Using a Drosophila transgenic system we investigated the ability of GAGA factor, a putative anti-repressor, to modulate transcription-related events in the absence or presence of a bona fide activator, the Adf-1 transcription factor. In contrast to previous in vitro and in vivo data linking the binding of GAGA factor to the acquisition of DNase hypersensitivity at heat shock promoters, we observed that inserting multiple GAGA binding motifs adjacent to a minimal alcohol dehydrogenase (Adh) promoter led to strongly elevated embryonic transcription without creation of a promoter-associated DNase-hypersensitive (DH) site. Establishment of DNase hypersensitivity required the presence of both GAGA and Adf-1 binding sites and was accompanied by a further, synergistic increase in transcription. Because Adf-1 is capable neither of establishing a DH site nor of promoting efficient transcription by itself in embryos, it is likely that DH site formation depends on a GAGA factor-mediated binding of Adf-1 to chromatin, perhaps facilitated by a locally remodeled downstream promoter region. More generally we suggest that GAGA factor-binding sequences may operate in a promoter-specific context, with transcriptional activation, polymerase pausing, and/or DH site formation critically dependent on the nature of the sequences (and their binding partners) linked in cis.

Control of eukaryotic transcription is a complex, tightly regulated process requiring the action of many distinct proteins, including chromatin-interacting/modifying proteins, transcriptional activators, and general transcription factors (GTFs) (for review see Refs. 1–3). In addition to recent progress in determining the role of chromatin-interacting proteins, unraveling the mechanisms by which sequence-specific, DNA-binding activators promote transcription has traditionally occupied a good deal of effort in the field. The consensus emerging from studies on many activators is that a significant activity of these proteins is to recruit components of the general transcription machinery and/or chromatin-interacting factors to promoters through direct protein-protein interactions (4).

The Drosophila GAGA factor is unusual in that its activity in transcription appears to be neither that of a purely sequence-specific transactivator nor that of an ATP-dependent chromatin-interacting factor. GAGA protein was first identified as a transcription factor that bound a repetitive GA element upstream of the engrailed (5) and Ultrabithorax (6) promoters. So-called GAGA elements have subsequently been identified in numerous promoters, including those controlling housekeeping, developmentally regulated, and inducible genes (7). GA repeats appear to be important in the acquisition of constitutive DNase I-hypersensitive (DH) sites found at the transcriptionally inactive but inducible hsp26 (8) and hsp70 (9) promoters in vivo. Moreover, in concert with the ATP-dependent nucleosome remodelling factor (NURF), GAGA factor aids in the local remodelling of GAGA element-containing chromatin templates in vitro (10–12), including the establishment of DH sites at an hsp70 promoter (10). Other in vitro studies have revealed that although GAGA factor stimulates transcription of repressed promoters, it does not activate transcription above basal (i.e. nonrepressed) levels (12–14). Null mutations of the Trithorax-like (Tri) gene, which encodes GAGA factor, result in larval lethality (15), whereas less severe alleles show enhancement of position effect variegation as well as deficiencies in chromosome condensation, segregation, and nuclear cleavage (15, 16). Taken together, the data suggest a role for GAGA factor in establishing or maintaining particular chromatin structures. A model has emerged where, when located in the vicinity of a promoter, GAGA factor facilitates promoter activation not by actively recruiting GTFs but rather by functioning as an anti-repressor molecule. GAGA factor binding is proposed to promote local displacement or disruption of nucleosomes in creating a DNase I-hypersensitive site, thereby allowing bona fide activators and/or the general transcription machinery to gain access to DNA at promoter regions (Refs. 10 and 17; for review see Ref. 18).

Because much of the aforementioned work on GAGA factor has employed complex, multi-factor binding promoters or in vitro conditions, we sought to clarify the activity of GAGA factor in vivo in the context of a defined promoter at the level of both transcription and chromatin perturbation. In addition, it was of interest to ask the same question of a bona fide activator for direct comparison. For this latter purpose we chose Adf-1, first identified as a regulator of the alcohol dehydrogenase (Adh) distal promoter (19) and subsequently shown to bind the antennapedia P1 and dopa decarboxylase promoters (20) and the fz-proximal enhancer (21). In our experiments, therefore, we have analyzed Drosophila carrying transgenes consisting of GAGA factor or Adf-1-binding elements, either alone or in combination, upstream of a minimal Adh distal promoter. Un-
expectedly, we find that GAGA factor alone is able to stimulate embryonic transcription markedly in vivo, whereas Adf-1 is a relatively poor activator when operating unaided in these cells. As might be anticipated, the highest levels of transcription are achieved through a synergistic contribution of these two independent activities. A second, unforeseen result is that a promoter-proximal DH site is established only in the presence of both activities rather than via the action of GAGA factor alone. Therefore, in the context of the Adh distal promoter, GAGA factor and Adf-1 synergize both at the level of activating transcription and of modulating chromatin structure.

