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NOTES

Characterization of the Mouse Adeno-Associated Virus *AAVS1* Ortholog

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The nonpathogenic human adeno-associated virus (AAV) has developed a mechanism to integrate its genome into human chromosome 19 at 19q13.4 (termed *AAVS1*), thereby establishing latency. Here, we provide evidence that the chromosomal signals required for site-specific integration are conserved in the mouse genome proximal to the recently identified *Mbs85* gene. These sequence motifs can be specifically nicked by the viral Rep protein required for the initiation of site-specific AAV DNA integration. Furthermore, these signals can serve as a minimal origin for Rep-dependent DNA replication. In addition, we isolated the mouse Mbs85 proximal promoter and show transcriptional activity in three mouse cell lines.

The nonpathogenic human parvovirus, adeno-associated virus type 2 (AAV), replicates only in cells coinfected with a helper virus (5, 6). In the absence of such helper conditions, AAV establishes latency, integrating its genome site-specifically into a region of human chromosome 19 called *AAVS1* (12, 17–19, 27).

Both AAV DNA replication and site-specific integration require the large nonstructural Rep proteins (Rep68/78) and motifs within the inverted terminal repeats (4, 28, 32). Rep68/78 specifically bind a sequence motif within the inverted terminal repeats, the Rep binding site (RBS) (10, 11, 24, 26), and cleave in a site- and strand-specific manner at the terminal resolution site (TRS) located 13 nucleotides (nt) upstream from the RBS (8, 15, 31, 40). The TRS/RBS sequence can act as a minimal origin for Rep-mediated DNA replication (29, 35). An almost identical sequence in chromosome 19 represents the minimal sequence necessary and sufficient for AAV site-specific integration (22, 23). Biochemical assays have demonstrated that Rep can specifically interact with both viral and cellular RBS-TRS motifs (9, 11, 37); these motifs are thereby thought to target integration of AAV DNA into *AAVS1* (4, 28, 32). It has recently become evident that the *AAVS1* RBS is located 17 nt upstream from the translation initiation site of the protein phosphatase 1 regulatory inhibitor subunit 12C gene (*PPP1R12C*), also called *MBS85* (myosin binding subunit 85) (33).

By using *MBS85* exon sequences, we analyzed the National Center for Biotechnology Information mouse database for similarities to the human *AAVS1* locus (1). This analysis revealed a homology of 90% between the 5′ end of the human *MBS85* cDNA and the 969-nt mouse cDNA clone AK010836, which contains a sequence homologous to the human TRS-RBS motifs as well as the Mbs85 initiation codon (separated by 25 nt) (Fig. 1A). Interestingly, a simian *AAVS1* locus containing the corresponding upstream region and a TRS-RBS motif has recently been isolated from the African green monkey genome (2). *AAVS1* is located 14.9 and 36 kb centromeric to the slow skeletal troponin T (*TNNT1*) and cardiac troponin I (*TNNI3*) genes, respectively (12). The mouse *Tnni3* and *Tnnt1* genes are located on chromosome 7 (13, 14), in a region previously shown to be syntenic to the human chromosome 19 region that contains *AAVS1* (7). We used the Celera discovery system to search the Celera mouse genome assembly with the mouse *Tnni3* and *Tnnt1* genes, the AK010836 cDNA, and the human *MBS85* genomic sequence. All of these sequences specifically matched the same scaffold (500 kb) in the Celera database. The mouse *Mbs85* is located on chromosome 7 and is separated by only 2.5 and 16 kb from the *Tnnt1* and *Tnni3* genes, respectively (Fig. 1B). The Celera map revealed a gene 3.1 kb downstream of *MBS85*, designated *DRC3*, the mouse homolog of which is located 2.1 kb downstream of the *Mbs85* gene (Fig. 1B).

