ISSN 0233-7657. Биополимеры и клетка. 1998. Т. 14. № 6

DNA-containing phages neutralizing with anti-MS2 serum

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The DNA-containing phages neutralizing with anti-MS2 serum have been detected in MS2-induced bacterial cultures, in the cells containing the recombinant plasmid as well as in preparations of transducing lambda and P1 phages. The identity of all these new developed bacteriophages permits to suppose the common mechanism of their origin.

We have earlier described the induction of MS2-resistant forms in the offspring of an *Escherichia coli* AB 259 Hfr 3000 cell having survived this phage infection [1]. The phage-induced mutants have been shown to be genetically unstable ones; they are able to segregate new mutant types; two of them, a granular mutant and a lysing one, have been studied in detail [2]. The cloning of the mutant region in a non-replicating Ap-fragment [3] has demonstrated the some possibility of integration of an MS2-specific sequence with the *E. coli* cell DNA; such a result induces to study these mutants from the new point of view of RNA phages-host cell interaction problem as origin and properties of these mutants suggested the presence at least of a part of phage genome or its DNA copy in their chromosome. The attempts to find the association of MS2-specific nucleic acid with the host cell chromosome has been made using the method of hybridization of MS2-induced mutant RNA and DNA with MS2 cDNA [4] and by the genetic transfer of the mutant properties into the lambda phage chromosome [5]. Despite of some positive results we have failed to detect the presence of MS2 phage in MS2-induced mutant cells or the phage secretion by these cells. Such phenomena do not correlate with the well-known traditional lysogeny conception as well as with the persistant state conception because in both these cases bacterial cultures contain some quantity of free phage. At the same time, such fact as DNA-containing MS2-derivative phages segregation in the course of transducing P1 and lambda phages reproduction [5] suggest the anti-MS2 serum neutralizing phages might have been detected in MS2-induced mutant cells of granular and lysing types. The aim of this work was revealing of DNA-containing phages with some MS2-like properties and comparison of these properties with ones of phage segregants described earlier [5].

Indeed a prolongated observation of wild type *E. coli* 3000 cells put in the medium together with lysing and granular type mutants permits to detect a phage whose development goes with the extremely low frequency. If a drop of an one-day-old *E. coli* 3000 or of another F-pili forming culture is put on the lawn formed by any of these two mutants a spot region of the wild type lawn becomes markedly clearer in several days. Some additional passages of the material isolated from the spot give us the possibility to detect the phages being identical and possessing the host range of the MS2 phage. Contrary to phages appearing in QB- and MS2-resistant fi + R *E. coli* cultures [6, 7] the phage particles found in our test-system are markedly different in their size and morphology from the MS2 phage; these particles are DNA-containing and consisted of heads and tails; they have been neutralized by the anti-MS2 serum, the neutralization level being the same as with the native MS2 phage. Neutralization constants were 285, 267, 230 and 293 for MS2, phage segregated from lysing bacterial mutant, phage segregated from granular mutant and phage segregated from recombinant plasmid-containing culture accordingly. Immunization

