MOLECULAR AND CELLULAR BIOLOGY, Sept. 1984, p. 1853–1863
0270-7306/84/091853-11$02.00/0

Vol. 4, No. 9

High-Resolution Mapping of DNase I-Hypersensitive Sites of *Drosophila* Heat Shock Genes in *Drosophila melanogaster* and *Saccharomyces cerevisiae*

NANCY COSTLOW AND JOHN T. LIS*

Section of Biochemistry, Molecular and Cellular Biology, Cornell University, Ithaca, New York 14853

Received 21 February 1984/Accepted 8 June 1984

High-resolution analysis of the chromatin structure of the promoter regions of five *Drosophila* heat shock genes showed a similar location for the hypersensitive sequences relative to the start of transcription. For each of the five genes examined—those coding for hsp27, hsp26, hsp23, hsp70, and hsp83—the DNase I-hypersensitive sites in *Drosophila melanogaster* nuclei mapped to two regions upstream of the coding region. These sites occurred on the average, 115 and 17 base pairs upstream from the start of transcription of the five heat shock genes examined. This latter site corresponded to sequences at or near the TATA consensus sequence. Sites even further upstream of the hsp27, hsp26, and hsp83 genes were also evident. Additionally, for the two genes examined—hsp70 and hsp83—the DNase I-hypersensitive sites were preserved, at least within this level of resolution (±10 base pairs), when the *Drosophila* genes were integrated into the *Saccharomyces cerevisiae* genome. This result indicates that the signals responsible for generating these hypersensitive sites are inherent in the DNA sequences and, in this case, are not highly species specific.

The ability of a eucaryotic gene to be transcribed in vivo has been correlated with discontinuities in the chromatin structure upstream of that gene. These discontinuities have been detected as containing DNA sequences that are extremely susceptible to double-stranded cleavage by DNase I, as well as by other types of nucleases (43). The accumulated evidence suggests that these structures, called DNase I-hypersensitive sites, are necessary but insufficient for eucaryotic gene expression (10). These structures may be a consequence of either a specific DNA configuration that prevents normal nucleosome structure or a specific protein-DNA interaction or both.

The relationship between the existence of DNase I-hypersensitive sites upstream of eucaryotic genes and the expression of those genes is correlative at present. Neither the molecular composition nor the function of these regions is clear. Examination and comparison of DNA sequences surrounding and composing DNase I-hypersensitive sites should help to clarify the involvement of the DNA sequence in their generation. Yet, to date, in only a few studies (13, 25, 31, 33) have hypersensitive sites been mapped with a resolution high enough that the DNA sequence itself could be correlated with their locations.

The *Drosophila* heat shock genes are members of a family of coordinately regulated genes. Heat shock induction of these genes occurs in all tissues examined, including *Drosophila melanogaster* tissue culture cells, upon a temperature shift from 23 to 37°C (2). Induction of subsets of the heat shock gene family at different developmental stages has also been reported (34, 44). DNase I-hypersensitive sites upstream of heat shock genes have been mapped previously with a resolution of ca. ±50 to 100 base pairs (bp) (20, 42, 43). The sequences upstream of these genes are hypersensitive to nucleases even at 23°C, which may be a reflection either of their ability to become active after heat shock induction or of their past transcriptional activity during an earlier developmental period.

* Corresponding author.

In this study we report the mapping at high resolution (±10 bp) of DNase I-hypersensitive sites upstream of five *Drosophila* heat shock genes, those coding for proteins hsp27, hsp26, hsp23, hsp70, and hsp83. We also show that, within this level of resolution, the DNase I-hypersensitive sites occur in the same positions on the hsp70 and hsp83 genes when these *Drosophila* genes are integrated into the genome of *Saccharomyces cerevisiae*. The correlation of the DNA sequences with the positions of the DNase I-hypersensitive sites points out some salient features of the DNA sequence that may be involved in the generation of hypersensitivity in chromatin.

MATERIALS AND METHODS

Enzymes. Restriction endonucleases were obtained commercially. DNase I (Worthington Diagnostics) was stored at a concentration of 5 mg/ml in 0.15 M NaCl–40% glycerol at −20°C. Units were determined as described by the manufacturer. DNA polymerase was obtained from New England Biolabs.

Isolation of 67B DNA. Phage banks containing embryonic *D. melanogaster* Oregon R DNA cloned into a lambda Sep6 vector (24) were screened with a cDNA clone specific for the hsp23 gene (22) by the procedure of Benton and Davis (3). Plaques giving positive signals on duplicate filters were purified, and DNA isolated from these phage was shown to contain heat shock genes from the 67B heat shock locus by RNA hybridization with heat shock and non-heat shock RNAs and by in situ hybridization to polytene chromosomes. The positions of the genes within this cloned segment are shown as mapped previously (7, 8, 34). Restriction fragments from this 17-kilobase segment of DNA were then subcloned into pBR322 for further use.

Plasmid DNA and fragment isolation and radioactive labeling. All plasmids used in this study were propagated in *Escherichia coli* K-12 strain HB101 (F^- *hsdS20* r_B^- m_B^- *recA13 ara-14 proA2 lacY1 galK2 rpsL20* Sm^r *xyl-15 mtl-1 supE44* λ^-) or SF8 (F^- *thr leu thi supI lacY tonA gal thy recB recC lop-11 hsdS*$_k$). Plasmid DNA was isolated by equilibrium gradient centrifugation (24). Fragments were

isolated from plasmid DNA by purification from 0.7% low-melting-point agarose gels run in 89 mM Tris-borate–3 mM EDTA, pH 8.0. Fragments were excised from the gels with scalpels, brought to 0.5 M NaCl, and heated at 80°C for 5 min until the agarose liquefied. Samples were extracted five times with phenol that had been equilibrated with 0.5 M NaCl–1 mM EDTA, pH 8.0. After each extraction, samples were heated to 65°C. The first two phenol phases were reextracted with 0.5 M NaCl–1 mM EDTA (pH 8.0), all aqueous layers were combined and extracted with ether, and DNA was precipitated with ethanol. Isolated fragment DNA was nick translated (30) to a specific activity of ca. 1×10^9 to 2×10^9 cpm/μg by using [α-^{32}P]dATP and [α-^{32}P]dCTP (Amersham Corp.) at 3,000 Ci/mmol.

Isolation and DNase I treatment of *D. melanogaster* nuclei and DNA. *D. melanogaster* nuclei were isolated and treated with DNase I, and DNA was purified as previously described (43) from Schneider line 2 tissue culture cells grown at 23°C in Shields and Sang medium (32).

Construction of *S. cerevisiae* strains. The hsp70 and hsp83 genes were each joined to the plasmid vector Yip33 (4), which carries the *leu2* gene of *S. cerevisiae*. This vector is propagated in *S. cerevisiae* only if it becomes integrated into the *S. cerevisiae* genome. Most integration events occur by homologous recombination between the *leu*$^+$ gene of the plasmid and the *leu*$^-$ gene of the *S. cerevisiae* recipient, yielding a stable *leu*$^+$ transformant. The characterized transformant containing the hsp70 gene was named Y70, and the one containing hsp83 was named Y83. The Y70 transformant was derived by cloning a *Bgl*II fragment from plasmid 132E3 (1) into the *Bam*HI site of the vector Yip33. This *Bgl*II fragment contains all *cis*-acting regulatory sequences required for expression in *D. melanogaster* (23). The resulting hybrid plasmid was introduced into *S. cerevisiae* cells by transformation (14). Transformants were obtained at higher than expected frequencies. These transformants produced sectored colonies indicative of unstable transformation. This behavior stems from the presence of an autonomously replicating sequence that is associated with the hsp70 genes (R. Hackett, personal communication). Occasionally, smooth fast-growing colonies arose from the initial transformants. These were shown to have the plasmid stably integrated into the yeast genome at the *leu2* locus by Southern (35) analysis of DNA.

With the same strategy, a *Bam*HI-*Bgl*II fragment carrying the entire hsp83 gene plus flanking sequences was ligated into the *Bam*HI site of Yip33. In contrast to the hsp70 hybrid, only stable transformants (large smooth colonies) occurring at the expected low frequencies were detected. Restriction-cut DNA from one transformant, Y83, showed the pattern expected from homologous recombination at the leucine gene.

