., Tamai, K., Tanimoto, K., Komatsu, K. (2002). NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. *Curr Biol* **12**, 1846-51.

Lieber, M. R., Ma, Y., Pannicke, U., Schwarz, K. (2003). Mechanism and regulation of human non-homologous DNA end-joining. *Nat Rev Mol Cell Biol* **4**, 712-20.

Little, J. B. (2000). Radiation carcinogenesis. *Carcinogenesis* **21**, 397-404.

MacCallum, D. E., Hall, P. A., Wright, E. G. (2001). The Trp53 pathway is induced in vivo by low doses of gamma radiation. *Rad Res* **156**, 324-7.

Marchetti, F., Bishop, J. B., Lowe, X., Generoso, W. M., Hozier, J., Wyrobek, A. J. (2001). Etoposide induces heritable chromosomal aberrations and aneuploidy during male meiosis in the mouse. *Proc Natl Acad Sci U S A* **98**, 3952-7.

Marchetti, F., Essers, J., Kanaar, R., Wyrobek, A. J. (2007). Disruption of maternal DNA repair increases sperm-derived chromosomal aberrations. *Proc Natl Acad Sci U S A* **104**, 17725-9.

Marchetti, F., Wyrobek, A. J. (2005). Mechanisms and consequences of paternally-transmitted chromosomal abnormalities. *Birth Defects Res C Embryo Today* **75**, 112-29.

Marchetti, F , Wyrobek, A. J. (2008). DNA repair decline during mouse spermiogenesis results in the accumulation of heritable DNA damage. *DNA Repair* **7**, 572-81.

Matsuda, Y., Seki, N., Utsugi-Takeuchi, T., Tobari, I. (1989a). Changes in X-ray sensitivity of mouse eggs from fertilization to the early pronuclear stage, and their repair capacity. *Int J Rad Biol* **55**, 233-56.

Matsuda, Y., Seki, N., Utsugi-Takeuchi, T , Tobari, I. (1989b). X-Ray- and mitomycin C (MMC)-induced chromosome aberrations in spermiogenic germ cells and the repair capacity of mouse eggs for the X-ray and MMC damage. *Mutat Res* **211**, 65-75.

Matsuda, Y., Tobari, I. (1989). Repair capacity of fertilized mouse eggs for X-ray damage induced in sperm and mature oocytes. *Mutat Res* **210**, 35-47.

Matsuda, Y., Tobari, I. (1995). Radiosensitivity and effects of repair inhibitors for X-ray-induced chromosomal damage in mouse zygotes in S and G2 phases. *Int J Rad Biol* **68**, 615-23.

McFadden, D., Friedman, J. (1997). Chromosome abnormalities in human beings. *Mutat Res* **396**, 129-140.

McGaughey, R. W., Chang, M. C. (1969). Meiosis of mouse egg before and after sperm penetration. *J Exp Zool* **170**, 397-410.

Meistrich, M. L. (1989). Histone and basic nuclear protein transitions in mammalian spermatogenesis. Histones and other basic nuclear proteins. L. S. Hnilica, G. S. Stein and J. L. Stein. Boca Raton, CRC Press**:** 165-182.

Niwa, O. (2003). Induced genomic instability in irradiated germ cells and in the offspring; reconciling discrepancies among the human and animal studies. *Oncogene* **22**, 7078-86.

Niwa, O., Kominami, R. (2001). Untargeted mutation of the maternally derived mouse hypervariable minisatellite allele in F1 mice born to irradiated spermatozoa. *Proc Natl Acad Sci U S A* **98**, 1705-10.

Nomura, T. (1982). Parental exposure to x rays and chemicals induces heritable tumours and anomalies in mice. *Nature* **296**, 575-7.

Nomura, T., Nakajima, H., Ryo, H., Li, L. Y., Fukudome, Y., Adachi, S., Gotoh, H., Tanaka, H. (2004). Transgenerational transmission of radiation- and chemically induced tumors and congenital anomalies in mice: studies of their possible relationship to induced chromosomal and molecular changes. *Cytogenet Genome Res* **104**, 252-60.

Nonchev, S., Tsanev, R. (1990). Protamine-histone replacement and DNA replication in the male mouse pronucleus. *Mol Reprod Dev* **25**, 72-6.

Olsen, A. K., Lindeman, B., Wiger, R., Duale, N., Brunborg, G. (2005). How do male germ cells handle DNA damage? *Toxicol Appl Pharmacol* **207**, 521-31.

