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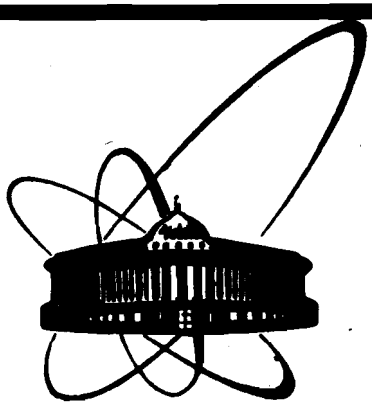

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INFLUENCE OF THE E.coli REPAIR SYSTEM ON THE SPONTANEOUS λ-PROPHAGE INDUCTION

1989

The induction of propage λ[1,2] is one of the expressions of the E.coli SOS-response to a variety of treatments that damage DNA[3]. DNA damage caused by the inducing agents blocks DNA synthesis[4] and generates an induction signal, probably single-stranded DNA plus an adenine nucleotide, which activates the latent ability of the RecA protein to promote proteolysis[2,5,6]. In a lysogen the activated RecA protein cleaves both the LexA-repressor, the common repressor of at least 17 genes scattered about the chromosome, which leads to the SOS-response, and the λ-repressor, which starts the lytic pathway of the cell growth[7,8].

Mutations in the recA and lexA genes of Escherichia coli eliminate or strongly reduce the λ-prophage induction[9,10,11]. Thus, the current understanding of SOS regulation reveals the biochemical steps of the λ-prophage induction. However, there is no investigations dealing with the mechanism of spontaneous prophage induction and it is unknown whether the SOS-repair system takes part in it. In this paper the spontaneous phage production in repair-deficient and wild type E.coli lysogenic strains has been studied.

MATERIALS AND METHODS

The E.coli strains used in our experiments are presented in the Table. The C^{r}_{str} strain has been used as an indicator of plaque forming units in the spontaneous phage production experiments.

Free-phage Production and Bacterial growth

The cells were growing overnight in liquid nutrient broth (Gamaleya Institute of Microbiology, Moscow), diluted 1:100 and incubated at 37°C. To determine the free-phage production, 0.1 ml of the appropriate diluted suspension was added to the TOP agar (7 g agar, 1 g yest exstract, 8 g NaCl, and 1M $MgSO_4$ per 1ℓ H_2O) together with 0.1 ml of the overnight indicator bacteria and 200 μg/ml of streptomycin, and poured on the plates containing nutrient agar. The growth of bacteria was checked by the colony forming unit determination. All the dilutions were made in 10^{-2} M $MgSO_4$.

Table

Bacterial strains

Strains	Genotype	Sourse
HfrH(λ)	thi	Institute of Genetics,
30SO(λ)	thi, lac, recA37	Acad. Sci., USSR
W3110(λ)	B1, thy36, deoC	Institute of Biophysics
JC5491(λ)	Hfr, Thr, Ile,	Ministry of Health
	val, B1, recBC	-"-
P3478(λ)	polA1, B1, thy	-"-
GC244(λ)	lexA4, his	-"-
C	str^r	-"-

RESULTS

The dependences of phage production on the incubation time are show in fig.1. As can be seen, for all strains except recA$^-$, the number of free phage particles increases during 3-4 hours after the beginning of the cell growth. Then the rate of the free phage production slows down and changes its sign. The maxima of the presented curves lie in the interval [3.10^5 - 2.10^6] phages/ml. Unlike this, the phage production of the recA$^-$ strain increases very slowly and reaches the maximum level of ~10^4 phages/ml during 8 hours of the cell grouth.

The ratios of free phages to bacteria per ml as a function of the incubation time for all strains are compared in fig.2. As is shown, all indicated functions, except that of the recA$^-$ strain, at first increase, then pass through a maximum, and fall after four hours of incubation. The behaviour of the recA$^-$ strain ratio of free phages to bacteria is entirely different. At first it falls down, goes through a minimum, and then increases.

DISCUSSION

The difference between the phage production levels of recA$^-$ and recA$^+$ strains suggests that the RecA protein is responsible for spontaneous λ-prophage induction. Following the absence of a natural RecA protein there is an excessive DNA degradation in the recA$^-$ strain. In this strain the cells can be