Isolation of *S. cerevisiae* nuclei, DNase I treatment, and purification of DNA. The procedure used was a modification of the method of Ide and Saunders (16). A 2-liter culture of *S. cerevisiae* was grown overnight in YEPD broth at 27°C. Cells were harvested at an optical density of 4.0 at 660 nm. Cells were pelleted by centrifugation at 2,000 × *g* in a Sorvall GS3 rotor for 5 min at 4°C, suspended in 60 ml of 0.5% β-mercaptoethanol, and pelleted again in a Sorvall HB4 rotor by centrifugation at 2,500 × *g* for 5 min at 4°C. Spheroplasts were prepared by gently shaking cells in 4 ml of buffer A (1.1 M sorbitol, 20 mM potassium phosphate [pH 6.5], 0.5 mM $CaCl_2$) per g (wet weight) of cells with 0.25% β-mercaptoethanol and 0.16 mg of zymolyase 60,000 (Kirin Brewery) per ml at 32°C until no intact cells could be detected microscopically and lysis occurred after Ficoll or sodium dodecyl sulfate was added to a portion of the preparation. Spheroplasts were harvested by centrifugation at 2,000 × *g* in an HB4 rotor for 5 min at 4°C, suspended in 10 ml of cold buffer A, and pooled into a single centrifuge tube for lysis and homogenization. They were pelleted as described above and suspended as a wet cell paste in 3 ml, and then in 13 ml more, of cold buffer (18% [wt/vol] Ficoll, 20 mM potassium phosphate [pH 6.5], 0.5 mM $CaCl_2$, 1 mM phenylmethylsulfonyl fluoride). They were lysed and homogenized with 10 strokes of a Teflon plunger—the plunger used here had about 4 mm of clearance. After homogenization, 20 ml of solution B (1 M sorbitol, 25 mM potassium phosphate [pH 6.5], 0.5 mM $CaCl_2$) was added, and the lysate was loaded onto four Percoll (Sigma Chemical Co.) gradients, which were made by mixing 17 ml each of solutions B and C (1 M sorbitol, 25 mM potassium phosphate [pH 6.5], 0.5 mM $CaCl_2$, 60% Percoll), adding phenylmethylsulfonyl fluoride to 1 mM, and centrifuging in a Sorvall SS34 rotor at 18,000 × *g* for 50 min at 4°C. The gradients and lysates were centrifuged at 5,500 × *g* in an HB4 rotor for 20 min at 4°C. After centrifugation, the nuclei appeared as a band in the center of the gradient and were extracted from the top of the tube with a pipette. The nuclei were diluted with 2 volumes of solution B, pelleted by centrifugation in an HB4 rotor at 2,500 × *g* for 5 min at 4°C, and suspended in 6 ml of buffer A plus 1 mM $MgCl_2$. They were divided into six aliquots; one was reserved as a control, and DNase I was added to the remaining five in a concentration range of 38 ng/ml to 5 μg/ml. Digests were incubated for 5 min at 25°C, and digestion was terminated by adding EDTA to 12.5 mM and sodium dodecyl sulfate to 0.5%. DNA was purified from the nuclei as described by Wu et al. (43).

DNase I treatment of purified genomic or plasmid DNA. Restriction-cut DNA was precipitated with ethanol and dissolved in the same buffer used in the DNase I digestions of *D. melanogaster* nuclei (43). DNase I was added over a range of concentrations. Digestion was for 1 to 2 min at 25°C and was terminated by adding EDTA to 12.5 mM. The digests were analyzed by agarose gel electrophoresis, and appropriate digests were chosen for further study.

Restriction digests of *D. melanogaster* and *S. cerevisiae* nuclei. Nuclei were prepared as described above, resuspended, and digested by the procedure of Sweet et al. (38). DNA was purified from the nuclei by the procedure of Wu et al. (43).

Agarose gel electrophoresis, blotting to nitrocellulose, and hybridizations. After restriction digestion, aliquots of DNA samples were routinely assayed on small agarose gels for completion of digestion. Before the samples were loaded onto the preparative gel they were adjusted to the same salt concentration. Electrophoresis was typically done for 36 h on an 800-ml 1.3 to 1.7% agarose gel (40 by 24 by 0.6 cm) in 89 mM Tris-borate–3 mM EDTA, pH 8.0. Gels were blotted to nitrocellulose sheets (BA85; Schleicher & Schuell, Inc.) by the procedure of Southern (35), except that the gels were placed on a large sponge in a reservoir of 20× SSC (1× SSC contains 0.15 M NaCl plus 0.015 M sodium citrate) during the transfer. The nitrocellulose was vacuum dried for 2 h at 75°C, pretreated, and hybridized at 40°C to nick-translated DNA by the procedure of Wahl et al. (41) for 6 to 15 h in a mixture containing 50% formamide. Routinely, 50 to 100 ng of purified fragment in 100 to 150 ml of hybridization buffer was used for one filter (35 by 24 cm). Washing was done for

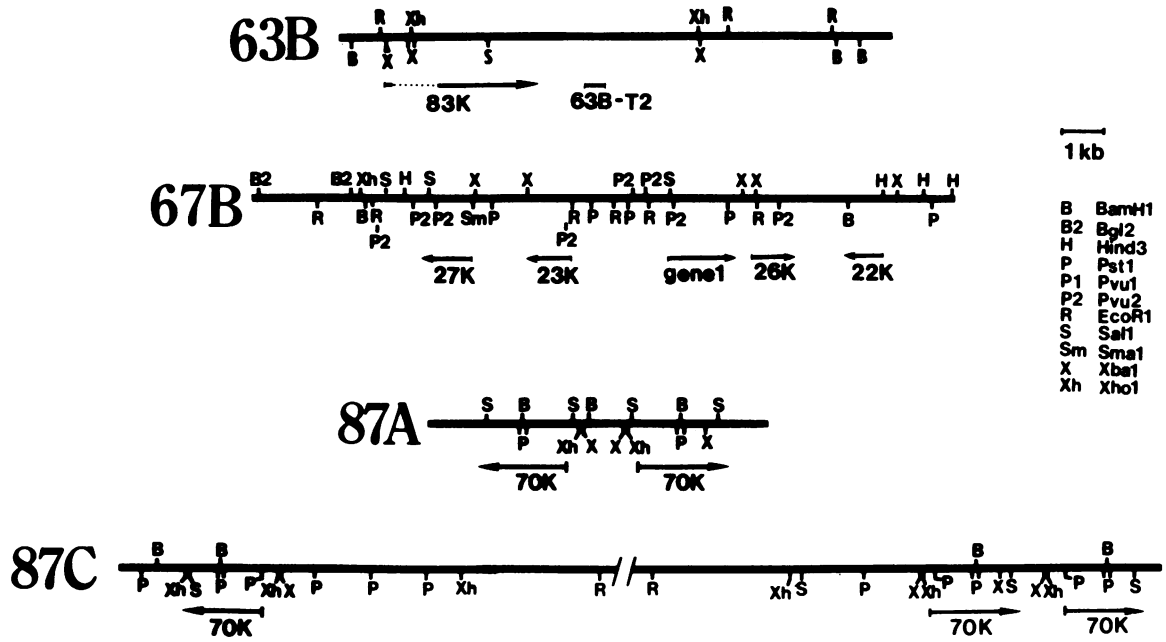

FIG. 1. Restriction maps of genes used in this study: 63B (26), 67B (7, 8, 34, 40; N. Costlow, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1984), and 87A and C (1, 9, 15, 19).

ca. 5 to 6 h at 60 to 65°C in 2× SSC–0.2% sodium dodecyl sulfate, with several changes of wash liquid. Blots were sandwiched between two sheets of preflashed X-ray film and two Cronex Lightning-Plus intensifying screens (Du Pont Co.), and autoradiography was done at −70°C for 3 days to 3 weeks. Densitometry was done on a Quick-Scan densitometer (Helena Laboratories), and controls were used to ensure that the scans were reproducibly linear with respect to distance scanned. The computer programs used were executed on an Apple IIe computer with the software described by Fristensky et al. (11) and in this paper.

RESULTS

Accuracy and error determination. DNase I-hypersensitive sites upstream of the five *Drosophila* heat shock genes, those coding for hsp27, hsp26, hsp23, hsp70, and hsp83 (Fig. 1), were mapped by the technique of indirect end labeling used by Wu (42). Hypersensitive sites upstream of these genes have been mapped previously with a resolution of ±50 to 100 bp (20, 42, 43). Our mapping, with a resolution of at least ±10 bp, was done by using 35-cm-long agarose gels, in which the differences in DNA fragment mobility were 6 to 13 mm per 100 bp. In addition, the DNase I-hypersensitive sites were mapped relative to restriction sites within and surrounding the DNA sequence being examined; that is, the molecular weight standards consisted of restriction fragments resulting from cleavage at the restriction site used to define one endpoint of the DNase I-generated fragments and from cleavage at other restriction sites chosen for their proximity to the expected DNase I-hypersensitive regions. For each experiment, samples of nuclei were digested with different concentrations of DNase I. The actual positions of the DNase I-hypersensitive sites were calculated from those digests by using the smallest amount of DNase I necessary to generate bands on the resulting autoradiograms. Thus, the cutting sites reported here represent the first cuts made by DNase I as well as could be determined in this type of analysis. The positions of the restriction and DNase I-generated fragments were derived from densitometry scans of autoradiograms. The error in these measurements was determined by plotting molecular weight against position and calculating the maximum deviation of the molecular weight markers used in each analysis from the least-squares-generated line. In no case was this deviation more than 10 bp from the theoretical value, and usually it was less.

