In vitro growth kinetics and gene expression analysis of the turkey adenovirus 3, a siadenovirus

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ABSTRACT

Turkey adenovirus 3 (TAdV-3) belongs to the genus Siadenovirus, family Adnoviridae. Previously, nucleotide sequencing and annotation of the Virginia avian strain (VAS) of TAdV-3 genome, isolated in our laboratory, indicated the presence of a total of 23 genes and open reading frames (ORFs). The goals of this study were 1) to delineate the growth kinetics of the virus using a (PCR-based infectivity assay), and 2) to determine the virus gene expression profile during the early and late phases of infection in target B lymphocytes. The one-step growth curve experiment demonstrated three phases of virus replication cycle: a lag phase lasted for 12-18 h post-infection (h.p.i.), in which the number of infectious virus particles increased over 20,000 fold, and a high decrease phase thereafter. Southern blot analysis indicated that the synthesis of new viral RNA began at 8 h.p.i. Gene-specific RT-PCR revealed the expression of mRNAs from the 23 TAdV-3 genes/ORFs. According to the temporal transcriptional profiling of TAdV-3 genome, genes could be divided into 3 groups based on the time of transcription initiation: group 1 showed detectable levels of transcription at 24 h.p.i. and included 7 genes, i.e., hsp, 3x, ps, p0, II, 100 K, and 33 K; group 2 included 12 genes whose mRNAs were detected for the first time at 48 h.p.i., i.e., ORF1, ORF2, pol, pIV, pR5, pSp, ORF7, and ORF8; group 3 of transcripts were detectable starting 8 h.p.i. and included only 4 genes, i.e., N2, 22 K, p01, and pVIII. Our data suggest that the transcriptional kinetics of genus Siadenovirus differ from that observed in other adenoviral genus; however, a few TAdV-3 genes showed similar expression patterns to their adenoviral homologs.

1. Introduction

Turkey adenovirus 3 (TAdV-3), more commonly known as turkey hemorrhagic enteritis virus (THEV), is the causative agent of a variety of clinical conditions in a number of avian species. TAdV-3 causes hemorrhagic enteritis in turkeys, marble spleen disease in pheasants, and avian adenovirus splenomegaly in chickens (Pierson and Fitzgerald, 1973). TAdV-3 targets B lymphocytes, inducing apoptosis and transient immunosuppression, which often results in additional mortality due to secondary bacterial infection (Pierson and Fitzgerald, 2013; Pierson et al., 1996; Rauteschlein et al., 2000; Saunders et al., 1993; Suresh and Sharma, 1995, 1996). TAdV-3 has a non-enveloped,icosahedral capsid of 70–90 nm in diameter, enclosing a linear, non-segmented, double-stranded DNA (dsDNA) genome of 26 kb (Beach et al., 2009; Bliet et al., 1977; Jucker et al., 1996; Tolin and Dormer, 1975; van den Hurk, 1992). TAdV-3 belongs to the family Adnoviridae, genus Siadenovirus, species Turkey siadenovirus A (Adams et al., 2014). TAdV-3 and frog adenovirus 1 (FrAdV-1) have been the only two members in the genus until 2009. Since then, several siadenovirus members have been recognized and associated with infections in different avian and non-avian species (Kato et al., 2009; Kovacs and Berks, 2009; Kovacs et al., 2010; Riviere et al., 2009; Welsham et al., 2009).

The first siadenoviral genome to be partially and fully sequenced was TAdV-3 (Beach et al., 2009; Jucker et al., 1996; Pitcovski et al., 1998), followed by FrAdV-1 (Davison et al., 2000) and other

Abbreviations: TAdV-3, turkey adenovirus 3; VAS, Virginia avian strain; ORF, open reading frames; h.p.i., hour(s) post-infection; pol, adenovirus DNA-dependent DNA polymerase; IFV, infectious viral particle(s)

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