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Parvovirus DNA Replication

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Parvoviruses are unique among all known viruses in having single-stranded DNA genomes which are linear. Virions are non-enveloped, containing a single copy of the small (4–6 kb) viral chromosome encapsidated in a rugged icosahedral protein capsid 18–26 nm in diameter. Although lacking associated enzymes or nucleosomal proteins, the particles have been shown, in some cases, to contain polyamines such as spermidine, spermine, and putrescine (Berns et al. 1995). The family Parvoviridae contains a broad spectrum of physically similar viruses that replicate in the nuclei of both invertebrate and vertebrate hosts. Viruses infecting mammalian cells form the subfamily Parvovirinae and include a number of helper-independent viruses, represented in this review by minute virus of mice (MVM), and the adeno-associated (AAV) viruses, represented here by AAV2, which, in general, only replicate in cells coinfected with a helper adenovirus or herpes virus. Further background information on the structure and biology of the parvoviruses can be obtained from reviews published elsewhere (Cotmore and Tattersall 1987; Berns 1990, 1995; Tjissen 1990; Muzyczka 1992). Space constraints allow only a representative selection of the most directly pertinent references to be cited herein.

Whereas AAV encapsidates, in separate virions, DNA strands of either sense, MVM selectively encapsidates (to 99%) strands that are minus sense with respect to transcription. Both viruses encode all of their known proteins from a single-sense DNA strand, and each encodes two separate gene complexes. Transcripts from one half of the genome, designated by convention the right-hand side, program synthesis of an overlapping set of capsid polypeptides, whereas the left half gives rise to a series of nonstructural proteins essential for viral DNA replication. These latter are designated the Rep proteins in AAV and the NS (nonstructural) proteins in MVM. Genome usage in all parvoviruses is remarkably efficient, so that some protein sequences are encoded in overlapping reading frames, and others contain regulatory elements for tran-

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scription or mRNA splicing. Nevertheless, the viruses' limited genetic capacity ultimately dictates that they adopt the replication machinery of the host cell, augmented and diverted by one or two specialized virally coded proteins.

ROLLING HAIRPIN REPLICATION

Parvoviral DNA replication in many ways resembles the single-strand-specific "rolling-circle" mechanisms previously characterized in prokaryotic replicons, such as the single-stranded coliphages and certain bacterial plasmids (Kornberg and Baker 1991). Incoming viral particles contain a single copy of the linear, non-permuted DNA genome which is first converted to a duplex replication intermediate. The relatively long single-stranded coding region in viral DNA is bracketed by short (121–421 base) palindromic terminal sequences capable of folding into hairpin duplexes. These telomeres play a central role in viral replication, containing most of the *cis*-acting information required for both replication and encapsidation, and their complexity and remarkable diversity in different viral genera suggest that they must serve multiple functions in the life cycle (Tjissen 1990; Berns et al. 1995). The hairpins pair the terminal 3′ nucleotide of incoming virion DNA with an internal base and, in so doing, create a DNA primer that allows a host polymerase to synthesize the first complementary DNA strand. Although unusual in eukaryotic replication, DNA primers created by the introduction of a single-strand nick into a duplex intermediate are the hallmark of prokaryotic rolling-circle mechanisms. The palindromic terminal sequences of parvoviruses allow this mechanism to be adapted for the replication of linear single-stranded progeny DNA, since a free 3′ hydroxyl group, generated in the previous host cell by the introduction of a site-specific nick into a duplex intermediate, is folded back and paired with an internal base in the progeny viral genome. Thus primed, complementary strand synthesis generates a monomer-length, duplex intermediate in which the two strands are covalently cross-linked at one end via a single copy of the viral 3′ telomere (step i in Fig. 1).

