MOLECULAR AND CELLULAR BIOLOGY, Aug. 1988, p. 3204–3214
0270-7306/88/083204-11$02.00/0

Vol. 8, No. 8

TATA Box-Dependent Protein-DNA Interactions Are Detected on Heat Shock and Histone Gene Promoters in Nuclear Extracts Derived from *Drosophila melanogaster* Embryos

DAVID S. GILMOUR,[1]* THOMAS J. DIETZ,[1,2] AND SARAH C. R. ELGIN[1]

Department of Biology[1] and Molecular Biology and Biochemistry Program, Division of Biology and Biomedical Sciences,[2] Washington University, St. Louis, Missouri 63130

Received 29 January 1988/Accepted 2 May 1988

We monitored protein-DNA interactions that occur on the hsp26, hsp70, histone H3, and histone H4 promoters in nuclear extracts derived from frozen *Drosophila melanogaster* embryos. All four of these promoters were found to be transcribed in vitro at comparable levels by extracts from both heat-shocked and non-heat-shocked embryos. Factors were detected in both types of extracts that block exonuclease digestion from a downstream site at ca. +35 and −20 base pairs from the start of transcription of all four of these promoters. In addition, factors in extracts from heat-shocked embryos blocked exonuclease digestion at sites flanking the heat shock consensus sequences of hsp26 and hsp70. Competition experiments indicated that common factors cause the +35 and −20 barriers on all four promoters in both extracts. The formation of the barriers at +35 and −20 required a TATA box but did not appear to require specific sequences downstream of +7. We suggest that the factors responsible for the +35 and −20 barriers are components whose association with the promoter precedes transcriptional activation.

The RNA polymerases of eucaryotes do not have the innate capacity to recognize promoter sequences in DNA. Instead, they appear to recognize particular nucleoprotein structures (see references 6 and 38 for discussion). Treatment of chromatin in nuclei with DNase I and other DNA cleavage reagents shows that the DNA in the immediate vacinity of the transcription start of many genes is particularly sensitive to digestion (for review, see references 12 and 35). Since this hypersensitivity often exists prior to, and always at the earliest stages of, gene activation, it is reasonable to hypothesize that this hypersensitivity might reflect a precursor to or the nucleoprotein structure recognized by RNA polymerase.

The promoter regions of the heat shock genes in *Drosophila melanogaster* are in a DNase I-hypersensitive configuration prior to transcriptional induction (9, 19, 40). High-resolution analyses indicate that proteins are associated with the TATA boxes (7, 41; G. H. Thomas and S. C. R. Elgin, EMBO J., in press); this interaction may be critical for the formation of the hypersensitive site. Following a heat shock treatment, transcription of the heat shock genes is dramatically and rapidly induced (1). This induction appears to involve the rapid activation of a protein that then binds to the heat shock consensus sequences (HSCS) located upstream of the transcriptional initiation sites (10, 34, 45).

It has recently been discovered, by using an in vivo protein-DNA cross-linking technique, that on average one RNA polymerase II molecule is associated with each hsp70 promoter prior to transcriptional induction by heat shock (15). The RNA polymerase II is bound within the region of DNase I hypersensitivity which surrounds the transcriptional initiation site. The presence of RNA polymerase II prior to gene activation raises the intriguing possibility that the regulatory event leading to transcriptional induction occurs subsequent to the RNA polymerase II-promoter interaction. This contrasts with models in which regulatory factors function by facilitating the association of RNA polymerase with the promoter.

To begin to identify proteins whose interactions with the promoter might precede transcriptional induction, we monitored the interaction of proteins with several *D. melanogaster* promoters in extracts from heat-shocked and non-heat-shocked *D. melanogaster* embryos. Two heat-shock-inducible promoters, those of hsp70 and hsp26, and two promoters whose activities are slightly repressed by heat shock, those of histones H3 and H4 (14), were examined so that interactions independent of transcriptional induction might be distinguished from those dependent on induction. The former class of interactions might be involved in the formation of the DNase I-hypersensitive sites or the RNA polymerase-promoter interactions that precede induction or both. The extracts were prepared from frozen *D. melanogaster* embryos because these provide an abundant, relatively inexpensive, and consistent source of material; as such, these extracts should serve well as the starting point for the isolation of proteins essential to promoter function.

MATERIALS AND METHODS

Description of clones. The promoter regions of the *D. melanogaster* heat shock and non-heat shock genes were cloned into vector pUC13 (25) so that end-labeled DNA fragments could be easily prepared for binding studies and transcription products could be monitored with commercially available oligonucleotide primers. *Sal*I and *Xho*I sites have also been positioned at each side of the promoter region so that the promoters could be polymerized into tandem arrays (44).

The hsp26 promoter fragment was derived originally from plasmid 88R6.1, a subclone of 88B13 (8) which was prepared in this laboratory. An *Eco*RI/*Pst*I fragment was isolated and digested with *Sau*3A. The mixture of fragments was ligated into *Bam*HI/*Eco*RI-cut pUC13, leading to the cloning of sequences from −170 to +7 (where +1 represents the start of transcription); the union of the *Bam*HI site of pUC13 with

* Corresponding author.

the *Sau*3A site at −170 of the promoter results in the creation of a *Bam*HI site at this junction. This clone was designated 88BE; its binding and transcriptional properties are presented in Fig. 7. The region from −170 to +7 was isolated as a *Bam*HI/*Eco*RI fragment, mixed with an isolated *Eco*RI/*Bam*HI fragment (derived from the clone which provides probe d in reference 33) encompassing the region of hsp26 from +7 to +47, and ligated into *Bam*HI-cut pUC13. A clone encompassing the region from −170 to +47 was isolated, and the expected sequence was confirmed by DNA sequencing. This clone was further modified by changing the *Xma*I site in the pUC13 polylinker region and immediately adjacent to −170 to an *Xho*I site; *Xma*I-cut DNA was treated with the Klenow fragment of DNA polymerase, ligated with *Xho*I linkers, recircularized with T4 ligase, and transformed into *Escherichia coli*. This clone, whose insert is flanked by *Xho*I and *Sal*I sites, is called hsp26 S/X.

The hsp70 promoter clone was derived from clone 132E3 (27). An *Xba*I/*Pst*I fragment containing the region from −254 to +87 was cloned into *Xba*I/*Pst*I-cut pUC13. The *Pst*I site was converted into a *Sal*I site by using *Sal*I linkers to produce hsp70 S/X. An *Xho*I/*Sal*I fragment, which encompasses sequences from −195 to +87, from this second clone was inserted into the *Xho*I/*Sal*I vector portion hsp26 S/X to create the clone called hsp70 S/X-1.

The histone H3/H4 promoter clone was derived from clone cDm500 (21) and was prepared essentially as described by Heiermann and Pongs (16). The ends of an *Hae*II fragment harboring the two promoters were made blunt with T4 DNA polymerase and cloned into the *Sma*I site of pUC13. The resulting clone was further modified by inserting an *Xho*I site at the *Eco*RI site flanking the insert and downstream of the H3 promoter. (*Eco*RI-cut DNA was treated with the Klenow fragment of DNA polymerase, ligated with *Xho*I linkers, recircularized with T4 ligase, and transformed into *E. coli*.) Insertion of the *Xho*I site produced *Eco*RI sites on both sides of the *Xho*I site. The resulting clone is called H3/H4 S/X.

Cloned promoter polymers were prepared by isolating the *Sal*I/*Xho*I fragments containing each promoter and ligating them together with T4 ligase. The mixture was cut with *Xho*I and *Sal*I, resulting in head-to-tail tandem arrays of the promoter fragments. Polymers larger than five copies in length were isolated on agarose gels and ligated into the *Sal*I/*Xho*I vector portion of the hsp26 S/X clone. Clones were obtained that contained six copies of the hsp26 promoter, six copies of the hsp70 promoter, or eight copies of the H3/H4 promoter.

To prepare the hsp70 TATA box deletion, the hsp70 promoter fragment was isolated as an *Xho*I/*Hin*dIII fragment from hsp70 S/X. A portion of the fragment preparation was ligated into *Sal*I/*Hin*dIII-cut M13mp10 to produce a "wild-type" promoter called hsp70 wt. Another portion of the fragment was cut with *Hae*II and was spermine precipitated (17) to deplete the fragment mixture of the small 20-base-pair TATA box-containing region (see Fig. 6). The mixture was then ligated into *Sal*I/*Hin*dIII-cut M13mp10, and a "TATA box deletion" was isolated.

