Similarity in genome organization between Molluscum contagiosum virus (MCV) and vaccinia virus (VV): identification of MCV homologues of the VV genes for protein kinase 2, structural protein VP8, RNA polymerase 35 kDa subunit and 3β -hydroxysteroid dehydrogenase

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Molluscum contagiosum virus (MCV) and vaccinia virus (VV) are serologically unrelated poxviruses with a disparate genome composition (MCV, 66% G+C; VV, 33% G+C). Molecular studies of MCV have been hindered by the inability to propagate the virus in cells cultured in vitro. We sequenced 7765 bp of MCV DNA cloned from four widely spaced regions throughout the MCV genome and identified a total of 11 potential open reading frames (ORF), designated CX1-11. These include MCV homologues of the VV genes encoding protein kinase 2, structural protein VP8, RNA polymerase 35 kDa subunit and 3β -hydroxysteroid dehydrogenase. The position and orientation of the MCV ORFs was collinear to the VV genome, with the exception of the region around ORF CX11 which is inverted in the MCV genome.

Molluscum contagiosum virus (MCV) is the only species of the genus *Molluscipoxvirus* and does not cross-react serologically with other poxviruses (Mitchell, 1953). MCV causes raised umbilicated lesions in the epidermis of human skin (Postlethwaite, 1970), specifically infecting differentiating keratinocytes. The virus has not been cultured *in vitro* although

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MCV replication has been demonstrated in human foreskin grafted to athymic mice (Buller et al., 1995). MCV infection is common in children, sexually transmitted between adults and is frequently seen in immunocompromised individuals (Cotton et al., 1987). In contrast to most poxviruses, the MCV genome has a high G + C content (66%). Restriction endonuclease analysis of the MCV genome shows no similarity to other poxviruses and there is considerable variability in restriction enzyme maps between three MCV subtypes (Porter & Archard, 1992). The genome has inverted terminal repeats (Porter & Archard, 1987; Bugert et al., 1989) containing tandemly repeated sequences (Bugert et al., 1993). Initial sequencing studies on MCV type I (MCV-I) identified an open reading frame (ORF) potentially encoding a 43 kDa protein, homologous to the vaccinia virus (VV) major envelope antigen (Blake et al., 1991). Further studies identified a number of ORFs potentially encoding limited homology to VV proteins (Bugert et al., 1993; Hadasch et al., 1993) and apparent MCV homologues of the VV genes encoding poly(A) polymerase and small (22 kDa) and large (147 kDa) subunits of the DNAdependent RNA polymerase (Sonntag et al., 1995). The latter occur within BamHI fragment A of MCV-I DNA and their arrangement is co-linear with the equivalent VV locus.

We now report the sequence of four widely spaced regions of the MCV-I genome and the identification of putative MCV genes by comparison to the VV genomic sequence (Goebel *et al.*, 1990; Johnson *et al.*, 1993).

MCV-I was purified from human skin lesions and virus DNA extracted as described by Porter & Archard (1987). The *Hind*III, *Bam*HI and *Cla*I restriction enzyme maps of MCV-I DNA are described by Porter & Archard (1992). *Hind*III fragments Q, C, E, I, J_L, N, M, H, K_R and J_R were eluted from agarose gels and cloned in the *Hind*III site of plasmid pUC1318 (Kay & McPherson, 1987) by standard methods. The recombinant *Hind*III C plasmid was digested with *Bam*HI and sub-

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fragments were recloned to give clones of MCV-I BamHI fragments M, N and L. Genomic MCV-I DNA was digested with ClaI and cloned randomly in the ClaI site of Bluescript KS(+) (Stratagene): recombinant plasmids containing ClaI fragments F, H and E were isolated. Each cloned MCV-I fragment was sequenced using commercially available pUC forward and reverse primers with the PRISM Ready Reaction DyeDeoxy Terminator Cycle sequencing kit and the Applied Biosystems model 373A DNA sequencing system. ORFs were identified using DNA Strider 1.2 and potential protein homologies were identified using BLAST searches. Sequences of interest were extended by custom primer walking or sequencing appropriate subclones with pUC forward and reverse primers. The sequences reported here were determined in both directions. Where sequences spanned two adjacent cloned fragments such as BamHI L and N, HindIII I and E or HindIII N and M, primers were designed to span the restriction sites. PCR sequencing using genomic DNA for the HindIII I and E or HindIII N and M junctions and a plasmid containing the HindIII C fragment including the BamHI L and N junction (see Fig. 1) confirmed that these fragments were indeed contiguous within the MCV genome. Repetitive sequencing in both directions revealed no ambiguities.

Four regions of MCV-I DNA totalling 7765 bp were sequenced (Fig. 1) and analysis of these revealed marked similarity between MCV and VV at the amino acid level. These regions of MCV-I DNA correspond to VV ORFs F9L, F10L, L3L to J3R, A28L-30L and A44L which include the VV genes known to code for protein kinase 2 (F10L), structural protein VP8 (L4R), RNA polymerase 35 kDa subunit (A29L) and the 3β -hydroxysteroid dehydrogenase (3β -HSD) (A44L: Goebel *et al.*, 1990; Johnson *et al.*, 1993). The sequences of the expanded regions of Fig. 1 are shown in Fig. 2. The 1850 bp MCV sequence shown in Fig. 2(*a*) comprises the entire *Bam*HI N fragment plus 251 bp of the left end of *Bam*HI M and reads from right to left in the genome. A complete ORF of 1329 bp (CX1) is present in position 110–1438, encoding a putative

protein of 443 amino acids (aa) which shows 60.2% identity and 80.5% similarity to VV protein kinase 2 (VVPK2). The catalytic domains I, II and VI of VVPK2 (Lin & Broyles, 1994) are conserved in the putative MCV protein sequence (Fig. 3 *a*), suggesting that a protein expressed from this ORF would have kinase activity. A partial ORF (CX1) extending from position 1422–1850 shows 44.4% identity and 72.2% similarity at the amino acid level to VV ORF F9L.

