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Radiation- and Drug-Induced DNA Repair in Mammalian Oocytes and Embryos[1]

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Summary

A review of studies showing ultraviolet- or drug-induced unscheduled DNA synthesis in mammalian oocytes and embryos suggests that the female gamete has an excision repair capacity from the earliest stages of oocyte growth. The oocyte's demonstrable excision repair capacity decreases at the time of meiotic maturation for unknown reasons, but the fully mature oocyte maintains a repair capacity, in contrast to the mature sperm, and contributes this to the zygote. Early embryo cells maintain relatively constant levels of excision repair until late fetal stages, when they lose their capacity for excision repair.

These apparent changes in excision repair capacity do not have a simple relationship to known differences in radiation sensitivity of germ cells and embryos.

Introduction

There is now direct and inferential evidence that male and female germ cells of mammals are capable of repairing DNA damage during gametogenesis. In males, repair has been demonstrated directly after ultraviolet (UV) or X-irradiation and after mutagen treatment of early spermatogenic stages but has not been detected in mature spermatozoa (10,29,39,68,69; reviewed by Sega, this volume). In females, repair of UV damage has been observed at all stages of germ cell maturation. We will review the evidence for repair in female mammalian germ cells and in early embryos, with particular emphasis on the mouse, the species used in most studies. Indirect evidence for repair capability in oocytes comes from dose-fractionation studies of specific locus mutations and dominant lethal effects. However, we will primarily consider cases in which direct observations of unscheduled DNA synthesis after UV or drug treatment indicate a capacity for excision repair. We will also consider how changing repair capacities compare to sensitivity for mutation induction or cell killing in mammalian oocytes and embryos.

In classical work Muller (53) determined that X-ray-damaged chromosomes in Drosophila spermatozoa did not rejoin broken ends before fertilization. Using similar split-dose studies, Dempster (16), Kaufmann (36), and Oster (54) confirmed Muller's discovery and concurred in suggesting that X-ray-induced breaks are rejoined after fertilization; this has been further demonstrated by Würgler and Maier (76). In other early work Henshaw (33) showed that sea urchin eggs recovered from X-ray-induced cleavage delay in

proportion to the time between irradiation and insemination with unirradiated sperm. Failla (24) extended this work by showing that the similar cleavage delay caused by irradiating sperm (34) could be reduced exponentially by allowing sperm to reside in the egg for 20-100 min before cleavage began. Failla (24,25) concluded that a radiation repair process occurs during sperm and egg recovery.

The initial evidence that mammalian oocytes were capable of radiation repair was the discovery that mouse oocytes exposed to chronic γ- or X-irradiation at low dose rates incurred substantially fewer specific locus mutations than those exposed to equivalent acute doses of X rays (46,61,62). Because there were no specific locus mutations induced in mouse oocytes exposed to neutron radiation more than 7 weeks before fertilization, Russell (63) suggested that early oocytes may have a more efficient repair mechanism than later-stage oocytes and spermatogonia (reviewed in 67 and by Russell, this volume). These early observations stimulated attempts to detect repair processes by direct observation of mouse oocytes and early embryos.

Oogenesis and Embryogenesis

The general features of oogenesis and early embryogenesis are similar in most mammalian species that have been studied. Primordial germ cells differentiate in the extraembryonic mesoderm and are then seen in yolk sac endoderm during early organogenesis (11). They subsequently migrate to the genital ridges via the hind gut endoderm and mesentery, where they proliferate until the entire cohort of

spermatogonia or oogonia is produced. In female mice and rats these enter meiotic prophase relatively synchronously (after completing their last pre-meiotic S phase) at 15-16 days of gestation. In males, initiation of meiosis occurs after birth. At birth, mammalian ovaries contain the female's lifetime supply of oocytes. In the mouse, these are initially "resting" or primordial dictyate oocytes, but beginning in the first neonatal week and increasing until the third week, large numbers of oocytes begin growth (58). The resting oocytes continue to be recruited into growth as follicles mature throughout the reproductive lifespan, although this occurs at a lower rate during pregnancy and with increasing age. In immature and pregnant mice, the growing oocytes are not ovulated, but instead they degenerate as fully grown oocytes; the time required for an oocyte to develop from its recruitment into a growing follicle until ovulation has been estimated at 19 days (58). In immature mice (0-35 days) there is extensive atresia, illustrated by the observation that 4,000-5,000 oocytes leave the resting pool but only about 850 develop into oocytes in growing follicles (58). By comparison, during the period from 1 to 12 months, 4,000-5,000 oocytes leave the resting oocyte pool and most of these enter the growth phase, although only 700-800 are actually ovulated in cycling mice (58). In mature mice, there are 100-300 growing oocytes at any time before 2 years of age, when oocytes are depleted and ovulation ceases.

At ovulation, meiotic maturation occurs. The germinal vesicle, which is about one-third the diameter of the oocyte, breaks down, chromosomes condense into metaphase I of meiosis, and the first polar body is emitted, with separation of homologous chromosomes;

oocytes progress to metaphase II, where they are arrested until fertilization. Oocytes acquire the capacity for spontaneous maturation in chemically defined medium after they reach approximately 60 μm in diameter (73). During the entire growth period until meiotic maturation, the oocyte is capable of extensive RNA and protein synthesis (reviewed in 51). Despite a report that some oocyte DNA synthesis occurs after birth (15), it seems likely that scheduled DNA synthesis in oocytes occurs only before the beginning of meiosis (44).