The numbers given as the centers of the hypersensitive regions were reproducible for any given sample from gel to gel, but the error measurement given does not take into account the variability obtained in digests with DNase I. We attempted to minimize this variability by using only those digests in which the smallest amount of DNase I was used to visualize bands on the resulting autoradiograms. Additional cutting by DNase I within the hypersensitive region tended to shorten any fragments observed. By isolating nuclei that were treated with DNase I within a very narrow range of concentrations, we were able to reproduce the values found for the hypersensitive sites to within 20 bp.

Comparison of the widths of bands or peaks generated by DNase I digestion at one endpoint with those generated by restriction enzyme digestion clearly showed that DNase I did not cut at a single site but made double-stranded breaks over a region of DNA. The interval over which DNase I made these double-stranded breaks in nuclear DNA was estimated from calculations involving the peak width at half-peak height. Peaks resulting from cleavage at a single base pair, i.e., cleavage by a restriction enzyme, had a defined and reproducible width at half-peak height. A computer program was used to superimpose such experimentally derived peaks on themselves at 1-bp intervals over a given interval size to obtain a plot of width at half-peak height versus interval size. The assumption was made in these calculations that there was an equal probability of cutting by DNase I at each base pair within a given interval. These data were then used to make a standard curve from which to derive interval sizes for the peaks obtained experimentally from the DNase I digests. These estimates were made only

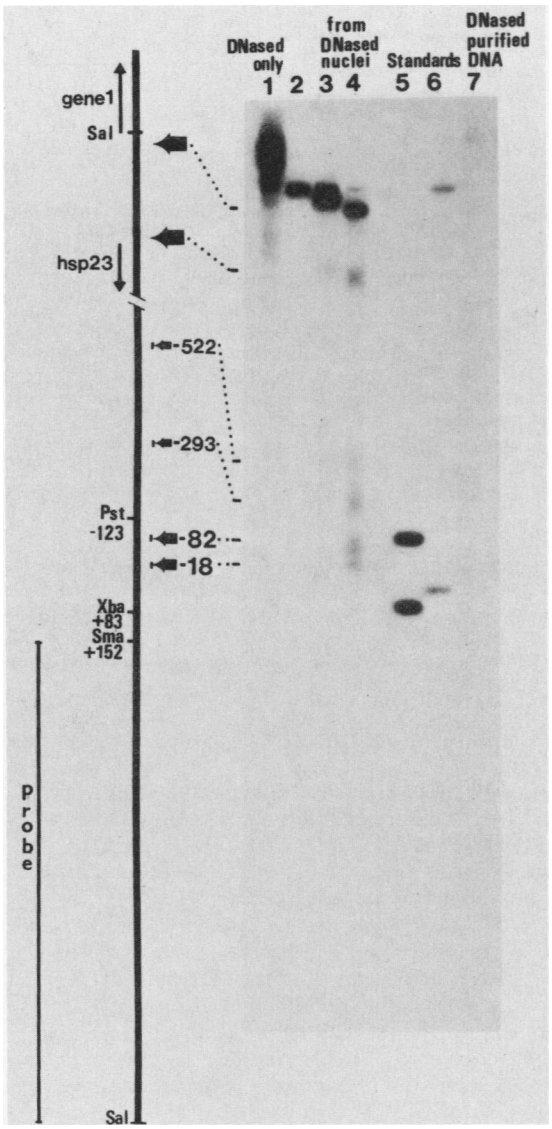

FIG. 2. Autoradiogram showing the mapping of DNase I-hypersensitive sites 5′ to the hsp27 gene. Electrophoresis was done on a 1.4% agarose gel. Lanes: 1, 10 μg of DNA from DNase I-treated (680 U/ml) nuclei; 2 through 4, 10 μg of *Sal*I-cut DNA purified from *D. melanogaster* nuclei that were treated with 0, 450, and 680 U of DNase I per ml, respectively; 5, 6 μg of *Sal*I-*Pst*I-cut and *Sal*I-*Sma*I-cut *D. melanogaster* DNA; 6, 6 μg of *Sal*I-*Xba*I-cut *D. melanogaster* DNA; 7, 10 μg of *Sal*I-cut purified *D. melanogaster* DNA treated with DNase I (300 U/ml). Hybridization was to the purified *Sal*I-*Sma*I fragment as shown. The *Sal*I restriction fragment homologous to this fragment is ca. 5.9 kb.

for those sites at which we could reasonably determine a width at half-peak height and are shown in brackets in Fig. 6. Since 80 to 90% of the area of any particular peak representing DNase I cleavages could be accounted for by the computer-generated simulation, and since these peaks represented the first cuts made by DNase I, this estimate is valid as a minimun interval within which DNase I cuts nuclear DNA. Comparison of the shapes of the computer-generated simulation with the experimentally observed DNase I peak showed that DNase I cut more efficiently near the center of the interval and less efficiently at positions farther from the center.

Genes for the small heat shock proteins: hsp27, hsp26, and hsp23. The hsp27, hsp26, and hsp23 genes are single-copy genes all located at the 67B locus (Fig. 1). The hypersensitive sites upstream of hsp27 were mapped from a *Sal*I site near the 3′ end of that gene (Fig. 2). In this and in all subsequent figures, the numbers on the restriction map refer to the distance (in bp) from the start of transcription, which is denoted as +1, with all coding sequences as positive numbers. The corresponding densitometry scans for this and the other autoradiograms are shown in Fig. 6. The major peaks of DNase I hypersensitivity upstream of hsp27 are centered at −82 and −18 bp relative to the start of transcription at +1 bp (17, 36). In addition, broader regions of sensitivity existed further upstream at −522 and −293 bp, ±20 bp. Comparison of DNase I only (Fig. 2, lane 1) and DNase I-*Sal*I digests (lane 4) show that all the fragments were bounded by a DNase I site at one end and a *Sal*I site at the other. Since these DNase I-*Sal*I fragments were all

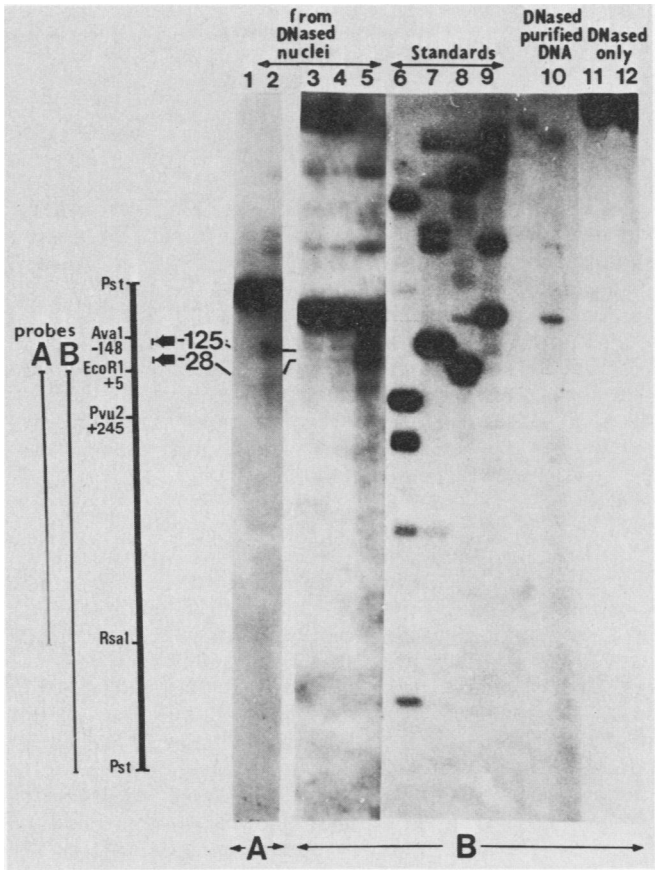

FIG. 3. Autoradiograms showing the mapping of DNase I-hypersensitive sites 5′ to the hsp23 gene. DNA at 15 μg on a 1.7% gel (lanes A) and 10 μg on a 1.3% gel (lanes B) was loaded in each lane. Lanes: 1 and 2, *Rsa*I-cut DNA from *D. melanogaster* nuclei treated with 0 and 340 U of DNase I per ml, respectively; 3 through 5, *Pst*-cut DNA from *D. melanogaster* nuclei treated with 180, 340, and 680 U of DNase I per ml, respectively; 6 through 9, *D. melanogaster* DNA cut with *Pst*I and *Pvu*II, *Pst*I and *Ava*I, *Pst*I and *Eco*RI, and *Pst*I, respectively; 10, *Pst*I-cut purified DNA treated with 300 U of DNase I per ml; lanes 11 and 12, DNA from *D. melanogaster* nuclei treated with 180 and 680 U of DNase I per ml, respectively. Hybridization was to either the *Rsa*I-*Eco*RI (lanes A) or the *Pst*I-*Eco*RI (lanes B) purified fragment as shown. The genomic restriction fragments homologous to these fragments are the 1.05-kb *Rsa*I (probe A) and the 2.4-kb *Pst*I (probe B) fragments.

