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Nucleotide Sequences at the φX Gene A Protein Cleavage Site in Replicative Form I DNAs of Bacteriophages U3, G14, and α3

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Gene A protein, a bacteriophage φX174-encoded endonuclease involved in φX replicative form (RF) DNA replication, nicks not only φX RFI DNA but also RFI DNAs of several other spherical single-stranded DNA bacteriophages. The position of the φX gene A protein nick and the nucleotide sequence surrounding this site in RF DNAs of the bacteriophages U3, G14, and α3 were determined. Comparison of the nucleotide sequences which surround the nick site of the gene A protein in RF DNAs of φX174, G4, St-1, U3, G14, and α3 revealed that a strongly conserved 30-nucleotide stretch occurred in RF DNAs of all six phages. However, perfect DNA sequence homology around this site was only 10 nucleotides, the decamer sequence CAACTTGATA. The present results support the hypothesis that, for nicking of double-stranded supercoiled DNA by the φX gene A protein, the presence of the recognition sequence CAACTTGATA and a specific gene A protein binding sequence upstream from the recognition sequence are required. The sequence data obtained so far from phages U3, G14, St-1, and α3 have been compared with the nucleotide sequences and amino acid sequences of both φX and G4. According to this comparison, the evolutionary relationship between phages G4, U3, and G14 is very close, which also holds for phages α3 and St-1. However, the two groups are only distantly related, both to each other and to φX.

The gene A protein of bacteriophage φX174 initiates φX replicative form (RF) DNA replication by cleaving the viral strand of RFI DNA next to the G-residue, which corresponds to nucleotide 4,305 of the φX DNA sequence (2, 3, 7, 10, 16, 19, 24). This creates a 3'-OH primer terminus for DNA synthesis and a 5' terminus, presumably the A residue at position 4,306 of the φX DNA sequence (24) to which the gene A protein remains covalently bound (8, 16, 19). Previous work from our laboratory has shown that RFI DNAs of bacteriophages G4 and St-1 are nicked by the φX gene A protein at only one site (15, 29). Comparison of the nucleotide sequences surrounding the φX gene A protein nick sites in φX RF DNA and G4 RF DNA has revealed the presence of a common 30-nucleotide stretch, the sequence

CAACTTG↓ATA*T*TAA*T*AACACTATAGACCAC

at the nick sites (indicated by the arrow) in these RF DNAs (9, 29). In St-1 DNA this nucleotide sequence occurs at the φX gene A protein nick site with two changes, T → A and A → G, respectively, at the positions marked with an asterisk in the above sequence (15). Therefore, perfect DNA sequence homology around the φX gene A protein nick sites in RF DNAs of φX, G4, and St-1 is only 10 nucleotides, the sequence CAACTTGATA, which suggests that the recognition sequence of the φX gene A protein lies within this decamer sequence. Van Mansfeld et al. (28) have shown that the single-stranded synthetic decamer sequence CAACTTGATA and single-stranded natural DNA which contains this sequence (i.e., the strand of polyoma virus DNA with the same polarity as the late mRNAs [26]) are cleaved by the φX gene A protein at the same site as the corresponding sequence of the φX origin region. Their results identify the recognition sequence of the φX gene A protein within the decamer sequence CAACTT-G↓ATA.

However, double-stranded supercoiled DNA, in which the recognition sequence CAACTT-G↓ATA is followed by a nucleotide stretch differing from the corresponding nucleotide sequence in φX (e.g., supercoiled polyoma virus DNA [26]), is not nicked upon incubation with

the φX gene A protein (28). Moreover, we have shown recently that the presence in double-stranded supercoiled recombinant plasmid DNA of the sequence CAACTTGATATTAATAA-CAC (i.e., a sequence of 20 consecutive nucleotides from the 30-nucleotide stretch, conserved in φX and G4 RF DNAs at the φX gene A protein nick site [see above]) is insufficient for specific nicking by the φX gene A protein. To explain these results, a model has been proposed for initiation of φX RF DNA replication that involves the presence of the recognition sequence CAACTTGATA of the φX gene A protein as well as a specific nucleotide sequence required for the binding of the φX gene A protein (14).

It has been shown that RFI DNAs of bacteriophages U3, G14, and α3 are nicked upon incubation with the φX gene A protein (15). The present study is concerned with the elucidation of the gene A protein nick sites and the nucleotide sequences surrounding these sites in U3, G14, and α3 RF DNAs to obtain further information about the nucleotide sequences in double-stranded supercoiled DNA, required for binding or cleavage or both by the φX gene A protein. The sequence data obtained from phages U3, G14, and α3 have been compared with the corresponding data of φX (24), G4 (13), and St-1 (15). The impact of this comparison on the evolutionary relationship among φX, G4, U3, G14, St-1, and α3 will be discussed.

A preliminary account concerning the determination of the φX gene A protein nick site in U3 RF DNA has already been published (1).

