Reprinted from Nature, Vol. 289, No. 5794, pp. 198-203, January 15 1981

Chromatin fine structure of active and repressed genes

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Study of the structural organization of chromatin during transcription and replication may reveal important aspects of these processes. At the lowest level of organization, chromatin consists of a repeating subunit, the nucleosome (for reviews see refs 1–3). Electron microscopy indicates that the nucleosomes are arranged helically[4–6] or form discrete superbeads[7], generating the familiar 250 Å–300-Å fibre[8]. It has been suggested that this fibre is further folded into loops containing up to several hundred nucleosomes[9,10]. Despite extensive study, the significance and fate of these nucleosomes remain obscure. We have used here micrococcal nuclease digestion to compare the structures of actively transcribing and inert chromatin of the genes coding for the major heat-shock protein of *Drosophila melanogaster*. The repressed *hsp 70* genes were considerably more resistant to cleavage by micrococcal nuclease than their flanking regions and the bulk of chromatin. The active genes, previously shown to be more sensitive than the repressed genes[11–13], are also more susceptible to the nuclease than their 3′-flanking regions and bulk chromatin.

The heat-shock genes of *D. melanogaster* provide an excellent system for studying different aspects of gene structure and regulation (reviewed in ref. 14). These genes are induced by transferring *Drosophila* tissues or cell cultures from their normal temperature at 25 °C to elevated temperatures above 30 °C. The major protein synthesized at 37 °C—*hsp 70*—has a molecular weight of 70,000. Six copies of the gene coding for this protein exist in the haploid genome of Kc cells[15]; each has been extensively characterized by cloning and whole-genome Southern analysis[15] (M.-E. Mirault, personal communication).

Nuclei of a *Drosophila* tissue-culture cell line were digested with micrococcal nuclease before or after heat shock. The DNA fragments obtained were separated according to size by agarose gel electrophoresis, denatured, transferred and bound covalently to diazobenzyloxymethyl (DBM)-paper[16]. By hybridization with radioactively labelled probes, the DNA sequences of interest were analysed selectively. Three different probes were used (Fig. 1)—the *Sal–Sal* fragment of 56H8 containing almost the entire coding region yet no flanking region, the *Sal–Bgl*II fragment of 56H8 adjacent to the 3′ end of the coding region and the *Bgl*II–*Bgl*II fragment to the left of the coding region in the genomic clone 132E3. The last probe contains moderately repetitive sequences[17] and was found to exhibit the same distribution of the nucleosomal repeat pattern (Fig. 2*C*) as bulk chromatin [revealed by staining the gel with ethidium bromide (Fig. 2*A*)] and hence is designated 'bulk'. Whenever the patterns revealed by these different probes are compared, they have been obtained by hybridizations with the same DBM-paper after release of the previous probe by denaturation.

The micrococcal nuclease pattern of the coding region (Fig. 2*B*) differs from that of bulk chromatin (Fig. 2*A*, *C*) in non-heat-shock conditions. The higher ratios of multimers to monomer in the lanes of the repressed gene (Fig. 2*B*) compared with the corresponding lanes representing bulk chromatin (control lanes in Fig. 2*A*, *C*) indicate that at least part of the coding region of the repressed gene is more resistant to micrococcal nuclease than bulk chromatin. This conclusion is corroborated by the observation that the average size of nick-translated DNA (bulk) obtained after nuclease digestion of non-heat-shocked nuclei is much smaller than that of the pattern revealed by hybridization of the same DNA with the coding region. (Compare lanes labelled 'nt' and 'c' of 6% acid-soluble DNA in Fig. 3. Considering that the label incorporated into nick-translated DNA is proportional to its mass whereas the label of the hybridized DNA is proportional to the number of DNA molecules, the observed difference is even greater than apparent from a mere comparison of the patterns of the autoradiogram.)

