

Nucleotide sequence of the *Galleria mellonella* nuclear polyhedrosis virus origin of DNA replication

V.M. Blinov, V.V. Gutorov, N.G. Holodilov, A.A. Iljichev, V.A. Karginov, N.N. Mikrjukov, V.A. Mordvinov, I.V. Nikonov, N.A. Petrov, I.H. Urmanov and S.K. Vasilenko

All-Union Research Institute of Molecular Biology, Koltsovo, Novosibirsk Region, USSR

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The initiation sites of the *Galleria mellonella* L. nuclear polyhedrosis virus (*G.m.* NPV) DNA replication were revealed. For this purpose SCLd 135 cells permitting the *G.m.* NPV productive reproduction were transformed by the recombinant plasmids containing the viral genome individual fragments in pRSF 2124 and pBR 322 vectors. It was revealed that 2 of the 32 recombinant plasmids can autonomously replicate in the eucaryotic cells. According to the Maxam-Gilbert method the DNA *G.m.* NPV fragment (1300 bp) primary structure of pHBR plasmid was determined. The structure analysis revealed the typical regulator signals as in the replicons. The possible regulation mechanism of the DNA *G.m.* NPV synthesis initiation was supposed.

Nuclear polyhedrosis virus Plasmid Culture cell Ori site Nucleotide sequence

1. INTRODUCTION

The initiation of DNA synthesis is a key stage of viral reproduction. Thus the most important problem of the functional mapping is the detection of the viral genome sites containing the DNA synthesis origin. To solve this problem we used a method based on the study of the ability of the recombinant plasmid containing the individual fragments of the viral genome to replicate in the cell system permissive for the virus-donor of DNA fragments.

The system of the culture cells (SCLd 135) permissive for the reproduction of the free nuclear polyhedrosis virus of *Galleria mellonella* (*G.m.* NPV) has been selected previously [1], and the collection of the recombinant plasmids containing the individual fragments of this viral genome was made [2]. Our purpose in these experiments was to locate and to determine the primary structure of the origin of replication of *G.m.* NPV DNA.

2. MATERIALS AND METHODS

Introduction of the plasmid DNA in SCLd 135 cells was made as in [3].

Treatment of the cellular monolayer was with DNaseI (Sigma) in the presence of 10 mM MgCl₂ at 3 mg enzyme/mg plasmid DNA. Isolation of the extrachromosomal DNA from SCLd 135 cells was carried out by the procedure in [4]. The *E.coli* C600 competent cells were transformed by the plasmid DNA as in [4].

Restriction endonucleases, polynucleotide kinase and DNA polymerase I were provided by Dr Yu.S. Nechaev. Alkaline phosphatase was kindly donated by Dr V.G. Korobko. [α -³²P]dNTP and [γ -³²P]ATP (spec. act. 1000–3000 Ci/mmol) and [6-³H]thymidine were supplied by Amersham (England) or Radiopreparate (Tashkent). Introduction of ³²P by means of polynucleotide kinase or DNA polymerase I was made as in [5]. DNA sequencing was done as in [5].

3. RESULTS AND DISCUSSION

The replicative ability of the recombinant plasmids containing the individual fragments, *G.m.* NPV DNAs, was studied in the eucaryotic cells in the following way.

The plasmids were introduced into SCLd 135 cells and cellular monolayer was treated with DNase I. Cells were incubated at 28°C for 24 h, then removed from the glass and lysed. Subsequently, the extrachromosomal DNA was isolated by the standard procedure. The *E.coli* C600 competent cells were transformed by the obtained material.

The 32 recombinant plasmids were studied using the given scheme. Twenty-seven of them contained *Eco*RI fragments of *G.m.* NPV DNA in pRSF 2124 (series-pNRSF) and the 5 harbored *Bam*HI fragments of this viral genome inserted in pBR 322 (series-pNBR).

The material that transforms *E.coli* C600 competent cells proved to be isolated from the cell culture introduced by plasmid pORSF and pHBR. Transformants obtained in the course of experiments were cultivated and used for isolating the plasmid DNAs. The restriction analysis data indicated that pORSF and pHBR retained the ability to transform *E.coli* cells after incubation for 24 h in SCLd 135 cell culture.