The 40-mer of the hsp70 HSCS was kindly provided by H. Xiao and J. T. Lis, Cornell University, New York, N.Y. It is plasmid pXM40 (44); we have referred to it as the HSCS 40-mer.

Isolation of promoter fragments for competition. DNA fragments containing promoter sequences were isolated from their respective promoter polymer clones. The region from −170 to +47 of hsp26 was derived from the polymer clone as a *Bam*HI fragment. The region from +103 downstream of H3 to +100 downstream of H4 was isolated as a *Bam*HI/*Eco*RI fragment. The region from −195 to +87 of the hsp70 promoter was isolated as a *Pva*II fragment; because the region from −195 to +87 is in tandem arrays, sequences from +65 to +85 are positioned adjacent to sequences at −195 in the isolated fragment. The region containing 40 tandemly repeated copies of the hsp70 HSCS was isolated as an *Eco*RI/*Hin*dIII fragment from plasmid pXM40.

Extract preparation. Embryos, 6 to 18 h old, were collected and flash frozen in liquid nitrogen as described by Miller and Elgin (26). For heat shock treatment preceding flash freezing, the embryos were suspended in *Drosophila* Ringer solution (130 mM NaCl, 5 mM KC1, 1.5 mM $CaCl_2$ [13]) at 25°C and were vigorously shaken in an air incubator for 1 h at 37°C.

Nuclei were isolated essentially as described by Miller and Elgin (26), with minor modifications. A 100 g portion of embryos was dechorionated in 300 ml of a freshly prepared solution of 50% Chlorox–1% Triton X-100–1% NaCl. The dechorionated embryos were broken by homogenization in 100 ml of buffer A {1 M sucrose, 4 mM $MgCl_2$, 0.1 mM EGTA [ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-*2*′-ethanesulfonic acid], pH 7.5, 2 mM β-mercaptoethanol,0.5 mM phenylmethylsulfonyl fluoride–5 μg of leupeptin per ml} and filtered through two layers of Miracloth (Miracloth reusable cloths are green, silicone-treated, nonwoven rayon cloths). The retentate was suspended in an additional 100 ml of buffer A, homogenized, and filtered through the Miracloth. A low-speed centrifugation at 400 × *g* for 10 min in a GSA rotor (Ivan Sorvall, Inc.) removed additional debris, and the nuclei were collected from the supernatant by centrifugation at 4,400 × *g* for 10 min in a GSA rotor. Nuclei were suspended in 20 ml of buffer A, and 10-ml portions were overlaid on 20 ml of high-sucrose buffer (1.75 M sucrose, 2 mM $MgCl_2$, 0.1 mM EGTA, 10 mM HEPES [pH 8.0], 2 mM β-mercaptoethanol, 0.5 mM phenylmethyl-sulfonyl fluoride, 5 μg of leupeptin per ml) and pelleted by centrifugation at 25,000 × *g* for 30 min in an HB4 rotor (Sorvall). The pelleted nuclei were suspended in 18 ml of 0.3 M sucrose–10 mM HEPES (pH 8.0)–2 mM $MgCl_2$–0.1 mM EGTA–0.1 M NaCl–0.5 mM dithiothreitol (DTT)–0.5 mM phenylmethylsulfonyl fluoride–0.1 mM leupeptin and separated into six 3-ml portions in AH65 ultracentifuge tubes (Sorvall).

The suspended nuclei were extracted with ammonium sulfate essentially as described by Parker and Topol (28). One-tenth volume of 4 M $(NH_4)_2SO_4$ was slowly added with mixing to each tube, and tubes containing the viscous lysate were rocked at 4°C for 30 min. The samples were centrifuged at 45,000 rpm for 1 h in a Sorvall AH65 rotor. The supernatant was transferred to a 50-ml tube, and 0.3 g of solid $(NH_4)_2SO_4$ per ml was added. The samples were left on ice for several hours, and then precipitates were collected by centrifugation in an HB4 rotor at 12,500 rpm for 30 min. The pellet was suspended in 8 ml of 40 mM KCl–25 mM HEPES (pH 7.6)–0.1 mM EDTA–1 mM DTT–10% glycerol and dialyzed against 2 liters of the same buffer for 4 h. The final conductivity was within 20% of that of 0.1 M KCl–25 mM HEPES (pH 7.6)–0.1 mM EDTA–1 mM DTT–10% glycerol. Insoluble material was removed by centrifugation at 10,000 rpm in an HB4 rotor for 15 min. The final protein concentration was 6 mg/ml (as determined by the Bradford assay [4], using bovine serum albumin as standard). Samples were stored frozen at −80°C.

In vitro transcription. A 5-μl amount of extract (6 mg/ml) was incubated with 1 μg of DNA in 25 μl. In addition to the components contributed by the extract, the buffer contained 50 mM KCl, 10 mM HEPES (pH 8.0), 5 mM $MgCl_2$, 0.5 mM each ATP, CTP, GTP, and UTP, 0.5 mM DTT, and 0.2 U of RNasin (Promega) per ml. Each transcription reaction was carried out at 25°C for 30 min and was stopped by the addition of 75 μl of 53 μg of yeast tRNA per ml–133 μg of proteinase K per ml–0.8% sodium sarkosine. After a 30-min incubation at 37°C, 10 μl of 3 M sodium acetate (pH 5.2) was added to the reaction mix and the sample was extracted once with phenol-chloroform-isoamyl alcohol (49.5:49.5:1). The underlying organic phase was removed, and nucleic acids in the remaining aqueous phase were precipitated with 2.5 volumes of ethanol. The precipitated nucleic acids were dissolved in 50 μl of 50 mM Tris chloride (pH 8.0)–10 mM $MgCl_2$–0.2 U of RNasin per μl and treated with 2 U of DNase I for 30 min at 37°C. A 4-μl portion of 5 M NaCl and 46 μl of H_2O were added, and each sample was again extracted with the phenol-chloroform-isoamyl alcohol mixture and precipitated with ethanol. The RNA pellet was dissolved in 6 μl of H_2O containing 50,000 Cherenkov counts of ^{32}P-end-labeled oligonucleotide primer as indicated in the figure legends. (The 17-base primer [no. 1201, New England BioLabs, Inc.] and the 24-base primer [no. 1224, New England BioLabs] were labeled with polynucleotide kinase [22] and purified on Nensorb 20 columns [Du Pont Co.] according to the manufacturer's instructions.) To the dissolved RNA was added 4 μl of 2.5 M NaCl–25 mM Tris chloride (pH 8.0)–0.25 mM EDTA; the samples were incubated overnight at 45°C for the 24-base primer or at 37°C for the 17-base primer. The sample was diluted 10-fold, and primer extension reactions were carried out as described by Williams and Mason (39). Nucleic acids were ethanol precipitated with 10 μl of 3 M sodium acetate (pH 5.2) and 250 μl of ethanol and, following solubilization, a second time with 50 μl of 0.4 M LiCl–4 mM Tris chloride (pH 8)–0.4 mM EDTA with the addition of 125 μl of ethanol. The final precipitate was dissolved in 5 μl of sequencing gel loading buffer (22) and run on an 8% polyacrylamide sequencing gel.

Molecular weight markers were prepared by annealling the radioactively labeled 24-mer primer to a single-stranded M13 clone containing the hsp70 promoter and carrying out primer extension reactions essentially as described by McGraw (24).

Exonuclease protection assay. The exonuclease protection assays were performed essentially as described by Wu (42). A 2.5-μl portion of extract was added to 10 μl of solution containing DNA end labeled with polynucleotide kinase (10,000 Cherenkov counts) in 93.8 mM KCl–6.25 mM $MgCl_2$–0.125 mM EGTA–18.8 mM Tris chloride (pH 7.6)–0.625 mM DTT–2.5 mM sodium phosphate–6.25% glycerol–50 μg of *Hae*III-cut *E. coli* DNA per ml–50 μg of mixed deoxynucleotide hexamers (Pharmacia, Inc.) per ml–25 μg of yeast tRNA per ml. A 200-ng amount of specific competitor DNA or additional *Hae*III-cut *E. coli* DNA was added to the reaction mix prior to the addition of nuclear extract. The mixture was incubated at 25°C for 15 min and then treated for 10 min at 30°C with 2 μl of freshly diluted exonuclease III (3 μl of exonuclease III [200 U/μl; Boehringer Mannheim Biochemicals] mixed with 24 μl of ice-cold 50 mM NaCl–20 mM Tris chloride [pH 8.0]–5 mM $MgCl_2$). The reaction was stopped with 25 μl of 10 mM EDTA–1% sodium dodecyl sulfate, and the mixture was diluted to 100 μl with H_2O. A 10-μl portion of 3 M sodium acetate (pH 5.3) was added, and the samples were phenol extracted, ethanol precipitated, and analyzed on 8% polyacrylamide sequencing gels.

