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Adjacent GATA and κB-like motifs regulate the expression of a *Drosophila* immune gene

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ABSTRACT

The GATA motif is a well known positive *cis*-regulatory element in vertebrates. In this work we report experimental evidence for the direct participation of a GATA motif in the expression of the *Drosophila* antibacterial peptide gene *Cecropin A1*. Previously we have shown that a κB-like site is necessary for *Cecropin A1* gene expression. Here we present evidence that the *Drosophila* Rel protein which binds to the κB-like site requires an intact GATA site for maximal Dif-mediated transactivation of the *Cecropin A1* gene. We show that a *Drosophila* blood cell line contains factors binding specifically to the GATA motif of the *Cecropin A1* gene. The GATA binding activity is likely to include member(s) of the GATA family of transcriptional regulators. We show that the promoters of several inducible insect immune genes possess GATA sites 0–12 base pairs away from κB-like sites in functionally important promoter regions. Clusters of GATA and κB sites are also observed in the promoters of two important mammalian immune genes, namely IL6 and IL3. The consistent proximity of GATA and κB sites appears to be a common theme in the immune gene expression of insects and mammals.

INTRODUCTION

An important component of the insect immune response is the rapid secretion of antibacterial peptides such as cecropins into the hemolymph (1–3). Cecropins, which have been isolated from a number of different insect species, constitute perhaps the most potent family of inducible antibacterial peptides (1,4). The *Drosophila Cecropin* (*Cec*) genes (*CecA1, A2, B* and *C*) have been cloned and the main sites of expression are fat body and hemocytes (5–7). Synthesis of these peptides is regulated at the transcriptional level, possibly via a common regulatory mechanism (reviewed in refs 8,9). It has been shown that a 760 base pair (bp) upstream region of *CecA1* gene contains elements necessary for its inducible and fat body-specific expression (10). A stretch of 40 nucleotides within this 760 bp upstream region, conserved among all four *Cec* genes, contains the well-known κB motif and two other DNA sequence elements referred to here as Region 1 (R1) and GATA (Fig. 1B). The κB-like site of the *CecA1* promoter functions as an immunoresponsive *cis*-acting element for expression in a *Drosophila* hemocyte cell line (10). This κB-like site is also necessary for activation of *CecA1* expression by the *Drosophila* Rel protein Dif (*Dorsal*-related immunity factor) (11,12). Similar κB-like motifs were shown to be necessary for the inducible expression of the *Drosophila diptericin* gene (13). Furthermore, κB-like motifs are present in the promoters of inducible antibacterial factors of *Hyalophora cecropia* (14), *Sarcophaga peregrina* (15), *Bombyx mori* (16) and *Drosophila virilis* (17). The R1 sequence element is present in several inducible genes from insects (9) but its functional relevance has not yet been investigated.

The vertebrate GATA motif, WGATAR, is a DNA sequence element initially defined in the promoters of erythroid cell globin genes (18,19). Analysis of erythroid-expressed genes consistently revealed GATA motifs in functionally important promoter regions (20). The GATA motif is now recognized as a positive *cis*-regulatory element in diverse vertebrate and invertebrate genes. Interestingly, similar GATA motifs are present in the promoters of the *Drosophila Cecropin* genes (Fig. 1B). Here we demonstrate for the first time the participation of a GATA site in the expression of an insect immune gene, *Drosophila CecA1*. We also show that the κB-specific Dif, which mediates *CecA1* expression, requires not only the κB-like site but also an intact GATA site for full *trans*-activation.

Proteins which interact with the GATA site constitute the GATA family of transcriptional regulators. GATA proteins have been identified from a number of organisms including *Drosophila* (21–30). We demonstrate the presence of a GATA-specific factor(s) in a *Drosophila* blood cell line, mbn-2. This cell line (31), shows several hemocyte-like characteristics including the capacity to phagocytose other cells and constitutes a useful system to study induction of the *Drosophila* immune system (4). Furthermore, mbn-2 cells express the *Drosophila Cec* genes upon stimulation with lipopolysaccharides (LPS) (32).

There are striking parallels between insect immunity and innate immunity in mammals, especially in the common utilization of transcription factors of the Rel family (reviewed in refs 2–4). It was proposed that mammalian and insect immunity share a common evolutionary origin. The three *Drosophila* Rel factors Dif, Dorsal and Relish translocate from the cytoplasm to the nucleus upon bacterial infection (11,33,34). In transfection experiments all three factors activated the expression of antibacterial genes (12,33,34).

