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Negative regulatory regions of the PAT1 promoter of Hz-1 virus contain GATA elements which associate with cellular factors and regulate promoter activity

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The persistence-associated transcript 1 (PAT1) is actively expressed during persistent infection with Hz-1 virus, while transcription of the rest of the viral genes is shut down. Previously, results of a series deletion of the PAT1 promoter suggested that the regions from nucleotides −312 to −212 and nucleotides −158 to −90 negatively regulate the promoter activity. Here, the negative regulatory effect of the −312/−90 fragment was confirmed using a heterologous IE0 promoter of Autographa californica multiple nucleopolyhedrovirus. Further, the negative regulation of the −312 to −212 region was orientation-independent. The results of electrophoresis mobility shift assays showed that cellular protein(s) bind specifically to DNA fragments −312/−212 and −158/−90. In each of these fragments, a GATA element was identified by computer-assisted analysis. Mutating both GATA elements in the −312/−90 fragment completely eliminated its negative effect on IE0 promoter activity, while mutating only one of these elements had little or no effect. Together, these results suggest that the GATA element has a negative regulatory role on the IE0 and PAT1 promoters.

Introduction

Hz-1 virus is a rod-shaped virus containing a circular double-stranded DNA genome of 228 kb (Huang *et al.*, 1982; Chao *et al.*, 1990). It was identified as a persistent virus infection in the *Heliothis zea* cell line IMC-Hz-1 (Granados *et al.*, 1978; McIntosh & Ignoffo, 1981; Ralston, 1981). Originally, Hz-1 virus was classified as a member of the *Nudibaculoviridae*, but it is currently an unclassified invertebrate virus as it is not able to produce occlusion bodies (Volkman *et al.*, 1995).

Hz-1 virus was the first invertebrate virus to be studied for temporal gene expression during productive and persistent virus infections (Chao *et al.*, 1992). It replicates in the nuclei of infected cells and produces numerous virions during productive infection in most cells. Persistent infection is established in only a very small proportion (0·01–0·05 %) of infected cells (Granados & Williams, 1986; Lin *et al.*, 1999). During a productive infection, Hz-1 virus produces more than 100 different transcripts, but only one transcript is predominantly detected in a persistent infection, the persistence-associated transcript 1 (PAT1) (Chao *et al.*, 1992). PAT1 is also expressed as early as 2 h post-infection (p.i.) in productive infection, and its expression level remains constant up to 12 h p.i. (Chao *et al.*, 1992). It has been suggested that PAT1 is involved in the establishment of Hz-1 virus persistence (Chao *et al.*, 1998).

Previous studies showed that PAT1 is a transcript with several unique features (Chao *et al.*, 1998). Sequence analysis of PAT1 reveals abundant direct and inverted repeats. No significant open reading frames (ORFs) can be detected in any reading frames. The lack of ORFs in the PAT1 sequence was confirmed by the fact that it is not associated with polysomes (Chao *et al.*, 1998). Furthermore, PAT1 is a nuclear RNA, as was concluded from subcellular fractionation and *in situ* hybridization (Chao *et al.*, 1998). The fact that the promoter sequence of PAT1 is adjacent to its transcription start site argues that PAT1 is not a spliced intron of a large transcript. In addition, the CAGT motif, an initiator element for early transcription of the baculovirus *ie-1* gene (Pullen & Friesen, 1995), is located at nucleotide (nt) +3 to +6 of the PAT1 transcript. All the abovementioned characteristics suggest that PAT1 does not encode a protein.

The minimal requirement of the PAT1 promoter has been determined by progressive deletions from the upstream region (Chao *et al.*, 1998). Deletions up to nt −212 resulted in a threefold increase in promoter activity as compared to deletions up to nt −315. Deletions up to nt −158 showed similar levels of promoter activity to the deletion up to nt

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−212. A further deletion to nt −90 caused a sevenfold increase in promoter activity as compared to the promoter with a deletion to nt −315 (Chao *et al.*, 1998). These results suggested that both −315/−212 and −158/−90 regions exert negative regulatory effects.

In this report, we confirm that the −312/−90 region of the PAT1 promoter exerts an inhibitory effect on expression from the heterologous IE0 promoter of Autographa californica multiple nucleopolyhedrovirus (AcMNPV). The AcMNPV IE0 promoter was used as it has been well studied (Kovacs *et al.*, 1991). Besides, both Hz-1 virus and baculovirus replicate in insect cells, and this lends strength to the fact that this study will be useful in understanding transcription in insect virus–insect cell systems. Our results show that the inhibitory effect is independent of its orientation towards the promoters. Computer-assisted analysis shows that two GATA elements are present in the −312/−90 region of the PAT1 promoter. GATA family transcription factors share one or two copies of a highly conserved zinc finger domain that binds with the core sequence WGATAR (W, A/T; R, A/G). Substitutions of the four core nucleotides in both GATA elements of the −312/−90 region eliminated its negative regulatory activity, suggesting that both GATA elements are involved in the inhibitory regulation.