of rabbits was made according to Adams [8] and preliminary purifying of MS2 phage in CsCl gradient was made according to Maniatis et al. [9]. Anti MS2-serum obtained in such a way did not neutralise phages P1 and λ as well as it did not agglutinate *E. coli* 3000 host cells. On the other side anti-serums against phages λ and P1 and to *E. coli* 3000 cells did not neutralise both MS2 phage and all the new DNA-contained phages obtained in the process of this work. Exhaustion of anti MS2-serum by MS2 phage preparations having different phage particles concentrations decreased neutralizing properties of serum both to MS2 phage and to DNA-containing phages described here; in the cases when the high-titre MS2 phage was used for exhaustion neutralizing properties of serum disappeared completely and all tested phages (both RNA- and DNA-containing ones) formed negative colonies in the presence of such exhausted anti MS2-serum. It should be noted that these data confirm only the activity of anti MS2-serum to new phages but don't exlude the possibility of the presence of some other antigens on the surface of these phages. This problem will be investigated by us in the further experiments. It is clear from our electron microscopic photoes of MS2 derivative phage particles having been mixed with the T4 phage (Fig. 1) and MS2 phage (Fig. 2) that the derivative particles are markedly larger comparing to the MS2 ones. Their icosahedrical head having a diameter about 50 nm, and their tails being about 150—160 nm long. Despite of these particles strict specificity for donor type cells, our electron microscopic investigations detect no derivative particles adsorption on F-pili. The cause of this phenomenon is supposed to be elucidated in further investigations. At the same time such particles form negative colonies belonging to the same type as the colonies formed by P1 and lambda transducing phages segregants [5]. Despite of the bacterial lawn ageing, the colonies of DNA-containing MS2 derivative phages become larger during several days, the demi-lysis zones becoming also wider and occuping the whole lawn. The cells containing the recombinant plasmid *pL34* constructed by us [3] also accumulate the DNA-containing phage identical to those one found in MS2-induced mutants from the point of view of its morphology, its neutralization with anti-MS2 serum, and its restriction obtained after restriction nucleases treatment (see Fig. 3). The identical DNA-containing particles are also found in MS2 preparations, such an identity having been proved by the markers already described above for other DNA-containing phages neutralizing with anti-MS2-serum.

Fig. 1. Electron micrograph of the DNA containing MS2-like phages mixed with T4 phage. Uranyl acetate (2 %) staining. X 120000

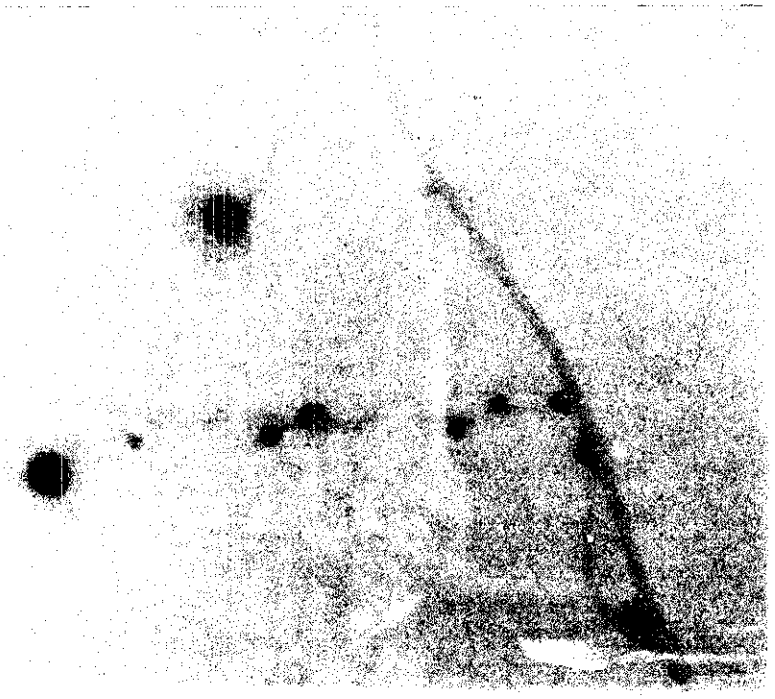

Fig. 2. Electron micrograph of the DNA-containing MS2-like phage particles mixed with MS2 phage. Uranyl acetate (2 %) staining. × 120000

