A heat-shock-activated cDNA encoding GAGA factor rescues some lethal mutations in the Drosophila melanogaster Trithorax-like gene

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A heat-shock-activated cDNA encoding GAGA factor rescues some lethal mutations in the *Drosophila melanogaster* *Trithorax*-like gene

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**Summary**

GAGA factor is an important chromosomal protein involved in establishing specific nucleosome arrays and in regulating gene transcription in *Drosophila melanogaster*. We developed a transgenic system for controlled heat-shock-dependent overexpression of the GAGA factor 519 amino acid isoform (GAGA-519) *in vivo*. Efficient production of stable protein from these transgenes provided genetic rescue of a hypomorphic *Trithorax*-like (*Trl*) lethal allele to adulthood. Nevertheless, supplemental GAGA-519 did not suppress position effect variegation (PEV), a phenomenon commonly used to measure dosage effects of chromosomal proteins, nor did it rescue other lethal alleles of *Trl*. The results suggest requirements for the additional isoforms of GAGA factor, or for more precise regulation of synthesis, to carry out the diverse functions of this protein.

**1. Introduction**

It is well established that generation of an appropriate chromatin structure is important for regulation of eukaryotic gene expression (for reviews, see Felsenfeld, 1992; Wallrath et al., 1994; Wolfe, 1998). While in many cases chromatin remodelling is part of the activation process, in other cases genes appear to be ‘pre-set’ in a precise nucleosome array that leaves key regulatory elements in nucleosome-free regions. Such nucleosome-free sites are hypersensitive to cleavage by nucleases, and are often referred to as DNase I hypersensitive sites (DH sites) (Elgin, 1988; Wallrath et al., 1994). A critical component that appears to be involved in the formation of pre-set DH sites in *Drosophila melanogaster* is the abundant DNA binding protein, GAGA factor. GAGA factor has been shown to bind specific DNA regulatory elements of many *Drosophila* genes and has been localized *in vivo* to many sites along the chromosome arms and to the centromeric regions of metaphase chromosomes (reviewed in Granok et al., 1995; Wilkins & Lis, 1997; Farkas et al., 2000; see also Raff et al., 1995; Platero et al., 1998).

Lu et al. (1992, 1993) observed that GAGA factor binding sites 5′ of the *hsp26* transcription start site are necessary for the formation of the two DH sites found at this promoter; these DH sites encompass the heat shock regulatory elements. In the absence of the native GAGA factor binding sites, the DH sites are not formed and the gene is not heat-shock inducible. Further studies using the *hsp70* gene have shown that the GAGA factor binding sites are critical in establishing the normal transcription complex, which includes a paused molecule of RNA polymerase II at the promoter *in vivo* (Lee et al., 1992; Shopland et al., 1995; reviewed in Lis & Wu, 1993). The involvement of GAGA factor in chromatin structure determination is supported by studies using reconstituted chromatin templates *in vitro*. When purified GAGA factor is added to a reconstituted *hsp70* promoter (Tsukiyama et al., 1994) or *hsp26* promoter (Wall et al., 1995), nucleosome disruption is observed over and immediately adjacent to the GAGA factor binding sites.

GAGA factor is encoded by the gene *Trithorax*-like (*Trl*) (Farkas et al., 1994). *Trl* alleles have been identified both due to their effects on *Ultrabithorax* (*Ubx*) expression and due to their dominant enhancement of position effect variegation (PEV) (Dorn et al., 1993a, b; Farkas et al., 1994). PEV, the clonal inactivation of genes placed adjacent to heterochromatin following a chromosomal rearrangement,
has been proposed to involve changes in chromatin packaging (Locke et al., 1988; Wallrath & Elgin, 1995; reviewed in Weiler & Wakimoto, 1995; Elgin, 1996). Flies homozygous for Trl mutations die mostly as third instar larvae (Farkas et al., 1994). One allele, Trl13C, is a functional hypomorph; flies homozygous for this mutation have reduced but detectable levels of GAGA factor and occasionally can survive under favourable conditions (Farkas et al., 1994; Bhat et al., 1995). Fertilized embryos produced from rare Trl13C/Trl13C females exhibit defects in the early mitotic divisions, including decondensed, fragmented and bridged chromosomes; specific effects on known target genes have also been observed (Bhat et al., 1996).