Perreault, S. D. (1992). Chromatin remodeling in mammalian zygotes. *Mutat Res* **296**, 43-55.

Redon, C., Pilch, D., Rogakou, E., Sedelnikova, O., Newrock, K., Bonner, W. (2002). Histone H2A variants H2AX and H2AZ. *Curr Opin Genet Dev* **12**, 162-9.

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. , Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* **273**, 5858-68.

Russell, L. B. (1999). Significance of the perigametic interval as a major source of spontaneous mutations that result in mosaics. *Environ Mol Mutagen* **34**, 16-23.

Russell, W. L., Bangham, J. W., Russell, L. B. (1998). Differential response of mouse male germ-cell stages to radiation-induced specific-locus and dominant mutations. *Genetics* **148**, 1567-78.

Saito, S., Goodarzi, A. A., Higashimoto, Y., Noda, Y., Lees-Miller, S. P., Appella, E. , Anderson, C. W. (2002). ATM mediates phosphorylation at multiple p53 sites, including Ser(46), in response to ionizing radiation. *J Biol Chem* **277**, 12491-4.

Schultz, R. M. (2002). The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. *Hum Reprod Update* **8**, 323-31.

Schultz, R. M. , Worrad, D. M. (1995). Role of chromatin structure in zygotic gene activation in the mammalian embryo. *Sem Cell Biol* **6**, 201-208.

Searle, A. G., Ford, C. E., Evans, E. P., Beechey, C. V., Burtenshaw, M. D., Clegg, H. M., Papworth, D. G. (1974). The induction of translocations in mouse spermatozoa. I. Kinetics of dose response with acute x-irradiation. *Mutat Res* **22**, 157-74.

Sega, G. A. (1974). Unscheduled DNA synthesis in the germ cells of male mice exposed in vivo to the chemical mutagen ethyl methanesulphonate. *Proc Natl Acad Sci U S A* **71**, 4955-9.

Sega, G. A. (1979). Unscheduled DNA synthesis (DNA repair) in the germ cells of male mice: its role in the study of mammalian mutagenesis. *Genetics* **92**, s49-s58.

Shimura, T., Inoue, M., Taga, M., Shiraishi, K., Uematsu, N., Takei, N., Yuan, Z. M., Shinohara, T., Niwa, O. (2002). p53-dependent S-phase damage checkpoint and pronuclear cross talk in mouse zygotes with X-irradiated sperm. *Mol Cell Biol* **22**, 2220-8.

Siliciano, J. D., Canman, C. E., Taya, Y., Sakaguchi, K., Appella, E., Kastan, M. B. (1997). DNA damage induces phosphorylation of the amino terminus of p53. *Genes Dev* **11**, 3471-81.

Smith, G. C., Jackson, S. P. (1999). The DNA-dependent protein kinase. *Genes Dev* **13**, 916-34.

Somers, C. M., Yauk, C. L., White, P. A., Parfett, C. L., Quinn, J. S. (2002). Air pollution induces heritable DNA mutations. *Proc Natl Acad Sci U S A* **99**, 15904-7.

Sotomayor, R. E., Sega, G. A. (2000). Unscheduled DNA synthesis assay in mammalian spermatogenic cells: an update. *Environ Mol Mutagen* **36**, 255-65.

Stiff, T., O'Driscoll, M., Rief, N., Iwabuchi, K., Lobrich, M., Jeggo, P. A. (2004). ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res* **64**, 2390-6.

Taneja, N., Davis, M., Choy, J. S., Beckett, M. A., Singh, R., Kron, S. J., Weichselbaum, R. R. (2004). Histone H2AX phosphorylation as a predictor of radiosensitivity and target for radiotherapy. *J Biol Chem* **279**, 2273-80.

Valerie, K. , Povirk, L. F. (2003). Regulation and mechanisms of mammalian double-strand break repair. *Oncogene* **22**, 5792-812.

van Gent, D. C., Hoeijmakers, J. H., Kanaar, R. (2001). Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet* **2**, 196-206.

van Loon, A. A., Sonneveld, E., Hoogerbrugge, J., van der Schans, G. P., Grootegoed, J. A., Lohman, P. H. , Baan, R. A. (1993). Induction and repair of DNA single-

strand breaks and DNA base damage at different cellular stages of spermatogenesis of the hamster upon in vitro exposure to ionizing radiation. *Mutat Res* **294**, 139-48.