We find that the GATA and κB motifs are located close to each other in the promoters of several inducible insect immune genes and in two immune-related mammalian genes, IL3 and IL6. This indicates a possible role for GATA motifs and GATA proteins in the evolutionary connection between insect and mammalian immunity.

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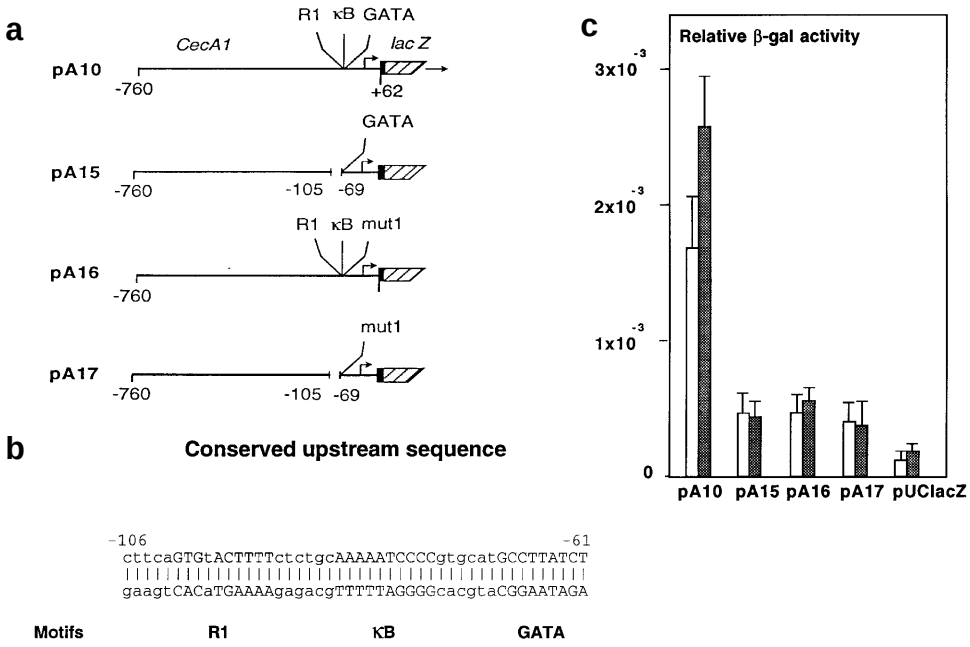

Figure 1. The GATA motif is necessary but not sufficient for *CecA1* activation. (**a**) Schematic representation of the *CecA1–lacZ* fusion genes. The constructs contain upstream regions of the *CecA1* gene and the transcriptional start site (arrow) fused to a SV 40 leader (filled box), providing a translational start site in frame with the *E.coli lac Z* coding sequence (hatched box). Numbers refer to positions relative to the CAP site. Plasmid pA16 carries mutations in the GATA core sequence (GATA to CGAG, see also Materials and Methods). The R1 and κB-like sites are deleted in pA15 and pA17. In addition, pA17 carries base substitutions in the GATA core sequence identical to those in pA16. (**b**) Upstream sequence of the proximal promoter of the *CecA1* gene. Numbers indicate the distances from the transcriptional start site and capital letters refer to sequences conserved in at least three of the *Cecropin* genes (7). (**c**) Relative β-gal activity in mbn-2 cell extracts from untreated (open bars) and LPS-treated (shaded bars) cells after transfection of 1 µg of the *Cec–lacZ* reporter plasmids. The results shown are the average of at least three independent experiments with standard deviation indicated by T-bars.

MATERIALS AND METHODS

Electrophoretic mobility shift assay (EMSA)

Deoxyoligonucleotides were labelled with [α-^{32}P]ATP and the Klenow DNA polymerase. The oligonucleotides used were
5′-d(tcgagacA**GATAA**GGCatgc) GATA-S;
5′-d(gacaaaatgacA**GATAA**GGCatgc) GATA;
5′-d(aacaaaatgacA**CGAGA**GGCatgc) mut1;
5′-d(aacaaaatgacA**GATAA**GTGatgc) mut2.
Capital letters refer to the *Drosophila CecA1* GATA site. Underlined bases in mut1 and mut2 indicate the altered nucleotides of the GATA site. We refer to the sequence GATAA indicated in bold as the GATA core sequence.

