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Analysis of the Monkeypox Virus Genome

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Monkeypox virus (MPV) belongs to the *orthopoxvirus* genus of the family *Poxviridae*, is endemic in parts of Africa, and causes a human disease that resembles smallpox. The 196,858-bp MPV genome was analyzed with regard to structural features and open reading frames. Each end of the genome contains an identical but oppositely oriented 6379-bp terminal inverted repetition, which similar to that of other orthopoxviruses, includes a putative telomere resolution sequence and short tandem repeats. Computer-assisted analysis was used to identify 190 open reading frames containing ≥60 amino acid residues. Of these, four were present within the inverted terminal repetition. MPV contained the known essential orthopoxvirus genes but only a subset of the putative immunomodulatory and host range genes. Sequence comparisons confirmed the assignment of MPV as a distinct species of orthopoxvirus that is not a direct ancestor or a direct descendent of variola virus, the causative agent of smallpox.

INTRODUCTION

The family *Poxviridae* consists of complex double-stranded DNA viruses that are distinguished by their replication in the cytoplasm of vertebrate or invertebrate cells (Moss, 2001). Poxviruses belonging to the *orthopoxvirus* genus include the closely related variola (VAR), monkeypox (MPV), cowpox (CPV), and vaccinia (VAC) viruses (Fenner *et al.,* 1989; Marennikova and Shchelkunov, 1998). VAR is the etiologic agent of human smallpox, an epidemic disease with mortality rates of 10–40% that was eliminated by a strategy of case identification and prophylactic vaccination of contacts with live VAC through the coordinated efforts of the world community under the aegis of the World Health Organization (Fenner *et al.,* 1988). Following smallpox eradication in 1977, vaccination ceased, resulting in a decline in immunity to other orthopoxviruses as well as VAR. Thus, there is now increased susceptibility to zoonotic orthopoxviruses including MPV, CPV, and strains of VAC (Damaso *et al.,* 2000; Esposito and Fenner, 2001). Potentially, increased circulation of these viruses in the human population could lead to adaptive increases in their pathogenicity or transmissibility. MPV is of greatest concern because human monkeypox, a sporadic disease in the tropical rainforest regions of Central and Western Africa, is similar to smallpox in its clinical manifestations and appears to be increasing in frequency (Breman, 2000; Hutin *et al.,* 2001; Jezek and Fenner, 1988; Marennikova *et al.,* 1972; Mukinda *et al.,* 1997).

The similar clinical manifestation of monkeypox and smallpox led to a hypothesis that MPV is the evolutionary ancestor of VAR (Fenner, 1977; Marennikova *et al.,* 1972; Noble, 1970). Comparisons of VAR and MPV, based on genomic restriction endonuclease maps (Esposito and Knight, 1985; Mackett and Archard, 1979) or nucleotide sequences of individual viral genes (Douglass and Dumbell, 1992; Esposito and Knight, 1984; Hutin *et al.,* 2001; Mukinda *et al.,* 1997), were interpreted by some as indicating that MPV and VAR evolved independently (Douglass and Dumbell, 1992) and by others that VAR is ancestral to MPV (Bugert and Darai, 2000). Because a reliable answer to this question could be obtained only through comparisons of complete genomes, we sequenced the DNA of a recent human MPV isolate, strain ZAI-96-I-16 (MPV-ZAI), and concluded that MPV was not the immediate ancestor or descendent of VAR (Shchelkunov *et al.,* 2001).

We carried out a detailed analysis of the 196,858-bp MPV-ZAI DNA sequence, comprising the entire genome with the exception of part of the covalently closed terminal hairpin loops, and compared this with the corresponding complete genome sequences of VAC (Antoine *et al.,* 1998; Goebel *et al.,* 1990), VAR (Massung *et al.,* 1994; Shchelkunov *et al.,* 1993d, 1995, 2000), and the partial sequence of CPV (Shchelkunov *et al.,* 1998).

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0042-6822/02

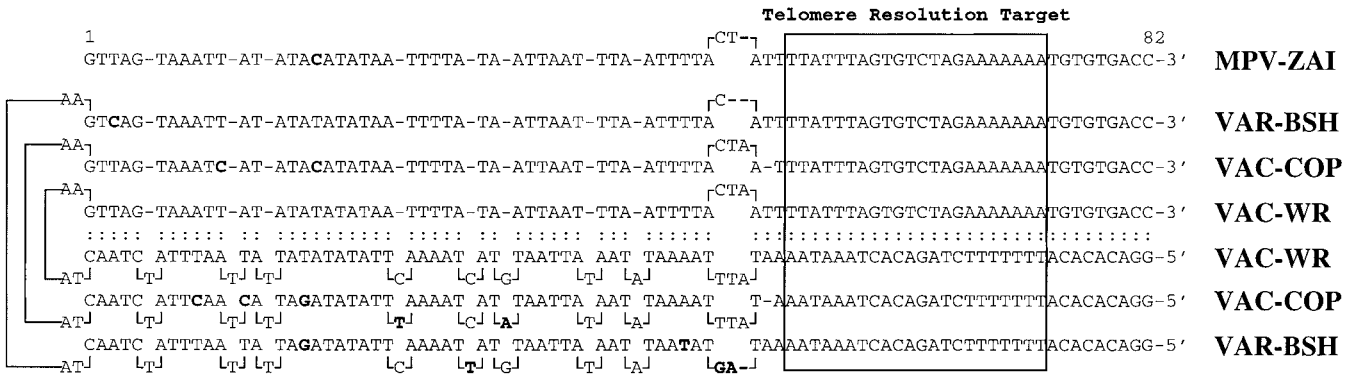

FIG. 1. Comparison of the terminal region of MPV and other orthopoxviruses. Upper line shows the sequenced part of the MPV-ZAI terminal loop. Sequences, which are necessary for telomere resolution, are boxed. Nucleotides, which differ from VAC-WR sequence, are printed in bold font. ZAI, BSH, and WR represent the virus strains Zaire-96-I-16, Bangladesh, and Western Reserve, respectively.

RESULTS AND DISCUSSION

Genome topography

The ends of orthopoxvirus genomes contain an identical but oppositely oriented sequence called a terminal inverted repetition (ITR) (Garon *et al.,* 1978; Wittek *et al.,* 1978), which includes a set of short tandem repeats (Wittek and Moss, 1980) and terminal hairpins (Baroudy *et al.,* 1982). The MPV-ZAI genome contains a 6379-bp ITR. Using S1 nuclease hydrolysis and DNA polymerase I repair, we suc-

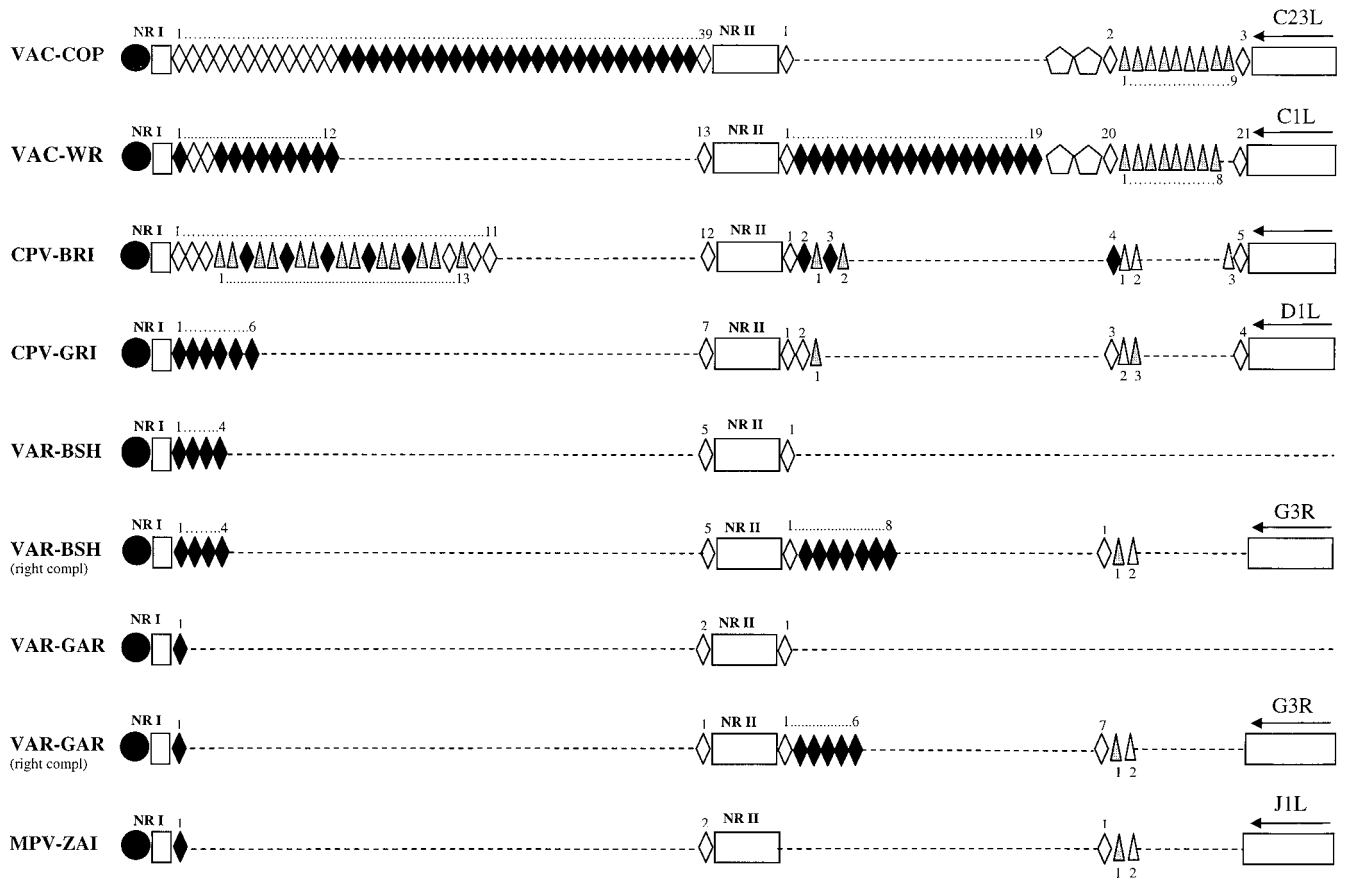

FIG. 2. Patterns of tandem repeats within the ITR region of MPV and other orthopoxviruses. White rectangles indicate unique ITR sequences: NR I and NR II and the coding region. The black diamonds correspond to 70-bp tandem repeats with their numbers indicated above. The gray triangles correspond to 54-bp repeats with their numbers indicated below. The pentagons in the ITR sequence of VAC-COP and VAC-WR indicate the 125-bp repeats. The open diamonds and triangles indicate that these repeats differ from the consensus sequence by deletions, substitutions, or inserts. The black circle denotes the terminal hairpin. Dotted lines indicate the absence of corresponding DNA.

