Activation of Replication Origins in \( \phi 29 \)-related Phages
Requires the Recognition of Initiation Proteins to Specific Nucleoprotein Complexes*

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Protein p6 of \textit{Bacillus subtilis} phage \( \phi 29 \) activates the initiation of viral DNA replication by forming a multimeric nucleoprotein complex at the origins of replication, located at both ends of the linear genome. This activation requires a precise positioning of the protein p6 array with respect to the initiation site. To investigate this activation mechanism, we have purified the \( \phi 29 \) protein p6 counterparts from the related phages Nf and GA-1 and analyzed the formation of complexes with DNA. In the homologous protein p6-DNA complexes the \( \phi 29 \) and Nf protein arrays showed an identical positioning, different than that of the GA-1 protein array. In contrast, in the heterologous complexes the protein showed a different arrangement except in the case of the Nf protein-\( \phi 29 \) DNA complex. We have also purified the proteins involved in the initiation of replication (terminal protein and DNA polymerase) from phages Nf and GA-1 and measured the ability of the different p6 proteins to activate homologous and heterologous replication origins. The results obtained indicate that the activation requires not only the formation of a specific nucleoprotein complex but also its specific recognition by the proteins involved in the initiation of DNA replication.

\textit{Bacillus subtilis} phage \( \phi 29 \) has a linear, double-stranded DNA with a terminal protein (TP)\(^1\) covalently linked to the 5’ ends. The \( \phi 29 \) genome replicates by a protein-priming mechanism in which the phage DNA polymerase interacts with a free TP molecule forming a heterodimer that recognizes the origins of replication located at both genome ends. The viral DNA polymerase catalyzes the covalent linkage of the 5’-terminal nucleotide (dAMP) to the TP, that acts as a primer. The initiation complex, TP-dAMP, is further elongated by the phage DNA polymerase by a strand displacement mechanism (reviewed in Ref. 1). Among the proteins required \textit{in vivo} for DNA replication (2, 3), protein p6 has been shown to activate \textit{in vitro} the initiation of \( \phi 29 \) DNA replication (4) by forming a multimeric nucleoprotein complex at the origins of replication (5, 6). In addition, protein p6 is able to bind to the whole \( \phi 29 \) DNA, and a role in genome organization has been proposed (7). Protein p6 forms dimers in solution (8) that interact with DNA through the minor groove every 24 bp, giving rise to a large multimeric complex in which a DNA right-handed superhelix wraps around a protein p6 core (9). The path followed by the DNA in the complex has been determined (10). When protein p6 binds to the origins of replication, it recognizes DNA signals located between positions 62–125 and 46–68 from the right and left termini, respectively, that act as nucleation sites from which the complex is extended by cooperativity covering approximately the terminal 250–300 bp (6). Protein p6 may, thus, provide the adequate structural framework to activate the initiation of \( \phi 29 \) DNA replication. The distance from the protein p6 nucleation site to the initiation site of DNA replication determines the phase in which the protein array is located. A precise protein p6 binding phase is crucial for the activation of the replication origins (9). Thus, protein p6 did not activate a DNA fragment with a 4-bp insertion between the nucleation and the initiation sites, in which the protein p6 moiety was located out of phase with respect to the replication origins, but it recovered the activity when the phase was restored in a construction with a 24-bp insertion (9). These results are consistent with a specific recognition of the protein p6 nucleoprotein complex by the proteins involved in the initiation of \( \phi 29 \) DNA replication, namely TP and/or DNA polymerase. In this paper we took advantage of \( \phi 29 \)-related phage systems to study the specificity of the activation of different replication origins by protein p6.

Based on serological properties, physical maps, peptide maps, and DNA terminal sequences, a number of \( \phi 29 \)-related phages have been classified in three groups. Phage \( \phi 29 \), the most thoroughly studied of them (for a review, see Ref. 11) belongs to group A, phages Nf and M2 belong to group B, and phage GA-1, evolutionarily less related (12), belongs to group C. All of them have linear double-stranded DNAs with short inverted terminal repeats and a TP required for transfection (13–15), and they share with \( \phi 29 \) a similar protein-priming mechanism for DNA replication\(^2\) (16). Preliminary studies showed that DNase I footprints of \( \phi 29 \) protein p6 with Nf and GA-1 DNA terminal fragments were different than those observed with \( \phi 29 \) DNA (17). Thus, we have purified the \( \phi 29 \) protein p6 counterparts from phages Nf and GA-1 to study the formation of nucleoprotein complexes with homologous and heterologous p6 proteins, at the origins of replication of \( \phi 29 \), Nf, and GA-1 DNAs. In addition, we have partially purified initiation proteins (TP and DNA polymerase) from Nf- and

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1 The abbreviation used is: TP, terminal protein; bp, base pair(s).

