

Mapping and Sequencing of a Gene from Myxoma Virus That Is Related to Those Encoding Epidermal Growth Factor and Transforming Growth Factor Alpha

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Myxoma virus, a *Leporipoxvirus* and agent of myxomatosis, was shown to possess a gene with the potential to encode an epidermal growth factorlike factor. Its relationship to other members of this family, including the poxvirus growth factors from Shope fibroma virus and vaccinia virus, was analyzed. Alignment of DNA sequences and related open reading frames of myxoma virus and Shope fibroma virus indicated colinearity of genes between these poxviruses.

Although poxvirus replication and assembly occur outside the host cell nucleus within viroosomes found in the cytoplasm of infected cells (6, 15), a number of poxviruses are known to be responsible for proliferative diseases. Examples

epidermal lesions in humans (4). It has recently been shown that vaccinia virus, a cytolytic *Orthopoxvirus* (6), and SFV, a tumorigenic *Leporipoxvirus* (7, 19, 28), encode gene products designated vaccinia growth factor (VGF) (2, 3, 12, 16,

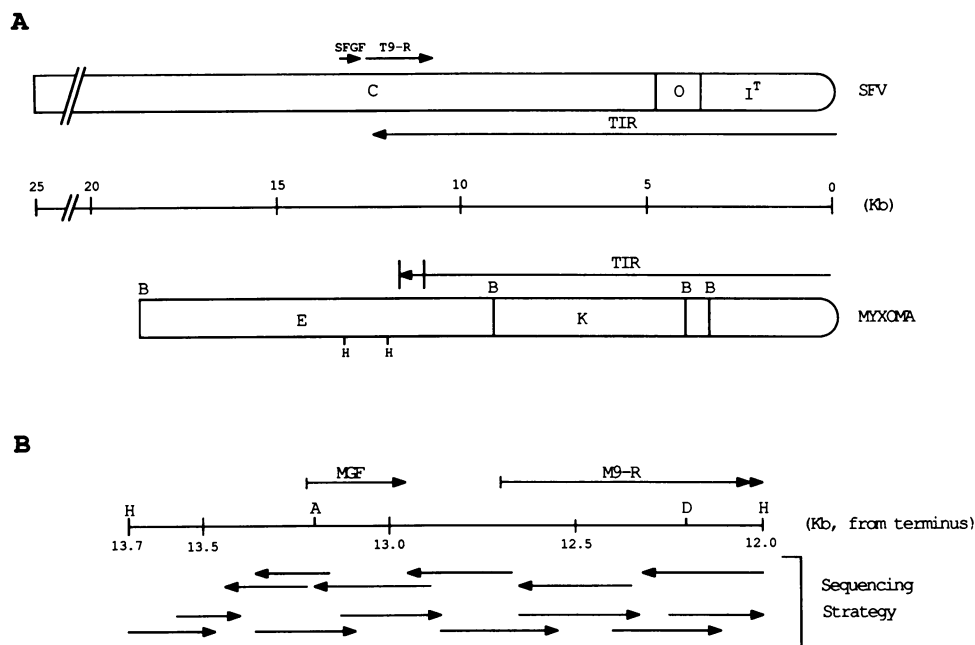


FIG. 1. (A) Alignment of the right termini of SFV and myxoma virus. *Bam*HI fragments I^T, O, and C are shown for SFV (7), and the positions of the SFGF gene, the T9-R ORF, and the 12.4-kb TIR are indicated by arrows. For myxoma virus, *Bam*HI fragments K and E (1) and the 1.7-kb *Hinc*II fragment which hybridized to the SFGF probe are indicated. Other *Hinc*II sites in the *Bam*HI E fragment of myxoma virus are not shown. The approximate location of the unique sequence-TIR junction for myxoma virus is indicated by vertical bars. Both the SFV and myxoma virus genomes are similarly oriented such that the right-hand hairpin termini (indicated by the closed loop) are at the right. B = *Bam*HI; H, *Hinc*II. (B) Sequencing strategy for the 1.7-kb myxoma virus *Hinc*II fragment containing the MGF gene. The locations for the complete MGF (single-headed arrow) and the start of ORF M9-R (double-headed arrow) are shown with approximate distances from the right-hand viral hairpin. A, *Ava*I; D, *Dde*I.