Cloning and conceptual translation of GAGA factor cDNAs (Soeller et al., 1993; Benyajati et al., 1997) reveals that the protein contains a single zinc finger with an adjacent basic region; this domain is responsible for the high-affinity DNA binding to (GA)\textsubscript{n} sequences (Pedone et al., 1996). In addition, GAGA factor contains a glutamine-rich carboxyl terminus, and an amino terminal BTB/POZ domain; both domains appear to be involved in protein–protein interactions (Zollman et al., 1994; Bardwell & Treisman, 1994; Chen et al., 1995; Agianian et al., 1999; Wilkins & Lis, 1999; Katsani et al., 1999; Espinás et al., 2000; reviewed in Granok et al., 1994; Wilkins & Lis, 1997; Farkas et al., 2000). Northern and western blot assays show that GAGA factor is represented by multiple transcripts and multiple isoforms at all stages of development (Gilmour et al., 1988; Biggin & Tjian, 1988; Soeller et al., 1993; Benyajati et al., 1997). The most prominent isoform, which is present at all developmental stages, is a 519 amino acid (aa) protein encoded by a 2.4 kb mRNA (GAGA-519). Recently, additional cDNAs for GAGA factor have been recovered that encode an alternate 581 aa isoform (GAGA-581) (Benyajati et al., 1997). Some of the additional isoforms observed may represent post-translational modifications of the GAGA factors synthesized using these two transcripts.

We desired to establish a system that would allow the amount of GAGA factor in the fly to be experimentally manipulated. Supplemental GAGA factor could be used to study the developmental requirements for GAGA factor and the dynamic interplay among GAGA factor, histones, and other non-histone chromosomal proteins in setting chromatin structure. In this report we describe a gene system, hsp70.GAGA (hG), that utilizes a heat shock promoter to drive \textit{in vivo} expression of a GAGA factor cDNA encoding GAGA-519. We show that these transgenic flies, under heat shock conditions, produced abundant and stable GAGA-519, and that over-expression of this transgene rescued Trl13C homozygous flies from lethality.

2. Materials and methods

(i) \textit{Drosophila} \textit{strains}

All fly stocks were maintained on standard corn meal medium (Ashburner, 1989). The following stocks were used: CyO; TM2, Ubx\textsuperscript{+30} ry\textsuperscript{y}/T(2;3), ap\textsuperscript{xw} (from J. Lis, Cornell University); Trl\textsuperscript{62}/TM3, Sb ry\textsuperscript{5,6k}e (from G. Reuter, Martin Luther University, Germany); Trl13C/TM2, Ubx\textsuperscript{+80}ry and Trl88S/TM3, Sb Ser (from F. Karch, University of Geneva, Switzerland); w; hsp83.GAGA and Trl\textsuperscript{62}/TM6B, Tb Hu\textsuperscript{e} (from A. Greenberg and P. Schedl, Princeton University); y w\textsuperscript{54} (from J. Kennison, NIH); red e (from T. Breen, Southern Illinois University); Df(3R) e\textsuperscript{64F}/TM2, Ubx\textsuperscript{1b}ry\textsuperscript{se} red e\textsuperscript{e} (from C. Nusslein-Volhard, Tübingen University, Germany). Additional stocks were either obtained from the \textit{Drosophila} Stock Center (Bloomington, IN), or were constructed using standard genetic techniques. All balancer chromosomes used in this study are described in Lindsley & Zimm (1992).

