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# The Complete Genome Sequence of Shope (Rabbit) Fibroma Virus

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We have determined the complete DNA sequence of the Leporipoxvirus Shope fibroma virus (SFV). The SFV genome spans 159.8 kb and encodes 165 putative genes of which 13 are duplicated in the 12.4-kb terminal inverted repeats. Although most SFV genes have homologs encoded by other *Chordopoxvirinae*, the SFV genome lacks a key gene required for the production of extracellular enveloped virus. SFV also encodes only the smaller ribonucleotide reductase subunit and has a limited nucleotide biosynthetic capacity. SFV preserves the *Chordopoxvirinae* gene order from S012L near the left end of the chromosome through to S142R (homologs of vaccinia F2L and B1R, respectively). The unique right end of SFV appears to be genetically unstable because when the sequence is compared with that of myxoma virus, five myxoma homologs have been deleted (C. Cameron, S. Hota-Mitchell, L. Chen, J. Barrett, J.-X. Cao, C. Macaulay, D. Willer, D. Evans, and G. McFadden, 1999, *Virology* 264, 298–318). Most other differences between these two Leporipoxviruses are located in the telomeres. Leporipoxviruses encode several genes not found in other poxviruses including four small hydrophobic proteins of unknown function (S023R, S119L, S125R, and S132L), an $\alpha$ 2,3-sialyltransferase (S143R), a protein belonging to the Ig-like protein superfamily (S141R), and a protein resembling the DNA-binding domain of proteins belonging to the HIN-200 protein family S013L). SFV also encodes a type II DNA photolyase (S127L). *Melanoplus sanguinipes* entomopoxvirus encodes a similar protein, but SFV is the first mammalian virus potentially capable of photoreactivating ultraviolet DNA damage. 

## INTRODUCTION

The Poxviridae comprise of a large family of double-stranded DNA viruses that infect vertebrates (Chordopoxvirinae) and insects (Entomopoxvirinae). These viruses are characterized by a complex capsid architecture, replicate in the cytoplasm of infected cells, and are of particular interest for their remarkable capacity to disarm or delay many different host antiviral responses. Poxviruses are endowed with some of the largest known viral genomes (139–260 kb), and thus few complete genomic DNA sequences have been reported to date. Those viral DNA sequences that have been completely elucidated include that of a single viral member of the Entomopoxvirinae family [*Melanoplus sanguinipes* entomopoxvirus, MSV (Afonso *et al.,* 1999)] as well as those of several Chordopoxvirinae. These latter viruses include the *Orthopoxviruses* vaccinia [VAC, (Goebel *et al.,* 1990; Antoine *et al.,* 1998)] and variola [VAR, (Massung *et al.,* 1994; Shchelkunov *et al.,* 1995)], as well as the *Molluscipoxvirus* molluscum contagiosum virus [MCV, (Senkevich *et al.,* 1997)].

Shope (or rabbit) fibroma virus (SFV) belongs to the *Leporipoxvirus* genus, a family of viruses whose host range includes rabbits, hares, and squirrels (reviewed in Digiacomo and Mare, 1994). SFV was originally shown to cause fibroxanthosarcoma-like tumours in its natural host, the eastern cottontail rabbit *Sylvilagus floridanus* (Shope, 1932b). Leporipoxviruses appear to be transmitted from rabbit to rabbit by biting insects, and the widespread prevalence of antibodies to the virus suggests that SFV infections may be endemic throughout US and Canadian rabbit populations (Digiacomo and Mare, 1994). Superficially similar disease symptoms and virus particles also have been reported in the African hare *Lepus capensis*, suggesting that the range of Leporipoxviruses may extend as far as African rabbit populations (Karstad *et al.,* 1977). Healthy adult rabbits mount an effective cell-mediated immune response, which typically starts to induce tumour regression within 10–12 days post infection (Scott *et al.,* 1981; Sell and Scott, 1981). However, SFV sometimes can cause a lethal disseminated infection in newborn rabbits and immunocompromised adults. Immunological studies (Shope, 1932a) and subsequent DNA sequence analyses have shown that SFV is closely related to myxoma virus (MYX), the causative agent of rabbit myxomatosis. The two kinds of viral infections are reportedly hard to differentiate in cottontail rabbits, but whereas SFV-induced disease in European rabbits (*Oryctolagus cuniculus*) closely resembles the disease in *S. floridanus*, myxoma causes a rapidly lethal disseminated infection in European rabbits that is strikingly different from the benign fibromas caused by SFV. This phenotype is partially explicable by the fact that myxoma virus, unlike SFV, can inhibit an

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apoptotic response in rabbit T lymphocytes and thus is able to replicate and spread through the lymphatic system (Macen *et al.,* 1996).

A number of studies have been directed at mapping and sequencing the genomes of SFV and myxoma viruses as well as what appears to be a natural recombinant called malignant rabbit fibroma virus (Strayer *et al.,* 1983; Block *et al.,* 1985; Upton *et al.,* 1988). Restriction sites are highly conserved amongst SFV strains and a detailed restriction map suggested a total genome size of ~160 kb with 12-kb terminal inverted repeats (TIR) (Wills *et al.,* 1983; Delange *et al.,* 1984; Cabirac *et al.,* 1985). DNA sequencing studies have shown that the SFV genome bears the hairpin tips characteristic of poxviruses (DeLange *et al.,* 1986) and encodes an array of genes thought to be required either for growth (Stuart *et al.,* 1993) or pathogenesis (Chang *et al.,* 1987, 1990; Upton *et al.,* 1987; Smith *et al.,* 1991a; Strayer *et al.,* 1991; Opgenorth *et al.,* 1993; Mossman *et al.,* 1995; Brick *et al.,* 1998). DNA transfection methods have been used to show that SFV catalyzes an efficient nonspecific DNA replication reaction (DeLange and McFadden, 1986), and this reaction has been exploited to study the mechanics of both SFV telomere resolution (DeLange *et al.,* 1986) and poxvirus genetic recombination (Evans *et al.,* 1988; Fisher *et al.,* 1991; Parks and Evans, 1991a,b). Biochemical and genetic studies also have characterized the reactions catalyzed by some of the most highly conserved of the replicative genes encoded by poxviruses. These include SFV topoisomerase (Upton *et al.,* 1990b; Palaniyar *et al.,* 1996, 1999), DNA ligase (Parks *et al.,* 1994, 1998), thymidine kinase (Upton and McFadden, 1986), and uracil-*N*-glycosylase (Upton *et al.,* 1993). However, these and other studies have yet to explain how or why SFV-infected cells catalyze such high levels of genetic recombination or *trans*-replication.

As a way of gaining further insights into these varied aspects of SFV biology, we have determined the complete genomic DNA sequence of the virus using a combination of random sequence analysis and primer walking. Together with a separate report on the genome sequence of myxoma virus (Cameron *et al.,* 1999) our work provides the first insights into the complete gene complement of *Leporipoxviruses*.

## RESULTS AND DISCUSSION

### Overview of the SFV genome

The SFV genome is 159,857 bp in length and encodes 60.5% (A+T) base pairs. The calculated *Bam*HI restriction map was in exact concordance with that determined experimentally (Delange *et al.,* 1984). The gene arrangement and direction of transcription closely resembles that seen in other Chordopoxviridae (Fig. 1) in that there is a pronounced tendency for genes located near the ends of the virus to be transcribed toward the telomeres. This can be demonstrated by arbitrarily dividing the genome into four 40,000-nt quarter sections. Within the left- and right-hand portions of the genome, only six genes in each of these two quarter sections disregard this rule. Throughout the rest of the genome, genes also tend to be arranged in transcriptional clusters with the occasional single gene periodically interrupting clusters of genes all transcribed in a common direction. The genes were numbered starting from the left end of the virus using a scheme similar to that used for molluscum contagiosum and *Melanoplus sanguinipes* viruses (Table 1) (Senkevich *et al.,* 1996; Afonso *et al.,* 1999). Several exceptions to the sequential number system were employed to maintain continuity with the earlier literature and with the gene order in myxoma virus. For example, we have assigned decimals to the genes previously called T-3A (S003.1L) and T-3C (S003.2L) to ensure that other telomeric genes (e.g., T-6 = S006L) retain their original numbering. Other features of the gene nomenclature are discussed in the accompanying paper (Cameron *et al.,* 1999)

The SFV gene order also closely matches that of the other sequenced Chordopoxviridae. Within the central core of the virus only a small number of genes have been deleted or replaced, and we can readily align nearly every SFV gene stretching from S019L to S126R with VAC and MCV homologs running from VAC F9L/MC016L to VAC A37R/MC149R. Thereafter the alignment can still be followed in vaccinia virus but becomes less obvious due to the absence or substitution of many genes. Nevertheless, it can still be traced out to S012L on the left end of the virus [which encodes a homolog of VAC F2L (dUTPase)] and out to S142R on the right side of the genome (which encodes a homolog of VAC B1R protein kinase). The rightmost portion of SFV also encodes a few genes found to the left of F2L in vaccinia virus. These include S144R, S146R, and S154R, which appear to be homologs of VAC C3L, N1L, and VAC M2L, respectively, and the different locations suggest that a translocation may have occurred sometime after the genetic isolation of Leporipoxviruses and Orthopoxviruses. SFV, like other Chordopoxviridae, shares no obvious conservation of gene order with the one sequenced Entomopoxvirus.

### Terminal inverted repeats

The SFV TIRs have been previously shown to span 12,397 nt. They are most notable for the complete absence of any repetitive DNA and a high density of encoded genes. An odd feature of the terminal inverted repeats is that the TIR junction falls within the boundaries of genes designated S009L (previously called T-9) and S155R. Because the C termini of these two proteins are located within the TIR, they share 443 amino acids of perfect amino acid identity. However, S009L encodes 67 more amino acids than does S155R, in a portion of the

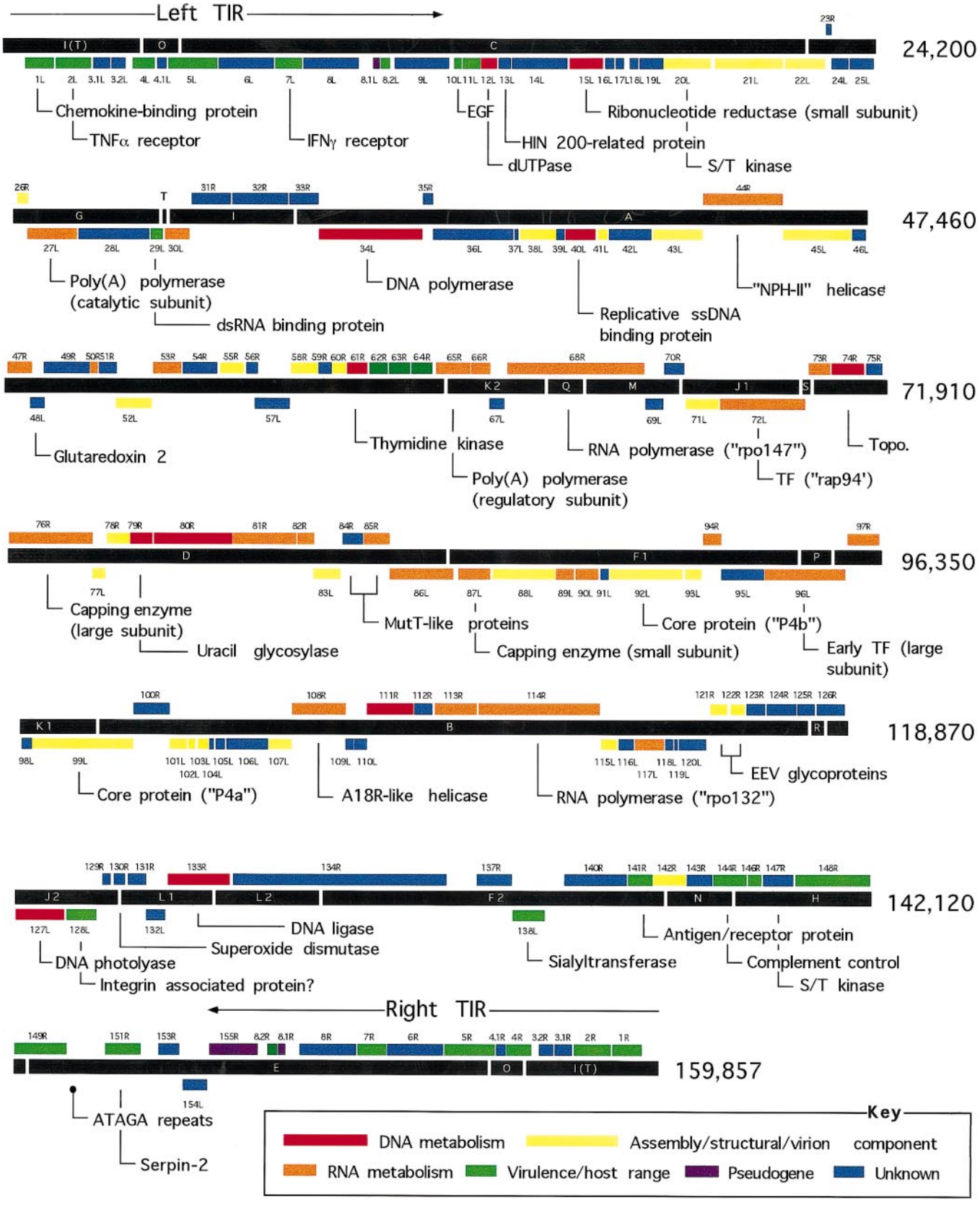


FIG. 1. SFV genetic map. Rectangles indicate open reading frames, and the direction of transcription is indicated by the box placement. The regions corresponding to each of the known *Bam*HI restriction fragments also have been located on the genetic map in white lettering. Where genes are transcribed in a leftward or a rightward direction ,they have been drawn below or above the line, respectively. There is a tendency for genes to be transcribed in commonly oriented blocks, interrupted by single-isolated genes. A selected subset of SFV genes and genome features also have been identified to illustrate points raised in the Discussion and to facilitate comparison with other poxvirus genetic maps.