of such tumorigenic poxviruses are Shope fibroma virus (SFV), which induces benign fibromas in rabbits (19), and *Molluscum contagiosum*, which causes benign tumorlike

22, 24, 28) and Shope fibroma growth factor (SFGF) (5), respectively, which share significant amino acid (aa) homology with epidermal growth factor (EGF) and transforming growth factor α . The role(s) of such growth factors in poxviral replication and virus-specific cytopathology is still

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10      20      30      40      50      60      70
TTAARACAGA TACRACATAC GGACGCCGCT ATGTTCTCGG AAGTCATAGA CCGTATTGTC GCGGAARGAC

80      90      100     110     120     130     140
AGCAGGTGAT TGGATTATTT CAGAAAAAAT GTAAATATATA CACGACATAC TACARTGTAC GTACGGCGCG

150     160     170     180     190     200
GTGTAAATA TCCGCTATC TARCCCGCGC AGTGTGTGGC TTTGTCGCAT ACGGARTACT AAA

218     233     248
ATG GTA CCG AGG GAC CTA GTC GCA ACT CTC TTA TGT GCG ATG TGT ATT GTA CAG
MET Val Pro Arg Asp Leu Val Ala Thr Leu Leu Cys Ala MET Cys Ile Val Gln

263     278     293     308
GCA ACG ATG CCT TCG TTG GAT AAT TAT CTG TAT ATT ATT AAA CGT ATT AAA CTA
Ala Thr MET Pro Ser Leu Asp Asn Tyr Leu Tyr Ile Ile Lys Arg Ile Lys Leu

323     338     353
TGT AAC GAC GAC TAT AAA AAC TAT TGT CTA AAT AAC GGA ACC TGT TTC ACC GTA
Cys Asn Asp Asp Tyr Lys Asn Tyr Cys Leu Asn Asn Gly Thr Cys Phe Thr Val

368     383     398     413
GCA TTA AAC AAT GTT TCA CTT AAC CCG TTT TGT GCG TGT CAT ATT AAC TAC GTG
Ala Leu Asn Asn Val Ser Leu Asn Pro Phe Cys Ala Cys His Ile Asn Tyr Val

428     443     458     471     481
GGA ACG CGA TGT CAG TTT ATT AAT CTA ATT ACC ATT AAG TAA CCCGTTTATC ATGTATAATA
Gly Ser Arg Cys Gln Phe Ile Asn Leu Ile Thr Ile Lys

491     501     511     521     531     541     551
ATACATACGT ATTTTITAGAT AACTTTAATA AATRAACATG TATAARACTTA CTTATCATAT ACGGTACACA

561     571     581     591     601     611     621
TAACGAATAA CACTACATGT TTTTATATAT ACATAGGTTT GGAAAAAATC TAATCACGAA CGTATCATTA

631     641     651     661     671     681     691
GACATGACT CCATCTAGGA GGGGTTTTGG GAACATCGTA CACGATATAT TCACATCGCG AAARCATAAA

701     711     731     746
TAATAATTTT TTACACGAT TCACG ATG TCG CGC ACT TTA TTG AGA TTT CTG GAA GAT
MET Ser Arg Thr Leu Leu Arg Phe Leu Glu Asp

761     776     791
GGT GCA ATG ATG AGC GAC GTA ACA GTC GTC GCC GGG GAC TCG ACG TTT CTC GGG CAT
Gly Ala MET Ser Asp Val Thr Val Val Ala Gly Asp Ser Thr Phe Leu Gly His

806     821     836     851
AAA GTT ATT TTA TCT CTT CAC TCG GAT TAC TTC TAT CGT CTG TTT ATG GGA GAC
Lys Val Ile Leu Ser Leu His Ser Asp Tyr Phe Tyr Arg Leu Phe Asn Gly Asp

866     881     896     911
TTT ACC TCG CCC GAT ACG GTT ACG CTG GAC GCG ACG GAC GAT GCC GTT CGT ACG
Phe Thr Ser Pro Asp Thr Val Thr Leu Asp Ala Thr Asp Asp Ala Val Arg Thr

926     941     956
GTG TTT ACG TAT ATG TAC GCG GGA TGT GAC GGG TTA AAC GAT CGT ACG ATA GAC
Val Phe Thr Tyr MET Tyr Ala Cys Asp Gly Leu Asn Asp Arg Thr Ile Asp