2 B. Illana, L. Blanco, and M. Salas (1996) \textit{J. Mol. Biol.}, in press.
GA-1-infected cells, to test the activity of the different protein p6-DNA complexes. The conclusion reached by these studies is 2-fold; first, the three p6 proteins, despite their amino acid sequence homology, form stereospecific complexes only with their own DNA and second, the optimal activation of the replication origins in the three viral systems requires both the formation of a specific nucleoprotein complex and the homologous TP and DNA polymerase, implying that specific interaction between the protein p6-DNA complex and TP and/or DNA polymerase plays an important role in the mechanism of activation.

**EXPERIMENTAL PROCEDURES**

**Chemicals, Nucleotides, Oligonucleotides, and Enzymes—**Gluutaraldehyde was obtained from Serva. Synthetic oligonucleotides for sequencing were obtained from Isogen. The fmol® DNA sequencing system and topoisomerase I were from Promega. Chloroquine was obtained from Sigma. [α-32P]dATP (3000 Ci/mmol) was purchased from Amer sham International plc. DNase I was obtained from Worthington; restriction endonucleases and Klenow fragment of Escherichia coli DNA polymerase I were obtained from New England Biolabs. Micrococcal nuclease was obtained from Pharmacia Biotech Inc.

**Purification of Protein p6 from Nf and GA-1—**P6 proteins were purified from *B. subtilis* strains 110NA and GRI, infected with phages Nf and GA-1, respectively. Cells were grown at 30°C up to an optical density of 0.5 and then infected to 10. Cells were harvested at 50 min after infection, disrupted by grinding with alumina, and suspended in buffer A (50 mM Tris-HCl, pH 7.5, 5% glycerol) containing 1 mM NaCl. DNA was removed by addition of polyethyleneimine up to 0.25%, after adjusting absorbance at 260 nm to 120 units/mI, and centrifugation for 10 min at 10000 × g. The supernatant was made 0.5% NaCl with buffer A and 0.4 and 0.6 μM NaCl, respectively. Protein samples were precipitated as above and chromatographed in a phosphocellulose column, where p6 proteins were recovered in the supernatant after centrifugation as above. After precipitation with ammonium sulfate up to 70%, the samples were dissolved in buffer A and applied to a heparin-agarose column. Protein p6 from Nf and GA-1 were eluted with buffer A containing 0.4 and 0.6 mM NaCl, respectively. Protein samples were precipitated as above and chromatographed in a phosphocellulose column, where p6 proteins were recovered in the flow-through. After this step both proteins were at least 90% homogeneous. N-terminal sequencing was performed in an Applied Biosystems 473A pulsed liquid protein sequence, for 35 and 6 cycles in the GA1 and the Nf protein, respectively.

**DNA Sequence of Gene 6 from GA1—**The N-terminal amino acid sequence of the p6 protein from GA1 protein and p6 protein from a degenerated 22-mer oligonucleotide complementary to the gene 6 region correspond to amino acids 14–21. DNA sequencing of GA1 gene 6 was done using genomic DNA as template and the dideoxynucleotide chain-termination method (18) with *Thermus aquaticus* DNA polymerase (19). The sequence of the beginning of gene 6 was obtained using a complementary 20-mer oligonucleotide corresponding to amino acids 40 to 34.

