DNA sequence requirements for generating paused polymerase at the start of *hsp70*

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RNA polymerase II is transcriptionally engaged but paused ~25 nucleotides from the start site of the *hsp70* gene of *Drosophila melanogaster* in uninduced (non-heat-shocked) flies. Here, we identify regions of the *hsp70* promoter that are required for formation of this paused polymerase. Various *hsp70* promoter sequences are substituted for promoter sequences of a yolk protein gene, *yp1*, which, in males, is normally not expressed and has no paused polymerase. Run-on assays with nuclei of male transgenic flies are used to measure the level of paused polymerase on the hybrid genes. Sequences that reside upstream of the *hsp70* TATA element, when fused upstream of the *yp1* TATA element, specify the formation of a paused polymerase on the 5' end of this hybrid gene. Within this region are multiple copies of the GAGA element, which is known to bind a constitutively expressed factor. This element appears to play a role in generating the pause. Also, in the absence of much of this upstream region, *hsp70* sequences in the vicinity of the transcriptional start and pause site participate in specifying the pause. Deletions of the pause site reduce the level of paused polymerase but do not lead to constitutive transcription. However, a connection between transcription and pausing is seen. The level of paused polymerase on the various hybrid *hsp70-yp1* promoters correlates with the promoter's potential to direct heat-induced transcription.

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Heat shock treatments of *Drosophila* cells trigger a rapid and dramatic increase in the transcription of the *hsp70* gene. This upsurge in transcription is detectable within several tens of seconds and the shift to the fully induced level of transcription is complete within 3 min (Belyaeva and Zhimulev 1975; T. O'Brien, E. Wong, and J.T. Lis, unpubl.). During this induction, the density of transcribing RNA polymerase II on the *hsp70* gene increases ~200-fold, as estimated from measurements of pulse-labeled transcripts (Lis et al. 1981b), amounts of cross-linking of RNA polymerase to the *hsp70* gene in vivo (Gilmour and Lis 1985), and quantification of transcripts produced by nuclear run-on assays (O'Brien and Lis 1991). The fully induced *hsp70* gene is packed with approximately one to two polymerases per 100 bp (Corces et al. 1981).

The *hsp70* promoter in uninduced cells appears poised for a rapid and intense change in transcription rate. The promoter is punctuated by nuclease hypersensitive sites located at known regulatory elements and also at the

transcription start and leader regions (Wu 1980; Costlow and Lis 1984). RNA polymerase II is already present on the 5' end of the uninduced *hsp70* gene as detected both in intact cells by UV cross-linking (Gilmour and Lis 1986) and in nuclei by nuclear run-on assays (Rougvie and Lis 1988). Nuclear run-on assays demonstrate further that in uninduced *Drosophila* cells, this RNA polymerase II complex synthesizes an ~25-nucleotide nascent RNA and then pauses or arrests. This polymerase complex can escape the pause and transcribe into the body of the gene if nuclei are treated with either 0.6% Sarkosyl or high salt (Rougvie and Lis 1988). In cells, the rate of escape from the pause is enhanced by heat shock. The recruitment of new polymerases to the *hsp70* promoter appears to be inherently fast and not rate limiting over the entire range of *hsp70* gene expression (O'Brien and Lis 1991; C. Giardina and J. Lis, unpubl.). Thus, a major control of *hsp70* expression appears to be at the level of early elongation, that is, the rate of escape of the paused polymerase into the body of the *hsp70* gene.

A full understanding of the mechanism of elongation control of the *hsp70* gene requires identification of the DNA sequences and proteins responsible for generating the paused RNA polymerase II complex. Known se-

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quence elements in the *hsp70* regulatory region include four separate heat shock elements (HSEs), which bind heat shock transcription factor (HSF) (Parker and Topol 1984), and multiple copies of the GAGA element, which bind a general, constitutively active transcription factor (Gilmour et al. 1989). In addition, the proximal promoter contains a TATA box. Sequences in the transcription start region extending through the first 30 nucleotides of the transcription unit are similar among *Drosophila* heat shock genes (Hackett and Lis 1983). In this paper we identify both distal and proximal regions of the *hsp70* promoter that function in setting up paused polymerase. Mutations of these regions assayed in transgenic flies implicate the GAGA element as a contributor to this function. Other constructs demonstrate that *hsp70* sequences from the transcriptional start region also contribute to the formation of paused polymerase. In addition, the level of paused polymerase appears to correlate with the promoter's potential to be induced by heat shock.

Results

Strategy for mapping sequences that set up paused polymerase

The paused RNA polymerase II at the start of the uninduced *hsp70* gene can be detected in nuclear run-on assays (Rougvie and Lis 1988). Although this engaged polymerase fails to transcribe in a standard run-on assay buffer, it does transcribe efficiently if either 0.6% Sarkosyl or 0.8 M KCl is included in the reaction. Both conditions block transcriptional initiation but allow transcriptionally engaged RNA polymerases to elongate (Hawley and Roeder 1985; Cai and Luse 1987). We reasoned that in vitro-mutated *hsp70* promoters could likewise be assayed for paused polymerase in the context of normal chromatin after the stable integration of these promoters into the *Drosophila* genome. We used P-element-mediated germ-line transformation (Rubin and Spradling 1982) to generate transgenic flies that contain single chromosomal inserts of these modified promoters. To distinguish the RNA products of the introduced promoter from those arising as a result of transcription of the five endogenous copies of the *hsp70* gene, we marked the modified *hsp70* promoters by fusion to the *Drosophila* yolk protein gene *yp1*. The endogenous *yp1* gene is transcribed only in females (Garabedian et al. 1986); therefore, run-on assays were performed only with male nuclei. The nontransformant control shows an undetectable level of *yp1* transcripts if run-on assays are done either in the absence or presence of Sarkosyl (data not shown). This demonstrates that the endogenous *yp1* gene of males contains little or no transcribing or paused polymerase and thus provides a low background for detecting transcription from the introduced *hsp70-yp1* genes.

The general strategy for identifying the DNA sequences that set up paused polymerase was to construct

various hybrid genes by fusing *hsp70* to the *yp1* sequences at positions within their respective promoter regions. Each construct was used to generate transgenic lines. Nuclei were isolated from adult males of each line and allowed to transcribe in the presence of radioactive NTPs. Sarkosyl was present (except in the controls) during the run-on transcription assays permitting the paused RNA polymerase, if present, to transcribe. The resulting radiolabeled, run-on transcripts were analyzed by hybridization to gel-separated restriction fragments of *yp1* and *hsp70* genes that had been blotted onto nylon membranes. The amount of radiolabeled RNA that hybridized to each fragment was quantified using a Betagen Betascope. The number of paused RNA polymerase II complexes on the fusion constructs was calculated by comparing the hybridization signal of the *yp1* gene to that of the *hsp70* gene standard. We have estimated that the uninduced *hsp70* genes have one RNA polymerase per gene for each of the five genomic copies (Gilmour and Lis 1986; O'Brien and Lis 1991). To minimize any effects of genomic position, we examined two or more independent transformants of each construct.