We sequenced and assembled three mouse expressed sequence tag clones (AA021750, AW911639, and BE847281) containing *Mbs85*. The resulting 3.1-kb mouse cDNA was 77% identical to the human *MBS85* cDNA. The mouse *Mbs85* gene spans 20 kb of genomic sequences, and the 2.3-kb predicted open reading frame is composed of 22 coding exons (20). Thus, the mouse and the human homologs of *MBS85* display the same overall genomic organization (Fig. 1B). The deduced mouse

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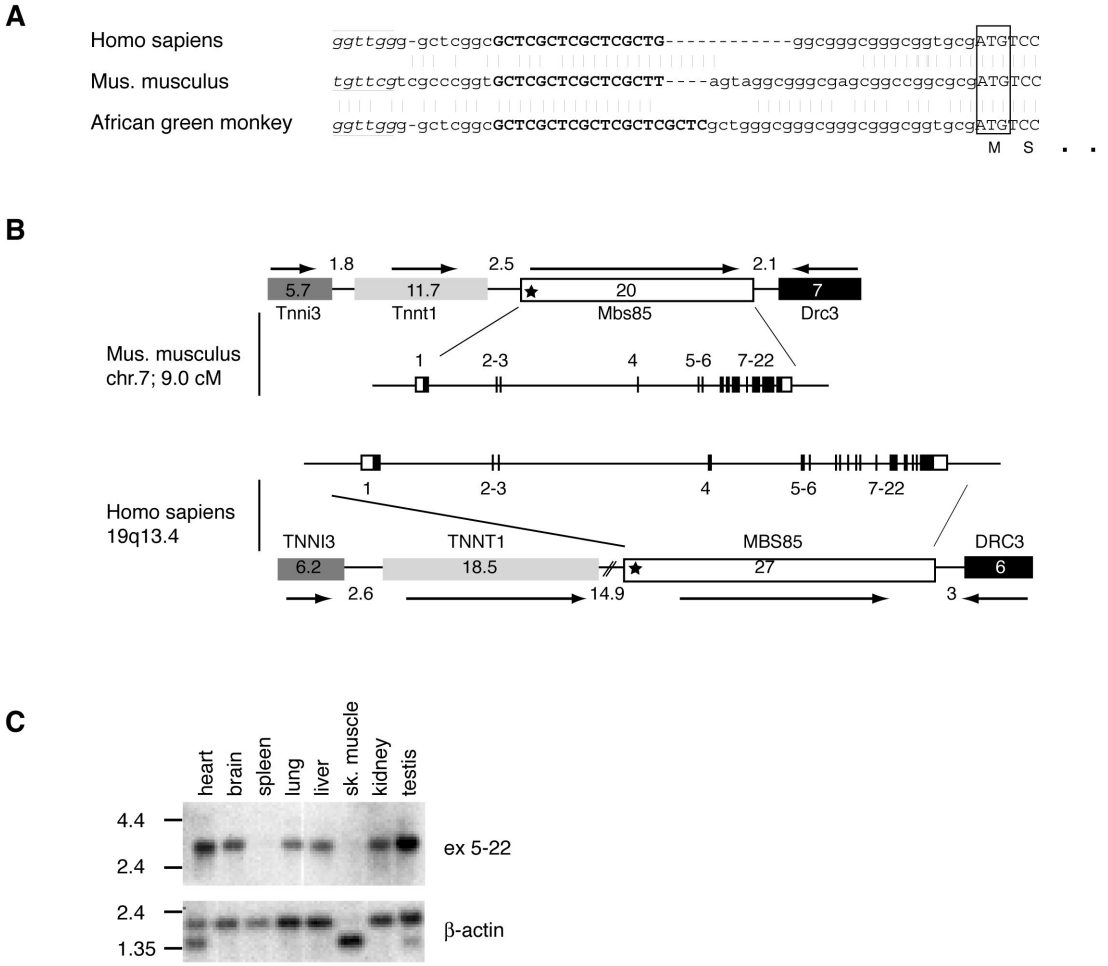


FIG. 1. (A) Nucleotide sequence alignment of the human, mouse, and monkey *AAVS1* sequences (MacVector; Oxford Molecular Limited). The TRS motif is italicized and underlined; the RBS motif is in bold. M and S indicate the translation initiation site and serine residue, respectively. Dashes indicate gaps between sequences. (B) Schematic representation of the genetic organization of the region surrounding the TRS and RBS motifs within the mouse (top) and human (bottom) genomes. The lengths and distances between the *TNNI3*, *TNNT1*, *MBS85*, and *DRC3* genes are shown in kilobases. The positions of the TRS-RBS motifs are indicated by stars. The arrows indicate the direction of transcription. The center section shows the genomic organization of the mouse and human *MBS85* transcripts. Black boxes indicate coding regions, white boxes indicate noncoding regions. (C) Tissue distribution of *Mbs85* mRNAs in the mouse. A multiple tissue Northern blot derived from mouse tissues was hybridized with a cDNA probe consisting of exons 5 to 22 (ex5-22) of the mouse *Mbs85* cDNA. The ex5-22 probe was generated by digestion of clone BF540586 with EcoRI/HindIII. The bottom panel shows hybridization with a β-actin cDNA probe. The blot was stripped between hybridizations. Sizes are indicated in kilobases.