The 3158 bp sequence at the right end of the centrally located MCV-I ClaI F fragment is shown in Fig. 2(*b*). This contains three complete ORFs of 762 bp (CX4), 438 bp (CX5) and 549 bp (CX6) and two partial ORFs of 88 bp (CX3) and 863 bp (CX7). These MCV ORFs correspond to VV genes L3L-J3R (Goebel *et al.*, 1990), with the exception of the thymidine kinase (TK) gene, J2R, not found at this position in MCV-I. The complete ORFs code for putative polypeptides of 254 (CX4), 146 (CX5) and 183 (CX6) aa, resembling the products of VV ORFs L4R (42.8% identity; 75.3% similarity), L5R (31.8% identity; 60.6% similarity) and J1R (44.5% identity; 74.2% similarity) respectively.

The partial 863 bp ORF (CX7) shows 57.2% aa identity and 80.7% aa similarity to VV J3R. VV ORF L4R encodes the structural protein VP8 (Yang et al., 1988) and VV J3R encodes the poly(A) polymerase stimulatory subunit (Gershon et al., 1991). The VV TK gene is located between ORFs J1R and J3R (Hruby & Ball, 1982) but an MCV-I homologue was not found in the corresponding location. Thymidine kinase activity has not been demonstrated in MCV and it is not known whether this gene is absent or situated in a different position in the MCV-I genome. Fowlpox virus (FPV) also lacks a TK gene in this position (Drillien et al., 1987) but it has been located in the region corresponding to VV I4L (Binns et al., 1992). Fig. 2(c) shows 1251 bp of MCV-I DNA, located at the left end of HindIII I and the right end of HindIII E and reading from right to left in the genome. It includes two complete ORFs of 201 bp (CX10) and 978 bp (CX9) at positions 80-280 and 183-1160 respectively. CX10 encodes a putative polypeptide of 67 aa

(a)	
1	CGCTCACAGCTTCGCGAGCCCGGATCAGCCCCGCGCCAGCAGTCCGCCGTCGCCGGCAGGCTCCCCGCTGTCGCGCGCG
	CX2
101	GCACAT <u>TAAAT</u> GGCATTCTCGGACAGTGCCTCCGCGGACGCGCCCTGGAGCGCAGTCCCAGCACCGCGCCGCGACGAGACCACGGTCCTGGGCGACGAAA
1	M A F S D S A S A D A P W S A V P A P R R D E T T V L G D E I
201	TCTACTTTAACTACGTGTACGGGCAGCTCGAGCTCGACGGCACAGTTGGATCCCTCACGTGCGCATGCTGCGCTACTTCCGCAACTTCTCGCGCGCCGCCGCCG
32	Y F N Y V Y G Q L E L S D S W I P H V R M L R Y F R N F S R A A L
301	GCTGCGCATCGCCAGCACGGAGTACGTGAACCCGTCCTATTTCCAGCAGAAGGACAAGCGCTTTGCGCCCGTCAACAACGACTTCTACCACCTGTCCACC
65	L R I A S T E Y V N P S Y F Q Q K D K R F A P V N N D F Y H L S T
401	GGCGGCTACGGCATTGTCTTCCGCGTGGAAGAGTACGTGGTCAAGTTCGTCTTCGAGCCTGGCAGCCAGTTCCACCCCATGGATCTCACGTCCGAATACA
98	G G Y G I V F R V E E Y V V K F V F E P G S Q F H P M D L T S E Y T
501	CCGTGCCGCGCTTCCTCTACAACAACCTGCGGGGCGACGAGCGCCCTGCTGGTGGTGGCGCGCGC
132	V P R F L Y N N L R G D E R L L V V R A L A M G L N Y K I G F L Y
601	CACGCTCTACAAGCGCGTGCTGCACATGGTCCTGCTACTGGCACGCATCCTGGACGGGCAGCCGCTGTCGCTAGCGTACTCGCGCCAGGTGGCCAAG
165	T L Y K R V L H H V L L L A R I L D G Q P L S L A Y S R R Q V A K
701	CTCTTCGCGGAGCGCAAGGACAGCGCCAAGTTCGTGCGCTTGCTGTCCTACTTCTACCCCGCCGTCATTAAGAGCAACCTCAACGTCATCAACCACTTCG
198	L F A E R K D S A K F V R L L S Y F Y P A V I K S N L N V I N H F G
801	GGCACATGATACATTTCTTCGAGCACGAGAAACGCGCCAACTACACCTATGACCGCGGCAACATCATCGTTTTTCCGCTGGCACGCTGCTCGGCGGAGAA
231	H M I H F F E H E K R A N Y T Y D R G N I I V F P L A R C S A E K
901	GGTCACCGCGGCGAACTGTGCCGAGTTCGGCTTCGGCTCAGTGGTGCACTACGTCAAGTTCCTCTTCCTACAGATGGCGCTGCTATACATCAAGATTTAC
264	V T A A N C A E F G F A S V V H Y V K F L F L G M A L L Y I K I Y
1001	GAACTGTCCTGCCACAACTTTATCCACGTGGACCTCAAGCCCCGACAACATCTTGCTCTTCGACTCGGAACGCGAGATGCGCATCCACGTGGGCGAGCGTA
297	ELSCHNFIHVDLKPDNILLFDSEREMRIHVGERS
1101	GCTACGTCTTCCGCGAGCCCGTACGCAGCGCGCGCGCACGATTTTGACTTCTCGCAGGTTTCCGAAATCCCCCAACAAGAAAATCACGGCCAGCCTGCGCGT
331	Y V F R E P V R S A L N D F D F S G V S F I P N K K I T A S L R V
1201	CGAGCAGAACTGGTTCTATGACTTCCACTTTTTCGTGCACACACTACTACTCAAGGTCTACCCCCGAGCCCGAGCGCGCGC
364	E Q N W F Y D F H F F V H T L L K V Y P F L F R D A A W S K A L G
1301	GAGTICETGGTCTGCTGCGCAACGCCGCAACGCCTGCGGGGAGAGGTTCCGCCTGCGCGCGC
397	
1401	ACCITITCICCGACTGGATAAATGGEGAGEGEGEGETTTAGFAFGCTCTAFGGGGGGFCTTTGGTGGGAGGTGGGAAGGTCGGGGGGGGGG
431	L F S D V I N G F P P *
1501	
1601	TAGGTICGTCIGCIGGTCGAAGGCTTCCCCAGAGAGCGTCGACGAGCGCCGAGATCGCCGAGATCGCCCGAGACGGCGCGAGGCGCGCGGCGGCGGCGGCGGCG
1701	GCCGCCAGCAACGACGACGACGCACGCACGCACGCACGC
	A A S H F F S S I F F F C B A H A A I V O S I D V O T I H V C T C I
1801	
	Fig. 2. (a) For legend see page 3117.