RESULTS

Extracts from non-heat-shocked and heat-shocked *D. melanogaster* embryo nuclei accurately initiated transcription from the hsp70, hsp26, histone H4, and histone H3 promoters. We were interested in identifying those components that interact with the promoter of a gene and might serve in establishing the preinitiated state. The four promoters that have been studied here are from the heat shock genes hsp26 and hsp70 and the histone genes H3 and H4 (Fig. 1). The heat shock gene promoters have been cloned individually, while the histone H3 and H4 promoters have been cloned together in their naturally occurring divergent orientation. Since the production of a properly initiated transcript would presumably require components which interact with the promoter before and after initiation of transcription, we have used the criteria of transcription in choosing an extract with which to begin our studies. The extracts from nuclei of frozen embryos accurately initiated transcription on the four promoters described above (Fig. 1). Transcripts initiating within the promoters extended into the pUC13 vector sequences and could be analyzed by "primer extension analysis" from a labeled pUC13-homologous probe by reverse transcriptase. Figure 1 shows that the major site of transcriptional initiation for each of these promoters is the same as reported for transcripts produced in vivo (16, 18, 36). The minor bands may reflect weak transcription initiation sites or sites where reverse transcriptase has prematurely terminated or both. No other strong transcription initiation sites could be detected within the cloned promoter regions (data not shown).

Specific protein-DNA interactions were detected on the hsp26 promoter by using an exonuclease protection assay. Protein-DNA interactions were analyzed by using an exonuclease protection assay that allows detection of sequence-specific interactions in the presence of numerous nonspecific interactions (42). A DNA fragment labeled at one end with polynucleotide kinase was first incubated with extract and then treated with exonuclease III. The presence of protein will impede the progress of the exonuclease, and its location was determined by sizing the resulting, partially digested DNA fragment on a sequencing gel. The pattern of barriers produced when purified DNA was treated with exonuclease III (Fig. 2, lane 1) differed dramatically from that produced on DNA in the presence of nuclear extract (Fig. 2, lanes 2 and 8). Prominent barriers were seen at positions +35 and −21, with an additional barrier at −41 when extract from heat-shocked embryos was used. Certain sequences in purified DNA themselves appeared to impede the progress of exonuclease III. Usually these barriers could be distinguished from those produced by bound protein by comparing the exonuclease III digestion pattern of protein-bound DNA with that of purified DNA. However, this comparison was not sufficient in our case because an endogenous nuclease contributed to the overall pattern of degraded DNA recovered following the incubations with extract (Fig. 2, lanes 13 and 14). To identify barriers that are due to bound protein, we carried out the binding reaction in the presence of different nonradioactive competitor DNA fragments and then subjected the mixture to exonuclease III treatment. If a barrier was due to the binding of a specific protein, the barrier should be reduced when excess, nonradioactive DNA containing the binding site was included in the reaction; a DNA fragment lacking the binding site should fail to

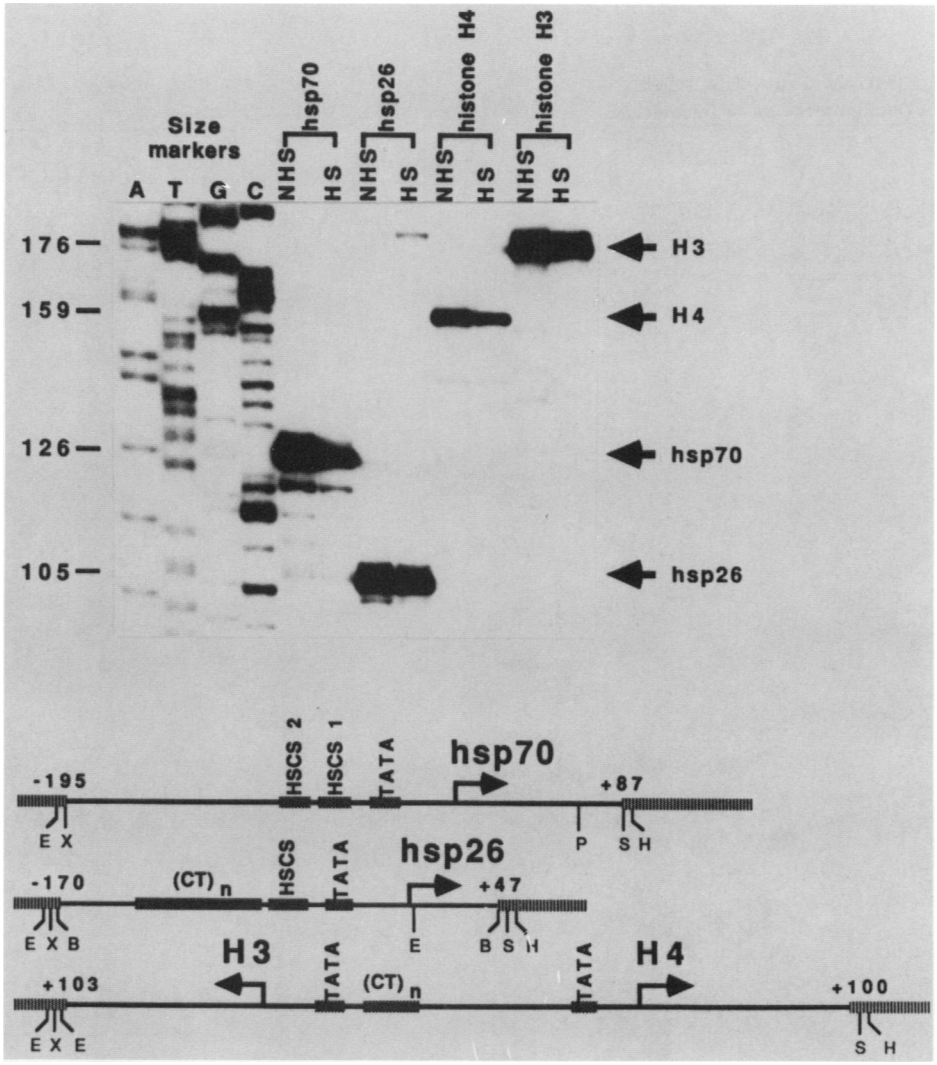

FIG. 1. Transcription from the hsp70, hsp26, histone H4, and histone H3 promoters in extracts from non-heat-shocked and heat-shocked embryos. In vitro transcription reactions were carried out on each of the plasmids containing the regions depicted by the restriction maps. A 1-μg portion of each plasmid was incubated with 5 μl of extract (protein concentration, 6 mg/ml) in a total of 25 μl for 30 min at 25°C. Transcripts from the hsp70, hsp26, and histone H4 genes were detected by primer extension with an end-labeled 17-base oligonucleotide, and histone H3 transcripts were detected with an end-labeled 24-base oligonucleotide. Sizes were determined by comparison with the sequencing reaction products from an M13 clone containing the hsp70 promoter; numbers on the left indicate the lengths of the polynucleotides. The maps describe the promoter clones used throughout much of this study. The numbers indicate positions relative to the transcription start site of each promoter. The positions of the HSCS are shown. TATA indicates the position of the TATA box, $(CT)_n$ indicates the position of a homopurine stretch of bases (33), and the large arrows indicate the start site of transcription in vivo with the heads pointed in the direction of transcription. The letters below the lines represent restriction enzyme sites: E, *Eco*RI; X, *Xho*I; S, *Sal*I; H, *Hin*dIII; B, *Bam*HI; P, *Pvu*II.

alter the barrier. When nonradioactive DNA fragments containing the promoters for hsp70, hsp26, or histones H3 and H4 (Fig. 2, lanes 3, 4, and 5, respectively) were included in the binding reactions, the barriers at positions +35 and −21 were greatly reduced, indicating that they are produced by the binding of promoter-specific DNA-binding proteins. These barriers were not altered by the presence of additional *E. coli* DNA (lane 2) or a fragment containing 40 copies of the hsp70 HSCS (lane 6).