Mbs85 protein sequence is 781 amino acids in length and is 86% identical to its human counterpart (33).

To assess the distribution of *Mbs85* mRNAs, a mouse poly(A) multiple tissue Northern blot (Clontech, Palo Alto, Calif.) was hybridized to a mouse *Mbs85* cDNA probe consisting of exons 5 to 22. As is observed in a human multiple tissue Northern blot (33), a single mRNA of approximately 3.1 kb is highly expressed in heart and testis, and to a lesser extent in kidney, brain, liver, and lung (Fig. 1C).

Current models of AAV integration predict that a Rep-mediated nick at the chromosomal TRS is a prerequisite (22). To determine if Rep68 can specifically nick the putative mouse TRS, double-stranded and partially single-stranded 5′ end-labeled origin substrates were incubated with purified His-tagged Rep68 proteins in a cell-free endonuclease assay as described previously (39). Rep68 nicked the AAV, human, and mouse TRS substrates releasing an expected 14-nt labeled fragment (Fig. 2). Nicking is Rep68 dependent since no cleavage of the AAV, human, or mouse origin substrates is observed when an endonuclease-negative mutant is used (Rep68Y156F) (30, 39). Substitution of the two thymidine residues within the mouse TRS sequence resulted in an expected loss of specific Rep-mediated cleavage (Fig. 2).

Origin interactions by Rep are thought to represent the initiating steps of integration (29, 34, 35). To test whether the mouse TRS-RBS sequence could also serve a similar function, cell-free DNA replication assays were performed as described previously (36). Linearized substrates containing the AAV, human, or putative mouse origin in a pBluescript backbone were incubated with HeLa cell extracts in the presence or the absence of purified His-tagged Rep68 protein (75 ng) and [α-^{32}P]dCTP. Rep68 initiates replication on templates contain-

