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Genetics

Genetic lesions induced by chemicals in spermatozoa and spermatids of mice are repaired in the egg

(repair in fertilized eggs/germ-cell mutagenesis/dominant-lethal mutations)

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ABSTRACT **Conclusive proof that the mouse egg is capable of carrying out repair of genetic lesions present in the male genome was obtained through dominant-lethal studies of chemically treated spermatozoa and spermatids and through cytological analysis of first-cleavage metaphases. The maximum difference in repair capability between stocks of females, found for isopropyl methanesulfonate treatment, was large; considerably smaller differences were found for ethyl methanesulfonate, triethylenemelamine, and benzo[*a*]pyrene treatments; and no difference was found for x-ray treatment.**

The ability of mouse oocytes to carry out repair of induced genetic damage has been suggested in several studies. W. L. Russell and coworkers (1–3) favored the hypothesis that the radiation dose rate and dose-fractionation effects and the effect of the irradiation-conception interval on the yield of specific-locus mutations after treatment of oocytes are attributable to the repair of mutational or premutational lesions. Repair in oocytes treated in the advanced stages of follicular development was also postulated as a possible reason for the large differences between the sensitivity of female mouse stocks to dominant-lethal induction with ethyl methanesulfonate (EtMes) or isopropyl methanesulfonate (iPrMes) (4, 5). More recently, several investigators have shown that resting, growing, and mature oocytes of mice are all capable of carrying out UV-light-induced unscheduled DNA synthesis (6–9). Unscheduled DNA synthesis was also demonstrated in oocytes treated with either methyl methanesulfonate or 4-nitroquinoline-1-oxide at the germinal vesicle, metaphase I, and metaphase II stages (9).

The oocyte is ovulated with its chromosomes in the metaphase of the second meiotic division. It remains in this state until stimulated to undergo further development by sperm entry. The question of whether the egg is also capable of carrying out repair of genetic damage present in the male genome is the subject of the present study. There is already evidence for this phenomenon from a study in which it was shown that the genotype of untreated female mice had some influence on the frequency of dominant-lethal mutations induced by thiophosphamide in spermatids (10). More conclusive proof is provided in the present report. It describes a case in which large differences in repair capabilities of eggs exist between stocks of mice.

MATERIALS AND METHODS

Repair in the fertilized egg was demonstrated through dominant-lethal mutation studies and cytological examination of first-cleavage metaphase. Dominant-lethal studies were performed by giving the chemical or radiation treatments to approximately 12-week-old male mice of one stock [either (101 × C3H)F_1 or the reverse hybrid, (C3H × 101)F_1], then mating them simultaneously to approximately 12-week-old untreated females from various stocks. In primary experiments (Exp. A), four stocks of females were used: T-stock, (SEC × C57BL)F_1, (C3H × 101)F_1, and (C3H × C57BL)F_1. In repeat experiments (Exps. B and C), two, three, or all four of the stocks were used. Each experimental or control male was caged with two females at the beginning of the respective mating periods. Females were checked for the presence of a vaginal plug each morning and were killed for uterine analysis 12–15 days after a plug was observed.

