THE CAPACITY OF THE BACTERIAL HOST FOR THE REPRODUCTION OF THE RNA PHAGE f2

Zdeněk Neubauer and Vojtěch Závada

Faculty of Science, Department of Microbiology, Charles University Prague

Received March 18, 1965

As the experiments with different inhibitors suggest, the reproduction of RNA phages is sensitive neither to the inhibition of DNA synthesis (Cooper & Zinder 1962; Knolle & Kaudewitz 1964) nor to that of DNA-primed synthesis of RNA (Haywood & Sinsheimer 1963). Thus the reproduction of bacterial viruses containing RNA seems to be independent on replication and transcription of the host genome. Such autonomy is characteristic for T-even phages (Stent 1958). One would, therefore, expect a high UV-resistance of capacity for RNA phage (defined as the ability of the UV irradiated host to give infective centre when infected with non-irradiated virus).

It is known that immediately after T-even phage infection the bacterial genome is blocked (Nomura & al. 1962). Thus the ability of RNA phage to replicate in a cell previously infected with T2 would prove its complete independence on bacterial genome. Consequently we were also interested whether and to what extent the capacity for f2 was affected by the preinfection of the host with UV inactivated phage T2r (UV-T2). The inactivated phage T2 was used to avoid its reproduction in treated cells. Like the active phage UV-T2 does not destroy the existing biosynthetic capacity of the cell either (Dirksen & al.1960).

The UV-Sensitivity of Capacity for f2 in E.coli C F^+:

Contrary to the previous assumption, the capacity for f2 (fig.1) differs remarkably as for its UV-sensitivity from the extremely UV-resistant capacity for autonomous virulent phages, resembling in this regard rather the capacity for temperate phage lambda (Marcovich 1956; Stent 1958) and for single-stranded DNA phage S 13 (Tessman & Ozaki 1960).

Our data are in good agreement with those reported by Nonoyama & Ikeda (1964). They found 8% survival of the capacity for RNA phage beta in cells irradiated with the UV-dose decreasing the colony forming ability (c.f.a.) by 5 $\log_{10}$. They observed the accumulation of double-stranded viral RNA in UV-irradiated bacteria and suggested that a host cell function which normally might prevent the accumulation of the double-stranded form was damaged by radiation. Fenwick & al.(1964) reported 5% yield of infective particles of an RNA phage R 17 in bacteria irradiated with a dose decreasing the c.f.a. by 5 $\log_{10}$. However, it is not clear from their result

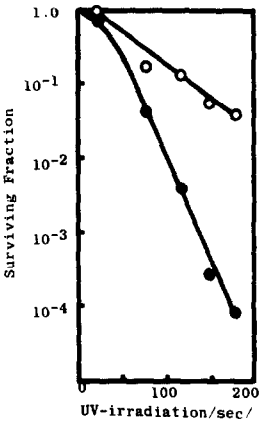

Fig. 1. The colony forming ability (closed circles) and capacity (open circles) of E.coli C F$^+$(streptomycin resistant) for RNA phage f2 after UV-irradiation

	Parameters of the reported curves	Parameters of curves in three other similar experiments		
Relation of the final slopes (c/c.f.a.)	0,31	0,51	0,30	0,34
Extrapolating number of capacity curve	1,2	2,2	1,0	1,7
Extrapolating number of c.f.a. curve	6,0	3,0	6,8	3,0

Cells were grown in tryptose medium (Bacto-Tryptose 0,5%, NaCl 0,5%, $CaCl_2$ 0,0025 M, pH 7) with aeration at 37°C to 2.10^8/ml, centrifuged once and resuspended at the same cell concentration in 0.5% NaCl. The chilled suspension (3,6ml) was irradiated in 10 cm Petri dish under germicidal lamp Philips TUV 15 (8 ergs/mm^{2}sec). The irradiated suspension was transferred to an equal volume of tryptose medium at 37°C and after 5 minutes phage f2 was added (input 5). After another 5 minutes the adsorption mixture was treated for 5 minutes with anti-f2 serum. Then the suspension was chilled, diluted in chilled tryptose medium and plated for infective centres. The number of cells surviving UV-irradiation was determined in the non-infected aliquot. After irradiation a red-yellow light was used to avoid photoreactivation.

to what extent this was due to the decrease in the average burst size or in the capacity in proper sense.

