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Sequence Analysis of the Termini of Virion and Replicative Forms of Minute Virus of Mice DNA Suggests a Modified Rolling Hairpin Model for Autonomous Parvovirus DNA Replication

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The nucleotide sequences of the terminal regions of monomer replicative form DNA, a pivotal intermediate species in the replication of minute virus of mice, were determined. The left (3′) terminus had a unique sequence on both strands and in both 3′-hairpin configurations. In contrast, the right (5′) terminus was sequence heterogenous and extended an additional 18 base pairs beyond that expected from the known sequence of the virion DNA. These data unambiguously establish the sequence complexity at the termini of both the single-stranded viral genome and the pool of replicative DNA. A comparison of the combined sequence information leads us to propose a modified rolling hairpin model for the replication of autonomous parvoviruses which is compatible with all available data.

The family Parvoviridae consists of two groups of mammalian parvoviruses: the defective (helper-dependent) subgroup which contains the adeno-associated viruses (AAV) and the autonomous (helper-independent) subgroup to which minute virus of mice (MVM) belongs (5). These viruses contain single-stranded DNA genomes 4.5 to 5.2 kilobases in size. The basic features of parvoviral DNA replication are now fairly well characterized (6, 20, 27), and it is clear that palindromic sequences at each genomic terminus play a major role in the replication process. AAV DNA contains both natural and inverted terminal repetitions (7, 16); the terminal repetition is 145 nucleotides long and within it is a palindromic sequence of 125 residues that forms a hairpin structure (17). Each 3′- and 5′-hairpin structure contains two sequences that are the inverted complement of the other (17). This observation is consistent with a hairpin transfer mechanism of replication (9, 23, 27) in which the palindromic region of the genome terminus is transferred from the parental to the progeny strand during replication. Since the palindromic sequence of AAV DNA is imperfect, two sequences are generated at each terminus during this process.

The autonomous parvoviruses also have hairpin duplexes at both the 3′ and 5′ ends of their genomes (8, 10, 20); however, they do not contain inverted terminal repetitions. In addition, the 3′- and 5′-hairpin structures differ both in size and in sequence (1, 2, 10, 20, 21). The 3′-hairpin structures of four serologically distinct rodent parvoviruses have essentially identical sequences (1) and contain 115 or 116 nucleotides that are totally unique; no sequence heterogeneity is detected, in contrast to AAV DNA. Since base mismatches occur within these palindromes, sequence heterogeneity would be expected if the autonomous parvoviral genomes are processed from replication intermediates in precisely the same manner as AAV. These observations support the initial proposal that these viruses replicate via an obligatory dimer replicative form (RF) molecule (27) but suggest that the maturation of progeny DNA must occur by an alternative mechanism that retains the same sequence orientation as the parental genome (1). It is still possible, however, that two sequence orientations are present in the pool of RF DNA intermediates but that only a single sequence orientation is packaged. Similar ambiguities can also arise from the fact that a protein is covalently bound to the 5′ ends of both strands of RF DNA (M. B. Chow, Ph.D. thesis, Yale University, New Haven, Conn., 1981; 3). Although only a single sequence is observed when the left (3′) terminus of monomer RF DNA is analyzed after kinasing the 5′ end of the complementary (C) DNA strand (Chow, Ph.D. thesis), it is still possible that only the "flip" orientation is labeled in the kinase reaction and that the "flop" orientation is selectively blocked by the protein.

To further define the processing events that occur during DNA replication, we have definitively established that the 5′ terminus of MVM DNA is sequence heterogenous, as predicted by a hairpin transfer process (3, 4). In this paper, we report the sequence of the 3′- and 5′-terminal regions of monomer RF DNA species, a predominant intermediate in MVM DNA replication. This study demonstrates conclusively that the left (3′) terminus of all monomer RF DNA molecules in the replication pool contain only one sequence orientation, identical to that of the parental viral genome. The right (5′) terminus contains two sequences, like the 5′ end of virion DNA. An additional surprising observation is that the 5′ end of the monomer RF is 18 base pairs (bp) longer than expected; thus, the viral (V) strand of DNA in the RF molecule contains 18 nucleotides that are not present in the V-strand DNA extracted from virus particles.

Taking these data into consideration, we now propose a modified rolling hairpin model for the replication of autonomous parvoviruses which is consistent with all the sequence and biochemical data available. This model is similar to that described briefly by Astell et al. (1), but it provides a mechanism for processing dimer RF DNA into two metabolically active monomer RF molecules that are identical at the 3′ terminus to the parental (monomer) RF. This model is also

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compatible with replication of the defective parvoviruses, such as AAV-2.

MATERIALS AND METHODS

Sources of materials were described previously (1). DNA polymerase I (Klenow fragment) was purchased from Boehringer Mannheim Biochemicals. Viral growth and DNA purification procedures have been summarized previously (8, 24), as have techniques for restriction endonuclease digestions, end labeling of DNA, sequencing, and acrylamide gel electrophoresis (1). Monomer RF was isolated from mouse A9 L cells infected with MVM(p) at a multiplicity of infection of 10. Cells were harvested at 20 tc 24 h postinoculation, and low-molecular-weight DNA was prepared by a modification of the Hirt extraction (19). RF DNA was purified by electrophoresis on 0.6% agarose gels.

