DNA sequence analysis *Plutella xylostella* granulovirus

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A *Plutella xylostella* granulovirus (PxGV) was used for the determination of the entire nucleotide sequence of the virus genome. The size of the PxGV genome was 100,999 base pairs (bp). One hundred and twenty ORFs of 50 amino acids or more were found. Among these ORFs, 57 ORFs were common to the five baculoviruses. Gene parity plot analysis of the ORFs on the genome between PxGV and four NPVs indicated that 19 PxGV ORFs located to 48.5 to 83.4 m.u. showed a very similar alignment. Four highly conserved homologous regions consisting of a repeat sequence were found.

Introduction

PxGV has a relatively small size genome, which suggests that the genetic information packed into this genome may be essential and evolutionarily fundamental for baculoviruses. The gene content of PxGV may tell us about the genetic information common to both the nucleopolyhedroviruses and granuloviruses, and also about genes that are specific to granuloviruses. Interest in using PxGV as a reference virus within the GV group and in the baculovirus family generally, and also as a virus pesticide, was strong enough to encourage us to undertake this sequencing project.

Materials and methods

The PxGV isolate we started with originated in cabbage fields in the Shiga Prefecture in Japan, which Dr. Kondo, a former student of Dr. Maeda now working in Shiga Agricultural Experiment Station, collected from diamondback moth larvae infected with granulosis. We cloned PxGV by serial passage through diamondback moth larvae, checking its genetic purity by restriction endonuclease analysis of virus DNA. We established a plasmid library of PxGV DNA fragments, which overlapped on the virus genome map. For sequencing plasmid DNA, we used conventional methods. Once we obtained a contiguous DNA sequence, the data were analyzed by the Genetyx-Win programs for detection of ORFs, determination of identity and homology among ORFs, sequence alignment and phylogenetic tree construction for multiple ORFs, and determining the AT content of DNA fragments. Homologous gene product searches, using PxGV ORFs with a minimum size of 50 amino acids, were done by BLASTP protocols using the SwissProt database. Gene Parity Plot analysis using a method from Dr. Vlak's group was also employed for aligning PxGV ORFs with their homologues in five individual baculovirus genomes.[1)]

Results and discussion

The genome has a size of 100,999 base pairs, and its AT content is 59 %. We found 120 ORFs of 50 or more amino acids, and four homologous regions were present on the genome. PxGV has the smallest genome among the baculovirues, which have been completely sequenced so far. The AT content of PxGV DNA is identical to that of AcMNPV,[2)] and much higher than those of two other NPVs.[3,4)] When we calculated the percentage of the genome occupied by ORF coding regions, homologous regions, and intergenic regions, we found that PxGV homologous regions accounted for a high percentage.

Genetic information in the first 50 map units includes 64 ORFs located in different reading frames and on both DNA strands (Fig. 1(A)). Known genes with designated names in other baculoviruses, which are homologous to PxGV ORFs, are shown in parentheses. AT content curves showed that only the regions containing hr1 and hr2 are sustained at a high level. The AT content in regions between these two hrs appears to be lower than that in other parts of the genome. The other 56 ORFs are also evenly distributed among each reading frame and DNA strand (Fig. 1(B)). Similarly in the first 50 m.u. region, the AT content between these two hrs seems to be lower than it is in other areas.

Functionally defined genes that are present or absent in PxGV and three NPVs were examined.[2-4)] For PxGV ORFs associated with virus late gene expression, and with virus DNA replication and repair, twenty-one genes with these functions previously found in three NPVs, PxGV possesses only 13. For four groups of the functionally defined genes such as very late expression, inhibition of apoptosis, modulation of insect growth and development, and viral enzymes, in PxGV, homologues for many viral enzymes in the last two of these categories are absent. For another four groups, such as specifying occlusion body production and structure, budded virus protein, and nucleocapsid protein, in contrast to the previous gene groups, PxGV possesses homologues to most of the genes previously identified in three NPVs, suggesting that baculovirus genes encoding structural components may be more highly conserved than the other genes.

When the alignment and orientation of the ORFs on the genome was compared between PxGV and four NPVs AcMNPV, OpMNPV, LdMNPV and BusuNPV, and also between PxGV and XcGV, using gene parity plot analysis, 19 PxGV ORFs located from 48.5 to 83.4 m.u. showed a very similar alignment to those of AcMNPV, OpMNPV and LdMNPV.[1)]

Immediate early gene 1 is the key gene for initiation of virus gene expression during baculovirus replication. Under the conditions of homologue searching that we have used, there was no PxGV ORF showing homology to *ie1* from any other