TABLE 1

SFV Gene Products

| ORF[a] | Translation Start | Translation Stop[b] | Size (aa) | MW (kDa)[c] | Prom. type[d] | Function/feature(s) | Homolog(s)[e] | BLASTP score[f] | Amino acid identity[g] | Reference(s)[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| S001L/R | 1251<br>158607 | 475<br>159383 | 258 | 28.4 | E | Chemokine binding protein | VAC C23L/B29R | 4E-32 | 74/164 (45) | Graham *et al.* (1997); Patel *et al.* (1990) |
| S002L/R | 2325<br>157533 | 1348<br>158510 | 325 | 35.1 | E | TNF-$\alpha$ Receptor | VAC C22L/B28R | 5E-25 | 154/320 (48) | Smith *et al.* (1990, 1991a) |
| S003.1L/R | 2861<br>156997 | 2406<br>157452 | 151 | 17.7 | E | Unknown | VAC B15R | 4E-08 | 37/120 (30) | |
| S003.2L/R | 3306<br>156552 | 2929<br>156929 | 125 | 14.4 | ? | Unknown | SHP T3C | 2E-17 | 44/104 (42) | Gershon and Black (1989) |
| S004L/R | 4211<br>155647 | 3534<br>156324 | 225 | 25.1 | E | Virulence factor (RDEL motif) | VAC B9R | 0.002 | 23/69 (33) | Barry *et al.* (1997) |
| S004.1L/R | 4493<br>155365 | 4251<br>155607 | 80 | 9.2 | L | Unknown | SPV C2L | 4E-17 | 44/92 (47) | Massung *et al.* (1993) |
| S005L/R | 5967<br>153891 | 4513<br>155345 | 484 | 55.8 | E | Virulence factor (ankyrin-like protein) | VAC B4R | 3E-10 | 90/427 (21) | Howard *et al.* (1991) Mossman *et al.* (1996a) |
| S006L/R | 7530<br>152328 | 6004<br>153854 | 508 | 58.2 | E | Kelch repeat | VAC A55R | 8E-32 | 124/506 (24) | |
| S007L/R | 8360<br>151498 | 7572<br>152286 | 262 | 30.6 | E | IFN-$\gamma$ receptor mimic | VAC B8R | 7E-12 | 48/171 (28) | Upton *et al.* (1992) Mossman *et al.* (1996b) |
| S008L/R | 9959<br>149899 | 8415<br>151443 | 514 | 59.4 | E | Kelch repeat | VAC A55R | 1E-30 | 120/502 (23) | |
| S008.1L/R | 10567<br>149291 | 10397<br>149461 | 56 | 6.3 | — | Serpin fragment ("SERP-1") | MYX MAP1 | 0.38 | 19/37 (51) | Upton *et al.* (1990a) |
| S008.2L/R | 10842<br>149016 | 10606<br>149252 | 78 | 9.1 | E | Interferon resistance | VAC K3L | 0.014 | 18/60 (30) | Beattie *et al.* (1991) Davies *et al.* (1992) |
| S009L | 12548 | 11016 | 510 | 58.3 | E | Kelch repeat | VAC A55R | 6E-27 | 125/511 (24) | Goebel *et al.* (1990) |
| S010L | 12936 | 12694 | 80 | 9.2 | L | EGF-like growth factor | VAC C11R | 0.22 | 15/51 (29) | Chang *et al.* (1987) |
| S011L | 13409 | 12918 | 163 | 18.5 | E | Membrane virulence factor | SPV C10L (?) | 2.5 | 20/78 (25) | Graham *et al.* (1992) Massung *et al.* (1993) |
| S012L | 13856 | 13425 | 143 | 15.4 | E | dUTPase | VAC F2L | 5E-40 | 82/138 (59) | Roseman and Slabaugh (1990) Roseman *et al.* (1996) |
| S013L | 14217 | 13894 | 107 | 12.7 | E | Resembles *M. musculus* interferon-inducible protein | IFN-203 | 0.007 | 22/83 (26) | Gribaudo *et al.* (1997) |
| S014L | 15802 | 14252 | 516 | 58.7 | E | Kelch repeat | VAC F3L | 2E-41 | 135/511 (26) | Roseman and Slabaugh (1990) |
| S015L | 16824 | 15856 | 322 | 37.5 | E | Ribonucleotide reductase (small subunit) | VAC F4L | 1E-143 | 243/319 (76) | Slabaugh *et al.* (1988) Roseman and Slabaugh (1990) |
| S016L | 17101 | 16853 | 82 | 9.4 | ? | Unknown | SPV C15L | 4E-9 | 29/74 (39) | Massung *et al.* (1993) |
| S017L | 17337 | 17104 | 77 | 8.8 | E | Unknown | | | | |
| S018L | 17726 | 17526 | 66 | 7.5 | E | Unknown | VAC F8L | 0.043 | 19/44 (43) | |
| S019L | 18427 | 17780 | 215 | 24.5 | L | Unknown | VAC F9L<br>MC016L | 2E-49<br>8E-36 | 100/217 (46)<br>78/214 (36) | |
| S020L | 19742 | 18405 | 445 | 52.5 | L | S/T/Y protein kinase 2 | VAC F10L<br>MC017L | 0.0<br>1E-165 | 300/440 (68)<br>264/439 (60) | Lin and Broyles (1994) Derrien *et al.* (1999) |
| S021L | 21728 | 19845 | 627 | 72.5 | E | EEV maturation (fowlpox) | VAC F12L<br>MC019L | 1E-101<br>2E-66 | 215/618 (34)<br>176/620 (28) | Ogawa *et al.* (1993) |
| S022L | 22876 | 21764 | 370 | 41.5 | L | Envelope antigen | VAC F13L<br>MC021L | 1E-115<br>6E-84 | 201/369 (54)<br>158/364 (43) | Jackson and Hall (1998) Hirt *et al.* (1986) |
| S023R | 22958 | 23064 | 35 | 3.8 | L | Unknown | | | | |
| S024L | 23488 | 23042 | 148 | 17 | E | Unknown | VAC F15L<br>MC025L | 1E-36<br>6E-35 | 65/148 (43)<br>63/147 (42) | |
| S025L | 24181 | 23552 | 209 | 24.5 | E | Unknown | VAC F16L<br>MC029L | 3E-30<br>1E-12 | 77/223 (34)<br>53/229 (23) | |

TABLE 1—*Continued*

| ORF[a] | Translation Start | Translation Stop[b] | Size (aa) | MW (kDa)[c] | Prom. type[d] | Function/feature(s) | Homolog(s)[e] | BLASTP score[f] | Amino acid identity[g] | Reference(s)[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| S026R | 24220 | 24525 | 101 | 11.2 | L | DNA-binding phosphoprotein (VP11) | VAC F17R<br>MC030R | 7E-28<br>5E-17 | 63/104 (60)<br>44/94 (46) | Kao *et al.* (1981)<br>Jackson and Hall (1998) |
| S027L | 25934 | 24522 | 470 | 53.8 | ? | Poly (A) polymerase catalytic subunit | VAC E1L<br>MC031L | 1E-175<br>1E-138 | 290/462 (62)<br>249/471 (52) | Gershon *et al.* (1991) |
| S028L | 28906 | 25931 | 721 | 83.9 | L | Unknown | VAC E2L<br>MC032L | 1E-151<br>1E-101 | 286/740 (38)<br>226/749 (30) | |
| S029L | 28485 | 28135 | 116 | 13.1 | E | dsRNA-dependent protein kinase inhibitor | VAC E3L | 6E-15 | 35/90 (38) | Chang *et al.* (1995) |
| S030L | 29215 | 28547 | 222 | 25.8 | E | RNA polymerase subunit (rpo30, VITF-1) | VAC E4L<br>MC034L | 6E-74<br>3E-58 | 130/192 (67)<br>102/180 (56) | Ahn *et al.* (1990)<br>Broyles and Pennington (1990) |
| S031R | 29315 | 30493 | 392 | 45.7 | E | Unknown | VAC E5R | 2E-12 | 79/314 (25) | |
| S032R | 30503 | 32200 | 565 | 66.7 | L | Unknown | VAC E6R<br>MC037R | 0.0<br>0.0 | 333/567 (58)<br>283/565 (50) | |
| S033R | 32203 | 33027 | 274 | 31.8 | E | Unknown | VAC E8R<br>MC038R | 1E-102<br>1E-89 | 177/264 (67)<br>160/269 (59) | |
| S034L | 36041 | 33024 | 1005 | 117.1 | E | DNA polymerase | VAC E9L<br>MC039L | 0.0<br>0.0 | 641/1008 (63)<br>490/1009 (48) | Earl *et al.* (1986)<br>Traktman *et al.* (1984) |
| S035R | 36075 | 36365 | 96 | 11.1 | ? | Unknown | VAC E10R<br>MC040R | 3E-38<br>3E-30 | 70/95 (73)<br>55/95 (57) | |
| S036L | 38431 | 36386 | 681 | 78.2 | E | Unknown | VAC O1L<br>MC042L | 1E-111<br>5E-48 | 231/677 (34)<br>113/488 (23) | |
| S037L | 38565 | 38467 | 32 | 3.8 | L | Unknown | FPV O3L<br>VAC O3L(?)<br>MC043.1L(?) | 0.32<br>>1<br>>1 | 13/27 (48) | |
| S038L | 39517 | 38576 | 313 | 36.4 | L | DNA-binding, late stage virion morphogenesis | VAC I1L<br>MC044L | 1E-117<br>2E-94 | 208/313 (66)<br>165/308 (53) | Klemperer *et al.* (1997) |
| S039L | 39742 | 39518 | 74 | 8.6 | L | Unknown | VAC I2L<br>MC045L | 1E-12<br>7E-9 | 35/71 (49)<br>29/73 (39) | |
| S040L | 40555 | 39743 | 270 | 30.3 | E | DNA-binding phosphoprotein | VAC I3L<br>MC046L | 1E-65<br>4E-49 | 126/228 (55)<br>102/237 (43) | Davis and Mathews (1993) |
| S041L | 40859 | 40623 | 78 | 8.9 | ? | Structural protein (VP13K) | MCO47L<br>VAC I5L | 2E-10<br>8E-9 | 29/76 (38)<br>25/69 (36) | Takahashi *et al.* (1994) |
| S042L | 42040 | 40880 | 386 | 44.7 | L | Unknown | VAC I6L<br>MC048L | 1E-104<br>6E-53 | 187/386 (48)<br>111/363 (30) | |
| S043L | 43322 | 42033 | 429 | 49.9 | L | Virion morphogenesis, cysteine protease? | VAC I7L<br>MC049L | 1E-163<br>1E-129 | 270/425 (63)<br>225/424 (53) | Kane and Shuman (1993)<br>Li and Hochstrasser (1999) |
| S044R | 43328 | 45364 | 678 | 78.2 | ? | NPH-II, RNA helicase | VAC I8R<br>MC050L | 0.0<br>1E-171 | 381/676 (56)<br>304/683 (44) | Koonin and Senkevich (1992)<br>Shuman (1992) |
| S045L | 47133 | 45361 | 590 | 68.4 | L | Metalloendo-proteinase?, virion morphogenesis | VAC G1L<br>MC056L | 0.0<br>1E-156 | 322/592 (54)<br>259/591 (43) | Whitehead and Hruby (1994) |
| S046L | 47465 | 47130 | 111 | 12.8 | E/L | Unknown | VAC G3L<br>MC057L | 4E-11<br>4.0 | 36/89 (40)<br>26/87 (29) | |
| S047R | 47459 | 48136 | 225 | 26.2 | E | IBT-dependent protein, late elongation factor (VLTF) | VAC G2R<br>MC058R | 5E-53<br>2E-25 | 102/225 (45)<br>62/235 (26) | Meis and Condit (1991)<br>Black *et al.* (1998) |
| S048L | 48480 | 48094 | 128 | 14.7 | L | Glutaredoxin 2, membrane protein | VAC G4L<br>MC059L | 5E-28<br>2E-13 | 56/121 (46)<br>36/121 (29) | Gvakharia *et al.* (1996)<br>Jensen *et al.* (1996) |
| S049R | 48483 | 49781 | 432 | 49.8 | ? | Unknown | VAC G5R<br>MC060R | 9E-74<br>2E-54 | 163/391 (41)<br>127/395 (32) | |

