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17. Gallbladder bile was analyzed immediately by visual inspection and by polarizing microscopy on a heated stage at 37°C. Biliary lipids were measured in bile as described by Carey and Small (*3*). The percentage of cholesterol saturation of bile was calculated from the total and relative biliary lipid compositions [M. C. Carey, *J. Lipid Res.* **19**, 945 (1978)].
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Repair of the Ultraviolet-Irradiated Male Genome in Fertilized Mouse Eggs

Abstract. *Unscheduled DNA synthesis occurred in both male and female pronuclei of the mouse zygote in response to irradiation with ultraviolet light, indicating a capacity for excision repair. Furthermore, damage to DNA of the male gamete before fertilization can be repaired after the sperm enters the egg cytoplasm.*

Environmental agents that damage DNA produce mutations and lead to malignancies in humans and laboratory animals (*1*). When genetic damage occurs in the germ cells, there is a risk of increased malignancy or genetic disease in the offspring (*2*). Although mammalian cells in general can repair damaged DNA, the human disease xeroderma pigmentosum is characterized by a deficiency in the repair of DNA (*3*). The high incidence of malignancies in this disease indicates that repair of DNA damage may play a significant role in controlling mutagenesis and carcinogenesis. Because the mature sperm of mice, rats, rabbits, and humans are also known to be deficient in excision repair (*4*), the period after the entry of sperm into the egg cytoplasm is the only stage at which damage to sperm DNA could be repaired before the zygote genome begins active DNA replication and cell division.

We studied the repair of mouse sperm DNA between entry into the egg cytoplasm and the beginning of the first S phase by means of autoradiographically detectable DNA synthesis in response to ultraviolet irradiation. Such non-S-phase, or unscheduled, DNA synthesis is thought to indicate the activity of a DNA repair system that removes damaged bases (*5*). In one approach, both male and female pronuclei were irradiated in one-cell embryos after fertilization in vivo; in another, mature sperm were irradiated before they were used for fertilization in vitro.

Embryos were obtained by superovulation (*6*) of ICR mice (Flow Labs, Dublin, Virginia) and cultured as described in (*6*). After removal of follicle cells, washed embryos were irradiated 15 to 16 hours after administration of human chorionic gonadotropin (hCG) in medium without bovine serum albumin (exposure, 1.25 J/m^2/sec), then incubated in medium containing [^{3}H]thymidine (20 μCi/ml; specific activity, 40 Ci/mmole, Amersham). After 1 hour, embryos were washed and incubated in medium containing nonradioactive thymidine, fixed, and air-dried on slides (*7*). Slides were exposed to Kodak NTB emulsion for 2 weeks, developed, and stained with Giemsa.

Unscheduled DNA synthesis occurred in both female and male pronuclei of one-cell mouse embryos that had been irradiated with ultraviolet light 16 hours after treatment with hCG (Fig. 1A). In unirradiated (control) embryos, nuclei showed up to 10 grains, a cellular background seen in mouse oocytes and embryos (*8*). About 10 percent of nuclei

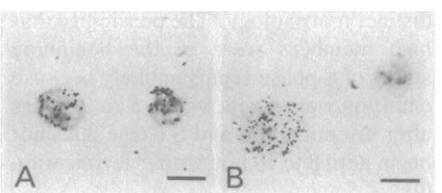

Fig. 1. (A) Unscheduled DNA synthesis in pronuclei of a one-cell mouse embryo fertilized in vivo and then exposed to ultraviolet light. (B) Unscheduled DNA synthesis in mouse embryo fertilized in vitro after sperm were exposed to ultraviolet light. Scale bars are 10 μm.

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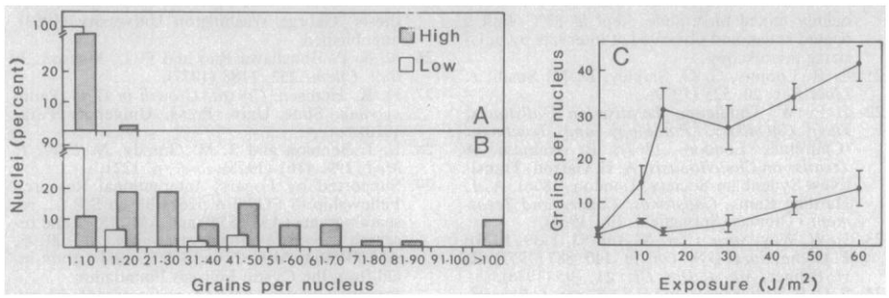

Fig. 2. Histograms of nuclear grain counts obtained from embryos fertilized in vitro (A) with unirradiated (control) sperm and (B) with sperm exposed to ultraviolet light at 45 J/m². (C) Unscheduled DNA synthesis in mouse embryos fertilized in vitro after sperm were exposed to ultraviolet radiation; the upper curve represents high members of pronuclear pairs, and the lower curve represents low members of pronuclear pairs (*11*); values are means ± standard errors.

