The Genome of Lipid-Containing Bacteriophage PRD1, Which Infects Gram-Negative Bacteria, Contains Long, Inverted Terminal Repeats

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The bacteriophage PRD1 is a lipid-bearing phage that infects a wide variety of gram-negative bacteria, including Escherichia coli and Salmonella typhimurium when they contain the appropriate plasmid. It contains a linear duplex DNA molecule that is covalently bound by its 5' ends to a terminal protein. We report here that the PRD1 genome contains a 111-base-pair terminal inverted repeat which does not bear homology to that of any known linear duplex DNAs with terminal proteins. We further report that its 3' termini are susceptible to enzymatic digestion by exonuclease III.

Bacteriophage PRD1 is a lipid-containing phage which infects Escherichia coli, Salmonella typhimurium, and a wide variety of gram-negative bacteria which harbor plasmids belonging to the P, N, or W compatibility type (3, 19). It has a linear, nonredundant, double-stranded genome of 14.7 kilobases which is covalently bound to a protein by a phophodiester bond between its 5' termini and a tyrosine residue (1, 16, 19). The ease with which large quantities of this phage can be isolated, as well as the existence of numerous conditionally lethal mutant phages and host strains, makes this phage an ideal system for studying the biochemistry and genetics of membrane biosynthesis and assembly (for a review see references 7 and 17). These same qualities also make PRD1 an attractive system with which to study the replication mechanisms of linear duplex DNA molecules.

Many other replicating linear duplex DNAs that have a terminal protein covalently bound to their 5' ends have been characterized (24, 27). PRD1 and related bacteriophages, however, are the only bacteriophages containing similarly organized genomes which infect E. coli or S. typhimurium. PRD1 also contains the smallest known linear duplex protein-bound genome. These attributes facilitate biochemical and genetic analyses of linear DNA replication. In this note, we report the existence of a 111-nucleotide terminal inverted repeat at the ends of the PRD1 genome. We also show that the 3' nucleotides are not blocked with respect to enzymatic digestion by exonuclease III.

To sequence the terminal nucleotides, PRD1 DNA was digested with several restriction enzymes. Terminal fragments were then identified by their inability to enter a polyacrylamide gel when bound to terminal protein (Fig. 1A). These fragments were isolated, labeled with either [γ-32P]ATP and kinase or [α-32P]dNTP and Klenow fragment and sequenced by the method of Maxam and Gilbert (15). In these cases, secondary restriction or strand separation was not required before sequencing because the terminal 5' ends are blocked by amino acid residues which remain after proteinase K digestion. Both strands of the left and right termini were sequenced (Fig. 2). The left and right terminal sequences were identified as such by comparing their sequences to that of the leftmost 420-base-pair HaeII fragment. A restriction map of HaeII sites has previously been established by McGraw et al. (16). The orientation of the left and right termini corresponds to that of the left and right sides of this restriction map, as well as to the genetic map which has also been established (16, 18).

The terminal *Taq*I (Fig. 1A, lane 2) is 180 bp long and originates from the right side of the PRD1 genome. The terminal *Hae*II and *Dde*I fragments (lanes 4 and 6) originate from the left side of the genome and have sizes of 420 and 120 bp, respectively. The left terminal *Taq*I fragment and the right terminal *Dde*I fragment are too large to be resolved with this gel system. The rightmost *Hae*II fragment is 280 bp.

FIG. 1. (A) Identification of terminal restriction fragments by their inability to enter a polyacrylamide gel when bound to the terminal protein. Phage and DNAs were prepared as described by McGraw et al. (16). Non-proteinase K-treated (odd numbers) and proteinase K-treated (even numbers) DNAs were restricted with *Taq*I, (lanes 1 and 2), *Hae*II (lanes 3 and 4), *Dde*I (lanes 5 and 6), or *Hin*II (lanes 7 and 8). (B) Exonuclease III digestion of the 3' termini of PRD1 DNA. A 20-μl reaction mixture containing 20 to 30 μg of DNA was digested with exonuclease III (270 U) in 50 mM NaCl-10 mM Tris chloride (pH 7.5)-10 mM MgCl2-1 mM dithiothreitol. Samples were removed at 0, 2, 4, 10, 30, and 60 min. The reaction was terminated with phenol and then ethanol precipitated and digested with *Hin*II. The terminal fragments which had been identified in panel A (lanes 7 and 8) disappeared with time. In both panels A and B, bands were resolved on a 5% polyacrylamide gel in 0.5x Tris-borate-EDTA buffer.