TABLE 1—*Continued*

| ORF[a] | Translation Start | Translation Stop[b] | Size (aa) | MW (kDa)[c] | Prom. type[d] | Function/feature(s) | Homolog(s)[e] | BLASTP score[f] | Amino acid identity[g] | Reference(s)[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| S050R | 49785 | 49976 | 63 | 7.1 | E/L | RNA polymerase subunit (rpo7) | VAC G5.5R<br>MC061R | 3E-23<br>4E-18 | 49/63 (77)<br>40/63 (63) | Meis and Condit (1991)<br>Amegadzie *et al.* (1992)<br>Johnson *et al.* (1993) |
| S051R | 49976 | 50500 | 174 | 20 | ? | Unknown | VAC G6R<br>MC062R | 2E-36<br>2E-18 | 75/164 (45)<br>58/171 (33) | |
| S052L | 51521 | 50469 | 350 | 39.4 | L | Structural protein (VP16K) | VAC G7L<br>MC065L | 8E-91<br>1E-52 | 175/372 (47)<br>108/250 (43) | Takahashi *et al.* (1994) |
| S053R | 51551 | 52333 | 260 | 29.9 | I | Late transcription factor (VLTF-1) | VAC G8R<br>MC067R | 1E-125<br>1E-107 | 214/260 (82)<br>186/260 (71) | Keck *et al.* (1990)<br>Wright *et al.* (1991) |
| S054R | 52354 | 53352 | 332 | 38.6 | L | Myristylprotein | VAC G9R<br>MC068R | 7E-78<br>7E-41 | 143/324 (44)<br>102/322 (31) | Martin *et al.* (1997) |
| S055R | 53353 | 54081 | 242 | 26.2 | L | Myristylprotein, IMV virion protein | VAC L1R<br>MC069R | 3E-89<br>5E-80 | 153/239 (64)<br>141/242 (58) | Franke *et al.* (1990)<br>Martin *et al.* (1997) |
| S056R | 54137 | 54436 | 99 | 11.8 | E | Unknown | VAC L2R<br>MC070R | 2E-8<br>2.0 | 26/68 (38)<br>19/80 (23) | |
| S057L | 55348 | 54386 | 320 | 37.5 | L | Unknown | VAC L3L<br>MC072L | 7E-86<br>5E-63 | 167/328 (50)<br>122/286 (42) | |
| S058R | 55373 | 56128 | 251 | 28.6 | L | DNA binding structural protein precursor (VP8) | VAC L4R<br>MC073R | 4E-87<br>6E-65 | 151/250 (60)<br>129/254 (50) | Yang and Bauer (1988)<br>Bayliss and Smith (1997) |
| S059R | 56147 | 56536 | 129 | 15 | L | Unknown | VAC L5R<br>MC074R | 2E-19<br>0.74 | 50/123 (40)<br>24/111 (21) | |
| S060R | 56490 | 56936 | 148 | 17.1 | L | Dimeric virion protein | VAC J1R<br>MC075R | 5E-35<br>5E-32 | 72/150 (48)<br>69/147 (46) | cited in Antoine *et al.* (1998) |
| S061R | 56969 | 57505 | 178 | 19.9 | E | Thymidine kinase | VAC J2R | 1E-62 | 116/175 (66) | Upton and McFadden (1986) |
| S062R | 57587 | 58063 | 158 | 18.5 | E | Host range, triplicated | VAC C7L | 2E-14 | 48/148 (32) | Perkus *et al.* (1990) |
| S063R | 58120 | 58728 | 202 | 22.8 | E | Host range, triplicated | VAC C7L (?) | 0.64 | 38/156 (24) | Perkus *et al.* (1990) |
| S064R | 58770 | 59366 | 198 | 23.3 | E | Host range, triplicated | VAC C7L | 3E-17 | 42/138 (30) | Perkus *et al.* (1990) |
| S065R | 59468 | 60484 | 328 | 39.7 | E/L | Poly (A) polymerase regulatory subunit (VP39) | VAC J3R<br>MC076R | 1E-129<br>8E-89 | 217/333 (65)<br>165/342 (48) | Jackson and Bults (1990)<br>Schnierle *et al.* (1992) |
| S066R | 60384 | 60941 | 185 | 21 | ? | RNA polymerase subunit (rpo22) | VAC J4R<br>MC077R | 2E-68<br>5E-59 | 123/185 (66)<br>107/187 (57) | Broyles and Moss (1986)<br>Jackson and Bults (1990) |
| S067L | 61345 | 60944 | 133 | 15.4 | ? | Unknown (essential) | VAC J5L<br>MC078L | 7E-45<br>2E-28 | 79/131 (60)<br>52/114 (45) | Zajac *et al.* (1995) |
| S068R | 61416 | 65276 | 1286 | 147.5 | E/L | RNA polymerase subunit (rpo147) | VAC J6R<br>MC079R | 0.0<br>0.0 | 1019/1288 (79)<br>918/1289 (71) | Broyles and Moss (1986) |
| S069L | 65803 | 65282 | 173 | 20.1 | L | S/Y phosphatase | VAC H1L<br>MC082L | 7E-58<br>3E-48 | 108/172 (62)<br>88/171 (51) | Mossman *et al.* (1995) |
| S070R | 65819 | 66391 | 190 | 21.6 | L | Unknown | VAC H2R<br>MC083R | 9E-74<br>1E-59 | 124/188 (65)<br>96/181 (53) | |
| S071L | 67368 | 66394 | 324 | 37.2 | L | IMV envelope protein | VAC H3L<br>MC084L | 9E-52<br>9E-20 | 109/326 (33)<br>83/315 (26) | Chertov *et al.* (1991) |
| S072L | 69765 | 67369 | 798 | 94.3 | L | RNA polymerase-associated TF (RAP 94) | VAC H4L<br>MC085L | 0.0<br>0.0 | 504/800 (63)<br>440/800 (55) | Ahn and Moss (1992b) |
| S073R | 69894 | 70490 | 198 | 22.3 | E/L | Late transcription factor (VLTF-4) | VAC H5R<br>MC086R | 6E-9<br>0.003 | 29/60 (48)<br>21/59 (35) | Kovacs and Moss (1996) |
| S074R | 70518 | 71462 | 314 | 37 | L | Topoisomerase I, (telomere resolvase) | VAC H6R<br>MC087R | 1E-113<br>6E-93 | 193/312 (61)<br>164/310 (52) | Upton *et al.* (1990b)<br>Palaniyar *et al.* (1999) |
| S075R | 71462 | 71914 | 150 | 17.6 | I | Unknown | VAC H7R<br>MC088R | 6E-26<br>1E-14 | 58/145 (40)<br>47/148 (31) | |

TABLE 1—*Continued*

| ORF[a] | Translation Start | Translation Stop[b] | Size (aa) | MW (kDa)[c] | Prom. type[d] | Function/feature(s) | Homolog(s)[e] | BLASTP score[f] | Amino acid identity[g] | Reference(s)[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| S076R | 71907 | 74417 | 836 | 97 | E | mRNA capping enzyme, large subunit | VAC D1R<br>MC090R | 0.0<br>0.0 | 540/832 (64)<br>467/819 (57) | Upton *et al.* (1991) |
| S077L | 74810 | 74379 | 143 | 16.6 | ? | Virion component | VAC D2L<br>MC091L | 9E-20<br>3E-13 | 51/150 (34)<br>46/161 (28) | Upton *et al.* (1991)<br>Dyster and Niles (1991) |
| S078R | 74816 | 75541 | 241 | 28 | ? | Structural protein | VAC D3R<br>MC092R | 2E-22<br>5E-8 | 79/247 (31)<br>49/218 (22) | Dyster and Niles (1991) |
| S079R | 75538 | 76194 | 218 | 25.6 | E | Uracil DNA glycosylase | VAC D4R<br>MC093R | 3E-93<br>2E-65 | 150/218 (68)<br>112/222 (50) | Upton *et al.* (1993) |
| S080R | 76228 | 78588 | 786 | 90.9 | E/L | Replication/recomb. NTPase | VAC D5R<br>MC094R | 0.0<br>0.0 | 498/785 (63)<br>419/787 (53) | Evans *et al.* (1995) |
| S081R | 78585 | 80492 | 635 | 73.2 | L | Early transcription factor (VETF-1) | VAC D6R<br>MC095R | 0.0<br>0.0 | 514/637 (80)<br>493/631 (78) | Strayer *et al.* (1991)<br>Broyles and Fesler (1990)<br>Gershon and Moss (1990) |
| S082R | 80523 | 81014 | 163 | 18.4 | E/L | RNA polymerase subunit (rpo18) | VAC D7R<br>MC097R | 6E-50<br>2E-44 | 97/161 (60)<br>88/158 (55) | Strayer *et al.* (1991)<br>Ahn *et al.* (1990)<br>Quick and Broyles (1990) |
| S083L | 81842 | 80985 | 285 | 32.4 | ? | Carbonic anhydrase-like structural protein | VAC D8L | 3E-28 | 82/224 (36) | Niles *et al.* (1986);<br>Niles and Seto (1988)<br>Strayer *et al.* (1991) |
| S084R | 81888 | 82508 | 206 | 24 | E | Mut-T like protein | VAC D9R<br>MC098R | 1E-58<br>4E-45 | 105/203 (51)<br>90/209 (43) | Niles *et al.* (1986)<br>Koonin *et al.* (1993) |
| S085R | 82505 | 83287 | 260 | 30.4 | E/L | Mut-T like protein, neg. regulates gene expression | VAC D10R<br>MC099R | 5E-56<br>1E-41 | 111/244 (45)<br>90/211 (42) | Niles *et al.* (1986)<br>Shors *et al.* (1999) |
| S086L | 85182 | 83284 | 632 | 72.8 | L | NTPase I, DNA helicase | VAC D11L<br>MC100L | 0.0<br>0.0 | 433/630 (68)<br>365/626 (58) | Christen *et al.* (1998)<br>Koonin and Senkevich (1992) |
| S087L | 86063 | 85200 | 287 | 33.2 | ? | mRNA capping enzyme small subunit, transcrip. initiation factor (VITF) | VAC D12L<br>MC101L | 1E-125<br>1E-106 | 207/287 (72)<br>176/284 (61) | Niles *et al.* (1989)<br>Vos *et al.* (1991)<br>Weinrich and Hruby (1986) |
| S088L | 87750 | 86092 | 552 | 62.3 | L | Rifampicin resistance IMV protein | VAC D13L<br>MC102L | 0.0<br>0.0 | 378/550 (68)<br>318/549 (57) | Tartaglia and Paoletti (1985)<br>Sodeik *et al.* (1994) |
| S089L | 88226 | 87777 | 149 | 16.8 | I | Trans-activator protein (VLTF-2) | VAC A1L<br>MC103L | 1E-52<br>9E-42 | 98/150 (65)<br>77/147 (52) | Keck *et al.* (1990)<br>Keck *et al.* (1993) |
| S090L | 88937 | 88263 | 224 | 26.2 | I | Trans-activator protein (VLTF-3) | VAC A2L<br>MC104L | 1E-112<br>2E-97 | 190/224 (84)<br>165/222 (74) | Keck *et al.* (1990)<br>Keck *et al.* (1993) |
| S091L | 89161 | 88934 | 75 | 8.8 | L | Unknown | VAC (MVA) 8.9K<br>MC105L | 9E-19<br>6E-7 | 42/76 (55)<br>24/65 (36) | Antoine *et al.* (1998) |
| S092L | 91131 | 89170 | 653 | 74.3 | L | Major core protein P4b precursor | VAC A3L<br>MC106L | 0.0<br>0.0 | 398/652 (61)<br>368/667 (55) | Rosel and Moss (1985) |
| S093L | 91627 | 91166 | 153 | 17.7 | L | Core protein? | MC107L<br>VAC A4L (?) | 6E-4<br>>1 | 25/73 (34)<br>19/64 (29) | Demkowicz *et al.* (1992)<br>Cudmore *et al.* (1996) |
| S094R | 91667 | 92161 | 164 | 18.8 | L | RNA polymerase subunit (rpo19) | VAC A5R<br>MC108R | 2E-36<br>2E-35 | 78/126 (61)<br>76/129 (58) | Ahn *et al.* (1992) |
| S095L | 93279 | 92158 | 373 | 43.4 | I | Unknown | VAC A6L<br>MC109L | 1E-114<br>1E-95 | 201/372 (54)<br>162/372 (43) | |
| S096L | 95444 | 93309 | 711 | 82.6 | L | Early transcription factor large subunit (VETF) | VAC A7L<br>MC110L | 0.0<br>0.0 | 495/711 (69)<br>449/708 (63) | Gershon and Moss (1990) |
| S097R | 95491 | 96351 | 286 | 33.8 | E | Intermediate transcription factor (VITF-3) | VAC A8R<br>MC111R | 1E-98<br>3E-68 | 175/286 (61)<br>129/291 (44) | Sanz and Moss (1999) |
| S098L | 96581 | 96348 | 77 | 8.7 | E/L | Unknown | VAC A9L<br>MC112L | 2E-20<br>1E-12 | 43/58 (74)<br>35/58 (60) | |