Nuclear and cytoplasmic extracts were prepared from 10^7 mbn-2 cells according to Grant *et al.* (35). The DNA binding reactions and subsequent EMSA on a 5% native polyacrylamide gel were performed using ^{32}P-containing deoxyoligonucleotide probes as described in (10). The dried gels were scanned using a PhosphorImager (Molecular Dynamics). Unlabelled oligonucleotides were added to the binding reaction mixture as competitors before the addition of extracts.

One microgram of Dif expression plasmid was translated *in vitro* using wheat germ extracts for coupled transcription and translation (Promega). The reaction was carried out in 50 µl according to the manufacturer's specifications. Two microlitres of the translated Dif protein was used for the EMSA experiment without further purification.

Recombinant DNA

The construction of the plasmids pA10, pA15 and pAct-*Dif* was described previously (10,12). Plasmid pA16 was constructed using site-directed mutagenesis by PCR (36). This introduced four base substitutions in the GATA core sequence (GATA→CGAG). These substitutions were the same as in mut1 shown underlined. The R1 and κB sites were intact in pA16. The construct pA17 was made by (i) removal of a small fragment containing R1 and κB-like sites from pA16, by cleaving it with *Bst*EII and *Sph*I; and (ii) religation after filling in the ends with Klenow DNA polymerase. Both pA16 and pA17 were sequenced to verify the mutations and the integrity of the remaining upstream region.

Cell cultures and transfection experiments

Drosophila mbn-2 cells (31) were grown at 25°C in Schneider's medium as described (12). Transfection by calcium phosphate precipitation and measurement of relative β-galactosidase activity (β-gal) were performed according to (10), except for the use of the CATELISA kit (Boehringer Mannheim). An immune response was activated by the addition of purified LPS (10 µg/ml) from the *E.coli* strain 055:B5 4 h prior to harvesting.

RESULTS

The GATA site is necessary but not sufficient for *CecA1* expression in a *Drosophila* blood cell line

Transfection and transient expression of reporter gene constructs in mbn-2 cells were used previously to identify a κB-like motif

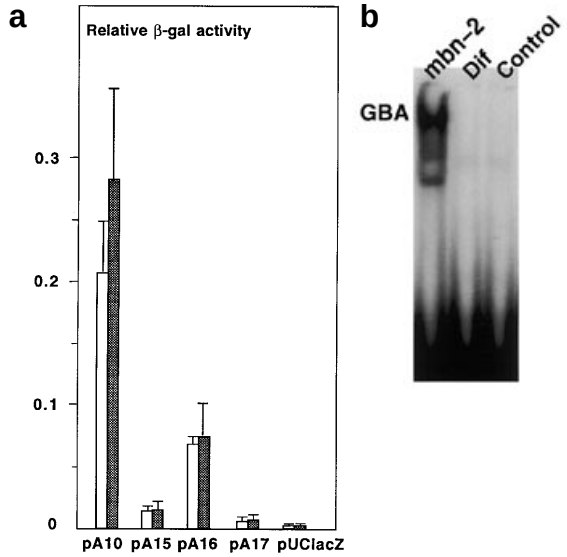

Figure 2. Dif requires both intact GATA and κB sites for proper transactivation of the *CecA1* gene. (**a**) Relative β-gal activity in mbn-2 cell extracts from untreated (open bars) and LPS-treated (shaded bars) cells after co-transfection of 1 µg of the *Cec–lacZ* reporter plasmids and 1 µg of the Dif expression plasmid pAct-*Dif*. The results shown are the average of at least four independent experiments with standard deviation indicated by T-bars. (**b**) Electrophoretic mobility shift assay using ^{32}P-labelled GATA probe (see Materials and Methods for sequence) and 2 µl of Dif translated *in vitro*, or nuclear extracts prepared from LPS-treated mbn-2 cells. Control lane has 2µl of a mock-translated reaction mixture in the absence of any expression plasmid. The band immediately below GBA is due to degradation of the DNA binding activity (compare this with the subsequent figures). The band with the highest mobility is unrelated to GBA.

