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Dynamics of potentiation and activation: GAGA factor and its role in heat shock gene regulation

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ABSTRACT

GAGA factor (GAF) binds to specific DNA sequences and participates in a complex spectrum of chromosomal activities. Products of the *Trithorax-like locus* (*Trl*), which encodes multiple GAF isoforms, are required for homeotic gene expression and are essential for *Drosophila* development. While homozygous null mutations in *Trl* are lethal, heterozygotes display enhanced position effect variegation (PEV) indicative of the broad role of GAF in chromatin architecture and its positive role in gene expression. The distribution of GAF on chromosomes is complex, as it is associated with hundreds of chromosomal loci in euchromatin of salivary gland polytene chromosomes, however, it also displays a strong association with pericentric heterochromatin in diploid cells, where it appears to have roles in chromosome condensation and segregation. At higher resolution GAF binding sites have been identified in the regulatory regions of many genes. In some cases, the positive role of GAF in gene expression has been examined in detail using a variety of genetic, biochemical, and cytological approaches. Here we review what is currently known of GAF and, in the context of the heat shock genes of *Drosophila*, we examine the effects of GAF on multiple steps in gene expression.

HEAT SHOCK GENES AS A MODEL SYSTEM

The heat shock genes of *Drosophila melanogaster* provide a powerful system for studying the mechanics of transcriptional regulation. Transcription from major heat shock loci can be increased >100-fold upon heat shock (1). This robust and rapid response triggers a high level of induction in as little as 3 min (2) and is mediated by factors present in cells prior to induction (3). The strength and speed of the heat shock response facilitates detection of specific regulatory components and is highly conducive to quantitative and mechanistic studies of these components. Extensive studies of heat shock genes from a number of laboratories provide a firm groundwork on the chromatin structure, key regulatory factors, and DNA sequence elements needed for efficient expression (4,5; Fig. 1). Since both the upstream sequence and core promoter elements of heat shock genes are functionally similar to and interchangeable with elements of other genes transcribed by RNA polymerase II (pol II), heat shock studies are likely to be extremely pertinent to general transcriptional regulation (6–8). One particular factor, the GAGA factor (GAF), has been implicated in numerous steps in heat shock gene regulation, as well as in the regulation of a variety of housekeeping and developmentally regulated genes in *Drosophila* (Table 1). This review will focus specifically on the contributions of GAF to the regulation of heat shock gene transcription and its function in the establishment of a 'potentiated' promoter.

GAGA FACTOR: ANTIREPRESSOR OF CHROMATIN STRUCTURE

GAF was originally identified by its ability to stimulate transcription from the *engrailed* (*en*) and *ultrabithorax* (*Ubx*) promoters *in vitro* (9,10). GAF binds to CT-rich sequences found in these promoters and those of numerous other *Drosophila* genes (11; Table 1). GAF elements are comprised of dinucleotide repeats, $(CT{\cdot}GA)_n$, with a consensus of 3.5 repeats, although some elements can be found composed of much longer arrays (28). GAF elements are often found interspersed or overlapping with other key regulatory elements and have been shown to contribute to transcription factor occupancy of these non-GAF elements *in vivo*. This is best demonstrated with respect to heat shock factor (HSF) occupancy of heat shock elements (HSE) on the *hsp70* gene (12). Though GAF appears to have many target genes, considerable effort has been directed towards zeroing in on the contribution of GAF to heat shock gene regulation, focusing specifically on the *hsp70* (12–14) and *hsp26* genes (15–17). GAF appears to have a major role in heat shock gene regulation, and studies of its function will most certainly have an impact on our general understanding of transcriptional regulation.