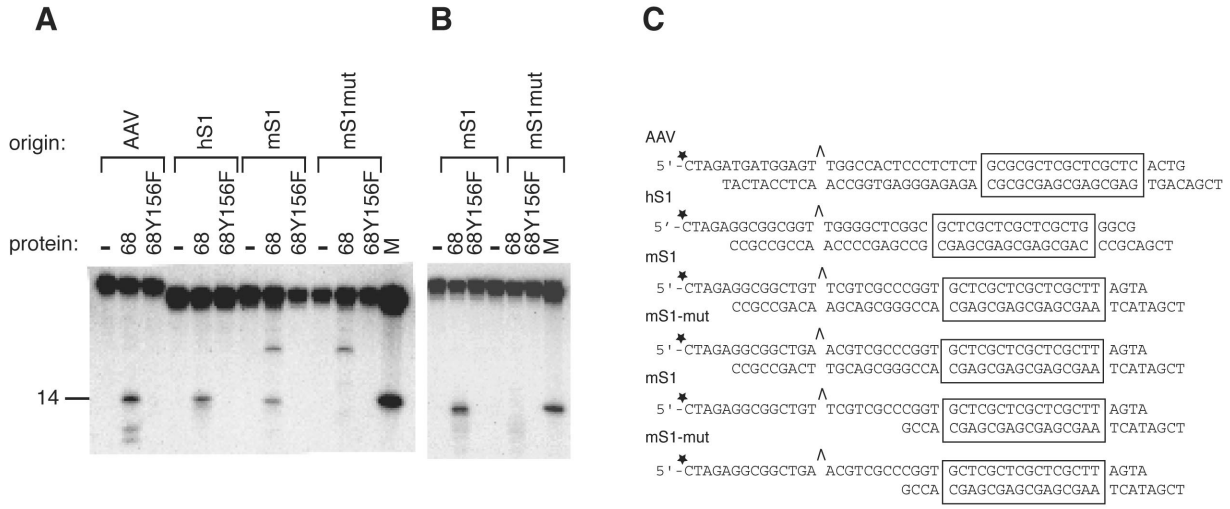


FIG. 2. Cell-free endonuclease assays. (A) Fully double-stranded and (B) partially single-stranded origin substrates (as a specificity control) (5 fmol) containing the AAV, human (hS1), mouse (mS1), and mouse origin mutant (mS1mut) sequences were incubated for 45 min at 37°C in either the absence (−) or presence of 1 pmol of AAV Rep68 protein (68) or 1 pmol of AAV Rep68 endonuclease mutant (Y156F). M, 5′ end-labeled marker oligonucleotides corresponding in sequence and length to the expected reaction product (14 nt). (C) Synthetic oligonucleotide substrates used in the nicking and replication assays. Locations of the TRS (^) and RBS (boxed) are indicated. The TRS-containing strand was first kinase labeled (positions of the 5′ radiolabel are indicated by stars) and then annealed to its complementary strand.

ing the AAV, human, or mouse origin but not on the vector DNA alone (Fig. 3). In all cases, replication is Rep dependent.

We further compared the human and mouse 5′ untranslated regions. It has been reported that the human *AAVS1* fragment located 74 to 426 nt upstream of the translation initiation codon is sufficient to drive the expression of a reporter gene following transient transfections in both 293 and HeLa cells (21).

Alignment of the human and mouse sequences upstream of the ATG revealed an overall 62% identity in the putative promoter region (Fig. 4A). Several conserved putative *cis*-acting DNA elements (i.e., *Sp1*, *CRE/ATF*) suggest the presence of a TATA-less promoter and common regulatory mechanisms for the expression of the human and mouse *MBS85* genes.

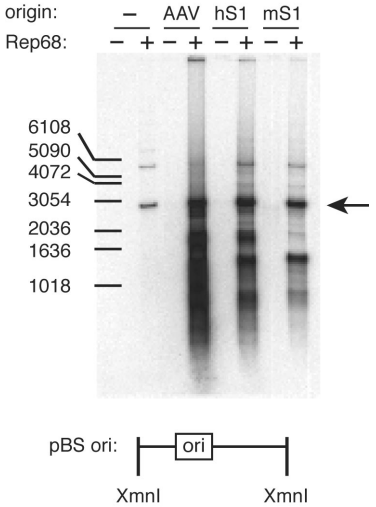


FIG. 3. Cell-free DNA replication assay. Schematic representation of the pBSori substrates used in this study (bottom). The AAV, human (hS1), and mouse (mS1) origins (consisting of the TRS and RBS sequences) were cloned into pBluescript via XbaI and SalI sites. Prior to the assay, plasmids were linearized with XmnI. Each linear, origin-containing substrate was incubated in the presence (+) or absence (−) of AAV Rep68 protein (top). An arrow indicates the position of the full-length replication product. Sizes are in base pairs.

We identified mouse cell lines expressing *Mbs85*. Total RNAs were extracted from C2C12, NIH 3T3, and N2A cell lines (Tel-Test, Friendswood, Tex.). Northern blots hybridized to the mouse *Mbs85* ex5-22 cDNA probe revealed a unique 3.1-kb transcript in all three cell lines (Fig. 4B).

To test the 324-bp NaeI fragment containing the RBS and TRS motifs for transcriptional activity, it was cloned into the pDsRed2.1 promoterless red fluorescent protein vector (Clontech) in both the sense and antisense orientation. C2C12, N2A, and NIH 3T3 cells were transfected and fixed 45 h posttransfection with 3.7% paraformaldehyde, and the slides were mounted in vectashield mounting medium with DAPI (4′,6′-diamidino-2-phenylindole) (Vector Laboratories, Burlingame, Calif.). The sense, but not the antisense, construct shows transcriptional activity in all three cell lines (Fig. 4C). These results were confirmed by fluorescence-activated cell sorter analysis (data not shown).

In this report, we show that the target site for AAV site-specific integration is not restricted to primates but is also present in the mouse genome in a region that is syntenic to the human chromosome 19 region containing *AAVS1* (Fig. 1).

