JOURNAL OF VIROLOGY, Oct. 1986, p. 224–229
0022-538X/86/100224-06$02.00/0
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Vol. 60, No. 1

Complete Sequence and Enhancer Function of the Homologous DNA Regions of *Autographa californica* Nuclear Polyhedrosis Virus

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Received 24 March 1986/Accepted 11 June 1986

The nucleotide sequence of the five regions of homologous DNA in the genome of *Autographa californica* nuclear polyhedrosis virus DNA was determined. The homology of repeated sequences within a region was 65 to 87%, and the consensus sequences for each region were 88% homologous to each other. Sequences proximal to the *Eco*RI sites were most conserved, while the distal sequences were least conserved. The *Eco*RI sites formed the core of a 28-base-pair imperfect inverted repeat. All homologous regions functioned as enhancers in a transient expression assay. A single *Eco*RI minifragment located between *Eco*RI-Q and -L enhanced the expression of 39CAT as efficiently as the regions containing numerous *Eco*RI repeats did.

The genome of the baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) is a circular double-stranded molecule of approximately 128 kilobase pairs. Although the genome consists primarily of unique sequences, five regions which share homologous sequences are interspersed along the length of the genome (6). Because the homologous regions (*hr*s) are rich in *Eco*RI sites, an *Eco*RI restriction digest of AcNPV DNA contains extramolar amounts of small fragments which range in size between 72 and 215 base pairs (bp). A similar pattern of interspersed homologous DNA is also found in another baculovirus, *Choristoneura fumiferana* nuclear polyhedrosis virus (21), suggesting that the *hr*s play an important role in the life cycle of baculoviruses.

The synthesis of viral proteins in AcNPV-infected cells occurs in successive stages, indicating temporal control of viral gene expression (5, 15, 20, 26, 37). At least some of this temporal control is exerted at the level of transcription, as evidenced by the presence of different RNA transcripts at different times of infection (1, 9, 30, 34). Overlapping transcripts have been mapped in several regions of the AcNPV genome (10, 23, 28). Some of these transcripts share a common 5′ end, while others are 3′ coterminal. It has been hypothesized that this type of transcriptional unit may regulate the expression of baculovirus genes, as the initiation of transcription at upstream promoters may repress initiation of transcription downstream (10). Activation of some of the delayed-early class of promoters may be due to an immediate-early gene which *trans* activates the expression of the 39K gene (17). Activation of the late class of transcripts may be linked to a virus-induced RNA polymerase (12).

Recently, we demonstrated that plasmids containing *hr* DNA stimulate the expression of the chloramphenicol acetyltransferase (CAT) gene under the control of the AcNPV 39K promoter (18). Although this *trans* effect was originally observed as part of our search for *trans*-active factors, we hypothesized that the stimulation of CAT activity was the result of in vivo recombination and the resultant *cis* activation. *hr*5 was shown to exhibit all the characteristics of an enhancer, including orientation independence and the abilities to function at a distance from the promoter, to activate heterologous promoters, and to increase RNA polymerase density on the linked gene.

Here we report the nucleotide sequence of the remaining homologous regions and present evidence that they function as enhancers.

MATERIALS AND METHODS

DNA sequencing. The sequencing strategy for the five regions is indicated in Fig. 1. The AcNPV restriction fragments *Pst*I-D (*hr*1), *Hin*dIII-L (*hr*2), *Hin*dIII-B (*hr*3), *Bgl*II-G (*hr*4), or *Hin*dIII-Q (*hr*5) were partially or completely digested with *Eco*RI and ligated with *Eco*RI-digested M13mp9 (27). Single-stranded recombinant phage was purified and sequenced by the dideoxy chain termination procedure (29). The restriction map data generated by the initial sequencing were used to design additional cloning and sequencing to determine the order of the *Eco*RI minifragments. For *hr*2, *Bal* 31 deletions of *Hin*dIII-L were constructed according to standard procedures (25) and sequenced to determine the order of the minifragments. The sequences were compiled and analyzed by the programs of Devereaux et al. (7).

