

Comparative analysis of genome sequences of three isolates of *Orf virus* reveals unexpected sequence variation

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Abstract

Orf virus (ORFV) is the type species of the *Parapoxvirus* genus. Here, we present the genomic sequence of the most well studied ORFV isolate, strain NZ2. The NZ2 genome is 138 kbp and contains 132 putative genes, 88 of which are present in all analyzed chordopoxviruses. Comparison of the NZ2 genome with the genomes of 2 other fully sequenced isolates of ORFV revealed that all 3 genomes carry each of the 132 genes, but there are substantial sequence variations between isolates in a significant number of genes, including 9 with inter-isolate amino acid sequence identity of only 38–79%. Each genome has an average of 64% G + C but each has a distinctive pattern of substantial deviation from the average within particular regions of the genome. The same pattern of variation was also seen in the genome of another parapoxvirus species and was clearly unlike the uniform patterns of G + C content seen in all other genera of chordopoxviruses. The availability of genomic sequences of three *orf virus* isolates allowed us to more accurately assess likely coding regions and thereby revise published data for 24 genes and to predict two previously unrecognized genes.

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1. Introduction

Poxviruses are large DNA viruses, the most notorious of which is *Variola virus* (VARV), the causative agent of smallpox. The family is divided into entomopoxviruses and chordopoxviruses with the latter further subdivided into eight genera (Moyer et al., 2000). The genomes of the chordopoxviruses range in size from 135 to 365 kbp but show conservation of both genomic organization and content. The central regions of both genomes contain 88 genes which are present in all chordopoxviruses and which mostly occur in the same order and orientation (Delhon et al., 2004; Upton et al., 2003). In contrast, the terminal regions are variable in genetic content. Genes in these near-terminal regions of the genome are frequently not essential for growth in cultured cells but often encode fac-

tors with important roles in viral–host interactions including modulating host responses to infection and determining host range.

The established species in the genus *Parapoxvirus* are *Bovine papular stomatitis virus* (BPSV) and *Pseudocowpoxvirus* (PCPV) which are maintained in cattle, *Parapoxvirus of red deer in New Zealand* (PVNZ) and the type species, *Orf virus* (ORFV), which is maintained in sheep and goats (Mercer and Haig, 1999). BPSV, PCPV and ORFV have all been shown to infect humans. Infection of animals or humans by ORFV occurs via broken or scarified skin giving rise to pustular lesions. Viral replication is confined to the epidermis. In immune competent individuals, lesions resolve after a few weeks but severe progressive lesions can occur in immunosuppressed individuals. Despite an apparently normal host immune response to ORFV infection, the virus can repeatedly infect previously exposed animals, albeit with reductions in both the size of the lesions and the time to resolution (Haig and McInnes, 2002; Haig and Mercer, 1998).

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The genomes of parapoxviruses are among the smallest of the chordopoxviruses and despite sharing the genome architecture typical of all chordopoxviruses, differences in G + C content, virion morphology and the presence of a substantial number of genes unique to parapoxviruses indicate a significant divergence from other poxvirus genera (Delhon et al., 2004; Mercer and Haig, 1999; Tikkanen et al., 2004). We present here the genome sequence of the NZ2 strain of ORFV, the most extensively studied strain of parapoxvirus.

2. Materials and methods

2.1. Viruses

ORFV strain NZ2 was isolated in New Zealand from sheep scab material and plaque purified twice in primary bovine testis cells (Robinson et al., 1982). Sheep were then inoculated with the virus and the scabs that formed used as the source of viral

DNA (Mercer et al., 1987). Other poxviruses used in sequence comparisons are listed in Table 1. These include ORFV strain SA00 isolated in Texas, USA, from a goat kid and propagated in Madin–Darby ovine kidney cells (Guo et al., 2003) and ORFV strain IA82 isolated in Iowa, USA, from a lamb and propagated in ovine fetal turbinate cells (Delhon et al., 2004). (Merchinsky, 1990) (Fig. 1

2.2. Viral DNA isolation, cloning, sequencing and analysis

NZ2 genomic DNA was fragmented by digestion with *Bam*HI, *Eco*RI, *Hind*III or *Kpn*I or partial digestion with *Sau*3A and cloned into appropriate vectors (Mercer et al., 1987, 1995, 1997). Sequencing was conducted by the dideoxy chain termination method using the primary clones or subclones generated by sonication or random transposon insertion. Gaps were closed by primer walking and by sequencing of PCR products. Reaction products were analyzed on ABI 373, 377 or 3700 automated DNA sequencers. The Lasergene (DNASTar Inc.) suite of

Table 1
Poxvirus complete genomic sequences used in this study

Genus	Species (abbreviation)	Isolate	NCBI Accession no.
<i>Parapoxvirus</i>	<i>Orf virus</i> (ORFV)	NZ2	DQ184476
		SA00	AY386264
		IA82	AY386263
	<i>Bovine papular stomatitis virus</i> (BPSV)	AR02	AY386265
<i>Orthopoxvirus</i>	<i>Vaccinia virus</i> (VACV)	Copenhagen	M35027
		WR	AY243312
		Tian Tan	AF095689
		Modified vaccinia Ankara	U94848
	<i>Variola virus</i> (VARV)	India-1967	X69198
		Bangladesh-1975	L22579
		Garcia-1966	Y16780
<i>Camelpox virus</i> (CMLV)	M-96	AF438165	
	CMS	AY009089	
<i>Cowpox virus</i> (CPXV)		Brighton Red	AF482758
		GRI-90	X94355
	<i>Ectromelia virus</i> (ECTV)	Moscow	AF012825
	<i>Monkeypox virus</i> (MPXV)	Zaire-96-I-16	AF380138
<i>Leporipoxvirus</i>	<i>Myxoma virus</i> (MYXV)	Lausanne	AF170726
	<i>Rabbit fibroma virus</i> (SFV)	Kasza	AF170722
<i>Capripoxvirus</i>	<i>Sheeppox virus</i> (SPPV)	TU-V02127	AY077832
		Niskhi	AY077834
		A	AY077833
	<i>Goatpox virus</i> (GTPV)	Pellor	AY077835
		G20-LKV	AY077836
	<i>Lumpy skin disease virus</i> (LSDV)	Neethling strain 2490	AF325528
		Neethling vaccine strain	AF409138
		Neethling Warmbaths	AF409137
<i>Suipoxvirus</i>	<i>Swinepox virus</i> (SWPV)	17077-99	AF410153
<i>Yatapoxvirus</i>	<i>Yaba monkey tumor virus</i> (YMTV)	Roswell Park-Yohn	AY386371
	<i>Yaba-like disease virus</i> (YLDV)		AJ293568
<i>Molluscipoxvirus</i>	<i>Molluscum contagiosum virus</i> (MOCV)	Subtype 1	U60315
<i>Avipoxvirus</i>	<i>Fowlpox virus</i> (FWPV)	Challenge virus	AF198100

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