In some viruses (such as AAV2), this cross-linked 3′ structure creates a replication origin that can be activated by the Rep initiator endonuclease in a process called "terminal resolution," discussed below. In others, such as MVM, this structure does not appear to function as a replication origin. In either case, major viral DNA amplification proceeds through a series of concatemeric duplex intermediates via a unidirectional, single-strand-specific mechanism dubbed "rolling-

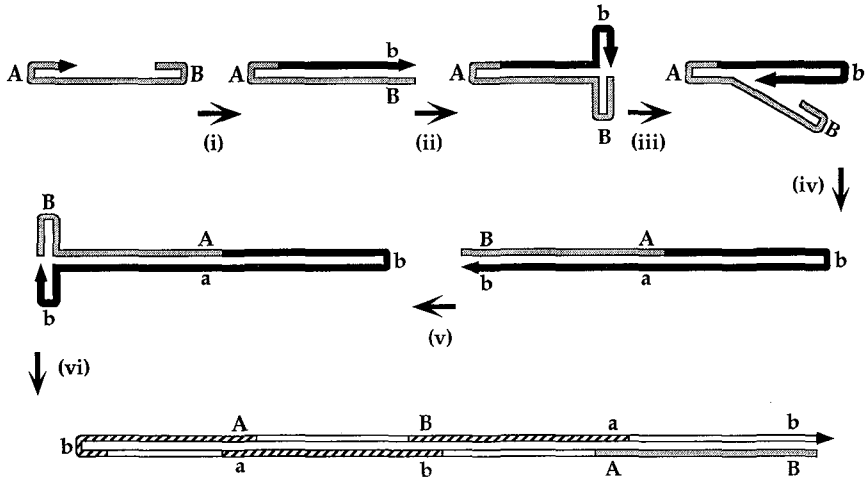

Figure 1 Rolling-hairpin replication. The sequence of the parental parvoviral genome is represented by a shaded bar. In steps *i* through *v*, newly synthesized DNA is shown as a black bar with an arrow at its 3′ end. *A* and *B* depict the palindromic sequences at each terminus, with their complements represented by *a* and *b*, respectively. Step *vi* produces a tetramer in which, for MVM, there are three progeny genomes, shown cross-hatched, in addition to the parental sequence. These overlap and are distributed throughout the molecule on alternate strands.

hairpin" synthesis. Instead of progressing continuously around circular templates as in prokaryotic rolling-circle systems, in rolling-hairpin synthesis the unidirectional replication fork appears to shuttle back and forth along the linear genome, changing direction as a result of the sequential synthesis and rearrangement of the palindromic viral termini, as diagrammed in Figure 1. Current models suggest that during this process, terminal hairpins are first unwound and copied by strand-displacement synthesis to create "extended-form" termini containing a single new DNA strand (step i) and these are then melted-out and reformed into hairpinned "rabbit-ear" structures (step ii) to provide the base-paired 3′ nucleotide needed to prime synthesis of additional linear sequences (step iii). As a result, the coding sequences of the virus are copied twice as often as the termini, and palindromic dimeric (step iv) and tetrameric (step vi) concatemers accumulate in which the unit-length duplex genomes within them are fused in left end:left end and right end:right end orientations.

Individual genomes are excised from these concatemers, and their telomeres are regenerated, by the introduction of single-strand nicks into

replication origins generated at either end of each genome during the rolling-hairpin process. Excision is accomplished by the pleiotropic virally coded initiator proteins Rep68 and Rep78 (AAV) or NS1 (MVM) and leads to the establishment of new unidirectional replication forks at the nick sites, which then duplicate the required terminal sequences. Extended-form termini created by this process are equivalent to those seen in Figure 1 (steps i and iv) and can theoretically be melted out and reformed into rabbit-ear structures capable of priming additional rounds of DNA synthesis. Subsequent displacement of progeny single strands requires ongoing viral DNA synthesis and only occurs in the presence of competent capsid proteins. Since all progeny DNA is found encapsidated, its synthesis likely involves direct sequestration of displaced strands into preformed, or partially formed, capsids.