Methods

■ **Cells.** SF9 cells, a clonal isolate of *Spodoptera frugiperda* IPLB-SF21-AE cells (Vaughn *et al.*, 1977), were maintained at 26 °C in TNM-FH (Gibco BRL) supplemented with 8% foetal bovine serum.

■ **PCR and construction of plasmids.** Primers with *Xho*I or *Hin*dIII restriction enzyme sites at their 5′ ends were used to amplify various PAT1 promoter regions: −727/−505, −312/−90, mut−312/−90, −312/mut−90 and −158/−90 (Table 1) from pHzEM, which contains the *Eco*RI M fragment of the Hz-1 genome harbouring the PAT1 coding region. The amplification reactions were performed with a thermocycler (Biometra) with 30 cycles of 94 °C for 40 s, 55 °C for 1 min, 72 °C for 2 min. The PCR-amplified fragments were purified after fractionation in low melting temperature agarose (Sea Plaque GTG, FMC Bio-Products), digested with *Xho*I or *Hin*dIII (NEB) and then ligated into the *Xho*I- or *Hin*dIII-linearized plasmid pTSV/IE0 containing a *lacZ* reporter gene under the control of a 589 bp fragment containing the IE0 promoter of AcMNPV (Lee *et al.*, 1995). The resulting plasmids, pTSV/IE0/−312/−90, pTSV/IE0/−312/−90R, pTSV/IE0/−727/−505, pTSV/IE0/−312/mut−90, pTSV/IE0/mut−312/−90 and pTSV/IE0/mut−312/mut−90 were sequenced to determine the direction of inserts. pTSV/IE0/−312/−90R is the construct containing the −312/−90 fragment in reverse orientation.

■ **DNA transfection.** SF9 cells (2×10^5) were seeded in a 24-well culture plate (Corning) and then transfected with 1 μg of appropriate plasmid DNA using lipofectin according to the manufacturer's protocol (Gibco BRL). For internal control of the transfection, pActin-CAT was used, which has a chloramphenicol acetyltransferase (CAT) reporter gene driven by a *Drosophila* actin promoter (kindly provided by Y. C. Chao, Institute of Molecular Biology, Academia Sinica, Taiwan).

■ **β-Galactosidase activity assay and CAT assay.** Cell lysates were prepared by three freeze–thaw cycles. For the determination of β-galactosidase (β-gal) activity, cell lysates were incubated with a reaction mixture containing 25 mM Tris, pH 7·5, 125 mM NaCl, 2 mM $MgCl_2$, 12 mM β-mercaptoethanol and 0·3 mM 4-methylumbelliferyl-β-D-galactoside at 37 °C for 30 min. To stop the reaction, TCA was added to the mixture and immediately chilled on ice. Subsequently, glycine carbonate reagent was added. The emitted fluorescence was detected by

Table 1. Oligonucleotide sequences (5′ → 3′)

The recognition site for the *Xho*I restriction enzyme is underlined; the *Hin*dIII site is double-underlined. Bold face indicates the GATA site (some of the GATA sequences are on the other strand or are mutated sequences).

Oligonucleotides for PCR amplification	
−727	AATCTCGAGTCCCGGCTATGCGCAAGAGT
−505	CCGCTCGAGTCTAACCGTGTGAATGAATT
−312	GGGCTCGAGTTTAAAGAGCTAGCGGTAAA
mut −312	AGACTCGAGAAAGAGCTAGCGGTAAAAAT**TCCG**AAAA
−158	TTACTCGAGATTAAGAGGAATCGTTCCCC
−90	GGGCTCGAGATAAAACTT**TATC**AGAAAAA
mut −90	GTTCTCGAGTAATAAAACTT**GCCT**AGAAAAACGTGC
+29	CACAAGCTTGGTATGAACACGACTCGAAT
Oligonucleotides for gel shift assays	
GATA(+)	CACGTTTTTCT**GATA**AAGTT
GATA(−)	AACTT**TATC**AGAAAAACGTG
mut GATA(+)	CACGTTTTTCT**AGGC**AAGTT
mut GATA(−)	AACTT**GCCT**AGAAAAACGTG

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a minifluorometer (Hoefer, TKO 100). Statistical analysis was carried out using the Mann–Whitney Rank Test in the SigmaStat statistics software package (Jandel Scientific Corp.). CAT assays were carried out according to Nissen & Friesen (1989).

