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An Insertion of Insect Cell DNA in the 81-Map-Unit Segment of *Autographa californica* Nuclear Polyhedrosis Virus DNA

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In this report, a transposonlike insertion of *Spodoptera frugiperda* insect cell DNA was analyzed in single-plaque isolate E of the insect baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV). The 634-base-pair insertion is characterized by an 18-base-pair terminal inverted repeat and carries an *Eco*RI site. This additional *Eco*RI site in the 81-map-unit segment of the DNA of plaque isolate E of AcNPV explains the differences between the *Eco*RI restriction map of the DNA from this isolate and those of the virus stocks used in other laboratories. Except for this insertion, the nucleotide sequence at the site of insertion in the DNA of plaque isolate E is identical to that of AcNPV E2 (G. E. Smith and M. D. Summers, Virology 89:517–527, 1978). The cellular DNA insertion in the AcNPV genome is represented many times in the *S. frugiperda* cell genome but has no detectable homology with DNAs from species other than lepidopteran insects. In *S. frugiperda* cells, the transposonlike insertion sequences are transcribed into cytoplasmic RNA. The transcription of these sequences is initiated within the cellular insertion element. As reported previously (C. Oellig, B. Happ, T. Müller, and W. Doerfler, J. Virol. 61:3048–3057, 1987), in *S. frugiperda* cells infected with plaque isolate E of AcNPV, at least nine different size classes of AcNPV-specific RNAs are synthesized; in AcNPV E2-infected cells, similar size classes have been detected. The cellular insertion of plaque isolate E provides the initiation site for the synthesis of an additional RNA size class which is transcribed off viral DNA.

The insect baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) has been used as a model to study the molecular biology of this group of viruses and has been developed into a very efficient eucaryotic expression vector system (33). The production of the occluded form of this virus, in which large numbers of virions are incorporated into polyhedra, is dependent on the synthesis of the AcNPV-encoded polypeptide polyhedrin. Nonoccluded, free virions can be synthesized even when the viral polyhedrin gene is nonfunctional (31). Upon continued serial, undiluted passage of AcNPV, fewer polyhedra per cell can be produced (13, 14, 18, 23, 26, 27). The DNAs of some of these fewer-polyhedra-producing AcNPV variants have been shown to contain insertions of host cell DNA in different segments of the viral genome (2, 3, 7, 12, 14, 15, 24). At the sites of insect cell DNA insertion into the viral genome, the sequence TTAA has frequently been observed to be duplicated (2, 7, 12). In one instance, the insertion has characteristics of a copialike transposable element (24). In two other examples, RNA initiation sites have been mapped inside the cellular insertion (2, 3, 15). Recently, the transcriptional initiation for a gene encoding a 25-kilodalton protein, often absent from fewer polyhedra-producing mutant AcNPV-infected cells (3) but present in cells infected with other strains of AcNPV, has been mapped in the region of a host cell DNA insertion (2, 3).

For nuclear-DNA viruses having constant encounters with cellular DNA and the nuclear DNA recombination machinery, recombinations between viral and cellular DNAs are likely to be frequent events. There are numerous well-documented examples of viral and cellular recombinants in bacteriophage λ, simian virus 40, and adenovirus type 12 (1, 5, 6, 9, 19). The cellular DNA sequences in these recombinants do not constitute transposonlike elements.

In previous work from our laboratory, the nucleotide sequence, the transcriptional patterns, and the potential open reading frames in the 81.2- to 85.0-map-unit region of the DNA from plaque isolate E of AcNPV have been determined (25). The organization in the corresponding segment and in other regions of the genome of the baculovirus *Orgyia pseudotsugata* nuclear polyhedrosis virus (OpNPV) has been found to be very similar to the AcNPV genome (16, 17). This conservation of genome organization has also been documented for other parts of the baculovirus genome (20, 28, 29). Judging from the nucleotide sequence in this segment of the OpNPV genome (16), it appears likely that a nucleotide sequence of 634 base pairs (bp) that is absent from the OpNPV genome has been inserted in the genome of plaque isolate E of AcNPV. This 634-bp sequence is flanked by a terminal repeat of 18 bp (Fig. 2 in reference 25) and hence carries a structure that is frequently found in transposable elements. It will be shown in this report that the presumptive transposable insert in the 81-map-unit segment of this AcNPV DNA has extensive sequence homologies with the DNA of *Spodoptera frugiperda* cells which have been used as hosts for the propagation of AcNPV. This cellular DNA sequence is transcribed in *S. frugiperda* cells. The 634-bp sequence in plaque isolate E of AcNPV apparently represents a cellular transposonlike insert. This insert contains an additional *Eco*RI restriction site which accounts for the different *Eco*RI restriction map of the DNA in plaque isolate E of AcNPV (21, 22, 25, 37) in comparison, e.g., with that of the DNA of the E2 isolate of AcNPV (32, 34). The viral nucleotide sequence flanking the host DNA insert in plaque isolate E of AcNPV has been shown to be identical to