(ii) Plasmid construction

All experimental manipulations of recombinant DNA were performed as described by Sambrook et al. (1989). The starting expression plasmid was pETGAGA, which contains the complete coding sequence of GAGA-519 (S. Alignay & J. T. Kadonaga, unpublished). A 1.9 kb XbaI/NheI fragment of pETGAGA (which contains the GAGA factor coding sequence, 41 bp of 5′ non-coding vector sequence, and 146 bp of 3′ non-coding vector sequence) was ligated into the XbaI site of pW8D44 (D. Johnson, unpublished). This recombinant plasmid was designated phsp70.GAGA. pW8D44 contains the hsp70 promoter, the multiple cloning site of pW8 (Klemenz et al., 1987), and approximately 2 kb of 3′ hsp70 sequences, including the polyadenylation site and transcription termination site (Amin et al., 1987). phsp70.GAGA was digested with SalI and a 2.2 kb restriction fragment containing the hsp70 promoter and the GAGA factor coding sequences was ligated into SalI-digested Carnegie 20T (Xiao & Lis, 1988). The resulting plasmid was designated pCarhsp70.GAGA (phG; Fig. 1).

(iii) \textit{Drosophila} germline transformation

Independent transformed lines (designated hG) were obtained following co-injection of phG and helper plasmid pr25.7wc into ry\textsuperscript{30e} embryos as described (Karess & Rubin, 1984; Spradling, 1986). The copy number and integrity of the transgenes were verified by Southern hybridization using probes for both GAGA factor and ry sequences (data not shown). The transformed lines were either made homozygous or
the transgenes were maintained over the appropriate balancer chromosome.

(iv) Antibody production and purification

Recombinant GAGA-519 (rGAGA factor) was purified from *E. coli* by cation exchange chromatography as described (Lu et al., 1993). rGAGA factor was further purified by SDS PAGE, and the excised gel slice was used to immunize rats as described (Amero et al., 1988). Antibodies were purified from serum by precipitation with ammonium sulphate followed by batch chromatography using DEAE cellulose (Harlow & Lane, 1988).

(v) Western blot analysis

Nuclei were isolated from 6- to 18-hour-old embryos as described (Gilmour et al., 1988) and the proteins were size-separated by SDS PAGE. The proteins were transferred to nitrocellulose and the filters were blocked with 3% non-fat milk in phosphate-buffered saline. Blocked membranes were incubated with a 1:2000 dilution of anti-GAGA factor antibody in Tris-buffered saline, pH 7.4, 0.05% Tween-20 (TBST) containing 1% bovine serum albumin (BSA). After washing, the membranes were incubated for 45 min with alkaline-phosphatase-conjugated goat anti-rat antibodies (Cappel/Organon Teknika, Durham, NC) in TBST/1% BSA. The secondary antibody was detected using 5-bromo-4-chloro-3-indolyi
phosphate/nitro blue tetrazolium (BCIP/NBT) according to the manufacturer’s instructions (Promega).

For analysis of heat-shock-induced expression, five flies carrying the hG transgene, or five *ry*<sup>506</sup> control flies, were heat shocked in a 37 °C incubator for 1 h and then frozen in liquid nitrogen. The flies were then homogenized in 100 µl of SDS PAGE sample buffer and the homogenates were boiled in a water bath for 3 min, chilled on ice, and centrifuged for 1 min to precipitate insoluble material. Twenty microlitres of the resulting supernatants were loaded onto a 12% polyacrylamide SDS gel for electrophoresis and western analysis as described above. In experiments in which the amount of induced GAGA-519 was quantified, purified rGAGA factor was added to control samples prior to boiling.

(vi) Genetic rescue of Trl alleles

Three transgenes – hG2 and hG14 on the second chromosome, and hG22 on the X chromosome – were tested for genetic rescue of Trl lethality by constructing stocks with hG/CyO; Trl/TM3, *Sb ry e* or hG22; Trl/TM3, *Sb ry e*, where Trl represents each of the alleles studied: *Trl<sup>62</sup>, Trl<sup>13C</sup>, Trl<sup>185</sup> or Trl<sup>667</sup>. The genotypes of these stocks were verified by genomic Southern blotting (data not shown). *Trl<sup>62</sup>* was used to test for rescue of heteroallelic combinations by crossing hG22; hG2/CyO; *Trl<sup>62</sup>/TM3, Sb ry e* females to males heterozygous for either *Trl<sup>185</sup>, Trl<sup>667</sup>* or *Trl<sup>13C</sup>*. A heteroallelic survival test of *Trl<sup>13C</sup>/Trl<sup>667</sup>* with hG2 was also performed. In all survival tests, vials were either maintained at room temperature or were heat shocked at 37 °C for 1 h per day. The presence of *Trl/Trl* progeny was assessed by the lack of dominant markers from the balancer chromosomes and was confirmed by Southern blot hybridization. Additional rescue tests were performed with two independent stocks in which *Trl<sup>62</sup>* had been recombinated onto a third chromosome marked with *red* and *e*. The genotypes of these recombinant stocks were verified by Southern blot hybridization. For larval rescue tests, *Trl<sup>62</sup>/CyO; Tb females were crossed with control *Trl<sup>62</sup>/Tb* males, or with hG22; hG2/CyO; *Trl<sup>62</sup>/Tb* or hG22; *Trl<sup>62</sup>/Tb* males. Pupating homozygous *Trl<sup>62</sup>* larvae (non-*Tubby* individuals) were counted and then removed from the vials over a period of 6 d. Between 200 and 300 pupae were scored for each cross.