Upon fertilization the fully grown oocyte, arrested in metaphase II, emits the second polar body, separating chromatids. Male and female pronuclei form within 10 hr, and DNA synthesis occurs (40,45). First cleavage takes place 18-24 hr after fertilization. In the mouse, subsequent cleavages occur at approximately 10-hr intervals *in vivo* or slightly longer intervals *in vitro*, with asynchrony appearing at the second cleavage (7,37). In late 8-cell-stage embryos, cell boundaries become less distinct as the process of compaction begins, coincident with the differentiation of cell contact and junctions (19). This process continues at the morula (12- to 16-cell) stage. Development of the blastocyst is apparent when trophectoderm (outer cells) begin to secrete the blastocoel fluid. At this stage the inner cell mass, which gives rise to the fetus, can be clearly distinguished by light microscopy from the trophectoderm, which gives rise to the placenta. When placed in a suitable nutritional environment *in vitro* the blastocyst attaches to the substrate, and the trophectoderm transforms into a sheet of giant trophoblast cells. This grows out on the substratum,

leaving the inner cell mass derivatives exposed directly to the culture medium (75). Both fetal and placental cells continue to proliferate and differentiate in culture for approximately 1 week beyond the blastocyst stage, and can develop into early organogenesis-stage embryos with a beating heart and active circulatory system (35).

Evidence for Excision Repair

UV irradiation can result in autoradiographically detectable DNA synthesis in cells that are not in S phase. This "unscheduled" DNA synthesis appears to reflect the removal and replacement of damaged bases, particularly thymine dimers (55). The excision repair system includes at least four enzyme activities, an endonuclease, an exonuclease, a polymerase, and a ligase, to accomplish the repair replication that is observed (reviewed by Setlow, this volume). Because UV light and certain mutagenic drugs induce substantial ("long-patch") repair reactions, they have generally been used to determine the excision repair capacity of mouse oocytes and embryos. Evidence for unscheduled DNA synthesis in the mouse has been obtained by exposing various stages of oocytes and embryos to 15-450 J/m^2 UV light from germicidal mercury lamps (254 nm) and then culturing the cells in ^{3}H-thymidine and exposing them to autoradiographic emulsion for grain counting. We have carried out a regression analysis of published data in order to estimate the response of various stages of oocytes and embryos to a UV dose of 60 J/m^2. This procedure allowed a rough comparison of data obtained with various dose regimens and autoradiographic exposure times, even though the small number of data points in each study (one to six) limited the confidence that could be placed on estimates from regression analysis.

In this comparison we disregarded differences in labeling conditions, thymidine permeability, and pool sizes because there were few data regarding the effect of these parameters on grain counts in the stages of cells examined. It is important to bear in mind that an accurate quantitative comparison of repair capacity between stages would require such information, and that the present comparisons are based only on available data for grain counts, dose, and autoradiographic exposure time.

The earliest stage of oocyte that has been studied, and the most important one from a genetic standpoint, is the resting or primordial oocyte. Pedersen and Mangia (57) obtained resting oocytes from newborn female mice and irradiated them with 15-60 J/m^2 UV light at a dose rate of 1.3 J/m^2/sec. The normalized value obtained by regression analysis of their data was approximately 14 grains/day of autoradiographic exposure for a dose of 60 J/m^2 (Fig. 1a; 2a-c). Growing oocytes (approximately 65 μm diameter) irradiated with 60 J/m^2 showed much higher ^{3}H-thymidine incorporation, approximately 118 grains/day of autoradiographic exposure (Fig. 1b; 2d-f). Seeking an explanation for this large difference in incorporation between stages, Pedersen and Mangia (57) determined the relative autoradiographic efficiency in the two stages by labeling oocytes with ^{3}H-thymidine during their pre-meiotic S phase. They found a 2.2-fold higher efficiency in the autoradiography of growing oocytes than of resting oocytes. Even correcting for this difference, however, they concluded that there was approximately a 6-fold greater ^{3}H-thymidine incorporation into UV-irradiated growing

oocytes than in resting oocytes. Other factors that may have contributed to the observed difference, such as thymidine permeability, precursor pool size, and the extent of UV damage to DNA, were not evaluated. The most obvious changes between the resting and the growing oocytes are the large increases in cytoplasmic and nuclear radii. These changes may affect the dose of UV light received, and the 10-fold increase in nuclear volume probably reflects changes in the chromatin configuration and may affect the accessibility of DNA to repair enzymes, as discussed later.

The only demonstration of X-ray-induced unscheduled DNA synthesis in mammalian oocytes was obtained with newborn guinea pigs exposed to 5,000 rads at 385 rads/min and labeled with ^{3}H-thymidine *in vivo* (14). Although absolute grain counts were not given, there was a 2- to 3-fold increase in grains above background in the irradiated oocytes, but not in the control oocytes. Crone (14) also exposed mouse oocytes to 200 rads of X rays but was unable to detect unscheduled DNA synthesis at that dose.

UV-induced unscheduled DNA synthesis has also been seen in fully grown mouse oocytes undergoing meiotic maturation. Masui and Pedersen (50) studied three stages of naturally ovulated oocytes from ICR mice, those just removed from the ovary (germinal vesicle stage), those cultured for 6-8 hr (metaphase I), and those cultured for 16-18 hr (metaphase II). The highest level of unscheduled synthesis occurred in germinal vesicle-stage oocytes exposed to 30,60, or 120 J/m^2 UV light at 1.3 J/m^2/sec. In these oocytes grain count increased with dose up to 60 J/m^2, then reached

a plateau (Fig. 1c). The regression of grain counts with dose for the linear portion of the curve gave approximately 39 grains/day of autoradiographic exposure for a dose of 60 J/m^2. Oocytes exposed at metaphase I had markedly lower grain counts (5 grains/day) and those irradiated at metaphase II had even lower counts (3 grains/day) (Fig. 1d). Polar body chromatin showed significant grains only at the lowest dose (30 J/m^2), and even then had fewer grains than any oocyte stage studied. In interpreting these results, Hasui and Pedersen (50) ruled out differences in permeability to isotope between stages by showing that thymidine uptake did not change during meiotic maturation; thymidine pool sizes were not determined. They considered, but did not evaluate, the possibility of a greater autoradiographic efficiency for germinal vesicles than for metaphase chromosomes owing to better spreading of the germinal vesicle chromatin. Despite these considerations, however, the large difference in grain count seems to indicate a substantial decrease in the oocyte's capacity for unscheduled DNA synthesis at the time of germinal vesicle breakdown.

