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Molecular characterization of the small nonstructural proteins of parvovirus Aleutian mink disease virus (AMDV) during infection

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ABSTRACT

Aleutian mink disease virus (AMDV) is the only member in genus *Amdovirus* of the family *Parvoviridae*. During AMDV infection, six species of viral transcripts are generated from one precursor mRNA through alternative splicing and alternative polyadenylation. In addition to the large non-structural protein NS1, two small non-structural proteins, NS2 and NS3, are putatively encoded (Qiu J, et al., 2006. J. Virol. 80 654–662). However, these two proteins have not been experimentally demonstrated during virus infection, and nothing is known about their function. Here, we studied the nonstructural protein expression profile of AMDV, and for the first time, confirmed expression of NS2 and NS3 during infection, and identified their intracellular localization. More importantly, we provided evidence that both NS2 and NS3 are necessary for AMDV replication.

Introduction

Aleutian mink disease virus (AMDV), an autonomous parvovirus, belongs to genus *Amdovirus*, in the family *Parvoviridae* (Tijssen et al., 2012). AMDV infection is known only to produce clinical manifestation in mink and ferrets (Aasted, 1985; Porter et al., 1982). AMDV is the only classified member in the genus *Amdovirus* (Tijssen et al., 2012). However, identification of an *Amdovirus* sequence in grey fox (GFADV) (Li et al., 2011) suggests the likelihood of more members in this genus. Aleutian mink disease affects mink farming worldwide, and has become the most significant threat to mink production in some area (Newman and Reed, 2006). AMDV infection in neonatal mink kits causes acute, rapidly progressing interstitial pneumonia with a high mortality rate (Alexandersen, 1990); whereas, in adult mink, AMDV undergoes a persistent infection which manifests with symptoms of glomerulonephritis, plasmacytosis, hypergammaglobinemia, arteritis, decreased fertility, and spontaneous abortion (Alexandersen et al., 1994; Gorham et al., 1976). The hypergammaglobulinemia that is characteristic of Aleutian mink disease is partly caused by massively increased level of anti-AMDV antibodies in blood. The antibody and virus complex is deposited within tissues of multiple organs in the body causing inflammation, which also facilitates the entry of virus into certain target cell populations (Kanno et al., 1993a). In vitro infection confirmed the antibody-dependent enhancement of AMDV infection (Bloom et al., 2001; Dworak et al., 1997; Kanno et al., 1993b). Therefore, it is difficult to develop a vaccine to prevent AMDV infection (Aasted et al., 1998). Currently, there are also no prevention or treatment methods available for Aleutian mink disease.

The ferret is another host of AMDV (Porter et al., 1982). Ferrets have been widely bred as pets and laboratory animals, for example, as an animal model of influenza virus (Barnard, 2009) and cystic fibrosis (Sun et al., 2010), but the impact of AMDV infection on the pathogenesis of these other infections has not been studied. Clinical symptoms in ferrets are renal failure, weight loss, splenomegaly, neurological symptoms like seizures and clotting abnormalities, and disease progression will result in death of the ferret within a few months. Recently evidence of AMDV infection was noted in two mink farmers who displayed disease symptoms similar to those of Aleutian mink disease. Hence, AMDV may be a zoonotic disease rarely capable of infecting humans (Jepsen et al., 2009).

The transcription profile of AMDV during infection displays features similar to those of parvoviruses in genera *Erythrovirus* and *Bocavirus* (Chen et al., 2010a; Ozawa et al., 1987; Qiu et al., 2006a). The six species of AMDV mRNA transcripts are generated from a single promoter, and are processed through alternative splicing and alternative polyadenylation (Fig. 1A) (Qiu et al., 2006a). R1 and R2 mRNAs encode the large non-structural protein NS1. The NS1 protein is assumed to function similarly to that of other autonomous parvoviruses. Parvovirus NS1 contains motifs of DNA

