Genomes of the Parapoxviruses Orf Virus and Bovine Papular Stomatitis Virus

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Bovine papular stomatitis virus (BPSV) and *orf virus* (ORFV), members of the genus *Parapoxvirus* of the *Poxviridae*, are etiologic agents of worldwide diseases affecting cattle and small ruminants, respectively. Here we report the genomic sequences and comparative analysis of BPSV strain BV-AR02 and ORFV strains OV-SA00, isolated from a goat, and OV-IA82, isolated from a sheep. Parapoxvirus (PPV) BV-AR02, OV-SA00, and OV-IA82 genomes range in size from 134 to 139 kbp, with an average nucleotide composition of 64% G+C. BPSV and ORFV genomes contain 131 and 130 putative genes, respectively, and share colinearity over 127 genes, 88 of which are conserved in all characterized chordopoxviruses. BPSV and ORFV contain 15 and 16 open reading frames (ORFs), respectively, which lack similarity to other poxvirus or cellular proteins. All genes with putative roles in pathogenesis, including a vascular endothelial growth factor (VEGF)-like gene, are present in both viruses; however, BPSV contains two extra ankyrin repeat genes absent in ORFV. Interspecies sequence variability is observed in all functional classes of genes but is highest in putative virulence/host range genes, including genes unique to PPV. At the amino acid level, OV-SA00 is 94% identical to OV-IA82 and 71% identical to BV-AR02. Notably, ORFV 006/132, 103, 109, 110, and 116 genes (VEGF, homologues of vaccinia virus A26L, A33R, and A34R, and a novel PPV ORF) show an unusual degree of intraspecies variability. These genomic differences are consistent with the classification of BPSV and ORFV as two PPV species. Compared to other mammalian chordopoxviruses, PPV shares unique genomic features with molluscum contagiosum virus, including a G+C-rich nucleotide composition, three orthologous genes, and a paucity of nucleotide metabolism genes. Together, these data provide a comparative view of PPV genomics.

Parapoxviruses (PPVs) represent one of the eight genera within the chordopoxvirus (ChPV) subfamily of the *Poxviridae* and include orf virus (ORFV), bovine papular stomatitis virus (BPSV), pseudocowpoxvirus (PCPV), PPV of red deer in New Zealand, and PPV of the grey seal (6, 51, 57, 65). Tentative members of the genus cause disease in camels and red squirrels (14, 68). Features that distinguish PPVs from other poxvirus genera are the ovoid virion shape, the crisscross pattern on the particle surface, and the relatively small size and high G+C content of the genome (55, 86; this report).

PPVs cause nonsystemic, eruptive skin disease in domestic and wild mammals. ORFV, the prototype species of PPV, is responsible for contagious ecthyma, an acute disease of sheep and goats. The disease, also known as orf, contagious pustular dermatitis, or scabby mouth, is characterized by proliferative lesions in the skin of the lips, around the nostrils, and in the oral mucosa (27). Lesions progress through a typical pattern of erythema, papula, pustule, and scab and usually resolve in 1 to 2 months (45). Although considered a mild disease, mortality rates up to 93% have been reported in kids (41). High mortality rates occur when lesions in lips and udders prevent in-

fected animals from suckling and grazing, resulting in rapid emaciation (13, 41, 58). Sheep can be repeatedly infected with ORFV, albeit with shorter times to recovery and less pronounced pathological changes than in a primary infection (45). A Th1-type immune response has been implicated in host immunity to ORFV infection (reviewed in reference 32). Attenuated orf vaccines can limit the severity of the infection but they are unable to completely prevent the disease (30).

BPSV infects cattle of all ages but clinical signs are usually seen in calves. The disease has a worldwide distribution and is characterized by papules, often mildly erosive, on the muzzle, oral mucosa, and udder and occasionally in the esophagus and forestomach (40). Like ORFV in sheep and goats, reinfection of cattle with BPSV is commonly observed, suggesting that virus infection does not confer significant immunity. Because of its clinical resemblance to foot-and-mouth disease, BPSV infections are reported to veterinary authorities for differential diagnosis.

Cocirculation of BPSV and ORFV in wild ruminants has been described (35), and PPV isolates from wild ruminants have been experimentally transmitted to sheep, goats, and cattle (59, 60). Both ORFV and BPSV cause occupational infections in humans with lesions characterized by large, painful nodules in the hands and, less frequently, the face (8, 47, 69).

Classification of PPVs has relied on natural host range,

pathology, and viral DNA restriction enzyme analysis. The latter revealed considerable genomic heterogeneity, even between isolates of the same virus (26, 35, 63, 64). Hybridization analysis of viral DNA indicates that internal but not terminal genomic regions are conserved between PPVs (26). Data concerning PPV genomics, largely obtained by using ORFV strain NZ2, has revealed (i) colinearity between the ORFV and other poxvirus genera genomes (21, 49, 50), (ii) the presence of a set of genes mostly located at the termini of the viral genome with putative or known roles in virulence or immunomodulation (15, 23, 38, 42, 76), and (iii) the occurrence of genomic rearrangements of the termini upon serial virus passage in vitro (12, 22). Less is known about the gene complement and genomic organization of other PPV. DNA sequencing of the right end of the BPSV strain B177 genome indicated an organization similar to that of the right end of the ORFV genome, except for the lack of a vascular endothelial growth factor (VEGF) gene in BPSV (67). Here we present the complete DNA sequences of two ORFV isolates and one BPSV isolate, thus providing an overview of PPV genomic organization and gene content as well as a comparison between the two viruses.

