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Protein-Primed DNA Replication: Role of Inverted Terminal Repeats in the *Escherichia coli* Bacteriophage PRD1 Life Cycle

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***Escherichia coli* bacteriophage PRD1 and its relatives contain linear double-stranded DNA genomes, the replication of which proceeds via a protein-primed mechanism. Characteristically, these molecules contain 5′-covalently bound terminal proteins and inverted terminal nucleotide sequences (inverted terminal repeats [ITRs]). The ITRs of each PRD1 phage species have evolved in parallel, suggesting communication between the molecule ends during the life cycle of these viruses. This process was studied by constructing chimeric PRD1 phage DNA molecules with dissimilar end sequences. These molecules were created by combining two closely related phage genomes (i) in vivo by homologous recombination and (ii) in vitro by ligation of appropriate DNA restriction fragments. The fate of the ITRs after propagation of single genomes was monitored by DNA sequence analysis. Recombinants created in vivo showed that phages with nonidentical genome termini are viable and relatively stable, and hybrid phages made in vitro verified this observation. However, genomes in which the dissimilar DNA termini had regained identical sequences were also detected. These observations are explained by a DNA replication model involving two not mutually exclusive pathways. The generality of this model in protein-primed DNA replication is discussed.**

Inverted terminal repeats (ITRs), typically several hundred base pairs long, have been found in various linear virus genomes as well as in linear plasmids of both prokaryotic and eukaryotic origin (28, 36–38). These double-stranded DNA molecules also contain 5′-covalently linked terminal proteins, and their replication takes place by a protein-primed mechanism in which the terminal protein serves as a primer in initiation (37). The ITRs contain the replication origins as the endmost part, and as a consequence the protein-primed DNA replication can start from either end of the linear molecule. ITRs have also been shown to facilitate binding of certain accessory proteins participating in DNA replication (see reference 37 for review). Other roles of the ITRs are unknown, and it is not known how such structures maintain their identical nucleotide composition in spite of their physical separation.

Bacteriophage PRD1 is the member of a family of very closely related plasmid-dependent viruses infecting various gram-negative bacteria, including *Escherichia coli* and *Salmonella typhimurium*. The other members of this virus group are PR3, PR4, PR5, PR772, and L17 (3, 7, 8, 32, 39, 44, 48). In vitro studies have established that PRD1 uses the protein-primed mode of DNA replication and that the replication can start from either end of its 14,925-bp genome (2, 6, 41, 42, 49). ITRs of the phage genome are 110 bp long (39), and they contain minimal DNA replication origins of 20 bp at the very end (50). It has also been shown that if a mutation has occurred at one end of the genome of PRD1 group phage, it can also be found at the other end (39). The PRD1 early genes coding for the proteins assumed to be involved in DNA replication are located at the genome ends: the terminal protein gene (*VIII*) and the DNA polymerase gene (*I*) at the left end (17, 20, 40) and two genes coding for DNA binding proteins (*XII* and *XIX*) at the right end (11, 35). The roles of the terminal protein and the DNA polymerase in PRD1 DNA replication are well established (1, 2, 41, 42, 49). It is not clear, however, what the roles of the DNA binding proteins in phage DNA metabolism are (29, 33, 34).

We report here evidence that two possible pathways are present in bacteriophage PRD1 DNA replication. The major, replicative pathway operates when initiation occurs at both ends of the genome, leading to the collision of the replication forks and concomitant separation of the replication products. This pathway does not facilitate transfer of sequence information between genome ends. The other sequence monitoring pathway involves DNA strand hybridization within the ITRs and leads to a sequence transfer between the molecule ends. A novel type of intermolecular strand hybridization, between a displaced single strand and a double-stranded molecule, is proposed as being at least as plausible as intramolecular strand hybridization of the displaced strand (a panhandle intermediate formation) to explain the features of the latter pathway.

MATERIALS AND METHODS

Nucleoside triphosphates and enzymes. Restriction endonucleases, RNase, pronase, exonuclease III, and T4 DNA ligase were from Boehringer Mannheim. [α-^{35}S]dATP (~1,000 Ci/mmol), [α-^{32}P]dCTP (~3,000 Ci/mmol), and [α-^{32}P]dGTP (~3,000 Ci/mmol) were obtained from Amersham. Modified T7 DNA polymerase (Sequenase, version 2.0) was from United States Biochemical Corporation.

