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# Nucleosomal Instability and Induction of New Upstream Protein-DNA Associations Accompany Activation of Four Small Heat Shock Protein Genes in *Drosophila melanogaster*

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**We investigated in detail the structural changes that occur in nuclear chromatin upon activation of the four small heat shock protein genes in *D. melanogaster*. Both the chemical cleavage reagent methidiumpropyl-EDTA· iron(II) [MPE· Fe(II)] and the nuclease DNase I revealed a complex pattern of four or five hypersensitive sites upstream of each gene before activation. In addition, MPE· Fe(II) detected a short positioned array of nucleosomes located on each coding region. Upon heat shock activation a number of changes in the patterns occurred. For each gene, at least one of the upstream hypersensitive regions was eliminated or substantially shifted in position. Regions were established which became highly refractile to digestion by either MPE · Fe(II) or DNase I and, as such, appeared as small "footprints" in the pattern. The location of these refractile regions relative to the cap site varied for each gene examined. The coding regions themselves became highly accessible to DNase I. The nucleosomal arrays detected by MPE·Fe(II) were characterized by a considerable loss of detail and significantly enhanced accessibility, the extent of which probably reflected the relative transcription rate of each gene. Careful mapping of the location and extent of each upstream footprint and comparison with the DNA sequence revealed the presence at each location of two (or more) contiguous or overlapping segments that bear high homology to the heat shock consensus sequence C-T-N-G-A-A-N-N-T-T-C-N-A-G. A specific protein factor (or factors) is most likely bound at or near these sequences in heat-shocked *Drosophila* cells.**

Elucidation of the distribution and specific nature of the sum of protein-DNA contacts in the chromatin surrounding transcription units under different conditions of gene expression represents an important initial step in attempts to understand the mechanisms of gene regulation in eucaryotes. Data regarding alterations in the structure of chromatin correlated with changing levels of (or commitment to) gene activity have been assembled over several years, and various inferences have been drawn. Genes programmed for expression, or actually being expressed, in a given cell are substantially more sensitive to nuclease digestion (notably by DNase I) than their counterparts in nonexpressing cells (54). The extent of this enhanced nuclease sensitivity has been reported to extend significantly beyond the borders of the transcribed region itself (4, 30, 51). It has usually been inferred that active genes and the domains in which they reside must somehow be more open or accessible on activation, probably as a result of rearrangements of DNA-protein contacts.

Some features of chromatin structure detected at active or activatable genes are particularly intriguing. Inactive genes are packaged in typical nucleosomal arrays; whether or not the nucleosomes are specifically positioned with regard to the underlying DNA sequence apparently depends on a number of factors (13a). Upon gene activation however, the array appears to be disrupted to various extents, perhaps dependent on the rate of transcription (2, 5, 14, 42). Whether passage of RNA polymerase causes the disruption or whether the extent of perturbation in a given case actually controls the rate of polymerase passage is not clear. The physical association of histone complexes with transcribing sequences has been questioned in many cases, as has the integrity of the octameric complex (40). Active or potentially active genes are also demarcated at their 5′ ends (and sometimes at their 3′ ends) by sites hypersensitive to a variety of nucleases, particularly DNase I, from which the operational term DNase I-hypersensitive sites derives (15). These sites, which appear to be necessary but not sufficient for transcriptional competence, are comprised of specific protein-DNA associations at the very least (17, 57) and may also involve altered secondary structures of DNA in some cases (16). The morphology and physical dimensions of DNase I-hypersensitive sites indicate that they are nucleosome-free regions (34).

We have been particularly interested in the chromatin structure of the 67B1 region of *Drosophila melanogaster* which encodes the small heat shock proteins hsp 28, hsp 26, hsp 23, and hsp 22 (9, 11, 53), as well as a variety of other developmentally regulated genes of unknown function (48). Previously we established a low-resolution pattern of DNase I hypersensitivity for five of the genes in this region in non-heat-shocked cells (27). A higher-resolution DNase I study for three of these genes was recently published (10).

In the present study, we used the chemical cleavage agent methidiumpropyl-EDTA· iron(II) [MPE· Fe(II)] (8, 19) and DNase I to map in detail, using high-resolution (±15 base pairs [bp]) agarose gels, through ca. 14.7 kilobases of chromatin at this locus under both non-heat shock (genes inactive) and heat shock (genes active) conditions. Both reagents identify a complex pattern of regions hypersensitive to cleavage, situated upstream of each of the genes examined. In addition, MPE· Fe(II) reveals the presence of nucleosomes at defined locations across portions of the region

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under non-heat shock conditions, a result which could not be obtained by using micrococcal nuclease at this locus (6, 28). Upon gene activation by elevation of the temperature, a considerable perturbation in this complex pattern of DNA-protein associations occurs. In particular, we observe that certain upstream regions that contain heat shock consensus sequences (35, 38) become refractile to MPE· Fe(II) and DNase I cleavage, presumably because of the establishment of specific DNA-protein contacts over these sequences. In addition, the nucleosomal arrays across each gene become particularly susceptible to cleavage by both reagents, consistent with a substantial disruption of the nucleosomal core-DNA interaction.

## MATERIALS AND METHODS

**Reagents.** MPE was obtained from Peter Dervan, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, Calif. It was stored in the dark at −20°C. Aqueous stock solutions were similarly stored frozen at −20°C. Restriction enzymes were supplied by New England BioLabs, Inc. (Beverly, Mass.) or Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) and were used according to the recommendations of the manufacturer. DNase I (grade DPFF) and micrococcal nuclease were products of Worthington Diagnostics (Freehold, N.J.). RNase A was from Sigma Chemical Co. (St. Louis, Mo.), and proteinase K was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.).

**Plasmid DNA.** DNA for probing genomic blots was derived from the recombinant plasmids 88B13, 88.2, 88.3, 88.4 (9), and 88RS1 (28). Bacteria harboring plasmids were grown at 37°C in Luria broth, and plasmids were amplified with chloramphenicol at 200 μg/ml. Large-scale purification of plasmid DNA was performed by the boiling protocol of Holmes and Quigley (21) followed by RNase treatment and banding in cesium chloride-ethidium bromide. DNA fragments suitable for nick translation were obtained by plasmid restriction, followed by agarose gel electrophoresis and immobilization of the DNA on a strip of NA-45 paper (Schleicher & Schuell, Inc., Keene, N.H.) inserted into the gel immediately ahead of the fragment of interest, with subsequent elution in 1 M salt and purification by standard procedures. Samples (100 ng) of DNA were labeled by nick translation (43) in the presence of $^{32}$P-labeled deoxynucleoside triphosphates, desalted on Sephadex G-50, and used directly in filter hybridizations.

**Nuclear isolation.** Nuclei were isolated from 6- to 18-h-old *Drosophila* embryos or Schneider line 2 tissue culture cells essentially as previously described (7, 59). As required, embryos were heat shocked for 45 min at 37°C under high-humidity conditions and then rapidly frozen in liquid nitrogen before nuclear isolation. Schneider cells were similarly shocked at 37°C for 45 min in tissue culture medium and then rapidly cooled to 4°C by swirling in an ice-salt bath before nuclear isolation.

**Nuclear digestions.** Nuclear digestions with MPE· Fe(II) were performed as described by Cartwright et al. (8) as modified by Cartwright and Elgin (7). In outline, the procedure involved preparation of an aqueous MPE· Fe(II) complex from separate MPE and ferrous ammonium sulfate solutions, followed by addition of the complex to a nuclear suspension in the presence of small quantities of dithiothreitol and hydrogen peroxide. Reactions were terminated by addition of bathophenanthroline disulfonate (a strong iron chelator), and nuclei were lysed in the presence of EDTA and sodium dodecyl sulfate (SDS). DNA from the nuclear lysates was prepared by standard procedures as previously described (59). Enzymatic digestions of nuclei with either micrococcal nuclease or DNase I and subsequent purification of DNA were performed exactly as described previously (59). When salt extraction of nuclei was performed before digestion, nuclei were incubated at 4°C with 0.35 M or 0.5 M KCl and processed exactly as previously described (7).