Currently, Rep interactions with a minimal origin are defined by specific binding to the RBS followed by site- and strand-specific nicking at the TRS (8, 11, 15, 16). We demonstrate that the TRS and RBS motifs present in the 5′ untranslated region of the mouse *Mbs85* gene can act as a substrate for Rep-mediated nicking and as a functional Rep-dependent origin (Fig. 2 and 3). Figure 2 also shows a second nick on the

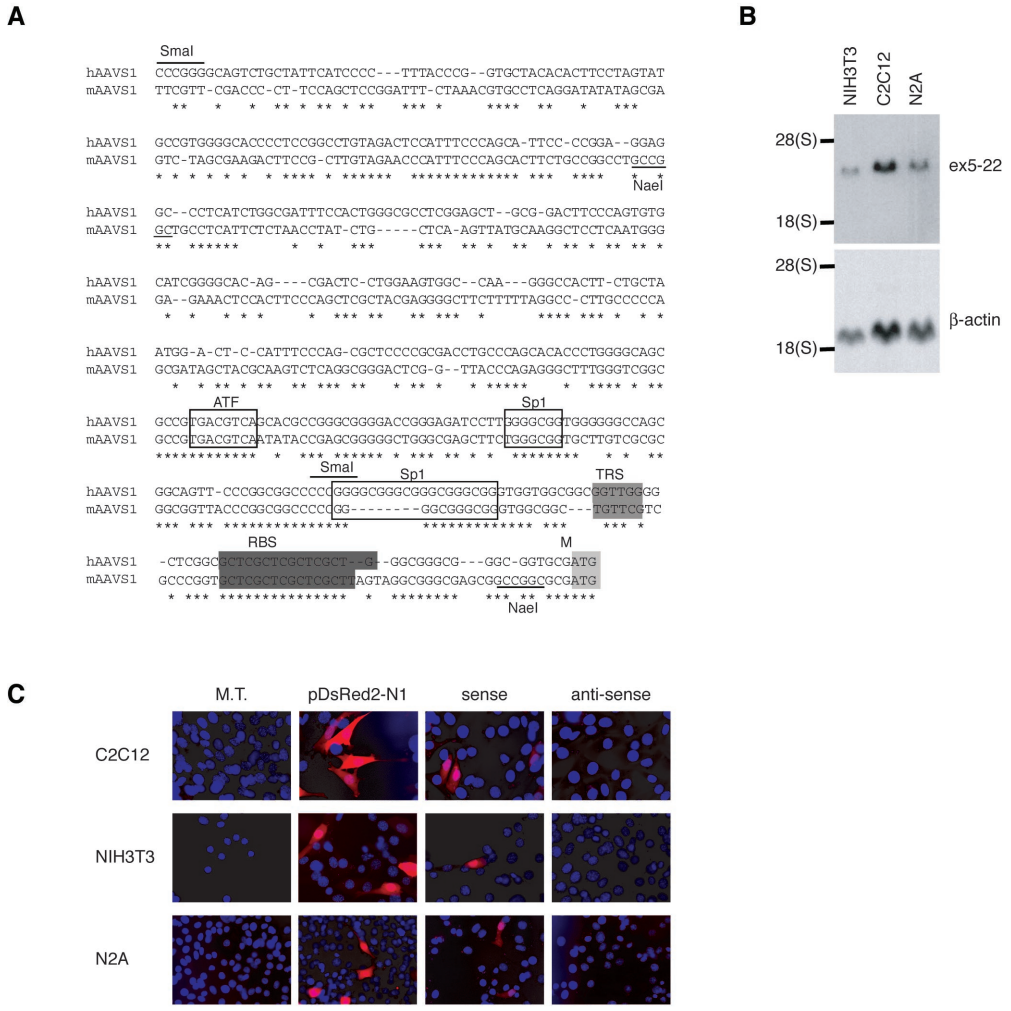


FIG. 4. (A) Nucleotide sequence alignment of the human and mouse *AAVS1* sequences upstream of the ATG (MacVector). The recognition sites for the TRS, RBS, translation initiation codon (M), and transcription factor binding elements for Sp1, CRE, and ATF-2 (white boxes) are indicated. Dashes indicate gaps between the human and mouse sequences. The SmaI sites present in the human *AAVS1* sequence are denoted by the line above, and the NaeI sites present in the mouse *AAVS1* sequence are underlined. (B) Expression of *Mbs85* by Northern blot analyses. The Northern blot of C2C12, N2A, and NIH 3T3 cells was hybridized with a cDNA probe consisting of exons 5 to 22 (top panel). The blot was stripped and rehybridized with a β-actin cDNA probe (bottom panel). Positions of the 28S and 18S rRNAs are indicated. (C) Transcriptional activity of the mouse Mbs85 proximal promoter. Plasmid pDsRed2-N1 (red fluorescent protein under the cytomegalovirus promoter; Clontech) and the sense and antisense plasmids were transfected into C2C12, NIH 3T3, and N2A cells. Forty-five hours posttransfection, the cells were visualized for red fluorescent protein expression and DAPI staining by using an epifluorescent microscope (Leica DMRA2) and a Hamamatsu digital camera. M.T., mock transfection.

mouse substrate which has been seen previously (34, 38), the significance of which is at present unclear.

