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Osaka University
Replication of Bacteriophage Lambda DNA

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Preface

The mechanisms of DNA chain initiation and elongation have been intensively investigated using small DNA phages or small plasmids as models of DNA replication in *Escherichia coli*. They studied these problems mainly by biochemical approaches, and resolved these mechanisms in molecular level (for a review, see Kornberg, 1978). We can imagine the feature of replicating DNA as below. At an unique origin site, primer RNA is formed by actions of dnaB protein (mobile replication promoter) and dnaG (primase) protein or RNA polymerase (Geider et al. 1978; Itoh and Tomizawa, 1980), and the RNA is switched to elongate DNA chain. Once the chain elongation initiates, the replication fork is advanced forming DNA chains discontinuously by dnaB protein, primase and DNA polymerase III holoenzyme. These are composed of multi-step reactions involving activities of many accessory replication proteins. In these reactions, the rate-limiting step is the initiation of replication, and its regulation controls overall replication process. Unless we understand how replication fork moves, the investigation of regulation at initiation step is scarcely progressed. The reason is that the study of the regulation requires not only biochemical but also genetical and molecular biological informations. How is each replication unit regulated independently while maintaining its own copy number? Jacob et al (1963) proposed one simple model to answer this question. They assumed "replicon" which consists of replicator (replication origin) and initiator. The initiator acts positively on replicator, allowing the initiation of replication from replicator. Actual mechanism of the regulation will be more complex, for example there would exist negative regulation mechanisms, but this model explains primary feature of the regulation on initiation.

Bacteriophage λ is a temperate phage which has a capacity either to replicate autonomously or to convert to a lysogenic (temperate) stage after the DNA enters *E.coli* cells. λ DNA is 48kb long and genes coding on this genome have been well analyzed (Szybaliski and Szybaliski 1979). Replication of λ DNA has been intensively studied as a model of the complex process of regulation in replication, and it is showed that λdv, a plasmid derivative of bacteriophage λ is a good model for studying "replicon" (Matsubara, 1981). Many investigators attempted to dissect
the biochemical process of the initiation reaction. But anyone did not success in purification of \( \lambda \) initiators nor in replication of \( \lambda \) \textit{in vitro}. I also attempted to analyze the regulation mechanism existing in the \( \lambda \) DNA replication, and successfully purified \( \lambda \) initiators and demonstrated the molecular interaction between \( \lambda \) replicator and initiator. In addition, I constructed an \textit{in vitro} replication system of \( \kappa_{dv} \) plasmid. I believe that these works removed difficulties to study biochemically the regulation in \( \lambda \) replication, and the replication of \( \lambda \) will become the most advanced system to understand the regulation mechanism acting in replication.
I. Replication of Bacteriophage \( \lambda \)

The mechanism of bacteriophage \( \lambda \) replication has been studied for a long time via genetic and molecular biological approaches (for a review, see Skalka 1977). The following features summarize our current understanding:

1) Circular DNA molecules of bacteriophage \( \lambda \) enter into the replication cycle. The replicative intermediates are in \( \Theta \) forms at an early stage of bacteriophage \( \lambda \) growth (Tomizawa and Ogawa 1968), but they are in rolling circle forms at late stages (Skalka et al. 1972; Bastia et al. 1975; Takahashi 1975). The switch from \( \Theta \)-type to rolling-circle-type replication has not been studied well.

2) Replication is initiated at a unique site of DNA called ori (Schnös and Inman 1970; Inman 1981).


4) Two \( \lambda \)-coded initiator proteins, \( Q \) and \( P \), are required for replication (Ogawa and Tomizawa 1968). There is evidence from genetic studies that ori is the action site of the \( Q \) protein (Furth et al. 1978). The \( Q \) protein interacts with \( P \) protein (Tomizawa 1971; Furth et al. 1978), which, in turn, is the target for action by host-coded proteins such as the dnaB product (Georgopoulos and Herskowitz 1971, Saito and Uchida 1977, Klinkert and Klein 1979). The \( Q \) protein is unstable and is degraded within a few minutes after production (Wyatt and Inokuchi 1974; Kuypers et al. 1980; Lipińska et al. 1980, Gottesman et al. 1981). It is possible, therefore, that the rate of production of this protein controls the rate of replication. \( P \) protein, in contrast, is stable.

5) The ori region or a DNA region nearby must be "activated" by transcription prior to or during replication (Dove et al. 1969, 1971; Furth et al. 1982). This phenomenon, called transcriptional activation, is independent of the production of initiator proteins. Its implication is not clear.

6) All the genes and genetic sites needed for \( \lambda \) DNA replication are clustered in the so-called early region of the \( \lambda \) genome. The cluster can be extracted as a DNA fragment and can be perpetuated in a plasmid state in Escherichia coli, since it can replicate autonomously. The
plasmid is called λdv (Matsubara and Kaiser 1968; Matsubara 1981). The genetic structures of λ and λdv are shown in Figure 1.

7) The entire genome of λdv is transcribed under the control of pRoR. The transcription is autoregulated and is kept at a low level (Murotsu and Matsubara 1980; Johnson et al. 1981; Matsubara 1981). Therefore, initiation of replication is under the control of this transcription, which simultaneously activates ori and provides the unstable Q protein.

8) The entire nucleotide sequence of λdv has been elucidated (Moore et al. 1978, Schwarz et al. 1980; for a review, see Matsubara 1981), and part of it is reproduced in Figure 2. Around the replication-initiation site are four characteristic 19-bp repeating sequences arranged tandemly (hereafter referred to as ori-repeats). To the right of the ori-repeats is an A+T-rich segment whose sequence is reminiscent of that of the primase action site in the genome of bacteriophage G4. Several cis-acting replication-defective mutations map in the ori-repeats and the A+T-rich region (Hobom et al. 1978; Moore et al. 1978). Further to the right is a region with a long palindromic sequence. To the left of the ori-repeats is a leftward coding sequence for an RNA with complete promoter and terminator sequences. The product RNA is 78 bases long, and is called oopRNA (Hayes and Szybalski 1973). Its role is not clear. All these unique features are retained among the λ-related phage genomes, although their sequences differ considerably.

9) A region around 500-bp to the left of the ori-repeats has been cloned. Under special circumstances this region replicated autonomously (Hobom et al. 1978; Lusky and Hobom 1979a,b). It was named ice, and was postulated to be a site of switching from primer RNA to DNA synthesis. However, further evidence to support this idea has not yet been obtained.
Figure 1. The genetic structures of bacteriophage \( \lambda \) and the \( \lambda dv \) genome.