(vii) PEV characterization

Male flies carrying the hG transgene, or *ry*<sup>506</sup> controls, were crossed to *y w<sup>ma</sup>* females. Control flies were raised at 25 °C while a test population was heat shocked at 37 °C for 1 h each day until eclosion. After eclosion, young adult flies were kept at 18 °C until further use. Eye pigment extraction was performed as...
previously described (Khesin & Leibovitch, 1978),

using extracts from \( y^w67c23 \) flies as a control. Five replicates were performed for each experiment.

3. RESULTS

(i) Heat-shock-inducible expression of hG transgenes

The \( P \)-element transformation plasmid, pCarhsp70.GAGA (phG) (Fig. 1), contains cDNA encoding the most abundant isoform of GAGA factor, GAGA-5. We recovered seven independent lines showing germline transmission of the \( ry^+ \) phenotype with a single, intact copy of the transgene. Western blot analysis was performed to verify that GAGA-5 is produced from the hG transgenes. Antibodies generated against purified, recombinant GAGA-5 (rGAGA factor) recognize a set of GAGA factor isoforms in embryo nuclei that range in size from 66 to 90 kDa (Fig. 2A). This pattern is consistent with that obtained using DNA affinity-purified GAGA factor from \( D. \) melanogaster embryo nuclei (Biggin & Tjian, 1988; Gilmour et al., 1988).

Adult flies containing the hG transgene, or \( ry^{106} \) controls, were heat shocked for 1 h at 37 °C. As expected, low but detectable levels of endogenous GAGA-519 were observed in extracts from adult flies in the absence of heat shock (Fig. 2B). Following heat shock, the protein band migrating with a molecular weight of approximately 66 kDa was observed to give a much stronger signal in extracts from transgenic flies (Fig. 2B). The size of this band corresponds precisely to that of the purified rGAGA factor used as the antigen (compare with lanes including rGAGA factor). Using known amounts of rGAGA factor for comparison (Fig. 2B, rGAGA factor lanes), we estimate that approximately 80 ng of GAGA-519 is induced per fly (total body mass of 0.7 mg). This is approximately a 100-fold increase over the level of

![Fig. 2. GAGA-519 can be overexpressed in hG flies. (A) Characterization of the anti-GAGA factor antibodies. A western blot analysis of \( D. \) melanogaster embryo nuclear proteins was performed using antisera raised against purified recombinant GAGA-519 (rGAGA factor). Each of two antisera tested (lanes 1 and 2), but not preimmune sera (lanes 3 and 4), recognizes a set of proteins ranging in apparent molecular weight from 66 to 90 kDa. The positions of the molecular weight markers are indicated at the left. (B) Heat-shock-induced GAGA-519. Adult hG flies, or \( ry^{106} \) controls, were either heat shocked for 1 h or maintained at room temperature. Crude homogenates from single flies were fractionated by SDS PAGE and analysed by western blotting. In the blot shown (line hG10), the location of GAGA-519 is marked with an arrow. The vertical bars mark a set of constitutive, cross-reacting proteins that appear to be unrelated to GAGA factor. The bottom portion of the filter was probed using an antibody against actin-capping protein \( \beta \) to verify equal loading in each well (data not shown). In the three lanes at the right of the blot, known amounts of rGAGA factor were mixed with homogenates from \( ry^{106} \) flies prior to SDS PAGE to provide a quantitative marker. (C) Stability of heat-shock-induced GAGA-519. hG2 flies, or \( ry^{106} \) controls, were heat shocked at 37 °C for 1 h and allowed to recover at 25 °C for the indicated lengths of time prior to homogenization and analysis by SDS PAGE and western blotting. The position of GAGA-519 is marked by an arrow. nhs, non-heat-shock control.](https://www.cambridge.org/core/terms). https://doi.org/10.1017/S0016672301005122
Table 1. Rescue of Trl mutants to adulthood by hG transgenes