were heavily labeled (more than 100 grains) and were considered to be entering the S phase of the first embryonic cell cycle, which may begin as early as 8 hours after fertilization (*9*); these were not included in the calculation of means. The nuclei of embryos that were exposed to ultraviolet light at 15 to 60 J/m² were labeled with intermediate numbers of grains, in addition to the low and high counts seen in the nuclei of unirradiated embryos. When embryos were irradiated with 15, 30, 45, and 60 J/m², the mean grain counts per pronucleus were 24.7 ± 3.8, 28.5 ± 3.4, 24.0 ± 2.1, and 36.6 ± 3.4, respectively. These values were significantly higher than those for controls (2.1 ± 0.5). The labeling in both pronuclei of the embryo (Fig. 1A) indicated that both the male and female genomes could be repaired when they were damaged after fertilization in vivo.

To determine whether damage to the male genome incurred before fertilization could be repaired in the egg, we irradiated isolated sperm and then used them for fertilization in vitro. Sperm were incubated in culture medium for 1.5 hours (*10*) and then irradiated while being continuously stirred in medium without bovine serum albumin. They were added (10^5/ml) to dishes containing freshly isolated eggs (13 to 14 hours after hCG treatment), which were incubated for 3 hours and then labeled and fixed. Grains were counted in embryos with both pronuclei intact and clearly visible (about 40 percent). Counts were corrected for background by subtracting grains in an equivalent area adjacent to the pronuclei. Incubation with deoxyribonuclease before autoradiography eliminated most of the label in pronuclei, indicating that it was in the DNA.

Unscheduled DNA synthesis occurred in only one of the two pronuclei of the embryos obtained by this method (Fig. 1B). Because we could not distinguish the male and female pronuclei by size or other morphologic criteria, we assigned each member of a pair of pronuclei to a high or low group according to relative grain count. In control embryos, which were fertilized with unirradiated sperm, most low and high members of pronuclear pairs had 0 to 10 grains (Fig. 2A). In embryos fertilized with irradiated sperm, most low members of pronuclear pairs had 0 to 10 grains; in high members, grain distribution was shifted markedly to higher values (Fig. 2B). High members of pronuclear pairs had mean grain counts significantly higher than controls at all ultraviolet doses ($P < .001$); the low members, by contrast, had grain counts that were similar to controls when exposed to ultraviolet light at 10 to 30 J/m² and only slightly above controls at exposures of 45 and 60 J/m² (Fig. 2C) (*11*). The mean grain counts for high members of pronuclear pairs were in the same range as mean grain counts obtained when both male and female pronuclei were irradiated after fertilization in vivo.

The most straightforward interpretation of these data is that ultraviolet light induces unscheduled DNA synthesis in the pronucleus derived from the irradiated sperm. This interpretation is further supported by the observation of unscheduled DNA synthesis in the irradiated sperm head during the initial stages of its decondensation, when it had increased in volume but still retained its distinct morphology. The possibility that high members were in the beginning stages of S phase seems unlikely because embryos were fixed within 5 to 7 hours after insemination, and S phase does not begin until 8 to 10 hours after fertilization (*9*).

Our results from fertilization both in vivo and in vitro thus indicate that damage to the DNA of the male genome induced by ultraviolet light undergoes repair within hours after fertilization. The amount of unscheduled DNA synthesis observed in the irradiated male pronucleus was similar to that observed in mature, unfertilized eggs and in preimplantation embryos (*12*); however, our method does not reveal the absolute amount of damage repaired.

Our data indicate that the quiescent genome of the male mouse becomes accessible for excision repair of pyrimidine dimers during the period between the entry of sperm into the egg cytoplasm and the beginning of S phase (*9*). The earliest time for genetic expression of the zygote genome that has been recognized in mouse embryos is the two-cell stage, when heterogeneous nuclear RNA can be detected (*13*). Our results indicate that repair of the damaged male and female genomes can precede their genetic expression in the developing embryo.

There is indirect evidence for similar repair processes in zygotes of some invertebrates and of mice. The recovery from x-ray damage of sperm in sea urchin embryos and strain variations in x-ray–induced X-chromosome loss in *Drosophila* may reflect maternal repair processes (*14*). In mice, the incidence of dominant lethality and chromosomal aberrations induced by exposing males to isopropyl methanesulfonate and other chemicals varies according to the strain of the female (*15*). This could be attributed to a differential repair capacity on the part of the fertilized egg.

The relation between DNA damage, repair, and mutagenesis is not yet well understood for cells of the germ line or other tissues in either mice or humans (*16*). Our data provide direct evidence that at least some damage to the DNA of the mature mammalian sperm can be repaired in the egg after fertilization.

