Formation of a Covalent Complex between the Terminal Protein of Pneumococcal Bacteriophage Cp-1 and 5'-dAMP

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Received 30 September 1985/Accepted 27 December 1985

Incubation of extracts of Cp-1-infected *Streptococcus pneumoniae* with [α -³²P]dATP produced a labeled protein with the electrophoretic mobility of the Cp-1 terminal protein. The reaction product was resistant to treatment with micrococcal nuclease and sensitive to treatment with proteinase K. Incubation of the ³²P-labeled protein with 5 M piperidine for 4 h at 50°C released 5'-dAMP, indicating that a covalent complex between the terminal protein and 5'-dAMP was formed in vitro. When the four deoxynucleoside triphosphates were included in the reaction mixture, a labeled complex of slower electrophoretic mobility in sodium dodecyl sulfate-polyacrylamide gels than the terminal protein-dAMP complex was also found, indicating that the Cp-1 terminal protein-dAMP complex can be elongated and, therefore, that it is an initiation complex. Treatment of the ³²P-labeled terminal protein-dAMP complex with 5.8 M HCl at 110°C for 2 h yielded phosphothreonine. These results, together with the resistance of the terminal protein-DNA linkage to hydroxylamine, suggest that the Cp-1 terminal protein is covalently linked to the DNA through a phosphoester bond between L-threonine and 5'-dAMP, namely, a O-5'-deoxyadenylyl-L-threonine bond.

Bacteriophage Cp-1 from *Streptococcus pneumoniae* contains a double-stranded linear DNA of about 18,000 base pairs (21) that has a terminal protein (TP) of 28,000 daltons at the two 5' ends (8) and a 236-base-pair long inverted terminal repeat (6). Several Cp-1-related pneumococcal phages have recently been isolated and characterized as also having a TP (14) and a long inverted terminal repeat (5). Bacteriophage ϕ 29 of *Bacillus subtilis* and adenovirus also have DNAs with inverted terminal repeats 6 base pairs (7, 28) and ~100 base pairs (25) long, respectively, and a specific viral protein covalently linked at the two 5' ends through a phosphodiester bond between serine and the 5'-terminal nucleotide (4, 9). PRD1, a bacteriophage that infects gram-negative hosts such as *Escherichia coli* and *Salmonella typhimurium*, also has a TP covalently bound to its DNA through a phosphodiester bond between tyrosine and 5'-dGMP (1). Other linear DNAs with TPs are plasmid pSLA2 from *Streptomyces* spp. (10), the killer plasmids pGKL1 and pGKL2 from yeasts (13), and S1 and S2 mitochondrial DNAs from maize (11). In addition, TPs are also present at the 5' ends of the RNAs of animal viruses such as poliovirus, foot and mouth disease, encephalomyocarditis, vesicular exanthema, and infectious pancreatic necrosis as well as plant viruses such as cowpea mosaic, tobacco ringspot, southern bean mosaic, tobacco etch, potato leafroll, and pea enation mosaic (3).

Rekosh et al. (20) first proposed a model in which the TP serves as a primer for the initiation of linear adenovirus DNA replication. Strong evidence in support of this model has been obtained in vitro for both ϕ 29 (22) and adenovirus (24). It has been shown that a free molecule of the TP (or its precursor in the case of adenovirus), in the presence of a viral DNA polymerase and the TP-DNA complex, reacts with the 5'-terminal deoxynucleoside triphosphate (dNTP)

to form a TP-deoxynucleoside monophosphate covalent complex that provides a 3'-OH group that is used as a primer for elongation by the DNA polymerase.

In this paper, we show that extracts from Cp-1-infected *S. pneumoniae* incubated with dATP catalyze the formation of an initiation complex between the TP and 5'-dAMP, the terminal nucleotide at both 5' ends of Cp-1 DNA (6). In the presence of the four dNTPs the initiation complex was elongated. We also show that the Cp-1 TP is linked to the 5'-terminal nucleotide dAMP through a phosphoester bond with threonine.

MATERIALS AND METHODS

Bacterial strains and phage. Strain R6, a derivative of *S. pneumoniae* R36A (Rockefeller University stock), was used as the indicator bacterium. A streptomycin-resistant strain, R6st, constructed by genetic transformation, was used for the growth of the phage. Bacteriophage Cp-1 has been described elsewhere (21).