■ **Preparation of nuclear extracts.** SF9 cells (2×10^7) were resuspended in ice-cold buffer A (15 mM KCl, 10 mM HEPES, pH 7·6, 2 mM $MgCl_2$, 0·1 mM EDTA, 1 mM DTT, 0·1% NP40 and 0·5 mM PMSF) and the mixture was incubated on ice for 10 min. The nuclei were collected by centrifugation at 500 ***g*** for 10 min and resuspended in buffer B (1 M KCl, 25 mM HEPES, pH 7·6, 0·1 mM EDTA, 1 mM DTT and 0·5 mM PMSF). The suspension was incubated at 4 °C for 15 min and vortexed every 3 min. The lysate was centrifuged at 20 800 ***g*** for 20 min (Eppendorf 5417R). The supernatant was transferred to a new tube and diluted with buffer C (20% glycerol, 25 mM HEPES, pH 7·6, 0·1 mM EDTA, 1 mM DTT and 0·5 mM PMSF) at a ratio of 1:3·75 (Inoue *et al.*, 1994). The protein concentration in the nuclear extracts was determined using a Protein Assay kit (Bio-Rad).

■ **Gel mobility shift assay and competition experiments.** These were performed according to Inoue *et al.* (1994) with some modifications. Both positive (+) and negative (−) strands of 20-mer oligonucleotides containing the GATA element (Table 1) were synthesized, annealed and end-labelled with [γ-^{32}P]ATP by T4 polynucleotide kinase (NEB). Labelled probe (1 ng) was incubated with 5 μg nuclear extract in binding buffer (12 mM HEPES–NaOH, pH 7·6, 4 mM Tris–HCl, pH 7·6, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 12% glycerol and 2·5 mM PMSF) in the presence of 1 μg of poly dI-dC (Gibco BRL) for 30 min at room temperature. DNA–protein complexes were analysed on a 6% native polyacrylamide gel using 1× TBE electrophoresis buffer at 100 V for 6 h. Gels were then dried and autoradiographed. Competition experiments were performed as described by Inoue *et al.* (1994), except that labelled probe was incubated with competitor oligonucleotides prior to the addition of nuclear extract. The percentage of competition was determined by measuring band intensities using an image analyser (FLA-1000, Fujifilm).

Results

Confirmation of the negative regulatory effect of the −312/−90 region

To confirm the negative effect of the −312/−90 region, we employed *lacZ* reporter constructs (pTSV/IE0) containing the *lacZ* gene transcribed by the heterologous IE0 promoter from AcMNPV. The −312/−90 region was inserted in both orientations upstream of the IE0 promoter in pTSV/IE0 to yield the reporter plasmids pTSV/IE0/−312/−90 and pTSV/IE0/−312/−90R. A control construct, pTSV/IE0/−727/−505, was constructed in the same manner since deletion of nt −727 to −505 of the promoter region of PAT1 did not show any effect on PAT1 transcription (Chao *et al.*, 1998). Plasmids were transfected into SF9 cells along with an internal control plasmid bearing CAT controlled by the *Drosophila* actin promoter to normalize the transfection efficiency in the transient assay. Cells were harvested at 48 h p.i., lysed and analysed for both β-gal and CAT activities. Results showed that the β-gal activity decreased to 40% when the fragment from −312 to −90 was inserted upstream of the IE0 promoter (pTSV/IE0/−312/−90) compared to the β-gal activity of pTSV/IE0, and it was reduced to 50% when the inserted fragment was in reverse orientation (pTSV/IE0/−312/−90R). Conversely, no inhibitory effect was detected with pTSV/IE0/−727/−505 (Fig. 1). Thus, the upstream region of the PAT1 promoter, −312/−90, has a negative regulatory effect on the heterologous IE0 promoter. The effect of the −158/−90 region on the IE0 promoter has been analysed in the same way as −312/−90. Similar inhibitory results were obtained with the −158/−90 region in the forward orientation, and this activity was abolished when the mutated −158/−90 region was used (data not shown).

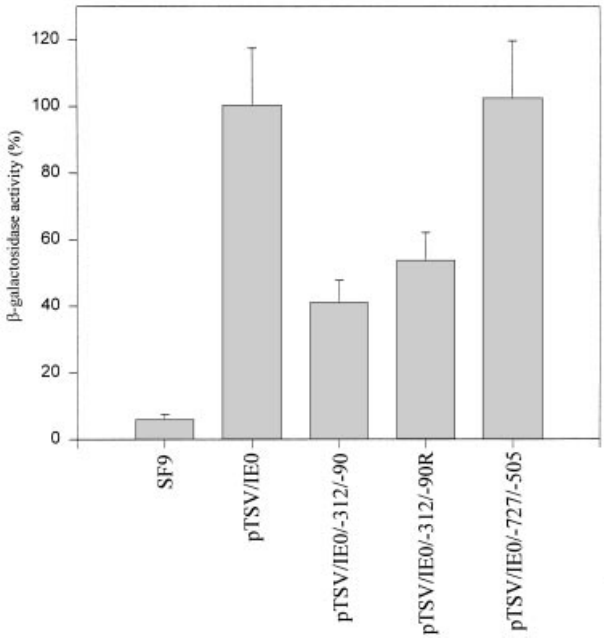

Fig. 1. β-Gal activities detected in cells transfected with different plasmids: pTSV/IE0, pTSV/IE0/−312/−90, pTSV/IE0/−312/−90R or pTSV/IE0/−727/−505. The β-gal activity was normalized to the CAT activity of the internal control per μm protein extract. Untreated SF9 cells were also analysed and served as a negative control. The expression level of β-gal activity in SF9 cells transfected with pTSV/IE0 is taken to be 100%. The data are the average of triplicates of each experiment, each of which was repeated at least three times.

