

A Cowpox Virus Gene Required for Multiplication in Chinese Hamster Ovary Cells

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Cowpox virus, in contrast to vaccinia virus, can multiply in Chinese hamster ovary cells. To study the genetic basis for this difference in host range, recombinants between vaccinia and cowpox viruses were isolated and their DNA restriction patterns were examined. The ability to multiply in Chinese hamster ovary cells could be correlated with the conservation of cowpox virus sequences mapping at the left end of the genome. This was further demonstrated by marker rescue of the host range phenotype with restricted cowpox virus DNA. Marker rescue with cloned restriction fragments of decreasing size enabled the fine localization of the host range function to a 2.3-kilobase-pair fragment. Nucleotide sequencing revealed that the fragment encoded a single major polypeptide of approximately 77,000 daltons. It is suggested that the role of the host range gene from cowpox virus is to prevent the early and extensive shutoff of protein synthesis that normally occurs in Chinese hamster ovary cells infected by vaccinia virus.

Most orthopoxviruses can multiply in cells derived from a variety of tissues established from numerous mammalian and several avian species. In vivo, all mammalian species studied to date are permissive for vaccinia virus (VV), at least at the site of inoculation and despite the fact that no natural host is known for VV. This broad host range may be explained in part by the relative autonomy the orthopoxviruses display with respect to the cells they infect, which is due in turn to the capacity of these viruses to code for many of the genes involved in viral DNA and RNA synthesis. The large genome of these viruses also endows them with the coding potential for functions that might allow adaptation to different cellular environments. One example is provided by a VV host range gene that we recently characterized (5). Cowpox virus (CPV), the member of the orthopoxvirus family with the largest genome, probably encodes more genes involved in cell adaptation than any of the other members. That this may indeed be the case is suggested by the finding that CPV can multiply in Chinese hamster ovary (CHO) cells, in contrast to VV (2, 6). CHO cells are one of the few established cell lines in which VV fails to replicate. To identify the CPV gene(s) that are essential for multiplication in CHO cells, we initially isolated recombinants between VV and CPV that multiplied in CHO cells and then correlated the segregation of host range phenotypes with their DNA restriction patterns. By using information obtained from such studies, DNA from a recombinant VV having integrated CPV DNA was cloned into a bacterial plasmid which was further assayed for its ability to transfer the host range phenotype to VV by marker rescue. Fine mapping of the host range gene was achieved by employing smaller and smaller fragments in subsequent marker rescue experiments. Finally, nucleotide sequencing revealed that the host range phenotype was encoded within a single long open reading frame.

MATERIALS AND METHODS

Viruses and cells. The Brighton strain of CPV was obtained from the American Type Culture Collection. The Copenhagen strain of VV and temperature-sensitive mutants or host range mutants derived from it have been previously described (1, 3). CHO cells, primary chick embryo fibroblasts (CEF), mouse L thymidine kinase (tk)-negative cells, and baby hamster kidney cells (BHK 21) were used as hosts for infection in Alpha medium, Eagle basal medium, Dulbecco medium, and BHK 21 medium, respectively, containing 5% fetal calf serum. Plaque assays on BHK 21 cells were carried out under liquid medium. Plaque assays on CHO cells were carried out under a 1% low-melting-temperature agarose overlay. Incubation of infected cells was for 2 days at 37°C, and plaques were visualized by staining with an agarose overlay containing neutral red.

Isolation of CPV-VV recombinants. CEF were coinfecting with approximately 5 PFU of the VV temperature-sensitive *tsN7* mutant and wild-type CPV. Infected cells were incubated at 33°C until complete necrosis of the cell monolayer, and the virus progeny was plated after serial dilution on CEF at 39.5°C under a solid medium. Isolated plaques were picked, and their titers were amplified on CEF.

Cloning procedures, plasmids, and sequencing. Cloning procedures and analysis of DNA were essentially as described by Maniatis et al. (11). The plasmid vector pAT153 (16) was propagated in *Escherichia coli* HB101. The VV transplacement vector pTG186poly (7) was similar to one previously described (10). Briefly, it consisted of a minimal *E. coli* replicon (pTG1H) into which had been inserted the VV *Hind*III J fragment. The latter was interrupted at the tk locus by the VV 7.5-kilodalton early late promoter (10) and a polylinker segment for convenient insertion of foreign genes. For nucleotide sequencing, DNA fragments were inserted into the single-stranded bacteriophage vectors M13 TG130 and M13 TG131 (8) and phage was propagated in *E. coli* JM103. Dideoxynucleotide sequencing was carried out by the method of Sanger et al. (15).