Paused polymerase on a single gene can be detected in nuclei isolated from adult flies

Run-on assays with nuclei from transgenic males are shown in Figure 1. Run-on transcription in the absence of Sarkosyl produced abundant histone *H1* RNAs but very little RNA homologous to *hsp70*. In contrast, when nuclei were treated with Sarkosyl and allowed to transcribe during a short (5 min) run-on reaction, synthesis of RNAs homologous to the 5' end of the *hsp70* gene was greatly stimulated. This result is like that seen with nuclei isolated from cell culture (Rougvie and Lis 1988) and demonstrates that the *hsp70* genes in tissues of adult flies, like those in cell culture, contain a paused polymerase.

The run-on assays with adult nuclei can also detect RNA polymerase on an *hsp70-yp1* hybrid. The nuclei used to generate the initial test results shown in Figure 1 were isolated from a transgenic line that contains a single-copy insert of the *hsp70-yp1* hybrid gene called *UP1x5*. This hybrid gene contains five tandem copies of an *hsp70* upstream sequence, nucleotides –89 to –38, fused to position –38 of the *yp1* gene promoter (see Materials and methods; Fig. 2). Like the *hsp70* gene, normal run-on reactions in the absence of Sarkosyl produce no detectable *yp1* transcripts from the hybrid gene, indicating that polymerase is not efficiently transcribing this gene in the absence of heat shock. This is also supported by analysis of RNAs by Northern blot hybridization (Kraus et al. 1988). However, in the presence of Sarkosyl, transcription is seen on the 5' but not the 3' fragment, indicating that a transcriptionally engaged polymerase is present on the 5' end of the gene. This transcription of *yp1* is from the hybrid gene and not the endogenous *yp1* gene as determined by the finding that nuclei from adult males of the ACR control (nontransformed line that is the parent of all the transformed lines described in this

Figure 1. The initial test for mapping sequences that set up paused polymerase. The run-on assay results shown were obtained using nuclei isolated from adult male flies transgenic for the *UP1x5* hybrid gene. This gene contains five copies in tandem of the upstream *hsp70* sequences from -89 to -38 fused to position -38 of the *yp1* gene (see Fig. 2 and Materials and methods). Run-on transcription assays were performed for 5 min with nuclei transcribing in the absence or the presence of 0.6% Sarkosyl, which blocks initiation completely but allows elongation of transcriptionally engaged RNA polymerase (Rougvie and Lis 1988). Both 5' and 3' DNA fragments of the *yp1*, *hsp70*, and histone *H1* genes are included on each blot, and their positions are as indicated in the key. In the case of *hsp70*, a fragment from the middle (m) of the gene is also present. Restriction maps of the fragments shown in the key are drawn below the hybridization results. The thick bars represent transcribed sequences.

paper) have consistently shown no detectable *yp1* transcription in run-on reactions done in either the absence or presence of Sarkosyl. This initial test demonstrates that *hsp70-yp1* promoter fusions can set up a Sarkosyl-releasable polymerase that can be detected in run-on reactions.

hsp70 upstream sequences can set up paused polymerase on the *yp1* gene and render it heat inducible

In the region between -89 and the *hsp70* TATA box are two HSEs, which bind HSF (Parker and Topol 1984; Wu 1984), and a GAGA element, which binds a general, constitutively active transcription factor (Gilmour et al. 1989). The fusion of a single copy of this *hsp70* upstream region between -89 and -38 to the *yp1* gene at -38 (the *UP1* construct in Fig. 2A) gives a hybrid gene that

has no detectable paused polymerase in uninduced transgenic flies, <12% (the limit of detection) of that found on a single *hsp70* gene (Fig. 2A,B). In contrast, an *hsp70-yp1* construct that contains additional *hsp70* upstream sequences to -256, *UP2*, has an easily detectable level of paused polymerase that is 78% that of a native *hsp70* gene. The paused polymerase is also generated in constructs that contain multiple tandem copies of the -89 to -38 region: a dimer (*UP1x2*) generates paused polymerase on the hybrid gene at a level 55% that of an *hsp70* gene, or, as mentioned in the initial test above, a pentamer (*UP1x5*) of this region fused to *yp1* sequence generates an even higher level (Fig. 2). Thus, *hsp70* sequences that reside entirely upstream of the TATA box can specify the formation of paused polymerase when fused upstream of the *yp1* gene.

The levels of paused polymerase on this series of *hsp70-yp1* hybrid genes in uninduced flies appear to correlate with their levels of expression in heat shock-induced flies (Fig. 2). The heat-induced expression of the hybrid gene *UP1*, which contains one copy of the -89 to -38 region of *hsp70* and has no detectable paused polymerase, is barely detectable in either assays of steady-state RNA (Kraus et al. 1988) or in nuclear run-on assays performed on transgenic lines that have been heat shocked (Fig. 2C). In contrast, *UP2*, an *hsp70-yp1* construct that contains additional *hsp70* upstream sequences to -256 and has paused polymerase, is expressed during heat shock at a >100-fold higher level than *UP1*, a level that is 23% that of a native *hsp70* gene (Fig. 2). In addition, the presence of multiple copies of only the -89 to -38 *hsp70* region in the *hsp70-yp1* hybrids also produces both paused polymerase and high levels of heat-induced transcription [cf. *UP1* with *UP1x5* and *UP1x2* (Fig. 2A,B)]. Thus, the pausing of RNA polymerase on these genes appears to be related to their ability to be induced by heat shock.

hsp70 sequences in the transcriptional start region can complement upstream sequences in setting up paused polymerase

The sequences in and around the pause and transcriptional start site also contribute to the RNA polymerase pausing. The short region of upstream *hsp70* sequences from -89 to -38 in the *UP1* construct cannot by itself, in a single copy, generate detectable paused polymerase. However, it can set up paused polymerase when present with additional upstream sequences (above) or with additional sequences from the transcriptional start region. The *L1* hybrid gene contains *hsp70* sequences from -89 to +62, which include the upstream sequence of -89 to -38, the TATA box, the start site, and the leader region where polymerase pauses. This hybrid programs the formation of an intermediate level (67% of an *hsp70* gene) of the paused polymerase (Fig. 3). A slightly lower level (44%) is seen when an additional 32 bp are deleted (*L1+30*, -89 to +30), whereas deletion of an additional 7 bp of the *hsp70* leader (*L1+23*) results in a distinctly lower level of paused polymerase (19%). Note that iden-