In another study, Ku et al. (41) irradiated metaphase II oocytes obtained by superovulation of (C3H x DBA 2) F_1 mice. Oocytes were exposed to 30-450 J/m^2 at 2, 5.2, or 15 J/m^2/sec and cultured in ^{3}H-thymidine of unspecified concentration or specific activity. At the lowest dose rate they found 5 grains per oocyte chromosome set/day of exposure (estimated for a dose of 60 J/m^2 from doses 1-120 J/m^2 in their figure). They carried out DNAse controls to show that grains were indeed contained in the DNA. At the higher dose rates they found significantly lower grain counts but could not account for this difference.

Recently, Brazill and Masui (8) studied UV- and drug-induced unscheduled DNA synthesis in random-bred CBL mouse oocytes using an experimental design that reduced the variation due to autoradiographic efficiency. After exposing oocytes at the germinal vesicle, metaphase I, or metaphase II stage, they cultured them for 2 hr in ^{3}H-thymidine followed by a cold thymidine chase and then continued the incubation in unlabeled medium until the oocytes reached metaphase II, when they were fixed for autoradiography. Oocytes exposed at the germinal vesicle stage to a single UV dose (50 J/m^{2}, dose rate not given) had 17 grains/day; when exposed at metaphase I or metaphase II they had 7 grains/day. This confirmed Masui and Pedersen's (50) finding of a decrease in unscheduled DNA synthesis during meiotic maturation. Brazill and Masui (8) showed that this change was not due to differential autoradiographic efficiency because they performed all grain counts on metaphase II chromosomes, regardless of the stage exposed. They also confirmed the very low level of unscheduled DNA synthesis when the first polar body was irradiated (at metaphase II) but showed that the polar body had grain counts comparable to the oocyte when irradiation and ^{3}H-thymidine incorporation occurred before polar body formation (germinal vesicle or metaphase I stage).

Further insight into the polar body's deficiencies came from Brazill and Masui's (8) data on oocytes treated with drugs. They exposed germinal vesicle, metaphase I, or metaphase II oocytes to either 10^{-5} M 4-nitroquinoline 1-oxide (4NQO) or 10^{-3} M methyl methanesulfonate (MMS). The 4NQO treatment of germinal vesicle-

stage oocytes induced 6 grains/day of autoradiographic exposure and induced fewer grains at metaphase I and II stages and in the polar body. The MMS treatment, however, induced approximately the same level of grains at all oocyte stages and in the polar body, 1 grain/day of autoradiographic exposure. There were essentially no grains on control oocytes after correction for background. Citing unpublished data that showed similar grain counts in oocytes and polar bodies when eggs were irradiated at the germinal vesicle stage and labeled with ^{3}H-thymidine at metaphase II, they proposed that the decreased capacity for unscheduled DNA synthesis in polar bodies treated with UV or 4NQO is due to loss of an endonuclease activity. Although there is no direct evidence that oocytes are deficient for this enzyme activity, their observation that MMS induced similar levels of unscheduled DNA synthesis in both oocytes and polar bodies indicates that the polar body is deficient for an early step in excision repair. In this regard the polar body resembles xeroderma pigmentosum cells, which cannot repair UV damage but can carry out unscheduled DNA synthesis in response to damage by MMS and other agents that induce short-patch repair (12,60,70).

A change in capacity for carrying out early steps in excision repair may also account for the large decrease in oocyte unscheduled DNA synthesis between the germinal vesicle stage and metaphase I or II. This intepretation is supported by the observation that MMS-induced repair in oocyte DNA remains unchanged during oocyte maturation (8). This point could be resolved with additional data from other agents that induce short-patch repair, such as X rays or ethyl methanesulfonate (60).

The change in oocyte organization at the time of germinal vesicle breakdown could also contribute to the observed differences in repair activity. The contents of the germinal vesicle, which occupy approximately 1/27 the volume of the oocyte, may be correspondingly diluted or redistributed upon germinal vesicle breakdown. Despite the observed decrease in unscheduled DNA synthesis during meiotic maturation, the remaining capacity in the meiotically mature metaphase II oocyte, or unfertilized egg, raises the possibility that egg cytoplasm may confer a repair capacity on the repair-incompetent male gamete after fertilization.

Our own recent work has revealed a capacity for unscheduled DNA synthesis in pronuclear-stage mouse embryos irradiated with UV light (Brandriff, B. and Pedersen, R.A., unpublished observations). Embryos from ICR mice were exposed to 15-60 J/m^2 UV light several hours after sperm penetration, then labeled with ^3H-thymidine and fixed for autoradiography at the pronuclear stage. After a 2-week exposure to emulsion, both pronuclei showed dose-dependent increases in grain counts similar to the grain numbers of irradiated, unfertilized metaphase II oocytes (approximately 3 grains/day at 60 J/m^2). These results indicate a capacity for excision repair during the interval between sperm penetration and the first embryonic S phase.

In an earlier attempt to determine whether mouse egg cytoplasm was able to repair drug-induced damage to sperm DNA, Sega et al. (69) examined eggs fertilized by sperm of MMS-treated males but found no autoradiographic evidence for unscheduled DNA synthesis. Differences in procedures, including mode of damage and strain of mice, may account for our different findings. Using another approach Generoso et al. (28 and this volume) have inferred that repair of alkylation damage

in sperm DNA occurs after fertilization in some strains of mice (see below).

The excision repair capacity of other preimplantation and early postimplantation-stage mouse embryos was studied by Pedersen and Cleaver (56) (Fig. 3). They irradiated embryos with 60 J/m^2 UV light at 1.3 J/m^2/sec and found the following grain counts/day of exposure: morula, 4; blastocyst, 4; postimplantation trophoblast, 5; and postimplantation inner cell mass, 2. Unlike oocytes, embryos had S phase nuclei at all stages studied, particularly in the early cleavage stages. The preponderance of S phase nuclei in 8-cell and earlier stages made it difficult to analyze cleavage-stage embryos for their repair capability (Pedersen, R.A., unpublished observations). Nevertheless, because similar levels of unscheduled DNA synthesis were observed in pronuclear embryos and morulae, it seems likely that early cleavage-stage embryos also have an excision repair capacity.