Marker rescue. As previously described (7), CEF were infected with a VV temperature-sensitive mutant (*tsN7*) for 2 h at the permissive temperature (33°C). The medium was then removed, and cells were transfected with a calcium

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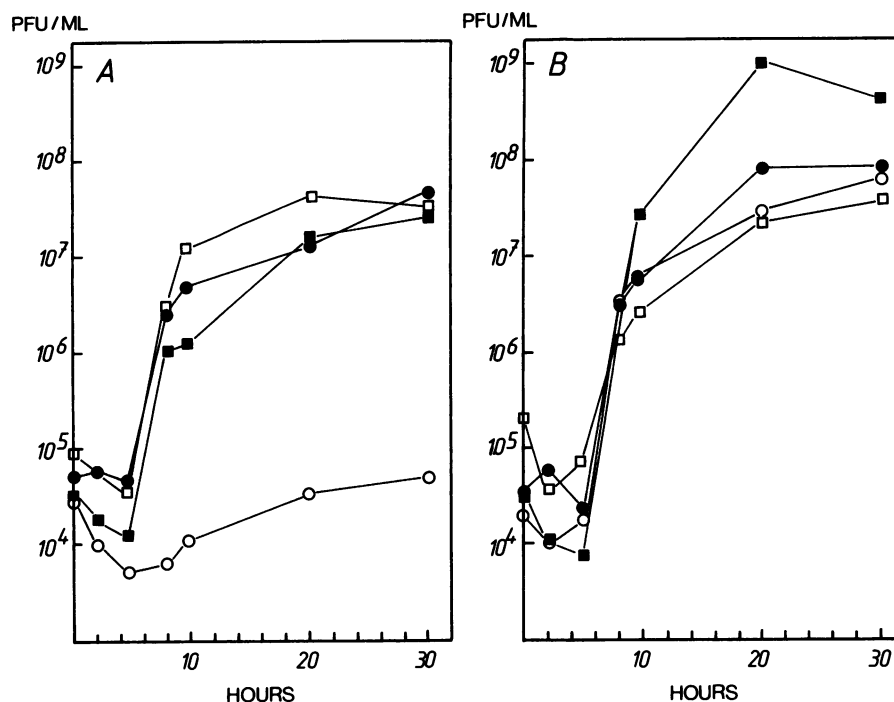


FIG. 1. Multiplication of VV, CPV, and recombinants in CHO and BHK 21 cells. Monolayers of CHO cells (A) or BHK 21 cells (B) were infected with approximately 0.1 PFU per cell of the following viruses: wild-type VV (○), wild-type CPV (●), a recombinant between VV and CPV (■; see Fig. 3, recombinant 6), or a VV tk-negative recombinant containing the insert from plasmid pEA36 (□). Virus was allowed to adsorb to the monolayers for 1 h at room temperature, and at various times thereafter, the cells were frozen. After several cycles of freezing and thawing, the virus samples were titrated on BHK 21 cell monolayers. The titers are expressed as PFU per milliliter in the cell culture.

phosphate precipitate containing VV wild-type DNA and the desired plasmid. After 1 h at room temperature, fresh medium was added to the cells, and incubation continued at the nonpermissive temperature (39.5°C) for 2 h. The calcium phosphate precipitate was then removed, and cells were treated for 1 min with 10% glycerol in culture medium and washed twice with phosphate-buffered saline. Fresh medium was added, and incubation was continued at 39.5°C for 2 days. Non-temperature-sensitive virus issued from the transfection was tested by the plaque assay for its ability to multiply on L tk-negative cells in the presence of 5'-bromodeoxyuridine or on CHO cells. In some experiments where indicated, CEF were infected with wild-type VV and transfected with DNA by using the protocol described above, except that incubation was continuous at 37°C.

RESULTS

Multiplication of CPV in CHO cells. Previous work has shown that VV Copenhagen and WR strains are unable to multiply in CHO cells (2, 6). Other VV strains available from the American Type Culture Collection, as well as the rabbitpox virus strain, were examined and found to behave in the same manner. CPV (Brighton), however, multiplied efficiently in CHO cells (Fig. 1A) and gave rise to plaques under an agar overlay (not shown). The yield obtained with CPV in BHK 21 cells (Fig. 1B) or CEF (not shown) was, on the other hand, poorer by about 1 order of magnitude than that with VV. To ascertain whether CPV could complement VV for multiplication in CHO cells, coinfection experiments were carried out (Table 1). As expected, in the single-control infections, there was no increase in the VV titer and a 30-fold increase in the CPV titer. The natural thermosensitivity of

CPV at 39.5°C was then used to detect VV in the mixed infection. Thus, the yield in the coinfection was 10-fold higher than the input virus titer, and most of this progeny corresponded to VV, since it lacked the thermosensitivity of CPV. As it is unlikely that such a high coinfection titer would be obtained simply through recombination, the results indicate that CPV encodes a function that could complement the VV defect for multiplication in CHO cells.

Isolation of recombinants between CPV and VV. An approximate localization of the region of the CPV genome that enables multiplication in CHO cells was first sought by isolating recombinants between VV and CPV and correlating their phenotypes with their DNA restriction profiles. Again, the natural thermosensitivity of CPV was used to select against wild-type CPV. In this case, CEF were coinfecting with CPV and a *ts* mutant of VV at 33°C. Recombinants were then selected by plating at 39.5°C, a nonpermissive temperature for both parental viruses. A total of 25 independent recombinants were amplified on CEF and assayed for their ability to multiply in CHO cells. Among these isolates, only

TABLE 1. Coinfection of CHO cells with CPV and VV^a

Virus	Adsorption titer at 33°C	36-h titer at:	
		33°C	39.5°C
VV	4.6×10^5	9.5×10^4	8.6×10^4
CPV	1.5×10^4	5.0×10^5	1.3×10^3
VV + CPV	2.5×10^5	2.2×10^6	1.8×10^6

^a CHO cell monolayers were infected at approximately 5 PFU per cell with the viruses indicated. The virus found in the infected cultures after a 1-h adsorption period or after 36 h of incubation at 33°C was determined by titration on BHK 21 cells at either 33 or 39.5°C.