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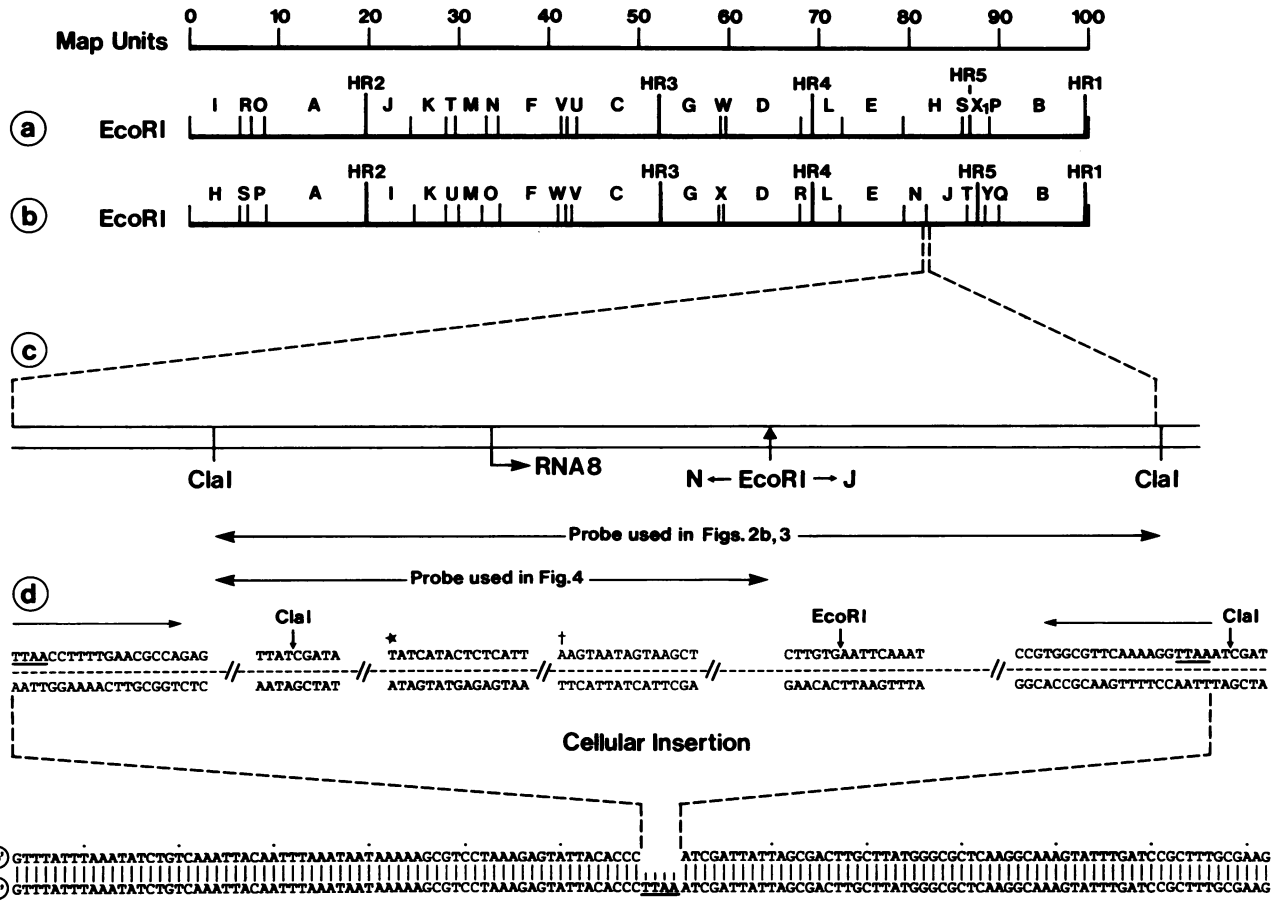