TABLE 1

The Potential Open Reading Frames of Monkeypox Virus Strain Zaire-96-I-16

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
J1L*	1611	871	246	E–L	Secreted CC-chemokine-binding protein	— VAR C23L VAC (244 aa) D1L CPV (255 aa)	— 86.4/242 86.7/255	Graham *et al.* (1997); Smith *et al.* (1997)
J2L*	2786	1740	348	E	Secreted TNF-binding protein	— VAR C22L VAC (122 aa) D2L CPV (351 aa)	— 83.5/91 90.3/351	Upton *et al.* (1991); Shchelkunov *et al.* (1993a); Hu *et al.* (1994)
J3L*	4640	2877	587		Ankyrin-like	— VAR C21L VAC (113 aa) C20L VAC (103 aa) C19L VAC (259 aa) D3L CPV (586 aa)	— 96.9/97 69.6/115 83.2/232 96.6/586	Shchelkunov *et al.* (1993b); Safronov *et al.* (1996)
D1L*	6110	4797	437		Ankyrin-like	— VAR — VAC C1L CPV (437 aa)	— — 97.5/437	Safronov *et al.* (1996); Shchelkunov *et al.* (1998)
D2L	7121	6927	64		VAC C7L-like	D1.5L VAR (128 aa) — VAC C4L CPV (170 aa)	89.2/65 — 95.4/65	Shchellkunov *et al.* (1998)
D3R	7556	7984	142	E	Secreted EGF-like growth factor	D2R VAR (140 aa) C11R VAC (142 aa) C5R CPV (138 aa)	86.3/139 91.5/141 91.3/138	Blomquist *et al.* (1984); Buller *et al.* (1988)
D4L	9081	8830	83	E		D3L VAR (330 aa) C10L VAC (331 aa) C6L CPV (331 aa)	94.0/83 95.2/83 96.4/83	Venkatesan *et al.* (1982)
D5R	9567	10,295	242	L	Zinc binding, virulence factor, inhibits UV-induced apoptosis	D4R VAR (242 aa) — VAC C7R CPV (242 aa)	95.5/242 — 97.5/242	Upton *et al.* (1994); Senkevich *et al.* (1994); Brick *et al.* (2000)
D6L	10,903	10,523	126	E	Secreted IL-18-binding protein	D5L VAR (126 aa) — VAC C8L CPV (124 aa)	92.1/126 — 79.7/123	Born *et al.* (2000); Smith *et al.* (2000); Calderara *et al.* (2001)
D7L	12,945	10,963	660	E	Host range; ankyrin-like	D6L VAR (452 aa) — VAC C9L CPV (668 aa)	92.2/451 — 92.4/669	Spehner *et al.* (1988); Shchelkunov *et al.* (1991, 1993b)
D8L	13,273	13,079	64			— VAR — VAC C10L CPV (62 aa)	— — 93.8/64	
D9L	15,325	13,433	630	E	Ankyrin-like	D6.5L VAR (91 aa) D7L VAR (153 aa) C9L VAC (634 aa) C11L CPV (614 aa)	90.1/91 70.3/138 86.0/634 67.9/633	Shchelkunov *et al.* (1993b)
D10L	16,432	15,980	150	E	Host range	D8L VAR (150 aa) C7L VAC (150 aa) C13L CPV (150 aa)	96.7/150 96.0/150 96.0/150	Chen *et al.* (1992)
D11L	17,122	16,661	153	E		D9L VAR (156 aa) C6L VAC (151 aa) C14L CPV (156 aa)	94.1/152 92.0/150 94.7/152	Cooper *et al.* (1981)
D12L	17,886	17,266	206	E	BTB domain of kelch-like protein	D10L VAR (134 aa) C5L VAC (204 aa) C15L CPV (205 aa)	90.0/130 95.9/193 97.0/197	Shchelkunov *et al.* (1998); Cooper *et al.* (1981)
D13L	18,878	17,931	315	E		D11L VAR (316 aa) C4L VAC (316 aa) C16L CPV (315 aa)	92.1/316 94.3/316 94.3/316	Cooper *et al.* (1981)
D14L	19,710	19,060	216	E	Secreted complement-binding protein	D12L VAR (263 aa) C3L VAC (263 aa) C17L CPV (259 aa)	89.4/218 93.1/218 93.0/214	Kotwal and Moss (1988)
D15L	20,151	19,834	105		Discontinuous ORF (MPV D15L–D18L) of kelch-like protein	D13L VAR (201 aa) C2L VAC (512 aa) C18L CPV (512 aa)	93.8/80 97.1/105 98.1/105	Shchelkunov *et al.* (1998)

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
D16L	20,438	20,205	77			D13L VAR (201 aa)	86.3/73	
						C2L VAC (512 aa)	97.3/73	
						C18L CPV (512 aa)	97.3/73	
D17L	20,736	20,440	98			D13.5L VAR (79 aa)	97.6/42	
						C2L VAC (512 aa)	86.5/89	
						C18L CPV (512 aa)	87.6/89	
D18L	21,142	20,819	107			— VAR	—	
						C2L VAC (512 aa)	98.1/106	
						C18L CPV (512 aa)	99.1/106	
D19L	22,010	21,366	214	E		D14L VAR (214 aa)	92.5/214	Belle Isle *et al.* (1981)
						C1L VAC (224 aa)	94.9/214	
						C19L CPV (231 aa)	95.8/214	
P1L	22,409	22,056	117	E–L	Secreted virulence factor	P1L VAR (117 aa)	92.3/117	Kotwal and Moss (1989)
						N1L VAC (117 aa)	89.7/117	
						P1L CPV (117 aa)	94.9/117	
P2L	23,069	22,536	177	E		P2L VAR (177 aa)	90.4/177	Tamin *et al.* (1988)
						N2L VAC (175 aa)	90.3/175	
						P2L CPV (175 aa)	93.7/175	
O1L	24,438	23,110	442	E–L	Ankyrin-like	O1L VAR (446 aa)	95.9/442	Tamin *et al.* (1988);
						M1L VAC (472 aa)	97.1/443	Shchelkunov *et al.*
						O1L CPV (474 aa)	95.7/443	(1993b)
O2L	25,170	24,508	220	E		O2L VAR (220 aa)	95.5/220	Morgan and Roberts
						M2L VAC (220 aa)	97.7/220	(1984)
						O2L CPV (163 aa)	97.5/163	
C1L	26,153	25,299	284	E	Host range; ankyrin-like	O3L VAR (70 aa)	90.5/63	Gillard *et al.* (1986);
						C1L VAR (66 aa)	89.4/66	Shchelkunov *et al.*
						K1L VAC (284 aa)	95.8/284	(1993b)
						M1L CPV (284 aa)	93.7/284	
C2L	27,511	26,384	375	E	Serine protease inhibitor-like, SPI-3, prevents cell fusion	C2L VAR (373 aa)	92.2/374	Law and Smith (1992);
						K2L VAC (369 aa)	92.2/374	Turner *et al.* (2000)
						M2L CPV (373 aa)	94.4/374	
C3L	27,803	27,672	43	E	IFN resistance, homolog of eIF-2α, inhibits eIF-2α phosphorylation	C3L VAR (88 aa)	78.6/42	Beattie *et al.* (1991);
						K3L VAC (88 aa)	95.2/42	Davies *et al.* (1991)
						M3L CPV (88 aa)	97.6/42	
C4L	29,133	27,859	424		Phospholipase D-like	— VAR	—	Cao *et al.* (1997); Sung *et*
						K4L VAC (424 aa)	98.3/424	*al.* (1997)
						M4L CPV (424 aa)	97.6/424	
C5L	29,990	29,160	276		Lysophospholipase-like	— VAR	—	Antoine *et al.* (1998)
						K5L VAC (136 aa)	91.7/108	
						K6L VAC (81 aa)	94.9/78	
						M5L CPV (276 aa)	97.5/276	
C6R	30,126	30,575	149		VAC B15R-like	C4R VAR (149 aa)	97.3/149	
						K7R VAC (149 aa)	94.0/149	
						M6R CPV (161 aa)	97.3/149	
C7L	31,278	30,619	219			C5L VAR (251 aa)	83.6/213	
						F1L VAC (226 aa)	83.6/226	
						G1L CPV (238 aa)	87.2/234	
C8L	31,745	31,290	151	E	Deoxyuridine triphosphatase	C6L VAR (147 aa)	98.0/147	McGeoch (1990)
						F2L VAC (147 aa)	98.6/147	
						G2L CPV (147 aa)	99.3/147	
C9L	33,225	31,762	487	E	Kelch-like	C7L VAR (179 aa)	93.3/179	Xue and Cooley (1993);
						F3L VAC (480 aa)	96.2/479	Shchelkunov *et al.*
						G3L CPV (485 aa)	97.3/484	(1998)
C10L	34,195	33,236	319	E	Ribonucleotide reductase, small subunit, R2	C8L VAR (333 aa)	97.5/319	Slabaugh *et al.* (1988)
						F4L VAC (319 aa)	98.4/319	
						G4L CPV (319 aa)	98.4/319	
C11L	35,258	34,227	343			C9L VAR (348 aa)	85.1/348	
						F5L VAC (321 aa)	92.8/321	
						G5L CPV (323 aa)	92.9/323	