Analysis of enhancer function. The conditions for cell culture, transfection, and CAT assays have been described previously (14, 17). To determine whether the *hr*s function as enhancers, we repaired the following fragments with the Klenow fragment of DNA polymerase I according to standard procedures (25) and cloned in both orientations into the *Hin*dIII (Klenow-repaired) site in the multiple cloning site upstream of 39CAT: for *hr*1, the *Cla*I fragment (Fig. 1); for *hr*2, a 1.5-kilobase-pair *Hin*dIII-*Sal*I fragment; for *hr*3, the *Mlu*I-*Ssp*I fragment (Fig. 1); and for *hr*5, the *Mlu*I fragment (Fig. 1). *hr*4*left* was cloned (in one orientation with an *Nsi*I-*Xba*I fragment) into the *Pst*I and *Hin*dIII sites of 39CAT. The resulting plasmid contains *hr*4 in an orientation opposite of that in the standard AcNPV genetic map (33).

RESULTS AND DISCUSSION

Nucleotide sequence of AcNPV homologous DNA. The sequencing strategy for the five homologous regions is shown in Fig. 1, and the nucleotide sequences are presented

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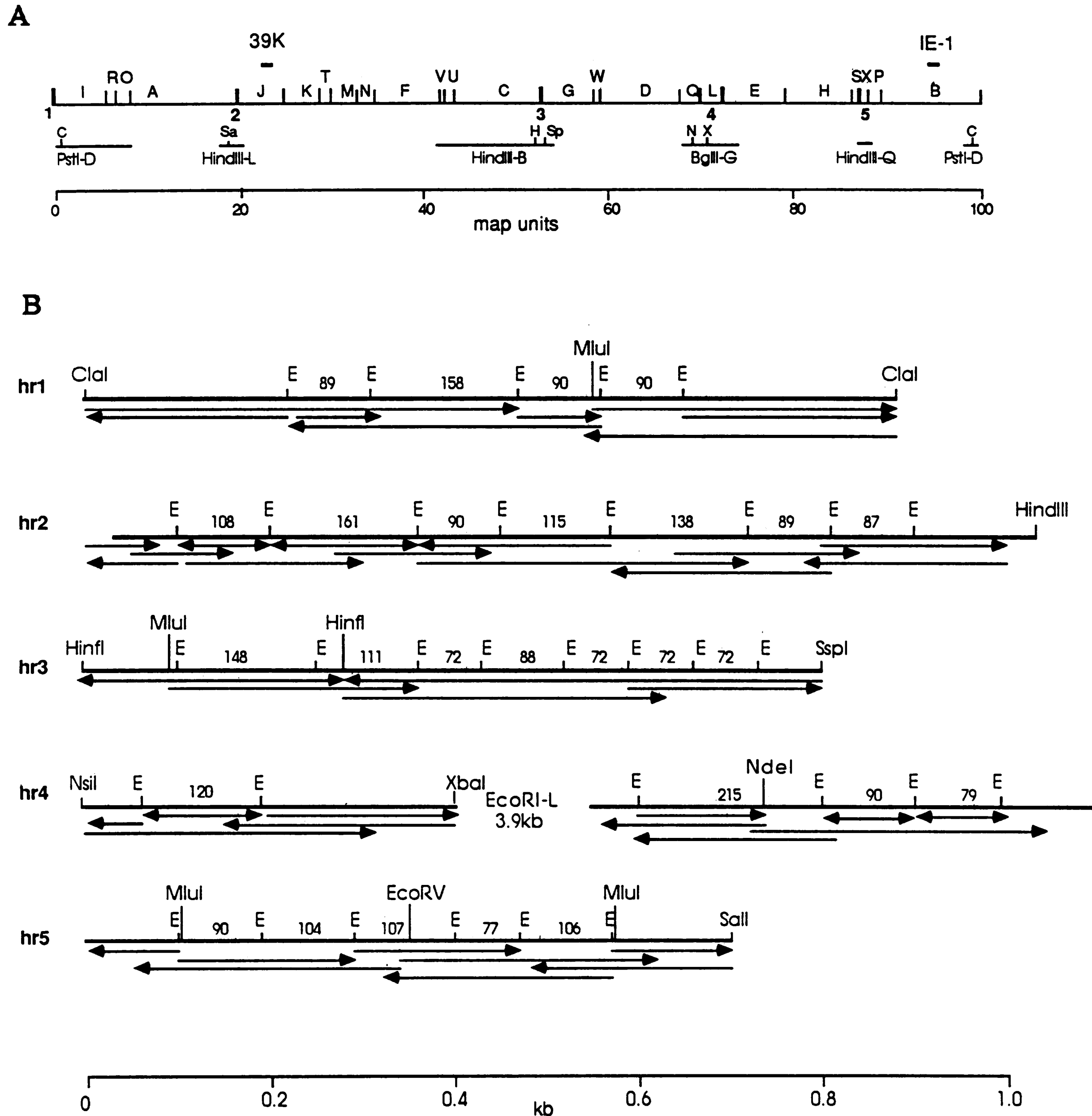

