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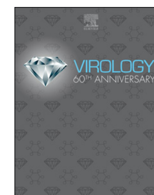

The 164 K, 165 K and 167 K residues in 160YPVVKKPKLTEE171 are required for the nuclear import of goose parvovirus VP1

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ABSTRACT

goose parvovirus (GPV) belongs to the *Dependoparvovirus* genus in *Parvovirinae* subfamily within *Parvoviridae* family, is the etiological agent of Derzsy's disease. Nuclear localization signal (NLS) is important for parvovirus lifecycle in the delivery of genomes and the structural protein of progeny virus into the nucleus. Here, NLS was first identified in GPV. By using the PSORT II program, a basic region (BR, 160YPVVKKPKLTEE171) in the N-terminus of VP1 was found, which predicted as putative NLS motif of goose parvovirus capsid. The GPV BR could transfer both small reporter proteins (EGFP) and large reporter protein (β-galactosidase) into the nucleus by Immunofluorescence assay. Furthermore, the K164A, or K165A, or K167A substitutions mutation of GPV VP1 did abolish its nuclear localization, suggesting that the 164 K, 165 K and 167 K residues in the 160YPVVKKP-KLTEE171 are required for its for nuclear import. Our finding may help us to gain a better understand of GPV lifecycle.

1. Background

Nuclear localization signals (NLSs) are usually composed of basic residues (K and R), tend to be hydrophilic, and are divided into classical (cNLS) and non-classical (ncNLS) types. The cNLSs are further divided into classical monopartite NLS and classical bipartite NLS. In the cytoplasm, the small proteins can be transported into the nucleus through the nuclear pore complex freely or by passive diffusion, while the larger proteins (> 50 kDa) require active transport. Large proteins can be transported into the nucleus with the help of an NLS (Macara, 2001). Notably, the functions of NLSs have been identified in some DNA viruses, such as herpesvirus (Abaitua et al., 2012; Hennig et al., 2014), circovirus (Liu et al., 2001; Xiang et al., 2013) and parvovirus (Boisvert et al., 2014; Grieger et al., 2006), as well as some RNA viruses (Cros et al., 2005 ; Walker and Lipkin, 2002).

Goose parvorirus (GPV) is the members of *Dependoparvovirus* genus of *Parvovirinae* subfamily within *Parvoviridae* family GPV, which is fetal for gosling and can cause enteritis and diarrhoea, is the etiological agent of Derzsy's disease (Brown et al., 1995a; Derzsy, 1967). GPV is a single-stranded DNA virus without envelop protein and its complete genome is about 5.1 kb in length (Zádori et al., 1995), which contains two major open reading frames (ORF) and the inverted terminal repeats (ITR) at the both genomic terminus. The left ORF encodes the non-structural protein required for both replication of viral genome and regulation of capsid gene expression (Li et al., 2009; Qiu et al., 2005), and the right ORF encodes the structural protein composed of three capsid protein which have common region of C-terminus (Zádori et al., 1995). During the life cycle of GPV, the genomes and progeny capsid proteins must been delivered into nucleus of host cells to complete its assembling. However, NLS has not been identified in GPV yet.

In this study, we predicted a putative basic region (BR) of GPV by using the PSORT II program, which is a classical NLS in the N-terminus (160YPVVKKPKLTEE171) of VP1. Comparison with the NLS of adeno-associated virus 2 (AAV2) (Grieger et al., 2006), the BR of GPV is similar to it. We illustrate here that the BR can target the GPV VP1 into

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Table 1
The oligonucleotide primer used in this study.

Primer	The sequences (5′-3′)
5′-GPV NLS	***C***GCTACCCGGTGGTTAAGAAGCCTAAACTTACCGAGGAGTAA***CCC***
3′-GPV NLS	***GGG***TTACTCCTCGGTAAGTTTAGGCTTCTTAACCACCGGGTAGC***GGATC***
5′-SV40 NLS	***C***GCCCAAAGAAGAAGAGAAAGGTCTAA***CCC***
3′-SV40 NLS	***GGG***TTAGACCTTTCTCTTCTTCTTTGGGC***GGTAC***
β-Gal-F	ATTCTGCAGTCGAC***GGTACC***GCATGAGCGAAAAATACATCG
β-Gal-R	TCTAGATCCGGTGGAT***CCCGGG***TTATTTTTGACACCAGACCAACTGG
GPV-NLS-β-Gal-F	AATTCTGCAGTCGAC***GGTACC***GCTACCCGGTGGTTAAGAAGCCTAAACTTACCGAGGAGATGAGCGAAAAATACATCG
SV40-NLS-β-Gal-F	AATTCTGCAGTCGAC***GGTACC***GCACACCACCTAAAAAGAAGAGAAAAGTAGAAATGAGCGAAAAATACATCG
VP1WT-F	GAATTCTGCAGTCGAC***GGTACC***GCTCTACTTTTTTAGATTCTTTTGAAGAG
VP1WT-R	CTAGATCCGGTGGAT***CCCGGG***TTACAGATTTTGAGTTAGATATCTGGT
VP1mNLS-F	TTGCAGCACCTGCACTTACCGAGGAGGTCAG
VP1mNLS-R	TGCAGGTGCTGCAACCACCGGGTAATGGTC
VP1K164A-F	TTGCAAAGCCTAAACTTACCGAGGAGGTCAG
VP1K164A-R	TTTAGGCTTTGCAACCACCGGGTAATGGTC
VP1K165A-F	TTAAGGCACCTAAACTTACCGAGGAGGTCAG
VP1K165A-R	TTTAGGTGCCTTAACCACCGGGTAATGGTC
VP1K167A-F	TTAAGAAGCCTGCACTTACCGAGGAGGTCAG
VP1K167A-R	TGCAGGCTTCTTAACCACCGGGTAATGGTC