In their study of mouse fetal stages, Peleg et al. (59) assessed excision repair and unscheduled DNA synthesis in primary cultures initiated at 13-15 or 17-19 days of gestation. At the first and second transfers 13- to 15-day fetal cells excise 50% of UV-induced thymine dimers within 24 hr; this decreases to 4% of dimers by the ninth transfer. Autoradiographically detected unscheduled DNA synthesis showed similar decreases during successive transfers. Cells grown from 17- to 19-day fetuses did not show excision repair in the first or subsequent transfers and had low levels of unscheduled DNA synthesis. These observations suggest that the low levels of excision repair seen in adult mouse cells, as compared with other mammalian species, are the result of a developmentally regulated

decline, rather than an inherently low level of excision repair in mice (6,32,43,55).

Other Evidence for Repair

In addition to the dose-rate effects for mutation induction cited earlier, inferential evidence for repair in oocytes and embryos comes from studies of chromosome aberrations, dominant lethality, and embryo radiosensitivity. Brewen et al. (9) measured aberrations in metaphase I chromosomes of CDI/CR mouse oocytes irradiated 8-14 days earlier with different X-ray dose regimens, and found a clear dose-rate effect for deletions and exchanges. They concluded that the different rates of aberrations caused by similar doses of chronic and acute X rays indicate a 2-track process. Furthermore, by fractionating the acute dose they demonstrated recovery within 135 min after exposure to 200 rads of X rays; they also concluded that this recovery or repair process was not significantly altered by increasing the initial dose from 100 to 300 rads.

Using another approach Generoso et al. (28) combined cytogenetic analysis of metaphase I chromosomes and dominant lethal analysis of embryos obtained from matings between mutagen-treated (101 x C3H) F_1 males and various strains of females. They found that matings with T stock females produced higher rates of isopropyl methanesulfonate-induced dominant lethality and chromatid aberrations than matings with other strains. They concluded that these differences were due to strain differences in capacity for repair of alkylation damage in pronuclear stage embryos. Interestingly, there were no strain differences with X-ray-induced damage and less obvious differences with ethyl

methanesulfonate, triethylenemelamine, and benzo(a)pyrene, suggesting that if repair is responsible for the maternal species-specific response, then different lesions have unique effects in the oocyte and early embryo and may be handled by different repair enzymes (see Generoso, this volume).

In an attempt to detect postreplication repair in early embryos Eibs and Spielmann (23,74) treated UV-irradiated NMRI mouse embryos with 0.1-0.5 mM caffeine. Although these concentrations of caffeine had no detrimental effect on control mouse embryo development in vitro to the blastocyst stage, caffeine potentiated the inhibitory effects of UV irradiation. The authors concluded that there is a capacity for postreplication repair in the preimplantation mouse embryo. This is the only report of such a capability in mammalian germ cells or embryos.

Radiation Sensitivity of Oocytes and Embryos

The effects of radiation on mammalian oocytes and embryos are complex. They vary between species and between strains and depend on age and stage of meiosis or mitosis. In addition, physical factors such as temperature, oxygen tension, and type and method of irradiation influence the outcome of exposure. For a meaningful discussion of radiosensitivity, it is important to specify which criterion is used for assessing effects (see 2,4,48 for reviews).

Primordial oocytes are more sensitive to killing by X-irradiation than later stages of oocytes in the juvenile and adult rat and mouse. Degenerative changes become apparent 3-6 hr after exposure,

and by 18 hr most affected oocytes have died. Exposure to 300 rads destroyed nearly the entire population of primordial oocytes in adult rats (49). The greatest sensitivity to radiation killing of mouse oocytes occurs during the third week after birth, when a dose of 20 rads leaves only 1% of the oocytes intact. In addition to X rays, tritium and aromatic hydrocarbons also have lethal effects on primordial mouse oocytes (3,17), with a peak sensitivity at 2-3 weeks after birth (18,26).

With dominant lethality as the criterion of damage, oocytes are most sensitive during metaphase I of meiosis (22,47,66). Mouse oocytes are more sensitive for specific locus mutation induction at growing and fully grown stages than at resting stages, as discussed previously.

Irradiation of pregnant mice during preimplantation stages results in extensive embryo death, but no malformations are induced in embryos that survive to birth (65). As judged by comparing subsequent preimplantation development, pronuclear stages of the mouse are more sensitive than 2-cell stages to UV and X-irradiation (20,23,42). One possible explanation for the considerable variability in studies of early mouse embryos might be the differential sensitivity of embryos as they progress through the cell cycle (21,31,64). Sensitivity is high shortly after fertilization and at early pronuclear stages and becomes lower in later pronuclear stages; early 2-cell stages are relatively resistant compared to 2-cell stages just before the second cleavage (64). It seems likely that similar variations in radiosensitivity occur also at later stages, when cleavage is asynchronous and the contribution of the cell

cycle differences to overall radiosensitivity cannot be readily determined. Irradiation at later stages indicates decreasing sensitivity to UV at the 8-cell stage (23) and complex changes in the sensitivity to X rays (1,27,30,38). Cells giving rise to the inner cell mass are more susceptible to tritium and X-ray damage than precursors of trophoblast (30,72).

Given the large number of variables in the studies described here it is difficult to ascertain the role of DNA repair in the radiation sensitivity in mammalian oocytes and embryos. The extreme sensitivity of early oocytes to cell killing occurs at a time of moderate excision repair capacity. The increase in sensitivity to specific locus mutation induction, which occurs as oocytes develop, coincides with an apparent increase in excision repair capacity. A change in opposite directions would be expected if repair capacity alone accounted for the mutation data. The increase in dominant lethal induction that occurs during meiotic maturation coincides with an apparent decrease in excision repair. Before attributing causality to this coincidence, however, we should consider the possibility that both excision repair and dominant lethality are regulated by other properties in the maturing oocyte. Finally, the changes in radiation sensitivity during early embryo development occur during a time of relatively constant excision repair capability.