**Purification of TP and DNA Polymerase from Nf and GA-1—**TP and DNA polymerase from Nf and GA-1 were partially purified until no activity can be restored by alkali removal of the peptide (25). Peptides from the corresponding TPs, covalently attached to their 5′ termini, that remained after proteinase K digestion of genomic DNAs, were removed by incubation for 2 h at 37°C with 0.5 mM piperidine (24). The reaction mixture in the initiation of replication assay contained, in 25 μL, 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 20 mM ammonium sulfate, 0.1 mM dithiothreitol, 0.25 μM [α-32P]dATP (0.25 μCi), 125 ng each of Nf DNA polymerase and TP, 6 fmol of the indicated DNA fragment or 500 ng of the corresponding TP-DNA, and the indicated amounts of the corresponding protein p6. The reaction was carried out for 5 min at 15°C and then stopped by adding EDTA up to 10 mM. The samples were incubated for 30 min at 37°C with 25 units of micrococcal nuclease in the presence of 35 mM Tris-HCl, pH 8.8, and 14 mM CaCl₂, and the reaction was stopped by addition of 20 mM EDTA and 0.1% SDS. The samples were filtered through Sephadex G-50 spin columns in the presence of 0.1% SDS. The initiation complex formed was analyzed by SDS-polyacrylamide gel electrophoresis as described (24). Densitometric scans of the exposed films were performed in a Molecular Dynamics 300A densitometer. Alternatively, the Fuji imaging plate type BAS III and the Bioimaging analyzer BAS L1500 were used for quantitation.

**RESULTS**

**Gene 6 from Phages Nf and GA-1: Nucleotide Sequence and Purification of Gene Products—**The nucleotide sequence of gene 6 from phage GA-1 was obtained by genomic sequencing, and the amino acid sequence of the corresponding protein was deduced. Fig. 1 shows the alignment of the amino acid sequence with those of p6 proteins from Nf (26) and Nf. The Nf and GA-1 sequences have 52 and 34% identity and 73 and 58% homology, respectively, with the *B. subtilis* cells. The N-terminal sequences of the purified proteins from Nf and from

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3 M. Monsalve, personal communication.

4 B. Nuez, unpublished results.
GA-1 (6 and 35 amino acids, respectively) were determined and shown to be identical to those obtained from the nucleotide sequence.

**Dimer Formation of Nf and GA-1 Protein p6**—Protein p6 from φ29 forms dimers in solution (8). Due to the high degree of sequence homology a similar behavior could be expected for the Nf and GA-1 proteins. We have directly assayed the formation of dimers by glutaraldehyde cross-linking (20); the results shown in Fig. 2 indicate that p6 proteins from Nf and GA-1 were able to form dimers. In fact, the percentage of Nf and GA-1 dimers was higher than in the case of φ29 protein p6, at the same protein concentration. We have also tested their ability to form heterodimers by mixing proteins from different sources. The presence of a band with electrophoretic mobility between φ29 and Nf dimers indicates that φ29 protein p6 is able to interact with the Nf protein. In contrast, the GA-1 protein did not form heterodimers either with the φ29 or the Nf proteins. These results are in agreement with the closer sequence homology between the φ29 and the Nf with respect to the GA-1 p6 protein.

**Complex Formation of Nf Protein p6 with φ29 DNA**—The structure of the φ29 protein p6-DNA complex at the genome ends was determined mainly from footprinting studies (9). Hydroxyl radical footprinting provided information, at high resolution, about the protein contacts with the DNA. The complex formed by the φ29 protein p6 with φ29 DNA terminal fragments gave rise to a repetitive pattern of 3–4-bp protections evenly distributed every 12 bp along the fragments, defining the binding periodicity of the monomer and the positioning of the protein array with respect to the site where initiation of replication takes place (9). The protein p6 binding phase has been shown to be identical in the two φ29 genome ends and critical for the activation of the replication origin (9). Hydroxyl radical footprinting experiments were performed with Nf protein p6 and a φ29 DNA right terminal fragment to study whether Nf protein p6 could form a nucleoprotein complex, and in that case, to compare its structure with that formed with φ29 protein p6. As shown in Fig. 3A, Nf protein p6 was able to bind to φ29 DNA. Moreover, the binding periodicity and the positioning of the monomers was the same as that of the φ29 protein.

On the other hand, DNase I footprinting of the φ29 protein p6 on φ29 terminal fragments revealed a pattern of strong hypersensitive bands, evenly distributed every 24 bp, reflecting strong structural distortions in the DNA structure, probably kinks, induced by the binding of the φ29 protein p6 dimers (9). Therefore, the location of the hypersensitive sites indicates the positioning of the protein dimers and defines the DNA binding phase. As shown in Fig. 3B, Nf protein p6 displays the same positioning at the φ29 DNA terminal fragment as the φ29 protein p6. However, the small differences observed in the hypersensitivities, specially at the genome end, suggest that the structure of the Nf and φ29 protein p6 dimers may slightly differ. Fig. 3B also shows that the binding affinities are different, 0.1 µg of the φ29 protein gave rise to complex formation with φ29 DNA, while 1.5 µg of the Nf protein did not change the DNase I digestion pattern. Thus, φ29 protein p6 binds to φ29 DNA with higher affinity than the Nf protein.