After mild digestions, the DNA of the repressed gene hybridizing to the coding region exhibits a relatively narrow size distribution with an average of about 2.5 kilobases (control lane of *a* in Fig. 2*B*). In a more extensive digestion, three sharp bands at 2.52, 2.34 and 2.16±0.05 kilobases appear above a background (control lane of *b* in Fig. 2*B*). These bands are more clearly visible when the background is reduced by a shorter exposure (lane *b* at far right in Fig. 2*B*). Therefore, a region of 2.5 kilobases, larger than the coding region[18] but which must

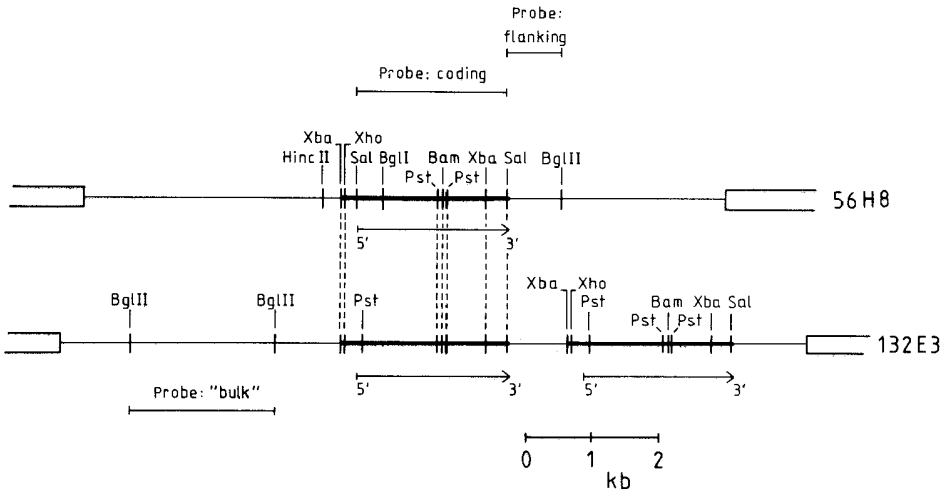
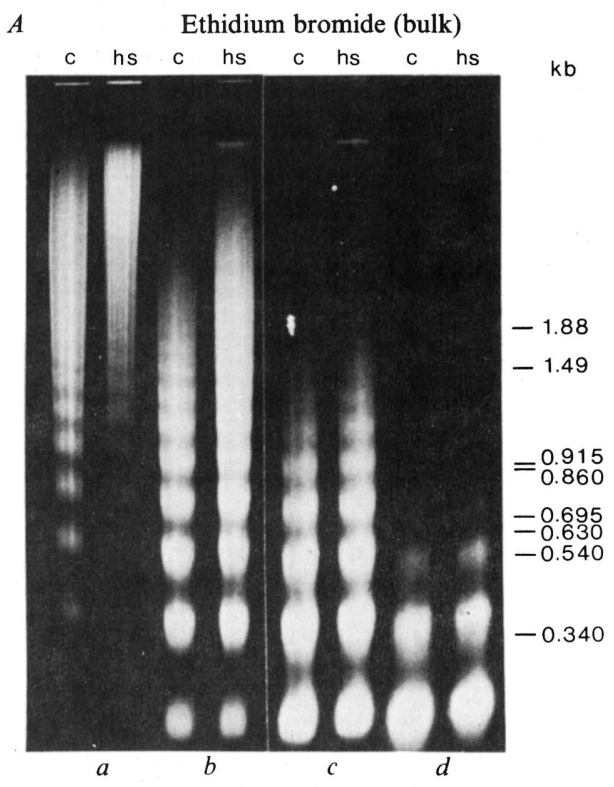
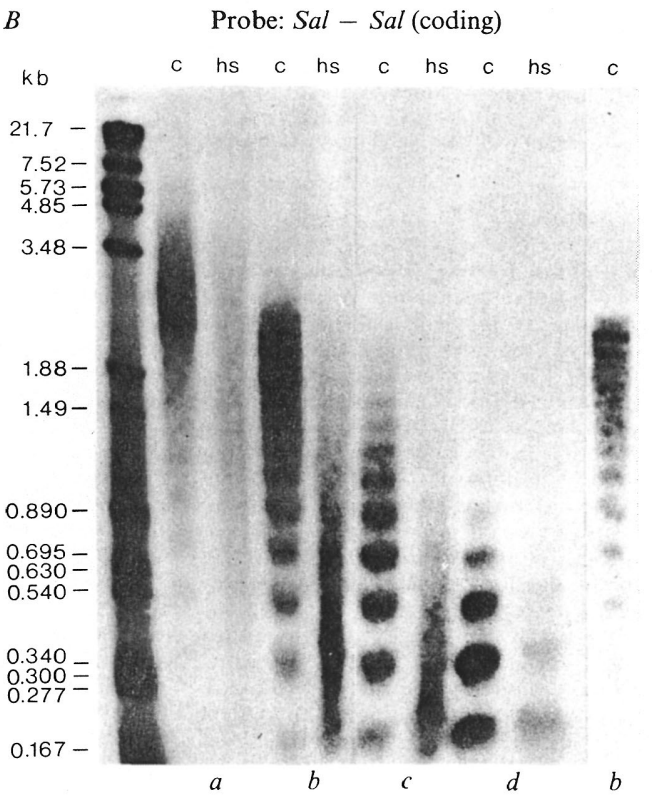
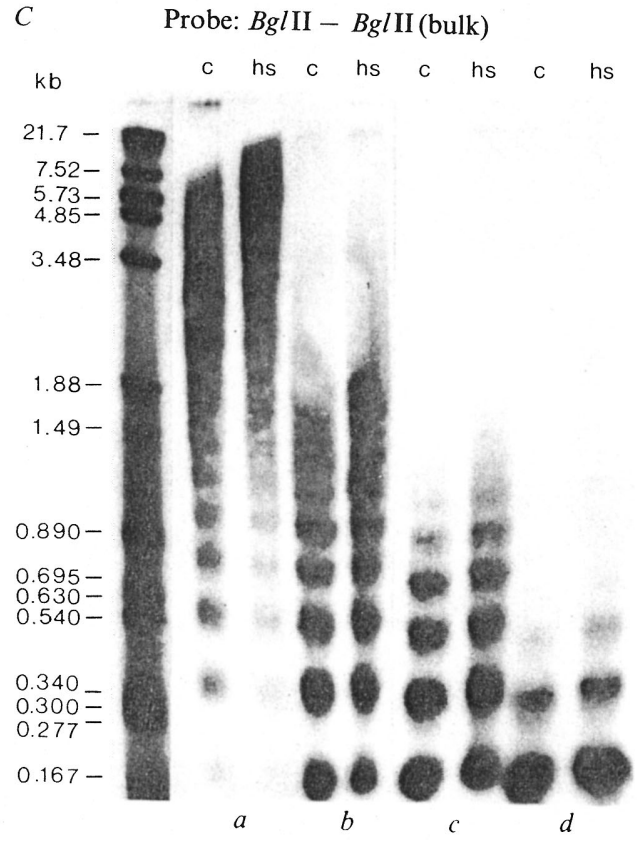