which is 44.9% identical and 75.4% similar to that of VV ORF A30L. CX9 encodes a putative 303 aa protein which shows 44.5% identity and 69.5% similarity to the RNA polymerase 35 kDa subunit encoded by VV A29L (Amegadzie *et al.*, 1991). Downstream of the MCV-I homologue of A29L is the start of ORF CX8 at position 1206, which shows 66.7% identity to VV ORF A28L over the first 15 aa.

Fig. 2(*d*) shows 1506 bp of sequence from the right end of MCV-I *Hind*III fragment N and the left end of *Hind*III M. This contains one complete ORF of 1248 bp at positions 70–1317 (CX11) encoding a putative product of 354 aa which is 44% identical and 70·3% similar to VV 3 β -HSD, the product of VV ORF A44L (Moore & Smith, 1992). This MCV-I ORF is in the opposite orientation to the VV gene and a sequence to the left of CX11 (data not shown) is similar to VV A45R, which is located to the right of VV A44L.

Analysis of the sequences 5' to MCV ORFs reveals motifs consistent with known poxvirus early and late promoters (Davison & Moss, 1989a, b) which are conspicuous amongst the G+C-rich coding regions of MCV. Elements with homology to VV early promoters were present upstream of ORFs CX9 and CX11 and the VV late motif TAAAT was present upstream of ORFs CX1–7 and CX10 (see Fig. 2). It has yet to be determined whether these motifs are active in transcription of MCV genes.

These results demonstrate collinearity in gene organization between MCV-I and VV in the region between VV ORFs F9L and A45R. Seven complete MCV-I ORFs have been identified which, when translated, would show 32-60% aa identity and 61-81% aa similarity to their respective VV homologues (see Fig. 3). Such a high degree of protein conservation in two viruses which do not cross-react serologically and which have genomes of disparate base composition implies the conservation of functions essential to poxvirus infectivity, such as the kinase, structural protein and RNA polymerase subunit. Conservation of a potential virulence factor, the 3β -HSD, thought to reduce host inflammatory response by inducing host steroid hormone synthesis (Moore & Smith, 1992), is