Media and chemicals. M3 medium containing tryptone (17 g/liter; Difco Laboratories), glucose (2.5 g/liter), NaCl (5 g/liter), neopeptone (5 g/liter; Difco), and 5 mM potassium phosphate buffer (pH 8.0) was used for the growth of the phage.

Growth and purification of the phage and preparation of cell extracts. Details of the preparation and purification of the phage have been described previously (8), except that M3 medium was used and the host bacteria were infected at a multiplicity of infection of 10. Cell extracts were prepared essentially as described by Watabe et al. (27) with slight modifications. *S. pneumoniae* R6st cells were grown at 37°C to 6.2×10^7 cells per ml in M3 medium. The cells were infected with Cp-1 at a multiplicity of infection of 10 and incubated at 30°C for 90 min. Infected cells were lysed as described previously (27), except that lysozyme and EDTA were not added. After high-speed centrifugation, solid am-

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FIG. 1. Labeling of the TP by incubation of extracts from Cp-1-infected cells with $[\alpha\text{-}^{32}\text{P}]\text{dATP}$. (A) Extracts from Cp-1-infected cells were incubated with $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ as described in Materials and Methods and subjected to SDS-polyacrylamide gel electrophoresis (lane a). Lane b, DNA-TP complex isolated from phage Cp-1, labeled with ^{125}I as described previously (8), and treated with micrococcal nuclease to degrade the DNA. Lane c, ^{35}S -labeled $\phi 29$ structural proteins as molecular weight markers. (B) Extracts from uninfected (lane a) or Cp-1-infected (lane b) cells were incubated with $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ as in panel A. Lane c, As for lane b, except that $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ was used instead of $[\alpha\text{-}^{32}\text{P}]\text{dATP}$. Lane d, ^{35}S -labeled $\phi 29$ structural proteins as molecular weight markers. K = 1,000.

monium sulfate was added to the supernatant to 70% saturation. Samples (1.5 ml) were centrifuged for 15 min in an Eppendorf microcentrifuge, and the pellets were stored at -20°C until used. Each pellet was suspended in 100 μl of 50 mM Tris hydrochloride (pH 7.5) before use. The protein concentration of the extracts was approximately 15 mg/ml. Control uninfected cells were processed the same way as the infected cells. The DNA-protein complex from phage Cp-1 was isolated by treatment of phage particles with guanidinium hydrochloride as described previously (18).

Standard assay for the formation of the Cp-1 TP-DNA complex. For analytical experiments, the standard 50- μl reaction mixture contained 50 mM Tris hydrochloride (pH 7.5), 10 mM MgCl_2 , 3 mM ATP, 1 mM dithiothreitol, 0.25 μM $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ (410 Ci/mmol), 0.4 μg of Cp-1 DNA-TP complex, and 10 μl of cell extract. After incubation for 20 min at 30°C , the samples were processed, treated with micrococcal nuclease, and subjected to electrophoresis in polyacrylamide slab gels containing 10% acrylamide and 0.1% sodium dodecyl sulfate (SDS) as described previously (18). After electrophoresis, the gels were dried, and the radioactive bands were visualized by autoradiography with intensifying screens at -70°C .

Identification of the nucleotide linked to the TP. The TP-nucleotide complex was prepared as described above, except that 0.75 μM $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ in a final volume of 0.1 ml was used. After removal of the $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ by gel filtration on a Sephadex G-50 column, the samples were treated with micrococcal nuclease and subjected to SDS-polyacrylamide gel electrophoresis. The radioactive bands were cut out, the gels were crushed, and the protein was eluted overnight at 25°C with 50 mM Tris hydrochloride (pH 7.5) containing 0.1% SDS. After centrifugation in an Eppendorf microcentrifuge, the proteins in the supernatant were precipitated with 4 volumes of acetone in the presence of bovine serum albumin (20 $\mu\text{g}/\text{ml}$) as the carrier. Alkaline hydrolysis of the samples was performed in 5 M piperidine for 4 h at 50°C . Degradation products were identified by thin-layer chroma-

tography on polyethyleneimine-cellulose plates as described previously (18).

Elongation of the Cp-1 TP-dAMP complex. The reaction mixture was as described above, except that 1.5 μM $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ (410 Ci/mmol) was used. After incubation for 5 min at 30°C , dATP, dGTP, dCTP, and dTTP were added to a final concentration of 10 μM each and the mixture was further incubated for 15 min. The samples were treated with micrococcal nuclease as described above and, after the addition of EDTA to 10 mM and SDS to 0.1%, the samples were centrifuged in a Sephadex G-50 column equilibrated with 0.01% SDS and analyzed by electrophoresis in gels containing 20% acrylamide and 0.1% SDS.