Fig. 1 Restriction map of *hsp 70* genes. Restriction maps of the *D. melanogaster* sequences in the hybrid plasmids 56H8 and 132E3 (ref. 26) have been published[27]. Only a few selected restriction sites are indicated here; the distances have been slightly corrected by calibration with additional low-molecular-weight markers (*Hae*III digest of PM2 DNA[28]). Sequences complementary to the *hsp 70* mRNA and the direction of transcription of these genes are indicated by arrows[18]. The protected domain of the repressed *hsp70* genes is represented by a thick bar comprising the coding region and most of the z_{nc} region[18]. The probes for the coding (*Sal–Sal*) and the flanking region (*Sal–Bgl*II), and the probe revealing a pattern similar to bulk chromatin (*Bgl*II–*Bgl*II) are shown above and below the maps. The names of the restriction enzymes have been abbreviated as follows: *Bam*, *Bam*HI; *Pst*, *Pst*I; *Sal*, *Sal*I; *Xba*, *Xba*I; *Xho*, *Xho*I. kb, Kilobases.


A
Ethidium bromide (bulk)
c hs c hs c hs c hs
kb
1.88
1.49
0.915
0.860
0.695
0.630
0.540
0.340
a b c d


B
Probe: Sal – Sal (coding)
c hs c hs c hs c hs c
kb
21.7
7.52
5.73
4.85
3.48
1.88
1.49
0.890
0.695
0.630
0.540
0.340
0.300
0.277
0.167
a b c d b


C
Probe: BglII – BglII (bulk)
c hs c hs c hs c hs
kb
21.7
7.52
5.73
4.85
3.48
1.88
1.49
0.890
0.695
0.630
0.540
0.340
0.300
0.277
0.167
a b c d

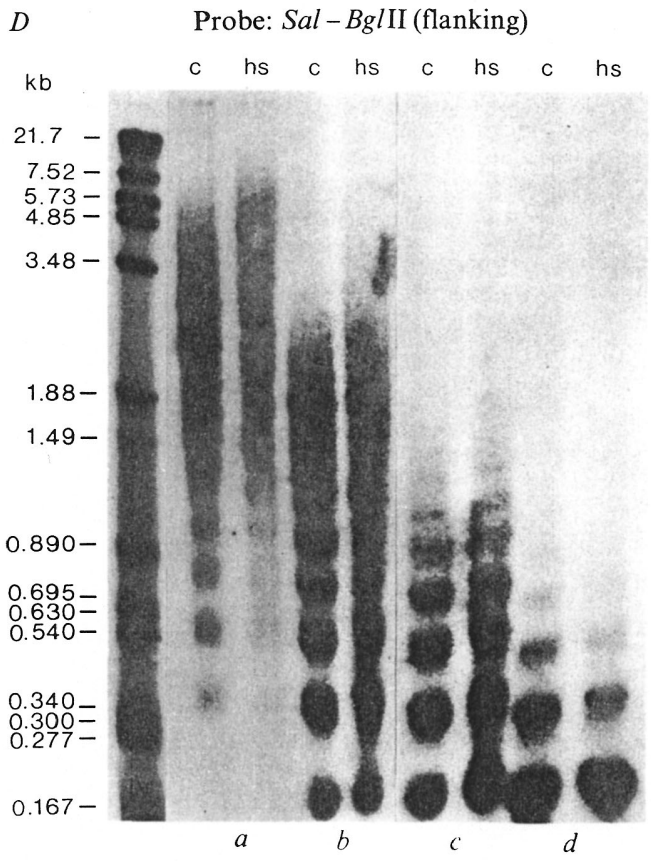

D
Probe: Sal – BglII (flanking)
c hs c hs c hs c hs
kb
21.7
7.52
5.73
4.85
3.48
1.88
1.49
0.890
0.695
0.630
0.540
0.340
0.300
0.277
0.167
a b c d

