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Short communication

Bovine adenovirus-3 E1A coding region contain *cis*-acting DNA packaging motifs

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Abstract

To elucidate further the regulation of E1 gene transcription and viral DNA packaging, we constructed and analyzed mutant BAdV-3s in which the deletion of sequences between left ITR and E1A ATG codon was combined with the functional blocking of E1A gene expression by introducing deletion mutations into E1A open reading frame (ORF). The results suggest that E1A coding region contains *cis*-acting packaging motifs for efficient encapsidation of BAdV-3 DNA into preformed empty capsids. In addition, E1A is not required for the transcription of E1B.

Keywords: BAdV-3; DNA packaging; E1A; *Cis*-acting packaging motifs; Transcription; E1B

The packaging of adenovirus genome involves the specific recognition of *cis*-acting viral DNA sequences named packaging domain by viral and/ or cellular proteins (Ostapchuk and Hearing, 2005). The *cis*-acting packaging domain of human adenovirus (HAdV)-5 is located at the left end of the viral genome between nucleotide (nt) 194 and 380 and contains at least seven functionally redundant elements termed A repeats (AI to AVII; Schmid and Hearing, 1997). Although the primary sequence of the packaging motifs of HAdV-5 (Schmid and Hearing, 1997), porcine adenovirus (PAdV)-3 (Xing and Tikoo, 2003, 2004) and canine adenovirus (CAdV)-2 (Soudais et al., 2001) appear to be different, the packaging domains of these adenoviruses are located between left inverted terminal repeat (ITR) and ATG of E1A.

Bovine adenovirus (BAdV)-3, also a member of *Mastadenovirus* genus is being characterized at the molecular level to develop it as a vector for the gene delivery to animals (Reddy et al., 1999; Zakhartchouk et al., 1998, 1999) and humans (Rasmussen et al., 1999). Using deletion strategy, earlier we reported that the part of the *cis*-acting packaging domain of BAdV-3 is located between nt 224 and 541 of BAdV-3 genome (Xing et al., 2003). Numbers indicate the nucleotide position (nt) relative to the left terminus of wild-type BAdV-3 genome (GenBank accession no. AF030154). However, deletion of these sequences could not lead to the loss of the viral viability (Xing et al., 2003) suggesting that the *cis*-acting packaging domain(s) exist beyond these sequences. Here, we report the construction and analysis of the additional mutant BAdV-3s in which deletions of the sequences between the left ITR and ATG codon of E1A were combined with the functional blocking of E1A (Fig. 1A) gene expression.

To this end, we constructed four BAdV-3 mutants (Fig. 1A) designated as Bav3-L1 (nts 641–690), Bav3-L2 (nts 225–559, 641–690), Bav3-L3 (nts 947–1054) and Bav3-L4 (nts 225–559,641–690, 947–1054) using primers (Fig. 1B) and plasmid pLtRtHind.Mod [containing the 4.8% left end (nt 1–1653) and 3.5% right end (nt 33235–34446) of viral genome (Xing et al., 2003)] as a DNA template. These manipulations also created a stop codon in E1A ORF, which results in the loss of the expression of E1A. The identity of the mutant BAdV-3s was confirmed by PCR using primers (PLB5, 5′-CCGcaattgAGTTCCGCACCCGCTACG -3′; PLB2, 5′-CACATAATGCCTGgaattcC-3′; Fig. 1B). As shown in Fig. 1C, the specific DNA fragments of expected size were observed for each mutant BAdV-3 viral DNA. Moreover, the deletions were confirmed by DNA sequence analysis of PCR products.

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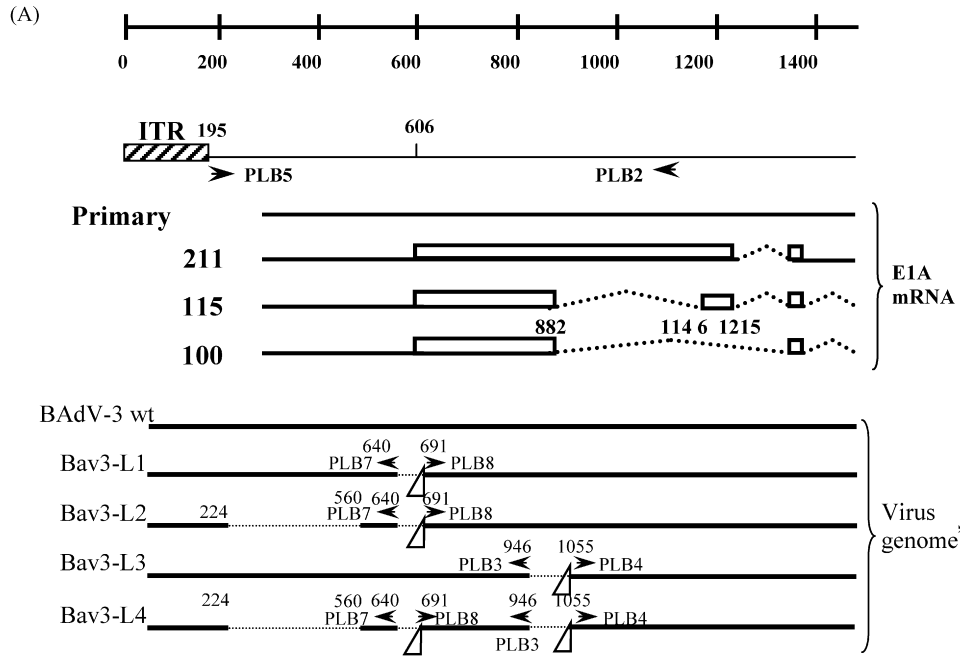
To determine the effect of the deletions on the expression of the viral early genes, the transcription and the protein expression were investigated by Northern hybridization and Western blot, respectively. Madin–Darby bovine kidney (MDBK) cells were infected with wild-type or mutant BAdV-3s at an multiplicity of infection (MOI) of 5 plaque-forming units (PFU). At 24 h and 48 h postinfection, the infected cells were harvested and the total RNA was isolated. After treating with DNase I to remove the contaminated DNA, the RNAs were separated on denaturing 1% agarose gel and then transferred onto hybridization membrane. Northern blot analysis was performed with [^{32}P]-labeled DNA probes for E1A (nt 560–1156), E1B (nt 1398–1651) or E3 (nt 27273–27959).