smaller than 4.8 kilobases, they must have originated from the *Sal*I site found at the 3′ end of the hsp27 gene. The hypersensitivity was not due to DNA sequence specificity of the enzyme but rather to the packaging of the DNA into chromatin, since fragments of like molecular weight were not observed with purified genomic DNA digested with *Sal*I and DNase I (lane 7). Also evident in lanes 3 and 4 is cutting at the hypersensitive sites 5′ to the hsp23 gene and to a developmentally regulated gene, gene 1 (34).

The DNase I-hypersensitive sites upstream of the hsp26 gene occurred at positions −118 and −15 bp (see Fig. 6). These sites were mapped from a *Cla*I site at +1,015 bp by using as probe the *Cla*I-*Eco*RI fragment from +1,015 to +7 bp. Cutting at the −15-bp position was more evident in samples with twice the amount of DNase I than was used in the scan shown in Fig. 6. In these samples (not shown), two peaks of hypersensitivity were evident: at −78 bp, which replaced the −118-bp peak, and at −15 bp. Hypersensitive sites also occurred further upstream, even in the samples with lower DNase I concentrations. These latter sites were located at positions −374 and −296 bp, ±20 bp. As for the hsp27 gene, none of the fragments visible in the autoradiograph corresponded to DNase I-DNase I cleavages, and none occurred as a result of any sequence preference of DNase I.

DNase I-hypersensitive sites upstream of the hsp23 gene were located at −125 and −28 bp from the start of transcription (Fig. 3, lanes 2 through 5). These sites were mapped from both an *Rsa*I (Fig. 3A) and a *Pst*I (Fig. 3B) endpoint. No fragments corresponding to DNase I-DNase I cleavage were found to comigrate with either the *Rsa*I-DNase I (data not shown) or the *Pst*I-DNase I fragments (lanes 11 and 12). Also, no sequence preference of DNase I was observed for this region of DNA (lane 10). A densitometer scan of lane 2 is shown in Fig. 6. Cleavage at the −28-bp site was more evident after longer exposure and in digests with greater amounts of DNase I (Fig. 3, lanes 4 and 5). In this and in all subsequent figures, splicing of gel lanes was done only to eliminate unnecessary tracks and conserve space or to include a longer exposure of an autoradiogram. All lanes shown as a single unit were run on the same gel.

hsp70. The restriction maps of the five copies of the hsp70 gene in *D. melanogaster* located at loci 87A and 87C are shown in Fig. 1 (1, 8, 15, 19). DNase I-hypersensitive sites exist upstream of each of these five copies (42). The DNase I sites upstream of the hsp70 genes in *D. melanogaster* mapped at higher resolution are shown in Fig. 4 and 6. Hypersensitive sites were mapped relative to a *Bam*HI site at +1,252 bp that was common to all five copies of this gene. The most sensitive region was evident in the digestions (Fig. 4, lanes 5 and 7) with the smallest amount of DNase I. Only one peak of sensitivity was observed under these conditions, centered at −128 bp relative to the start of transcription (39). In digestions with more DNase I, the sequences sensitive to DNase I encompassed a larger region, comprising three peaks of sensitivity centered at −89, −2, and +42 bp (lane 6). These sites did not result from DNase I-DNase I cleavages, since no bands are evident in the lanes containing DNase I-treated *D. melanogaster* DNA (lanes 12 and 13). Plasmid DNA containing a *Bgl*II fragment from plasmid 132E3 (1) was linearized with *Bam*HI and then cut with different concentrations of DNase I. These digests did produce some fragments that comigrated with those generated in digests of nuclei (Fig. 4B, lanes 4 through 6, and Fig. 6). However, accessibility to DNase I at these positions in chromatin is greatly enhanced over that in plasmid DNA. Furthermore, none of the many other fragments observed in the DNase I digests of plasmid DNA appeared in the digests of nuclei, indicating that there is a special feature of the chromatin structure that allows the associated DNA sequences to be especially susceptible to DNase I cleavage in nuclei. For the hsp70 and hsp83 genes, the controls for the sequence specificity of DNase I were performed with plasmid DNA containing cloned genomic fragments rather than with purified genomic DNA; this was done to avoid the possibility of cleavage by endogenous nucleases at the hypersensitive sites.

The hypersensitive sites 5′ to the *Drosophila* hsp70 gene were analyzed in *S. cerevisiae* transformant Y70, which contained the *Drosophila* hsp70 gene integrated into the genome at the *leu2* locus. In *S. cerevisiae* Y70, two upstream regions were the most sensitive (Fig. 4A, lanes 2 and 3) and, from the densitometer scans of the autoradiogram, were centered at −96 and −2 bp from the start of transcription of *D. melanogaster*. These peaks of hypersensitivity can be considered identical within this level of resolution (±10 bp) to the −89- and −2-bp peaks observed in digests of *D. melanogaster* nuclei (Fig. 6). The difference between the cleavages at the −128- and −96-bp positions may be due to the variability of DNase I digests of nuclei, as discussed above. It is also possible that the sequence specificity that DNase I displays for this region influences the exact center of the peaks shown in Fig. 6.

hsp83. The *Drosophila* hsp83 gene, located at locus 63B, is a single-copy gene that is expressed at a low level in non-heat-shocked cell cultures. After heat shock, its expression increases 11-fold (26). The pattern of hypersensitivity appears at first to differ from that of the other heat shock genes that we have examined in that there are five rather than two or four hypersensitive regions near the 5′ end of the gene at −451, −213, −120, −20, and +20 bp (Fig. 5, lane 7, and Fig. 6). The pattern of hypersensitivity in *S. cerevisiae* transformant Y83, containing the integrated copy of the *Drosophila* hsp83 gene, was nearly the same except that the region at −451 bp in *D. melanogaster* was less sensitive to DNase I in *S. cerevisiae* nuclei. The major peaks of sensitivity in the yeast DNA were centered at −229, −120, −14, and +20 bp (Fig. 5, lanes 2 through 4). DNase I treatment of *Xho*I-linearized plasmid DNA, aDm4.46 (26), generated discrete fragments that were reproducibly obtained on the 35-cm-long gels (lanes 12 and 13). The fragments generated in DNase I digests of plasmid DNA were not entirely identical to those generated in digests of nuclear DNA—one major difference was the complete absence of a fragment from the plasmid that comigrated with the fragment generated by cleavage at the hypersensitive site at −20 bp. Thus, with certainty, the hypersensitive site at −20 bp is indeed a consequence of the chromatin structure, and cleavage there is not due to any sequence preference of DNase I. Whether the other fragments observed in digests of nuclei resulted from a sequence preference of DNase I or from the existence of a DNase I-hypersensitive structure in chromatin is less obvious. Clearly, the microdensitometer scans of the autoradiogram show that the peaks of sensitivity obtained in plasmid and nuclear digests could not be exactly superimposed.