Fig. 2 Chromatin structure of *hsp 70* genes: comparison with structure of bulk and flanking regions. Digests of control (labelled 'c') and heat-shocked nuclei ('hs') were compared pairwise in bulk chromatin (*A*), in the coding region of the *hsp 70* gene (*B*), in a region containing a middle-repetitive sequence (*C*) and in the 3'-flanking region of the *hsp 70* gene (*D*). Four levels of digestion are shown in each panel corresponding to about 2.3% (*a*), 7% (*b*), 12% (*c*) and 18% (*d*) acid-soluble DNA. For preparation of nuclei, *Drosophila* Kc cells (adapted to growth in low serum) were grown in suspension at 25 °C in Echalier's medium[29] supplemented with 1% fetal calf serum (Gibco). Cells heat-shocked for 35 min at 36 °C, or control cells not subjected to heat shock, were collected in mid-log phase at a density of about 8×10^6 cells ml^{-1}. The cells were quickly chilled by pouring them on to half a volume of frozen balanced salt solution (BSS), and all subsequent steps were carried out on ice or at 4 °C. The cells were pelleted, washed twice in BSS and resuspended in Mg^{2+}-buffer (0.33 M sucrose, 20 mM HEPES, *p*H 7.5, 80 mM NaCl, 1 mM $MgCl_2$) at 4×10^8 cells ml^{-1}. To lyse the cells, the cellular suspension was made 0.2% in Triton X-100, incubated for 15 min on ice and passed four times through a hypodermic needle (G-18). The nuclei were washed (1,400*g* for 10 min) two or three times in Mg^{2+}-buffer and finally resuspended at a concentration of 1.2×10^9 nuclei ml^{-1}. For digestion, $CaCl_2$ to 1 mM was added to the nuclear suspension, and 0.5-ml aliquots were incubated for 2 min at 37 °C with 20 (*a*), 60 (*b*), 180 (*c*) and 360 units (*d*) of micrococcal nuclease (Worthington). The DNA was extracted and treated with a mixture of RNase A and T_1 RNase. Samples containing 5 μg (*A*) or 50 μg of DNA (*B–D*) were subjected to electrophoresis in two 1.7% agarose slab gels in 50 mM KNa_2PO_4, *p*H 6.5, 4 mM NaAc, 0.5 mM EDTA. One gel was stained with ethidium bromide (*A*), while the DNA from the other gel was transferred to DBM-paper and analysed by hybridization with various probes (Fig. 1); *Sal–Sal* of 56H8 (obtained as subclone *Sal*-O; *B*), *Bgl*II–*Bgl*II of 132E3 (*C*) and *Sal–Bgl*II of 56H8 (*D*). Before transfer, the DNA was denatured by washing the gel twice for 15 min in 0.5 M NaOH, neutralized in 0.5 M Na-phosphate, *p*H 5.5, for 15 min, and washed twice for 10 min in 11.5 mM Na-phosphate-citrate, *p*H 4.0, first at room temperature, then at 4 °C. For preparation of DBM-paper, a published procedure[16] was modified to achieve an efficiency of DNA transfer of at least 40% over a size range of 40 bases to 21 kilobases. Using this modification, we can transfer at least twice as much DNA as was used in the experiments reported here before the binding capacity of the DBM-paper is reached[19]. Hybridization with the nick-translated[30], ^{32}P-labelled probe was carried out in 0.02% each of Ficoll, polyvinylpyrrolidone and bovine albumin[31], 5 × SSC, 1 mM EDTA, 0.1% SDS, 2 mg ml^{-1} salmon sperm DNA. After hybridization, the paper was washed four times for 1 h at 65 °C in 2 × SSC, 0.5% SDS, 17 mM Na-phosphate, *p*H 7.5, 0.05% $Na_4P_2O_7$, 1 mM EDTA and finally briefly in water at room temperature. For autoradiography, preflashed[32] Kodak XR-5 film was used with a Kyokko intensifying screen. All three autoradiograms have been obtained from one transfer of DNA to DBM-paper. After autoradiography, the hybridized probe was removed by four 30-min washes in 99% formamide at 80 °C, the DBM-paper was washed in water and the remaining ^{32}P-radioactivity (<3%) further reduced during 3–4 half lives to less than 0.3%. For calibration, the far-left lanes of each autoradiogram show *Hae*III fragments of PM2 DNA[28] and *Eco*RI fragments of λ DNA[33] labelled at their 3' ends by 'Klenow' DNA polymerase I (Boehringer Mannheim). The lane on the far right in (*B*) represents a shorter exposure of the fourth lane from the left in (*B*). kb, kilobases.