Figure 2. *hsp70* upstream sequences can set up paused polymerase on the *yp1* promoter. (A) The four different constructs shown (*UP1*, *UP2*, *UP1x2*, *UP1x5*) were analyzed for their levels of engaged RNA polymerase in both uninduced and heat-induced transgenic flies. The *yp1* gene portion is illustrated as a stippled bar; the *hsp70* sequence is shown as a thinner solid line. The coordinates above the construct refer to the ends of the *hsp70* segment where +1 is the start of transcription in *hsp70*; the number below (–38) refers to the fusion point in *yp1* relative to its start site. The positions of the HSE and GAGA sequences in the *hsp70* sequence are shown by the wide hatched rectangles and solid triangles, respectively. The position of the TATA sequence is indicated by an oval. The number of paused RNA polymerase II complexes on the hybrid constructs was calculated from run-on assays with nuclei of uninduced transgenic male flies, as described in Fig. 1, using nuclear run-on data, an example of which is shown in B. The average value of arrested polymerase of each construct was obtained by averaging three to four independent run-on experiments using two or more independent transformants. The individual run-on results are shown in parenthesis below the average value, and the letters designate a particular transgenic line. Heat-induced transcription was determined by nuclear run-on assays using nuclei isolated from heat-shocked, male, transgenic adults. The heat-induced transcription of each hybrid gene was expressed as a ratio of signal from each hybrid gene to that from an endogenous *hsp70* gene after correcting for the differences in copy number and fragment length. (B) Southern blots of restriction-cut plasmids containing the *yp1* or *hsp70* genes that were probed with radiolabeled run-on RNAs from uninduced males. Run-on reactions were performed in the presence of Sarkosyl. (C) Southern blots of restriction-cut plasmids containing the *yp1* or *hsp70* genes that were probed with radiolabeled run-on RNAs from heat shock-induced males.

tical *yp1* sequences are present in the *L1* + 23 and *L1* + 30 hybrid genes (Fig. 3), indicating that the difference in pausing is a consequence of the deletion of *hsp70* sequences. Further deletion of *hsp70* sequence to –12 (*L1* – 12) lowers the level beyond detection (Fig. 3). Thus, the gradual loss of the start region sequences in this 3' deletion series results in a corresponding decrease in the level of paused polymerase.

It is intriguing that two very different types of additions to the –89 to –38 sequence of *hsp70*, either more upstream sequences (additional copies of the –89 to –38 region or sequences upstream of –89) or sequences around the transcription start, can generate paused polymerase. The upstream and start region may provide redundant functions such that the deletion of one region is complemented by the presence of the other region. Fig-

A

Constructs	Paused Polymerase YP1/hsp70	Heat-Shock Transcription YP1/hsp70
<p>UP1</p>	<0.12	<0.002
<p>L1-12</p>	<0.12	0.012 (0.015) E (0.009) F
<p>L1+23</p>	0.19 (0.3, 0.19) C (0.13, 0.15) D	0.06 (0.054) C (0.066) D
<p>L1+30</p>	0.44 (0.22, 0.62) A (0.54, 0.36) D	0.11 (0.11) A (0.10) D
<p>L1</p>	0.67 (0.68, 0.64) A (0.68) B	0.23 (0.20) A (0.26) B

B

Constructs	Paused Polymerase YP1/hsp70	Heat-Shock Transcription YP1/hsp70
<p>L2+23</p>	1.31 (0.99, 1.44, 1.51) A	0.39 (0.39) A
<p>L2+30</p>	1.44 (1.27, 1.92) A (1.12) B	0.62 (0.72) A (0.51) B
<p>L2</p>	2.04 (1.95, 2.36) A (1.8) I	0.77 (0.70) A (0.83) I

Figure 3. The *hsp70* start and leader region can complement upstream sequences in setting up paused polymerase. (A) The four different constructs shown (UP1, L1-12, L1+23, L1+30, and L1) that have progressively more of the *hsp70* start and leader sequence. The symbols and analysis are as in Fig. 2. (B) The three constructs (L2+23, L2+30, and L2) are related to the L1 constructs described in A, but have an additional copy of the *hsp70* sequence -89 to -38. The transcription starts on the parental L1 and L2 constructs at the normal *hsp70* start site (Kraus et al. 1988).

ure 3B shows the results obtained with another series of constructs in which an additional copy of the *hsp70* sequence from the -89 to -38 region is fused upstream of 3' deletion series described above (*L2* is derived by adding this sequence to *L1*; *L2* + 30 is likewise derived from *L1* + 30, etc.). The additional -89 to -38 sequence enhances the formation of paused polymerase on all of these constructs (Fig. 3B). The addition of the -89 to -38 sequence to promoters with -89 to $+62$ (*L2*) or -89 to $+30$ (*L2* + 30) results in high levels of paused polymerase, similar to levels seen with five copies of the -89 to -38 region (*UP1x5*). Addition of the -89 to -38 region also compensates for the significant decrease in the level of paused polymerase with increasing deletion of the leader sequence (cf. *L1* and *L1* + 23 with *L2* and *L2* + 23). This suggests that the additional -89 to -38 sequence functionally substitutes for the sequences around the *hsp70* start in generating paused polymerase.

The progressive deletion of sequences from the start/leader region also results in a corresponding progressive decrease in heat shock-induced transcription as measured both with nuclear run-on assays (Fig. 3A) or with Northern blots (Fig. 4). Deletion of the sequences between $+30$ and $+62$ results in a twofold decrease in transcription rate (cf. *L1* + 30 with *L1*). The additional deletion of sequences between $+23$ and $+30$ further reduces transcription approximately another twofold. Still further removal of promoter sequence to -12 (*L1* - 12) drops expression ~ 20 -fold compared with *L1*. Apparently, sequences within each of these intervals contribute to the transcriptional efficiency of the gene upon heat shock.

Deletion of the pause region in *hsp70* does not result in constitutive expression

The RNA polymerase pause site in *hsp70* has been mapped to approximately $+25$ (Rougvie and Lis 1988). Thus, the region between $+23$ and $+30$ contains or is in the immediate vicinity of the pause site. If a specific sequence element at or downstream of the pause site normally acts to block progress of RNA polymerase into the fusion gene, then the removal of such sequences by deletion should result in expression of the downstream

yp1 gene sequences even in the absence of heat shock. Two independent assays of transcriptional activity demonstrate that none of the pause region deletions leads to such constitutive expression. First, the 3'-deletion constructs generate very low steady-state levels of *yp1* RNA in uninduced male transgenic flies (Fig. 4A). This reflects a low transcription rate rather than an instability of *yp1* RNA in males, because we have shown previously that *yp1* RNA in males is as stable as it is in females (Kraus et al. 1988). Second, run-on assays of transcriptionally engaged polymerase in isolated nuclei demonstrate that in the absence of heat shock, transcription is undetectable on the 3' fragments of all of the *hsp70*-*yp1* hybrid genes (Fig. 2B and data not shown). Thus, none of the deletions that extend into the *hsp70* pause region leads to constitutive transcription of the fusion gene.

GAGA element plays a key role in setting up paused polymerase

Because the duplication of the -89 to -38 region is sufficient to generate a paused polymerase complex, as shown by *UP1x2* and *UP1x5*, we analyzed the function of sequence elements in this short interval in more detail. As shown in Figure 5A, the known sequence elements in the -89 to -38 region include HSEs (for review, see Lis et al. 1990) and a GAGA element (Biggin and Tjian 1988). We have examined the effects of introducing mutations into the HSE or GAGA elements of *L1* (Fig. 5A). The introduction of a pair of point mutations into two critical bases of the promoter proximal HSE1 (located between -62 and -47) has only a modest effect, reducing paused polymerase 30% in uninduced cells (Fig. 5B). However, this mutation reduces the level of heat shock-induced transcription by ~ 20 -fold relative to the parental *L1* gene. In contrast, the introduction of multiple point mutations into the GAGA element (located between -74 and -67) reduces the level of paused polymerase more than fourfold. This mutation also has an effect of similar magnitude on heat-induced transcription, reducing the level of polymerase on the induced gene fivefold to sixfold.