<table>
<thead>
<tr>
<th>Parental genotype</th>
<th>Trl/Trl flies</th>
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</thead>
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<tr>
<td></td>
<td>25 °C</td>
</tr>
<tr>
<td><strong>Homoallelic stocks</strong></td>
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</tr>
<tr>
<td>hG2/CyO; Trl62/TM3</td>
<td>0 (n &gt; 1000)</td>
</tr>
<tr>
<td>hG14/CyO; Trl62/TM3</td>
<td>0</td>
</tr>
<tr>
<td>hG22; Trl62/TM3</td>
<td>0</td>
</tr>
<tr>
<td>hG22; hG2/CyO; Trl62/TM3</td>
<td>0</td>
</tr>
<tr>
<td>hG2/Trl62/TM3</td>
<td>0</td>
</tr>
<tr>
<td>hG2/CyO; Trl4085/TM3</td>
<td>0</td>
</tr>
<tr>
<td>hG2/CyO; Trl13C/TM3</td>
<td>0</td>
</tr>
<tr>
<td>hG2/CyO; Trl13C/TM3*</td>
<td>0</td>
</tr>
<tr>
<td>hG2/CyO; Trl1087/TM3</td>
<td>0</td>
</tr>
<tr>
<td>hsp83.GAGA; Trl13C/TM3</td>
<td>134 (n = 1307)</td>
</tr>
<tr>
<td>hsp83.GAGA; Trl13C/TM3b</td>
<td>118 (n = 948)</td>
</tr>
<tr>
<td><strong>Recombinant crosses</strong></td>
<td></td>
</tr>
<tr>
<td>Trl62 red e/TM3×hG2/CyO; Trl62/TM3</td>
<td>nd</td>
</tr>
<tr>
<td>Trl62 red e/TM3×hG22/Y; Trl62/TM3</td>
<td>nd</td>
</tr>
<tr>
<td>hG22; Trl62/TM3×Trl62 red e/TM3</td>
<td>nd</td>
</tr>
<tr>
<td>hG22; hG2/CyO; Trl62/TM3×Trl62 red e/TM3</td>
<td>nd</td>
</tr>
<tr>
<td><strong>Heterallelic crosses</strong></td>
<td></td>
</tr>
<tr>
<td>hG22; hG2/CyO; Trl62/TM3×Trl4085/TM3, Sh Ser</td>
<td>nd</td>
</tr>
<tr>
<td>hG22; hG2/CyO; Trl62/TM3×Trl4085/TM3</td>
<td>nd</td>
</tr>
<tr>
<td>hG22; hG2/CyO; Trl62/TM3×Trl13C/TM2, Ubx ry</td>
<td>nd</td>
</tr>
<tr>
<td>Trl4085/TM3×hG2/CyO; Trl13C/TM3</td>
<td>0 (n = 1273)</td>
</tr>
</tbody>
</table>

nd, not determined.
a Strain hG2/CyO; Trl13C/TM3 after c. 2 yr of culture (showing loss of heat-shock-inducibility of the transgene).
b Strain hsp83.GAGA; Trl13C/TM3 after c. 2 yr of culture.
c Maternal genotype appears first.

endogenous GAGA-519 in the adult fly. Similar results were obtained with all seven hG lines.

