Expression of *Drosophila melanogaster* gene encoding transcription factor GAGA is tissue-specific and temperature-dependent

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Abstract The GAGA factor (GAF) of *Drosophila melanogaster* encoded by the Trithorax-like gene is known to maintain expression of many *Drosophila* genes including homeotic ones, through configuration remodeling of local chromatin. The complicated transcript pattern of the GAF gene has been revealed at all stages of development. The study of GAF gene expression in whole flies and in salivary glands and in the brains with adjacent imaginal disks of the third instar larvae showed tissue-specific variations in transcript patterns and dependence of these patterns on the temperature of development (14–37°C).

Key words: *Drosophila*; Trithorax-like gene; GAGA factor; Tissue-specific gene expression; Temperature dependence

1. Introduction

The *Drosophila melanogaster* transcription factor GAGA (GAF) is encoded by the Trithorax-like gene, which is required for the normal expression of homeotic genes [1]. Some mutant alleles of the gene additionally act as dominant enhancers of position effect variegation [2]. The GAF factor binds to (GA)n-rich sites in promoter regions of several *D. melanogaster* genes. These include such well-known genes as Ultrabithorax (Ubx) [3], Adh [4], engrailed, fushi tarazu [5], Kruppel [6], heat shock genes (hsp26 [7], hsp70 [8]) and those encoding histones H3 and H4 [9]. The binding of GAF to specific elements leads to chromatin structure remodeling in the promoter region (dynamic disruption of nucleosomes, formation of DNase I-hypersensitive regions) [10], thus the GAF is suggested to allow other transcription factors access to DNA [11] and to help keep on target genes by maintaining a 'open' configuration in the local chromatin [12]. The expression of the GAF gene in the course of *Drosophila* ontogenesis has been described by Soeller et al.: multiple transcripts with sizes and numbers varying at different developmental stages of *Drosophila* were observed on Northern blots containing poly(A)$^+$ RNA from whole individuals [5]. We have reported the expression of the gene in several larval and imaginal tissues: as was demonstrated by in situ hybridization on histological sections, the GAF gene was actively expressed in neural structures of adult flies and in the imaginal disks of larvae [13]. The gene being actively expressed in neural structures of *Drosophila* and highly conserved during evolution, we termed it Nc70F (a neural conserved gene located within region 70F of chromosome 3L of *D. melanogaster*) in our initial paper [14]. For further studies of GAF gene expression, we analyzed its transcript pattern in several tissues of third instar larvae of *Drosophila* developed at different temperatures using different portions of GAF cDNA as hybridization probes.

2. Materials and methods

The wild-type Canton S strain of *D. melanogaster* was used in the experiment.

2.1. RNA isolation

Total RNA from *Drosophila* larval tissues was isolated with the phenol-chloroform method [15]. Both salivary glands and brain with adjacent imaginal disks were dissected from each larva and separately collected in liquid nitrogen. Each sample contained the RNA from the organs of 40 larvae and was divided into two even portions to perform A and B blots. Total RNA from the flies was purified using the urea-LiCl method [16] with subsequent isolation of poly(A)$^+$ RNA on oligo(dT)-cellulose.

2.2. Northern blot analysis

The RNA samples were electrophoresed through 1.2% agarose gel following glyoxal and DMSO denaturation and transferred to nylon membranes (Zeta-probe blotting membrane, Bio-Rad) according to the manufacturer's protocol. Hybridization was carried out at 42°C in the presence of 50% formamide according to the manufacturer's protocol. Transcript size was approximately estimated using: (a) a position of ribosomal 18S RNA after staining of gel by ethidium bromide (1.9 kbp) and (b) rehybridization of blots with ^{32}P-labeled fragment of the hsp83 gene that reveals the 3.05 kbp hsp83 transcript [17].

2.3. Probes

The cDNA of GAF gene (accession number X59784 [13]) was isolated and sequenced by us previously. This cDNA sequence overlaps with the cDNA reported on by Soeller et al. (accession number L22205 [5]) from position 582 bp to the polyA track. Different portions of GAF gene cDNA were used as probes (Fig. 1): probe A, 684 bp *Pst*I-*Xho*I fragment; probe B, 550 bp *Xho*I-*Eco*RI fragment; probe C, 798 bp *Nco*I-*Eco*RI fragment. Following appropriate digestion of the clone, all fragments were purified by agarose gel electrophoresis and labeled by nick-translation reactions routinely.