Symbols for genes and genetic sites are those used by Szybalski and Szybalski (1979). Numbers represent the number of nucleotides from the right end of \( pRoR \) (see Fig. 2 for details). Horizontal arrows at the bottom represent origin and direction of transcription. \( \lambda dv021 \), a typical \( \lambda dv \) (Chow et al. 1974) was used, and called as \( \lambda dv \) in this work, except when noted otherwise. Its unit length is 3.1 kilo base pairs and codes the sequence from -830 to +2300 (the precise end points are not clear).
Figure 2. The nucleotide sequence covering λAdv genome (Schwarz et al. 1980; Matsubara, 1981).

The genes, tof, cll, O and P are boxed. The genetic sites are boxed with double lines at the top and bottom. Particular origin structures, ori-repeats, A+T-rich and palindromic are indicated. Heavy underlines represent the ribosome-binding (Shine-Dalgarno) sequences. Position numbers are those used by Schwarz et al. (1980); Position 1 in this system corresponds to position no. 38,844 in the system of Daniels et al. (1980).
II. Purification of \( \lambda \) O and P proteins

Purification of a \( \lambda \)ori-binding protein from \( \lambda dv \) carrier cells. By genetical evidence, \( \lambda \) O protein was predicted to bind to \( \lambda \) ori sequence (Furth et al. 1978). I searched such an activity that binds to \( \lambda \)ori among proteins from \( E. coli \) cells carrying \( \lambda dvAj5 \) (Tsurimoto and Matsubara 1981a). \( \lambda dvAj5 \) is a copy-mutant \( \lambda dv \) which accumulates 500-1000 copies/cells, and it was expected that proteins coded for by the genes of \( \lambda dv \) would accumulate in its carrier cells (Murotsu et al. 1977). One protein fraction contained the activity to bind specifically to \( \lambda dv \) DNA or dv-DNAs from \( \lambda \)-related phages, \( \lambda imm434 \) and \( \lambda imm21 \) (Matsubara and Otsuji 1978), whose ori region is the same as that of \( \lambda \). It does not bind to other DNAs, e.g., \( \phi 80dv \) or pBR322. I concluded that the \( \lambda \) ori binding protein is the Q protein from following lines of evidence: 1) The protein could be purified from cells carrying \( \lambda dv \) or a recombinant plasmid coding the intact Q protein but not from cells without plasmids or carrying a recombinant plasmid coding the incomplete Q protein. 2) The most purified \( \lambda \) ori binding protein from \( \lambda dv \) carrier cells showed a molecular weight of 32,000 that is in good agreement with the value previously reported for the Q protein made in an in vitro protein synthesizing system (Yates et al. 1977), or in minicells carrying \( \lambda dv \) or those infected with \( \lambda \) phage (Lipinska et al. 1980).

The binding of the Q protein can be observed with DNA in the linear duplex form or in closed circular form, as long as it carries \( \lambda \) ori region. Figure 3 shows mapping of the binding site among \( \lambda dv \) restriction fragments. \( \lambda dv021 \) DNA was digested with AvaII and EcoRI, and 5' ends of the digests were labeled with \( ^{32}P \). These DNA fragments were mixed with the Q protein and filtered through a nitrocellulose filter. Only a 164-bp fragment (AvaII at position 983 to EcoRI at position 1147) which carries ori-repeats, A+T-rich stretch, and part of a palindromic structure was trapped on the filter mediated by the Q protein. \( \lambda dv \) DNAs carrying other regions, such as \( ci \) and \( pRoR, ice \) and \( oop \), did not show this interaction.

The Q protein is extremely unstable in vivo and scarce amounts of it accumulates in cells. This point prevented us from obtaining large amounts of the Q protein needed for further analyses. I could prepare only 5\( \mu \)g of near homogeneous Q protein (the purity is 65%) from 50g of
Figure 3 Autoradiogram showing that the purified Φ protein specifically interacts with λ ori fragment.

λdv021 DNA, digested with Avaii and EcoRI, and 5'-end-labeled with 32p (1.0x10^{-3} pmol each fragment), was mixed with the Φ protein fraction (which possessed binding activity to 1.5x10^{-3} pmol DNA carrying ori site) in 200μl of Buffer B (10mM Tris-HCl [pH7.4], 10mM Mg(CH₃COO)₂, 20mM KCl, 0.1mM EDTA, 6mM 2-mercaptoethanol, and 50μg/ml BSA) and incubated at 0°C for 30 min. The reaction mixture was filtered through a nitrocellulose filter, and the filtrate was collected. The retained DNA was recovered from the filter by washing with 1ml of 1 M NaCl. The DNAs were precipitated with ethanol and analyzed by polyacrylamide gel electrophoresis and autoradiographed. A control sample filtered in the absence of protein was made, and treated similarly. (1) Control sample, all the fragments produced by Avaii and EcoRI digestion exist. a: 2.8-kb fragment carrying from Avaii (1453) to Avaii (983); b: 164-bp from Avaii (983) to EcoRI (1147); c: 269-bp from EcoRI (1147) to Avaii (1416); d: 42-bp from Avaii (1416) to Avaii (1453). (2) Filtrate with the Φ protein. (3) The DNA retained on filter with the Φ protein.
\textit{\lambda dvAj5} carrier cells using this system. Therefore, it was desirable to improve the efficiency of its production, and I constructed a system overproducing the Q and P proteins as described in the next section. 

**The O, P overproducer system.** To prepare the O and P proteins in large quantities, I constructed a pBR322-based recombinant plasmid (Fig. 4) that carries the O and P genes under direct transcriptional control by two tandemly arranged promoters, pR and pL (Tsurimoto et al. 1982). These two promoters are repressed at low temperature (32°C) by a thermosensitive repressor ci857, whereas at high temperature (42°C) they are active. The structure of this recombinant plasmid may be reminiscent to that of \textit{\lambda dv}. However, it does not carry an autorepressor gene tof (Murotsu and Matsubara 1980), or the transcriptional termination signal tR1 (Rosenberg et al. 1978), both of which are located in between the pRoR and the O gene in \textit{\lambda dv}, modulating transcription of O and P genes. Thus, the recombinant plasmid allows strong expression of the O and P genes upon transfer the carrier cells from low to high temperature (thermal induction).