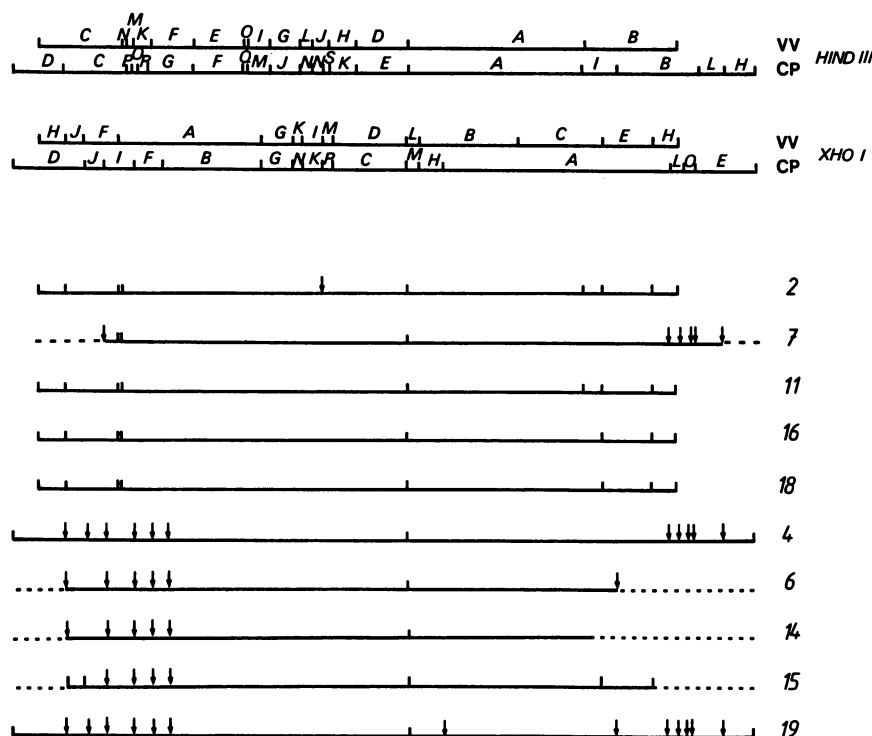


FIG. 2. DNA restriction maps for VV-CPV recombinants. The top four lines represent the restriction maps for VV and CPV (CP) obtained upon digestion with *Hind*III or *Xho*I. The bottom lines are maps for five recombinants unable to multiply in CHO cells (2, 7, 11, 16, and 18) or able to multiply in CHO cells (4, 6, 14, 15, and 19). Arrows indicate restriction sites (*Xho*I or *Hind*III) that could only be derived from CPV DNA, and vertical lines indicate sites derived only from VV DNA. The horizontal dashes show regions of DNA which could not clearly be attributed to either of the parental viruses.

five multiplied in CHO cells. Total DNA from CEF infected with either these five recombinants or five others was prepared and digested with *Hind*III or *Xho*I. A summary of the restriction profiles of the recombinants showing sites known to be found in only one of the parent viruses is presented in Fig. 2. The five recombinants capable of multiplying in CHO cells (recombinants 4, 6, 14, 15, and 19) all retained restriction sites originating from the left-hand end of the CPV genome. One recombinant, unable to multiply in CHO cells, retained sites from the CPV right-hand end. Taken together, these results suggested that the left-hand end alone contained the region involved in the host range phenotype.

Marker rescue of the host range phenotype with intact or restricted CPV DNA. Marker rescue of the host range phenotype was undertaken to more precisely map the region of the genome encoding this function. CPV DNA was cut with different restriction enzymes and transfected into cells previously infected with wild-type VV. Virus issued from the transfection was assayed for the ability to multiply in CHO cells. Transfection with CPV DNA restricted with either *Hind*III, *Eco*RI, *Bam*HI, or *Sal*I, but not *Xho*I, yielded the recombinants expected (Table 2). Therefore, it could be predicted that only the *Xho*I site would be close to or within the host range sequence. Several recombinants that arose from another transfection experiment similar to the one described above with *Hind*III-digested CPV DNA were isolated, and their DNAs were prepared. The restriction profiles obtained for these recombinants after digestion of their DNA with *Eco*RI were more closely comparable to a VV profile than to a CPV DNA profile (Fig. 3A). However,

in all of the recombinants, the *Eco*RI C fragment typical of VV was missing. It could thus be deduced that recombination between VV DNA and a CPV fragment had led to a rearrangement of the VV *Eco*RI C fragment. To identify the rearranged fragment(s) thought to contain the *Eco*RI C sequences and expected to be linked to the host range sequence, DNA from the recombinants was blotted to a nitrocellulose filter and hybridized with a nick-translated VV *Sal*II K fragment (Fig. 3, a restriction fragment within *Eco*RI-C). Hybridization revealed *Eco*RI-C in VV DNA, two different restriction fragments in CPV DNA containing sequences repeated at both ends of the CPV genome, and a single fragment in the DNA from the recombinant viruses that comigrated with the large *Eco*RI A and B fragments

TABLE 2. Marker rescue of the host range phenotype with intact or restricted CPV DNA^a

DNA in transfection	Enzyme	Titer on CHO cells
None	None	0
CPV DNA	None	440
	<i>Bam</i> HI	120
	<i>Xho</i> I	0
	<i>Eco</i> RI	630
	<i>Sal</i> I	1,400
	<i>Hind</i> III	1,400

^a CEF were infected with wild-type VV and transfected with no DNA, intact CPV DNA, or CPV DNA that had been restricted to completion with the indicated enzymes. After 48 h, the cells were disrupted by ultrasonic treatment, and the virus titers were determined on CHO cell monolayers.

