DNase I hypersensitive sites in Drosophila chromatin occur at the 5' ends of regions of transcription
(chromatin structure/Drosophila melanogaster/heat shock)

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ABSTRACT By using a map of the unique region of DNA encoding the four small heat-shock proteins of Drosophila melanogaster (hsps 22, 23, 26, and 28), and a simple mapping technique, the positions of the DNase I hypersensitive sites of chromatin in the vicinity of these genes have now been determined. The major chromatin-specific sites occur at the 5' ends of each of the four heat-shock protein genes in embryo nuclei. These genes are not active in the nuclei analyzed but can be quickly induced in these cells by the heat-shock stimulus. The chromatin structure indicated by DNase I hypersensitivity may be a necessary factor in the general mechanism of gene activation.

During the last few years, considerable progress has been made in the analysis of chromatin structure through the use of various nucleases. Recently it has been observed that there are sites in chromatin that are hypersensitive to cleavage by DNase I (1–4). Such sites occur at specific positions along the chromatin fiber at intervals of about 2–10 kilobases (kb) in Drosophila (2). The positions of such sites relative to the restriction map of a unique, cloned region of the genome may be determined by a simple mapping procedure (5). We have studied the region encoding the four small heat-shock proteins (hsps 22, 23, 26, and 28) of Drosophila at locus 37B, which has recently been cloned and mapped in detail (6). The cloned region is present in only one copy per haploid genome. There is no evidence for the presence of repeated sequences of any kind. We observe a closely spaced pair of DNase I hypersensitive sites located at the 5' end of each of the four heat-shock genes. Two minor sites occur between the heat-shock encoding regions, but none occurs completely within the regions of transcription. Because no corresponding preferential cleavage sites exist in the purified DNA, we infer that a differential accessibility or liability of the DNA occurs at these sites in vivo as a consequence of chromatin structure.

MATERIALS AND METHODS

Isolation of Nuclei from Drosophila Embryos. Nuclei were isolated by a method optimized for an in vitro transcription assay in this laboratory. Embryos (6–18 hr old) were collected from a laboratory population of D. melanogaster (Oregon R) maintained at 25°C as described by Elgin and Miller (7). Aliquots (30–40 g) of embryos were sealed in plastic tubes, frozen in liquid nitrogen, and kept at −80°C until used. Embryos were dechorionated at room temperature by stirring in 50% Chlorox/1% NaCl/1% Triton X-100 for 5 min and then washed extensively in cold water on a Nitex filter. All subsequent operations were performed at 4°C. The embryos were homogenized in a type C Teflon/glass homogenizer filled with buffer A (1 M sucrose/1 mM NaCl/2.5 mM ammonium acetate/0.5% Triton X-la/0.25 M sucrose/0.025 M NaCl/10 mM EDTA/10 mM Tris base) and purified as above.

DNase I Hypersensitivity. Nuclei were washed in a hemocytometer, counted, and resuspended at 109/ml in digestion buffer, adjusted to 5 × 109/ml for immediate use.

Digestion of Chromatin with DNase I. Parallel aliquots of nuclei in digestion buffer (60 mM KCl/15 mM NaCl/15 mM Tris-HCl, pH 7.4/0.5 mM dithiothreitol/0.25 M sucrose/0.055 M CaCl2/3 mM MgCl2) were incubated at 25°C for 3 min with various dilutions (1–30 units/ml, final concentration) of DNase I as described (2).

Primary restriction digests of the DNA were performed according to the manufacturer’s conditions (New England BioLabs), and the DNA was repurified by precipitation from 2.5 M ammonium acetate as above.

High molecular weight Drosophila embryo DNA was prepared by shaking freshly prepared nuclei (see above) into extraction buffer (2% NaDodSO4/7 M urea/0.35 M NaCl/1 mM EDTA/10 mM Tris base) and purifying as above.