Males used in dominant-lethal studies were treated with one of the following: 65 or 125 mg of iPrMes per kg, 200 mg of EtMes per kg, 0.2 mg of triethylenemelamine (TEM) per kg, 500 mg of benzo[*a*]pyrene (Bz*a*P) per kg, or 550 R (1 R = 2.58 × 10^{-4} C/kg) of acute x-rays. X-rays were delivered at 87.2–92.3 R/min to the lower portion of the body. All doses, with the exception of 125 mg of iPrMes per kg, were chosen because they were estimated to induce approximately 50% dominant-lethal mutations in treated (101 × C3H)F_1 males mated to (C3H × 101)F_1 females at the respective periods after treatment. The chemicals were prepared in Hanks' balanced salt solution and administered intraperitoneally in a maximum volume of 1 ml, with the exception of Bz*a*P, which was prepared in corn oil and given in a maximum volume of 0.5 ml. Experimental males were mated at the following intervals after treatment: iPrMes (65 mg/kg) or x-rays (550 R), 0.5–3.5 days; iPrMes (125 mg/kg), 3.5–7.5 days; EtMes (200 mg/kg), 6.5–9.5 days; TEM (0.2 mg/kg), 4.5–7.5 days; and Bz*a*P (500 mg/kg), 3.5–6.5 days. Gametes used in fertilization during 0.5–6.5 days after treatment were treated as spermatozoa, while the ones used later were treated as spermatids. In some experiments a single control group served as the control for two mutagens. When males treated with these mutagens were mated at different intervals after treatment, control males were mated during an interval that covered part of each interval in the experimental groups.

Cytological analysis of first-cleavage metaphase was conducted in order to determine whether the large differences between stocks of females in their yield of dominant-lethal mutations induced in spermatozoa by iPrMes are truly attributable to differences in the repair capabilities of the egg or to differential survival of affected embryos in different maternal environments. (101 × C3H)F_1 males treated with 65 mg of iPrMes per kg were mated to either (SEC × C57BL)F_1 or (C3H × C57BL)F_1 females during the first 4 days after treatment. First-cleavage metaphases were prepared by our unpublished method. The important feature of this method is that the eggs are ovulated normally—i.e., without the influence of exogenous hormone. Thus, the pertinent experimental conditions under which the cytological study was conducted are similar to that

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Abbreviations: EtMes, ethyl methanesulfonate; iPrMes, isopropyl methanesulfonate; TEM, triethylenemelamine; Bz*a*P, benzo[*a*]pyrene.

in the dominant-lethal study. Ovulation was partially synchronized by maintaining females on a short dark phase (5 hr) for at least 5 weeks prior to the start of an experiment. To limit the period of sperm entry, we caged the females with males for only 30 min, beginning 3 hr after the end of the dark period. At this time, ovulated eggs were in the ampulla. Fertilized eggs were collected between 9 and 11 hr after mating and incubated in a medium containing a small amount of colchicine. The eggs were processed for air-dry preparation of first-cleavage metaphase chromosomes 20 hr after mating.

RESULTS

Differences between Stocks of Females in Their Yield of Dominant-Lethal Mutations. Because only one stock of males was exposed to the mutagen in each experiment, any measurable difference in the yield of dominant-lethal mutations between the different stocks of females strongly suggests differences in the ability of the egg to repair premutational lesions carried by the male genome. Two experiments were performed for each chemical and three for x-rays (Table 1). The repeatability of results with the four chemicals strongly indicates that the differences in the responses of various stocks of females as described below are real. Data show different arrays of responses to the mutagens by the four stocks and indicate various degrees of repair of premutational damage produced by the chemical mutagens.

The biggest difference between stocks of females in the yield of dominant-lethal mutations was observed for treatment with the alkylating compound iPrMes. At the lower dose of 65 mg/kg, a marginal effect of 9% dominant-lethal mutations was observed for (C3H × C57BL)F_1 females; a low, but more clear-cut, dominant-lethal effect of 18% was also observed for (C3H × 101)F_1 females. In marked contrast to these two stocks, much higher dominant-lethal frequencies were observed for (SEC × C57BL)F_1 and T-stock females: 50 and 81%, respectively. The repeat experiment at a dose of 125 mg/kg did not include T-stock females for the obvious reason, but results clearly show similar relative differences between the other three stocks as at the lower dose.

Differences between stocks of females were also observed for treatments with the other two alkylating chemicals, EtMes and TEM, but the biggest differences observed between any two stocks are considerably smaller than those observed for iPrMes treatment. In general, results obtained for treatment with EtMes are similar to those obtained with TEM, although the biggest difference observed for EtMes treatment seems to be slightly higher than that for TEM. In both cases, slightly lower frequencies of induced dominant-lethal mutations were observed with (C3H × 101)F_1 females than with females of the other three stocks (the frequencies of induced dominant-lethal mutations were about the same in these stocks).