In order to recognise the ability of plasmids pORSF and pHBR to replicate in SCLd 135 cells the kinetics of the plasmid conservation was determined as follows.

The recombinant plasmids were introduced into the cell monolayer. Then, the cells were removed from the glass, lysed and the plasmid DNA was isolated at 6-h intervals. The *E.coli* C600 competent cells were transformed by the prepared material.

All recombinant plasmids were used in these experiments. The *G.m.* NPV DNA, pBR 322 and pRSF 2124 were used for the control of the conservation of the plasmid DNAs in SCLd 135 cells and the transformation of *E.coli* cells.

The reliable increasing of the transforming activity of the material, isolated 24 h after introduction of plasmids pORSF and pHBR into SCLd 135 cells, was observed in comparison with the activity, determined after 6 and 12 h incubation in the culture.

We then investigated the degree of incorporation of [^3H]thymidine into the acid-insoluble fractions, obtained after isolation of extrachromosomal DNA from SCLd 135 cells, treated with plasmids pORSF and pHBR. As a result, accumulation of the labelled plasmids was found to correlate with the accumulation of the transforming activity in SCLd 135 cells introduced by pORSF and pHBR.

The two bands, concentrating both the radioactivity and the transforming activity, are formed from the SCLd 135 cells introduced by these plasmids after the centrifugation of clear lysate in the CsCl density gradient. The simulation of this experiment, i.e., the centrifugation of the plasmids produced in the bacterial cells in the CsCl density gradient, made it possible to establish the similarity of density CsCl, favouring the formation of the bands containing the plasmid DNA in the principal and simulated experiments (fig.1).

Two bands obtained on agarose gel by electrophoresis of DNA in the simulated experiment were shown to represent the circular and supercircular forms of the plasmid DNA.

The results of all presented experiments are conclusive evidence that plasmids pHBR and pORSF

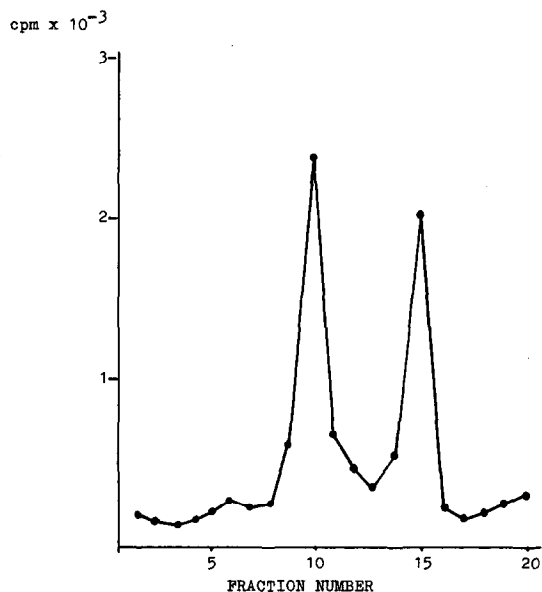


Fig. 1. CsCl density gradient centrifugation of the material isolated from CSLd 135 cells 24 h after introduction by the plasmid pHBR.

GATCCAACAC ACGCGTCGAA GAAACCATGA AAACGGTGAA TGTGGGGCAA TGTGGGGCAA GAAGATTTCC TCATGTGGAG CATCAGGCAG CAGTCGGAG TGGGGGAAGA 100
 CTAGGTTGTG TCGCGAGCTT CTTTGGTACT TTTGGGACTT ACACCGGTTT CTTCTAAACG AGTACACCTC GTACTCGGTG GTACAGCTCC ACCCGTTCT

GGTGATGGAC CGTTGGGCA GTGACAGGA CGACTGTMTT CCGGACAACG AGCGCGGCG CAGTGGGTGA AAGCAAAAG GTTGTGTGAAG CGGCAGAATA 200
 CGACTAGGTG GCAACCCTGT CACTGTCTCT GCTGCACAAA GGCTGTCTCC TCCGGGGCGG GTACACCCACT TTTCCGTTTCT CAACCACTTC GCCGTCCTAT