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long but cannot be identified in Fig. 1A because it is obscured by two other HaeII fragments of equal size (16).

The genome of PRD1 contains a 111-bp inverted repeat at its termini (Fig. 2). The G+C content of the inverted terminal repeat is 55%.

The susceptibility of the 3' ends of the PRD1 genome to digestion by exonuclease III was of interest because previous reports indicated that the 3' ends are protected from digestion with terminal deoxynucleotidyl transferase (1). PRD1 DNA was digested with exonuclease III for various lengths of time and restricted with HindIII. The terminal HindIII fragments which had been identified previously (Fig. 1A) disappeared with time (Fig. 1B), indicating that they are susceptible to exonuclease III digestion.

Bamford and Mindich (2) have shown that the 5' termini of the PRD1 genome are covalently bound to a protein by a phosphodiester bond between a guanine and a tyrosine residue of the terminal protein. Exonuclease III digestion of the termini indicated that the 3' ends are not protected by a protein. The sequence of the termini shows that they are blunt ended. Coetzee and Bakker (5) have suggested that bacteriophage PR4, which is serologically related to PRD1, has unique cohesive ends. They based this conclusion on the observation of concatenates and circles when they examined PR4 DNA by electron microscopy. Concatenation and circularization, however, are probably caused by interactions between the terminal proteins (20, 23).

The terminal sequences of all replicating, non-redundant linear duplex DNAs that are covalently linked to terminal proteins also contain inverted terminal repeats. The functional significance of these inverted repeats remains unclear. The terminal nucleotide sequences of the bacillus phages φ29, φ15, Nf, M2Y, GA1, and PZA maintain a high degree of homology, and their terminal 6-bp inverted repeats are identical (21, 29). The terminal repeats of the pneumococcus phages Cp-1, Cp-5, and Cp-7 have also been highly conserved through evolution; the first 39 nucleotides from the ends are identical (8). A high degree of homology has also been observed with respect to the terminal repeats of all adenovirus serotypes which have been analyzed. Its first 50 bp are highly conserved and 14-bp sequences starting 9 bp from the ends are identical (25). The terminal repeats of PRD1 exhibit little or no homology to those of any other linear DNAs mentioned above.

Replication of PRD1, φ29, CP-1, and the adenovirus genomes is initiated by a protein-priming mechanism (2, 4, 9, 10, 22, 26). Subsequent DNA synthesis by φ29 and adenovirus has been shown to proceed by strand displacement (11–13, 24, 28). The mechanism by which the displaced, single-stranded DNA acts as a template for complementary strand synthesis, however, is not known at present. Danielle (6) and Lechner and Kelly (14) have proposed a model for adenovirus replication in which the inverted terminal repeats of the displaced parental strand hybridize to form a panhandle structure that allows the terminal protein to prime the synthesis of the second daughter strand. If this model is correct, then it is likely to apply to PRD1, which has terminal repeats of a length comparable to that of adenovirus. Inverted repeats at the termini of linear duplex DNAs of viruses that infect similar hosts but belong to different serotypes have been highly conserved. This suggests that terminal repeats have a function other than simply facilitating possible formation of a panhandle replication intermediate. Since the PRD1 genome is the smallest known linear duplex DNA and because all of the genetic tools which are available in E. coli and S. typhimurium are applicable to PRD1, this phage is an attractive model system for studying the function of the terminal repeats and the molecular mechanisms of linear non-redundant duplex DNA replication in general.

We are grateful to Leonard Mindich for bringing bacteriophage PRD1 to our attention, as well as for supplying phage and strains. This investigation was supported by Public Health Service grant GM28013 from the National Institutes of Health.

ADDITIONAL PROOF

We recently learned that H. Savilahti and D. H. Bamford, University of Helsinki, Helsinki, Finland, found similar terminal inverted repeats in PRD1 and related phage genomes.

LITERATURE CITED