To measure the stability of the heat-shock-induced GAGA-519, hG2 flies were heat shocked for 1 h at 37 °C and were then allowed to recover at room temperature for different lengths of time prior to analysis by SDS PAGE and western blotting (Fig. 2C). Heat-shock-induced GAGA-519 was still easily detectable in these flies after 24 h. From these data we conclude that line hG2 produces large amounts of stable GAGA-519 following a 1 h heat shock. We therefore employed a heat shock protocol of 1 h per day at 37 °C in subsequent experiments to test the biological role of this isofrom.

(ii) Heat-shock-induced overexpression of GAGA-519 rescues some Trl mutants from lethality

Trl mutant homozygotes die mostly as late third instar larvae (Farkas et al., 1994; Bhat et al., 1996). As a functional assay for the heat-shock-induced GAGA-519, we tested whether hG transgenes were able to rescue both homo- and heteroallelic Trl mutants from lethality. Four alleles of Trithorax-like were used in this analysis. Trl62 has a transposable element inserted 5′ of the transcription start site; Trl13C has a transposable element inserted into an intron; Trl857 has a deletion within the transcribed region; and Trl4085 has a large deletion of the 5′ transcribed and translated region (Farkas et al., 1994). We constructed stocks that carry an hG transgene on the first (hG22) or second chromosome (hG2, hG14) as well as balanced lethal Trl mutations on the third chromosome (Table 1). The progeny were allowed to develop at room temperature or were heat shocked 1 h per day to induce hG. At no time did we observe surviving adults among Trl homozygous flies that did not contain the transgene (thousands of individuals examined; data not shown). Several investigators (Farkas et al., 1994; Bhat et al., 1996) have reported the recovery of homozygous Trl13C animals at a low frequency; however, under the growth conditions of our laboratory we have not observed any survivors. Of those stocks containing the transgene, Trl13C/Trl13C adults were recovered as 0-41% of the total progeny from heat-shocked bottles (Table 1). The absence of a wild-type copy of Trl on the third chromosome was confirmed by genomic Southern blots of the DNA from the rescued animals (data not shown). The number of rescued flies was much lower than the theoretically expected value of 33%.

In contrast to the results with Trl13C, we were unable to obtain genetic rescue of animals homozygous for Trl62, Trl857 or Trl4085 (Table 1, homoallelic
stocks). To eliminate the possibility that the Trl mutant chromosomes contain additional lethal mutations that are preventing the recovery of homozygous individuals, we examined heteroallelic combinations; in addition, we recovered lines with Trl82 recombined onto an unrelated third chromosome marked with red and e. Two such independent recombinant lines with Trl82 were tested with hG2 or hG22; however, we again failed to observe homozygous Trl82 animals (Table 1, recombinant crosses). Trl82/ TM3, Sb ry females carrying two copies of hG were crossed to males heterozygous for other Trl alleles. No Trl/Trl animals were observed in the progeny, indicating a lack of complementation among these Trl alleles and with the hG transgene (Table 1, heteroallelic crosses). However, when Trl867 females were crossed to Trl13c males carrying the hG transgene, Trl13c/Trl867 adults were recovered as 0·79% of the progeny under heat shock conditions (c. 5% of the theoretically expected number; Table 1). Trl13c/Trl13c and Trl867/Trl13c rescued adults can survive at room temperature no longer than 3 d and are sterile. From these data we conclude that GAGA-59 produced from hG rescues only hypomorphic Trl13c mutants.

Trl82 homozygotes normally die as late third instar larvae, although a small number pupate. Therefore, we examined whether one or two copies of hG could increase the number of homozygous Trl82 pupae. For this assay, the Trl82 allele was balanced over TM6B, Tb to facilitate scoring in larvae and pupae. In control crosses, with and without heat shock treatment (37 °C, 1 h per day), homozygous Trl82 pupae appeared on the second day following pupation of their Trl82/Tb siblings. After 4 d, approximately 4% of the total pupae were homozygous Trl82 animals. Trl82 homozygotes carrying two copies of hG pupated earlier than larvae lacking the hG transgenes. The total percentage of homozygous Trl82 pupae carrying hG22 and hG2 on the fourth day was approximately 7% without heat shock and was increased to 12% with heat shock treatment. Similar results were obtained with Trl82/TM6B, Tb larvae carrying hG22 alone. Eclosion of homozygous animals was not observed. From these data we conclude that although heat-shock-induced GAGA-59 does not rescue homozygous Trl82 flies to adulthood, the additional protein does shift the lethal phase.