Cellular proteins bind to −158/−90 and −312/−212 regions

To investigate whether any cellular proteins bind to the −312/−90 region and stimulate its negative regulatory effect, both −312/−212 and −158/−90 fragments were evaluated for cellular protein binding by electrophoresis mobility shift assays (EMSAs) using nuclear extracts prepared from SF9 cells. Two major DNA–protein complexes (a and f) and four minor complexes (b, c, d and e) were detected with the −158/−90 fragment (Fig. 2*a*, lane 2). Results of EMSA with the −158/−90 fragment suggested that several proteins might bind to the −158/−90 region or that there were protein–protein interactions between the DNA–protein complexes. Three DNA–protein complexes (a, b and c) were

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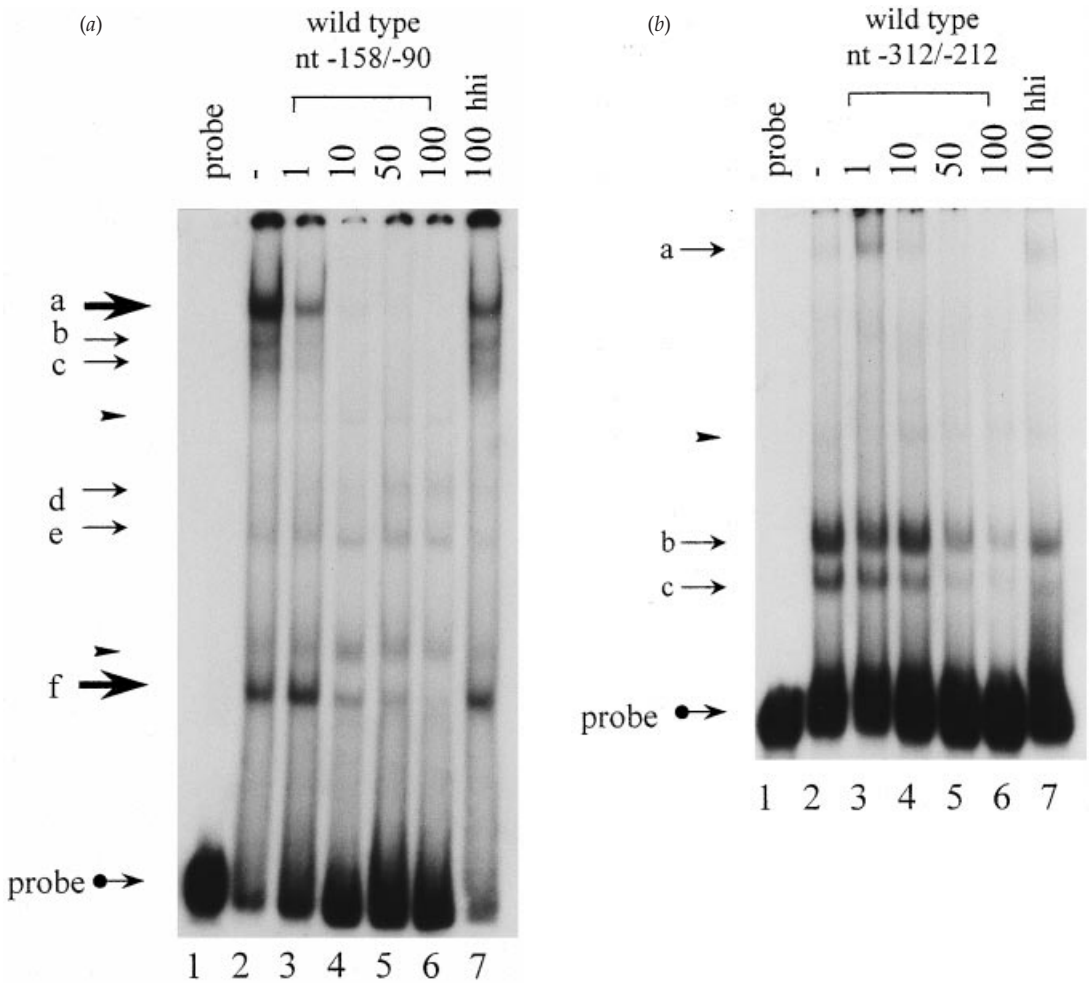