In an attempt to clarify the involvement of a DNA sequence preference of DNase I in cleavage at the hypersensitive sites, we used restriction endonucleases to digest intact nuclei by the procedure of Sweet et al. (38) (Fig. 7). The upstream sequences about −220 bp upstream of the hsp83 gene were more accessible to nucleases than were

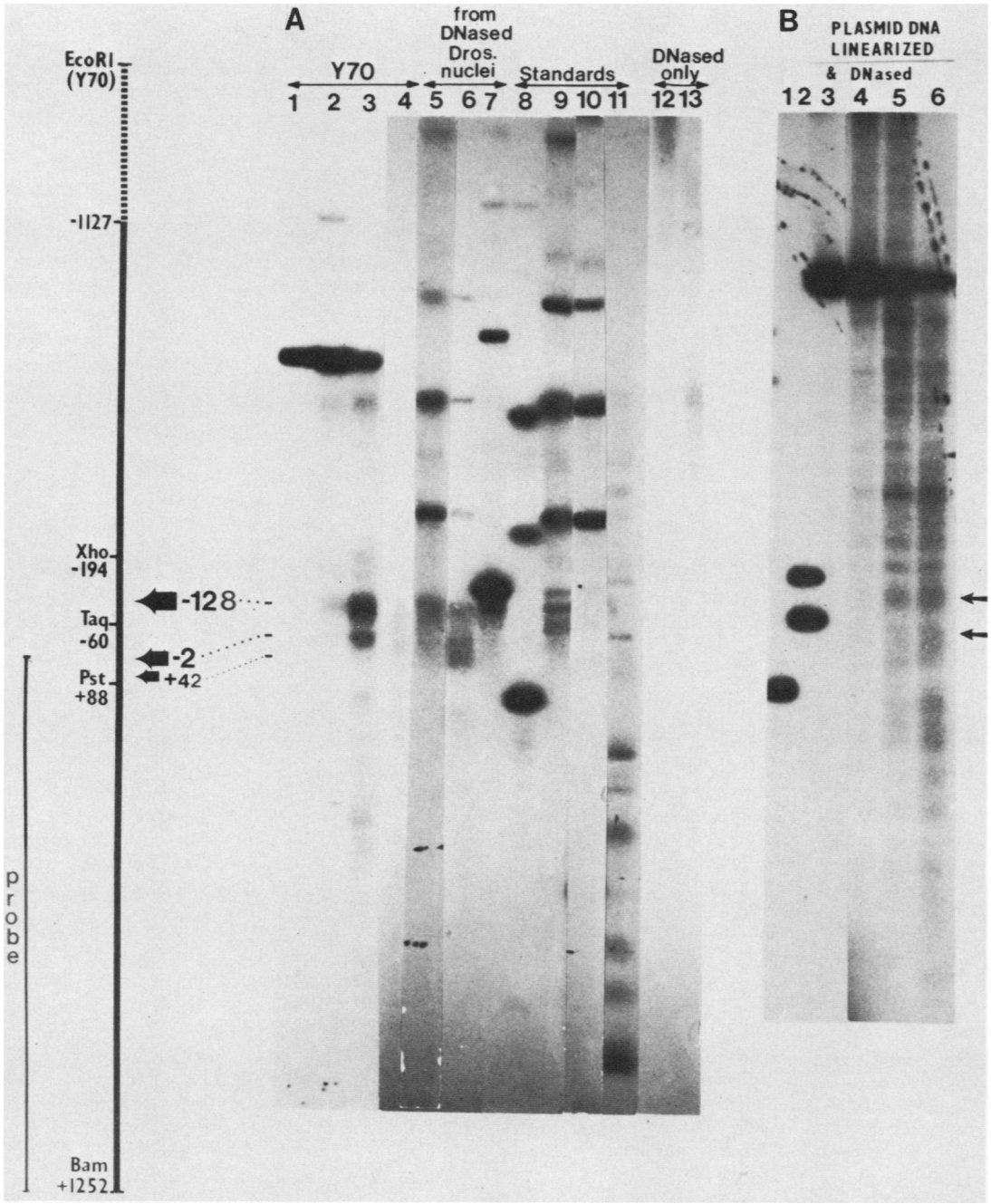

FIG. 4. Autoradiograms showing the mapping of DNase I-hypersensitive sites 5′ to the *Drosophila* hsp70 gene in *D. melanogaster* and in *S. cerevisiae* transformant Y70. Electrophoresis was done on 1.4% agarose gels. (A) Lanes: 1 through 4, *Bam*HI-cut *S. cerevisiae* Y70 DNA from nuclei treated with 0, 4, 103, and 308 U of DNase I per ml, respectively; 5 through 7, DNA from *D. melanogaster* nuclei treated with 340, 680, and 340 U of DNase I per ml, respectively, and restriction-cut with *Bam*HI-*Eco*RI (lanes 5 and 6) or *Bam*HI-*Xho*I (lane 7); 8 through 11, DNA from *D. melanogaster* nuclei restriction digested with *Bam*HI and *Pst*I, *Bam*HI and *Eco*RI and partially with *Taq*I, *Bam*HI and *Eco*RI, and *Bam*HI and *Eco*RI and partially with *Msp*I, respectively; 12 and 13, DNA from *D. melanogaster* nuclei treated with 340 and 680 U of DNase I per ml, respectively. (B) Lanes: 1 through 6, 10 μg of sonicated calf thymus DNA loaded with 20 ng of *Bam*HI-*Pst*I (lane 1), *Bam*HI-*Xho*I plus *Bam*HI-*Nru*I (lane 2), and *Bam*HI (lane 3) digested with plasmid DNA containing the *Bgl*II fragment from plasmid 132E3 (1); lanes 4 through 6, 200 ng of *Bam*HI-cut purified plasmid DNA treated with 25, 50, and 100 U of DNase I per ml, respectively. Arrows mark the −128- and −2-bp positions. The heavy line shows *D. melanogaster* DNA; the dashed line shows DNA which in *S. cerevisiae* Y70 is pBR322 DNA. Hybridization was with the purified *Bam*HI-*Sal*I fragment from a cloned 87A copy of hsp70 (1), which hybridizes with a 2,755-bp *Bam*HI-*Eco*RI restriction fragment in *S. cerevisiae* Y70 and 9.5-, 8-, 3.2-, 2.25-, and 1.6-kb restriction fragments in *D. melanogaster*. Hybridization to the larger fragments is not shown.

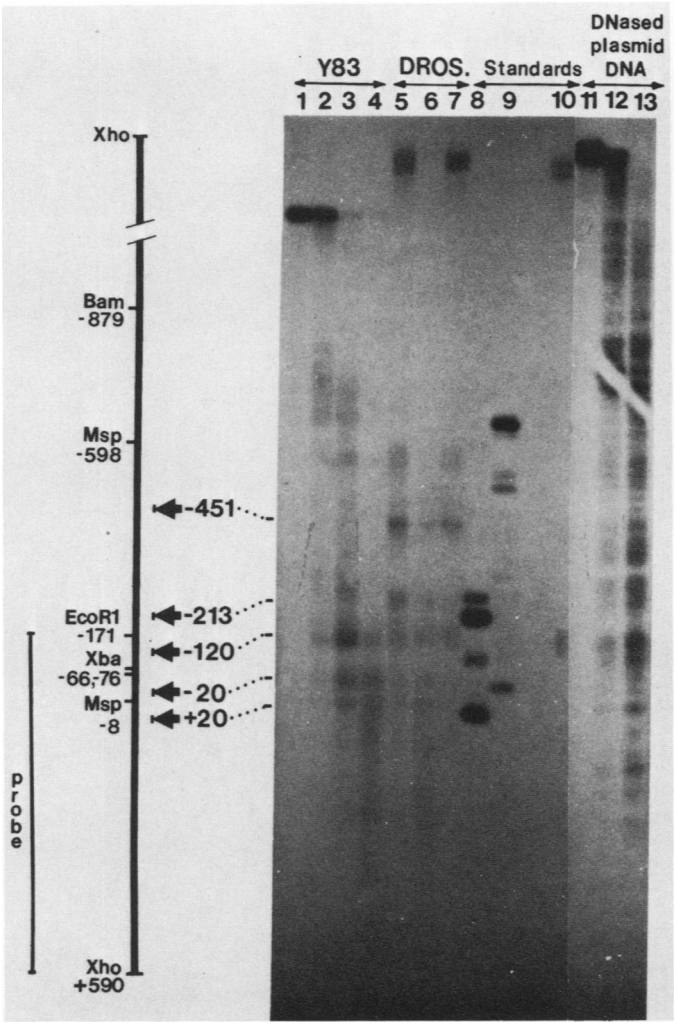

FIG. 5. Autoradiogram showing the mapping of hypersensitivity 5′ to the *Drosophila* hsp83 gene in *D. melanogaster* and in *S. cerevisiae* transformant Y83. Electrophoresis was done on a 1.7% gel. Lanes: 1 through 4, 10 μg of *Xho*I cut DNA from *S. cerevisiae* Y83 nuclei treated with 0, 103, 308, and 513 U of DNase I per ml, respectively; 5 through 7, 10 μg of cut DNA from *D. melanogaster* nuclei treated with 480, 340, and 180 U of DNase I per ml, respectively; 8, 6 μg of *Xho*I and *Bam*HI and a partial *Xba*I digest of *D. melanogaster* DNA, plus *Xho*I-*Eco*RI-digested *D. melanogaster* DNA; 9, 6 μg of *Xho*I and *Bam*HI and partial *Msp*I digest of *D. melanogaster* DNA; 10, *Xho*I-cut *D. melanogaster* DNA; 11 through 13, 4 ng of *Xho*I-cut purified plasmid DNA, aDm4.46 (26), treated with 0, 25, and 50 U of DNase I per ml, respectively, loaded with 10 μg of sonicated calf thymus DNA. Hybridization was with the purified *Xho*I-*Eco*RI fragment shown, which hybridizes with a restriction fragment of ca. 14 kb in *D. melanogaster*.