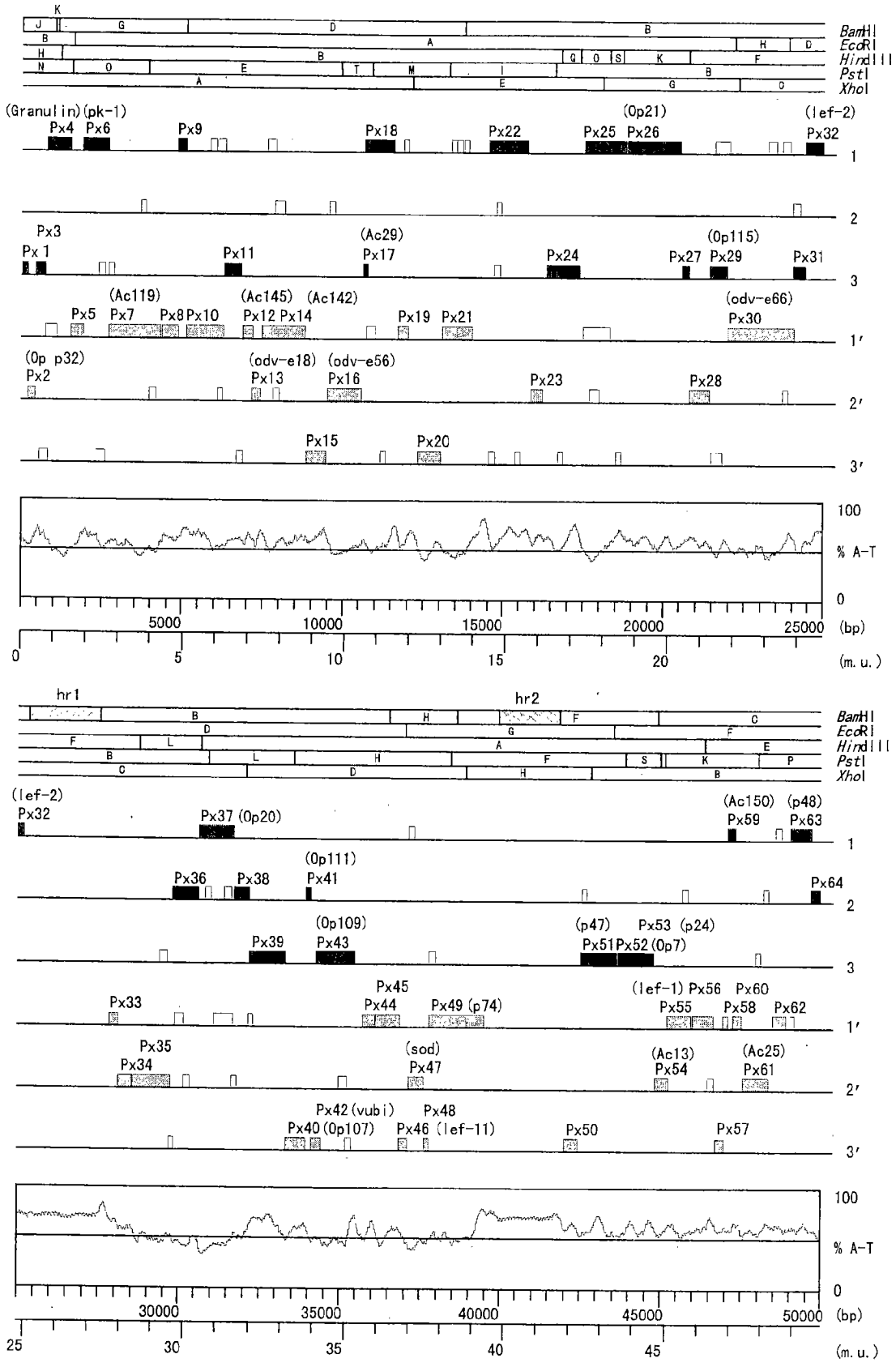

Fig. 1(A). Physical map, ORF organization and AT content curve of first 50 map units region of PxGV genome.

