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The Complete DNA Sequence of Myxoma Virus

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Myxomatosis in European rabbits is a severely debilitating disease characterized by profound systemic cellular immunosuppression and a high rate of mortality. The causative agent, myxoma virus, is a member of the poxvirus family and prototype of the *Leporipoxvirus* genus. As a major step toward defining the genetic strategies by which the virus circumvents host antiviral responses, the genomic DNA sequence of myxoma virus, strain Lausanne, was determined. A total of 171 open reading frames were assigned to cover the 161.8-kb genome, including two copies each of the 12 genes that map within the 11.5-kb terminal inverted repeats. Database searches revealed a central core of approximately 120 kb that encodes more than 100 genes that exhibit close relationships to the conserved genes of members of other poxvirus genera. Open reading frames with predicted signal sequences, localization motifs, or homology to known proteins with immunomodulatory or host-range functions were examined more extensively for predicted features such as hydrophobic regions, nucleic acid binding domains, ankyrin repeats, serpin signatures, lectin domains. and structural cysteine spacings. As a result, several novel, potentially immunomodulatory proteins have been identified, including a family with multiple ankyrin-repeat domains, an OX-2 like member of the neural cell adhesion molecule family, a third myxoma serpin, a putative chemokine receptor fragment, two natural killer receptor-like species, and a variety of species with domains closely related to diverse host immune regulatory proteins. Coupled with the genomic sequencing of the related leporipoxvirus Shope fibroma virus, this work affirms the existence of a conserved complement of poxvirus-specific core genes and expands the growing repertoire of virus genes that confer the unique capacity of each poxvirus family member to counter the immune responses of the infected host.

INTRODUCTION

Myxoma virus is a member of the *Leporipoxvirus* genus, Chordopoxvirinae subfamily, and Poxviridae family (Fenner, 1979). Other members of the *Leporipoxvirus* genus include Shope fibroma virus (SFV), hare fibroma virus, and squirrel fibroma virus. Like all members of the poxvirus family, myxoma virus possesses a large, linear double-stranded DNA genome with terminal inverted repeats (TIRs) and covalently closed hairpin loops at each end (Moss, 1996). The genome is contained within a characteristic brick-shaped virion, and viral replication occurs solely in the cytoplasm of infected cells. Myxoma virus, like other members of the poxvirus family, has the ability to successfully and strategically circumvent or disrupt critical facets of the host antiviral responses (McFadden *et al.,* 1995; Nash *et al.,* 1999).

Myxoma virus causes a mild, benign infection in its evolutionary host, the North American brush rabbit (*Sylvilagus californicus*) or the South American tapeti (*Sylvilagus brasiliensis*), but it causes a rapid systemic and lethal infection known as myxomatosis in European rabbits (*Oryctolagus cuniculus*) with mortality rates up to virtually 100% (Fenner, 1983). Myxomatosis is an extensively characterized veterinary disease that provides a well-defined *in vivo* model for the study of virus-encoded virulence factors, including those involved in immunomodulation. The symptoms and mortality rates associated with myxomatosis are believed to be the result of multiorgan dysfunction coupled with uncontrolled secondary gram-negative bacterial infections due to a progressive impairment of the host cellular immune response. Myxomatosis is transmitted via arthropod vectors, most notably the mosquito (Fenner and Ratcliffe, 1965). In the early 1950s, myxoma virus was released in Australia in an effort to reduce the problematic feral European rabbit populations. This biological approach to pest control proved to be ineffective due to the combination of increased host resistance in the surviving rabbit populations and genetic attenuation of field virus strains (Fenner and Ratcliffe, 1965; Kerr and Best, 1998).

The well-characterized pathogenic relationship between myxoma virus and the European rabbit host provides an ideal model system in which to analyze the

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ability of a large DNA virus to modulate the antiviral responses of the host immune system. To better understand this phenomenon, we undertook the sequencing of the entire DNA genome of myxoma virus (strain Lausanne) to enable a systematic survey of the functions of all the putative immunomodulatory genes. This strategy has been used successfully by a number of other groups to identify novel immunomodulatory virus genes. For example, the complete genomic sequence analyses of vaccinia (VAC), variola (VAR), and *Molluscum contagiosum* revealed a plethora of potential immunomodulatory genes (Antoine *et al.,* 1998; Goebel *et al.,* 1990; Massung *et al.,* 1994; Senkevich *et al.,* 1997; Shchelkunov *et al.,* 1995). The first molecular characterization of the myxoma virus genome was carried out by Russell and Robbins (1989) in which restriction enzyme maps for three different strains of myxoma virus were determined. Since then, however, only fragmentary sequencing studies have been carried out (see Jackson and Hall, 1998; Nash *et al.,* 1999 for references). We present here a comprehensive analysis of the myxoma virus genomic sequence, focusing primarily on prospective novel immunomodulatory genes and their possible functions. In the companion paper (Willer *et al.,* 1999), the sequence of the related leporipoxvirus, SFV, is presented, with discussion centered primarily on genes involved in replication and repair.

RESULTS AND DISCUSSION

General features of the myxoma genome

The myxoma virus genome (strain Lausanne) was determined to be 161774 nucleotides in length and has an A/T content of 56.4%, which is less A/T rich than the known orthopoxvirus genomes (Goebel *et al.,* 1990). The myxoma genome was assembled from approximately 2000 individual nucleotide sequencing reactions representing both strands with an overall 5.6-fold redundancy. The myxoma virus TIRs are 11.5 kb in length, which is shorter than the 12.4-kb TIRs of SFV (Cabirac *et al.,* 1985; Upton *et al.,* 1987). The boundary between the myxoma TIRs and unique central core sequences was found to exist between the M008.1L/R and the M009L/M156R genes (Fig. 1). Therefore, M009L is not a part of the TIR, as is the case of S009L in SFV (Willer *et al.,* 1999). Seven polymorphic loci were found to exist at nucleotides: 209 (T/A), 40404 (C/T), 65028 (C/T), 111824 (A/C), 112369 (A/G), 112588 (A/G), and 161566 (A/T). Of these polymorphic nucleotides, three (at positions 209, 40404, and 161566) are within intergenic regions, and a fourth (at 65028) is within M068R but maintains the amino acid (aa) leucine at that position. The remaining three polymorphisms are all within a single open reading frame (ORF), M114R (the RNA polymerase 132-kDa subunit). The first polymorphic site results in a lysine or threonine at aa position 858, the second polymorphic site results in either an arginine or glycine residue at position 1040, and the final polymorphic site results in a threonine or alanine at aa position 1113.

Our deduced genomic length falls slightly short of the predicted 163.6 ± 0.2 kb length based on restriction enzyme mapping of myxoma virus, strain Lausanne (Russell and Robbins, 1989). We compared the fragments predicted from the deduced sequence with those observed in the publication of Russell and Robbins, and the restriction enzyme fragment profiles were consistent with the published map (not shown).

Myxoma virus ORF arrangements

With all available reading frames, the number of possible myxoma virus ORFs encoding proteins of a minimum length of 50 aa was calculated to be 425. We based our gene assignment strategy on the fact that poxviruses tend to have a compact and efficiently organized genome, with the genes aligned closely one after another or slightly overlapping. Based on homologies to known poxvirus genes (as established by BLASTp scores) and comparison with the ORFs predicted in the recently sequenced SFV genome (Willer *et al.,* 1999), we have assigned 159 myxoma ORFs predicted to be colinear expressed genes. One gene (M037L) encoding only a 32-aa putative protein was included in the list of assigned genes. Thus a total of 159 assigned genes span the myxoma virus genome, of which 12 are present in diploid in the TIRs, giving a total of 171 genes from the full-length genome.

Our gene nomenclature was based loosely on those used for *Melanoplus sanguinipes* and *M. contagiosum* (Afonso *et al.,* 1999; Senkevich *et al.,* 1997) and was devised to correspond closely with that of the SFV genome (Willer *et al.,* 1999). Beginning at the left-most end of the genome, the TIR genes were designated in a fashion consistent with the names used in previous articles and then continued in increasing numerical order from the left-most genes. Thus M-T1 was renamed M001R/L, M-T2 became M002R/L, and so on until M156R (with the exclusion of M145). Four notable exceptions to the numeric scheme are M000.5 R/L, which is an ORF found only in the termini of the myxoma genome; M-T3A and M-T3C, which became M003.1 R/L and M003.2 R/L; SERP-1, which became M008.1 R/L; and M153.1R, which is uniquely found in myxoma. All ORFs were assigned the designation R (right) or L (left) to represent the direction of transcription.

All of the assigned myxoma ORFs are represented in a linear fashion in Fig. 1. A color scheme is provided to functionally assign the ORFs with regard to their predicted functions. The most obvious feature of the myxoma genome is the presence of genes with putative, conserved housekeeping (represented in red) or structural functions (yellow) in the central portion of the ge-

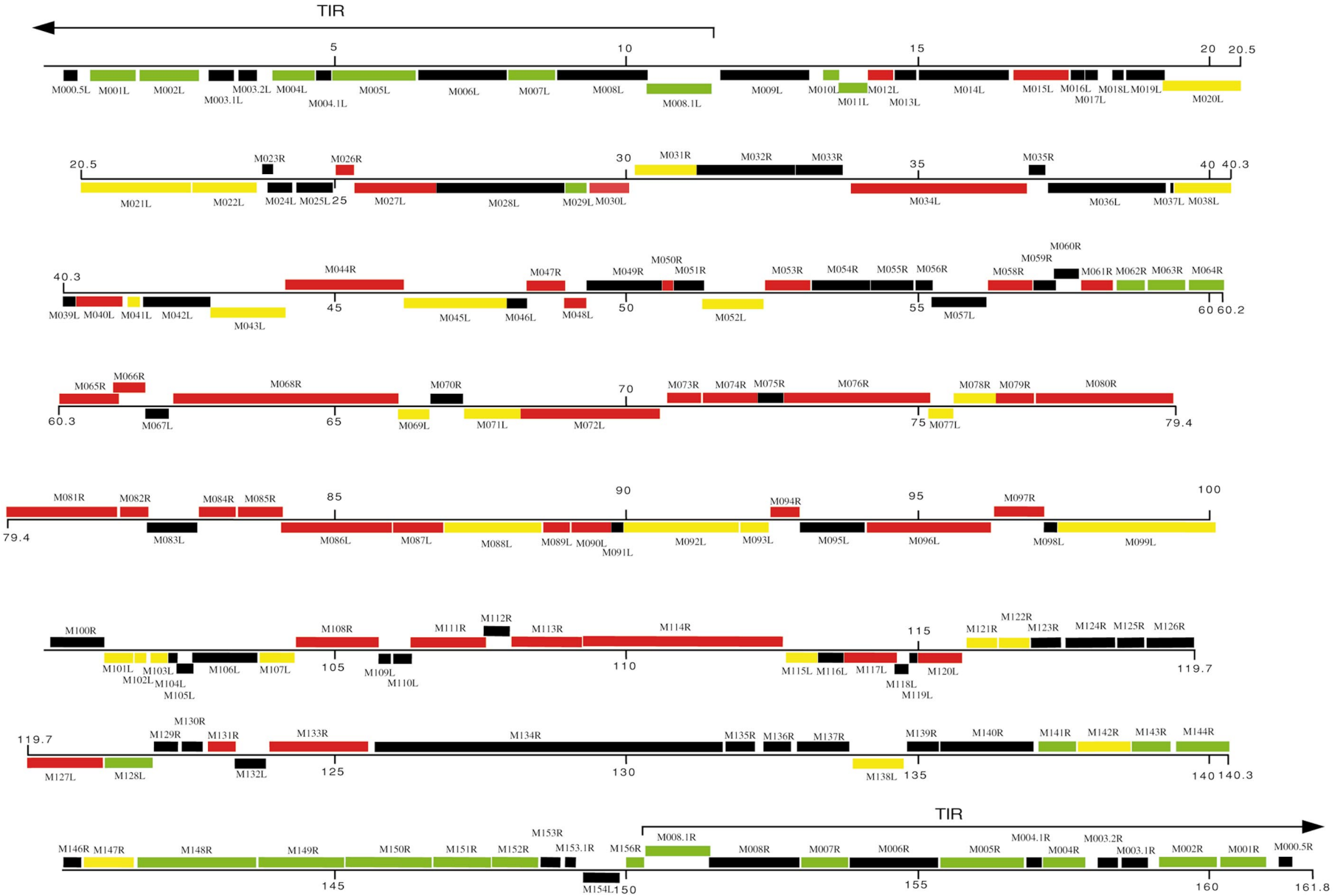

FIG. 1. Linear genomic map of myxoma virus. ORFs are indicated by differentially colored rectangles corresponding to the following: ORFs of unknown function are in black, ORFs with potential immunoregulatory or host-range functions are in green, ORFs involved in housekeeping functions (e.g., mRNA transcription/translation, and so on) are in red, and those ORFs that are structural or involved in either morphogenesis or protein modification are in yellow. Genes transcribed in the leftward direction are shown below the line, and those transcribed to the right are above the line. The terminal inverted repeat (TIR) sequences are demarcated by arrows at either end of the genome.