MATERIALS AND METHODS

Bacterial strains, viruses, and preparations of DNAs. The bacterial strains and the procedures for growth of bacteriophages φX174, U3, G14, and α3 and for preparation of viral DNAs were as described previously (15). RF DNA (i.e., double-stranded circular RF DNA) was prepared according to the method of Jansz et al. (17) with modifications as described by Baas et al. (4). Pure RFI DNA (i.e., supercoiled RF DNA with both strands closed) was isolated by sucrose gradient centrifugation followed by CsCl-buoyant density centrifugation in the presence of 200 μg of ethidium bromide per ml (23).

Enzymes. φX174 gene A protein was purified from φX174 *am3*-infected *Escherichia coli* HF4704 cells according to the method of Eisenberg and Kornberg (8) with modifications as described by Langeveld et al. (18). The various restriction endonucleases used in this work were from New England Biolabs, Inc. (Beverly, Mass.) with the exception of *Dde*I, which was purchased from Bethesda Research Laboratories, Inc. (Rockville, Md.). Assay conditions for restriction endonucleases were as described by the manufacturers. Bacterial alkaline phosphatase (grade F) was from Worthington Diagnostics (Freehold, N.J.), T4 polynucleotide kinase was purchased from Boehringer Mannheim (Mannheim, West Germany), and proteinase K was from E. Merck AG (Darmstadt, West Germany).

φX gene A protein incubation. RFI DNA was incubated with φX gene A protein for 45 min at 37°C in a 1.0-ml reaction mixture containing 50 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 5 mM dithiothreitol, 125 mM NaCl, 30 μg of RFI DNA, and an appropriate amount of φX gene A protein.

In control experiments the gene A protein was omitted from the mixtures. The reaction was terminated by adding 0.25 M EDTA (pH 8.0) to 25 mM and 100 μl of a proteinase K solution (5 mg of proteinase K per ml in 50 mM Tris-hydrochloride, pH 7.5) which had been preincubated for 60 min at 37°C. The incubation was continued for 60 min at 37°C, and the conversion of RFI DNA into RFII DNA (i.e., RF DNA with one or more discontinuities in either strand) was analyzed after electrophoresis of 20-μl samples from the reaction mixtures for 2 h at 200 V on horizontal 1% agarose slab gels containing 2 μg of ethidium bromide per ml.

Preparation and analysis of restriction DNA fragments, chemicals used, and DNA sequence analysis. The preparation of restriction DNA fragments, 5'-end-labeling of DNA fragments, the isolation of single-stranded DNA fragments from nicked double-stranded 5'-end-labeled restriction DNA fragments, the chemicals used in this study, and details about polyacrylamide gel electrophoresis were as described previously (15). The preparation of 5'-end-labeled DNA fragments suitable for DNA sequence analysis according to the chemical degradation method of Maxam and Gilbert (21, 22) has been described (14, 15).

RESULTS

Strategy used for localization of the φX gene A protein nick in RF DNAs of bacteriophages U3, G14, and α3. To obtain information about the nucleotide sequence specificity of the φX gene A protein, we have determined the position of the φX gene A protein nick and the nucleotide sequence surrounding this site in RF DNAs of bacteriophages U3, G14, and α3. The exact position of the φX gene A protein nick site and the nucleotide sequence at the 3'-OH end of the nick were determined by DNA sequence analysis of the single-stranded DNA subfragment obtained from the corresponding nicked double-stranded restriction DNA fragment. DNA sequence analysis of the corresponding unnicked double-stranded restriction DNA fragment yielded the nucleotide sequence surrounding the φX gene A protein nick site. The experimental approach used is shown in Fig. 1.

Position of the φX gene A protein nick site and the nucleotide sequence surrounding this site in U3 RF DNA. Previous work from our laboratory has shown that the φX gene A protein nick in U3 RF DNA is located in a 205-base pair (bp) *Hpa*II-*Hin*cII restriction DNA fragment at about 130 nucleotides upstream from the 5' *Hpa*II end (1). To determine the exact position of the φX gene A protein nick site in RF DNA of bacterio-

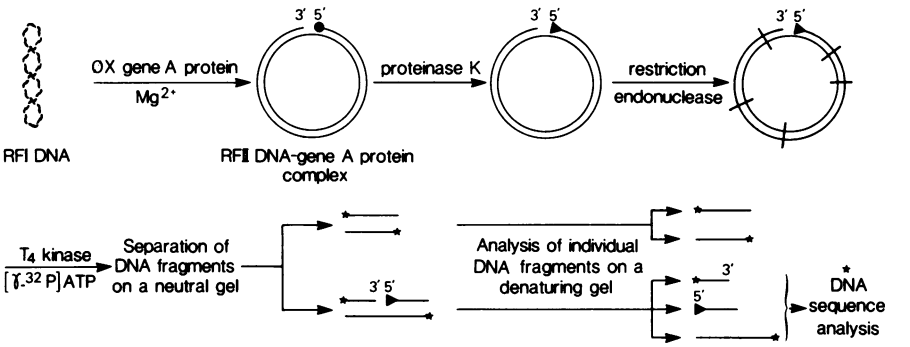

FIG. 1. Strategy for localization of the ϕX gene A protein nick in RF DNAs of bacteriophages U3, G14, and α3. Symbols: ●, ϕX gene A protein; ▲, residual ϕX gene A protein peptide(s).