THE DEPENDOVIRUS REPLICATION STRATEGY

The AAV Genome

The AAV2 genome is 4675 nucleotides in length, with a long single-stranded coding region bracketed between identical, but inverted, 145-nucleotide terminal repeat sequences. The distal 125 bases of each repeat form a complex palindrome capable of folding into the T-shaped hairpin structure shown in Figure 2. In the absence of a coinfecting herpesvirus or adenovirus, the infecting AAV genome integrates into the host genome with high efficiency and remains latent (for review, see Berns 1995). Viruses with an intact REP gene frequently integrate into a small region of chromosome 19q13-qter (Kotin et al. 1991), which contains a strong Rep:DNA-binding site (Weitzman et al. 1994), whereas viruses deleted for the REP gene integrate at many sites in the host genome (Walsh et al. 1993). Rescue from the latent state requires coinfection with a helper virus, not primarily because the helper supplies unique gene products required by AAV, but rather because it modifies the internal cellular environment in such a way as to permit productive AAV replication. Indeed, limited replication in the absence of a helper virus can be achieved if host cells are exposed to various forms of genotoxic stress (Yakobson et al. 1987; Yakinoglu et al. 1988). Model systems suggest that excision from the host DNA is mediated by Rep proteins (Samulski et al. 1983; Hong et al. 1992), which are expressed to high level only in the presence of a helper virus (Beaton et al. 1989).

The virus has transcriptional promoters at map units 5 (P5) and 19 (P19), accessing a single REP open reading frame. An intron within the 3′ end of the gene allows expression of either a spliced or unspliced

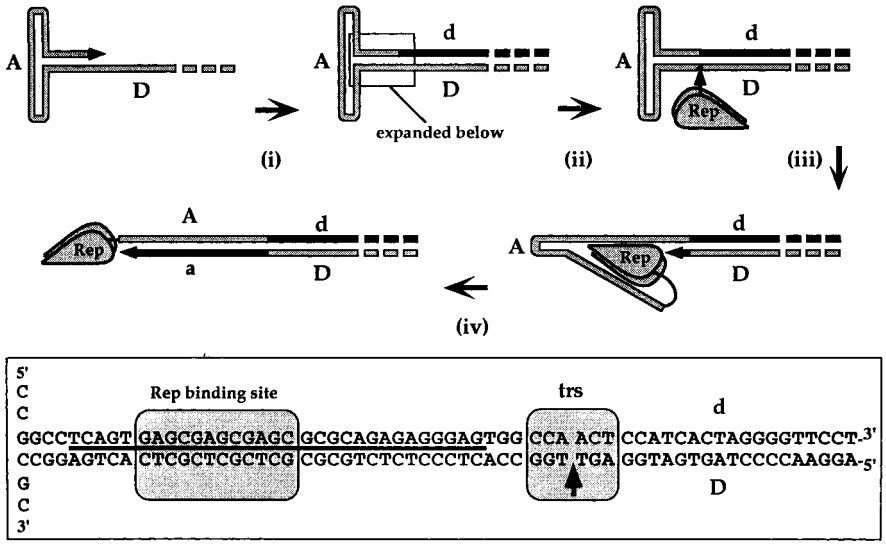

Figure 2 Terminal resolution reaction for AAV. The diagram is structured as in Fig. 1. In addition, *D* and *d* denote sequences that are contained within the terminal repeat but not within the hairpin. The double image of the Rep protein indicates that the active form is believed to be a dimer. In the expanded box, the sequence protected from DNase I digestion by Rep in an isolated linear origin (Chiorini et al. 1994) is indicated by the underline. The terminal resolution site is denoted *trs*, and the vertical arrow indicates the position of the Rep-induced nicking and 5′-attachment site.

version of each transcript, resulting in a total of four REP gene products. The two larger proteins, Rep78 and Rep68, are pleiotropic replication initiator proteins (Im and Muzyczka 1990, 1992), which appear to function almost identically in vitro (Ni et al. 1994). They are site-specific DNA-binding proteins, recognizing the sequence $(GAGC)_3$ in the stem region of the viral hairpin (Chiorini et al. 1994; McCarty et al. 1994) and variations on this sequence both within and outside the viral genome (Weitzman et al. 1994). Binding involves homodimers, if not higher-order multimers, of Rep, and such complexes can bind simultaneously to more than one DNA-binding site (McCarty et al. 1994). These proteins initiate replication by serving as site-specific endonucleases and are thought to be retained in the replication fork where they provide the necessary helicase activity (Im and Muzyczka 1990, 1992). The smaller Rep proteins, Rep52 and Rep40, are not required for duplex DNA replication but are implicated in progeny single-strand synthesis (Chejanovsky and Carter 1989).