**Site-specific cleavage analyses.** The location of cleavages introduced by the various agents into the DNA of nuclear chromatin was analyzed by the indirect end-labeling procedure (36, 56). DNA samples were digested to completion with a suitable restriction endonuclease and loaded on to a long (40-cm) agarose gel, and fragments were resolved by electrophoresis in Tris acetate-EDTA (pH 7.75) buffer in a cold room at 2.5 V/cm. Typical runs took approximately 40 h. In addition to DNA samples derived from chromatin digests, all gels were loaded with control lanes of protein-free DNA that had been digested to similar extents with MPE· Fe(II), DNase I, or micrococcal nuclease as appropriate. Size markers were prepared both from genomic DNA digested with restriction enzymes (internal markers) and from suitable restriction digests of plasmids 88B13 (9) and pBR322. Preparation of the gel for transfer to nitrocellulose and subsequent blotting were performed as described previously (59). Filters were hybridized in a slit well constructed from two long glass plates spaced at the base and sides with strips of thin silicone rubber sheeting; the apparatus was placed in an incubator at 65°C for hybridization and subsequent washings. Background was always low under these conditions; use of Seal-a-meal bags gave variable backgrounds. Preincubation of the filter was for 2 to 3 h in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–5× Denhardt solution–0.25% SDS. Hybridization for ca. 12 h was performed in 5× SSC–5× Denhardt solution–0.25% SDS–250 μg of denatured salmon sperm DNA per ml–10% dextran sulfate and ca. $10^7$ dpm of $^{32}$P-labeled probe. Washing of filters was performed in 5× SSC–5× Denhardt solution–0.25% SDS (once for 30 min), 2× SSC–0.25% SDS (twice for 30 min each), and finally in 0.5× SSC–0.25% SDS (twice for 30 min each), all at 65°C. Filters were dried and exposed to Kodak X-AR5 X-ray film with a Du Pont Cronex Lightning-Plus screen at −80°C.

## RESULTS

High-resolution chromatin structural data were obtained for each of the four small heat shock genes hsp 28, hsp 23, hsp 26, and hsp 22 depicted on the map from left to right in Fig. 1. In each case, data were obtained for both embryonic and tissue culture nuclei under non-heat shock and heat shock conditions. Probes used for the indirect end-labeling analysis are shown on the map (Fig. 1).

**hsp 28.** Figure 2A provides a detailed comparison of the type of information obtainable with the three probes of chromatin structure used in this study. As a general observation, it is important to note, for any given reagent, the contrast (or lack thereof) between data obtained upon chromatin (nuclear) digestion and that obtained by digestion of the purified, protein-free DNA. While MPE· Fe(II) showed a relatively (although not completely) sequence-neutral cleavage pattern in DNA, DNase I had considerably more sequence specificity, and micrococcal nuclease, as has been previously well documented (12, 22), showed a very high degree of DNA cleavage specificity. hsp 28 displayed a very strong complex of 5′ hypersensitive sites (denoted by black squares), detected in non-heat-shocked embryos with

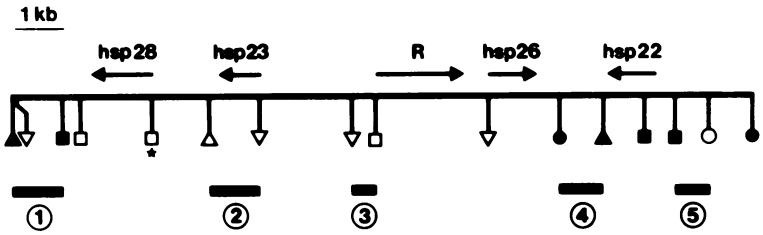


FIG. 1. Partial restriction map showing relevant landmarks in the region of locus 67B1 that encodes the four small heat shock proteins of *D. melanogaster*. More complete maps of the region can be found in references 9, 11, and 53. The *Sal*I site near the 5′ end of hsp 28 (starred) is present as a polymorphism in a proportion of our fly population and is not present in Schneider 2 tissue culture cells. Segments designated 1, 2, 3, 4, and 5 represent DNA fragments isolated from plasmids and nick translated for use as probes in the indirect end-labeling experiments. The genes are designated by lines over the map; the polarity of transcription is denoted by the arrowheads. Symbols: ▲, *Bam*HI; ▽, *Eco*RI; ■, *Hin*dIII; □, *Sal*I; △, *Xba*I; ●, *Sst*II; ○, *Bgl*I.

DNase I (lane 5) and MPE· Fe(II) (lane 8). While there are some new chromatin-specific bands in this upstream region detected with micrococcal nuclease, the majority of cleavages produced by this enzyme are found in both DNA and chromatin, both in this region and throughout the locus in general (see also Fig. 4, 5, 6). Figure 2B shows the generally similar pattern of hypersensitivity toward MPE· Fe(II) of this hsp 28 upstream region in Schneider 2 cell nuclei (lane 3) compared with embryonic nuclei (lane 1). Careful mapping of the MPE · Fe(II) cuts in Schneider 2 cells allows positioning of these sites with a certainty of ±10 bp. Relative to the hsp 28 cap site (24), these are at −23, −85, −310, −525, −725 (weak), and −893 bp. Of these, DNase I detects the first four also [although not with relative intensities equal to those of MPE· Fe(II)]. The upstream MPE sites may represent nucleosomal linker regions. In the inactive state the coding region of hsp 28 was highly inaccessible to DNase I. However, MPE· Fe(II) detected accessible regions occurring in fixed locations throughout the coding sequence. Moreover, these strongly accessible regions, which occur with a fairly regular spacing (starting at a position ca. 140 bp downstream of the cap site) of 180 to 200 bp, would appear to represent a positioned array of four to five nucleosomes across the coding region of hsp 28 (denoted by black circles in Fig. 2A, lane 8, and Fig. 2B, lanes 1 and 3). The sequence specificity of micrococcal nuclease is such that this array goes undetected in such an analysis (Fig. 2A, lanes 1 and 2). As reported previously, few, if any, prominent micrococcal nuclease cleavage sites occur in coding regions of genes (28).

Upon heat shock activation, several profound changes detected by both DNase I and MPE· Fe(II) were observed in the chromatin pattern of embryos. The major change in the hsp 28 hypersensitive region was the virtual disappearance of the strong site at −310 bp, suggesting that it is no longer accessible by virtue of a newly established DNA-protein interaction. Other sites in the region were relatively less affected (Fig. 2A, lanes 6, 9, and 10). The pattern in Schneider cells was more severely disrupted, with significant diminutions in the accessibility of virtually all of the 5′ sites. The region around −310 bp appeared to be so inaccessible that it "footprinted" as a region now deficient in both DNase I or MPE· Fe(II) cleavage (marked with arrowheads in Fig. 2A, lane 6, and Fig. 2B, lanes 2 and 4). In addition to these alterations, the pattern of distinct nucleosomes throughout the coding region was somewhat smeared, as detected by MPE· Fe(II). The overall enhanced accessibility of the coding region was most noticeable with DNase I cleavage (Fig. 2A, lane 6); the pattern was now quite similar to that detected by MPE· Fe(II) after heat shock (Fig. 2A, lanes 9 and 10).

We tested whether extraction of nuclei with salt would disrupt the chromatin pattern. After treatment with 0.35 M KCl for 30 min at 4°C and subsequent digestion with MPE· Fe(II), little change in the overall pattern was detected (Fig. 2A, lane 11). However, extraction with 0.5 M KCl severely reduced the intensity of the strong cleavage sites and led to generally more even levels of digestion in both spacer and coding sequences (Fig. 2A, lane 12). This latter treatment appears to have labilized a substantial portion of the specific DNA-protein contacts and led to a randomizing of the nucleosome associations in hsp 28.

Figure 3 shows embryonic samples digested with DNase I and MPE· Fe(II) and Schneider cell samples digested with MPE· Fe(II) probed from the opposite side of the hsp 28 gene. As expected, the major cleavages detected were all consistent with those just described; the nucleosomal array across hsp 28 in non-heat-shocked cells was especially apparent (denoted by black circles in Fig. 3, lane 4). A very strong hypersensitive region situated some 1.8 kilobases downstream of the hsp 28 cap site was detected by MPE· Fe(II) in Schneider cells (denoted by the asterisk in Fig. 3, lane 6). This disappeared upon heat shock (lane 7). The site can be detected in Fig. 2B, lane 3, near the bottom of the autoradiogram (starred), but was not prominent in embryos (Fig. 2A, lane 8; Fig. 3, lanes 1 and 4).

**hsp 26.** Figures 4A and B show the detailed chromatin structure surrounding hsp 26 as well as the developmentally regulated gene R. Once again, micrococcal nuclease showed little chromatin specificity either pre- or post-heat shock compared with protein-free DNA (lanes 1 to 4). However, both MPE· Fe(II) and DNase I revealed strong hypersensitive regions upstream of the hsp 26 cap site and in the vicinity of the R gene transcription initiation site (denoted by black squares). MPE· Fe(II) revealed strong sites at −20, −105 (more the center of a dispersed region of hypersensitivity), −300, −390, −585, and −640 bp for hsp 26 (Fig. 4A, lane 8, and Fig. 4B, lanes 1 and 3) relative to the cap site (24). DNase I showed some qualitative differences with bands at −20, −75 (very strong), −300, and −390 bp (Fig. 4A, lane 5). The most distal MPE· Fe(II) hypersensitive sites were not detected by DNase I. Through the hsp 26-coding region MPE· Fe(II) (but not DNase I) detected an ordered array of four to five nucleosomes with approximately 180 bp spacing (denoted by black circles in Fig. 4A, lane 8, and Fig. 4B, lanes 1 and 3). However, the distance from the cap site to the first intragenic band is noticeably longer than this (ca. 245 bp).