A similar analysis has been carried out for the hsp26 promoter fragments added to nuclear extracts from heat-shocked embryos (Fig. 2, lanes 8 to 12). The patterns of barriers produced by the extracts from non-heat-shocked and heat-shocked embryos under corresponding reaction conditions were very similar. An important exception was a barrier produced in the extract from heat-shocked embryos at −41 which flanks the HSCS. This barrier was reduced when either the hsp70 or the hsp26 competing promoter fragment was present or when a fragment containing 40 copies of the two HSCS of hsp70 was present (Fig. 2, lanes 9, 10, and 12) but not when *E. coli* DNA or the histone H3/H4 promoter fragment was present (lanes 8 and 11). The results indicate that the barrier at −41 is caused by the binding of protein to the HSCS.

A complex pattern of barriers within the HSCS region was produced in extracts from both heat-shocked and non-heat-shocked embryos. Most of these fragments appeared to be the end products of digestions in which two exonuclease molecules digesting from each end of the DNA molecule met roughly in the middle of the fragment. As the digestion proceeded from both ends of the DNA, eventually the duplexed region became so small that the two strands

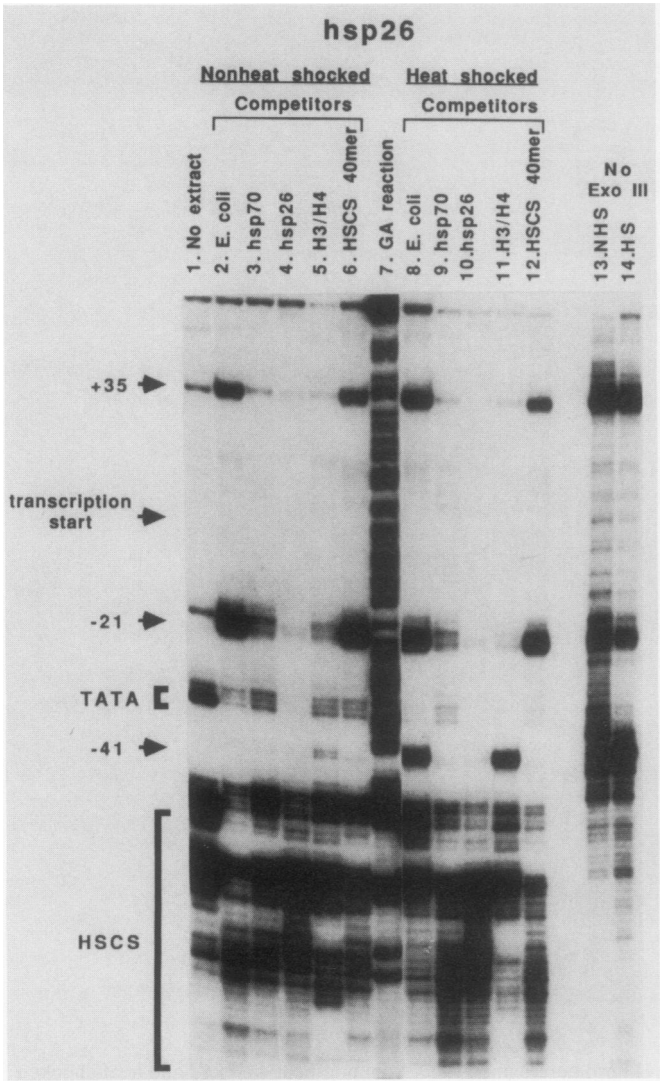

FIG. 2. Exonuclease protection assays of the hsp26 promoter region in nuclear extracts from non-heat-shocked and heat-shocked embryos. Nuclear extracts from non-heat-shocked (lanes 2 through 6 and 13) and heat-shocked (lanes 8 through 12 and 14) embryos were incubated with an hsp26 promoter fragment that was radioactively labeled at the *Xho*I site (see map in Fig. 1). In addition, 200 ng of *Hae*III-cut *E. coli* DNA (lanes 2 and 8), hsp70 (lanes 3 and 9), hsp26 (lanes 4 and 10), H3/H4 (lanes 5 and 11), or HSCS DNA fragments (lanes 6 and 12) was present as a competitor. Following a 15-min incubation at 25°C, all reactions except those in lanes 13 and 14 were treated with exonuclease III at 30°C for 10 min; samples in lanes 13 and 14 were incubated without exonuclease III at 30°C for 10 min. DNA was purified and analyzed on an 8% sequencing gel. The naked DNA control (lane 1) is end-labeled DNA treated only with exonuclease III. Lane 7 contains the purine cleavage reaction products of the end-labeled DNA. The transcription start site is given; TATA indicates the TATA box. The numbered arrows indicate positions where protein-DNA interactions impede the digestion by exonuclease (numbering relative to the transcription start).

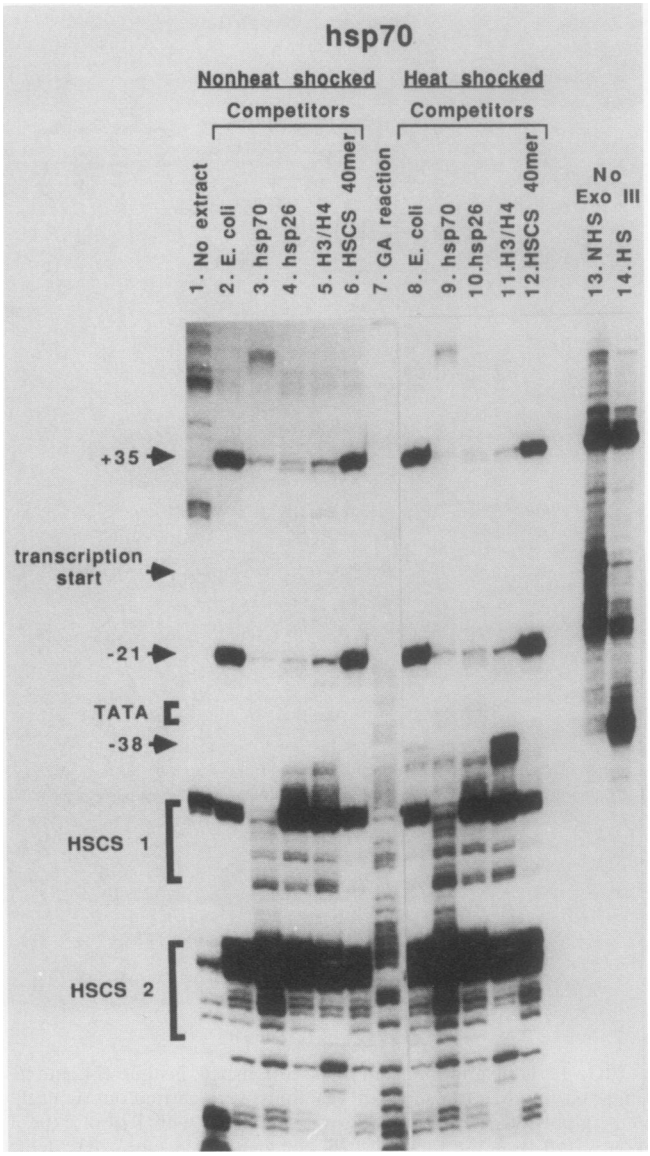

FIG. 3. Exonuclease protection assays of the hsp70 promoter region in nuclear extracts from non-heat-shocked and heat-shocked embryos. Binding studies with an hsp70 promoter fragment end-labeled at the *Eco*RI site (see map in Fig. 1) were carried out similarly to those described in the legend to Fig. 2.

separated. The resulting single-stranded DNA was not a suitable substrate for exonuclease III. Variations in the pattern (contrast lanes 8 and 11 with lanes 9, 10, and 12) suggest that the presence of the HSCS-binding protein or other proteins modulates this effect.

