THE CAPACITY OF THE BACTERIAL HOST FOR THE REPRODUCTION OF THE RNA PHAGE f2
II. ITS UV SENSITIVITY IN 5-BU LABELLED HOST AND THE EFFECT OF THE THYMINELESS DEATH

Vojtěch Závada, Jana Fikotová, Eva Koutecká, Zdeněk Neubauer and Marie Rašková

Faculty of Science, Department of Microbiology, Charles University Prague, Viničná 5

Received January 24, 1966

The capacity of E.coli to reproduce RNA phages was found to be quite sensitive to UV irradiation (Neubauer & Závada 1965; Rappaport 1965) and to be lost in cells preinfected with UV irradiated phage T2 (Neubauer & Závada 1965). In an attempt to specify the nature of the "capacity target" we investigated how the UV sensitivity of the capacity was affected by the sensitization of the host DNA to ultraviolet light. This was accomplished by the incorporation of 5-bromouracile (5-BU) into DNA of the thymine requiring bacteria (Opara-Kubinska & al. 1961). The capacity was found to be sensitized approximatively in the same extent as the colony-forming ability (c.f.a.) of the cells (Fig.1). Moreover, even in the non-irradiated control the capacity of the 5-BU cells was reduced about five-fold.

As the above result had suggested that the "capacity target" resided in host DNA we investigated the effect of thymineless death (Barner & Cohen 1955) on the capacity for f2, since the thymineless death is known to be due to chromosomal alterations (Lark & Lark 1964, Lark & Lark 1965). As can be seen (Fig.2), the decrease of colony-forming ability and capacity in the course of thymine starvation follows the same kinetics. This is an indication that the cells which lost the viability due to thymine starvation lost the capacity to reproduce f2 as well.

These results are in some contradiction to the experience so far obtained, as they indicate some sort of host DNA dependence of the capacity for f2. This finding raises some new problems: The reproduction of RNA bacterial viruses is known to be insensitive to mitomycin C, 5-FUDR and actinomycin D (Cooper & Zinder 1962 ; Haywood & Sinsheimer 1963; Knolle & Kaudewitz 1964). Thus it seems to be independent from both the replication and transcription of the host genome. Moreover, it was found (Mattern & al. 1965) that the UV sensitivity of capacity for f2 and other RNA phages was the same both in the wild type and several hcr^- and dar^- mutants of K 12 unable of a dark repair of UV lesions in DNA. Either the apparent contradiction between the independency of the UV sensitivity of capacity from the dark repair reported by Mattern & al. and its sensitization by 5-BU (our results) reflects some unsuspected difference between the strains used (derivatives of K 12 and C respectively) or it implies that only irreparable lesions in DNA destroy the capacity of the

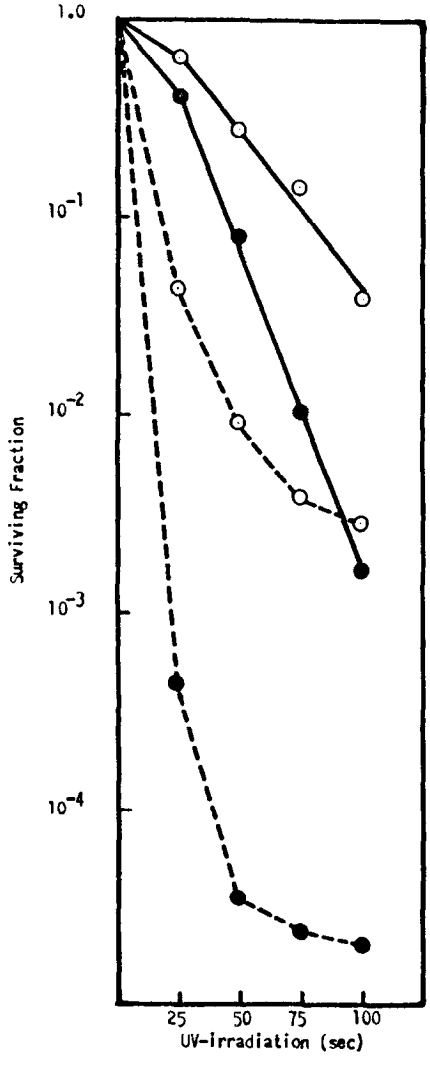

Fig. 1 UV sensitivity of colony-forming ability (closed circles) and capacity for f2 (open circles) in normal (solid line) and 5-BU (broken line) cells

$$\text{Relation of the final slopes} \frac{D}{D}\frac{\log 10 (\text{5-BU cells})}{\log 10 (\text{normal cells})}$$

	the reported experiment	three other similar experiments		
c.f.a.	0,23	0,40	0,23	0,20
capacity	0,29	0,31	0,27	0,26