FIG. 1. Arrangement of homologous sequences on the AcNPV genome (A) and the sequencing strategy of the *hr*s (B). In panel A, the location of the *hr*s in the *Eco*RI restriction map is indicated by heavy vertical lines 1 to 5. The indicated clones which overlap the *hr*s were subcloned for sequencing. Some relevant restriction sites are indicated: C, *Cla*I; H, *Hin*fI; Ss, *Ssp*I; N, *Nsi*I; X, *Xba*I; Sa, *Sal*I. By convention, the genetic map of AcNPV is presented with the left end of *Eco*RI-I at position 0 (33). In panel B, the indicated restriction fragments were sequenced. In *hr*2 Bal 31 deletions were constructed and sequenced. The number of bases between the *Eco*RI sites (E) is indicated.

in Fig. 2. The sequence of *hr*5 has been reported elsewhere (18). The sizes of the *Eco*RI minifragments in *hr*1, *hr*2, *hr*4, and *hr*5 were previously determined by Cochran and Faulkner (6) by polyacrylamide gel electrophoresis. Our sequence data indicated that their analysis was essentially correct for *hr*2 and *hr*5. *hr*1 contains four *Eco*RI minifragments, one each of 158 and 89 bp and two of 90 bp. This is one more minifragment than previously reported (6). *hr*4 was reported to contain four *Eco*RI fragments between *Eco*RI-Q and -L (6). DNA sequencing showed that only one fragment of 120 bp was located in that site; this is referred to here as *hr4left*. The other three fragments (215, 90, and 79 bp) are located between *Eco*RI-L and -E. This region is referred to here as *hr4right*. The size of the *Eco*RI minifragments in *hr*3 was not determined by Cochran and Faulkner. However, on the basis of hybridization data, *hr*3 was expected to be one of the smaller regions. Sequencing data indicated that *hr*3 is the second largest in overall size, with seven *Eco*RI fragments: one each of 148, 111, and 88 bp and four of 72 bp. The poor hybridization of *hr*3 in the previous study may be attributable to the fact that *hr*3 contains four 72-bp minifragments which are smaller than the probes used for hybridization (6).

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hr1

```
  1 ATCGATGATT GACCCCAACA AAAGATTTAT AATTAATCAT AATCACGAAC
 51 AACAACAAGT CAATGAAACA AATAAACAAG TTGTCGATAA AACATTCATA
101 AATGACACAG CAACATACAA TTCTTGCATA ATAAAAATTT AAATGACATC
151 ATATTTGAGA ATAACAAATG ACATTATCCC TCGATTGTGT TTTACAAGTA
201 GAATTCTACC CGTAAAGCGA GTTTAGTTTT GAAAAACAAA TGACATCATT
251 TGTATAATGA CATCATCCCC TGATTGTGTT TTACAAGTAG AATTCTATCC
301 GTAAAGCGAG TTCAGTTTTG AAAACAAATG AGTCATACCT AAACACGTTA
351 ATAATCTTCT GATATCAGCT TATGACTCAA GTTATGAGCC GTGTGCAAAA
401 CATGAGATAA GTTTATGACA TCATCCACTG ATCGTGCGTT ACAAGTAGAA
451 TTCTACTCGT AAAGCCAGTT CGGTTATGAG CCGTGTGCAA AACATGACAT
501 CAGCTTATGA CTCATACTTG ATTGTGTTTT ACGCGTAGAA TTCTACTCGT
551 AAAGCGAGTT CGGTTATGAG CCGTGTGCAA AACATGACAT CAGCTTATGA
601 GTCATAATTA ATCGTGCGTT ACAAGTAGAA TTCTACTCGT AAAGCGAGTT
651 GAAGGATCAT ATTTAGTTGC GTTTATGAGA TAAGATTGAA AAGCGTGTAA
701 AATGTTTCCC GCGCTTGGCA CAACTATTTA CAATGCGGCC AAGTTATAAA
751 AGATTCTAAT CTGATATGTT TTAAAACACC TTTGCGGCCC GAGTTGTTTG
801 CGTACGTGAC TAGCGAAGAA GATGTGTGGA CCGCAGAACA GATAGTAAAA
851 CAAAACCCTA GTATTGGAGC AATAATCGAT
```