We further demonstrate that a region containing the TRS-RBS motif upstream of the mouse *Mbs85* ATG contains regulatory elements sufficient to drive the expression of a reporter gene in vitro (Fig. 4). The fact that the TRS-RBS motif and its position relative to the ATG of the *MBS85* gene are conserved between human and mouse suggests that a cellular protein might interact with these sequences to potentially regulate *MBS85* transcription or translation. It is likely that integration of AAV DNA into *MBS85* affects *MBS85* expression and thereby the function of the gene product.

Site-specific integration by AAV might make possible targeted therapeutic gene transfer, thereby minimizing the risk of insertional mutagenesis. Knowledge of the effects of AAV-mediated site-specific integration is a prerequisite for achieving this goal. Therefore, there have been considerable efforts to develop rodent models containing the human *AAVS1* region (3, 25, 41). Rep-dependent site-specific nicking and DNA replication at the mouse TRS-RBS motifs suggests the possibility of AAV Rep-targeted integration at the mouse locus. The advantage of a mouse model with an innate *AAVS1* will be that the overall organization of the integration site, and thus potential effects of AAV-mediated disruption, would be similar to what occurs at the human locus. However, attempts at isolating viral-cellular junctions from latently infected mouse cell lines have not yet been successful. Currently, we are engaged in a comprehensive study aimed at comparing the efficiency of

AAV integration into the mouse genome at the innate *AAVS1* to that described for human cells at the human *AAVS1*, to evaluate, in a meaningful manner, the resemblance and/or differences of these two systems.

Sequences were retrieved from GenBank: *Homo sapiens MBS85* cDNA, AF312028; MBS85 protein, AF312028_1; *AAVS1*, S51329; *TNNT1* gene, AJ011712/AJ011713; *TNNI3* gene, X90780; *DRC3* gene, AF282168; *Homo sapiens* chromosome 19 clone CTD-2587H24, AC010327; *Mus musculus Tnnt1* gene, U92882; *Tnni3* gene, Z22784; 969-nt DNA clone, AK010836; *Mus musculus* BAC clone RP23-313M20, AC079521.