MATERIALS AND METHODS

Virus strains. ORFV strain SA00 (OV-SA00) was isolated in Texas from scab material collected from a kid with severe multifocal, proliferative dermatitis and propagated in Madin-Darby ovine kidney cells (29). ORFV strain IA82 (OV-IA82) is a field isolate obtained from nasal secretions of a lamb at the Iowa Ram Test Station during an orf outbreak in 1982 and was passaged in ovine fetal turbinate cells. BPSV strain AR02 (BV-AR02) was isolated from a 3-week-old calf with oral lesions in Arkansas and passaged in primary lamb kidney cells.

Viral DNA isolation, cloning, sequencing, and sequence analysis. Viral genomic DNA was extracted from infected primary lamb kidney cell cultures as previously described (82). Random DNA fragments were obtained by incomplete enzymatic double digestion with *Aci*I and *Taq*I endonucleases (New England Biolabs, Beverly, Mass.), and DNA fragments larger than 1.0 kbp were cloned and used in dideoxy sequencing reactions as previously described (2). Reaction products were analyzed on an ABI Prism 3700 automated DNA sequencer (Applied Biosystems, Foster City, Calif.). Sequence data were assembled with the Phrap and CAP3 software programs (18, 19, 33), and gaps were closed as described previously (1). The final DNA consensus sequences for each genome represented on average seven- to ninefold redundancy at each base position and a Consed estimated error rate of 0.01 per 10 kbp (18, 19, 28).

Genome DNA composition, structure, repeats, and restriction enzyme patterns were analyzed as previously described (1) with the Genetics Computer Group GCG version 10 software package (16). Pairwise genomic alignments were done with WABA (http://www.cse.ucsc.edu/~kent/), and multiple genomic alignments were done with Dialign (54) and Clustal (77) alignment programs. Open reading frames (ORFs) longer than 30 codons were evaluated for coding potential and ORFs greater than 60 codons were subjected to homology searches as previously described (1, 2). In addition, Framefinder (http://www.hgmp.mrc.ac.uk/~gslater) was used to evaluate coding potential. Based on these criteria, 131 (BPSV) and 130 (ORFV) putative genes were annotated and orthologous ORFs were similarly numbered. Phylogenetic comparisons were done with the PHYLO_WIN software package (25) and Puzzle (75).

Nucleotide sequence accession number. The genome sequences of ORFV strains IA82 and SA00 and BPSV strain AR02 have been deposited in GenBank under accession no. AY386263, AY386264, and AY386265, respectively.

RESULTS AND DISCUSSION

BPSV and ORFV genomes. Genome sequences of BV-AR02, OV-SA00 and OV-IA82 were assembled in contiguous sequences of 134431, 139962, and 137241 bp, respectively. This agrees with previous restriction enzyme-based size estimates for both viruses (26, 48, 63). Variable genome sizes are common between PPV isolates, especially in BPSV, where differ-

ences up to 17 kb have been reported (26, 64). Hairpin loop sequences at the end of the genomes were not sequenced, and the left-most nucleotide of each assembled genome was arbitrarily designated base 1. Nucleotide composition averaged 64% G+C for each of the three isolates analyzed here. This content is not uniformly distributed along the entire genome, with a G+C content lower than 50% being found in both coding (e.g., ORFs 127 and 006/132) and intergenic regions.

Like other poxviruses, BPSV and ORFV genomes contain a large central coding region bounded by two identical inverted terminal repeat (ITR) regions (12, 26, 48). Assembled ITRs of BV-AR02, OV-SA00, and OV-IA82 contain 1,161, 3,936, and 3,092 bp, respectively. The differences in length between the ITRs of OV-SA00 and OV-IA82 strains are in agreement with previous work, indicating natural intrastrain variations in this genomic region (64). Only one ORF (001), previously described for ORFV strains NZ2 and NZ7 (20, 24) and in BPSV strain B177 (67), initiates and is completely located within the ITRs in the three virus isolates. This ORF of unknown function is unique to BPSV and ORFV, sharing 63% amino acid identity. Putative transcription control elements similar to those described for the ORFV strain NZ2 homologue are found flanking BPSV 001, suggesting early gene expression, as is the case for ORFV NZ2 (20). A second ORFV gene of unknown function (002), not present in BPSV, initiates within the unique region and terminates within the ITR.

Despite the high G+C content and paucity of stop codons, which yield 362 and 345 methionine-initiated ORFs of at least 60 codons in BPSV and ORFV genomes, respectively, coding potential analysis and similarity to known proteins led us to conservatively predict 131 genes in BPSV and 130 genes in ORFV. These genes, which encode proteins of 53 to 1289 amino acids, represent an approximate coding density of 90% for BPSV and 95% for ORFV (Table 1). The central genomic core region (ORFs 009 to 111) contains homologues of conserved poxvirus genes involved in basic replicative mechanisms and structure and morphogenesis of intracellular mature and extracellular enveloped virions (EEV) (55) (Table 1). Homologues of vaccinia virus (VACV) F9L and F10L, which are located at the left end of the conserved core in most ChPVs, are located at the right end of PPV genomes (ORFs 130 and 131). Terminal genomic regions (ORFs 001 to 008 and 112 to 134) represent approximately 20% of the viral genome and contain genes likely affecting pathogenesis. These include genes potentially involved in host range (ankyrin repeat proteins; ORFs 003, 004, 008, 118, 123, 126, 128, and 129), immune evasion (ORF 127), and immune modulation (ORF 117) and genes affecting virulence (ORF 006/132). Notably, PPVs contain a dUTPase gene previously characterized in ORFV (22, 43) but lack homologues of other ChPV genes likely involved in nucleotide metabolism, making this class of genes underrepresented in PPVs.