contain at least part of the coding region, is more resistant to micrococcal nuclease digestion than its flanking regions when the gene is not expressed. To determine the right boundary of this protected domain, the *Sal–Bgl*II probe specific for the 3'-flanking region of one of the genes (Fig. 1) was used. As evident from Fig. 2*D*, the patterns resemble those of the bulk DNA (Fig. 2*A*, *C*) more closely than those of the coding region (Fig. 2*B*). The three bands between 2 and 2.5 kilobases are barely visible (control lane of *b* in Fig. 2*D*), and digestion is slightly inhibited in the repressed gene (ratio of monomer to oligomer DNA in lanes of control in Fig. 2*D*) compared with the bulk (Fig. 2*C*). This suggests that the protected domain and the *Sal–Bgl*II region overlap only slightly. In other words, the protected region of the repressed gene ends at a site close to the 3' end of the mRNA coding region[18] (Fig. 1).

From the size of the protected domain of about 2.5 kilobases, we predict that its left end reaches beyond the 5' end of the mRNA coding region and maps close to the left boundary of the z_{nc} region[18]. A more precise localization of the 5' limit by direct mapping is complicated by the presence of repetitive sequences in this region.

No bands are ever observed between 2 and 2.5 kilobases in heat-shock conditions, even when digestion is very mild (<0.2% acid-soluble DNA in Fig. 3), so that the DNA containing the coding region exhibits about the same average size as the protected domain of the repressed gene. (The size of the bulk DNA in such digests is on average 15 kilobases, as shown in the two lanes on the left in Fig. 3.)

During more extensive digestions, beyond 7% acid-soluble DNA, the protected domain of the inactive *hsp 70* genes is eventually degraded to smaller multiples of the nucleosomal repeat which are resolved significantly better than in bulk chromatin (control lane of *c* in Fig. 2*B*, and 11% acid-soluble DNA in Fig. 3). The largest fragments observed correspond to 12, 13 and 14 nucleosomes and co-migrate with the three bands of 2.16, 2.34 and 2.52 kilobases visible after milder digestions, whereas nucleosomal repeats larger than 10 are not resolved in bulk chromatin. It follows that the average spacing of nucleosomes is more uniform in the inactive gene than in bulk chromatin. Calibration with markers of known lengths shows that the nucleosomal repeat of the inactive gene is 180 ± 4 base pairs (Fig. 3).

The fragment sizes resulting from digestion of active genes are not distributed randomly, yet differ from those obtained after micrococcal nuclease digestion of bulk chromatin or of inactive genes. This results in bands ('hs' lanes of *b* and *c* in Figs 2*B* and 4) at positions between those of the familiar nucleosomal repeat. These bands are clearer in Fig. 4 where the DNA has been analysed in denaturing conditions. As the bands appear at roughly equivalent positions and intensities in denaturing (Fig. 4) and non-denaturing gels (Fig. 2*B*), the DNA fragments produced are largely double-stranded. These bands do not originate from the presence of preferential cleavage sites in the free DNA because digestion of free DNA of a mixture of 56H8 and 132E3 does not produce such bands after hybridization to the same *Sal–Sal* probe. Nor are they oligonucleosomes of which the DNA has been degraded from the ends because no such monosomes are observed. Moreover, in contrast to bulk chromatin, these structures do not show any metastable intermediates at any level of digestion but are readily degraded to very short pieces. This is demonstrated in Fig. 4 in which DNA fragments as short as 57 nucleotides have been transferred efficiently (shortest marker indicated on the left), yet very little hybridization is observed below 160 bases. The conditions of hybridization used permit detection of hybrids as short as 40 base pairs[19].