Interactions similar to those on hsp26 occurred on hsp70 in nuclear extracts from non-heat-shocked and heat-shocked embryos. We examined the interactions that occurred on an end-labeled hsp70 fragment in nuclear extracts from non-heat-shocked and heat-shocked embryos. Two prominent barriers were produced at positions +35 and −21 relative to the transcription start site in the presence of both extracts (Fig. 3, lanes 2 and 8). These two barriers were eliminated by nonradioactive DNA fragments containing the hsp70, hsp26, or histone H3 and H4 promoters (lanes 3 to 5 and 9 to 11). An additional weak barrier flanking HSCS1 was produced in the extract from heat-shocked embryos at position −38. This barrier did not form when the HSCS polymer fragment or the hsp26 or hsp70 promoter fragment was present, indicating that it was due to the same protein that generated the barrier at −41 on the hsp26 promoter. We do not understand why the −38 barrier is stronger in the presence of the H3/H4

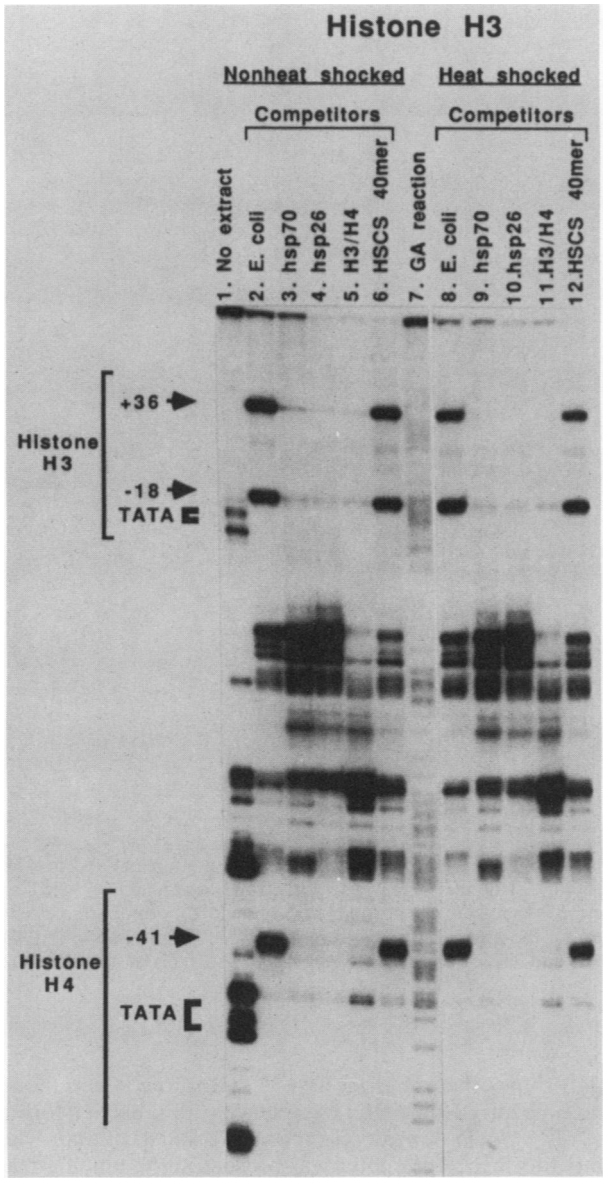

FIG. 4. Exonuclease protection assays of the histone H3 promoter region in nuclear extracts from non-heat-shocked and heat-shocked embryos. Binding studies with the H3/H4 promoter fragment endlabeled at the *Sal*I site (see map in Fig. 1) were carried out similarly to those described in the legend to Fig. 2. The numbered arrows indicate points where protein-DNA interactions impeded the digestion by exonuclease, and the numbers indicate the positions of the barriers relative to the transcription start.

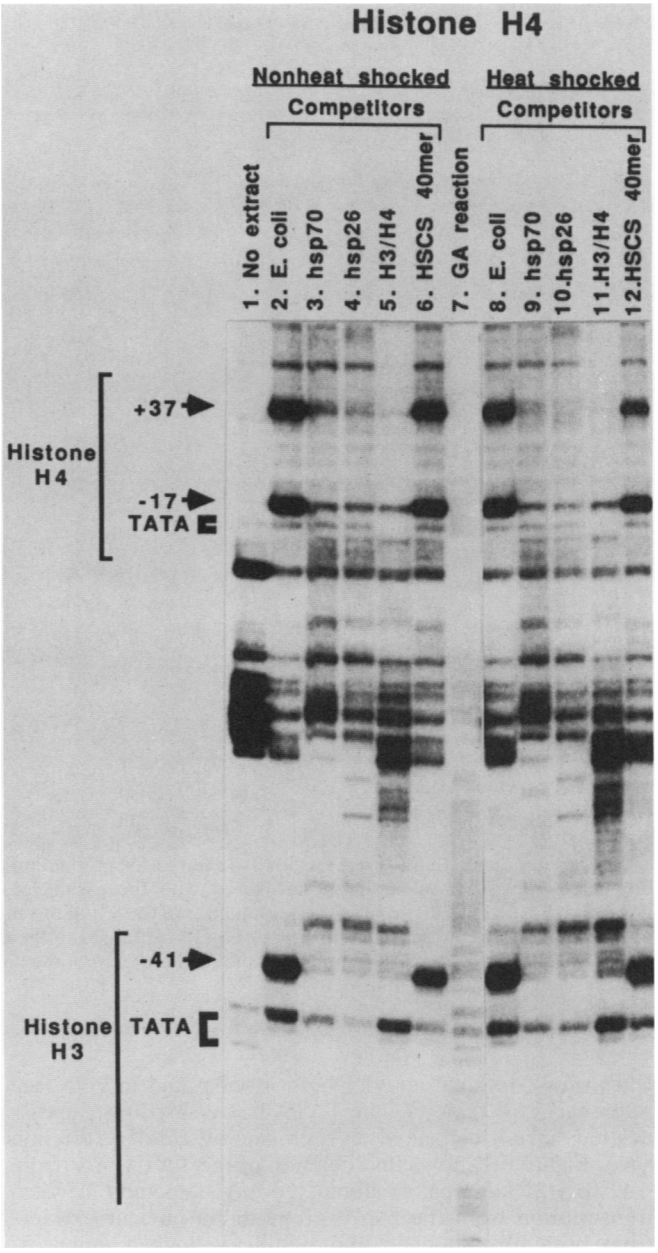

FIG. 5. Exonuclease protection assays of the histone H4 promoter region in nuclear extracts from non-heat-shocked and heat-shocked embryos. Binding studies with the H3/H4 promoter fragment endlabeled at the *Xho*I site (see map in Fig. 1) were carried out similarly to those described in the legend to Fig. 2.

competitor than in the presence of the *E. coli* DNA competitor, but this result is very reproducible.

Two barriers similar to those on the heat shock promoters were produced on the histone H3 and H4 promoters. Since the H3/H4 promoter fragment inhibited the formation of the barriers at +35 and −21 on the hsp70 and hsp26 promoter fragments, we wanted to determine whether or not similar barriers formed on the histone promoter fragment in nuclear extracts from non-heat-shocked and heat-shocked embryos. Figures 4 and 5 (lanes 2 and 8) show that barriers at approximately +35 and −20 formed on both histone promoters. These barriers were inhibited by the presence of nonradioactive promoter fragments (lanes 3 to 5 and 9 to 11). In addition, barriers at −41 were detected that were also inhibited by the presence of competing promoter fragments. The barrier at −41 flanked the "backside" of the TATA box of the proximal gene and appeared to be due to the protein bound at the second TATA box on this fragment. We observed a similar barrier on the hsp70 promoter when the radioactively labeled end of the DNA was downstream of the transcription start site (data not shown).

The TATA box, required for transcription in vitro, was required for binding of factors which caused the −20 and +35 barriers. The TATA box has been shown to be a critical element in many promoters since its deletion greatly dimin-