Results for Bz*a*P treatment also showed clear-cut differences in the yield of induced dominant-lethal mutations between stocks of females. As for EtMes and TEM treatments, the biggest difference between any two stocks is considerably smaller than that found for iPrMes treatment. The most noticeable contrast between treatments with three alkylating chemicals and Bz*a*P is the relatively higher yield in the Bz*a*P-induced dominant-lethal mutations observed in (C3H × 101)F_1 females compared with (C3H × C57BL)F_1 and (SEC × C57BL)F_1 females.

Results of three experiments with x-ray treatment show no detectable differences between the four stocks of females. These results are consistent with those from similar x-ray studies by Frölen (11) and Storer (12) (except in one case, in which better survival of affected heterozygous than affected homozygous embryos was suggested).

Cytological Analysis of First-Cleavage Metaphase. The largest difference between any two stocks of females was observed between T-stock and (C3H × C57BL)F_1 in the experiment with 65 mg of iPrMes per kg. Ideally, the cytological study should have been performed on the two stocks that showed the highest difference. However, because (SEC × C57BL)F_1 females, which showed the second highest response, were more available than T-stock females at the time of the study, this stock was compared with (C3H × C57BL)F_1. Results of the cytological study (Table 2) clearly parallel those of the dominant-lethal study, although the proportions of cells with aberrations in the two stocks are lower than expected from the frequency

Table 1. Differences between stocks of untreated female mice in their yield of dominant-lethal mutations induced in male germ cells*

		(C3H × C57BL)F_1			(C3H × 101)F_1			(SEC × C57BL)F_1			T-stock		
Treatment	Exp.	PF†	LE‡	DL§	PF	LE	DL	PF	LE	DL	PF	LE	DL
iPrMes, 65 mg/kg	A	33	9.0	9	22	6.3	18	26	5.0	50	23	1.5	81
125 mg/kg	B	26	5.5	42	17	3.3	58	13	0.2	98	—	—	—
EtMes, 200 mg/kg	A	30	3.2	70	17	3.2	55	29	3.1	69	30	1.8	75
	B	—	—	—	44	3.9	47	—	—	—	40	2.4	69
TEM, 0.2 mg/kg	A	29	4.5	58	19	3.6	49	29	4.2	58	32	2.8	62
	B	26	4.0	66	50	3.8	49	—	—	—	24	2.7	62
Bz*a*P, 500 mg/kg	A	26	8.0	23	13	4.1	41	24	6.6	35	19	3.8	51
	B	23	7.5	21	14	3.9	50	20	7.0	27	—	—	—
X-ray, 550 R	A	33	5.9	40	18	4.2	45	21	4.4	56	29	3.7	53
	B	29	5.4	48	17	4.3	39	26	5.3	48	26	3.8	51
	C	26	5.7	51	45	3.9	48	—	—	—	26	3.8	46

* Numbers of pregnant females in control groups were generally similar to those in experimental groups, except for the 125 mg/kg dose of iPrMes; in this case fewer pregnancies were observed than in the control group, presumably due to the high incidence of preimplantation losses. In all experiments differences between stocks were also expressed in terms of postimplantation losses.

† Number of pregnant females.

‡ Number of living embryos per pregnant female.