sequences within the coding region at +222 bp. Control partial digests with *Ava*II cuts showed that *Ava*II cuts at sites −220 and +222 bp in purified DNA, with no preferential digestion occurring at either site (Fig. 7, lanes 3 and 4). However, in *Ava*II digests of *D. melanogaster* nuclei (lanes 1 and 2), only the −220-bp site was cleaved. Fragments of this and lower molecular weights transferred and hybridized to the radioactively labeled DNA on the same Southern blot, indicating that loss of low-molecular-weight fragments during transfer cannot account for these results. These results show that in nuclei, the −220-bp site is at least 4 times more accessible to *Ava*II than is the +222-bp site. *Ava*II digests of *S. cerevisiae* nuclei yielded the same result (lanes 5 to 7), but also evident in these digests were fragments generated by cleavage at the −120-bp hypersensitive site by endogenous nucleases present in *S. cerevisiae* nuclei. Cleavage by these endogenous nucleases was observed only under the conditions used for the restriction endonuclease digestions and not under those used for the DNase I digestions described above. That these nucleases cleaved at the −120-bp position supports the idea that this site is hypersensitive as a result of the chromatin configuration of those sequences and not merely as a consequence of DNase I sequence specificity. As a control in this experiment, *Xho*I-cut plasmid DNA containing homologous sequences was added in 100-fold molar excess over the genomic DNA and was incubated with *S. cerevisiae* Y83 nuclei under conditions in which the endogenous nuclease(s) is active. This digest yielded a continuous smear rather than discrete fragments on long

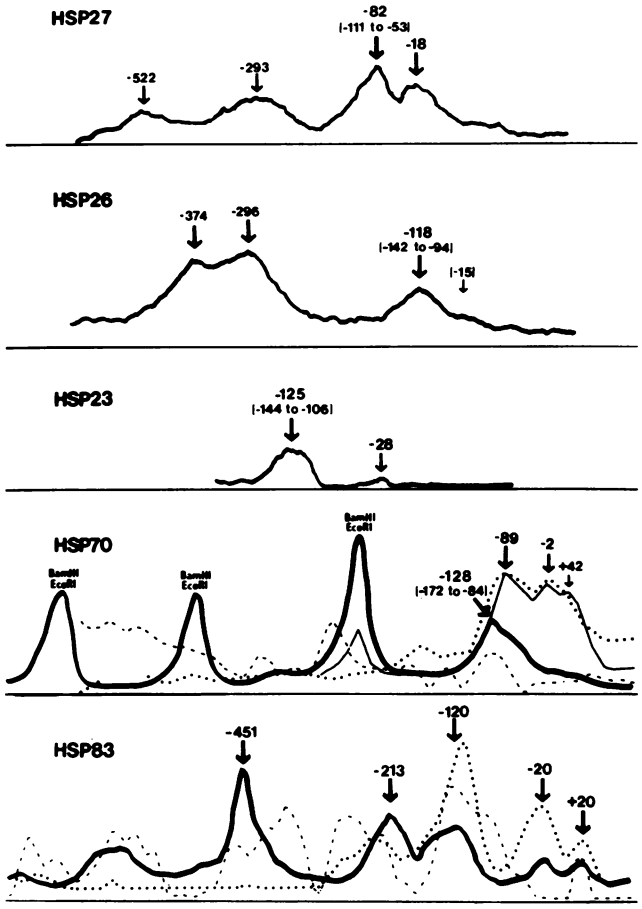

FIG. 6. Densitometer scans of restriction enzyme digests of DNA samples from *D. melanogaster* nuclei treated with 340 (heavy line) or 680 (light line) U of DNase I per ml, from *S. cerevisiae* nuclei (dotted line), and DNase I digests of restriction-cut purified plasmid DNA (dashed line). Numbers in brackets refer to the interval sizes, estimated as described in the text. Digests with increased amounts of DNase I showed greater hybridization at the −15- and −28-bp positions for the hsp26 and hsp23 gene, respectively. The densitometer scan shown for hsp23 was that of a shorter exposure of lane 2 from Fig. 3A.

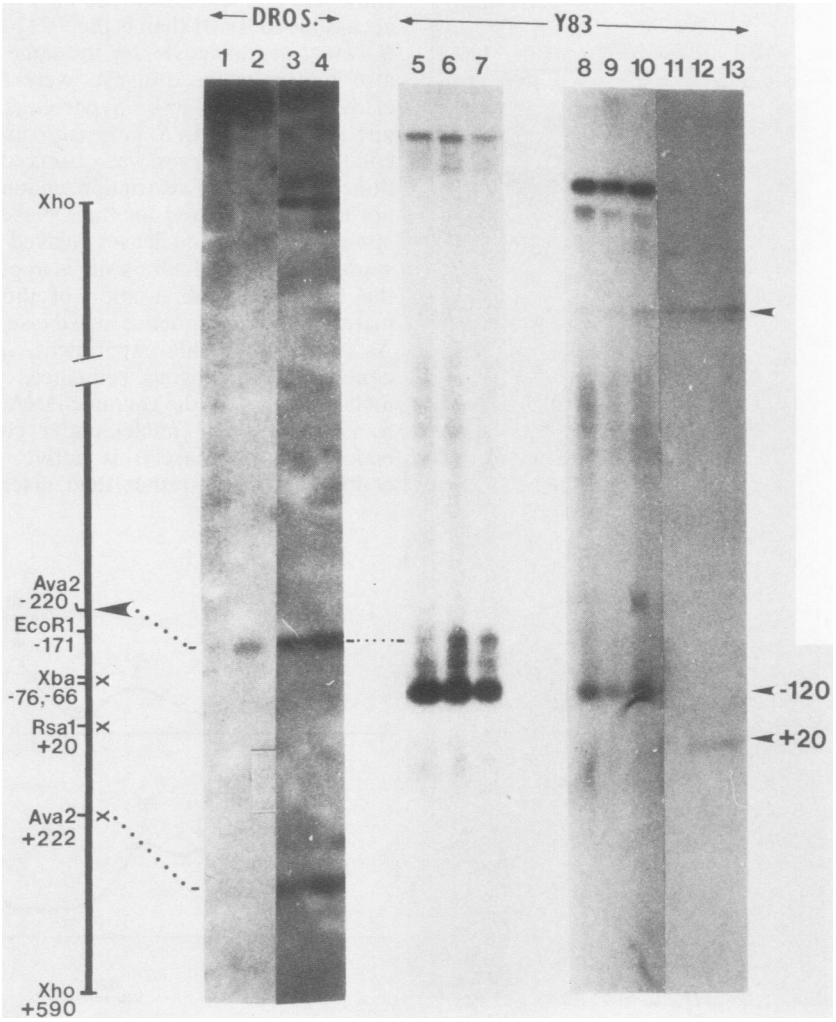

FIG. 7. Autoradiograms showing restriction digests of *D. melanogaster* and *S. cerevisiae* nuclei. All samples were digested to completion with *Xho*I, electrophoresed on a 1.7% gel, blotted, and hybridized to the *Xho*I-*Eco*RI fragment from the cloned hsp83 gene (Fig. 5); 5 μg of DNA was loaded per lane. Lanes: 1 and 2, *Ava*II digests of *D. melanogaster* nuclei treated with 10 and 20 U of DNase I per ml, respectively, for 2 h at 37°C; 3 and 4, *Ava*II digests of *D. melanogaster* genomic DNA, at 1 U/μg for 5 and 10 min, respectively; 5 through 7, *Ava*II digests of *S. cerevisiae* Y83 nuclei, at 2, 10, and 20 U/ml, respectively, for 2 h at 37°C; 8 through 10, *Rsa*I digests of *S. cerevisiae* Y83 nuclei, at 1, 25, and 50 U/ml, respectively, for 1 h at 37°C; 11 through 13, *Rsa*I digests of *S. cerevisiae* Y83 genomic DNA at 1 U/μg for 5, 10, and 15 min, respectively. On the restriction map, the arrow denotes cleavage at the *Ava*II site at −220, and × marks sites where cleavage did not occur.

gels, suggesting that the endogenous nucleases do not have a sequence preference for the sequences at or near −120 bp.