phage U3, 30 μg of U3 RFI DNA was incubated with as well as without the ϕX gene A protein; subsequently, 5'-^{32}P-labeled *Hpa*II restriction DNA fragments were prepared from U3 RF DNA, using the experimental approach indicated in Fig. 1.

The restriction DNA fragments were separated by electrophoresis on a neutral polyacrylamide gel. In both cases, after digestion of U3 RF DNA with the restriction endonuclease *Hpa*II, five restriction DNA fragments were obtained (data not shown). Each DNA fragment was eluted from the gel and subsequently analyzed on a 4% polyacrylamide gel in 98% formamide. In this gel system the two strands of a restriction DNA fragment are not separated (20). Restriction DNA fragments that contain nicked DNA can yield two extra labeled single-stranded DNA fragments. However, for ϕX174, G4, and St-1 it has been shown that the 5' end of the ϕX gene A protein nick cannot be labeled by the combined action of alkaline phosphatase and polynucleotide kinase because the gene A protein is covalently bound to the DNA at the 5' end of the nick (8, 15, 16, 19, 29). Therefore, only one extra labeled band is observed in the denaturing gel, representing the DNA at the 3'-OH end of the nick.

Also for U3, only one restriction DNA fragment (P2, ca. 1,650 bp) showed one extra ^{32}P-labeled band on the denaturing gel (Fig. 2; data for *Hpa*II restriction DNA fragments P1 and P3 to P5 not shown), with a length of approximately 130 nucleotides.

Redigestion of the nicked, end-labeled restriction DNA fragment P2 with restriction endonuclease *Hinc*II yielded two labeled restriction DNA subfragments with approximate lengths of 185 and 205 bp, respectively (data not shown). The 130-nucleotide single-stranded DNA fragment was isolated from the nicked 205-bp restriction DNA fragment by electrophoresis of this double-stranded DNA fragment on a 10% polyacrylamide gel in 7 M urea. After elution from this gel the single-stranded DNA fragment was subjected to DNA sequence analysis according to the chemical degradation method of Maxam and Gilbert (21, 22), which yielded the nucleotide sequence at the 3'-OH end of the ϕX gene A protein nick in U3 RF DNA (Fig. 3).

The nucleotide sequence surrounding the ϕX gene A protein nick site in U3 RF DNA was determined as follows. The unnicked end-labeled U3 *Hpa*II restriction DNA fragment P2 was redigested with restriction endonuclease *Hinc*II, which was followed by electrophoretic separation of the cleavage products on a neutral polyacrylamide gel. The 205-bp restriction DNA fragment (see above) was eluted from this gel and subjected to chemical degradation, which was followed by separation of the reaction products on DNA sequence gels (21, 22). Autoradiography of these gels revealed the nucleotide sequence surrounding the ϕX gene A protein nick site in U3 RF DNA (Fig. 4).

Localization of the ϕX gene A protein nick site in RF DNAs of bacteriophages G14 and α3. To determine the position of the ϕX gene A protein nick in RF DNAs of bacteriophages G14 and α3, both G14 RFI DNA(30 μg) and α3 RFI DNA (30 μg) were incubated with and without ϕX gene A protein, and subsequently RF DNAs were digested with restriction endonuclease *Hae*III. Following the experimental steps indicated in Fig. 1, ^{32}P-end-labeled *Hae*III restriction DNA fragments were separated by electrophoresis on neutral polyacrylamide gels. Restriction endonuclease *Hae*III cleaves G14 RF DNA into 12 restriction DNA fragments and α3 RF DNA into 10 restriction DNA fragments. Each restriction DNA fragment was subsequently analyzed on polyacrylamide gels in 98% formamide.