nome extending from M012L to M142R, whereas genes containing motifs that imply a role in immunomodulation or host range (green) tend to be closer to the termini. Genes with undefined functions are shown in black. Thus the central 120 kb of the myxoma genome represents most of the essential genetic information common to all poxviruses, whereas the flanking 40 kb (approximately 15 kb at the left and 25 kb at the right) are enriched for genes that are more demonstrably specific for the *Leporipoxvirus* genus.

The map of the myxoma virus genome illustrates the efficient use of coding capacity in poxviruses (Fig. 1). There is little evidence of wasted space, and in contrast to other poxviruses, there is no indication of noncoding DNA or significant stretches of repetitive DNA within the TIR, with the exception of nine tandem copies of a conserved nonamer/decamer between M002L/R and M003.1L/R. Although the TIR region of sequenced poxviruses is variable in length and sequence, the regions generally encode a small number of genes and include significant stretches of noncoding DNA. For example, there is almost 6 kb of noncoding DNA at the termini of vaccinia Ankara strain (Antoine *et al.,* 1998), 3 kb in entomopoxvirus (*M. sanguinipes*) (Afonso *et al.,* 1999), 3 kb in *M. contagiosum* virus (Senkevich *et al.,* 1997), and 4 kb in vaccinia Copenhagen (Johnson *et al.,* 1993). In contrast, there are contiguous ORFs right up to the termini in myxoma.

All 159 myxoma ORFs are presented in Table 1. For each ORF, the nucleotide span within the myxoma genome and number of aa encoded are provided. Also indicated are the best protein matches in the public domain database in terms of the best smallest sum probability BLASTp score and, in certain cases, a second relevant match in the database. As a reference for each of the matches, SWISS-PROT or GENBANK accession numbers are provided. Based on the relevant matches, putative functions and/or predicted activities are given for most of the ORFs. The degree of conservation of each ORF with its closest poxvirus gene homolog is indicated as U (unique, defined as having less than 20% aa identity with a previously sequenced poxvirus gene outside the *Leporipoxvirus* genus), S (semiconserved, having identity of 20–50%), or C (conserved, having identity of greater than 50%). As commonly observed in other poxviral genomes, the ORFs closest to the ends of the genome are transcribed preferentially toward the nearest terminus.

Poxvirus promoters have been divided into the early, intermediate, and late temporal categories. Strong early and late promoters have been defined by mutational analysis, as have intermediate promoters, which were first identified with poxvirus genes encoding late-transcription factors (Baldick *et al.,* 1992; Davison and Moss, 1989, 1990). A comparison of the promoter sequences from *M. contagiosum* and vaccinia virus identified consensus sequence patterns for each of the three promoter types (Senkevich *et al.,* 1997). Strong early promoters are characterized by a 15-nucleotide A-rich tract, often with a G nucleotide in the middle. Transcription initiation takes place 15–20 nucleotides downstream of the promoter. The transcriptional initiation sites for two myxoma early genes M001L/R and M011L have been mapped (Graham *et al.,* 1992; Macaulay and McFadden, 1989), and both sites show corresponding A-rich domains 10–20 nucleotides upstream of the transcription start sites. In the case of M011L, the A-rich domain lies approximately 100 bp upstream of the ORF, indicating that the early promoters are not necessarily adjacent to the ORFs. Late promoters are defined by the transcription initiation site TAAAT sequence, which often forms part of the initiating methionine codon by being followed by a G. In strong late promoters, the TAAAT is preceded by a short spacer of 4–10 nucleotides and then a T-rich tract of 5–15 nucleotides. Intermediate promoters have a $TA^{3-6}T$ transcriptional initiation site that is similar to the late promoters but are preceded by an AAANAA motif about 12–15 nt upstream of the TAAA. These promoter elements have been conserved among the leporiviruses and orthopoxviruses, and this is reflected by the identification of very similar consensus promoter sequences in SFV (Willer *et al.,* 1999). Using these promoter sequence signatures, we manually inspected the regions upstream of each ORF in the myxoma virus genome and tentatively classified each of the promoter elements (Table 1).

An interesting feature of the myxoma genome organization revealed in Fig. 1 and Table 1 is that the basic order of centrally located genes in vaccinia virus, strain Copenhagen (Johnson *et al.,* 1993), is fairly well maintained in the core 120 kb of the myxoma virus genome. In fact, myxoma M012L–M140R are shown to have significant homology to VAC F2L through A55R, with certain noteworthy exceptions. No myxoma homologs are found to VAC F5L, F6L, F11L, F14L, E11L, O2L, I4L, A25L, A26L, A31R, A36R, A39R–A49R, A53R, and A54L. In most of these cases, the function of the vaccinia virus homolog missing from myxoma is unknown. In the case of the missing VAC O2L, it appears that myxoma virus lacks this glutaredoxin homolog (Ahn and Moss, 1992), although it still possesses M048L, a homolog of the related VAC glutaredoxin-2, G4L (Gvarkharia *et al.,* 1996). The lack of a myxoma homolog of VAC I4L (the large subunit of ribonucleotide reductase) and A48R (thymidylate kinase) is also noteworthy because it implies that myxoma is likely more dependent than is vaccinia on the host cell for nucleotide pools (Beaud, 1995). A more in-depth discussion of the functional relevance of these particular deletions is found in the companion SFV publication (Willer *et al.,* 1999).

Some of the other interesting genetic arrangements include several insertions, duplications, rearrangements, and even a translocation of myxoma ORFs with respect to the order of their vaccinia counterparts. Three contig-

TABLE 1

Assigned Genes of Myxoma Virus

ORFs[a]	Nucleotide position[b]	aa[c]	Promoter type[d]	Relevant matches/homologs[e]	Putative function/structure[f]	BLASTP[g]	Conservation within poxviruses[h]		
							U	S	C
M000.5L/R	581–366 161194–161409	72	L				U		
M001L/R	1585–806 160190–160969	260	E	VAC 35K major secreted protein B29R/C23L (sp P21090)	Secreted chemokine binding protein (Table 4) (M-T1, gi 2076755)	4.00e-36		S	
M002L/R	2645–1668 159130–160107	326	E	MPV TNF-R homolog (gi 2738073)/VAC B28R homolog (gi 439102)	Soluble TNF receptor homolog (M-T2, sp P29825) (Table 4)	6e-94/6e-93		S	
M003.1L/R	3279–2827 158496–158948	151	E	CAP T3A (gi 74384)/VAC B15R (sp P21089)		3e-26/1e-10		S	
M003.2L/R	3690–3352 158085–158423	113	?	CAP T3C (sp P18388)		6.00e-21		S	
M004L/R	4636–3926 157139–157849	237	E	CAP T4 (sp P18385)/VAC B9R (sp P21005)	ER-localized apoptosis regulator (Table 5) (M-T4, gi 2897907)	7e-34/6e-3		S	
M004.1L/R	4912–4643 156863–157132	90	L?	SPV C2L (sp P32230)		7.00e-15	U		
M005L/R	6383–4935 155392–156840	483	E	VAC B4R (sp P21001)/human ankyrin (sp Q01485)	Ankyrin-like host range (Table 5) (M-T5, gi 1421732)	3e-14/2e-7		S	
M006L/R	7948–6422 153827–155353	509	E	SPV C4L (sp P32228)/VAC A55R (sp P21073)	Kelch ring canal protein homolog (M-T6 gi 4186093)	8e-70/1e-31		S	
M007L/R	8776–7988 152999–153787	263	E	SPV C6L (sp P32226)/VAC B8R (sp P21004)	IFNγ receptor homolog, α chain (Table 4) (M-T7, gi 332308)	5e-22/5e-8		S	
M008L/R	10374–8830 151401–152945	515	E	SPV C4L (sp P32228)/VAC A55R (sp P21073)	Kelch ring canal protein homolog (M-T8, gi 332306)	5.3e-81/4e-32		S	
M008.1L/R	11461–10355 150314–151420	369	L	bovine plasminogen activator inhibitor (sp P13909)	Secreted serpin (Table 4) (SERP-1, sp P12393)	4.00e-45	U		
M009L	13130–11604	509	E	SPV C4L (sp P32228)/VAC A55R (sp P21073)	Kelch ring canal protein homolog (M-T9, sp P08073)	2e-67/4e-29		S	
M010L	13643–13389	85	E	TGFα (sp P01134)/VAC growth factor (sp P20494)	EGF-like growth factor (Table 4) (MGF, sp P08072)	1e-3/7e-3		S	
M011L	14125–13628	166	E	SPV C10L (sp P32222)	Integral membrane protein/ apoptosis regulator (Table 5) M11L (gi 279830)	7.00e-05		S	
M012L	14584–14141	148	E	VAC F2L (sp P21035)	Deoxyuridine 5′ triphosphate nucleotidohydrolase	8.00e-41			C
M013L	14984–14607	126	E	mouse IFN-inducible protein (gi 2465727)	IFN-inducible protein homolog	7.70e-02	U		
M014L	16565–15015	517	E	SPV C13L (sp P32206)/VAC F3L (sp P21013)	Kelch-like protein	7e-98/3e-40		S	
M015L	17586–16621	322	E	VAC F4L (sp P20493)	Ribonucleotide reductase, small subunit	1.00e-45			C
M016L	17848–17618	77	?	SPV C15L (sp P32220)		2.00e-04		S	
M017L	18088–17861	76	E				U		
M018L	18513–18316	66	E	VAC F8L (sp P21017)	Nonessential/cytoplasmic protein	4.20e-02		S	
M019L	19216–18572	215	L	SPV C19L (sp P32207)/VAC F9L (sp P21018)		3e-68/1e-57			C
M020L	20531–19197	445	L	SPV C20L (sp P32216)/VAC F10L (sp P21095)	Serine/threonine protein kinase	0.00e00/1e-180			C
M021L	22526–20652	625	E	VAC F12L (sp P21053)	EEV maturation	1.00e-04		S	
M022L	23673–22561	371	L	VAC F13L (sp P20638)	Palmitylated envelope protein/EEV antigen	1.00e-17			C
M023R	23755–23937	61	L				U		
M024L	24285–23842	148	E	VAC F15L (sp P21020)		4.00e-36		S	