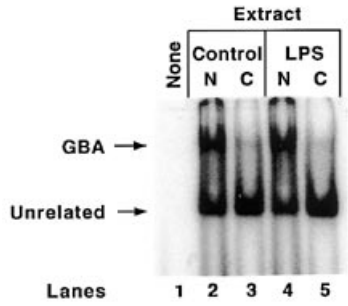

Figure 3. mbn-2 cells contain GATA-binding activity (GBA). EMSA using ^{32}P-labelled GATA-S probe with untreated (Control) or LPS-treated (LPS) nuclear (N) and cytoplasmic (C) extracts from mbn-2 cells. The DNA sequences for the probes are given in Materials and Methods.

as a necessary *cis*-regulatory element for *CecA1* expression (10). Here we employed this approach to directly test the role of a GATA site in *CecA1* gene expression. Figure 1A illustrates the constructs used in transfections. The *Cec-lacZ* reporter plasmid pA10 contains the necessary *cis*-acting elements for expression of the *CecA1* gene, including the three conserved DNA sequence motifs R1, GATA and the κB-like element. pA10 confers high levels of reporter gene expression upon stimulation with LPS (Fig. 1C; ref. 10). In contrast, transfection of the pA16 construct (which is identical to pA10 except for four base substitutions in the GATA core sequence) reduced the β-gal activity to 15–20% of the original LPS-induced activity (Fig. 1C). The level of β-gal expression from pA16 was the same in untreated cells (open bars) and LPS-treated cells (shaded bars). Thus, the GATA site is necessary for high levels of LPS-inducible *CecA1* expression in mbn-2 cells. The residual β-gal activity from pA16 is not due to the R1 or κB-like sites since similar values were obtained from pA17 which lack all three elements (Fig. 1A and C). There may be contributions from the remaining upstream sequence or from the TATA element itself. The empty expression vector, on the other hand, does not carry either of these.

In contrast to pA16, pA15 possesses the intact GATA site but lacks the R1 and κB-like sites (Fig. 1A). The β-gal expression from pA15 is also reduced to near background level (pA15, Fig. 1C). This indicates that the GATA site is not sufficient for *CecA1* expression in mbn-2 cells.

Cells transfected with the pA10 construct had β-gal activity even in the absence of LPS. This observation is consistent with our previous results that transfection itself stimulates endogenous *CecA1* gene expression (10). Such an effect was not observed when the pA15, pA16 and pA17 constructs were used for transfection experiments. The present data indicate that the GATA and κB-like sites are both necessary for transfection-induced *CecA1* expression.

Dif requires an intact GATA site for maximal transactivation of the *CecA1* gene

Petersen *et al.* (12) have shown that the *Drosophila* Rel protein Dif mediates transcriptional activation of the *CecA1* gene in co-transfection assays. Furthermore, Dif-mediated transcriptional activation requires the κB-like site of the *CecA1* promoter. In view of the proximity of a functionally important GATA site to the κB-like site, we asked whether the GATA site is important for Dif *trans*-activation. If so, does Dif bind directly to the GATA site? Firstly, we carried out co-transfection assays with the constructs shown in Figure 1A and the Dif expression plasmid pAct-*Dif*. Overexpression of Dif resulted in much higher levels of β-gal activity from the pA10 construct than in the absence of co-transfected Dif [as shown in Fig. 3A and as reported by Petersen *et al* (12)]. The induction of β-gal expression in pA15, which has no κB-like site, was only 5% of that in pA10 (Fig. 2A). Interestingly, the reporter construct pA16, in which the core sequence of the GATA site is mutated, gave considerably lower levels of β-gal expression (30%) than pA10. This suggests that Dif requires not only the κB-like site but also the GATA site for its maximal function in *CecA1* expression.

Next we tested whether Dif binds directly to the GATA site when activating the *CecA1* gene. Dif protein was expressed *in vitro* using a coupled transcription–translation system. The correct translation of Dif was confirmed by both SDS–PAGE of ^{14}C-labelled Dif and by western blotting using an antibody raised against a peptide within the Rel domain of Dif (data not shown). The *in vitro* translated Dif was used for DNA binding experiments with a ^{32}P-labelled oligonucleotide probe containing the *Drosophila* GATA site of *CecA1* promoter (GATA-S, Materials and Methods). The results showed that Dif does not bind to the GATA site directly (Fig. 2B). However, nuclear extracts from mbn-2 cells gave rise to a DNA–protein complex with the GATA-S probe (mbn-2 lane of Figs 2B and 3). We conclude that Dif's dependence on the GATA site for *CecA1* expression is indirect, probably through GATA-specific factors.