RESULTS

The 3′ and 5′ ends of monomer RF DNA refer, respectively, to the 3′ and 5′ regions of the V strand.

Sequence of the 3′ end of monomer RF DNA: 3′ end labeling the terminus of the V strand. Monomer RF DNA was 3′ end labeled at both termini with [α-^{32}P]dTTP by using DNA polymerase I (Klenow fragment). The DNA was subsequently restricted with *Rsa*I, and labeled fragments were separated on a polyacrylamide gel (Fig. 1, lane A). Two fragments of ~90 and ~680 bp were observed. From restriction endonuclease mapping and previous sequencing data on the viral genome (4), we expected a terminal *Rsa*I fragment of ~700 bp from the 3′ end and a smaller fragment (~68 bp) from the 5′ end of the monomer RF. We tentatively identified the 680-bp band as the 3′-terminal fragment and the 90-bp band as the 5′-terminal fragment. The discrepancy in the predicted size of the 5′ terminus (~68 bp) and its observed size (~90 bp) is significant and is discussed in more detail below. The 3′-terminal (680-bp) fragment (Fig. 1, lane A) was sequenced by the method of Maxam and Gilbert (18). The sequence of this fragment (data not shown) was unique and was identical to the sequence of the 3′ terminus of V-strand DNA (1, 4).

Sequence of the 3′ end of monomer RF DNA: 3′ end labeling the *Hin*fI site at nucleotide 225. To sequence the C strand of the 3′-terminal region, monomer RF was restricted with *Hin*fI and labeled with [α-^{32}P]dGTP. From the complete sequence of MVM (4), we were able to predict that four fragments (1,750, 1,700, 290, and 225 bp) should be labeled. Of these, the 225-bp fragment should contain the 3′-terminal palindrome region in the extended configuration (see Fig. 6). All of these fragments should be uniquely labeled at one end of the fragment. In fact, five fragments were observed (Fig. 1, lane C), the additional fragment being ~185 bp. To characterize these fragments, all were sequenced. The 225-bp fragment, which indeed corresponds to the extended configuration of the 3′ terminus, contained a unique sequence (Fig. 2A) that agreed completely with the sequence obtained from the 680-bp fragment (see above) and with previous restriction endonuclease analysis of monomer RF DNA (1). Of particular interest is the 185-bp fragment (Fig. 2B). It is also a 3′-terminal fragment and, as expected, shares sequence with the 225-bp fragment (Fig. 2A). The fact that this fragment is ~180 bp long and contains ~360 nucleotides indicates it is the "loop end" fragment of covalently linked V and C strands, i.e., the 3′-terminal sequence in the hairpin or "joined" configuration (see Fig. 6).

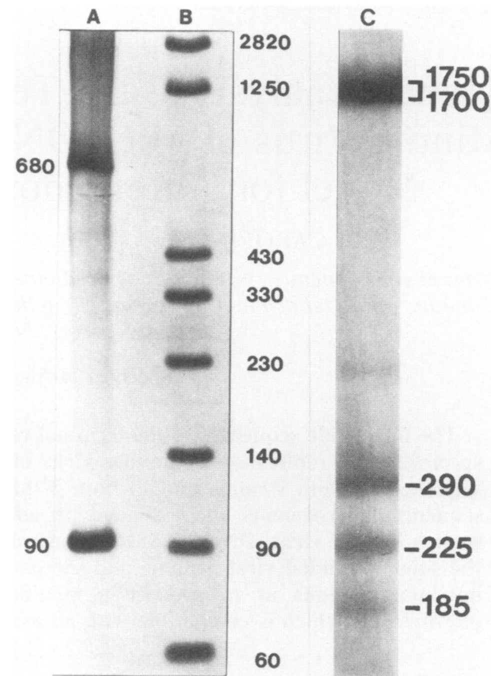

FIG. 1. Terminal *Rsa*I and *Hin*fI fragments from monomer RF. In lane A, monomer RF was 3′ end labeled with [α-^{32}P]dTTP by using DNA polymerase I (Klenow fragment) cut with *Rsa*I, and the digested DNA was electrophoresed in a 5% polyacrylamide gel. The 90-bp fragment is from the left (3′) end, and the 680-bp fragment is from the right (5′) end. Lane B is ϕX174 *Taq*I marker fragments. In lane C (run on a different gel), *Hin*fI-digested monomer RF DNA was 3′ end labeled with cold dATP and [α-^{32}P]dGTP. From other restriction mapping data, the 225-bp fragment is the 3′-terminal fragment, the 1,750-bp fragment is from the 5′ terminus, and the 1,700- and 290-bp fragments are internal fragments. The 185-bp fragment is a 3′-terminal fragment in which the viral and complementary strands are covalently linked (see Fig. 6E and F).