Figure 5A shows the time course of change in bulk cellular proteins upon thermal induction. Two proteins with molecular weights 34,000 and 26,000 accumulated noticeably; these are the O and P proteins. Each O and P proteins accounts for 2-3% of the bulk cellular proteins at 2 hours after induction. No such protein bands were detected in induced cells that did not carry the recombinant plasmid (Fig. 5B). The overproduced O protein was stable in vivo (its half-life was more than 30 min; data not shown), which was unexpected in view of the short half-life of O protein (1.5 min; Gottesman et al. 1981) that has been observed with \textit{\lambda} phage-infected cells or \textit{\lambda dv} carrier. The stability may have been instrumental in the accumulation of this protein. It could be that proteinases that degrade O protein are insufficient or that such proteinases are inactive in the thermoinduced cells.

**Purification of O and P proteins.** I purified the O and P proteins from an induced bacterial strain using electrophoresis to monitor purity (Tsurimoto et al. 1982). Cells were lysed in Buffer D (50mM Tris-HCl [pH7.4], 0.3M KCl, 0.1mM EDTA, 12mM 2-mercaptoethanol) in the presence of EDTA (5mM), lysozyme (125μg/ml) and Triton X-100 (1%) at 0°C, and then sonicated and centrifuged at high speed. The supernatant
Figure 4. Structure of an O, P-overproducing recombinant plasmid, pMY17-3.

Origins of DNA segments: (---) from pBR322; (■■■) fragment carrying the O and P genes from λadv021; (■■) fragment of λ carrying cI857 and the pR promoter; (■■) fragment of λ carrying the pL promoter. (⇒) Location of pR and its transcriptional orientation; orientation of transcription from pL is also shown by its tapered end. Arrowheads indicate the cutting sites of BamHI (Ba), BglII (Bg), EcoRI (E), HindIII (H), and PstI (Ps). The locations of O, P and the truncated cII gene (cII') are shown outside of the λadv sequence. pMY17-3 was selected as a plasmid conferring λ-immunity to its carrier cell, Km723 (Tsurimoto and Matsubara 1981a). Construction of the plasmid is described in Tsurimoto et al. (1982).
Figure 5. A: Accumulation of O and P proteins in induced overproducing cells.

Km723(pMY17-3) was grown in PBB medium (Tsurimoto and Matsubara, 1981a) at 32°C to OD_{660}=0.4 and then transferred to 42°C (time 0). Samples (0.25ml) were taken at the times indicated (in hours), cells were collected by centrifugation at 10,000g for 3 min, suspended with 10μl of sample buffer (62.5mM Tris-HCl [pH6.8], 2% SDS, 0.07M 2-mercaptoethanol, 0.5mg/ml bromophenol blue and 10% glycerol) and heated at 100°C for 2 min. Proteins were separated on a 15% polyacrylamide gel containing 0.1% SDS and stained with Coomassie blue. B: Comparison of proteins from Km723 cells with or without pMY17-3. Cells that had been incubated at 42°C for 2 hours were treated as in A. Arrowheads indicate the O- and P-protein bands (34,000 mw. and 26,000 mw., respectively).
containing some Q protein was saved, and the precipitate was extracted with Buffer D containing 2M urea. The combined extracts contained most of the Q protein. The P protein was extracted from the 2M urea-extracted residue with Buffer D containing 4M urea. Both initiator proteins were hydrophobic and this three-step extraction using denaturing reagents, viz. Triton X-100 and urea were necessary for effective extraction. These results suggest that the initiator proteins of λ may be bound to the cell membrane (Kuypers et al. 1980, Zylicz and Taylor, 1981) and replicating λ DNA become associated with the bacterial membrane.

The Q protein was purified by column chromatography using DEAE-cellulose, hydroxyapatite, phenyl-Sepharose, and CM-Sephadex. The P protein was purified by column chromatography using Bio-Gel P-6, DEAE-cellulose, phosphocellulose, and hydroxyapatite. These purification schemes yeilded homogeneous Q and P proteins (the purity of each proteins is more than 95%, respectively), as shown in Figure 6. I prepared 3mg of the Q protein and 1mg of the P protein from 1g of the thermoinduced Km723(pMY17-3). This recovery of the Q protein means that this protein was amplified to 1-10 thousand-fold from λdv system by this overproducing system.

Genes coding for the Q and P proteins. I determined the amino acid compositions and the amino- and carboxyl-terminal sequences of the purified Q and P proteins (Tsurimoto et al. 1982). The observed composition of each protein (Table 1) agrees well with that predicted from the nucleotide sequences. The amino-terminal sequences were determined up to the 4th residue by Edman degradations and were found to be Thr-Asp-Thr-Ala- for Q protein, and Met-Lys-Asn-Ile- for P protein. The carboxyl-terminal sequences, determined by carboxypeptidase A digestion are Leu and Val for Q and P proteins, respectively. These sequences agree perfectly with those predicted by nucleotide sequence analyses (Fig. 7). The first fMet in the Q protein is missing, possibly as a result of processing. In P protein, the first f-Met is deformylated. The termination codon for the Q protein and initiation codon for the P protein overlapped. This may mean a cooperative translation of these closely related initiation proteins. From the amino acid sequence and the nucleotide sequence data, the precise molecular
Figure 6. Electronphoretic profiles of purified Q protein (lane 1) and P protein (lane 2).

Purification procedures are described in the text and Tsurimoto et al. (1982). Each protein (3µg) was subjected to SDS-polyacrylamide gel electrophoresis. Note that no extra bands are detectable under these conditions.
Table 1. Amino acid compositions of the purified Q and P proteins

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<sup>a</sup>) Amino acid compositions predicted from nucleotide sequences (Denniston-Thompson et al. 1977; Scherer 1978; Schwarz et al. 1980)

<sup>b</sup>) Amino acid compositions obtained for the purified proteins (Tsurimoto et al. 1982). Each value represents the average of two determinations after 24 and 72 hr hydrolysis. Values represent residues per molecule. Asn, Gln, Trp and Cyc were not determined.
Figure 7. Comparison of terminal amino acid sequences of O and P proteins predicted from DNA sequence and determined from amino analyses of the purified proteins.

(A) Part of the λ DNA sequence covering the amino- and carboxyterminal regions of the O and P genes. (■■■) Start codons (ATG); (●●●) stop codons (TGA). Numbers above the sequence are explained in Fig. 2. (B) Partial amino acid sequences predicted from the nucleotide sequence. Upper and lower rows are O-protein and P-protein sequences, respectively. (C) Partial amino acid sequences determined by analyses of the purified proteins. Upper and lower rows are the O-protein and P-proteins, respectively. The total number of amino acid residues and molecular weights calculated from these data are shown. Locations of the O and P genes in the λ nucleotide sequecne are indicated at the top.
weights of the Q and P proteins are calculated to be 33,700 and 26,500, respectively.