Compared with the majority of transcription factors that have been characterized, the role of GAF is a bit unconventional. *In vitro* studies indicate that GAF seems to exert its effect not by activating or repressing the transcriptional machinery directly, but by mitigating the effects of histones. Unlike the transcription activators Sp1 and Gal4, GAF cannot activate transcription from a naked DNA template. It can, however, do so in the presence of

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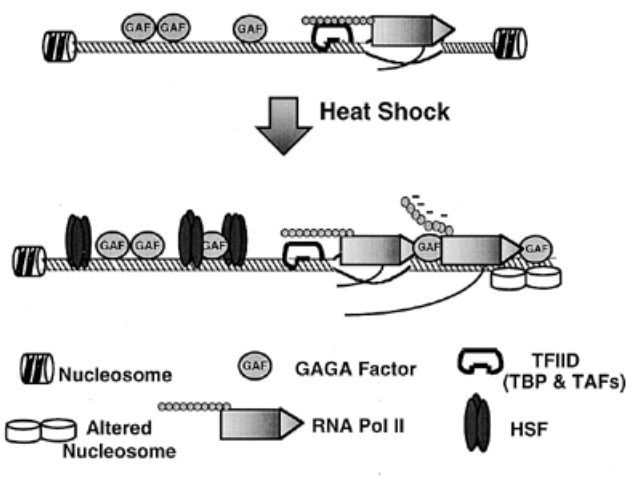

Figure 1. Architecture of a heat shock promoter before and after heat shock. A model showing important components of heat shock gene expression in *Drosophila*. An idealized heat shock gene promoter is pictured both before and after induction by heat shock. Pictured before heat shock are GAGA factor (GAF), TFIID (TBP and TAFs), and a paused RNA pol II complex. After heat shock GAF and TFIID associations persist, and heat shock factor (HSF) now binds the promoter, GAF association spreads from the promoter throughout the body of the gene, and paused pol II escapes efficiently into productive elongation concomitant with phosphorylation of its C-terminal domain.

histone H1, indicating that GAF can relieve or 'antirepress' the inhibitory effects of H1 (18). Furthermore, when the remaining histones are added to an *in vitro* chromatin reconstitution experiment, GAF cannot only 'antirepress', but, in conjunction with an ATP-dependent remodeling complex termed NURF, can also restructure chromatin at specific regions within the promoter of *hsp70* (14,19). Currently not much is known about the mechanism of NURF-based remodeling, nor have additional factors been identified with which it may interact. However, HSF has also been shown to be capable of restructuring nucleosomes on the *hsp26* promoter *in vitro* (20), and there are numerous genes without GAF binding elements, indicating that the function of GAF may be neither unique nor ubiquitous. There may, in fact, be a class of factors involved in promoter restructuring and, in the case of HSF, these factors may also have the ability to directly activate transcription. Given the growing knowledge of chromatin remodeling in *Drosophila* and yeast (19,21,22), there will most certainly be numerous methods and factors capable of restructuring chromatin architecture at gene promoters, and those factors which can alleviate this repression will therefore be key players in efficient expression of their target genes (for a review see 23).

Consistent with a function in chromatin remodeling, GAF is also a modifier of position effect variegation (PEV) (24). Mutations in GAF enhance PEV, thereby reducing the variegated expression of euchromatic genes in rearrangements subject to heterochromatinization. GAF is just one of several factors in a growing family of *Drosophila* proteins which have been implicated in regulation of chromatin architecture through PEV (Table 2). GAF may also be important in maintaining chromatin structure, as it is found associated with heterochromatin throughout the cell cycle (25). The association of GAF with heterochromatic repeats during mitosis (AAGAG) is suggested to be necessary for proper packaging of these repeats in mitotic chromosomes (J.S.Platero and S.Henikoff, personal commmunication). Consistent with this, defects are observed in chromosome condensation and segregation in some GAF mutants (26). The role of GAF may therefore extend beyond disrupting chromatin for specific gene expression, to maintaining chromatin structure throughout the cell cycle. Analyzing factors such as GAF whose mutations enhance PEV, and potentially chromosome structure, will nevertheless prove important in determining which genes are available in a chromatin context receptive to transcription.