G14 *Hae*III restriction DNA fragment Z1 (ca. 1,660 bp) and α3 *Hae*III restriction DNA frag-

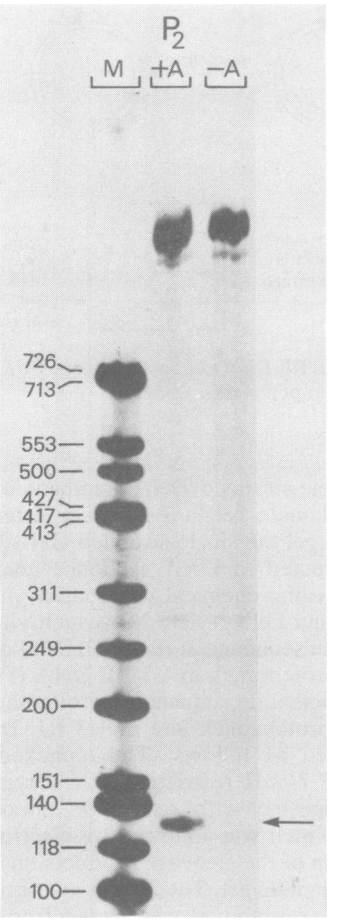

FIG. 2. Autoradiograph of a denaturing gel (4% polyacrylamide in 98% formamide) showing the U3 *Hpa*II restriction DNA fragment P2 obtained from φX gene A protein-nicked U3 RF DNA (+A) and the restriction DNA fragment P2 obtained from the control experiment in which the φX gene A protein was omitted (−A). The arrow marks the position of the DNA at the 3′ end of the φX gene A protein nick. In lane M 5′-^{32}P-labeled φX *Hin*fI restriction DNA fragments were run (lengths indicated in base pairs).

ment Z4 (ca. 725 bp) showed one extra labeled band on the denaturing gels (Fig. 5A [results for G14 restriction DNA fragments Z4 to Z12 not shown] and B [results for α3 restriction DNA fragments Z1 to Z3 and Z5 to Z10 not shown]). The length of the G14 single-stranded DNA fragment (ca. 1,200 nucleotides) and the length of the α3 single-stranded DNA fragment (ca. 600 nucleotides) do not allow an accurate determination of the nucleotide sequences at the 3′ ends of these fragments starting from the labeled 5′ ends. Therefore, we have looked for other restriction sites in the region around the φX gene A protein nick site in both G14 and α3 RF DNAs.

G14 restriction DNA fragment Z1 and α3 restriction DNA fragment Z4 were redigested with several restriction endonucleases. Length estimates of the redigestion products have led to cleavage maps of G14 restriction DNA fragment Z1 and α3 restriction DNA fragment Z4 (Fig. 6). By analysis of end-labeled DNA subfragments of both the nicked G14 restriction DNA fragment Z1 and the nicked α3 restriction DNA fragment Z4 on denaturing gels, the position of the φX gene A protein nick site in these restriction DNA fragments could be indicated unambiguously.

According to the cleavage maps (Fig. 6), the φX gene A protein nick site in G14 RF DNA is located in a 110-bp *Hin*cII restriction DNA fragment, whereas the φX gene A protein nick site in α3 RF DNA is located in a 190-bp *Hin*fI-*Hae*III restriction DNA subfragment of *Hae*III restriction DNA fragment Z4. The exact positions of these sites in G14 and α3 RF DNAs were determined as follows. G14 RFI DNA (30 μg) was incubated both with and without φX gene A protein. Subsequently, 5′-^{32}P-labeled *Hin*cII restriction DNA fragments were prepared from G14 RF DNA. The nicked 110-bp *Hin*cII restriction DNA fragment R9 showed one extra labeled band after electrophoresis on a denaturing gel, with the expected length of about 40 nucleotides (Fig. 7A).

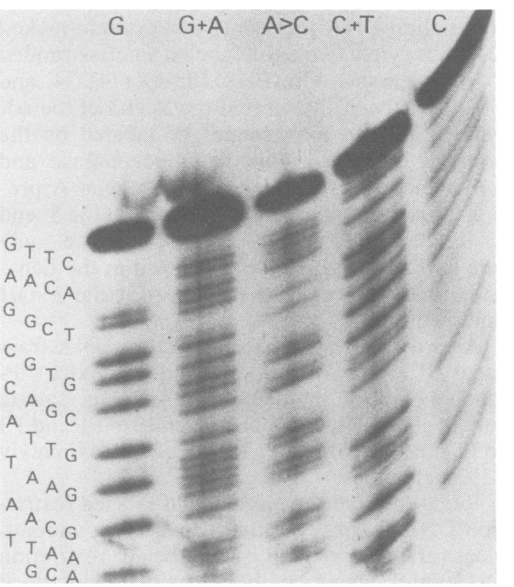

FIG. 3. Autoradiograph of the DNA sequence gel of the single-stranded P2 DNA subfragment (see text), showing the nucleotide sequence at the 3′ end of the φX gene A protein nick in U3 RF DNA.

