LYSOGENY BY MS2 PHAGE. ANALYSIS OF A RECOMBINANT PLASMID CONTAINING MS2 RNA-LIKE SEQUENCE

pL34 recombinant plasmid coding MS2-like RNA synthesis in plasmid-containing cells has been studied using physical mapping and blot-hybridisation with \( P^{32} \)-labelled pMS27 plasmid fragments including a DNA copy of MS2 phage RNA. The authors have determined the size of a fragment containing MS2-like sequence and its localisation inside the hybrid plasmid.

Introduction. Despite of RNA-containing phages being among the best studied biosystems, some of their properties, especially phage-host interactions, have not been completely investigated. One of the problems is a cause of multiple resistant cells development in \( E. coli \) cultures infected by RNA-containing phages in the conditions not favorable for quick cell division. In some previous papers concerning RNA-containing phages biology this phenomenon has been discussed from the point of view of possible preexisting mutants selection. Nevertheless, such an interpretation does not seem to be completely convincing because of large scale phage resistance development. In our previous paper [1] we have shown that more than 1% of infected bacterium offspring receives MS2-resistance marker which may not be due to pre-existing mutants selection. We interpret such a fact as a non-direct proof of direct interactions between phage genome and cell host DNA; our interpretation has been confirmed by non-stability of primary MS2-induced mutations and derivative forms segregation from these ones [2]. In order to prove that these mutants properties are caused not by a persistent MS2 infection but due to phage genome integration into the host cell chromosome we have created a genetic library of \( EcoRI \) fragments of a segregant obtained by us—a lysing \( E. coli \) AB 259 Hfr 3000 mutant [3]. A total RNA preparation isolated from plasmid-forming cells has been hybridised both with pMS27 DNA containing MS2 phage cDNA and with a part of this cDNA without vector sequence. The results obtained prove phage specific RNA to be transcribed from chromosomal DNA of the MS2-induced \( E. coli \) mutant; so we have really demonstrated that MS2 phage is able to establish true lysogeny as a state based on physical integration of phage and cellular DNA's. In the present paper we present our first data on our study of cloned host cell DNA fragment having the goal to determine how large is MS2-specific region and which is its localisation on the genetic map of our recombinant plasmid; we aim also to find there some recognition sites for several popular restrictive enzymes.

Materials and methods. Plasmids. Recombinant plasmid pL34 has been constructed by us using non-replicative Ap-fragment of pCV16 plasmid and described in the previous paper [3] as pL26 plasmid. A strain containing pCV16 plasmid [4] has been received from the Institute of Biochemistry and Physiology of Micro-organisms (Russian Academy of Sciences, Pushchino-na-Okie, Moscow district, Russian Federation). A strain carrying pMS27 plasmid [5, 6] is a friendly present of Dr R. Devo (Gent University, Belgium).
Nutrient media. We have mostly used 0.6% and 1.2% amino peptide (AP) containing agar and AP-containing broth. Plasmid isolation, restriction analysis, and blot-hybridisation have been perfected according to [7]; physical mapping of plasmid DNA sequences has been made with mutual and single digestion approaches [8].

Results and discussion. To determine the size of a recombinant DNA plasmid fragment carrying MS2-like sequence and its localisation in the recombinant plasmid we have used physical mapping and blot-hybridisation. Our probes were both the whole pMS27 plasmid containing MS2 cDNA and a fragment of this DNA copy without vector sequence. The results of an experiment with EcoRI and PstI treatment of pL34 and pCV16 plasmids are shown in the Fig. 1, a.

It is clear from the Fig. 1, b that the labelled pMS27 having been used as a probe gives hybridisation with all the fragments of both plasmids (pCV16 and pL34). It may be due to pCV16 origin, i.e. to the presence of some homologies with pBR322 sequences in the vector part of pMS27. In the next blot-hybridisation experiment (see Fig. 1, c) where
cDNA copies of phage MS2 RNA have been used hybridisation lines correspond to pCV16 fragments of the size 5.1 kbs and to pL34 fragments as large as 5.1 and 4.8 kbs in single and double treatments. The smaller pCV16 fragments (their sizes are about 1.1 and 0.7 kbs) corresponding to Ap-fragment of the total size 1.8 kbs do not hybridise with pMS27 fragment, i.e. a region of the recombinant plasmid able to hybridise with pMS27 fragment is not a part of pCV16 plasmid.