Table 1. Putative targets of GAF

Gene	GAGA elements important for expression (references)	GAF binding *In vitro*	*In vivo*	Expression affected in GAFmutant Trl^{13C}
hsp70	(12,13)	+(27,29)	+(29)	ND
hsp26	(15–17,54)	+ (27)	+ (29)	ND
hsp27	ND	ND	ND	ND
engrailed	(9)	+ (9)	ND	+ (24)
ultrabithorax	(10)	+ (10)	+ (10)	ND
actin 5C	(64)	ND	+ (29)	ND
kruppel	(65)	+ (65)	ND	ND
even-skipped	(66)	ND	ND	ND
E74	(67)	+(67)	ND	ND
Adh distal promoter	(28)	+ (28)	ND	ND
α*1-tubulin*	(68)	+ (68)	ND	ND
Histone genes, *his3* and *his4*	ND	+ (27)	+ (29)	ND
fushi tarazu	(24)	(69)	ND	+ (24)

Genes currently identified that contain putative GAGA elements. Genes are categorized and referenced by those containing GAGA elements which have been demonstrated as important for expression, those shown to bind GAF *in vitro*, and/or *in vivo* and/or those effected by a mutation in GAF (Trl^{13C}; 24). Those categories where information is lacking are designated ND, for not determined.

Table 2. Modifiers of PEV in *Drosophila*

Supressors	Enhancers
Histone gene cluster	Trithorax-like or GAF (24)
Su(var)205 or HP1 (heterochromatin protein 1)	E(var)3-164 or E2F (elongation factor 2F) (70)
Su(var)3-7	E(var)3-93D (71)
Su(var)3-9 (73)	Histone deacetylase RPD3 (72)
Su(var)231	Zeste (74)
mus 209 or PCNA (proliferating cell nuclear antigen)	Putative ubiquitin-specific protease (75)
Su(var)3-6 or PP1 (catalytic subunit of a type 1 protein phosphatase)	Modifier of mdg4 or mod(mdg4) (76)
Su(var)2-1, Su(var)2-10 and Su(var)3-3 (histone deacetylases)	
S-Adenosylmethionine synthetase [Su(z)5] (77)	
Modifier of white (mow) (78)	
Suppressor of hairy wing [Su(Hw)] (76)	

Known modifiers of position effect variegation (PEV) in *Drosophila*. The list is separated into enhancers and suppressers of PEV as assayed by mutant analysis. Unless otherwise specified, discussion of modifiers and the phenomena of PEV is included in the following reviews and references therein (46,62,63). Remaining entries were found from a Biosis search of the literature, using the following strings: Su(var), E(var), PEV, or position effect.

GAF: ONE LOCUS, MANY PROTEINS

GAF is encoded by the *Trithorax-like* (*Trl*) gene, which is required for normal expression of several homeotic genes in *Drosophila*. Null alleles of the *Trl* locus result in larval lethality, indicating that the product of the *Trl* locus is essential during development (24). Less severe mutations in *Trl* implicate GAF in gene expression and chromatin architecture. In mutants with the hypomorphic Trl^{13C} allele, expression of the *Ubx*, *Ftz*, *en* and *Abd-B* genes is compromised, resulting in phenotypes consistent with a partial loss of function (24,26). Since these mutations have been found to enhance PEV as well, it is reasonable to assume that *in vivo*, as seen *in vitro*, GAF is responsible for maintaining an open chromatin architecture, thereby facilitating expression of target genes. The range of phenotypes associated with different *Trl* alleles (11) and the distribution of GAF at hundreds of loci on polytene chromosomes (14; Wilkins and Lis, unpublished results) suggests that the list of genes regulated by GAF (Table 1) is far from exhaustive. GAF also appears to have an even more global role in chromatin structure, as suggested by the spectrum of nuclear cleavage cycle defects observed in Trl^{13C} embryos (24,26).