TABLE 1—*Continued*

ORFs[a]	Nucleotide position[b]	aa[c]	Promoter type[d]	Relevant matches/homologs[e]	Putative function/structure[f]	BLASTP[g]	Conservation within poxviruses[h]		
							U	S	C
M025L	24971–24345	209	E	VAC F16L (sp P21021)		7.00e-30		S	
M026R	25012–25317	102	L	VAC F17R (sp P07397)	DNA-binding phosphoprotein (vp11-like)	1.00e-27			C
M027L	26729–25320	470	L	VAC E1L (sp P21079)	Poly(A) polymerase catalytic subunit	0.00e00			C
M028L	28921–26729	731	L	VAC E2L (sp P21080)		1.00e-62		S	
M029L	29307–28963	115	E	VAC E3L (sp P21081)	IFN-resistance/PKR inhibitor/ host range (Table 5)	2.00e-20		S	
M030L	30037–29372	222	E	VAC E4L (sp P21082)	RNA polymerase subunit (rpo30)/VITF-1	4.00e-76			C
M031R	30138–31316	393	E	VAC E5R (sp P21046)	Virosome component	9.00e-15		S	
M032R	31329–33023	565	L	VAC E6R (sp P21047)		0.00e00			C
M033R	33029–33844	272	E	VAC E8R sp P21049)		1.00e-05			C
M034L	36864–33847	1006	E	VAC E9L (sp P20509)	DNA polymerase	0.00e00			C
M035R	36898–37185	96	L, E?	VAC E10R (sp P21050)		3.00e-38			C
M036L	39251–37212	680	E	VAC O1L (sp P21093)	Leucine zipper motif	1.00e-20		S	
M037L	39385–39290	32	L	FPV ortholog of VAC 03L and MC043.1L (gi 3123527)		2.40e-01	U		
M038L	40337–39399	313	L	VAC I1L (sp P20498)	Essential gene/DNA-binding/ virion morphogenesis	1.00e-17			C
M039L	40562–40341	74	L	VAC I2L (sp P12922)		5.00e-11		S	
M040L	41375–40566	270	I?	VAC I3L (sp P20499)	DNA-binding phosphoprotein	3.00e-71			C
M041L	41688–41455	78	L?	VAC I5L (sp P20500)	structural protein (vp13K)?	2.00e-10		S	
M042L	42869–41712	386	I	VAC I6L (sp P12925)		1.00e-12			C
M043L	44151–42865	429	L	VAC I7L (sp P20501)	Core protein/morphogenesis factor	1.00e-73			C
M044R	44157–46190	678	L?, I?	VAC I8R (sp P20502)	RNA helicase/ nucleophosphohydrolase II	0.00e00			C
M045L	47962–46193	590	I	VAC G1L (sp P21022)	Putative 68K protein/virion morphogenesis	0.00e00			C
M046L	48294–47962	111	L	VAC G3L (sp P21024)		1.00e-23		S	
M047R	48288–48962	225	E	VAC G2R (sp P21023)	Isatin-β-thiosemicarbazone-dependent late gene expression regulator	3.00e-56		S	
M048L	49309–48926	128	L	VAC G4L (sp P21025)	Glutaredoxin 2 homolog/membrane protein	3.00e-27		S	
M049R	49312–50604	431	E?	VAC G5R (sp P21026)		3.00e-87		S	
M050R	50611–50799	63	E	VAC G5.5R (gi 335676)	RNA polymerase subunit (rpo7)	1.00e-23			C
M051R	50805–51326	174	?	VAC G6R (sp P21027)		4.00e-36		S	
M052L	52350–51301	350	L	VAC G7L (sp P21028)	Structural protein	3.00e-92		S	
M053R	52380–53159	260	I	VAC G8R (sp P21029)	Late *trans*-activator protein VLTF-1	1.00e-26			C
M054R	53183–54178	332	L	VAC G9R (sp P21030)	Myristylated protein	2.00e-86		S	
M055R	54182–54907	242	L	VAC L1R (sp P20540)	Myristylated virion protein (IMV)	6.00e-96			C
M056R	54965–55261	99	E	VAC L2R (sp P20843)		2.00e-06		S	
M057L	56176–55217	320	L	VAC L3L (sp P21031)		3.00e-86			C
M058R	56201–56953	251	L	VAC L4R (sp P20981)	Major core protein (vp25K-like)/nucleic acid-binding protein	9.00e-87			C
M059R	56975–57361	129	L	VAC L5R (sp P07615)		3.00e-27		S	
M060R	57318–57761	148	L	CAP F7 (sp P19746)/VAC J1R (sp P21032)	Virion protein dimer	5e-46/3e-36			C
M061R	57797–58330	178	E	VAC J2R (sp P03297)	Thymidine kinase	3.00e-65			C
M062R	58406–58879	158	E	CAP CF8A (sp P19747)/VAC C7L (sp P17363)	Host range/virulence factor (Table 5)	2e-30/7e-13		S	

TABLE 1—*Continued*

ORFs[a]	Nucleotide position[b]	aa[c]	Promoter type[d]	Relevant matches/homologs[e]	Putative function/structure[f]	BLASTP[g]	Conservation within poxviruses[h]		
							U	S	C
M063R	58939–59583	215	E	CAP CF8A (sp P19747)	Host range/DAXX-like motif (Table 5)	1.00e-14		S	
M064R	59631–60239	203	E	CAP CF8A (sp P19747)/VAC C7L (sp P17363)	Host range/virulence factor (Table 5)	7e-26/7e-11		S	
M065R	60284–61297	338	L?	VAC J3R (sp P21033)	Poly(A) polymerase regulatory subunit/mRNA methyltransferase	1.00e-154			C
M066R	61200–61754	185	E?	VAC J4R (sp P07391)	RNA polymerase subunit (rpo22)	5.00e-69			C
M067L	62161–61763	133	L?	VAC J5L (sp P21083)	Essential	9.00e-47			C
M068R	62235–66092	1286	E, (I?)	VAC J6R (sp P20504)	RNA polymerase subunit (rpo147)	0.00e00			C
M069L	66614–66081	178	L	VAC H1L (sp P20495)	Tyrosine/serine phosphatase	7.00e-60			C
M070R	66630–67199	190	L?	VAC H2R (sp P20496)		3.00e-72			C
M071L	68179–67208	324	L	Lumpy skin disease virus p32 (gi 4884704)/VAC H3L (sp P20497)	Immunodominant envelope protein (IMV)	1e-107/2e-62		S	
M072L	70570–68183	796	L	VAC H4L (sp P07241)	RNA polymerase-associated transcription factor RAP94	0.00e00			C
M073R	70698–71279	194	E	VAC H5R (sp P20538)	Late *trans*-activator protein VLTF-4 virosome component	2.00e-27		S	
M074R	71310–72254	315	E, L	VAC H6R (sp P08585)	DNA topoisomerase I	1.00e-14			C
M075R	72257–72697	147	E?	VAC H7R (sp P20539)		2.00e-26		S	
M076R	72702–75206	835	E	VAC D1R (sp P20979)	mRNA capping enzyme, large subunit	0.00e00			C
M077L	75602–75174	143	E?	VAC D2L (sp P21008)	Structural protein	3.00e-21		S	
M078R	75608–76327	240	E?	VAC D3R (sp P21009)	Structural protein	3.00e-20		S	
M079R	76327–76980	218	E	VAC D4R (sp P20536)	Uracil-DNA glycosylase	2.00e-94			C
M080R	77017–79374	786	E	VAC D5R (sp P21010)	Nucleoside triphosphatase	0.00e00			C
M081R	79374–81278	635	L	VAC D6R (sp P20634)	Early transcription factor subunit/VETF-1	0.00e00			C
M082R	81314–81802	163	E or I	VAC D7R (sp P21034)	RNA polymerase subunit (rpo 18)	1.00e-60			C
M083L	82636–81779	286	E?	*O. cuniculus* carbonic anhydrase I (gi 164840)	Carbonic anhydrase-like/virion membrane protein	5.00e-40		S	
M084R	82685–83302	206	E	VAC D9R (sp P21011)	25K mutT-like protein	1.00e-58			C
M085R	83302–84078	259	L	VAC D10R (sp P21012)	mutT-like protein/down-regulator of viral gene expression	2.00e-54		S	
M086L	85980–84085	632	L	VAC D11L (sp P20637)	Nucleoside triphosphatase I/DNA helicase	0.00e00			C
M087L	86861–86001	287	L?	VAC D12L (sp P20980)	mRNA capping enzyme, small subunit/VITF	e-123			C
M088L	88545–86884	554	E, L?	VAC D13L (sp P04321)	Rifampicin resistance protein virion protein (IMV)	0.00e00			C
M089L	89021–88575	149	I	VAC A1L (sp P20982)	*trans*-Activator protein (late gene)/VLTF-2	2.00e-54			C
M090L	89729–89058	224	E, I?	VAC A2L (sp P07609)	*trans*-Activator protein (late gene)/VLTF-3	e-111			C
M091L	89953–89729	75	L	VAC A3L fragment (WR) (sp P07608)	Putative 8.9K protein	2.00e-19			C
M092L	91923–89965	653	L	VAC A3L (sp P20643)	Major core protein/P4b	0.00e00			C
M093L	92438–91962	159	L	MC107L (gi 1492050)	Core protein	3.00e-05	U		
M094R	92477–92968	164	L	VAC A5R (sp P20984)	RNA polymerase subunit (rpo 19)	1.00e-44			C
M095L	94089–92971	373	I	VAC A6L (sp P20985)		e-119			C
M096L	96252–94120	711	L?	VAC A7L (sp P20635)	Early transcription factor 82K subunit/VETF	0.00e00			C

