Faisst S, Rommelaere J (eds): Parvoviruses. From Molecular Biology to Pathology and Therapeutic Uses. Contrib Microbiol. Basel, Karger, 2000, vol 4, pp 163–177

Molecular Biology of Autonomous Parvoviruses

Bernhard Hirt

Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland

Introduction

Several members of the family of autonomous parvoviruses are known for a long time to cause serious disease, like feline panleukopenia virus (FPLV), canine parvovirus (CPV) or aleutian disease of mink virus (see preceding chapter).

A less pathogenic virus, minute virus of mice (MVM), however, has been studied by many scientists as a model in order to find out how parvoviruses function. MVM is easy to grow in cell culture and it is not known to cause epidemic disease, even if some strains seem to infect laboratory mouse colonies and alter their immune response (F. Fitch, personal communication). In the following, the structure and the life cycle of autonomous parvoviruses will be discussed with the main emphasis on the model virus MVM.

The most characteristic feature of parvoviruses is the structure of their DNA. It is linear, single-stranded and about 5,000 nucleotides long. Both ends of the DNA molecule are folded back to form a short double-stranded, hairpin-like structure.

As their name says, parvoviruses are among the smallest known viruses. The virion consists of a icosahedral protein capsid, about 26 nm in diameter, that contains one DNA molecule. In MVM, like in most autonomous parvoviruses, only the DNA strand that is complementary to viral messenger RNA, is encapsidated. The virions are very resistant. They can survive alcohol or ether treatment, temperatures of up to 56 °C and pH changes between 3 and 9. This explains for instance why the spread of canine parvovirus has not been restricted by measures of quarantine.

When autonomous parvoviruses infect a cell, they attach to the membrane and enter the cell by endocytosis. The virus particles are transported to the cell nucleus. It is not known where exactly the DNA is liberated from the capsid. In the nucleus, the 3′ hairpin structure of the DNA serves as primer for the synthesis of a complementary strand. The double-stranded DNA is a prerequisite for the transcription of viral genes. The assembly of progeny virions occurs in the nucleus. The infection by autonomous parvoviruses is lytic and leads to the death of the cell. Persistent infections of cell populations have been described with a minority of cells being infected and producing virus and a majority of cells being resistant to infection.

Koering et al. [1] recently described a rat cell line that continuously produces MVM. Cells have been cloned, though not in the presence of antibodies, and they continue to produce virus. The authors state that the virus does not lyse cells, but they have not studied how the virus is released from the cells and whether all cells in a population contain virus. The persistence they describe is very different from that observed with adeno-associated viruses which can integrate their DNA into that of the host cell and survive in a proviral state.

Structure of Autonomous Parvoviruses

The analysis of virus particles by electron microscopy revealed their small size and their icosahedral structure. The surface of the particles being rather smooth, no details could be observed that would allow an unambiguous identification of parvoviruses.

The first crystallographic studies on parvoviruses were made by the group of Rossmann who determined the structure of canine parvovirus at high resolution [2]. Based on this work, several other autonomous parvoviruses have been studied and their structure solved to a resolution of 3.3 Å [3].

The major capsid protein is VP2. A minority of VP1 molecules (for sequence relations see below) are also part of the capsid, but empty capsids can be assembled from VP2 alone. Proteolytic enzymes can shorten some of the VP2 molecules in the virion to produce a third polypeptide VP3. The virus has a diameter of about 26 nm. The capsids are $T = 1$ icosahedrons consisting of 60 molecules of VP1, VP2 and VP3. As expected, the epitopes that can induce neutralizing antibodies have been localized at the outer surface of the capsids. So have amino acid residues been identified at the surface of the virus that differ between strains of related viruses.

Some ordered DNA was detected in each of the 60 subunits of the cap-

sids accounting for about 13% of the total DNA. It is not known yet which nucleotide sequences interact with the capsid proteins, except for the 3′ hairpin structure.

Genome Organization and Transcription of MVM

Minute Virus of Mice was discovered by L. Crawford [4] when he was studying murine Adenovirus. This strain, now called MVMp (for prototype) grows well in cultured mouse fibroblasts. It is not known to cause disease.

The nucleotide sequence of MVMp DNA has been completely determined by Astell and coworkers [5] and a lot can be learned from it. The open reading frames show that the messenger RNA for viral proteins must be complementary to the DNA contained in the virions.

The sequence also revealed that the double-stranded 3′ end of the viral DNA has a Y-type structure and that there is a site where a couple of nucleotides are not complementary (fig. 1 A). Surprisingly, two slightly different sequences were found for the 5′ end of the DNA, both being present in half of the molecules, and they are designated ‘flip’ and ‘flop’ in figure 1 B. Each molecule is folded back on itself and forms a double strand, again with a site where a couple of nucleotides are not complementary. The DNA sequence of a flip DNA fragment is complementary to that of a flop one, and together they can form a perfect double helix. The turnaround region of the hairpin becomes then the center of an approximately 200 bp long, almost perfect palindrome. This structure has an important function in DNA replication.

