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 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 DNA-dependent RNA polymerase (Sonntag *et al.*, 1995). The latter occur within *Bam*HI 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 *Hin*dIII, *Bam*HI and *Cla*I restriction enzyme maps of MCV-I DNA are described by Porter & Archard (1992). *Hin*dIII fragments Q, C, E, I, J_L, N, M, H, K_R and J_R were eluted from agarose gels and cloned in the *Hin*dIII site of plasmid pUC1318 (Kay & McPherson, 1987) by standard methods. The recombinant *Hin*dIII C plasmid was digested with *Bam*HI and sub-

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The GenBank accession numbers of sequences reported in this paper are: U32423 (region CX1-CX2); U32424 (region CX3-CX7); U32425 (region CX8-CX10); U32426 (region CX11).

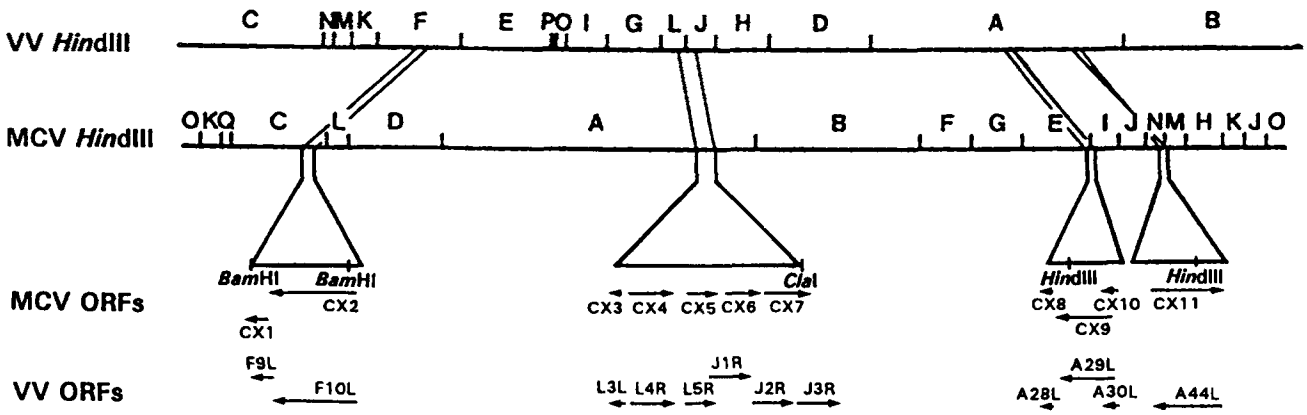

Fig. 1. *Hin*dIII restriction enzyme maps of VV and MCV-I, showing the regions of MCV-I sequenced. Open reading frames are indicated by arrows. The MCV-I ORFs are designated CX1–CX11 reading from left to right in the genome and are compared with the corresponding ORFs in VV.

fragments were recloned to give clones of MCV-I *Bam*HI fragments M, N and L. Genomic MCV-I DNA was digested with *Cla*I and cloned randomly in the *Cla*I site of Bluescript KS(+) (Stratagene): recombinant plasmids containing *Cla*I 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 *Bam*HI L and N, *Hin*dIII I and E or *Hin*dIII N and M, primers were designed to span the restriction sites. PCR sequencing using genomic DNA for the *Hin*dIII I and E or *Hin*dIII N and M junctions and a plasmid containing the *Hin*dIII C fragment including the *Bam*HI 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 *Hin*dIII I and the right end of *Hin*dIII 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*)