TABLE 1—*Continued*

ORFs[a]	Nucleotide position[b]	aa[c]	Promoter type[d]	Relevant matches/homologs[e]	Putative function/structure[f]	BLASTP[g]	Conservation within poxviruses[h]		
							U	S	C
M097R	96302–97159	286	E	VAC A8R (sp P20986)	VITF-3 subunit	1.00e-04			C
M098L	97393–97166	76	L	VAC A9L (sp P20987)		1.00e-25			C
M099L	100099–97397	901	L	VAC A10L (sp P20642)	Major core protein precursor/ P4a	0.00e00			C
M100R	100114–101052	313	L	VAC A11R (sp P20988)		9.00e-89			C
M101L	101537–101055	161	L	VAC A12L (sp P20989)	Virion protein	1.00e-32			C
M102L	101776–101573	68	L	VAC A13L (sp P20990)	Virion membrane protein/p8 (IMV)	1.40e-02		S	
M103L	102123–101836	96	L	VAC A14L (sp P20991)	Structural membrane protein/ p16 (IMV)	2.00e-21			C
M104L	102301–102143	53	L	MC119L (gi 1492062)	Receptor-like fragment (Table 4)	5.00e-09		S	
M105L	102575–102294	94	L	VAC A15L (sp P20992)		1.00e-19		S	
M106L	103689–102562	376	L	VAC A16L (sp P20993)	35K myristylated protein	e-111			C
M107L	104307–103708	200	L	VAC A17L (sp P16711)	Morphogenesis factor membrane protein (IMV)	1.00e-32		S	
M108R	104322–105755	478	I	VAC A18R (sp P20534)	DNA helicase/negative transcriptional regulator	e-161			C
M109L	105960–105742	73	L	VAC A19L (sp P20994)		5.00e-17			C
M110L	106302–105964	113	L?	VAC A21L (sp P20996)		2.00e-34			C
M111R	106301–107593	431	E	VAC A20R (sp P20995)	DNA polymerase processivity	3.00e-96		S	
M112R	107547–108038	164	E?	VAC A22R (sp P20997)		1.00e-54			C
M113R	108069–109223	385	E	VAC A23R (sp P20998)	Intermediate transcription factor/VITF-3 subunit	1.00e-124			C
M114R	109252–112716	1155	E	VAC A24R (sp P19798)	RNA polymerase 132K subunit/IBT resistance	0.00e00			C
M115L	113289–112726	188	L	CAP HM2 (sp P16717)/VAC A27L (sp P20535)	Fusion protein/EEV formation	1e-25/2e-4		S	
M116L	113712–113293	140	L	CAP HM3 (sp P16718)/VAC A28L (sp P21086)		7e-52/5e-36			C
M117L	114626–113721	302	E	VAC A29L (sp P21087)	RNA polymerase subunit (rpo 35)	1.00e-101			C
M118L	114825–114598	76	L	VAC A30L (sp P21088)		4.00e-11			C
M119L	114993–114844	50	E?	no hits			U		
M120L	115764–115000	255	L?	VAC A32L (sp P21055)	ATPase	7.00e-85			C
M121R	115849–116376	176	E	VAC A33R (sp P21056)	EEV glycoprotein/lectin-like (Table 4)	2.00e-22		S	
M122R	116386–116901	172	L	VAC A34R (sp P21057)	EEV glycoprotein (Table 4)	7.00e-41		S	
M123R	116937–117473	179	E	VAC A35R precursor (sp P21058)		3.00e-22		S	
M124R	117513–118370	286	E	MC144R (gi 1492087)	Canavalin precursor-like	2.00e-11	U		
M125R	118387–118869	161	E				U		
M126R	118914–119726	271	E	VAC A37R (sp P21060)	EEV protein	3.00e-19		S	
M127L	121053–119719	445	E	MSV235 (gi 4049783)	Putative photolyase	1.00e-25	U		
M128L	121901–121059	281	E?	rat CD47 (gi 2394318)/VAC A38L (sp P21061)	Integrin-associated protein (CD47) homolog (Table 4)	3.00e-08/ 6.00e-05	U		
M129R	121900–122307	136	E?	VAC E7R (sp P21048)	Myristylated protein	2.80e-01		S	
M130R	122377–122742	122	E				U		
M131R	122812–123300	163	L	bovine Cu, Zn superoxide dismutase (sp P00442)	Superoxide dismutase-like	4.00e-38		S	
M132L	123814–123290	175	E				U		
M133R	123886–125574	563	L	VAC DNA ligase A50R (sp P20492)	DNA ligase	1.00e-167			C
M134R	125694–131693	2000	E	MC035R (gi 1491978)	Surface glycoprotein	0.00e00		S	
M135R	131699–132232	178	E	VAC B19R (sp P21077)	IL-1/IL-6 receptor-like (Table 4)	9.00e-08		S	
M136R	132368–132904	179	L?	VAC A52R (sp P21070)		1.00e-03	U		
M137R	132908–133837	310	L	VAC A51R (sp P21069)		1.00e-34		S	

TABLE 1—*Continued*

ORFs[a]	Nucleotide position[b]	aa[c]	Promoter type[d]	Relevant matches/homologs[e]	Putative function/structure[f]	BLASTP[g]	Conservation within poxviruses[h]		
							U	S	C
M138L	134746–133877	290	E	Mouse α-2,3-sialyltransferase (gi 558532)	α-2,3-Sialyltransferase	1.00e-48	U		
M139R	134806–135369	188	E	VAC A52R (sp P21070)		9.00e-28		S	
M140R	135375–137033	553	E?	VAC A55R (sp P21073)	Kelch ring canal protein (nonessential)	4.00e-66		S	
M141R	137069–137722	218	E	*X. laevis* NCAM2 (sp P36335)/ human OX-2 (sp P41217)	Immunoglobulin domain/OX-2 homolog (Table 4)	2.00e-06/ 3.00e-3	U		
M142R	137731–138648	306	E	VAC B1R (sp P20505)	Ser/Thr protein kinase	2.00e-76		S	
M143R	138665–139366	234	L	VAR D4R (gi 297187)/CPV D7R (gi 3096983)/EMV 28K (gi 397980)	Zinc ring finger protein/apoptosis regulator (Table 5)	2.00e-21/ 2e-21/ 5.00e-20		S	
M144R	139411–140310	300	E?	*A. trivirgatus* membrane cofactor (gi 2330890)/VAC C3L (sp P10998)	Complement control protein homolog/CD46 homolog (Table 4)	7.00e-23/ 1.00e-17		S	
M146R	140335–140658	108	E?	VAC N1L (sp P21054)	Nonessential virulence factor (Table 4)	1.20e-01		S	
M147R	140700–141563	288	E	FPV kinase homolog (gi 2393890)	Ser/Thr protein kinase	1.00e-17	U		
M148R	141626–143650	675	E	Human ankyrin (gi 4803663)/ VAC B4R (sp P21001)	Ankyrin-like/host range (Table 5)	2.00e-18/ 1.00e-10		S	
M149R	143655–145124	490	E	VAC B4R (sp P21001)	Ankyrin-like/host range (Table 5)	9.00e-13	U		
M150R	145191–146672	494	E	VAC C9L (sp P21042)	Ankyrin-like/host range (Table 5)	4.00e-07	U		
M151R	146684–147682	333	E	Human leukocyte elastase inhibitor (sp P30740)/VAC WR SPI-2 (sp P15059)	Serpin/SERP-2 (Table 4)	1.00e-47/ 6.00e-42		S	
M152R	147688–148485	266	E?	Human leupin (gi 2118384)	Serpin-like/SERP-3 (Table 4)	9.00e-03	U		
M153R	148526–148897	124	E	SPV C7L (sp P32225)/murine herpesvirus 68 IE1 homolog (gi 2317971)	Herpesvirus IE1-like zinc ring finger protein	1e-12/2e-9		S	
M153.1R	148944–149144	67	E	Beet soil-borne mosaic virus 75K (gi 3172384)		9.30e-02	U		
M154L	149884–149243	214	E	VAC M2L (sp P21092)	Nonessential gene	1.00e-55			C
M156R	149998–150303	102	L	SPV C8L (sp P32224)/VAC K3L (sp P20639)	Interferon resistance/eIF2α homolog (Table 5)	5.00e-06/ 3.00e-03		S	

[a] See Fig. 1 for diagramatic representations.

[b] Stop codon is not included (nucleotide 1 is defined in Materials and Methods).

[c] Length of each ORF given in amino acids.

[d] Promoter type: early (E), intermediate (I), or late (L); if no consensus promoter was found immediately upstream of the ORF, a question mark is shown.

[e] The most relevant match found for each ORF on BLASTp analysis is indicated; the closest vaccinia virus (strain Copenhagen) homolog is indicated for some ORFs; accession numbers are provided as references for each match. The vaccinia version is indicated for cases where similarity is high among multiple poxviruses. SFV genes are not included.

[f] Putative functions or structures were assigned according to functions of host proteins and/or other poxviruses.

[g] BLASTp scores are indicated for each match in the relevant column (see Materials and Methods). Only values less than e − 2 are included for similarities with nonpoxvirus proteins.

[h] The final three columns indicate whether an ORF is unique (U) <20% identical; semiconserved (S) (20–50% identical), or conserved (C) (>50% identical) with respect to at least one gene within the poxvirus family outside the leporipoxvirus genus (see text for details).

Abbreviations: CAP: capripox virus; EMV: ectromelia virus; FPV: fowlpox virus; MC: *Molluscum contagiosum*; MPV: monkeypox virus; MSV: entomopoxvirus, *Melanoplus sanguinipes*; SPV: swinepox virus; VAC: vaccinia virus (strain Copenhagen, unless otherwise indicated); TNF-R: tumour necrosis factor receptor; ER: endoplasmic reticulum; IFN: interferon; EGF: epidermal growth factor; MGF: myxoma growth factor; EEV: extracellular enveloped virus; IMV: intracellular mature virus; PKR: double-stranded RNA-dependent protein kinase; VITF: vaccinia intermediate transcription factor; VETF: vaccinia early transcription factor; VLTF: vaccinia late transcription factor.

uous myxoma genes, M062R–M064R, are related to the vaccinia host-range gene C7L. These three putative myxoma host-range genes have been inserted between M061R and M065R, which corresponds to their adjacent vaccinia homologs VAC J2R and VAC J3R. Three C7L-like host range genes are also found in SFV (Willer *et al.,* 1999), suggesting they may have arisen via recombination events in an ancestral *Leporipoxvirus* virus, resulting in the triplication of a C7L-like precursor gene (Oguiura *et al.,* 1993).

Two myxoma ORFs in two different positions in the myxoma genome reveal similarity to VAC I6L in $BLAST_p$ searches. M042L, which is relatively well conserved with VAC I6L, lies between the myxoma counterparts of VAC I5L and I7L, whereas the much less conserved M130R lies at the opposite end of the myxoma genome. M130R is a much smaller ORF than VAC I6L, and most of the similarity between the two proteins occurs at the N-terminus of M130R. A closer similarity is found between M130R and the human immunodeficiency virus tat protein (not shown). A second duplication is seen with M091L, which resembles VAC A3L, and is inserted directly downstream of M092L, itself a homolog of VAC A3L (p4b major core protein).

Three sets of myxoma ORFs at first glance appear to be rearranged with respect to their order in the VAC genome: M046L/M047L (which correspond to the reversed order of VAC G3L and G2R, respectively), M110L/M111R (which correspond to the reversed order of VAC A21L and A20R, respectively), and M136R/M137R (which correspond to the reversed order of VAC A52R and A51R, respectively). However, inspection of the physical arrangements of the vaccinia ORFs reveal that their nomenclature, which derives from the nucleotide number of the ATG initiating codon, is sometimes at variance with the order of the main coding sequences themselves. Thus these myxoma genes are in fact colinear with their vaccinia counterparts despite their apparently flipped numbering.

Finally, M129R may be a translocation (to the opposite end of the genome) and subsequent mutation of a VAC E7R-like gene, in a fashion similar to M130R. The overall sequence conservation of M129R with its closest vaccinia virus cousin, VAC E7R, is rather low. Thus we consider it to be a only a semiconserved gene in the myxoma genome.

Genetic differences between myxoma and SFV

The entire genomic sequence of another leporipoxvirus, SFV, is presented (Willer *et al.,* 1999) as a companion article to this one. It is immediately obvious that the two viruses share a great degree of similarity at the genetic level, however, there are several noteworthy differences. Although a future publication will more fully analyze the similarities and differences between myxoma and SFV, the information given in Table 2 was compiled to give a brief outline of those genes that differ significantly between the two viruses. Only one gene, M000.5L/R in myxoma virus, is completely absent in SFV. M000.5L/R maps at the very ends of the myxoma TIRs and is predicted to encode a protein 72 aa in length. Due to the lack of a significant cellular homolog of known function, it is difficult to speculate on the possible functions of this protein.

TABLE 2

Myxoma Genes Distinct from SFV

Myxoma	aa	SFV	Predicted function in myxoma
M000.5L/R	72	Missing	?
M008.1L/R	369	Fragmented	SERP-1
M023R	61	S23R (35 aa)	Late protein
M129R	136	S129R (78 aa)	Myristylated protein
M135R	178	Fragmented	IL-1/6-receptor homolog
M136R	179	Fragmented	VAC A52R-like
M139R	188	Fragmented	VAC A52R-like
M150R	494	Fragmented	Ankyrin repeats/host range
M152R	265	Fragmented	SERP-3
M153R	124	S153R (187 aa)	Zinc finger
M153.1R	67	S153R (187 aa)	?