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REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Amiss, T. J., and R. J. Samulski.** 2001. Methods for adeno-associated virus-mediated gene transfer into muscle. Methods Mol. Biol. **175:**455–469.
3. **Bakowska, J. C., M. V. Di Maria, S. M. Camp, Y. Wang, P. D. Allen, and X. O. Breakefield.** 2003. Targeted transgene integration into transgenic mouse fibroblasts carrying the full-length human AAVS1 locus mediated by HSV/AAV rep(+) hybrid amplicon vector. Gene Ther. **10:**1691–1702.
4. **Balague, C., M. Kalla, and W. W. Zhang.** 1997. Adeno-associated virus Rep78 protein and terminal repeats enhance integration of DNA sequences into the cellular genome. J. Virol. **71:**3299–3306.
5. **Berns, K. I.** 1996. Parvoviridae: the viruses and their replication, p. 2173–2197. *In* B. N. Fields, D. M. Knipe, and P. M. Howley (ed.), Fields virology, 3rd ed., vol. 2. Lippincott-Raven, Philadelphia, Pa.
6. **Berns, K. I., and R. M. Linden.** 1995. The cryptic life style of adeno-associated virus. Bioessays **17:**237–245.
7. **Blake, J. A., J. T. Eppig, J. E. Richardson, M. T. Davisson, et al.** 2000. The mouse genome database (MGD): expanding genetic and genomic resources for the laboratory mouse. Nucleic Acids Res. **28:**108–111.
8. **Brister, J. R., and N. Muzyczka.** 1999. Rep-mediated nicking of the adeno-associated virus origin requires two biochemical activities, DNA helicase activity and transesterification. J. Virol. **73:**9325–9336.
9. **Chiorini, J. A., M. D. Weitzman, R. A. Owens, E. Urcelay, B. Safer, and R. M. Kotin.** 1994. Biologically active Rep proteins of adeno-associated virus type 2 produced as fusion proteins in *Escherichia coli*. J. Virol. **68:**797–804.
10. **Chiorini, J. A., S. M. Wiener, R. A. Owens, S. R. Kyostio, R. M. Kotin, and B. Safer.** 1994. Sequence requirements for stable binding and function of Rep68 on the adeno-associated virus type 2 inverted terminal repeats. J. Virol. **68:**7448–7457.
11. **Chiorini, J. A., L. Yang, B. Safer, and R. M. Kotin.** 1995. Determination of adeno-associated virus Rep68 and Rep78 binding sites by random sequence oligonucleotide selection. J. Virol. **69:**7334–7338.
12. **Dutheil, N., F. Shi, T. Dupressoir, and R. M. Linden.** 2000. Adeno-associated virus site-specifically integrates into a muscle-specific DNA region. Proc. Natl. Acad. Sci. USA **97:**4862–4866.
13. **Guenet, J. L., D. Simon-Chazottes, M. Gravel, K. E. Hastings, and S. Schiaffino.** 1996. Cardiac and skeletal muscle troponin I isoforms are encoded by a dispersed gene family on mouse chromosomes 1 and 7. Mamm. Genome **7:**13–15.
14. **Huang, Q. Q., and J. P. Jin.** 1999. Preserved close linkage between the genes encoding troponin I and troponin T, reflecting an evolution of adapter proteins coupling the Ca(2+) signaling of contractility. J. Mol. Evol. **49:**780–788.
15. **Im, D. S., and N. Muzyczka.** 1990. The AAV origin binding protein Rep68 is an ATP-dependent site-specific endonuclease with DNA helicase activity. Cell **61:**447–457.
16. **Im, D. S., and N. Muzyczka.** 1992. Partial purification of adeno-associated virus Rep78, Rep52, and Rep40 and their biochemical characterization. J. Virol. **66:**1119–1128.
17. **Kotin, R. M., R. M. Linden, and K. I. Berns.** 1992. Characterization of a preferred site on human chromosome 19q for integration of adeno-associated virus DNA by non-homologous recombination. EMBO J. **11:**5071–5078.
18. **Kotin, R. M., J. C. Menninger, D. C. Ward, and K. I. Berns.** 1991. Mapping and direct visualization of a region-specific viral DNA integration site on chromosome 19q13-qter. Genomics **10:**831–834.
19. **Kotin, R. M., M. Siniscalco, R. J. Samulski, X. D. Zhu, L. Hunter, C. A. Laughlin, S. McLaughlin, N. Muzyczka, M. Rocchi, and K. I. Berns.** 1990. Site-specific integration by adeno-associated virus. Proc. Natl. Acad. Sci. USA **87:**2211–2215.
20. **Krogh, A.** 1997. Two methods for improving performance of an HMM and their application for gene finding. Proc. Int. Conf. Intell. Syst. Mol. Biol. **5:**179–186.
21. **Lamartina, S., E. Sporeno, E. Fattori, and C. Toniatti.** 2000. Characteristics of the adeno-associated virus preintegration site in human chromosome 19: open chromatin conformation and transcription-competent environment. J. Virol. **74:**7671–7677.