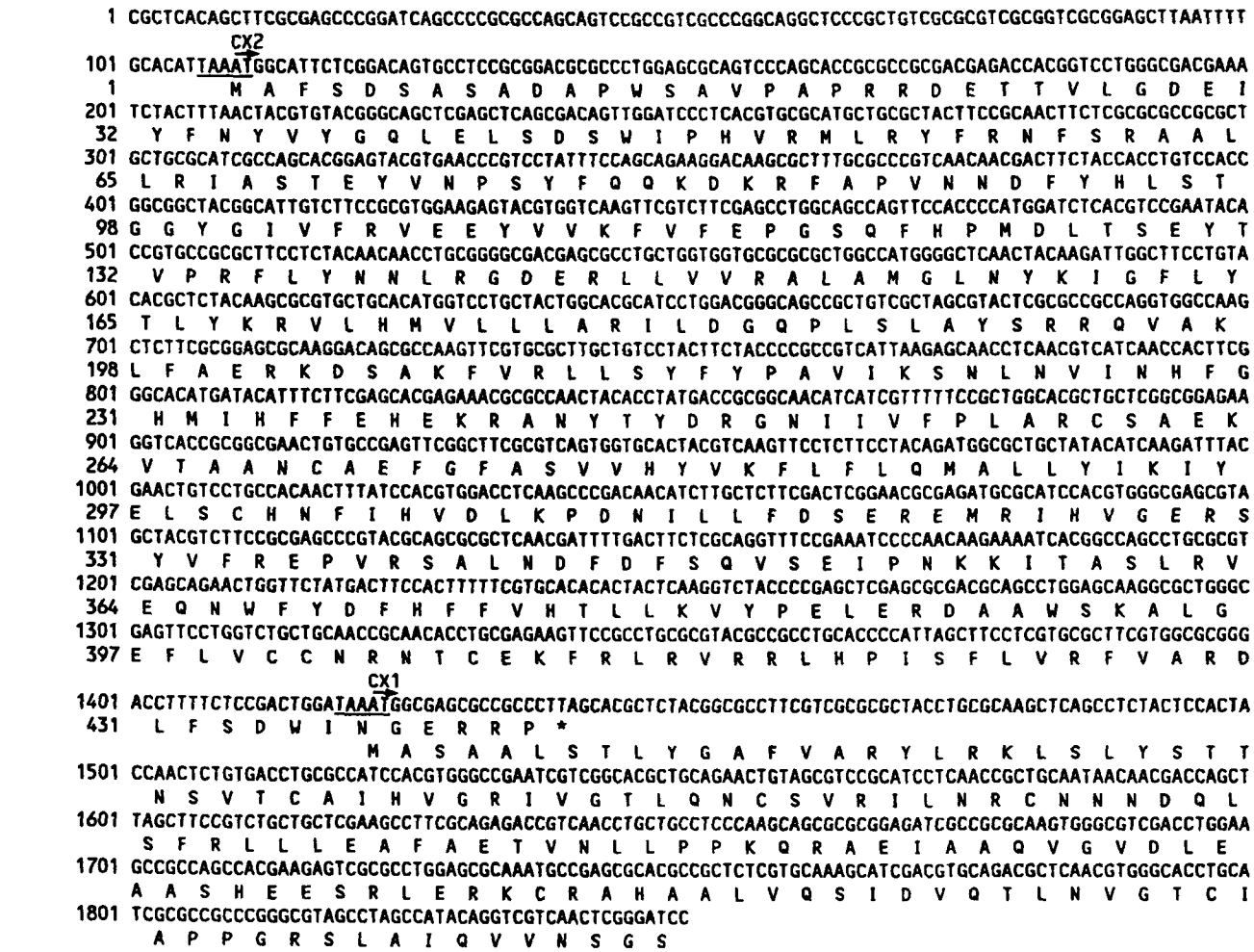

Fig. 2. (*a*) For legend see page 3117.

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 *Hin*dIII fragment N and the left end of *Hin*dIII 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 3β-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, 1989*a*, *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