(b) CX3 R S E L F H A H P K P A D L H A H P K Q G D G G A N Q K N _M 1 CGCGACTCGAGGAAGTGCGCGTGCGGGTTTGGGCGCGCCCAAGTGCGCGTGAGGCTTTTGCCCGTCGCCGCCGCGTTCTGCTTGTTCA CX4 101 AAAAACCACTC<u>TAAAT</u>GAACGCCCTCTTCGAGAACCTCTTCGACGACGACGCCGTCTGCGCCGGCAGCGTCTCGCGCGAAGACTTCCTGCTGGTCGTCGC M N A L F E N L F D E D A V C A G S V S R E D F L L V V A 1 201 CGGCGCCAAGGTCAAGTTCCCCGCGTTCGCTGCTGTCCATGTACCGCGTGGTGCCGCCGCCACCATGTCGCGGTTACGAGCTCGCGCTCGTGCAGTCCGAGACG 30 G A K V K F P R S L L S M Y R V V P R T M S R Y E L A L V Q S E T 301 GTCACGGGCGTGGTCTTCACCACCGTGTACAACGTGCGCCCCAACCTGGGGCTGGAGGAGCGCGGAGGCGCTGAGCCTGCCCGCGCTCGAGAAGTACTACC 63 V T G V V F T T V Y N V R R N L G L E E R E A L S L P A L E K Y Y L 401 TGGACAAGGCGAACGACGTGCTTACGCTCATGGTCAACAACACCAACCTGGAGCACATAGCGGCCTACCGCATGCGCAGCCGGCCCTGCTCAACCCCCGT 97 DKANDVLTLMVNNTNLEHIAAYRMRSRRLLNPV 130 V F R A G A V P L A L V F T S R K K L S I Y R E D T S Q A A E D S 601 ACCTACACCAAGATCGCGGCCAATGTGGCGGCGGGCAAATACGCAGGGCTGCTGCTGCTGATGTGCACACGCCCGGGACGGCGCTCATGCTGACCG 163 TYTKIAANVALAGKYAGLLLLDVHTPGTALMLTA 701 CCGTGTACGGGCTGGACGATCGGCGCGAGCTGCGCAAGCTCGCGGACAGCACCGCCCTCGAGAACCACCAGCAGAGCGGCGCGCTCTCGGAAGCCATGAA VYGLDDRRELRKLADSTALENHQQSGALSEAMK LSDFRAVFEGLKKSVPLTNLEMINE* CX5 1101 CCTCGAATTTCGAGAGAATAATGAGCAGCACCGCACGCCAGCAGGAGGAGGAGGAGCAGCGCGCGCGCGCGCGCTTTTTATCGAGCCGCGCTTCGAGCACG 1 M S S T A R Q Q Q R S S V R L Q P V F I E P R F E H A 1201 CTTTCCTTTGCGGCGAGCGCTATCTCTGGATTGTCTTTTTCGAGGCACTCGTCGCGCTGCTTCTGCGCTGGTTTCTGGGCTCGGAGTTGCGCGCCGC 28 FLCGERYLWIVFFEALVALLLLRWFLGSELRAA 61 F S R R P R A P E D P L R Q M V A G K R L A C A G E R L M I L G L 93 H G G P Q A A L N L D G S E V R L P N C E A F L R G A G R A N T D S 1501 GCGCGGGCGAGGCAGGAACGGATGCGGGGACCGGCACGGACGTGGGCGCTCTCCTTGTCTAACGCCCTCCTCACGCCCTCTCGAGGCGGAAAGTCGTG 127 A G E A G T D A G T G T D V G A L L V * 1601 CGCGTTCGGAACGCGTGAGGAGGCGCGCACTGCTAGCGACGCGGACACATTGCGAGGCACCGCCCCGCCCCGCGCTCGACAACGACAGGAACGCACCACGGCC CX6 