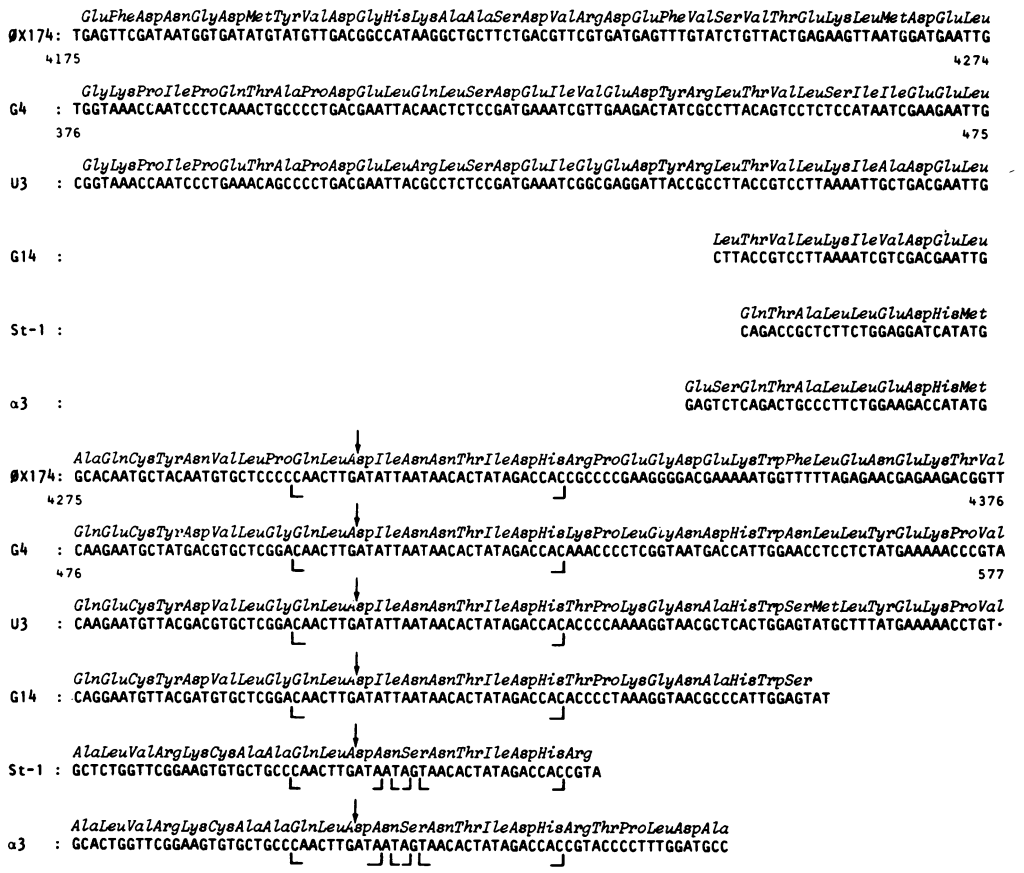

FIG. 4. Nucleotide sequences in RF DNAs of ϕX, G4, U3, G14, St-1, and α3 in the region of the ϕX gene A protein nick (indicated by vertical arrows) with the known amino acid composition of part of the gene A proteins of ϕX and G4 and the amino acid sequences which the DNA sequences of U3, G14, St-1, and α3 are presumed to code for. The sequence data and the numbering of ϕX174 are from Sanger et al. (24), those of G4 are from Godson et al. (13), and the sequence data of St-1 were taken from reference 15.

*Hae*III restriction DNA fragment Z4 was prepared from ϕX gene A protein-nicked and unnicked α3 RFI DNA (30 μg). DNA fragment Z4 was redigested with restriction endonuclease *Hin*fI which was followed by 5′-^{32}P-labeling of the DNA subfragments. Electrophoresis of the nicked 190-bp *Hin*fI-*Hae*III restriction DNA fragment in denaturing conditions yielded one extra labeled band with the expected length of 65 nucleotides (Fig. 7B).

The G14 40-nucleotide fragment and the α3 65-nucleotide fragment were eluted from the denaturing gels and subjected to DNA sequence analysis according to the chemical degradation method (21, 22), which yielded the nucleotide sequence at the 3′-OH end of the ϕX gene A protein nick in G14 and α3 RF DNAs (Fig. 4).

Nucleotide sequence surrounding the ϕX gene A protein nick site in G14 and α3 RF DNAs. To determine the nucleotide sequence surrounding the ϕX gene A protein nick site in G14 RF DNA, the unnicked G14 restriction DNA fragment Z1 was digested with restriction endonuclease *Hpa*II. After 5′-^{32}P-labeling, the DNA fragments were redigested with restriction endonuclease *Hin*fI. The 315-bp DNA fragment (Fig. 6A) was eluted from a neutral polyacrylamide gel on which the *Hin*fI cleavage products had been separated. DNA sequence analysis of this fragment yielded the nucleotide sequence surrounding the ϕX gene A protein nick site in G14 RF DNA (Fig. 4).