**Binding of Q protein to the ori-repeats.** Purified Q protein binds specifically to a 164-bp fragment that coding λ ori sequence. I studied further the interaction of Q protein and λ ori fragment using a footprinting assay (protection against DNase I digestion) and a protection assay against exonuclease III (ExoIII). The results of these experiments demonstrate that the binding site of the Q protein consists of the four ori-repeats.

A 387-bp λ fragment carrying the ori-repeats was labeled with 32p at the 5' end of the left-hand side (AvaII; 983) of the molecule (the 5' end of the right-hand side [TaqI; 1370] is unlabeled). It was mixed with different concentrations of Q protein, and treated with low concentration of DNase I. As DNase I randomly cuts DNA, the resulting set of fragments form a ladder in gel electrophoreses (Fig. 8 lane 1). In the presence of the Q protein, region 1 is covered and thus protected from DNase I attack (lane 4). Upon addition of more Q protein (lane 5), the protected region is further expanded (region 2). The protected regions 1 and 2 correspond precisely to the inner two ori-repeats and all four ori-repeats, respectively (Fig. 11).

Experiments of a protection assay against ExoIII were carried out using 5'-end-labeled λ ori fragments: AvaII (983) to EcoRI (1147) labeled at the EcoRI end; and AvaII (983) to HinfI (1157) labeled at the AvaII end. Without Q protein, these fragments were digested by ExoIII to some points that stopped the nuclease naturally (Fig. 9, lane 2 and Fig. 10, lane 3). When the Q protein binds to these fragments, the digestion of ExoIII was stopped at 3' border of the binding sites and generated two protected bands on each strand (Fig. 9, lane 3,4,5 and Fig. 10, lane 6,7). These results show that 3' borders of the binding on r- and l-strands were determined to locate at left ends of ori-repeats 1 and 2, and at right ends of ori-repeats 3 and 4, respectively. At a low concentration of Q protein, ExoIII did not digest the region covered by ori-repeats 2 and 3, and outer two repeats also protected in increasing Q protein amounts (Fig. 11).

Experiments of these two types of protection assays gave exactly the same result. The Q protein binds to a 19-bp ori-repeats covering
Approximately 2.5x10^{-15} moles of λori DNA fragments were prepared by Tacl (1370) and AvaII (983) digestion (see Fig. 2). The AvaII 5' terminus was end-labeled with 32P. The DNA was incubated at 70°C for 20 min with varying amounts of Q protein in 100μl of Buffer B (see Fig. 4). DNase I was then added to a final concentration of 0.01 mg/ml and the mixture was incubated at 20°C for 2.5 min. The DNA was precipitated with 70% ethanol, dissolved with 10μl of 0.1M NaOH plus 0.01M EDTA, mixed with 10μl of urea-dye solution (8M urea, 0.1% bromophenol blue and 0.1% xylene cyanol), heated at 90°C for 1 min, and subjected to electrophoresis in 10% polyacrylamide gel with Tris-borate buffer containing 8M urea. After electrophoresis, the gel was autoradiographed.

Lane 1, DNase I digestion in the absence of Q protein. Lanes 2, 4, and 5, DNase I digestion in the presence of 3, 6 and 12ng Q protein/100μl, respectively. Brackets 1 and 2 at the right illustrate the regions protected against DNase I attack by 6ng and 12ng of Q protein. Numbers at the side of each bracket represent the 3' and 5' borders of the protected regions as estimated from size standard in lane 3 prepared by guanine degradation of the fragment according to the method of Maxam and Gilbert (1977). These are expressed in number of nucleotides from the AvaII end.
Figure 9. The O protein protects ρ-strand against ExoIII digestion. Approximately $3 \times 10^{-15}$ mols of 165-bp fragment (AvaII [983] to EcoRI [1147] and $^{32}$P-end-labeled at the EcoRI terminus) was incubated in 100 µl of Buffer B at 37°C with varying amounts of O protein for 12 min and then digested with ExoIII (60 units/ml) at 37°C for 5 min. After digestion the samples were electrophoresed and autoradiographed as Fig. 8. Lane 1, undigested fragment. Lane 2, digested with ExoIII in the absence of O protein. Lanes 3, 4, 5, digested with the same amount of ExoIII after incubation of the DNA with 3, 6 and 12 ng of O protein, respectively. Numbers represent fragment sizes expressed in nucleotides from the EcoRI terminus.
Figure 10. The O protein protects 1-strand against ExoIII digestion. Experiments were done as Fig. 9, except that approximately $2 \times 10^{-15}$ mols of 174-bp fragment [AvaiI (983) to HinfI (1157) and $^{32}$P-end-labeled at the AvaII terminus] and 180 units/ml of ExoIII were used. Lane 1, undigested fragment. Lane 2, length standard same as that used in Fig. 8 lane 3. Lane 3, ExoIII digestion of the fragment without O protein. Lane 4, 5, 6 and 7, ExoIII digestions of the fragment after incubation with 1.5, 3, 6 and 12ng of O protein, respectively. Numbers indicate fragment sizes expressed in nucleotides from the AvaII terminus. Arrowheads indicate G-clusters exist in ori-repeats.
Figure 11. Sequence of the ori region of λ DNA protected by the O protein against digestion by DNase I and exonuclease III. Upper and lower rows of nucleotide sequences represent the l- and r-strands of λ DNA, respectively, from AvaII (983) to EcoRI (1147). Slanted arrows indicate the boundaries of the region protected against exonuclease III digestion. Protected 1 and protected 2 correspond to protection boundaries by low and high concentrations, respectively, of O protein. Brackets 1 and 2 indicate regions of the l-strand protected against DNase I cleavage. Bracket 3 indicates region of the r-strand protected against DNase I cleavage at high concentration of O protein (data not shown). The four boxes represent the 19-bp ori-repeats (repeats 1 through 4). Note that the boundaries of the ori-repeats match well with the boundaries of the region protected by the O protein.
both $l$- and $r$-strands. The $Q$ protein preferentially binds to the inner two ori-repeats when its concentration is low. At high concentration, all four ori-repeats are bound.

I next constructed a series of deletion derivatives from a pBR322-based recombinant plasmid pOA-4 (Fig. 12) that carries the ice-opo-ori region of $\lambda$ but not the palindromic region (see also Fig. 2). In these derivatives, the A+T-rich region and some of the ori-repeats in the cloned $\lambda$ DNA fragment have been deleted to various extents by progressive digestion with BAL31 exonuclease. Figure 12 shows the deletion end points and the ability of these derivatives to bind to the $Q$ protein (Tsurimoto and Matsubara 1981c). Deletion of the palindromic and A+T-rich regions did not affect the binding ability. Deleting the right-most repeat did not affect the result, but loss of two repeats lowered the binding activity to 38% of normal. Deletion of all but one unit practically abolished the binding activity. These observations are in accord with the previous observation that among the four ori-repeats, the inner two are the preferential sites of $Q$-protein binding. The palindromic and A+T-rich regions apparently do not affect the binding activity.