Phages, plasmids, and bacterial strains. Wild-type phages PRD1, PR4, PR5, L17, and PR772 (3, 8) were grown on *S. typhimurium* LT2(pLM2) (30) or DS88(pLM2) (4). Amber mutant viruses PRD1 *sus*2 (gene *I* mutant [31]) and PR4 *sus*1100 (this study) were grown on *S. typhimurium* suppressor strain PSA(pLM2) (29). Recombinant phages were

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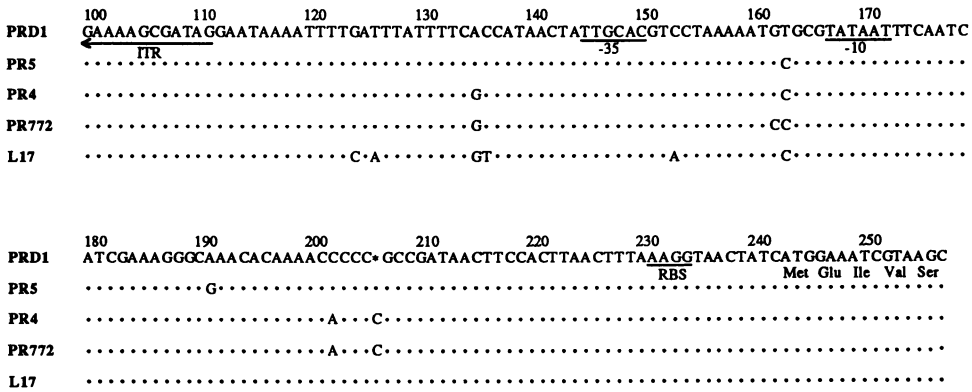

FIG. 1. The right-end nucleotide sequences following the ITRs of phages PRD1, PR5, PR4, PR772, and L17. The −35 and −10 consensus right early promoter sequences and gene *XIX* ribosome binding site are underlined (see reference 35 for overall gene organization). The arrow indicates the location of an ITR. An asterisk indicates a single base pair deletion.

grown on *S. typhimurium* LT2(pLM2) and on *E. coli* DH5α(pLM2) (24). Plasmid pLM2 (30) encodes the receptor for PRD1. Phage PR4 *sus*1100 was isolated by following the previously described procedure (29). By marker rescue analysis with cloned PRD1 genome fragments (27, 31) and by complementation analysis with PRD1 mutant phages (29, 31), the mutation in this phage was assigned to a gene equivalent to gene *III* of PRD1 (23).

Phage production and DNA isolations. Large-scale phage production and purification were performed as described previously (4). DNA was isolated from these virus preparations without pronase treatment essentially as previously described (5), except that extractions with phenol and ether were performed two and three times, respectively. The DNA was resuspended in water and stored at −20°C. Small-scale phage production and the DNA isolation method for dideoxy sequencing with phage terminus-specific primers were dependent on the host strain used and are described as follows.

(i) ***S. typhimurium* LT2(pLM2).** A five-milliliter culture was grown to an optical density of 1.6 at 37°C with aeration, infected by a single plaque, and shaken additionally for 1 h 50 min. Cells were removed by centrifugation (Sorvall SS-34 rotor, 15 min, 15,000 rpm). Three milliliters of 20% (wt/vol) polyethylene glycol 6000–2.5 M NaCl was added to the supernatant, which was then incubated on ice for 30 min. Virus precipitate was collected by centrifugation (see above), and the pellet was rinsed with water and resuspended in 400 μl of TE buffer (6 mM Tris [pH 8.0], 1 mM EDTA). The sample was transferred to a microcentrifuge tube, and 120 μl of 10% sodium dodecyl sulfate and 75 μl of predigested pronase (stock solution, 5 mg/ml) were added. After a 45-min incubation at 37°C, the sample was boiled for 1 min and immediately cooled on ice. After 15 min of centrifugation in an Eppendorf centrifuge, the DNA-containing supernatant was mixed with 500 μl of ice-cold isopropanol. The sample was kept at −20°C for 15 min and centrifuged for 15 min. The pellet was washed with 70% ethanol and dried. The DNA was resuspended in 400 μl of TE buffer, and 10 μl of RNase (stock solution, 10 mg/ml) was added. After 1 h at 37°C, the DNA was extracted with phenol, extracted with chloroform, extracted with ether three times, precipitated with ethanol, dried, and resuspended in water.

(ii) ***E. coli* DH5α(pLM2).** Five milliliters of Luria-Bertani broth was added onto a semi-confluent phage plate, and the Luria-Bertani soft-agar layer was removed. Phage in the preparation were additionally grown at 37°C for 1 h 50 min with aeration. Cells were removed by centrifugation, and DNA was isolated as described above for *S. typhimurium* LT2(pLM2). DNA was further purified by Magic Minipreps (Promega) DNA purification columns as recommended by the manufacturer.