hr2

```
  1 TGAGCAAAAC ACAACCGGCA AATTCTCGGC GGCCGTTTGG GAATGCGGAA
 51 TAATTGCCAT ATGTAAATGA TGTCATCGGT TCTAACTCGC TTTACGAGTA
101 GAATTCTACG TGTAAAACAT AATCAAGAGA TGATGTCATT TGTTTTTCAA
151 AACTGAACTC AAGAAATGAT GTCATTTGTT TTTCAAAACT GAACTGGCTT
201 TACGAGTAGA ATTCTACTTG TAAAACACAA TCGAGAGATG ATGTCATATT
251 TTGCACACGG CTCTAATTAA ACTCGCTTTA CGAGTAAAAT TCTACTTGTA
301 ACGCATGATC AAGGGATGAT GTCATTGGAT GAGTCATTTG TTTTTCAAAA
351 CTAAACTCGC TTTACGAGTA GAATTCTACT TGTAAAACAC AATCAAGGGA
401 TGATGTCATT ATACAAATGA TGTCATTTGT TTTTCAAAAC TAAACTCGCT
451 TTACGGGTAG AATTCTACTT GTAAAACAGC AACTCGAGGG ATGATGTCAT
501 CCTTTACTCG ATGATTATAA ACGTGTTTAT GTATGACTCA TTTGTTTTTC
551 AAAACTAAAC TCGCTTTACG AGTAGAATTC TACTTGTAAC GCACGATCAA
601 GGGATGATGT CATTTATTTG TGCAAAGCTC GATGTCATCT TTTGCACACG
651 ATTATAAACA CAATCCAAAT AATGACTCAT TTGTTTTCAA AACTGAACTC
701 GCTTTACGAG TAGAATTCTA CTTGTAAAAC ACAATCAAGG GATGATGTCA
751 TTTTCAAAAT GATGTCATTT GTTTTTCAAA ACTAAACTCG CTTTACGAGT
801 AGAATTCTAC TTGTAAAACA CAATCAAGGG ATGATGTCAT TTTAAAAATG
851 ATCATTTGTT TTTCAAAACT AAACTCGCTT TACGAGTAGA ATTCTACGTG
901 TAAAACACAA TCAAGGGATG ATGTCATTTA CTAAATAAAA TAATTATTTA
951 AATAAAACTG TTTTTTATTG TCAAATACAC ATTGATTCAC
```

hr3

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  1 ACGCGTAGAA TTCTACTTGT AAAGCAAGTT AAAATAAGCC GTGTGCAAAA
 51 ATGACATCAG ACAAATGACA TCATCTACCT ATCATGATCA TGTTAATAAT
101 CATGTTTTAA AATGACATCA GCTTATGACT AATAATTGAT CGTGCGTTAC
151 AAGTAGAATT CTACTCGTAA AGCGAGTTTA GTTTTGAAAA CAAATGAGTC
201 ATCATTAAAC ATGTTAATAA TCGTGTATAA AGGATGACAT CATCCACTAA
251 TCGTGCGTTA CAAGTAGAAT TCTACTCGTA AAGCGAGTTC GGTTTTGAAA
301 AACAAATGAC ATCATTTCTT GATTGTGTTT TACACGTAGA ATTCTACTCG
351 TAAAGTATGT TCAGTTTAAA AAACAAATGA CATCATTTTA CAGATGACAT
401 CATTTCTTGA TTATGTTTTA CAAGTAGAAT TCTACTCGTA AAGCGAGTTT
451 AGTTTTAAAA AACAAATGAC ATCATCTCTT GATTATGTTT TACAAGTAGA
501 ATTCTACTCG TAAAGCGAGT TTAGTTTTGA AAAACAAATG ACATCATCTC
551 TTGATTATGT TTTACAAGTA GAATTCTACT CGTAAAGCGA GTTTAGTTTT
601 GAAAAACAAA TGACATCATC CCTTGATCAT GCGTTACAAG TAGAATTCTA
651 CTCGTAAAGC GAGTTGAATT TTGATTACAA TATT
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hr4left