φ29 protein p6 does not recognize a specific sequence, since the main recognition signals mapped in φ29 DNA do not show any sequence homology (6). Considering that protein p6 induces strong bends in the complexed DNA (66° every 12 bp) (10), it has been proposed that protein p6 would recognize regions with bendable properties that would favor the formation of the complex (6). The impaired cutting of hydroxyl radicals to DNA narrowed minor grooves provides an experimental tool to sense bent (and bendable) sequences (27). We have compared the hydroxyl radical footprints of naked and protein p6-complexed φ29 DNA to see whether there is any similar cutting tendency. Fig. 3C shows densitometric scans of the footprints where some regions of φ29 DNA (shaded areas) show a digestion pattern that resembles that of the complex with φ29 protein p6. Therefore, the region that spans from positions 70 to 120 could be a candidate to be a signal for protein p6-DNA recognition; in fact, it is comprised in the previously mapped main recognition signals (6). Thus, this approach could be of help to localize putative recognition signals for protein p6.

In summary, Nf protein p6 forms a nucleoprotein complex with a φ29 DNA terminal fragment in which the protein binds to the same positions as the φ29 protein, although the detailed structure of the complexes, specially at the initiation of replication site, may not be exactly the same.

**Complex Formation of Protein p6 from Nf and φ29 with Nf DNA**—We have also studied the complex formation of Nf protein p6 with a Nf DNA terminal fragment and compared with that of φ29 protein p6. The hydroxyl radical footprinting of Nf protein p6 with Nf DNA (Fig. 4A) indicates that the protein array is identically positioned as that of the φ29 protein in the

![Fig. 1. Amino acid sequences of p6 proteins from phages φ29, Nf, and GA-1.](image)

![Fig. 2. Dimer formation by p6 proteins from φ29, Nf, and GA-1.](image)
Complex formation with φ29 DNA right terminal fragment. Hydroxyl radical (A) and DNase I (B) footprints of p6 proteins from φ29 and NF with a φ29 DNA right terminal fragment (273 bp). Footprints were performed as described under “Experimental Procedures” with the indicated amounts of p6 proteins. Numbers are nucleotide positions from the genome end. The diagrams are schematic representations of the nucleoprotein complexes. Ellipsoids represent protein p6 dimers and rectangles the φ29 DNA fragment. The vertical lines inside the rectangles indicate the 24-bp binding phase of the dimers of φ29 protein p6 in φ29 DNA. Empty and cross-hatched symbols indicate φ29 and NF, respectively. The genome end is located at the left of the rectangle. C, densitometric scan of the hydroxyl radical footprint of φ29 DNA in the absence and presence of φ29 protein p6, shown in A. Numbers indicate nucleotide positions from the genome ends.

φ29 DNA (Fig. 3A). In contrast, the protections of φ29 protein p6 monomers on NF DNA showed a ~4-bp shift with respect to those of the NF protein, and therefore, the φ29 and NF proteins lay on the same DNA with a different positioning, implying that, despite the high degree of homology between the two proteins, they recognize different signals in the same DNA.

These results were further confirmed by DNase I footprinting. The DNase I hypersensitivities pattern indicates that the binding phase of the NF protein to NF DNA (Fig. 4B) was the same as that of the φ29 protein to φ29 DNA (Fig. 3B) and also that the binding of φ29 and NF protein p6 dimers to NF DNA was shifted ~8 bp (Fig. 4B). In addition, they corroborate that the two proteins have different nucleation sites in NF DNA. Thus, 0.7 μg of the NF protein p6 bound to the terminal region of the DNA fragment (up to position ~140) but not to the more distal region (from position ~140 on), while with the same amount of φ29 protein p6 preferential binding to the distal region (from position ~150 on) was observed. Therefore, the main recognition signals for the binding of both proteins, and thus the nucleation sites for the formation of the complexes, lay at different regions in the NF DNA terminal fragment. We have used the method described above to predict DNA signals that could be recognized by NF protein p6. Fig. 4C shows densitometric scans of hydroxyl radical footprints; two regions in the NF DNA fragment (shaded areas) present similar cutting tendency than in the complex with the NF protein. One of them is located within the ~150 terminal nucleotides, where NF protein p6 preferentially binds to NF DNA.