Upon heat shock the features expected from the data analyzed at hsp 28 were generally maintained. Hence, the coding region was strongly perturbed and became very

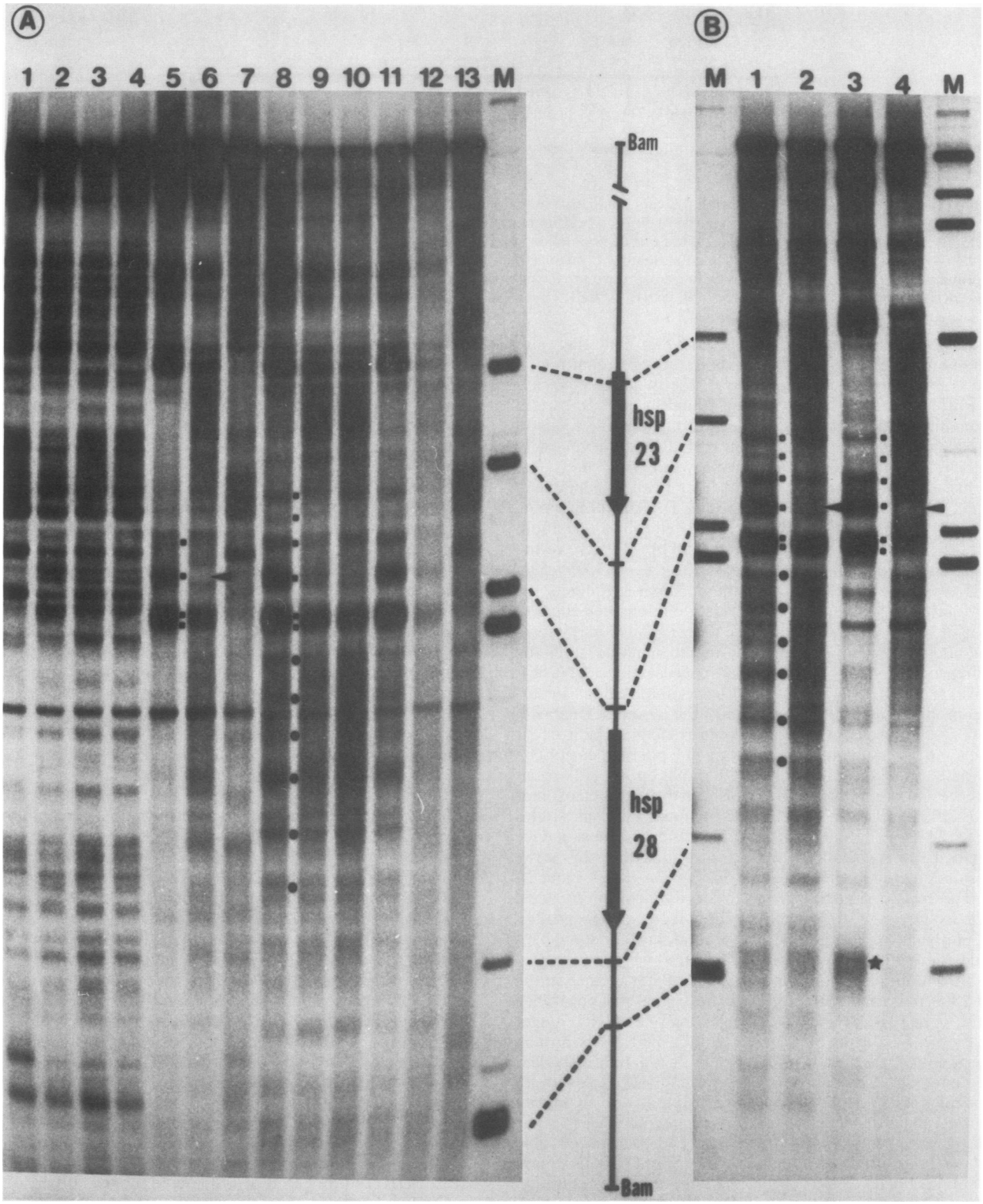


FIG. 2. Chromatin fine structure in the vicinity of hsp 28. Samples (9 μg) of DNA were digested to completion with *Bam*HI and fractionated on a 1.2% agarose gel. NHS and HS denote nuclei from non-heat-shocked and heat-shocked cells or embryos, respectively. (A) All samples were of embryonic origin. Micrococcal nuclease digestions at 25°C were on protein-free genomic DNA (lane 1) at 1 U/ml, NHS nuclei (lane 2) at 8.8 U/ml, HS nuclei (lanes 3 and 4) at 8.8 and 11.7 U/ml, respectively; DNase I digestions at 25°C were on NHS nuclei (lane 5) at 11.7 U/ml, HS nuclei (lane 6) at 15.6 U/ml, and genomic DNA (lane 7) at 0.1 U/ml; MPE · Fe(II) digestions at 25°C were on NHS nuclei (lane 8) at $2.5 \times 10^{-5}$ M for 15 min, HS nuclei (lanes 9 and 10) at $2.5 \times 10^{-5}$ M for 20 and 30 min, respectively, 0.35 M KCl-extracted NHS nuclei (lane 11) and 0.5 M KCl-extracted NHS nuclei (lane 12) digested as in lane 8, and genomic DNA (lane 13) at $5 \times 10^{-6}$ M for 2 min. All nuclei were digested at a concentration of $10^9$/ml. (B) Comparison of samples from embryos and tissue culture cells. MPE · Fe(II)

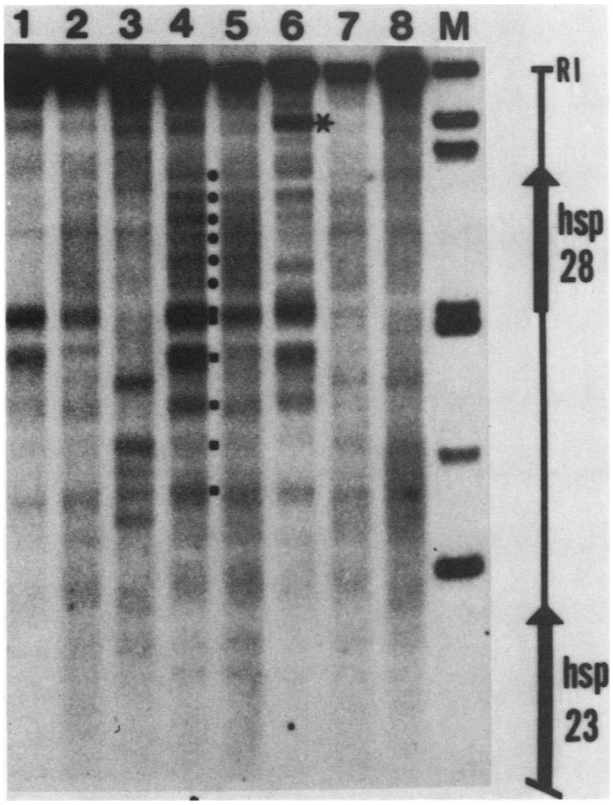


FIG. 3. Chromatin structure probed from the 5′ side of hsp 28. Samples (5 μg) of DNA were digested to completion with *Eco*RI. DNase I digests of embryonic nuclei in lane 1 (non-heat shocked [NHS]), lane 2 (heat shocked [HS]) and of genomic DNA (lane 3) were as described in the legend to Fig. 2. MPE · Fe(II) digests of nuclei from embryos (lane 4, NHS; lane 5, HS) and from Schneider 2 cells (lane 6, NHS; lane 7, HS) and of genomic DNA (lane 8) were as described in the legend to Fig. 2. M denotes markers. DNA samples were fractionated on a 1.3% agarose gel and blotted to nitrocellulose, and the filter was hybridized with probe 2 (Fig. 1) and subsequently washed and autoradiographed as described in Materials and Methods. Black squares and circles denote hypersensitive regions and nucleosomal linkers, respectively. The asterisk in lane 6 denotes a Schneider 2 cell-specific hypersensitive region (see text).

accessible to both DNase I and MPE· Fe(II) (Fig. 4A, lanes 6, 9, and 10, and Fig. 4B, lanes 2 and 4). Indeed the extent of increased hybridization in the region indicates this is the most heavily perturbed of all the four small heat shock genes upon heat shock. Of particular interest is the observation that DNase I now cuts the coding-region chromatin at quite frequent intervals, on the order of 100 bp between cleavage sites (denoted by open arrowheads in Fig. 4A, lane 6). While there was some DNA background apparent (lane 7), many of the cuts appeared chromatin specific or enhanced or both. At the hypersensitive sites the most obvious change was a region of heat shock-induced chromatin inaccessibility (a footprint) that mapped in the region from about −20 to −75 bp from the cap site (marked by arrowheads in Fig. 4A, lanes 6, 9, and 10, and Fig. 4B, lanes 2 and 4). An obvious effect was to shift the center of one of the 5′ DNase I hypersensitive sites from −75 bp to around −95 bp.