FIG. 1. Restriction maps of DNA of E2 (a) and E (b) isolates of AcNPV, part of the nucleotide sequence of the presumptive insertion sequence in DNA of plaque isolate E of AcNPV and nucleotide sequence comparison in the region flanking the host DNA insertion between isolates E2 (a') and E (b') of AcNPV DNA. The *Eco*RI restriction maps of plaque isolates E (21, 22, 37) and E2 (32, 34, 38) of AcNPV were compared. The restriction map of the *Eco*RI N-*Eco*RI J fragment transition in the DNA of plaque isolate E is enlarged in panel c. The nucleotide sequence in panel d represents parts of the presumptive insert sequence in plaque isolate E of AcNPV DNA (25). The inverted terminal repetition (horizontal arrows in panel d) and the additional *Eco*RI site are also indicated (c and d). Symbols: ↳ and †, site of transcription initiation of RNA 8 (25) in infected *S. frugiperda* cells; ★, site of transcription initiation of a cellular RNA in uninfected cells. The dagger and star symbols designate the block but not the exact nucleotide of transcription initiation. The nucleotide sequence in panel a' was determined in the *Xho*I I fragment of the DNA from isolate E2 by using the double-stranded pIBI24 clone (courtesy L. Guarino, College Station, Tex.) as the template and a synthetic oligodeoxynucleotide of 23 nucleotides as the primer. Its nucleotide sequence was that of positions 11 to 33 of the sequence published in Fig. 2 of reference 25. A bacteriophage T7 sequencing kit (Pharmacia LKB) with [α-^{35}S]dATP was used, and the dideoxy sequencing method (30) was employed. DNA fragments were separated on a 7% polyacrylamide gel containing 8 M urea. After electrophoresis, the gel was immersed in 10% acetic acid for 20 min, dried at 80°C, and autoradiographed.

the corresponding nucleotide sequence in the E2 strain of AcNPV.

Details of the propagation of *S. frugiperda* cells, the isolation of plaque isolate E of AcNPV, the multiplication and purification of the isolate, and the isolation of its DNA were previously reported (8, 37). The results of the structural analyses of the pBR322-cloned *Eco*RI J and N fragments and of the corresponding subclones were detailed elsewhere (25). Standard methods of molecular biology, the preparation of cellular DNAs (36), Southern blot hybridization (35), oligolabeling of DNA (11), DNA-DNA and DNA-RNA hybridization experiments under stringent conditions (39), and nuclease S1 analysis of RNA (4, 25) were reported earlier and will not be described here.

The nucleotide sequence comparison of the 81-map-unit segment of AcNPV DNA (25) and the corresponding segment in the genome of the closely related baculovirus OpNPV (16) revealed that the two viral genomes had similar sequences in that segment, except for an insertion of 634 nucleotide pairs in the genome of plaque isolate E of AcNPV (Fig. 1). The insert in the AcNPV genome was characterized by a perfect terminal inverted repeat of 18 nucleotide pairs with the sequence 5'-TTAACCTTTTGAACGCCA-3' (Fig. 1d). The element 5'-TTAA-3' in this sequence was of viral origin and was previously observed at the junctions between viral DNA and other transposonlike insertions (2, 3, 7, 12). The presence of this repeat sequence rendered it likely that the inserted sequence constituted a transposon-type insertion. It was also important to note that this insertion sequence carried an *Eco*RI site (Fig. 1b to d) which represented the demarcation between the *Eco*RI N and J fragments in the restriction map (Fig. 1b and c) of plaque isolate E of AcNPV (21, 22). This restriction map differed from that of the E2 isolate of AcNPV (32, 34, 38) by exactly this one additional *Eco*RI site (Fig. 1a to d).