Comparison of BPSV with ORFV. At the genomic level, BPSV and ORFV genomes share 67 to 75% nucleotide identity (versus 94% between the two ORFV strains) and contain 127 genes with the same relative order and orientation, of which 15 are unique to PPVs. These features support the inclusion of BPSV and ORFV in the same genus. BV-AR02 and OV-SA00 demonstrate average amino acid identities of 71% (versus 94% between OV-SA00 and OV-IA82), consis-

TABLE 1. ORFV and BPSV ORFs

ORF	ORFV OV-SA00 Nucleotide position	OV-SA00 Length[a]	OV-IA82 Length[a]	OV-IA82 % Id[b] vs. OV-SA00	OV-IA82 Accession no.[c]	BPSV BV-AR02 Nucleotide position	BPSV Length[a]	BPSV % Id[b] vs. OV-SA00	BPSV Accession no.	Predicted structure/function[d]	VACV ORF	VACV % Id vs. OV-SA00	MOCV ORF	MOCV % Id vs. OV-SA00
001	3611–3165	149		73	AY186732	956–516	147	63	AY186733	Unknown				
002	4125–3781	115	117	92	M30023	Not present				Unknown				
003	Not present					2587–1100	496			Ankyrin repeat protein	M1L	28		
004	Not present					4215–2659	519			Ankyrin repeat protein	M1L	27		
005	5110–4874	79	71	90	M30023	4627–4334	98	45		Unknown				
006	Present in RT					5362–4907	152	38		VEGF				
007	5700–5194	169	159	90	AF056304	5949–5461	163	72		dUTPase	F2L	57		
008	7331–5742	530	516	91	S78516	7581–6028	518	62		Ankyrin repeat protein	B4R	22	MC018L	25
009	8829–7474	452	452	96	U34774	9110–7716	465	56		Unknown	F11L	29	MC019L	34
010	10785–8857	643	453	98	U34774	11078–9150		74		Actin tail, EEV maturation	F11L	28		
011	11993–10860	378		97	U06671	12291–11158		83		EEV phospholipase	F13L	44	MC021L	45
012	12291–12025	89		87	AY231125	12569–12315	85	45		Unknown				
013	12601–12837	79		93		12910–13128	73	50		Unknown				
014	13163–12885	93		93		13493–13215		68		Modified RING finger			MC026L	46
015	14785–13169	539		96		15110–13503	536	61		Unknown			MC027L	23
016	15633–14857	259		91	AY283523	15947–15201	249	57		DNA-binding phosphoprotein	F16L	22	MC029L	24
017	15893–16207	105		93	U30337	16259–16573		71		Unknown	F17R	49	MC030R	48
018	17652–16237	472		98		18040–16598	481	81		Poly(A) polymerase catalytic subunit	E1L	42	MC031L	47
019	19837–17663	725		97	AF380126	20228–18054		73		Unknown	E2L	28	MC032L	32
020	20458–19910	183		93		20876–20292	195	54		dsRNA-binding PKR inhibitor	E3L	29		
021	21069–20491	193		97	AY299390	21482–20886	199	79		RNA polymerase subunit RPO30	E4L	51	MC034L	48
022	21156–22856	567		98		21585–23282	566	84		Unknown	E6R	48	MC037R	55
023	22880–23695	272		100		23315–24130		88		Membrane protein	E8R	58	MC038R	57
024	23758–24633	292		92	AY283522	24187–24867	227	63		Unknown				
025	27675–24640	1,012	288	99	U49979	27901–24875	1,009	86		DNA polymerase	E9L	56	MC039L	53
026	27693–27980	96		100		27931–28221	97	89		IMV redox protein, virus assembly	E10R	62	MC040R	65
027	28393–27983	137		98	AY231124	28634–28224		82		Virion core protein	E11L	38	MC041L	40
028	30509–28383	709	718	95	AY267341	30723–28624	700	66		Unknown	O1L	22	MC042L	27
029	32972–30555	806	811	95		33190–30770	807	65		Unknown			MC043L	25
030	34128–33166	321		99		34360–33395	322	78		DNA-binding protein	I1L	47	MC044L	44
031	34350–34141	70		100		34578–34372	69	72		Unknown	I2L	34	MC045L	40
032	35217–34363	285		95	AY231127	35451–34588	288	67		DNA-binding phosphoprotein	I3L	40	MC046L	43
033	35486–35253	78		94		35725–35468	86	76		IMV membrane protein	I5L	36	MC047L	40
034	36656–35490	389		99		36900–35734		80		Unknown	I6L	31	MC049L	38
035	37945–36656	430		99		38189–36900		85		Virion core protease	I7L	56	MC050R	61
036	37951–39999	683		98		38195–40246	684	78		NPH-II, RNA helicase	I8R	47		51
037	41788–39980	603		99		42032–40227	602	76		Metalloprotease, virion morphogenesis	G1L	44	MC056L	47
038	42125–42817	231		98	AY254902	42376–43074	233	74		Late transcription elongation factor	G2R	32	MC058R	37
039	42131–41802	110		92		42382–42050	111	71		Unknown	G3L	33	MC057L	34
040	43158–42748	137		98		43412–42999	138	80		Glutaredoxin 2, virion morphogenesis	G4L	39	MC059L	44
041	43161–44516	452		96	AY267343	43415–44737	441	67		RNA polymerase subunit RPO7	G5R	33	MC060R	36
042	44521–44709	63		100		44740–44928		84		Unknown	G5.5R	68	MC061R	57
043	44731–45285	185		97		44943–45518	192	70		Unknown	G6R	35	MC062R	36
044	46481–45288	398		98		46665–45493	391	66		Virion core protein	G7L	30	MC065L	36
045	46514–47311	266		100		46699–47496		93		Late transcription factor VLTF-1	G8R	65	MC067R	69
046	47322–48323	334	340	93		47511–48512		76		Myristylated protein	G9R	38	MC068R	37
047	48327–49058	244		98		48516–49247		87		Myristylated IMV envelope protein	L1R	59	MC069R	59