Hypersensitive sites were detected both by DNase I and MPE· Fe(II) in the vicinity of the 5′ end of the developmentally regulated R gene. The major site was a doublet (Fig. 4A, lanes 5 and 8; Fig. 4B, lanes 1 and 3) and mapped around 500 bp upstream of the *Sal*I site that is considered to be at (or very close to) the start site for R gene transcription (47, 48). Both MPE· Fe(II) and DNase I saw three mildly hypersensitive sites within the R gene spaced about 150 bp apart approximately 950 bp downstream of the *Sal*I site (denoted by open squares in Fig. 4A, lanes 5 and 8, and Fig. 4B, lane 1). In addition, MPE· Fe(II) detected an ordered series of cleavage sites upstream of this region, i.e., within the R gene (Fig. 4A, lane 8, and Fig. 4B, lanes 1 and 3), that appeared spaced with about 190-bp periodicity on average. The array was particularly noticeable in Schneider 2 cells (denoted by open circles in Fig. 4B, lane 3). It should be noted that the R gene is expected to be nontranscribed in both embryos and Schneider cells. The effect of heat shock on the R gene in embryos is minimal (Fig. 4A, lanes 6, 9, and 10, and Fig. 4B, lane 2); however, the extent of digestion of the R-coding region in Schneider cells appeared less than before heat shock (Fig. 4B, compare lanes 3 and 4). The salt extractions shown in Fig. 4A, lanes 11 and 12, provided the same general conclusion as for the hsp 28 region, i.e., little perturbation at 0.35 M KCl, quite substantial perturbation after 0.5 M KCl extraction.

A final point concerns the long spacer between R and hsp 23. Although in general MPE· Fe(II), and to a lesser extent DNase I, show relatively sequence-neutral patterns of cleavage of protein-free DNA, this particular sequence contained sites of particularly strong cleavage by MPE· Fe(II) (Fig. 4A, lane 13; also Fig. 5, lane 11) and by DNase I (Fig. 4A, lane 7; also, Fig. 5, lane 6). No sequence data has been reported yet for this region, but it seems likely to be particularly AT rich.

**hsp 23.** By use of a probe upstream to hsp 23, details of the hypersensitive site and coding-region nucleoprotein organization were obtained (Fig. 5). As before, micrococcal nuclease revealed little beyond an array of strong sequence-specific cleavages in both chromatin and protein-free DNA. Both DNase I and MPE· Fe(II) revealed a complex pattern of hypersensitive sites 5′ to the hsp 23 initiation site that differ somewhat in their details, while agreeing in overall morphology (denoted by black squares in lanes 4 and 7). In embryos MPE· Fe(II) mapped strong cleavage sites at −23, −98, −258 and −293 (tight doublet), −358, and −558 (weaker) bp (lanes 7 and 9) relative to the mRNA cap site (24). DNase I revealed a substantial area of heightened accessibility immediately 5′ to the cap site that spanned an area of some 100 bp or so. Within this there was a weak peak of intensity at about −45 bp and a strong peak at −105 bp (lane 4). Additional DNase I hypersensitive regions were centered at −290 and −350 bp; the weaker site at −550 bp appeared to be a DNA-specific cleavage. In embryos, an array of cleavages, spaced at approximately 180-bp intervals, was detected through the hsp 23-coding region by

digestions on embryonic nuclei (lane 1, NHS; lane 2, HS) were as in panel A (lanes 8 and 10, respectively); Schneider line 2 tissue culture cell nuclei ($5 \times 10^8$ per ml) were digested with MPE · Fe(II) at $2.5 \times 10^{-5}$ M for 10 min at 25°C with NHS (lane 3) or HS (lane 4) nuclei as substrate. M denotes lanes loaded with various restriction digests of genomic DNA to serve as markers. Gels were blotted to nitrocellulose, and the filters were hybridized to probe 1 (Fig. 1) and washed and autoradiographed as described in Materials and Methods. Arrowheads denote heat shock-induced regions of chemical or enzymatic inaccessiblity. Black squares denote hypersensitive sites, while black circles mark linker regions in nucleosomal arrays. The star in lane 3 of panel B denotes a Schneider 2 cell-specific region of hypersensitivity (see text).