Fig. 2. Gel mobility shift assays with −158/−90 (*a*) and −312/−212 (*b*) fragments. (*a*) Lane 1, without nuclear extract; lanes 2–7, with SF9 nuclear extracts. The positions of shifted DNA–protein complexes for the −158/−90 fragment are labelled as complexes a, b, c, d, e and f. Major bands are indicated by bold arrows (lane 2). (*b*) Three complexes were formed with the −312/−212 fragment, and marked as complexes a, b and c. Competition experiments were performed in the presence of 1× (lane 3), 10× (lane 4), 50× (lane 5) and 100× (lane 6) cold probe, and 100× *hhi* fragment (nonspecific competitor, lane 7). Arrowheads indicate additional complexes formed by nonspecific binding of cellular factors.

observed with the −312/−212 fragment (Fig. 2*b*, lane 2). Poly dI-dC was included in these binding experiments to exclude nonspecific binding.

To determine the binding specificity of these complexes, competition experiments were carried out in the presence of the specific competitor, the unlabelled −158/−90 fragment, ranging from 1- to 100-fold molar excess, or with 100-fold molar excess of a nonspecific competitor, a 222 bp fragment of *hhi*, which is an immediate early gene of Hz-1 virus. DNA–protein complexes a and f observed with the −158/−90 region were almost abolished with 10-fold or more molar excess of the specific competitor (Fig. 2*a*, lanes 3–6). Conversely, complexes b and c disappear in the presence of molar equivalent specific competitor, and complex e remained unaffected. Complex d is only formed in the presence of excessive amounts of the −158/−90 fragment. A 100-fold molar excess of nonspecific competitors had no effect on any of the complexes (Fig. 2*a*, lane 7). Two additional bands (Fig. 2*a*, arrowheads) were not competed for even with 100-fold molar excess of the −158/−90 region, suggesting that they were associated with nonspecific cellular factors. Complexes formed with the −312/−212 fragment were also specific since 100-fold molar excess of the *hhi* fragment did not compete. On the contrary, the non-radiolabelled −312/−212 fragment did compete for complex a at 10-fold molar excess, and complexes b and c at 50-fold molar excess (Fig. 2*b*). A minor complex (Fig. 2*b*, arrowhead) is formed by nonspecific binding of cellular factors, since it is not competed for with 100-fold molar excess by the −312/−212 fragment.

To identify the protein-binding motifs within the −158/−90 and the −312/−212 regions, the sequences of these fragments were compared with the database of the Human

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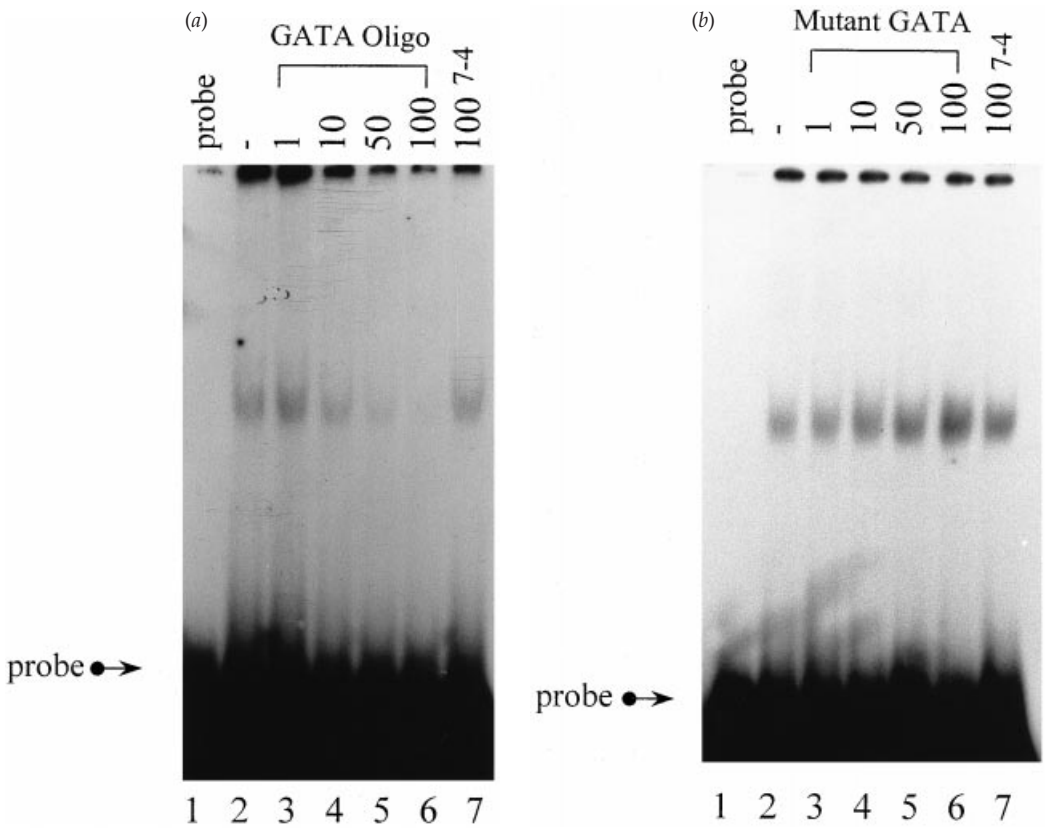