Sequence of the right (5′) end of monomer RF DNA: 3′ end labeling of the C strand. The 90-bp fragment, 3′ end labeled on the C strand with [α-^{32}P]dTTP (Fig. 1A), was sequenced and shown to be heterogenous from the first nucleotide on (data not shown). This suggests that the C strand at the 5′ end of the RF molecules is frayed. Attempts to kinase the 5′ end of the V strand in monomer RF DNA also gave multiple bands on sequencing (Chow, Ph.D. thesis); hence, the 5′ end of the V strand of monomer RFs is also frayed. Thus, neither strand at the 5′ end of monomer RF DNA contains a unique terminal nucleotide. The 5′ terminus of monomer RF was, however, found to be labeled uniquely when the 3′ end of the C strand was labeled with dTTP and [α-^{32}P]dGTP by using DNA *Pol*I (Klenow fragment). The 90-bp fragment product of this reaction was sequenced by the method of Maxam and Gilbert (Fig. 3). Of particular interest is that this fragment is 18 bp longer than predicted from the sequence of the viral strand (3). The 5′ end of the V-strand DNA isolated from mature virus particles terminates with the sequence 3′-CATTATGATTA-5′, whereas the V strand of monomer RF DNA terminates in the sequence 3′-CATTATGATTACCA AGTCAACCAAGTGAC-5′. The implications of this observation with respect to the mechanism of MVM DNA replication are discussed below.

Structure of the 5′ terminus of monomer RF. Although we have not separated the two sequence orientations at the right

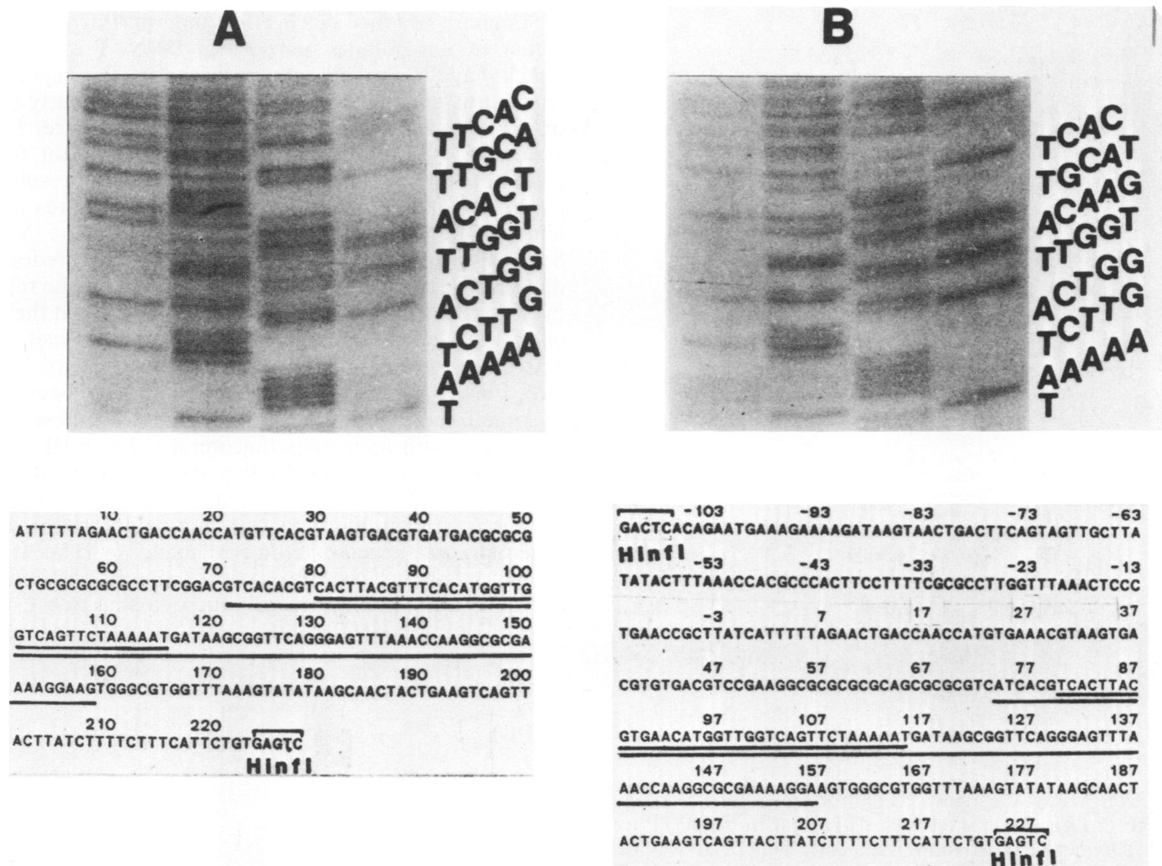

FIG. 2. Sequence of the 225- and 185-bp *Hin*fI fragments from monomer RF DNA (Fig. 1, lane C). The sequences of the 225-bp fragment (extended 3′-end conformation) and the 185-bp fragment (V and C strands covalently linked) which were read from these gels are underlined once, and the nucleotides displayed in A and B are underlined twice. The entire sequence of each fragment is also shown.