Conclusions

A review of studies showing UV or drug-induced unscheduled DNA synthesis in mammalian oocytes and embryos suggests that the female gamete has an excision repair capacity from the earliest stages of oocyte growth. The oocyte's demonstrable excision repair capacity decreases at the time of meiotic maturation for unknown reasons, but the fully mature oocyte maintains a repair capacity in contrast to the mature sperm and contributes this to the zygote. Early embryos maintain relatively constant levels of excision repair until late fetal stages, when primary fibroblast cultures, at least, lose their capacity for excision repair.

It must be borne in mind that this comparison between stages rests on several unverified assumptions, including constant thymidine pool sizes, permeability, and a constant relationship between autoradiographically demonstrated unscheduled DNA synthesis and excision repair. Furthermore, because unscheduled DNA synthesis is usually measured after UV irradiation, there is little information about the oocyte or embryo's ability to repair other types of lesions, such as those induced by drugs or ionizing radiation. Thus it is not surprising that oocyte and embryo radiosensitivity appears not to be related to repair capacity. Sega et al. (69) recently came to a similar conclusion about the relationship between excision repair and radiosensitivity in male germ cells.

Although the role of repair processes in mutation induction and cell killing is unclear, some avenues for further research are open. The analysis of different types of excision repair processes and even specific enzyme activities is imperative if we are to understand the role of repair in the quantitative relationship between

damage and mutation induction. Long-patch and short-patch repair involve different enzyme activities (60), and additional enzymes are involved in repairing base damage (Friedberg et al., this volume).

In addition, the role of chromatin compaction in repair needs to be clarified, particularly for germ cells. Recent reports (5,13,71) indicate that the excision repair that occurs after UV irradiation or treatment with alkylating agents is in the linker regions between nucleosomes at early times after treatment. The inaccessibility of DNA to repair enzymes may also be involved in some complementation groups of xeroderma pigmentosum because repair-deficient cell lines can remove thymine dimers from purified DNA but not from chromatin (52). It is interesting in this regard that mouse oocyte chromatin is organized into nucleosomes (Bakken, A., unpublished observations).

Our ultimate concern is the relevance of repair in model mammalian systems to human cells. This is important for establishing risks of environmental radiation and chemical exposure of human beings. A thorough understanding of agent-specific and strain-specific variability in repair processes in model rodent systems will go a long way toward determining the effectiveness of DNA repair in alleviating human genetic hazards.

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Table 1

UV-Induced DNA Synthesis

Stage	Response at 60 J/m^2			
	Observed Grains	Grains from Regression Analysis[a]	Grains/Day[a]	Reference
Resting oocyte	216 ± 38	198	14	57
Crowing oocyte	855 ± 53	827	118	57
Germinal vesicle stage	605 ± 114	544	39	50
	240 ± 12[b,c]		17	8
Metaphase I	77 ± 11	63	5	50
	100 ± 10[b,c]		7	8
Metaphase II	37 ± 11	36	3	50
	120[c]	77	5	41
	100 ± 10[b,c]		7	8
Morula	91 ± 7		4	56
Blastocyst	76 ± 6		4	56
Trophoblast	94 ± 7		5	56
Inner cell mass	46 ± 5		2	56

To compare data in these studies we computed grain counts/day of autoradiographic exposure, based where possible on the regression of grains with dose. The correlation coefficient (r) was calculated for both linear (grains = m[dose] + b) and nonlinear (grains = b[dose]^m) regression to determine the best fit. A dose of 60 J/m^2 was used for comparison because this was the dose most commonly used.

[a]Data normalized to 1 day of autoradiographic exposure.

[b]Dose = 50 J/m^2.

[c]Data estimated from figures.

Figure Legends

Fig. 1. UV-induced unscheduled DNA synthesis in mouse oocytes. a, Resting stage. Data from ref. 57. b, Growing stage. Data from ref. 57. c, Fully grown germinal vesicle stage. Data from ref. 50. d, Fully grown metaphase I stage. Data from ref. 50.

Fig. 2. Unscheduled DNA synthesis in mouse oocytes. a, Resting oocyte (12-14 μm diameter). Differential interference contrast microscopy. b, Autoradiograph of control resting oocyte. Arrow indicates boundary of nucleus. c, Autoradiograph of resting oocyte exposed to 60 J/m^2 UV light. d, Growing oocyte (60 μm diameter). Differential interference contrast microscopy. e, Autoradiograph of control growing oocyte. f, Autoradiograph of growing oocyte exposed to 60 J/m^2 UV light. Magnifications: a,d = 400X; b,c,e,f = 512X. Reprinted with permission, ref. 57.

Fig. 3. Unscheduled DNA synthesis in mouse embryos. a, Morula stage of a pre-implantation mouse embryo, cultured from the 2-cell stage. Differential interference contrast. b, Autoradiographs of S phase and interphase nuclei of control morula. c, Autoradiographs of S phase and interphase nuclei of a UV-irradiated morula. d, Blastocyst stage pre-implantation mouse embryo cultured from the 2-cell stage. Differential interference contrast. e, Autoradiographs of S phase and interphase nuclei of control blastocyst. f, Autoradiographs of S phase and interphase nuclei of UV-irradiated blastocyst. Magnifications: a,d = 600X; b,c, e,f = 720X. Reprinted with permission, ref. 56.