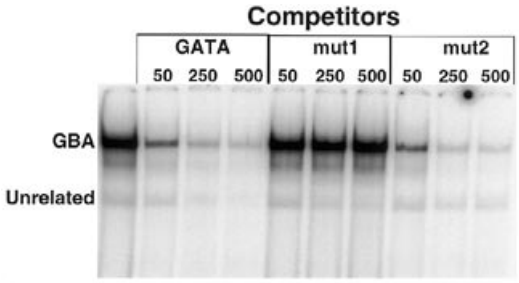

Figure 4. The GATA binding activity is specific for the GATA motif. EMSA using nuclear extracts from LPS-treated mbn-2 cells in the absence or presence of competing oligonucleotides. The sequences for the ^{32}P-labelled GATA probe and the competing oligonucleotides (GATA, mut1 and mut2) are given in Materials and Methods.

The mbn-2 cells contain a nuclear GATA-binding activity

Nuclear factors interacting with the GATA site were identified by electrophoretic mobility shift assays (EMSA) of extracts from mbn-2 cells before (control) and after exposure to LPS. Figure 3 shows the presence of a GATA-binding activity (GBA) in nuclear extracts from both control and LPS-stimulated cells (lanes 2 and 4). Cytoplasmic extracts did not reveal any substantial GBA, neither before nor after LPS-stimulation (lanes 3 and 5). The fastest migrating complex on the gel (unrelated, Fig. 3) is due to single-stranded DNA-binding proteins in mbn-2 extracts (data not shown). The GATA-binding activity (GBA) is distinct from the previously identified κB-binding activity (κBA, also referred to as DIF in ref. 10). Competition experiments confirmed that the κBA did not bind to the GATA site directly (data not shown).

Non-specific binding of nuclear proteins to the ^{32}P-labelled GATA probe was ruled out by competition experiments. The binding experiments were conducted in the presence of excess unlabelled oligonucleotides containing the GATA, R1 or κB-like sites. The extracts were added to a solution containing the labelled probe and the unlabelled competitors. The unlabelled GATA oligonucleotide competed efficiently with the labelled probe (Fig. 4) and only 10–20% of the binding activity resided at a 50-fold excess of the unlabelled probe. On the other hand, even a 500-fold excess of the R1 and κB motif-containing oligonucleotides were unable to displace the ^{32}P-GATA probe from its complex (data not shown).

We used EMSA to assess the relative importance of the core sequence (GATAA) and the additional conserved bases for GBA complex formation. The two competitors used were mut1 and mut2. In mut1, the first four nucleotides of the core sequence were substituted (GATA to CGAG). In mut2, on the other hand, two bases 3′ to the core sequence were substituted (GC to TG), keeping the core sequence intact. While mut1 was unable to compete with ^{32}P-GATA probe for GBA formation even at a 500-fold excess, mut2 competed as efficiently as the wild type sequence (Fig. 4). This demonstrates that the four bases in the core sequence are important for its interactions with the GATA-binding factor(s). The consensus nucleotides outside the core sequence are dispensable. In contrast to the case of GBA formation, the unrelated complex was competed by all three oligonucleotides in a non-specific manner (Fig. 4 and data not shown). By virtue of its specific interaction with the GATA site, we propose that all or some components of the GATA-binding activity are members of the GATA family.

EDTA inhibits the formation of the GATA-binding activity

All the known members of the GATA family of transcription factors are Zn finger proteins which require Zn ions in order to bind to DNA (20). To test whether Zn is required for GBA complex formation, the GATA binding experiment was carried out in the presence of excess amounts of EDTA, which competes for bound Zn^{2+} (37). Incubation of the GATA probe with mbn-2 nuclear extracts in the presence of 10 mM EDTA suppressed DNA–protein complex formation to 27% of its former value (Fig. 5B). On the other hand, 120 mM NaCl had no effect on the complex formation. We conclude that EDTA has an inhibitory effect on GBA complex formation. This is probably because GBA is a Zn finger protein(s) and requires Zn^{2+} for the formation of stable complexes with DNA.

The GATA motif is present in many insect and two mammalian immune genes

The strong conservation of the GATA motif in the proximal promoters of *Drosophila Cec* genes prompted us to examine its occurrence in the upstream region of other immune genes. Table 1 lists inducible insect immune genes which have the GATAA sequence (the core sequence) in their upstream region. Comparison of this sequence and its flanking bases within each species led to a species-specific consensus for the GATA site. The T/AGATAA sequence is well conserved between different insect species. In *Drosophila* and *Sarcophaga* the consensus sequence extends by three nucleotides at the 3′ end. Many of the inducible immune genes shown in Table 1 have a GATA site between positions –35 and –65. They also contain κB-like motifs in their promoter regions (6,7,17,38–42).