1701 GACGGCACGGGCACGCGCGCGCGCCTTTCCGGCCTG<u>TAAAT</u>GGACCACAAGCAGTACCTGCTAACTATGTTCTTCGCAGAGGACAGCTCCTTCTTCAAGTAC M D H K Q Y L L T M F F A E D S S F F K Y 1 1801 CTGTCGGAGGACGACGACGACGACGCCCTGGACGACGTAATGATTGTCAAGCACTACATGGACGTGCTGCCGGCCCTGCTAGTGCGCGCCCAAGAACAAGC 22 L S E Q D D D T A L D D V M I V K H Y M D V L L A L L V R A K N K L 1901 TCGAGGCGCTTGGGCACTGCTACGAGCCGCTGTCCGAGGACTTCCGCGCGCTCTTGCATGTGCGCCAGCTGCGAGAACTACGCCAGGTGCACGACCGCGC 56 EALGHCYEPLSEDFRALLHVRQLRELRQVHDRA 2001 GCTGCTGCGCCTGGACGCAGAGCCCGTGCATGTGAGCCATGGCTACCTTGCGGACTTCGTGCCTAGCCTAGTACGGCTGGCGCGAGCTGGGGGAGCTG 89 L L R L D A E P V H V S H G Y L A D F V L S L V R L A R E L G E L 2101 TGCGTGCCACCGCGCACGCGCTACGTGGACCCGCGCGACGACCCTACGCTGGCCTACGTGCTGGAGATCCTGCACGGCACGGACGTCGACTCTGGCGCGG 122 C V P P R T R Y V D P R D D P T L A Y V L E I L H G T D V D S G A G CX7 156 AYALARPEAEKISPVRRALPGCSPSRRP* ME

3 A Q A M E R P L L Y F H E L T Q T Q E Y D A E V E R A A R S R F P 2401 GCACAAGGGCAGCTCAAGCTGCTCATCGGCGAGCTCTTTTTCCTGAACAAGCTGCACCGGCGCGAGATGCTCGCCGGCACCACGGTGGTTTACATCGGCT 36 A Q G Q L K L L I G E L F F L N K L H R R E M L A G T T V V Y I G S 2501 CCGCGCCCGGCGGGCACATCCGCTACCTGGTGGAGCACTTCCGCGCGCCTAGGGGTGCCGCTGCGCTGGATGCTGCTCGACGGGCGCACCACCACCGCCG 70 A P G G H I R Y L V E H F R A L G V P L R W M L L D G R S H D S R 103 L Q G L S D V T L V T R F V D E R Y L M R M R Q A L R G A R V V L 2701 ATCTCCGACATCCGCTCGCGTCGCGGCAGCGAGCGCCGGCAGGAGGACCTGCTGTACGACTACGCGTTGCAAAACTCCATGCTGAGCATCCTGAAGCCCG 136 I S D I R S R R G S E P S T E D L L Y D Y A L Q N S M L S I L K P V 170 ASSLKWRCPFPDQWLHNFYVVCGKELLQPFAPP 203 F S A E L R L L S V H A G A P R L R C I T L A A A R D Y E K K M F 3001 TATCTCAATAACGTGATCCGGCGCCCGCATCGTGCTCAACTTCGACTACCCGAACCAGGAGTACGACTTCTTTCACATGTTCCACCTCCTAAATACGGTAC 236 Y L N N V I R R R I V L N F D Y P N Q E Y D F F H M F H L L N T V L 3101 TGTGTCCTCGCAGCTTCGACAGCCCCACCAAGAAGGTGCTCTTCTTGCAGCAATCGAT 270 CPRSFDSPTKKVLFLQQS