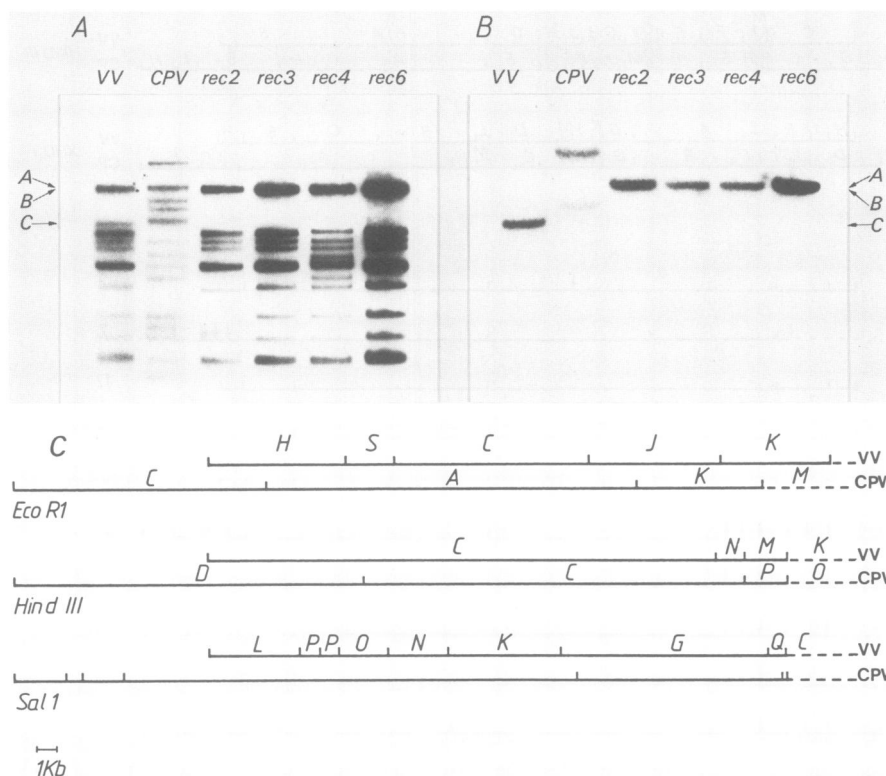


FIG. 3. DNA restriction pattern of recombinants isolated after transfection with *Hind*III-restricted CPV DNA. (A) *Eco*RI restriction profiles of VV DNA, CPV DNA, and DNA from four recombinants capable of multiplying in CHO cells. (B) Autoradiogram of the same DNAs as in panel A after transfer to a nitrocellulose filter, hybridization with the nick-translated VV *Sal*I K fragment, and washing to remove nonhybridizing material. The fragments designated A and B in the margins are the top two comigrating *Eco*RI fragments of VV DNA. The VV *Eco*RI C fragment is missing from all of the recombinants. (C) Restriction maps for the left-hand end of VV and CPV DNA.

from VV (Fig. 3B). The result could be explained by an insertion of about 4 kilobase pairs of CPV DNA into the *Eco*RI C fragment of VV via homologous recombination between corresponding regions of the two genomes.

Marker rescue of the host range phenotype with cloned DNA. The enlarged *Eco*RI C fragment from one of the recombinants described above (Fig. 3, recombinant 6) was ligated to *Eco*RI-digested pAT153, and *E. coli* HB101 competent cells were transformed to ampicillin resistance with the ligation mixture. Colonies were then screened by hybridization with the nick-translated VV *Sal*I K fragment previously used. Two recombinant plasmids designated pEA1 and pEA2 containing the *Eco*RI fragment in either of the two possible orientations were isolated and analyzed to establish a restriction map (Fig. 4), as well as to assay for their ability to rescue VV for multiplication in CHO cells. When CEF infected with VV *ts*N7 were cotransfected with pEA1 or pEA2 and VV wild-type DNA, a portion of the progeny multiplied in CHO cells. The insert in these plasmids was therefore further subcloned into a VV tk transplacement vector (pTG186poly) by using various restriction sites (Fig. 4) to generate plasmids pEA5a, pEA5b, pEA6, pEA7, pEA8, pEA9, and pEA36. The purpose of using the tk-containing vector was to allow homologous recombination to occur at the tk locus even after possible removal of sequences flanking the host range gene that might otherwise have been required for recombination within the VV *Eco*RI C fragment at the left end of the VV genome. After each plasmid construction, marker rescue was carried out to assay for the transfer of host range phenotype and to detect the generation