DISCUSSION

The present study addresses the functional relevance of a putative *cis*-acting element, namely the GATA site, in insect immune gene expression. This site is present in the upstream region of many inducible insect immune genes (Table 1). We provide experimental evidence for the participation of the GATA site in *Drosophila CecA1* gene expression. Four base substitutions in the GATA core sequence significantly reduced the function of an otherwise normal *CecA1* promoter (Fig. 1). We also show that the *Drosophila* hemocyte cell line mbn-2 contains a DNA-binding activity (GBA), specific for the GATA site (Figs 3 and 4). Future experiments should address the importance of the GATA site for *Cec* gene expression in different tissues like fat body and hemocytes.

LPS induces nuclear κB-binding activity (κBA) in *Drosophila* mbn-2 cells (10). This is consistent with the fact that Rel proteins are translocated to the nucleus in response to an external signal, such as LPS, prior to their binding to κB sites. In contrast, the nuclear GBA is constitutive and is not dependent on LPS (Fig. 3). However, mutations in the GATA core sequence interfered with the LPS-inducibility of *CecA1* gene expression (pA16, Fig. 1C). Thus, the GATA site is necessary for high levels of LPS-induced *CecA1* expression in transfection experiments. Our observations point towards a plausible cross-talk between Rel and GATA proteins. Albeit Dif did not bind to the GATA sequence (Fig. 2B), Dif *trans*-activation was not efficient when analysed on a *CecA1* promoter construct mutated in the GATA site (Fig. 2A). This

Table 1. GATA sites in the 5′- upstream region of inducible immune related genes in insects

Immune genes	Position*	Sequence	Immune genes	Position*	Sequence
Drosophila melanogaster			*Hyalophora cecropia*		
CecA1	-62	cAGATAAGGC	attacin A	-62	tTGATAAtaa
CecA2	-58	cTGATAAGGC		-122	cTGATAAAat
	-268	gcGATAAGcC		-163	cTGATAAtgc
	-322	cTGATAATGT		-239	tTGATAAtga
CecΨ1	-55	cAGATAAGaC		-462	cAGATAAtgc
CecΨ2	-55	gAGATAAcGC		-642	aTGATAAtgt
CecB	-685	tgGATAATGC	attacin Ψ	-15	gAGATAAGtt
	-708	ggGATAATGa		-38	aTGATAAGaa
	-717	tgGATAAatg		-69	tAGATAAGag
CecC	-48	ccGATAAGGC	attacin B	-38	aAGATAAGta
diptericin	-69	tAGATAAGGT		-135	gcGATAAAtt
	-492	gTGATAATTg	cecropin A	-8	gTGATAAAta
	-925	gTGATAAGaC	cecropin B	-111	cTGATAAccg
				-162	tAGATAAAtg
Consensus		TGATAATGC	cecropin D	-54	cTGATAAAca
		A G T		-212	tTGATAAAgt
			lysozyme	-45	agGATAAAtt
Drosophila virilis				-105	gcGATAAGga
Cec1	-55	cTGATAAGCC		-222	cAGATAAAca
Cec2A	-55	cTGATAAGCC		-350	aTGATAActt
Cec2B	-55	cTGATAAGCC			
Cec3	-54	gTGATAAGCC	Consensus		TGATAAA
					A G
Consensus		TGATAA			
Sarcophaga peregrina					
lectin	-120	aTGATAAgcA	*Bombyx mori*		
	-229	aTGATAAtgg	*CecB1*	-102	aAGATAAcgg
	-389	cTGATAAcgt		-162	cTGATAAaag
sarcotoxin 1b	-179	gTGATAAgAA		-275	tAGATAAcca
	-323	aTGATAAatA	*CecB2*	-102	aAGATAAcgg
	-336	agGATAAttA		-158	cTGATAAaag
sarcotoxin II				-270	tAGATAAcca
unit 1	-55	cTGATAAcAA	attacin	-94	aTGATAAgaa
	-85	cTGATAAttt		-230	tTGATAAact
	-119	aTGATAAtAA			
	-192	cTGATAAaAA	Consensus		TGATAA
unit 2	-10	gAGATAAtcA			A
	-55	cTGATAAcAA			
unit 3	-10	gAGATAAtcA			
	-55	cTGATAAcAA	Mammalian Consensus#		TGATAA
	-93	aTGATAAtAA			A G
Consensus		TGATAANAA			
		A			

The sequence for the genes listed in this table are taken from the EMBL data bank (EMBL/DDJB/GenBank) database.
Capital letters in the sequences below indicate conserved nucleotides.
*Position from the transcriptional start site to the G in the GATA core sequence.
#Taken from ref. 18.

suggests that Dif needs the cooperation of the GBA for full *trans*-activation and LPS response.