Only remnant fragments of six other myxoma genes, namely M008.1L/R, M135R, M136R, M139R, M150R, and M152R, are observed in the SFV genome. M008.1L/R (SERP-1), a secreted serine protease inhibitor, has a demonstrated potent anti-inflammatory action, and its fragmentation is thought to be at least partially responsible for the decreased virulence of SFV in European rabbits (Upton *et al.,* 1990). M152R is also predicted to be serpin-like because it contains structural elements of the serpin signature (and is thus designated SERP-3); however, its function has not yet been elucidated. The fragmentation in SFV of the M135R gene, whose closest vaccinia homolog (B19R) has similarity to the interleukin-1β receptor (Smith and Chan, 1991; Spriggs *et al.,* 1992), is another notable difference between SFV and myxoma. M136R and M139R share some similarity with VAC A52R, but the homology between M136R and VAC A52R is much lower. M150R shares a low identity with VAC C9L but also includes multiple ankyrin repeats, which makes it a likely candidate for a host-range gene (Johnson *et al.,* 1993).

Two myxoma genes, M023R and M129R, are found in truncated form in the SFV genome. M023R does not have significant homology to any viral proteins, and M129R only has an extremely low similarity to VAC E7R, which is a myristylated late protein (Martin *et al.,* 1997). M153R is related to the N-terminal region of S153R, whereas M153.1R has homology to the C-terminal region. Under normal circumstances, this would suggest that the myxoma version is a truncated gene; however, a promoter-

TABLE 3

Myxoma Virus Proteins with Predicted N-Terminal Signal Sequences

Myxoma ORFs	Predicted cleavage site	Predicted no. of aa after cleavage	Predicted transmembrane domains	Predicted N-glycosylation sites	Predicted location
M001R/L (M-T1)	16–17	244	None	2	Secreted
M002R/L (M-T2)	16–17	310	None	4	Secreted/intracellular
M003.2R/L	20–21	93	None	1	Secreted
M004R/L (M-T4)	16–17	221	TM type 2 (154–175)/C-terminal RDEL retention motif	1	ER
M004.1R/L	37–38 (?)	53?	None	0	Secreted
M007R/L (M-T7)	18–19	244	None	2	Secreted
M008.1R/L (SERP-1)	15–16	354	TM type 1 (32–53)	3	Secreted
M010L (MGF)	19–20	66	None	2	Secreted
M016L	37–38 (?)	40?	None	1	Secreted
M035R	21–22	75	TM type 2 (65–83)	0	Transmembrane
M037L	20–21	12	None	0	Secreted
M041L	24–25	54	TM type 2 (49–73)	0	Transmembrane (VP 13K)
M046L	20–21	91	None	1	Secreted
M098L	22–23	54	TM type 1 (46–66)	0	Transmembrane
M102L	24–25	44	None	0	Secreted
M103L	29–30	67	TM type 1 (46–65)	1	Transmembrane (p16)
M104L	22–23	31	TM type 2 (32–53): possible uncleaved leader sequence?	0	Transmembrane
M110L	23–24	90	None	0	Secreted
M116L	25–26	115	None	1	Secreted
M121R	8 to 9	168	TM type 1 (46–64)	1	Transmembrane (EEV?)
M122R	37–38 (?)	135?	TM type2 (15–36): possible uncleaved leader sequence?	1	Transmembrane (EEV?)
M125R	18–19	143	None	3	Secreted
M128L	15–16	266	5 TMs (113–132, 142–165, 174–191, 212–231, 242–265)	6	Transmembrane
M134R	16–17	1984	6 TMs (171–191, 977–999, 1003–1028, 1328–1346, 1517–1537, 1944–1961)	33	Transmembrane
M135R	18–19	160	TM type 1 (162–178)	1	Transmembrane/secreted
M141R	16–17	202	TM type 2 (162–178)	8	Transmembrane
M144R	20–21	280	TM type 2 (257–279)	3	Transmembrane
M153.1R	14–15	53	None	0	Secreted
M154L	18–19	196	TM type 1 (58–76)	1	Transmembrane

like region appears to exist upstream of the M153.1R, at the 3′ end of the M153R gene. Expression analysis of the M153.1R ORF will determine whether it is a pseudogene or a bona fide novel ORF distinct from SFV. The difference in virulence observed between myxoma and SFV infections of European rabbits may thus be partly attributed to the divergence of these viruses, largely at their near-right TIR genomic regions. Nevertheless, there is sufficient variation elsewhere between the two genomes that makes the accurate assignment of the "virulence loci" for myxomatosis difficult based on sequence analysis alone.

Signal sequence predictions

The elucidation of the entire genomic sequence of the virulent Lausanne strain of myxoma virus has provided a spectrum of novel ORFs to analyze for potential immunoevasive roles in the *in vivo* system. As a prelude to this investigation, we analyzed all 159 ORFs for the presence of putative signal peptides, glycosylation sites, and transmembrane regions (Table 3). From this analysis, 29 ORFs were predicted to contain N-terminal signal peptides, and of these, approximately half (15 ORFs) include one or more putative transmembrane domains. One cautionary note is the possibility that signal peptides (in particular, those longer than 30 aa), as well as predicted transmembrane regions, can sometimes actually be hydrophobic stretches buried in the interior of folded proteins. The other possibility is that a putative signal sequence may not be cleaved, as we suspect might be the case for M104L (discussed later), thus providing an N-terminal transmembrane spanning domain. Taking into account the predicted localization of the ORFs in Table 3, 14 of the ORFs are predicted to be either transmembrane (cell surface or virion envelope associated) or endoplasmic reticulum localized. One ORF, M008.1

TABLE 4

Myxoma Proteins with Potentially Immunomodulatory Motifs

ORF	aa	Calc (obs) MW (kDa)[a]	Homologs	Proposed function/motif	aa identity	Predicted localization
M001R/L	260	28.4 (40)	VAC 35-kDa protein B29R	Secreted chemokine binding protein (M-T1)	94/260 (36%)	Extracellular
M002R/L	326	35.2 (55–60)	Monkeypox TNF-R homolog	Soluble TNF-R homolog/apoptosis regulator (M-T2)	156/315 (49%)	Intracellular and extracellular
M007R/L	263	30 (37)	VAC B8R	IFNγ receptor homolog, α chain (M-T7)	49/192 (25%)	Extracellular
M008.1R/L	369	41.6 (55)	Bovine plasminogen activator inhibitor 1	Secreted anti-inflammatory serpin (SERP-1)	121/363 (33%)	Extracellular
M010L	85	9.6	Rat transforming growth factor-α	EGF-like growth factor (MGF)	28/83 (28%)	Extracellular
M104L	53	6.3	Ateline herpesvirus 3 ORF 74 (gi 4019301)	Receptor-like fragment	17/40 (42%)	Membrane associated
M121R	176	19.7	Human NKG2-D (sp P26718)	NK cell receptor homolog?/EEV component C-type lectin superfamily?	18/52 (34%)	Type 2 membrane
M122R	172	19.7	Mouse Ly-49C (gi 1330631)	NK cell receptor homolog?/EEV component C-type lectin domain (47–167)	29/127 (22%)	Type 2 membrane
M128L	281	32.1	CD47 (integrin-associated protein) (gi 2394318)	CD47-like	55/229 (24%)	Transmembrane (5-TM)
M135R	178	20.4	VAC B19R	IL-1/IL-6 receptor-like	40/149 (26%)	Membrane associated
M141R	218	23.7	Human OX-2 (sp P41217)	OX-2 homolog/Ig domain (32–105)	24/85 (28%)	Membrane associated
M144R	300	34.2	VAC C3L	Complement binding protein homolog/CD46-like 3-CCP (22–79, 84–144, 149–205)	46/130 (35%)	Membrane associated
M146R	108	12.5	VAC N1L	Virulence factor	28/101 (27%)	Cytoplasmic?
M151R	326	38	VAC SPI-2	Serpin (SERP-2)	116/346 (33%)	Cytoplasmic
M152R	266	30	Leupin (gi 2118384)	Zinc finger (217–254)/RNA binding (61–68) (SERP-3)	47/228 (20%)	Cytoplasmic

[a] Calculated molecular weights predicted from aa sequence are compared with observed molecular mass values (when available, in parentheses) of the fully modified glycoprotein.

(SERP-1), demonstrated at least one discrepancy: M008.1 is predicted by the TMpredict program to contain a single TM type 1 (from aa 32–53), whereas it is known experimentally to be a secreted glycoprotein (Nash *et al.,* 1997).

Immunomodulatory proteins

A number of the strategies used by myxoma virus to modulate the host immune response have already been identified and well characterized (Jackson *et al.,* 1999; Messud-Petit *et al.,* 1998; Nash *et al.,* 1999; Petit *et al.,* 1996). Myxoma virus encodes a number of proteins that have been shown experimentally to function as secreted viroceptors or virokines. For example, M-T1 (M001L/R) binds and inhibits the activity of CC chemokines, M-T2 (M002L/R) is a tumor necrosis factor receptor homolog, M-T7 (M007L/R) is an interferon (IFN)-γ receptor homolog, myxoma growth factor (M010L) is an epidermal growth factor-like growth factor, and SERP-1 (M008.1L/R) is a secreted serpin with anti-inflammatory activity (Nash *et al.,* 1999). Myxoma virus also uses important intracellular strategies to mediate host range or circumvent diverse antiviral pathways: for example, M-T2 (M002L/R), M-T4 (M004L/R), M-T5 (M005L/R), M11L (M011L), and SERP-2 (M151R) serve to inhibit various apoptotic pathways that are triggered by viral infection in certain cell types (reviewed in McFadden and Barry, 1998; Turner and Moyer, 1998). Myxoma virus has also been shown to down-regulate CD4 and class I MHC expression in infected cells, but the genes responsible for this have not yet been identified (Barry *et al.,* 1995). We expect that many more virulence genes, in addition to those containing obvious predicted immunomodulatory or host-range domains (Tables 4 and 5), will be uncovered in future gene disruption studies. In the next few sections, several candidates for novel immunomodulatory or host-range proteins listed in Tables 4 and 5 are considered in greater detail.