The molecular events involved in the sequential binding of $Q$ proteins and the structure of $Q$ protein-DNA complex are not yet clear.

Interaction of $P$ protein with $\lambda$ protein. Genetic studies have shown that $\lambda$ $P$ protein interacts with $\lambda$ dnaB protein of the host (Georgopoulos and Hershowitz 1971). This was confirmed in vitro by Wickner (1978) who showed that a DNA-independent ATPase activity of $\lambda$ dnaB protein is inhibited by the $P$ protein. Klein et al. (1980) demonstrated the physical interaction between the $P$ and $\lambda$ dnaB proteins. Figure 13 shows that my purified $P$ protein inhibits the ATPase activity of purified $\lambda$ dnaB protein, in confirmation of Wickner's observation. The inhibition of $\lambda$ dnaB ATPase activity by the $P$ protein depended on the molar ratio of $P$ protein to $\lambda$ dnaB protein; at a ratio of 2, complete inhibition was attained. No inhibition was observed with the $Q$ protein, and the $P$ protein did not bind to the ori-repeats. I have tried to demonstrate molecular interactions between the purified $Q$ and $P$ proteins, but so far no evidence has not been obtained.
Figure 12. Structure and Q-protein-binding activity of a set of deletion derivatives of pOA-4.

pOA-4 is the parental plasmid that carries the \lambda \text{ori} fragment (AluI at position 432 to EcoRI at position 1147; see Fig. 2) in place of the smaller EcoRI-BamHI segment of plasmid pBR322 (Tsurimoto et al. 1982). Only the sequence of the inserted \lambda DNA fragment is shown. Boxed areas 1 through 4 indicate the ori-repeats. Four deletion derivatives, pOri-D1, pOriD-4510, pOriD-9010 and pOriD-9011, were constructed from pOA-4 by BAL31 digestion starting from EcoRI site (Tsurimoto and Matsubara, 1981c). The deletion endpoint of each plasmid is indicated by an arrow, and the region to the right of the arrow is deleted. These plasmid DNAs were labeled with \[^{3}H\]thymidine and linearized by AvaI digestion. Each \(^{3}H\)-labeled linear DNA (25ng) was mixed with 8, 16 and 24ng of purified Q protein in 1ml of Buffer B (see Fig. 3). The mixture was incubated at 37°C for 15 min and filtered through a nitrocellulose filter. The Q-protein-binding activity to each plasmid DNA was measured by counting the amount of \(^{3}H\) retained on the filter. The results were averaged and expressed as relative values to that observed with intact pOA-4 (=1.0).
Figure 13. Inhibition of dnaB ATPase activity by purified P protein. The assay was carried out essentially as described by Wickner and Hurwitz (1975). The assay mixture (30μl) contained 20mM Tris-HCl (pH7.9), 0.4mM MgCl₂, 1mM dithiothreitol, 30mM KCl, 0.5mM [γ-³²P]ATP (4x10³ cpm/nmol; Amersham), 50μg/ml bovine serum albumin, and 9.3μg/ml dnaB protein was incubated at 30°C for 30 min. The purified dnaB protein was a gift from N. Nakayama and Y. Kajiro (Tokyo University). Inorganic phosphate released from ATP by dnaB protein was determined by assaying charcoal-nonadsorbing ³²P in the presence of 10mM HCl and 2mM potassium phosphate (Tsurimoto et al, 1982). Under my assay conditions, the dnaB protein released 4nmol of inorganic phosphate. To examine the inhibition by P protein, various amounts of P protein or heated P protein were added to the mixture. ○; intact P protein, ▲ P protein heated at 65°C for 5 min.
III. In vitro replication of λdv DNA

The in vitro replication system. The purified Q and P proteins initiate λdv DNA synthesis in vitro when combined with an ammonium sulfate fraction (fraction II) of E.coli lysate developed by Fuller et al. (1981). Their system allowed in vitro replication of an oriC plasmid bearing the chromosomal origin in the presence of fraction II, along with hydrophilic polymers and an ATP-regeneration system. I prepared fraction II from E.coli YS1 (Tomizawa et al. 1975) that did not carry bacteriophage λ or λdv. λdv was inactive when the template added to the assay mixture without Q and P proteins, but addition of Q and P proteins to this system promoted its DNA synthesis (Fig. 14). The same system synthesizes DNA when pBR322 is added. In this case, however, the DNA synthesis is not dependent on the Q and P proteins. Omission of one of the two initiators from the reaction mixture lost the activity of λdv DNA synthesis (Table 2). Therefore, both Q and P proteins are absolutely required for the activity. Figure 15 shows that the level of DNA synthesis increased when the Q protein concentration was increased and that it reached a plateau at 20μg/ml, when the molar ratio of λdv DNA to the Q protein is about 1/100. Binding studies have shown that under these conditions, the Q protein saturates the binding sites of λ DNA. When Q protein was added at 10μg/ml, maximal DNA synthesis was attained at a concentration of about 8μg/ml of P protein, where the Q:P ratio is about equal.

Table 2 demonstrates requirements of this system which is fortified with the hydrophilic polymer polyethylene glycol (PEG) 20,000 and an ATP-regeneration system. Nalidixic acid and novobiocin, which inhibit DNA gyrase, inhibited the reaction. Chloramphenicol, an inhibitor of protein synthesis, showed no effect, and rifampicin, which inhibits RNA polymerase, showed an intermediate effect. I studied further the intermediate effect of the drug on λdv replication. Rifampicin was added at various times after initiation of the reaction of λdv and pBR322. Figure 16 demonstrates that the template-dependent pBR322 DNA synthesis was inhibited by the addition of rifampicin at any stage, as was previously shown for CoIE1 (Sakakibara and Tomizawa 1974). These plasmids require an RNA-polymerase-dependent primer RNA synthesis for replication. λdv DNA synthesis, on the other hand, was inhibited by
Figure 14. Kinetics of λadv and pBR322 DNA synthesis with and without Q and P proteins.