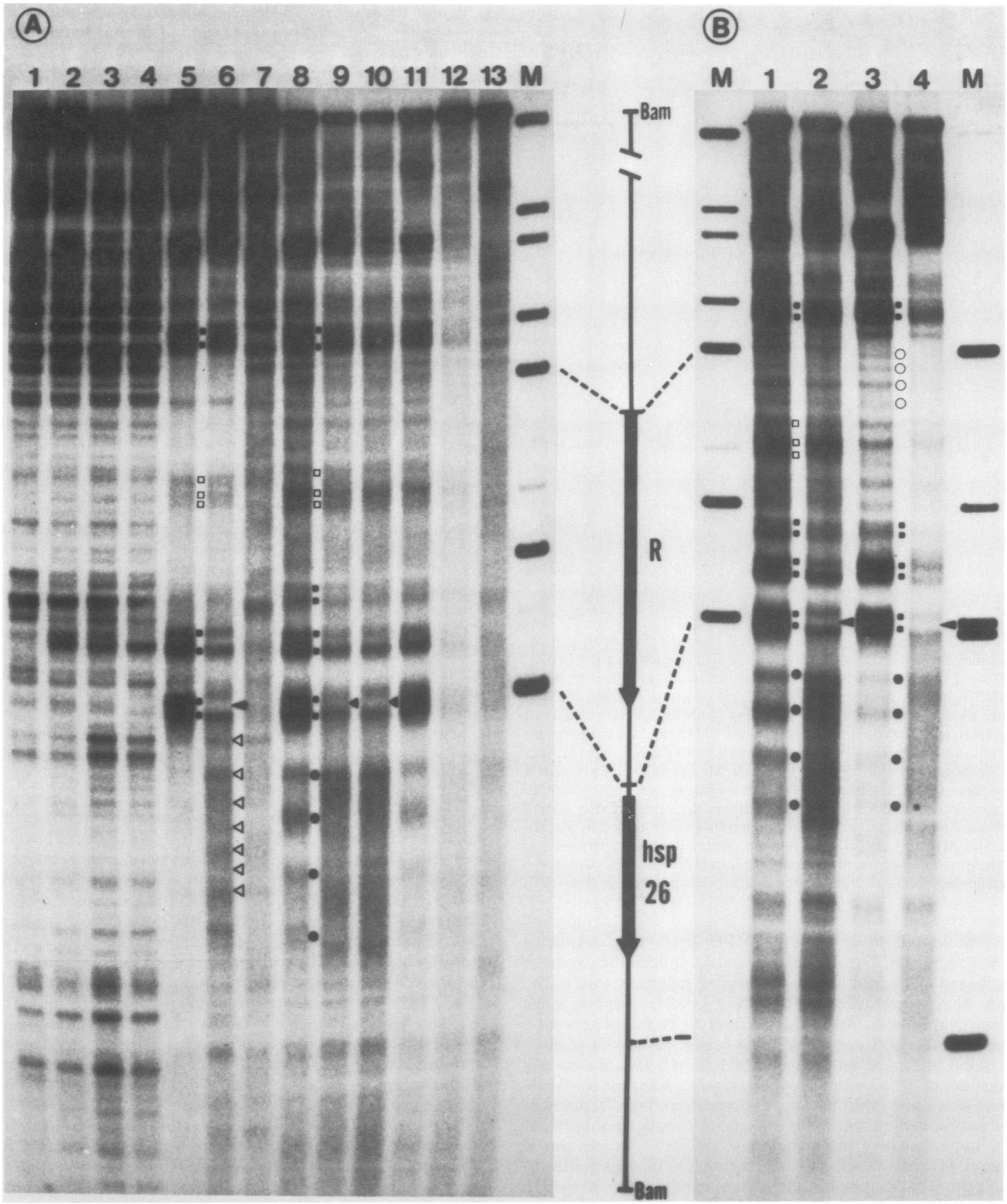


FIG. 4. Chromatin fine structure in the vicinity of hsp 26. Both panels A and B were obtained by reprobing the blots from Fig. 2 with probe 4 (Fig. 1), and hence the lane designations and samples are exactly as detailed in the legend to Fig. 2. The blots from Fig. 2 were stripped of probe by washing each filter in 0.15 M sodium hydroxide at room temperature (twice for 15 min each), followed by washing in 1 M Tris hydrochloride (pH 8.0) (once for 15 min) and finally in 10 mM Tris hydrochloride–1 mM EDTA (pH 8.0) (once for 10 min). Filters were soaked briefly in 5× SSC and then preincubated in the normal manner. Hybridization, washing, and autoradiography of the filters was as described in Materials and Methods. Black arrowheads denote heat shock-induced regions of chemical or enzymatic inaccessibility. Black squares and circles denote hypersensitive sites and nucleosomal linkers, respectively. Open squares and circles denote hypersensitive sites and nucleosomal linkers, respectively, within the R gene (see text). Open arrowheads show DNase I cutting sites within the active hsp 26-coding region.

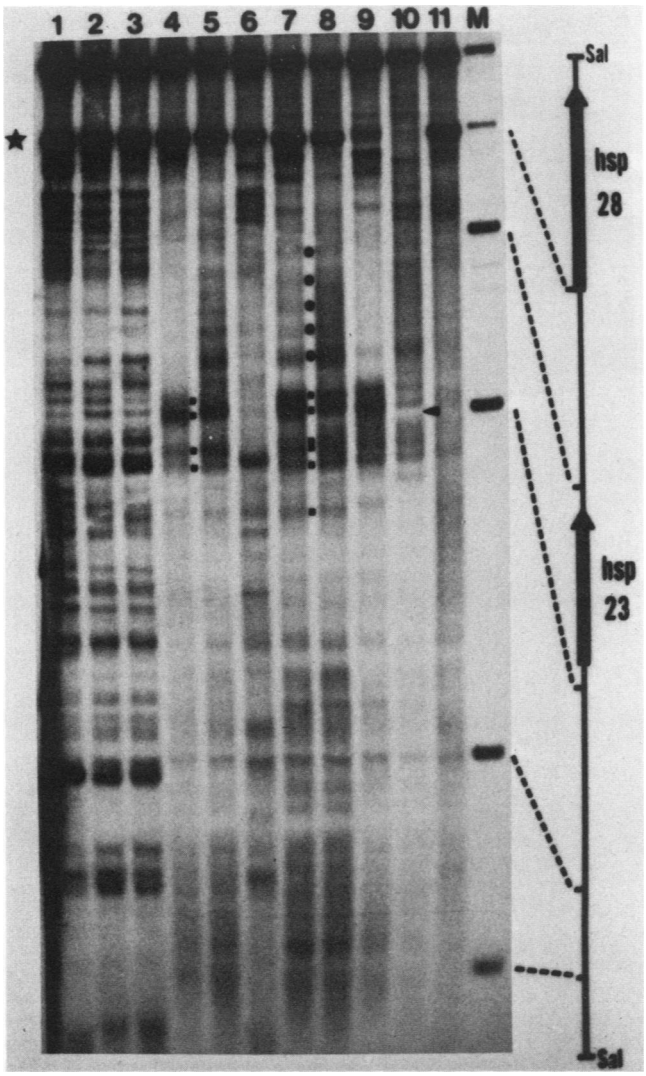


FIG. 5. Chromatin fine structure in the vicinity of hsp 23. Samples (9 μg) of DNA were digested to completion with *Sal*I and fractionated on a 1.4% agarose gel. Conditions of digestion were as described in the legend to Fig. 2. Micrococcal nuclease digestions were on genomic DNA (lane 1), non-heat-shocked (NHS) embryonic nuclei (lane 2), and heat-shocked (HS) embryonic nuclei (lane 3); DNase I digestions were on NHS embryonic nuclei (lane 4), HS embryonic nuclei (lane 5), and on genomic DNA (lane 6); MPE · Fe(II) digestions were on NHS embryonic nuclei (lane 7), HS embryonic nuclei (lane 8), NHS Schneider cell nuclei (lane 9), HS Schneider cell nuclei (lane 10), and on genomic DNA (lane 11). M denotes markers. The gel was blotted to nitrocellulose, hybridized with probe 3 (Fig. 1), and washed and autoradiographed as described in Materials and Methods. The arrowhead denotes a heat shock-induced region of MPE · Fe(II) inaccessibility. Black squares and circles denote hypersensitive sites and nucleosomal linkers, respectively. The star at the top left marks the location of a polymorphic *Sal*I site (see text).

MPE· Fe(II) (denoted by black circles in lane 7); the array lost its distinctly nucleosomal character before the 3′ end of the gene was reached. In the long spacer lying between hsp 23 and the developmentally regulated gene R (gene 1 of reference 48), MPE· Fe(II) revealed an ordered nucleoprotein structure, but with irregularly spaced cleavages (mostly spaced at less than 100 bp) not characteristic of a uniquely positioned nucleosome array (lane 7, from the hypersensitive region to the bottom of the gel). These results were substantially similar in Schneider 2 cells (Fig. 5, lane 9) for all the regions examined. It should be noted that the fly population that we used for embryo harvesting appears to have a polymorphism for the restriction enzyme *Sal*I. A proportion of the embryos have an additional site located virtually at the hsp 28 cap site which disrupts some of the pattern over hsp 28 (Fig. 5, lanes 1 to 8, 11). Schneider 2 cells lack this *Sal*I site, and the general features of the hsp 28 hypersensitive region can be clearly seen in lane 9.

Heat shock activation of hsp 23 led to alterations similar to those for hsp 28 and hsp 26. However, the degree of smearing and enhanced accessibility in the hsp 23-coding region to both DNase I and MPE· Fe(II) (Fig. 5, lanes 5, 8, and 10) was somewhat greater than that seen for hsp 28 (Fig. 2 and 3) but rather less than that observed for hsp 26 (Fig. 4). Changes in the hypersensitive region were less marked than for the other genes, but Schneider cells in particular revealed a newly induced highly inaccessible region detected as a footprint by MPE· Fe(II) (marked with an arrowhead in Fig. 5, lane 10). This region covers nucleotides from about −100 to −175 bp followed by a smear to a single hypersensitive site at about −290 bp. DNase I also revealed some changes; the strong site centered at −105 bp was shifted downstream to about −90 bp as if a new protein-DNA contact were being induced in this upstream region (Fig. 5, lane 5).

**hsp 22.** Both MPE· Fe(II) and DNase I detected a highly ordered hypersensitive region upstream of hsp 22 (sites marked by black squares in Fig. 6).DNase I (lane 4) reported sites at −10 (quite broad), −85, −215 (weak), −290 (weak), and −345 bp relative to the hsp 22 cap site (24). MPE· Fe(II) saw qualitatively identical sites; in addition, extra distal sites were seen at −585 and −700 bp (Fig. 6, lanes 7 and 9). The coding region was highly resistant to DNase I (lane 4) but appeared to be well organized by DNA-protein contacts, as revealed by MPE· Fe(II) digestion (lane 7). There is a substantial (ca. 400-bp) gap to the first strong cut within the gene, and following this, the pattern reveals two (perhaps three) positioned nucleosomes (denoted by black circles). At the 3′ end of the gene, the banding pattern becomes more complex, with spacing between cleavages frequently occurring at intervals of 100 bp or less. Heat shock activation led to increased DNase I and MPE· Fe(II) accessibility in this region (lanes 5, 8, and 10); however, the pattern was not as disrupted as in the cases of hsp 26 and hsp 23, and the DNase I pattern now quite closely resembled the MPE·Fe(II) digestion pattern. The most noticeable effect of heat shock in the hypersensitive domain upstream of the gene was introduction of a strongly inaccessible region located at approximately −10 to −90 bp relative to the cap site (marked by arrowheads in Fig. 6). This resulted in new DNase I hypersensitive sites at +10 and −110 bp. Otherwise the hypersensitive site pattern seemed relatively unaffected (lane 5). MPE· Fe(II) digestion revealed substantially similar features (lanes 8 and 10). In Fig. 7 we further resolved the hsp 22 hypersensitive region (spanning about 400 bp). The pattern revealed is quite complex. Close inspection shows two regions that are revealed as footprints by DNase I upon heat shock activation. The very strongly protected region (solid arrowhead) already detected in Fig. 6 is augmented by a second, more distal, inaccessible region (marked by open arrowhead), which maps around −175 bp (limits, −155 to −190 bp). The second site was not detected well in embryonic nuclei by MPE· Fe(II). The summary (Fig. 8) shows the more detailed mapping information from Fig. 7.