Construction of hybrid phages in vitro. Restriction endonuclease *Bst*XI has a temperature optimum of 55°C. Because of that, we studied the functionality of terminal-protein-containing DNA (P8-DNA) as a function of temperature. This was done by incubating 100 ng of native PRD1 genome in different temperatures for 2 h, electrotransfecting the treated P8-DNA into DH5α cells, and plating them on a lawn of DH5α(pLM2). Up to 45°C, the transfection yielded 3×10^6 to 4×10^6 PFU. At higher temperatures (50 and 55°C), the values were 2×10^6 and 0.5×10^6 PFU, respectively. Subsequently, both PRD1 and PR4 DNA were cut with *Bst*XI at 44°C for 2 h, extracted with phenol, extracted with ether three times, precipitated with ethanol, and resuspended in water. The same amount of each digested phage genome was ligated as described previously (25) for 1 h at room temperature with 1 U of T4 DNA ligase. One microliter of the ligation mixture (100 ng of DNA) was electrotransfected into competent cells as described previously (24). Transfected cells were plated on a lawn of the corresponding bacterial host containing plasmid pLM2 to allow scoring as plaques. The phage yield was roughly 4×10^5 PFU/100 ng of ligated DNA. From the control experiments, it was estimated that at least 99% of the parental genomes were cleaved by *Bst*XI and that approximately 10% of the cleaved molecules could be rescued by ligation.

DNA sequencing. By using the strategy described earlier (39), DNA sequencing of the terminal restriction fragments of PRD1 phage group (Fig. 1) was performed by the method of Maxam and Gilbert (26).

The phage DNA template for dideoxy sequencing of the recombinants was prepared by incubating the pronase-treated phage DNA with exonuclease III (20 U/μg of DNA) in 66 mM Tris (pH 8.0)–6.6 mM $MgCl_2$ for 15 min. The DNA was extracted with phenol, extracted with ether two times, precipitated with ethanol, dried, and resuspended in water. Dideoxy sequencing was performed according to the United States Biochemical's Sequenase protocol, except that the 5× Sequenase buffer was 200 mM Tris (pH 7.4)–2.5 mM $MnCl_2$. Primers used were p9040 (5′-GCGCAATTTGGGG TAGTGGT-3′) and p9039 (5′-GTGTTTGCCCTTTCGATG

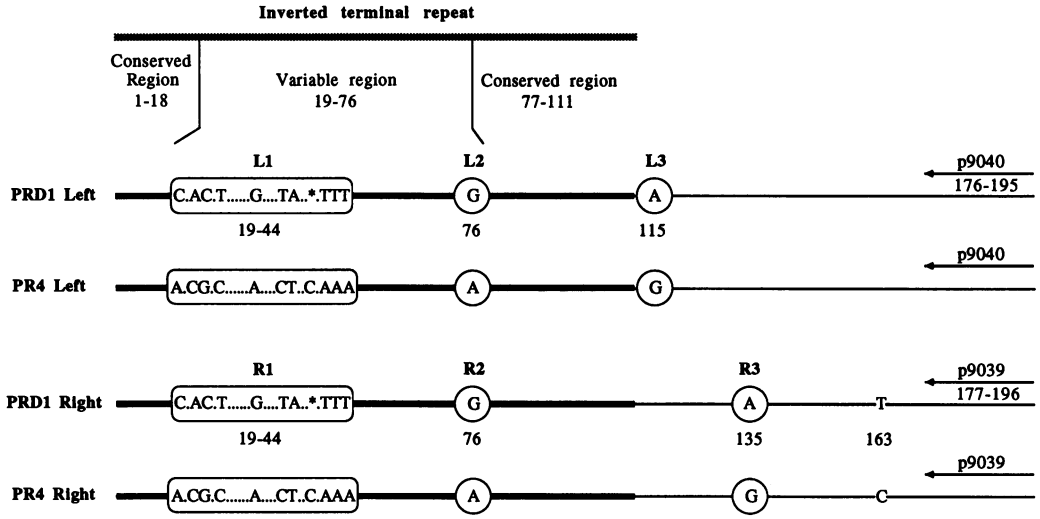

FIG. 2. Nucleotide differences at the ends of the genomes of PRD1 and PR4. The numbering follows that of reference 39 and is different from the PRD1 genome numbering (6) because of a 1-nt deletion at position 40 in the PRD1 genome compared with the PR4 genome (indicated by asterisks in the left and right ends). Only divergent nucleotides are indicated. Circled sequences denote the informative identification tags used in the study. They are labelled L1, L2, and L3 on the left end and R1, R2, and R3 on the right end (tags L1 and R1 contain several nucleotides). Primers used in DNA sequencing are indicated on the right.