Log phase cells of E.coli C $F^{+}sm^{r}thy^{-}$ grown on GS medium (Okada & al. 1962) supplemented with 100 ug/ml thymine were collected on membrane filter (porosity 0,1-0,3 u), washed and resuspended in GS medium supplemented with either 50 ug/ml thymine or 50 ug/ml 5-BU + 10 ug/ml thymine at a cell concentration 5.10^{7}/ml. After two generations of growth at 37°C with aeration the cells were collected on the filter again, washed and resuspended in ice-chilled 0,5% NaCl at a concentration about 3.10^{8} cells/ml. UV irradiation of 3,6 ml samples of the suspension was performed in 10 cm diameter Petri dishes with a partially shielded Philips TUV 15 germicidal lamp that had an

intensity of about 8 ergs $mm^{-2}sec^{-1}$ at 31,5 cm distance. The irradiated suspension was transferred to an equal volume of prewarmed tryptose medium (Bacto-Tryptose 0,5%, $CaCl_2$ 0,005M, NaCl 0,5%) at 37°C and phage f2 was added (input 0,2). After 5 minutes the adsorption mixture was treated for another 5 minutes with anti-f2 serum. Then the suspension was chilled, diluted in chilled tryptose medium and plated for surviving cells (c.f.a.) and for infective centres (capacity). Yellow light was used to avoid photoreactivation.

E.coli C $F^+sm^rthy^-$ was derived from E.coli C (kindly supplied by prof.R.L.Sinsheimer) by means of F infection of a sm^r mutant using a K 12 S F^+ donor (obtained by Institut Pasteur). The thy^- mutant of C F^+sm^r was isolated using the method of Okada & al. 1962.

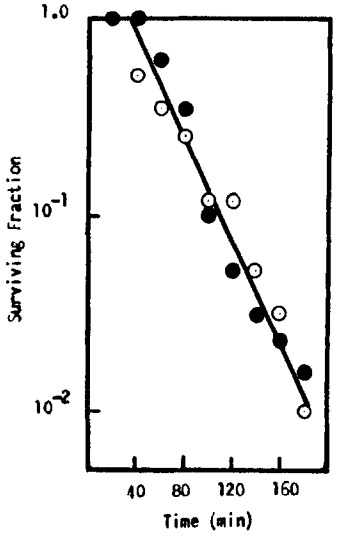

Fig. 2 Thymineless death of the host E.coli C $F^+sm^rthy^-$ (closed circles) and of its capacity for f2 (open circles)

Log phase cells of E.coli C $F^+sm^rthy^-$ grown on the aerated GS medium supplemented with 100 ug/ml thymine were collected on filter, washed with and resuspended in the prewarmed GS medium at a concentration of about 3.10^7 cells/ml. The aeration was restored and the suspension was incubated at 37°C. Samples were withdrawn at intervals, mixed with equal volume of prewarmed tryptose medium and infected with f2 (input 1). After 5 minutes the adsorption mixture was treated for another 5 minutes with anti-f2 serum. Then the suspension was chilled, diluted in chilled tryptose medium and plated for surviving cells (c.f.a.) and for infective centres (capacity).

host for RNA phage and that 5-BU incorporated in DNA increases the probability of such UV lesions. It was suggested that the UV sensitizing effect of 5-BU on c.f.a. consists mostly in interfering with the dark repair and photoreactivation mechanisms of the cells (Haynes 1964; Hotz 1964; Stahl & al. 1961). This would indicate that the mechanisms of the UV sensitization of viability and capacity of the cell are not identical. Some other mechanisms might be involved in the UV sensitization

of the DNA by 5-BU as indicated by experiments with single-stranded DNA phage ØX174 and double-stranded lambda and T1 which were sensitized even when assayed on bacteria lacking the host cell reactivation capacity (Kozinski & Szybalski 1959; Hotz 1964; Dennhardt & Sinsheimer 1965).

Experiments are now in progress investigating the effect of DNA damage upon f2 reproduction in various periods of the lytic cycle.

REFERENCES

Barner,H.D., Cohen,S.E., J.Bacteriol., 71, 149 (1955).
Cooper,S., Zinder,N.D., Virology, 18, 405 (1962).
Dennhardt,D.T., Sinsheimer,R.L., J.Mol.Biol., 12, 674 (1965)
Haynes,R.H., Photochem.Photobiol., 3, 429 (1964).
Haywood,A.M., Sinsheimer,R.L., J.Mol.Biol., 6, 247 (1963).
Hotz,G., Z.Vererbungsl., 95, 211 (1964).
Knolle,P., Kaudewitz,F., Abstracts Vol.of the 6th Internat.Congress Biochem. 1964.
Kozinski,A.W., Szybalski,W., Virology, 9, 260 (1959).
Lark,C., Lark,K.G., J.Mol.Biol., 10, 120 (1964).
Lark,K.G., Lark,C., J.Mol.Biol., 13, 105 (1965).
Mattern,I.E., Winden,van,M.P., Rörsch,A., Mutation Res., 2, 111 (1965).
Neubauer,Z., Závada,V., Biochem.Biophys.Res.Comm., 20, 1 (1965).
Okada,T., Homma,J., Sonohora,H., J.Bacteriol., 84, 602 (1962).
Opara-Kubinska,Z.,Lorkiewicz,Z.,Szybalski,W., Biochem.Biophys.Res.Comm., 4, 288 (1961).
Rappaport,I., Biochem.Biophys.Acta, 103, 481 (1965).
Stahl,F.W., Crasemann,J.M., Okun,L., Fox,E., Laird,C., Virology, 13, 98 (1961).