M141R: Member of the OX-2/NCAM family

One of the myxoma genes encoding a predicted membrane-associated protein, M141R, shares aa homology with the *Xenopus laevis* neural cell adhesion molecule (NCAM) and OX-2 membrane proteins from various spe-

TABLE 5

Predicted Apoptosis Regulators or Host Range Proteins of Myxoma Virus

ORF	aa	calc (obs) MW (kDa)[a]	Homolog	aa identity	Proposed function/motif	Predicted localization
M004R/L (M-T4)	237	26.5 (30)	CAP T4	82/225 (36%)	ER-localized apoptosis regulator/RDEL; helix-loop-helix (127–135)	Endoplasmic reticulum
M005R/L (M-T5)	483	55.5 (55)	Human ankyrin	68/266 (26%)	Apoptosis regulator; 7 ankyrin repeats; RNAse3 domain (218–292)	Cytoplasmic
M011L (M11L)	166	18.8 (18)	SPV C10	41/155 (26%)	Anti-apoptotic integral membrane protein/ TM (140–160); helix-loop-helix (82–90)	Membrane associated
M029L	115	12.8	VAC E3L	45/102 (44%)	PKR (ds RNA-dependent protein kinase) inhibitor; IFN resistance/host range	Cytoplasmic
M062R	158	18.4	VAC C7L	47/148 (31%)	Virulence factor/host range	Cytoplasmic
M063R	215	24.6	DAXX (gi 2648018)	27/88 (30%)	Domain with similarity to Fas-binding death associated protein/apoptosis regulator?	?
M064R	203	23.8	VAC C7L	35/137 (25%)	Virulence factor/host range	Cytoplasmic
M143R	234	27.9	EMV 28K protein	65/235 (27%)	Apoptosis regulator/zinc finger (173–218)	Cytoplasmic
M148R	675	77.4	Human ankyrin	161/642 (25%)	4 TM (215–235, 251–271, 439–459, 477–497); 9 ankyrin repeats	Cytoplasmic ?
M149R	490	56.7	VAC B4R	90/435 (20%)	8 Ankyrin repeats	Cytoplasmic
M150R	494	57.8	VAC C9L	91/447 (20%)	5 Ankyrin repeats	Cytoplasmic
M156R	102	12	VAC K3L	20/60 (33%)	eIF2α homolog; interferon resistance/S1 RNA binding	Cytoplasmic

[a] Calculated molecular weights predicted from aa sequence are compared with observed molecular mass values (when available, in parentheses) of the fully modified glycoproteins.

cies (Table 4). A potential OX-2 homolog has also been identified in human herpes virus 8 (HHV8) (Russo *et al.,* 1996), but information regarding its characterization has yet to be reported. In comparison with the rat, human, and HHV8 OX-2 molecules, the myxoma homolog shares sequence identity of 20–22% and sequence similarity of 34–42%. The translated sequence of the M141R gene is predicted to encode a protein of 218 aa with a molecular weight of approximately 24 kDa, containing an immunoglobulin domain and sites for *N*-glycosylation. The M141R protein shares features with the cellular OX-2, including a predicted N-terminal signal sequence and a C-terminal transmembrane domain. The putative myxoma OX-2 homolog contains the two highly conserved cysteine residues involved in the formation of the V-like immunoglobulin domain (Fig. 2). In addition, it contains several critical residues that are almost invariably conserved in immunoglobulin domains: Asp111 and Gly113 near the C-terminal conserved Cys117 residue and Trp60 near the N-terminal conserved Cys47 residue. A striking

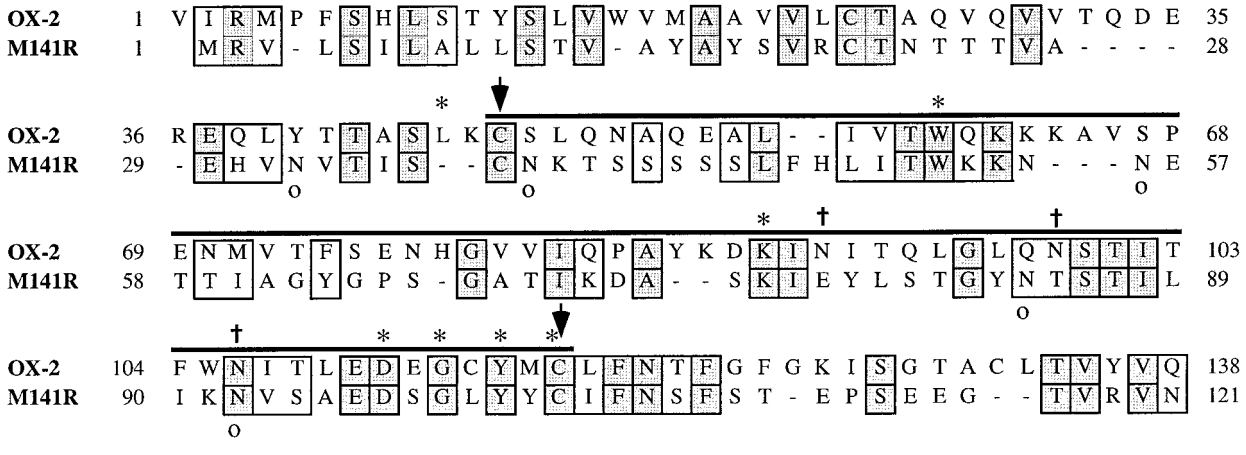

FIG. 2. Alignment of N-terminal regions of human OX-2 and M141R. Bold line above the OX-2 residues denotes the V (variable)-like immunoglobulin (Ig) domain in OX-2 and M141R. Arrows denote the two conserved cysteines that define the Ig domain boundary; asterisks identify Ig superfamily consensus residues, including those conserved in V-like Ig domains; crosses signify conserved N-linked glycosylation sites in OX-2; and predicted sites in M141R are denoted with circles. Amino acid identities are in shaded boxes, and similarities are in open boxes.

homology exists around the C-terminal conserved Cys117 residue, which is known to be a region of strong homology in V-like immunoglobulin domains. Recently, it was shown that expression of murine OX-2 prolongs renal allograft survival by modulating T cell stimulation (Gorczynski *et al.,* 1999, 1998). We propose that the myxoma OX-2 homolog could play a role in the development of immune tolerance by acting as a costimulator for T-cell anergy, thus providing an immunoprivileged environment for myxoma virus infection.

M128L: Homolog of CD47

Another myxoma gene that is predicted to encode a membrane-associated protein with possible immunomodulatory function is M128L (Table 4). The best BLASTp match was obtained when M128L was compared with the rat CD47 (integrin associated protein) molecule, and considerably less similarity was demonstrated to the closest poxvirus homolog, vaccinia A38L (Table 1). Vaccinia A38L is expressed as an integral membrane glycoprotein involved in the influx of extracellular calcium (Parkinson *et al.,* 1995; Sanderson *et al.,* 1996), but its *in vivo* function is unknown. M128L is 281 aa residues, with a predicted molecular weight of 32 kDa, including an N-terminal signal sequence, five to six predicted transmembrane domains, and a short cytoplasmic tail. Cellular CD47 is a plasma membrane protein with an extracellular immunoglobulin variable-like domain, five transmembrane domains, and a short cytoplasmic tail (Porter and Hogg, 1998). Although M128L is not predicted by conventional programs, such as SMART and Prosite, to have a classic immunoglobulin-like domain, there is significant conservation of cysteine residues and flanking aa that map to the CD47 immunoglobulin boundary domain (Fig. 3A). Moreover, the two molecules are strikingly similar in the overlapping location of their predicted membrane-spanning domains (underlined in Fig. 3A), as predicted by von Heijne transmembrane analyses (Fig. 3B). Given the importance of CD47 in the host defense system, as evident in the decreased resistance to bacterial infection and the granulocyte defects observed in CD47-deficient mice (Lindberg *et al.,* 1996), we predict that M128L is a cell-surface immunomodulator. Recently, it has been shown that under appropriate conditions, CD47 activation results in the rapid death of T cells via a novel apoptotic pathway (Pettersen *et al.,* 1999). Thus M128L protein could act either as a cell surface decoy for the CD47 ligand that initiates an apoptotic response or as an intracellular sink for signaling molecules of this apoptotic cascade.

M104L: A truncated receptor-like fragment

The myxoma gene M104L is predicted to encode a small 53 aa protein with significant aa similarity to an important domain of a member of the G protein-coupled receptor family (Table 4). In particular, M104L shows closest similarity to the interleukin-8 receptor-like species encoded by the Ateline herpesvirus 3 ORF 74 (Fig. 4), a member of the ORF74-family of herpesvirus-encoded chemokine receptors (Bais *et al.,* 1998; Rosenkilde *et al.,* 1999). Chemokine receptors are seven-transmembrane domain G protein-coupled receptors that are involved in immune cell activation and trafficking (Locati and Murphy, 1999; Premack and Schall, 1996; Strader *et al.,* 1994). Although M104L has no vaccinia or variola homologs, it does show significant homology to the MC119L gene of *M. contagiosum*. Interestingly, MC119L also shares similarity to a *C. elegans* gene that appears to encode a member of the seven-transmembrane rhodopsin receptor family.

M104L has a predicted molecular weight of 6.3 kDa and shares an aa identity of 42% over a 40-residue region of alignment with the ORF 74 (Fig. 4). Although M104L includes a predicted signal sequence cleavage site adjacent to the N-terminal hydrophobic domain (Table 3), it is unclear whether this motif (which corresponds to transmembrane domain 5 of the ORF 74 protein) represents a true signal sequence or an N-terminal transmembrane region. The fact that the M104L protein sequence aligns to the fifth and sixth transmembrane domains and intervening intracellular loop 3 of the chemokine receptor is particularly relevant, as this loop is among the receptor domains implicated in chemokine receptor signaling (Berson and Doms, 1998; Murphy, 1994) and the sixth transmembrane domain has been implicated in receptor dimerization that could play a role in signal transduction (Hebert and Bouvier, 1998; Rodriguez-Frade *et al.,* 1999). Thus we speculate that inhibition of chemokine receptor signaling by M104L could potentially occur by heterodimerization with chemokine receptors to prevent functional dimers or through the sequestration of downstream receptor signaling molecules.

M121R/M122R: Potential similarities to the NK-receptor superfamily

Two of the predicted secreted/cell surface proteins, M121R and M122R, exhibit homology to members of the natural killer cell (NKC) receptor family: NKG2 (human) and Ly-49 (murine), respectively (Table 4). These particular members of the NKC receptor family bind to class I MHC on target cells and inhibit NKC-mediated cytotoxicity, which is normally responsible for immune surveillance to eliminate cells with decreased class I MHC expression levels (Biron *et al.,* 1999; Lanier, 1998; Lopez-Botet and Bellon, 1999). Many viruses, including myxoma, have the ability to down-regulate cell surface expression of class I MHC, to escape detection by T lymphocytes via class I MHC-mediated antigen presentation (Boshkov *et al.,* 1992). Thus specific viral anti-NK cell mechanisms are presumed to exist, and cell surface