TABLE 1—*Continued*

| ORF[a] | Translation Start | Translation Stop[b] | Size (aa) | MW (kDa)[c] | Prom. type[d] | Function/feature(s) | Homolog(s)[e] | BLASTP score[f] | Amino acid identity[g] | Reference(s)[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| S099L | 99290 | 96582 | 902 | 103.7 | L | Major core protein P4a precursor | VAC A10L<br>MC113L | 0.0<br>0.0 | 433/907 (47)<br>385/908 (42) | Van Meir and Wittek (1988)<br>Vanslyke *et al.* (1991) |
| S100R | 99305 | 100249 | 314 | 35.5 | L | Unknown | VAC A11R<br>MC114R | 2E-81<br>4E-66 | 161/323 (49)<br>140/325 (43) | |
| S101L | 100716 | 100246 | 156 | 16.9 | L | Structural protein | MC115L<br>VAC A12L | 6E-30<br>1E-27 | 75/151 (49)<br>78/182 (42) | Takahashi *et al.* (1994) |
| S102L | 100955 | 100749 | 68 | 7.8 | L | Structural protein, IMV membrane protein p8 | VAC A13L | 0.00067 | 25/69 (36) | Takahashi *et al.* (1994)<br>Jensen *et al.* (1996) |
| S103L | 101296 | 101009 | 95 | 10.4 | L | IMV membrane protein, assembly factor | VAC A14L<br>MC118L | 2E-24<br>2E-17 | 52/93 (55)<br>40/95 (42) | Jensen *et al.* (1996)<br>Rodriguez *et al.* (1998) |
| S104L | 101474 | 101313 | 53 | 6.3 | L | Unknown | VAC A14.5L[i]<br>MC119L | 2E-14[j]<br>9E-9 | 34/51 (66)<br>24/53 (45) | |
| S105L | 101748 | 101464 | 94 | 10.9 | L | Unknown | VAC A15L<br>MC120L | 9E-20<br>6E-8 | 43/93 (46)<br>33/94 (35) | |
| S106L | 102862 | 101732 | 376 | 43.9 | L | 35K myristylprotein | VAC A16L<br>MC121L | 1E-110<br>4E-85 | 185/381 (48)<br>148/359 (41) | Martin *et al.* (1997) |
| S107L | 103478 | 102879 | 199 | 22.4 | L | IMV membrane protein, morphogenesis factor | VAC A17L<br>MC122L | 5E-32<br>5E-28 | 76/170 (44)<br>76/190 (40) | Rodriguez *et al.* (1995)<br>Krijnse-Locker *et al.* (1996) |
| S108R | 103493 | 104929 | 478 | 55.2 | I | Transcript release factor, ATP-dep. DNA helicase | VAC A18R<br>MC123R | 1E-162<br>1E-128 | 280/490 (57)<br>220/459 (47) | Bayliss and Condit (1995)<br>Xiang *et al.* (1998) |
| S109L | 105131 | 104910 | 73 | 8 | L | Unknown | VAC A19L<br>MC124L | 9E-17<br>4E-10 | 44/72 (61)<br>35/75 (46) | |
| S110L | 105473 | 105132 | 113 | 13.3 | ? | Unknown | VAC A21L<br>MC125L | 3E-32<br>3E-19 | 66/117 (56)<br>48/111 (43) | |
| S111R | 105472 | 106770 | 432 | 50.8 | E | DNA polymerase processivity factor | VAC A20R<br>MC126R | 2E-79<br>1E-45 | 154/410 (37)<br>109/383 (28) | McDonald *et al.* (1997) |
| S112R | 106733 | 107215 | 160 | 18.8 | ? | Unknown | VAC A22R<br>MC127R | 1E-52<br>1E-33 | 97/154 (62)<br>71/142 (50) | |
| S113R | 107243 | 108400 | 385 | 44.9 | E | Intermediate transcrip. factor (VITF-3) | VAC A23R<br>MC128R | 1E-120<br>1E-95 | 221/386 (57)<br>184/385 (47) | Sanz and Moss (1999) |
| S114R | 108426 | 111893 | 1155 | 132.5 | E/L | RNA polymerase subunit (rpo132) | VAC A24R<br>MC129R | 0.0<br>0.0 | 944/1155 (81)<br>862/1156 (74) | Hooda-Dhingra *et al.* (1990)<br>Amegadzie *et al.* (1991b) |
| S115L | 112448 | 111909 | 179 | 20.6 | L | Fusion protein | VAC A27L<br>MC131L/MC133L | 2E-4<br>0.18 | 28/88 (31)<br>22/68 (32) | Rodriguez and Esteban (1987)<br>Gong *et al.* (1990)<br>Rodriguez and Smith (1990) |
| S116L | 112871 | 112449 | 140 | 16.1 | L | Unknown | VAC A28L<br>MC134L | 1E-30<br>5E-29 | 71/148 (47)<br>59/141 (41) | |
| S117L | 113785 | 112877 | 302 | 35 | E | RNA polymerase subunit (rpo35) | VAC A29L<br>MC135L | 1E-102<br>9E-81 | 172/300 (57)<br>146/298 (48) | Amegadzie *et al.* (1991a) |
| S118L | 113984 | 113754 | 76 | 8.5 | L | Unknown | VAC A30L<br>MC136L | 4E-12<br>2E-9 | 35/60 (58)<br>35/69 (50) | |
| S119L | 114122 | 114003 | 39 | 4.4 | L | Unknown | | | | |
| S120L | 114924 | 114157 | 255 | 29.1 | E/L | Putative ATPase | MC140L<br>VAC A32L | 4E-90<br>1E-84 | 158/249 (63)<br>151/255 (59) | Koonin *et al.* (1993) |
| S121R | 115011 | 115529 | 172 | 19.2 | E | EEV glycoprotein | VAC A33R<br>MC142R | 4E-18<br>0.027 | 57/190 (30)<br>33/131 (25) | Roper *et al.* (1996) |
| S122R | 115539 | 116054 | 171 | 19.8 | L | Lectin-like EEV glycoprotein | VAC A34R<br>MC143R | 2E-40<br>8E-4 | 76/172 (44)<br>40/173 (23) | Duncan and Smith (1992)<br>McIntosh and Smith (1996) |
| S123R | 116088 | 116627 | 179 | 20.4 | E | Unknown | VAC A35R<br>MC145R | 2E-21<br>1E-11 | 53/172 (30)<br>43/170 (25) | |
| S124R | 116664 | 117524 | 286 | 32.6 | E/L | Unknown | MC144R | 9E-12 | 52/221 (23) | |
| S125R | 117533 | 118027 | 164 | 18.9 | ? | Unknown | SHP HM1 (?) | 0.64 | 33/143 (23) | Gershon *et al.* (1989) |

TABLE 1 — *Continued*

| ORF[a] | Translation Start | Translation Stop[b] | Size (aa) | MW (kDa)[c] | Prom. type[d] | Function/feature(s) | Homolog(s)[e] | BLASTP score[f] | Amino acid identity[g] | Reference(s)[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| S126R | 118067 | 118882 | 271 | 31.5 | E | Unknown | VAC A37R<br>MC149R | 1E-21<br>4E-11 | 69/262 (26)<br>46/191 (24) | |
| S127L | 120206 | 118869 | 445 | 52.5 | ? | Type II CPD photolyase (PL) | *M. domestica* PL<br>MSV235 PL | 1E-125<br>1E-124 | 212/435 (48)<br>211/451 (46) | Kato *et al.* (1994)<br>Afonso *et al.* (1999) |
| S128L | 121081 | 120209 | 290 | 33 | ? | CD47-like transmemb. integrin assoc. protein | Human OA3-312<br>VAC A38L (?) | 3E-4<br>7.0 | 53/268 (19)<br>24/99 (24) | Campbell *et al.* (1992)<br>Sanderson *et al.* (1996) |
| S129R | 121224 | 121460 | 78 | 8.8 | E | Myristylprotein | VAC E7R (?) | 0.79 | 13/50 (26) | Martin *et al.* (1997) |
| S130R | 121545 | 121859 | 104 | 11.6 | ? | Unknown | | | | |
| S131R | 121931 | 122422 | 163 | 17.8 | L | Cu-Zn superoxide dismutase (SOD) | *O. cuniculus* SOD<br>VAC A45R | 2E-37<br>4E-06 | 80/150 (53)<br>35/199 (29) | Reinecke *et al.* (1988)<br>Smith *et al.* (1991b) |
| S132L | 122933 | 122406 | 175 | 20.6 | E | Unknown | MC085L (?) | 2.1 | 30/91 (32) | |
| S133R | 123023 | 124702 | 559 | 63.2 | L | ATP-dependent DNA ligase | VAC A50R | 1E-162 | 283/557 (50) | Parks *et al.* (1994) |
| S134R | 124813 | 130632 | 1939 | 219.8 | E | Unknown | MC035R | 0.0 | 411/944 (43) | |
| "S135R" | | | | | | (deleted in SFV, retained by MYX) | | | | Cameron *et al.* (1999) |
| "S136R" | | | | | | (deleted in SFV, retained by MYX) | | | | Cameron *et al.* (1999) |
| S137R | 131457 | 132386 | 309 | 35.8 | L | Unknown | VAC A51R | 8E-34 | 89/263 (33) | |
| S138L | 133285 | 132413 | 290 | 33.7 | E | $\alpha$ 2,3-sialyltransferase (ST) | MYX MST3N<br>*M. musculus* ST | 1E-132<br>3E-49 | 225/290 (77)<br>105/243 (43) | Jackson *et al.* (1999) |
| "S139R" | | | | | | (deleted in SFV, retained by MYX) | | | | Cameron *et al.* (1999) |
| S140R | 133842 | 135503 | 553 | 63.1 | ? | Kelch repeat | VAC A55R | 7E-65 | 166/499 (33) | |
| S141R | 135538 | 136209 | 223 | 24.4 | E | Fam. of surface antigen/receptor proteins | MYX MA56<br>*X. laevis* N-CAM | 2E-76<br>0.029 | 156/224 (69)<br>16/47 (34) | Jackson *et al.* (1999)<br>Tonissen and Krieg (1993) |
| S142R | 136203 | 137123 | 306 | 35.5 | E | S/T protein kinase | VAC B1R | 1E-75 | 138/290 (47) | Banham and Smith (1992)<br>Lin *et al.* (1992) |
| S143R | 137135 | 137839 | 234 | 28.1 | L | RING finger protein, localizes to viral factories | CPX D7R | 1E-19 | 60/218 (27) | Brick *et al.* (1998);<br>Upton *et al.* (1994)<br>Safronov *et al.* (1996) |
| S144R | 137878 | 138777 | 299 | 34.7 | ? | Complement control protein (VCP) precursor | VAC C3L | 1E-17 | 51/160 (31) | Kotwal and Moss (1998b) |
| S145R | | | | | | (omitted during sequence anotation) | | | | |
| S146R | 138784 | 139110 | 108 | 13.7 | E | Virulence factor, secreted protein | VAC N1L | 0.025 | 31/101 (30) | Kotwal and Moss (1988a)<br>Kotwal *et al.* (1989) |
| S147R | 139169 | 140032 | 287 | 33.5 | E | S/T protein kinase? | FPV protein kinase | 1E-18 | 66/259 (25) | Hertig *et al.* (1997) |
| S148R | 140100 | 142121 | 673 | 77.2 | E | Host range, ankyrin-like | VAC B18R | 2E-12 | 119/551 (21) | Mossman *et al.* (1996a)<br>Smith *et al.* (1991b) |
| S149R | 142123 | 143595 | 490 | 56.7 | E | Host range, ankyrin-like | VAC B4R | 8E-12 | 79/392 (20) | Howard *et al.* (1991)<br>Mossmann *et al.* (1996a)<br>Smith *et al.* (1991b) |
| "S150R" | | | | | | (deleted in SFV, retained by MYX) | | | | Cameron *et al.* (1999) |
| S151R | 144598 | 145599 | 333 | 38.3 | E | Serpin ("SERP-2") | VAC C12L | 5E-40 | 114/356 (32) | Petit *et al.* (1996) |

TABLE 1—*Continued*

| ORF[a] | Translation Start | Translation Stop[b] | Size (aa) | MW (kDa)[c] | Prom. type[d] | Function/feature(s) | Homolog(s)[e] | BLASTP score[f] | Amino acid identity[g] | Reference(s)[h] |
|---|---|---|---|---|---|---|---|---|---|---|
| "S152R" | | | | | | (deleted in SFV, retained by MYX) | | | | Cameron *et al.* (1999) |
| S153R | 146088 | 146693 | 187 | 23.1 | E | Unknown, RGD motif, may bind metals (see text) | SPV C7L<br>BHV4-IE1 | 2E-19<br>3E-10 | 47/158 (29)<br>27/82 (32) | Massung *et al.* (1993)<br>Cameron *et al.* (1999) |
| S154L | 147428 | 146784 | 214 | 24.2 | E | Unknown | VAC M2L | 1E-56 | 109/218 (50) | |
| S155R | 147511 | 148842 | 443 | 50.8 | — | Truncated duplicate of S009L, probably inactive | VAC C2L | 5E-25 | 90/376 (23) | |

*Note.* CPX, cowpox virus; FPV, fowlpox virus; MC, molluscum contagiosum virus (subtype-1); MSV, *Melanoplus sanguinipes* entomopoxvirus; MYX, myxoma virus; SHP, sheeppox virus; SPV, swinepox virus; VAC, vaccinia virus (strain Copenhagen unless otherwise noted).

[a] The open reading frame numbering system is described in the text. Several numbers were not used to maintain congruence with the myxoma gene notation.

[b] Including the last base of the stop codon.

[c] Predicted molecular weight for an unmodified polypeptide.

[d] E, early; I, intermediate; L, late.