of tk-negative recombinants. This procedure delineated the smallest fragment (2.3 kilobase pairs) contained on pEA36 that was capable of rescuing VV for multiplication in CHO cells. Further attempts to subclone smaller fragments of pEA36, which still retained biological activity, failed. The ability of one of the recombinants containing this minimal information to multiply in CHO cells is illustrated by the multiplication curve in Fig. 1. Interestingly, all of the plasmids capable of marker rescue (pEA5a, pEA5b, pEA6, pEA9, and pEA36), except for pEA36, gave rise to both tk-negative and tk-positive recombinants. For example, of 27 recombinants isolated for their ability to multiply in CHO cells after transfection with pEA9, 16 were tk negative and 11 were tk positive. This indicated that the pEA9 plasmid still contained enough sequence homology to allow recombination at the left-hand end of the VV genome. However, rescue with pEA36 yielded only tk-negative recombinants, suggesting that very little or no homology with the left-hand end of the VV genome had been retained on this plasmid.

Nucleotide sequence of the host range gene. The 2.3-kilobase-pair fragment contained in pEA36 was subcloned into the single-stranded phage vector M13 TG130 or M13 TG131 and sequenced by the dideoxynucleotide method with universal primers or synthetic oligonucleotides as primers (Fig. 5). The nucleotide sequence contained a single long open reading frame running from right to left on the CPV genome. The calculated molecular weight of the protein that could be encoded by the 2,004-nucleotide open reading frame was 77,000. The promoter for transcription of the gene appeared to be maintained within the portion sequenced,

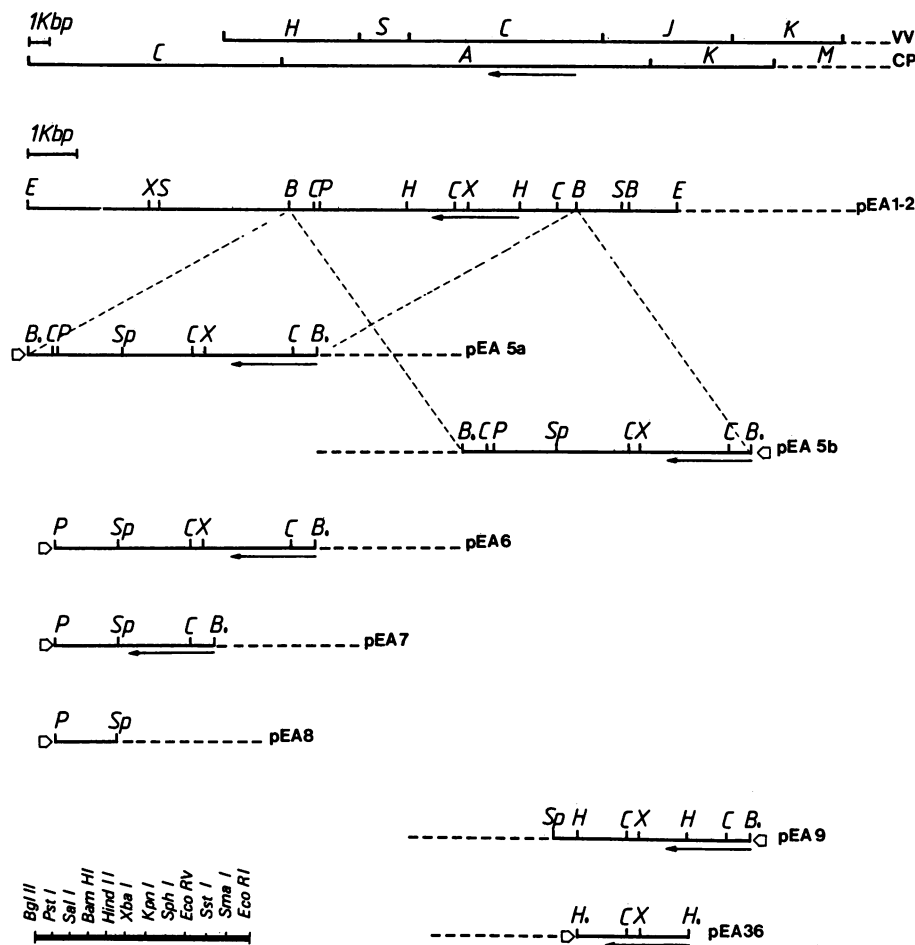


FIG. 4. Restriction maps for the recombinant plasmids used to delineate the CPV host range gene. The top two horizontal lines represent the *EcoRI* maps of the left-hand end of VV and CPV (CP) DNAs (letters indicate the restriction fragments according to size). A restriction map of plasmids pEA1 and pEA2 is shown underneath, with the pAT153 bacterial-vector portion symbolized by a dashed line. The large *Bgl*II (B) fragment from pEA1 was inserted in either of the two possible orientations into the *Bam*HI (B_0) site of the transplacement vector pTG186poly to give rise to pEA5a and pEA5b. For these two and the following vectors, the dashed lines represent pTG186poly schematically, the short thick arrows symbolize the VV 7.5-kilodalton promoter used to obtain transcription of foreign genes inserted into the vector, and the long thin arrows indicate the direction of transcription of the host range gene deduced from subsequent sequencing. The polylinker segment at the bottom of the figure shows the restriction sites positioned at the 3' end of the 7.5-kilodalton promoter. The pEA6 plasmid was derived from pEA5a by cutting it with *Pst*I (P) and religating the largest fragment. Plasmid pEA7 was derived from pEA6 by cutting it with *Cla*I (C) and religating the largest fragment. Plasmids pEA8 and pEA9 were derived from pEA6 and pEA5b, respectively, by cutting with *Sph*I (Sp) and religating. Plasmid pEA36 was constructed in two steps. First, the small *Hpa*I (H) fragment from pEA9 was isolated and inserted into the *Sma*I site of the phage vector M13 TG130 (same polylinker segment as indicated at the bottom of the figure). The fragment was then excised from M13 by digestion with *Eco*RI and *Pst*I and inserted into pTG186poly digested with the same enzymes. E, *Eco*RI; X, *Xho*I; H_0 and B_0 , loss of the *Hpa*I or *Bgl*II site, respectively.