Characterization of the bond between the Cp-1 TP and 5'-dAMP. The complex between the Cp-1 TP and 5'-dAMP was prepared as described above, except that the samples were not subjected to SDS-polyacrylamide gel electrophoresis. After micrococcal nuclease treatment, the samples were hydrolyzed in 5.8 M HCl in a sealed tube for 2 h at 110°C and subjected to bidimensional thin-layer analysis on cellulose plates (10 by 12 cm; Merck). In the first dimension, electrophoresis was performed in 2.5% formic acid–7.8% acetic acid (pH 2.0) (2) at 600 V. In the second dimension, chromatography in isopropanol-HCl- H_2O (70:15:15) (17) was developed. Xylene cyanol FF and acid fuchsin were used as visual markers, and phosphothreonine, phosphoserine, and phosphotyrosine (40 μg each; Sigma Chemical Co.), identified by ninhydrin staining, were used as phosphoamino acid markers.

RESULTS

Initiation of Cp-1 DNA replication in vitro. Extracts prepared from Cp-1-infected *S. pneumoniae* containing endogenous Cp-1 DNA allowed the incorporation of $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ into acid-insoluble material. When the reaction products were analyzed by SDS-polyacrylamide gel electrophoresis, a ^{32}P -labeled band was found (Fig. 1A, lane a). This band migrated to the same position as the ^{125}I -labeled TP of Cp-1 (8) (Fig. 1A, lane b). The ^{32}P -labeled band disappeared after treatment with proteinase K (results not shown). Uninfected extracts did not produce any band labeled at the Cp-1 TP position (Fig. 1B, lane a). On the other hand, no ^{32}P -labeled band was detected when $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ was used in place of $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ (Fig. 1B, lanes c and d). The maximum amount of ^{32}P -labeled Cp-1 TP band was obtained after 20 min at 30°C (Fig. 2A). The optimal temperature for this reaction was 30°C ; incubation at 37°C decreased to about 40% the amount of complex formed (Fig. 2B). The optimal Mg^{2+} concentration was about 10 mM, and no complex was detected when MgCl_2 was omitted from the reaction mixture (Fig. 2C). The effect of the ATP concentration is shown in Fig. 2D. Although ATP was not absolutely required, the addition of 3 mM ATP stimulated the reaction about four-fold.

Characterization of the nucleotide moiety covalently linked to the Cp-1 TP. To further characterize the ^{32}P -labeled TP complex formed in vitro, we treated the radioactive product eluted from the SDS-polyacrylamide gel with alkali under conditions in which the phosphoester bond between the nucleotide and the protein would be hydrolyzed, leaving the phosphate group involved in the linkage attached to the nucleotide. After complete hydrolysis, obtained by incubation with 5 M piperidine for 4 h at 50°C , the degradation products were analyzed by thin-layer chromatography on

FIG. 2. Requirements for the formation of the ^{32}P -labeled Cp-1 TP complex. Extracts from Cp-1-infected cells were incubated with $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ as described in Materials and Methods, except that the incubation time (A), the temperature of the incubation (B), the MgCl_2 concentration (C), or the ATP concentration (D) was changed as indicated. The different samples were subjected to SDS-polyacrylamide gel electrophoresis, and the labeled band at the position of the Cp-1 TP, detected by autoradiography, was quantitated by densitometry.

polyethyleneimine-cellulose plates. The only labeled product had the same mobility as dAMP (Fig. 3).

Elongation of the Cp-1 TP-dAMP complex. To determine whether the Cp-1 TP-dAMP complex obtained in vitro can serve as a primer for elongation, the following experiment was carried out. The Cp-1 TP-dAMP complex was formed in the presence of $1.5\ \mu\text{M}$ $[\alpha\text{-}^{32}\text{P}]\text{dATP}$, chased after 5 min by the addition of $10\ \mu\text{M}$ concentrations of the four dNTPs, and further incubated for 15 min. After micrococcal nuclease digestion, the reaction products were analyzed by SDS-polyacrylamide gel electrophoresis. A fast-moving band corresponding to the position of the Cp-1 TP-dAMP complex and ^{32}P -labeled material of slower mobility that could correspond to elongated material protected by the terminal protein from the micrococcal nuclease digestion were revealed (Fig. 4, lane a). After treatment of the sample with piperidine under conditions that would hydrolyze the Cp-1 TP-dAMP linkage but not the DNA, no radioactive material was observed (Fig. 4, lane b), since the protected elongation products should be small and, therefore, should run off the gel.