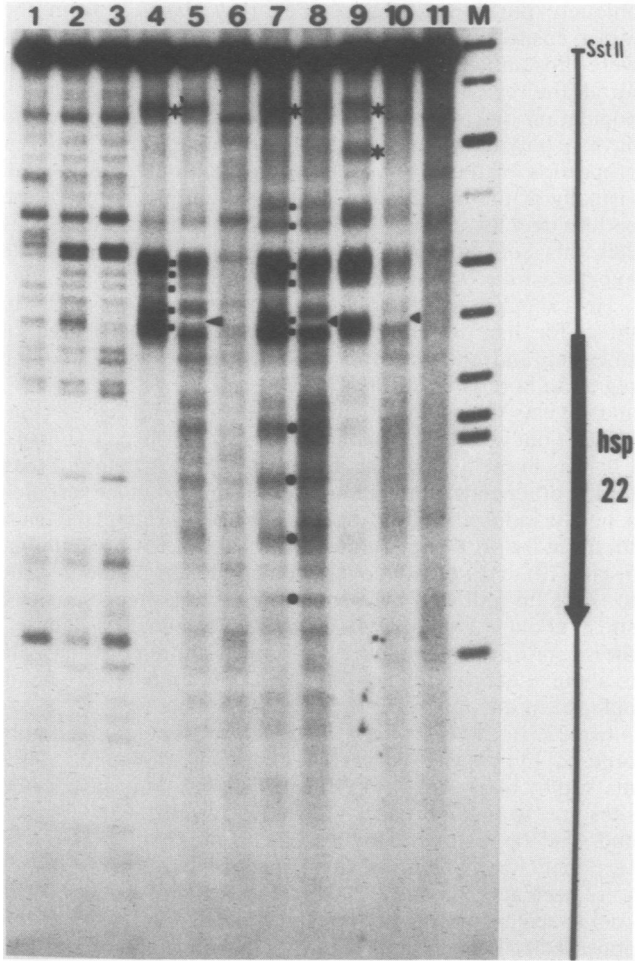


FIG. 6. Chromatin fine structure in the vicinity of hsp 22. DNA samples (9 μg) were digested to completion with *Sst*II and fractionated on a 1.35% agarose gel. Conditions of digestion were as described in the legend to Fig. 2. Micrococcal nuclease digestions were on genomic DNA (lane 1), non-heat-shocked (NHS) embryonic nuclei (lane 2), and heat-shocked (HS) embryonic nuclei (lane 3). DNase I digests were performed on NHS embryonic nuclei (lane 4), HS embryonic nuclei (lane 5), and on genomic DNA (lane 6). MPE · Fe(II) digestions were performed on NHS embryonic nuclei (lane 7), HS embryonic nuclei (lane 8), NHS Schneider 2 cell nuclei (lane 9), HS Schneider 2 cell nuclei (lane 10), and on genomic DNA (lane 11). M denotes genomic markers. The gel was blotted to nitrocellulose, and the filter was hybridized with probe 4 (Fig. 1). Subsequent filter washing and autoradiography were as described in Materials and Methods. The arrowheads denote heat shock-induced regions of chemical and enzymatic inaccessibility. Black squares and circles denote hypersensitive sites and nucleosomal linkers, respectively. The stars locate regions of hypersensitivity not investigated further (see text).

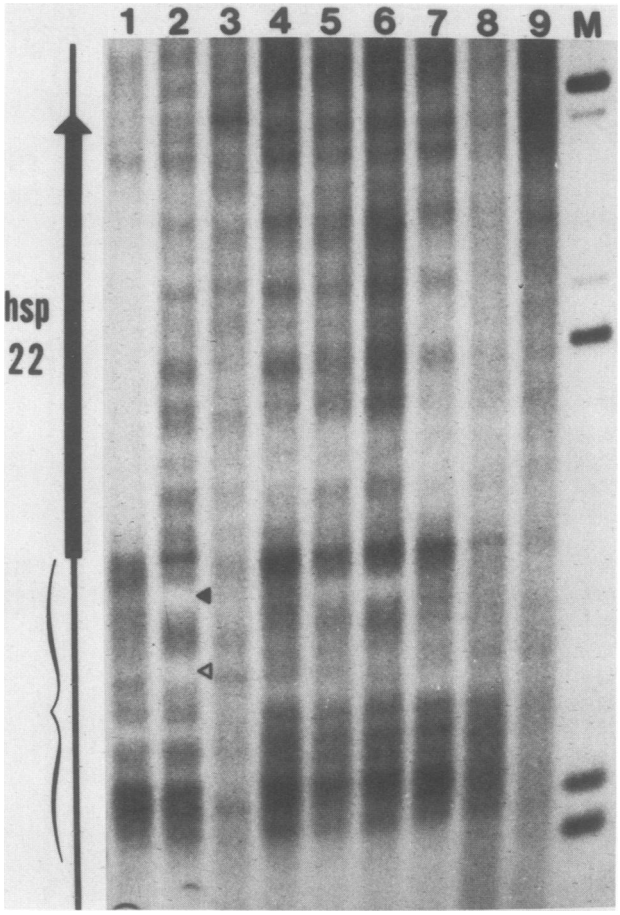


FIG. 7. High-resolution analysis of the nucleoprotein organization at hsp 22. Samples (9 μg) of DNA were digested to completion with *Bgl*I and fractionated on a 1.4% agarose gel. The region presented spanned a distance of approximately 20 cm from the dye front (at the bottom of the 40-cm gel) toward the origin. Conditions of digestion were as described in the legend to Fig. 2. DNase I digestions were performed on non-heat-shocked (NHS) embryonic nuclei (lane 1), heat-shocked (HS) embryonic nuclei (lane 2), and on genomic DNA (lane 3). MPE · Fe(II) digestions were performed on NHS embryonic nuclei (lane 4), HS embryonic nuclei (lanes 5 and 6), 0.35 and 0.5 M KCl-extracted NHS embryonic nuclei (lanes 7 and 8, respectively), and on genomic DNA (lane 9). M denotes genomic markers. The gel was blotted to nitrocellulose, hybridized with probe 5 (Fig. 1), and subsequently washed and autoradiographed as described in Materials and Methods. The filled and open arrowheads denote regions of heat shock-induced inaccessiblity to DNase I (see text). The hypersensitive region spans the distance within the bracketed section at the lower left of the autoradiogram.

Figure 6 also reveals DNase I- and MPE· Fe(II)-hypersensitive regions far upstream of hsp 22 in both embryonic and Schneider 2 cell nuclei (marked by stars in lanes 4, 7, and 9). These sites fall within a region which has been previously reported to code for a developmentally regulated transcript (gene 4 of reference 48), but neither precise extents of transcription nor the direction were mapped. We do not find it possible to make a prediction concerning the possible orientation of gene 4 owing to some size discrepancy in comparing our restriction maps with others published (47, 48) for this region. In Schneider cells, heat shock appeared to completely eliminate these sites, as detected by MPE · Fe(II) (lane 10).

## DISCUSSION

**Complex pattern of hypersensitivity at the 5′ end of *Drosophila* heat shock genes.** We carefully mapped the positions and extents of the various hypersensitive regions and collected the data in schematic form for the small heat shock genes in Fig. 8. The gel conditions used and the internal restriction fragment markers allowed up to map positions with a certainty of ±15 bp and in some cases ±10 bp. Each of the four small heat shock genes possesses a complex pattern of hypersensitive sites, detected in nearly every

instance by both MPE· Fe(II) and DNase I cleavage. For hsp 26 and hsp 28 these data agree well with those reported recently by Costlow and Lis (10). Thus, both genes contain four regions hypersensitive to DNase I upstream of the gene. In addition MPE · Fe(II) detects other singularities in the chromatin structure further upstream of these strongly hypersensitive regions. We do not know if these are important with regard to regulatory features. In some instances, the additional MPE· Fe(II) sites are spaced at approximately 200-bp intervals and may represent nucleosomal linker regions. For hsp 23 and hsp 22 we observed similar complex patterns of hypersensitivity upstream of each gene. It is apparent that these four heat shock genes have quite a different hypersensitive-site morphology than do the hsp 70 and hsp 83 genes of *Drosophila* (56, 57). These latter genes appear to contain just two regions of hypersensitivity located within 100 bp upstream of the respective cap sites. The present data show that the small heat shock genes contain hypersensitive sites that in some cases map out beyond 500 bp of the cap site, and additional components detected by MPE· Fe(II) out to 700 or 800 bp distal may be of relevance to regulatory control. One reason for this complex morphology may be that these four genes are known to be under dual control, i.e., in addition to heat shock induction, the genes undergo developmental regulation in larval and adult stages, with elements of tissue-specific expression also seen (33, 48, 60). In addition, the genes appear to be inducible in certain lines of tissue culture cells by ecdysterone (25, 26). No such level of regulation has been reported for either the hsp 70 or hsp 83 gene.