since the predicted transcriptional orientation of the open reading frame on the pEA36 plasmid was in the opposite direction of the 7.5-kilodalton VV promoter. Moreover, a 100-base-pair segment located 50 base pairs preceding the presumed translation initiation codon was as much as 85% AT rich, a typical characteristic of orthopoxvirus promoters. The predicted amino acid sequence displayed no remarkable features that might help in understanding the biological activity of the protein. Neither did comparison of the amino acid sequence with previously published poxvirus sequences or sequences available in data bases reveal any significant similarities.

DISCUSSION

Our results demonstrate that CPV contains a gene, apparently missing in VV, that extends its host range in tissue

culture. Examination of the previously published restriction maps for CPV (14) enabled us to localize this gene at approximately 27 kilobase pairs from the left-hand end of the genome, overlapping the *Xho*I J and I fragments. The insertion of this gene into the VV genome by recombination with a restriction fragment from CPV suggests that the CPV host range gene is flanked by sequences common to both viruses. What role the gene plays *in vivo* is not yet known, although one may speculate that it extends the CPV host range to other as yet unidentified cell types. In this respect, it is interesting to recall that CPV or viruses closely related to it, as deduced from their DNA restriction maps (4, 9), are the most widespread orthopoxviruses in nature. This is in contrast to VV which has not yet been clearly demonstrated to occur independently of vaccination. Sequences found in CPV that are missing in the VV genome, including the gene