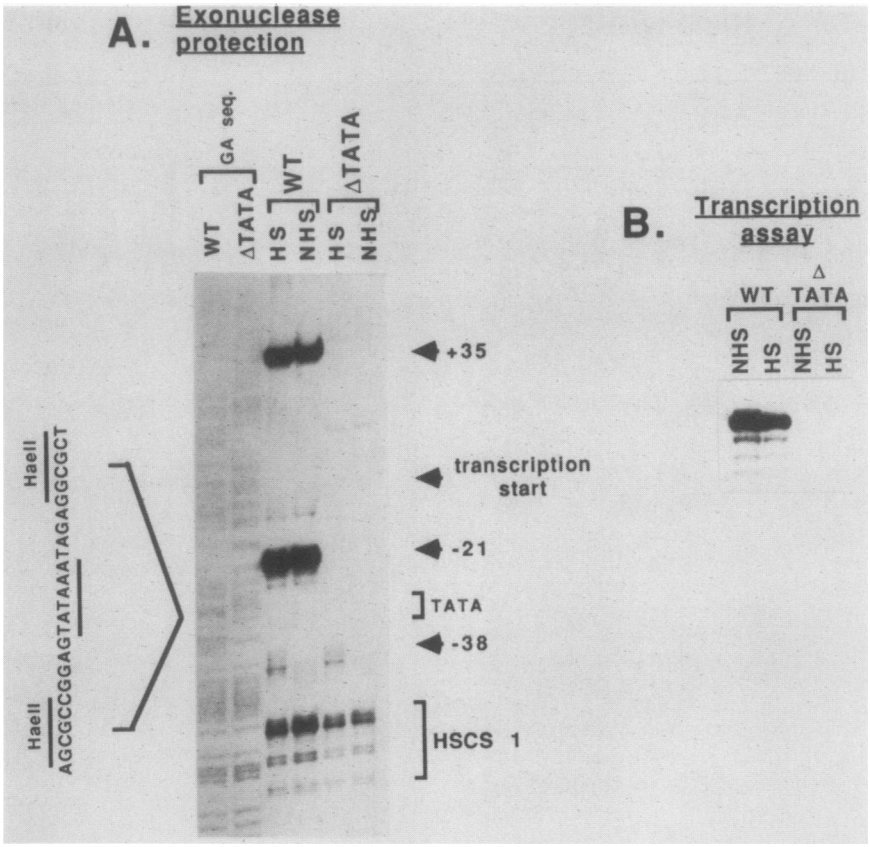

FIG. 6. Evidence that the TATA box is required for production of the barrier at +35. (A) Protein interactions on a normal and a TATA box-deleted hsp70 promoter were measured with the exonuclease protection assay in nuclear extracts from heat-shocked (HS) and non-heat-shocked (NHS) embryos. The sequence to the left of the autoradiogram is that from the normal (wild-type [WT]) promoter. Excision of the region between the two *Hae*II sites (−42 to −22) led to the production of the TATA box deletion (ΔTATA). (B) Transcription with extracts from heat-shocked and non-heat-shocked embryos of the normal (WT) and TATA box-deleted (ΔTATA) hsp70 promoter, measured as described in Materials and Methods.

ishes transcriptional activity both in vitro and in vivo (see reference 5 for review). The TATA box of hsp70 was easily deleted since it is flanked on each side by *Hae*II restriction sites. Figure 6B shows that deletion of the TATA box (from −42 to −22 base pairs) eliminated any detectable in vitro transcription from the hsp70 promoter in nuclear extracts from both heat-shocked and non-heat-shocked embryos. Removal of the TATA box also dramatically altered the protein-DNA interactions in extracts from both heat-shocked and non-heat-shocked embryos. Figure 6A shows that the barriers at −21 and +35 were both eliminated when the TATA box was deleted. This occurred despite the remaining presence of the sequences from −21 to +35. The interaction of heat shock factors with the HSCS on the TATA box-deleted promoter was still seen (−38 arrow). The properties of this deletion established a strong correlation between the barriers at ca. −20 and +35 and the transcriptional activity of the promoters.

The +35 barrier to exonuclease formed on cloning vector sequences when they were juxtaposed to the hsp26 transcription start site. We were curious to determine whether the barrier at +35 depended on the underlying sequences in addition to the TATA box. An hsp26 construction called 88BE, which replaces sequences downstream of +7 with pUC13 sequences, was tested in both the exonuclease protection assay and transcription assays. Figure 7A (lanes 2 and 4) shows that a barrier at +35 still formed even though it now occurred on pUC13 sequences; this barrier formed when *E. coli* DNA was present (lanes 2 and 4) but not when competitor hsp26 promoter was present in the binding reaction (lanes 3 and 5). In addition, Fig. 7B shows that this promoter was transcribed in vitro; direct comparison with the larger hsp26 promoter fragment shown in Fig. 1 indicated that the two were transcribed at nearly equivalent efficiencies. It would appear that a specific DNA sequence is not required downstream of the +7 site for protection of sequences to 35 bases downstream.

DISCUSSION

The promoter regions of many genes are in DNase I-hypersensitive configurations even prior to transcriptional induction (12, 35). This suggests that, for certain promoters, specific nonhistone chromosomal proteins might bind to the promoter in the absence of a complete transcriptional complex. High-resolution analyses indicate that chromosomal proteins are associated with the TATA box region of hsp70 (41) hsp26 (Thomas and Elgin, in press) prior to their activation. Lower-resolution analyses also indicate that the histone H3 and H4 promoters are in non-nucleosomal configurations prior to transcriptional induction (31). Here, we describe the detection of two binding activities in extracts

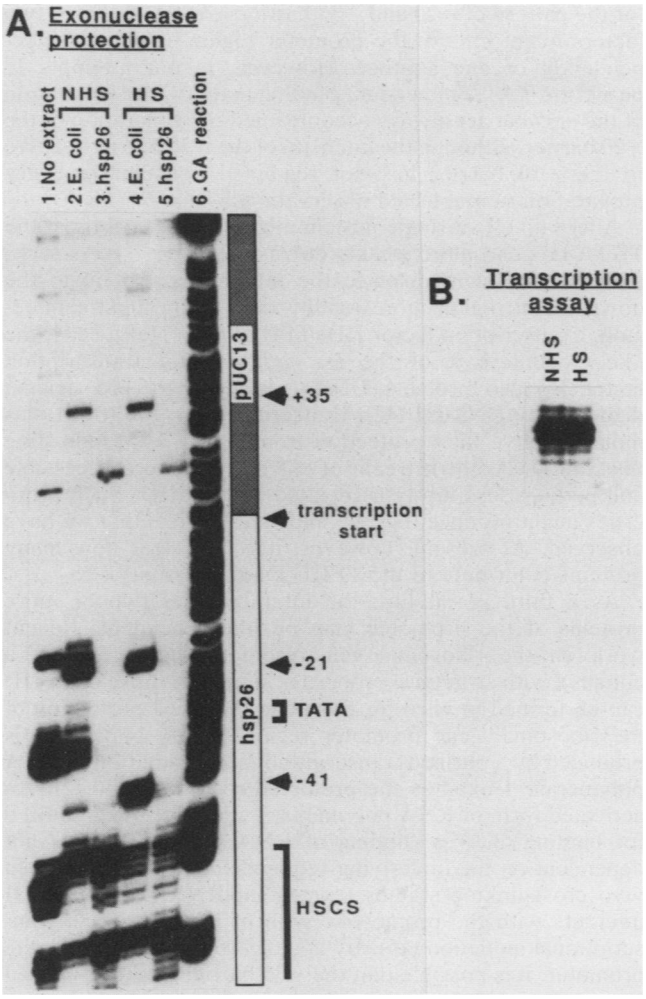

FIG. 7. Evidence that sequences downstream of +7 are not required for formation of the +35 barrier. (A) Protein interactions on an hsp26 promoter fused at position +7 to pUC13 sequences (clone 88BE) were measured with the exonuclease protection assay in nuclear extracts from non-heat-shocked (NHS) and heat-shocked (HS) embryos. The fragment was labeled at the *Bam*HI site at −170 (Fig. 1) and extended to the *Pvu*II site located in pUC13, 97 base pairs downstream from the hsp26 transcription start. A 200-ng amount of either *Hae*III-cut *E. coli* (lanes 2 and 4) or hsp26 (lanes 3 and 5) DNA was included as a competitor to identify sequence-specific interactions. Lane 1 contained purified, end-labeled DNA that was treated only with exonuclease III, and lane 6 contained the purine cleavage reaction products of this DNA. (B) Transcription with nuclear extracts from heat-shocked and non-heat-shocked embryos of the hsp26-pUC13 promoter fusion (clone 88BE) used in panel A.

derived from frozen *D. melanogaster* embryonic nuclei that depended upon the TATA box region. These binding activities may represent factors involved in the early preparation of the promoter for transcription. A second type of factor, one which interacts with the heat shock consensus sequences of the heat shock gene promoters, was detected in nuclear extracts from heat-shocked embryos.

An essential step towards understanding the transcription process involves the identification and isolation of transcription factors. For the most part, cultured cells have served as the source of these proteins. The observations we present here indicated that frozen *D. melanogaster* embryos should serve as a good alternative to cultured cells. There are significant advantages in using this source of starting material. By using large populations of flies, it is possible to obtain >500 g of embryos every 2 weeks; the bulk of this is collected over a 4-day period. Since our extracts were from frozen embryos, large stockpiles of starting material could be maintained. We estimate that 500 g of embryos can provide an equivalent amount of material as approximately 10 liters of *D. melanogaster* cells grown to a density of 10^{10} cells per liter. This estimate is derived by considering either the amount of DNA or the amount of chromosomal protein obtained from either starting source. We have observed that approximately 100 mg of DNA is recovered from 500 g of embryos or from 10 liters of cells. Alternatively, we find that 300 mg of nuclear protein extract is recovered from 500 g of embryos, and similar amounts have been reported from approximately 10 liters of cells (28, 42).