Identification of the amino acid in the Cp-1 TP linked to 5'-dAMP. The identification of the Cp-1 TP amino acid involved in the linkage to 5'-dAMP was accomplished by acid hydrolysis of the Cp-1 TP-dAMP complex formed in vitro, followed by thin-layer electrophoresis and chromatography. After heating at 110°C in $5.8\ \text{M}$ HCl for 2 h, three radioactive spots were detected (Fig. 5); one of them corresponded to the phosphothreonine position, the faster-

moving one corresponded to inorganic phosphate, and the one with the lowest mobility probably was a peptidyl-phosphate hydrolysis intermediate, since it disappeared after heating for 165 min under the same conditions, whereas the other two spots remained (results not shown).

DISCUSSION

Several viral genomes contain a protein covalently linked to their 5' termini, providing a new way to solve the problem of the initiation of replication of linear DNA ends. The protein-priming mechanism was confirmed by the identification of a TP-dAMP complex in phage $\phi 29$ (22) and a precursor TP-dCMP complex in adenovirus (24). More recently, a covalent complex between the TP of phage PRD1 and 5'-dGMP was found when extracts from PRD1-infected *E. coli* were incubated with $[\alpha\text{-}^{32}\text{P}]\text{dGTP}$ (1).

When extracts from Cp-1-infected *S. pneumoniae* were incubated with $0.25\ \mu\text{M}$ $[\alpha\text{-}^{32}\text{P}]\text{dATP}$, a ^{32}P -labeled protein complex with the same electrophoretic mobility as the phage Cp-1 TP was detected by SDS-polyacrylamide gel electrophoresis. As expected, this complex was not formed when uninfected extracts of *S. pneumoniae* or $[\alpha\text{-}^{32}\text{P}]\text{dCTP}$ was used in the reaction mixture, indicating the viral origin of the labeled protein and the specificity of the nucleotide, which is complementary to that at the 3' ends of Cp-1 DNA (6). The nucleotide moiety of the ^{32}P -labeled Cp-1 TP complex was characterized as 5'-dAMP, the nucleotide at the 5' termini of Cp-1 DNA (6), indicating that under the conditions used ($0.75\ \mu\text{M}$ dATP), no elongation occurred, since there are

FIG. 3. Thin-layer chromatography on polyethyleneimine-cellulose plates of the ^{32}P -labeled Cp-1 TP complex hydrolyzed with piperidine. The in vitro ^{32}P -labeled Cp-1 TP complex was hydrolyzed with 5 M piperidine for 4 h at 50°C and subjected to thin-layer chromatography as described in Materials and Methods. Unlabeled 5'-dAMP, detected by UV absorbance, was used as an internal standard. ^{32}P was run in parallel.

three consecutive As at the 5' ends of Cp-1 DNA. When the extracts were incubated with $10\ \mu\text{M}$ concentrations of the four dNTPs, labeled material with an electrophoretic mobility lower than that of the Cp-1 TP-dAMP complex was found. The fact that the labeled material disappeared after treatment with piperidine under conditions that hydrolyze the Cp-1 TP-dAMP linkage suggests that the radioactive material was covalently linked to the Cp-1 TP. These results

indicate that the Cp-1 TP-dAMP complex can be elongated and, therefore, that it is the initiation complex of Cp-1 DNA replication. A similar result was obtained when extracts of $\phi 29$ -infected *B. subtilis* were incubated with low ($0.25\ \mu\text{M}$) or high ($40\ \mu\text{M}$) concentrations of dATP (18).

To characterize the amino acid involved in the linkage to 5'-dAMP, we performed acid hydrolysis of the Cp-1 TP-dAMP complex synthesized in vitro. Under the experimental conditions used, the amino acid phosphoester bond was slightly more stable than the peptidic bond. Phosphothreonine, but not phosphoserine or phosphotyrosine, was identified among the products of hydrolysis. This result, together with the fact that the Cp-1 DNA-TP complex was resistant to treatment with hydroxylamine (8) (which rules out the possibility of a phosphoamine bond), suggests that the linkage between the TP and the DNA in phage Cp-1 is a O-5'-deoxyadenylyl-L-threonine bond. This is in agreement with the fact that the Cp-1 DNA-TP complex (6, 8) and the Cp-1 TP-dAMP complex (this paper) are more resistant to alkaline hydrolysis than the corresponding complexes in phage $\phi 29$ (7, 18), since it is known that phosphothreonine and phosphothreonine peptides are more resistant to alkaline hydrolysis than are phosphoserine and phosphoserine peptides (12, 19) and that nucleotide (5'-O)-peptides with a threonine residue located within the peptide chain are more stable than those with a serine residue so located (23). This is the first reported case in which a protein is covalently linked to DNA through a threonine residue.