22. **Linden, R. M., P. Ward, C. Giraud, E. Winocour, and K. I. Berns.** 1996. Site-specific integration by adeno-associated virus. Proc. Natl. Acad. Sci. USA **93:**11288–11294.
23. **Linden, R. M., E. Winocour, and K. I. Berns.** 1996. The recombination signals for adeno-associated virus site-specific integration. Proc. Natl. Acad. Sci. USA **93:**7966–7972.
24. **McCarty, D. M., D. J. Pereira, I. Zolotukhin, X. Zhou, J. H. Ryan, and N. Muzyczka.** 1994. Identification of linear DNA sequences that specifically bind the adeno-associated virus Rep protein. J. Virol. **68:**4988–4997.
25. **Rizzuto, G., B. Gorgoni, M. Cappelletti, D. Lazzaro, I. Gloaguen, V. Poli, A. Sgura, D. Cimini, G. Ciliberto, R. Cortese, E. Fattori, and N. La Monica.** 1999. Development of animal models for adeno-associated virus site-specific integration. J. Virol. **73:**2517–2526.
26. **Ryan, J. H., S. Zolotukhin, and N. Muzyczka.** 1996. Sequence requirements for binding of Rep68 to the adeno-associated virus terminal repeats. J. Virol. **70:**1542–1553.
27. **Samulski, R. J., X. Zhu, X. Xiao, J. D. Brook, D. E. Housman, N. Epstein, and L. A. Hunter.** 1991. Targeted integration of adeno-associated virus (AAV) into human chromosome 19. EMBO J. **10:**3941–3950. (Erratum, **11:**1228, 1992.)
28. **Shelling, A. N., and M. G. Smith.** 1994. Targeted integration of transfected and infected adeno-associated virus vectors containing the neomycin resistance gene. Gene Ther. **1:**165–169.
29. **Smith, D. H., P. Ward, and R. M. Linden.** 1999. Comparative characterization of Rep proteins from the helper-dependent adeno-associated virus type 2 and the autonomous goose parvovirus. J. Virol. **73:**2930–2937.
30. **Smith, R. H., and R. M. Kotin.** 2000. An adeno-associated virus (AAV) initiator protein, Rep78, catalyzes the cleavage and ligation of single-stranded AAV *ori* DNA. J. Virol. **74:**3122–3129.
31. **Snyder, R. O., R. J. Samulski, and N. Muzyczka.** 1990. In vitro resolution of covalently joined AAV chromosome ends. Cell **60:**105–113.
32. **Surosky, R. T., M. Urabe, S. G. Godwin, S. A. McQuiston, G. J. Kurtzman, K. Ozawa, and G. Natsoulis.** 1997. Adeno-associated virus Rep proteins target DNA sequences to a unique locus in the human genome. J. Virol. **71:**7951–7959.
33. **Tan, I., C. H. Ng, L. Lim, and T. Leung.** 2001. Phosphorylation of a novel myosin binding subunit of protein phosphatase 1 reveals a conserved mechanism in the regulation of actin cytoskeleton. J. Biol. Chem. **276:**21209–21216.
34. **Urcelay, E., P. Ward, S. M. Wiener, B. Safer, and R. M. Kotin.** 1995. Asymmetric replication in vitro from a human sequence element is dependent on adeno-associated virus Rep protein. J. Virol. **69:**2038–2046.
35. **Ward, P., M. Falkenberg, P. Elias, M. Weitzman, and R. M. Linden.** 2001. Rep-dependent initiation of adeno-associated virus type 2 DNA replication by a herpes simplex virus type 1 replication complex in a reconstituted system. J. Virol. **75:**10250–10258.
36. **Ward, P., E. Urcelay, R. Kotin, B. Safer, and K. I. Berns.** 1994. Adeno-associated virus DNA replication in vitro: activation by a maltose binding protein/Rep 68 fusion protein. J. Virol. **68:**6029–6037.
37. **Weitzman, M. D., S. R. Kyostio, R. M. Kotin, and R. A. Owens.** 1994. Adeno-associated virus (AAV) Rep proteins mediate complex formation between AAV DNA and its integration site in human DNA. Proc. Natl. Acad. Sci. USA **91:**5808–5812.
38. **Wu, J., M. D. Davis, and R. A. Owens.** 2001. A Rep recognition sequence is necessary but not sufficient for nicking of DNA by adeno-associated virus type-2 Rep proteins. Arch. Biochem. Biophys. **389:**271–277.
39. **Yoon, M., D. H. Smith, P. Ward, F. J. Medrano, A. K. Aggarwal, and R. M. Linden.** 2001. Amino-terminal domain exchange redirects origin-specific interactions of adeno-associated virus rep78 in vitro. J. Virol. **75:**3230–3239.
40. **Yoon-Robarts, M., and R. M. Linden.** 2003. Identification of active site residues of the adeno-associated virus type 2 Rep endonuclease. J. Biol. Chem. **278:**4912–4918.
41. **Young, S. M., Jr., D. M. McCarty, N. Degtyareva, and R. J. Samulski.** 2000. Roles of adeno-associated virus Rep protein and human chromosome 19 in site-specific recombination. J. Virol. **74:**3953–3966.