971     986     1001     1016
GAT TTA CAA TCC ATT ATC GTA TTG GCG GAC TAC CTG GGT ATA ACG AAA CTG GTG
Asp Leu Gln Ser Ile Ile Val Leu Ala Asp Tyr Leu Gly Ile Thr Lys Leu Val

1031     1046     1061
GAC GAA TGC GTA CGT CGT ATC GTA TCT AAA GTG GAC GTA TTA AAC TGC GTA GGG
Asp Glu Cys Val Arg Arg Ile Val Ser Lys Val Asp Val Leu Asn Cys Val Gly

1076     1091     1106     1121
GTA TAT ACG TTT GCG GAG CAC TAT CAT ATA ACG GAC TTG CAG CGG GCG GCC AAA
Val Tyr Thr Phe Ala Glu Thr Tyr His Ile Thr Asp Leu Gln Arg Ala Ala Lys

1136     1151     1166     1181
ACG TTT TTA ACA GAA CTA CTG GGG TCT AAA GAA GCG TTC GAA GAA CTA TCC CAA
Thr Phe Leu Thr Glu Leu Leu Gly Ser Lys Glu Ala Phe Glu Glu Leu Ser Gln

1196     1211     1226
GAC GAT GCG GTT ATC GCG TTA AGG GAA ACG CGT AAC ATT GTC GAT ACG GAA TCC
Asp Asp Ala Val Ile Ala Leu Arg Glu Thr Arg Asn Ile Val Asp Arg Arg Ser

1241     1256     1271     1286
ATT CTT AGA GCG ATC CTG TTA TGG GTT CGA AAA TGT CCA GAT CGT ATC GAA CAA
Ile Leu Arg Ala Ile Leu Leu Trp Val Arg Lys Cys Pro Asp Arg Ile Glu Gln

1301     1316     1331
CTA ARG GTG TTA GTC GCC GCC GTA GAC GAC GTA GAC GAT GAC AAC GTA TAT
Leu Lys Val Leu Val Ala Ala Val Asp Asp Val Asp Asp Asp Asp Asn Val Tyr

1346     1361     1376     1391
ACG ATC TAC GAG AGA TAC GCT GAA GAA CTA AAG GAT ATG ATC GCG TGT CCA TTA
Thr Ile Tyr Glu Arg Tyr Ala Glu Leu Lys Asp MET Ile Ala Cys Pro Leu

1406     1421
TCC TAT AAT TGC GTC GTT GTG GTC
Ser Tyr Asn Cys Val Val Val

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FIG. 2. Partial DNA sequence of the myxoma virus 1.7-kb *HincII* fragment which hybridized to the SFGF probe. Translated regions are MGF (nucleotides 204 to 458) and a partial sequence of ORF M9-R (nucleotides 717 to 1421), which encodes the N-terminal 235 aa of the putative M9-R polypeptide.

undefined, but two possibilities are that such gene products could (i) facilitate S-phase independence of viral replication by stimulating increases in cellular functions such as nucleotide pool levels and ribosome availability, and (ii) mediate the cellular proliferation often observed concomitantly with poxviral infections, albeit to markedly different extents with different poxviruses. The notion that poxviral growth factors may play a central role in virus-cell interactions is supported by recent studies on malignant rabbit virus (MRV) (1, 20, 21, 25). The genome of MRV, a natural recombinant between SFV and myxoma virus, induces SFV-like fibromas during the initial stages of infection of domestic rabbits (20, 21). Mapping and sequencing studies indicate that MRV contains only a few kilobases (kb) of SFV DNA sequences (1, 25) transferred from the junction region between the internal unique sequences and the terminal inverted repeat (TIR) sequences at the right terminus of SFV and results in the replacement of a comparable extent of myxoma virus sequences. This region of the SFV genome has been shown to include four intact major open reading frames (ORFs T6 to T9) plus the intact SFGF gene (5, 25). Because of the unusual pathology of MRV infections and the fact that myxoma virus itself induces fibromatous dermal lesions in its natural host (rabbits of the genus *Sylvilagus*) and a marked degree of proliferation of endothelial cells in the myxoma lesions in *Oryctolagus cuniculus* (8-10, 13, 23), it was of interest to determine whether myxoma virus also possesses an EGF-like growth factor gene and, if so, to examine its relationship to SFGF and VGF.