Terminal Resolution and the Structure of AAV Origins

Duplex forms of the AAV genome that are covalently continuous at one end by a single copy of the terminal palindrome can be resolved to an extended-form configuration containing two copies of the palindrome by a process dubbed "terminal resolution." First suggested by Cavalier-Smith (1974) as a theoretical solution to the problem of maintaining the sequence of linear chromosome ends during DNA replication, this type of resolution has been recapitulated in vitro using AAV substrates (Im and Muzyczka 1990; Snyder et al. 1990a,b), and a similar process is thought to occur at the MVM 5′, but not the 3′, terminus (Cotmore and Tattersall 1992).

The AAV terminus, shown in Figure 2, is composed of three palindromic sequences which fold into a T-shaped structure in virion DNA in a way that allows the 3′ nucleotide to prime complementary strand synthesis. Rep68 and Rep78 bind to sequences centered on the $(GAGC)_3$ repeat in the hairpin stem and introduce a nick 20 bases away, at a specific site termed the *trs* (Snyder et al. 1993). The sequence boxed in Figure 2 can support limited Rep-mediated, site-specific nuclease activity in vitro, but a third recognition element, involving the arms of the hairpin and generally referred to as the "secondary structure" element, is also required for efficient initiation (Chiorini et al. 1994; McCarty et al. 1994). These T-fork sequences, which contain degenerate forms of the $(GAGC)_n$ motif, appear to alter the affinity of the substrate for Rep and to change the DNase I protection pattern, but not the number of molecules bound (McCarty et al. 1994), presumably by providing second-site interactions within multimeric Rep:DNA complexes. Cleavage results in the formation of a phosphodiester bond between the phosphoryl group of the 5′ terminal thymidine residue and an aromatic hydroxyl group from a Rep tyrosine, in a process that requires ATP (Snyder et al. 1990a, 1993). The nick effectively inverts the original complex palindrome onto the progeny strand (Fig. 2, step iii) while providing a new base-paired 3′ hydroxyl to prime synthesis of its complement. This process thus replaces the original sequence of the hairpin (dubbed the "flip" sequence) with its inverted complement, "flop" (Fig. 2, step iv). Since this inversion is repeated with every round of resolution, progeny genomes contain equal numbers of termini in both sequence orientations. The terminal palindromes of all parvoviruses are imperfect, so that flip and flop sequence orientations can be readily identified. The absence of such heterogeneity at the 3′ terminus of MVM first alerted Astell and her colleagues (1983a) to the existence of an alternate mechanism for telomere regeneration, dubbed "junction resolution," discussed below.

THE MVM REPLICATION STRATEGY

The MVM Genome

The negative-sense MVM genome is 5172 nucleotides long, with 4805 nucleotides of single-stranded DNA bracketed between unique terminal palindromes of 121 (3′) and 246 (5′) nucleotides. These are capable of folding into Y-shaped (Fig. 3) and cruciform hairpin structures, respectively. MVM does not require coinfection with a helper virus for its own productive replication, but it is unable to induce resting cells to enter S phase. However, such viruses can remain in infected G_0 cells for prolonged periods without causing apparent toxicity, and these cells can still enter S phase upon induction (for review, see Tjissen 1990). Viral transcription is not activated until after entry into S phase (Clemens and Pintel 1988) and, as the early viral gene products accumulate, host cell DNA replication is terminated. Progression through the cell cycle is suspended, and cells continue actively synthesizing viral DNA until subsequent lysis or apoptosis results in the release of progeny virus, generally within 24 hours (Cotmore and Tattersall 1987).