(5′) end of monomer RF, we do know these exist. For example, monomer RF DNA was 5′ end labeled with $^{32}PO_4$ by polynucleotide kinase, and the extended conformation of the 1.5-kilobase *Eco*RI B (right-end) fragment was isolated after electrophoresis on a 1% agarose gel. This fragment was digested with *Hha*I, which cuts within the 5′-terminal palindromic sequence, and the DNA digest was analyzed on a 12% polyacrylamide gel. Two discrete fragments of approximately 100 and 145 bp were observed in equimolar amounts (Fig. 4). A single end fragment would be expected if the RF DNA contained a unique terminal sequence. The presence of two end fragments also cannot be attributed to incomplete digestion, since the large excess of simian virus 40 carrier DNA present in the same reaction was digested to completion. An additional point to note is that the known sequence of the 5′-terminal sequence of virion DNA (3, 4) would predict that the *Hha*I fragments from the flip and flop sequence orientations would be 80 and 125 bp, respectively. The observed fragment sizes (~100 and 145 bp) are entirely consistent with the sequence data in Fig. 3 which demonstrate that the 5′ terminus of the V strand in monomer RF DNA contains an additional 18 nucleotides that are not present on the V-strand DNA in mature virus particles.

Additional evidence for sequence heterogeneity at the 5′ terminus comes from the following experiment. Monomer RF contains two *Msp*I (*Hpa*II) sites within the 5′-terminal region (4). A double digest of RF DNA with *Msp*I-*Alu*I and 3′ end labeling with [α-^{32}P]dCTP generated three labeled fragments, a 570-bp *Msp*I-*Alu*I fragment, a 9-bp *Msp*I-*Msp*I fragment, and a 119-bp *Msp*I–5′-terminal fragment. Sequence analysis of the 119- and 570-bp fragments showed that both sequences were initially homogenous but then became heterogenous (two sequences which are readable) (Fig. 5). The region of heterogeneity of both fragments is consistent with that predicted from the heterogenous sequence of the V strand (4).

DISCUSSION

Both conformations at the 3′ end of monomer RF DNA contain a unique sequence. Previous sequence studies (1) demonstrated only a unique 3′ structure for V-strand DNA; hence, we concluded that autonomous parvoviruses replicated their 3′ terminus in a different manner to the defective (e.g., AAV) genomes, which do exhibit two sequence orientations within the 3′-hairpin structure. However, it was still possible that two sequences were present in the pool of MVM DNA replicative intermediates but that only a single sequence orientation, the flip sequence, was packaged. In an attempt to demonstrate a unique or heterogenous structure at the 3′ terminus of monomer RF, the 3′-terminal end of monomer RF DNA, ^{32}P-labeled at the 5′ terminus of the C strand, was digested with restriction endonucleases which cut within the 3′ hairpin (*Hha*I and *Fnu*DII), and the labeled fragments were sized. The results were consistent with only one sequence orientation at the 3′-end monomer RF (Chow, Ph.D. thesis). However, it was possible that only the flip orientation was labeled in the kinase reaction and that the

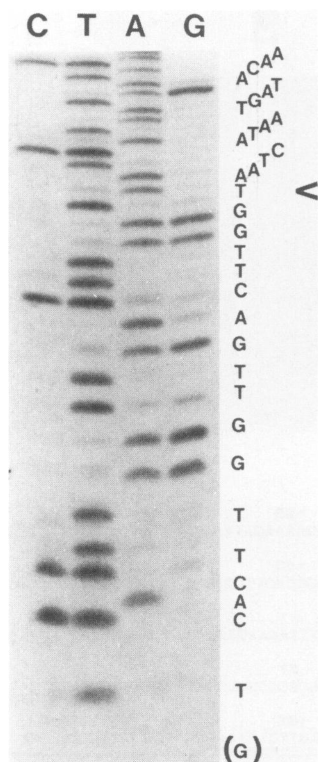

FIG. 3. Sequence of the right (5′) end of monomer RF DNA. Monomer RF (2 μg) was labeled with dTTP and [α-^{32}P]dGTP by using DNA polymerase I (Klenow fragment). The DNA was digested with *Rsa*I, and the 90-bp fragment corresponding to the 5′ end of the viral RF was sequenced by the method of Maxam and Gilbert (18) and analyzed on a 20% acrylamide (7 M urea) gel. The arrow denotes the beginning of the sequence TAATC, which is complementary to sequence ATTAG found at the 5′ terminus of mature V-strand DNA that is encapsidated in virions.

opposite orientation remained unlabeled because it was blocked with a protein. Indeed, a protein covalently attach at the 5′ ends of both strands of MVM monomer RF DNA has been detected (Chow, Ph.D. thesis; 3). To resolve these uncertainties about the true complexity at the 3′ genomic terminus, we cut monomer RF with *Hin*fI and labeled the terminal fragment at its internal (*Hin*fI) site. The sequence of the entire 225-nucleotide fragment was found to be unique; hence, we can conclude unambiguously that there is no hairpin transfer process as proposed in the original rolling hairpin model (25) for replication of the 3′ terminus of MVM DNA. This observation must be included in any model for the replication of autonomous parvovirus DNA.