Mapping and sequencing of the MGF gene. A 594-base-pair *Sau3A* fragment encompassing the complete 240-bp SFGF coding sequence plus 213-base-pair 5' noncoding sequences upstream and 141 base pairs downstream (5) was labeled with [³²P]dATP by nick translation and used to probe a Southern blot of myxoma virus DNA digested with *Bam*HI under conditions of moderate stringency such that the genomes of SFV and myxoma virus cross-hybridized (1). The *Bam*HI E fragment of myxoma virus was identified as being homologous to the SFGF probe, and further mapping analysis localized the region of homology to a 1.7-kb *Hinc*II fragment within the myxoma *Bam*HI E fragment (data not shown). Figure 1A indicates the position of the SFGF gene and the related *Hinc*II fragment of myxoma virus with respect to the TIRs at the right end of the two viral genomes. This 1.7-kb *Hinc*II fragment was blunt ended with T4 DNA polymerase and cloned into the *Sma*I site of M13mp18 (27) in both orientations; nested deletions created for each orientation of the fragment (Fig. 1B) with exonuclease III (11) were sequenced with the dideoxy chain termination method (18), and the DNA sequence was analyzed with the Core Library programs of the BIONET computer facility (IntelliGenetics Inc., Palo Alto, Calif.) as previously described (25). Translation of 1.4 kb of the DNA sequence closest to the viral TIR (Fig. 2) revealed two ORFs (Fig. 1B), one closely related to and in the same orientation as that of SFGF (designated here as myxoma virus growth factor [MGF]) and another partial ORF (M9-R) that is homologous to T9-R of SFV (25).

Analysis of the MGF gene. The MGF gene encodes a polypeptide of 85 aa, 5 more than SFGF. Therefore both SFGF and MGF are similar in size to the 77-aa cleaved product of the VGF gene (Fig. 3). Both MGF and SFGF contain 12 of the 13 aa residues that are conserved among VGF, EGF, and transforming growth factor α , whereas the single variant residue (Asn-70) is the same in MGF and SFGF (Fig. 3). The MGF and SFGF polypeptides each have a signallike stretch of hydrophobic residues at their N