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
C12L	35,436	35,215	73			C10L VAR (72 aa) F6L VAC (74 aa) G6L CPV (74 aa)	84.9/73 94.4/72 94.4/72	
C13L	35,676	35,452	74	E		C11L VAR (79 aa) F7L VAC (92 aa) G7L CPV (80 aa)	81.6/76 79.5/88 93.4/76	Panicali and Paoletti (1982)
C14L	36,022	35,828	64			C12L VAR (65 aa) F8L VAC (65 aa) G8L CPV (65 aa)	93.8/65 96.9/65 96.9/65	
C15L	36,717	36,079	212			C13L VAR (212 aa) F9L VAC (212 aa) G9L CPV (212 aa)	97.6/212 99.1/212 99.1/212	
C16L	38,023	36,704	439	L	Serine/threonine protein kinase 2, VPK2, regulation of virion morphogenesis	C14L VAR (439 aa) F10L VAC (439 aa) G10L CPV (439 aa)	98.4/437 98.9/439 99.1/439	Lin and Broyles (1994); Traktman *et al.* (1995); Wang and Shuman (1995); Betakova *et al.* (1999)
C17L	39,110	38,046	354	E		C15L VAR (354 aa) F11L VAC (354 aa) G11L CPV (354 aa)	93.5/354 97.2/354 97.2/354	Golini and Kates (1984)
C18L	41,061	39,154	635	E–L	Actin tail formation	C16L VAR (635 aa) F12L VAC (635 aa) G12L CPV (634 aa)	95.0/636 97.2/636 98.6/635	Zhang *et al.* (2000)
C19L	42,222	41,104	372	L	Major envelope antigen of EEV, wrapping of IMV to form IEV, phospholipase D-like	C17L VAR (372 aa) F13L VAC (372 aa) G13L CPV (372 aa)	97.3/372 98.7/372 98.7/372	Hirt *et al.* (1986); Baek *et al.* (1997); Sung *et al.* (1997); Roper and Moss (1999)
C20L	42,461	42,240	73	E–L		C18L VAR (73 aa) F14L VAC (73 aa) G14L CPV (73 aa)	78.1/73 97.3/73 98.6/73	Golini and Kates (1984)
C21L	43,209	42,733	158	E		C19L VAR (161 aa) F15L VAC (158 aa) G15L CPV (158 aa)	98.0/153 98.1/158 98.7/158	Golini and Kates (1984)
C22L	43,911	43,216	231	E		C20L VAR (231 aa) F16L VAC (231 aa) G16L CPV (231 aa)	95.2/231 96.5/231 97.0/231	Golini and Kates (1984)
C23R	43,973	44,278	101	L	Virion core DNA-binding phosphoprotein	C21R VAR (101 aa) F17R VAC (101 aa) G17R CPV (101 aa)	94.1/101 97.0/101 96.0/101	Kao and Bauer (1987)
F1L	45,714	44,275	479	E	Poly(A) polymerase, catalytic subunit	E1L VAR (479 aa) E1L VAC (479 aa) F1L CPV (479 aa)	97.7/479 99.0/479 99.4/479	Gershon *et al.* (1991)
F2L	47,924	45,711	737			E2L VAR (737 aa) E2L VAC (737 aa) F21 CPV (737 aa)	97.7/737 98.0/737 97.8/737	
F3L	48,509	48,048	153	E	IFN resistance, dsRNA-binding, inhibits dsRNA-dependent protein kinase, and 2-5A-synthetase	E3L VAR (190 aa) E3L VAC (190 aa) F3L CPV (190 aa)	85.6/153 86.3/153 86.3/153	Chang *et al.* (1992); Rivas *et al.* (1998)
F4L	49,449	48,670	259	E–L	RNA polymerase, 30-kDa subunit, intermediate stage transcription factor, VITF-1	E4L VAR (259 aa) E4L VAC (259 aa) F4L CPV (259 aa)	96.5/259 98.5/259 98.1/259	Ahn *et al.* (1990a); Rosales *et al.* (1994)
F5R	50,477	52,180	567			E6R VAR (567 aa) E6R VAC (567 aa) F6R CPV (567 aa)	97.2/567 98.9/567 98.9/567	
F6R	52,262	52,762	166	L	Soluble myristylated protein	E7R VAR (76 aa) E7R VAC (166 aa) F7R CPV (166 aa)	86.7/60 94.0/166 94.6/166	Martin *et al.* (1997)

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
F7R	52,862	53,683	273			E8R VAR (273 aa) E8R VAC (273 aa) F8R CPV (273 aa)	97.1/273 98.5/273 98.5/273	
F8L	56,711	53,691	1006	E	DNA polymerase	E9L VAR (1005 aa) E9L VAC (1006 aa) F9L CPV (1006 aa)	97.7/1006 98.7/1006 98.6/1006	Earl *et al.* (1986)
F9R	56,743	57,030	95	L	Protein disulfide bond-forming enzyme	E10R VAR (95 aa) E10R VAC (95 aa)	94.7/95 96.8/95	Senkevich *et al.* (2000)
F10L	57,414	57,025	129	L	Virion core protein	E11L VAR (129 aa) E11L VAC (129 aa)	96.1/129 97.7/129	Wang and Shuman (1996)
Q1L	59,398	57,401	665	E		Q1L VAR (666 aa) O1L VAC (666 aa)	93.1/666 97.4/666	
Q2L	59,771	59,445	108	L	Virion-associated glutaredoxin	Q2L VAR (108 aa) O2L VAC (108 aa)	96.3/108 98.1/108	Ahn and Moss (1992a); Rajagopal *et al.* (1995)
I1L	60,856	59,918	312	L	Virosomal protein essential for virus multiplication	K1L VAR (312 aa) I1L VAC (312 aa)	96.8/312 99.4/312	Ryazankina *et al.* (1993); Schmitt and Stunnenberg (1988)
I2L	61,084	60,863	73	L		K2L VAR (73 aa) I2L VAC (73 aa)	98.6/73 98.6/73	Schmitt and Stunnenberg (1988)
I3L	61,894	61,085	269	E-I	ssDNA-binding P-protein interacts with R2 subunit of ribonucleotide reductase	K3L VAR (269 aa) I3L VAC (269 aa)	98.1/269 98.1/269	Davis and Mathews (1993); Rochester and Traktman (1998)
I4L	64,291	61,976	771	E	Ribonucleotide reductase, large subunit, R1	K4L VAR (771 aa) I4L VAC (771 aa)	97.7/771 98.7/771	Tengelsen *et al.* (1988)
I5L	64,559	64,320	79	L	IMV surface membrane protein	K5L VAR (79 aa) I5L VAC (79 aa)	93.7/79 94.9/79	Takahashi *et al.* (1994)
I6L	65,726	64,578	382			K6L VAR (382 aa) I6L VAC (382 aa)	97.6/382 98.2/382	
I7L	66,990	65,719	423	L	Virion core protein, DNA topoisomeras II homolog	K7L VAR (423 aa) I7L VAC (423 aa)	98.8/423 99.1/423	Kane and Shuman (1993)
I8R	66,996	69,026	676	E–L	Nucleoside triphosphate phosphohydrolase II, NPH-II, DNA, and RNA helicase	K8R VAR (676 aa) I8R VAC (676 aa)	96.7/676 98.1/676	Shuman (1992); Bayliss and Smith (1996); Gross and Shuman (1998)
G1L	70,805	69,030	591	L	Putative proteinase	H1L VAR (591 aa) G1L VAC (591 aa)	97.1/591 98.5/591	Whitehead and Hruby (1994)
G2L	71,137	70,802	111	E		H2L VAR (111 aa) G3L VAC (111 aa)	95.5/111 98.2/111	Meis and Condit (1991)
G3R	71,131	71,793	220	L		H3R VAR (220 aa) G2R VAC (220 aa)	96.8/220 98.6/220	Meis and Condit (1991)
G4L	72,137	71,763	124	L	Virion-associated glutaredoxin, required for disulfide bonds and assembly	H4L VAR (124 aa) G4L VAC (124 aa)	99.2/124 99.2/124	Gvakharia *et al.* (1996); White *et al.* (2000)
G5R	72,140	73,444	434	E–L		H5R VAR (434 aa) G5R VAC (434 aa)	96.3/434 98.2/434	Meis and Condit (1991)
G6R	73,452	73,643	63	E–L	RNA polymerase, 7-kDa subunit	H5.5R VAR (63 aa) — VAC	96.8/63 —	Amegadzie *et al.* (1992)
G7R	73,643	74,140	165			H6R VAR (165 aa) G6R VAC (165 aa)	94.5/165 93.9/165	
G8L	75,220	74,105	371		Virion protein	H7L VAR (371 aa) G7L VAC (371 aa)	98.4/371 98.7/371	Takahashi *et al.* (1994)
G9R	75,251	76,033	260	I	Late gene transcription factor, VLTF-1	H8R VAR (260 aa) G8R VAC (260 aa)	99.6/260 100/260	Keck *et al.* (1990)
G10R	76,053	77,075	340	L	Myristylated protein	H9R VAR (340 aa) G9R VAC (340 aa)	97.9/340 98.5/340	Martin *et al.* (1997)
M1R	77,076	77,828	250	L	Myristylated IMV surface membrane protein	M1R VAR (250 aa) L1R VAC (250 aa)	99.2/250 98.8/250	Ravanello and Hruby (1994)
M2R	77,860	78,138	92			M2R VAR (87 aa) L2R VAC (87 aa)	96.3/82 98.8/82	