The E1A-specific DNA probe (nt 560–1156; Fig. 1A) detected two major RNA bands (3.2 kb and 1.3–0.8 kb) at 24–48 h post infection of cells with wild-type BAdV-3 (Fig. 1D(i)). However at 48 h post infection, the RNA band between 1.3 kb and 0.8 kb displayed the stronger signal than that of 3.2 kb. In contrast, only 3.2 kb band was detected at 24–48 h postinfection of cells with mutant Bav3-L1, Bav3-L2, Bav3-L3, or Bav3-L4. These results suggest that production of functional E1A transcripts was blocked in mutant BAdV-3 infected cells. It is possible that the deletions altered the structure required for proper splicing of the primary E1A transcript.

The E1B specific DNA probe (nt 1398–1651; Fig. 1A) detected an E1B specific 2.1 kb band and two additional RNA



(B)

Primer	Sequence	Nucleotide position (nt)	
LZP53:	5'-CGTCTTCAAGGATCCGAA-3'	minus nt 22-39	*BamH*I, sense
PLB1:	5'-**TTCAAAGCTTCATTCCCTG**-3'	1642-1657	*Hind*III, antisense
PLB2:	5'-**CACATAATGCCTGGAATTCC**-3'	1155-1174	*EcoR*I antisense
PLB3:	5'-CCGGGTACC**GCATTCAGACACTCATCCT**-3'	928-946	*Kpn*I, antisense
PLB4:	5'-CCGGGTACC**GATAGCGATGTAGAAGAGG**-3'	1055-1074	*Kpn*I, sense
PLB5:	5'-**CCGCAATTGAGTTCCGCACCCGCTACG**-3'	205-222	*Mfe*I, sense
PLB7:	5'-CCGTCTAGA**TGCCGTCGTTGAGAACGAG**-3'	621-640	*Xba*I, antisense
PLB8:	5'-CCGTCTAGA**AGTGTCATGAGGTACTTCC**-3'	691-709	*Xba*I, sense

Fig. 1. Viruses and deletions. **(A)** Schematic diagram showing 1450 bp left end genome of BAdV-3. The left ITR (hatched box), location of primers PLB5 and PLB2 (arrow heads) used in PCR are depicted. Schematic diagram showing E1 mRNAs. The sizes and left end structures of spliced 3.2 kb, 1.3 kb, and 1.1 kb E1A mRNAs [encoding 211, 115, and 105 amino acid residue (211R, 115R, and 105R) protein, respectively]. Schematic diagram of mutant viruses. The deletions (dotted lines) and translation stop codons (open triangles) introduced in mutant BAdV-3 genome. Open boxes and dotted lines in mRNA represent protein coding region and intron region, respectively. The designated name of each mutant BAdV-3 is shown on the left. Numbers in mRNA or virus genome indicate the nucleotide position (nt) of either ends of exon or undeleted virus genome relative to the left terminus of wt BAdV-3 genome (GenBank accession no. AF030154). (B) List of primers used for PCR. The restriction endonuclease cleavage sites are underlined. Numbers indicate the nucleotide position (nt) relative to the left terminus of BAdV-3 (GenBank accession no. AF030154) genome. BAdV-3 nucleotide sequences are indicated in boldface type. (C) PCR amplification. The amplification of viral genomic DNAs using primers PLB2/PLB5. The numbers on the bottom designate the expected size (in bp) of PCR products. (D) Northern blot analysis of E1A mRNA (i), E1B mRNA (ii) and E3 mRNA (iii). The RNA isolated from virus infected cells at indicated times postinfection was analyzed by Northern blot using [^{32}P]-labeled probes corresponding to (i) nt 560–1156, (ii) nt 1398–1651 and (iii) nt 27273–27959. As a control, the RNAs stained with ethidium bromide in denaturing formaldehyde agarose gel were photographed. 18s and 28s rRNAs are indicated. Results from one representative of the two similar experiments are shown. The sizes of RNA bands (in kb) are indicated on the right of the panels. (E) Western blot analysis of E2A. Proteins from the lysates of the virus-infected cells were separated by SDS-PAGE, transferred onto nitrocellulose membrane and analyzed by Western blot (WB) using polyclonal antibodies against BAdV-3 DBP. Coomassie R250 blue (CB) staining protein loading control.