Based upon quantitative S1 measurements (data not shown), we estimate that the transcriptional activity of our nuclear extract from frozen embryos is similar to that of extracts from fresh embryos described by Heiermann and Pongs (16). This level is approximately 10-fold lower than crude nuclear extracts from *Drosophila* KcH cells (28). However, since the transcription reaction is influenced by many inhibitory and stimulatory factors, it is difficult to evaluate crude extracts solely by this criterion. Moreover, Price et al. (30) have found that, to obtain high-quality nuclear extracts from cultured cells, one must carefully maintain optimal growth conditions. The growth conditions for embryos are easily maintained at a constant state, and we have observed that the extracts from frozen embryos are of a very consistent quality.

The binding studies presented here indicated that potentially important factors are easily detected in the crude nuclear extracts. A TATA-binding factor was detected in 12 μg of nuclear protein from embryos; a factor with similar properties is detected in approximately the same amount of nuclear protein derived from cultured cells (42). The binding of a second factor, which interacted with the HSCS, was easily detected on hsp26 and more weakly detected on hsp70 in extracts from heat-shocked embryos.

It was not surprising that the crude nuclear extract from heat-shocked embryos did not exhibit selective or enhanced transcription of the heat shock genes in comparison to extracts from non-heat-shocked embryos; commonly, crude extracts fail to exhibit features of regulated transcription (11, 23, 29). We anticipate that stimulation of transcription will require at a minimum that the TATA-binding factors and the HSCS-binding factor occupy the DNA simultaneously. Under our present binding conditions, this requirement was not achieved. Such conditions will be more easily achieved following the isolation of the different factors.

By using an exonuclease III protection assay, Wu (41, 42) detected a factor in extracts from both non-heat-shocked and heat-shocked cells that protects the TATA box region of hsp70 between −21 (when exonuclease approaches from the downstream side) and −41 (when exonuclease approaches from the upstream side). This is similar to a protection that we observed in extracts from non-heat-shocked and heat-shocked *D. melanogaster* embryos. This factor from embryos interacted not only with the hsp70 and hsp26 promoters, but also with the histone H3 and H4 promoters. That the same factor interacted with these different promoters was indicated by the effective competition for binding and by the observation that the region of DNA protected from exonuclease digestion was similar on each promoter. It has been

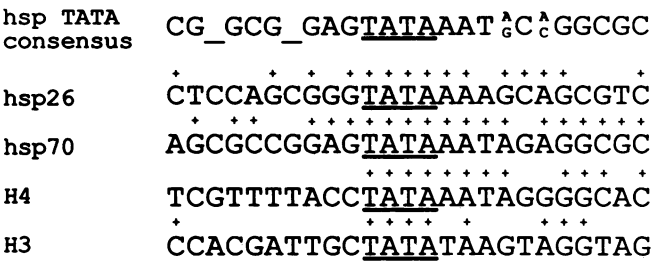

FIG. 8. Sequence comparison of the TATA box region. The sequence on the top line represents a TATA region consensus sequence for the heat shock gene family described by Wu (42). Below are the corresponding sequences from the hsp26, hsp70, histone H4, and histone H3 promoters. Pluses above each base indicate homology with the consensus sequence.

suggested (42) that all TATA-binding functions might be governed by a family of proteins, each recognizing a unique consensus sequence that surrounds the TATA box core (Fig. 8). A consensus sequence has been derived from the heat shock genes which could specify the interaction of heat shock gene promoters with a particular member of the TATA-binding family. However, since we observed competition for binding with the TATA region of both the heat shock and histone gene promoters, then at least one limiting component of the TATA factor must interact with a broader spectrum of promoters. Comparison of the TATA region for the histone genes with the TATA region for the heat shock genes indicated that there is a good match from the TATA to the downstream portion of the consensus, but no match with the consensus upstream of TATA (Fig. 8).

We detected a novel exonuclease barrier at position +35 that was produced when exonuclease approaches from downstream of this site. This barrier was not detected by Wu (42) in the nuclear extracts prepared from cultured cells; however, the methods used for preparing nuclear extracts differ. It appears that this barrier requires the TATA box region since it was not detected when the sequences from −42 to −22, which contain the TATA box, were deleted. The +35 barrier coincides with the extent of protection produced by transcription factor B from cultured *Drosophila* cells previously described by Parker and Topol (28, 29). It may be significant that we have followed an essentially similar procedure for extracting the isolated nuclei and both extracts accurately initiate transcription on the hsp70, histone H3, and histone H4 promoters. After several purification steps, transcription factor B has been shown to produce a DNase I footprint that extends from −40 to +35 (28, 29). Parker and Topol (29) reported that the levels of binding activity were lower in fractionated extracts from heat-shocked cells than in those from non-heat-shocked cells and suggested that this decrease could somehow contribute to the general repression of transcription that accompanies heat shock treatment. In contrast to the DNase I footprinting assay, the exonuclease protection assay provided a measure of DNA-binding activity without requiring extensive purification. With the exonuclease protection assay, the intensity of the +35 barrier was found to be essentially the same for heat shock and histone gene promoters after binding in nuclear extracts from non-heat-shocked and heat-shocked embryos. This indicates that the level of TATA-binding activity may not be different in non-heat-shocked and heat-shocked cells but that apparent differences may arise during the fractionation of the nuclear extract.

Three different types of interactions might be responsible for the pattern of −20 and +35 barriers observed. First, two factors might bind in the promoter region essentially independently of one another. However, in our attempts to purify the TATA-dependent binding activity, the generation of the +35 barrier always accompanied the generation of the −20 barrier, although the intensity of the +35 barrier relative to the −20 barrier has not remained constant in every preparation (unpublished observations).

Alternatively, a single protein might interact tightly at the TATA box and more weakly downstream to +35. As such, exonuclease might displace the interacting protein in the downstream region more readily than in the upstream region. Transcription factor IID (TFIID) from HeLa cells, the likely counterpart of the *D. melanogaster* transcription factor B, also protects DNA from −40 to +35 against digestion by DNase I (32). Protection from −40 to +1 was more effective than protection from +1 to +35, indicating that the DNA downstream of +1 may be more accessible and perhaps less firmly associated with TFIID. Such properties might produce the +35 and −20 barriers that we have observed. At present, however, it is not clear how many proteins contribute to the TFIID-binding activity.

As a third possibility, the interaction of two or more proteins at the promoter may be interdependent. Recent work on the ribosomal gene promoter indicates that a complex with structural properties similar to those of TFIID can be formed in vitro for this gene. DNase I protection of the ribosomal gene promoter region from −14 to −67 is produced by a purified transcription factor; addition of RNA polymerase I extends the protected region to +20 (2). An activated form of RNA polymerase I appears to be required for binding (3, 37). Binding of RNA polymerase I is not dependent on the underlying sequence composition (20). In vivo cross-linking studies reveal that RNA polymerase II interacts with the promoter region of hsp70 prior to transcriptional induction (15). By analogy to the ribosomal gene promoter, it is possible that the +35 barrier might be caused by a subunit of RNA polymerase II while the −20 barrier might be caused by a TATA-binding factor. As observed in the case of RNA polymerase I, the protection of the region flanking the +35 barrier is not dependent on the underlying sequence (Fig. 7).

In addition to the TATA-binding activities, we have detected a binding activity that protects the heat shock consensus sequences of hsp26 and more weakly that of hsp70 from digestion when exonuclease approaches from the downstream side. Much higher levels of this HSCS-binding activity were present in the nuclear extract from heat-shocked embryos than in those from non-heat-shocked embryos. Similar proteins probably interact with these two heat shock promoters as the barriers on both promoters were removed by competition with a polymer of the hsp70 heat shock consensus sequence. The protein involved in this interaction is probably the same as that recently purified from cultured cells by Wu et al. (43); they have demonstrated a binding activity which is higher in extracts from heat-shocked than in those from non-heat-shocked cultured cells or embryos. Close examination of our results from binding studies with hsp26 reveal that low levels of protein in extracts from non-heat-shocked embryos bind to the HSCS (Fig. 2, lanes 3 and 5). These low levels of activity are variable (data not shown); we suspect that they are due to inadvertent stress produced during collection of the embryos.

The results of vivo cross-linking studies indicate that RNA polymerase II interacts with the hsp70 promoter region even

prior to gene activation (15). Potentially, then, RNA polymerase II might interact with some promoters in the absence of a regulatory protein; regulation of transcription must then occur subsequent to this interaction. That the hsp70 promoter and many other promoters are associated with nonhistone chromosomal proteins prior to gene activation is implied by their DNase I hypersensitivity. We suspect that the factors responsible for the −20 and +35 exonuclease III barriers provide (at least some of the) important interactions that precede the transcriptional regulatory event. Testing this hypothesis will require the isolation of these components. These factors may be obtained from frozen *D. melanogaster* embryos, which provide an abundant, relatively inexpensive, and consistent source of material.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grant DCB-8601449 and Public Health Service BRSG grant 507 RR07054-22 from the National Institutes of Health to S. C. R. Elgin and by Public Health Service Postdoctoral Fellowship F32 GM107982 from the National Institutes of Health to D. S. Gilmour. T. J. Dietz is also supported by the Division of Biology and Biomedical Sciences, Washington University, St. Louis, Mo.