A closer look at the *Trl* locus yields another level of complexity. *In vivo* GAF exists as a series of isoforms ranging in size from 66 to 120 kDa, with a major species of 67 kDa (27,28). These isoforms differ slightly in domain composition (29) and are the products of multiple RNAs from the *Trl* locus (30,31). A major subset of these polypeptides appear encoded by two GAF cDNAs that have been recently characterized (31). These two cDNAs code for proteins of 519 and 581 amino acids which share a common N-terminus and binding domain, while differing in their C-terminal domains. Interestingly, the resultant polypeptides not only bind the same sequences in the *Adh* distal promoter, but also have identical distributions on polytene chromosomes, similar nucleosome remodeling properties, and appear to form both homo- and heteromultimeric complexes *in vitro* and *in vivo* (31). It will be intriguing to find out exactly what contributions the various isoforms of GAF make toward its overall function(s) and what implications possible heteromultimers will have on our understanding of the maintenance of chromatin structure.

A major consideration in future studies will have to address the function of these multiple isoforms of GAF. What function and what possible interactions these isoforms have with each other and various sequence elements remains poorly understood. To date, the majority of studies have focused specifically on the 67 kDa isoform of GAF and, for the purpose of this review, all discussions of GAF will refer to the 67 kDa species unless otherwise specified. Study of the remaining GAF species is, however, imperative, as expression of heat shock promoter-driven transgenes containing the sequences coding for the 67 kDa isoform have to date only been able to rescue some allelic combinations of *Trl* and have been unable to rescue null mutations (Greenberg and Schedl, personal communication; Granok, Leibovitch and Elgin, personal communication). Multiple GAF isoforms are likely to be needed to recover the full functional activity of GAF.

GAF: PROTEIN DOMAINS

The 67 kDa isoform of GAF is composed of three major recognizable domains: the POZ/BTB domain, a zinc finger domain, and a glutamine-rich domain (Fig. 2). The POZ/BTB domain defines a growing family of factors in *Drosophila* (32) and is believed to be a protein interaction domain, which in the case of the ZID, Ttk, and Bric-a-Brac proteins, is capable of mediating dimerization *in vitro* (33,34). The POZ/BTB domain of GAF, however, does not appear to mediate homodimer formation *in vitro* (34). While GAF may not dimerize with itself *in vitro* via a POZ–POZ interaction, recent studies indicate that the 67 kDa isoform of GAF is a multimer in *Drosophila* nuclear extracts (Wilkins and Lis, unpublished results), and that different isoforms of GAF appear to form homo- and heteromultimers *in vivo* (31). Whether multimerization involves the POZ domain interacting with some additional region or is totally independent of POZ remains to be seen. The myriad of factors that might multimerize with GAF, whether it be another isoform or some heterologous POZ-containing protein, also remains to be determined.

Deleting the POZ/BTB domain does not have appreciable negative effects on DNA binding of GAF. In fact, deleting all the sequences save the single zinc finger and some N-terminal basic residues leaves a protein still capable of binding a consensus element *in vitro* (35). Similar sequences are also capable of binding to and footprinting the *hsp70* promoter *in vitro* in a manner identical to that of the full-length protein (Wilkins and Lis, unpublished results). Most zinc finger proteins described have multiple fingers; therefore GAF is fairly unique in that it has but

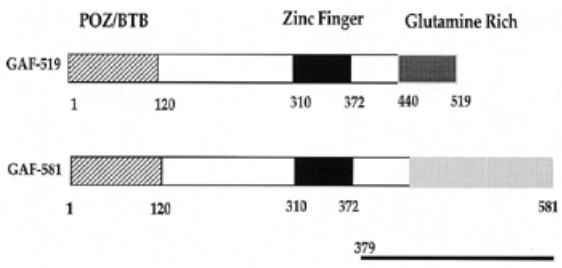

Figure 2. Domain representation of GAGA factor. The three recognized domains of the 519 and 581 amino acid forms of GAF are labeled and set apart by shading with amino acid residue locations listed. The POZ/BTB domain is represented by diagonal line shading (residues 1–120), the zinc finger motif is represented as a black shaded area (residues 310–372), and the glutamine-rich domain is indicated by a lighter grayish shaded region (residues 425–519 of the 519 isoform and 445–581 of the 581 isoform). The 581 amino acid form is also listed with similar labeling. The 519 and 581 isoforms are identical to the end of the zinc finger (residue 378) and diverge in those regions at the C-terminus of the proteins, inclusive of the glutamine-rich domains. The regions of divergence are delineated by a dark line. For a more detailed comparison and explanation see Benyajati *et al.* (31).