The analysis revealed other sites of hypersensitivity not apparently associated with the four heat shock genes. Strong hypersensitivity is associated with the developmentally regulated gene R, as previously reported (27). Sites at or near the 5′ end were mapped with DNase I, and additional regions were detected with MPE· Fe(II). Sites were also detected within the gene. The biological significance of the latter remains obscure. Other developmentally regulated genes were reported upstream of hsp 22 (48), and we observed patterns of hypersensitivity mapping in the relevant region. An intriguing observation was made in Schneider 2 tissue culture cells, which revealed a strongly hypersensitive region downstream of hsp 28 (Fig. 3, lane 6). MPE· Fe(II) detected it as a closely spaced doublet (Fig. 2B, lane 3). The site was only weakly detected (if at all) in embryos (Fig. 3, lane 4). Transcription from sequences adjacent to this region in third instar larvae has recently been detected (J. C. Eissenberg and S. C. R. Elgin, unpublished observations).

**Nucleosomes occupy distinct positions on inactive heat shock gene-coding sequences.** Digestion of either embryos or Schneider line 2 tissue culture cells with MPE· Fe(II) leads to the production of oligonucleosomal fragments and has allowed unambiguous detection of positioned nucleosome arrays over certain sequences (8). We found previously that micrococcal nuclease was highly unsuitable for this purpose at locus 67B1 by virtue of strong, regular cleavages throughout the locus in protein-free DNA (6, 28). The current experiments confirmed this amply. There were only a few locations where chromatin-specific micrococcal nuclease cleavages were detected (principally near the 5′ ends of the genes). The implication is that most sequences at this locus are not protected from micrococcal nuclease digestion even when binding to the histone core. Similar observations have been reported in analyses of satellite DNA sequences (23, 49). MPE· Fe(II) does, as expected (20), show a very low degree of sequence specificity on most sequences at 67B1,

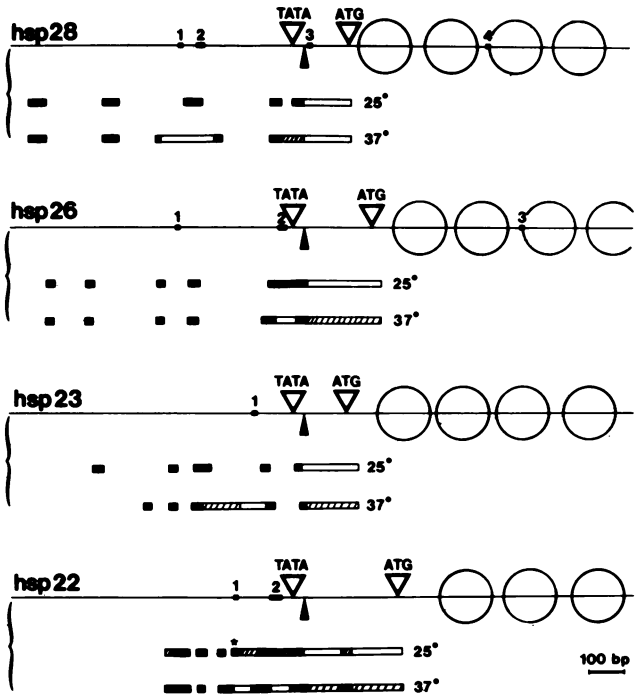


FIG. 8. Schematic summary of hypersensitive site and nucleosome positioning data for each of the four heat shock genes at 67B1. The maps are drawn such that the transcription start sites for each gene (denoted by the solid black arrowhead) are aligned directly above one another. Transcription polarity is from left to right for all genes for purposes of comparison. Regions that contain homologies to the heat shock consensus sequence (at least an 8/10 match to nondegenerate bases in the sequence) are denoted by numbered boxes on the horizontal line for each gene. Hypersensitive sites are shown as filled boxes beneath each transcription unit; regions of intermediate sensitivity are hatched; while regions that display a strong footprint are shown as open boxes. Data for both 25 and 37°C are given. Nucleosome locations on the coding regions at 25°C are depicted by circles representing 145-bp cores. The hypersensitive region marked with the star at hsp 22 appears as a DNase I-sensitive region in protein-free DNA also.

and the nucleosome positions which it reveals on coding regions are therefore expected to represent strongly preferred locations. In some cases there appears to be a large gap (250 to 400 bp) before the first strong nucleosome linker cleavage is seen. The gap could represent a region in which both histone and nonhistone proteins are bound to DNA and, as such, might represent a boundary for nucleosome placement (see reference 13a, for a discussion of boundary constraints for nucleosomal locations). In support of this view is the observation that the positioned array only persists for three or four nucleosomes before significant shifts in the spacing of cleavage sites (to a range of 50 to 100 bp) occur. Such a result might be predicted on the basis of statistical placement of nucleosomes adjacent to a boundary (29). On the other hand, we do not rule out the posssibility that these short nucleosomal arrays are so positioned by virtue of specific DNA-histone interactions; these appear to be important in setting a defined nucleosomal position both in vitro (41, 46) and in vivo (52). No arrays of positioned nucleosomes were detected in the spacer regions between genes, including the long spacer between the cap sites of hsp 23 and R. There does appear to be quite a long array of positioned nucleosomes located over the coding sequence

TABLE 1. Regions footprinted upon heat shock induction show homologies to the heat shock consensus sequence[a]

| Gene | Site[b] | Sequence[c] |
|---|---|---|
| hsp 28 | 1 | -368 CAAGAGAACTCCAGAAAGAAATGTCAAGAAGTTTCTGG -331 |
| | 2 | -295 CTAGAAAGAACCAGAAGATGCGAG -272 |
| hsp 26 | 2 | -71 CTGTCACTTTCCGGACTCTTCTAG -48 |
| hsp 23 | 1 | -174 GCGGCAAATTCGAGAACTCTGCGATATTTTCAGCCCGAGAAGTTTCGTG -126 |
| hsp 22 | 1 | -195 CCAGAAACTTCCAC -182 |
| | 2 | -94 GAAGAAAATTCGAGAGAGTGCCGGTATTTTCTAG -61 |

[a] Sequences were derived from reference 50 for hsp 26, hsp 23, and hsp 22 and courtesy of E. Hoffman and V. Corces (unpublished observations) for hsp 28. Regions with a minimum of 7/10 homology to the nondegenerate bases in the consensus sequence are shown.

[b] Site designation refers to Fig. 8.

[c] Numbering is relative to transcription start sites previously identified (24). Extents of homology to the canonical consensus sequence are shown by lines above or below the sequence.

for the R gene itself. This is particularly noticeable in Schneider cells (Fig. 4B, lane 3).

**Footprints are detected over heat shock consensus sequences upon heat shock activation.** Upon heat shock activation of either embryos or tissue culture cells profound structural changes take place in regions both flanking and within the four small heat shock genes. The most noticeable feature upstream of each gene is the suppression or displacement of at least one of the cluster of hypersensitive sites. In each case this is supplanted by a highly MPE · Fe(II)- or DNase I-resistant region upstream of each gene, the extent of which can be mapped with some precision. These apparent footprints are highly reproducible from gel to gel and do not represent spurious regions of imperfect hybridization. The slit-well hybridization protocol used here (see Materials and Methods) produces consistently low backgrounds and reproducible hybridization patterns, in contrast to our experience with hybridization in plastic bags. For both hsp 22 and hsp 26 a new region of some 30 to 40 bp becomes inaccessible after heat shock, centered around positions −60 to −70 bp relative to the cap site. Sequence data for this position (50) in both genes reveals two good matches in each case to nondegenerate bases in the heat shock consensus sequence 5′-C-T-N-G-A-A-N-N-T-T-C-N-A-G-3′ (35, 38) (Fig. 8; Table 1). For hsp 26, the two consensus sequences (one 8/10 and one 7/10 homologous) overlap, while at hsp 22, the two sequences (both 8/10 homologous) are contiguous. In Fig. 7 a high-resolution picture of the hsp 22 hypersensitive region is shown. In the heat-shocked condition DNase I reveals a second newly induced region of inaccessibility centered around −180 bp. Inspection of the sequence data for this region revealed a single copy match of high homology (8/10) to the consensus sequence, centered at about 185 bp upstream of the cap site (Fig. 8; Table 1).