The sequence of the loop end of covalently linked V and C strands in monomer RF DNA is also unique (Fig. 2B) and is precisely that predicted for the parental monomer RF from the known sequence of virion DNA. Although the sequence presented in Fig. 2B is the flop sequence orientation, this represents the C-strand sequence (i.e., the *Hin*fI fragment was 3′ end labeled on the C strand). Because this type of intermediate is a major one in the RF pool (from labeling *Hin*fI-digested monomer RF we estimate approximately 40 to 50% of the RF molecules are covalently linked) (Fig. 1, lane C), it also must be included as a major intermediate in any model of V-strand DNA replication.

Structural features at the 3′ and 5′ ends of V-strand DNA and monomer RF DNA are summarized in Fig. 6.

Summary of other biochemical data pertinent to the replication of autonomous parvovirus DNA. Previous studies which have identified intermediates in the replication of parvovirus genomes have been reviewed recently (15), and only three salient points will be mentioned here. First, the synthesis of parental RF DNA occurs within 6 to 8 h postinfection via a self-priming mechanism, resulting in a parental monomer RF in which V and C strands are covalently linked through the 3′ terminus of the V strand. Secondly, concatemeric RF DNA forms (predominantly dimer RF molecules) are detected throughout the replicative cycle, and pulse-chase experiments indicate that these molecules, as well as monomer RF species, are metabolically active and give rise to single-stranded progeny V strands (26). Finally, partially replicated dimers have been detected in some infected cell lines. The structure of these dimers is consistent with molecules that initiate dimer RF formation (step 3, Fig. 7) but terminate this synthesis prematurely near the 3′-terminal hairpin (12). Faust and Gloor suggest that these strong-stop intermediates may be due to the intervention of a site-specific telomeric nuclease (12). It is also possible that the DNA polymerase simply has difficulty in extending through the guanine- and cytosine-rich 3′-terminal

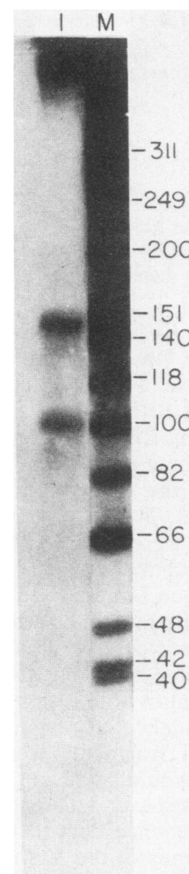

FIG. 4. *Hha*I digest of 5′-end-labeled *Eco*RI B fragment of RF DNA. MVM RF DNA was 5′ end labeled with T4 polynucleotide kinase and digested with *Eco*RI, and the fragments were separated on a 1% agarose gel. The *Eco*RI B fragment in the extended configuration (see Fig. 6) was isolated from the gel and further incubated with *Hha*I. The ^{32}P-labeled DNA was then run on an 8% polyacrylamide gel (lane 1). ϕX174 RF DNA digested with *Hin*fI is shown in lane M.

hairpin. Certainly, this is observed in vitro with *Escherichia coli* DNA polymerase Klenow fragment in DNA sequencing (Astell, unpublished data) and cloning (P. T. Tattersall, personal communication).

Modified rolling hairpin model for replication of autonomous parvovirus genomes. A model consistent with the available sequence and biochemical data on DNA intermediates is presented in Fig. 7. In this model, the incoming V strand (step 1) is extended by a host DNA polymerase, most likely the α enzyme (13), and the 5′ hairpin is displaced (step 2). At step 3, a rabbit-eared structure is formed (25, 27), and displacement synthesis occurs to produce the dimer RF (step 4). At this point, we propose that a site-specific nick is introduced as indicated by the arrow and extension of the (parental) V strand occurs in a 5′→3′ direction (step 5). This displaces the 3′ palindrome, enabling it to fold up into a hairpin configuration. It is also proposed that the same site-specific nickase remains covalently attached to the 5′ end of the nicked DNA and, after a second nick opposite the hairpin, that this nickase reseals a complete 3′ hairpin (step 6, left diagram). V-strand synthesis occurs by repeated displacement of V strands on a C-strand template (step 6). Note that this process will retain the sequence orientation at the 3′ end of V strands, whereas hairpin transfer will result in two sequence orientations at the 5′ end. These two sequences at the 5′ end will be related in that one will be the inverted complement of the other (steps 7 and 8). At step 6 (left diagram), it is proposed that a nick is introduced as indicated and that strand displacement generates a monomer RF with covalently linked V and C strands. This molecule (step 7, left diagram) is identical to the parental RF (step 2), with the exception of the 5′ end which has undergone hairpin transfer and is also 18 nucleotides longer (see below).