EXPERIMENTAL PROCEDURES

Construction of the P Element Constructs and Transgenic Lines—All recombinant DNA procedures were performed using standard techniques (22); full details can be provided upon request. In outline, a minimal derivative of the Drosophila distal Adh promoter (~41 to +20) driving a luciferase reporter gene was isolated from p41ADHLUC (kind gift from A. Brasier) and subcloned into the EcoRV site of pBlueScriptSKII –) (Stratagene) to generate pSKL. The su(Hw)-binding element of the gypsy retrotransposon was isolated by Sall digestion of pGBaX (23) and inserted at the XhoI site of pSKL to generate pSKLS. A basic GAGA element, lacZ gene flankned by promoter and terminator sequences, and T7 RNA polymerase was cloned into the BamHI site of pSKLS, in a divergent orientation relative to the Adh-luciferase transcription unit, to generate pSKZLS. For the GAGA construct, single-stranded synthetic oligonucleotides comprising the proximal GAGA element of Drosophila hsp26 (~125 to ~80) (along with appropriate flanking restriction sites) were annealed and inserted between the PstI and Smal sites of pSKZLS, i.e., in the region between the T7 RNA polymerase and Adh promoters, to generate pSKZGLS. For the Adf-1 and GAGA+Adf-1 constructs, we performed polymerase chain reaction using p89ADHLUC (kind gift from A. Brasier) to generate an approximately 180-bp product spanning regions ~89 to +20 of the Adh distal promoter fused to the luciferase gene and used this product to replace the ~41Adh promoter derivative in both pSKZLS and pSKZGLS to generate pSKZALS and pSKZGLS. Luciferase activity was measured using a luminometer (Lumat LB 9500; Berthold). Theraf-1 construct contains the binding element for Adf-1 in its natural context upstream of the Adh promoter (~89 to ~42). The GAGA construct contains the proximal GAGA element (~125 to ~80) of the Drosophila hsp26 regulatory region. The GAGA+Adf-1 construct consists of both elements upstream of the promoter. Pertinent restriction sites: B, BamHI; P, PstI; A, AvaII. The angled arrows above each construct indicate the approximate positions of transcription start sites, whereas open arrows below each construct indicate the additional GAGA sites present in the Adh promoter (see “Discussion”).

in 50% bleach for 3 min, washed thoroughly, placed into a microcentrifuge tube containing 100 µl of lysis buffer (1% Triton X-100, 25 mM glycylyglycine, 15 mM MgSO4, 4 mM EGTA, 1 mM dithiothreitol, pH 7.8), and homogenized using a Kontes pestle. For adult extracts, 10 anesthetized flies were homogenized in 100 µl of lysis buffer. Both extracts were cleared by 5 min of centrifugation at 4°C. 10–20 µl of extract was analyzed for luciferase activity using the luciferase reporter assay system (Promega). Luciferase activity for each sample was normalized for protein concentration and adjusted for background luminescence by subtracting the signal obtained from samples derived from the non-transgenic parental line.

RESULTS

Strategy for Investigating the Role of GAGA Factor in Gene Activation in Vivo—To systematically investigate the in vivo activity of GAGA repeats and presumably GAGA factor binding, our approach was to add an extensive GAGA element upstream of either a minimal promoter or a promoter containing the binding site for an activator, linked to a reporter gene. We assayed homozygous transgenic lines generated by P element-mediated transformation (25) carrying one of four possible constructs for their effects on luciferase reporter gene activity and perturbations in chromatin structure.