ORF	Position	aa	aa	%	GenBank no.	Position	aa	%	Predicted function	VACV	%	MCV	%
048	49107–49376	90		98	AY231128	49299–49565	89	62	Unknown	L2R	32	MC070R	37
049	50639–49389	417	418	98		50699–49572	376	65	Unknown	L3L	39	MC072L	43
050	50669–51445	259		97		50729–51496	256	83	DNA-binding virion core protein VP8	L4R	50	MC073R	48
051	51471–51854	128		99		51530–51916	129	75	Putative membrane protein	L5R	38	MC074R	43
052	51811–52263	151		99		51873–52325		76	Membrane protein, morphogenesis	J1R	32	MC075R	38
053	52336–53343	336		98	AY254905	52414–53424	337	82	Poly(A) polymerase small subunit VP39	J3R	55	MC076R	59
054	53261–53818	186		98		53342–53899		84	RNA polymerase subunit RPO22	J4R	53	MC077R	54
055	54275–53775	167		99		54356–53856		83	Late membrane protein	J5L	55	MC078L	52
056	54358–58224	1,289		99		54446–58312		90	RNA polymerase subunit RPO147	J6R	67	MC079R	67
057	58816–58274	181		97		58851–58315	179	71	Protein phosphatase, virus assembly	H1L	41	MC082L	41
058	58446–59408	321		98	AY040082	58863–59444	194	86	Unknown	H2R	50	MC083R	56
059	60442–59417	342	338	97		60479–59460	340	68	IMV protein VP55, morphogenesis	H3L	31	MC084L	29
060	62857–60446	804		99	S62819	62891–60483	803	86	RNA polymerase-associated protein, RAP94	H4L	54	MC085L	55
061	62968–63648	227	228	91	AY231123	62996–63721	242	51	Late transcription factor VLTF-4	H5R	30	MC086R	30
062	63676–64629	318		99	U12401	63754–64713	320	87	DNA topoisomerase I	H6R	56	MC087R	54
063	64625–65038	138		97	U12401	64709–65125	139	64	Unknown	H7R	24	MC088R	34
064	65076–67598	841		99		65164–67689	842	85	mRNA capping enzyme, large subunit	D1R	56	MC090R	59
065	68033–67566	156		98		68130–67657	158	73	Virion protein	D2L	32	MC091L	42
066	67807–68679	291		97		68111–68785	225	62	Virion protein	D3R	35	MC092R	35
067	68882–69374	231		98	AY231122	68733–69473	247	86	Uracil DNA glycosidase	D4R	63	MC093R	63
068	69391–71751	787		98	AY267342	69490–71853	788	88	NTPase, DNA replication	D5R	57	MC094R	58
069	71761–73665	635		99		71837–73786	650	92	Early transcription factor VETFs	D6R	67	MC095R	67
070	73705–74274	190		97		73827–74354	176	82	RNA polymerase subunit RPO18	D7R	56	MC097R	58
071	74307–74978	224		99		74389–75057	223	79	NPH-PPH downregulator	D10R	35	MC099R	38
072	76887–74974	638		100		76966–75053		87	NPH-I	D11L	57	MC100R	58
073	77518–76955	188		94		77580–76999	194	63	Unknown				
074	78467–77568	300		98	AY254904	78505–77636	290	86	mRNA capping enzyme, small subunit	D12L	57	MC101L	52
075	80112–78478	545		99		80184–78550		85	Rifampin resistance, IMV assembly	D13L	57	MC102L	55
076	80585–80136	150		98		80657–80208		84	Late transcription factor VLTF-2	A1L	40	MC103L	49
077	81298–80627	224		100		81358–80687		91	Late transcription factor VLTF-3	A2L	71	MC104L	71
078	81546–81298	83	82	96		81608–81369	80	72	Thioredoxin-like protein	A2.5L	41	MC105L	44
079	83584–81560	675		98		83664–81616	683	74	P4b precursor	A3L	45	MC106L	44
080	84586–83603	328	324	89	AY231126	84414–83683	244	44	Virion core protein, virion assembly	A4L	24	MC107L	45
081	84625–85143	173	172	98	AY254903	84455–84967	171	84	RNA polymerase subunit RPO19	A5R	47	MC108R	49
082	86288–85155	378		97		86126–84975	384	73	Unknown	A6L	42	MC109L	42
083	88849–86327	841		99		88287–86170	706	88	Early transcription factor VETFL	A7L	56	MC110L	60
084	88512–89420	303		99	AY254900	88354–89274	307	83	Intermediate transcription factor VITF-3	A8R	45	MC111R	46
085	89673–89395	93		97	U30340	89527–89240	96	88	Late virion membrane protein	A9L	65	MC112L	53
086	92405–89691	905		98		92269–89546	908	76	P4a precursor	A10L	40	MC113L	47
087	92420–93427	336		99		92284–93318	345	87	Unknown	A11R	45	MC114R	48
088	94213–93434	260	261	91		93996–93328	223	56	Virion core protein	A12L	42	MC115L	38