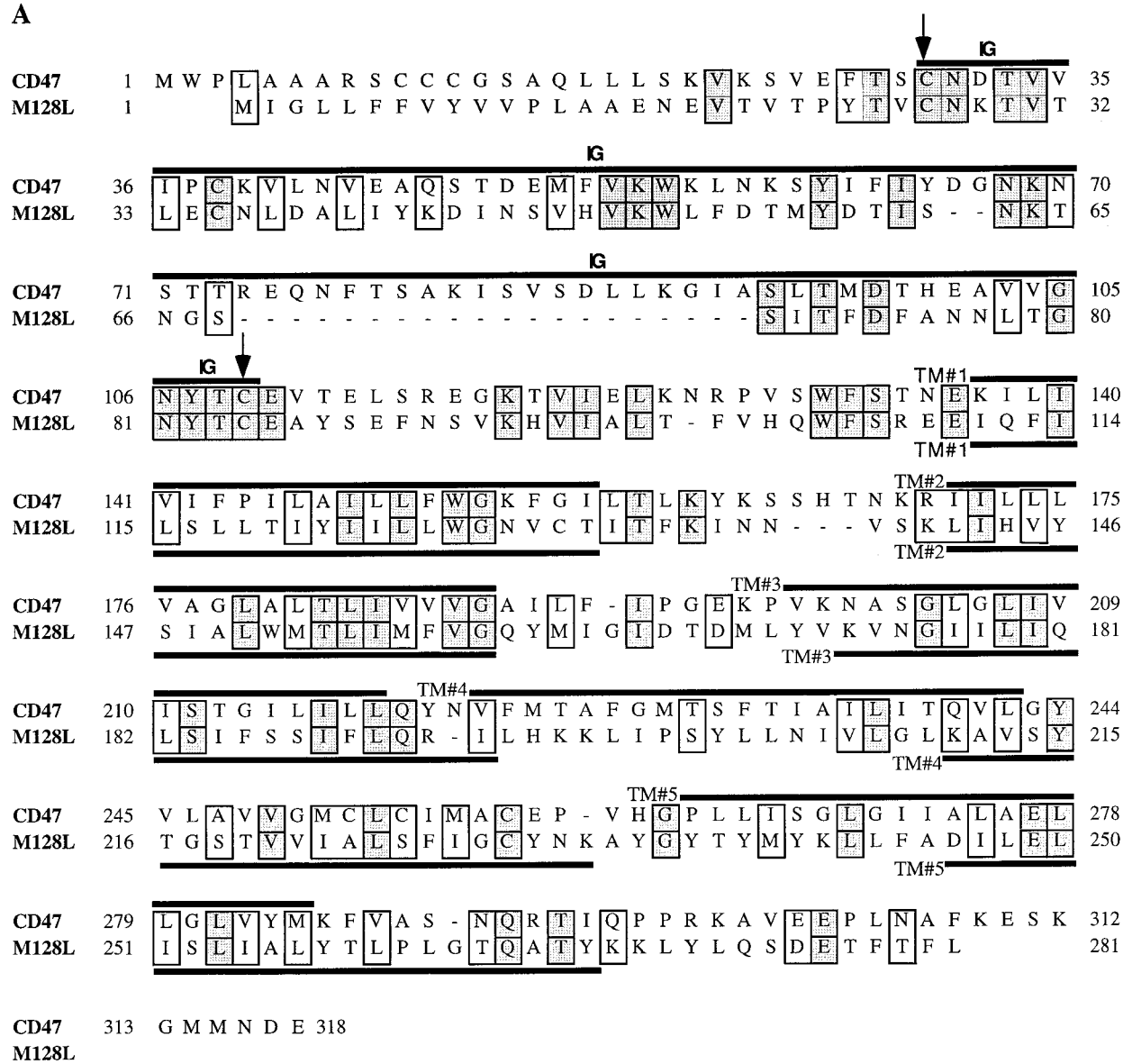

FIG. 3. Alignment of rat CD47 (integrin-associated protein) with M128L. (Panel A) Bold lines denote the following domains: IG specifies the immunoglobulin domain of CD47, and TMs 1–5 identify the five predicted transmembrane domains. The two conserved cysteines involved in the Ig domain formation are indicated by arrows. Amino acid identities are in shaded boxes, and similarities are in open boxes. (Panel B) von Heijne transmembrane plots of M128L and rat CD47.

NKC decoy receptors would be a reasonable strategy to prevent NK cell recognition. M121R shares aa sequence similarity to the C-type lectin superfamily, with highest similarity to the human NKG2-D type II integral membrane protein. The corresponding gene for M121R in vaccinia, A33R (Table 1), also shares sequence similarity with genes thought to encode C-type lectin superfamily members, according to $BLAST_p$ analysis. The M121R gene is predicted to encode a protein of 176 residues in length, with a molecular weight of 19.7 kDa, including a predicted transmembrane domain from residues 46–66. The NKG2 family members (NKG2-A, C, E, and D/F) are found at the cell surface as part of a disulfide-bonded heterodimer in association with the invariant CD94 molecule (Lanier, 1998; Weis *et al.,* 1998). M122R is predicted to encode a protein 172 aa in length, with a molecular weight of 19.7 kDa, including a predicted transmembrane segment from residues 16–36 and, perhaps most interesting, a C-type lectin domain from aa 47–167. The closest cellular homolog for M122R is the murine Ly-49C molecule (Fig. 5), which is another C-type lectin superfamily receptor (Lanier, 1998; Weis *et al.,* 1998). VAC A34R, the vaccinia counterpart of M122R (Table 1), also shares sequence similarity with rat and mouse NKC receptor NKR-P1, according to $BLAST_p$ analysis. It is possible that these two myxoma NKC receptor homologs

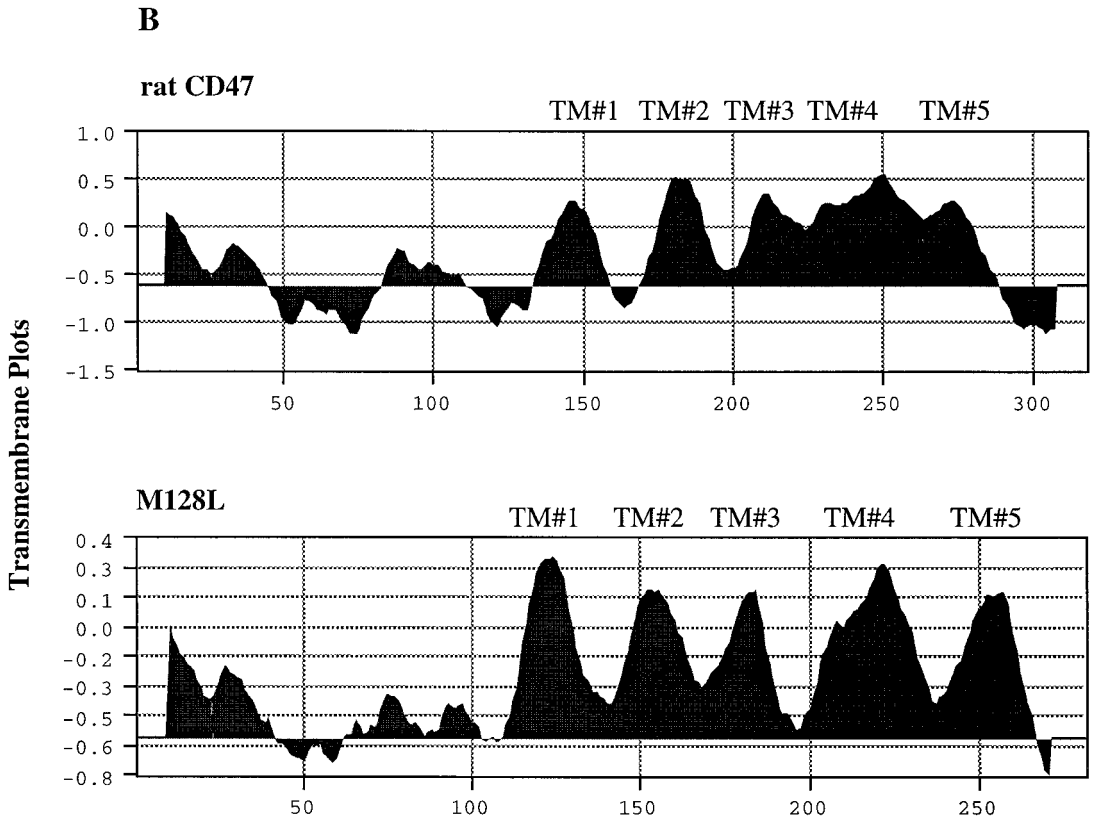

FIG. 3—*Continued*

function as constitutively active NKC receptors inhibiting cell-mediated cytotoxicity if expressed at the cell surface. The closest vaccinia homologs to M121R and M122R, A33R, and A34R, respectively, are extracellular enveloped virus (EEV) glycoproteins that regulate the release of EEV particles from virus-infected cells by facilitating actin tail assembly on intracellular virions (Blasco *et al.,* 1993; Duncan and Smith, 1992; McIntosh and Smith, 1996; Roper *et al.,* 1996, 1998; Röttger *et al.,* 1999; Sanderson *et al.,* 1998). Thus a role for M121R/M122R in myxoma virus dissemination via EEV particles is also a distinct, but not mutually exclusive, possibility.

Putative novel regulators of host range or apoptosis

Table 5 summarizes the myxoma genes that are predicted to play a role in host-range determinations or the regulation of apoptosis. Some genes (M-T4, M-T5, and M11L) have already been demonstrated to be required for myxoma infection of T-lymphocytes by modulating the apoptotic response to myxoma virus infection (McFadden and Barry, 1998). Others are related to poxvirus genes with known roles in host range, such as M062R, M063R and M064R, which are similar to the VAC C7L gene that is required for vaccinia replication in human cells (Oguiura *et al.,* 1993; Perkus *et al.,* 1990). In the case of M143R, the closest poxvirus homolog is the 28-kDa RING zinc finger protein of ectromelia virus, which is essential for growth in murine peritoneal macrophages (Senkevich *et al.,* 1995). Interestingly, the homologous protein in SFV perturbs the DNA fragmentation component of apoptosis (Brick *et al.,* 1998). At least one novel myxoma gene is a candidate modulator of a relevant

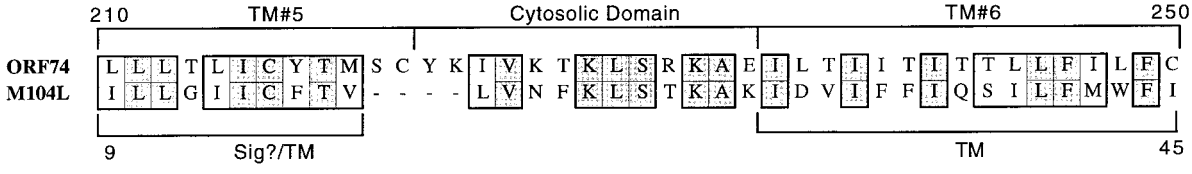

FIG. 4. Alignment of Ateline herpesvirus 3 ORF 74 (interluekin-8 receptor family member) and M104L. TM denotes predicted transmembrane regions, and Sig? identifies a region in M104L that could act as a cleaved signal sequence or an uncleaved transmembrane. The region of homology spans the fifth and sixth transmembranes and intervening cytosolic loop 3 of ORF 74. Amino acid identities are in shaded boxes, and similarities are in open boxes.

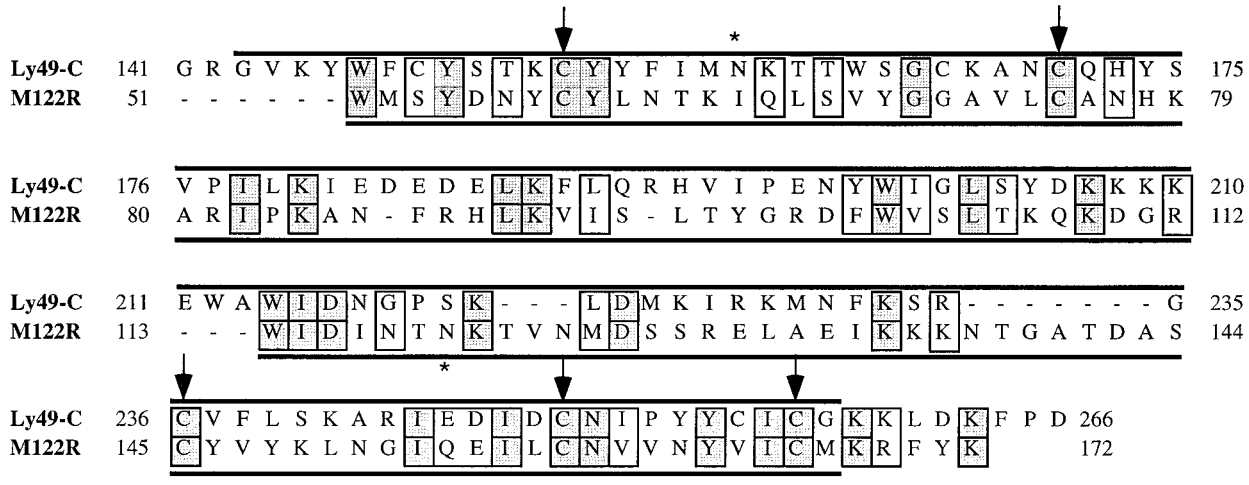

FIG. 5. Alignment of C-terminal regions of murine Ly-49C and M122R. Bold lines are shown above and below predicted C-type lectin domains. Asterisks denote predicted N-glycosylation sites, and arrows identify conserved cysteines in C-type lectin domains. Boxes are as in Fig. 2.

apoptosis pathway. The M063R protein exhibits similarity to a glutamate-rich domain of human DAXX, a FAS-binding death associated protein (Chang *et al.,* 1998, 1999). DAXX and M063R share significant aa identity (30%) and similarity (46%) over an 88-aa stretch at the C-terminal half of M063R.