The reactions were carried out in a standard reaction mixture (25μl) containing 40mM HEPES (pH8.0); 80mM KCl; 2mM ATP; 0.5mM each of GTP, CTP and UTP; 50μg/ml bovine serum albumin; 21.6mM creatine phosphate; 11mM Mg(CH$_3$COO)$_2$; 100μg/ml creatine kinase; 6% (W/V) polyethylene glycol (PEG) 20,000; 0.1mM each of dATP, dGTP, dTTP and dCTP with [α-32P]dCTP (Amersham) at 100cpm/pmol of dCTP; 8μg/ml λadv021 DNA (○,●) or pBR322 DNA (□,■); and 120μg of E.coli YS1 (Tomizawa et al. 1975) protein fraction (fraction II) prepared according to the method of Fuller et al. (1981). The mixture was incubated at 30°C. Open symbols represent the activities with a combination of Q protein (20μg/ml) and P protein (16μg/ml); solid symbols represent activities without the proteins. The extent of DNA synthesis was expressed as total dNMP incorporated.
Table 2. Requirements for in vitro replication of \( \lambda_dv \) DNA.

<table>
<thead>
<tr>
<th>Omission and Additions</th>
<th>DNA synthesis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>pmol</td>
</tr>
<tr>
<td>complete</td>
<td>221.2</td>
</tr>
<tr>
<td>- Mg((CH_3COO)_2)</td>
<td>4.4</td>
</tr>
<tr>
<td>- ATP, creatins phosphate and creatine kinase</td>
<td>0.6</td>
</tr>
<tr>
<td>- creatine phosphate and creatine kinase</td>
<td>99.8</td>
</tr>
<tr>
<td>- PEG 20,000</td>
<td>28.5</td>
</tr>
<tr>
<td>- ( \lambda_dv ) DNA</td>
<td>8.5</td>
</tr>
<tr>
<td>- O protein</td>
<td>2.0</td>
</tr>
<tr>
<td>- P protein</td>
<td>5.3</td>
</tr>
<tr>
<td>+ Nalidixic acid (500(\mu g/ml))</td>
<td>18.8</td>
</tr>
<tr>
<td>+ Novobiocin (20(\mu g/ml))</td>
<td>8.0</td>
</tr>
<tr>
<td>+ Chloramphenicol (200(\mu g/ml))</td>
<td>187.2</td>
</tr>
<tr>
<td>+ Rifampicin (20(\mu g/ml))</td>
<td>42.4</td>
</tr>
</tbody>
</table>

The reactions were carried out in the standard reaction mixture (described in Fig. 14) with 20\(\mu g/ml\) O protein and 16\(\mu g/ml\) P protein. Inhibitors were added at the indicated concentrations just prior to starting the reaction.
Figure 15. Requirement for the exogenously added Q and P proteins in the replication of λdv DNA in vitro.

The reactions were carried out in a standard reaction mixture with 8μg/ml of λdv DNA at 30°C for 30 min. (A) DNA synthesis in the presence of 9μg/ml P protein and various amounts of Q protein as indicated. (B) DNA synthesis in the presence of 10μg/ml Q protein and various amounts of P protein as indicated.
Figure 16. Effect of rifampicin on the kinetics of DNA synthesis in vitro with pBR322 (A) or λdv (B).

Plasmid DNA was incubated beginning at -10 min under the standard conditions described in Fig. 14, except that dNTPs and O and P proteins were omitted. At time 0, dNTPs with [α-32p]dCTP were added in (A) and dNTPs with [α-32p]dCTP, 20μg/ml O protein, and 16μg/ml P protein were added in (B). The mixtures were incubated up to 30 min. (O), no rifampicin addition; (●), rifampicin (20μg/ml) added at various times as indicated.
rifampicin only when this drug was added at the preincubation stage. Rifampicin added at later times exerted almost no effect. In Table 2, rifampicin and initiators were added together at the time of starting the reaction without preincubation. These observations suggest strongly that RNA synthesis by RNA polymerase effective for \( \lambda \) replication is limited only at a stage prior to, or at the onset of, \( \lambda_{adv} \) replication. However, the role of the rifampicin-sensitive reaction on \( \lambda_{adv} \) replication must be analyzed further.

The products of in vitro DNA synthesis. From the amount of radiolabeled precursors incorporated, I calculated that the amount of DNA synthesized is equal to 80% of the exogenously added \( \lambda_{adv} \) DNA. The products were analyzed by gel electrophoresis (data not shown). Most of the molecules were found in open circular form and limited amount was in covalently closed circular form. This may indicate low activities of DNA ligase or DNA gyrase in fraction II. Small fractions migrated behind the covalently closed circular form and at position of catenate. These presumably represent replicative intermediates.

To analyze the products of in vitro DNA synthesis, I used \(^3\text{H}-\)labeled \( \lambda_{adv} \) DNA as template and \(^{32}\text{P}-\)labeled BrdU triphosphate as one of the four deoxyribonucleoside triphosphates for incorporation (Fig. 17). The products were centrifuged in a neutral CsCl-density gradient. About 86% of added template \( \lambda_{adv} \) DNA (\(^3\text{H}\)) and all the newly synthesized DNA (\(^{32}\text{P}\)) were in the half-heavy position. No full-heavy \(^{32}\text{P}\) was detected. Upon denaturation, all of the \(^3\text{H}\) and \(^{32}\text{P}\) were recovered as fully light and fully heavy single-stranded DNA, respectively. These observations indicate that in my in vitro system, DNA synthesis represents replication and that the externally added \( \lambda_{adv} \) DNA carries out one round of replication.
Figure 17. Analysis of density-labeled λcv DNA synthesized in vitro with BrdUMP.

3H-labeled λdvo21 in covalently closed circular form was incubated in the standard reaction mixture (200μl) (see Fig. 14) containing 0.1mM each BrdUTP, dGTP and dATP with [α-32p]dCTP; 20μg/ml of Q protein; and 16μg/ml of P protein. The reaction was stopped by addition of 50μl of 0.25 M EDTA and 2.5% SDS, followed by incubation at 37°C for 1 hr with 150μl of 5mg/ml proteinase K. The DNA was purified by phenol extraction and Sephadex G-100 (Pharmacia) gel filtration. The sample (3ml) in 10mM Tris-HCl (pH7.4), 20mM NaCl, and 1mM EDTA was mixed with 4g of CsCl. (A) Neutral CsCl density gradient profiles measured after centrifugation at 36,000 rpm for 48 hr at 15°C in a SW 50.1 rotor. HL and LL indicate the density of the half heavy fraction (1.755g/cm³), and light fraction (1.715g/cm³), respectively. The DNAs in fractions No. 15-18 (bracket) were pooled and dialyzed against 10mM Tris-HCl (pH7.4), 1mM EDTA. The sample was treated with 0.3M NaOH, 0.2M K2HPO4 and 3mM EOTA at 90°C for 5min, adjusted to 0.15M NaCl, 0.015M Na3citrate, 0.1M NaOH, 5mM EDTA, and 0.04% sodium N-lauroylsarcosinate (3ml), and mixed with 4.24g CsCl for alkaline CsCl density gradient analysis. (B) Alkaline CsCl density gradient profiles measured after centrifugation as in A. (−−−) Newly synthesized 32P- and BrdU-labeled DNA; (−−−) 3H-labeled parental DNA.
IV. Site of replication initiation and the minimal region required for initiation