These interpretations were made by determining the viral

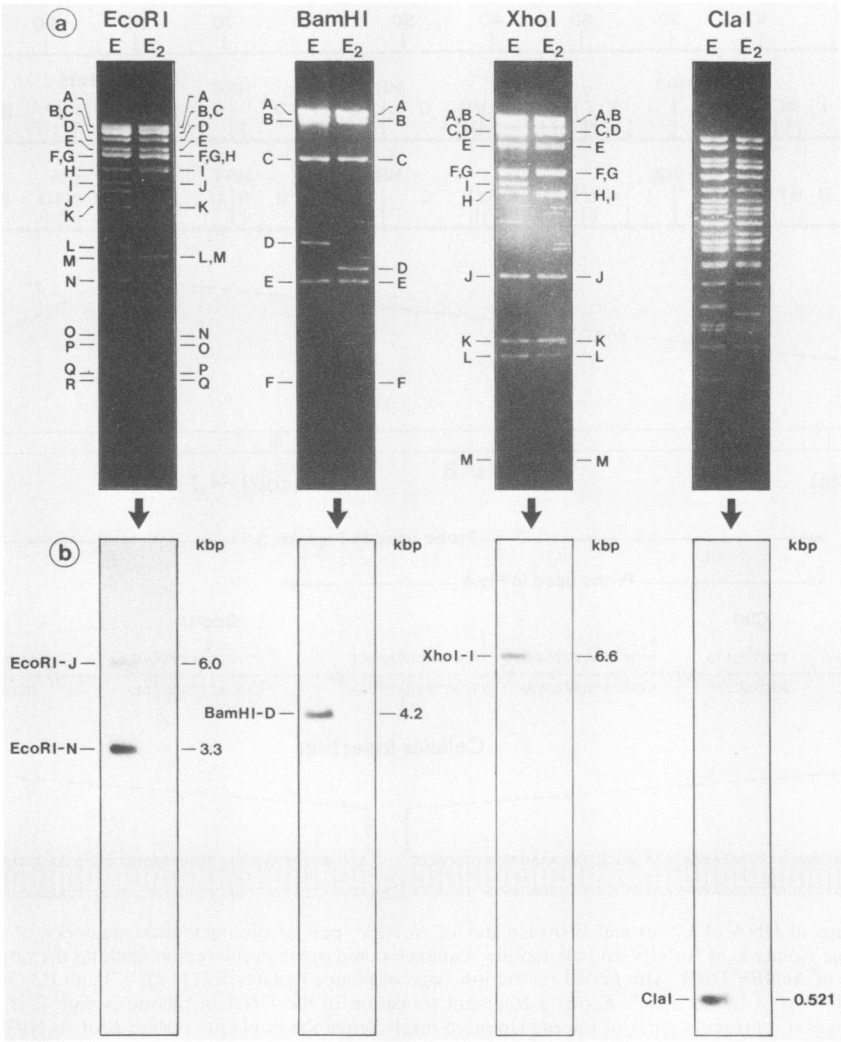

FIG. 2. Comparison of different restriction patterns of DNAs from plaque isolates E (21, 22, 37) and E2 (32, 34, 38) of AcNPV. DNA from AcNPV isolate E or E2 was cleaved with restriction endonuclease *Eco*RI, *Bam*HI, *Xho*I, or *Cla*I as indicated. The fragments were separated by electrophoresis on a 0.8% agarose gel. DNA fragments were ethidium bromide stained, and photographs with UV light were made (a). Subsequently, the DNA fragments were transferred by Southern (35) blotting to a nitrocellulose filter, and the insert-containing fragments were visualized by hybridization to the ^{32}P-labeled (11), 521-nucleotide *Cla*I fragment (Fig. 1c and d) and then autoradiographed (b). Designations of lanes in panel b correspond to those in panel a. Individual restriction fragments (a and b) and their sizes (b) in kilobase pairs (kbp) are indicated in the left and right margins. In panel a, the order of the *Xho*I H and I fragments is reversed for the DNA of plaque isolate E. The *Xho*I I fragment of this isolate contains the 634-nucleotide-pair insert, and as a consequence, its size exceeds that of the *Xho*I H fragment in the isolate E DNA.

nucleotide sequences in the two regions flanking the cellular-DNA insert of 634 nucleotide pairs in the DNA of isolate E2 of AcNPV (Fig. 1a′). The results of this analysis unequivocally show that the nucleotide sequence in this region of plaque isolate E DNA (25) (Fig. 1b′) is identical to that in the corresponding segment in the E2 isolate of AcNPV (Fig. 1a′). Thus, except for the cellular DNA insertion, the DNA sequence in this segment of viral DNA was not altered by the insertional recombination event.