Vilarino-Guell, C., Smith, A. G., Dubrova, Y. E. (2003). Germline mutation induction at mouse repeat DNA loci by chemical mutagens. *Mutat Res* **526**, 63-73.

Wahl, G. M., Carr, A. M. (2001). The evolution of diverse biological responses to DNA damage: insights from yeast and p53. *Nat Cell Biol* **3**, E277-86.

Ward, I. M., Chen, J. (2001). Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *J Biol Chem* **276**, 47759-62.

West, S. C. (2003). Molecular views of recombination proteins and their control. *Nat Rev Mol Cell Biol* **4**, 435-45.

Wouters-Tyrou, D., Martinage, A., Chevaillier, P., Sautiere, P. (1998). Nuclear basic proteins in spermiogenesis. *Biochimie* **80**, 117-28.

Wyman, C. , Kanaar, R. (2006). DNA Double-Strand Break Repair: All's Well that Ends Well. *Annu Rev Genet* **40**, 363-83.

Wyrobek, A. J., Eskenazi, B., Young, S., Arnheim, N., Tiemann-Boege, I., Jabs, E. W., Glaser, R. L., Pearson, F. S., Evenson, D. (2006). Advancing age has differential effects on DNA damage, chromatin integrity, gene mutations, and aneuploidies in sperm. *Proc Natl Acad Sci U S A* **103**, 9601-6.

Xu, G., Spivak, G., Mitchell, D. L., Mori, T., McCarrey, J. R., McMahan, C. A., Walter, R. B., Hanawalt, P. C., Walter, C. A. (2005). Nucleotide excision repair activity varies among murine spermatogenic cell types. *Biol Reprod* **73**, 123-30.

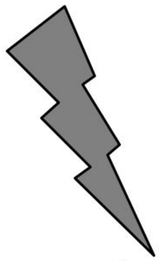
Yauk, C., Polyzos, A., Rowan-Carroll, A., Somers, C. M., Godschalk, R. W., Van Schooten, F. J., Berndt, M. L., Pogribny, I. P., Koturbash, I., Williams, A., Douglas, G. R., Kovalchuk, O. (2008). Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. *Proc Natl Acad Sci U S A* **105**, 605-10.

Yauk, C. L., Dubrova, Y. E., Grant, G. R., Jeffreys, A. J. (2002). A novel single molecule analysis of spontaneous and radiation-induced mutation at a mouse tandem repeat locus. *Mutat Res* **500**, 147-56.

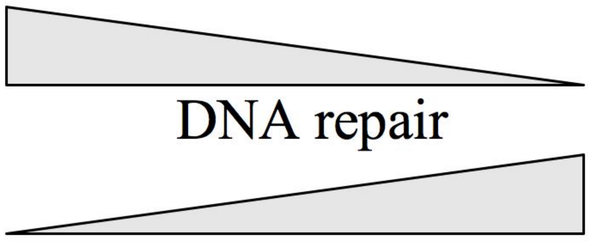
Zeng, F., Baldwin, D. A., Schultz, R. M. (2004). Transcript profiling during preimplantation mouse development. *Dev Biol* **272**, 483-96.

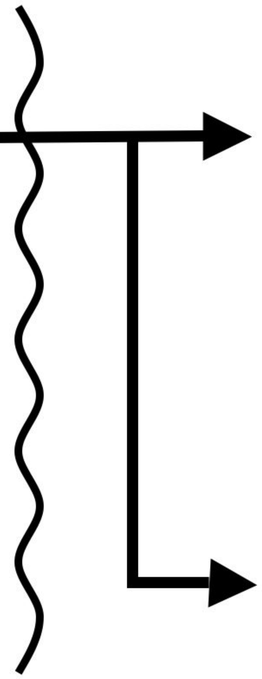
Figure 1: Exposures to ionizing radiation and other germ cell mutagens lead to both immediate and delayed genomic damage in the offspring. The amount of DNA damage that is transmitted to the zygote is dependent on the DNA repair capacity of the exposed germ cell stage. As DNA repair capacity declines during spermatogenesis, DNA lesions can accumulate unrepaired in the maturing sperm. The extent of de novo genomic damage that is transmitted to the offspring is dependent upon the maternal genotype and its ability to repair the damage.

Exposure



Spermatogenesis





Fertilization

Susceptibility depends on maternal genotype

Immediate effects

- *de novo* gene mutations
- *de novo* chromosomal aberrations

Delayed effects

- genomic instability
- mosaicism