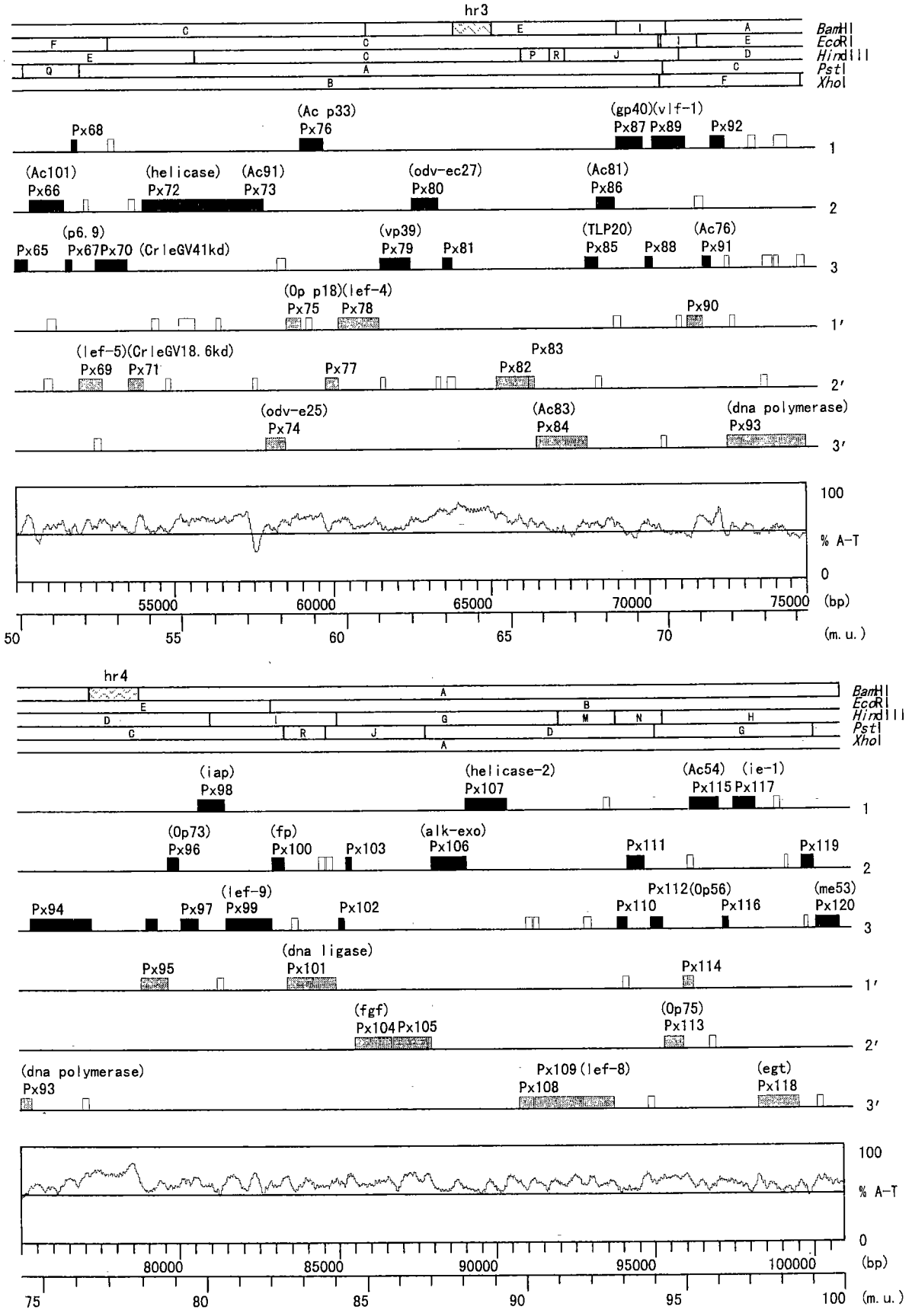

Fig. 1(B). Physical map, ORF organization and AT content curve of second 50 map units region of PxGV genome.

baculovirus. However, at the lower probability score of 0.21, PxGV ORF117 showed homology to AcMNPV *ie1*. PxGV ORF117 encodes a 249 amino acid residue polypeptide, which is much smaller than the size of 568 amino acid residues for AcMNPV *ie1*. AcMNPV *ie1* has three functionally defined domains: a transcription activation region, a DNA binding region and an oligomerization region. The region of homology shown here corresponds to a part of the DNA binding region in AcMNPV IE-1. We cannot say yet whether the PxGV ORF117 gene product performs the same role as AcMNPV IE-1.

PxGV has 4 hrs and they have high AT contents and a highly conserved repeat unit with a size of 105 base pairs. Hr1 and hr2 consist of 22 and 23 repeats, respectively, and have similar sizes. Similarly, hr3 and hr4 each consist of 10 repeats and have similar overall sizes. The distance between hr1 and hr2 and their inverted orientations are comparable in the case of hr3 and hr4. The AT contents of the regions between these two sets of hrs are lower than those of the regions between hr4 and hr1 and between hr2 and hr3. The sizes of consensus DNA repeat sequences in hr1 to 4 are 105 base pairs, identical to each other. The orientation of repeats in hr1 and hr3 is opposite to that in hr2 and hr4. These properties may be important for aspects such as packaging or replication mechanisms.

To see whether PxGV hrs may act as autonomously replicating sequences, plasmids containing PxGV hrs were transfected into Sf9 cells, which had previously been infected with AcMNPV as a helper virus or co-transfected with PxGV DNA.[5] In this assay, replicated plasmid DNA molecules are confirmed by the presence of unmethylated *DpnI* recognition sequence.[6] The results show that none of the PxGV hr-containing plasmid DNA replicated in Sf9 cells with the aid of an AcMNPV replication system. This suggests either that PxGV hrs do not act as origins of DNA replication, or that expression of PxGV genes necessary for DNA replication did not occur in Sf9 cells.

Analysis of the PxGV DNA genome sequence provides a lot of genetic information about this virus, which is distantly related to previously described NPVs whose genomes have also been sequenced and analyzed. Gene homologues detected in PxGV, and a highly conserved hr structure, are major features of this virus, and may indicate genetic properties common among baculoviruses that are important for investigating baculovirus evolution. DNA sequences of viruses closely related to PxGV should be helpful in future towards establishing the genetic divergence between this GV and the NPVs.

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