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  1 ATGCATATAA TTGTGTACAA AATATGACTC ATTAATCGAT CGTGCGTTAC
 51 AAGTAGAATT CTACTGGTAA AGCAAGTTCG GTTGTGAGCC GTGTGCAAAA
101 CATGACATCA TAACTAATCA TGTTTATAAT CATGTGCAAA ATATGACATC
151 ATCCGACGAT TGTGTTTTAC AAGTAGAATT CTACTCGTAA AGCGAGTTTA
201 AAAATTTTGT GACGTCAATG AAACAACGTG TAATATTTTT TACAATATTT
251 AAGTGAAACA TTATGACTTC CAATAATTTT GTGGATGTGG ATACGTTTGC
301 AAGACAATTG ATTACAGATA AATGTAGTGC TCTAATCGAA AGATGCGGAT
351 CTGTTGCCGG CAAACATTTT AGAGATTAGT AGAGAAAGGC CAGAGACAAG
401 TATTTTGAGG TGCCAACTCA AAAAAACTAT GAATACATTA AAAAATTATT
451 TTTACGAACA AAATATATGG ACGATTCGAT AGATTATAAA GATTTTAACA
501 GACGCATCCT ATTGATAGTT TTTAAATTCG CTTTAAACAA GAGCACCAAC
551 TACTTTCCAT CGTACTAAAG AGATCATCGA GGTGGCCATT AAACGTTTAA
601 ACAAAATTAA CCCCGATTTA AAGAGTTCTC CGCGCAATGC TTCAGCATTA
651 CAAATGAATG TTTGGAAAAT CTAGA
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hr4right

```
  1 AACTGGCTTT ACGAGTAGAA TTCTACTTGT AAAACACAAT CAAGAAATGA
 51 TGTCATTTTT GTACGTGATT ATAAACATGT TTAAACATGG TACATTGAAC
101 TTAATTTTTG CAAGTTGATA AACTAGATTA ATGTATGACT CATTTGTTTG
151 TGCAAGTTGA TAAACGTGAT TAATATATGA CTCATATGTT TGTGCAAAAA
201 TGGTGTCATC GTACAAACTC GCTTTACGAG TAGAATTCTA CTTGTAAAAC
251 ACAATCGAGG GATGATGTCA TTTGTAGAAT GATGTCATTT GTTTTTTCAA
301 AACCGAACTC GCTTTACGAG TAGAATTCTA CTTGTAAAAC ACAATCGAGG
351 GATGATGTCA TTTGTAGAAT GATGTCATCG TACAAACTCG CTTTACGAGT
401 AGAATTCTAG TAAAACAC
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FIG. 2. Nucleotide sequence of the *hr*s. The sequences are presented so that they read from left to right on the genetic map (genome orientation).

Analysis of the *hr* nucleotide sequence. The homology of the repeated sequences in *Eco*RI minifragments is demonstrated in Fig. 3. In this analysis, gaps were inserted for optimal alignment of homologous sequences. Much of the sequence heterogeneity within each region can be attributed to the additional bases in the longer minifragments. The sequences surrounding the *Eco*RI sites were the most conserved, and the sequences distal to the *Eco*RI sites were the least conserved.

Homologous sequences extending to the left and right of

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hr1

201  TCGATTGTGT TTTACAAGTA GAATTCTACC CGTAAAGCGA GTTTAGTTTT (22) .......... .......... .......... ..TATAATGA CATCATCCC
269  CTGATTGTGT TTTACAAGTA GAATTCTATC CGTAAAGCGA GTTCAGTTTT (59) AAGTTATGAG CCGTGTGCAA AACATGAGAT AAGTTTATGA CATCATCCA
428  CTGATCGTGC GTTACAAGTA GAATTCTACT CGTAAAGCCA GTTC......      .GGTTATGAG CCGTGTGCAA AACATGACAT CAGCTTATGA C.TCATAC.
518  TTGATTGTGT TTTACGCGTA GAATTCTACT CGTAAAGCGA GTTC......      .GGTTATGAG CCGTGTGCAA AACATGACAT CAGCTTATGA .GTCATAA.
619  TTAATCGTGC GTTACAAGTA GAATTCTACT CGTAAAGCGA GTT
con  ttgattgtgt tttacaagta gaattctact cgtaaagcga gttc------      .ggttatgag ccgtgtgcaa aacatgacat cagcttatga catcat-c.

hr2

101  TTTACGAGTA GAATTCTACG TGTAAAACAT AATCAAGAGA TGATGTCATT TGTTTTTCAA ( 8) TCAAGAAATG ATGTCATTTG TTTTTCAAAA CTGAACTGGC
209  TTTACGAGTA GAATTCTACT TGTAAAACAC AATCGAGAGA TGATGTCCAT ATTTTGCACA (63) GTCATTGGAT GAGTCATTTG TTTTTCAAAA CTAAACTCGC
371  TTTACGAGTA GAATTCTACT TGTAAAACAC AATCAAGGGA TGATGTCATT ..........      .ATACAAATG ATGTCATTTG TTTTTCAAAA CTAAACTCGC
459  TTTACGGGTA GAATTCTACT TGTAAAACAG CAACTCGAGG GATGATGTCA TCCTTTACTC (14) TGTTATGTAT GACTCATTTG TTTTTCAAAA CTAAACTCGC
575  TTTACGAGTA GAATTCTACT TGTAACGCAC GATCAAGGGA TGATGTCATT TATTTGTGCA (39) TCCAAATAAT GACTCATTTG .TTTTCAAAA CTGAACTCGC