Analysis of DNA Fragments. Gel electrophoresis and Southern transfer were performed as described (2), except that a uniform sample size of 10 μg DNA was loaded per gel well in all cases. The preparation and labeling of recombinant plasmids, hybridization of the filters, and autoradiography were carried out essentially as described (2), but a final concentration of 1 μg of DNA per ml was used for nick-translation reactions. The size of DNA fragments was determined relative to standard fragments from pBR322. Work with recombinant DNA plasmids was performed under E. coli in the presence of ampicillin, in accordance with current National Institutes of Health guidelines.

Abbreviations: kb, kilobase(s); ECTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.

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Corces et al. (6) is presented in Fig. 1 to facilitate description of the experiments presented here.

Probe 88.3 is a recombinant clone in a pBR322 vector, containing the 2.2-kb fragment abutting the right-hand BamHI site shown. When Oregon R embryo DNA was cleaved completely with BamHI, sized on an agarose gel, and blotted, only a single fragment 12.1 kb long hybridized with 88.3, as expected from the restriction map in Fig. 1. However, when DNA from nuclei lightly digested with DNase I was so treated, a set of four discrete doublet bands appeared (Fig. 2, lanes C and D). These bands are neither restriction fragments nor the fragments produced in nuclei by digestion with DNase I alone (shown in lanes F and G). Therefore, they are bounded at one end by the Bam site and at the other end by a DNase I site; the DNase I sites can readily be located on the restriction map from the size of the fragments. The fact that no bands smaller than 2.4 kb are observed indicates that no fragments with a right-hand end other than the Bam site have been generated. Lanes A and B of Fig. 2 contained purified Drosophila embryo DNA digested with DNase I to an equivalent average molecular weight and similarly digested with BamHI. No such bands are seen. Consequently, these bands cannot have been generated by sequence-specific cleavage of the DNA by DNase I; these hypersensitive sites must be a consequence of chromatin structure.

**RESULTS**

A restriction map of the region of the Drosophila genome encoding the four small heat-shock proteins as determined by

![Figure 1: Partial restriction map of the cloned region encoding the four small heat-shock proteins.](image)

**Fig. 1.** Partial restriction map of the cloned region encoding the four small heat-shock proteins. The genes encoding hsp22, 23, 26, and 28 are clustered in a 12-kb interval. Regions homologous to heat-shock RNA were mapped and the identity of the coding sequences were determined by Corces et al. (6). Arrows indicate the direction of transcription. 88.1 and 88.3 are the subcloned restriction fragments used as probes in these experiments.

![Figure 2: Hybridization of probe 88.3 to a Southern blot containing samples from DNase I-digested Drosophila embryo nuclei and DNA.](image)

**Fig. 2.** Hybridization of probe 88.3 to a Southern blot containing samples from DNase I-digested Drosophila embryo nuclei and DNA. Each lane of a 0.9% agarose gel (except for those containing molecular weight markers) was loaded with 10 μg of DNA. Lanes A and B, purified embryo DNA digested with BamHI and DNase I at 0.025 and 0.05 unit/ml; C and D, DNA from nuclei digested with DNase I (1 and 2 units/ml), the DNA then purified and digested with BamHI; F and G, same samples as in C and D, without BamHI digestion; I and J, same samples, digested with EcoRI; K and L, as in A and B, but digested with EcoRI; E and H, size markers, made up of digested pBR322 and plasmids homologous to the probe.
The two smallest fragments, which can be most precisely measured, averaged 2.4 and 2.6 kb in size. The bands were not as sharp as those produced with restriction fragments, and typically they spanned 50–100 base pairs. When a series of samples generated by increasing extents of DNase I digestion were examined (data not shown), the smaller band always was the more prominent of the two. Thus, it appears that one can distinguish two classes of such DNase I hypersensitive sites, which we will refer to as major and minor.