The *Rsa*I site at +20 bp was not cleaved in yeast nuclei (lanes 8 through 13); however, an upstream *Rsa*I site was cleaved, indicating that this enzyme was active within yeast nuclei. This upstream site does not exist in *D. melanogaster* nuclei; thus, we had no positive control for *Rsa*I digests in *D. melanogaster* nuclei. *Xba*I did not cleave at −76 or −66 bp upstream of the hsp83 gene, a region shown to be relatively DNase I resistant in nuclei (Fig. 5 and 6), but an *Xba*I site that is contained within a hypersensitive region upstream of the hsp26 gene was cleaved in intact nuclei (data not shown). In summary, we found that, except for the lack of cleavage at the *Rsa*I site at +20 bp, the relative sensitivity to restriction endonuclease digestion paralleled that to DNase I cleavage.

DISCUSSION

Nuclease-sensitive structures upstream of several eucaryotic genes have been detected and correlated with the transcriptional activity of genes in both a tissue-specific and a temporal-specific way (6, 25, 33, 37). These correlations suggest that changes in chromatin structure may play a role in the regulation of gene expression by determining the accessibility of certain sequences to RNA polymerase or to regulatory proteins in the cell. Alternatively, these changes in chromatin structure may simply reflect different states of gene activity.

We mapped DNase I-hypersensitive sites upstream of five *Drosophila* heat shock genes, members of a family of coordinately regulated genes, with a resolution of no less than ±10 bp. We found that in all five genes, the two DNase

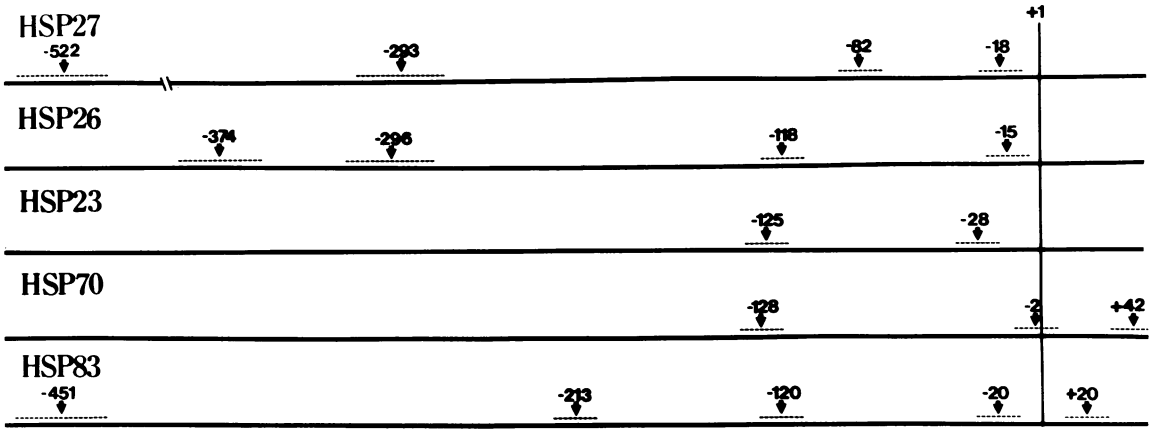

FIG. 8. Summary of DNase I-hypersensitive sites 5′ to five *Drosophila* heat shock genes. Arrows, Positions of the center of each peak of hypersensitivity, determined as described in the text. The dashed line below each arrow indicates the error in that particular measurement. The heat shock genes are shown aligned at the start of transcription (+1 bp).

I-hypersensitive sites most immediately upstream of the genes occur at similar positions relative to the starts of transcription, at ca. −115 and −17 bp upstream of the starts of transcription. The −17-bp site is positioned at or near the TATA consensus sequence that is common to all eucaryotic genes transcribed by RNA polymerase II (5). In addition to these two nuclease-sensitive regions, the hsp27, hsp26, and hsp83 genes also have hypersensitive sites further upstream (Fig. 8). Transcription of these three genes has been reported in *D. melanogaster* ovaries, under non-heat shock conditions (44). These additional hypersensitive regions may reflect this differential regulation. Other DNase I-hypersensitive sites which have been mapped to a high resolution include those upstream of the *Drosophila* glue protein gene, *Sgs-4* (33), the chicken adult β-globin gene (25), and those at the origin-proximal region of polyoma virus (13) and simian virus 40 (31). Although these genes, like the heat shock genes, do show hypersensitivity at their 5′ termini, the detailed structure of the heat shock genes, with their two distinct sites at, on the average, −115 and −17 bp, seems to be a feature characteristic of the heat shock genes and may reflect a common mode of regulation.

For the small heat shock genes, hsp27, hsp26, and hsp23, these hypersensitive upstream sites are a consequence of the packaging of the DNA into chromatin. These results agree with those of Keene et al. (20). In contrast to other previously reported results (42, 43), however, we found that the digests of purified DNA containing either hsp70 or hsp83 did show some sequence-specific cutting by DNase I at the same sequences found to be hypersensitive in nuclear DNA. That the earlier reports failed to find any sequence-specific cutting at these sites may be because digests of purified DNA appear to run as a continuous smear on the shorter gels used in those studies. However, for the hsp70 gene, the efficiency of cutting at these sequences in chromatin was greatly enhanced over that seen in purified DNA. In fact, most of the cleavages observed in digests of purified DNA were not seen in digests of nuclear DNA. This implies that these sequences assume a special nuclease-sensitive configuration in chromatin. We conclude that some component of chromatin is responsible for the increased efficiency of cutting at these sites upstream of the hsp70 gene in nuclei. For the hsp83 gene, the results obtained with digests of nuclear DNA with restriction enzymes support the conclusion that, for this gene, sequences upstream of the start of transcription are more sensitive to nuclease digestion than are sequences further downstream within the coding portion of this gene. Since we detected no cleavage by *Rsa*I at the +20-bp site, this site may appear to be hypersensitive because of the sequence specificity of DNase I. Omission of the hypersensitive site at +20 bp would make the pattern of hypersensitivity at the hsp83 gene closely parallel to that found at the hsp27 and hsp26 genes.

It has been shown that the *Drosophila* hsp70 and hsp83 genes are transcribed in *S. cerevisiae* (21). We show here that when the *Drosophila* hsp70 and hsp83 genes are integrated into the *S. cerevisiae* genome, the hypersensitivity of the upstream sequences is preserved. This indicates that the information necessary for specifying the hypersensitive structure in chromatin is contained within the DNA sequence. Furthermore, the signals or components involved in generating a DNase I-hypersensitive configuration of these sequences in chromatin do not appear, at least in this case, to be species specific.

By comparing the DNA sequences adjacent to and composing the DNase I-hypersensitive sites, we hoped to find a correlation of particular sequence elements with the DNase I cleavages that would suggest a molecular model for this hypersensitivity. A search for conserved sequences among these five heat shock genes showed several similarities relative to the DNase I-hypersensitive sites. Hackett and Lis (12) reported that the sequences from −47 through +30 bp are relatively conserved for the heat shock family. In addition, Pelham (28) derived a consensus sequence centered ca. 55 bp upstream of the start of transcription of the hsp70 gene, which is found in approximately the same region for the other heat shock genes, except hsp23, and is required for heat shock induction of the *Drosophila* hsp70 gene in monkey COS cells (28). This sequence also confers heat shock inducibility on the herpes simplex virus thymidine kinase gene when placed upstream of this gene (29). The DNase I-hypersensitive sites bracket this consensus sequence for each of the genes examined in this study except hsp23, for which only a poor match with the consensus sequence was found. If the consensus sequence represents a binding site for a regulatory protein, the hypersensitive sites may represent an entry site for the regulatory protein or for RNA polymerase; alternatively, the sites may be hypersensitive

merely by virtue of neighboring a protein-binding site. Parker and co-workers (27) isolated a protein fraction from *D. melanogaster* that binds to sequences upstream of the heat shock genes which are bracketed by the hypersensitive sites as mapped in this study.