Interestingly, after the stocks carrying the hG transgene (with and without Trl mutations) had been propagated for c. 2 y, the lines lost their ability to synthesize high levels of additional GAGA-59 in response to heat shock (data not shown). Further, these transgenic lines were no longer able to rescue flies homozygous for Trl13c, indicating a loss of inducible GAGA-59 activity (Table 1). We have also tested the efficiency of rescue by transgene hsp83.GAGA, in which a GAGA-59 cDNA has been placed under the control of the hsp83 promoter (Greenberg & Schedl, 1999). Initially, we obtained rescue of Trl13c homozygotes by hsp83.GAGA at a frequency of 10·3%, even in the absence of heat shock (Table 1, hsp83.GAGA: Trl13c/TM3). In a rescue experiment 2 years later, 12·4% of the progeny were homozygous for the Trl13c allele. This indicates that, unlike hsp70.GAGA, the efficiency of rescue by this transgene was unaffected by prolonged culture.

(iii) Expression of hG does not suppress PEV
Several Trl alleles have been shown to act as dominant enhancers of position effect variegation (PEV) (Dorn et al., 1993a, b; Farkas et al., 1994). We have also observed strong enhancement of PEV by Trl867 using different wmd4 stocks (data not shown). Several genes affecting PEV have been observed to show antipodal dosage effects, e.g., if one dose of the gene acts as an enhancer of PEV, three doses act as a suppressor (Wustmann et al., 1989; Sinclair et al., 1992; Eisenberg et al., 1992). Therefore we examined whether or not overexpression of GAGA-59 might suppress PEV of the wmd4 allele. As expected, the results showed that elevated temperature per se suppresses PEV (Spofford, 1976; Weiler & Wakimoto, 1995) (Fig. 3). The wmd4, Ry506 flies subjected to heat shock showed a 30% increase in eye pigmentation. However, neither hG2 nor hG14 suppressed PEV relative to the Ry506 control. Under non-heat-shock conditions, the hG chromosome may have slightly enhanced eye pigment variegation, reducing levels approximately 20%. E elevating the growth temperature of the wmd4; hG2 and wmd4; hG14 flies suppressed
PEV by 38% and 20%, respectively. This level of suppression was comparable to that obtained by elevating the growth temperature of the control flies.

4. Discussion

Using a cDNA transgene to produce GAGA-519 in a heat-shock-dependent fashion, we have demonstrated genetic rescue of flies with the Trl13c allele (Table 1). Other alleles could not be rescued; however, the lethal phase of Trl62 homozygous larvae was shifted to later stages. The Trl13c allele is a hypomorph, with decreased amounts of GAGA factor present in the flies. Under our laboratory conditions, heat shock induction of the transgene was essential for rescue. This result is consistent with the observation that the loss of heat-shock inducible GAGA-519 synthesis following prolonged culture (c. 2 yr) of the hG flies was accompanied by a loss of heat-shock-inducible rescue.

Several transcripts are produced from the Trl locus (Soeller et al., 1993; Farkas et al., 1994), and some of these transcripts are alternately spliced to encode different isoforms of GAGA factor. GAGA-519 and GAGA-581 differ in the glutamine-rich C-terminal domain (Benyajati et al., 1997). No major differences have been reported concerning the behaviour of these isoforms in vitro (Benyajati et al., 1997). However, western blots specific for GAGA-519 or GAGA-581 do show different developmental and tissue-specific profiles (Benyajati et al., 1997), indicating specific regulation in their synthesis and suggesting specificity in function. That the two isoforms are functionally different in vivo is further suggested by experiments with hsp83.GAGA cDNA transgenes, encoding either the 519 aa or 581 aa isoforms expressed using the Drosophila hsp83 gene promoter. The hsp83.GAGA-519 transgene is more effective than the hsp83.GAGA-581 transgene in reversing the Ubx phenotype and in preventing sterility, although the two transgenes rescue flies from lethality with similar efficiency (Greenberg & Schedl, 1999; A. Greenberg & P. Schedl, personal communication).