What is the nature of the GBA? The binding to the GATA core sequence and the sensitivity to Zn ions suggest that the GBA is a member of the Zn-finger containing GATA family of transcription factors. There are three known GATA proteins in *Drosophila*, dGATAa (28,29), dGATAb (previously known as ABF) (27) and dGATAc (30). The proteins dGATAa and dGATAc are proposed to be involved in determining dorsal cell fate (28,29) and in embryonic development (30) respectively. The protein dGATAb is involved in the development of the fat body (27). Rehorn *et al.* showed recently that dGATAb is encoded by the *serpent (srp)* locus (43). The *srp* gene is expressed both in fat body and hemocytes, and embryos mutant for *srp* lack mature fat body and hemocytes (43). Like the *srp* gene, the *Cec* genes are also expressed in the fat body and hemocytes. The overlapping expression pattern of *srp* and *Cec* genes makes dGATAb protein an interesting candidate for the GATA-binding activity (GBA).

Computer-assisted analysis revealed that at least one GATA element is located very close to a κB-like site in many insect immune genes. For example, the GATA site is located 8 bp from the functionally important κB-like site in the *Cecropin* and diptericin genes of *Drosophila melanogaster* (Fig. 6). An exception is the proximal κB-like site of the *CecB* promoter which has no neighbouring GATA site. The *CecB* gene promoter does, however, have neighbouring GATA and κB-like sites 656 nucleotides upstream from the CAP site. A deletion construct of *CecB* gene which lacks these distal elements, but carries the proximal conserved sequences including the κB-like site, did not confer any reporter gene activity in mbn-2 cells (Roos, E., Björklund, G. and Engström, Y., submitted). This is in agreement with the hypothesis that the κB site needs a neighbouring GATA site to induce *Cec* gene

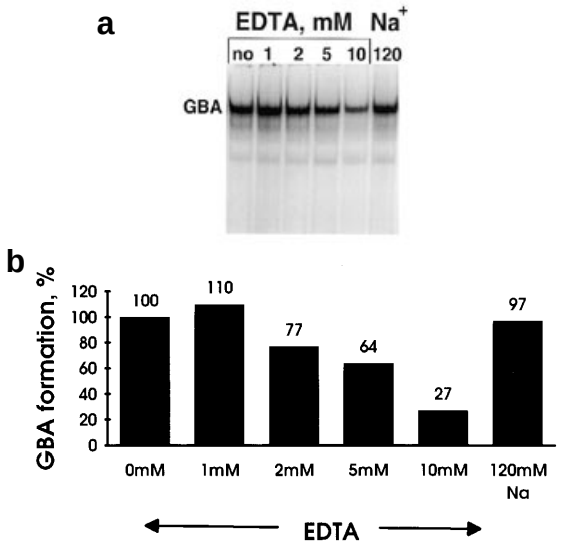

Figure 5. Effect of EDTA on GBA complex formation (**a**) EMSA was carried out using a ^{32}P-labelled GATA probe and LPS-treated mbn-2 cell nuclear extracts in the presence of varying concentrations of Na_2EDTA as indicated. The DNA binding reaction was carried out in a reaction buffer containing 100 mM NaCl. EDTA was added prior to the addition of the nuclear extract. The lane labelled 120 mM Na^+ had no EDTA in the DNA binding reaction mixture. It serves as a control where NaCl was used to obtain the same concentrations of Na^+ as in the sample with the highest concentration of Na_2EDTA (10 mM). (**b**) PhosphorImager scannings of the gel in (a) was used to calculate the percentage of GBA complex formation with increasing concentrations of EDTA. The intensity of the band with no EDTA (100 mM NaCl in the reaction buffer) was taken as 100% in order to estimate the DNA–protein complex formation in the other lanes.