AT-3') hybridizing to the nucleotides (nt) 176 to 195 and 177 to 196 at the left and right ends of the phage genomes, respectively.

RESULTS

We wanted to create hybrid PRD1 group phages and after propagation examine the terminal nucleotide sequences of their genomes. Five very closely related phages, PRD1, PR4, PR5, L17, and PR772, were available for our study. Some of the essential DNA sequence data have been previously published (6, 39, 40). Additional necessary information was obtained by sequencing the right ends of the phage genomes up to nt 257 (Fig. 1). On the basis of the data, we chose PRD1 and PR4 for hybrid phage construction. The differences between the terminal DNA sequences of these phages were designated L1, L2, and L3 (at the left terminus) and R1, R2, and R3 (at the right terminus), and these phage-specific markers (tags) were monitored by sequence analysis (Fig. 2).

First, we constructed PRD1-PR4 hybrid phages in vivo. This was accomplished by coinfecting nonsuppressing *S. typhimurium* LT2(pLM2) with PRD1 *sus*2 and PR4 *sus*1100 mutants. The former has a mutation in gene *I* (coding for the DNA polymerase, nt 1016 to 2677, 6.8 to 17.9% map position) and the latter has a mutation in the gene equivalent to gene *III* of PRD1 (coding for the major capsid protein, nt 8595 to 9782, 57.6 to 65.5% map position, PRD1 nomenclature). As a control, LT2(pLM2) was also infected separately with *sus*2 and *sus*1100. Progeny phage titers were determined on suppressor (PSA) and nonsuppressor (LT2) hosts (Table 1). Phage yield in coinfection was over 10 times higher than that in single-phage infections. This was indicative of complementation and/or recombination. Recombinants were scored on a nonsuppressor host, and the recombination frequency was found to be roughly 1.3%.

Twenty-three putative hybrid phage plaques from the coinfection were picked and grown on LT2(pLM2), after which their DNA was isolated and subjected to analysis of the terminal sequences (Fig. 3). The observed tag sequences divide the phages into six categories, the formation of which can be explained by a single (class I), double (classes II, III, and VI), or triple (classes IV and V) crossover(s). Reversion to wild type, even though possible, is not likely because of the low reversion frequencies of the mutant viruses (see Table 1). In classes V and VI, one of the putative crossovers could be mapped by sequence analysis to a defined region (between two tags) in the DNA. All of the rearrangements in this phage cross can be explained by homologous recombination. However, as described in Discussion, a special end-restoring mechanism may have been operational in creating the class VI configuration.

We studied class I recombinant phage viability in a RecA$^-$ host by plating out one class I mutant on DH5α(pLM2). Unlike in the previous experiment, in this case only one DNA molecule entered each cell to start clonal propagation. Seven individual plaques were picked and grown on DH5α(pLM2); their DNA was isolated and subjected to sequence analysis. The class I genotype had remained the same in all seven isolates, confirming that phages with dissimilar end sequences are viable and reasonably stable.

We also constructed hybrid DNA molecules in vitro. This strategy allowed us to bypass any potential rearrangements made in a RecA$^+$ host during the construction of the hybrid

TABLE 1. Coinfection with phage amber mutants[a]

Mutant	PFU	
	LT2(pLM2)	PSA(pLM2)
*sus*2	5.8×10^4	2.7×10^9
*sus*1100	1.3×10^5	2.1×10^9
*sus*2 + *sus*1100	6.4×10^8	5.0×10^{10}

[a] LT2[pLM2] was inoculated to an optical density of 0.5 and was grown to an optical density of 1.6. Five milliliters of the cells was infected with amber mutant phages by using a multiplicity of infection of 10. After 1 h 50 min, the cell debris was removed and PFU of the supernatants was determined on restrictive [LT2 (pLM2)] and permissive [PSA (pLM2)] hosts.