Exons accessed from the MVM P4 promoter give rise to a series of alternatively spliced transcripts encoding two types of nonstructural proteins, designated NS1 and NS2. NS1, the replication initiator, is an abundant and long-lived nuclear phosphoprotein of 83 kD, with helicase and ATPase activities (Wilson et al. 1991), and is the only NS protein which is essential for productive replication in all cell types (Naeger et al. 1990; Li and Rhode 1991). It is a site-specific DNA-binding protein, recognizing the sequence $(ACCA)_{1\text{-}3}$, present in the viral origins, and reiterated at multiple sites throughout the viral genome (Cotmore et al. 1995). Since most MVM sequences of 100 bp or more contain at least one copy of this recognition sequence, and some regions contain multiple tandem and inverted reiterations, NS1 can bind throughout MVM replicative-form DNA (S.F. Cotmore and P. Tattersall, unpubl.). This not only suggests a potential role for NS1 in viral nucleosome structure and progeny strand packaging, but also indicates that previously identified *cis*-acting sequences thought to interact with cellular proteins to instigate transcriptional *trans*-activation (Gu and Rhode 1992; cf. Christensen et al. 1995) or to enhance replication (Tam and Astell 1993) must be reassessed for their ability to bind and sequester NS1.

Unlike Rep:DNA binding, association and dissociation of the NS1:DNA complex is markedly dynamic under physiological conditions, modulated by the binding of ATP. Experimentally, the interaction can only be demonstrated in the presence of ATP or by cross-linking the NS1 molecules with antibodies directed against their amino- or carboxy-

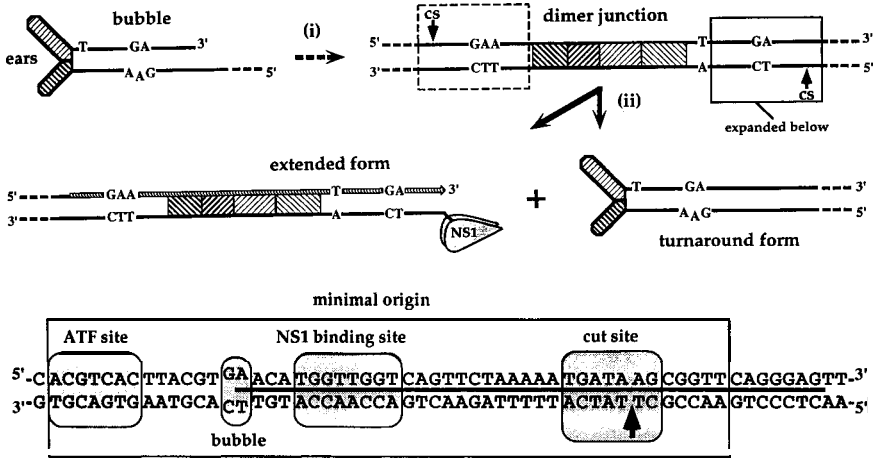

Figure 3 Junction resolution reaction for the left end of MVM. Step *i* condenses the in vivo steps *i–iv* of Fig. 1, showing greater detail of the organization of the left-end hairpin sequences within the dimer junction. Cross-hatched boxes represent the palindromic sequences that fold to give the internal "ears" in the hairpin form of the 3′ end of the genome. Step *ii* represents the in vitro resolution reaction described in the text. The potential cut sites are denoted *cs*, and newly synthesized DNA is depicted as a hatched bar with an arrowhead at its 3′ end. The domains within the minimal origin described in the text are boxed, and the underline represents the sequences protected by NS1 from DNase I digestion (Christensen et al. 1995).