Fig. 2. (b) For legend see facing page.

(c)CX10 MEDVDEA 101 AACCTCCTGCACCTGCTGGAACGCTTGGCGGGGAGGGCGGCGACGACGACGACGCTGGCCGCCATCCGCGAGCTAATCAGCGCCCATCAATTCAA N L L H L L E R L A G S G D D D F G A T L A A I R E L I S A I N S K CX9 201 AGGTGCTCACCCTTAATAAAAATCAAAAAAAGTGCGCGTGCGGGCGAGCATGTACCGAGAAAAACGCTTCGCATTGACCTCCCGCCCAGCGTGG VLTLNKKSKKSARAGEHVPRRENASH* MYREEKTLR IDL PPS 301 CCAGCTTCATCAAGCACGGCTTCCGGCACCACGTGCGCTGGCCCCACGCTGGCGTGGTGGTGGCCAACACTACCACAGCCATTAACGAAGAGTG SFIKHGFRHHVRWPTLALGVVLANTTTAINEEW L T A V E S M P T R K V F H A F V E P V L E G T L H N C V H L K K 501 ACGCAGAGCGAGGGCGACGCGTACGTGTCCATGCACGACTTTGACTACGTGGTGCGCGACGACGGCACGCTGAGCAAGCTCAAGAAGCCCAAGGATC T Q S E G D A Y V S M H D F D Y Y V V R D D G T L S K L K K P K D L 601 TGCGCGAGACGCTGCTGCACAGCTTCCTGGAGTACCGGCTCAAGAACACCAAGAGCATCGAGCTGGTGGCCTTTAGCAGCGGCACGCAGATCCGCGAGGA R E T L L H S F L E Y R L K N T K S I E L V A F S S G T Q I R E E 701 GCTGCTGACGCACCTGGCCGGGGTGCTGGACATCGAGGTCTTCACGCGCGAGCACGCCAACGTGAAGGTGACCTTCCCCGAGGAGCCTCGCAGCACGTGT L L T H L A G V L D I E V F T R E H A N V K V T F P E E P R S T C P F G V I A P R G Q L R I F F E A Y P W V D T H Q H L H A V L R L L 901 TGGAGAGGAAGCTTGTGGCCGACGTGCGCAGCAGCAGCCGGATCCTTGTCACGCCGGAACTGGACTTCGAGGGCGGCGTGTCCAAGTACGACCCCGCGAGCCG E R K L V A D V R S S Q I L V T P E L D F E G G V S K Y D P A S R M L L V R D M V T M S I V N F F G A R A Q L D T Y H D F D M R V V D T E R F L S A L A E A F A T L R A L V CX8 M D P L S V F F L V V A A A A (d) 1 AGTCTCATGGTCATTTTTGGTAGCAAGTTTCACGCGTGAGTAGCAACAAGAGGAAAAGAAACCCCCTAGCAGACAACTGCGTCTCAAAGACAAGTCACACT 101 CTTGTTTGTTGTCCCACTCTCGAAAGTCGCGCTCTTTGCTTTCCTTGGTGAAGAGCCACGCCGCACTTGACAGGCACGTTCTTCCGAGACTGTCAAGTGT CX11 201 CCGGCT<u>CCAAAGTGAAAAAGAA</u>CTTCTCTTAGCACGGCACGCCCAAACTGCGGGCATGAAGGTGTACGCGGTGACGGGTGGCGGTGGCTTCATCGGCAGC M K V Y A V T G G G F I G S 301 TACATTGTACGCGCACTGCTGCAATGTGAGCGCACGCTCATTGAGCTGCGCGTGATTGACGTCCGATGGGGGACAAAAGTCTCCTCGCGGAACGTGAACG Y I V R A L L Q C E R T L I E L R V I D V R W G T K V S S R N V N V 401 TGGTCTACATCTACTGCGACGTGTGCGACACTGCGCGCCTGTGTGCCGCGCTCGAGGGAGTGGACGTGCTCATCCACACTGCGGGGGCTAGTCGACGTGAT VYIYCDVCDTARLCAALEGVDVLIHTAGLVDVM G E Y S E D E I Y R A N V H G T H S A L S A C V C A G V R F V V Y 601 ACCAGCAGTATGGAGGTCGTTGGCCCGAACATGCGCGGGGGGGCCCTTCGTTGGCGACGAGAAAACCGAGTACGAGTCTTGCCACCAGCACTGCTACCCGC TSSMEVVGPNMRAEPFVGDEKTEYESCHQH' CYPR 701 GCAGCAAGGCGGAAGCCGAGGAGCTAGTGCTCAGTTCCAACGGGCGCCGAGTACGCGGGGGGTCAGCGCATGCTTACATGCGCGTTGCGCCCGGGGGGGTGT SKAEAEELVLSSNGRRVRGGQRMLTCALRPPGV 801 CTACGGTGAGGGCAACCAGCTGCTGCTGCGGCTAGCAAAGAACTACGTGCGCATGGGCTTGCACGTGCCACGTACCGTGCGAGAACGCTCTGCAAAGC Y G E G N Q L L L R L A K N Y V R M G L H V P R T V C E N A L Q R V Y V G N V A W M H V L A A R A L Q E P D S R L P G N A Y F C Y D 1001 ACCACTCTCCGTGCATGGACTACGAAGCTTTCAATGTGATGCTCTTACGCTCGTCGGCGTGGGGCGGTCCGCGGCTCCCGCGCGCCTTACTGAC H S P C M D Y E A F N V M L L R S F G V E L G G P R L P R A L L T 1101 CGTGGCGGCGTATACCAACGCCGCACTGCAGTGGCTGCTCCGCCAGCTGGGCATCCGCCTCTGCTCACCCCTTACACGCTCGCCGTTGCCAAT V A A Y T N A A L Q W L L R Q L G I R F S P L L N P Y T L A V A N 1201 GCCTGCTTCGTCATACGCACGCGCAAGGCACGCGAGCACATGGGCTACGAGCCGATCCACAACTGGAAGCAGTCGCGCAAAAAACACCACGCGATGGCTGC A C F V I R T R K A R E H M G Y E P I H N W K Q S R K N T T R W L R SQLAS 1401 AAGTEGETAECAAGAACAGAGAAAGGEAECCEAECAGGAACAGGGAACAATEECECEGECAGGAAEGGGAAAGGEAEAECEETGTAAEAAGGAAAAGGEAE