Fig. 3. Gel mobility shift assay confirming the presence of GATA-binding proteins in the SF9 nuclear extracts. Interaction of nuclear extract prepared from SF9 cells with the end-labelled GATA element and competed with either (*a*) wild-type GATA oligonucleotide or (*b*) mutated oligonucleotide. Competition experiments were performed in the presence of 1× (lane 3), 10× (lane 4), 50× (lane 5) or 100× (lane 6) cold probe, and 100× 7-4 fragment (nonspecific competitor, lane 7).

Genome Program of Japan. Sequence analysis revealed that each fragment had one GATA element and binding motifs for transcription factors such as heat shock factor (HSF) (Sistonen *et al.*, 1994; Morano & Thiele, 1999), c-Myc and Cdx A (Margalit *et al.*, 1993; Frumkin *et al.*, 1993).

Analysis of the GATA elements in the −312/−90 region

Among the proteins that have binding motifs in the −312/−90 region, both HSF and c-Myc are positive regulators, and only the GATA-binding protein has been shown to have both positive and negative regulatory effects on gene expression (Orkin, 1992; Yang & Evans, 1995). Thus, the GATA element was most likely to exert the negative effect on PAT1 promoter activity. Therefore, we investigated whether the GATA element was responsible for the observed gel-retarded complexes in EMSAs with the −158/−90 region. A 20-mer oligonucleotide duplex containing the GATA element based on the −158/−90 region (Table 1) was used as a probe for EMSAs. A DNA–protein complex was detected with the GATA element probe (Fig. 3*a*, lane 2). This complex was specific, since the binding decreased upon addition of 10- to 100-fold molar excess of the unlabelled GATA oligonucleotide (Fig. 3*a*, lanes 4–6). However, the complex formation could not be outcompeted for by a 100-fold molar excess of non-specific competitor 7-4, a 20 bp fragment derived from the *hhi1* gene of Hz-1 virus (Fig. 3*a*, lane 7). These results suggest that a specific protein or proteins recognize the GATA element located within the −158/−90 region.

To rule out any effect of the remaining 16 nucleotides surrounding the four nucleotides of the core GATA element in complex formation, a competition experiment was carried out using the 20-mer oligonucleotides containing a substitution of sequence GATA to AGGC (Table 1). Results showed that the mutated oligonucleotides did not compete for the factors bound to the wild-type oligonucleotides (Fig. 3*b*, lanes 3–6). The original complex formed with wild-type GATA oligonucleotide could not be detected when the mutated GATA oligonucleotide was used as probe. These results suggest that the DNA–protein complex formed with the 20-mer oligonucleotide was mainly related to the GATA element.

To confirm that the GATA element was responsible for the observed DNA–protein interaction within the −158/−90 region, competition experiments were performed with frag-

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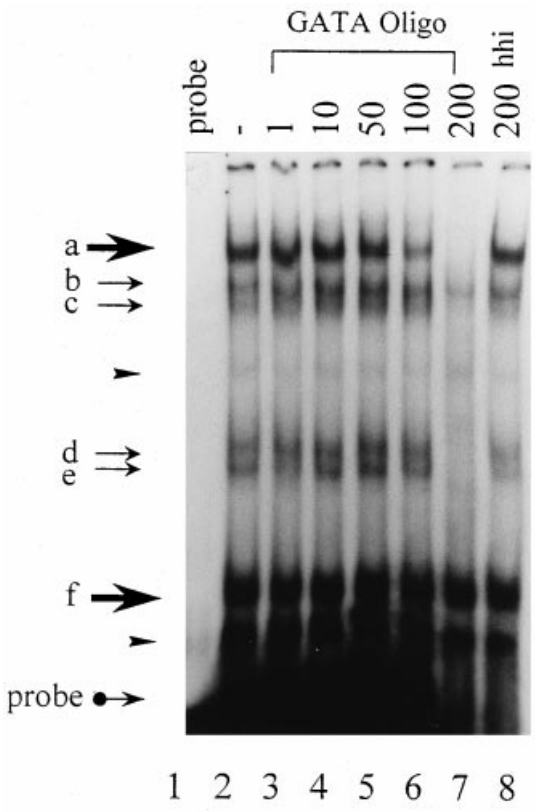