GAGA Factor and Adf-1 Synergize to Activate Transcription—Both GAGA factor and Adf-1 are known to be players in the transcriptional activation of specific RNA polymerase II-regulated genes in Drosophila. Adf-1 is believed to recruit specific GTFs to the promoter (27). GAGA repeats have been shown to be important for transcription in vivo of the hsp26 (28), hsp70 (29), and Ultrabithorax (30). For our study we utilized the hsp26-proximal GAGA element. This element is one of the longest GAGA repeats characterized, with 35 nucleotides protected in footprinting assays, most likely reflecting the binding of multiple GAGA proteins along its length (31, 32). We created the following transgenic lines (Fig. 1): control, consisting of a TATA box-containing promoter derived from the distal tran-
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**FIG. 2.** GAGA factor and Adf-1 work synergistically to activate embryonic transcription in vivo. Whole cell extracts derived from 0–12-h transgenic embryos were analyzed for luciferase activity. Bars represent activity from a minimum of three independent experiments for each indicated transgenic line. RLU, relative light units.

**TABLE I**

Summary of transgene-specific transcriptional activity

| Transgenic construct | Average RLU per mg (~10⁻⁶) | Fold activation |
|----------------------|-----------------------------|-----------------|
| **Embryo**           |                             |                 |
| Control              | 2.15 ± 0.70                 |                 |
| Adf-1                | 7.02 ± 2.84                 | 3.3             |
| GAGA                 | 32.32 ± 3.98                | 15.0            |
| GAGA + Adf-1         | 62.57 ± 10.07               | 29.1            |
| **Adult**            |                             |                 |
| Control              | 0.03 ± 0.007                |                 |
| Adf-1                | 0.19 ± 0.009                | 6.4             |
| GAGA                 | 0.14 ± 0.002                | 4.9             |
| GAGA + Adf-1         | 1.89 ± 0.057                | 64.4            |

In whole cell extracts of 0–12-h embryos from the various transgenic lines, luciferase transcription is increased slightly (3-fold) in the transgenic lines, luciferase transcription is increased slightly in the multiple lines analyzed (34).

In vivo Effects of GAGA Factor on Nucleosome Integrity—Because the GAGA transgenes exhibited a strong stimulation of transcription but no detectable DH site formation, we sought to determine what kind of more subtle changes, if any, in chromatin structure might be associated with the presence of GAGA factor adjacent to the Adh promoter. Brief treatment of chromatin with MNase followed by gel analysis of purified DNA reveals a pattern of DNA fragments increasing in size by approximately 200-base pair increments derived from digestion within linker DNA at mono-, di-, tri-nucleosomal, etc., intervals. MNase analysis of the hsp70 (10), hsp26 (11), and ftz (12) promoters reconstituted into chromatin in vitro showed that addition of GAGA factor, in the presence of both embryonic nuclear extract and ATP, led to a localized disruption of the nucleosomal array at the GAGA sequences. Because both cofactors were essential, it was suggested that the observed re-modeling depended critically on the presence of NURF in the extract. To assess the ability of GAGA factor to locally remodel nucleosomal arrays in vivo on a simple model promoter, we conducted MNase digestion analysis on embryonic nuclei derived from each of the transgenic lines.

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Short probes located immediately adjacent to (and on either side of) the insertion site can reveal the local effects of individual inserts across the various lines. Using a sequence probe derived from directly downstream of the insertion (encompassing both the Adh promoter and the immediately 5’ portion of the luciferase gene), those lines that contain the GAGA element reveal an altered, much less distinct, local nucleosomal pattern relative to the control and Adf-1 lines (Fig. 4A, compare lanes 5–8 with lanes 1–4; densitometric tracing of lanes 2, 4, 6, and 8 is shown in Fig. 4D). Probing immediately upstream of the insertion site reveals rather little in the way of obvious perturbation in comparing any of the lines (Fig. 4B; see Fig. 4E for a densitometric trace). Moreover, when the same blot is probed with a DNA sequence derived from the endogenous...
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**Fig. 3.** A promoter-proximal DNase I-hypersensitive site is present only in the GAGA+Adf-1 line. DH sites (indicated by asterisks) were mapped by indirect end labeling in embryos of the following transgenic lines: control (lane 1), Adf-1 (lane 4), GAGA (lane 3), and GAGA+Adf-1 (lane 11). A, purified DNA was digested to completion with SstI and EcoR I09I and analyzed by Southern blot with a probe consisting of the 593-bp EcoRI-EcoR I09I fragment of luciferase as indicated. Amount of DNase per sample: 0 units (lanes 3, 4, 7, and 10), 30 units (lanes 1, 6, 9, and 12), or 60 units (lanes 1, 6, 9, and 12). Lanes 13 and 14 contain restriction-digested control plasmid DNA as location markers (M); BamHI and AvaII sites adjacent to the promoter are indicated by arrows at the side. B, DNA samples identical to those in A were digested to completion with BamHI and analyzed by Southern blot with a probe consisting of an ~2-kilobase BamHI-EcoRI fragment derived from the endogenous hsp26 gene. Amount of DNase per sample: 0 units (lanes 1, 5, 9, and 13), 10 units (lanes 2, 6, 10, and 14), 30 units (lanes 3, 7, 11, and 15), and 60 units (lanes 4, 8, 12, and 16).