terminal peptides (Christensen et al. 1995). Since NS1 appears to form multimers in vivo (Nüesch and Tattersall 1993), this suggests that ATP may induce NS1 to oligomerize and that only multimeric forms of the protein bind efficiently to its cognate site. NS1 is the initiator endonuclease for MVM DNA replication (Cotmore et al. 1992, 1993; Nüesch et al. 1995), containing critical sequence motifs encoding a putative metal coordination site and an active-site tyrosine that have been recognized in prokaryotic rolling-circle initiators (Koonin and Ilyina 1993). These two elements are thought to comprise the catalytic site of the nickase. NS1 becomes linked to the 5′ end of the displaced strand via a tyrosine residue at amino acid position 210 (Nüesch et al. 1995) and, like Rep, is presumed to remain in the replication fork providing the necessary helicase activity. NS2 polypeptides are only required for productive replication in cells of murine origin. In these cells, NS2 clearly influences multiple steps in the viral life cycle, including DNA replication, by currently undefined mechanisms (Naeger et al. 1993).

Structure of the MVM 3′ Origin

MVM termini are unique, differing from each other in size, primary sequence, and predicted structure (Astell et al. 1983b). At the 5′ end of the genome, a hairpin resolution process, thought to resemble the one recapitulated in vitro for AAV, results in the generation of equal numbers of termini in flip and flop configurations, whereas at the 3′ telomere a more restricted mechanism, dubbed junction resolution, generates termini in a single orientation (Astell et al. 1985). Although all parvoviruses probably use junction resolution to resolve concatemeric intermediates, for MVM a critical asymmetry in the stem of the 3′ hairpin restricts this mechanism to the synthesis of a single sense DNA strand (Cotmore and Tattersall 1994).

In MVM, the 121-nucleotide 3′ telomere (Fig. 3) exists as a single flip sense sequence containing internal palindromes designated the "ears" and an asymmetric, mismatch "bubble" in the stem where the triplet GAA on the inboard arm is opposed to the doublet GA on the outboard strand (Astell et al. 1979, 1983b). During rolling-hairpin replication (Fig. 1) this sequence is extended and copied to form the palindromic duplex junction that links unit-length genomes in the dimer intermediate, and it is this duplex structure, rather than the terminal hairpin, that contains the replication origin. Figure 3 shows how the asymmetries in the hairpin are organized in the junction sequence. Since it contains two copies of the terminal palindrome, one in each sequence orientation, it also contains two candidate cut sites (cs) located on opposite DNA strands and on each arm of the palindrome. However, only the cut site on the arm carrying the GA/TC doublet functions as a replication origin (Cotmore and Tattersall 1994). The use of such a replication strategy in MVM appears to be an adaptation which, by restricting NS1-mediated nicking to the outboard arm of the telomere, allows similar configurations of these same sequences on the inboard arm, and in the mismatched, hairpinned configuration of the terminus, to be dedicated to transcription (Faisst et al. 1994; Gu et al. 1995). Since the replication fork initiated on the TC arm is unidirectional and supports the synthesis of a single, continuous DNA strand (S.F. Cotmore and P. Tattersall, unpubl.), use of a single cut site inevitably results in the synthesis of DNA in a single sequence orientation. However, as discussed below, a resolution process in which replication initiated at a nick site on the TC/TTC strand leads to the selective synthesis of a GAA/GA strand must be complex and likely involves rearrangement into a cruciform structure.

The minimal active MVM 3′ origin (boxed in Fig. 3) is a multidomain structure of approximately 50 bp derived from the stem region of