The ori-repeats or a near-by region is the site for initiation of \( \lambda \text{Adv} \) DNA replication. To investigate the initiation site of \( \lambda \text{Adv} \) DNA, I used the "ddCTP method" which blocks chain elongation at an early phase of replication (Conrad et al. 1979). \( \lambda \text{Adv} \) DNA was synthesized in the presence of \([\alpha-^{32}\text{P}]dCTP\), and ddCTP. The DNA was purified, cleaved with DdeI or DdeI plus EcoRI, and then analyzed by electrophoresis in a polyacrylamide gel. The result of autoradiography is shown in Figure 18A, and the cleavage map of \( \lambda \text{Adv} \) is shown in Figure 18B. At a concentration of 25\( \mu \text{M} \) ddCTP, the isotope was incorporated almost uniformly into each fragment. However, at a concentration of 100\( \mu \text{M} \) ddCTP, some fragments were labeled specifically. Relative radioactivities among these fragments were measured by scanning the intensity of radioactivity and dividing by the number of nucleotides, followed by normalization as described in Figure 18B. The result clearly demonstrates that some fragments located in the \( \sigma \) gene are preferentially labeled at 100\( \mu \text{M} \) ddCTP. Since DNA elongation is halted randomly by ddCTP, the relative amount of radioactivity in a fragment should reflect its distance from the starting point of DNA synthesis. Thus, I can conclude that DNA synthesis of \( \lambda \text{Adv} \) starts at a point in, or very closed to, the 112-bp fragment, which is located to the left of the ori-repeats. The gradual decrease of radioactivity on both sides of the starting point indicates that DNA synthesis proceeds bidirectionally. This result is in good agreement with those obtained from in vivo study (Schnös and Inman 1970, Inman 1981). It should be noted that the result of this experiment shows the DNA elongation occurred in population of replicated DNA molecules. To analyze the DNA elongation process in each molecule, other experiments, such as an electron microscope analysis, would be necessary.

Minimal DNA structure for initiation of \( \lambda \text{Adv} \) replication. To determine the essential region for replication, I used the following pBR322-based recombinant plasmids, which carry all or part of the replication origin: pOri-1 carries the four ori-repeats and the A+T-rich region (Tsurimoto and Matsubara 1981a), pOA-4 carries the \( \text{ice} \) and \( \text{oopRNA} \) region in addition to the pOri-1, and pOri-D1 is similar to pOA-4, but
Figure 18. Determination of the initiation site in λDNA using ddCTP method.

(A) Distribution of newly incorporated radioactivity among various cleavage fragments from λdv021 DNA. The standard reaction mixture (25μl) contained 20μg/ml O protein, 16μg/ml P protein; 500cpm per pmol [α-32P]dCTP and 25μM ddCTP (lane 1) or 100μM ddCTP (lanes 1, 2). After incubation, DNA was purified as described in Fig. 16, precipitated with 70% ethanol three times and digested with DdeI (lanes 1, 2), or DdeI plus EcoRI (lane 3). The digested fragments were electrophoresed in 8% polyacrylamide gel with Tris-borate buffer containing 8M urea, and autoradiographed. Numbers at the side of the bands indicate their size (bp). Locations of these fragments along the λdv genome are shown in B.

(B) Relative intensity of radioactivity. The intensity of each band in A was measured by scanning, divided by the size of the fragment; each value was then normalized by taking the value for 383-bp fragment, located around cI and pr, as 1.0. (□) 25μM ddCTP; (□) 100μM ddCTP. In A, two 315-bp bands overlap, and the relative intensities of these two fragments were taken to be equivalent. In lane 3, 150-bp and 280-bp fragments were generated by EcoRI cleavage of the 430-bp fragment on lane 2. The 150-bp fragment overlaps the 157-bp fragment and the intensity of this fragment was determined by subtracting the value of the 157-bp fragment in lane 2 from that of the overlapped bands in lane 3. The sites of the genetic markers cI, tof, cII, pr, ori, O and P are indicated below the graph (see Fig. 2).
lacks the A+T-rich region (see Fig. 12 and Table 3). pBR322 was used as a control. These DNAs were added to the in vitro replication system in the presence or absence of Q and P proteins. The results are summarized in Table 3. Without Q and P proteins, \( \lambda \)\textsubscript{dv} was not able to synthesize DNA, but the pBR322-based recombinant plasmids allowed incorporation to some extent. Addition of the Q and P proteins stimulated DNA synthesis of pOA-4 and pOri-l, but not of pBR322 or pOri-D1. The initiation position of these plasmids was assayed using the ddCTP method as in Figure 18 (Figs 19A,B). The DNA synthesis of pOA-4 and pOri-l is initiated from a position near the ori-repeats, as observed with intact \( \lambda \)\textsubscript{dv}. In both cases where 100\( \mu \)M ddCTP was used, DNA synthesis initiated from the pBR322 origin was minimal, if any. On the other hand, pOri-D1 initiated its DNA synthesis only at a low ddCTP concentration apparently from pBR322 origin (Sutcliffe 1978), as its profile was the same as that of pBR322. These results demonstrate that a minimal \( \lambda \) structure necessary for the Q- and P-dependent initiation of replication resides in the 350-bp fragment of pOri-l, which carries the ori-repeats together with an A+T-rich stretch. Deleting a 46-bp A+T-rich region abolished the initiation ability, even though the remaining region retains the ability to bind the Q protein (Fig. 12).
### Table 3. Structure of hybrid plasmids containing λ ori fragments and Their replication activities in vitro.