Experiments are now in progress on 5BU-labeled cells to investigate if there is any correlation between the extent of the UV-damage in the host's DNA and the decrease of its capacity for f2. Recently (Schuster & al. 1964) the capacity for phage lambda has been reported to have the same UV-sensitivity both in uvr$^+$ and uvr$^-$ hosts and therefore not to be susceptible to repair mechanism of the cell. Thus even with the temperate phage lambda the capacity does not seem to be affected directly by UV-lesions in host DNA. The UV-sensitivity of capacity for f2 might perhaps reflect some direct hazard for the viral genome entering the irradiated host in which some mechanisms triggered by UV-irradiation may persist (see Devoret & George 1964) and cause some lethal damage on the single-stranded viral RNA chromosome.

The Capacity for f2 in Bacteria Preinfected with UV-T2

In experiments with bacteria preinfected with UV-T2 the c.f.a. and the capacity for f2 decreased in about the same degree (Tab.I). Thus it seems that only those cells which survived the UV-T2 infection are able to reproduce f2. This result does not necessary suggest the dependence of the capacity for f2 on the activity of host genome, possibly some special mechanism of the sort of mutual exclusion phenomena is in action here.

TABLE I

Effect of UV-T2 preinfection of E.coli C F^+ upon its colony forming ability and capacity for the reproduction of f2 phage

Input of UV-irradiated T2 (surviving fraction: 10^{-1})	Decrease of c.f.a. (surviving fraction in $\log_{10}$)	Decrease of capacity for f2 (surviving fraction in $\log_{10}$)
12	- 2,0	- 1,9
6	- 1,1	- 1,6

The cells (2.10^8/ml) were infected with f2 (input 5) 2 minutes after adding UV-T2. After another 5 minutes necessary for adsorption the mixture was treated for 5 minutes with anti-f2 and anti-T2 serum. Then the suspension was chilled, diluted and plated for infective centres and the number of cells surviving UV-irradiation was determined.
In the control experiment the bacterial growth ceased immediately after the addition of UV-T2 (input 10) and the O.D. remained constant for at least 100 minutes. When induced by 0,001 M TMG 3 minutes after the addition of UV-T2 the culture synthetized within the following 30 minutes less than 3% of beta-galactosidase as compared with 100% in the untreated control. The residual synthesis can be attributed to surviving cells.

In experiments both with UV-irradiated and UV-T2 treated cells about 10-50% (the exact ratio varying on parallel plates) of chloroforme-sensitive infective centres gave rise to minute plaques which never appeared in untreated controls. This feature was not hereditary and may be due e.g. to the strongly delayed lysis of some plated infective centres derived from treated cells.

Phage f2 was kindly supplied by Dr.Zinder from the Rockefeller Institute.

REFERENCES

Cooper,S., Zinder,N.D. (1962): Virology, 18, 405.
Devoret,R., George,J.(1964): C.R.Acad.Sci.PARIS, 258, 2227.
Dirksen,M.L., Wiberg,J.S., Koerner,J.F., Buchanan,J.M. (1960): Proc.N.A.S., 46,1425.
Fenwick,M.L., Erikson,R.L., Franklin,R.M. (1964): Science, 146, 527.
Haywood,A.M., Sinsheimer,R.L. (1963): J.Mol.Biol., 6, 247.
Knolle,P., Kaudewitz,F. (1964): Abstracts Vol.of the 6th International Congress of Biochemistry 1964.
Marcovich,H. (1956): Ann.Inst.Pasteur, 91, 511.
Nomura,M., Matsubara,K., Okamoto,K., Fujimura,R. (1962): J.Mol.Biol., 5, 535.
Nonoyama,M., Ikeda,Y. (1964): J.Mol.Biol., 9, 763.
Schuster,B., Schuster,H., Weigle,J.J.: Biol.Ann.Rep.1964. California Inst. of Technol. (109).
Stent,G.S. (1958): Adv.Virus.Res., 5, 95.
Tessman,E.S., Ozaki,T. (1960): Virology, 12, 431.