The nucleotide sequence surrounding the ϕX gene A protein nick site in α3 RF DNA was determined as follows. The unnicked ^{32}P-labeled 190-bp *Hin*fI-*Hae*III restriction DNA fragment (prepared as described above) was digested with restriction endonuclease *Hpa*II. The 135-bp DNA fragment (Fig. 6B) was eluted from a neutral polyacrylamide gel on which the *Hpa*II

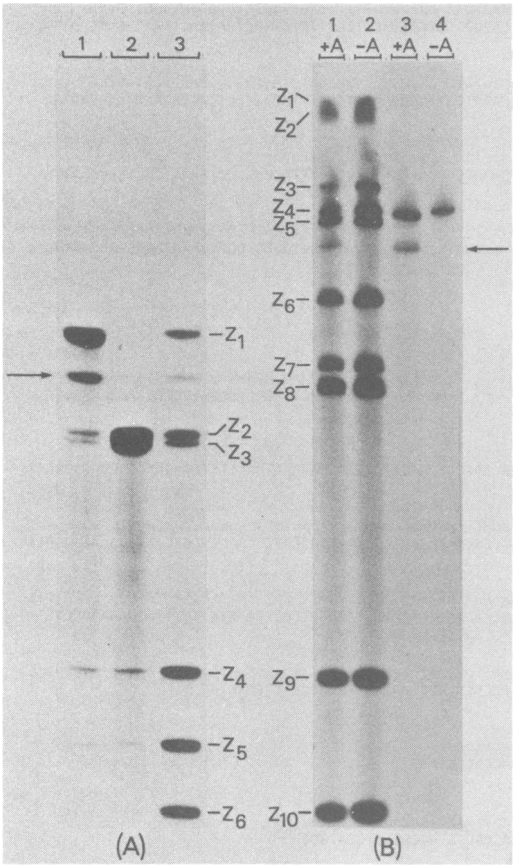

FIG. 5. Autoradiographs of denaturing gels which show (A, lane 1) G14 *Hae*III restriction DNA fragment Z1 obtained from ϕX gene A protein-nicked G14 RF DNA; (A, lane 2) restriction DNA fragments Z2 and Z3 obtained from ϕX gene A protein-nicked G14 RF DNA; (A, lane 3) part of the *Hae*III digest of ϕX gene A protein-nicked G14 RF DNA. (B) shows: (lane 1) *Hae*III digest of ϕX gene A protein-nicked α3 RF DNA; (lane 2) *Hae*III digest of α3 RFI DNA (ϕX gene A protein incubation omitted); (lanes 3 and 4) α3 restriction DNA fragment Z4 obtained from ϕX gene A protein-nicked α3 RF DNA (+A) or from α3 RFI DNA (−A). The arrows mark the position of the DNA at the 3′ end of the ϕX gene A protein nick in G14 RF DNA (A) and in α3 RF DNA (B).

cleavage products had been separated. DNA sequence analysis of this fragment yielded the nucleotide sequence surrounding the ϕX gene A protein nick site in α3 RF DNA (Fig. 4).

DISCUSSION

Earlier studies from our laboratory and elsewhere have shown that the nucleotide sequences surrounding the ϕX gene A protein cleavage site in the viral strands of ϕX RF DNA and G4 RF DNA have a stretch of 30 nucleotides in common (9, 13, 19, 24, 29). The origin of ϕX RF DNA replication is contained in this 30-nucleotide sequence. In St-1 RF DNA this 30-nucleotide stretch occurs at the ϕX gene A protein nick site with two changes, namely, T (nucleotide 4,309 in ϕX; nucleotide 510 in G4) → A (in St-1) and A (nucleotide 4,312 in ϕX; nucleotide 513 in G4) → G (in St-1) (15). The question of which nucleotide sequences in this strikingly conserved 30-nucleotide sequence are involved in initiation of RF DNA replication formed the basis for the present study on bacteriophages U3, G14, and α3.

The isometric phages have been classified in several groups due to differences in host range (5), antiserum specificity (11, 27), and requirements for host DNA synthesis proteins (6). Representatives of the various groups have been studied in this paper.

The results show that the ϕX gene A protein nick in RF DNAs of bacteriophages U3, G14, and α3 creates in all cases, as has been observed with ϕX, G4, and St-1 RF DNAs (15, 19, 29), a 3′-OH-G terminus and a 5′ terminus, presumably an A residue to which the gene A protein is covalently bound.

The nucleotide sequences surrounding the ϕX gene A protein nick site in RF DNAs of bacteriophages ϕX, G4, U3, G14, St-1, and α3 are shown in Fig. 4. Comparison of these nucleotide sequences reveals that the 30-nucleotide sequence surrounding the ϕX gene A protein nick site in ϕX and G4 RF DNAs is also present in the DNA sequences of U3 and G14. In α3 RF DNA this 30-nucleotide sequence occurs at the ϕX gene A protein nick site with the same two base changes observed in the St-1 DNA sequence. Consequently, perfect DNA sequence homology around the ϕX gene A protein nick site in ϕX, G4, U3, G14, St-1, and α3 RF DNAs is only 10 nucleotides, the decamer sequence CAACTTGATA.