Tattersall and coworkers succeeded in cloning MVM [6]. They introduced the double-stranded replicative form DNA into a plasmid. The cloned DNA proved to be infectious, despite the fact that a fragment of the 5′ end of the genome was missing. Because of the palindromic structure of the double-stranded 5′ end, the information contained in the sequence of the missing fragment was still present. They found that after transfection progeny virus was produced with complete DNA. Once an infectious cloned DNA was available, site directed mutagenesis became possible and this promoted the studies of the viral functions.

A rough map of the viral genome is provided by the open reading frames. The determination of the promoters and of the splice sites, however, was needed to precisely locate the different viral proteins onto the genetic map (see fig. 2) [7].

The template for transcription is double-stranded viral DNA in the nucleus of the infected cell. This DNA is not associated to histones and does

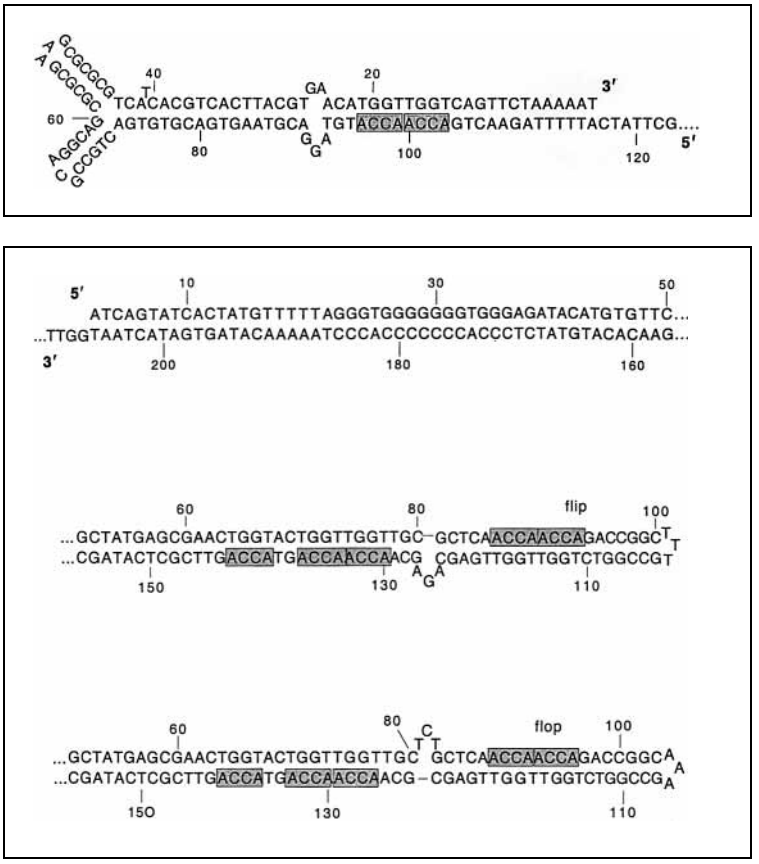

Fig. 1. Nucleotide sequence of the ends of the DNA of MVMi. *A*: 3′end. *B*: 5′ end. The multiple ACCA sites which can bind NS 1 are shaded. The sequences shown in this paper are those of MVMi and they are taken from Sahli et al. [46].

not form nucleosomes, but it is bound to unidentified cellular proteins [8]. Transcription goes from left to right and starts at two promoters, P4 and P39, at 4 and 39 map units (percent of the genome length from the left end), respectively. Termination of transcription occurs close to the right end of the DNA, where one can find polyadenylation signals. The two capsid proteins VP1 and VP2 are coded by the right half of the genome, both from the same reading frame and under the control of the P39 promoter. This means that splicing is needed to remove the VP1 translation start site from the transcript in order to produce the mRNA of the most abundant capsid protein, VP2. Therefore the amino acid sequence of VP2 is identical to the