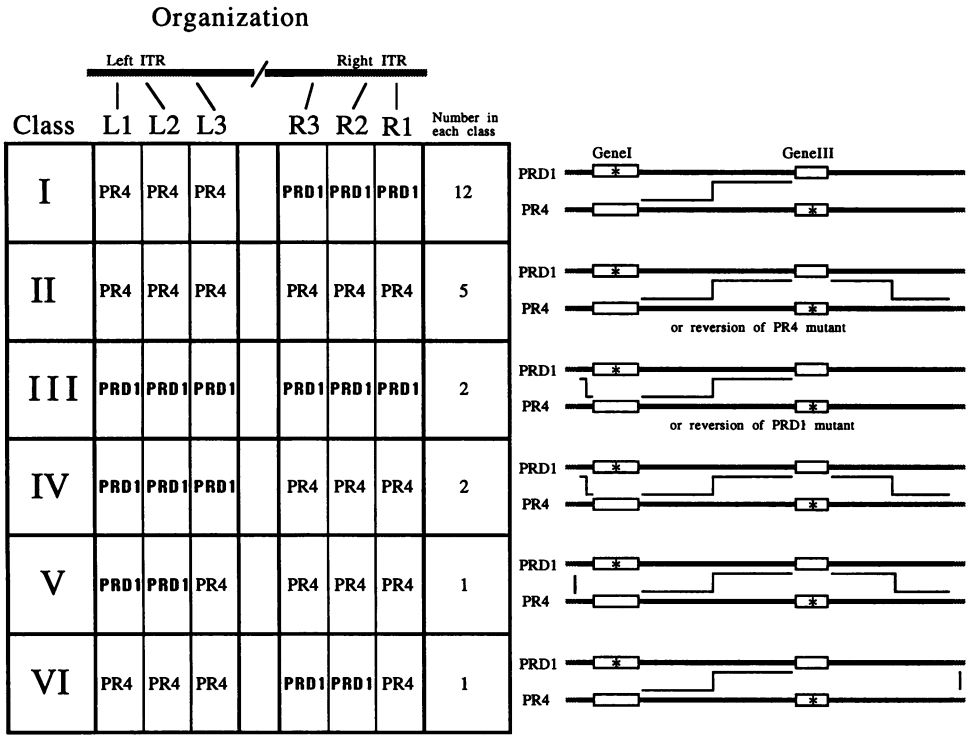

FIG. 3. Organization of the genomes obtained in a coinfection experiment with PRD1 and PR4 mutant phages. On the left is shown the observed tag composition of possible hybrid phages (see Fig. 2). On the right are depicted the proposed crossover events for formation of each class.

phages by recombination in vivo. Wild-type PRD1 and PR4 genomes were cut with *Bst*XI and ligated without prior purification of the fragments. Ligation mixtures were subsequently electroporated into LT2 and DH5α backgrounds (see Materials and Methods). A total of 104 individual plaques were picked, grown on the corresponding host for DNA isolation, and subjected to restriction enzyme analysis with *Hae*II. This enzyme distinguishes (in addition to parental configurations) between the PR4-PRD1 and the PRD1-PR4 configurations (Fig. 4). The clonal character of each phage isolated, as judged from restriction enzyme analysis, suggested that only one genome entered each cell. This was expected, because in these conditions, of every 10^4 cells approximately 1 is transfected (reference 24 and this study). The restriction analysis revealed 32 recombinants, 31% of the total plaques analyzed. All of the recombinants exhibited the restriction pattern consistent with ligation at the *Bst*XI site. Twenty-six of the recombinants were also subjected to DNA sequence analysis (Fig. 5). Thirteen had a full set of three tags in the PR4-PRD1 configuration and 12 had a full set of three tags in the PRD1-PR4 configuration; all of them were also consistent with *Hae*II restriction enzyme analysis. However, one clone formed by ligation at the *Bst*XI site (PR4-PRD1 configuration) and originally electroporated into RecA$^-$ background (DH5α) showed a unique tag composition (see bottom row in Fig. 5). This composition may have arisen by a mechanism described in Discussion. An alternative explanation would involve the unlikely entry of two different molecules into the same cell combined with recombination or damage and repair.

Phages which showed a unique tag combination in sequence analysis were further propagated. Class V and class VI phages from the in vivo experiment (grown on *S. typh-*