[e] Although many poxviral homologs were often detected, normally only the vaccinia and molluscum contagiosum gene homologs are described where such genes exist. When no vaccinia and/or molluscum contagiosum homolog(s) were found, the closest alternative poxvirus and/or cellular homolog was identified. In cases where BLASTP scores exceed 0.1, synteny and amino acid identity were also used to identify homologs.

[f] BLAST expectation scores depend upon the size of the sequence database. These BLASTP scores were determined on July 21–23, 1999.

[g] Percentages shown in parentheses.

[h] See Goebel *et al.* (1990) and Senkevich *et al.* (1996) for a description of the vaccinia and molluscum contagiosum virus genes listed in Table 1.

[i] A 14.5L is a small vaccinia gene (53 aa, nucleotides 127,154–127,306) not annotated in M35027. A late promoter motif preceeds A14.5L and homologs are also encoded by variola (data not shown).

[j] Tblastn score determined August 25–27, 1999.

gene extending beyond the TIR junction and clearly preceded by an early poxviral promoter. Close examination of the two ORFs suggests that S155R is probably a pseudogene in which the N terminus has been disrupted by mutation. This is evidenced by the fact that the identity with S009L can be traced back from the putative ATG start codon to the TIR junction, where it disappears without encountering an in-frame coding element or an upstream promoter. Moreover, the missing portion of S155R encodes only part of the BTB/POZ domain encoded by S009L. Because this domain mediates protein–protein interactions (Bardwell and Treisman, 1994), it seems unlikely that the encoded protein would be functional were it to be expressed.

## Repetitive DNA

SFV contains only a single example of simple repetitive DNA. This is ~150 bp of DNA located immediately 3′ of the S149R gene and encoding ~30 copies of the pentanucleotide repeat 5′ -ATAGA- 3′ (nt. 143,592–143,746). The repeat is located within a 1-kb ORF-free gap in the coding sequence from which the SFV homolog of the myxoma M150R gene also appears to have been deleted (see below). The function of the ATAGA repeat is unknown, although BLASTN searches also detected identical repeats in clones of varied metazoan origin. The repeat is not detected in myxoma virus or in any other poxviruses.

## Transcriptional regulation

Conserved sequence motifs were found immediately upstream of many SFV genes, which closely resembled poxviral early and/or late promoter elements. Figure 2 presents histograms illustrating the two conserved motifs, and the relative probability of encountering particular bases at different positions within these two sequence motifs. Although it is not shown in Fig. 2, the TATAAATG late motif also was preceded by a highly A/T rich upstream consensus motif again characteristic of Chordopoxviral late promoters. Using these consensus sequences, we can tentatively identify those genes that are most likely regulated by early, late, or hybrid early/late promoters (Table 1). Intermediate promoters are more difficult to identify due to the limited number encoded by Chordopoxviruses. We do find sequences resembling an intermediate promoter immediately upstream of the

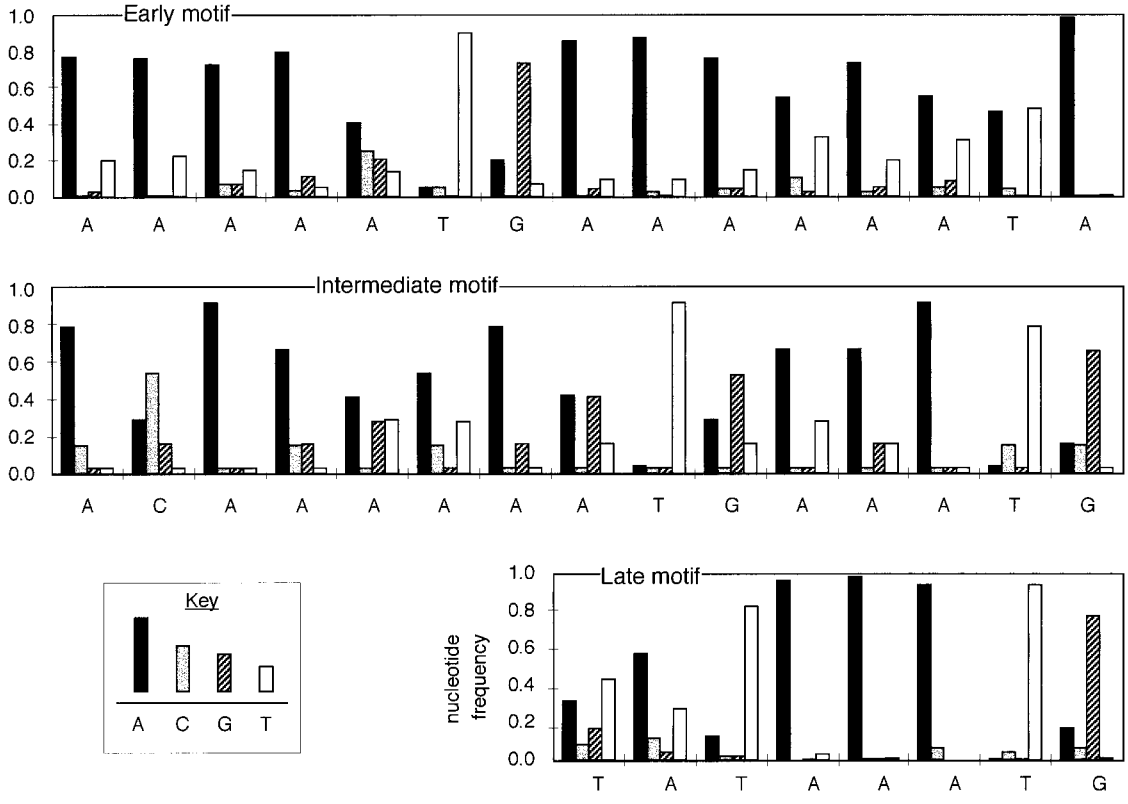


FIG. 2. SFV promoter motifs. Three types of promoter motifs were found immediately upstream of many SFV open reading frames along with a fourth, AT-rich pattern, usually found upstream of the TATAAATG late motif (not shown). The histograms illustrate the frequency of occurrence of each of the four bases within these three conserved motifs. The early and late promoter consensus patterns are found upstream of ~70 and 85 genes, respectively, and the statistics derive from an analysis of these two gene families. The intermediate motif was discovered through an analysis of seven SFV genes whose VAC and MCV gene homologs are thought to be under intermediate promoter control.

seven SFV genes that are homologs of known or putative VAC and MCV intermediate genes [S053R (VAC G8R), S075R (VAC H7R), S087L (VAC D12L), S089L (VAC A1L), S090L (VAC A2L), S095L (VAC A6L), S108R (VAC A18R)] (Fig. 2). However, the consensus is not sufficiently robust to identify other examples with any degree of confidence.

We also noted that a $T_5$NT motif is usually found near the 3′ end of putative SFV early genes. The same $T_5$NT early-transcription-termination motif appears to be randomly distributed throughout the coding regions of putative late genes. This distribution pattern was first noted in vaccinia virus (Yuen and Moss, 1987) and has been used as a sequence clue that helps to differentiate early and late poxvirus genes. However, we noted numerous exceptions to the rule that $T_5$NT motifs are not expected near the 5′ ends of early genes. The S033R, S034L, S064R, S065R, S068R, S079R, S080R, S085R, S111R, S114R, and S117R genes all encode early or hybrid early/late promoters (Fig. 2) in addition to a "precocious" $T_5$NT motif. In most such cases, the motif is located a few hundred nucleotides downstream of the probable transcription initiation site. There are no other obvious associated genetic features, and it seems unlikely that we might have erroneously assigned the promoters, since most of these genes encode typical poxvirus early proteins (replicative enzymes and RNA polymerase components). It is difficult to say whether these genes are subject to a special form of transcription regulation or whether the elements are inactive due to sequence context effects. However, it is apparent that one cannot use the distribution of $T_5$NT motifs to predict Leporipoxvirus gene expression kinetics with confidence.

### Similarity among SFV, vaccinia, and molluscum contagiosum proteins

When compared with other fully sequenced non-Leporipoxviral genomes, the individual genes of SFV most closely resemble genes encoded by vaccinia and variola viruses. When these same gene products are compared with those encoded by MCV and MSV, the SFV proteins produced alignments displaying consistently lower alignment scores. This point is illustrated in Fig. 3 where we summarize those SFV genes which generated the 20 highest BLASTP alignment scores. The preponderance of putative transcription and replicative proteins amongst this selected set of SFV genes is also noteworthy, as are the many proteins required for viral assembly. Figure 3 also illustrates a singular exception to the rule that highly conserved SFV genes most closely resemble their Or-

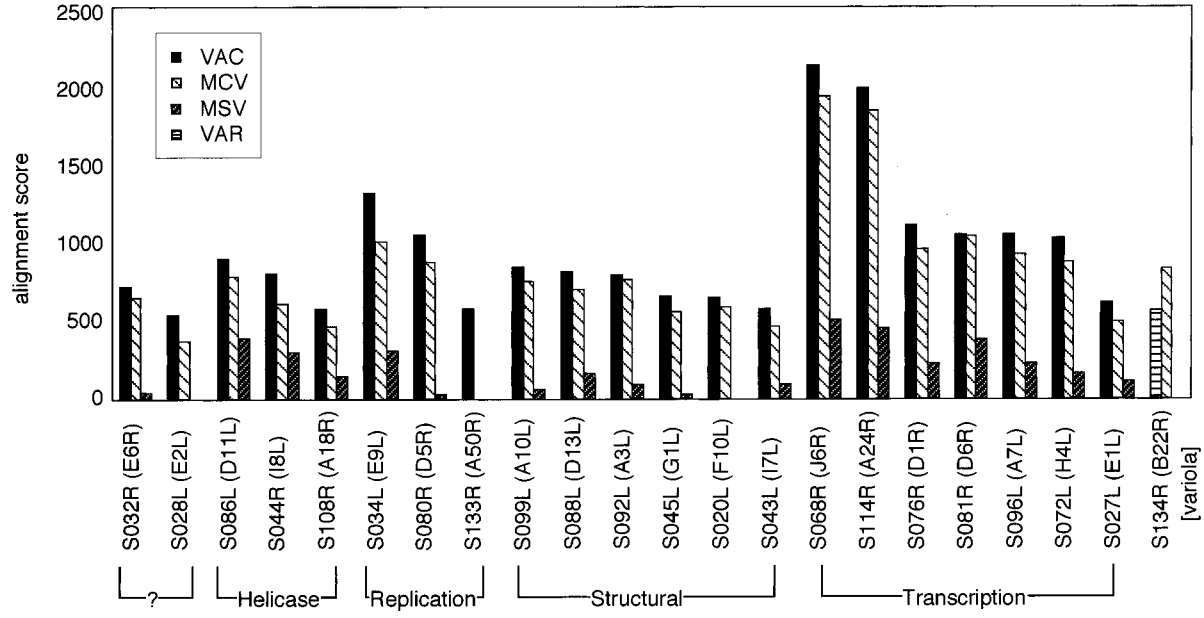


FIG. 3. Conservation of amino acid sequence between SFV, VAC, MCV, and MSV gene products. BLASTP alignment scores were calculated for all of the proteins encoded by SFV and used to identify the 20 gene products showing the highest conservation of amino acid sequence over the greatest peptide distance. The identity of the vaccinia virus gene homolog also is listed to aid in the identification of each SFV gene product. No MSV homologs of S028L, S133R, S020L, or S134R can be detected, or a MCV homolog of S133R (DNA ligase). Most VAR alignment scores are essentially identical to VAC scores and have been omitted for brevity. However, S134R encodes a homolog of the VAR B22R and MC035R gene products, a gene not encoded by vaccinia virus.

thopoxviral homologs. The exception is S134R, which encodes the largest protein in the SFV genome (220 kDa). The S134R gene encodes a transmembrane protein of unknown function and more closely resembles the MC035R rather than the VAR B22R gene product. Moreover there is no VAC homolog of this gene.

The closer resemblance to Orthopoxviridae also can be illustrated by a more comprehensive comparison of the similarity between less highly conserved genes. Figure 4 illustrates a pairwise comparison of BLASTP expectation scores (E-values) obtained through alignments of SFV, VAC, MCV, and MSV gene homologs. A clear trend is noted wherein the logarithm of the expectation scores more closely favours vaccinia over MCV or MSV gene products. Parenthetically this approach provides a method of determining whether viruses such as MCV or MSV really are missing certain genes or whether the presence of the homolog has been obscured by gene-specific patterns of amino acid sequence divergence. We note that points lying along the abscissa indicate SFV gene homologs encoded by vaccinia virus, but apparently absent in MCV and/or MSV. Clearly the greater the distance from the regression line, the greater the statistical likelihood that the gene is truly absent. A more formal analysis of this methodology will be detailed in a later communication.

### Vaccinia, VAR, and MCV genes absent in SFV

Most of the SFV genes located within the genomic core interval bounded by S012L and S142R appear to have syntenic VAC, VAR, and/or MCV homologs. However, there are a few "common" genes whose absence from the SFV genome serves to further delimit the minimal gene complement of Chordopoxviruses (Table 2). One of these genes has recently been shown to be mutable in modified vaccinia strain Ankara (Antoine *et al.,* 1998) and thus the absence of F11L/C15L/MC018L gene homologs is not entirely surprising. SFV also lacks a homolog of the gene encoding a steroid dehydrogenase/isomerase (A44L/A47L/MC152R), although this conserved virulence factor is also not required for growth in culture (Moore and Smith, 1992).