Fig. 2. Electrophoregram of pL34 plasmid DNA (a) and radio autograph obtained during hybridisation of the corresponding filter with P32-labelled pMS27 fragment DNA (b): lanes: 1 — pL34 DNA+PstI; 2 — pL34 DNA+PstI+BamHI; 3 — pL34 DNA+BamHI+HindIII; 4 — pL34 DNA+BamHI+HindIII+PstI; 5 — pL34 DNA+HindIII; 6 — pL34 DNA+HindIII+PstI; 7 — pL34 DNA+PstI+EcORI; 8 — pL34 DNA+EcORI; 9 — pL34 DNA+EcORI+BamHI; 10 — pL34 DNA+EcORI+BamHI+HindIII; 11 — lambda phage DNA+EcORI+HindIII (marker lane)

Fig. 3. Physical maps of pCV16 and pL34 plasmids. A shaded part corresponds to the fragment including ampicilline resistance gene, an unshaded and wider one corresponds to a fragment containing MS2-like sequence

Our data obtained in the experiments of recombinant and vector plasmids hybridisation with pMS27 plasmid carrying MS2 cDNA and with this cDNA copy permit us to conclude that the sequence homologous to MS2 RNA is situated in the large pL34 fragment (5.1 kbs) limited by PstI sites. The hybridisation observed with pCV16 DNA is thought to be most probably due to random short sequences, pCV16 DNA being non-hybridisable both with MS2 RNA and also total RNA isolated from a strain containing a recombinant plasmid and being able to hybridise both with labelled pMS27 plasmid and with its fragment [3]. These data suggest that on the contrary to recombinant plasmid pCV16 plasmid con-
tains no sequence coding MS2-like RNA synthesis and forming hybrids with such RNA. So the hybridisation observed in pCV16 experiments may be explained by a presence of a random sequence or of a sequence with the biological function analogue to lysogenic phage integration site in host cell chromosome. Electrophoregrams of the recombinant plasmid after PstI, BamHI, HindIII, and EcoRI restrictive digests as well as blot-hybridisation of fragments obtained with pMS27 fragment are demonstrated in the Fig. 2.

Our results lead to the conclusion that MS2-like sequence in most probably localised inside the fragment as large as 3.9 kbs limited by PstI and BamHI cleavage sites. The localisation of this fragment is shown on the recombinant plasmid map given in the Fig. 3.

The undoubtful hybridisation of this fragment with a fragment of MS2 cDNA fragment and almost total coincidence of its size (3.9 kbs) with MS2 RNA size (3.569 kbs) [9] permit to think that MS2-like sequence integrated into pL34 recombinant plasmid is localised inside this fragment. It should be noted, however, that this region contains no EcoRI and Sall sites detected inside MS2 cDNA [10]. A Sall site situated inside of PstI-BamHI fragment (see Fig. 4) is localised not far from one of its termini corresponding in its localisation to none of two Sall sites detected inside DNA copy of the phage RNA [10]. So the region of recombinant plasmid being correspondent to MS2 sequence according to hybridisation experiments is probably a fragment of the structure more complex than a double-stranded one. Such a conclusion is not contradictory to our previous results demonstrating that labelled recombinant plasmid DNA fails to hybridise with MS2 RNA [3]. The absence of recombinant plasmid DNA and MS2 RNA hybridisation accompanied by MS2-like RNA synthesis in plasmid-containing cells may be most probably explained by a hypothesis that this plasmid contains a plus-chain of MS2 RNA-like DNA or even MS2 RNA and has a three-stranded structure in the fragment of the size about 3.9 kbs. Our point of view is the last version is also probable because of being able to explain our negative results obtained when we have attempted to detect some restrictive sites inside pL34 plasmid PstI-BamHI fragment with size of 3.9 kb, these ones being undoubtfully present in the DNA copy of MS2 RNA.

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ЛІЗОГЕШЯ У ФАГА MS2. АНАЛІЗ РЕКОМБІНАНТНОЇ ПЛАЗМІДИ, ЩО МІСТИТЬ MS2 РНК-ПОДІБНУ ПОСЛІДОВНОСТЬ

Резюме

Рекомбінантну плазміду pL34, що кодує синтез MS2-подібної РНК у плазмідозвісних клітинах, вивчили методом фізичного картування та блот-гібридизації. Як зонд використовували фрагмент плазміди pMS27, що містить ДНК-копію РНК фага MS2. Було виявлено розміри фрагменту, який включає MS2 РНК-подібну послідовність, та його локалізацію в гібридній плазміді.
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