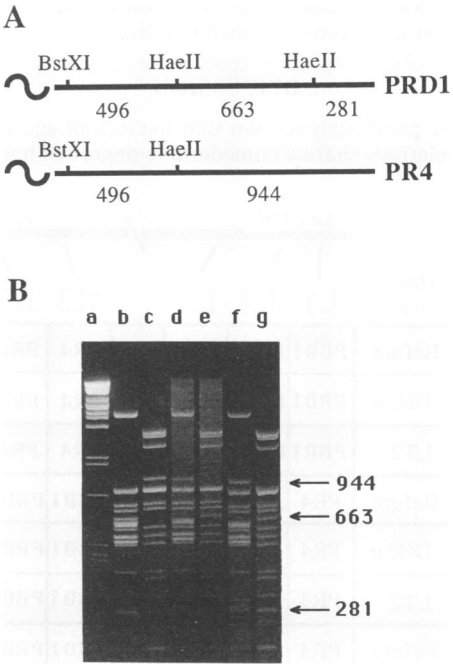

FIG. 4. *Hae*II digestion of in vitro-produced recombinant phage genomes. (A) *Hae*II and *Bst*XI restriction map of the right ends of the genomes of PRD1 and PR4. (B) Analysis of *Hae*II restriction fragments by electrophoresis in a 2% agarose gel. Lanes: a, lambda DNA digested with *Pst*I as a molecular weight marker; b, wild-type PRD1; c, wild-type PR4; d and f, hybrid PRD1-PR4 configuration; e and g, hybrid PR4-PRD1 configuration. Lanes d and e show DH5α as a host; lanes f and g show LT2 as a host.

Host strain	Left ITR L1	L2	L3	Right ITR R3	R2	R1	Number obtained
LT2	PR4	PR4	PR4	PRD1	PRD1	PRD1	4
DH5α	PR4	PR4	PR4	PRD1	PRD1	PRD1	9
LT2	PRD1	PRD1	PRD1	PR4	PR4	PR4	9
DH5α	PRD1	PRD1	PRD1	PR4	PR4	PR4	3
DH5α	PR4	PR4	PR4	PRD1	PRD1	PR4	1

FIG. 5. Number and organization of in vitro-created phage genomes as revealed by sequence analysis and judged by tags (see Fig. 2).

imurium LT2) and having PRD1-PRD1-PR4=PR4-PR4-PR4 and PR4-PR4-PR4=PRD1-PRD1-PR4 tag compositions, respectively, as well as in vitro-created phage (grown on *E. coli* DH5α) with a tag composition of PR4-PR4-PR4=PRD1-PRD1-PR4 were plated out on LT2 and DH5α backgrounds. One plaque of each clone (clones A, B, and C) was picked and grown on a corresponding host for DNA isolation and sequence analysis (Fig. 6). The sequences of the progeny phages were the same as those of their progenitors except for the right end of the clone A when plated on LT2. In this case, a novel combination of tags, PRD1-PRD1-PR4=PR4-PR4-PRD1, was detected. The reorganization may have occurred early in this LT2 infection. Alternatively, the phage (with reorganized ends) was already present in the phage stock as a minor population not detectable by DNA sequence analysis. If this was the case, the formation of the observed tag combination must have occurred earlier.

DISCUSSION

We have previously shown that bacteriophage PRD1 and its close relatives share a considerable degree of homology at

Clone	Host strain	Left ITR L1	L2	L3	Right ITR R3	R2	R1
A	Before	PRD1	PRD1	PR4	PR4	PR4	PR4
	DH5α	PRD1	PRD1	PR4	PR4	PR4	PR4
	LT2	PRD1	PRD1	PR4	PR4	PR4	PRD1
B	Before	PR4	PR4	PR4	PRD1	PRD1	PR4
	DH5α	PR4	PR4	PR4	PRD1	PRD1	PR4
	LT2	PR4	PR4	PR4	PRD1	PRD1	PR4
C	Before	PR4	PR4	PR4	PRD1	PRD1	PR4
	DH5α	PR4	PR4	PR4	PRD1	PRD1	PR4
	LT2	PR4	PR4	PR4	PRD1	PRD1	PR4

FIG. 6. Genotypes of phages having reorganized end sequences before and after propagation in DH5α and LT2 hosts. The tags are those depicted in Fig. 2.

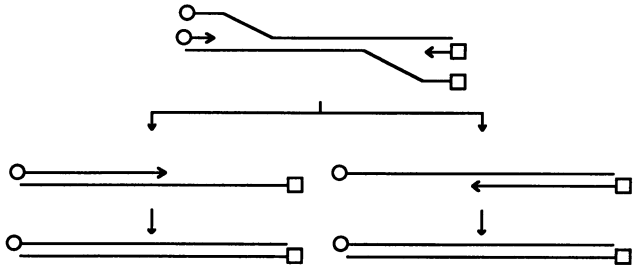

FIG. 7. Replicative pathway of PRD1. Initiation at both ends (○, left; □, right) of the genome leads to a collision of replication forks and concomitant separation of the products. As a consequence, the terminal sequences retain their identity.

their ITRs (39). The ITRs between phages are noticeably divergent, but the ITRs of a particular phage are identical, except for one mismatch in the L17 genome (nt 91). This observation implies that mutations at one end of the genome have been copied to the other end during evolution. We wanted to study whether this phenomenon was common and whether it could be reconstituted experimentally. Consequently, we made hybrid phages with dissimilar end sequences in vivo as well as in vitro and followed the terminal sequences of the progeny phage genomes.