Several absent genes suggest that SFV may not utilize the same infection route(s) as do other Chordopoxviruses. SFV appears to lack homologs of the A-type inclusion proteins. These genes have been shown to be mutable in a variety of Orthopoxviruses, but most viruses produce at least a truncated form of the protein that may promote infection of phagocytic cells by intracellular mature virions (IMV) (Ulaeto *et al.,* 1996). The complete absence of any SFV gene homolog is curious and predicts that Leporipoxvirus infections might produce relatively small amounts of IMV. It is also noteworthy that SFV lacks an A36R/A38R/MC146R gene homolog. Even though the gene is not essential for growth, disruption of VAC A36R creates a small plaque phenotype and reduces extracellular enveloped virus production (EEV) three- to fivefold (Parkinson and Smith, 1994). Recent studies have shown that A36R gene products are found on the surface of intracellular enveloped viruses (IEV)

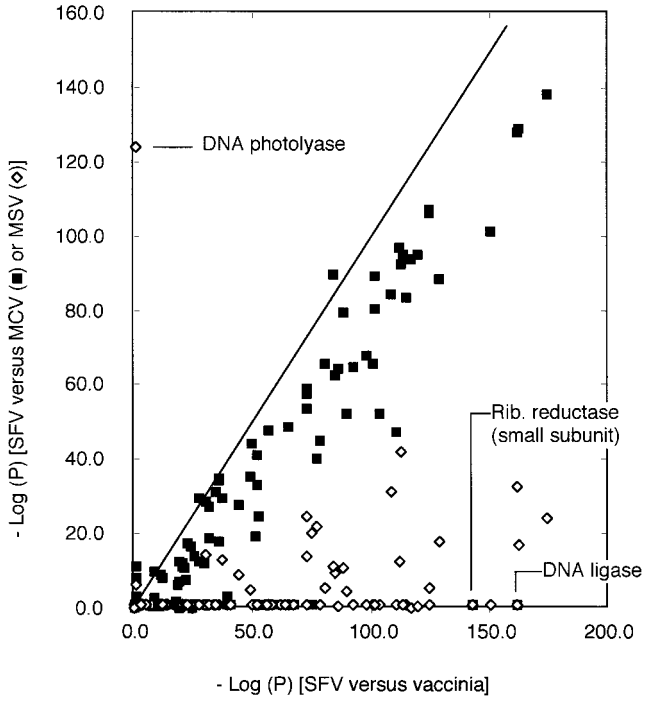


FIG. 4. Similarities between SFV, VAC, MCV, and MSV genomes. A BLASTP score was calculated for each SFV gene *vs* its putative VAC-, MCV-, and/or MSV-encoded gene homolog. The negative logarithm of the resulting SFV-VAC score (E-value) then was plotted on the abscissa *vs* the corresponding MCV (■) or the MSV (◇) score. The data points would be expected to distribute along the line where $\log_{10}(x) = \log_{10}(y)$ were SFV no more closely related to vaccinia than to MCV or MSV. However, most comparisons favour VAC over MCV (i.e., SFV genes more closely resemble vaccinia genes) and greatly favour VAC over MSV. The SFV DNA photolyase gene, S127L, is an obvious exception to this trend. MSV235 encodes a homolog of S127L (Fig. 5), but no such gene seems to be encoded by VAC or MCV.

where they serve to nucleate actin and thus facilitate EEV dissemination. Because SFV encodes homologs of most of the other proteins thought to be required for vaccinia IEV assembly (S022L/F13L, S115L/A27L, S121R/A33R, S122R/A34R, but probably not B5R) (Vazquez *et al.,* 1998; Rottger *et al.,* 1999), it seems likely that although SFV can form intracellular enveloped virions, the virus is not subsequently spread by a route dependent upon actin-driven EEV production. This is certainly consistent with the very small plaques produced by the virus. Experience suggests that most infectious SFV particles are cell associated, but it is presently unknown whether these are IEV, or cell-associated enveloped viruses.

Two additional genes are conserved among VAC, VAR, and MCV even though there appear to be no SFV-encoded homologs. One is A31R/A34R/MC138R and no mutations have yet been shown to map to the gene. Synteny predicts that S119L might encode a homolog of these genes, but the SFV peptide is too small (39 *vs* 124 amino acids) and shows little resemblance to the Orthopoxviral and Molluscipoxviral proteins. SFV also lacks a homolog of the E11L/E11L/MC041L gene. Vaccinia E11L encodes a late protein that is encapsidated within the vaccinia virus core, and a conditionally lethal temperature-sensitive mutation has been mapped to the gene (Wang and Shuman, 1996). What biological function is served by VAC E11L and its homologs is uncertain.

Another peculiar feature of the SFV genome is the partial absence of most of the genes involved in nucleotide biosynthesis. In this respect the virus lies midway between vaccinia and variola viruses, which encode several such genes, and MCV, which encodes none at all. For example, SFV seemingly lacks thymidylate and guanylate kinases (VAC A48R and VAC A57R, respectively) while still encoding a thymidine kinase (S061R) (Upton and McFadden, 1986). It also lacks the putative cytidine kinase encoded by fowlpox virus (Koonin and Senkevich, 1993). SFV also encodes only the smaller R2 subunit of the ribonucleotide reductase (S015L = VAC F4L), presumably rendering it dependent upon host cell functions for the synthesis of DNA biosynthetic precursors. A similar situation is observed in human cytomegalovirus except that this virus encodes only the larger R1 subunit (Bankier *et al.,* 1991). Why SFV should retain only one-half of an $\alpha_2\beta_2$ protein is unclear. It has been observed that heterodimers composed of a vaccinia R1 subunit and mouse R2 subunit are catalytically active (Hendricks and Mathews, 1998), and it is quite possible that SFV has

TABLE 2

Selected VAC, VAR, and MCV Genes Missing from the SFV Chromosome

| Virus | | | |
|---|---|---|---|
| VAC | VAR | MCV | Predicted function or properties |
| F11L | C15L | MC018L | Non-essential (Antoine *et al.,* 1998) |
| E11L | E11L | MC041L | Essential virion component (Wang and Shuman, 1996) |
| O2L | Q2L | — | Glutaredoxin 1 (Ahn and Moss, 1992a) |
| I4L | K4L | — | Ribonucleotide reductase (large subunit) (Tengelsen *et al.,* 1988) |
| — | — | MC066L | Glutathione peroxidase (Shisler *et al.,* 1998) |
| A25L | A26L | MC130L | A-type inclusion protein (Funahashi *et al.,* 1998) |
| A26L | A30L | MC131L | A-type inclusion protein (Funahashi *et al.,* 1988) |
| A31R | A34R | MC138R | Function unknown |
| A36R | A38R | MC146R | Intracellular enveloped virion protein (Rottger *et al.,* 1999) |
| A44L | A47L | MC152R | Steroid dehydrogenase/ isomerase (Moore and Smith, 1992) |
| A48R | J2R | — | Thymidylate kinase (Smith *et al.,* 1991b) |
| A57R | J8R | — | Guanylate kinase (Smith *et al.,* 1991b) |

TABLE 3

SFV Genes Exhibiting Shared Structural Motifs

| Family | Genes | Comment[a] |
|---|---|---|
| Ankyrin-like repeat | S005L/R | 5 ankyrin-like repeats |
| | S148R | 5 ankyrin-like repeats |
| | S149R | 4 ankyrin-like repeats |
| Kelch repeat | S006L/R | 4 kelch motifs plus BTB/POZ domain |
| | S008L/R | 4 kelch motifs plus BTB/POZ domain |
| | S009L | 4 kelch motifs plus BTB/POZ domain |
| | S0014L | 4 kelch motifs plus BTB/POZ domain |
| | S140R | 6 kelch motifs plus BTB/POZ domain |
| | S155R | 4 kelch motifs plus a portion of BTB/POZ domain |
| C7L-like proteins | S062R | VAC C7L homolog |
| | S063R | VAC C7L homolog (distant) |
| | S064R | VAC C7L homolog |

[a] The number of repeat motifs varies depending upon cut-off score. We set E = 10 when searching the Pfamm HMM (v. 4.1) database.

evolved to exploit the opposite arrangement consisting of the SFV R2 and host R1 subunits (C. Mathews, personal communication). If this was the case, one would expect the small subunit to be the one retained by SFV because this is the subunit that, in vaccinia virus, permits a virus-specific interaction with the replicative single-strand DNA binding protein (Davis and Mathews, 1993). The DNA sequence suggests that S015L encodes a catalytically active protein because the gene still encodes the residues required to coordinate a diferric iron centre as well as the tyrosine involved in stable free radical formation ($Y_{110}$) and all, except the very last, of the residues postulated to transport electrons from the centre of the R2 dimer to the nucleotide-binding R1 dimer (Jordan and Reichard, 1998). If S015L does encode an active protein, the electron donor remains to be identified because SFV lacks a gene homologous to VAC O2L. The O2L-encoded glutaredoxin 1 protein is thought to donate electrons to VAC ribonucleotide reductase (Rajagopal *et al.,* 1995) and yet the only glutaredoxin encoded by SFV (S048L) most closely resembles the glutaredoxin 2 protein encoded by VAC G4L.

## SFV gene families

Several SFV genes share common structural features that permit their assignment to viral gene families. These are summarized in Table 3. Perhaps most striking are the number of encoded proteins displaying ankyrin-like and kelch motif repeats. Also noteworthy are the three homologs of VAC C7L encoded by SFV genes S062R, S063R, and S064R. Nothing is known concerning the function of these SFV genes, but the C7L gene product is required for vaccinia replication in certain cell types and therefore has been categorized as a host-range function (Oguiura *et al.,* 1993). SFV has a very limited host range, growing only on rabbit and a few selected primate cells, and it is thus somewhat surprising that it encodes such an abundance of putative host-range genes.

## Leporipoxvirus genes not encoded by other poxviruses

A Leporipoxvirus-specific SFV gene is difficult to define because novelty will depend upon the size of the poxvirus sequence database and upon the cut-off point for alignment scores. Futhermore, alignment scores are dependent upon protein size, and one can miss homologous genes if they happen to be very small. [This point is clearly illustrated by comparing BLASTP and amino acid identity scores for ORFs S008L and S008.1L (Table 1)]. We have taken a conservative approach that uses a combination of semiquantitative factors to decide whether a gene should be considered unique to Leporipoxviruses. These factors include the BLASTP expectation score (which should normally exceed 0.01–0.1 except in the case of very small proteins) and no evidence of a possible syntenic relationship between the SFV gene and other poxvirus genes. The degree of amino acid identity also was taken into consideration, with amino acid identities exceeding 20–25% being considered probable evidence of homology. By these criteria SFV and myxoma encode a number of genes seemingly unique to Leporipoxviruses that are summarized in Table 4. Several small hypothetical genes were discovered whose size renders similarity searches problematical (Table 4). A disproportionate number of these new proteins are predicted to encode transmembrane helices including S023R, S119L, S125R, and S132L, but little else is known of their function. S130R encodes an 11.6-kDa

TABLE 4

Novel Leporipoxvirus Genes

| SFV gene | Predicted or known encoded function |
|---|---|
| S013L | Resembles DNA binding domain of HIN-200 protein family |
| S017L | Possibly a homolog of VAC F7L |
| S023R | Unknown, predicted transmembrane helix |
| S119L | Unknown, predicted transmembrane helix |
| S125R | Unknown, predicted transmembrane helix |
| S127L | Type II CPD photolyase |
| S130R | Unknown |
| S132L | Unknown, predicted transmembrane helices |
| S138L | $\alpha$ 2,3-sialyltransferase (Jackson, Hall, and Kerr, 1999) |
| S141R | Ig-like protein superfamily, predicted transmembrane helices |