The proximal GAGA sequence element and a portion of HSE2 overlap; therefore, the introduction of multiple

Figure 4. Northern blot analysis of RNA isolated from uninduced (A) or heat-shock-induced (B) male transgenic flies. The ACR(f) and ACR(m) lanes contain RNA isolated from female and male ACR flies, respectively, the stock used to generate the transgenic lines. The names of the transgenic lines are written above each lane, and the structure of hybrid genes in these lines is diagrammed in Fig. 3. Total RNA (30 μ g) was fractionated and hybridized with *yp1* DNA as a probe. As a control for the amounts of RNA loaded, a duplicate filter was hybridized with actin 5C DNA.

A

B

Figure 5. Effect of multiple point mutations in the GAGA element or the HSE on paused polymerase. (A) The *mmGAGA-L1* and *dmHSE-L1* genes contain multiple point mutations in the GAGA element (positions -74 and -71 to -68, Δ) or two point mutations in HSE1 (positions -59 and -52, $\bullet$) respectively (see Materials and methods). (B) The level of paused polymerase and heat-induced transcription of the *mmGAGA-L1* and *dmHSE-L1* genes in transgenic flies were compared with that of *L1*. The symbols are as in Fig. 2.

mutations into GAGA must also introduce mutations into a portion of HSE2. In making the multiple mutation *mmGAGA-L1* (Fig. 5A), we attempted to minimize perturbation of the HSE2 by preserving the highly conserved and critical G at -72 of the heat shock consensus (Lis et al. 1990) and the preceding A residue at -73, which is also known to be important in binding HSF from methylation interference assays (Shuey and Parker 1986; Perisic et al. 1989). Also, we note that HSE2 is a weaker transcription element and binds HSF less tightly than HSE1 (Parker and Topol 1984). The predicted minimal effect of this multiple mutation on HSE2 function is borne out in the heat-shock response of the *mmGAGA-L1* hybrid gene, which is much more robust than that of the hybrid containing the double mutation in HSE1 (*dmHSE-L1*). The level of effect is comparable to that of a mutation of a discrete GAGA element (separate from HSEs) that resides in the regulatory region of another

heat shock gene, *hsp26* (Glaser and Lis 1990). Thus, the main effect of the multiple mutation on pausing and expression appears to be through its effect on GAGA element function, rather than through the disruption of HSE2 function. Furthermore, because HSF does not appear to bind HSEs in uninduced cells (Zimarino and Wu 1987; Thomas and Elgin 1988), it is hard to envision how an HSE might serve to set up a paused polymerase.

The level of paused polymerase on most hybrid genes examined correlates with the potential for transcriptional activation upon heat shock (Figs. 2 and 3). The exception is the gene *dmHSE-L1*, which contains a pair of point mutations in the proximal HSE. The *dmHSE-L1* gene has an intermediate level of paused polymerase, but upon heat shock, it is transcribed at a much lower level than the *L1* gene from which it was derived (Fig. 5). The failure of this gene to be highly expressed during heat shock is consistent with previous studies showing that

mutations in either of the proximal HSEs dramatically reduce induced expression [Xiao and Lis 1988]. Therefore, the pausing of polymerase appears to be a feature of these promoters that is necessary, but not sufficient, for high levels of heat-induced transcription of *hsp70*. Not surprisingly, interactions of HSF with the HSEs are also required to induce the higher transcription rates.

Discussion

The goal of this study was to identify regions of the *hsp70* promoter that participate in generating the paused RNA polymerase on the 5' end of the *hsp70* gene and to examine the relationship of this pausing to heat-inducible expression. To this end, we generated and analyzed transgenic fly lines carrying a variety of fusions between *hsp70* and *yp1* gene promoters. The endogenous *yp1* gene normally has no detectable paused or transcribing polymerases in the adult male. The replacement of *yp1* promoter sequences with sequences upstream of the *hsp70* gene can, in some cases, generate a paused polymerase at the 5' end of the *yp1* gene. A large segment of the *hsp70* upstream sequence, from -256 to -38 , when fused to position -38 of the *yp1* gene, can program the formation of paused polymerase, and transcription initiates at the normal start of the *yp1* gene [Kraus et al. 1988]. Thus, sequences upstream of the *hsp70* TATA box can specify the formation of the paused polymerase on the *yp1* sequence, indicating that this pausing does not obligatorily require sequences from the *hsp70* transcriptional start region or from the *hsp70* pause site.

The sequences in the -256 to -38 interval contain multiple binding sites for heat shock transcription factor [Parker and Topol 1984] and the general GAGA factor [Gilmour et al. 1989], and perhaps other, as yet unidentified elements. The search for critical elements that specify the pause was narrowed by examining constructs that contain a shorter upstream interval, -89 to -38 , containing two HSEs and one GAGA factor-binding site. This segment on its own fails to generate detectable paused polymerase when fused to -38 of the *yp1* gene. However, two copies of the -89 to -38 region in tandem can specify the pause. In addition, the segment from -89 to $+62$, which has only one copy of the -89 to -38 region plus the *hsp70* start region, can also specify formation of the pause, suggesting a redundancy or a cooperation in the activities of the start region and upstream sequences. Derivatives of this *L1* construct that contain multiple point mutations in either the proximal HSE or the GAGA element are telling. While the introduction of a pair of point mutations into the proximal HSE has little effect on the formation of the paused polymerase, multiple point mutations in the GAGA element reduce the level of paused polymerase by more than fourfold.

The GAGA element binds a 66-kD protein that appears to be a general transcriptional activator [Biggin and Tjian 1988; Gilmour et al. 1989]. The GAGA element is found in the regulatory regions of a variety of heat shock and developmentally expressed genes and has been shown to act positively on the expression of the

hsp26 gene [Glaser and Lis 1990; Glaser et al. 1990] and the *Ultrabithorax* (*Ubx*) gene [Biggin and Tjian 1988]. Furthermore, DNase I footprinting experiments have shown that GAGA factor binds strongly to the GAGA element spanning from -67 to -74 between HSE1 and HSE2 of *hsp70*. Recently, an analysis of transgenic lines containing *hsp26* transgenes with various upstream deletions identified a CT-rich region, which contains multiple GAGA elements, as a key player in the formation of DNase I hypersensitive sites in the *hsp26* promoter [Q. Lu, L. Wallrath, B. Allan, R. Glaser, J. Lis, and S. Elgin, in prep.]. Insertion of the -89 to -38 sequence of *hsp70* upstream of an *hsp26* gene deleted of the CT-rich region restored these hypersensitive sites. These observations are particularly intriguing because the *hsp26* gene, like *hsp70*, has paused polymerase in uninduced cells [Rougvié and Lis 1990]. Thus, the positively acting GAGA element plays a role in specifying both the paused polymerase complex and the DNase I hypersensitive region of related heat shock promoters.