At step 6 in both diagrams, a nick is required that would cut the C strand near its 3′ end (i.e., near the 5′ hairpin of the V strand). The arrow is bent to indicate that the cut does not occur at the C-strand–hairpin boundary but is inside of this by some 18 nucleotides. We believe this to be the case because our data show clearly that monomer RF molecules are 18 bp longer at the 5′ end than V-strand DNA. Also, the nickase, which is possibly the same enzyme as that required in step 4 (3), becomes covalently linked to the 5′ end of the V strand. This could then be processed off before or during packaging (albeit not with 100% efficiency) such that most of the mature V strands do not have a protein attached. Evidence for a protein linked to the 5′ ends of H-1 RF DNA (19), MVM RF DNA (3), and MVM V-strand DNA (5′ end) (Chow, Ph.D. thesis) is in agreement with this proposal.

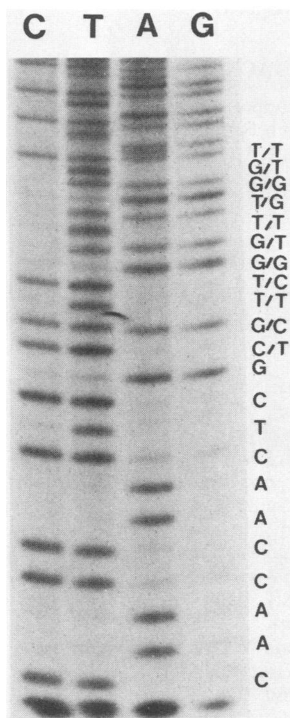

FIG. 5. Sequence of the 119-bp *Msp*I–5′-terminal fragment from monomer RF DNA. RF DNA (2 μg) was digested with *Msp*I and *Alu*I and labeled with [α-^{32}P]dCTP by using DNA polymerase I (Klenow fragment). The radioactive fragments were separated on a 5% acrylamide gel (100 V for 16 h), and the 119- and 570-bp fragments were electroeluted and sequenced. The nucleotide sequence of the 119-bp fragment is illustrated. The 570-bp fragment contained the same sequence and heterogeneity as seen with the 119-bp fragment. (The 9-bp *Msp*I-*Msp*I fragment ran off the bottom of the gel and was not recovered in this experiment.)

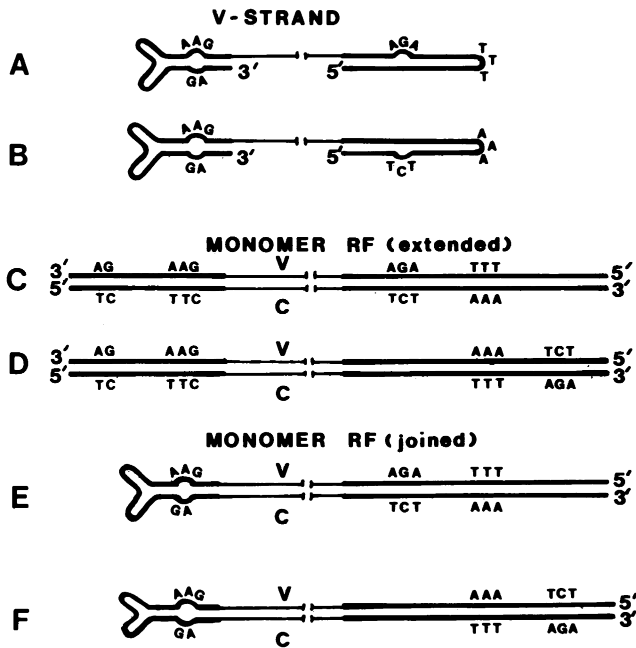

FIG. 6. Schematic illustration of the structures at the 3′ and 5′ ends of V-strand DNA, monomer RF DNA (extended), and monomer RF DNA (joined). The heterogeneity of the nucleotide sequence organization of the 3′ and 5′ hairpins of V-strand DNA is illustrated in A and B. The nucleotide sequence heterogeneity of the 3′- and 5′-terminal regions of monomer RF (extended form) is shown in C and D, and the structure of the 3′ loop which covalently links V and C strands of monomer RF is given in E and F. For simplicity, the asymmetry at the 3′ end is limited to the AAG bubble. The asymmetry in the arms of the hairpin are omitted here, but the data in all cases are consistent with a unique 3′ end.

Site-specific nuclease predicted to nick the V strand should have properties similar to the gene-A protein of φX-174. In the modified rolling hairpin model described above, we propose that an enzyme responsible for nicking the dimer RF (Fig. 7, steps 4 and 5) has an additional function, that of ligating the displaced 3′ hairpin to the lower V strand (step 5). The multipurpose enzyme is similar in function, then, to a group of enzymes, the topoisomerase I enzymes (14), and in particular the gene-A protein of φX174 (11). (As yet we