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
M3L	79,148	78,114	344			M3L VAR (349 aa) L3L VAC (350 aa)	95.1/349 96.6/350	
M4R	79,173	79,928	251	L	Virion core protein, ssDNA binding, stimulation of I8R helicase activity	M4R VAR (251 aa) L4R VAC (251 aa)	98.4/251 98.8/251	Yang *et al.* (1988); Bayliss *et al.* (1996)
M5R	79,938	80,324	128			M5R VAR (128 aa) L5R VAC (128 aa)	99.2/128 99.2/128	
L1R	80,281	80,739	152			L1R VAR (159 aa) J1R VAC (153 aa)	97.4/152 96.7/152	
L2R	80,759	81,292	177	E	Thymidine kinase	L2R VAR (177 aa) J2R VAC (177 aa)	95.5/177 96.6/177	Weir and Moss (1983)
L3R	81,358	82,359	333	E	Poly(A) pol stimulatory subunit, cap-specific mRNA (nucleoside-$O^{2'}$-)-methyltransferase	L3R VAR (333 aa) J3R VAC (333 aa)	97.9/333 98.8/333	Gershon *et al.* (1991); Schnierle *et al.* (1992)
L4R	82,274	82,831	185	E	RNA pol 22-kDa subunit	L4R VAR (185 aa) J4R VAC (185 aa)	97.8/185 100/185	Broyles and Moss (1986)
L5L	83,292	82,891	133	L	Essential for virus multiplication	L5L VAR (133 aa) J5L VAC (133 aa)	100/133 98.5/133	Zajac *et al.* (1995)
L6R	83,399	87,259	1286	E	RNA pol 147-kDa subunit	L6R VAR (1286 aa) J6R VAC (1286 aa)	98.6/1286 98.6/1286	Broyles and Moss (1986)
H1L	87,771	87,256	171	L	Tyrosine/serine protein phosphatase, blocks IFN-γ signal transduction	I1L VAR (171 aa) H1L VAC (171 aa)	98.8/171 98.8/171	Guan *et al.* (1991); Najarro *et al.* (2001)
H2R	87,785	88,354	189			I2R VAR (189 aa) H2R VAC (189 aa)	100/189 98.9/189	
H3L	89,332	88,358	324	L	IMV heparan-binding surface membrane protein	I3L VAR (325 aa) H3L VAC (324 aa)	93.2/3239 3.8/324	Chertov *et al.* (1991); Lin *et al.* (2000); da Fonseca *et al.* (2000)
H4L	91,720	89,333	795	L	RNA pol-associated protein, RAP 94, provides specificity for early promoters	I4L VAR (795 aa) H4L VAC (795 aa)	97.0/795 98.1/795	Ahn and Moss (1992b); Kane and Shuman (1992); Zhang *et al.* (1994)
H5R	91,905	92,546	213	E–L	Virosome-associated, late gene transcription factor, VLTF-4, Ca^{2+}-binding motif	I5R VAR (221 aa) H5R VAC (203 aa)	92.8/222 93.9/213	Kovacs and Moss (1996); Shchelkunov *et al.* (1993c)
H6R	92,547	93,491	314	E	DNA topoisomerase	I6R VAR (314 aa) H6R VAC (314 aa)	98.7/314 99.7/314	Shuman and Moss (1987)
H7R	93,529	93,969	146	L		I7R VAR (146 aa) H7R VAC (146 aa)	95.2/146 97.3/146	Rosel *et al.* (1986)
E1R	94,013	96,550	845	E	mRNA capping enzyme large subunit; RNA 5′ triphosphatase and RNA guanylyl transferase activities	F1R VAR (844 aa) D1R VAC (844 aa)	98.3/845 98.6/845	Morgan *et al.* (1984); Shuman and Morham (1990)
E2L	96,949	96,509	146	E–L	Virion core protein	F2L VAR (146 aa) D2L VAC (146 aa)	97.9/146 98.6/146	Dyster and Niles (1991)
E3R	96,942	97,643	233	L	Virion core protein	F3R VAR (237 aa) D3R VAC (237 aa)	94.9/237 96.6/237	Dyster and Niles (1991)
E4R	97,643	98,299	218	E	Uracil DNA glycosylase required for DNA replication	F4R VAR (218 aa) D4R VAC (218 aa)	96.8/218 98.6/218	Upton *et al.* (1993); Stuart *et al.* (1993)
E5R	98,331	100,688	785	E–L	Nucleic acid independent nucleoside triphosphatase, required for DNA replication	F5R VAR (785 aa) D5R VAC (785 aa)	98.5/785 99.5/785	Evans *et al.* (1995)
E6R	100,728	102,641	637	L	Early transcription factor, VETF, small subunit	F6R VAR (637 aa) D6R VAC (637 aa)	99.2/637 99.7/637	Gershon and Moss (1990); Broyles and Fesler (1990)
E7R	102,668	103,153	161	E	RNA pol 18-kDa subunit	F7R VAR (161 aa) D7R VAC (161 aa)	96.9/161 96.9/161	Ahn *et al.* (1990b)

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
E8L	104,030	103,116	304	L	IMV surface membrane 32 kDa protein, binds cell surface chondroitin sulfate, IMV adsorption to cell surface	F8L VAR (304 aa) D8L VAC (304 aa)	92.8/304 94.7/304	Niles and Seto (1988); Maa *et al.* (1990); Hsiao *et al.* (1999)
E9R	104,072	104,713	213	E	Mut-like	F9R VAR (213 aa) D9R VAC (213 aa)	97.2/213 97.2/213	Koonin (1993); Lee-Chen *et al.* (1988)
E10R	104,710	105,456	248	L	Down regulation of gene expression, Mut-like	F10R VAR (248 aa) D10R VAC (248 aa)	98.0/248 98.0/248	Shors *et al.* (1999); Koonin (1993); Lee-Chen *et al.* (1988)
E11L	107,352	105,457	631	L	Nucleoside triphosphate phosphohydrolase I, NPH I, DNA-dependent ATPase, early gene transcription termination	N1L VAR (631 aa) D11L VAC (631 aa)	98.1/631 98.6/631	Rodriguez *et al.* (1986); Broyles and Moss (1987); Christen *et al.* (1998)
E12L	108,250	107,387	287	E–L	mRNA capping enzyme small subunit, mRNA (guanine-N^7-)-methyl transferase	N2L VAR (287 aa) D12L VAC (287 aa)	98.6/287 99.0/287	Niles *et al.* (1989); Shuman and Morham (1990)
E13L	109,936	108,281	551	L	Needed for immature IMV surface membrane	N3L VAR (551 aa) D13L VAC (551 aa)	98.9/551 98.9/551	Zhang and Moss (1992)
A1L	110,413	109,961	150	I	Late gene transcription factor, VLTF-2	A1L VAR (150 aa) A1L VAC (150 aa)	100/150 98.7/150	Keck *et al.* (1990, 1993b)
A2L	111,108	110,434	224	I	Late gene transcription factor, VLTF-3, zinc binding	A2L VAR (224 aa) A2L VAC (224 aa)	99.1/224 99.1/224	Keck *et al.* (1990, 1993a)
A3L	111,338	111,105	77			A2.5L VAR (76 aa) — VAC	88.3/77 —	
A4L	113,287	111,353	644	L	Major virion core protein p4b	A3L VAR (644 aa) A3L VAC (644 aa)	98.6/644 99.1/644	Rosel and Moss (1985)
A5L	114,185	113,340	281	L	39-kDa immunodominant virion core protein	A4L VAR (271 aa) A4L VAC (281 aa)	87.6/282 95.0/281	Maa and Esteban (1987); Williams *et al.* (1999)
A6R	114,223	114,708	161	E–L	RNA pol 22 and 21 kDa subunits	A5R VAR (164 aa) A5R VAC (164 aa)	97.0/164 97.6/164	Ahn *et al.* (1992)
A7L	115,823	114,705	372	L		A6L VAR (372 aa) A6L VAC (372 aa)	98.4/372 98.7/372	Weinrich and Hruby (1986)
A8L	117,979	115,847	710	L	Early transcription factor, VETF, large subunit, needed for morphogenesis	A7L VAR (710 aa) A7L VAC (710 aa)	97.2/710 98.3/710	Gershon and Moss (1990); Hu *et al.* (1998)
A9R	118,033	118,911	292	E	Intermediate transcription factor, VITF-3, 34-kDa subunit	A8R VAR (288 aa) A8R VAC (288 aa)	98.3/288 98.6/288	Sanz and Moss (1999)
A10L	119,194	118,892	100			A9L VAR (95 aa) A9L VAC (99 aa)	94.8/97 97.0/100	
A11L	121,870	119,195	891	L	Major virion core protein p4a	A10L VAR (892 aa) A10L VAC (891 aa)	97.5/892 97.1/891	Van Meir and Wittek (1988)
A12R	121,885	122,841	318			A11R VAR (319 aa) A11R VAC (318 aa)	98.4/319 99.4/318	
A13L	123,415	122,843	190	L	Virion core protein	A12L VAR (189 aa) A12L VAC (192 aa)	98.4/190 96.9/192	Whitehead and Hruby (1994)
A14L	123,651	123,439	70	L	IMV inner and outer membrane protein	A13L VAR (68 aa) A13L VAC (70 aa)	88.4/69 92.8/69	Takahashi *et al.* (1994); Salmons *et al.* (1997)
A15L	124,029	123,757	90	L	IMV inner membrane protein	A14L VAR (90 aa) A14L VAC (90 aa)	97.8/90 100/90	Takahashi *et al.* (1994); Salmons *et al.* (1997)
A16L	124,481	124,197	94			A15L VAR (94 aa) A15L VAC (94 aa)	96.8/94 98.9/94	
A17L	125,598	124,465	377	L	Soluble myristylated protein	A16L VAR (377 aa) A16L VAC (378 aa)	95.8/377 96.3/378	Martin *et al.* (1997)
A18L	126,215	125,601	204	L	IMV surface membrane protein, early function in virion morphogenesis	A17L VAR (203 aa) A17L VAC (203 aa)	97.1/204 97.1/204	Ichihashi *et al.* (1994); Rodriguez *et al.* (1995); Wolffe *et al.* (1996)