Hybrid phage construction by recombination in vivo was accomplished by coinfection with PRD1 and PR4 amber mutants and plating the progeny on a nonsuppressor host. Because the reversion frequencies of the mutant phages were negligible compared with the recombination frequency, the progeny should theoretically be nearly 100% recombinants. Indeed, this was the case because, in various similar crosses, almost all phages studied by restriction analysis showed the hybrid genotype (23). Moreover, the detectable hybrid genotype pattern is dependent on the restriction enzyme used in the analysis. Therefore, the number obtained represents the minimum estimate of actual crossovers (and thus recombinants). All other PRD1 group phages could also be recombined with PRD1 (23), apparently via homologous recombination. No obvious phenotypic differences in the hybrid phages compared with their parental phages could be detected. In addition, complementation was observed among members of the whole phage group, which enabled us to map mutations in each phage to their corresponding PRD1 gene counterparts. For these reasons, we consider all of these phages as belonging to the same phage species.

Phages with nonidentical genome end sequences could be isolated, indicating that such genomes are relatively stable. The majority of the molecules created in vivo and in vitro retained the expected end sequence configuration. This showed that the viability of the phage does not depend on the identical ITRs. Thus, the pathway that operates when initiation occurs at both ends of the genome and does not regenerate identical ITRs must be favored (Fig. 7). However, if this mode of replication is exclusively the only operational pathway, the observed coevolution of the ITRs in PRD1-group phages (39) cannot be explained. Therefore, we envision that there must be a mechanism which keeps the genome ends identical (at least in evolutionary time scale). Interestingly, in this study, we observed three different phage isolates with reorganized ITRs. One of them originated in a RecA$^-$ host. Even though one may envision alternative explanations for their generation in each individual case, the simplest assumption is that they arose as a consequence of the same mechanism.

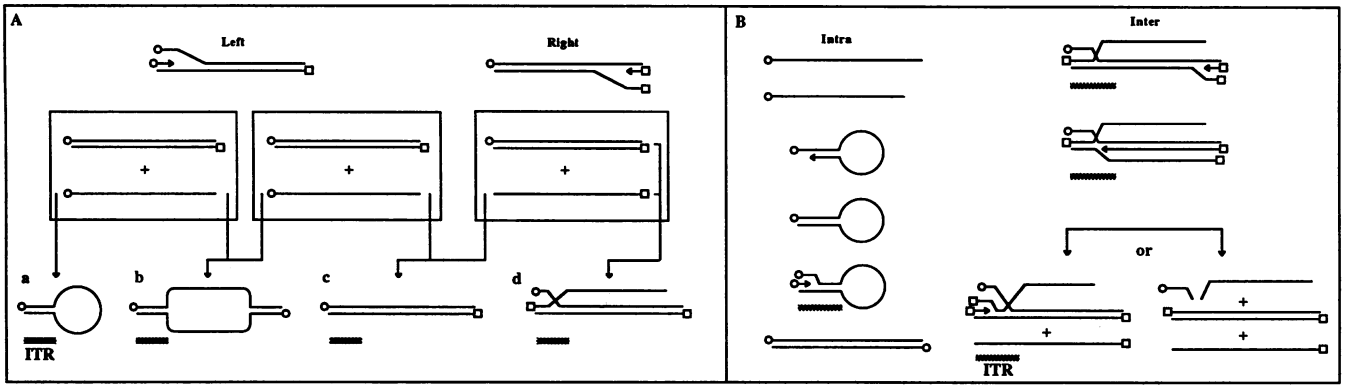

FIG. 8. (A) Possible interactions of a displaced single strand(s). Initiation at only one end of the genome (left [○] or right [□]) leads to a displacement of a single strand. (a to c) Single strand-single strand interactions; (d) single strand-double strand interactions. The situations illustrated by a, b, and d can facilitate the communication between genome ends. (B) Sequence monitoring pathway of PRD1. On the left is the intramolecular model. Note also the analogy to intermolecular situation b depicted in panel A. On the right is the intermolecular model with single strand-double strand interactions. The figure depicts the 5′-strand invasion. However, the 3′-strand invasion is also possible if complete displacement of the single strand occurs. See Discussion for the details. Shaded blocks denote the ITRs (for clarity they are depicted only at the left end). Terminal proteins at the 5′ ends of DNA strands are depicted with either a circle or a square.