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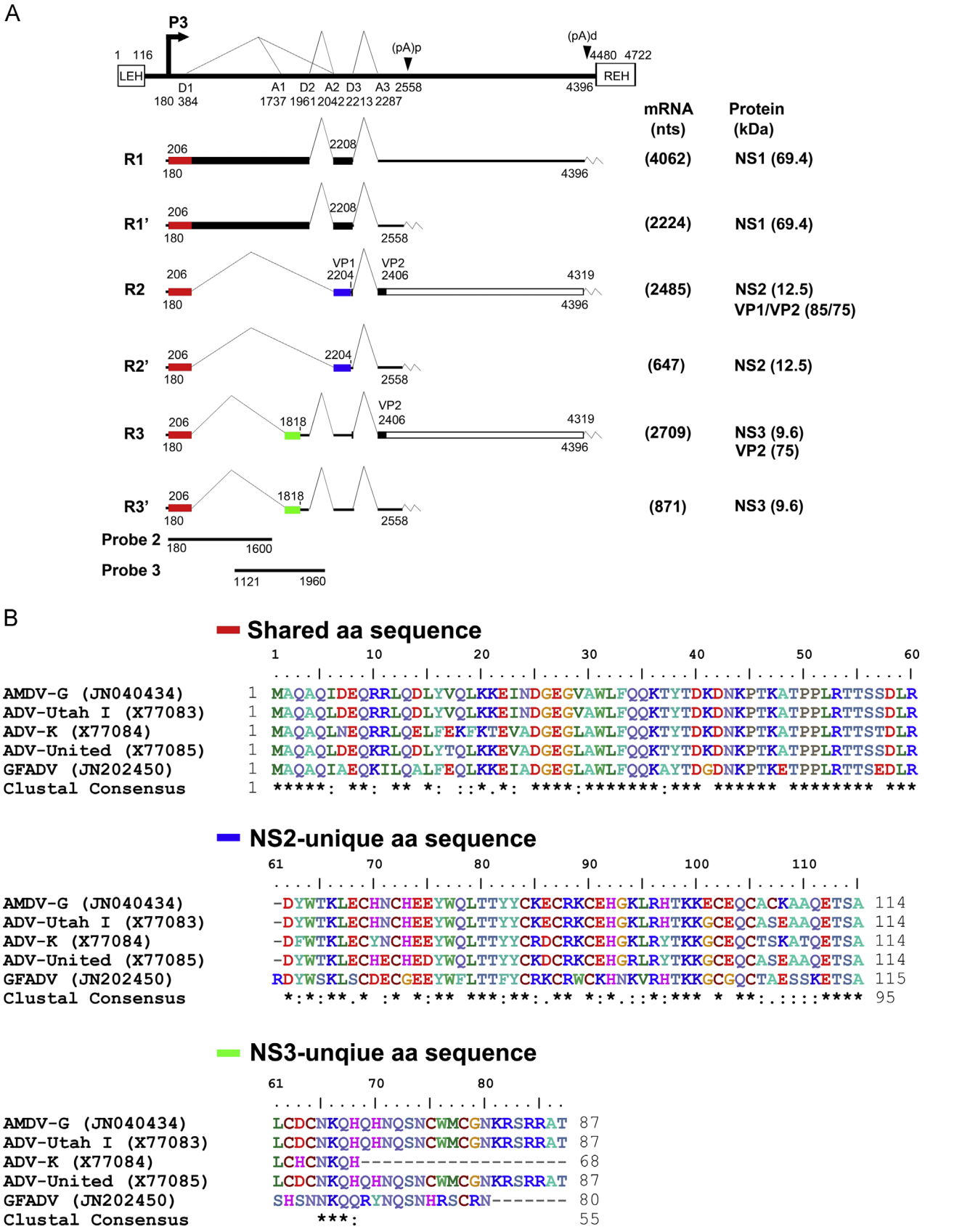

Fig. 1. Genetic map of AMDV and the amino acid sequence alignment of the AMDV NS2 and NS3 proteins among members in genus *Amdovirus*. (A) Genetic map of AMDV. The genome of AMDV-G is depicted to scale with indicated transcription units, including the end hairpin termini (LEH and REH), P3 promoter, splice donor site (D1–D3), splice acceptor sites (A1–A3), internal proximal polyadenylation site [(pA)p], and internal distal polyadenylation site [(pA)d]. The major six mRNA transcripts are shown with R1-3 polyadenylated at the (pA)d site and R1′-3′ polyadenylated at the (pA)p site. Viral NS1, NS2, NS3 and VP1/VP2 proteins encoded from each mRNA are indicated. (B) Amino acid sequence alignment of AMDV NS2 and NS3 among members in genus *Amdovirus*. Amino acid sequence alignment of AMDV NS2 and NS3 among four AMDV isolates (AMDV-G, ADV-Utah, ADV-K, and ADV-United) and the newly identified GFADV were chosen to align using ClustalW2. The shared N-terminus, the unique C-termini of NS2 and NS3 are shown. Identical amino acids are shown as a "star" symbol, while homologous amino acids shown as two dots.