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
A19R	126,230	127,708	492	E–L	DNA helicase, postreplicative negative transcription elongation factor	A18R VAR (493 aa) A18R VAC (493 aa)	95.3/493 96.1/493	Simpson and Condit (1995); Xiang *et al.* (1998)
A20L	127,922	127,689	77			A19L VAR (76 aa) A19L VAC (77 aa)	93.5/77 96.1/77	
A21L	128,270	127,923	115			A20L VAR (117 aa) A21L VAC (117 aa)	95.7/117 97.4/117	
A22R	128,269	129,549	426	E	Processivity factor for viral DNA pol	A21R VAR (426 aa) A20R VAC (426 aa)	96.7/426 97.4/426	Ishii and Moss (2001); Klemperer *et al.* (2001)
A23R	129,479	130,042	187			A22R VAR (187 aa) A22R VAC (176 aa)	95.2/187 98.8/173	
A24R	130,062	131,210	382	E	Intermediate transcription factor, VITF-3, 45-kDa subunit	A23R VAR (382 aa) A23R VAC (382 aa)	97.9/382 98.7/382	Sanz and Moss (1999)
A25R	131,207	134,701	1164	E–L	RNA pol 132-kDa subunit	A24R VAR (1164 aa) A24R VAC (1164 aa)	98.5/1163 98.8/1164	Hooda-Dhingra *et al.* (1990); Amegadzie *et al.* (1991b)
A26L	135,700	135,473	75			— VAR — VAC	— —	
A27L	137,905	135,815	696	L	N-terminal of A-type inclusion body protein of CPV	A28L VAR (702 aa) — VAC	93.9/691 —	Funahashi *et al.* (1988); Shchelkunov *et al.* (1994)
A28L	139,513	137,951	520	L	Major component of IMV surface tubules, p4c	A29L VAR (498 aa) A26L VAC (322 aa) A27L CPV (518 aa)	89.8/520 92.9/212 95.8/520	Sarov and Joklik (1972)
A29L	139,896	139,564	110	L	IMV surface membrane 14-kDa fusion protein, binds cell surface heparan	A30L VAR (110 aa) A27L VAC (110 aa) A28L CPV (110 aa)	93.6/110 93.6/110 95.5/110	Rodriguez and Esteban (1987); Chung *et al.* (1998)
A30L	140,337	139,897	146			A31L VAR (146 aa) A28L VAC (146 aa) A29L CPV (146 aa)	96.6/146 95.9/146 97.3/146	
A31L	141,255	140,338	305	E	RNA pol 35-kDa subunit	A32L VAR (305 aa) A29L VAC (305 aa) A30L CPV (305 aa)	95.7/305 98.0/305 98.0/305	Amegadzie *et al.* (1991a)
A32L	141,451	141,218	77			A33L VAR (77 aa) A30L VAC (77 aa) A31L CPV (77 aa)	96.1/77 96.1/77 96.1/77	
A33R	141,611	142,039	142			A34R VAR (140 aa) A31R VAC (124 aa) A32R CPV (145 aa)	95.8/142 86.6/142 96.6/145	
A34L	142,908	142,006	300	E–L	DNA packaging into virion, NTP-binding motif A	A35L VAR (270 aa) A32L VAC (300 aa) A33L CPV (300 aa)	97.8/270 98.0/300 99.0/300	Koonin (1993); Cassetti *et al.* (1998)
A35R	142,936	143,481	181	L	EEV envelope glycoprotein, needed for formation of actin-containing microvilli and cell-to-cell spread	A36R VAR (184 aa) A33R VAC (185 aa) A34R CPV (185 aa)	92.8/180 96.1/180 96.1/180	Roper *et al.* (1996, 1998)
A36R	143,486	143,992	168	L	EEV envelope glycoprotein, lectin-like, required for infectivity of EEV, formation of actin-containing microvilli, and cell-to-cell spread	A37R VAR (168 aa) A34R VAC (168 aa) A35R CPV (168 aa)	95.8/168 95.2/168 98.2/168	Blasco *et al.* (1993); McIntosh and Smith (1996); Wolffe *et al.* (1997)
A37R	144,036	144,566	176			A38R VAR (60 aa) A35R VAC (176 aa) A36R CPV (176 aa)	91.7/60 97.7/176 98.3/176	
A38R	144,611	145,249	212	E–L	IEV but not CEV envelope protein, tyrosine phosphorylated for actin tail formation	A39R VAR (216 aa) A36R VAC (221 aa) A37R CPV (223 aa)	91.7/204 94.6/204 95.1/204	Parkinson and Smith (1994); Wolffe *et al.* (1998); Frischknecht *et al.* (1999); van Eijl *et al.* (2000)

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
A39R	145,363	146,169	268			A40R VAR (68 aa)	89.6/67	
						A37R VAC (263 aa)	96.2/262	
						A38R CPV (268 aa)	95.9/268	
A40L	147,254	146,421	277		Integral membrane glycoprotein, Ig-like, regulates influx of extracellular Ca^{2+}	A41L VAR (277 aa)	95.7/277	Parkinson *et al.* (1995); Sanderson *et al.* (1996)
						A38L VAC (277 aa)	96.0/277	
						A40L CPV (277 aa)	99.3/277	
A41L	148,646	147,981	221	E–L	Secreted protein reduces influx of inflammatory cells	A46L VAR (218 aa)	91.4/222	Ng *et al.* (2001)
						A41L VAC (219 aa)	94.1/222	
						A43L CPV (219 aa)	94.1/222	
A42R	148,842	149,243	133	L	Profilin-like	A47R VAR (133 aa)	99.2/133	Goebel *et al.* (1990); Blasco *et al.* (1991)
						A42R VAC (133 aa)	97.7/133	
						A44L CPV (133 aa)	100/133	
A43R	149,281	149,874	197	E–L	Membrane glycoprotein	A48R VAR (195 aa)	92.9/196	Duncan and Smith (1992)
						A43R VAC (194 aa)	92.9/197	
						A45R CPV (196 aa)	94.4/197	
A44R	149,894	150,118	74			— VAR	—	
						— VAC	—	
						A46R CPV (78 aa)	95.9/73	
A45L	151,252	150,212	346	E	3-β-Hydroxy-delta5-steroid dehydrogenase	A49L VAR (210 aa)	94.9/197	Moore and Smith (1992)
						A50L VAR (61 aa)	88.1/59	
						A44L VAC (346 aa)	98.3/346	
						A47L CPV (346 aa)	98.8/346	
A46R	151,299	151,676	125	L	Superoxide dismutase-like, virion core protein	A51R VAR (125 aa)	96.8/125	Goebel *et al.* (1990); Almazan *et al.* (2001)
						A45R VAC (125 aa)	96.0/125	
						A48R CPV (125 aa)	98.4/125	
A47R	151,666	152,388	240			A52R VAR (240 aa)	95.8/240	
						A46R VAC (214 aaa)	90.6/203	
						A49R CPV (240 aa)	96.3/240	
A48R	152,404	152,658	84			— VAR	—	
						— VAC	—	
						— CPV	—	
A49R	153,468	154,082	204	E	Thymidylate kinase	J2R VAR (205 aa)	98.5/205	Smith *et al.* (1989a); Hughes *et al.* (1991)
						A48R VAC (204 aa)	99.0/204	
						A51R CPV (227 aa)	99.0/204	
A50R	154,648	156,312	554	E	DNA ligase	J4R VAR (552 aa)	96.4/554	Kerr and Smith (1989); Smith *et al.* (1989b)
						A50R VAC (552 aa)	97.8/554	
						A53R CPV (552 aa)	97.8/554	
A51R	156,366	157,370	334			J5R VAR (334 aa)	92.2/334	
						A51R VAC (334 aa)	95.5/334	
						A54R CPV (334 aa)	96.1/334	
B1R	158,318	158,530	70		Kelch-like	J8R VAR (172 aa)	77.4/62	Xue and Cooley (1993); Shchelkunov *et al.* (1998)
						A55R VAC (564 aa)	86.7/60	
						A57R CPV (564 aa)	86.7/60	
B2R	158,617	159,558	313	E–L	EEV membrane glycoprotein hemagglutinin, prevents cell fusion	J9R VAR (313 aa)	81.2/319	Shida (1986); Seki *et al.* (1990); Brown *et al.* (1991)
						A56R VAC (315 aa)	93.0/315	
						A58R CPV (314 aa)	91.7/315	
B3R	160,325	161,224	299	E	Serine/threonine protein kinase, essential for DNA replication, intermediate transcription factor	B1R VAR (300 aa)	93.6/297	Banham and Smith (1992); Lin *et al.* (1992); Rempel and Traktman (1992); Kovacs *et al.* (2001)
						B1R VAC (300 aa)	97.0/298	
						B1R CPV (300 aa)	97.3/298	
B4R	161,299	162,810	503		Schlafen-like	— VAR	—	Schwarz *et al.* (1998); Shchelkunov *et al.* (2000)
						B2R VAC (219 aa)	90.7/214	
						B3R VAC (124 aa)	81.3/123	
						B2R CPV (503 aa)	89.3/503	
B5R	163,058	164,743	561		Ankyrin-like	B6R VAR (558 aa)	91.8/562	Shchelkunov *et al.* (1993b, 1998)
						B4R VAC (558 aa)	93.1/562	
						B3R CPV (558 aa)	94.5/562	

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
B6R	164,845	165,798	317	E–L	Palmitylated 42-kDa EEV glycoprotein required for efficient cell spread, complement control protein-like	B7R VAR (317 aa) B5R VAC (317 aa) B4R CPV (317 aa)	93.0/316 95.9/317 98.4/317	Engelstad and Smith (1992, 1993); Isaacs *et al.* (1992); Wolffe *et al.* (1993)
B7R	165,891	166,421	176			B8R VAR (56 aa) B6R VAC (173 aa) B5R CPV (183 aa)	87.0/46 87.6/177 85.8/183	
B8R	166,458	167,006	182			— VAR B7R VAC (182 aa) B6R CPV (182 aa)	— 96.7/182 96.7/182	
B9R	167,061	167,864	267	E	Secreted IFN-γ binding protein	B9R VAR (266 aa) B8R VAC (272 aa) B7R CPV (271 aa)	90.9/263 97.4/267 97.3/263	Alcami and Smith (1995); Seregin *et al.* (1996)
B10R	167,957	168,622	221		Shope fibroma virus T4 protein-like	— VAR B9R VAC (77 aa) B8R CPV (221 aa)	— 97.1/69 97.3/221	Shchelkunov *et al.* (1998)
B11R	169,744	170,592	282	E	Protein kinase-like	B12R VAR (134 aa) B12R VAC (283 aa) B11R CPV (283 aa)	77.5/71 97.2/283 96.1/283	Banham and Smith (1993)
B12R	170,707	171,741	344	E	Serine protease inhibitor-like, SPI-2, inhibits IL-1β converting enzyme	B13R VAR (344 aa) B13R VAC (116 aa) B14R VAC (222 aa) B12R CPV (345 aa)	93.6/344 95.6/114 90.5/220 97.1/344	Kotwal and Moss (1989); Ray *et al.* (1992); Kettle *et al.* (1997)
B13R	171,868	172,317	149		VAC B15R	B14R VAR (149 aa) B15R VAC (149 aa) B13R CPV (149 aa)	94.6/149 93.3/149 94.0/149	
B14R	172,403	173,383	326	E	Secreted, IL-1β binding, inhibits virus induced fever	B15R VAR (63 aa) B17R VAR (69 aa) B16R VAC (290 aa) B14R CPV (326 aa)	88.5/61 83.8/68 95.5/290 95.4/326	Spriggs *et al.* (1992); Alcami and Smith (1992); Alcami and Smith (1996)
B15L	173,665	173,429	78			B18L VAR (340 aa) B17L VAC (340 aa) B15L CPV (340 aa)	93.3/75 96.0/75 95.9/74	
B16R	174,203	175,261	352	E	Cell surface antigen and secreted IFN-α/β-binding protein	B20R VAR (354 aa) B19R VAC (353 aa) B17R CPV (351 aa)	85.6/353 93.8/353 92.0/351	Ueda *et al.* (1990); Symons *et al.* (1995)
B17R	175,330	177,711	793		Ankyrin-like	B21R VAR (787 aa) B20R VAC (127 aa) B18R CPV (795 aa)	87.9/795 95.9/123 95.9/800	Shchelkunov *et al.* (1993b, 1998)
B18R	177,857	178,069	70		Kelch-like	— VAR — VAC B19R CPV (557 aa)	— — 90.9/55	Shchelkunov *et al.* (1998)
B19R	178,963	180,036	357	E	Serine protease inhibitor-like, SPI-1, apoptosis inhibition	B25R VAR (372 aa) C12L VAC (353 aa) B20R CPV (375 aa)	93.0/357 95.2/357 93.9/358	Kotwal and Moss (1989); Smith *et al.* (1989c)
B20R	180,215	180,787	190			— VAR — VAC B21R CPV (190 aa)	— — 96.3/190	
B21R	181,046	186,685	1879	E	Putative membrane-associated glycoprotein, cadherin-like domain	B26R VAR (1896 aa) — VAC B22R CPV (1933 aa)	85.9/1896 — 92.5/1935	Shchelkunov *et al.* (1994); Marennikova and Shchelkunov (1998)
K1R	187,567	187,779	70		Tumor necrosis factor receptor-like	— VAR — VAC K3R CPV (167 aa)	— — 91.9/62	Shchelkunov *et al.* (1998)
R1R	188,372	188,689	105			— VAR — VAC S1R CPV (210 aa)	— — 82.5/103	