ORF	OV-SA00 position	OV-SA00 length[a]	OV-IA82 length[a]	% Id[b]	Accession[c]	BPSV position	BPSV length[a]	% Id[b]	Function[d]	VACV ORF[e]	% Id[b]	MOCV ORF[e]	% Id[b]
089	94508–94233	92		97		94286–94053	78	71	Virion membrane protein	A13L	25	MC117L	33
090	94807–94535	91	90	97		94591–94322	90	76	IMV phosphorylated membrane protein	A14L	42	MC118L	46
091	94985–94827	53		100		94769–94611	84	84	IMV membrane protein, virulence factor	A14.5L	50	MC119L	40
092	95255–94989	89		98		95037–94762	92	66	Unknown	A15L	22	MC120L	32
093	96318–95245	358		99		96100–95024	359	84	Myristylated protein	A16L	41	MC121L	47
094	96935–96348	196		100		96750–96148	201	81	Phosphorylated IMV membrane protein	A17L	37	MC122L	43
095	96950–98413	488		99		96765–98231	489	88	DNA helicase, transcription elongation	A18R	47	MC123R	52
096	98660–98391	90		96		98457–98209	83	71	Unknown	A19L	41	MC124L	43
097	98996–100282	429		98	AY254901	98799–100076	426	71	DNA polymerase processivity factor	A20R	29	MC126R	36
098	98997–98674	108		97		98965–98474	164	77	Unknown	A21L	39	MC125L	42
099	100282–100719	146		100		100084–100521		95	Holiday junction resolvase	A22R	55	MC127R	54
100	100745–101884	380		98	AY283521	100548–101690	381	84	Intermediate transcription factor VITF-3	A23R	51	MC128R	57
101	101912–105394	1,161		99	U33419	101718–105200		92	RNA polymerase subunit RPO132	A24R	69	MC129R	70
102	107099–105540	520	518	71		106895–105336	519	76	A type inclusion protein	A26L	24	MC131L	25
103	108692–107145	516	522	53	P26654	108480–106924	89	57	A type inclusion protein	A26L	25	MC131L	27
104	109004–108735	90		93		108798–108532		74	Fusion protein, virus assembly	A27L	40	MC133L	35
105	109466–109047	140		99		109250–108831		89	Unknown	A28L	41	MC134L	46
106	110426–109485	314		98	AY299389	110230–109274	319	79	RNA polymerase subunit RP035	A29L	39	MC135L	49
107	110608–110429	60		95		110415–110230	62	75	Virion morphogenesis	A30L	36	MC136L	44
108	111601–110780	274	266	94		111404–110628	259	94	DNA packaging, ATPase	A32L	50	MC140L	71
109	111686–112177	164	159	56	AY231121	111489–111974	162	49	EEV glycoprotein	A33R	29	MC142R	30
110	112191–112691	167	165	49		112004–112504		50	EEV glycoprotein	A34R	25	MC143R	23
111	112723–113259	179	198	95	AY231120	112527–113078	184	62	Unknown	A35R	26	MC145R	27
112	113486–114349	288	286	82	AY231119	113173–114063	297	41	Putative chemokine binding protein	C23L	21		
113	114424–115023	200	211	81		114159–114755	199	41	Unknown				25
114	115070–116101	344	346	94		114805–115797	331	64	Unknown			MC149R	25
115	116225–116671	149	143	79		115859–116254	132	34	Unknown				
116	116743–117360	206	234	54		116254–117027	258	29	Unknown				
117	117539–118333	265		94	AF192803	117203–117994	264	40	GM-CSF/IL-2 inhibition factor	A41L	25		
118	118588–118893	102	221	94	AY231118	Not present			Unknown				
119	119303–119920	206	204	93	AY326433	118278–118625	140	56	Unknown				
120	120376–120957	194	199	81		118862–119281	269	34	Unknown				
121	121089–121994	302	300	88	AY231129	119398–120204	322	41	Unknown				
122	122050–123018	323		94	AY283520	120301–121266		56	Unknown				
123	123113–124687	525		95	AY186732	121371–122921	517	61	Ankyrin repeat protein	A51R	23		
124	124726–126321	532		96		122964–124481	506	57	Unknown	M1L	24		
125	126418–126936	173		93	AY231117	124623–125153	177	64	Unknown				
126	127051–128541	497	186	96	AY186732	125230–126747	506	58	Ankyrin repeat protein	M1L	31		
127	128622–129173	184		94	U60552	126898–127452	185	77	IL-10				
128	129357–130859	501	527	95	AY186732	127480–129030	517	57	Ankyrin repeat protein	B4R	21		
129	130924–132471	516	520	91	AY186732	129107–130651	515	59	Ankyrin repeat protein	B4R	22		
130	132555–134048	498		98	U29944	130698–132134	479	87	Protein kinase	F10L	52	MC017L	35
131	134011–134688	226	225	90	U29944	132100–132771	224	73	Putative membrane protein	F9L	33	MC016L	40
132	134777–135223	149	137	40	S67522	Present in LT	149	37	VEGF				
133	Not present					132821–133267	147	63	Unknown				
134	136352–136798	149		73	AY186732	133476–133916			Unknown				

[a] Length of ORF in codons. OV-IA82 and BPSV lengths are presented only if different from lengths of OV-SA00 homologues. RT and LT, right and left terminal genomic regions, respectively.

[b] % Id, percent amino acid identity.

[c] GenBank database accession numbers of homologous PPV sequences.

[d] Function was deduced from the degree of similarity to known genes and from Prosite signatures. PKR, protein kinase R; NPH, nucleophosphohydrolase; PPH, pyrophosphohydrolase; VLTF, vaccinia virus late transcription factor; VETF, vaccinia virus early transcription factor; VITF, vaccinia virus intermediate transcription factor.

[e] Best matching ORF from VACV strain Copenhagen genome (accession no. M35027) or from the MOCV genome (accession no. U60315).

tent with the classification of BPSV and ORFV as two PPV species. BPSV and ORFV share 44, 58, and 27 genes with 81 to 100%, 61 to 80%, and 29 to 60% amino acid identity, respectively. About half of the most similar ORFs (81 to 100% amino acid identity) are associated with transcription, transcription regulation, or RNA processing (Table 1).

BPSV and ORFV contain 15 and 16 ORFs, respectively, which share no significant homology to known proteins and are primarily located at the right end of the genome. Fourteen of these ORFs (ORFs 001, 005, 012, 013, 024, 073, 113, 115, 116, 119 to 121, 124, and 125) are present in both BPSV and ORFV, with amino acid identities ranging from 29 to 64%, two (ORFs 002 and 118) are present only in ORFV, and one (ORF 133) is unique to BPSV. Consistent with a possible host range function, homologues of six of these (ORFs 012, 024, 118, 119, 121, and 125) are transcribed at early times in cells infected with ORFV strain orf-11 (Table 1).