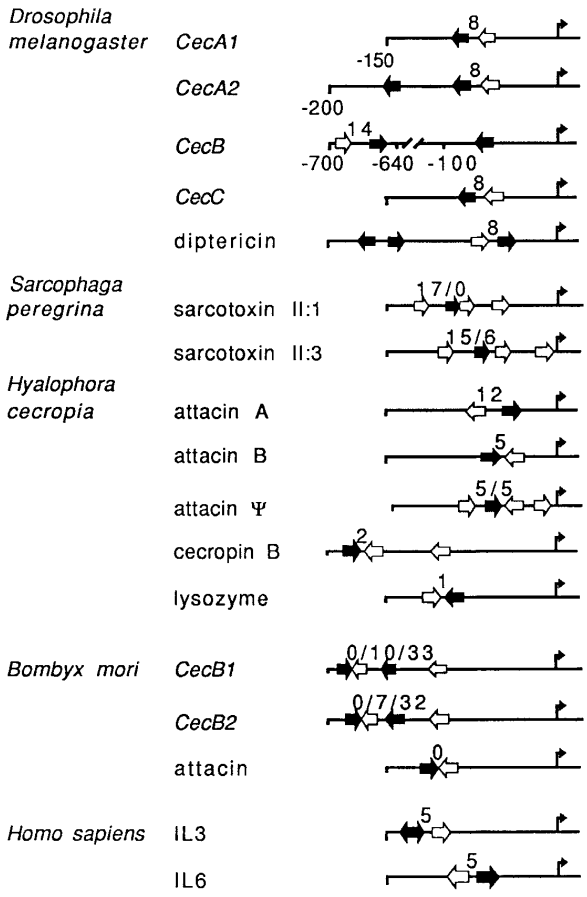

Figure 6. GATA and κB-like motifs in the upstream regions of inducible immune genes in insects and mammals. The analysis was carried out using the 'Find Pattern' and other relevant programs of the GCG package to identify κB and GATA motifs in the upstream sequences of the genes available in the EMBL data bank (EMBL/Gene Bank/DDBJ data base). The κB consensus sequence GGGRNNYYCC for mammals (47) and GGGRAYYYYY for insects (4) were used to identify the κB sites. For identifying GATA sites the sequence T/AGATAA/G was used for both mammalian and insect genes. In the case of the IL3 gene one mismatch was allowed in the κB site. The κB site of IL3 here, TGGAGGTTCC, is not the same as, but includes part of, the CK1 site of ref. (48). The shortest distance (in nucleotides) separating the κB motifs (open arrows) and the GATA motifs (filled arrows) regardless of their orientation is indicated above the arrows. The orientation of the arrows refer to the 5′ to 3′ direction of the site as defined by the consensus sequence. The upstream regions are drawn to scale and the transcriptional start site is indicated by a thin arrow.

expression in response to infection. Accordingly, the gene for the antibacterial protein andropin, which is upstream from the *CecA1* gene in the *Cecropin* locus (44), does not respond to infection and has neither a GATA nor a κB-like site in its upstream region.

We also found GATA and κB-like sites separated by 0–6 bp in the inducible immune genes of *Hyalophora cecropia* and *Sarcophaga peregrina* (Fig. 6). Two *Cecropin* genes, *CecB1* and *CecB2*, and an *Attacin* gene of *Bombyx mori* contain two LPS-responsive elements in their upstream promoters (16,45). These elements resemble the κB-like motif and are close to GATA sites (1–12 bp). The *Cecropin* locus in *Drosophila virilis* was recently cloned and the four *D.virilis Cecropin* genes were also found to contain κB-like and GATA sites in their proximal promoter region (17). Interestingly, the proximity of GATA and κB sites is not limited to insect immune genes. We observed GATA elements located 5 nucleotides away from κB motifs in two important immune-related human genes, IL3 and IL6. The spacing between κB and GATA sites in the upstream region of mouse, rat, cattle and sheep IL3 and IL6 genes is identical to that in human genes (Fig. 6). In *Drosophila*, the relative orientation of the two motifs is conserved between the immune genes analysed so far. However, other species listed in Figure 6 have their GATA and κB-like sites in a different relative orientation. It appears therefore, that their proximity is more important than their relative orientation.

The consistent proximity of the GATA and κB motifs in insect and mammalian immune gene promoters is intriguing (Fig. 6). The factors which interact with these two *cis*-acting elements may cooperate in immune gene expression. Consequently, the evolutionary relationship between mammalian and insect immune reactions may include GATA sites and GATA proteins, in addition to the previously proposed κB-Rel connections (3,10,11,33,46). A deeper understanding of the role of GATA-specific factors in the immune response of insects may add to the knowledge of immune gene induction in general.

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