one of the C_2H_2 variety, first characterized in the transcription factor TFIIIA in *Xenopus* (36). Work done with several zinc finger proteins indicate that each individual finger can interact specifically with a set of three bases (37,38). *In vitro* band shift and DNase I footprinting show that a trinucleotide sequence (GAG) is sufficient for GAF binding (29; Wilkins and Lis, unpublished results), although with a lower affinity than typical elements which usually average 3.5 $(CT{\cdot}GA)_n$ repeats (11). Recently the NMR structure of a GAF binding domain–DNA complex has been published indicating that a pentamer (GAGAG) constitutes the GAF binding consensus, with the zinc finger core binding the first three bases of the consensus, and basic regions N-terminal of the finger (BR1 and BR2) interacting with the remaining two bases of the consensus. Interestingly, only the central G is essential for GAF binding, with the protein tolerating minor changes at other positions within the consensus (39).

Of the three GAF domains, least is known about the glutamine-rich domain. Factors rich in glutamine residues, such as SP1, have been shown to be potent activators of transcription (40). In fact, the glutamine-rich regions of SP1 have been shown to interact with the general transcriptional machinery itself, targeting TFIID through TAF110 (41). These same glutamine-rich domains of Sp1 also govern multimer formation and can mediate transcriptional synergism from promoters containing multiple Sp1 binding sites (42). Glutamine repeats have also been shown to mediate stable multimerization *in vitro*, with X-ray diffraction and molecular modeling suggesting they form polar zippers of antiparallel β-strands linked by hydrogen bonds between amide groups (43,44). It is apparent that the glutamine-rich domain of GAF and/or flanking regions may have some function in multimerization (Wilkins and Lis, unpublished results), but GAF is not believed to stimulate the transcriptional machinery directly, instead functioning to alleviate the repressive effects of chromatin (18). Though GAF can restructure chromatin with the help of NURF (19), the molecular contacts that give rise to this antirepression are unknown. Could interactions with the general transcription factors (GTFs) or pol II be integral to this mechanism? Transcription requires a complex interplay between numerous cofactors, TAFs, GTFs, activators, and pol II (for a review see 45). While the function of the glutamine-rich domain remains poorly understood, it is still possible that it and other domains of GAF may make multiple contacts with the transcription machinery which could affect chromatin structure.

POTENTIATION: THE ROLE OF GAF IN CHROMATIN ARCHITECTURE AND RAPID ACTIVATION OF HEAT SHOCK GENES

Genes can exist in a variety of different chromatin states, and their corresponding levels of expression can vary greatly. Genes can be inactivated by inclusion in heterochromatin, with the extent of heterchromatinization in the genome depending on the competing activities of a variety of factors (Table 2). Some of these act positively, like GAF, to increase expression or decrease the tendency of DNA sequences to form heterochromatin (24). Other factors act negatively through chromatin, such as HP1 (46), to decrease gene expression. The repressive state of chromatin structure is therefore a consequence of the dynamic interplay of both positively and negatively acting factors.

On a more local level, the effects of GAF on chromatin structure at the regulatory regions of specific genes have also been examined. GAF has been implicated in the regulation of a subset of genes that are primed for rapid induction through the establishment of an open chromatin architecture at the promoter. These 'potentiated' promoters have been extensively studied in the context of the heat shock genes of *Drosophila*. Studies of the *hsp70* and *hsp26* genes have shown that pol II has access to the gene prior to induction and can initiate transcription, but is impeded from progressing beyond early elongation (47,48). Pol II is therefore present and awaiting the appropriate cues to proceed (Fig. 1). Resumption of elongation by this paused polymerase appears to be the rate limiting step in *hsp70* transcription and therefore a key target for regulation (49). Regulation during early elongation is not unique to the heat shock genes and has been shown on other developmentally expressed genes in *Drosophila* (47), the human c-*myc* gene (50,51), the mouse *transthyretin* gene (52), and HIV (53). It will be interesting to determine how widespread pausing is as a method of post-initiation transcriptional control, and what factors play a pivotal role in its regulation.