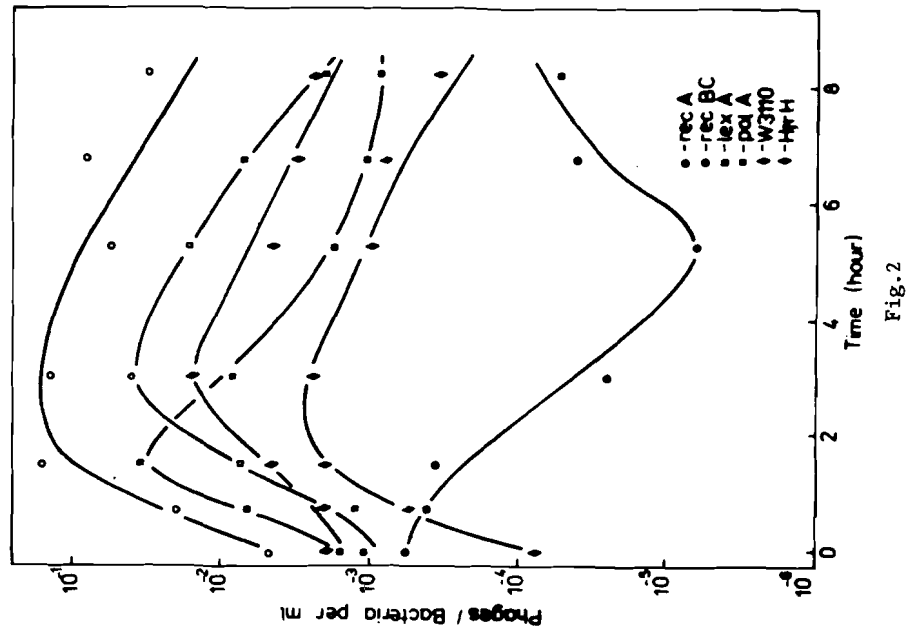

Fig.2

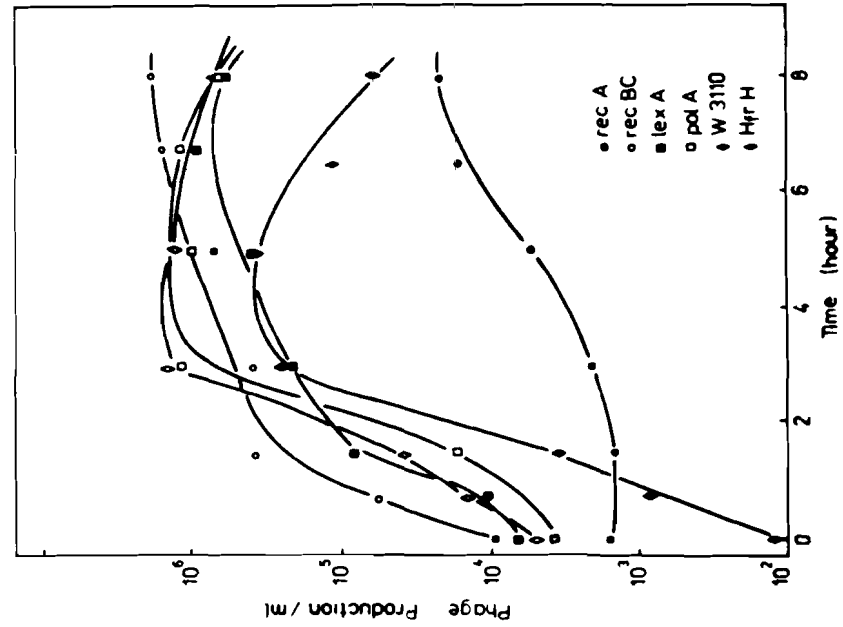

Fig.1

separated into two classes: nondividing and normal viable cells[12]. It has been shown that recA⁻ nondividing cells are incapable of synthesizing both DNA and protein[13]. This can be the result of an inability to transcribe and replicate DNA because of excessive degradation of DNA. Following this, both the level of the phage production and the colony forming units of the recA⁻ mutant will be lower in comparison with that of the wild type strain. In this case, the free phages/bacteria ratio of the recA⁻ strain must not differ from that of the recA⁺ strains. However as is shown in fig.2, there is a significant difference between these functions of the two genotypes. This fact suggestes that the explanation of the difference between the phage production levels of recA⁻ and recA⁺ strains by excessive DNA degradation is not right. In addition, there is the following support: it has been shown that in recB⁻C⁻ cells like in the recA⁻ cells, a class of nondividing cells can be singled out[14]. Despite of presence of such metabolic inactive cells both the phage production and the free phages/bacteria ratio of the recB⁻C⁻ strain are similar to those of the rest recA⁺ strains, but not to the recA⁻ (fig.1,2). We suppose that the additional level of spontaneous phage production in recA⁺ strains may by caused by a clevage of the λ-repressor from the recA protein and that the Okazaki's fragments are these sites of DNA where the activation of the RecA protein is taking place. However, it is not clear, how is the spontaneous induction going on in the recA⁻ cells? The explanation of this phenomenon can be based on (i) diminution of the λ-repressor concentration in the daughter cells after fission, and (ii) ability of the λ-repressor to bind single-stranded NDA gaps arising during the normal DNA replication.

The hypothesis that the diminution of the λ-repressor concentration in the daughter cells after fission can cause spontaneous prophage induction in the recA⁻ strain has led to a simple mathematical model based on the assumption: in daughter cells after cell-division the λ-repressor macromolecules submit to normal distribution. It has been ascertained that in a lysogenic cell there are ~200 (10^{-7} M) monomers of the λ-repressor[15] and the equilibrium dissociation constant K_d describing dimer formation is 2.10^{-8} M[16]. Simple mathematical calculations give about 500 λ-repressor dimers in one cell. Immediately after cell-division the average number of λ-repressor dimers in each of the two daughter cells is ~250. There is evidence that inactivation of 90% of the λ-repressor (i.e., below the level of about 50 λ-repressor dimers in a lysogenic cell) produces highly efficient induction[15]. The probability

(obtained from the normal distribution) that only ~50 dimers will fall into one of the daughter cells is $\sim 4.10^{-11}$. This result is far from the experimental data.

The last hypothesis presented here in order to explain λ-prophage induction in the recA⁻ strain is based on the capability of the λ-repressor dimer to act like a single-strand binding protein[17] . It has been shown[17] that in the presence of limiting amounts of the ind⁺ repressor, nonoperator DNA containing gaps effectively competes against λ DNA for binding the repressor. The recA⁻ spontaneous induction may go on by the following scheme: the λ-repressor will bind the single-strand regions of the chromosome (i.e., replication fork), lowering the concentration of the free repressor in the cell and thus derepressing the viral operon.

The dissociation constant of the repressor complexed to nonoperator DNA in vivo and the number of binding sites formed during growth have to be accurately determined to check whether they agree with the experimental level of spontaneous phage production in the recA⁻ cells.

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Received by Publishing Department
on June 6, 1989.