Fig. 4. Confirmation of the GATA-1 motif responsible for the interaction of the −158/−90 fragment. Nuclear extracts prepared from SF9 cells were allowed to bind to the end-labelled −158/−90 fragment (lane 2), competed with GATA-1-binding motif (1× to 200×, lanes 3–7), or competed with 200-fold molar excess of a nonspecific competitor, *hhi* fragment (lane 8) and analysed in a 4% PAGE. Lane 1, reactions performed without nuclear extract; lanes 2–8, reactions performed with SF9 nuclear extracts.

ment −158/−90 as probe and the 20-mer GATA element oligonucleotide as competitor. As indicated in Fig. 4, the amount of complex a formed with the −158/−90 fragment was reduced by 40% as measured by an image analyser using a 100-fold molar excess of GATA oligonucleotide as a specific competitor. A 200-fold molar excess of GATA oligonucleotide can compete for the complexes a, c, d and e, while complexes b and f were unaffected (Fig. 4). Two extra complexes are visible, one minor and one major. The minor one is located between complex c and d, and the major one is located between complex f and the probe. These two complexes were not detected in previous results using the −158/−90 region as probe (Fig. 2*a*). Occasionally, this occurred with different nuclear extract preparations. Besides, these two extra bands were not competed for even at a 200-fold molar excess of GATA oligonucleotide, suggesting that they are non-specifically binding complexes.

Effects of GATA element on reporter gene expression in SF9 cells

In order to investigate the importance of the GATA element in the negative regulatory activity of the −312/−90 region, either single or double mutants with substitutions of the four core nucleotides of the GATA element were generated (Table 1 and Fig. 5*a*). The effects of both the wild-type and mutated −312/−90 regions on *β*-gal activity driven by the IE0 promoter were compared. Results show that the inhibitory activity of the −312/−90 region was abolished when the core nucleotides 'GATA' were mutated in double mutants (Fig. 5*b*), suggesting that the GATA element within the −312/−90 region was necessary and sufficient for the

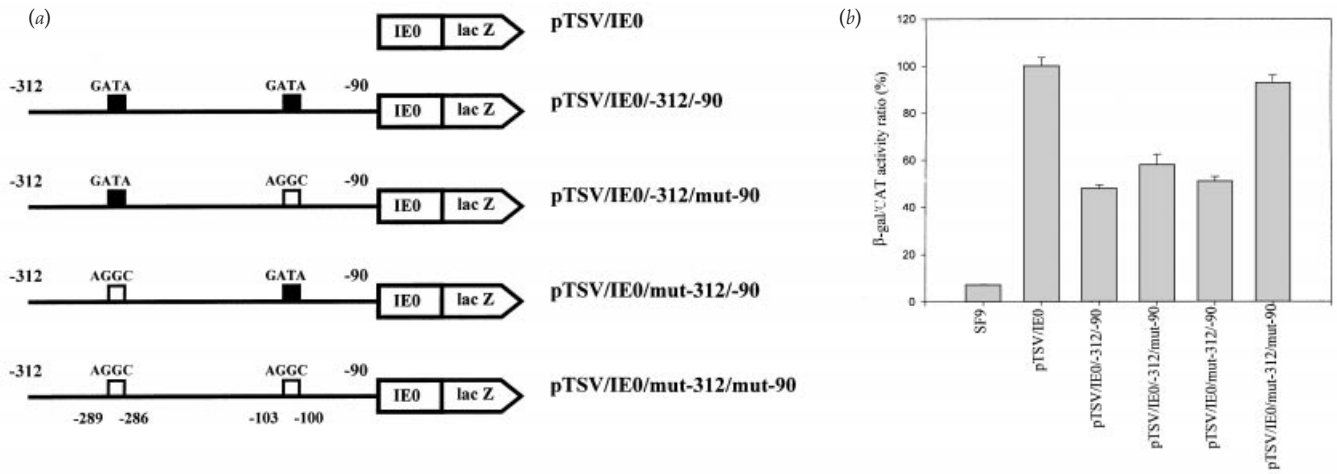

Fig. 5. Effect of the mutated −312/−90 fragment with substitutions of the four core nucleotides of the GATA element on IE0 promoter activity. The mutated −312/−90 fragments with a substitution of the four core nucleotides from GATA to TCCG (nt −289/−286) or AGGC (nt −103/−100) at either one GATA element or at both GATA elements (*a*) were tested for their negative regulatory effects on the *β*-gal expression level driven by the IE0 promoter (*b*). The *β*-gal expression level in SF9 cells transfected with pTSV/IE0 was 100%. The experiments were carried out in triplicate and repeated twice.