We thank John T. Lis and Hua Xiao for the HSCS 40-mer plasmid pXM40.

LITERATURE CITED

1. **Ashburner, M., and J. J. Bonner.** 1979. The induction of gene activity in Drosophila by heat shock. Cell **17**:241–254.
2. **Bateman, E., I. T. Calvin, P. Kownin, and M. R. Paule.** 1985. Footprinting of ribosomal RNA genes by transcription initiation factor and RNA polymerase I. Proc. Natl. Acad. Sci. USA **82**: 8004–8008.
3. **Bateman, E., and M. R. Paule.** 1986. Regulation of eukaryotic ribosomal RNA transcription by RNA polymerase modification. Cell **47**:445–450.
4. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Breathnach, R., and P. A. Chambon.** 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
6. **Brown, D. D.** 1984. The role of stable complexes that repress and activate eucaryotic genes. Cell **37**:359–365.
7. **Cartwright, I. L., and S. C. R. Elgin.** 1986. Nucleosomal instability and induction of new upstream protein-DNA associations accompany activation of four small heat shock protein genes in *Drosophila melanogaster*. Mol. Cell. Biol. **6**:779–791.
8. **Corces, V., R. Holmgren, R. Freund, R. Morimoto, and M. Meselson.** 1980. Four heat shock proteins of Drosophila melanogaster coded within a 12-kilobase region in chromosome subdivision 67B. Proc. Natl. Acad. Sci. USA **77**:5390–5393.
9. **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.
10. **Dudler, R., and A. A. Travers.** 1984. Upstream elements necessary for optimal function of the hsp70 promoter in transformed flies. Cell **38**:391–398.
11. **Dynan, W. S., and R. Tjian.** 1983. Isolation of transcription factors that discriminate between different promoters recognized by RNA polymerase II. Cell **32**:669–680.
12. **Eissenberg, J. C., I. L. Cartwright, G. H. Thomas, and S. C. R. Elgin.** 1985. Selected topics in chromatin structure. Annu. Rev. Genet. **19**:485–536.
13. **Ephrussi, B., and G. W. Beadle.** 1936. A technique of transplantation for Drosophila. Am. Nat. **70**:218–225.
14. **Farrell-Towt, J., and M. M. Sanders.** 1984. Noncoordinate histone systhesis in heat-shocked *Drosophila* cells is regulated at multiple levels. Mol. Cell. Biol. **4**:2676–2685.
15. **Gilmour, D. S., and J. T. Lis.** 1986. RNA polymerase II interacts with the promoter region of the noninduced hsp70 gene in *Drosophila melanogaster* cells. Mol. Cell. Biol. **6**:3984–3989.
16. **Heiermann, R., and O. Pongs.** 1985. In vitro transcription with extracts of nuclei of Drosophila embryos. Nucleic Acids Res. **13**:2709–2730.
17. **Hoopes, B. C., and W. R. McClure.** 1981. Studies on the selectivity of precipitation by spermine. Nucleic Acids Res. **20**: 5493–5504.
18. **Ingolia, T. D., and E. A. Craig.** 1981. Primary sequence of the 5′ flanking regions of the Drosophila heat shock genes in chromosome subdivision 67B. Nucleic Acids Res. **9**:1627–1642.
19. **Keene, M. A., V. Corces, K. Lowenhaupt, and S. C. R. Elgin.** 1981. DNase I hypersensitive sites in Drosophila chromatin occur at the 5′ ends of regions of transcription. Proc. Natl. Acad. Sci. USA **78**:143–146.
20. **Kownin, P., E. Bateman, and M. R. Paule.** 1987. Eukaryotic RNA polymerase 1 promoter binding is directed by protein contacts with transcription initiation factor and is DNA sequence-independent. Cell **50**:693–699.
21. **Lifton, R. P., M. L. Goldberg, R. W. Karp, and D. S. Hogness.** 1977. The organization of the histone genes in Drosophila melanogaster: functional and evolutionary implications. Cold Spring Harbor Symp. Quant. Biol. **42**:1047–1051.
22. **Maniatis, T., E. R. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Mathis, D. J., and P. Chambon.** 1981. The SV40 early region TATA box is required for accurate in vitro initiation of transcription. Nature (London) **290**:310–315.
24. **McGraw, R. A., III.** 1984. Dideoxy DNA sequencing with end-labeled oligonucleotide primers. Anal. Biochem. **143**:298–303.
25. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
26. **Miller, D. W., and S. C. R. Elgin.** 1981. Transcription of heat shock loci of Drosophila in a nuclear system. Biochemistry **20**: 5033–5042.
27. **Moran, L., M.-E. Mirault, A. Tissieres, J. Lis, P. Schedl, S. Artavanis-Tsakonas, and W. J. Gehring.** 1979. Physical map of two D. melanogaster DNA segments containing sequences coding for the 70,000 dalton heat shock protein. Cell **17**:1–8.
28. **Parker, C. S., and J. Topol.** 1984. A Drosophila RNA polymerase II transcription factor contains a promoter-region-specific-DNA-binding activity. Cell **36**:357–369.
29. **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.
30. **Price, D. H., A. E. Sluder, and A. L. Greenleaf.** 1987. Fractionation of transcription factors for RNA polymerase II from Drosophila K_c cell nuclear extracts. J. Biol. Chem. **262**:3244–3255.
31. **Samal, B., A. Worcel, C. Louis, and P. Schedl.** 1981. Chromatin structure of the histone genes of D. melanogaster. Cell **23**:401–409.
32. **Sawadogo, M., and R. G. Roeder.** 1985. Interaction of a gene-specific transcription factor with the Adenovirus major late promoter upstream of the TATA box region. Cell **43**:165–175.
33. **Siegfried, E., G. H. Thomas, U. M. Bond, and S. C. R. Elgin.** 1986. Characterization of a supercoiled-dependent S1 sensitive site 5′ to the Drosophila melanogaster hsp26 gene. Nucleic Acids Res. **23**:9425–9444.
34. **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.
35. **Thomas, G. H., E. Siegfried, and S. C. R. Elgin.** 1986. DNase I hypersensitive sites: a structural feature of chromatin associated with gene expression, p. 77–101. *In* G. R. Reeck, G. H. Goodwin, and P. Puigdomenech (ed.), Chromosomal proteins and gene expression. Plenem Publishing Corp., New York.
36. **Torok, I., and F. Karch.** 1980. Nucleotide sequences of heat shock activated genes in Drosophila melanogaster. I. Sequences in the region of the 5′ and 3′ ends of the hsp70 gene in the hybrid plasmid 56H8. Nucleic Acids Res. **8**:3105–3123.

37. **Tower, J., and B. Sollner-Webb.** 1987. Transcription of mouse rDNA is regulated by an activated subform of RNA polymerase I. Cell **50:**873–883.
38. **Travers, A.** 1983. Protein contacts for promoter location in eukaryotes. Nature (London) **303:**755.
39. **Williams, J. G., and P. J. Mason.** 1985. Hybridisation in the analysis of RNA, p. 139–160. *In* B. D. Hames and S. J. Higgins (ed.), Nucleic acid hybridisation: a practical approach. IRL Press, Arlington, Va.
40. **Wu, C.** 1980. The 5′ ends of Drosophila heat shock genes in chromatin are hypersensitive to DNase I. Nature (London) **286:** 854–860.
41. **Wu, C.** 1984. Two protein-binding sites in chromatin implicated in the activation of heat-shock genes. Nature (London) **309:**229–234.
42. **Wu, C.** 1985. An exonuclease protection assay reveals heat-shock element and TATA box DNA-binding proteins in crude nuclear extracts. Nature (London) **317:**84–87.
43. **Wu, C., S. Wilson, B. Walker, I. Dawid, T. Paisley, V. Zimarino, and H. Ueda.** 1987. Purification and properties of Drosophila heat shock activator protein. Science **238:**1247–1253.
44. **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.
45. **Zimarino, V., and C. Wu.** 1987. Induction of sequence-specific binding of Drosophila heat shock activator protein without protein synthesis. Nature (London) **327:**727–730.