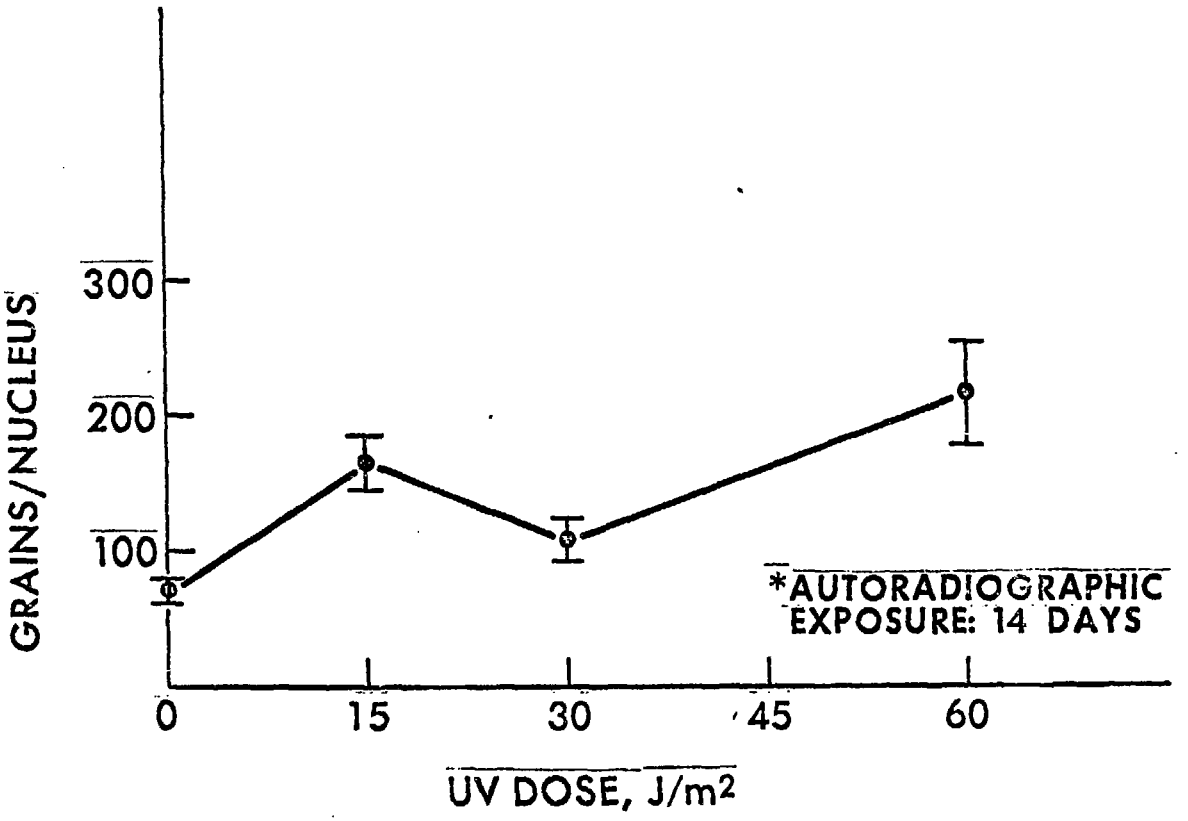
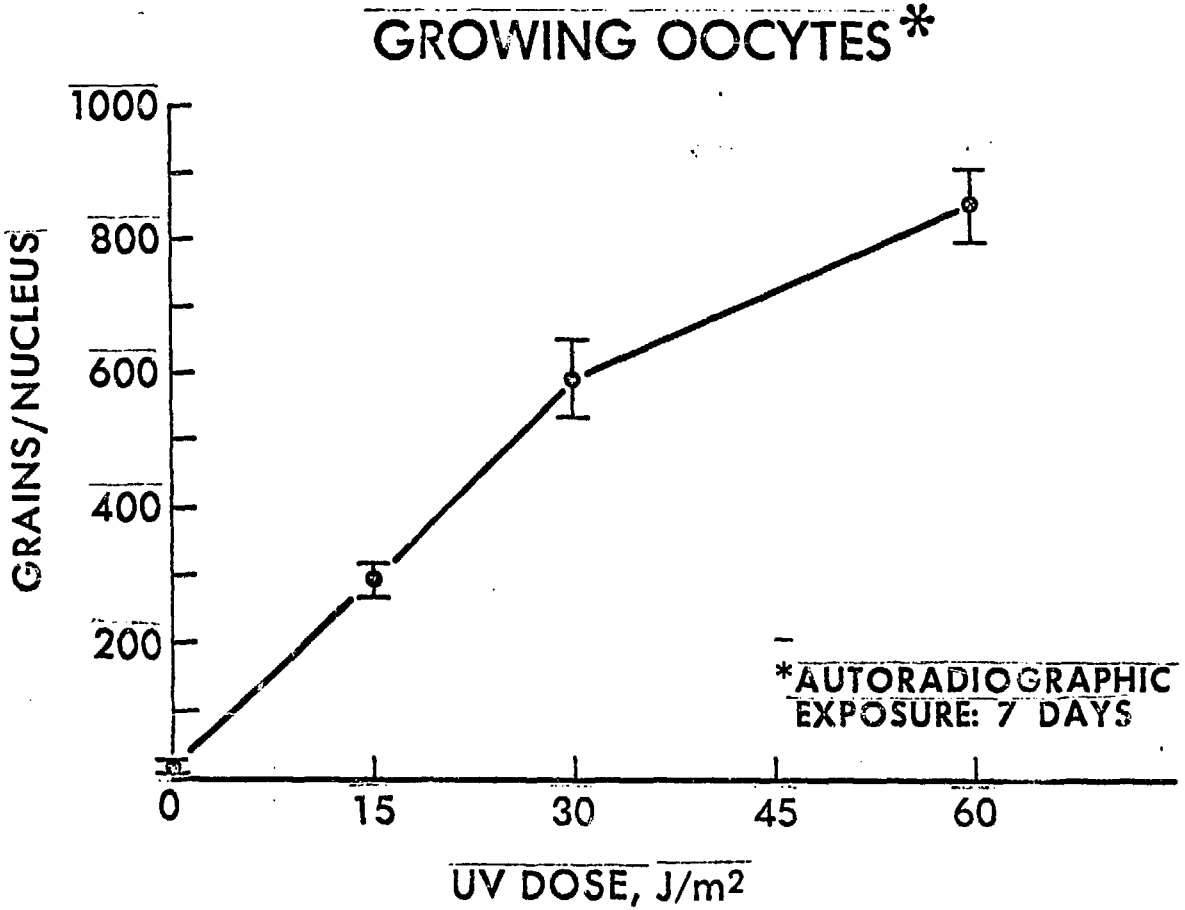