The GAGA element clearly is not the only sequence element that participates in generating a paused polymerase. The sequences around the transcriptional start region can contribute as well. This can be seen in constructs that contain one copy of *hsp70* sequences that extend no farther upstream than the second HSE at position -89 . In these constructs, the region of the *hsp70* start between -12 and $+23$, which includes the initiation site, is critical for the formation of detectable paused polymerase. Initiator elements have been implicated in the assembly of a transcription complex in at least some eukaryotic genes [Smale and Baltimore 1989; Pellman et al. 1990]; thus, it is not surprising that they may play a role in generating the paused polymerase at the promoter region as well. In addition, the *hsp70* sequences in the region of the pause itself, $+23$ to $+30$, also appear to contribute, albeit modestly (Fig. 3), to the formation of the pause. The precise position of the pause in the 5' *yp1* sequences in the hybrid genes is not addressed in this paper, but preliminary mapping indicates that it is occurring at a specific location [C. Giardina and J. Lis, unpubl.]. It remains to be determined how dependent pausing is on the specific sequences of the *hsp70* and *yp1* leader regions.

The level of paused polymerase on an *hsp70-yp1* hybrid promoter correlates, in general, with promoter activity upon heat shock. Those constructs that have the highest levels of paused RNA polymerase in uninduced cells are also expressed at the highest levels during heat shock while those with intermediate levels of paused polymerase are expressed at intermediate levels. The constructs having no detectable paused polymerase are expressed very poorly during heat shock. The one exception to this correlation is the construct with a double point mutation in the strong proximal HSE (*dmHSE-L1*). Although this construct has a moderately high level of arrested polymerase, its expression is only a few percent of the parental construct (*L1*). This apparent aberration can be easily understood if the establishment of a paused polymerase is a hallmark of the potential for activation

by heat shock. The activation also requires strong binding sites for the activator HSF and, in this construct, a pair of base substitutions disrupt the proximal HSE (Xiao and Lis 1988).

Why do some constructs have an intermediate level of paused polymerase? Forming paused polymerase may be a stochastic process in which some copies of the gene have a level of paused polymerase like that seen on the native *hsp70* gene while others have none. Various factors that are involved in forming paused polymerase may compete with nucleosomes and participate in determining whether the promoter is open and has paused polymerase or is masked by histones. The intermediate level of heat-induced expression in constructs that have an intermediate level of paused polymerase may be a consequence of inducing only those genes with paused polymerase. Precedent for all or none expression of a gene has been reported recently for the inducible *interleukin-2-lacZ* hybrid gene. When T cells that were stably transfected with a single copy of the gene were induced and assayed individually, the resulting expression of the hybrid gene showed a clear bimodal pattern of expression (Fiering et al. 1990).

Native *hsp70* genes appear to have approximately one paused polymerase per gene in the uninduced state, as calculated by comparison with the number of elongating RNA polymerases on *hsp70* genes during heat shock (Gilmour and Lis 1986; O'Brien and Lis 1991). Thus, all copies of the *hsp70* gene in a cell appear poised for activation. The level of paused polymerase on hybrid genes *L2*, *L2+30*, and *UP1x5* was calculated to be about twice that of the native *hsp70* gene (Figs. 2 and 3). This is not a consequence of multiple, well-separated start sites, as Kraus et al. (1988) examined the transcription initiation site of the construct *L2* by primer extension experiments and found that transcription initiates from one site only. Perhaps, these constructs have more than one paused polymerase that reside different distances from a common initiation site. Alternatively, the amount of RNA polymerase on our standard gene, *hsp70*, may be overestimated. Although in cell cultures the average level of paused polymerase per *hsp70* gene has been estimated to be one (O'Brien and Lis 1991), perhaps not all tissues of the adult flies, which are used in this study, have an arrested polymerase on each copy of the *hsp70* gene. In fact, at least one cell type, primary spermatocytes in adults, does not have heat-inducible *hsp70* expression (Bonner et al. 1984).

The formation of the paused polymerase on the *hsp70* gene occurs in the absence of heat shock and is dependent on the GAGA element and the transcriptional start region sequences, both of which interact with constitutively active factors (Parker and Topol 1984; Wu 1984; Gilmour et al. 1989). The importance of the GAGA element in pausing suggests that the constitutively active GAGA factor may provide a key function to *hsp70* in the uninduced cell. Two mechanisms for the GAGA element/factor interaction can be readily envisioned in the recruitment of RNA polymerase to the *hsp70* promoter. The binding of GAGA factor may exclude nucleosomes

or histone H1, rendering the promoter accessible for the assembly of the transcription complex (Croston et al. 1991; Workman et al. 1991). Alternatively, GAGA-binding proteins may actively facilitate assembly of the uninduced transcription complex by interacting with proteins that bind TATA or other components of the start region complex.

We speculate that the features of the promoter that attract RNA polymerase are also responsible for holding the polymerase at the promoter even after it has initiated transcription. Therefore, RNA polymerase can initiate transcription and transcribe two or three turns of the DNA helix but pauses at approximately +25, perhaps without having broken all of its contacts with the proteins and sequences of the strong *hsp70* promoter. In such a model, the escape of RNA polymerase in response to heat shock is facilitated by the binding of activated HSF.

The potential advantage of generating a paused polymerase in uninduced cells can be understood in terms of having the gene poised, ready to immediately release a transcribing polymerase in response to heat shock or other cellular stress signals. In addition, the combination of constitutively bound transcription factors, such as GAGA and TFIID, and the large, paused RNA polymerase II together can create highly accessible regions, detected as hypersensitive sites, that allow the binding of the activator HSF to HSEs upon heat shock. Maintenance of the heat shock regulatory regions in an accessible configuration is particularly important, because it is known that HSF (at least human HSF) fails to bind HSEs if the HSEs are associated with nucleosomes (Taylor et al. 1991). Once active HSF binds, we envision that it accelerates the rate at which RNA polymerase breaks its contacts with the promoter and escapes into a productive elongation mode. This escape may be tightly coupled to the recruitment of the next polymerase, because the pause site remains occupied even when the gene is induced (O'Brien and Lis 1991; C. Giardina and J. Lis, unpubl.). The paused polymerase may act as the "foot in the door" that maintains the promoter/regulatory region accessible to the RNA polymerase and factors required for both initial and subsequent rounds of transcription.

Materials and methods

Construction of plasmids

Except where mentioned, standard cloning methods were used (Berger and Kimmel 1987; Sambrook et al. 1989). To create *UP1*, *L1-12*, *L1+23*, *L1+30*, and *L1* (Figs. 2 and 3), 5' deletions of the *yp1* gene from its unique *StuI* site of pYP1 (Barnett et al. 1980) and 3' deletions of the *hsp70* gene from its unique *PstI* site at +89 of pXT1 (Xiao and Lis 1986) were made using *Bal 31* nuclease (Legerski et al. 1978; Kraus et al. 1988). *SalI* linkers were added to the ends of each deleted gene. The *hsp70* 3' deletions were excised from the pXT1 derivatives as *XhoI-SalI* fragments (*XhoI* is at -89 of *hsp70* in pXT1) and ligated into the *SalI* site of the P-element transformation vector cp20.1, a Carnegie 20 derivative (Simon et al. 1985). This leaves a *SalI* site at the 3' end of the *hsp70* deletion. The 5'-deleted *yp1* gene was then

moved as a *Sall* fragment (using the newly generated *Sall* site and the other *Sall* site of pYP1, which is ~2.2 kb past the 3' end of the *yp1* gene) into the *Sall* site at the 3' end of the *hsp70* deletion.

