IDENTIFICATION AND ANALYSIS OF A PUTATIVE RNA POLYMERASE SUBUNIT GENE OF ORF VIRUS

ABSTRACT

The nucleotide sequence of fragment HindIII-G of orf virus genome from a Brazilian isolate has been determined. Sequence analysis identifies a putative RNA polymerase subunit gene and a zinc finger peptide motif into this genomic fragment.

The orf virus (Contagious Pustular Dermatitis, Contagious Ecthyma virus, scabby mouth) belongs to the poxvirus family and frequently causes an exanthematic dermatitis in goats and sheep. The lesions are initially characterized by macules, which progress through papules, vesicles and pustules, until scab formation, within 3 to 4 weeks. These scabs, that are rich in viral particles and disappear upon lesion healing, are considered important in viral transmission and may be infective for long periods of time, under favorable circumstances. The lesions are generally mild and commonly occur in young animals, mainly in the leap (15), nevertheless, some outbreaks may be marked with exacerbated virulence, causing significant mortality and morbidity in suckling animals (8). The orf virus can infect man and is considered an occupational zoonosis (12, 15).

While we analyzed different isolates of Brazilian orf virus, we noticed that, based on restriction endonuclease analysis of genomic DNA, some bands differed in size among these isolates (11). One of these bands that distinguished isolates was the 5.3 Kb HindIII-G fragment of orf-A isolate. The isolate called orf-A is a Rio de Janeiro field isolate characterized in a previously published work (9). Its identification was confirmed against standard sera (Plum Island Disease Center), electron microscopy and biological assays. We set out to determine the coding potential of the HindIII-G fragment of orf-A, therefore, this fragment was cloned, sequenced and analyzed.

Scab suspensions of orf-A lesions were inoculated in young caprines in order to obtain viral stocks. Viral DNA was extracted directly from scabs, as described (10). For cloning procedures,
purified viral DNA was digested with HindIII endonuclease and fractionated by agarose gel electrophoresis. The agarose slice containing the HindIII-G viral genome fragment was cut out and the DNA purified by Gelase enzyme protocol (Epicentre Technol). This HindIII-G DNA was ligated to the plasmid pGEM-2 (PROMEGA) and the ligation mix used to transform competent MC1061 E.coli strain cells (18). A clone containing the entire HindIII-G fragment was identified after restriction analysis and named pB38.

The complete nucleotide sequence was directly derived from pB38 and from 10 sub clones thereof, based on a restriction endonuclease map. The sequence of 5261 nucleotides of the orf-A virus genome fragment (HindIII-G) was determined (Genbank accession number U33419), using the dideoxy termination method (19). In order to confirm the alignment of clones, synthetic oligonucleotides were used to derive sequences overlapping their boundaries.

Nucleotide sequence analysis was performed on an IRIX SYSTEM V.4 (gene) workstation, using the UWGCG (University of Wisconsin Genetics Computer Group) software. Computer analysis of the HindIII-G nucleotide sequence was initially based on the alignment to vaccinia virus, Copenhagen strain, genomic nucleotide sequence, GenBank accession number M35027 (4).

Significant homology between the sequence obtained and the A24R gene of vaccinia virus was noted. Gene A24R of vaccinia virus codes for a protein of 1164 amino acids, characterized as one of the 9 RNA polymerase subunits (6). Significant homology with A25L variola major, Bangladesh –1975, gene (7), rpo132 cowpox virus gene (14), ORF KS-1 goatpox virus (3) was also detected. A unique large open reading frame (called ORF-RPA) was found between nucleotides 161 and 3365 of the HindIII-G fragment, corresponding to 1168 amino acids. Alignment of the ORF-RPA amino acid sequence against the SWISS-PROT bank confirmed the significant homology with the same genome viral sequences identified by nucleotide sequence comparisons.

Analysis of the HindIII-G fragment nucleotide sequence showed typical poxvirus gene structures as TAAAT 5' leader sequence and a contiguous ATG starting codon; the large ORF-RPA and, downstream in the 3’ extreme, a TGA signaling the end of translation and a TTTTCTC indicating the transcription termination (5, 2). Based on the genetic elements found in the Poxvirus genome (4, 13), it was possible to derive the putative structure for the ORF-RPA gene (Figure 1).

**Figure 1.** Putative gene structure of RNA polymerase subunit in ORF-RPA. This scheme represents the HindIII-G fragment and its structural genetic elements: a. late promoter, nucleotides 177-181; b. initial translation codon, nucleotides 182-184; c. terminal translation codon, nucleotides 3366-3670; d. Transcriptional termination sequence, nucleotides 4098-4104.
The search for peptide motifs resulted in the localization of a zinc finger sequence (C-X₁₂-X-X₁₂)-C-X₂-C) (1, 17) in the nucleotide sequence of ORF-RPA. This motif is considered highly conserved among RNA polymerase subunit genes of Poxvirus or in RNA polymerases in general, possibly due to a bounding structure.

The GC composition was also analyzed. The genome of orthopoxvirus is AT rich, whereas in the parapoxvirus a high GC content has been observed (16). Accordingly, the HindIII-G fragment sequence showed a high GC content (60%) in contrast to only 36% in the A24R vaccinia virus gene sequence. This is mainly due to the third base variation, usually G or C in the ORF-RPA codons and A or T in A24R vaccinia virus gene codons.

The poxvirus genome encodes its own polymerases and its replication is cytoplasmatic, independent of cellular polymerases. Homology analysis, based on the alignment of the nucleotide sequence obtained with vaccinia virus genomic nucleotide sequence pointed to one of the 9 RNA polymerase subunits. The gene identified here is likely to represent a late gene, the product of which would be packaged in the viral particle and be important for transcription of early genes.

Another point of interest is the divergent GC composition among parapox, orthopox and capripoxvirus genus members, making unclear the type of selection pressure on these viruses. However, this divergence can be measured and used for phylogenetic analysis. In the case of this RNA polymerase subunit gene, the phylogenetic analysis performed is consistent with present generic taxonomy (Figure 2).

Finally, plasmid pB38 can be used as a molecular probe for diagnostic or epidemiological purposes, as we were able to identify and characterize an ORF isolate, based on its hybridization profiles to viral DNA from field viral isolates (9).

**Figure 2.** Phylogenetic analysis of the poxvirus family members. The nucleotide sequences homologous to ORF-RPA found in the Genbank were translated and compared to: a. RNA polymerase gene of vaccinia virus (4); b. RNA polymerase gene of cowpox virus (14); c. RNA polymerase gene of variola major virus (7); d. An ORF of goatpox virus (3); e. The ORF described in this article.
REFERENCES


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