hsp26 (Fig. 4, C and F), a regular and distinct nucleosomal ladder is present in all four of the transgenic lines, showing that the perturbations observed in the GAGA and GAGA+Adf-1 lines in Fig. 4A were genuine and not an experimental artifact. Because the same blot was used to produce the clearly differing data of Fig. 4 (compare A with B), it appears that the perturbations seen are extremely localized and directed downstream of the insertion site over the promoter and/or transcribed region. Lastly, because these downstream perturbations appear to be unequivocally associated with the presence of the GAGA element (none seen in the control or Adf-1 lines), they exist independently of the presence of both the Adf-1-binding site in DNA and the DH site in chromatin.

In a further attempt to measure the extent and location of GAGA factor-associated chromatin structure perturbation, we investigated restriction enzyme accessibility in and around the

**Fig. 4.** Effect of the hsp26-proximal GAGA element on the local nucleosomal array. Following MNase treatment of embryonic nuclei derived from various transgenic lines (as in Fig. 3), genomic DNA was isolated and analyzed by Southern blot. A single filter was probed with three different DNA fragments: a 220-bp fragment encompassing the Adh promoter and 5’ region of the luciferase gene (A), a 140-bp fragment from the immediately upstream region (B), and a fragment derived from the coding region of the endogenous hsp26 gene (as in Fig. 3B) (C). Units of MNase: 7.5 units (lanes 1, 3, 5, and 7) and 15 units (lanes 2, 4, 6, and 8). D–F, a densitometric trace was made of the nucleosomal array for each of the various transgenic lines shown in A–C, respectively. Lanes 2, 4, 6, and 8 from each autoradiogram were analyzed using NIH Image software. A schematic of the control transgene with probe size and location indicated by the heavy black bars is shown below; the location of the various insertions is indicated by the open triangle.
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**DISCUSSION**

Role of GAGA Factor in Trans-activation versus Chromatin Perturbation—GAGA factor has been classified as a chromatin anti-repressing factor rather than a conventional activator (for review see Ref. 18). Carefully controlled in vitro experiments showed that GAGA factor had no overt transcription activating ability on naked DNA templates (13, 14) but could relieve the repression of basal transcription at a linked promoter assembled into chromatin when the remodeling factor NURF was present (12). The observed stimulation of transcription by GAGA factor in crude extracts or transient assays (35, 36, 42) might then reflect its ability to partake in the suppression or destabilization of the many nonspecific DNA-protein interactions that are likely to occur on DNA templates in such assays.

With GAGA factor characterized purely as an anti-repressor, therefore, we were intrigued to find that the placement of high affinity GAGA-binding sites adjacent to a minimal Adh TATA promoter was sufficient for robust stimulation of transcription in an in vivo chromatin context (Fig. 2 and Table I). Two alternative, although not mutually exclusive, explanations could account for this activity. First, localized chromatin destabilization or nucleosome sliding in the vicinity of the TATA promoter, modulated in part by GAGA factor binding to its adjacent recognition site (presumably aided by the remodeling activity of the NURF complex Refs. 10 and 43), might allow access of the general transcription machinery to the promoter via binding of TATA-binding protein and the GTFs. In other words the stimulation of in vivo transcription purely by GAGA factor, as seen here, would be analogous to its in vitro ability to establish basal transcription. Considered in this light, the presence of promoter-proximal GAGA elements might be a way of achieving a measure of constitutive transcription in vivo against the normally repressive chromatin background. The data presented in Figs. 4A and 5A show a perturbation of the promoter region and/or downstream nucleosomal array in the presence of the GAGA element, but because the transcription machinery is clearly also present and functional in these cases, it is not possible unambiguously to ascribe the cause of this downstream chromatin disruption to GAGA factor binding alone (though neither can it be ruled out on this evidence). The reduction in accessibility of the upstream BamHI site when GAGA elements are present (Fig. 5B) would be consistent with a GAGA factor-induced sliding of a nucleosome into this location, an effect of GAGA factor binding to an in vitro reconstituted chromatin template that was noted by others (11). However, when we attempted to map nucleosomal positions in the promoter region by a relatively low resolution indirect end labeling analysis of MNase digested samples, we found no clear difference in the cleavage patterns of the control lines compared with the GAGA lines (data not shown), suggesting the effects of GAGA factor on transcription were not related to a major repositioning of the nucleosomal array at the promoter. Only in the GAGA+Adf-1 line did we observe an obvious change in the promoter-proximal MNase cleavage pattern, consistent with the unique establishment of a DH site in these lines (shown in Fig. 3). A second interpretation of the GAGA activity seen here is that GAGA factor acts as a conventional activator that (directly or indirectly) leads to active recruitment of the general transcription machinery. GAGA factor does have a glutamine-rich region, a domain found in one class of activator proteins (44), and whereas this domain can clearly lead to self-aggregation of GAGA factor (45, 46), the only transcription factor so far known to interact with it is heat shock factor (47). More interestingly perhaps, this GAGA domain has recently been found to promote distortion of downstream DNA (45), a perturbation that could facilitate the binding of TATA-