RESTING OOCYTES*
GRAINS/NUCLEUS
300
200
100
0
15
30
45
60
UV DOSE, J/m2
*AUTORADIOGRAPHIC EXPOSURE: 14 DAYS

Fig 1A


GROWING OOCYTES*
GRAINS/NUCLEUS
1000
800
600
400
200
0
15
30
45
60
UV DOSE, J/m2
*AUTORADIOGRAPHIC EXPOSURE: 7 DAYS

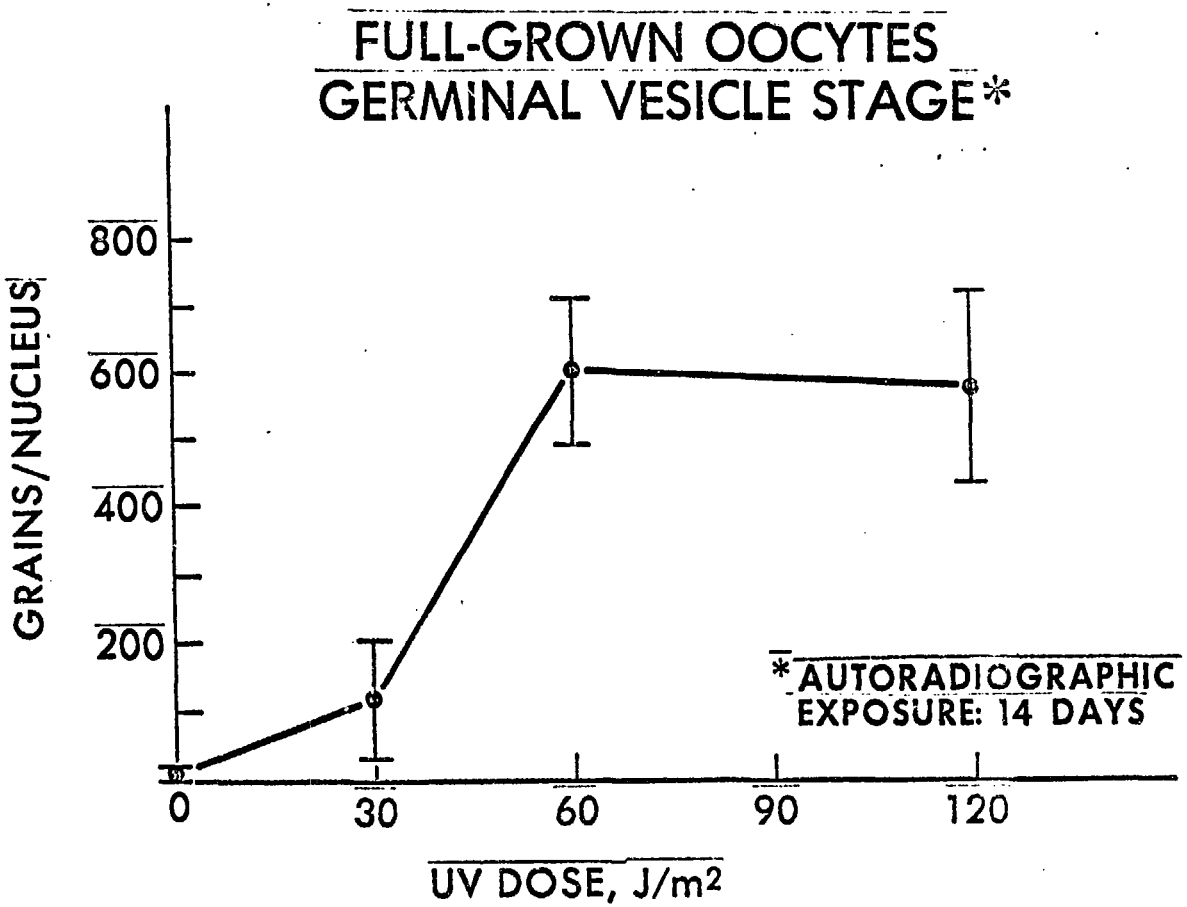
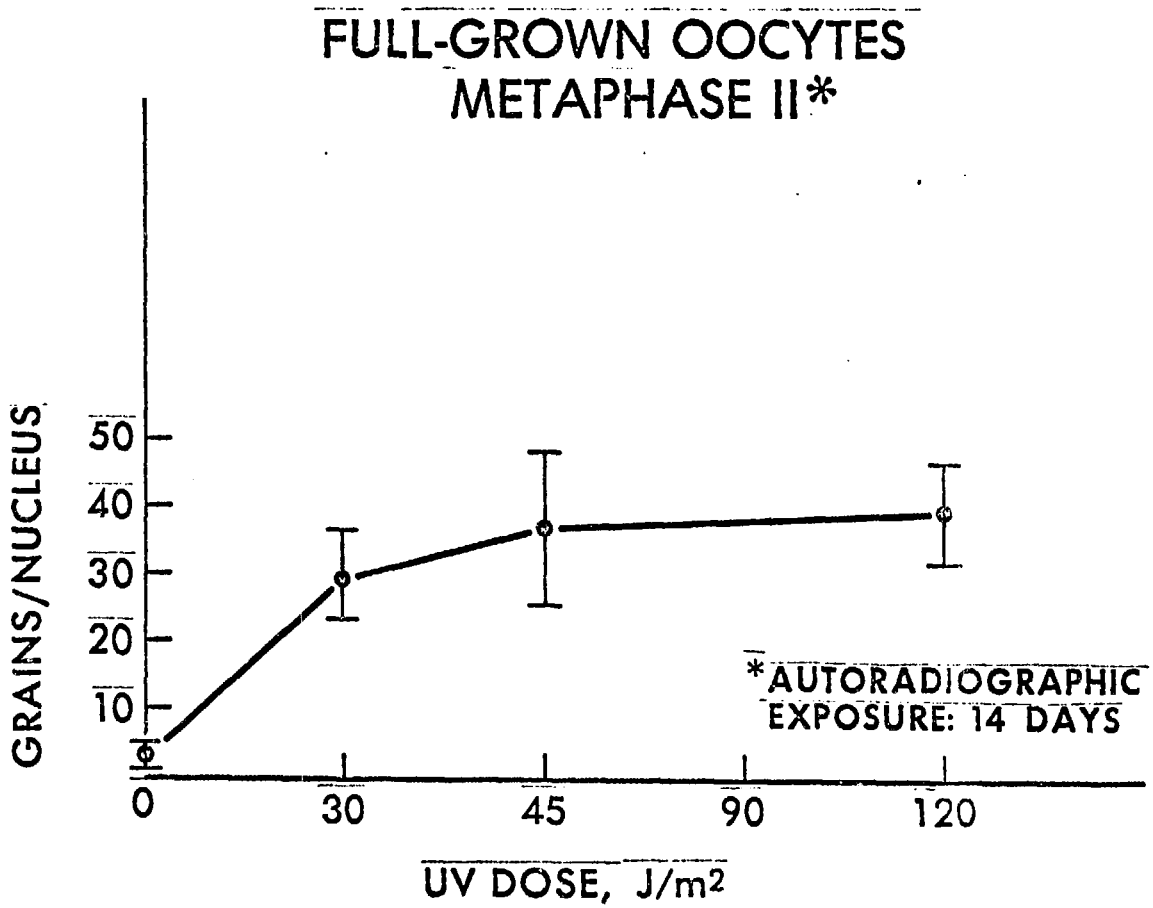