1501 ACCCCG

Fig. 2. MCV-I DNA sequences with deduced amino acid sequences below. Potential promoter sequences are underlined. (*a*) 1850 bp of MCV-I DNA spanning the left end of *Bam*HI L and the entire *Bam*HI N fragment, reading from right to left in the genome. (*b*) 3158 bp from the right end of MCV-I *Cla*I F, reading from left to right. (*c*) 1251 bp from the left end of MCV-I *Hind*III I and right end of *Hind*III E, reading from right to left in the genome. (*d*) 1506 bp from the right end of *Hind*III N and the left end of *Hind*III F, reading from left to right in the MCV-I genome.

<i>(a)</i>	
MCV CX1 VV F9L	MASA-ALSTLYGAFVARYLRKLSLYSTTNSVTCAIHVGRIVGTLQNCSVRILNRCNNNDQLSFRLLLEAFAETVNLLPPKQRAEIAAQVGVDLEAASHEE MAETKEFKTLYNLFIDSYLGKLAQHSIPTNVTCAIHIGEVIGQFKNCALRITNKCMSNSRLSFTLMVESFIEVISLLPEKDRRAIAEEIGIDLDDVPSAV ** *** *. **.**. * .******* .****.**
MCV CX1 VV F9L	SRLERKCRAHAALVQSIDVQTLNVGTCIAPPGRSLAIQVVNSGS SKLEKNCNAYAEVNNIIDIQKLDIGECSAPPGQHMLLQIVNTGSAEANCGLQTIVKSLNKIYVPPIIENRLPYYDPWFLVGVAIILVIFTVAICSIRRNL *.*** * * **.* ** * *.**
MCV CX1 VV F9L	ALKYRYGTFLYV
kinase MCV CX2 VV F10L	MAFSDSASADAPWSAVPAPRRDETTVLGDEIYFNYVYGQLELSDSWIPHVRMLRYFRNFSRAALLRIASTEYVNPSYFQQKDKRFAPVNNDFYHLSTGGY MGVANDSSPEYQWMSPHRLSDTVILGDCLYFNNIMSQLDLHQNWAPSVRLLNYFKNFNRETLLKIEENDYINSSFFQQKDKRFYPINDDFYHISTGGY **. * * * .** .*** .*** .*
MCV CX2 VV F10L	GIVFRVEEYVVKFVFEPGSQFHPMDLTSEYTVPRFLYNNLRGDERLLVVRALAMGLNYKIGFLYTLYKRVLHMVLLLARILDGQPLSLAYSRRQVAKLFA GIVFKIDNYVVKFVFEATKLYSPMETTAEFTVPKFLYNNLKGDEKKLIVCAWAMGLNYKLTFLHTLYKRVLHMLLLLIQTMDGQELSLRYSSKVFLKAFN
MCV CX2 VV F10L	ERKDSAKFVRLLSYFYPAVIKSNLNVINHFGHMIHFFEHEKRANYTYDRGNIIVFPLARCSAEKVTAANCAEFGFASVVHYVKFLFLQMALLYIKIYELS ERKDSIKFVKLLSHFYPAVINSNINVINYFNRMFHFFEHEKRTNYEYERGNIIIFPLALYSADKVDTELAIKLGFKSLVQYIKFIFLQMALLYIKIYELP ***** *** *** ***********************
MCV CX2 VV F10L	C-HNFIHVDLKPDNILLFDSEREMRIHVGERSYVFREPVRSALNDFDFSQVSEIPNKKITASLRVEQNWFYDFHFFVHTLLKVYPELERDAAWSKALGEF CCDNFLHADLKPDNILLFDSNEPIIHLKDKKFVFNERIKSALNDFDFSQVAGIINKKIKNNFKVKHNWYYDFHFFVHTLLKTYPEIEKDIEFSTALEEF * **********************************
MCV CX2 VV F10L	LVCCNRNTCEKFRLRVRRLHPISFLVRFVARDLFSDWINGERRP IMC-TKTDCDKYRLKVSILHPISFLEKFIMRDIFSDWINGER * *.*.**.* ******** .*. **.******
(1.)	
(b) VP8 MCV CX4 VV L4R	MNALFENLFDEDAVC-AGSVSR-EDFLLVVAGAKVKFPRSLLSMYRVVPRTMSRYELALVQSETVTGVVFTTVYNVRRNLGLEEREALSLPALEKYYLDK MSLLLENLIEEDTIFFAGSISEYDDLQMVIAGAKSKFPRSMLSIFNIVPRTMSKYELELIHNENITGAMFTTMYNIRNNLGLGD-DKLTIEAIENYFLDP *. *.***** ***.* .* .*. **.*** ***** *****.*** *******
MCV CX4 VV L4R	ANDVLTLMVNNTNLEHIAAYRMRSRRLLNPVVFRAGAVPLALVFTSRKKLSIYREDTSQAAEDSTYTKIAANVALAGKYAGLLLLDVHTPGTALMLTAVY NNEVMPLIINNTDMTAVIPKKSGRRKNKNNVIFRQGSSPILCIFETRKKINIYKENMESASTEYTPIGDNKALISKYAGINVLNVYSPSTSMRLNAIY *.*. **** *. * *.** *. * ** .***. * *. *
MCV CX4 VV L4R	GLDDRRELRKLADSTALENHQQSGALSEAMKLSDFRAVFEGLKKSVPLTNLEMINE GFTNKNKLEKLSTNKELESYS-SSPLQEPIRLNDFLGLLECVKKNIPLTDIP-TKD * * ** **. *****
MCV CX5 VV L5R	MSSTARQQQRSSVRLQPVFIEPRFEHAFLCGERYLWIVFFEALVALLLLRWFLGSELRAAFSRRPRAPEDPLRQMVAGKRLACAGERLMILGLHGGPQ MENVPNVYFNPVFIEPTFKHSLLSVYKHRLIVLFEVFVVFILIYVFFRSELNMFFMPKRKIP-DPIDRLRRAN-LACEDDKLMIYGLPVMTTQT ********* * ** .**.** .**** * *** * *** * *** **
MCV CX5 VV L5R	AALNLDGSEVRLPNCEAFLRGAGRANTDSAGEAGTDAGTGTDVGALLV SALSINSKPIVYKDCAKLLRSINGSQPVSLNDVLRR .**
MCV CX6 VV J1R	MDHKQYLLTMFFAEDSSFFKYLSEQDDDTALDDVMIVKHYMDVLLALLVRAKNKLEALGHCYEPLSEDFRALLHVRQLRELRQVHDRALLRLDAEPVHVS MDHNQYLLTMFFADDDSFFKYLASQDDESSLSDILQITQYLDFLLLLLIQSKNKLEAVGHCYESLSEEYRQLTKFTDSQDFKKLFNKVPI-VTDGRVKLN ***.*********************************
MCV CX6 VV J1R	HGYLADFVLSLVRLARELGELCVPPRTRYVDPRDDPTLAYVLEILHGTDVDSGAGAYALARPEAEKISPVRRALPGCSPSRRP KGYLFDFVISLMRFKKESSLATTAIDP-IRYIDPRRDIAFSNVMDILKSNKVNNN

Fig. 3. (a, b) For legend see facing page.