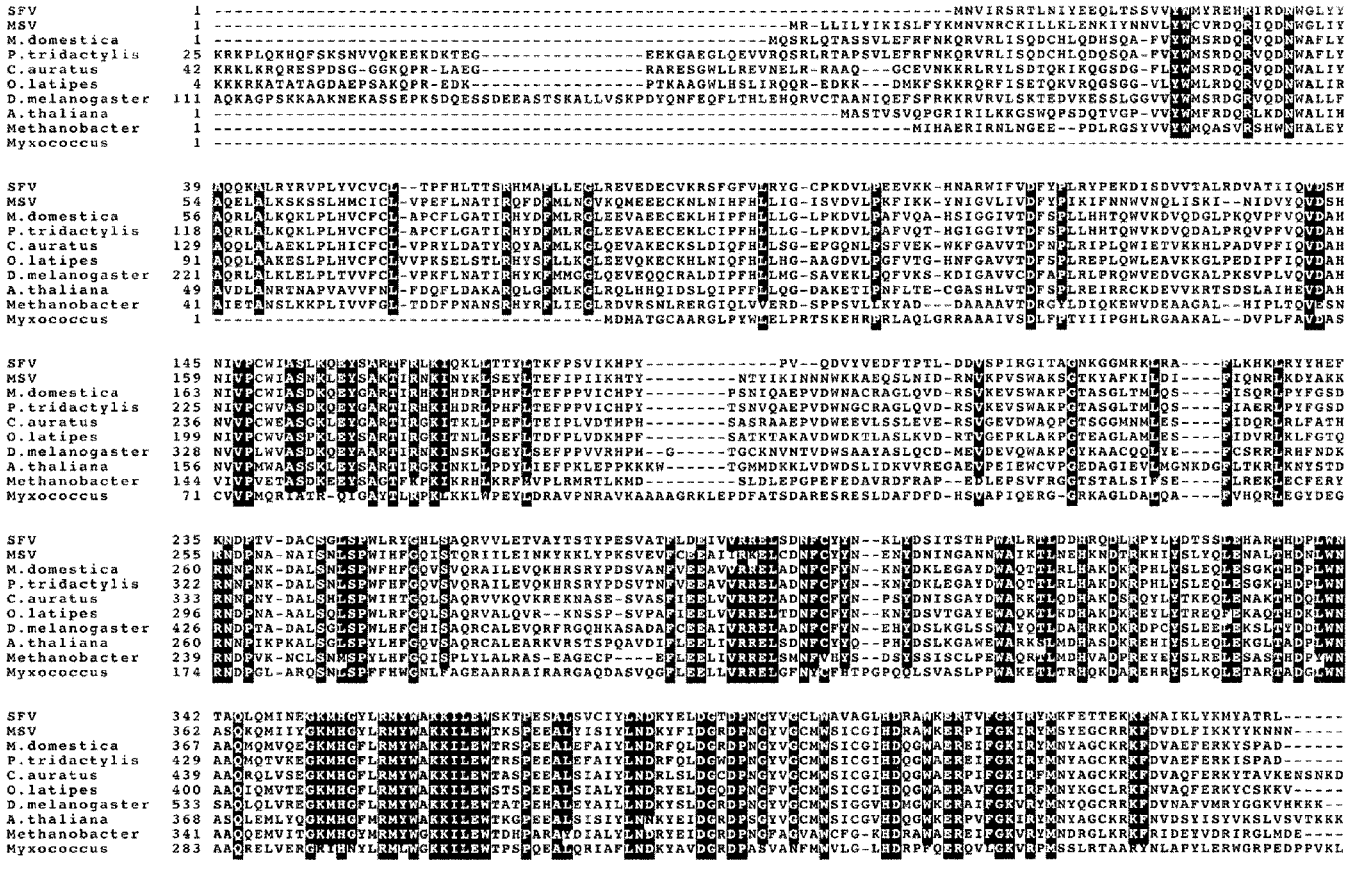


FIG. 5. ClustalW alignment of the S127L-encoded protein with other Type II CPD photolyases. GenBank protein sequence IDs were: g4049783 (MSV235), g1079459 (*Monodelphis domestica*), g1401039 (*Potorous tridactylus*), g464378 (*Carassius auratus*), g1401038 (*Oryzias latipes*), g1079066 (*Drosophila melanogaster*), g1617219 (*Arabidopsis thaliana*), g1197525 (*Myxococcus xanthus*), and g639674 (*Methanobacterium thermoautotrophicum*). Black boxes indicate identical residues found at ≥9 sites amongst the 10 aligned proteins.

protein displaying no significant similarity to any other protein in the sequence databases.

The S013L gene encodes a small protein (12,729 Da, pI = 7.91) whose expression appears to be regulated by a canonical poxvirus early promoter. The gene product resembles the N-terminal DNA-binding portion of proteins belonging to the hemopoietic IFN-inducible nuclear 200-amino acid repeat (HIN-200) protein family (Dawson and Trapani, 1995, 1996). Expression of these proteins can be induced by interferons $\alpha$ and $\gamma$, and one protein family member, IFI16, has been shown to be a transcriptional repressor that may regulate myelopoiesis (Johnstone *et al.,* 1998). A interesting feature of S013L is that it appears to have lost the 200 amino acid repeats that are required if IFI16 is to repress transcription but still encodes a putative SV40 T-antigen-like nuclear localization signal (PKKQRNR) near the C terminus (Mattaj and Englmeier, 1998). Although the function of S013L is uncertain, its structure suggests that the encoded protein could compete with the activity of certain transcriptional repressors and perhaps interfere in the differentiation of hemopoietic cells.

Perhaps the most unusual protein encoded by Leporipoxviruses is the S127L gene product. S127L encodes a 52.5-kDa protein that is clearly homologous to proteins belonging to the family of type II cyclobutane pyrimidine dimer (CPD) photolyases (Fig. 5). Photolyases catalyze a light-dependent reaction that returns CPD adducts back to their component pyrimidines and thus reverses the DNA damage caused by ultraviolet light. Although a similar gene is encoded by MSV (Afonso *et al.,* 1999), this is the first DNA repair gene of its kind found to be encoded by a Chordopoxvirus or, indeed, any mammalian virus. The gene is particularly curious in that whilst marsupials such as the common opossum (*Monodelphis domestica)* are known to encode photolyases (Yasui *et al.,* 1994), no placental mammal has yet been shown to have retained the gene. Because it is generally believed that poxviruses originally acquired most genes from their hosts, this discovery raises interesting questions concerning the evolutionary history of a New World virus now thought to infect only rabbits. Preliminary experiments show that the gene can complement an *Escherichia coli phr* (photolyase deficient) mutation *in vivo* (D. Willier, M. Moon, and D. Evans, unpublished data) and imply that Leporipoxviruses can repair the damage


FIG. 6. ClustalW alignment of the S131R-encoded protein with bovine and vaccinia superoxide dismutases. GenBank protein sequence IDs were: g134601 (bovine Cu/Zn SOD) and g134630 (VAC A45R gene product). The bovine SOD active site arginine is residue $R_{142}$.

caused by ultraviolet light exposure either before and/or during viral infection.

Two additional SFV genes are homologous to previously identified myxoma virus genes, but Leporipoxviruses otherwise appear to be the only poxviruses encoding such genes. The S138L gene is predicted to encode a 34-kDa $\alpha$ 2,3-sialyltransferase nearly identical to the myxoma enzyme recently characterized by Jackson and colleagues (Jackson *et al.,* 1999). SFV sialyltransferase presumably catalyzes an identical reaction and likewise promotes viral virulence. The S141R gene product also has a previously identified myxoma gene homolog (MA56) (Jackson *et al.,* 1999). S141R encodes a 24-kDa protein that is predicted to contain two transmembrane helices and resembles the immunoglobulin-like domains of a large family of eukaryotic cell-surface proteins. Further commentary on the possible function of these two Leporipoxvirus gene products can be found in the accompanying paper (Cameron *et al.,* 1999).

### Other unusual genes—SFV superoxide dismutase (S131R) and S153R

Although all of the sequenced Chordopoxviruses encode genes resembling some type of superoxide dismutase (SOD), the SFV-encoded superoxide dismutase homolog is peculiar in just how closely it still resembles mammalian cytoplasmic Cu/Zn superoxide dismutases. Figure 6 shows an alignment of the SFV gene product along with the proteins encoded by vaccinia virus (Smith *et al.,* 1991b) and by cattle (Steinman *et al.,* 1974). A 1.9 Å high-resolution X-ray crystal structure of bovine erythrocyte SOD provides insights into the structure of these proteins (Rypniewski *et al.,* 1995). The SFV gene encodes a nearly intact SOD, whereas the vaccinia A45R gene product has lost portions of the protein encoding all of the bovine protein's Zn-binding domain plus several small surface peptide loops (Fig. 6). Still retained by both proteins are many of the glycine residues which, in the crystal structure, form the SOD dimer interface. Thus the poxvirus proteins are potentially capable of forming heterodimers with cellular SOD or of complexing with a cellular copper chaperone (Casareno *et al.,*1998). Closer examination of the SFV gene suggests that while SFV SOD must have some biological function, the protein is unlikely to still catalyze superoxide dismutation. This is suggested by an R-to-V substitution at the SOD active site (residue $V_{154}$ in the SFV gene), and by preliminary experiments which have thus far failed to detect any residual superoxide dismutase activity in either vaccinia or SFV recombinant proteins (A. Winter, M. Moon, and D. Evans, unpublished results). Why so many poxviruses encode seemingly "inactive" superoxide dismutase homologs remains an intriguing and yet elusive question.

S153R encodes a 23-kDa gene that resembles swinepox C7L and a portion of some gamma herpes virus IE1 proteins (Table 1). The protein encodes a highly acidic tail (pI = 4.5) as well as an RGD cell-adhesion motif. The alignment between S153R and C7L spans most of the two genes, although the RGD motif and C-terminal acidic tail are unique features of S153R (data not shown). A common property of S153R and C7L is that both may be metal-binding proteins. For example S153R encodes an imperfect zinc-finger motif (Prosite PDOC00449), and it is this region of the protein that aligns with a zinc-finger motif encoded by a family of alternatively spliced herpes virus immediate-early proteins. C7L encodes a similar cysteine-rich motif characteristic of metallothioneins (Prosite PDOC00180). What function might be served by S153R is uncertain.

### Similarities and differences between Shope fibroma and myxoma viruses

A later publication will focus on the relationship between these two viruses in greater detail. At this point, we will simply highlight some of the more obvious genetic rearrangements and speculate as to the function of some of the SFV genes that have been subjected to these mutational processes. The probable function of genes found in myxoma virus, but seemingly mutated in SFV, is more appropriately discussed in the accompanying paper (Cameron *et al.,* 1999).

SFV and myxoma viruses encode a nearly identical

TABLE 5

Genetic Differences between SFV and Myxoma Viruses

| SFV gene | Myxoma gene | SFV arrangement | Myxoma gene properties[a] |
|---|---|---|---|
| — | M000.5L/R | No trace of ORF | — |
| S008.1L/R | M008.1L/R | Fragmentary remains | — |
| S009L/S155R | M009L | Partially duplicated | Single gene |
| — | M135R | Fragmentary remains | — |
| — | M136R | Fragmentary remains | — |
| — | M139R | Fragmentary remains | — |
| — | M150R | Fragmentary remains | — |
| — | M152R | Fragmentary remains | — |
| S153R | M153R/M153.1R | SPV C7L homolog | Probably disrupted by frameshift |
| S008.2L/R | M156R | Duplicated gene | Single gene |

[a] Myxoma gene properties are described in the accompanying paper (Cameron *et al.,* 1999).

complement of homologous genes. The similarity is sufficiently high that it was used in our analysis of the two viruses to differentiate start codons from fortuitous in-frame ATG codons, identify frameshifting sequence errors, and detect genes encoding very small peptides. It also permitted using a single common system of gene nomenclature. Despite this high degree of sequence conservation, SFV and myxoma are still clearly distinct viruses as judged by the criteria currently used to categorize poxvirus "species" (Douglas and Dumbell, 1996). This can be demonstrated by examining phylogenetic trees constructed using the sequence of such widely distributed poxvirus proteins as the thymidine kinase. Such phylogenies show that there is a greater degree of sequence divergence between SFV and myxoma than between most Orthopoxviruses (data not shown).

Closer inspection of the two genomes detects a few gene rearrangements and a number of genes deleted in SFV relative to myxoma (Table 5). The terminal inverted repeats (TIR) encode several of these changes. First, SFV lacks a homolog of a small gene encoded at the very ends of the myxoma genome about which nothing is yet known (M000.5L/R). Myxoma also encodes a 41,557-Da serpin (M008.1L/R, SERP-1) (Upton *et al.,* 1990a) of which only 6356 Da of fragmentary remains can still be detected in SFV (S008.1L/R). Both SFV and myxoma still encode divergent homologs of the VAC K3L gene (S008.2L/R and M156R, respectively), but the SFV gene is duplicated within the TIR whereas myxoma (like vaccinia) encodes only a single copy of the gene located immediately adjacent to the boundary with the myxoma right TIR (Cameron *et al.,* 1999). The function of these Leporipoxvirus K3L gene homologs is unknown. However, the VAC protein resembles the N-terminal portion of eIF-2$\alpha$ and inhibits interferon activity by preventing phosphorylation of eIF-2$\alpha$ (Beattie *et al.,*, 1991; Carroll *et al.,* 1993). A somewhat similar rearrangement also pertains to S009L/S155R. These genes are partially duplicated in SFV, as discussed previously, whereas myxoma encodes only a single gene homolog located immediately adjacent to the left TIR border (M009L) (Cameron *et al.,* 1999).

Although these telomeric rearrangements appear complex, they can be fairly easily rationalized. We suggest that the TIRs of a hypothetical "ancestral" Leporipoxvirus once most closely resembled that of SFV, except that these terminal inverted repeats were longer than modern SFV TIRs and still encoded two complete copies of the genes now called S/M009L, S008.2L/R, and S/M008.1L/R. SFV, strain Kasza, has likely undergone a partial deletion of the hypothetical ancestral "S009R" gene (leaving a pseudogene now called S155R) and a second excision of most of the S/M008.1L/R gene homolog by a well documented, but still poorly understood, mirror-deletion mechanism (McFadden and Dales, 1979). Myxoma, strain Lausanne, has probably suffered a complete deletion of the ancestral "M009R" gene, followed by a deletion of the ancestor of the gene now called S008.2L from the left TIR. The resulting myxoma TIR now encodes only one copy of M009L, and M156R actually derives from a gene originally syntenic with S008.2R. These genetic models are outlined in Fig. 7.