UP2 (Fig. 2) was constructed by ligating the *XbaI*–*Bss*HII fragment (containing the sequences from –256 to –65 of the *hsp70* gene) to the *Bss*HII–*Sall* fragment from the *hsp70*–*yp1* *UP1* hybrid gene (containing the sequences from –65 of the *hsp70* gene to 2.2 kb downstream of the 3' end of the *yp1* gene) and cloning into *XbaI*–*Sall*-cut cp20.1.

UP1x2, *L2* + 23, *L2* + 30, and *L2* were generated by first moving the *XhoI*–*MspI* fragment (–89 to –38) of pXM1 (Xiao and Lis 1986) into the *XhoI*–*Sall* site of pUC13X (Xiao and Lis 1986). The *XhoI*–*Sall* fragment from this latter plasmid was moved into the *Sall* site of Carnegie 20. The *hsp70* fragment containing the sequences from –89 to various 3'-deletion points was then added as an *XhoI*–*Sall* fragment. Finally, the *Sall* fragment containing 5'-deleted *yp1* gene (as described in the *UP1* construct) was ligated into the *Sall* site downstream of the *hsp70* promoter region.

To create *UP1x5* (Fig. 2), the *XhoI*–*MspI* fragment (–89 to –38) of pXM1 was cloned into the *XhoI*–*Sall* site of pUC13X. The *XhoI*–*Sall* fragment (containing –89 to –38) from this latter plasmid was then polymerized and characterized (Xiao and Lis 1986). A *XhoI*–*Sall* fragment containing five copies of –89 to –38 sequence arranged in head-to-tail orientation was cloned into the *Sall* site of Carnegie 20. The *Sall* fragment containing 5'-deleted *yp1* gene (as described in the *UP1* construct) was then ligated into the *Sall* site downstream of the *hsp70* promoter region.

mmGAGA-L1 (Fig. 5) was generated by mutating 4 nucleotides of the GAGA consensus sequence in the *L1* construct (Fig. 3). Two complementary 28-base oligonucleotides were synthesized which, when annealed, contained the region from –89 to –64 of the *hsp70* gene, including the mutated GAGA consensus sequence (Fig. 5A). The 28-base oligonucleotide has *XhoI* and *Bss*HII sites on its ends. A plasmid containing *hsp70* sequences from –89 to +62 of the *hsp70* (used in *L1*) was cut with *XhoI* and *Bss*HII, and the 28-mer was cloned into these sites. The 5'-deleted *yp1* gene (the 5' end is at –9) was cloned as a *Sall* fragment into the *Sall* site (at the 3' end of the *hsp70* sequence) of the plasmid that contains the inserted 28-nucleotide synthetic DNA fragment. The resulting *hsp70*–*yp1* fusion gene was cut with *SacI* (one *SacI* site is in the vector; the other is 1.6 kb downstream of the 3' end of the *yp1* gene), the ends of the fragments were filled in by Klenow treatment, and the fragment containing the fusion gene was moved into the *HpaI* site of the Carnegie 20 vector to generate *mmGAGA-L1*.

dmHSE-L1 (Fig. 5) was generated by mutating 2 specific nucleotides of HSE1 of construct *L1*. It was shown previously that a double point mutation of HSE1 abolished heat shock inducibility of the promoter when it was fused to the *lacZ* gene (Xiao and Lis 1988). Two complementary oligonucleotides, 13 bases (spanning from –51 to –63 of the *hsp70* gene) and 17 bases (spanning from –67 to –51) in length, which include the double-mutated HSE1, were synthesized. The 13-mer and 17-mer were annealed to produce a fragment that has *HpaI* and *Bss*HII sites on its ends. A plasmid of *hsp70* 3' deletions containing –89 to +62 sequence of the *hsp70* (used in *L1*) was cut with *NruI* and *Bss*HII, and the annealed 13-mer/17-mer were cloned into the site. The 5'-deleted *yp1* gene (5' end is at –9) was cloned as a *Sall* fragment into the *Sall* site (at the 3' end of the *hsp70* deletion) of the latter plasmid, which contains the newly cloned mutated HSE sequence. The *hsp70*–*yp1* fusion gene was moved into Carnegie 20 vector, as described for *mmGAGA-L1*, to generate *dmHSE-L1*.

The base sequence of modified regulatory regions was confirmed by DNA sequencing. Restriction fragments were cloned into M13 and sequenced by the dideoxy chain termination method using Sequenase (U.S. Biochemical).

Isolation of nuclei from adult flies

The protocol is a modification of that of Love and Minton (1985). Everything that came into contact with the flies or their extracts was prechilled at the start. The 1–1.5 gram of adult flies were collected in a prechilled bottle and transferred into an Omni mixer (Du Pont). Then, 15 ml of ice-cold buffer A [0.3 M sucrose, 2 mM MgOAc, 3 mM CaCl₂, 10 mM Tris-HCl (pH 8), 0.3% Triton X-100, 0.5 mM DTT] was added, and the flies were ground in the buffer for 1–2 min at the maximum speed. This procedure took place on ice. The mixture was filtered through two layers of 100-μm nylon mesh. The filtrate was put into a 15-ml glass/Teflon homogenizer and stroked ~40 times while on ice. The mix was filtered through two layers of 35-μm nylon mesh twice and mixed with an equal volume of buffer B [1.75 M sucrose, 5 mM MgOAc, 10 mM Tris-HCl (pH 8), 0.5 mM DTT]. The mixture was layered over a sucrose gradient containing 8 ml of buffer B over 4 ml of buffer B' [1.9 M sucrose, 5 mM MgOAc, 10 mM Tris-HCl (pH 8), 0.5 mM DTT], and spun down for 20 min at 15,000 rpm using the SW28 rotor at 4°C. After the supernatant was discarded, the walls of the centrifuge tubes were wiped with Kimwipes. The pellet was resuspended in 1.5 ml of buffer C [25% glycerol, 5 mM MgOAc, 50 mM Tris-HCl (pH 8), 5 mM DTT, 0.1 mM EDTA] and spun down for 1 min in a cold microcentrifuge. After the supernatant was discarded, the pellet was frozen quickly in liquid nitrogen and stored at –70°C.

Nuclear run-on transcription assays

Nuclear run-on transcription assays were performed essentially as described previously (Love and Minton 1985). The 100 μl of nuclei in buffer C [25% glycerol, 5 mM MgOAc, 50 mM Tris-HCl (pH 8), 5 mM DTT, 0.1 mM EDTA] were mixed with 117 μl of buffer D [1 mM ATP, 1 mM CTP, 1 mM GTP, 24 μM UTP, 4.4 mM MgOAc, 1.7 mM MnCl₂, 7.2 mM Tris-HCl (pH 8), 150 mM KCl, 1 μl of RNasin (40 U/μl), 10 μl of [³²P]UTP (100 μCi at 3000 Ci/mmol)]. For reactions performed in the presence of Sarkosyl, this detergent was added to buffer D to 1.2% (wt/vol). Unlabeled nucleoside triphosphates were purchased from Pharmacia or Sigma, and [³²P]UTP was from Amersham.