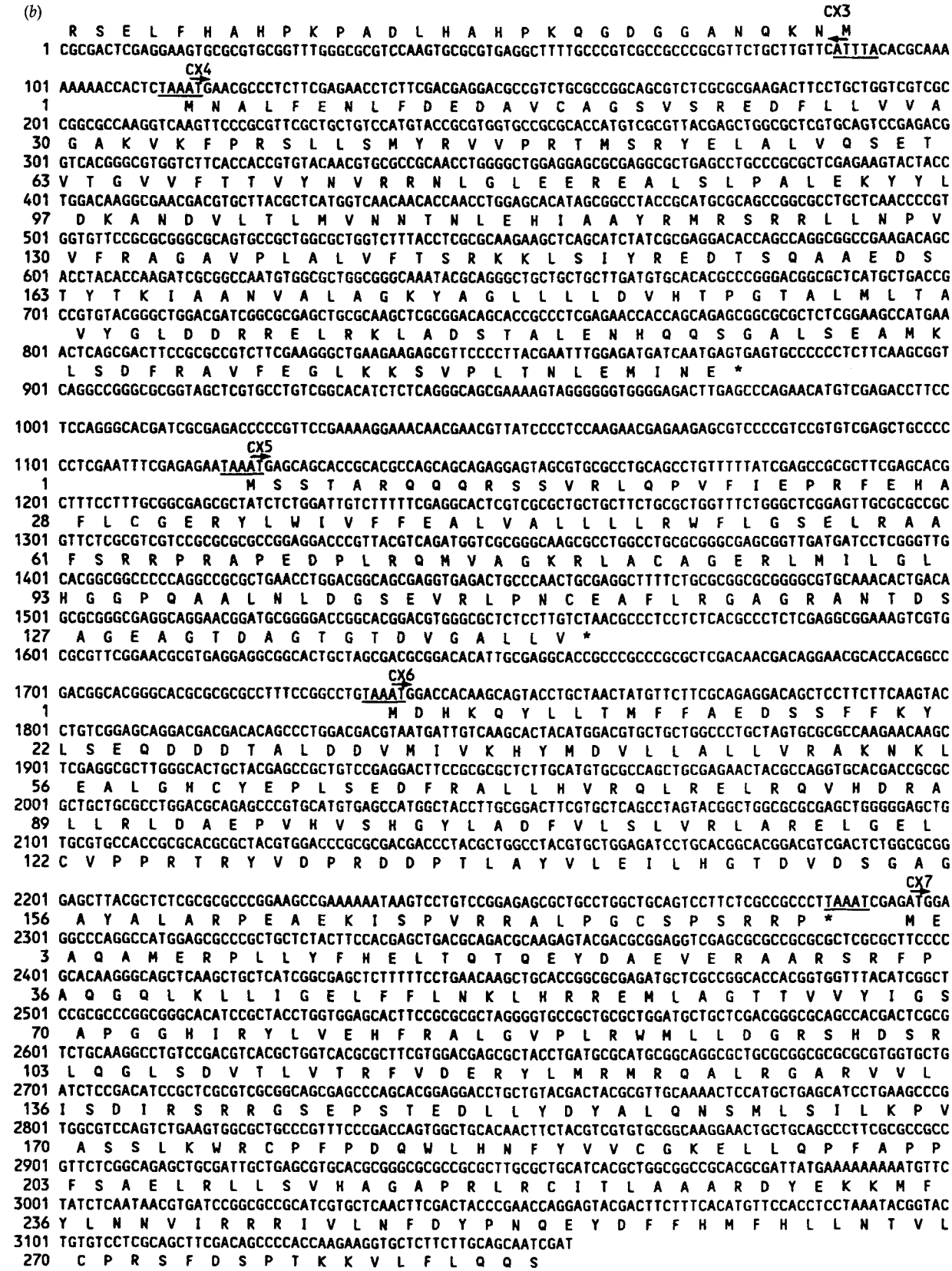

Fig. 2. (*b*) For legend see facing page.

(*c*)

CX10
1 AACGCGCTGCTTACGCTGGCTGCCCGACGGCGGCGAAGAATGCGAGCGACGCCAGTCCGTACTTATTTTTCCGTTTTAAATGGAAGACGTGGACGAGGCC
M E D V D E A
101 AACCTCCTGCACCTGCTGGAACGCTTGGCGGGCAGCGGCGACGACGACTTCGGCGCAACGCTGGCCGCCATCCGCGAGCTAATCAGCGCCATCAATTCAA
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 AGGTGCTCACCCTTAATAAAAAATCAAAAAAAAGTGCGCGTGCGGGCGAGCATGTACCGAGAAGAGAAAACGCTTCGCATTGACCTCCCGCCCAGCGTGG
V L T L N K K S K K S A R A G E H V P R R E N A S H *
M Y R E E K T L R I D L P P S V A
301 CCAGCTTCATCAAGCACGGCTTCCGGCACCACGTGCGCTGGCCCACGCTGGCGCTCGGCGTGGTGCTGGCCAACACTACCACAGCCATTAACGAAGAGTG
S F I K H G F R H H V R W P T L A L G V V L A N T T T A I N E E W
401 GCTCACAGCCGTGGAGTCCATGCCCACGCGCAAGGTCTTCCACGCCTTCGTGGAGCCCGTGCTGGAGGGCACGCTGCACATGTGCGTGCACCTCAAGAAG
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 M C V H L K K
501 ACGCAGAGCGAGGGCGACGCGTACGTGTCCATGCACGACTTTGACTACTACGTGGTGCGCGACGACGGCACGCTGAGCAAGCTCAAGAAGCCCAAGGATC
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
801 CCGTTTGGCGTGATCGCGCCGCGCGGGCAGCTGCGCATCTTCTTCGAGGCCTACCCCTGGGTTGACACGCACCAGCACCTGCACGCCGTGCTGCGCCTGC
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 TGGAGAGGAAGCTTGTGGCCGACGTGCGCAGCAGCCAGATCCTTGTCACGCCGGAACTGGACTTCGAGGGCGGCGTGTCCAAGTACGACCCCGCGAGCCG
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
1001 CATGCTGCTGGTCCGCGACATGGTGACCATGAGCATCGTTAACTTTTTCGGCGCGCGTGCGCAGCTCGACACCTACCACGATTTCGACATGCGTGTCGTG
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
1101 GACACCGAGCGCTTCCTGTCCGCGCTCGCGGAGGCTTTTGCTACCCTGCGCGCGCTCGTCTAATGGTGCGCGCTAGAGCGCGGCGCACAATCTCGCCGGC
D T E R F L S A L A E A F A T L R A L V *
CX8
1201 TGTAAATGGACCCGCTCTCGGTCTTCTTCCTCGTGGTCGCCGCGGCCGCCG
M D P L S V F F L V V A A A A