712  TTTACGAGTA GAATTCTACT TGTAAAACAC AATCAAGGGA TGATGTCATT TT........      ...CAAAATG ATGTCATTTG TTTTTCAAAA CTAAACTCGC
802  TTTACGAGTA GAATTCTACT TGTAAAACAC AATCAAGGGA TGATGTCATT TT........      .....AAAAA TGATCATTTG TTTTTCAAAA CTAAACTCGC
889  TTTACGAGTA GAATTCTACG TGTAAAACAC AATCAAGGGA TGATGTCATT T
con  tttacgagta gaattctact tgtaaaacac aatcaaggga tgatgtcatt ttttt.....      ---aaaaaat g-gtcatttg tttttcaaaa ctaaactcgc

hr3

  1  TTTACGCGTA GAATTCTACT TGTAAAGCAA GTTAAAATAA GCCGTGTGCA AAAATGACAT CAGACAAATG (48) CATCAGCTTA TGACTAATAA TTGATCGTGC
146  GTTACAAGTA GAATTCTACT CGTAAAGCGA GTTTAGTTTT GAAAA.CAAA TGAGTCATCA ATTAAACATG (13) ATAAAGGATG ACATCATCCA CTAATCGTGC
257  GTTACAAGTA GAATTCTACT CGTAAAGCGA GTTCGGTTTT GAAAAACAAA TGACATCATT ..........      .......... ........TC TTGATTATGT
329  TTTACAAGTA GAATTCTACT CGTAAAGTAT GTTCAGTTT. AAAAAACAAA TGACATCATT ..........      .......... ..ATCATTTC TTGATTATGT
417  TTTACAAGTA GAATTCTACT CGTAAAGCAA GTTTAGTTTT AAAAAACAAA TGACATCA.. ..........      .......... ......TCTC TTGATTATGT
489  TTTACAAGTA GAATTCTACT CGTAAAGCGA GTTTAGTTTT GAAAAACAAA TGACATCA.. ..........      .......... ......TCTC TTGATTATGT
561  TTTACAAGTA GAATTCTACT CGTAAAGCGA GTTTAGTTTT GAAAAACAAA TGACA..... ..........      .......... ...TCATCCC TTGATCATGC
633  GTTACAAGTA GAATTCTACT CGTAAAGCGA G.TGAATTTT GA
con  tttacaagta gaattctact cgtaaagcga gtttagtttt gaaaaacaaa tgacatca.. ..........      .......... ..atcatctc ttgattatgt

hr4

  1  AACTGGCTTT ACGAGTAGAA TTCTACTTGT AAAACACAAT CAAGAAATGA TGTCATTTTT GTACGTGATT ATAAACATGT TTAAACATGG (115) TCATCGTACA
216  AACTCGCTTT ACGAGTAGAA TTCTACTTGT AAAACACAAT CGAGGGATGA TGTCATTTGT AGAATGATGT CATTTGTTTT TTCAAAACCG       ..........
306  AACTCGCTTT ACGAGTAGAA TTCTACTTGT AAAACACAAT CGAGGGATGA TGTCATTTGT AGAATGATGT CAT....... ..........       ....CGTACA
385  AACTCGCTTT ACGAGTAGAA TTCTAGT.GT AAAACAC
con  aactcgcttt acgagtagaa ttctacttgt aaaacacaat cgagggatga tgtcatttgt agaatgatgt cat....t.t tt.aa.a..g       ....cgtaca

hr5

 69  TTAAAATTGA ACTCGGCTTT ACGAGTAGAA TTCTACGCGT AAAACACAAT CAAGT.ATGA ( 7) .......... .....GCTGA TGTCATGTTT TGCACACGGC
165  TCATAACCGA ACT.GGCTTT ACGAGTAGAA TTCTACTTGT AACGCACGAT CGAGTGGATG (10) GTTTTTCAAA TCGA.GATGA TGTCATGTTT TGCACACGGG
273  CTCATAAACT .....GCTTT ACGAGTAGAA TTCTACGTGT AACGCACGAT CGATTGATGA (11) TTTTGCAATA TGATATCATA CAATATGACT CATTTGTTTT
376  TCAAAACCGA ACTTGA.TTT ACGGGTAGAA TTCTACTCGT AAAGCACAAT CAA.......      .......... ..AAAGATGA TGTCATTTGT TTT.......
449  TCAAAACTGA ACTCGGCTTT ACGAGTAGAA TTCTACGTGT AAAACACAAT CAAGAAATGA ( 8) GTTATAAAAA TAAAAGCTGA TGTCATGTTT TGCACATGGC
559  TCATAACTAA ACTC.GCTTT ACGGGTAGAA TTCTACGCGT AAAACA
con  tcaaaactga actcggcttt acgagtagaa ttctacgtgt aaagcacaat caagtgatga      -tt--.-a-a t.aaagctga tgtcatgttt tgcaca-ggc
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FIG. 3. Alignment of homologous sequences. The nucleotide sequences of the five *hr*s are presented with gaps (.) inserted for optimal alignment of homologous sequences. Some bases from the longer fragments are not shown; the number of bases is indicated in parentheses. *Eco*RI restriction sites are underlined. The numbers on the left refer to the base numbers in Fig. 2. con, Consensus sequence for each region; —, where the consensus can be more than 1 base.

the first and last *Eco*RI sites were also included. The number of homologous bases beyond the *Eco*RI sites was variable in both directions, and there was apparently no sequence common to all 5′ or 3′ ends of the *hr*s.