TABLE 1—*Continued*

ORF	Translation Start	Translation Stop	Size (aa)[a]	Type[b]	Function/feature(s)[c]	VAR-IND, VAC-COP, and CPV-GRI isologs	Identity[d]	References
N1R	188,894	189,355	153		VAC B15R-like	— VAR	—	
						B22R VAC (181 aa)	91.5/153	
						H1R CPV (153 aa)	92.8/153	
N2R	189,703	189,924	73		C-terminal part of CPV C3L	— VAR	—	
						— VAC	—	
						— CPV	—	
N3R	190,103	190,633	176		CPV C2L	— VAR	—	
						— VAC	—	
						— CPV	—	
N4R*	190,749	192,062	437		CPV C1L, ankyrin-like	— VAR	—	
						— VAC	—	
						— CPV	—	
J1R*	192,219	193,982	587		Ankyrin-like	G1R VAR (585 aa)	89.4/584	Shchelkunov *et al.* (1993b)
						B25R VAC (259 aa)	83.2/232	
						B26R VAC (103 aa)	69.6/115	
						B27R VAC (113 aa)	96.9/97	
						H3R CPV (586 aa)	96.6/586	
J2R*	194,073	195,119	348	E	Secreted TNF-binding protein	G2R VAR (349 aa)	85.1/349	Upton *et al.* (1991);
						B28R VAC (122 aa)	83.5/91	Shchelkunov *et al.*
						H4R CPV (351 aa)	90.3/351	(1993a); Hu *et al.* (1994)
J3R*	195,248	195,988	246	E–L	Secreted CC-chemokine-binding protein	G3R VAR (253 aa)	83.5/254	Graham *et al.* (1997);
						B29R VAC (244 aa)	86.4/242	Smith *et al.* (1997)
						H5R CPV (255 aa)	86.7/255	

Note. A dash indicates a deletion in the coding sequence of one virus relative to the other. An asterisk denotes ORFs from the inverted terminal repeats of the viral genome. IV, IMV, IEV, CEV, and EEV are immature virion, intracellular mature virion, intracellular enveloped virion, cell-associated extracellular enveloped virion, and extracellular enveloped virion, respectively.

[a] Number of deduced amino acids (aa) encoded within an ORF.

[b] Expression time of the corresponding VAC genes revealed experimentally is presented. E, early; E–L, early–late; I, intermediate; L, late. Putative expression time presupposed from sequence data is in parentheses.

[c] Experimentally revealed functions of viral proteins or homologies based on searching of PIR and SWISS-PROT databases.

[d] Values of amino acid sequence identity (in percent) are presented and calculated by FASTA analysis (Pearson and Lipman, 1988) for overlapping regions of homologous ORFs.

ceeded in cloning one of the two complementary, incompletely base-paired hairpin loop strands of MPV-ZAI DNA. Alignment of this sequence with those of other orthopoxviruses indicated considerable conservation and suggested that only the four nucleotides comprising the loop were missing (Fig. 1). Interestingly, the putative telomere resolution sequence of MPV (Fig. 1) is identical to that of VAC and VAR (Merchlinsky, 1990). The region of tandem repeats adjacent to the terminal hairpin is rather short in the MPV-ZAI genome and comprises NR I (85 bp) and NR II (322 bp), separated by two 70-bp repeats, one element of 70 bp, and two elements of 54 bp, located between NR II and the ITR coding sequence (Fig. 2). The organization of this region of MPV-ZAI DNA is most similar to that of VAR-GAR (Fig. 2). However, the terminal regions of all the VAR strains have very short ITRs that lack ORFs and the sets of repeats at the right and left termini differ (Fig. 2) (Massung *et al.,* 1995).

Coding region

Computer-assisted analysis identified the 190 open reading frames (ORFs) containing ≥60 amino acid residues listed in Table 1. The corresponding ORFs of VAR-IND, VAC-COP, and CPV-GRI are indicated along with their predicted amino acid lengths. Four ORFs at the left side of the MPV-ZAI genome (Fig. 3A) are located within the ITR and thus have counterparts on the right side of the genome (Fig. 3B). As expected, all genes known to be essential in other orthopoxviruses are present in MPV and occupy the central region of the genome (ORFs C10L to A25R). These ORFs have greater than 90% sequence identity with those of other orthopoxviruses. The majority of species- and strain-specific differences between orthopoxviruses reside in the left and right terminal regions, as can also be seen for MPV-ZAI (Figs. 3A and 3B). MPV-ZAI has no unique genes. On the contrary, MPV-ZAI is missing 25 ORFs that are found in CPV-GRI (Shchelkunov *et al.,* 1998) and 19 potential ORFs calculated for VAR-IND (Shchelkunov *et al.,* 1993d). While the roles for some of these genes remain entirely unknown, they include many that are probably involved in immune evasion, host range, and cell proliferation (Alcami and Koszinowski, 2000; Moss and Shisler, 2001). We previously

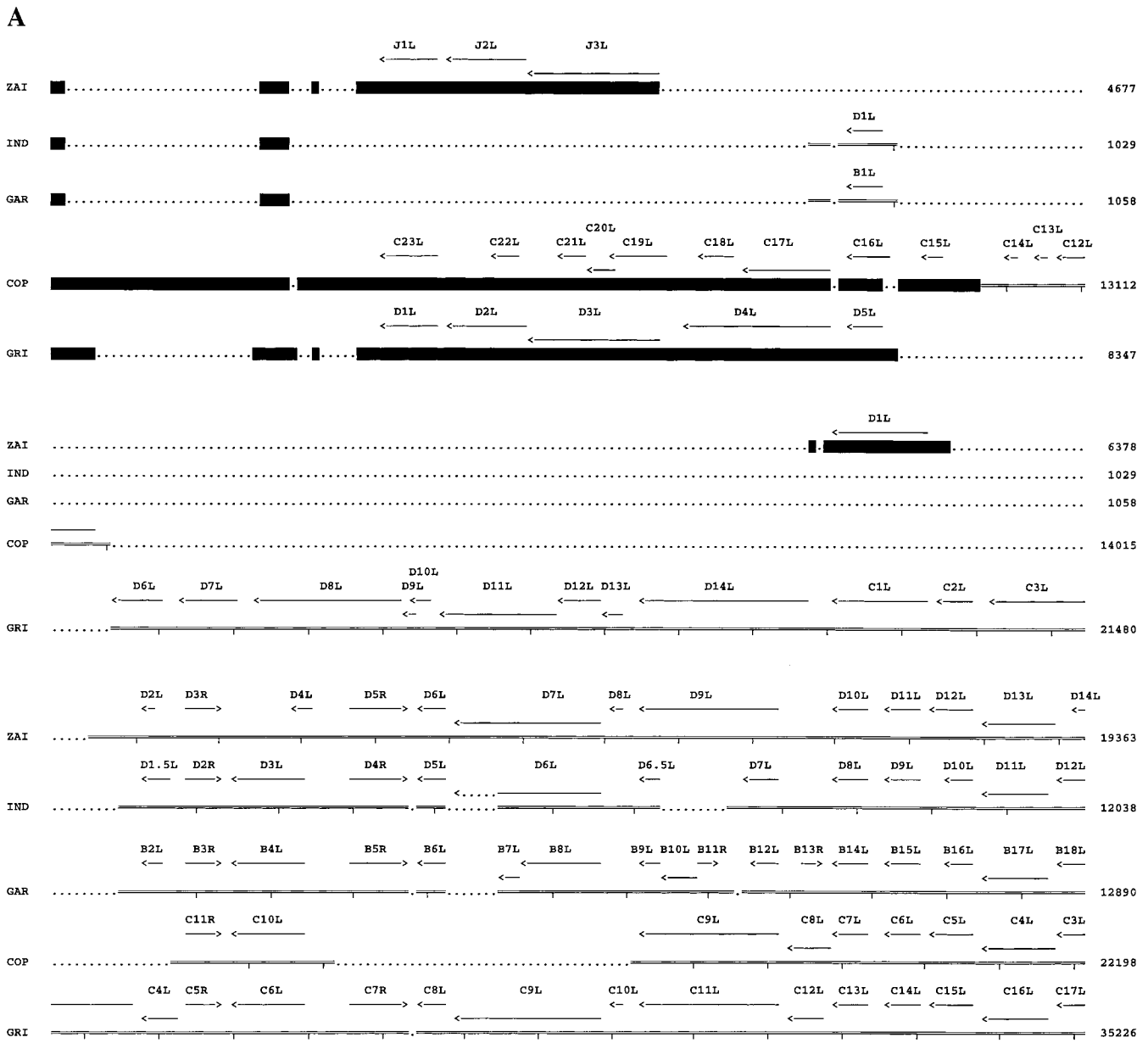

FIG. 3. Graphic alignment of the ORFs within the left (A) and right (B) terminal variable genomic region of MPV with corresponding genome segments of other orthopoxviruses. The sizes and directions of ORFs are marked with arrows. Deletions within the DNA and proteins of one virus relative to another, exceeding 150 bp and 50 amino acids, respectively, are marked with dots. The black blocks mark the sequences within the ITR regions. Nucleotide numbers are shown on the right.

compared the genes in these categories from MPV and VAR (Shchelkunov *et al.,* 2001), but we have added the growth factor and immune evasion genes of VAC and CPV in Table 2 because the latter contains all such known to be present in orthopoxviruses. Based on the number of intact and not extensively truncated virulence genes (indicated in parentheses), we obtain the following: CPV-GRI (17) > VAR-IND (11) = VAR-GAR (11) > MPV-ZAI (10) > VAC-COP (9) > VAC-MVA (6). MPV-ZAI also contains fewer ankyrin repeat genes than CPV, as previously noted (Shchelkunov *et al.,* 2001).