When the analogous experiment was performed with EcoRI, only one doublet was seen, the major site mapping at 3.3 kb and the minor site at 3.6 kb (Fig. 2). In addition a cleavage site was observed 6.4 kb from the EcoRI site, which, by comparison with results from a control experiment using purified DNA (lanes J and K), can be identified as a site specified by the sequence preference of the enzyme.

Similar experiments have been carried out with 88.1 as a probe. This plasmid contained a 2.6-kb fragment bordered by EcoRI and Pst sites. When the same samples used above (cleaved with DNase I and BamHI) were probed with 88.1, a set of four discrete doublet bands was observed. Assignment of map locations showed that the position of the cleavage sites corresponds exactly to those mapped from the other direction. The data are shown in Fig. 3, lanes A and B. The major and minor sites in proximity to hsp 28 and hsp 23 can now be accurately measured as 2.8/3.1 kb and 4.9/5.1 kb, respectively, from the Bam site.

These findings have been confirmed through the use of two additional probes that flank a Sal I site located midway between hsp 23 and hsp 26, 7.0 kb from the left-hand BamHI site. 88RS1 contains a 0.6-kb fragment to the left of this site (bordered by Sal I and EcoRI sites) and 88RS2 contains the 2.0-kb fragment to the right of this site (bordered by the Sal I site and the EcoRI site at the 5' end of hsp 26). The map locations of the DNase I hypersensitive sites found with these probes are in excellent agreement with those found by using 88.1 and 88.3. The data are summarized in Table 1.

When these measurements are correlated with the transcriptional data on the restriction map of this region, the striking result shown in Fig. 4 is obtained. The midpoint of each of the four major DNase I hypersensitive sites is almost exactly at the 5' end of one of the hsp genes; the site extends 25–50 base pairs in

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**Table 1. Summary of evidence for position of DNase I hypersensitive sites**

<table>
<thead>
<tr>
<th>DNase I site</th>
<th>Restriction enzyme</th>
<th>Probe</th>
<th>Distance to DNase I site, kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsp 22</td>
<td>Major EcoRI</td>
<td>88.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Minor</td>
<td>EcoRI</td>
<td>88.3</td>
<td>3.6</td>
</tr>
<tr>
<td>hsp 23</td>
<td>Major BamHI</td>
<td>88.1</td>
<td>4.9</td>
</tr>
<tr>
<td>Minor</td>
<td>Sal I</td>
<td>88RS1</td>
<td>2.1</td>
</tr>
<tr>
<td>hsp 26</td>
<td>Major BamHI</td>
<td>88.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Minor</td>
<td>Sal I</td>
<td>88RS2</td>
<td>1.9</td>
</tr>
<tr>
<td>hsp 28</td>
<td>Major BamHI</td>
<td>88.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Minor</td>
<td>Sal I</td>
<td>88RS1</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Map distances are: BamHI left to Sal I, 7.0 kb; Sal I to BamHI right, 4.2 kb; BamHI left to Pst I, 2.7 kb; BamHI left to EcoRI, 9.0 kb.

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**Fig. 3.** Hybridization of probe 88.1 to a Southern blot containing samples from DNase I-digested Drosophila embryo nuclei and DNA. Lanes: A, purified embryo DNA digested with DNase I (0.05 unit/ml) and BamHI; B, DNA from nuclei digested with DNase I (2 units/ml) and the purified DNA then digested with BamHI; D, same sample as in B, but without restriction endonuclease; C, size markers.

**Fig. 4.** Correlation of DNase I hypersensitive sites with regions of transcription at locus 67B. Vertical arrows indicate the positions of DNase I hypersensitive sites, located as given in the text; the larger arrow indicates the major site in each case.
each direction. The midpoint of the minor site is located \( \approx 300 \) base pairs upstream from that point. The measured center-to-center distance is 270 base pairs for the two sites at hsp 28 and hsp 26, 300 for hsp 22, and 190 for hsp 23. Two minor sites are observed in the region between the hsp 23 and hsp 26 genes. Although there is no hsp gene in this region, the possibility that this region is transcribed in some cell type under some conditions cannot be ruled out.