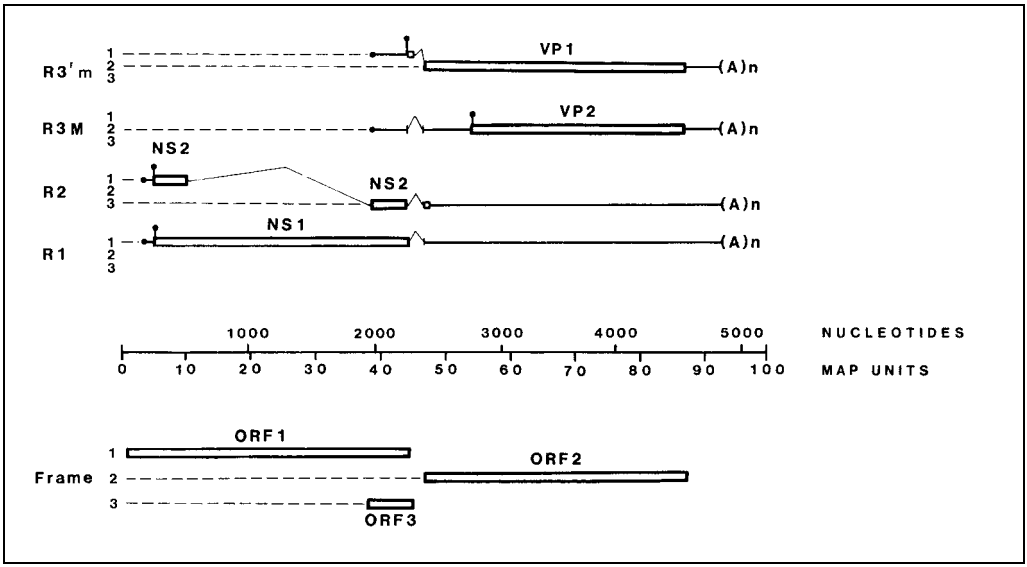

Fig. 2. Coding schemes for the four MVMi viral proteins. (Bottom) The three largest open reading frames found in MVMi and the frames in which they are read. (Top) The manner in which blocks of coding dequence are assembled in the four viral mRNAs to make up the sequences of the four viral polypeptides. The position of the blocks of coding sequence indicates from which frame they are read, and broken lines help in the alignment. Vertical markers indicate the positions of the initiation codons used for each protein, and knobs mark the cap sites of the mRNAs, from Jongeneel et al. [7] with permission.

amino acid sequence of the C-terminal part of VP1. The molecular weights of VP1 and VP2 are 80 and 64 kD respectively.

The left half of the genome is under the control of the P4 promoter and codes for the nonstructural proteins. NS1 has a molecular weight of 83 kD and NS2 of 24 kD. They have their N-terminal sequence in common. The C-terminal half of NS2 is coded in a different reading frame by a region of about 350 bp. This same region of the genome codes for NS1 in the original reading frame.

No genes for polymerases were found in the viral DNA and host enzymes are therefore needed for transcription and replication. RNA polymerase II transcribes the viral DNA.

Control of Transcription

As we will see later, NS1 is needed for the replication of viral DNA. It was therefore expected that transcription starts first at the P4 promoter and this happens at the beginning of S-phase [9]. The P4 promoter occurs in two different forms. Early on, as soon as the viral DNA gets converted to the double-stranded form, the Y-type structure at the left end of the viral DNA becomes part of a promoter and transcription can begin at about nucleotide 200. Once the DNA replication has started, a large proportion of DNA molecules will be in a tail to tail dimer form (see below), and the nucleotides from 1–200 will be in extended form in the interior of a linear double-stranded DNA molecule. A series of binding sites for transcription factors have been localized on the two forms of the promoter (fig. 3 A) [10–12].

An important role is played by the S-phase specific transcription factor E2F. If the binding site of E2F in the P4 promoter is inactivated by mutation, one observes that transcription is reduced 4-fold. Virus replication, however, is very strongly inhibited since the titre obtained with mutated virus is 10^4 times lower than that of the control [13]. The inactivation of other binding sites of transcription factors in the promoter P4 reduces the transcription up to 2-fold, but has no strong effect on virus replication. It was concluded that E2F is needed during infection for the production of a critical level of NS1 protein and that in the absence of E2F binding, virus replication is very low indeed. Adding of NS1 in trans restored the full virus production [13].

But NS1 is not only essential for DNA replication, it also functions as transcription factor for the viral promoters P4 and P39 (see below) [14, 15], and in addition it can activate cellular promoters [16]. The binding site for NS-1 that has been found in P4 (fig. 1 A) is important for DNA replication [17]. Whether binding of NS1 to this site also has a function in the regulation of transcription is not known.

The RNA synthesized from P4 is spliced. The NS1 protein is coded by a region upstream of the splice site of the R1 mRNA (fig. 2). Three different splicing reactions in the region of 46 map units have been observed to produce three slightly different R1 molecules with identical coding region. The biological significance of these differences is not understood.

R2, the mRNA for the minor early protein NS2 is also under the control of P4. It results from an alternative splicing that removes about 1 kb of the RNA in the middle of the region coding for NS1. In addition, a minor RNA fragment is spliced out close to the C-terminus of NS2. Three variants have been observed for the small splice leeding to three NS2 molecules that differ in their C-termini. The biological significance of these variants re-