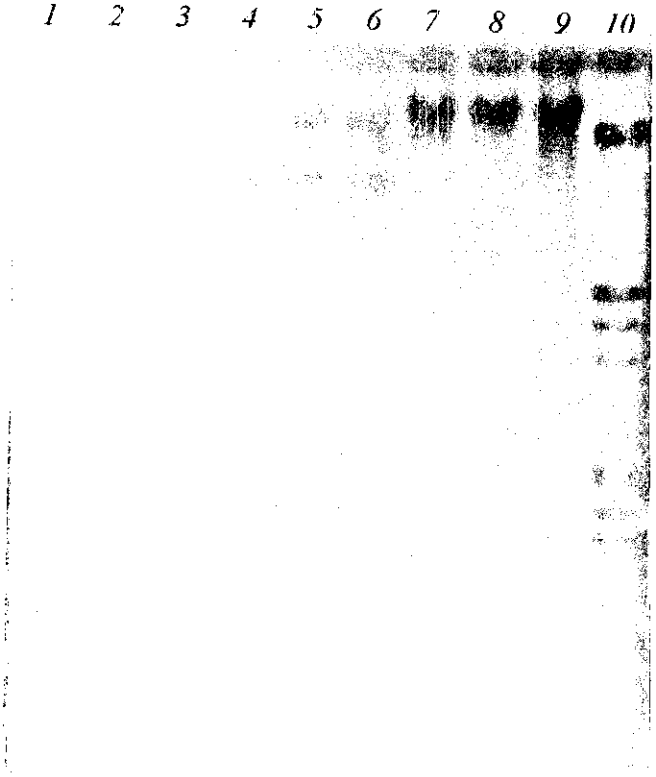

Fig. 3. Electrophoregram of the DNAs isolated from MS2-like phages and digested with restrictases *HaeIII* (*1—3*), *PstI* (*4—6*), and *BamHI* (*7—9*). (*1, 4, 7* — DNA of the phage segregated from *E. coli* 3000 lysing type mutant; *2, 5, 8* — DNA of the phage segregated from *E. coli* granular mutant; *3, 6, 9* — DNA from the phage segregated from *pL34*-containing cells); *10* — lambda phage DNA + *EcoRI* + *HindIII* (marker lane)

The formation of DNA-containing phages in MS2-induced *E. coli* mutants suggests once more that some processes well known and investigated in detail may be accompanied with some other events of a very low frequency followed by new biological forms birth being new virus forms in our case. Contrary to retrovirus-cell systems, no DNA-containing structures taking part in RNA-containing phages reproduction and phage RNA replication have been yet described. Additionally, the construction of revertase-target MS2 DNA *in vitro* is completely impossible without artificial connection of the poly(dA)·poly(dT)-linker to MS2 phage RNA [10] being inaccessible for this enzyme without such a supplement. So it cannot be ruled out that the process of formation of DNA-containing phages antigenically related or identical to RNA-containing ones may be due to other mechanisms of RNA-DNA interactions perhaps more similar to those described previously for procaryotic cells [11]. It cannot be ruled out that the presence in the bacterial cell of RNA-phage specific information independently of its form (phage multiplication or some other form specific for MS2-induced cell mutants) induces prophages presented in *E. coli* cells chromosomes and plasmids. Such a prophage sequence may form hybride forms with RNA phages components both on the level of genomes and MS2-specific protein coating of phage particles with new properties.

In any case, we have data enough to assume the new virus-host cell interaction ideas are acceptable for

RNA-containing bacteriophages, at least at the structural level, and may be successfully detected in a system of MS2-induced cellular and phagic mutants.

Acknowledgement. The author thanks Dr. F. Tovkatch for the preparation of electron microscopic photoes, Dr. Anna Miriuta for the restrictive analysis of the phage DNAs, and Dr. Ella Zherebtsova having prepared the anti-phage serum and helped to prepare this manuscript.

Т. П. Перерва

ДНК-вмісні фаги, що нейтралізуються анти-MS2 сироваткою

Резюме

ДНК-вмісні фаги, які нейтралізуються анти-MS2 сироваткою, виявлено в культурах MS2-індукованих мутантів, у клітинах, що містять рекомбінантну плазміду, та в препаратах фагів MS2 і P1, здатних до трансдукції фагів лямбда. Тотожність новоутворених бактеріофагів дозволяє припустити загальний механізм їхнього походження.

Т. П. Перерва

ДНК-содержащие бактериофаги, нейтрализующиеся анти-MS2 сывороткой

Резюме

ДНК-содержащие фаги, нейтрализующиеся анти-MS2 сывороткой, обнаружены в MS2-индуцированных бактериальных культурах, в клетках, содержащих рекомбинантную плазмиду, в препаратах фагов MS2, P1 и лямбда-трансдуцирующих фагов. Идентичность новообразованных бактериофагов позволяет предположить общий механизм их происхождения.

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Received 24.10.97