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Spontaneous and Induced Mutagenesis in Western Equine Encephalomyelitis Virus in Chick Embryo Cells with Different Repair Activity

(excision repair system/RNA-containing virus/mammalian cell culture)

N. P. DUBININ, G. D. ZASUKHINA, V. A. NESMASHNOVA, AND G. N. LVOVA

Institute of General Genetics, USSR Academy of Sciences

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ABSTRACT **Evidence was obtained indicating differences in the survival rate of Western equine encephalomyelitis virus after exposure to ultraviolet radiation and methyl methanesulfonate in commercial and leukosis-free chick embryo cells that differed in repair activity. The levels of spontaneous mutagenesis (on the basis of the yield of small plaque variants of the encephalomyelitis virus) did not essentially change when the virus was passaged in leukosis-free chick embryo cells, whereas an increase in the number of small plaque variants was observed in the cells of commercial chick embryos. A 10-fold increase in the number of induced virus variants was observed in commercial chick embryo cells in experiments with methyl methanesulfonate as compared with the control, whereas the induction of virus variants was not noted in leukosis-free cells.**

We have shown earlier that primary and continuous cells of the golden hamster differ in their ability to excise thymine and uracil dimers formed after UV irradiation in DNA and RNA of cells. The differences in the survival rate of RNA virus after treatment with some mutagens were demonstrated in this cell system (1). In addition, we have discovered other cells that differ in the system of DNA repair; leukosis-free and commercial (contaminated with avian leukosis virus) chick embryo cells. Study of the reparative activity of commercial and leukosis-free chick embryo cells, carried out by the method of Setlow *et al.* (2), revealed that leukosis-free chick embryo cells possessed reparative activity, whereas, commercial chick embryo cells lacked the capacity for excising, from DNA thymine dimers formed after UV irradiation (3).

The present paper reports the results of the study of the survival rate of Western equine encephalomyelitis (WEE) virus after treatment with mutagens and spontaneous variability of the virus in these cells.

MATERIALS AND METHODS

The large plaque mutant of RNA virus (WEE virus) obtained in 1943 from the depository of the Rockefeller Institute was used. When passaged in white mice, the large plaque mutant of this virus produced mainly large plaques, 4–6 mm in diameter, in chick embryo cells.

Treatment of virus with mutagens

Experiments with Methyl Methanesulfonate. Commercial and leukosis-free chick embryo cells were grown in a medium consisting of 0.5% lactalbumin hydrolysate with 10% bovine serum. Cells were infected with the virus at a multiplicity of infection of 1–5 plaque-forming units (PFU) per cell. Infected cells were incubated for 1 hr at 37°, then flushed twice with medium , and supplemented with a culture medium containing methyl methanesulfonate at a concentration of 2×10^{-3} M. Two hours later the medium with mutagen was decanted, cells were washed off, refed with a fresh medium, and incubated at 37° for 3 hr. Thereafter, the cells were resuspended in the culture medium. Infected cells supplemented with a mutagen-free medium served as the control.

Experiments with UV Irradiation. Irradiation of infected cells was conducted in the dark. UV radiation was generated by a BUV-15 lamp operated at 4.6 erg/mm² irradiation efficiency, 253.7 nm wave length, and 3 min of exposure. Irradiated cells were washed, refed with a fresh medium, incubated at 37° for 3 hr, and then resuspended. Infected non-irradiated cells served as control.

Titration of Virus. After cells were destroyed by freezing and thawing, the virus-containing materials were titrated in commercial chick embryo cells by the plaque assay technique. The virus survival rate was expressed as the difference between the titers (log PFU/ml) of control and mutagen-treated virus. Small (less 1.5 mm) plaques were enumerated in progeny of mutagen-treated and control virus.

RESULTS

Spontaneous mutability of the virus in commercial and leukosis-free chick embryo cells was analyzed within five passages made every 24 hr (which corresponds approximately to 40 virus cycles). The results revealed that 14% of small plaque variants had been produced by the end of the first passage in leukosis-free chick embryo cells (Table 1). Their number did not essentially change within the five passages made. Small plaque variants were examined for the stability of the plaque size marker. They were found to maintain the small plaque characteristics within 40 subsequent virus generations.

The number of small plaque variants in commercial chick embryo cells increased with passages and reached 76% by the fifth passage. Therefore, the appearance of virus variants was observed in every subsequent virus generation, which resulted in their accumulation in a virus population.

The analysis of the levels of the virus survival rate in experiments with UV irradiation revealed that the virus survival rate (log PFU/ml) in commercial chick embryo cells

Abbreviations: WEE virus, Western equine encephalomyelitis virus; PFU, plaque-forming units.

was 3.6, whereas it was 0.7 in leukosis-free cells (Fig. 1.) It should be noted that commercial and leukosis-free chick embryo cells possess the same susceptibility to the virus.

We obtained analogous data in experiments with MMS when an enhanced survival rate of the virus in leukosis-free chick embryo cells was also observed. If the difference between the titers (log PFU/ml) of control and treated virus in commercial chick embryo cells was 1.8, the titers of control and treated virus in leukosis-free chick embryo cells were the same.