In summary, both hydroxyl radical and DNase I footprinting indicate that protein p6 from NF and φ29 form complexes with a NF DNA right terminal fragment. Although the location of the NF protein is the same as that of the φ29 protein on φ29 DNA, the positioning of both proteins with respect to the site of initiation of NF DNA replication is different.

Complex Formation of GA-1 Protein p6 with GA-1, φ29, and NF DNAs—Protein p6 from GA-1 formed also a complex with GA-1 DNA, as shown by hydroxyl radical footprinting (Fig. 5A). Strikingly, the periodicity of the protected regions was 11 bp, instead the 12 bp previously observed with φ29 and NF p6 proteins. We can conclude, consequently, that the structure of the GA-1 complex is different from that of the NF or φ29 complexes. This different arrangement of GA-1 protein p6 on GA-1 DNA was confirmed by DNase I footprinting, as the pattern of hypersensitive bands induced by the protein dimers showed a periodicity of 22 bp, instead of 24 bp (Fig. 5B). Densitometric scans of hydroxyl radical footprints of GA-1
DNA showed short regions in which the cutting tendency was the same as in the presence of the protein (shaded areas) and, as described above, could correspond to putative recognition signals.

It was interesting to know whether the GA-1 protein p6 could bind to φ29 and Nf DNA, and if so, what was its binding periodicity. The DNase I footprints of the GA-1 protein p6 to φ29 and Nf DNA right terminal fragments shown in Fig. 5B indicated changes in the DNase I digestion pattern, although the lack of a clear periodicity of protections and hypersensitivities indicates that they do not form a homogeneous and regular multimeric complex (see Figs. 3B and 4B for comparison). On the other hand, we also studied the binding of both φ29 and Nf p6 proteins to GA-1 DNA. DNase I footprinting studies revealed that both proteins formed complexes with a binding periodicity of dimers of 24 bp. Moreover, the protein moieties did not show a single binding phase, and they were shifted with respect to the one observed with their homologous DNA (data not shown).

In summary, GA-1 protein p6 forms a nucleoprotein complex with the GA-1 right origin of replication structurally different than those of φ29 and Nf p6 proteins with their own DNA. In contrast, GA-1 protein p6 does not form a clearly structured complex with φ29 and Nf DNA.

**Induction of Positive Supercoiling by Protein p6 from Nf and GA-1**—One of the most striking structural features of the φ29 nucleoprotein complex is the right-handed sense of the superhelical path followed by the DNA. This was shown by the induction of positive supercoiling by protein p6 in covalently closed circular DNA in the presence of topoisomerase I (5, 10). Due to the structural similarities between the φ29 and the Nf nucleoprotein complexes, it could be expected that Nf protein p6 would induce also positive supercoiling; although in the case of the GA-1 protein, where the binding periodicity was lower, the sign of the induced supercoiling could not be anticipated. As shown in Fig. 6 both Nf and GA-1 protein p6 induced positive supercoiling in a covalently closed circular DNA. In fact, the induction of positive supercoiling was higher with 6 μg of GA-1 protein p6 than with 10 μg of Nf protein p6, but lower than with 3 μg of φ29 protein p6. The different amounts required reflect, most probably, the binding affinity to the plasmid DNA. Therefore, although the structural parameters defining the GA-1 nucleoprotein complex differ from those of φ29 and Nf, the overall path of the DNA has the same sense in all of them.

**Activation of Initiation of DNA Replication**—The activity of homologous and heterologous protein p6-DNA complexes was tested using, in a first approach, the TP-containing viral genomes. Since TP-DNA templates are only active with homologous TPs, both in vivo (28, 29) and in vitro (25), we partially purified the initiation proteins (TP and DNA polymerase) from Nf- and GA-1-infected *B. subtilis* cells, until no contaminant protein p6 was detected. Thus, we measured the activity of the different p6 proteins in an assay of initiation of DNA replication with TP-DNAs from φ29, Nf, and GA-1 and their own initiation proteins. The results, shown in Fig. 7, indicate that φ29, Nf, and GA-1 p6 proteins activated only homologous TP-DNAs. The lack of activation of heterologous p6 proteins with Nf and GA-1 TP-DNAs could be expected, since the p6 proteins were not correctly positioned in the nucleoprotein complexes. However, the activation of Nf protein p6 using φ29 TP-DNA as template was very poor, despite the fact that the binding phase of the Nf protein p6 in the φ29 DNA complex is apparently the same as that of the φ29 protein p6 (see Fig. 3A). An explanation consistent with this finding could be that activation requires specific recognition of initiation proteins to the nucleoprotein complex.