After incubation (from 2 to 5 min) at room temperature, reactions were stopped by the addition of 1 ml of stop buffer [2% SDS, 7 M urea, 350 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl (pH 8)], transferred to a 15-ml tube containing an additional 4-ml stop buffer, extracted three times with Leder phenol (phenol/chloroform/isoamyl alcohol at 50 : 50 : 1), and ethanol-precipitated. Pellets were rinsed with 70% ethanol, dried, and resuspended in H₂O. CaCl₂ and MgCl₂ were added to 6 mM, and the DNA was digested with 1 unit of RNase-free DNase (RQ1 DNase; Promega) for 20 min at 37°C. After ethanol precipitation, the pellets were resuspended in H₂O. The radiolabeled RNA was added to a hybridization solution for probing Southern blots.

DNA (Southern) blots

Restriction-digested DNA (2 μg per lane) was separated on 0.7–1.2% agarose gels in TAE buffer (Sambrook et al. 1989) and transferred to GeneScreen Plus (Du Pont) membranes or BioTrace RP (Gelman) membranes in 0.4 N NaOH for 12 hr. Following transfer, DNA was UV cross-linked to the mem-

branes by placing the membrane DNA-side down on a short-wave UV transilluminator for 45 sec. The membranes were then neutralized in 0.2 M Tris-HCl (pH 7.5) and 2× SSC, and air-dried. Filters to be probed with radiolabeled RNA were prehybridized for 2–5 hr in 50% formamide, 6× SSC, 1% SDS, and 10% dextran sulfate. Probe, which had been preincubated with blank filters overnight, was added to the prehybridized filters, and the hybridizations were incubated in roller bottles at 42°C for 24–48 hr. Following hybridization, the blots were washed twice, for 10 min each, in 2× SSC, 0.2% SDS, at room temperature, followed by two washes, for 30 min each, of 0.1× SSC, 0.1% SDS, at 55°C. After washing, the filters were dried and counted using the Betascope for quantitation.

Estimating the number of paused RNA polymerase II molecules on hsp70–yp1 fusion constructs

The numbers of paused RNA polymerase II molecules on the fusion constructs were estimated by comparing the hybridization signal of *yp1* 5' fragment to that of *hsp70* 5' fragment. The level of polymerase on an uninduced *hsp70* gene was taken as one, based on estimates from both in vivo UV cross-linking assays (Gilmour and Lis 1986) and nuclear run-on assays (O'Brien and Lis 1991). The values are expressed in Figures 2, 3, and 5 as the ratio of polymerases on the *yp1* sequences of the hybrid gene divided by the polymerases per copy of the *hsp70* gene and, thus, are corrected for the fact that the signals for *hsp70* are from five copies of this gene per genome, whereas the *yp1* signals are from a single copy of the *hsp70–yp1* gene.

In all of the hybridization assays in this paper, the 5' fragment of *yp1* that is blotted to filters includes the start of the *yp1* gene, whereas the 5' fragment of the *hsp70* gene lacks the sequence of from +1 to +62. This use of only sequences downstream of +62 avoids cross-hybridization of RNA from the γ element (Brown and Ish-Horowicz 1981; Lis et al. 1981a). The γ element is a repetitive sequence interspersed with $\alpha\beta$ repeats at the 87C locus, and γ has 99% sequence identity to the *hsp70* upstream sequences and downstream sequences to +62 (Hackett and Lis 1981). Because of this sequence identity, the γ sequence may have a paused polymerase on its promoter like the *hsp70* gene. We found that the removal of the +1 to +62 sequence from the 5' fragment of *hsp70* caused significant reduction (about threefold) of the hybridization signal to the 5' fragment, suggesting that the γ element contributes to the signal. However, the removal of the +1 to +62 sequence would also eliminate the contribution of short nascent *hsp70* RNAs hybridizing upstream of the +62 region. Because *Drosophila simulans* flies do not have γ or $\alpha\beta$ elements in their genome, we performed run-on assays using nuclei isolated from *D. simulans* to determine the relative contribution of the +1 to +62 region of *hsp70* to the hybridization signal. Any reduction in the hybridization signal of the 5' fragment missing the sequences upstream of +62 would be due solely to the loss of the contribution from short *hsp70* RNAs that hybridize upstream of the +62 sequence. In *D. simulans*, the hybridization signal decreased ~1.5-fold after removal of the +1 to +62 sequence, indicating that the signal from the *hsp70* 5' fragment in *D. melanogaster* is under-represented ~1.5-fold as a result of the lack of that sequence in the fragment. This correction value was applied to the calculation of the number of paused polymerases on each construct.

RNA (Northern) blots

To prepare heat shock RNA, adult flies were heat shocked by immersing a glass vial containing the flies in a water bath at

36.5°C for 1 hr. RNA was prepared as described previously (Simon et al. 1985). Non-heat-shock RNA was prepared similarly but without heat shock treatment of flies. RNA samples were electrophoresed and transferred to GeneScreen Plus (Du Pont) essentially as described in the manual. Ethidium bromide staining revealed the presence of approximately equivalent amounts of RNA in all lanes.

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References

- Barnett, T., C. Pahl, J.P. Gergen, and P.C. Wensink. 1980. The isolation and characterization of *Drosophila* yolk protein genes. *Cell* **21**: 729–738.
- Belyaeva, E.S. and I.F. Zhimulev. 1975. RNA synthesis in the *Drosophila melanogaster* puffs. *Cell Differ.* **4**: 415–427.
- Berger, S.L. and A.R. Kimmel, eds. 1987. Guide to molecular cloning techniques. *Methods Enzymol.* **152**.
- Biggin, M.D. and R. Tjian. 1988. Transcription factors that activate the Ultrabithorax promoter in developmentally staged extracts. *Cell* **53**: 699–711.
- Bonner, J.J., C. Parks, J. Parker-Thornburg, M.A. Mortin, and H.R.B. Pelham. 1984. The use of promoter fusions in *Drosophila* genetics: Isolation of mutations affecting the heat shock response. *Cell* **37**: 979–991.
- Brown, A.J.L. and D. Ish-Horowicz. 1981. Evolution of the 87A and 87C heat-shock loci in *Drosophila*. *Nature* **290**: 677–682.
- Cai, H. and D.S. Luse. 1987. Transcription initiation by RNA polymerase II in vitro. *J. Biol. Chem.* **262**: 298–304.
- Corces, V., A. Pellicer, R. Axel, and M. Meselson. 1981. Integration, transcription, and control of a *Drosophila* heat shock gene in mouse cells. *Proc. Natl. Acad. Sci.* **78**: 7038–7042.
- Costlow, N. and J.T. Lis. 1984. High-resolution mapping of DNase I-hypersensitive sites of *Drosophila* heat shock genes in *Drosophila melanogaster* and *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **4**: 1853–1863.
- Croston, G.E., L.A. Kerrigan, L.M. Lira, D.R. Marshak and J.T. Kadonaga. 1991. Sequence-specific antirepression of histone H1-mediated inhibition of basal RNA polymerase II transcription. *Science* **251**: 643–649.
- Fiering, S., J.P. Northrop, G.P. Nolan, P.S. Mattila, G.R. Crabtree, and L.A. Herzenberg. 1990. Single cell assay of a transcription factor reveals a threshold in transcription activated by signals emanating from the T-cell antigen receptor. *Genes & Dev.* **4**: 1823–1834.
- Garabedian, M.J., B.M. Shepherd, and P.C. Wensink. 1986. A tissue-specific transcription enhancer from the *Drosophila* yolk protein 1 gene. *Cell* **45**: 859–867.