CPV-GRI encodes six ~500 amino acid proteins of unknown function with 22–26% identity to each other that belong to the kelch superfamily (Kumar *et al.,* 1993; Shchelkunov *et al.,* 1998). Although VAC-COP encodes three full-size kelch-like proteins, which are homologous to the corresponding CPV-GRI proteins (99.4, 97.9, and 98.6% identity, respectively), the MPV-ZAI genome encodes only one (C9L) with 97.3% identity to CPV-GRI protein G3L. In the genome of VARs, these ORFs all appear to be disrupted, indicating that they are nonessential for replication.

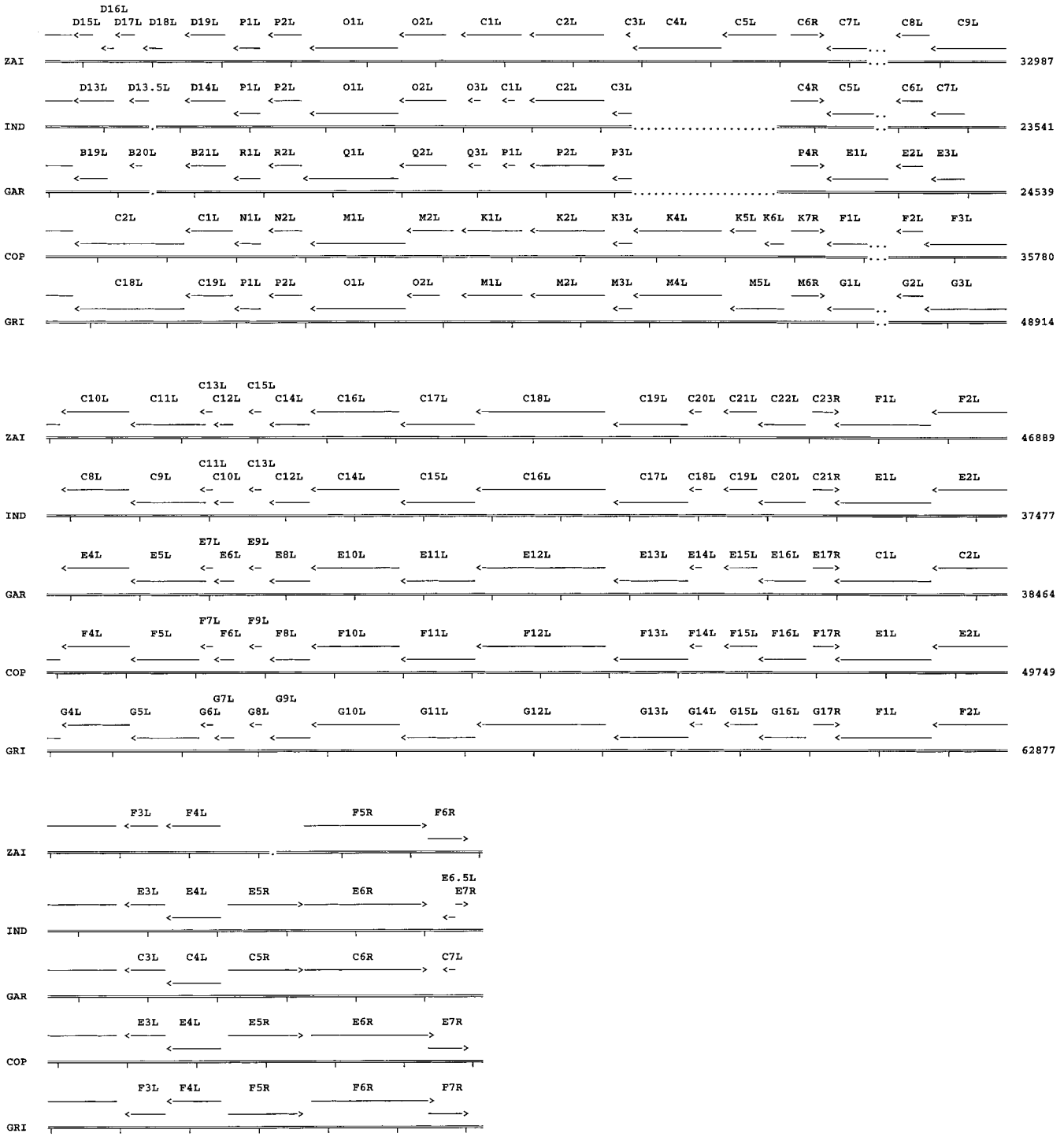

FIG. 3—*Continued*

VAC contains two ORFs with phospholipase D motifs: F13L is necessary for the formation of extracellular virus and efficient cell-to-cell spread of infection, whereas K4L is dispensable for replication in tissue culture (Blasco and Moss, 1991; Cao *et al.,* 1997; Sung *et al.,* 1997). CPV contains both of these ORFs as well as an additional putative lipid metabolizing enzyme that is homologous to lysophospholipase, which is mutated in VAC strains (Antoine *et al.,* 1998). MPV-ZAI contains intact versions of these three ORFs (C4L, C5L, and C19L, Table 1), whereas VAR strains lack C4L and C5L homologs as a result of deletion of a DNA segment (Fig. 3A). The functions of the C4L and C5L proteins are not yet known, although both are encoded by the two orthopoxviruses, MPV and CPV, with relatively wide host ranges.

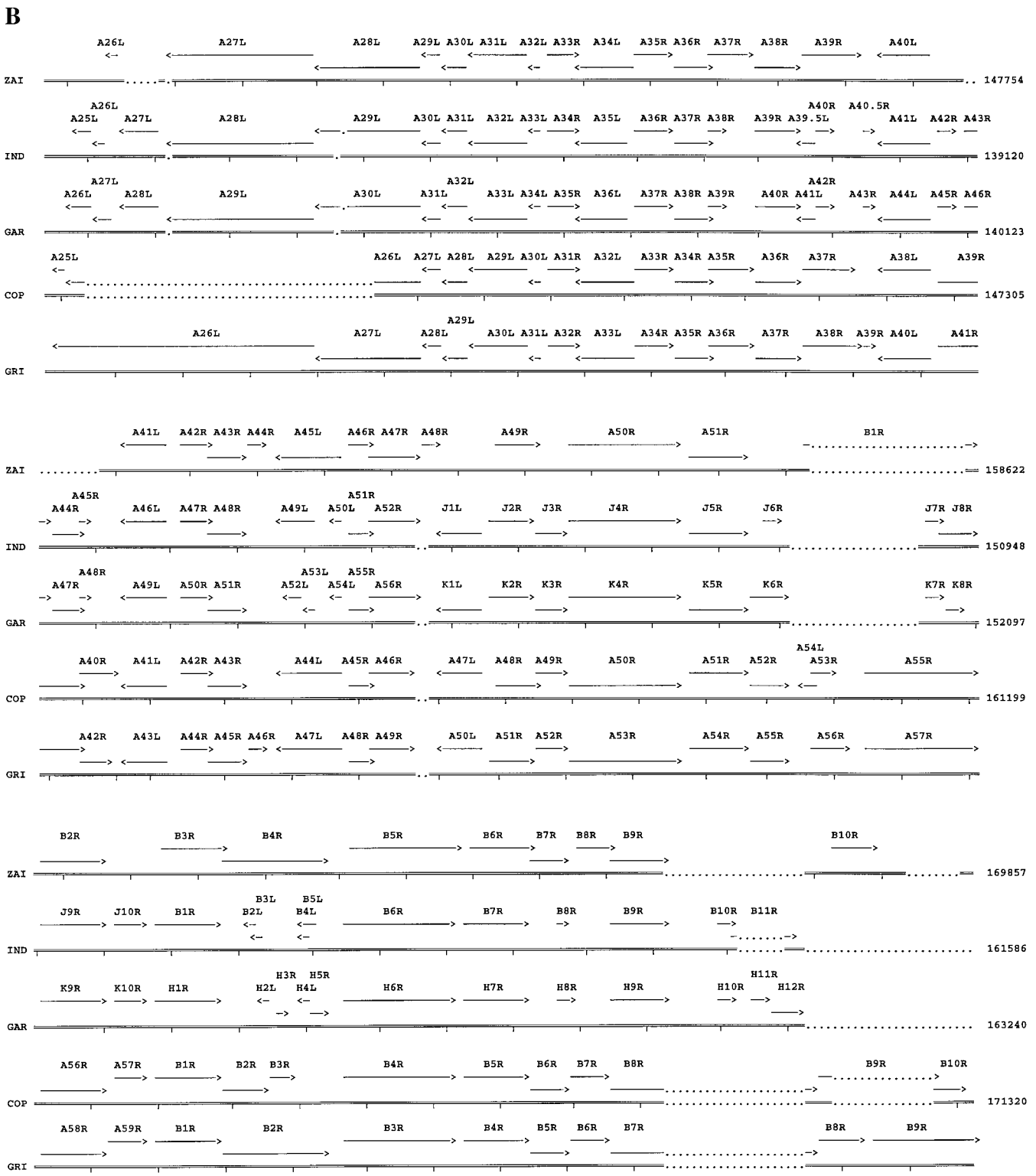

FIG. 3—*Continued*

Phylogenetic analysis

Based on the DNA sequences of the terminal variable regions, we considered that the organization of CPV most closely resembles that of the ancestral orthopoxvirus species and that VAR was slightly more distant from MPV than from VAC (Shchelkunov *et al.,* 2001). By analyzing the left and right variable regions separately, however, the situation appears more complex. The VAC strains appear closer to MPV-ZAI with respect to their left