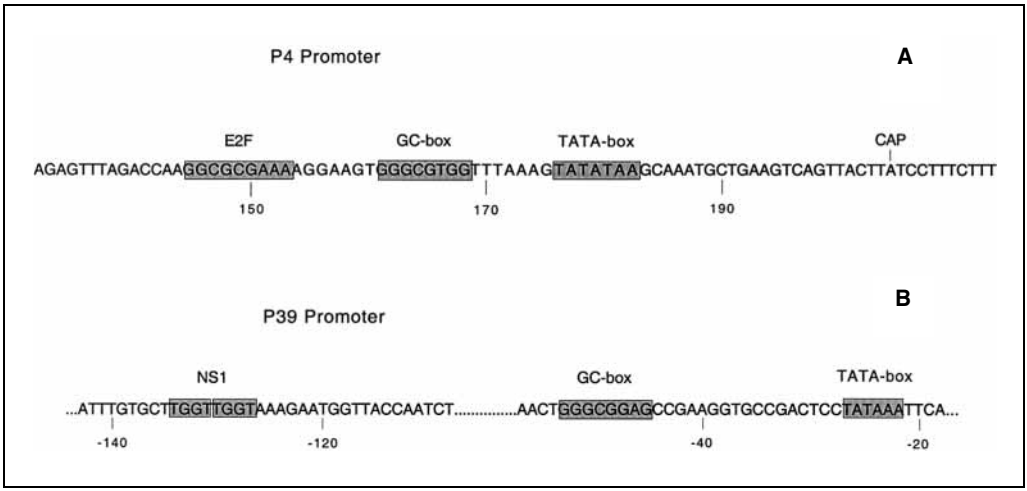

Fig. 3. Nucleotide sequences of the promoter regions of MVMi. Here the sequences are not those of virion DNA but of its complement which is analogous to the viral transcripts. The boxes represent binding sites for transcription factors. A: P4 promoter. The numbering starts at the left end of the genome. The binding site for NS1 is visible in Fig. 1 A. B: P39 promoter: The numbering is backwards from the transcription start site at nucleotide 2004.

mains to be elucidated. The splicing of MVM RNA has been studied in detail by Pintel and coworkers [18].

The promoter P39 directs the synthesis of the mRNAs for the capsid proteins. R3M, the messenger for the major capsid protein VP1, is produced by splicing out 97 nucleotides, including an AUG, at map position 45. This puts the start site for protein synthesis at position 55. The mRNA for VP1, R3′m, results from alternative splicing at map position 46 that removes 83 nucleotides and changes the reading frame. The start site for VP2 is at map position 45. As indicated in figure 2, the amino acid sequence of VP2 is contained in that of the C-terminal part of VP1.

From other viruses we know that nonstructural early proteins stimulate the transcription of the genes for the late, structural proteins. It was therefore no surprise when it was found that NS1 is a potent activator of the promoter responsible for the capsid proteins. The P39 promoter is illustrated in figure 3. It contains a TATA box and 20 nucleotides upstream of a GC box, to which the transcription factor SP1 binds. In the absence of NS1 the basic activity of this promoter is very low [19]. Addition of NS1 stimulates transcription more than 100-fold. The promoter contains the sequence $(ACCA)_2$

which can bind NS1 in the presence of ATP [20], a sequence that is also found in the P4 promoter, 100 bp upstream of the start site. A transactivation responsive element has also been mapped and it overlaps with the NS1 binding site [14]. Despite these interesting findings, the mode of action of NS1 remains controversial, since other researchers reported that the proximal promoter region containing only the TATA and GC boxes is sufficient for the full activation by NS1 [21, 22].

DNA Replication

As mentioned above, the viral genes do not code for polymerases and therefore the replication of the viral DNA depends on host enzymes. As a consequence, the DNA can replicate only in the nucleus and only during S-phase. For this reason, cell damage induced by the virus can be seen only in proliferating cells (see preceding chapter). Other small DNA viruses that also depend on host enzymes for their replication, like for instance SV40, have the capability to stimulate resting host cells and to push them to replicate their DNA. This is not the case for parvoviruses, who can infect resting cells but then have to wait for the next S-phase in order to synthesize new DNA.

The first step in the replication of viral DNA is the conversion of the single-stranded DNA liberated from the virion into the double-stranded replicative form (RF). The Y-shaped 3′ hairpin region described above is an excellent primer for DNA polymerases of the host, which can copy the full length of the DNA molecule, resulting in a monomer RF with a hairpin at its left end and an extended double-stranded right end (fig. 5, 2). This is now a double-stranded, linear DNA molecule and there is no simple way to replicate linear DNA molecules. All known DNA polymerases are incapable to start the synthesis of a polynucleotide chain, i.e. they need a primer and add nucleotides to its 3′ OH end. The primer, which normally is RNA, will later be degraded. After such a replication of a linear DNA molecule, removal of the primers would make the 5′ ends of the daughter strands shorter than those of the template and the molecules would get shorter with each round of replication.

Cavalier-Smith [23] has proposed a replication mechanism that can prevent the shortening of linear DNA molecules during replication and this was on purely theoretical grounds. This model is illustrated in figure 4. It postulates that the linear DNA has a palindrome at both ends. After replication the 5′ end is shorter and the 3′ end single-stranded. Since it is a palindrome, it can fold back on itself and form a double-stranded hairpin. This structure is a primer for DNA polymerase that can fill in the gap. DNA