- Gilmour, D.S. and J.T. Lis. 1985. In vivo interactions of RNA polymerase II with genes of *Drosophila melanogaster*. *Mol. Cell. Biol.* **5**: 2009–2018.
- . 1986. RNA polymerase II interacts with the promoter region of the noninduced *hsp70* gene in *Drosophila melanogaster* cells. *Mol. Cell. Biol.* **6**: 3984–3989.
- Gilmour, D.S., G.H. Thomas, and S.C. Elgin. 1989. *Drosophila* nuclear proteins bind to regions of alternating C and T residues in gene promoters. *Science* **245**: 1487–1490.
- Glaser, R.L. and J.T. Lis. 1990. Multiple, compensatory regulatory elements specify spermatocyte-specific expression of the *Drosophila melanogaster hsp26* gene. *Mol. Cell. Biol.* **10**: 131–137.
- Glaser, R.L., G.H. Thomas, E. Siegfried, S.C.R. Elgin, and J.T. Lis. 1990. Optimal heat-induced expression of the *Drosophila hsp26* gene requires a promoter sequence containing (CT)_n(GA)_n repeats. *J. Mol. Biol.* **211**: 751–761.
- Hackett, R.W. and J.T. Lis. 1981. DNA sequence analysis reveals extensive homologies of regions preceding *hsp70* and alpha-beta heat shock genes in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **78**: 6196–6200.
- . 1983. Localization of the *hsp83* transcript within a 3292 nucleotide sequence from the 63B heat shock locus of *D. melanogaster*. *Nucleic Acids Res.* **11**: 7011–7030.
- Hawley, D.K. and R.G. Roeder. 1985. Separation and partial characterization of three functional steps in transcription initiation by human RNA polymerase II. *J. Biol. Chem.* **260**: 8163–8172.
- Kraus, K.W., Y. Lee, J.T. Lis, and W.F. Wolfner. 1988. Sex-specific control of *Drosophila melanogaster* yolk protein 1 gene expression is limited to transcription. *Mol. Cell. Biol.* **8**: 4756–4764.
- Legerski, R.J., H.L. Hodnett, and H.B. Grey. 1978. Extracellular nucleases of *Pseudomonas* Bal 31. III. Use of the double-strand deoxyribonuclease activity as the basis of a convenient method for the mapping of fragments of DNA produced by cleavage with restriction enzymes. *Nucleic Acids Res.* **5**: 1445–1463.
- Lis, J.T., D. Ish-Horowicz, and S.M. Pinchin. 1981a. Genomic organization and transcription of the alpha-beta heat shock DNA in *Drosophila melanogaster*. *Nucleic Acids Res.* **9**: 5297–5310.
- Lis, J.T., W. Neckameyer, R. Dubensky, and N. Costlow. 1981b. Cloning and characterization of nine heat-shock-induced mRNAs of *Drosophila melanogaster*. *Gene* **15**: 67–80.
- Lis, J.T., H. Xiao, and O. Perisic. 1990. Modular units of heat shock regulatory regions: Structure and function. In *Stress proteins in biology and medicine* (ed. R. Morimoto, A. Tissières, and C. Georgopoulos), pp. 411–428. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Love, J.D. and K.W. Minton. 1985. Screening of lambda library for differentially expressed genes using in vitro transcripts. *Anal. Biochem.* **150**: 429–441.
- O'Brien, T. and J.T. Lis. 1991. RNA polymerase II pauses at the 5' end of the transcriptionally induced *Drosophila hsp70* gene. *Mol. Cell. Biol.* **11**: 5285–5290.
- Parker, C.S. and J. Topol. 1984. A *Drosophila* RNA polymerase II transcription factor binds to the regulatory site of an *hsp70* gene. *Cell* **37**: 273–283.
- Pellman, D., M.E. McLaughlin, and G.R. Fink. 1990. TATA-dependent and TATA-independent transcription at the *HIS4* gene of yeast. *Nature* **348**: 82–85.
- Perisic, O., H. Xiao, and J.T. Lis. 1989. Stable binding of *Drosophila* heat shock factor to head-to-head and tail-to-tail repeats of a conserved 5 bp recognition unit. *Cell* **59**: 797–806.
- Rougvié, A.E. and J.T. Lis. 1988. The RNA Polymerase II molecule at the 5' end of the uninduced *hsp70* gene of *D. melanogaster* is transcriptionally engaged. *Cell* **54**: 795–804.
- . 1990. Post-initiation transcriptional control in *Drosophila melanogaster*. *Mol. Cell. Biol.* **10**: 6041–6045.
- Rubin, G.M. and A.C. Spradling. 1982. Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**: 348–353.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Shuey, D.J. and C.S. Parker. 1986. Binding of *Drosophila* heat-shock gene transcription factor to the *hsp70* promoter: Evidence for symmetric and dynamic interactions. *J. Biol. Chem.* **261**: 7934–7940.
- Simon, J.A., C.A. Sutton, R.B. Lobell, R.L. Glaser, and J.T. Lis. 1985. Determinants of heat shock-induced chromosome puffing. *Cell* **40**: 805–817.
- Smale, S.T. and D. Baltimore. 1989. The "initiator" as a transcription control element. *Cell* **57**: 103–113.
- Taylor, I.C.A., J.L. Workman, T.J. Schuetz, and R.E. Kingston. 1991. Facilitated binding of GAL4 and heat shock factor to nucleosomal templates: Differential function of DNA-binding domains. *Genes & Dev.* **5**: 1285–1298.
- Thomas, G.H. and S.C.R. Elgin. 1988. Protein/DNA architecture of the DNase I hypersensitive region of the *Drosophila hsp26* promoter. *EMBO J.* **7**: 2191–2201.
- Workman, J.L., I.C.A. Taylor, and R.E. Kingston. 1991. Activation domains of stably bound GAL4 derivatives alleviate repression of promoters by nucleosomes. *Cell* **64**: 533–544.
- Wu, C. 1980. The 5' ends of *Drosophila* heat shock genes in chromatin are hypersensitive to DNase I. *Nature* **286**: 854–860.
- . 1984. Two protein-binding sites in chromatin implicated in the activation of heat-shock genes. *Nature* **309**: 229–234.
- Xiao, H. and J.T. Lis. 1986. A consensus sequence polymer inhibits in vivo expression of heat shock genes. *Mol. Cell. Biol.* **6**: 3200–3206.
- . 1988. Germline transformation used to define key features of heat-shock response elements. *Science* **239**: 1139–1142.
- Zimarino, V. and C. Wu. 1987. Induction of sequence-specific binding of *Drosophila* heat shock activator. *Nature* **327**: 727–730.

DNA sequence requirements for generating paused polymerase at the start of hsp70.

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References

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