3. Results

The GAF gene, present in the *Drosophila* genome in a single copy [4,5], was shown to generate multiple developmentally regulated transcripts [5]. To date at least two cDNA copies have been identified to encode proteins with different carboxy termini [18], since we used different portions of GAF cDNA as probes generated for Northern blot hybridizations. Probe A was a 684 bp *Pst*I-*Xho*I (subclone A) fragment, probe B was a 550 bp *Xho*I-*Eco*RI (subclone B) fragment and probe C was a 798 bp *Nco*I-*Eco*RI (subclone C) fragment (Fig. 1). The 120 bp fragment that encodes the N-terminal domain and is called 'POZ' or 'BTB' was absent from the probes,

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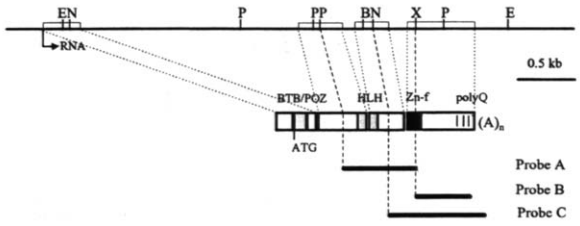

Fig. 1. Molecular map of GAF (Trithorax-like) gene. The top line represents GAF genomic DNA. Sites for endonucleases: E, *Eco*RI; P, *Pst*I; B, *Bam*HI; N, *Nco*I; X, *Xho*I. The arrow under the top line indicates the transcription start site. Rectangles represent the exons; regions encoding putative the conserved N-terminal BTB/POZ domain, the HLH and zinc finger motifs and stretches of polyglutamine residues are also shown. Three solid lines below indicate the probes used for Northern analysis. The scheme is compiled after the data by [2,13] and our data (in preparation).

because the domain was found highly homologous in GAF and trimtrack and BR-C products [5]. Expression of the GAF gene was monitored in the salivary glands and in the brains with the adjacent imaginal disks of third instar larvae of *D. melanogaster* developed at different temperatures, namely 14, 16, 18, 20, 25, 30 and 37°C and in whole flies at 20–25°C.

3.1. GAF gene expression in the salivary glands of D. melanogaster third instar larvae

Northern blots with total RNA isolated from salivary glands of larvae developed at different temperatures were probed with subclone A (Fig. 2A) and subclone B (Fig. 2B). As can be seen from Fig. 2A, only one transcript, 2.0 kbp in size, is identified in the salivary glands of larvae developed at 18–30°C and of those exposed to 37°C for 2–5 h. Those forced to develop at 16°C express an additional transcript 3.0 kbp in size, and those at 14°C express two additional transcripts 2.5 kbp and 3.0 kbp. In contrast, no temperature-dependent transcripts are identified in the same RNAs probed with subclone B (Fig. 2B). The 2.0 transcript is the only one found. We propose that the temperature-dependent 2.5 and 3.0 kbp transcripts do not include the 3′ exon in question.

3.2. GAF gene expression in the brain and the adjacent imaginal disks of D. melanogaster third instar larvae

In the next experiment total RNA from the brain and the adjacent imaginal disks of larvae developed at different temperatures was probed with subclone A (Fig. 3A) and subclone B (Fig. 3B). As can be seen from these figures, the larvae developed at 18–30°C show the same RNA profile when probed with either subclone. There are three prominent mRNAs (1.8, 2.0 and 2.5 kbp) with two larger messages in reduced amounts. In contrast, those developed at 14°C or 16°C display only one transcript (2.5 kbp) when probed with subclone A and two (2.0 kbp and 2.5 kbp) when probed with subclone B. These results suggest that at least one of the internal exons but always the last one is included in the 1.8, 2.0 and 2.5 kbp transcripts synthesized at 18–30°C. As far as the larvae developed at 14 and 16°C are concerned, it is striking to see the 2.0 kbp transcript in blot B (Fig. 3B) and not in blot A (Fig. 3A). We assume that the larvae developed at 14–16°C appear to express a new 2.0 kbp mRNA which uses only the last, 3′ exon, and none of the internal GAF gene exons involved. The larvae exposed to 37°C for 2–5 h reveal only the transcript of 2.0 kbp in either blot.