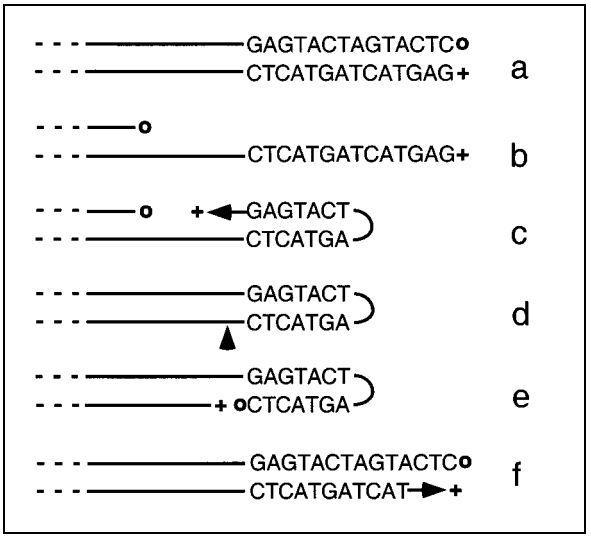

Fig. 4. The Cavalier-Smith model for the replication of DNA ends. a: The terminal region of the DNA is a palindrome (symbolized by an arbitrary nucleotide sequence). b: After replication the 5′ end is shortened and the 3′ and is single stranded. c: The 3′ end is folded back on itself and can serve as primer for DNA polymerase. d: The gap has been filled and the DNA ligated. The hairpin structure is a template for a site specific endonuclease. e: The nicked molecule is a primer for DNA polymerase that can reconstitute the original DNA end (f). Symbols: o = 5′ phosphate end; + = 3′ OH end; arrow = DNA polymerase; arrow-head: Site specific endonuclease.

ligase is present and can join the ends. The model now requests a specific nuclease that introduces a nick at the 5′ end of the palindrome sequence. DNA polymerase can now extend the DNA at the 3′ end at the nick site and copy the opposite strand to its end. The result is a DNA molecule with complete ends. Cavalier-Smith has proposed this model to explain the replication of the ends of eukaryotic chromosomes. Telomers, however, were found to maintain the lengths of chromosomes by a different mechanism. But the model does explain a main principle of the replication of parvovirus DNA, i. e. that at each cycle of replication the terminal palindromes get inverted. For MVM, we have seen that the palindromes of the 5′ ends are not perfect (flip and flop would be identical for a perfect palindrome, see fig. 1 B). The inversion of the ends during replication explains why the viral DNA is always a mixture of 50% each of flip and flop molecules, even if the DNA originates from a cloned virus.

The covalent linkage of the complementary strands predicted by the Cavalier-Smith model has been observed since MVM DNA molecules con-

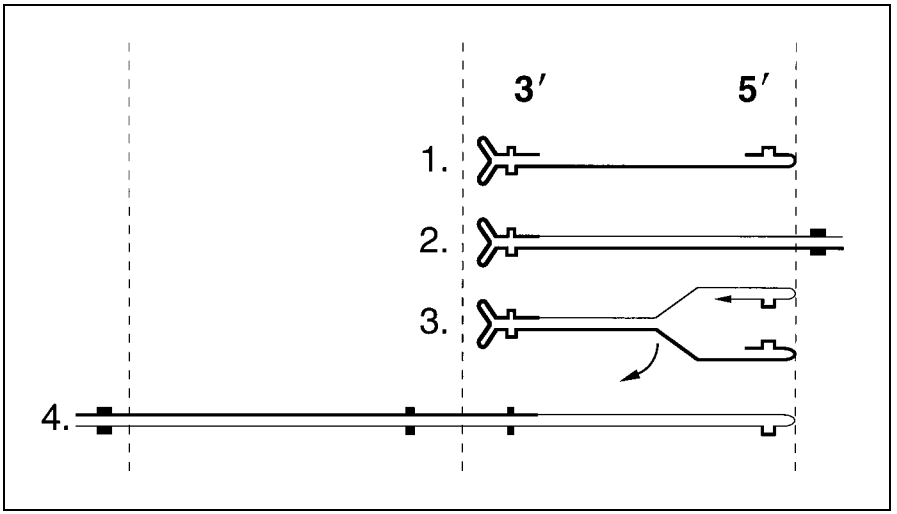

Fig. 5. Schematic representation of MVM DNA and its replicative forms. 2. Single-stranded DNA with hairpin ends as found in virions. It is a primer-template for DNA polymerase that can copy the DNA resulting in a replicating form monomer (2.). Hairpin transfer is represented in 3. The upper hairpin is a primer for DNA polymerase. The action of the polymerase leads to a replicative form dimer (4.). The unpaired bases are represented by deviations from the DNA strand. In the extended form these same bases are paired and they are symbolized by black rectangles. Thick line: Virion DNA; thin line: Newly synthesized DNA.

sisting of a single circular chain have been isolated from infected cells [24]. They can be produced when DNA polymerase converts viral DNA to double strands and stops at the point where it reaches the 5′ hairpin. DNA ligase can now link the new strand to the hairpin and the circle is closed. Other particularities of the replication of parvovirus DNA cannot be explained by the model of Cavalier-Smith: the appearance of double-stranded dimers and tetramers of viral DNA in nuclei of infected cells and the presence of unpaired bases in the terminal hairpin DNA.

Bourguignon et al. [25] have described the structure of the DNA extracted from MVM virions with its 5′ hairpin and a fold back at the 3′ end that can serve as primer. Based on this and on their previous findings, Tattersall and Ward proposed a model for DNA replication [26]. Polymerase begins at the 3′ hairpin and extends it until the DNA is in completely double-stranded form with a palindrome at its right end (fig. 5). Since it is one continuous strand, it renatures spontaneously after denaturation and this had been observed with DNA extracted from infected cells [27].