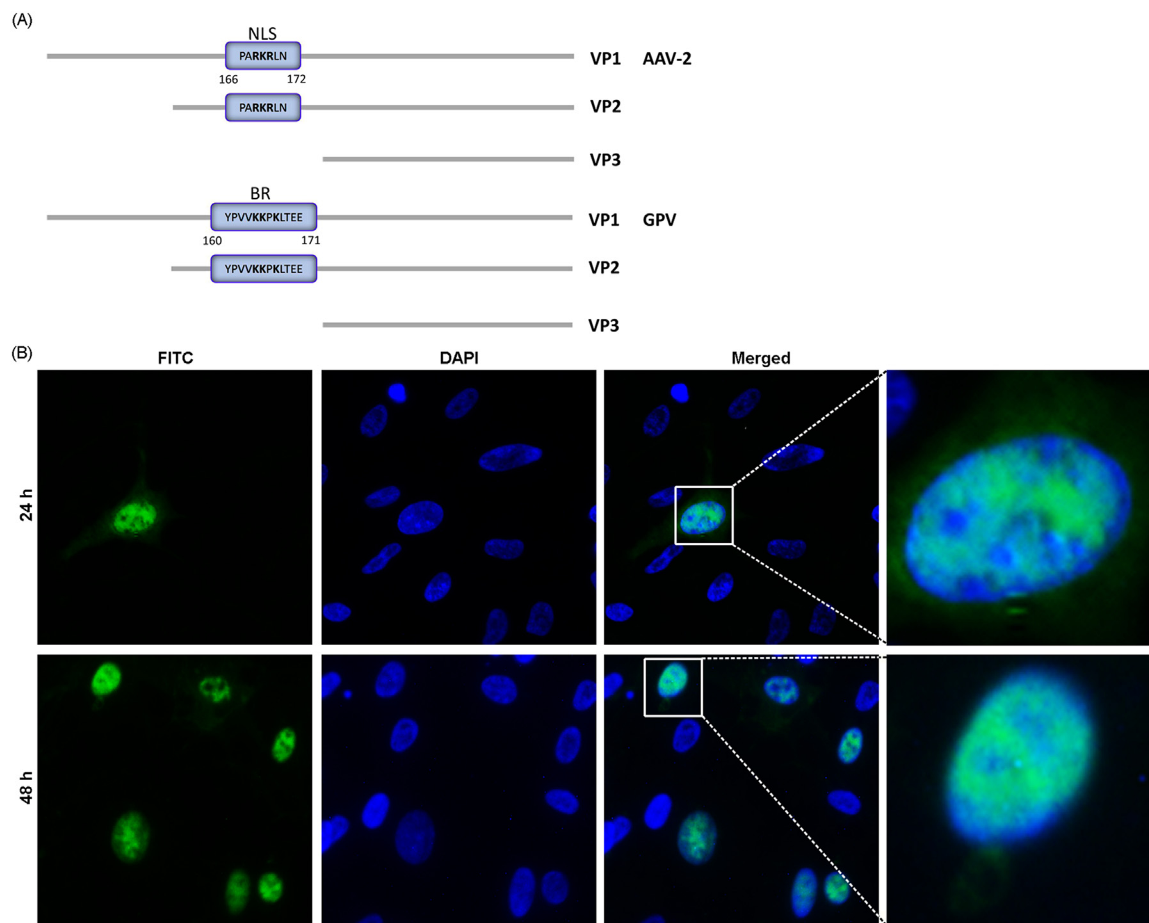

Fig. 1. (A) The reported NLS of AAV2, and the predicted BR motif of GPV. (B) Localization of GPV in the infected GEF cells. The GPV capsid proteins were detected by indirect immunofluorescence analysis at 24 h and 48 h post infection using mouse anti-GPV polyclonal antibodies as primary antibody.

the nucleus of cells. The BR was also found to lead the EGFP and EGFP-beta-Gal into to nucleus of transfected BHK21 cells. However, mutations of K164, K165 and K167 can abolish the activity of nuclear localization of GPV VP1. These results indicate that the BR play an important role in translocation of VP1 into the nucleus and may help us to gain a better understand of GPV life cycle.

2. Materials and methods

2.1. Virus, cells culture and transfection

GPV viruses were kindly provided by the Research Center of Avian Disease, College of Veterinary Medicine of Sichuan Agricultural University.

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