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Structure of cloned λ fragment</th>
<th>DNA synthesis (pmoles)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>O and P proteins +</td>
</tr>
<tr>
<td>A433</td>
<td>733B B793 E1147</td>
<td></td>
</tr>
<tr>
<td>pOA-4</td>
<td>ice oop ori-repeats</td>
<td>74.4 150.4</td>
</tr>
<tr>
<td>pOri-D1</td>
<td>E1101</td>
<td>80.8 89.6</td>
</tr>
<tr>
<td>pOri-1</td>
<td></td>
<td>58.4 134.4</td>
</tr>
<tr>
<td>pBR322</td>
<td></td>
<td>72.0 80.8</td>
</tr>
<tr>
<td>λadv021</td>
<td></td>
<td>1.1 248.0</td>
</tr>
</tbody>
</table>

As indicated, λ fragments were inserted in pBR322 by substituting the BamHI-EcoRI region. A, B and E represent cutting sites of AluI, BglII and EcoRI on the λ fragment, and the numbers indicate their positions on the λ DNA sequence (see Fig. 2). DNA structure of the ice (○), oopRNA-coding region (△), and ori-repeats (●●●) are indicated. DNA synthesis was measured as described in legend to Fig. 11, using 8µg/ml of each plasmid DNA in the presence or absence of O (20µg/ml) and P (16µg/ml) proteins. Results are expressed as total dNMP incorporated.
Figure 19. Initiation site of DNA synthesis in recombinant plasmids carrying various λ fragments around ori.

(A) Plasmid DNAs (8μg/ml) were incubated in 25μl of standard reaction mixture containing Q protein (20μg/ml), P protein (16μg/ml) and 25μM ddCTP (for pBR322 and pOri-D1) or 100μM ddCTP (for pOA-4 and pOri-1). Different concentrations of ddCTP were used, as the optimal conditions for using ddCTP were different among the plasmids used here. The DNAs were treated as in Fig. 18, except that they were digested with DdeI plus SalI (lanes 1,2) or DdeI Plus EcoRI and SalI (lanes 3,4). The digests were electrophoresed and autoradiographed. Lanes 1 and 2 and lanes 3 and 4 are the results obtained from independent electrophoreses. (1) pOri-1; (2) pOA-4; (3) pOri-D1; and (4) pBR322 (see Table 3). Arrowheads indicate the most highly labeled fragments. Numbers represent the size (bp) of the fragments. Bands 166 and 163 overlap in all the samples; in addition, bands 112 and 105 overlap in lane 3. (B) relative intensity of radioactivity calculated from A (see Fig. 18B). At the bottom of the figure are shown cleavage sites of DdeI (Δ), SalI (△) and EcoRI (▪), the origin and direction of replication (arrow), locations of tetracycline (Tet) and ampicillin (Amp) resistance genes (data from Sutcliffe, 1978). The regions corresponding to the inserted λ fragments are indicated by heavy lines, and the positions of Ice and ori are shown under these lines. For cleavage sites in λ DNA, see Fig. 18A. Numbers indicate the locations of the fragments in A on the inserted λ fragment.
In this paper I reported purification and some properties of the two λ-coded initiator proteins, Q and P, and in vitro initiation of λdv DNA replication in the presence of these initiator proteins.

The initiation site for DNA replication in vitro was shown to be located at, or very close to, the four 19-bp ori-repeats in the λ genome. An another site, named ice, was once proposed as a possible site for switching from primer RNA to DNA synthesis (Lusky and Hobom, 1979a,b); however, this was not found to be the case.

The minimal region of λ DNA that can replicate covers the ori-repeats and A+T-rich segment to the right of it. The ori-repeats comprise the site of Q-protein binding. The A+T-rich segment has a nucleotide sequence similar to that found in the primase action site in the bacteriophage G4 genome. No region further to the right of it, including the palindromic structure (see Fig. 2), was necessary for replication, nor was the region covering ice and oop. This does not necessarily mean, however, that such structures are unnecessary for in vivo replication. It is possible that an autonomously replicating DNA carries many accessory structures that enhance or control replication of the genome.

The E.coli extract used in the in vitro replication system (fraction II) allows replication of oriC DNA, a minichromosome of E.coli. Addition of the λ-coded Q and P proteins diverts this system to act upon λ DNA, in analogy with the event occurring in vivo. Since λ DNA replication depends on all the known host functions needed for E.coli chromosome replication, except for dnaA and dnaC functions (for review, see skalka 1977), there is a good possibility that the λ-coded Q and P proteins substitute for the dnaA and dnaC functions. Further studies on the functions of these proteins will clarify this point. Among the complex host factors, DNA gyrase and RNA polymerase have been shown to play a role in λ replication by this work.

The Q protein interacts with the 19-bp ori-repeats located at, or very close to, the initiation site of λ dv DNA synthesis. The ratio of added Q protein to ori DNA needed to maximally initiate DNA replication was approximately the same as that needed to saturate the four ori-repeats in binding studies. Genetic data suggest that the Q and P
proteins carry out molecular interactions. I have not been able to demonstrate physically the $O-P$ interaction, nor to obtain any sign of such an interaction by adding $P$ protein to the $O-\text{ori}$ binding system or by adding $Q$ protein to the $P-\text{dnaB}$ interaction. Thus, the $O-P$ interaction does not seem to be stable even if it occurs. I found that approximately equal amounts of the $Q$ and $P$ proteins were needed for maximal replication \textit{in vitro}. The $P$ protein interacts with $\text{dnaB}$ protein. Further studies, including detection of complex formation, and analyses of their functions, are awaited.

A rifampicin-sensitive reaction, RNA polymerase action, was shown to be involved in the early stage of $\lambda$ replication initiation \textit{in vitro}. The rifampicin sensitivity is lost during a short preincubation before the addition of $Q$ and $P$ proteins and nucleoside triphosphates. If the reaction involves an indispensable RNA synthesis, the product must be a very efficient RNA molecule or its promoter must have higher affinity to RNA polymerase than that of primer RNA in ColEl. Alternatively, if it represents formation of a rifampicin-resistant complex, it must be more stable than the complex used for primer RNA synthesis in ColEl. The RNA-polymerase-dependent RNA synthesis is not a prerequisite for the $Q$ protein and ori-repeats interaction. On the other hand, we do not know whether or not the binding of the $Q$ protein and ori-repeats interaction. It must be noted that the origin region of the $\lambda$ DNA in the recombinant plasmids (e.g., pOA-4 and pOri-l) studied in this work are transcribed only by readthrough transcription originating from the vector DNA region. These plasmids carry a promoter of the $\beta$-lactamase gene at about 180-bp apart from the EcoRI site corresponding to the position 1320 on $\lambda$DNA sequence. The transcription proceed away from $\lambda$ sequence and does not pass through $\text{ori}$ region. However, these conformation is analogous to the $\text{riC5b}$ mutant in which a new promoter is formed by mutations located within the $Q$ gene at position 1246 and 1270 (Furth et al. 1982). This is a replication-active mutant, even when $\text{pR}$ promoter is inactive. The relationship between primer RNA synthesis, transcriptional activation, and the complex formation around $\text{ori}$ region of DNA is an important problem that awaits further elucidation.

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Acknowledgments

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