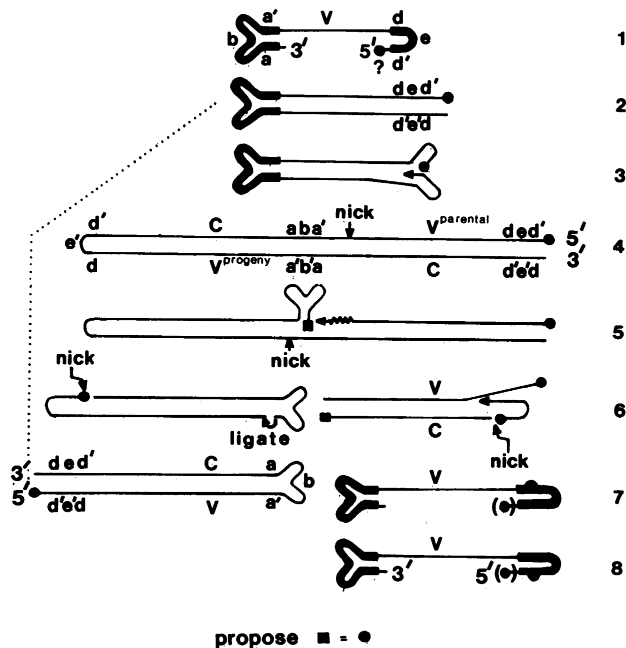

FIG. 7. Modified rolling hairpin model for autonomous parvovirus DNA replication. The circles attached to the 5′ ends of V-strand DNAs represent the blocking group (protein or its remnant?) that is presumed to be the site-specific nuclease. The squares are also presumed to be a site-specific nuclease and may be identical to the nuclease represented by the circles (3). However, this protein and approximately 18 nucleotides are processed from the 5′ end of most but not all V-strands; hence, the circle is bracketed. The question mark with parental V strand indicates that the blocking group has properties unlike that of the protein attached to the 5′ ends of RF DNA (see text for additional details).

have no biochemical evidence to suggest that the enzyme exists and that it is a topoisomerase.) The gene-A protein (presumed to be a dimer with two functional subunits) has been shown to nick the plus strand of φX RF at a specific site, and in doing so, one subunit of the enzyme becomes covalently attached to the 5′ end of the molecule. The plus strand is then extended around the circle, with the gene-A protein complex remaining associated with the replication site. As the gene-A protein encounters the regenerated double-stranded nick site, the second active subunit of the complex cuts the plus strand. The first gene-A protein subunit is then able to ligate the newly generated 3′ OH to the 5′ end of the displaced V strand, releasing the circular V strand.

In our model, the nickase is postulated to carry out analogous functions. If the nickase is a dimer of two active subunits, one subunit could cut the dimer RF preferentially at the site indicated in step 4 (we believe this site is specified by sequence; 3). As the 3′ OH is extended, the 3′ hairpin is displaced, although the 5′ end of the hairpin remains closely associated with the replication site. When the extension reaches the second nick site (which is very similar although not identical in sequence to the first), the second active subunit of the nickase could cut the lower strand as indicated (step 5). The dimer RF is now really two monomer RFs held together by the nickase subunits. At this point, and again with analogy to the gene-A protein complex, the 3′ OH end of the lower strand is ligated to the 5′ end of the displaced 3′ hairpin, releasing the first nickase subunit. (This may remain associated with the second subunit, which is attached to the 5′ end of the C strand [step 6, right diagram].)

It is intriguing that preliminary studies indicate that the protein associated with the 5′ end of MVM RF molecules is linked via a bond which appears to have the stability of a phosphotyrosine link (Chow and Ward, unpublished data). In several cases, topoisomerase I enzymes have been shown to be covalently attached to the 5′ end of nick sites via phosphotyrosine linkages (14). This linkage has also been demonstrated for the gene-A protein of φX174 (22).

Is the modified rolling hairpin model compatible with the replication of defective parvoviruses? Defective parvoviruses, such as AAV-2, have inverted terminal repetitions at the ends of the genome. These ends have been sequenced and found to contain a palindrome of some 145 nucleotides (17). This hairpin is also an imperfect palindrome and hence the 3′ and 5′ ends consist of two sequences, related in that one is the inverted complement of the other. This implies then that both the 5′ and 3′ ends of the genome undergo hairpin transfer. In a modified rolling hairpin model (Fig. 7), if the sequence of DNA near two nick sites at steps 4 and 5 were identical (and they are in AAV-2), then the nickase should bind to either the upper strand or lower strand with equal frequency. In this case, both 3′ and 5′ ends will undergo hairpin transfer. The difference in hairpin maturation in autonomous and defective parvovirus replication then may be due to the presence or absence of the bubble at 25 in the stem of the 3′ hairpin which generates a minor sequence heterogeneity near the nick sites. Experiments to test this proposal are in progress.