(*d*)

1 AGTCTCATGGTCATTTTTGTTTCGAAGTTTCACGCGTGAGTAGCAACAAAGAGGAAAAGAAACCCCTAGCAGACAACTGCGTCTCAAGACAAGTCACACT

101 CTTGTTTGTTGTCCCACTCTCGAAAGTCGCGCTCTTTGCTTTCCTTGGTGAAGAGCCACGCCGCACTTGACAGGCACGTTCTTCCGAGACTGTCAAGTGT
CX11
201 CCGGCTCCAAAGTGAAAAAGAACTTCTCTTAGCACGGCACGCCCAAACTGCGGGCATGAAGGTGTACGCGGTGACGGGTGGCGGTGGCTTCATCGGCAGC
M K V Y A V T G 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 TGGTCTACATCTACTGCGACGTGTGCGACACTGCGCGCCTGTGTGCCGCGCTCGAGGGAGTGGACGTGCTCATCCACACTGCGGGGCTAGTCGACGTGAT
V Y I Y C D V C D T A R L C A A L E G V D V L I H T A G L V D V M
501 GGGGGAGTATAGCGAGGACGAGATTTACCGCGCGAACGTGCACGGGACACACAGCGCGCTTAGCGCCTGCGTCTGCGCGGGCGTGCGCTTTGTGGTGTAC
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 ACCAGCAGTATGGAGGTCGTTGGCCCGAACATGCGCGCGGAGCCCTTCGTTGGCGACGAGAAAACCGAGTACGAGTCTTGCCACCAGCACTGCTACCCGC
T S S M E V V G P N M R A E P F V G D E K T E Y E S C H Q H C Y P R
701 GCAGCAAGGCGGAAGCCGAGGAGCTAGTGCTCAGTTCCAACGGGCGCCGAGTACGCGGGGGTCAGCGCATGCTTACATGCGCGTTGCGCCCGCCGGGTGT
S K A E A E E L V L S S N G R R V R G G Q R M L T C A L R P P G V
801 CTACGGTGAGGGCAACCAGCTGCTGCTGCGGCTAGCAAAGAACTACGTGCGCATGGGCTTGCACGTGCCACGTACCGTGTGCGAGAACGCTCTGCAAAGC
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 S
901 AGGGTCTACGTTGGCAACGTGGCCTGGATGCACGTACTTGCCGCACGCGCGCTGCAGGAACCGGACTCGCGCCTGCCGGGCAACGCATATTTCTGTTACG
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 ACCACTCTCCGTGCATGGACTACGAAGCTTTCAATGTGATGCTCTTACGCTCGTTCGGCGTGGAGCTGGGCGGTCCGCGGCTCCCGCGCGCTTTACTGAC
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 CGTGGCGGCGTATACCAACGCCGCACTGCAGTGGCTGCTCCGCCAGCTGGGCATCCGCTTCTCGCCTCTGCTCAACCCTTACACGCTCGCCGTTGCCAAT
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 GCCTGCTTCGTCATACGCACGCGCAAGGCACGCGAGCACATGGGCTACGAGCCGATCCACAACTGGAAGCAGTCGCGCAAAAACACCACGCGATGGCTGC
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
1301 GCTCGCAGCTCGCGAGCTAACTGCACCTTGCTAGAGCGAGCGCGGGCGGCGGACACTGGCACCGGAAGCATCGACAAGGAAAGGCACCCACCAGGAACGA
S Q L A S *
1401 AAGTCGCTACCAAGAACAGAGAAAGGCACCCACCAGGAACAGGGAACAATCCCCCGCCAGGAACGAGGAAAGGCACACCCCTGTAACAAGGAAAAGGCAC

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 *Hin*dIII I and right end of *Hin*dIII E, reading from right to left in the genome. (*d*) 1506 bp from the right end of *Hin*dIII N and the left end of *Hin*dIII M, reading from left to right in the MCV-I genome.