3.3. GAF gene expression in D. melanogaster adults

Previous experiments have revealed differences in the combinations of the three exons among the GAF transcripts in larvae under cold-shock conditions (14–16°C). However, transcripts with different structure can also be observed in adult flies at normal temperature (20–25°C). The respective patterns of GAF transcription revealed by hybridization of the blots containing poly(A)$^+$ RNA of adult flies with probe A and with probe C are presented in Fig. 4A,B. Probe A brings up one transcript (2.5 kbp), probe C two (2.5 and 3.0 kbp). Our conclusion is that the 3.0 kbp transcript expressed by the adult flies developed at 20–25°C does not appear to use the two internal exons.

4. Discussion

Some details of the complicated pattern of GAF gene ex-

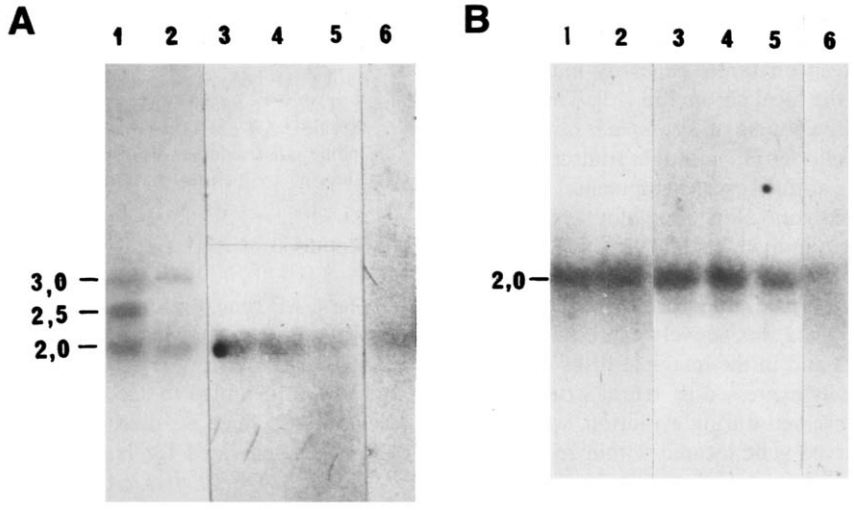

Fig. 2. Northern hybridizations to total RNAs from salivary glands of *D. melanogaster* third instar larvae developed at: lane 1, 14°C; lane 2, 16°C; lane 3, 18°C; lane 4, 25°C; lane 5, 30°C; lane 6, 25°C and treated by heating to 37°C for 2–5 h. Northern blots were probed with (A) the 684 bp *Pst*I-*Xho*I fragment (subclone A); (B) the 550 bp *Xho*I-*Eco*RI fragment (subclone B) of GAF gene cDNA. The lengths of the hybridizing transcripts are indicated. Equal loading was verified by ethidium bromide staining (not shown).