Of the 26 most distantly related ORFs between BPSV and ORFV (amino acid identity < 60%), 10 are unique to PPVs (ORFs 005, 012, 013, 113, 115, 116, 119 to 121, and 124), 3 are characterized ORFV NZ2 strain genes with putative (ORFs 020 and 117) or known (ORF 006/132) roles in pathogenesis, 10 show homology with VACV genes of known structure or function (ORFs 061, 080, 088, 103, 109, 110, 112, 126, 128, and 129), and 3 show homology with VACV virus ORFs of unknown function (ORFs 009, 016, and 122).

Highly variable PPV proteins include homologues of the VACV proteins H5R, A4L, A12L, A26L, A33R, A34R, M1L, and B4R. BPSV and ORFV 061, orthologues of VACV H5R gene, are only 51% identical. H5R encodes a late transcription factor (VLTF-4) which is synthesized before and after DNA synthesis, is phosphorylated by viral kinases, and is hypothesized to have multiple roles in the viral replicative cycle (5, 36). Notably, in closely related capripoxviruses, sheeppox virus and goatpox virus (genomes which share 96% nucleotide identity), VLTF-4 homologues are among the least conserved genes. It is tempting to speculate that PPV ORF 061 may play a role in host range during virus infection.

BPSV and ORFV 080 encode homologues of VACV A4L, a gene with significant variability in other poxvirus genera. A4L encodes an immunodominant late protein associated with the virion core and necessary for viral morphogenesis (84). OV-SA00 and OV-IA82 080 encode products that are 84 and 80 amino acids longer than the BPSV 080 product, respectively, due in part to the lack of four Cys-(Pro-Ala)$_3$ motifs separated by additional Pro/Ala-rich sequences in BPSV 080. Similar Pro/Ala-rich repeats are present in the molluscum contagiosum virus (MOCV) orthologue MC107L but not in A4L. Tandem repeat motifs in A4L-like proteins are thought to be involved in protein-protein interactions and antigenicity (7).

BPSV and ORFV 088, orthologues of VACV A12L virion core protein, share only 56% amino acid identity, an unusual degree of intragenus variability for A12L orthologues (e.g., >90% amino acid identity within orthopoxvirus [OPV], leporipoxvirus, and capripoxvirus). Notably, BPSV and ORFV 088 encode proteins of 223 and 260 amino acid, respectively, while non-PPV ChPV A12L orthologues are 156 to 195 amino acids. The difference in length is partially due to a positively charged 20-residue insertion immediately downstream of the predicted

VNA/GG cleavage site (position 170 in ORFV 088) and might suggest specific PPV core structure requirements (83).

PPV 102 and 103 are variable ORFs, with PPV 102 being more conserved between species than 103 (67 to 76% versus 57 to 58% amino acid identity, respectively). PPV 102 and 103 share similarity to each other (32 to 37% amino acid identity) and to homologues of OPV genes involved in formation of virus-filled A-type inclusions (ATIs) (46, 80). Both 102 and 103 are most similar to homologues of OPV P4c, an intracellular mature virion (IMV)-specific protein which helps direct IMV into ATIs. The carboxy termini of PPV 102 and OPV P4c proteins share sequences found in the VACV A27L fusion protein. PPV 103 has weak similarity to OPV ATI proteins, which constitute the crystalline matrix of OPV ATIs. Given the variable nature of these genes in different ChPV genera, PPV homologues may affect genera-specific and species-specific mechanisms of retaining or disseminating IMVs.

PPV 109 and 110 are orthologues of VACV A33R and A34R, respectively, genes encoding envelope type II glycoproteins expressed in intracellular enveloped virions and in EEV (44, 66). Mutations in these genes affect EEV formation (A33R and A34R), cell-to-cell spread of virus (A33R), and infectivity and virulence (A34R) (reviewed in reference 74). PPV 109 and 110, although distantly related to the VACV orthologues, are predicted to contain amino-terminal transmembrane regions and external Cys residues suggesting a similar protein topology and structure. Notably, OV-SA00 109 and 110 are as distantly related to OV-IA82 orthologues (56 and 49% amino acid identity) as they are to BPSV proteins (49 to 50% amino acid identity) (Table 1), with amino acids differences being largely concentrated in the predicted external domain. An explanation for this intraspecies variability is not immediately obvious. Alignment of available ORFV 109 sequences grouped OV-IA82, NZ2, and Orf-11 A33R homologues in a single cluster with 80 to 97% amino acid identity, excluding OV-SA00 A33R, which showed 51 to 55% amino acid identity relative to other ORFV sequences. A similar situation is observed when available ORF 110 sequences are compared. OV-SA00 is the only strain originally isolated from goats, whereas OV-IA82, NZ2, and ORF-11 strains were isolated from sheep. Thus, there appears to be a correlation between the species from which the virus was isolated and clustering of ORFV 109 and 110 amino acid sequences. This raises the possibility that differences in the external domain of both A33R and A34R are associated with host-specific requirements during virus infection by EEV. Differences in disease course have been shown following experimental infection with ORFV isolated from sheep or goats (87).

Variable PPV 126, 128, and 129 correspond to the Ank-1, Ank-2, and Ank-3 genes previously described for the BPSV B177 and ORFV D1701 strains (67). These ORFs are 58, 57, and 50% identical between BPSV and ORFV, respectively, and encode ankyrin repeat-containing proteins (ARPs). Cellular ARPs engage other proteins to form regulatory complexes which are involved in the control of processes such as cell cycle and cell differentiation (71). Many poxviruses encode ARPs, several of which have been linked to host range functions, apoptosis inhibition, and virulence (34, 56). Notably, BPSV contains two additional ARPs in the left terminal genomic

region (ORFs 003 and 004) which are not present in ORFV (Table 1).