the hairpin. It extends from an activated transcription factor (ATF) consensus binding site to a position some 7 bp beyond the cut site. An $(ACCA)_2$ NS1-binding sequence is positioned 17 nucleotides from the cut site, separated from the ATF consensus by the TC bubble dinucleotide. The actual sequence of the bubble dinucleotide appears unimportant, suggesting that it is not an essential part of the binding site for a cellular replication factor, but insertion of any third residue here, as in the GAA arm of the dimer junction, totally inactivates the origin (Cotmore and Tattersall 1994). This suggests that the bubble represents a critical spacer element, presumed to control the interaction of a cellular protein, binding at the ATF consensus, with $(ACCA)_2$-bound NS1. This precisely controlled interaction appears to activate the endonuclease function of NS1, since purified NS1 alone does not nick the viral origin efficiently (Nüesch et al. 1995). Mutations in the ATF consensus impair origin function, but purified homodimers of CREB protein or ATF 1, 2, or 3, despite binding avidly to this site, actually impair, rather than stimulate, origin-specific replication in vitro. In contrast, a highly enriched cellular protein fraction from 293 cells contains a factor(s) that can cooperate with NS1 to mediate 3′ origin-specific nicking and replication in vitro (J. Christensen et al., in prep.). This fraction, parvovirus initiation factor (PIF), contains a 120-kD protein that shows sequence-specific cross-linking to a double-stranded oligonucleotide containing the MVM ATF-binding site region. PIF is the subject of much interest, but its identity and its role in the biology of the host cell have yet to be determined.

Relatively little is known about which other cellular replication proteins are required by MVM. Since the replication fork initiated on the 3′ origin is aphidicolin-sensitive, unidirectional, and supports the synthesis of a single, continuous DNA strand, it is likely that polymerase-δ and its accessory proteins are required for its synthesis. This is supported by the observation that neutralizing monoclonal antibodies directed against polymerase-α:primase fail to inhibit MVM replication in vitro, whereas biochemical studies clearly indicate an absolute requirement for PCNA and RP-A (J. Christensen et al., in prep.). Since MVM gene expression coincides with a rapid reduction in the rate of cellular DNA replication (Cotmore and Tattersall 1987), it seems likely that the virus inactivates a critical cellular replication protein which it does not itself require. This hypothesis is currently under active investigation.

Junction Resolution at the MVM 3′ Origin

As shown in Figure 3, MVM left-end junction sequences are resolved and replicated asymmetrically in vitro into two viral telomeres, one in the

extended configuration containing a duplex copy of the entire palindrome and one containing a single copy of the palindrome in the covalently continuous, turnaround configuration (Cotmore et al. 1993; Liu et al. 1994). One of the strands of the extended-form terminus is newly synthesized, whereas its unreplicated complement is now covalently attached through its 5′ end to an NS1 molecule. How such resolution occurs is uncertain. It was originally suggested that NS1 might carry out a sequence of nicking and joining reactions analogous to those performed by the gene A protein of bacteriophage ϕX174 (Astell et al. 1983a). According to this model, the initiating nick would need to be introduced at the GAA-proximal cut site (shown as cs in Fig. 3 in the dotted box), thus liberating a 3′ hydroxyl capable of priming synthesis of the required flip-sense DNA strand across the axis of the palindrome. The NS1 molecule attached to the 5′ end of the displaced strand, remaining in the replication fork as it traversed the junction, would then encounter the "latent" nick site on the opposite, TC, strand and terminate the reaction at this second site by exchanging itself across analogous phosphodiester bonds (Astell et al. 1983a, 1985). This was an attractive model, since it simply recapitulated processes employed in prokaryotic rolling-circle replicons. Unfortunately, it is incompatible with the finding that replication initiates in the TC arm of the palindrome, since use of this cut site in the above scheme would lead to the synthesis of DNA strands with the flop (TC/TCC) sequence, rather than the flip (GAA/GA) sequence observed in vivo.

In order to synthesize a DNA strand in the flip configuration from a fork initiating on the TC arm, we suggest that the junction is reconfigured into a cruciform structure at a point soon after establishment of the replication fork, as shown in Figure 4, step ii. This would allow the fork to copy sequences in the flop orientation, producing a new flip-sense strand via a series of discrete linear and cruciform intermediates (step i and steps ii–v, respectively). The limited experimental data available tend to support such a mechanism. Replication forks can be established on linear sequences derived from a single arm of the junction (Cotmore and Tattersall 1994), but resolution of dimer junction substrates in murine cell extracts leads to the accumulation of nicked and partially replicated junction intermediates having structures compatible with those produced by steps i and iv in Figure 4 (Cotmore et al. 1993). Moreover, resolution of structures containing one copy of the GAA arm of the junction only occurs if this sequence is palindromic with, and hence able to form a cruciform with, the opposed, origin-containing arm (S.F. Cotmore and P. Tattersall, unpubl.). Since NS1 binds equally well to its cognate