Conventional restriction endonuclease analyses were performed with the DNAs of plaque isolates E and E2 (32, 34, 38) of AcNPV by using the restriction endonucleases *Eco*RI, *Bam*HI, *Xho*I, and *Cla*I (Fig. 2a). A comparison of the different restriction patterns of the DNAs from the two subtypes of AcNPV, E and E2, revealed that the 634-nucleotide insertion in the 81-map-unit segment of the DNA from plaque isolate E altered the sizes of the restriction endonuclease fragments which were located in that segment. Since the inserted DNA sequence contained an additional *Eco*RI recognition site (Fig. 1b to d), cleavage with this enzyme generated *Eco*RI J and N fragments in plaque isolate E DNA (Fig. 2a). Similarly, cleavage of isolate E DNA with the restriction endonucleases *Bam*HI and *Xho*I created *Bam*HI D and *Xho*I I fragments with lengths augmented in comparison with those of the corresponding fragments from isolate E2 DNA (Fig. 2a). The DNA fragments with altered lengths in the plaque isolate E genome had homology with the cellular DNA insert, as was shown by Southern blotting (35) of the DNA fragments (Fig. 2) and by hybridization (39) to the *Cla*I fragment straddling the *Eco*RI-J–*Eco*RI-N border in the DNA from plaque isolate E (Fig. 1). This *Cla*I fragment contained most of the 634-bp insert (Fig. 1d). The

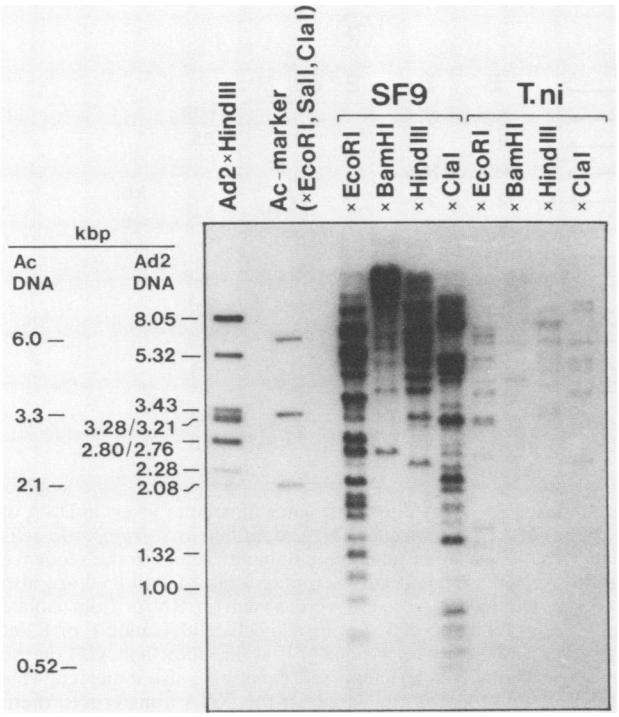

FIG. 3. Homology of *Cla*I fragment of cellular insert in DNA of plaque isolate E of AcNPV with sequences in DNA of *S. frugiperda* cells. DNAs from the sources indicated were cut with restriction endonucleases as indicated. Abbreviations: Ad2, adenovirus type 2; Ac marker, plaque isolate E of AcNPV; SF9, *S. frugiperda* cells (ATCC 1711-CRL; American Type Culture Collection); T. ni, *Trichoplusia ni* cells. Fragments were separated by electrophoresis on a 0.9% agarose gel and transferred to a nitrocellulose membrane (35), and the insert-specific DNA fragments were visualized by hybridization (39) to the isolated, gel-purified, ^{32}P-labeled (11) *Cla*I insert fragment (Fig. 1c and d) and then autoradiographed. The leftmost filter strip was cut off, and the DNA was hybridized to ^{32}P-labeled adenovirus type 2 DNA under similarly stringent conditions. After the filters were washed and dried, this strip was fitted exactly back to the main part of the filter for autoradiography. Sizes of AcNPV and adenovirus type 2 DNA fragments are designated in kilobase pairs (kbp) on the left.