On the *Drosophila* heat shock genes the establishment of this potentiated state is facilitated by GAF. Studies have suggested that GAF may exert some of its function at the level of chromatin structure, helping to maintain the promoter in an open conformation. Consistent with this, GAF tends to localize *in vivo* to regions of polytene chromosomes that are not highly condensed (25; Wilkins and Lis, unpublished results) and does not appear to overlap the localization of HP1, a non-histone chromosomal protein found in highly condensed regions of chromatin (23). On the *hsp26* gene the CT-rich sequences that bind GAF have been shown to be important for maintenance of an open promoter conformation. GAF is responsible for maintaining DNase I hypersensitive sites in the promoter, sites which are critical for heat-induced and developmentally triggered *hsp26* expression (16,17,54). Analysis of *hsp70* promoter sequences indicates that GAF, in conjunction with NURF, can actually disrupt chromatin on *in vitro* assembled templates. Disruptions in chromatin structure are detected upon GAF addition in regions at and immediately adjacent to GAGA binding sites (14,19). Similar remodeling of chromatin has also been observed on the *hsp26*

gene in *vitro* (20). By targeting the restructuring of chromatin, GAF can open up the promoter and make regulatory sequences available for association of additional factors which are unable to penetrate the nucleosomal structure. Presumably, without GAF, key regulatory sequences remain inaccessible, explaining why some developmental genes are unresponsive to activation in the context of a GAF mutant (24,26).

Two other critical components of the transcriptional machinery, TFIID and paused pol II, are found on the *hsp70* gene prior to induction. TFIID and pol II contribute to the promoter architecture and may aid in keeping promoter sequences in an open conformation. These promoter associations are dependent upon GAF, as mutations in GAF elements in the promoter of *hsp70* reduce both establishment of the paused polymerase (13) and TBP occupancy of the TATA element (12). In contrast, in the absence of TFIID and pol II, GAF appears able to establish moderate DNase I hypersensitivity in the promoter of *hsp70 in vivo* (55) and can be found to bind near the start site of transcription *in vitro* (Wilkins and Lis, unpublished results). It therefore appears that GAF is necessary and perhaps sufficient to keep promoters open, thereby facilitating factor access, but is it also needed specifically for the recruitment and stable association of pol II and other key transcription factors? Is GAF function limited merely to the top of this regulatory cascade, or does it function downstream to include interactions with general and specific regulatory factors and even pol II itself?

FROM POTENTIATION TO ACTIVATION: HSF ASSOCIATION AND POLYMERASE ESCAPE

Establishment of a potentiated promoter paves the way for rapid induction of heat shock genes. Upon heat shock, or other physiological stresses, HSF trimerizes, is phosphorylated and binds to HSEs in the promoter (56). Concomitant with HSF binding, polymerase now rapidly escapes from its early elongational pause and proceeds into productive elongation (57). This induction is dependent upon binding of HSF to its target elements (HSEs), which in turn is dependent upon GAF and the establishment of the potentiated promoter. Mutations and deletions in GAF elements severely reduce the level of HSF on promoters and subsequent heat shock gene expression (12,58). This may be a consequence of limited access of HSF to HSEs in chromatin, but may also involve GAF–HSF interactions, as GAF modestly aids the binding of HSF to HSEs *in vitro* (Mason and Lis, unpublished results).