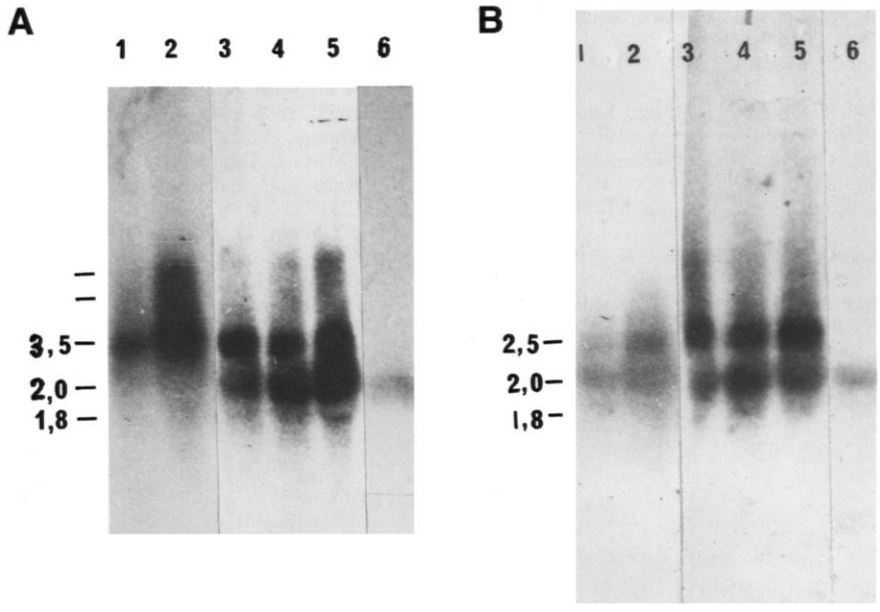

Fig. 3. Northern hybridizations to total RNAs from brain and adjacent immaginal disks of *D. melanogaster* third instar larvae developed at: lane 1, 14°C; lane 2, 16°C; lane 3, 18°C; lane 4, 25°C; lane 5, 30°C; lane 6, 25°C and treated by heating to 37°C for 2 5 h. Northern blots were probed with (A) the 684 bp *Pst*I-*Xho*I fragment (subclone A); (B) the 550 bp *Xho*I-*Eco*RI fragment (subclone B) of GAF gene cDNA. The sizes of the transcripts are indicated. Ethidium bromide-stained agarose gels demonstrated comparable loading (not shown).

pression have been revealed in the course of the three experiments described above. Firstly, the pattern varies during *Drosophila* ontogenesis, which is in agreements with the conclusion drawn by Soeller et al. [5]. Secondly, the GAF gene generates various tissue-specific transcripts: on the larvae developed at 18–30°C, we observed only one (2.0 kbp) in their salivary glands (Fig. 2), yet three (1.8, 2.0 and 2.5 kbp) in their brain and the adjacent imaginal disks (Fig. 3).

Thirdly, the transcriptional activity of the gene depends upon temperature so that 14°C is crucial for this characteristic. In the salivary glands of the *Drosophila* larvae, two additional transcripts occur at 14°C and never do so at normal temperatures (18–30°C) (Fig. 2A). Of the three observed at normal temperatures, only the 2.5 kbp transcript occurs at 14°C in the brain and imaginal disks (Fig. 3A). The pattern of GAF gene expression seems to be noteworthy in the light of investigation of position effect variegation (PEV). Indeed, the GAF gene falls into class II of modifiers of PEV [19]; PEV itself was found to be enhanced at lower temperature [20]. But the connection between the temperature-dependent changing in GAF transcripts pattern and the enhancement of PEV yet to be studied.

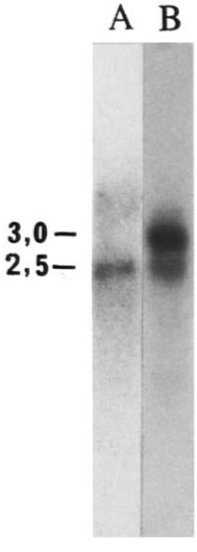

Fig. 4. Northern hybridizations to poly(A)$^+$ RNA (2 μg per lane) from *D. melanogaster* adults developed at 20–25°C with (A) the 684 bp *Pst*I-*Xho*I fragment (subclone A); (B) the 798 bp *Nco*I-*Eco*RI fragment (subclone C) of GAF gene cDNA. The sizes of the transcripts are indicated.

Finally, there is another noteworthy aspect of the temperature dependence of GAF expression: if third instar larvae are exposed to 37°C for 2–5 h, most transcripts, except for that 2.0 kbp in size, are missing from RNA preparations, no matter whether from the salivary glands, or the brain or the imaginal disks; not even these conditions can prevent revealing the 2.0 kbp transcript. This is an interesting fact, as GAF was reported to exert regulation upon the activity of heat shock genes [8,9].

A neurospecific character of GAF gene expression should be mentioned. Previously we have demonstrated the high transcription level of the GAF gene in neural structures of *Drosophila* by in situ hybridization in histological sections [14]. The results we present here evidence the complex, environment-depending spectrum of GAF transcripts in RNA from the brain and the adjacent imaginal disks of *Drosophila* larvae.

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