The next step in their model is a novel type of reaction called hairpin transfer: the right end of the RF molecule is partially melted and the ends of each strand then can fold back and form hairpin structures (fig. 5, 3). The 3′ hairpin can now serve as primer and the polymerase copy the entire DNA strand. This results in a double-stranded molecule of twice genome size (fig. 5, 4). It is a RF dimer with the genomes linked in tail-to-tail conformation and this structure has been confirmed by restriction fragment analysis [28]. With the same mechanism, replication can progress from dimers to tetramers (they were also found in infected cells) and so on. The model of Tattersall and Ward postulates an enzyme that introduces a specific nick at the 5′ end of the viral DNA (not shown on figure). Both functions that are specific for parvovirus DNA replication in their model can be accomplished by the nonstructural protein NS1. Its DNA-helicase activity is able to separate the strands for the hairpin transfer [29, 30] and its endonuclease activity [31] introduces specific nicks at the 5′ end.

As one can see in figure 1 A, the left end of the viral DNA contains a Y-shaped double-stranded fold back region, with a couple of nucleotides which are not paired. As opposed to the mixture of two forms (flip and flop) that one finds at the 5′ hairpin, only one form (flip) is found at the 3′ end of the DNA extracted from virions. To explain this finding, a more complex model for DNA replication is required. In this context it is interesting to know that NS1 can also introduce a nick into the extended form of the left terminus of MVM DNA, and that it does it in an asymmetric way [32]. A detailed description of the endonucleolytic action of NS1 and of its effects on DNA replication has been published by Cotmore and Tattersall [33].

So far, we have considered only the synthesis of double-stranded DNA. The single strands for the progeny viruses are being synthesized during encapsidation of the DNA. To produce progeny DNA, NS1 nicks at the 5′ end of a viral genome and stays covalently linked to it [34, 35]. The resulting 3′ end at the nick site serves as primer and the strand opposite to the nick is copied by DNA polymerase, deplacing from the template the strand to be packaged. It was expected that the 5′ end should be recognized by capsid proteins and that these could then progressively wrap the nascent single strand. But it was found that empty capsids bind to the Y-type hairpin at the 3′ end of the DNA and not to the 5′ end [36]. This interaction is specific for the secondary structure of the DNA rather than for the nucleotide sequence. It is still not clear how this interaction can lead to the encapsidation of the DNA. The NS1 molecule that is covalently bound to the 5′ end stays there also in the virus particle and it can be found at the outside of the capsid [37]. If it is cleaved off the virion by nuclease, together with the first few nucleotides, the infectivity is not reduced.

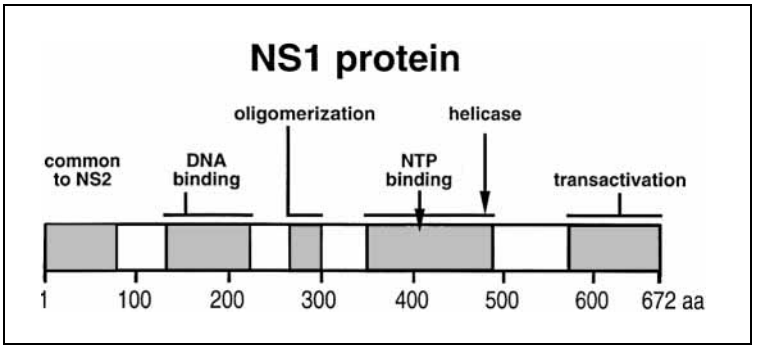

Fig. 6. Linear representation of the amino acid sequence of the NS1 protein. Arrows and shaded areas indicate functional regions that have been mapped.

The progeny DNA has also to be cleaved at its 3′ terminus, a reaction that is also catalysed by NS1.

As we have seen, NS1 has varied functions that are essential for transcription and replication. It also can inhibit the cell cycle if expressed in animal cells and can even be toxic (see below). It is a phosphoprotein of 83 kD and a linear diagram with the different functions attributed to regions of the protein is given in figure 6.

The much smaller NS2 proteins are mainly located in the cytoplasm. They are required for an efficient virus synthesis in mouse fibroblasts but not in some other permissive cells [38]. The mechanisms of their action are not understood yet.

The Life Cycle of MVM

Two different strains of MVM have been studied extensively, MVMp that grows in murine fibroblasts and MVMi that is specific for lymphoid cells. Little is known about the life cycles of these viruses in vivo. They have been isolated from tissue culture samples and propagated in vitro for many years.

When virus is added to cells, it is bound and taken up irrespective of whether the cells are permissive or not [39]. There must be intracellular specificity factors that determine whether the virus replicates or not. This is peculiar, because for many viruses the cell type specificity is determined by receptors at the cell surface.

In order to confer on the lymphotropic strain MVMi the capacity to grow in fibroblasts, it was sufficient to substitute by mutation two specific amino acids in the coat protein [40]. It is still not known what mechanism is responsible for this change of permissivity.

MVMp was converted to a virus capable to grow in lymphocytes by replacing two segments of its DNA by that of MVMi [41]. One of the regions was coding for capsid proteins, the other, however, was in the early region and contained the splice acceptor site for NS2. The lymphotropic viruses produced more NS2 mRNA than the virus that grows in fibroblasts. Whether this correlation is of biological importance is not known.