A strongly conserved stretch of 30 nucleotides is present at the ϕX gene A protein nick site in RF DNAs of all six phages, although some changes within this region are permitted (cf. St-1, α3). These results support and confirm our current model for initiation of ϕX RF DNA replication (Fig. 8) (1a, 4, 14).

According to this model, a region of approximately 30 nucleotides around the ϕX gene A protein nick site in ϕX RF DNA is involved in this process. Within this origin region, two separate conserved domains should be distinguished: a recognition sequence for the gene A protein, the decamer sequence CAACTTGATA (28), and a key or binding sequence for the gene A protein. These two domains are separated by an adenine-thymine-rich sequence in which varia-

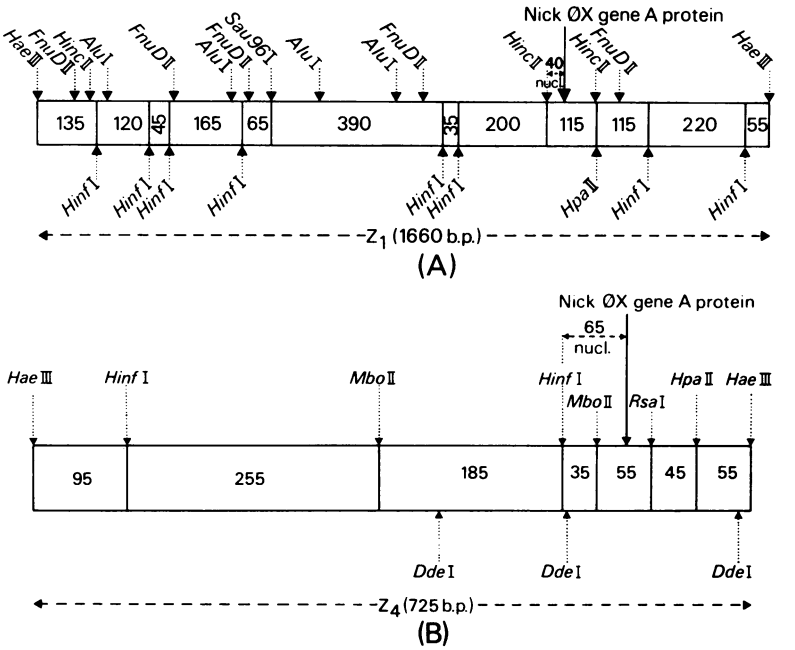

FIG. 6. Restriction endonuclease cleavage maps of G14 restriction DNA fragment Z1 (A) and α3 restriction DNA fragment Z4 (B).

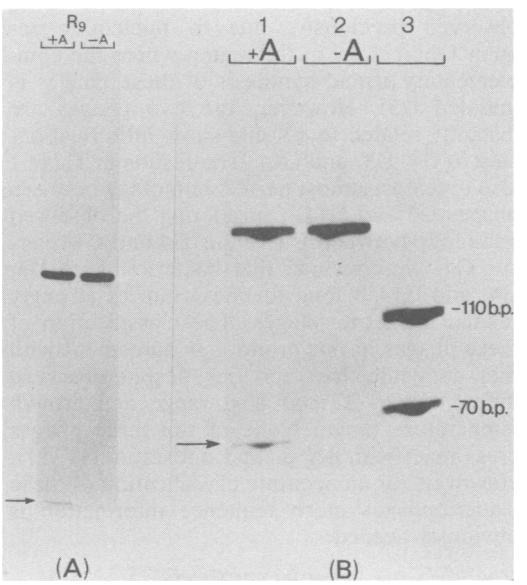

FIG. 7. Autoradiographs of denaturing gels showing: (A) G14 *Hin*cII restriction DNA fragment R9 obtained from the experiment with (+A) or without (−A) ϕX gene A protein incubation on G14 RFI DNA; (B, lanes 1 and 2) the 190-bp restriction DNA fragment (see text) obtained from nicked α3 RF DNA (+A) or unnicked α3 RFI DNA (−A). In (B) lane 3, ^{32}P-labeled marker DNA fragments were run. The arrows mark the position of the DNA at the 3′ end of the nick in G14 RF DNA (A) and α3 RF DNA (B).

tion may occur. In supercoiled ϕX RFI DNA the gene A protein binds to the key sequence, causing local denaturation in the DNA helix and exposure of the recognition sequence in a single-stranded form. This local denaturation is facilitated by the adenine-thymine-rich sequence and supercoiling of the DNA. Then the gene A protein cleaves the single-stranded recognition sequence CAACTTGATA next to the G-residue at position 4,305 of the ϕX DNA sequence, which allows DNA replication to start.