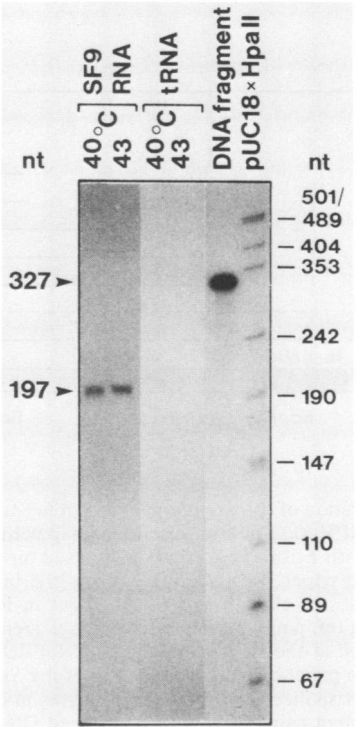

FIG. 4. Cellular insert DNA transcribed in uninfected *S. frugiperda* cells. The sources of RNAs analyzed in protection experiments or control DNA fragments were as indicated. Abbreviations: SF9 RNA, cytoplasmic RNA from *S. frugiperda* cells; tRNA, yeast tRNA; DNA fragment, *Cla*I-*Eco*RI fragment of the plaque isolate E of AcNPV insert (Fig. 1c); nt, nucleotide. Most experimental conditions are described in the text. The DNA probe was 5′ labeled at the *Eco*RI site and excised with *Hin*dIII from a pUC19 clone which contained the *Cla*I-*Eco*RI fragment (Fig. 1c). DNA-RNA hybridizations were performed at 40 or 43°C under previously published conditions (25). The hybrids were treated with 150 U of S1 nuclease (Boehringer GmbH) at 20°C for 45 min. Protected fragments, the ^{32}P-labeled, denatured *Cla*I-*Eco*RI fragment probe, and the denatured, 5′-terminal ^{32}P-labeled *Hpa*II fragments of pUC18 DNA (which were used as molecular weight markers) were coelectrophoresed on 5% polyacrylamide gels in 8 M urea–0.1 M Tris hydrochloride–0.0025 M EDTA–0.077 M sodium borate. After electrophoresis, the gel was dried and autoradiographed on XAR5 film for 2 days. Yeast tRNA used as a control did not yield any signals.

*Eco*RI J, *Eco*RI N, *Bam*HI D, and *Xho*I I fragments all hybridized with the insert fragment (Fig. 2b). Cleavage of the DNA from plaque isolate E with *Cla*I liberated a 521-nucleotide-pair fragment that carried the insert (Fig. 2b). The *Cla*I restriction pattern (Fig. 2a) was not analyzed further, since the *Cla*I fragments had not been mapped. It was also shown that the DNA of isolate E2 did not contain the 634-nucleotide-pair insertion (Fig. 2b).

Was the AcNPV DNA insertion in the 81-map-unit segment of cellular origin? As was apparent from the restriction map and nucleotide sequence data (25) (Fig. 1c and d and 2b), a major part of the presumptive insertion sequence could be excised from a pUC18-derived subclone by *Cla*I. The *Cla*I DNA fragment was purified by three cycles of electrophoresis on agarose gels (10). The DNA was eluted from the gel and ^{32}P labeled by the oligolabeling procedure (11). DNAs from *S. frugiperda* cells, the usual laboratory host for AcNPV propagation, or from *Trichoplusia ni* cells were cleaved with different restriction endonucleases (Fig. 3), blotted, and hybridized to the ^{32}P-labeled insert as the probe. A mixture of AcNPV DNA fragments generated by *Eco*RI, *Sal*I, or *Cla*I was coelectrophoresed as the size marker. The hybridization data, obtained under stringent conditions (39), demonstrated extensive sequence homologies between the *Cla*I fragment of the 634-nucleotide-pair insert in AcNPV DNA and *S. frugiperda* DNA and much weaker homologies with *T. ni* DNA. A multitude of cellular DNA fragments hybridized to the transposonlike insertion from the DNA of plaque isolate E of AcNPV. Thus, the insertion sequence was represented in *S. frugiperda* DNA in many copies and possibly constituted middle repetitive cellular DNA. Moreover, it was likely that the transposonlike sequence had been derived from *S. frugiperda* cells and might have been acquired by plaque isolate E of AcNPV during propagation in *S. frugiperda* cells. The weak homology with *T. ni* DNA likely reflected the relatedness of DNA sequences between the two lepidopteran species. In further control experiments, the insert DNA did not hybridize under stringent conditions (i.e., in 50% formamide and at 42°C [39]) with the DNAs of *Escherichia coli*, *Saccharomyces cerevi-*