§ Percent dominant-lethal mutations, calculated as

$$\left[1 - \frac{\text{living embryos/pregnant female (experimental)}}{\text{living embryos/pregnant female (control)}}\right] \times 100.$$

Table 2. Cytological analysis of first-cleavage metaphase

Stock of females	Treatment*	No. of cells scored	No. of cells with chromatid or isochromatid deletion	No. of cells with chromatid exchange	No. of cells with chromatid or isochromatid gap
(SEC × C57BL)F_1	iPrMes, 65 mg/kg	168	45	6	10
	Control	194	1	0	0
(C3H × C57BL)F_1	iPrMes, 65 mg/kg	138	3	0	0
	Control	259	1	0	0

* Males received the treatment; they were mated to untreated females during the first 4 days after treatment.

of induced dominant-lethal mutations. For 65 mg of iPrMes per kg, about 30% of the cells in (SEC × C57BL)F_1 females had aberrations (mostly deletions with few interchanges) compared with about 2% in (C3H × C57BL)F_1 females. Controls in both cases had less than 1% of the cells with aberrations.

DISCUSSION

The magnitude of the difference between T-stock and (C3H × C57BL)F_1 females in their yield of dominant-lethal mutations induced in iPrMes-treated spermatozoa is very remarkable. That this large difference is truly attributable to differences in the repair capabilities of fertilized eggs is supported conclusively by the parallel results of the cytological analysis of first-cleavage metaphase. Repair activity must, therefore, occur between the time of sperm entry and first-cleavage metaphase. There seems to be no alternative explanation. Furthermore, although no similar cytological analysis was performed for treatments with EtMes, TEM, and Bz*a*P, the smaller differences between stocks of females in the yield of dominant-lethal mutations may, likewise, be attributable to differences in repair capabilities of the eggs. The differences between stocks found by Malashenko and Surkova (10) in the thiophosphamide study are of the same magnitude as the differences found for these three compounds.

Another interesting finding in this study is that only one of the four stocks of females, T-stock, responded in the same relative order for each of the four chemicals tested, while the rest responded either in opposite ways from one another or in different relative orders in specific instances. For example, (C3H × 101)F_1 females gave relatively low yields of dominant-lethal mutations for treatment with EtMes and TEM and relatively high yields with Bz*a*P, whereas (C3H × C57BL)F_1 gave diametrically opposite results. This phenomenon suggests differences in the types of lesions produced by the respective chemical mutagens and is consistent with the concept that a number of different repair systems exist.

It seems unlikely that the various repair systems in the egg are a consequence of enzyme induction brought about by small amounts of the mutagen. It may be recalled that in the experiment with iPrMes at a dose of 125 mg/kg, and in all experiments with TEM and Bz*a*P, females were mated beginning at 3.5 days after males were treated; in the experiments with EtMes, they were mated beginning at 6.5 days after males were treated. Because of the long intervals between treatment of males and mating and because of the high reactivity of these compounds, it seems unlikely that a significant amount of unbound mutagen is transferred to the egg through the sperm or seminal fluid. Although the possibility cannot be ruled out that repair enzyme induction was brought about by the chromosome lesions themselves, it seems more likely that the repair enzymes existed in the egg even prior to sperm entry.

The present results raise many questions that have bearing on general problems regarding the nature of repair systems and the role of repair in mutation induction in mammalian germ cells. One intriguing question is why the maximum repair differences between stocks is so remarkably large for iPrMes treatment and small for the other two alkylating chemicals, EtMes and TEM.

Because the present results clearly demonstrate the significant role the genotype of the egg may have in the processing of premutational lesions that are carried in the chromosomes of mutagen-treated male germ cells of mammals, this phenomenon should be taken into consideration in practical screening activities, particularly in the use of the dominant-lethal test in which treated males are mated to untreated females. It is not known how prevalent the iPrMes-type situation is, but even the repair differences observed for treatment with EtMes, TEM, and Bz*a*P appear to be large enough to be of consequence because, in practical screening, small effects are probably the general rule. Without addressing this problem, negative results may simply mean that treated males were mated to females from repair-competent stocks. T-stock females consistently gave relatively high dominant-lethal frequencies for all the four compounds studied. We do not know yet how far this extends, but it seems likely that suitable tester stocks of females can be on hand for use in practical testing. Thus, a search for such stocks seems essential in order to improve the dominant-lethal procedure.

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