The results suggest that other GAGA factor isoforms, in addition to GAGA-519, may be required for viability. Given the genomic changes of the mutations (Farkas et al., 1994), one can infer that Trl62, Trl13c and Trl185 may eliminate several or all of the known transcripts, whereas some production of all isoforms might persist in Trl13c (having a P-element insertion into an intron). In fact, Bhat et al. (1996) have shown that all the GAGA factor isoforms can be observed in ovarian tissue from Trl13c homozygotes, although the levels of these proteins appear to be reduced compared with wild-type flies. The production of GAGA-519 from hG presumably supplies critical amounts of that isoform, allowing rescue of some Trl13c homozygotes. However, hG apparently fails to compensate for the reduction or loss of other GAGA factor isoforms inferred to occur in response to other Trl mutations.

Alternatively, the amount or timing of synthesis of GAGA-519 from the hG transgene might be insufficient for the needs of the animal and may, in fact, be deleterious. The ability of GAGA factor in vitro to protect weak minimal GAG binding sites, as well as the size of the DNase 1 footprint on natural promoters, is concentration dependent (Katsani et al., 1999). The 100-fold increase in GAGA-519 levels following heat shock of hG lines may result in the misexpression of many genes. In addition, over-expression of GAGA-519 might cause inappropriate interactions between GAGA-519 and other nuclear proteins. Numerous studies have shown that protein–protein interactions are mediated by GAGA factor’s BTB/POZ domain and by the glutamine-rich C-terminal domain (Bardwell & Treisman, 1994; Chen et al., 1995; Espinás et al., 1999; Katsani et al., 1999; Wilkins & Lis, 1999). GAGA factor from D. melanogaster embryo nuclei is recovered within the void volume following gel filtration chromatography (Gilmour et al., 1989; H. Granok & S. C. R. Elgin, unpublished observations). This indicates that GAGA factor is part of a large multiprotein complex and does indeed participate in protein–protein interactions in vivo. Thus, overproduction of GAGA-519 might improperly change the activity of many genes by both direct and indirect mechanisms.

Support for this possibility comes from comparing the results obtained using the hsp70.GAGA transgenic lines described above with results obtained using the hsp83.GAGA transgenic line (Table 1). The hG transgenes produced a 100-fold increase in GAGA-519 levels following heat shock. Although the hsp83.GAGA transgene does not produce a measurable change in GAGA-519 levels, even upon heat shock (A. Greenberg & P. Schedl, personal communication; B. Leibovitch & S. C. R. Elgin, unpublished observations), this transgene provides 25-fold better survival of both Trl13c/Trl13c and Trl13c/Trl185 flies (Table 1) than does the hsp70.GAGA transgene. A negative impact from excess GAGA-519 might contribute to the observed loss of activity in the hG lines upon prolonged culture; such loss is not observed with the hsp83.GAGA line (Table 1). Clearly, the hsp83.GAGA transgene is more effective in rescue experiments than the hsp70.GAGA transgene, due either to the amount of GAGA-519 produced or to the pattern of expression.

The results shown in Fig. 3 indicate that supplemental GAGA-519 does not suppress PEV. It is possible that GAGA factor is already present above stoichiometric amounts in wild-type flies, so no suppressive effects on PEV are observed upon over-
expression of GAGA-519. Alternatively, the lack of effect of supplemental GAGA-519 on PEV may simply be the consequence of misexpression of this protein, as mentioned above. In particular, supplemental GAGA-519 might be sequestered in inclusion bodies (Agianian et al., 1999) rather than forming productive complexes within chromatin.

We conclude that while artificial expression of the 519 aa isoform of GAGA factor is sufficient to compensate for a partial loss of the protein in vivo, it is not sufficient to perform all the required functions of this important protein. Further, D. melanogaster appears to be sensitive to the level of GAGA-519 produced; whether this reflects the total amount of protein, the ratio of isoforms, or both, is not yet resolved. Modifications of the expression system or of the expressed products may allow for the dissection of some of these requirements.

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References