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

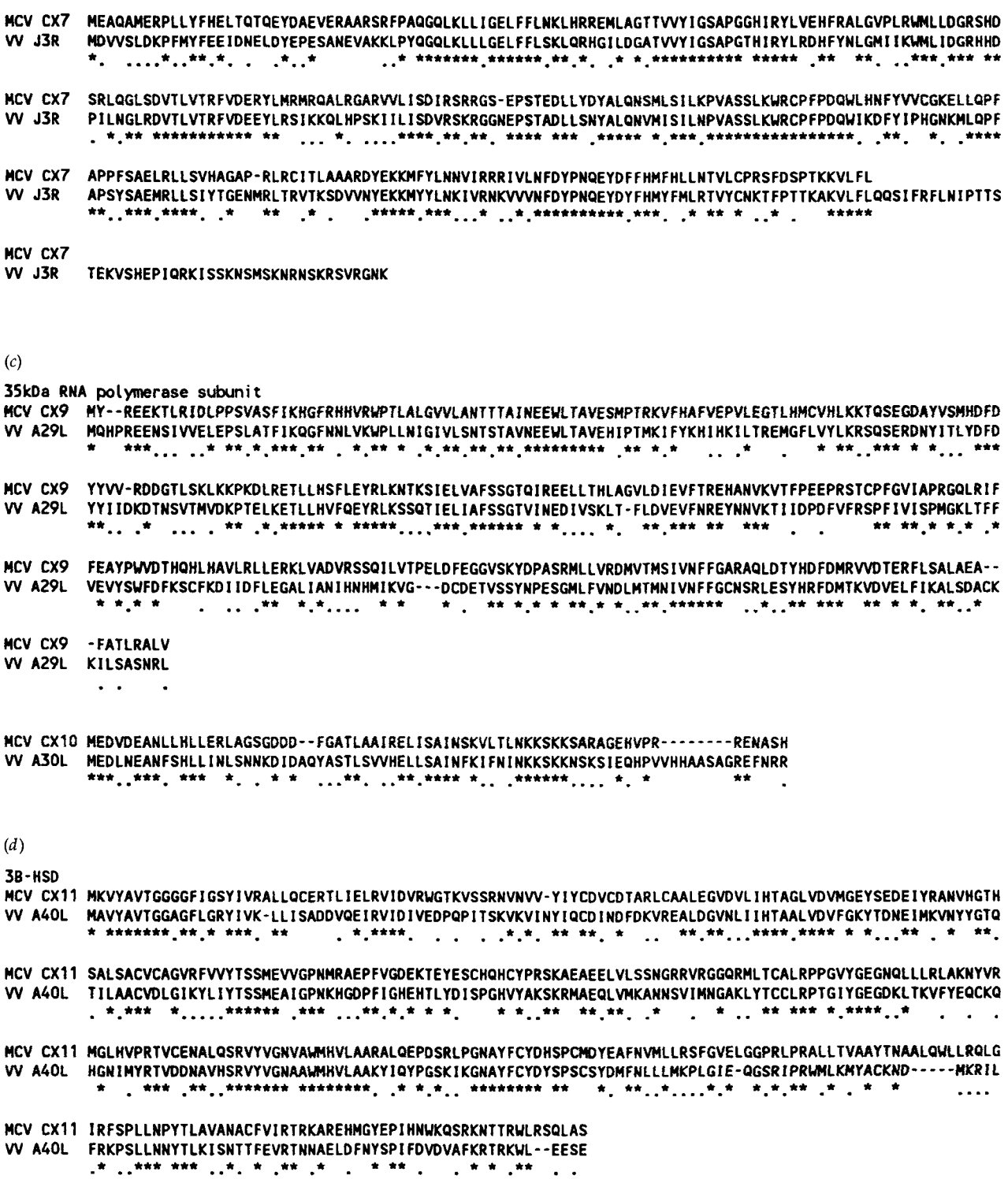

Fig. 3. Amino acid alignments of the translated sequences from Fig. 2 and their VV counterparts. MCV-I coding sequences of < 100 bp have not been included in the alignments. Asterisks indicate identical amino acid residues and dots similar amino acid substitutions. Dashes represent deletions. MCV-I CX1 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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