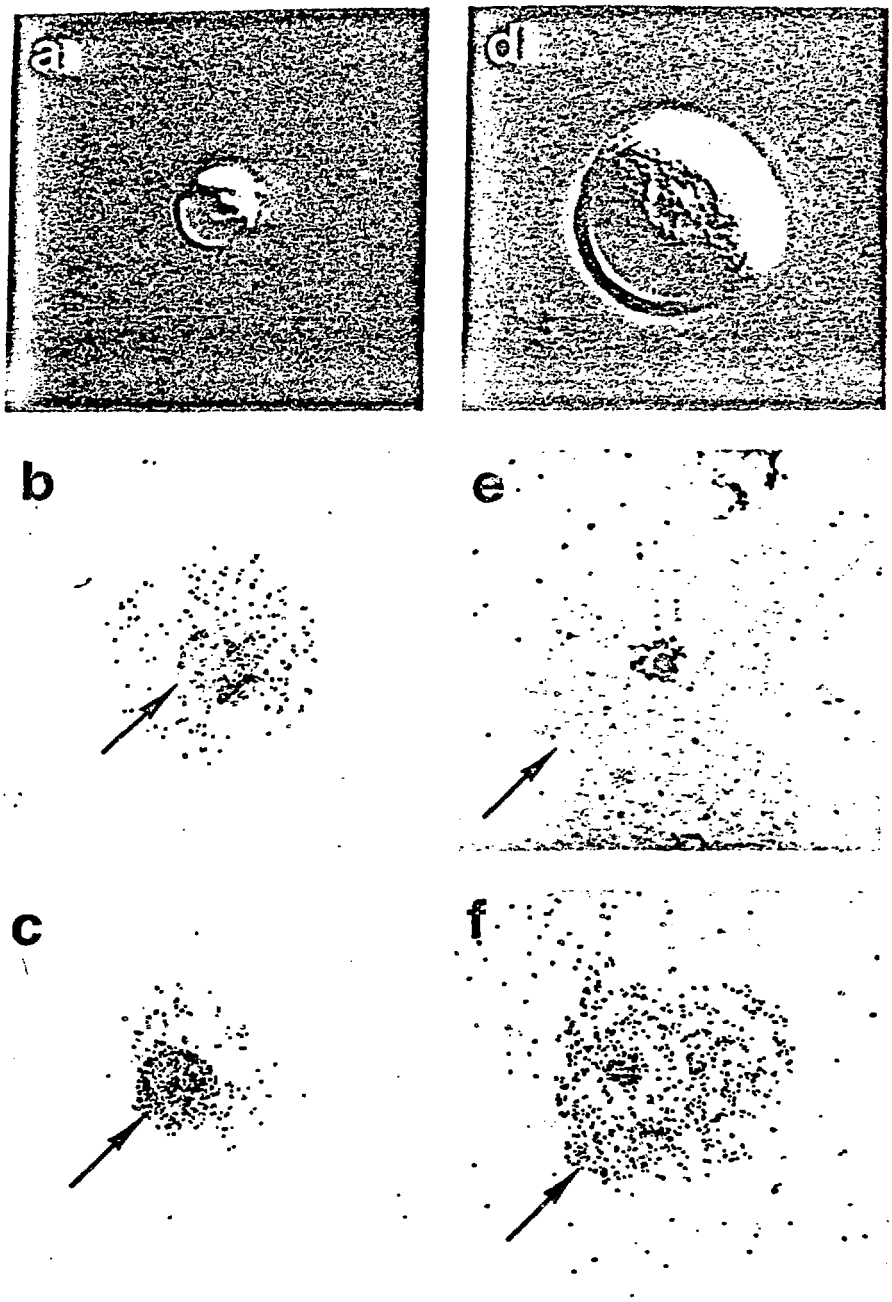

FULL-GROWN OOCYTES
GERMINAL VESICLE STAGE*
GRAINS/NUCLEUS
800
600
400
200
0
30
60
90
120
UV DOSE, J/m2
*AUTORADIOGRAPHIC EXPOSURE: 14 DAYS

Fig. 1C


FULL-GROWN OOCYTES
METAPHASE II*
GRAINS/NUCLEUS
50
40
30
20
10
0
30
45
90
120
UV DOSE, J/m2
*AUTORADIOGRAPHIC
EXPOSURE: 14 DAYS


a
d
b
e
c
f