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**FIG. 5. Accessibility of chromatin to restriction enzymes in the transgenic lines.** Isolated embryonic nuclei were incubated with increasing amounts of the indicated restriction enzyme. Purified DNA was subjected to indirect end labeling analysis as described in Fig. 3A; the location of the relevant restriction sites is depicted in Fig. 1. A, PstI digestion: 0 units (lanes 1, 4, and 7), 150 units (lanes 2, 5, and 8), and 350 units (lanes 3, 6, and 9). B, BamHI digestion: 0 units (lanes 1, 4, and 7), 150 units (lanes 2, 5, and 8), and 350 units (lanes 3, 6, and 9). The percentage site accessibility was calculated as the density ratio of the digested fragment relative to the sum of the parent band (P) plus the digested fragment using a Molecular Dynamics PhosphorImager and ImageQuant software. Each individual line was analyzed a minimum of three times, and the ratio given (indicated below the bar under the appropriate samples) represents the average site accessibility across a minimum of two independent lines for each transgenic construct. (The control samples in lanes 1–3 of A are relatively overloaded, but the density ratio shown is directly comparable with those of the other samples in A.)

A promoter region of the transgenes. A PstI site lies directly 3’ of the GAGA element, whereas a BamHI site is approximately 60 base pairs upstream of its 5’ end (Fig. 1). In embryonic nuclei, PstI accessibility within the transgene increases moderately in the GAGA lines and is highest in the GAGA+Adf-1 lines (Fig. 5A). In contrast, the level of BamHI (Fig. 5B) accessibility is, if anything, moderately reduced in the GAGA element-containing lines. These results signify a fairly localized and modest downstream perturbation effect and are in general agreement with the MNase data. In addition, we monitored the ability of T7 RNA polymerase (T7RP) to transcribe from its promoter located around 30 bp upstream of the insertion site (Fig. 1). T7RP is a single polypeptide enzyme that has been used previously as a probe for monitoring DNA accessibility in chromatin (for examples, see Refs. 40 and 41). The addition of T7RP to nuclei isolated from a GAGA+Adf-1 line produced no increase in RNA transcripts relative to the amount generated in nuclei from the control line (data not shown), confirming that the chromatin perturbation in the GAGA+Adf-1 line is localized to the Adh promoter and/or the luciferase transcribed region and does not extend into a closely adjacent upstream region.
binding protein and/or GTFs in accord with the general mechanism envisioned by the first model.

It is particularly important to consider our in vivo transcription data in the light of extensive transcription studies performed on GAGA element-containing Drosophila heat shock promoters. A distinctive property of these promoters is that, in the absence of an activated transcription factor (i.e. the heat shock factor protein), RNA polymerase II is present on the promoter in a transcriptionally initiated, but “paused” condition, a feature that is critically influenced by the presence of upstream GAGA elements (9, 17, 29). To a limited extent then, our data are in agreement, inasmuch as we observe GAGA-dependent loading of polymerase (measured by strongly increased transcription in the GAGA lines) in the absence of an overt transactivator known to function via recruitment. However, a crucial difference is that although the minimal Adh promoter used here clearly allows passage of RNA polymerase II into productive elongation under the influence of adjacent GAGA elements, the initiated polymerase at heat shock promoters falls into arrest immediately downstream and can only be released into elongation upon binding of the activated heat shock factor protein to sites upstream from the promoter. Recent in vitro reconstitution of a paused polymerase on the Drosophila hsp70 gene strongly suggests that an important element of pausing is inherent to the sequence contained within the heat shock promoter itself (48, 49). The absence of such sequence elements (or, conversely, presence of different elements) in the minimal Adh promoter, provides a plausible (and testable) explanation for why productive elongation is able to take place in the presence of GAGA factor alone in the experiments reported here. We are currently conducting promoter swap experiments to assess this potential promoter-specific contribution to the in vivo activity of GAGA factor.