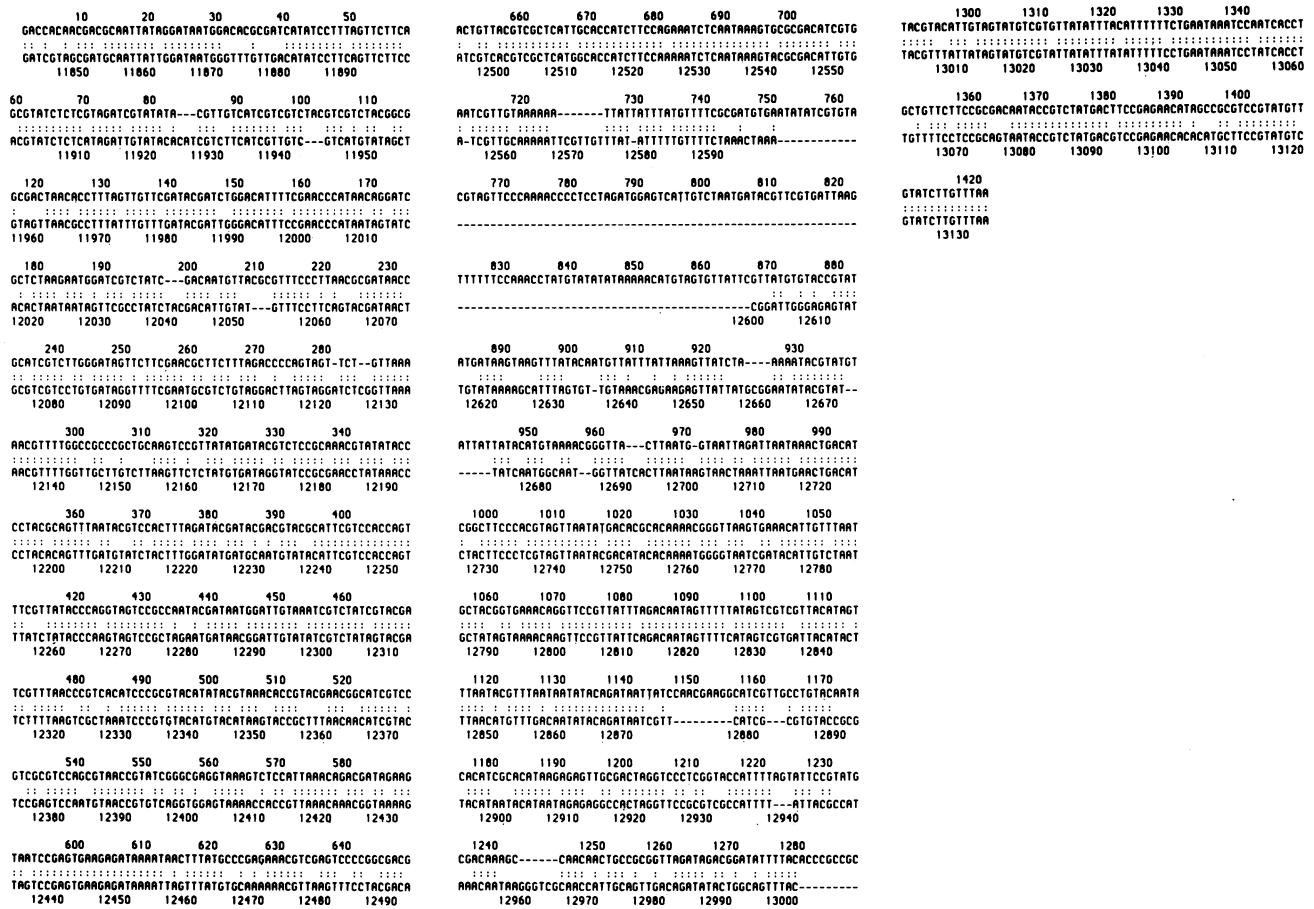


FIG. 4. Alignment of 1,421 nucleotides from the 1.7-kb *HincII* fragment of myxoma virus (top line) with the homologous SFV DNA sequence. The SFV sequence (bottom line) is numbered such that nucleotide no. 1 is at the mung bean nuclease cut site near the viral terminus used to clone the terminal I^T fragment (7, 28) and extends to the unique sequence-TIR junction (nucleotide no. 12,397) at the right end of the virus. Since the complete myxoma TIR sequence is not available, nucleotide no. 1 of the myxoma virus sequence is defined within the *HincII* site closest to the terminus (Fig. 1). The myxoma DNA sequences corresponding to MGF and the incomplete ORF M9-R are nucleotides 1218 to 964 and 705 to 1, respectively, and are numbered in the opposite direction to Fig. 2. The SFV sequences for SFGF and the incomplete ORF T9-R are nucleotides, 12,936 to 12,697 and nucleotides 12,548 to 11,841, respectively.

scription may proceed through one or more downstream SFV genes before terminating.

Since SFV contains two other TIR ORFs, T6 and T8, with extensive sequence homology to T9-R (25), it was necessary to measure the relationships among these three SFV ORFs and M9-R to determine the extent of colinearity between the myxoma virus and SFV genomes within this region that was suggested by alignment of the DNA sequences and ORFs. From the homology scores, it was evident that the myxoma M9-R ORF is more closely related to the SFV T9-R ORF (68.9%) than to the other two SFV ORFs, T8 (53.2%) and T6 (29.8%) (T8 and T9-R showed 31.5% homology). The DNA sequence alignments of all four ORFs (data not shown) and the fact that scores between myxoma M9-R and SFV T8/T6 were only slightly less than the scores for alignment of SFV T9-R with SFV T8-T6 suggested that there has been conservation of specific sequences within these polypeptides.

We showed that myxoma virus possesses a gene capable of encoding a polypeptide related to the EGF-like family of growth factors. This MGF gene shares extensive homology with SFGF, but neither is more closely related to VGF than to the other members of the group. Analysis of the myxoma-SFV recombinant MRV indicates that, although myxoma

sequences which include the MGF gene have been deleted, a small region of the SFV genome which contains the SFGF gene in addition to several other ORFs has been inserted (5, 25). However, both SFGF and MGF must be examined by site-specific mutagenesis before definitive conclusions can be made about the interplay between these viral growth factors and their respective target cells.

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