We propose here that there is, indeed, a potential alternative pathway in the PRD1 life cycle that mediates the communication between the genome ends and can explain the homology-restoring phenomenon. We call it the sequence monitoring pathway, and it may operate in either an intramolecular or an intermolecular fashion (Fig. 8). The intramolecular strand-hybridization model (Fig. 8B) implies the following. (i) A strand displaced during strand displacement replication can pair itself within the complementary ITR region. (ii) 3′-5′-exonuclease activity degrades the 3′ end of this single-stranded DNA molecule before or after pairing. (iii) DNA polymerase activity corrects the sequence by using the 5′ end of the molecule as template. (iv) Replication can start from the paired (double-stranded) part and continue around the otherwise single-stranded molecule. A priori, however, there is no reason to assume that hybridization must occur exclusively intramolecularly. A special intermolecular strand hybridization model (Fig. 8B) is also compatible with the results obtained. The key features of this model are as follows. (i) A displaced single strand invades the double-stranded molecule in a manner such that opposite polarity is achieved. (ii) This single-stranded DNA molecule pairs within the ITR to the double-stranded DNA molecule. (iii) To resolve the intermediate, strand displacement replication by DNA polymerase switches the template within the ITR or, alternatively, a strand transfer, facilitated by another protein(s), precedes replication. Even though Fig. 8 depicts the mechanism after strand displacement for clarity, we envision that the strand invasion may well be intrinsically coupled to replication. In this model, the only hypothetical event to be postulated is a template switching or, alternatively, a strand transfer. The model is intrinsically nonreciprocal and postulates the sequence-independent requirement of inverted repeats. It also separates the function of the minimal replication origin and the rest of the ITR. We favor this new model because of its independence of correction via nuclease and concomitant DNA polymerase activities (obligatory for the intramolecular mechanism). One can imagine that DNA binding proteins may be involved in the process. Interestingly, PRD1 encodes two DNA binding proteins (P12 and P19) abundantly expressed early in the phage infection (29, 33, 34), the roles of which in PRD1 DNA replication are unknown. They may be involved in the proposed homology-restoring process, ensuring that the ITRs are monitored during phage propagation. However, the possible role of the host factors must also be considered.

The question of repair in ITRs has been addressed in two other well-studied protein-primed DNA replication systems: bacteriophage ϕ29 and adenovirus. With ϕ29, no evidence has been gained in favor of any pathway involving DNA strand hybridization (10, 12, 14, 19, 43). It has been suggested that initiations at both ends of the ϕ29 genome occur before the displacement of the full-length single strand (12). Because in this phage the ITRs are only 6 bp long the sequence monitoring pathway may not be eligible and the phage is compelled to follow exclusively the replicative pathway. On the contrary, in the adenovirus system it has been shown that viral genomes missing part of sequences

TABLE 2. Summary of protein-primed DNA replication systems[a]

Replication system	Minimal origin of replication (bp)[b]	ITRs (bp)	Nature of pathway	
			Replicative	Sequence monitoring
Adenovirus	20	~100[c]	Yes	Yes; high activity
ϕ29	12	6	Yes	No
PRD1	18	110	Yes	Yes; low activity
Linear plasmids	?	Various	Yes	Most probably

[a] Data were compiled from references 37 and 50 and references therein.
[b] From in vitro studies.
[c] Depending on the adenovirus serotype.

from one ITR can be repaired (45). In addition, viruses with dissimilar end sequences are viable, but the ends of the molecules are rapidly converted to contain identical ITRs (22). An attractive model to explain these results has been that of the panhandle intermediate formation (9, 21), which we call intramolecular hybridization (Fig. 8B). The results obtained in various adenovirus studies postulating the obligatory communication between the ends of the genome have been interpreted with this model (13, 15, 16, 18, 46, 47). However, the panhandle replication intermediates have not been detected directly. We point out that the adenovirus results are also compatible with the proposed intermolecular hybridization model. In all linear plasmids studied to date, there exist ITRs long enough to facilitate the hybridization of DNA strands. We would expect that in linear plasmids also there is a pathway operating to maintain the identical ends. A summary of the most-studied DNA molecules utilizing a protein-primed DNA replication strategy is shown in Table 2. It seems plausible to assume that a minimal origin of replication in protein-primed DNA replication is sufficient for the replicative pathway. Longer ITRs, in addition to containing potential binding sites for replication accessory proteins, however, may facilitate an additional sequence monitoring pathway. Both of these pathways are present in PRD1, suggesting that an inherent control mechanism may be operational, the nature of which remains to be established.

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