Mutations in GAF elements and other sequences in the leader region have not only been shown to reduce HSF promoter association and establishment of paused polymerase, but they have also been shown to reduce the level of polymerase on the induced gene (13). The fact that these mutations do not lead to constitutive expression indicates that pausing is not merely a manifestation of negative regulation. In fact, pol II is still required to pass through this kinetically slow step after induction. Pausing can still be detected on the induced *hsp70* gene by $KMnO_4$ mapping (59), even with polymerases firing once every 6 s (49). Therefore, pausing appears to be an integral part of the activation mechanism and not simply representative of repression. So, what is retaining pol II near the promoter? Is it awaiting subsequent modification or interactions to make it transcriptionally competent, or is it being held through interactions with other promoter factors, breakable by subsequent interactions or modifications? A target for modification could be the C-terminal domain (CTD) of pol II, which is unphosphorylated in the paused, and highly phosphorylated in the elongating polymerase (60; Fig. 1). It remains to be demonstrated if GAF participates in this tethering, and if it directly cooperates in the escape of paused polymerase during activation.

The interactions of GAF are not restricted to the promoter. Although the association of GAF with heat shock genes is constrained to the promoter prior to heat shock, concomitant with induction its binding now extends throughout the gene. *In vivo* cross-linking studies have shown that GAF actually progresses through the body of the gene after induction with similar kinetics to that of the polymerase (60), possibly performing some as yet unknown additional role throughout elongation (29). GAF may be functioning to open up chromatin ahead of an elongationally competent pol II, or it may be responsible for maintaining an open conformation in its wake. Since the *hsp70* transcription unit appears devoid of high affinity sites, GAF presumably binds to low affinity trinucleotide sites (GAG) which become available as chromatin structure is altered during transcription. Two *Drosophila* homeodomain proteins, Eve and Ftz, have also been shown to bind over the length of their target genes and may also influence chromatin architecture throughout the transcription unit (61). Consistent with a more global function in chromatin architecture, GAF has also been implicated in the maintenance of nucleosomal structure at distant regulatory elements of the *Abd-B* gene, emphasizing that the function of GAF may not be relegated strictly to the promoter (24).

GAF is clearly a major player in regulation of heat shock gene expression, whether its effects are felt directly or indirectly. However, GAF is still only a small piece of the puzzle. In addition to GAF, HSF access to HSEs is also determined by TFIID and RNA pol II association with the promoter (12). Clearly there is a complex interplay between GAF, TFIID, paused pol II, and HSF that dictates an appropriate promoter architecture capable of supporting rapid and robust activation.

SUMMARY

GAF is a member of a growing family of factors that affect gene expression by influencing chromatin structure (Table 2). In so doing, it becomes a vital component in the mechanism of gene regulation. Clearly the function of GAF is not limited to gene expression, as it has been implicated in several global aspects of chromosome structure and function. GAF, however, does have a specific role in the architecture and function of heat shock gene promoters, as it has been implicated in nearly every aspect of heat shock gene transcription. Does GAF aid the association of other transcription factors by simply exposing DNA binding sites, or are there additional interactions of GAF with these factors to hasten recruitment or stability of both potentiated and activated complexes? In either case, it appears that the effects of GAF are far reaching. It effects the establishment and maintenance of the paused polymerase and is critical in maintaining the chromatin architecture of the uninduced and induced states. Without GAF the association of TBP, HSF, and polymerase are all substantially compromised. Not only does GAF appear to act prior to and during the early steps in transcription, but it may also exert effects throughout transcription elongation.

Further dissection of promoter architecture will be necessary to determine all the ways GAF prepares the promoter for efficient expression and possibly how this information is maintained during cell proliferation. The relationship of GAF to paused polymerase also warrants direct analysis, in the hope of detecting both its role in

pausing and its function during elongation. Does GAF interact with RNA polymerase in any of these states and how does it propagate through the active gene? An analysis of DNA as well as protein interactions will be necessary to elucidate this. GAF is an extremely important factor, but much is still a mystery concerning the actual nature of its interactions, and how they specifically dictate chromatin structure and, more specifically, promoter architecture.

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