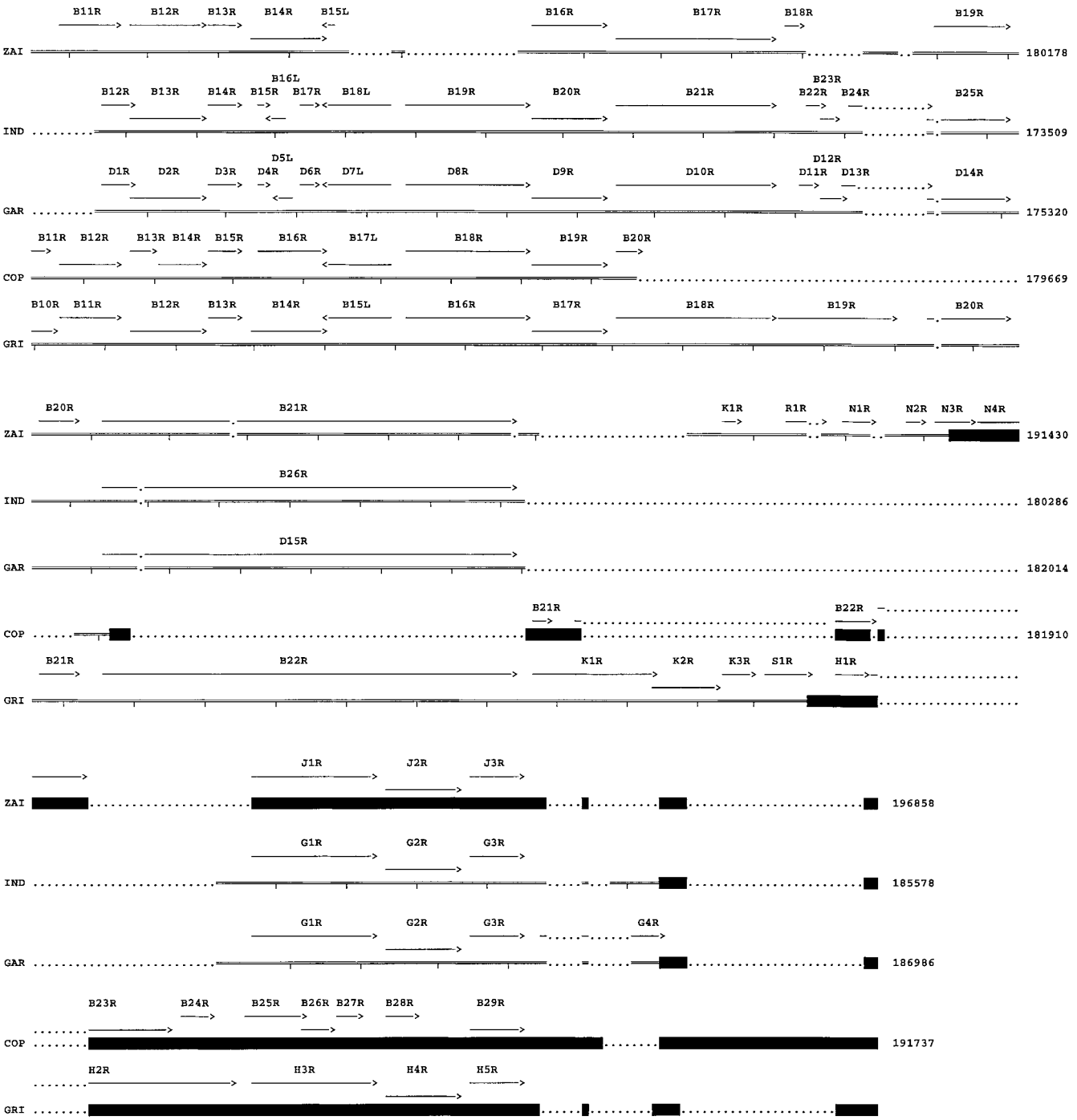

FIG. 3—*Continued*

terminal variable region and to VAR strains with respect to the right terminal variable region (Fig. 4). This discrepancy may be due to a complex recombinational origin of VAC, as suggested by others (Bedson and Dumbell, 1964; Marennikova and Shchelkunov, 1998).

Concluding remarks

In summary, the genome of MPV appears to be typical of orthopoxviruses, including a central conserved region, more variable left and right end regions, and an ITR with tandem repeats. Comparative analysis of the MPV, VAR, CPV, and VAC genomes confirmed that MPV is a discrete species exhibiting multiple differences from other orthopoxviruses pathogenic for humans. It seems likely that the orthopoxviruses analyzed thus far evolved independently from a cowpox-like ancestor virus, although recombination between species in the case of VAC is possible.

TABLE 2

Orthopoxviral Growth Factor and Immune Evasion Genes

	VAR-IND		VAR-GAR		MPV-ZAI		CPV-GRI		VAC-COP		VAC-MVA	
	ORF	Size (aa)	ORF	Size (aa)	ORF	Size (aa)	ORF	Size (aa)	ORF	Size (aa)	ORF	Size (aa)
Viral growth factor	D2R	140	B3R	140	D3R	142	C5R	138	C11R	142	005R	140
IL-18 binding protein	D5L	126	B6L	126	D6L	126	C8L	124	—†	—	008L	120
Complement binding protein	D12L	263	B18L	263	D14L†	216	C17L	259	C3L	263	—†	—
Interferon resistance factor, homolog of eIF-2α	C3L	88	P3L	88	—†	—	M3L	88	K3L	88	024L	88
Interferon resistance factor, dsRNA-binding protein	E3L	190	C3L	192	F3L†	153	F3L	190	E3L	190	050L	190
3-β-Hydroxy-delta5-steroid dehydrogenase	A50L†	61	A54L†	61	A45L	346	A47L	346	A44L	346	157L	346
Interferon-γ binding protein	B9R	266	H9R	266	B9R	267	B7R	271	B8R	272	176R†	226
Serine protease inhibitor homolog, SPI-2, inhibition of IL-1β converting enzyme, apoptosis inhibition	B13R	344	D2R	344	B12R	344	B12R	345	B13R†	116	181R†	116
Interleukin-1β-binding protein	B15R†	63	D4R†	63	B14R	326	B14R	326	B16R†	290	184R	326
Interferon-α/β-binding protein	B20R	354	D9R	355	B16R	352	B17R	351	B19R	353	187R†	234
Serine protease inhibitor homolog, SPI-1, apoptosis inhibition	B25R	357	D14R	357	B19R	357	B20R	375	C12L	353	—†	—
Tumor necrosis factor binding protein, CrmB	—	—	—	—	J2L*	348	D2L*	351	C22L*,†	122	002L*,†	176
	G2R	349	G2R	349	J2R*	348	H4R*	351	B28R*,†	122	192R*,†	176
Tumor necrosis factor binding protein, CrmC	—†	—	—†	—	—†	—	A56R	186	A53R†	103	—†	—
Tumor necrosis factor binding protein, CrmD	—†	—	—†	—	—†	—	K2R	322	—†	—	—†	—
Tumor necrosis factor binding protein, CrmE	—†	—	—†	—	K1R†	70	K3R	167	—†	—	—†	—
Chemokine binding protein	—	—	—	—	J1L*	246	D1L*	255	C23L*,†	244	001L*,†	136
	G3R	253	G3R	253	J3R*	246	H5R*	255	B29R*,†	244	193R*,†	136
Semaphorin-like	A42R†	74	A45R†	74	—†	—	A41R	402	A39R	403	150R†	83

Note. An asterisk denotes an ORFs that are duplicated in left and right inverted terminal repeat regions (ITR). ORFs that have functionally important differences from the corresponding ORFs of CPV-GRI are denoted by a dagger.

MATERIALS AND METHODS

The genome sequence of MPV-ZAI was determined as described (Shchelkunov *et al.,* 2001) and deposited with GenBank under Accession No. AF380138. Sequences were analyzed using software developed at the State Research Center for Virology and Biotechnology "Vector," Koltsovo, Russia (Resenchuk and Blinov, 1995). Protein homology searches were carried out by BLAST analyses (Altschul *et al.,* 1997) using NCBI internet resources. Sequences alignments were prepared using Clustal W (Thompson *et al.,* 1994). Phylogenies were calculated with the neighbor-joining (NJ) method (Saitou and Nei, 1987) using the MEGA package of software (Kumar *et al.,* 1993). For this analysis we used the 47.5-kb right-terminal and 50.3-kb left-terminal genomic fragments of MPV-ZAI and the corresponding DNA fragments of VAR-IND, VAR-BSH, VAR-GAR, VAC-COP, VAC-MVA, and CPV-GRI. The reliability of the phylogenetic relationship was statistically evaluated from 1000 bootstrap replicates.

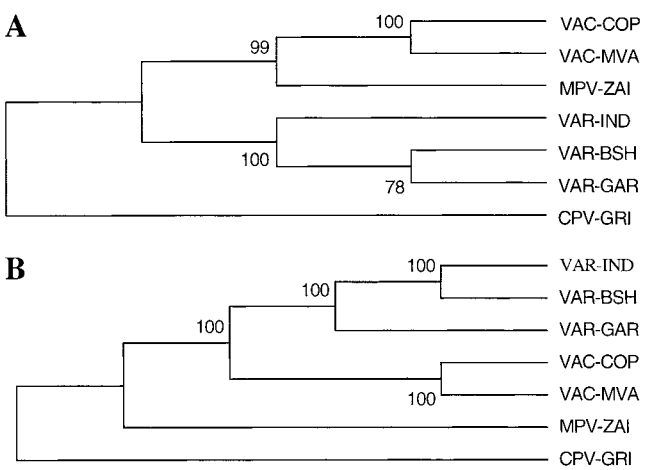

FIG. 4. Phylogenetic analysis of the left (A) and right (B) terminal variable genome regions of indicated orthopoxviruses.

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