M005R/L and M148R–M150R: Ankyrin-repeat family members

Ankyrin repeats are thought to be involved in intermolecular or intramolecular protein–protein interactions (Lambert *et al.,* 1990). Viruses have taken extensive advantage of this particular motif for protein–protein interactions. More than 508 ankyrin domains can be mapped in a total of 99 viral proteins using the standard predictive programs (Schultz *et al.,* 1998). A large proportion of viral proteins containing ankyrin-like repeats are present in the poxvirus family.

In myxoma virus, there are four ankyrin-containing proteins: M005R/L, M148R, M149R, and M150R (Table 5). Interestingly, SFV has counterparts for three of these myxoma ankyrin proteins but lacks a complete homolog of M150R (Willer *et al.,* 1999). The deletion of M-T5 (M005R/L) from wild-type myxoma virus results in a complete loss of virulence in a susceptible rabbit strain, and it was shown that this M-T5 knock-out virus caused apoptotic death of *in vitro* infected rabbit lymphocytes (Mossman *et al.,* 1996). Thus M-T5 (M005R/L) can be classified as an ankyrin-containing, host-range-determining virulence factor of myxoma virus.

The three newest members of the myxoma ankyrin family, M148R, M149R, and M150R, are predicted to be cytoplasmic in terms of cellular localization (Table 5). M148R, the largest of the three with a calculated molecular weight of 77.4 kDa, contains nine putative ankyrin repeats. M149R, with a calculated molecular weight of 56.7 kDa, contains eight putative ankyrin repeats, and M150R, at 57.8 kDa, is predicted to have five ankyrin repeats. M148R is the only one of the four ankyrin-like myxoma proteins for which transmembrane regions are predicted.

M008.1R/L, M151R, and M152R: SERP-1, -2, and -3

There are three ORFs in the myxoma genome that encode proteins with serpin signatures (Table 4). M008.1R/L (SERP-1) has already been characterized extensively as a secreted serpin with anti-inflammatory capabilities (Macen *et al.,* 1993; Nash *et al.,* 1999, 1997). A SERP-1 knock-out virus was shown to be less virulent than the wild-type counterpart (Macen *et al.,* 1993), and purified SERP-1 protein has been shown to be a potent anti-inflammatory agent in arterial restenosis (Lucas *et al.,* 1996). Among the *in vitro* proteinase targets of SERP-1 characterized are urokinase plasminogen activator, tissue-type plasminogen activator, plasmin, and thrombin, all of which bind SERP-1 with high kinetic rate constants (Nash *et al.,* 1998). M151R (SERP-2) is a weak intracellular inhibitor of interleukin-1β-converting enzyme (ICE) and granzyme B (Petit *et al.,* 1996; Turner *et al.,* 1999). SERP-2 has also been demonstrated to be a virulence factor: a SERP-2 knock-out virus resulted in reduced mortality rates of infected European rabbits (Messud-Petit *et al.,* 1998).

We have named the M152R protein, the new putative serpin, "SERP-3" to fit in with the nomenclature shared by the two other myxoma serpins. SERP-3 is calculated to be a 30-kDa intracellular protein with 20% identity to a cellular serpin named leupin or squamous cell carcinoma antigen 2 (Table 4), although the predicted P1-P1′ (reactive center of the serpin) of leupin is not conserved in M152R (not shown). It is predicted that M152R/SERP-3 has a zinc finger motif (Table 4) and an RNP-1 signature

(aa 61–68) that would suggest RNA-binding activity. A final intriguing point about SERP-3 is that like M008.1R/L (SERP-1), it is found in fragmented form in the SFV genome (Willer *et al.,* 1999).

Although caution must be exercised in assigning functions to potentially immunomodulatory domains, previous experience has indicated that even lower similarity scores can be significant. For example, the myxoma IFN-γ receptor homolog (M007L/R) demonstrates only 20% sequence identity to the ligand-binding domain of the cellular receptor counterparts, yet it binds and inhibits its IFN-γ with similar affinity to that of the host receptors (Upton *et al.,* 1992).

Concluding Remarks

This myxoma sequence, in conjunction with the concurrent publication of the SFV sequence (Willer *et al.,* 1999), provides a comprehensive look at a distinctive genus of the poxvirus family, the *Leporipoxviruses*. Myxoma virus encodes many of the housekeeping and structural genes that are conserved among the fully sequenced poxviruses and shares certain virulence factors possibly as the result of independent gene-capture events, such as M-T1 (M001R/L), which is closely related to the 35-kDa orthopoxvirus family of chemokine-binding proteins. In general, we predict that the genes that appear to be most divergent from other sequenced poxvirus family members are likely enriched for novel immunomodulatory factors. Knowledge gained from studies of the *in vivo* effects of immunomodulatory molecules such as those discussed here will provide a more comprehensive perspective of poxvirus–host immune interactions.

MATERIALS AND METHODS

Myxoma virus DNA isolation, cloning, and sequencing

Viral genomic DNA was prepared using the method outlined elsewhere (Esposito *et al.,* 1981). Briefly, roller bottles of baby green monkey kidney cells were infected with myxoma virus (Lausanne strain, from ATCC), viral cores were isolated with sucrose cushion centrifugation, and viral DNA was extracted with phenol–chloroform.

Random genomic fragments of viral DNA were generated by sonication at 0°C. The sheared DNA was then ethanol precipitated, and blunt ends were generated using both T4 and Klenow polymerases. After phenol–chloroform extraction and ethanol precipitation, the DNA was separated on a 0.8% agarose gel and fragments of 1.5–3.0 kb were excised and purified. With a 3:1 ratio of insert to vector ends, the myxoma DNA fragments were then blunt end ligated into the *Eco*RV site of calf intestinal phosphatase-treated pBluescript II (KS+) vector (Stratagene) by incubation at 16°C overnight with T4 DNA ligase (GIBCO). The ligation mixture was then transformed into *Escherichia coli* ElectroMAX DH10B electrocompetent cells (GIBCO). Polymerase chain reaction (PCR), using T3 and T7 primers (Promega), was performed directly on positive colonies to identify clones containing inserts of the appropriate size for sequence analysis. Cycle sequencing reactions were performed at the DNA Sequence Facility at The John P. Robarts Research Institute, which uses the ABI377 DNA sequencer. Sequencing reactions were performed initially with M13F/R primers and subsequently with uniquely tailored primers, when additional sequencing information was required from a given clone.

The nucleotide sequences obtained from the sheared fragments of myxoma virus DNA were manipulated using Sequencher 3.0 software (GeneCodes Corporation). Contigs were generated by assembling incoming nucleotide sequences automatically, with an 80% minimum match of overlapping nucleotide sequences and a 40-bp minimum overlap used as the contig building parameters. This ensured a random but stringent association of DNA sequence.

At the end of the first phase of sequencing, five large contigs of overlapping sequence were generated. To orient these five sections of the myxoma genome with respect to one another, the predicted restriction enzyme profiles for *Eco*RI, *Kpn*I, *Bam*HI, *Sal*I, *Hin*dIII, *Bgl*I, *Pst*I, and *Pvu*I were compared with those previously published for the myxoma virus Lausanne strain (Russell and Robbins, 1989). In this way, the contigs could be aligned in the correct order to fit the restriction map of myxoma Lausanne.

A second myxoma DNA library containing larger inserts was screened to close the gaps between the five major contigs. Random viral DNA fragments of 5–10 kb were generated by partial *Sau*3A restriction enzyme digestion and size selected on a 1% agarose gel. The purified DNA was ligated into calf intestinal phosphatase-treated *Bam*HI digested pMJ601 plasmid vector (Davison and Moss, 1990) and transformed into Electromax DH10B electrocompetent *E. coli* cells (GIBCO). Dot-blot hybridizations with probes to DNA sequence at the edge of each contig were carried out to identify potential gap region-containing clones. Sequence analysis with primers designed to bind and span the gap region was performed.

A single gap remained in the assembled contig sequence after probing our two libraries, so specifically designed internal primers were used to amplify the gap region using PCR. Multiple independent reactions were carried out directly on myxoma DNA, and the resulting PCR products were sent directly for sequence analysis, with custom-designed primers. This approach was successful in obtaining the sequence that bridged the gap between the two large remaining contigs.

The terminal nucleotide sequence was obtained by ligating a blunt-ended adapter (29-base upper strand, AATTCTAGAAGCTTCGGATCCCGGGTACC; 25-base

lower strand, GGTACCCGGGATCCGAAGCTTCTAG) to mung bean-treated myxoma genomic DNA. Subsequently, PCR amplification of the terminal sequence was performed using specifically designed primers complementary to (1) a region adjacent to the M001L/R gene and (2) the upper strand of the adapter sequence. Three separate PCR products were sequenced in both directions. The resulting sequence was found to contain the conserved terminal resolution sequence that in SFV maps next to the extrahelical bases at the hairpin terminus (Upton *et al.,* 1987).

Computer-based nucleotide and aa analysis

The final edited myxoma virus consensus sequence was subjected to an ORF analysis in all six possible reading frames using MacVector 6.0.1 software (Oxford Molecular Ltd.). Using a minimum cut-off of either 50 aa or, in one instance, 25 aa, each candidate ORF was translated to its primary aa sequence using MacVector. Traditional eukaryotic translational start and stop codons were used to delineate the ORFs. The resultant aa sequences were analyzed for homologies to other proteins contained in public domain databases by unfiltered BLASTp (Altschul *et al.,* 1997). This allowed for the assignment of putative functions to most ORFs. All BLASTp searches reflect the contents of protein and public nucleotide databases as of June 16, 1999.

Further analyses were conducted on the aa sequence of selected ORFs. Cell sorting predictions were performed using PSORTII (Horton and Nakai, 1996, 1997), which determines the likelihood of the presence of a signal peptide in a given ORF and subcellular localization of the putative proteins. Upstream nucleotide sequences were examined to determine whether early-, intermediate-, or late-type promoters may be present. MacVector was used to calculate the molecular weights and pIs of selected ORFs and to plot the hydrophilicity of their aa sequences using the scale of Kyte and Doolittle (1982). Both ProfileScan (Bucher and Bairoch, 1994; Hofmann *et al.,* 1999) and SMART (Schultz *et al.,* 1998) were used to search for specific motifs in particular ORFs, including transmembrane regions, ring finger domains, ankyrin repeats, and immunoglobulin-like domains.

Certain ORFs with interesting homologies and predicted functions were subjected to both CLUSTALW multiple alignments (with a gap penalty of 10) with the help of MacVector or the GAP function of GCG (Wisconsin Genetics Computer Group) (gap penalty of 12) to their cellular or viral counterparts. This allowed for both the visualization and the calculation of aa identities and similarities. The accession number for the entire myxoma sequence is AF170726.

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