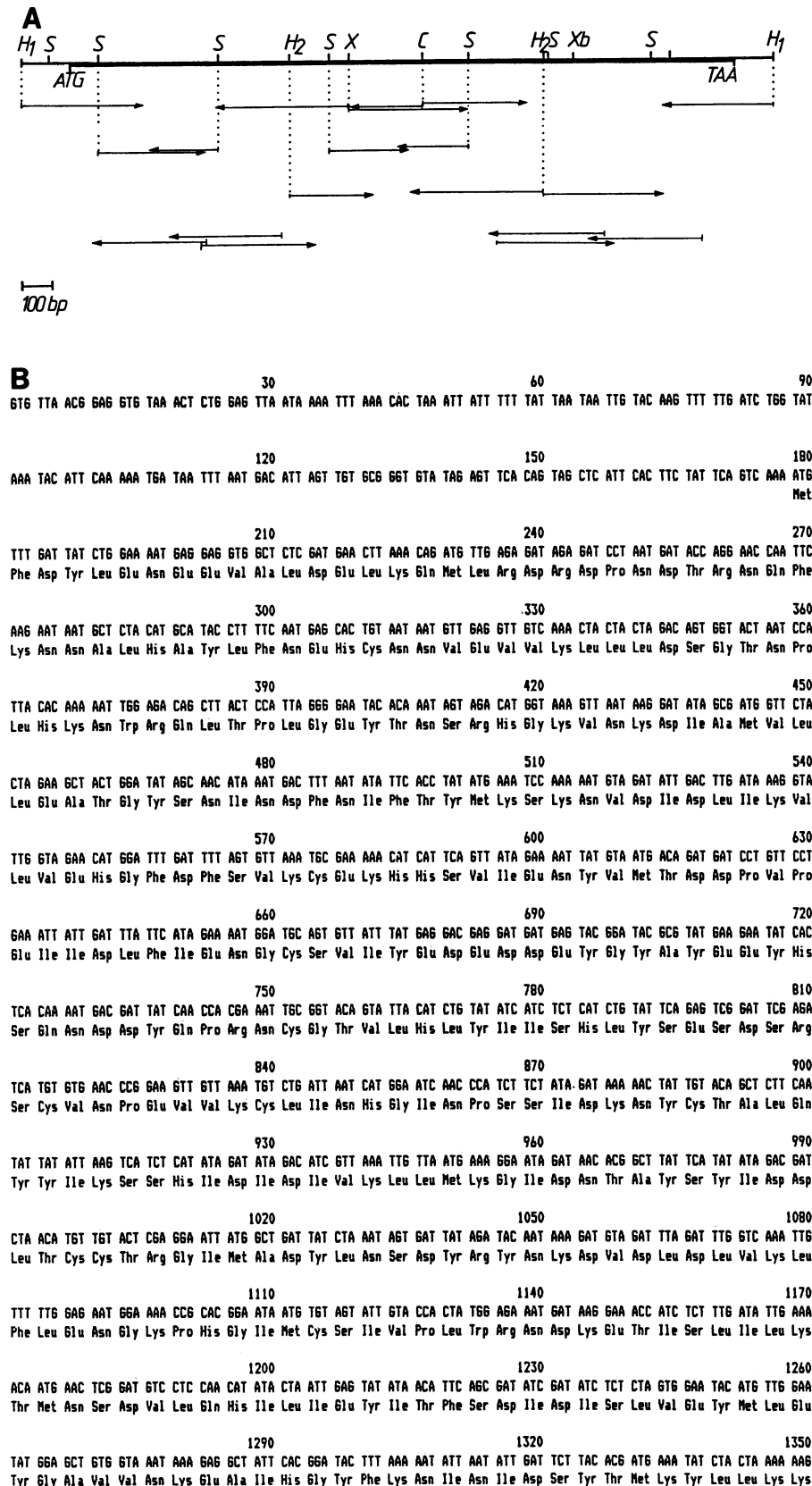


FIG. 5. Nucleotide sequence and deduced amino acid sequence of the CPV host range gene. (A) Sequencing strategy employed. The *Hpa*I (*H*₁ fragment corresponding to the *Hpa*I fragment inserted into pEA36 was subcloned in various orientations into M13 TG130 or M13 TG131 by using the restriction sites indicated by the dotted lines. S, *Sau*3A; H₂, *Hpa*II; X, *Xho*I; C, *Cl*aI; Xb, *Xba*I. M13 universal primers were used for dideoxynucleotide sequencing. The sequence was completed by using synthetic oligonucleotides in regions shown by the arrows under the restriction map. The bold line indicates the coding portion of the sequence. (B) Nucleotide and deduced amino acid sequence.

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1380                                1410                                1440
GAA GGG GGA GAT GCC GTC AAT CAT CTC GAT GAT GGA GAG ATC CCG ATT GGA CAC CTA TGT AAA TCC AAC TAT GGA CBT TAT AAT TTC TAC
Glu Gly Gly Asp Ala Val Asn His Leu Asp Asp Gly Glu Ile Pro Ile Gly His Leu Cys Lys Ser Asn Tyr Gly Arg Tyr Asn Phe Tyr

1470                                1500                                1530
ACT GAT ACA TAC AGA CAG GGT TTT CBT GAT ATG TCT TAT GCT TGC CCA ATT CTT AGT ACT ATA AAC ATT TGC CTA CCT TAT CTT AAA BAC
Thr Asp Thr Tyr Arg Gln Gly Phe Arg Asp Met Ser Tyr Ala Cys Pro Ile Leu Ser Thr Ile Asn Ile Cys Leu Pro Tyr Leu Lys Asp

1560                                1590                                1620
ATT AAC ATG ATT GAC AAA CBA GGA GAA ACA CTT CTT CAC AAG GCT GTT AGA TAT AAT AAA CAA TCT CTA GTG TCT TTA CTG CTA GAA TCC
Ile Asn Met Ile Asp Lys Arg Gly Glu Thr Leu Leu His Lys Ala Val Arg Tyr Asn Lys Gln Ser Leu Val Ser Leu Leu Leu Glu Ser

1650                                1680                                1710
GGT TCA GAT GTC AAC ATT AGA TCA AAT AAC GGA TAT ACA TGT ATA GCC ATT GCA ATC AAC GAA TCT AGA AAC ATT GAA CTG CTG AAC ATG
Gly Ser Asp Val Asn Ile Arg Ser Asn Asn Gly Tyr Thr Cys Ile Ala Ile Ala Ile Asn Glu Ser Arg Asn Ile Glu Leu Leu Asn Met

1740                                1770                                1800
CTA TTA TGT CAT AAA CCT ACA TTA GAT TGT GTG ATT GAT TCA TTG AGA BAA ATA TCT AAC ATA GTA GAT AAT GCC TAT GCT ATA AAA CAA
Leu Leu Cys His Lys Pro Thr Leu Asp Cys Val Ile Asp Ser Leu Arg Glu Ile Ser Asn Ile Val Asp Asn Ala Tyr Ala Ile Lys Gln

1830                                1860                                1890
TGT ATT AGA TAT GCC ATG ATT ATA GAT GAC TGT ATA TCG TCT AAG ATT CCA GAG TCC ATA AGT AAA CAC TAT AAT GAT TAT ATA GAT ATT
Cys Ile Arg Tyr Ala Met Ile Ile Asp Asp Cys Ile Ser Ser Lys Ile Pro Glu Ser Ile Ser Lys His Tyr Asn Asp Tyr Ile Asp Ile

1920                                1950                                1980
TGC AAT CAA GAA TTG AAC GAG ATG AAA AAA ATA ATA GTG GGA GGC AAC ACT ATG TTC TCA TTA ATA TTT ACT GAT CAT GGA GCT AAA ATT
Cys Asn Gln Glu Leu Asn Glu Met Lys Lys Ile Ile Val Gly Gly Asn Thr Met Phe Ser Leu Ile Phe Thr Asp His Gly Ala Lys Ile

2010                                2040                                2070
ATT CAT CCG TAT GCC AAT AAT CCA GAA TTA CBT GCG TAT TAT GAG TCA AAA CAA AAT AAA ATA TAC GTG BAA GTA TAT GAT ATT ATT TCC
Ile His Arg Tyr Ala Asn Asn Pro Glu Leu Arg Ala Tyr Tyr Glu Ser Lys Gln Asn Lys Ile Tyr Val Glu Val Tyr Asp Ile Ile Ser