**DISCUSSION**

The positions of the DNase I hypersensitive sites of chromatin located in the vicinity of the genes for the four small heat-shock proteins hsp 28, hsp 26, hsp 23, and hsp 22 at locus 67B in *Drosophila* have been mapped by using digestion of isolated nuclei. The four major hypersensitive sites fall at the 5' ends of these genes, with secondary sites \( \approx 300 \) base pairs upstream. Two minor sites are observed in the region between the genes for hsp 23 and hsp 26. No sites have been observed within regions of transcription, nor have sites been observed at or near the 3' ends of these genes.

As in any analysis of chromatin structure, the validity of the results rests on the integrity of the starting material, in this instance nuclei isolated from *Drosophila* embryos. In several cases, nuclei prepared for these experiments were also used in an in *vitro* transcription study (D. W. Miller, personal communication). On addition of labeled rXTP, such nuclei were found to be faithful templates for transcription of specific products synthesized by RNA polymerases II and III in *vitro*. Consequently, it appears likely that the chromatin structure within these nuclei was preserved in *vitro*.

The results presented here are in general agreement with those recently obtained in several different systems. The multiple-copy hsp 70 and unique hsp 83 genes of *Drosophila* have been examined and, in both cases, DNase I hypersensitive sites were observed in chromatin at the 5' end of the region of transcription. In some instances, multiple cleavages were observed (5). Position-specific DNase I hypersensitive sites have also been observed in chicken chromatin (4, 9). In particular, sites were located near the 5' end of the embryonic \( \beta \)-globin gene, 2 and 6 kb upstream from the 5' end of the adult \( \beta \)-globin gene, and near the 3' end of the \( \alpha \)-globin gene. The presence of such sites was reported to be correlated with the pattern of transcription in this system (chicken erythrocytes) (4). In the present study we examined genes inactive at the time but capable of being activated very quickly (within 5 min) after receiving the heat-shock stimulus (see ref. 10 for review). One is tempted therefore to speculate that such a DNase I hypersensitive site at the 5' end of the region of transcription is typical of the chromatin of genes that can be expressed in the cell under study. Sites may be found at other locations as well but have not been reported to occur entirely within regions of transcription. One might argue that the heat-shock protein genes are already "on," and that the DNase I hypersensitive sites are a consequence of, rather than necessary for, transcription. This seems unlikely in view of the facts that a major general shift in the DNase I sensitivity of heat-shock protein genes is observed on activation (11) and that genes active at low and high levels of transcription have been reported to be equally sensitive to DNase I (12). At present the structure responsible for such a DNase I hypersensitive site in chromatin is unknown. In some cases, but not all, the DNase I hypersensitive site in *Drosophila* chromatin is also a site of cleavage by micrococcal nuclease, suggesting a gap in the array of nucleosomes (ref. 5; unpublished data). The existence of a region of DNase I hypersensitivity in the simian virus 40 minichromosome has also been established (1, 4, 13). This region is located at map position 0.67–0.74, roughly coincident with the origin of replication and site of initiation of transcription of the late genes. Under appropriate conditions a gap in the nucleosome array can be observed by electron microscopy at 0.67–0.74 on the simian virus 40 minichromosome map (14, 15). It is logical to suppose that a lack of nucleosome structure could contribute to increased DNase I sensitivity; however, it should be noted that these special sites are considerably more sensitive than linker regions between nucleosomes in general. Furthermore, we do not have sufficient evidence to state that all such sites have a common structural basis. It will be of interest to pursue the question of the developmental specificity of such DNase I hypersensitive sites. Such a DNase I hypersensitive site may represent a region of DNA relatively accessible to macromolecules in the nucleus. Given that eukaryotic RNA polymerases and affector proteins are capable of recognizing specific sequences on naked DNA (16), the relative accessibility of such sites may be of critical importance in the control of gene expression.

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