Elsewhere in the SFV genome we find deletions that seem to have eradicated a further five genes relative to myxoma virus and a frameshift in myxoma that seems to have disrupted that virus's homolog of an SFV gene. Most of these rearrangements are located toward the right end of the virus, which seems to be an unusually dynamic poxvirus genetic region. The missing genes are the SFV homologs of myxoma M135R, M136R, M139R, M150R, and M152R. That these are SFV deletions, and not myxoma gene insertions, is suggested by the presence of DNA fragments in SFV that still exhibit patchy DNA homology to the genes retained by myxoma virus. No simple pattern of repeats or other sequence motifs can be discerned at the putative break points in the myxoma genome, but sequence drift during the course of SFV and myxoma evolution may have long since erased such features (data not shown). This is in contrast to the

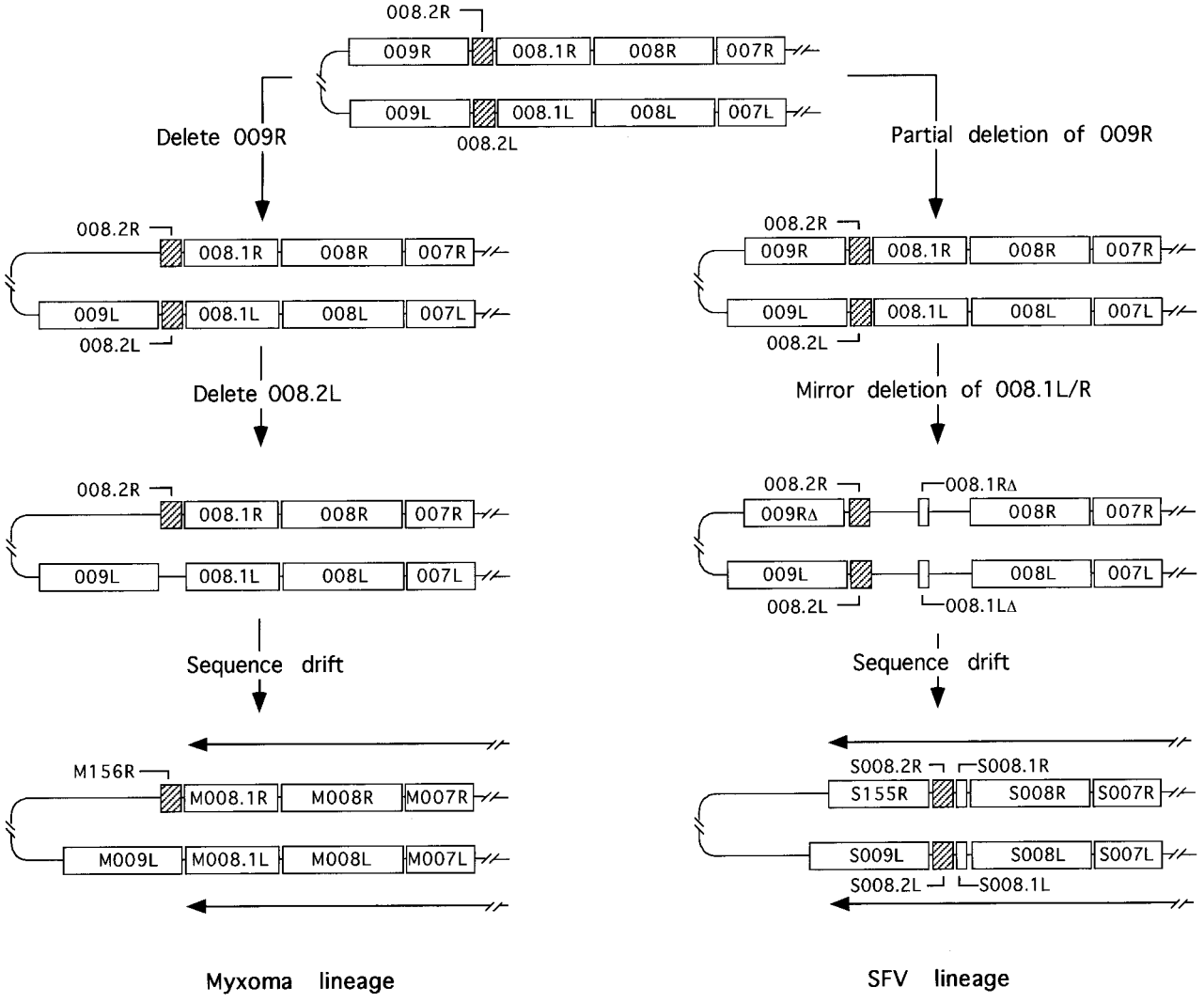


FIG. 7. Hypothetical rearrangements underlying the structure of SFV and myxoma telomeres. SFV and myxoma are proposed to have derived from a common ancestor strain that once encoded a longer TIR (arrows) and a greater complement of duplicated genes. Sequential deletions coupled with sequence drift have obscured the hypothetical common origin of the genes now called M156R and S008.2L/R (hatched boxes). The precise order in which these events might have taken place cannot be predicted with certainty.

small deletions observed in vaccinia and variola that sometimes still show evidence of having occurred between small directly repeated sequences (Shchelkunov and Totmenin, 1995). As described above, the S153R gene encodes a protein of unknown function that resembles swinepox virus C7L as well as a portion of genes encoded by some herpes viruses. A frameshift appears to have split the myxoma homolog of S153R into two open-reading frames, somewhere near the middle of the gene.

Inspection of the genes altered in SFV and myxoma viruses provides only limited insights into the altered virulence of SFV compared with myxoma. The most obvious deficiency is that SFV seems to be missing the two serpin-like genes encoded by M008.1L/R and M152R, one of which (M008.1) is known to be important for virulence (Upton *et al.,* 1990a; Cameron *et al.,* 1999). The biological and genetic properties of the other missing genes are less obvious. One of the mutated genes ("S135R") resembled a portion of the VAC B19R putative interleukin-1 receptor (Smith and Chan, 1991), two others ("S136R" and "S139R") resembled VAC A52R, and "S150R" appears to have once encoded several ankyrin-like repeats as do three other genes still retained by SFV (Table 3) (Cameron *et al.,* 1999). The impact these genetic alterations might have on Leporipoxvirus virulence is discussed further in the accompanying paper (Cameron *et al.,* 1999).

In conclusion, the DNA sequence of SFV provides further insights into the evolutionary history and minimal essential gene complement of Chordopoxviruses. Through comparative analysis of the genome sequences of myxoma and Shope fibroma viruses we expect to gain insights into how the two viruses can cause such dis-

tinctly different diseases. Furthermore this work has identified all of the genes that are collectively responsible for catalyzing high-frequency recombination and *trans*-replication in SFV-infected cells. The discovery of a novel DNA repair gene and a curious new superoxide dismutase homolog also will provide fascinating insights into how poxviruses avoid the genotoxic effects of ultraviolet light and protect themselves from the DNA, lipid, and protein-damaging effects of reactive oxygen species.

## MATERIALS AND METHODS

### SFV, myxoma virus, and other genome sequences

The completed nucleotide sequence of SFV has been submitted to GenBank and assigned Accession No. AF170722. The sequence of myxoma virus can be retrieved from GenBank using Accession No. AF170726 (Cameron *et al.,* 1999). Where we have compared gene sequences "vaccinia" refers to the Copenhagen strain (GenBank ID M35027), "variola" refers to variola major strain Bangladesh-75 (GenBank ID L22579), MCV refers to Molluscum contagiosum virus subtype 1 (GenBank ID U60315), and MSV refers to Melanoplus sanguinipes entomopoxvirus (GenBank ID AF063866), unless otherwise indicated.

### Materials, cell, and virus culture

SIRC cells and the Kasza strain of SFV (Kasza, 1974) were originally obtained from the American Type Culture Collection. SIRC cells were grown at 37° in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 1% nonessential amino acids and 10% fetal bovine serum (CanSera) in a 5% $CO_2$ atmosphere.

### Virus purification and DNA extraction

SFV virions were isolated essentially as described (Dales and Mosbach, 1968). Briefly, SIRC cells were infected with virus at a m.o.i. of 1 and incubated for 3 days at 37°C, and cell-associated virus particles ($\sim 1.5 \times 10^{10}$ plaque forming units) were extracted by Dounce homogenization at 4°C in 80 mL of 1:10 diluted phosphate-buffered saline. The cell debris were removed by low-speed centrifugation (1000 *g* for 10 min at 4°C), resuspended in 40 mL of serum-free DMEM, sonicated, and recentrifuged, and the two supernatents pooled. The virus was recovered by centrifugation (7000 *g* for 10 min at 4°C), treated with trypsin (15 min at 37°C), diluted to 10 mL with serum-containing DMEM, pelleted again, and resuspended in 2 mL of buffer containing 0.25M sucrose and 10 mM Tris—HCl pH 8.5. Viral particles then were applied to the top of a 20–50% (w/v) potassium tartrate gradient, and centrifuged at 32,000 *g* for 2.5 h at 4°C. The virus band was retrieved by side puncture, diluted two-fold with water, centrifuged at 13,000 *g* for 30 min at 4°C, and the viral pellet resuspended in 40 mL of buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.5). Virion proteins were eliminated by overnight digestion with 0.6 mg/mL proteinase K at 37°C in the presence of 0.1% (w/v) sodium dodecyl sulfate. High-molecular-weight viral DNA was recovered by phenol-chloroform extraction followed by precipitation with ethanol.

### Acquisition of primary sequence data and sequence assembly

Random DNAs were produced by fragmenting virus DNA on ice with a probe sonicator. The ends of the sheared DNA fragments were repaired using T4 and Klenow polymerases, and then fragments ranging in size from 1.5 to 3.0 kb were separated by electrophoresis and recovered. A pBlueScript II (KS+) plasmid vector (Stratagene) was digested with *Eco*RV and treated with shrimp alkaline phosphatase, ligated to the SFV DNA fragments using T4 ligase, and transformed into *E. coli* JM105 cells (Promega). Suitably sized plasmid clones were identified using the PCR and purified using a "High Pure" plasmid kit (Roche), and the double-stranded templates were sequenced from both ends of the insert using T3 and T7 primers. A cycle-sequencing protocol, AmpliTaq DNA polymerase (Perkin–Elmer), dye terminators, and an Applied Biosystems PRISM 377 sequencer that had been modified to provide extended sequence reads were used to obtain all of the raw sequence data. Data were collected using ABI data collection (v 1.1) and DNA sequencing software (v 3.3). As contigs started to assemble, we switched to a more efficient primer walking strategy using, as templates, a library of *Bam*HI restriction fragments previously cloned from SFV strain Kasza (Wills *et al.,* 1983; Delange *et al.,* 1984) plus custom sequencing primers (Gibco BRL). Primers were designed using RightPrimer (v 1.2.5).

Sequence traces were manually edited to correct errors in automated base calls and to trim the ill-defined sequences found at the 5′ and 3′ ends of each fragment. The final contig then was assembled from these fragmentary elements using the program Sequencher (v 3.1). After a completed sequence had been acquired, each base call within the assembly was manually inspected, and several portions of the genome were resequenced on the opposite strand, using new sequencing primers, to confirm the integrity of the sequence. This final sequence was overdetermined with about threefold redundancy at each base pair from 565 separate sequence fragments. Although portions of the SFV genome have been previously sequenced, including the TIRs, TIR junctions, and several internal *Bam*HI restriction fragments, we nevertheless resequenced all of these regions to confirm the accuracy of the final assembly. We did not resequence the very end of the virus. As defined by the an *Afl*II site located at the hairpin centre of inverted

repeat symmetry (DeLange *et al.,* 1986), the first base of our contig is located either 34 or 40 bases from the end of the genome (nt 125 or 198 in GenBank entry M14003.1). The exact distance depends upon the number of intervening extrahelical bases.

### Sequence analysis and assignment of open-reading frames (ORFs)

The SFV genome is generally syntenic with the genomes of Orthopoxviridae so the open reading frames were easily identified using a combination of BLAST analyses (Altschul *et al.,* 1997) and visual inspection. In some locations, we encountered gaps between genes that were too large to be occupied by the accompanying poxviral promoters (30–40 bp), and this was taken to be indicative of the possible presence of a small ORF. Where such ORFs were found on both SFV and myxoma viruses, they were provided with gene designations. The gene numbering starts from the revised left end of the virus [the right end of the virus in the original restriction map (Delange *et al.,* 1984)] and throughout most of the genome was incremented in steps of one. Where SFV clearly lacks a gene found in myxoma virus at the syntenic position (e.g., "S139") we have skipped that number to maintain consistency in the naming of homologous genes (Table 1).

Putative early and late promoters were identified using internet implementations of MEME version 2.2 (Bailey and Elkan, 1994) and MAST version 2.2 (Bailey and Gribskov, 1998). An iterative process was used whereby small collections of putative early and late promoter elements were used as training sets in a search for additional examples of conserved sequence motifs. Once genes had been roughly categorized as encoding either or both promoter motifs, histograms were assembled illustrating the likelihood of encountering particular bases at given positions within each conserved motif. BLAST scores (Altschul *et al.,* 1997) were calculated using NCBI internet resources and sequence alignments prepared using Macintosh or internet versions of MACAW (Schuler *et al.,* 1991) and Clustal W (Thompson *et al.,* 1994). Phylogenies were calculated using Felsenstein's PHYLIP package of software (Felsenstein, 1989). Protein motifs were identified by searches against the Pfamm HMM database.

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