MCV CX7 VV J3R	MEAQAMERPLLYFHELTQTQEYDAEVERAARSRFPAQGQLKLLIGELFFLNKLHRREMLAGTTVVYIGSAPGGHIRYLVEHFRALGVPLRUMLLDGRSHD MDVVSLDKPFMYFEEIDNELDYEPESANEVAKKLPYQGQLKLLLGELFFLSKLQRHGILDGATVVYIGSAPGTHIRYLRDHFYNLGMIIKUMLIDGRHHD ************************
MCV CX7 VV J3R	SRLQGLSDVTLVTRFVDERYLMRMRQALRGARVVLISDIRSRRGS-EPSTEDLLYDYALQNSMLSILKPVASSLKWRCPFPDQWLHNFYVVCGKELLQPF PILNGLRDVTLVTRFVDEEYLRSIKKQLHPSKIILISDVRSKRGGNEPSTADLLSNYALQNVMISILNPVASSLKWRCPFPDQWIKDFYIPHGNKMLQPF . *.** *********** ** * ****.**.
MCV CX7 VV J3R	APPFSAELRLLSVHAGAP-RLRCITLAAARDYEKKMFYLNNVIRRRIVLNFDYPNQEYDFFHMFHLLNTVLCPRSFDSPTKKVLFL APSYSAEMRLLSIYTGENMRLTRVTKSDVVNYEKKMYYLNKIVRNKVVVNFDYPNQEYDYFHMYFMLRTVYCNKTFPTTKAKVLFLQQSIFRFLNIPTTS *****.***** ** .* .* .* .* .* .*****
MCV CX7 VV J3R	TEKVSHEPIQRKISSKNSMSKNRNSKRSVRGNK
(C) 35402 PN	A polymonace symplet
MCV CX9 VV A29L	MYREEKTLRIDLPPSVASFIKHGFRHHVRWPTLALGVVLANTTTAINEEWLTAVESMPTRKVFHAFVEPVLEGTLHMCVHLKKTQSEGDAYVSMHDFD MQHPREENSIVVELEPSLATFIKQGFNNLVKWPLLNIGIVLSNTSTAVNEEWLTAVEHIPTMKIFYKHIHKILTREMGFLVYLKRSQSERDNYITLYDFD * **** **.*.*********************
MCV CX9 VV A29L	YYVV-RDDGTLSKLKKPKDLRETLLHSFLEYRLKNTKSIELVAFSSGTQIREELLTHLAGVLDIEVFTREHANVKVTFPEEPRSTCPFGVIAPRGQLRIF YYIIDKDTNSVTMVDKPTELKETLLHVFQEYRLKSSQTIELIAFSSGTVINEDIVSKLT-FLDVEVFNREYNNVKTIIDPDFVFRSPFIVISPMGKLTFF *** ** .**. ** .*. ****** * ******
MCV CX9 VV A29L	FEAYPWVDTHQHLHAVLRLLERKLVADVRSSQILVTPELDFEGGVSKYDPASRMLLVRDMVTMSIVNFFGARAQLDTYHDFDMRVVDTERFLSALAEA VEVYSWFDFKSCFKDIIDFLEGALIANIHNHMIKVGDCDETVSSYNPESGMLFVNDLMTMNIVNFFGCNSRLESYHRFDMTKVDVELFIKALSDACK * *.* *** *.* * * . ** . **
MCV CX9 VV A29L	-FATLRALV KILSASNRL
MCV CX10 VV A30L	MEDVDEANLLHLLERLAGSGDDDFGATLAAIRELISAINSKVLTLNKKSKKSARAGEHVPRRENASH MEDLNEANFSHLLINLSNNKDIDAQYASTLSVVHELLSAINFKIFNINKKSKKNSKSIEQHPVVHHAASAGREFNRR ******. *** **************
(<i>d</i>)	
3B-HSD MCV CX11 VV A40L	MKVYAVTGGGGFIGSYIVRALLQCERTLIELRVIDVRWGTKVSSRNVNVV-YIYCDVCDTARLCAALEGVDVLIHTAGLVDVMGEYSEDEIYRANVHGTH MAVYAVTGGAGFLGRYIVK-LLISADDVQEIRVIDIVEDPQPITSKVKVINYIQCDINDFDKVREALDGVNLIIHTAALVDVFGKYTDNEIMKVNYYGTQ * ***********************************
MCV CX11 VV A40L	SALSACVCAGVRFVVYTSSMEVVGPNMRAEPFVGDEKTEYESCHQHCYPRSKAEAEELVLSSNGRRVRGGQRMLTCALRPPGVYGEGNQLLLRLAKNYVR TILAACVDLGIKYLIYTSSMEAIGPNKHGDPFIGHEHTLYDISPGHVYAKSKRMAEQLVNKANNSVIMNGAKLYTCCLRPTGIYGEGDKLTKVFYEQCKQ
MCV CX11 VV A40L	MGLHVPRTVCENALQSRVYVGNVAWMHVLAARALQEPDSRLPGNAYFCYDHSPCMDYEAFNVMLLRSFGVELGGPRLPRALLTVAAYTNAALQWLLRQLG HGNIMYRTVDDNAVHSRVYVGNAAWMHVLAAKYIQYPGSKIKGNAYFCYDYSPSCSYDMFNLLLMKPLGIE-QGSRIPRWMLKMYACKNDMKRIL * *** ***.******** *******************
MCV CX11 VV A40L	IRFSPLLNPYTLAVANACFVIRTRKAREHMGYEPIHNWKQSRKNTTRWLRSQLAS FRKPSLLNNYTLKISNTTFEVRTNNAELDFNYSPIFDVDVAFKRTRKWLEESE -**** ****. *** .* * ** * **

Fig. 3. Amino acid alignments of the translated sequences from Fig. 2 and their VV counterparts. MCV-I coding sequences of < 100 bp have not been included in the alignments. Asterisks indicate identical amino acid residues and dots similar amino acid substitutions. Dashes represent deletions. MCV-I CX1 and CX7 are partial ORFs. Amino acid alignments were performed using ClustalV. The conserved catalytic domains of VV protein kinase 2 are shown in (*a*).

particularly interesting. A 3β -HSD gene has also been reported in FPV (Skinner *et al.*, 1994) and its presence in three distinct genera suggests a fundamental role for this enzyme in poxvirus replication *in vivo*.

MCV has a restricted host range and cell tropism and is less pathogenic than the orthopoxviruses. Genes responsible for host range and pathogenicity are generally located near the termini of poxvirus genomes and sequencing of MCV terminal regions (Bugert *et al.*, 1993, and our unpublished data for *Hin*dIII fragments C, H and K) indicates that the terminal regions of MCV and VV differ considerably in coding potential, suggesting that the determinants of restricted host range and limited pathogenicity of MCV are located there.

The development of a system to culture MCV will aid the functional analysis of these putative MCV proteins and may reveal the presence of novel and biologically interesting genes in this unusual virus.

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