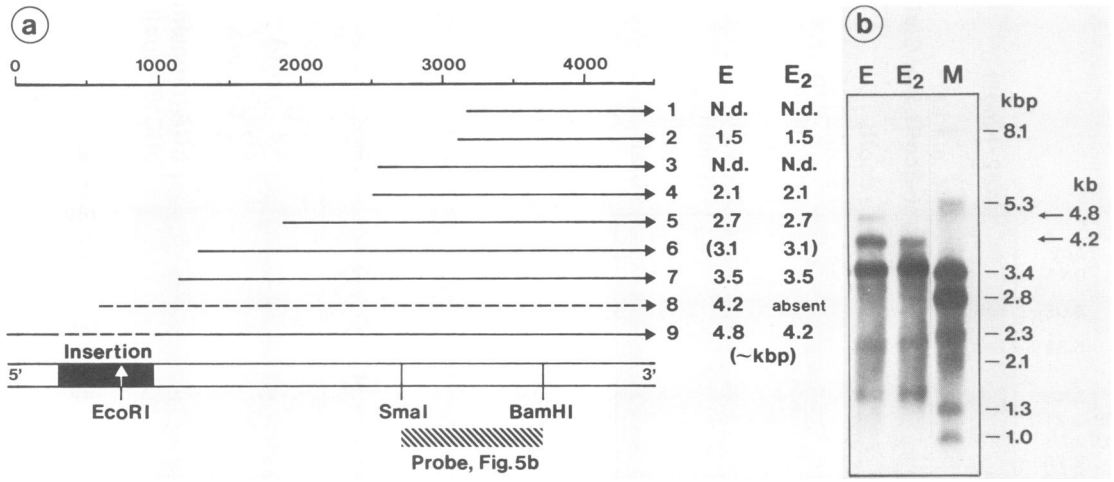

FIG. 5. Alteration of transcriptional pattern in 81.2- to 85.0-map-unit region in AcNPV DNA due to presence of cellular insert in DNA of isolate E of AcNPV. (a) The transcriptional map with the nine different size classes of AcNPV-specific RNAs identified in *S. frugiperda* cells after infection with E isolate of AcNPV is based on previously published data (25). The scale of nucleotide numbers refers to the sequence published in that paper. Restriction sites and the inserted cellular DNA in the AcNPV segment between map units 81.2 to 85.0 are also presented. RNA size class 8 and the segment in RNA 9 corresponding to the cellular-DNA insert were absent in RNAs from isolate E2-infected cells (cf. panel b). RNA size classes identified in the cytoplasm 24 h after infection of *S. frugiperda* cells with isolate E or E2 of AcNPV are listed at the right margin. N.d., Not detected. RNA size classes 1, 3, and 5 were very poorly, if at all, detectable (25) on the autoradiogram in panel b. The map location of the *Sma*I-*Bam*HI probe for the hybridization experiments (cf. panel b) is also indicated. This probe fragment visualizes all 9 RNA size classes in cells infected with isolate E of AcNPV. (b) The details of this RNA transfer (Northern blotting) experiment using the above-mentioned DNA fragment as the hybridization probe are described in the text. Lanes: E, RNA from isolate E-infected *S. frugiperda* cells; E_2, RNA from isolate E2-infected *S. frugiperda* cells (for details, see text); M, the marker of alkali-denatured *Hin*dIII fragments of adenovirus type 2 DNA. An autoradiogram is shown. RNA size classes 8 and 9 (about 4.2 and 4.8 kb, respectively) are indicated. kbp, Kilobase pairs.

siae, *Zea mays* L., *Drosophila* spp., *Ephestia* spp., salmon sperm, quail, hamster BHK21 cells, or human HeLa cells (data not shown).