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Molecular Basis of Bunyavirus Transmission by Mosquitoes: Role of the Middle-Sized RNA Segment

Abstract. *In an examination of the molecular basis of oral transmission of bunyaviruses by mosquitoes, La Crosse (LAC), snowshoe hare (SSH), and LAC-SSH reassortant viruses were compared in their ability to be transmitted to laboratory mice by the natural mosquito vector of LAC virus,* Aedes triseriatus. *Both LAC virus and the reassortment viruses containing the middle-sized (M) segment from the LAC parent were efficiently transmitted. In contrast, SSH virus and reassortment viruses containing the M RNA from the SSH parent were inefficiently transmitted. Thus, the M RNA segment, which codes for the virion glycoproteins, may be a major determinant of oral transmission of bunyaviruses by mosquitoes.*

The tripartite genome of the California group of bunyaviruses (Bunyaviridae) is composed of a large, a middle-sized, and a small (L/M/S) RNA segment (*1*). The RNA segments of certain bunyaviruses have been reassorted in vitro (*2*), providing new genomes of known segment composition. Analyses of wild-type La Crosse (LAC) and snowshoe hare (SSH) viruses and certain LAC-SSH reassortant viruses have been used to determine gene-coding assignments (*3*); similar analyses were done previously with influenza viruses and reoviruses (*4*). In this study we report the use of reassortant viruses to delineate the molecular basis of oral transmission by *Aedes triseriatus* mosquitoes.

Serologically, LAC and SSH viruses are very closely related; they are distinguishable, however, by highly specific virus neutralization tests (*5*). The LAC virus was first isolated in 1964 from brain tissues obtained from a child who had died of meningoencephalitis in 1960 (*6*). Subsequently, the virus was shown to be transmitted by a mosquito vector, *A. triseriatus*, and the principal vertebrate hosts were shown to be chipmunks and squirrels (*7*). In spite of its serologic similarity to LAC virus, SSH virus is maintained in nature in different *Aedes* species and different vertebrate hosts (*7*).

We compared LAC, SSH, and six LAC-SSH reassortant viruses (Table 1) for their ability to be transmitted by *A. triseriatus*, the natural vector of LAC virus (*7*). The origins of the plaque-cloned LAC and SSH viruses and the reassortant viruses have been described (*1, 2*). The six different LAC-SSH reassortants were originally produced by dual infection of BHK-21 cells with temperature-sensitive mutants of the parental viruses (*2*). The identities of the RNA segments of each reassortant virus were determined by oligonucleotide mapping (*2*). *Aedes triseriatus* were obtained from the colony at the Yale Arbovirus Research Unit. This colony originated from larval collections made in Connecticut during 1974, and it has been continuously maintained since then. Immunofluorescence (IF) was used to detect viral antigen in mosquito tissues (*8*). High titers of antibodies to LAC virus were prepared by hyperimmunization of mice. Ascitic fluids were collected by paracentesis. Antibodies were precipitated with $(NH_4)_2SO_4$ and conjugated with fluorescein isothiocyanate (*8*). The capacity of the conjugated antibodies to bind to wild-type LAC and SSH viruses in mosquito tissues and mouse brain impression smears was equal to that of the unconjugated antibodies. The high degree of cross-reactivity of the conjugate was expected because of the serologic similarities between the two viruses (*5*).

The oral transmission potential of the viruses was determined in four trials by inoculating *A. triseriatus* with ≤1000 plaque-forming units (pfu) of wild-type or reassortant virus. The viruses were inoculated intrathoracically in order to bypass the mesenteron, thereby precluding possible confounding variables asso-

Table 1. Transmission of bunyavirus reassortants by *Aedes triseriatus*. The data show the number of transmissions (T) compared to the number of attempted transmissions (A). N.D., not done.

Genome RNA segment composition	Trial 1		Trial 2		Trial 3		Trial 4		Total	
	T/A	Percentage	T/A	Percentage	T/A	Percentage	T/A	Percentage	T/A	Percentage
LAC/LAC/LAC	20/20	100	13/13	100	17/17	100	10/10	100	60/60	100
SSH/LAC/LAC	8/8	100	5/5	100	9/10	90	N.D.		22/23	96
SSH/LAC/SSH	13/15	87	9/10	90	13/14	93	N.D.		35/39	90
LAC/LAC/SSH	0/6	0	0/7	0	0/17	0	9/14	64	9/14	64*
Total LAC M RNA									126/136	93
LAC/SSH/LAC	N.D.		N.D.		N.D.		8/19	42	8/19	42
LAC/SSH/SSH	N.D.		N.D.		N.D.		5/14	36	5/14	36
SSH/SSH/LAC	6/17	35	2/5	40	3/13	23	N.D.		11/35	31
SSH/SSH/SSH	5/15	33	3/11	27	4/10	40	N.D.		12/36	33
Total SSH M RNA									36/104	35

*Two different LAC/LAC/SSH viruses were studied. The virus used in trials 1, 2 and 3 was replaced with an alternate LAC/LAC/SSH virus in trial 4. Since the first virus was defective (see text), only the results obtained with the alternative virus are included in the total.

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