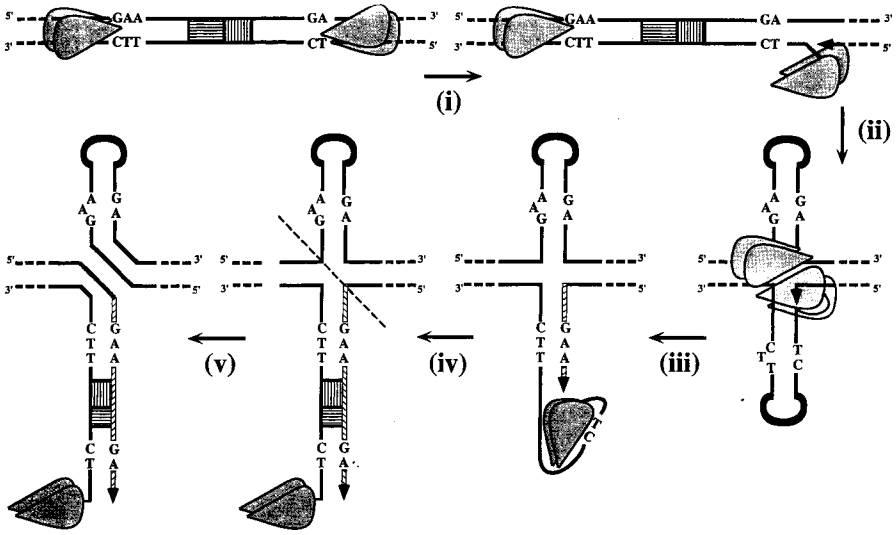

Figure 4 Hetero-cruciform model for MVM dimer junction resolution. Duplex forms of the internal "ear" palindromes are represented by hatched boxes, and hairpinned forms of these sequences are shown as thick black lines. NS1 proteins are shown bound to each arm of the junction as dimers, and from step *iii* on, the complex on the "inactive" arm is omitted for clarity. The dashed diagonal line indicates the preferred resolution axis of the cellular recombinase hypothesized to recognize the hetero-cruciform formed in step *iv* as a "Holliday structure" substrate and to resolve it in step *v*.

site on both arms of the junction palindrome (Cotmore et al. 1995), multimerization of this protein could well drive cruciform formation. Although it thus seems probable that resolution proceeds through a cruciform intermediate, how this structure is subsequently resolved is a matter of speculation. We suggest the recombinase-mediated strategy shown in Figure 4, step v, to provide what Crick has called a "don't worry" demonstration (Crick 1989). This implies that although we don't know how the strategy works, cellular mechanisms exist that could easily explain the observed products.

CONCLUDING REMARKS

In several important ways, parvoviral DNA replication resembles the single-strand-specific rolling-circle mechanisms previously characterized in prokaryotic systems. The single-stranded viral genome is replicated

through multimeric duplex replication intermediates by a quasi-circular, continuous-strand synthesis mechanism. As in prokaryotic rolling-circle synthesis, the next phase is initiated by a replicon-encoded endonuclease, rather than by RNA priming. The nuclease introduces a single-strand nick into specific origin sequences, itself becoming covalently attached to the 5′ end of the DNA in the process via a phosphodiester bond. This process liberates a base-paired 3′ deoxynucleotide that primes synthesis of the daughter DNA strand via a leading-strand-specific replication fork. Similarity with prokaryotic replicons extends to details such as protein sequence motifs involved in the catalytic site of the nickase and the nucleotide requirements for nicking, but there is as yet no evidence to suggest that parvoviral initiators retain the ability to carry out the type of nicking/joining reaction exhibited by their prokaryotic counterparts.

ACKNOWLEDGMENT

The authors were supported by U.S. Public Health Service grants AI-26109 and CA-29303 from the National Institutes of Health.

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