Previous experiments that have employed transfection of the small heat shock genes of *Drosophila* into monkey COS cells revealed that the transfected hsp 28 genes were either uninducible or inducible to only very low levels by heat treatment; hsp 23 was constitutive at low levels (3, 39). Moreover, no heat shock consensus sequence was evident in 250 bp of DNA flanking hsp 28, while the consensus found at hsp 23 was in a very different location (−150 bp) compared with hsp 26 and hsp 22. We find that heat shock activation of hsp 23 leads to protection of a region extending from about −105 to −175 bp from the cap site. In this region are two matches (one 8/10 and one 7/10) to the consensus sequence, centered at positons around −133 and −166 bp (Fig. 8; Table 1). Activation of hsp 28 leads to strong protection of the hypersensitive region at around −310 bp from the cap site. There are, as yet, no published sequence data this far upstream. However, sequences in the spacer between hsp 23 and hsp 28 have recently been determined (E. Hoffman and V. Corces, unpublished observations). We found two overlapping heat shock consensus sequence matches (both 8/10 homologous) in this sequence centered at around −283 bp. Slightly upstream of this location at around −350 bp are three more good matches (one 8/10 and two 7/10) that exist in a pattern of one consensus sequence contiguous to two overlapping sequences (Fig. 8; Table 1). These regions coincide rather well with the broad region centered around −310 bp that becomes highly refractile to cleavage after heat shock.

The most likely explanation for this set of observations is that a protein (or proteins) is newly bound to these particular heat shock consensus sequences upstream of each of the genes after heat shock activation. An obvious candidate for this role is the protein fractionated by Parker and Topol (37) which protects the heat shock consensus sequences of hsp 70 from DNase I digestion and is capable of conferring transcriptional activity on heat shock genes in vitro. The same (or a similar) factor may be involved in the protection from exonuclease III digestion of heat shock consensus sequences upstream of hsp 83, as reported by Wu (58). It is particularly interesting to note that for each gene studied in the present work the major region protected always contains a minimum of two copies of the consensus sequence (using a baseline of a 7/10 match); at least one copy is always an 8/10 match. We have, however, at high levels of gel resolution, identified a weak footprint on a single consensus sequence located upstream of hsp 22 at about −185 bp (Fig. 7 and 8). The significance of the paired consensus sequence motif on these genes might well be resolved by embryo germ line transformation experiments. Experiments of this kind with hsp 70 gene constructions have demonstrated the importance of a second copy of the heat shock consensus sequence (13, 45). Similar observations have been made by transfection of *Drosophila* tissue culture cells with hsp 70 constructs (1). Constructions that eliminated the upstream copy of the hsp 23 consensus region identified in the present work were also non-heat shock inducible in such transfected cells (31).

It should be noted that a computer search of the sequence data available for this locus suggests that there are more heat shock consensus sequences present than are relevant. At a stringency requiring a 8/10 match, there are sites centered

around −343 and +595 bp for hsp 26 and centered around +17, +504, and +1321 bp for hsp 28 in addition to those discussed above (Fig. 8). Various transformation experiments indicate that at least those within the genes are not important in heat shock-regulated expression. Careful chromatin structure analysis (as exemplified here) clearly can aid in the interpretation of DNA sequence patterns. Sequence level resolution of protected regions in nuclei is now practicable and should provide a complementary approach to sequence deletion analyses in identifying putative regulatory sequences.

As mentioned above, a number of heat shock gene-associated hypersensitive sites appear unaffected by heat shock activation. These are located upstream of the heat shock consensus sequences for hsp 22, 23, and 26, but (at least for two of the hypersensitive sites) downstream of the relevant sequences for hsp 28. We noted the potential that these sites might hold as landmarks for regulatory regions responsive to developmental control (e.g., in response to ecdysterone). We speculate that for hsp 28, the physical disposition of these different regulatory regions relative to the gene may be reversed. Similar conclusions have been derived by E. Hoffman and V. Corces based on a deletion analysis (personal communication).

**Nucleoprotein order is disrupted through the genes upon heat shock activation.** Two major changes occur on the heat shock gene-coding sequences upon gene activation. First, the previously inaccessible coding region becomes highly accessible to the enzyme DNase I. It is presumed that this occurs because of a substantial unfolding of the chromatin fiber across the gene, mediated by relaxed (or at least quite different) DNA-protein interactions. DNase I is quite a bulky enzyme and is unable to detect the positioned nucleosomes revealed by MPE· Fe(II) on the inactive gene. After activation, however, DNase I produces a cleavage pattern through the gene that seems partially dictated by the prevailing chromatin structure and partially by some sequence cutting preferences (compare with DNA controls). Second, in this configuration the active gene appears to have lost some or most of its ordered nucleosomal structure. The MPE· Fe(II) pattern becomes distinctly smeared in some cases, and the overall sensitivity to cleavage by MPE· Fe(II) is enhanced in every case. Intriguingly, however, in at least two cases (hsp 26 in Fig. 4 and hsp 22 in Fig. 7), the DNase I pattern reveals an approximately 100-bp repeating unit after heat shock which might be the result of partial nucleosome unfolding in active chromatin into heterotypic tetramers, as previously hypothesized (55). Recently, the existence of half-nucleosome particles on "active chromatin" preparations assembled in *Xenopus* oocytes was reported (44). Unfolded nucleosomes have also been observed by electron microscopy in spreads of active rDNA chromatin from *Physarum* sp. (40).

The overall effect of heat shock on the structure of this domain at locus 67B1 seems to be somewhat more profound in the Schneider 2 tissue culture cells. In many regions large parts of the pattern become rather indistinct, even in regions where no induction of genes is known to occur, e.g., at the R gene, at the hypersensitive sites downstream of hsp 28 (Fig. 2 and 3), and at the hypersensitive region upstream of hsp 22 (Fig. 6). The implication is that the whole domain, and not just the heat shock genes and their regulatory sequences, is unfolding and becoming highly disordered in terms of the previously static DNA-protein interactions. The effect appears to be a real one, since there is no detectable effect on the chromatin structure of other non-heat shock loci that we have examined in Schneider 2 cells subjected to heat shock (data not shown). Why there should be a rather more dramatic effect in the tissue culture cells subjected to heat shock compared with 6- to 18-h-old embryos is not clear. It is possible that the methods used shocked the tissue culture cells more rapidly and efficiently. There is also the possibility that the embryo, which contains a diverse collection of cell types, responds much less uniformly to heat shock than does the homogeneous tissue cell population.

A final observation concerns the relative increase in accessibility of the four heat shock genes one to another upon heat shock activation. The data indicate that the coding region for hsp 26 in embryos is the most sensitive to MPE· Fe(II) digestion after heat shock. This is followed by hsp 23, while those for hsp 22 and hsp 28 show the least relative increase in accessibility. This observation is most intriguing because it parallels the relative levels of mRNA that accumulate after a 35°C heat shock (32). While there are no direct data on the subject, the half-lives of heat shock messages appear to be such that the levels of mRNA accumulated in a relatively short heat shock are expected to be a reflection of the relative transcription rate (S. Lindquist, personal communication). When these data are coupled with our observations, we conclude that the increase in accessiblity toward MPE· Fe(II) of each gene upon heat shock may be directly correlated with the transcription rate for each gene. This observation is not necessarily in contradiction to the earlier observation that genes transcribed at different rates display the same level of DNase I sensitivity (18). General DNase I sensitivity (as opposed to hypersensitivity) seems to be a global property associated with active, activatable, and previously active genes; the proviso in the latter case is that the genes in question have a history of transcriptional activity in their particular cell lineage. While there is a clear increase in DNase I sensitivity observed across each of the heat shock gene-coding regions upon activation, the relative changes appear approximately similar. This may be a reflection of the large size of this enzyme, which inhibits the disclosure of structural changes that may be more readily detected by smaller, less sterically hindered reagents. There are some reports that micrococcal nuclease (a substantially smaller enzyme) reacts to changes in accessibility of chromatin that are probably correlated with transcription rate (2, 5, 14, 42). The particularly small size of the MPE· Fe(II) molecule compared with most enzymes may allow it to reveal more subtle changes of both chromatin unfolding and the nucleosomal or other DNA-protein reorganization that accompanies gene activation. It is not unreasonable to suppose that increasing levels of transcription are reflected at the structural level as a relatively more disordered nucleoprotein fiber.

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