The percent homology between the consensus sequences for each region and the individual *Eco*RI minifragments was 80% for *hr*1, *hr*2, and *hr*3; 65% for *hr4right*; and 87% for *hr*5 in Table 1. By this calculation, *hr4right* showed the least homology within a single region. This was a reflection of the large difference in size between the 215-bp minifragment and the two smaller fragments.

The homology between the consensus sequences of each region is shown in Fig. 4. The homology of the consensus sequences was 88% when two regions (*hr*1 and *hr*3) were compared in the genome antisense orientation. The homology between the consensus sequences was only 75% (not

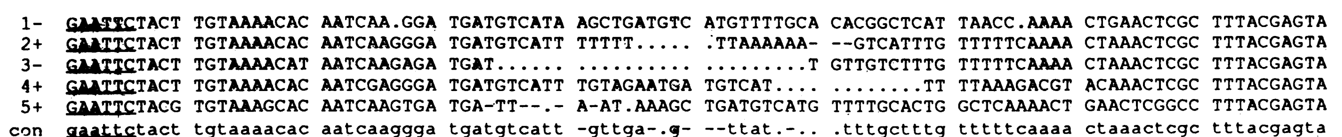

FIG. 4. Alignment of consensus sequences for each *hr*. In some cases the dashes have been changed to the base which best fits the overall consensus. The sequences for *hr*1 and *hr*3 are presented in the opposite (−) orientation as in the standard AcNPV genetic map; the other *hr*s are shown in the standard (+) orientation. ., Gap in the known sequence.

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TABLE 1. Homologous DNA-enhanced expression of 39CAT[a]

Transfected plasmids	CAT activity (pmol/min per 10^6 cells)	Fold stimulation
39CAT	1.2	1
39CAT-hr1$^+$	1,173	978
39CAT-hr1$^-$	2,075	1,729
39CAT-hr2$^+$	1,306	1,088
39CAT-hr2$^-$	2,352	1,960
39CAT-hr3$^+$	347	289
39CAT-hr3$^-$	243	202
39CAT-hr4*left*$^-$	1,622	1,351
39CAT-hr5$^+$	1,272	1,060
39CAT-hr5$^-$	1,259	1,049

[a] *Spodoptera frugiperda* cells were transfected with 1 μg of the indicated plasmids and 0.1 μg of pIE-1. The cells were harvested, and the CAT activity was measured 24 h posttransfection. Cell lysates were diluted so that less than 30% of the input chloramphenicol was acetylated. This experiment was repeated three times.

shown) when all regions were compared in the genome sense orientation. This indicated that there was a polarity to the sequence. However, the function of the AcNPV enhancers is apparently not polar (Table 1) (18). Nonpolar function is a characteristic of enhancers (13).

The 60 bp surrounding the *Eco*RI sites are highly conserved. This conserved region is separated by nonhomologous sequences of variable length between 12 and 155 bp. Both the conserved and nonhomologous sequences of the *hr*s are rich in adenine and thymine. The average A+T content of the *hr*s is 67%, significantly higher than the 58% average A+T content of the viral genome (32).

The most conserved feature of the *hr*s was a 28-bp imperfect inverted repeat (palindrome). The consensus sequences for the five palindromes are shown in Fig. 5. The *Eco*RI sites form the core of this palindrome. In four regions, there is a 1-bp mismatch in the consensus palindrome. In *hr*5, there is a 2-bp mismatch in the consensus sequence and the palindrome is 30 bp. It is interesting that while the sequence of the individual minifragments may differ from the consensus sequence, there is always a mismatch in the same location in every palindrome. There are no instances of a perfect 28-bp palindrome, suggesting a functional significance for the mismatch. Deletion analysis of *hr*5 (18) indicated that the minimal sequence requirement for the enhancer function is one copy of the inverted repeat.

***hr* DNA-enhanced expression of 39CAT.** To determine whether all of the *hr*s enhance delayed-early gene expression, we cloned DNA fragments containing *hr* sequences upstream of the 39K promoter in the plasmid 39CAT. All five