PPV genes involved in pathogenesis. ORFV encode several proteins with known or putative roles in pathogenesis: 020, an orthologue of vaccinia E3L that functions in interferon (IFN) resistance; 127, a viral homologue of IL-10; 117, a secreted granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibitor (GIF); 112, a putative chemokine-binding protein; and 132, a viral homologue of VEGF. These genes are present in BPSV with predicted amino acid identities ranging from 37 to 77%.

E3L encodes a double-stranded-RNA (dsRNA)-binding protein kinase inhibitor with orthologues in all ChPV genera except *Avipoxvirus* and *Molluscipoxvirus*. The E3L gene product provides IFN resistance to VACV-infected cells and broad host range to virus infection in tissue culture and is a virulence determinant (4, 9). The ORFV NZ2 strain E3L homologue is also involved in IFN resistance (31) and is 93% and 53% identical to its OV-SA00 and BPSV counterparts, respectively. ORFV and BPSV 020 are most similar at the carboxy-terminal half of the protein, which is predicted to bind dsRNA through a conserved binding motif (10). The amino-terminal half of the protein is less conserved (45% amino acid identity) and includes two deletions of six and four amino acids in ORFV. In VACV, the amino-terminal half of E3L is dispensable for replication in cell culture and is not required for IFN resistance. However, this region is required for full virulence in a mouse intranasal inoculation model (9). It is thus possible that differences between ORFV and BPSV in the amino-terminal half of 020 are significant for host range and pathogenesis in their respective hosts.

OV-SA00 and OV-IA82 127 are orthologues (95% amino acid identity) of the previously described ORFV NZ2 and NZ7 strain IL-10 genes (23). BV-AR02 127 is divergent from ORFV homologues (77% amino acid identity) with most amino acid differences concentrated in the amino terminal third of the protein (27% amino acid identity in the first 50 amino acids). Nevertheless, a putative signal peptide is present in the amino terminus of all three IL-10 homologues presented here. The carboxy two-thirds of PPV IL-10 are highly conserved with cellular IL-10, with all ORFV interleukin 10 (IL-10) proteins sequenced to date being most similar to ovine IL-10 (23; this work). Notably, and in agreement with previous results, BV-AR02 127 shares in this conserved region six residues identical to bovine but not to ovine and ORFV IL-10, including a His residue at position 75 predicted to interact with the IL-10 receptor chain 1 (67). These features may reflect specific adaptation to the natural host.

PPVs 117 are orthologues of ORFV GIF, a protein which binds and inhibits GM-CSF and IL-2 in vitro and may function as an immunomodulatory factor in vivo (15). OV-SA00, OV-IA82, and NZ2 strain GIF homologues are very similar to each other (94% amino acid identity), containing at least six potentially structurally significant Cys residues and an amino-terminal signal peptide. While BV-AR02 GIF shares these structural features, it is only 38 to 40% identical to ORFV GIF, an unexpected divergence considering the similarity between ovine and bovine GM-CSF and IL-2 (83 and 96% amino acid identity, respectively). Notably, PPV 117 shares 22 to 25% amino acid identity with PPV 112, a gene also predicted to

encode a signal peptide and expressed at early times postinfection in ORFV strain Orf-11 (Table 1). Both PPV 117 and 112 share limited sequence similarity and/or Cys patterns with VACV C23L and myxoma virus MT-1 chemokine binding proteins and VACV A41L virulence factor. Taken together, these data suggest that PPV 117 and 112 may be members of a divergent family of poxviral chemokine- and cytokine-binding virulence factors.

ORFV 132 and BPSV 006 encode homologues of mammalian VEGFs, angiogenic factors that bind receptor tyrosine kinases to affect embryonic development and tumor neovascularization (11, 53, 62, 85). ORFV, PCPV, and BPSV encode the only known viral VEGFs (vVEGFs), all of which contain a characteristic cystine knot motif, a potential signal sequence, potential N- and O-linked glycosylation sites, a carboxy-terminal Thr/Pro-rich motif unique to vVEGFs, and an Asp residue (position 85 in BPSV VEGF) associated with specific VEGF receptor binding (38, 79; this paper). All vVEGFs are flanked by similar putative transcriptional control elements (38, 79; this paper) suggesting that, as is the case for ORFV (38), these genes are expressed at early times postinfection. ORFV VEGF is known to play a role in ORFV pathogenesis associated with vascularization and epidermal lesion proliferation (70).

OV-SA00 and OV-IA82 VEGF are 38% identical to each other and most similar to NZ7 and NZ2-like VEGFs (90 and 80% amino acid identity, respectively). Previous sequence analysis of ORFV isolates from diverse geographic origins segregated VEGFs into two divergent groups, a more conserved NZ7-like group and a more variable NZ-2 group (52). The data presented here for U.S. ORFV isolates further supports the notion that VEGF type is independent of the geographic origin (52).

BV-AR02 006 represents a novel variant of PPV VEGF, previously not found in BPSV strain B177 by DNA hybridization (67). BV-AR02 006 is located in a BPSV-specific, left terminal genomic region contrasting the right terminal location of ORFV and PCPV VEGFs. BV-AR02 VEGF is 35 to 50% identical to other vVEGFs and contains a unique charged pentapeptide located at positions 34 to 39. This suggests that, as for PCPV, BPSV VEGF is distinct from ORFV VEGF prototypes. Sequence divergence revealed here may explain the lack of hybridization when BPSV B177 strain DNA was probed with ORFV VEGF probe (67). Alternatively, terminal genomic variability observed for BPSV isolates (26) may have resulted in loss of the gene from the B177 strain. The presence of vVEGF in BPSV suggests its importance in PPV pathogenesis; however, the divergent nature of BPSV VEGF may imply functions or binding specificities distinct from other vVEGFs.