The formation of a covalent complex between the Cp-1 TP and 5'-dAMP is a new case in support of the protein-priming mechanism for initiating the replication of linear double-stranded DNAs, in addition to phage $\phi 29$ (22), the $\phi 29$ -related phage M2 (15), adenovirus (24), and phage PRD1 (1). Recent evidence indicates that poliovirus (16) and encephalomyocarditis virus (26) form in vitro a TP-UMP complex that probably acts as a primer for further elongation. There-

FIG. 4. Elongation of the Cp-1 TP-dAMP complex. Extracts from Cp-1-infected cells were incubated for 5 min at 30°C with $1.5\ \mu\text{M}$ [$\alpha\text{-}^{32}\text{P}$]dATP and then for an additional 15 min with $10\ \mu\text{M}$ concentrations of the four dNTPs to allow elongation, and the samples were processed and subjected to SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. Lanes: a, untreated sample; b, sample incubated with 5 M piperidine for 4 h at 50°C ; c, ^{125}I -labeled $\phi 29$ TP; d, ^{35}S -labeled $\phi 29$ structural proteins as molecular weight markers. K = 1,000.

FIG. 5 Thin-layer analysis of acid hydrolysis products of the ^{32}P -labeled Cp-1 TP-dAMP complex. The in vitro ^{32}P -labeled Cp-1 TP-dAMP complex was treated with acid, and the hydrolysis products were subjected to bidimensional thin-layer analysis as described in Materials and Methods. Electrophoresis was run in the first dimension, and chromatography was run in the second one. O-phospho-L-serine (P-Ser), O-phospho-L-threonine (P-Thr), and O-phospho-L-tyrosine (P-Tyr), detected by ninhydrin staining, were used as internal markers.

fore, the protein-priming mechanism seems to be a new way to initiate replication in linear nucleic acids which contain a TP at their 5' ends.

ACKNOWLEDGMENTS

This investigation was aided by Public Health Service research grant 5 R01 GM27242-05 from the National Institutes of Health, by grants 847 and 3325 from the Comisión Asesora para el Desarrollo de la Investigación Científica y Técnica, and by a grant from Fondo de Investigaciones Sanitarias. P.G. and J.A.G. were the recipients of a postdoctoral fellowship from the Spanish Research Council.