When the fate of MVMp was studied after the infection of EL4 lymphocytes, it was found that the virus enters the cells and migrates towards the nucleus. Since neither viral transcription nor DNA replication could be observed, it was concluded that there was a block in decapsidation. When lymphocytes are transfected with cloned MVMp DNA, this DNA is transcribed, replicated and progeny virus is produced [42]. This confirms the hypothesis that the restriction occurs at the level of decapsidation.

The time course of the viral life cycle depends on the cells entering S-phase and therefore the virus production in a cell population is not synchronous. The first replicating DNA can be observed at about 10 h after infection and the first progeny virus after about 1 day.

Autonomous parvoviruses grow better in transformed than in normal cells. This observation is interesting, especially in the view of using viruses or viral genes to preferentially kill cancer cells. Research in this field has been reviewed [43]. Treatment of tumors with oncolytic viruses is likely to run into a problem well known from chemotherapy: even when most tumor cells are killed, resistant cells will be selected which continue to grow.

Since parvoviruses replicate only in S phase, it was not surprising that they grow well in transformed cells. But this preference is not only based on the increased cycling of tumor cells. An analysis of different lines of transformed cells has shown that certain oncogenes strongly stimulate the expression of NS1 and through this effect render the cells permissive for virus growth and this leads to the lysis of the cell [44]. It was also found that NS1 is toxic for those cells that express an oncogene [45]. This specific toxicity for transformed cells could be useful for the treatment of tumors.

Acknowledgment

The author thanks the University of Lausanne for support.

References

1 Koering CE, Geuskens M, Rommelaere J: Continuous production of minute virus of mice by an untransformed variant of Fisher rat fibroblast (FR3T3). J Gen Virol 1996;77(Pt 3):447–452.
2 Tsao J et al: The three-dimensional structure of canine parvovirus and its functional implications. Science 1991;251(5000):1456–1464.
3 Agbandje M, Parrish CR, Rossmann MG: The structure of parvoviruses. Seminars in Virology, 1995;6:229–309.
4 Crawford LV: A minute virus of mice. Virology, 1966;29(4):605–612.
5 Astell CR et al: The complete DNA sequence of minute virus of mice, an autonomous parvovirus. Nucleic Acids Res, 1983;11(4):999–1018.
6 Merchlinsky MJ et al: Construction of an infectious molecular clone of the autonomous parvovirus minute virus of mice. J Virol 1983;47(1):227–232.
7 Jongeneel CV et al: A precise map of splice junctions in the mRNAs of minute virus of mice, an autonomous parvovirus. J Virol 1986;59(3):564–573.
8 Doerig C et al: Nucleoprotein complexes of minute virus of mice have a distinct structure different from that of chromatin. J Virol 1986;58(3):817–824.
9 Clemens KE, Pintel DJ: The two transcription units of the autonomous parvovirus minute virus of mice are transcribed in a temporal order. J Virol 1988;62(4):1448–1451.
10 Ahn JK et al: Transcriptional analysis of minute virus of mice P4 promoter mutants. J Virol 1989;63(12):5425–5439.
11 Pitluk ZW, Ward DC: Unusual Sp1-GC box interaction in a parvovirus promoter. J Virol 1991;65(12):6661–6670.
12 Perros M et al: Upstream CREs participate in the basal activity of minute virus of mice promoter P4 and in its stimulation in ras-transformed cells. J Virol 1995;69(9):5506–5515.
13 Deleu L et al: Activation of promoter P4 of the autonomous parvovirus minute virus of mice at early S phase is required for productive infection. J Virol 1999;73(5):3877–3885.
14 Rhode SLd, Richard SM: Characterization of the trans-activation-responsive element of the parvovirus H-1 P38 promoter. J Virol 1987;61(9):2807–2815.
15 Doerig C et al: Minute virus of mice non-structural protein NS-1 is necessary and sufficient for trans-activation of the viral P39 promoter. J Gen Virol 1988;69(Pt 10):2563–2573.
16 Vanacker JM et al: Transactivation of a cellular promoter by the NS1 protein of the parvovirus minute virus of mice through a putative hormone-responsive element. J Virol 1996;70(4):2369–2377.
17 Cotmore SF et al: The NS1 polypeptide of the murine parvovirus minute virus of mice binds to DNA sequences containing the motif [ACCA]2-3. J Virol 1995;69(3):1652–1660.
18 Haut DD, Pintel DJ: Inclusion of the NS2-specific Exon in minute virus of mice mRNA is facilitated by an intronic splicing enhancer that affects definition of the downstream small intron. Virology 1999;258(1):84–94.
19 Lorson C, Pintel DJ: Characterization of the minute virus of mice P38 core promoter elements. J Virol 1997;71(9):6568–6575.
20 Christensen J, Cotmore SF, Tattersall P: Minute virus of mice transcriptional activator protein NS1 binds directly to the transactivation region of the viral P38 promoter in a strictly ATP-dependent manner. J Virol 1995;69(9):5422–5430.
21 Ahn JK, Pitluk ZW, Ward DC: The GC box and TATA transcription control elements in the P38 promoter of the minute virus of mice are necessary and sufficient for transactivation by the nonstructural protein NS1. J Virol 1992;66(6):3776–3783.
22 Lorson C et al: An Sp1-binding site and TATA element are sufficient to support full transactivation by proximally bound NS1 protein of minute virus of mice. Virology, 1998;240(2):326–337.
23 Cavalier-Smith T: Palindromic base sequences and replication of eukaryote chromosome ends. Nature 1974;250(5466):467–470.
24 Cotmore SF, Gunther M, Tattersall P: Evidence for a ligation step in the DNA replication of the autonomous parvovirus minute virus of mice. J Virol 1989;63(2):1002–1006.
25 Bourguignon GJ, Tattersall PJ, Ward DC: DNA of minute virus of mice: self-priming, nonpermuted, single-stranded genome with a 5′-terminal hairpin duplex. J Virol 1976;20(1):290–306.