Multiple GAGA Elements Are Insufficient for DH Site Establishment—In considering previous studies on the structural consequences of GAGA factor binding conducted on in vitro reconstituted chromatin templates (particularly at heat shock promoters), the absence of a DH site in vitro at the promoter of the transcriptionally active transgenic GAGA lines (Fig. 3, lanes 8 and 9) is a further unexpected result. Its absence is unlikely to be related to the demonstrated requirement for three or more independent GAGA elements to generate a DH site in vitro (10). In our constructs, there are two short GAGA sites in the minimal Adh promoter itself that are known to be binding targets both in vitro (33) and in vivo (50), as well as the 42-bp GAGA element derived from hsp26, a sequence that appears capable of binding multiple copies of the GAGA factor (33). Although GAGA elements contribute to the full manifestation of DNase I hypersensitivity at heat shock promoters, it appears that other promoter-proximal sequence elements and nonhistone trans-acting factors play some role in its establishment (see Refs. 8–11, 43, and 51). Again we suggest that one or more such sequence elements are absent from the minimal Adh promoter studied. Furthermore, it is important to reiterate that our data reflect the results of a true in vivo situation, where the levels of GAGA factor are natural and not achieved by in vitro manipulation or use of a purified or recombinant protein. Our results show that gene “activation,” mediated here by GAGA factor, is not necessarily accompanied by DH site formation at the promoter either as a necessary prerequisite for or consequence of transcription. In fact, we feel strongly that the differential manifestations of GAGA activity seen here, in comparison with studies on heat shock promoters in particular, are rather convincing indicators of a promoter-specific context to the function of GAGA factor, with the nature of cis-linked sequences playing a critical role in the process.

The promoter-proximal DH site seen in the GAGA + Adf-1 lines (Fig. 3) may reflect a DNA structural perturbation created by the efficient binding of Adf-1 in the presence of GAGA factor. Gao and Benyajati (33) have noted that Adf-1 binding to DNA in vitro creates a strong structural perturbation 3’ of the Adf-1-binding site, but neither the binding of Adf-1 nor the DNA distortion are influenced by adjacent GAGA factor binding; apparently there is no direct interaction between the proteins. We propose that in the context of the multiple GAGA element-containing chromatin template studied here, both transcriptional synergy and DH site establishment are readily explained by cooperative binding of Adf-1 and GAGA factor to chromatin. This occurs independently of protein-protein contacts between the two species (33) but may be facilitated by altered histone-DNA interactions or downstream DNA distortions mediated either by the GAGA factor itself or in conjunction with NURF (Fig. 4). A similar cooperative mechanism dependent on structural perturbation has been advanced to account for high levels of activation in model yeast extrachromosomal plasmid templates (52).

GAGA Factor Activities in Context—These observations highlight the point that gene activation may be achieved by a variety of complementary, often synergistic, mechanisms, and it will usually be the case that a hierarchy of different activities are assembled at individual promoters to allow fine tuning of the level of gene expression. It is certainly intriguing to note that GAGA elements are found linked to a number of different types of promoter in Drosophila, some inducible, some developmentally regulated, and some constitutively active (7, 18). Moreover, GAGA factor plays other roles in chromosomal metabolism that are not obviously related to transcription (53, 54). Our results, together with those of others, show that the transcriptional effects of linking GAGA elements to a promoter and the corresponding influence on the local structure of chromatin may depend rather critically on the promoter context from which the data are obtained. The biochemical activities and potential interacting partners of the various GAGA factor isoforms are at present either ill-defined or undefined. Others have described transcription factors, e.g. GAL4-VP16 or NF-κB, that are most likely able to combine both an anti-repressing and a transactivation function in a single entity (55, 56). It may be that GAGA factor, given a particular promoter or other cis-linked sequence context, can display one or more such capabilities depending on the combinatorial opportunities presented by that promoter sequence.

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