```
             *          *
hr1     TTTACAAGTAGAATTCTACTCGTAAA
        AAATGTTCATCTTAAGATGAGCATTT

             *          *
hr2     TTTACGAGTAGAATTCTACTTGTAAA
        AAATGCTCATCTTAAGATGAACATTT

             *          *
hr3     TTTACAAGTAGAATTCTACTCGTAAA
        AAATGTTCATCTTAAGATGAGCATTT

             *          *
hr4     TTTACGAGTAGAATTCTACTTGTAAA
        AAATGCTCATCTTAAGATGAACATTT

             **         **
hr5    CTTTACGAGTAGAATTCTACGTGTAAAG
       GAAATGCTCATCTTAAGATGCACATTTC
```

FIG. 5. Highly conserved palindromes in *hr* DNA. *, Mismatch.

regions enhanced CAT activity, although to various degrees (Table 1).

*hr*1 and *hr*2 consistently showed a polar effect of orientation. It is possible that this was due to the presence of other promoters adjacent to the *hr* sequences. A polar effect was also observed when *Hin*dIII-Q was cloned upstream of 39CAT (18). However, this polarity was not evident with the *Mlu*I fragment containing the *hr*5 enhancer.

The enhancement seen with *hr*3 was significantly lower than that observed with the other enhancers. This result was unexpected, as the sequence for *hr*3 was not significantly different from that of the other regions, except for the large number of small *Eco*RI fragments. It seems unlikely that this would account for the poor level of enhancement, as there were three larger fragments and, as discussed below, a single fragment is sufficient for the enhancer function.

The results with *hr*4*left* indicated that a single *Eco*RI minifragment stimulates as efficiently as regions containing multiple repeats. This indicates that the AcNPV *hr* enhancers are similar to other enhancers (11, 13, 36) which contain repeated sequences, although the repeats are not essential for enhancer activity.

The AcNPV enhancers differ significantly from the enhancers of the vertebrate DNA viruses with respect to their physical location in the viral genome. In the vertebrate viruses, the enhancers are located within a few hundred base pairs of major immediate-early genes (2, 4, 8, 16, 19, 22, 24, 35). Apparently the function of these enhancers is to *cis* activate the transcription of regulatory genes, which in turn *trans* activate delayed-early transcription. However, in AcNPV the enhancers are interspersed throughout the viral genome and are located at least 3 kilobase pairs from the immediate-early regulatory gene, IE-1. The transcription of IE-1 was not affected by linked *hr*5 sequences, whereas the expression of a delayed-early gene was enhanced 1,000-fold.

It has been hypothesized that the AcNPV *hr*s serve as origins of DNA replication (6). In simian virus and polyomaviruses, the enhancer sequences also function as the origin of replication (3, 31). It will be of interest to determine whether the AcNPV enhancers are also bifunctional.

ACKNOWLEDGMENTS

We thank David Baker for excellent technical assistance.

This research was supported by grant DNB-8510270 from the National Science Foundation and a grant from the Texas Agricultural Experiment Station.

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