26 Tattersall P, Ward DC: Rolling hairpin model for replication of parvovirus and linear chromosomal DNA. Nature, 1976;263(5573):106–109.
27 Tattersall P, Crawford LV, Shatkin AJ: Replication of the parvovirus MVM. II. Isolation and characterization of intermediates in the replication of the viral deoxyribonucleic acid. J Virol 1973;12(6):1446–1456.
28 Rhode SL, III: Replication process of the parvovirus H-1. VI. Characterization of a replication terminus of H-1 replicative-form DNA. J Virol 1977;21(2):694–712.
29 Wilson GM et al: Expression of minute virus of mice major nonstructural protein in insect cells: purification and identification of ATPase and helicase activities. Virology 1991;185(1):90–98.
30 Jindal HK et al: Mutations in the NTP-binding motif of minute virus of mice (MVM) NS-1 protein uncouple ATPase and DNA helicase functions. J Biol Chem 1994;269(5):3283–3289.
31 Cotmore SF, Nuesch JP, Tattersall P: *In vitro* excision and replication of 5′ telomeres of minute virus of mice DNA from cloned palindromic concatemer junctions. Virology, 1992;190(1):365–377.
32 Cotmore SF, Tattersall P: An asymmetric nucleotide in the parvoviral 3′ hairpin directs segregation of a single active origin of DNA replication. Embo J 1994;13(17):4145–4152.
33 Cotmore SF, Tattersall P: DNA replication in autonomous parvoviruses. Seminars in Virology 1995;6:271–281.
34 Cotmore SF, Tattersall P: The NS-1 polypeptide of minute virus of mice is covalently attached to the 5′ termini of duplex replicative-form DNA and progeny single strands. J Virol 1988;2(3):851–860.
35 Nuesch JP, Cotmore SF, Tattersall P: Sequence motifs in the replicator protein of parvovirus MVM essential for nicking and covalent attachment to the viral origin: identification of the linking tyrosine. Virology 1995;209(1):122–135.
36 Willwand K, Hirt B: The major capsid protein VP2 of minute virus of mice (MVM) can form particles which bind to the 3′-terminal hairpin of MVM replicative-form DNA and package single-stranded viral progeny DNA. J Virol 1993;67(9):5660–5663.
37 Cotmore SF, Tattersall P: A genome-linked copy of the NS-1 polypeptide is located on the outside of infectious parvovirus particles. J Virol 1989;63(9):3902–3911.
38 Cater JE, Pintel DJ: The small non-structural protein NS2 of the autonomous parvovirus minute virus of mice is required for virus growth in murine cells. J Gen Virol 1992;73(Pt 7):1839–1843.
39 Spalholz BA, Tattersall P: Interaction of minute virus of mice with differentiated cells: strain- dependent target cell specificity is mediated by intracellular factors. J Virol 1983;46(3):937–943.
40 Ball-Goodrich LJ; Tattersall P: Two amino acid substitutions within the capsid are coordinately required for acquisition of fibrotropism by the lymphotropic strain of minute virus of mice. J Virol 1992;66(6):3415–3423.
41 Colomar MC, Hirt B, Beard P: Two segments in the genome of the immunosuppressive minute virus of mice determine the host-cell specificity, control viral DNA replication and affect viral RNA metabolism. J Gen Virol 1998;79(Pt 3):581–586.
42 Previsani N et al: Growth of the parvovirus minute virus of mice MVMp3 in EL4 lymphocytes is restricted after cell entry and before viral DNA amplification: cell-specific differences in virus uncoating *in vitro*. J Virol 1997;71(10):7769–7780.
43 Rommelaere J, Cornelis JJ: Antineoplastic activity of parvoviruses. J Virol Methods 1991;33(3):233–251.
44 Salome N et al: Sensitization of transformed rat cells to parvovirus MVMp is restricted to specific oncogenes. Oncogene 1990;5(1):123–130.
45 Mousset S et al: The cytotoxicity of the autonomous parvovirus minute virus of mice nonstructural proteins in FR3T3 rat cells depends on oncogene expression. J Virol 1994;68(10):6446–6653.
46 Sahli R, McMaster GK, Hirt B: DNA sequence comparison between two tissue-specific variants of the autonomous parvovirus, minute virus of mice. Nucleic Acids Res 1985;13:3617–3633.

Dr. Bernhard Hirt, Swiss Institute for Experimental Cancer Research, Ch. des Boveresses 155, CH-1066 Epalinges s/Lausanne (Switzerland)