Apparently, the difference in the virus survival rate in commercial and leukosis-free chick embryo cells after treatment with mutagens is associated with the fact that leukosis-free chick embryo cells possess not only the active system of DNA repair but that of RNA repair as well, and potential damage of the virus genome is partially restored by the cell system that is actively functioning.

Table 2 summarizes the data on the yield of small plaque virus variants induced by methyl methanesulfonate. As is apparent from the table, no increase in the number of small plaque variants in leukosis-free chick embryo cells was observed under the given conditions of the experiment as compared with the control. At the same time, the number of small plaque variants of the virus induced by methyl methanesulfonate in commercial chick embryo cells was 10 times higher than in the control. In experiments with UV irradiation, the induction of variants was not observed in both cell systems at the given stage of investigations.

DISCUSSION

Reports on the repair of damage induced by UV irradiation in the genome of DNA viruses by means of the reparative mechanism of host cells have been published (4). We obtained analogous results with an RNA virus, WEE virus, in primary and continuous hamster kidney cells that differed in the system of RNA repair (1).

In another cell system, commercial and leukosis-free chick embryo cells, we also found evidence for differences in the virus survival rate after exposure to UV irradiation and methyl methanesulfonate. It should be emphasized that no difference between the levels of the survival rate of control virus and those of virus treated with methyl methanesulfonate in leukosis-free chick embryo cells was observed. The possible explanation for this fact would be that potentially sublethal damage of the virus genome is restored within one virus cycle. Under these conditions of the experiment the absence of the induction of small plaque variants was also noted: the level of small plaque variants in leukosis-free chick embryo cells after treatment with methyl methanesulfonate

TABLE 1. *Yield of small plaque variants when WEE virus was passed in leukosis-free and commercial chick embryo cells*

Passage history	% of small plaque variants* in chick embryo cells	
	Commercial	Leukosis-free
1	40 ± 2.0	14 ± 0.5
3	62 ± 3.0	15 ± 1.0
5	76 ± 2.0	18 ± 2.0

* For calculation of the percentage of variants, 250–300 plaques were enumerated. Values are ± SEM.

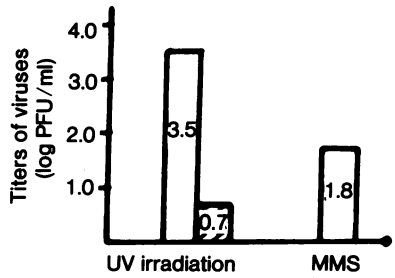

FIG. 1. The viability of Western equine encephalomyelitis virus in commercial and leukosis-free chick embryo cells after UV irradiation and methyl methanesulfonate (MMS) treatment. □, Commercial chick embryo cells. ▨, Leukosis-free chick embryo cells.

and in the control was the same. As we had shown earlier (5), the system of RNA repair in commercial chick embryo cells is in an inactive state, which also influences the levels of the survival rate and induced mutability of the virus.

It is noteworthy that spontaneous mutability of the virus also differed sharply in leukosis-free and commercial chick embryo cells. It is known that some mutants of bacteria with genetic defects in the system of repair possess an enhanced spontaneous mutability (6). Our data indicate that leukosis-free chick embryo cells contribute to a certain stability of the original genetic characteristics of the virus on the basis of plaque size, whereas in commercial chick embryo cells the induction of spontaneous mutation occurs in every virus cycle. This event results in the accumulation of virus variants in virus progeny. These findings are consistent with our earlier data on spontaneous mutability of the virus in primary and continuous golden hamster kidney cells possessing the active and defective system of RNA repair, respectively (7).

It has been also demonstrated in this cell system that spontaneous mutability of the virus on the basis of the plaque size marker is maintained at the same level within 40 virus cycles in the cells with the active system of repair. When the virus was passaged in continuous cells with the defective system of RNA repair, the accumulation of small plaque variants was observed. The yield of virus variants induced by methyl methanesulfonate was also higher in the cells with the defective system of repair, and the survival rate of the virus after treatment with the mutagen was lower in these cells by one order of magnitude than that in primary cells possessing the active system of repair.

Thus, the evidence for differences in the levels of spontaneous and induced mutability in an RNA virus was obtained both in the system of primary cells (commercial and leukosis-free chick embryo cells) and the system of primary

TABLE 2. *Yield of small plaque variants of WEE virus induced by methyl methanesulfonate in commercial and leukosis-free chick embryo cells*

Experiment	% of small plaque variants in chick embryo cells	
	Commercial	Leukosis-free
Methyl methanesulfonate	49 ± 1.0	2 ± 0.2
Control	5 ± 0.2	2 ± 0.3

Values are ± SEM.

and continuous cells (golden hamster kidney cells) which differ in reparative activity. One may assume that the effect of some chemical therapeutic preparations and inactivators of viruses might essentially vary owing to the potency of the system of repair.

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