AAATATCACT TCCGCAACAC AGTGCAACA AATCGTGGCG ATCGGGCAAT TCCACTTCGA AAATGTACAG CAAGCTCGAG TCCAGGGAG ACACGGACGA 300
 TGTTAGTGA AGCGGTGGTG TGCAGCTGT TGCAGCCGCA TAGCCGGTAA AGTGAAGT TTTACATCTC GTTCGAGCTC AGGTCCTGC TGTGGCTGCT

CysGlnVal TyrIleLeu AspAlaGluGly AsnProIle AsnValThr ValAspThrVal LeuHisArg AspGlyVal SerMetIleLeu LysGlnIlys
 GTGCCAGTA TACATTTGG ACGCTGAGG CAACCCCATC AAGTGTACCG TGGACACCTGT GCTTCATCGA GACGGCGTGA GTATGATCT CAACAAAAAG 400
 CAGGGTGCAT ATGTAAAACC TCGGACTGCG GTTGGGGTAC TTTGCAGTCC ACCTGTGACA CGAAGTACCT CTGCGCCACT CATACTAAGA GTTTCTTTTC

SerThrPhe ThrThrArgGln IleLysAla AlaCysLeu LeuIleLysAsp AspLysAsn AsnProGlu SerValThrArg GluHisCys LeuIleAsp
 TGTACGGTCA CACGGGCA AATAAAAGCT GCGTGTCTTC TCATTAAGA TGACAAAAAT AACCCGACT CGGTGCACCG CGAACACTGT TGTATGACA 500
 AGATGCCAGT GGTGGGGGT TTTTTCGA CGCACAGAG AGTAATTTCT ACTGTTTTTA TTTGGGGCTCA GCCACTGTGC GCTTGTGACA AACTAACCTGT

AsnAspIleTyr AspLeuSer LysAsnThr TrpAsnCysLys PheAsnArg CysIleLys ArgLysValGlu HisArgVal LysLysArg ProProThrTrp
 ATGATATATA TGATCTTCT AAAAAACGCT GGAACCTGCA GTTAAACGA TGGATTAAC GCAAAGTCA GCACCGAGTC AAGAAAGCGG CGCCCACTTG 600
 TACTATAT ACTAGAAAAGA TTTTGTGCA CCGTGCAGCT CAAATTTGCT CAAATTTGCT ACCTAATTTG CGTTCAGCT GCTTGGCTCAG TTTCTTCCCG CGGGGTGAAC

ArgHisAsn ValArgAla LysTyrThrGlu GlyAspThr AlaThrLys GlyAspLeuMet HisIleGln GluGluLeu MetTyrGluAsn AspLeuLeu
 GGCACACAC GTTAGAGCA AGTACACAGA GGGAGACACT GCCACCAAG GGCACCTGAT GCATATCAA GAGGAGCTGA TGTACGAAA CGATTTGCTG 700
 CGGGGTGTG CAATCTCGCT TCATGTGTCT CCGTCTGTGA CGCTGGACTA CGTATAAGTT CTCCTCGACT ACATGCTTTT GCTAAACGAC

LysMetAsn IleGluLeuMet HisAlaHis IleAsnLys LeuAsnAsnMet LeuHisAsp LeuIleVal SerValAlaLys ValAspGlu ArgLeuIle
 AAAATGAACA TTGAGCTCAT GCATGGGCAC ATCAACAACC TAAACAATAT GCTGCACGAC CTGATAGTCT CCGTGGCGAA GGTGGACGAG CGTTTGATTTG 800
 TTTTACTTGT AACTCGACTA CGTACCGCTG TAGTTGTTCG ATTTGTTATA CGACTGTCT GACTATACA GGCACCGGTT CCACCTGCTC CCAAACTAAC

GlyAsnLeuMet AsnAsnSer ValSerSer ThrPheLeuSer AspAspThr PheLeuLeu MetProCysThr AsnProPro AlaHisThr SerAsnCysTyr
 GCAATCTCAT GAACAAATCT GTTCTTCAA CATTTTCTC GGACGACAG TTTTGTCTGA TCGCGTGCAC CAATCCGCGG GCACACCCA GTAATGCTA
 CGTTAGATA CTTGTTAAGA CAAAAGACTT GFAAAAACAG CCGTCTGTTC AAAAAAGACT ACGGCACGTT GTTAGCGCGG CCTGTGTGGT CATTAACGAT 900