In comparing the nucleotide sequences shown in Fig. 4, it is clear that, outside the 30-nucleotide stretch which is conserved at the ϕX gene A protein nick sites in ϕX, G4, U3, G14, St-1, and α3 RF DNAs, many nucleotide changes occur in the DNA sequences of these six phages. The only other nucleotide sequence shared by these six phages is the pentamer sequence GTGCT and is found 15 to 16 nucleotides downstream from the ϕX gene A protein nick sites. We cannot exclude the possibility that this pentamer sequence is another important element in the interaction between the ϕX gene A protein and the origin region.

Because in ϕX and G4 the origin of RF DNA replication is located within viral gene A, it is reasonable to assume that this is also the case for U3, G14, St-1, and α3. If we further assume that the ϕX gene A protein-cleaved strands of the respective RFI DNAs are the viral strands

TABLE 1. Nucleotide sequence homology and amino acid sequence homology among the isometric phages φX, G4, U3, G14, St-1, and α3, in part of the A genes (shown in Fig. 4)[a]

Phage	% Nucleotide sequence homology						% Amino acid sequence homology					
	φX	G4	U3	G14	St-1	α3	φX	G4	U3	G14	St-1	α3
φX	100.0						100.0					
G4	54.0	100.0					41.8	100.0				
U3	51.2	81.1	100.0				37.3	83.6	100.0			
G14	65.5	79.6	92.9	100.0			56.8	81.1	97.3	100.0		
St-1	56.5	43.5	47.1	47.1	100.0		46.4	28.6	32.1	32.1	100.0	
α3	55.2	43.8	42.9	44.1	94.1	100.0	42.9	22.9	28.6	29.4	100.0	100.0

[a] Except for φX versus G4 (see reference 12), the numbers have not been corrected for possible deletions and insertions occurring in one sequence relative to the other.

and that the complementary strands are the template strands for mRNA synthesis, then only one of the possible reading frames in the DNA sequences of U3, G14, St-1, and α3 contains no stop codons. This reading frame is the same one that is used in φX (24) and G4 (13). The nucleotide sequence data and the corresponding amino acid sequences so far obtained from bacteriophages U3, G14, St-1, and α3 have been compared with the corresponding sequence data for φX and G4 (Fig. 4). The results of this comparison, expressed in percent nucleotide sequence homology and amino acid sequence homology, have been compiled in Table 1.

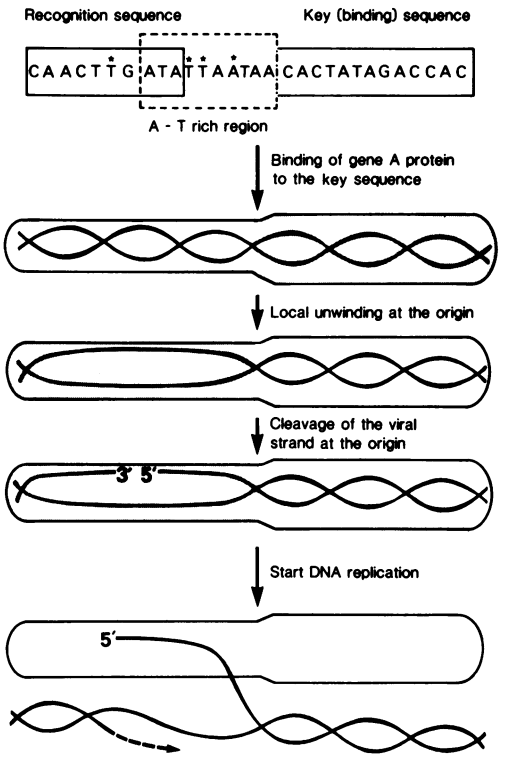

FIG. 8. Model for interaction of the φX gene A protein with the origin region in bacteriophage φX174 RF DNA. Nucleotides at positions indicated by the asterisks can be changed without effect on φX gene A protein nicking or DNA replication ability (4, 15). See text for further explanation. A-T, Adenine-thymine.

From the results (Table 1) it is clear that the relationship between St-1 and α3 is very close. Compared with St-1, the observed five nucleotide changes in the α3 DNA sequence all are third-base changes in the amino acid codons, which results in identical amino acids in the gene A proteins of St-1 and α3. A close relationship between bacteriophages St-1 and α3 has been observed previously, due to nucleotide sequence homology in the region where the complementary strand synthesis of these phages is initiated (25). However, the two phages are distantly related to φX and show little relationship to G4, U3, and G14. The results in Table 1 also reveal an almost perfect homology between phages U3 and G14. Considering the observed homology between U3 versus G4 and G14 versus G4, we conclude that bacteriophages U3, G4, and G14 belong to one group of strongly related isometric phages. The classification of these phages in one group is in agreement with their antigenic characteristics, despite observed differences in *E. coli* host range and growth temperature range. None of the three phages cross-react with φX or St-1 antiserum (11, 27). However, for an accurate classification of these bacteriophages more sequence information is obviously needed.

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LITERATURE CITED

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