We also tested whether DNA sequences corresponding to the cellular insert in plaque isolate E of AcNPV were transcribed in uninfected *S. frugiperda* cells growing in culture. The total cytoplasmic RNA was isolated from these cells. The RNA was hybridized to the denatured 327-nucleotide S1 probe comprising the *Cla*I-*Eco*RI fragment (Fig. 1c) of the 634-bp insert. The hybrids were treated with S1 nuclease, and the protected fragments were analyzed by gel electrophoresis. The results (Fig. 4) demonstrated that a 197-nucleotide DNA fragment was protected by the RNA from *S. frugiperda* cells but not by yeast tRNA. This finding indicated that RNA transcription was initiated inside the insertion sequence, and this site (Fig. 1d) corresponded to a position about 50 nucleotides 5′ from the transcription start site of RNA 8 (25) in the *Eco*RI N fragment of AcNPV DNA. The cytoplasmic RNA from AcNPV plaque isolate E-infected *S. frugiperda* cells (24 h postinfection) was also analyzed. In addition to the expected protected fragments which indicated the site of initiation of RNA 8 (Fig. 1d) and the presence of RNA 9 which spanned this segment (25), a weak 197-nucleotide signal was also observed (data not shown). This result indicates that the initiation sites in both the cellular insert in plaque isolate E DNA and the corresponding segments of cellular DNA were utilized in *S. frugiperda* cells which had been infected with plaque isolate E of AcNPV.

It was conceivable that the spectrum of RNA size classes with homologies to the 81.2- to 85.0-map-unit segment of plaque isolate E DNA was influenced by the presence of the cellular insert in this DNA and by the availability of a new cellular initiation site for transcription inside this cellular insert. *S. frugiperda* cells were infected with the E or E2 isolate of AcNPV. At 24 h after infection, the total cytoplasmic RNA was isolated and the polyadenylated sequences were selected, size fractionated by electrophoresis on 0.8% agarose gels containing 2.2 M formaldehyde, and transferred to a nitrocellulose filter by Northern (RNA) blotting. Technical details were as described elsewhere (25). The *Sma*I-*Bam*HI fragment illustrated in Fig. 5a was used as the ^{32}P-labeled (11) hybridization probe. The results of the hybridization analyses of the RNAs from isolate E-infected cells revealed size classes almost identical to those previously described (25) (Fig. 5). The scheme in Fig. 5a suggests that the altered size class pattern of AcNPV-specific RNAs transcribed from this viral DNA segment was due to the presence of the cellular DNA insert in DNA from plaque E isolate. This insert carried an initiation site of transcription which gave rise to the 4.2-kilobase (kb) RNA size class 8 and simultaneously augmented the length of RNA size class 9 from 4.2 to 4.8 kb (Fig. 5b). In RNA from isolate E-infected cells, RNA size classes 9 (4.8 kb) and 8 (4.2 kb) were identified, whereas RNA from isolate E2-infected cells contained a shorter size class 9 RNA of only about 4.2 kb (Fig. 5b). The patterns of RNA size classes 1 to 7 seemed to be identical in RNAs from *S. frugiperda* cells infected with isolate E or E2.

The data presented here are consistent with the notion that the genome of AcNPV plaque isolate E (37) had incorporated a transposonlike segment of *S. frugiperda* cell DNA into the 81-map-unit segment of its genome. The presence of a 4-bp duplication at the site of insertion of cellular DNA and of an 18-bp perfect inverted repeat in the insert supports the interpretation of a transposonlike element. There is, how-

ever, no direct proof for this contention. The insect DNA apparently contains this cellular insert DNA sequence in multiple copies. The additional *Eco*RI site in the inserted DNA segment explains the *Eco*RI map of the DNA in plaque isolate E of AcNPV. Isolate E2 of AcNPV (32, 34, 38) did not carry this insertion in its DNA. In uninfected and AcNPV plaque isolate E-infected *S. frugiperda* cells, the insert DNA was transcribed into cytoplasmic RNA. Infection of *S. frugiperda* cells with the E isolate of AcNPV generated an additional viral RNA size class which was absent from isolate E2-infected cells and was initiated inside the cellular insert in the DNA of plaque isolate E.

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