Comparison of PPV with other poxvirus genera. BPSV and ORFV contain 16 and 17 ORFs, respectively, which have no significant homology to genes from other poxvirus genera, and with the exception of VEGF, to other known proteins (Table 1). Although six of them are transcribed in ORFV strain Orf-11-infected cell cultures, their functions are not known. BPSV and ORFV contain a total of 113 and 111 genes, respectively, with homology to genes from other poxvirus genera. These include homologues of 88 of the 90 genes conserved within all other ChPVs, with 7 of the 11 most similar (≥60% amino acid identity; Table 1) involved in transcription, indicating that PPVs utilize basic ChPV replicative mechanisms (81). PPVs

are unique within the ChPV subfamily in that they lack homologues of VACV F15L, a gene of unknown function, and VACV D9R, a gene encoding a putative nucleoside triphosphate pyrophosphohydrolase containing a mutT motif similar to that in VACV D10R, a protein affecting viral transcription (55).

PPVs, although distinct, share a number of features with MOCV, the sole member of the molluscipoxvirus genus. PPV and MOCV are the only characterized poxviruses with genomes rich in G+C (64%), while others are rich in A+T. Homology searches revealed that 46 of 104 PPV proteins were most similar to MOCV orthologues, while 26 proteins were more similar to OPV orthologues (Table 1 and data not shown).

PPV 014, 015, and 029 are putative orthologues of MOCV MC026L, 027L, and 043L, respectively, based on amino acid identity and similar genomic location (72, 73). These ORFs of unknown function have no counterparts outside PPV and MOCV and are 61 to 68% identical between ORFV and BPSV. PPV 029 is transcribed at early times postinfection in Orf-11-infected cells (Table 1). PPV 014 and MC026L contain a RING-H2 motif which is present in proteins from diverse organisms. RING-H2 proteins are subunits of heteromeric ubiquitin ligases (E3s) which affect multiple cellular processes including cell cycle regulation and immune response (37).

PPVs and MOCV both lack genes present or conserved in other poxviruses. These comprise homologues of most poxviral genes likely involved in nucleotide metabolism, including homologues of OPV ribonucleotide reductase, thymidine kinase, guanylate kinase, thymidylate kinase, and a putative ribonucleotide reductase cofactor, VACV glutaredoxin O2L (3). PPVs, MOCV, and *Melanoplus sanguinipes* entomopoxvirus are the only poxviruses known to lack a thymidine kinase gene. In contrast, PPV 007, a gene not essential for growth of ORFV in vitro, is a dUTPase gene missing in MOCV (22, 43, 72, 73). Notably, PPVs and MOCV are the only ChPVs lacking homologues of VACV B1R, a Ser/Thr protein kinase similar to cellular VRK1 homologues and giving a temperature-sensitive DNA-negative phenotype (61). PPVs and MOCV lack homologues of VACV A50R DNA ligase, a gene also absent in yatapoxviruses (81). Also absent in PPV and MOCV are serine protease inhibitor and kelch-like gene families present in other ChPVs. These gene families are known to affect host responses, including inflammation, apoptosis, complement activation, and coagulation (39), and are associated with virulence (78). The lack of ChPV-like genes in both PPVs and MOCV may reflect adaptation for specific tissue tropism, which is notable considering that PPVs and MOCV appear to replicate in cycling cells (17, 45).

Features similar in both PPV and MOCV—nucleotide composition, amino acid similarity, and gene content—suggest that they are distinct from other known mammalian poxvirus genera. Phylogenetic analysis of protein sequences also supports the idea that, although divergent, PPVs and MOCV are distinct from other known mammalian ChPVs (Fig. 1).

Conclusions. PPV resembles other poxviruses in genome organization and gene content, sharing specific genomic features only with MOCV. Genome sequences of a BPSV strain and two ORFV strains described here provide a comparative view of PPV genomics and basic knowledge of viral functions
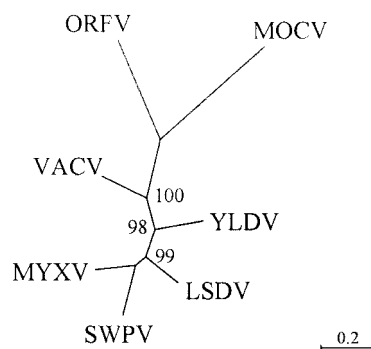

FIG. 1. Phylogenetic analysis of PPV proteins. PPV025 DNA polymerase and homologous sequences were aligned using ClustalW. Unrooted trees were generated using the neighbor-joining algorithm with Poisson correction for multiple substitutions. Bootstrap values greater than 95% after 1,000 replicates are indicated at appropriate nodes. Homologous protein sequences from the following viruses and accession numbers were compared: ORFV, AY386264; MOCV, MCU60315; VACV, M35027; yaba-like disease virus (YLDV), YDI293568; lumpy skin disease virus (LSDV), AF325528; myxoma virus (MYXV), AF170726; and swinepox virus (SWPV), AF410153. Similar results were obtained for 16 additional conserved PPV proteins, with 15 maintaining 100% bootstrap support for separation of PPV and MOCV from other mammalian ChPVs. Similar results were also obtained for these 17 proteins using the maximum likelihood algorithm with Dayhoff correction for multiple substitutions and for whole genomic nucleotide alignment utilizing amino acid translation as implemented by using Dialign (54).

associated with virus replication and manipulation of cellular responses. Significant differences occur between BPSV and ORFV genomes, and these may account for differences in host range. An improved understanding of PPV biology will permit the engineering of novel vaccine viruses and expression vectors with enhanced efficacy and greater versatility.

ADDENDUM

Since the completion of the analysis presented here, an additional ORFV genomic sequence has been deposited in the patent database (accession no. AX754989).

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