To test whether the protein p6 activation requires specific initiation proteins, we have assayed homologous p6-DNA complexes with heterologous initiation proteins. We have used TP-free DNA terminal fragments, since TP-DNA is not an

**Fig. 6. Induction of positive supercoiling by φ29, Nf, and GA-1 p6 proteins.** Plasmid pUC 19 Ω was relaxed in the presence of topoisomerase I, incubated with the indicated amounts of p6 proteins, and then further treated with topoisomerase I. Samples were analyzed by two-dimensional gel electrophoresis as described under “Experimental Procedures.”
The detailed mechanism by which φ29 protein p6 activates the initiation of φ29 DNA replication is not known. The re-
Fig. 8. Activation of initiation of DNA replication by p6 proteins using TP-free DNA terminal fragments from φ29, Nf, and GA-1. Initiation complex formation with TP-free DNA terminal fragments from φ29 (A, D), Nf (B, E), and GA-1 (C, F), and the indicated amounts of protein p6 was performed as described under “Experimental Procedures.” The source of the initiation proteins is also indicated. The initiation complexes formed were analyzed by SDS-polyacrylamide gel electrophoresis and quantitated by densitometric scans of exposed films. Activation values were normalized to that obtained in the absence of protein p6. Diagrams are schematic representations of the different protein p6-DNA complexes, in which p6 dimers are represented by ellipsoids and DNA fragments by rectangles. Empty, cross-hatched, and shaded symbols indicate φ29, Nf, and GA-1, respectively. Vertical lines in rectangles represent the 24-bp binding phase of protein p6 dimers from φ29 in φ29 DNA. The genome end is located at the left of the rectangles.
requirement of a precise positioning of the protein $p_6$ in the complex with respect to the DNA end (6) could be explained by two nonmutually exclusive hypothesis: (i) a direct interaction between protein $p_6$ and the initiation proteins, TP and/or DNA polymerase, in a way similar to that proposed for protein NF1 in adenovirus (30, 31) and (ii) a local melting at the replication origin induced by protein $p_6$, as it is the case of the DNA protein at ori C (32), the O protein at the $\lambda$ origin of replication (33), or the T antigen at the SV40 origin of replication (34).

The results obtained here on the activation of the replication origins support the hypothesis of a specific protein-protein interaction. Activation of the $\phi 29$ replication origin by $\psi 29$ protein $p_6$ occurred with $\phi 29$, but not with $Nf$ initiation proteins. Similarly, a small activation of GA-1 replication origin by GA-1 protein $p_6$ was observed with GA-1, but not $\phi 29$, initiation proteins. In the case of the $Nf$ origin of replication, the same level of activation required about a 3-fold higher amount of $Nf$ protein $p_6$ with $\phi 29$ than with $Nf$ initiation proteins. These results indicate that protein $p_6$ specifically recognizes homologous initiation proteins in the $\phi 29$ and GA-1 cases; however, $Nf$ protein $p_6$ was able to recognize also $\phi 29$ initiation proteins, although with lower affinity. The low activation of $\phi 29$ DNA by $Nf$ protein $p_6$ could be due to structural differences in the nucleoprotein complex with respect to that of the $\phi 29$ protein $p_6$, as shown by the small differences observed in the Dnase I footprints (see Fig. 3B). This difference could avoid an efficient contact with the $Nf$ initiation proteins.

The high number of protein $p_6$ copies, nearly a million per cell at late times after infection, together with the fact that protein $p_6$ can form complexes in vitro with almost the entire $\phi 29$ DNA (7), led us to propose that protein $p_6$ could be a histone-like protein playing a structural role in organizing the genome (34). In addition to the structural role forming large nucleoprotein complexes, the results presented in this paper suggest that protein $p_6$, by means of specific recognition of initiation proteins, may recruit them to the replication origins.

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