AsnAsnSer IleTyrIlys GluGlyArgTrp ValAlaAsn ThrAspSer SerGlnCysIle AspPheSer AsnTyrLys GluLeuAlaIle AspAspAsp
 CAACAACAC ATCTACAAG AAGCGGTG GGTGGCCAC ACGACTCTT CGCAATGCAT AGATTTTAC AACPAACAGG AACTAGCAAT TGACGACGAC 1000
 GTTGTGTGCG TAGATGTTTC TTCCCGCAAC CCACCGGTTG TGCTGTGAGA CGGTTAGTA TCTAAAAATCG TTAGTGTTC TTAGTGTTC ACTGTGTGCTG

ValGluIle Leu
 GTCGAGATTT TG
 CAGCTCTAAA ACCTAG

Fig. 2. Primary structure of the *G.m.* NPV DNA fragment isolated from plasmid pHBR. Repeated sequences are indicated by arrows (→). (---) Goldberg-Hogness box, (---) ribosome site, (...) initiation codon, (----) polyadenylation signal.

can be autonomously replicated in SCLd 135 cells. Analogous results were obtained in studying the eucaryotic systems, precisely the replicative characteristics of the recombinant plasmids containing ori *Xenopus laevis* [6], *Physarum* [7]. Thus, ori *G.m.* NPV in the plasmids pHBR and pORSF was considered to be cloned.

The *G.m.* NPV DNA fragment of plasmid pHBR was sequenced as in [5]. The determined primary structure is shown in fig.2. Screening of the structural elements characteristic of the DNA replication origins resulted in the following.

Four initiating codons possessing potential ribosome sites [8] in the preceding sequence were recognised. They are located in positions 263, 383, 658 of the upper chain and 73 of the lower one. Thus, the untranslated region may be situated within the segment 74-262, 74-382 or 74-657.

There is a set of direct repeats with an evident homology in the region from 64 to 314 bp (fig.2). Three of these repeats (263-277, 280-294, 301-315) overlap with the sequence having the initiating codon in position 263. The hypothetical protein X can be expected to be translated from this codon. An interesting property of this protein is the presence of the cluster of charged amino acids coded by the sequence from 521 to 622. Thirteen of the 34 amino acids in this region are positively charged (fig.2). Such clusters are characteristic of many DNA binding proteins such as π -protein coded by plasmid pR6K [9] or T-antigen of SV40 virus [10].

In positions 199-205, 309-315, 505-511 there are sequences similar to the Goldberg-Hogness box

TATA^{TT}_{AA} [11] which are usually situated 25-30

bases upstream from a transcription initiation site. The polyadenylation signal AATAAAA [12] was found in position 421-426. These structural elements may be important for regulation of the synthesis of hypothetical protein X.

The features of the established sequence are typical for DNA replication sites. Direct repeats are known to be present in many replicons [13-15]. Such sequences are supposed to be recognition sites for the proteins taking part in the initiation of DNA replication [16]. The direct repeats marked in the primary structure of the *G.m.* NPV DNA fragment overlap with the hypothetical protein region

and one of the possible Goldberg-Hogness boxes. The proximity of a replication initiation site and gene coding for the protein responsible for initiating DNA replication was shown for phage ϕ X174 [17] and λ [15], SV40 virus [18], and plasmid pR6K [9].

The hypothetical protein X may play a similar part in the replication of *G.m.* NPV DNA. The plasmid pHBR does not include the C-terminal region of this protein gene, but replicates in eucaryotic cells. Probably, the N-terminal part of the protein involving a cluster of the positively charged amino acids is enough for its function. Similar facts are described in the literature. There exists a replicable plasmid, pR1, containing the ori site and the N-terminal region of the gene coding for the protein π , which is sufficient for the replication [9].

Our results can be useful for further investigations of the replication mechanism of NPV DNA.

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