2100                                2130                                2160
AAT GCG ATA GTG AAG CAT AAT AAA ATT CAT AAA AAC ATA GAA TCA GTT GAT GAC AAT ACC TAC ATT TCT AAT TTG CCT TAT ACC ATC AAA
Asn Ala Ile Val Lys His Asn Lys Ile His Lys Asn Ile Glu Ser Val Asp Asp Asn Thr Tyr Ile Ser Asn Leu Pro Tyr Thr Ile Lys

2190                                2220                                2250
TAC AAA ATA TTC GAG CAA CAA TAA GTA TTT TTT ATA CCT TTA AAA TTG ATA AAT AAA TTT TTT CTA GTG ATA TTT TGG CAA GAT GAG AAT
Tyr Lys Ile Phe Glu Gln Gln End

2280
CCT ATT TCT CAT CCG TTT CAT GTA TGG GTG TGT TCA CTC ATA TGT TAA C

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FIG. 5—Continued.

identified in this work, are most likely responsible for the different behavior of the two viruses in vivo. Another gene mapping to the right-hand end of the genome and responsible for the typical hemorrhagic lesions induced by CPV also is lacking in VV (13), and of course, many others still remain to be identified.

Although the exact function of the CPV host range gene is as yet unknown, the phenotype displayed by VV in CHO cells suggests a possible role. VV infection of CHO cells leads to a very extensive and early shutoff of both host cell and viral protein synthesis (2, 12). The CPV host range gene could provide a means for specific shutoff of only cellular mRNA and thereby allow infection to become productive. Such a function would of course require that the protein be synthesized very early in infection, before shutoff is established. This role is similar to the one postulated for a previously characterized VV host range gene (5). Since CPV also encodes the latter gene (unpublished), it would thus encode two genes with related activities that are essential for virus replication in different cell types. The molecular characterization of the two different host range genes, one from CPV and the other found in both CPV and VV, provides tools to further explore their biological roles.

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LITERATURE CITED

1. Drillien, R., and D. Spehner. 1983. Physical mapping of vaccinia virus temperature sensitive mutations. *Virology* **131**:385–393.
2. Drillien, R., D. Spehner, and A. Kirn. 1978. Host range restriction of vaccinia virus in Chinese hamster ovary cells: relationship to shutoff of protein synthesis. *J. Virol.* **28**:843–850.
3. Drillien, R., D. Spehner, and A. Kirn. 1982. Complementation and genetic linkage between vaccinia virus temperature-sensitive mutants. *Virology* **119**:372–381.
4. Esposito, J. J., and J. C. Knight. 1985. Orthopoxvirus DNA: a comparison of restriction profiles and maps. *Virology* **143**:230–251.
5. Gillard, S., D. Spehner, R. Drillien, and A. Kirn. 1986. Localization and sequence of a vaccinia virus gene required for multiplication in human cells. *Proc. Natl. Acad. Sci. USA* **83**:5573–5577.
6. Hruby, D. E., D. L. Lynn, R. Condit, and J. R. Kates. 1980. Cellular differences in the molecular mechanisms of vaccinia virus host range restriction. *J. Gen. Virol.* **47**:485–488.
7. Kieny, M. P., R. Lathe, R. Drillien, D. Spehner, S. Skory, D. Schmitt, T. Wictor, H. Koprowski, and J. P. Lecocq. 1984. Expression of rabies virus glycoprotein from a recombinant vaccinia virus. *Nature (London)* **312**:163–166.
8. Kieny, M. P., R. Lathe, and J. P. Lecocq. 1983. New versatile cloning and sequencing vectors based on bacteriophage M13. *Gene* **26**:91–99.
9. Mackett, M., and L. C. Archard. 1979. Conservation and variation in Orthopoxvirus genome structure. *J. Gen. Virol.* **45**:683–701.
10. Mackett, M., G. L. Smith, and B. Moss. 1982. Vaccinia virus: a selectable eucaryotic cloning and expression vector. *Proc. Natl.*

- Acad. Sci. USA **79**:7415–7419.
11. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 12. **Njayou, M., R. Drillien, and A. Kirn.** 1982. Characteristics of the inhibition of protein synthesis by vaccinia virus in non-permissive Chinese hamster ovary cells. *Ann. Virol.* **133E**: 393–402.
 13. **Pickup, D. J., B. S. Ink, W. Hu, C. A. Ray, and W. K. Joklik.** 1986. Hemorrhage in lesions caused by cowpox virus is induced by a viral protein that is related to plasma protein inhibitors of serine proteases. *Proc. Natl. Acad. Sci. USA* **83**:7698–7702.
 14. **Pickup, D. J., B. S. Ink, B. L. Parsons, W. Hu, and W. K. Joklik.** 1984. Spontaneous deletions and duplications of sequences in the genome of cowpox virus. *Proc. Natl. Acad. Sci. USA* **81**:6817–6821.
 15. **Sanger, F. S., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
 16. **Twigg, A. J., and D. Sherratt.** 1980. Trans-complementable copy-number mutants of plasmid Col E1. *Nature (London)* **283**:216–218.