LITERATURE CITED

1. Bamford, D. H., and L. Mindich. 1984. Characterization of the DNA-protein complex at the termini of the bacteriophage PRD1 genome. *J. Virol.* **50**:309-315.
2. Bitte, L., and D. Kabat. 1974. Isotopic labeling and analysis of phosphoproteins from mammalian ribosomes. *Methods Enzymol.* **30**:563-590.
3. Daubert, S. D., and G. Bruening. 1984. Detection of genome-linked proteins of plant and animal viruses. *Methods Virol.* **8**:347-379.
4. Desiderio, S. V., and T. J. Kelly, Jr. 1981. Structure of the linkage between adenovirus DNA and the 55,000 molecular weight terminal protein. *J. Mol. Biol.* **145**:319-337.
5. Escarmís, C., P. García, E. Méndez, R. López, M. Salas, and E. García. 1985. Inverted terminal repeats and terminal proteins of the genomes of pneumococcal phages. *Gene* **36**:341-348.
6. Escarmís, C., A. Gómez, E. García, C. Ronda, R. López, and M. Salas. 1984. Nucleotide sequence of the termini of the DNA of *Streptococcus pneumoniae* phage Cp-1. *Virology* **133**:166-171.
7. Escarmís, C., and M. Salas. 1981. Nucleotide sequence at the termini of the DNA of *Bacillus subtilis* phage ϕ 29. *Proc. Natl. Acad. Sci. USA* **78**:1446-1450.
8. García, E., A. Gómez, C. Ronda, C. Escarmís, and R. López. 1983. Pneumococcal bacteriophage Cp-1 contains a protein bound to the 5' termini of its DNA. *Virology* **128**:92-104.
9. Hermoso, J. M., and M. Salas. 1980. Protein p3 is linked to the DNA of phage ϕ 29 through a phosphoester bond between serine and 5'-dAMP. *Proc. Natl. Acad. Sci. USA* **77**:6425-6428.
10. Hirochika, H., and K. Sakaguchi. 1982. Analysis of linear plasmids isolated from *Streptomyces*. Association of protein with the ends of the plasmid DNA. *Plasmid* **7**:59-65.
11. Kemble, R. J., and R. D. Thompson. 1982. S1 and S2, the linear mitochondrial DNAs present in a male sterile line of maize possess terminally attached proteins. *Nucleic Acids Res.* **10**:8181-8190.
12. Kemp, B. E. 1980. Relative alkali stability of some peptide o-phosphoserine and o-phosphothreonine esters. *FEBS Lett.* **110**:308-312.
13. Kikuchi, Y., K. Hirai, and F. Hishimuna. 1984. The yeast linear DNA killer plasmids, pGLK1 and pGLK2, possess terminally attached proteins. *Nucleic Acids Res.* **12**:5685-5692.
14. López, R., C. Ronda, P. García, C. Escarmís, and E. García. 1984. Restriction cleavage maps of the DNAs of *Streptococcus pneumoniae* bacteriophages containing protein covalently bound to their 5' ends. *Mol. Gen. Genet.* **197**:67-74.
15. Matsumoto, K., T. Saito, and H. Hirokawa. 1983. In vitro initiation of bacteriophage ϕ 29 and M2 DNA replication: genes required for the formation of a complex between the terminal protein and 5' dAMP. *Mol. Gen. Genet.* **191**:26-30.
16. Morrow, C. D., J. Hocko, M. Navab, and A. Dasgupta. 1984. ATP is required for initiation of poliovirus RNA synthesis in vitro: demonstration of tyrosine-phosphate linkage between in vitro-synthesized RNA and genome-linked protein. *J. Virol.* **50**:515-523.
17. Nishimura, S. 1972. Minor components in transfer RNA: their characterization, location and function. *Prog. Nucleic Acid Res. Mol. Biol.* **12**:49-85.
18. Peñalva, M. A., and M. Salas. 1982. Initiation of phage ϕ 29 DNA replication *in vitro*: formation of a covalent complex between the terminal protein p3 and 5'-dAMP. *Proc. Natl. Acad. Sci. USA* **79**:5522-5526.
19. Plimmer, R. H. A. 1941. Esters of phosphoric acid. Phosphoryl hydroxyamino acids. *Biochem. J.* **35**:461-469.
20. Rekosh, D. M. K., W. C. Russell, and A. J. D. Bellet. 1977. Identification of a protein linked to the ends of adenovirus DNA. *Cell* **11**:283-295.
21. Ronda, C., R. López, and E. García. 1981. Isolation and characterization of a new bacteriophage, Cp-1, infecting *Streptococcus pneumoniae*. *J. Virol.* **40**:551-559.
22. Salas, M. 1983. A new mechanism for the initiation of replication of ϕ 29 and adenovirus DNA: priming by the terminal protein. *Curr. Top. Microbiol. Immunol.* **109**:89-106.
23. Shabarova, Z. A. 1970. Synthetic nucleotide-peptides. *Prog. Nucleic Acid Res. Mol. Biol.* **10**:145-182.
24. Stillman, B. W. 1983. The replication of adenovirus DNA with purified proteins. *Cell* **35**:7-9.
25. Tolun, A., P. Aleström, and U. Pettersson. 1979. Sequence of inverted terminal repetitions from different adenoviruses: demonstration of conserved sequences and homology between SA7 termini and SV40 DNA. *Cell* **17**:705-713.
26. Vartapetian, A. B., E. V. Koonin, V. I. Agol, and A. A. Bagdanov. 1984. Encephalomyocarditis virus RNA synthesis *in vitro* is protein-primed. *EMBO J.* **3**:2593-2598.
27. Watabe, K., M. F. Shih, A. Sugino, and J. Ito. 1982. *In vitro* replication of bacteriophage ϕ 29 DNA. *Proc. Natl. Acad. Sci. USA* **79**:5245-5248.
28. Yoshikawa, H., T. Friedmann, and J. Ito. 1981. Nucleotide sequences at the termini of ϕ 29 DNA. *Proc. Natl. Acad. Sci. USA* **78**:1336-1340.