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negative regulation of PAT1 promoter activity. The single mutant showed a similar inhibitory effect as the wild-type −312/−90 fragment on promoter activity, albeit with differential inhibitory strength. However, the β-gal activity between the two single mutants did not differ significantly ($P = 0{\cdot}052$, Mann–Whitney Rank Sum Test). The GATA element within the −158/−90 fragment showed a moderately higher inhibitory effect than that within the −312/−212 region (Fig. 5*b*). These results suggest that the individual GATA element was functional per se. We conclude that the negative regulatory effect of −312/−90 is due to the binding of insect GATA-like proteins recognizing the GATA element on the −312/−90 fragment.

Discussion

The GATA proteins play important roles in the development and differentiation of all eukaryotic organisms ranging from yeast to fungi to humans. In invertebrates, GATA transcription factors were identified in *Drosophila*, silkworm and nematode. Multiple forms of GATA factor genes were identified in invertebrates. *Drosophila* GATA factor dGATAa/*pannier* is highly restricted to late embryogenesis and regulates the proneural genes *achaete* and *scute* (Ramain *et al*., 1993). dGATAb plays a role in fat body development and also in oogenesis (Lossky & Wensink, 1995). dGATAc is vital to the development of multiple organ systems in *Drosophila* embryos (Lin *et al*., 1995). The silkworm BmGATAβ gene encodes transcription factor *Bombyx* chorion factor 1, which regulates the expression of a class of chorion genes expressed during the late stage of choriogenesis and recognizes the GATA motif on promoter regions (Drevet *et al*., 1994). BmGATAβ is spatially and temporally regulated by alternative splicing to produce three isoforms: BmGATAβ1, gonad specific, BmGATAβ2, expressed in larva and pupal tissues, and BmGATAβ3, detected in pupal but not in larval tissue (Drevet *et al*., 1995).

Both positive and negative regulatory effects on the different genes have been identified for GATA-1 transcription factor (Orkin, 1992; Yang & Evans, 1995). In mouse erythroleukaemia cells, GATA-1 can activate γ-globin gene expression (Amrolia *et al*., 1995), while it has a negative regulatory effect on the ε-globin gene (Raich *et al*., 1995). In human K562 cells, GATA-1 has a positive effect on the human ferrochelatase gene (Turgores *et al*., 1994). *Drosophila pannier*, a GATA-1-like protein, plays a negative regulatory role on the *achaete* and *scute* genes involved in bristle formation (Ramain *et al*., 1993).

Our report here demonstrates that two GATA elements were identified in nt −312/−90 of the PAT1 upstream promoter region of Hz-1 virus. This fragment exerted a negative regulatory effect on transcription from both the PAT1 promoter and the heterologous AcMNPV IE0 promoter. Either GATA element in the −312/−90 region was adequate for the inhibition of promoter activity. Both −315/−212 and −158/−90 regions contribute to the negative regulatory effects equally. Interestingly, we found that the negative regulatory effect of the −312/−90 fragment was independent of its orientation to the promoter, which resembles the situation found with the silencer in yeast and other organisms (Ogbourne & Antalis, 1999).

There are six complexes (complex a to f) formed with the −158/−90 region (Fig. 2*a*). Among them, complex a is most likely related to the GATA oligonucleotide, since it was reduced by 40% using a 100-fold molar excess of GATA oligonucleotide, and complexes c, d and e were competed for with 200-fold molar excess of GATA oligo, while complexes b and f were unaffected. It is possible that complexes c, d and e might originate from protein–protein interactions between cellular proteins that recognize sequences within the −158/−90 region and the GATA–protein complex, or from the cellular proteins binding to the GATA–protein complex.

The GATA motif has been shown to be present in several baculovirus genes for binding of the insect GATA-like proteins. The promoter of PE38, a very early gene of AcMNPV encoding transcription factors containing two DNA-binding motifs, a zinc finger and a leucine zipper, contains a GATA motif recognized by *Spodoptera frugiperda* nuclear factor 1. Besides, computer analysis also showed that the A/TGATAT/C sequences are present in promoters of the immediate early genes of baculovirus, including 35K, ME53 and IEN of AcMNPV and IE1 of Orgyia pseudotsugata multiple nucleopolyhedrovirus (OpMNPV) (Krappa *et al*., 1992). It has been suggested that the insect GATA-binding proteins can recognize the GATA motif of 35K and ME53 promoters. However, point mutations in the GATA-1-binding site of PE38 did not affect its promoter activity, suggesting that GATA-1 is not essential for the expression of the PE38 gene (Krappa *et al*., 1992). In contrast, the removal of GATA abolishes the binding of host transcription factor to the *gp64* gene of OpMNPV and reduces transcription activation, suggesting that the GATA-1-binding sequence has a regulatory role on *gp64* (Kogan & Blissard, 1994).

In conclusion, two GATA elements on the −312/−90 region of the PAT1 promoter are related to the negative regulation effect of this fragment. The identification of these elements facilitates the understanding of the regulation of PAT1 transcription.

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