A simple explanation of the existence of hypersensitive sites is that the binding of this or of some other protein prevents nucleosomes from forming over sequences immediately adjacent to its binding site; these adjacent sequences would then become hypersensitive. If this model is correct, then a similar binding protein must exist in yeast to account for the similarity of the hypersensitive pattern between *S. cerevisiae* and *D. melanogaster*. Many organisms, including *S. cerevisiae*, have been shown to possess a heat shock gene family. In fact, the *S. cerevisiae* homolog to the *Drosophila* hsp70 gene has been extracted from a yeast sequence bank by using the *Drosophila* gene as a probe (18). It is conceivable that sequence elements are sufficiently conserved between the upstream regions of the *S. cerevisiae* and *D. melanogaster* sequences to allow interaction between the *Drosophila* hsp70 gene and transcription factors of yeast. Consistent with this model, we detected no cleavage by *Xba*I at the −76- and −66-bp positions of hsp83 in *D. melanogaster*. These restriction sites are contained within the consensus sequence derived by Pelham (28) and are bracketed by the two hypersensitive sites at −120 and −20 bp.

The role that any of these sequence elements plays in generating DNase I-hypersensitive sites remains unclear. We are addressing this question further by assaying deletions and subclones of the *Drosophila* hsp70 gene in yeast cells to assess the function and importance that any particular sequence may have in generating these hypersensitive sites in *S. cerevisiae*.

ACKNOWLEDGMENTS

We thank Volker Vogt and Joseph Calvo for critical appraisal of the work and the manuscript, Carl Parker for communicating his results before publication, and Melissa Stucky for preparing the manuscript.

This work was supported by Public Health Service grant no. GM25232 from the National Institutes of Health.

LITERATURE CITED

1. **Artavanis-Tsakonas, S., P. Schedl, M. E. Mirault, L. Moran, and J. Lis.** 1979. Genes for the 70,000 dalton heat shock protein in two cloned D. melanogaster DNA segments. Cell **17**:9–18.
2. **Ashburner, M., and J. J. Bonner.** 1979. The induction of gene activity in Drosophila by heat shock. Cell **17**:241–254.
3. **Benton, W. D., and R. W. Davis.** 1977. Screening λgt recombinant clones by hybridization to single plaques *in situ*. Science **196**:180–182.
4. **Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis.** 1979. Sterile host yeast (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8**:17–24.
5. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
6. **Burch, J. B. E., and H. Weintraub.** 1983. Temporal order of chromatin structural changes associated with activation of the major chicken vitellogenin gene. Cell **33**:65–76.
7. **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. U.S.A. **77**:5390–5393.
8. **Craig, E. A., and B. J. McCarthy.** 1980. Four Drosophila heat shock genes at 67B. Nucleic Acids Res. **8**:4441–4457.
9. **Craig, E. A., B. J. McCarthy, and S. C. Wadsworth.** 1979. Sequence organization of two recombinant plasmids containing genes for the major heat shock-induced protein of D. melanogaster. Cell **16**:575–588.
10. **Elgin, S. C. R.** 1981. DNase I-hypersensitive sites of chromatin. Cell **27**:413–415.
11. **Fristensky, B., J. Lis, and R. Wu.** 1982. Portable microcomputer software for nucleotide sequence analysis. Nucleic Acids Res. **10**:6451–6463.
12. **Hackett, R. W., and J. T. Lis.** 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.
13. **Herbomel, P., S. Saragosti, D. Blangy, and M. Yaniv.** 1981. Fine structure of the origin-proximal DNase I-hypersensitive region in wild-type and EC mutant polyoma. Cell **25**:651–658.
14. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**:1929–1933.
15. **Holmgren, R., K. Livak, R. Morimoto, R. Freund, and M. Meselson.** 1979. Studies of cloned sequences from four Drosophila heat shock loci. Cell **18**:1359–1370.
16. **Ide, G., and C. Saunders.** 1981. Rapid isolation of yeast nuclei. Curr. Genet. **4**:85–90.
17. **Ingolia, T. D., and E. A. Craig.** 1981. Primary sequence of the Drosophila heat shock genes in chromosome subdivision 67B. Nucleic Acids Res. **9**:1627–1642.
18. **Ingolia, T. D., M. R. Slater, and E. A. Craig.** 1982. *Saccharomyces cerevisiae* contains a complex multigene family related to the major heat shock-inducible gene of *Drosophila*. Mol. Cell. Biol. **2**:1388–1398.
19. **Ish-Horowicz, D., S. M. Pinchin, P. Schedl, S. Artavanis-Tsakonas, and M. E. Mirault.** 1979. Genetic and molecular analysis of the 87A7 and 87C1 heat-inducible loci of D. melanogaster. Cell **18**:1351–1358.
20. **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. U.S.A. **78**:143–146.
21. **Lis, J., N. Costlow, J. deBanzie, D. Knipple, D. O'Connor, and L. Sinclair.** 1982. Transcription and chromatin structure of Drosophila heat shock genes in yeast, p. 57–62. *In* M. T. Schlessinger, M. Ashburner and A. Tissieres (ed.), Heat shock: from bacteria to man. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Lis, J. T., W. Neckameyer, R. Dubensky, and N. Costlow.** 1981. Cloning and characterization of nine heat-shock-induced mRNAs of Drosophila melanogaster. Gene **15**:67–80.
23. **Lis, J. T., J. A. Simon, and C. A. Sutton.** 1983. New heat shock puffs and β-galactosidase activity resulting from transformation of Drosophila with an hsp70-lacZ hybrid gene. Cell **35**:403–410.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **McGhee, J. D., W. I. Wood, M. Dolan, J. D. Engel, and G. Felsenfeld.** 1981. A 200 base pair region at the 5′ end of the chicken adult β-globin gene is accessible to nuclease digestion. Cell **27**:45–55.
26. **O'Connor, D., and J. T. Lis.** 1981. Two closely linked transcription units within the 63B heat shock puff locus of D. melanogaster display strikingly different regulation. Nucleic Acids Res. **9**:5075–5092.
27. **Parker, C. S., and J. Topol.** 1984. A Drosophila RNA polymerase II transcription factor specific for the heat shock gene binds to the regulatory site of an hsp70 gene. Cell **37**:273–283.
28. **Pelham, H. R. B.** 1982. A regulatory upstream promoter element in the Drosophila Hsp70 heat-shock gene. Cell **30**:517–528.
29. **Pelham, H. R. B., and M. Bienz.** 1982. A synthetic heat-shock promoter element confers heat-inducibility on the herpes simplex virus thymidine kinase gene. Eur. Mol. Biol. Org. J. **1**:1473–1477.
30. **Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
31. **Saragosti, S., S. Cereghini, and M. Yaniv.** 1982. Fine structure

of the regulatory region of simian virus 40 minichromosomes revealed by DNase I digestion. J. Mol. Biol. **160**:133–140.
32. **Schneider, I., and A. B. Blumenthal.** 1978. Drosophila cell and tissue culture, p. 266–315. *In* M. Ashburner and T. R. F. Wright (ed.), The genetics and biology of Drosophila, vol. 2a. Academic Press, Inc., New York.
33. **Shermoen, A. W., and S. K. Beckendorf.** 1982. A complex of interacting DNase I-hypersensitive sites near the Drosophila glue protein gene, Sgs4. Cell **29**:601–607.
34. **Sirotkin, K., and N. Davidson.** 1982. Developmentally regulated transcription from Drosophila melanogaster chromosomal site 67B. Dev. Biol. **89**:196–210.
35. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
36. **Southgate, R., A. Ayme, and R. Voellmy.** 1983. Nucleotide sequence analysis of the Drosophila small heat shock gene cluster at locus 67B. J. Mol. Biol. **165**:35–57.
37. **Stalder, J., A. Larsen, J. D. Engel, M. Dolan, M. Groudine, and H. Weintraub.** 1980. Tissue-specific DNA cleavages in the globin chromatin domain introduced by DNase I. Cell **20**:451–460.
38. **Sweet, R. W., M. V. Chao, and R. Axel.** 1982. The structure of the thymidine kinase gene promoter: nuclease hypersensitivity correlates with expression. Cell **31**:347–353.
39. **Török, 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.
40. **Wadsworth, S., E. Craig, and B. McCarthy.** 1980. Genes for three Drosophila heat-shock-induced proteins at a single locus. Proc. Natl. Acad. Sci. U.S.A. **77**:2134–2137.
41. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.
42. **Wu, C.** 1980. The 5′ ends of Drosophila heat shock genes in chromatin are hypersensitive to DNase I. Nature (London) **286**:854–860.
43. **Wu, C., P. M. Bingham, K. J. Livak, R. Holmgren, and S. C. R. Elgin.** 1979. The chromatin structure of specific genes. I. Evidence for higher order domains of defined DNA sequence. Cell **16**:797–806.
44. **Zimmerman, J. L., W. Petri, and M. Meselson.** 1983. Accumulation of a specific subset of D. melanogaster heat shock mRNAs in normal development without heat shock. Cell **32**:1161–1170.