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LITERATURE CITED

1. **Astell, C. R., M. Smith, M. B. Chow, and D. C. Ward.** 1979. Structure of the 3′ hairpin of four rodent parvovirus genomes: nucleotide sequence homology at origins of DNA replication. Cell **17**:691–703.
2. **Astell, C. R., M. Smith, M. B. Chow, and D. C. Ward.** 1979. Sequence of the 3′ terminus of the genome from Kilham rat virus, a nondefective parvovirus. Virology **94**:669–674.
3. **Astell, C. R., M. Thomson, M. B. Chow, and D. C. Ward.** 1983. Structure and replication of minute virus of mice DNA. Cold Spring Harbor Symp. Quant. Biol. **47**:751–762.
4. **Astell, C. R., M. Thomson, M. Merchlinsky, and D. C. Ward.** 1983. The complete DNA sequence of minute virus of mice, an autonomous parvovirus. Nucleic Acids Res. **11**:999–1018.
5. **Berns, K. I. (ed.).** 1984. The parvoviruses, Plenum Publishing Corp., New York.
6. **Berns, K. I., and W. W. Hauswirth.** 1979. Adeno-associated viruses. Adv. Virus Res. **25**:407–432.
7. **Berns, K. I., and T. J. Kelley, Jr.** 1974. Visualization of the inverted terminal repetition in adeno-associated virus DNA. J. Mol. Biol. **82**:267–271.
8. **Bourguignon, G. J., P. J. Tattersall, and D. C. Ward.** 1976. DNA of minute virus of mice: self-priming, nonpermuted, single-stranded genome with a 5′-terminal hairpin duplex. J. Virol. **20**:290–306.
9. **Cavalier-Smith, T.** 1974. Palindromic base sequences and replication of eukaryotic chromosome ends. Nature (London) **250**:467–470.
10. **Chow, M. B., and D. C. Ward.** 1978. Comparison of the terminal nucleotide structures in the DNA of nondefective parvoviruses,

p. 205–217. *In* D. C. Ward and P. Tattersall (ed.), Replication of mammalian parvovirus. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

11. **Eisenberg, S., J. Girffiths, and A. Kornberg.** 1977. φX174 cistron A protein is a multi-functional enzyme in DNA replication. Proc. Natl. Acad. Sci. U.S.A. **74:**3198–3202.
12. **Faust, E. A., and G. Gloor.** 1984. Characterization of a metastable, partially replicated dimeric intermediate of minute virus of mice. J. Virol. **49:**621–625.
13. **Faust, E. A., and C. D. Rankin.** 1982. *In vitro* conversion of MVM parvovirus single-stranded DNA to the replicative form by DNA polymerase α from Ehrlich ascities tumor cells. Nucleic Acids Res. **10:**4181–4201.
14. **Gellert, M.** 1981. DNA topoisomerases. Annu. Rev. Biochem. **50:**879–910.
15. **Hauswirth, W. W.** 1984. Autonomous parvovirus DNA structure and replication, p. 129–152. *In* K. I. Berns (ed.),The parvoviruses. Plenum Publishing Corp., New York.
16. **Koczot, F. J., B. J. Carter, C. F. Garon, and J. A. Rose.** 1973. Self-complementarity of terminal sequences within plus or minus strands of adeno-associated virus DNA. Proc. Natl. Acad. Sci. U.S.A. **70:**215–219.
17. **Lusby, E., K. H. Fife, and K. I. Berns.** 1980. Nucleotide sequence of the inverted terminal repetition in adeno-associated virus DNA. J. Virol. **34:**402–409.
18. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. **74:**560–564.
19. **Revie, D., B. Y. Tseng, R. H. Graftsom, and M. Goulian.** 1979. Covalent association of protein with replicative form DNA of parvovirus H1. Proc. Natl. Acad. Sci. U.S.A. **76:**5539–5543.
20. **Rhode, S. L., III, and B. Klaassen.** 1982. DNA sequence of the 5′ terminus containing the replication origin of parvovirus replicative form DNA. J. Virol. **41:**990–999.
21. **Rhode, S. L., III, and P. R. Paradiso.** 1983. Parvovirus genome: nucleotide sequence of H-1 and mapping of its genes by hybrid-arrested translation. J. Virol.**45:**173–184.
22. **Sanhueza, S., and S. Eisenberg.** 1984. Cleavage of single-stranded DNA by the φX174 A* protein: the A*-single-stranded DNA covalent linkage. Proc. Natl. Acad. Sci. U.S.A. **81:**4285–4289.
23. **Straus, S. E., E. Sebring, and J. Rose.** 1976. Concatemers of alternating plus and minus strands are intermediates in adeno-virus-associated virus DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **73:**742–746.
24. **Tattersall, P., P. J. Cawte, A. J. Shatkin, and D. C. Ward.** 1976. Three structural polypeptides coded for by minute virus of mice, a parvovirus. J. Virol. **20:**273–289.
25. **Tattersall, P., and D. C. Ward.** 1976. Rolling hairpin model for replication of parvovirus and linear chromosomal DNA. Nature (London) **263:**106–109.
26. **Ward, D. C., and D. Dadachanji.** 1978. Replication of minute virus of mice DNA, p. 297–313. *In* D. C. Ward and P. Tattersall (ed.), Replication of mammalian parvoviruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Ward, D. C., and P. Tattersall.** 1982. Minute virus of mice, p. 313–332. *In* H. L. Foster, J. D. Small, and J. G. Fox (ed.), The mouse in biomedical research, vol. 2. Academic Press, Inc., New York.