The observed changes in chromatin structure depend completely on the level of transcription. After returning heat-shocked cells to the normal temperature (25 °C), there is a gradual disappearance of the transcription-specific structures (not shown). In some experiments (not shown), after micrococcal nuclease cleavage in the mRNA coding region of the active gene, we observed a smeared hybridization pattern initially which then gave way to a pattern reflecting the nucleosomal repeat. We could clearly correlate this smearing effect with a suboptimal heat-shock response. It thus seems crucial that transcription-specific structures be observed in heavily transcribed genes.

If the digestions after heat shock are relatively extensive ('hs' lanes of *d* in Figs 2*B*, 4), most of the hybridized DNA disappears, and only faint bands at the monomer and dimer positions of the inactive gene are visible. The residual monomer and dimer DNA can be explained if a few cells do not respond to heat shock or if a minor fraction of active genes contains small regions of a structure similar to that of inactive genes. Inactivity of one or more of the six *hsp 70* genes in all cells after heat shock is unlikely because, as the milder digestions show, the quantitative differences between the active and repressed genes exceed a ratio of 6 : 1 (compare, for example, the region between 2 and 2.5 kilobases in the two lanes of *b* in Fig. 2*B*).

As even bulk chromatin was more sensitive to micrococcal nuclease than the repressed gene, it was of interest to compare the sensitivity of the active gene with that of bulk chromatin. Comparison of the corresponding lanes of digests obtained after heat shock in Fig. 2*B* with *C* shows a smaller average DNA size and a reduced amount of hybridization in the former. Thus the DNA of active genes is more sensitive than the DNA of bulk chromatin.

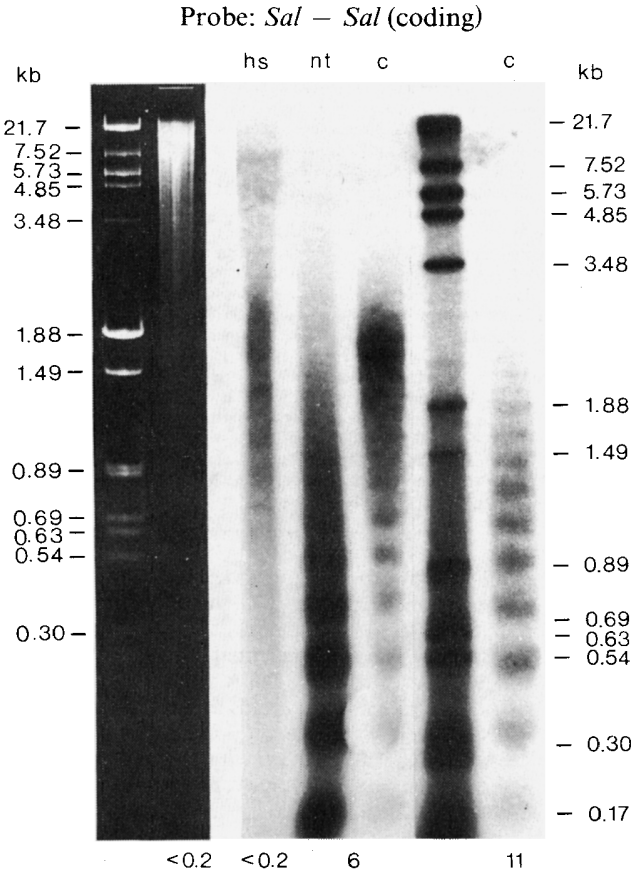


Fig. 3 Nucleosome spacing in repressed *hsp 70* genes and relative sensitivities of active and repressed *hsp 70* genes to micrococcal nuclease. On the left of the autoradiogram, the relative sensitivities to micrococcal nuclease of the active *hsp 70* gene ('hs'), of bulk chromatin ('nt') and of the repressed *hsp 70* gene ('c'; 6% acid-soluble DNA) are compared. At the far right, the uniform nucleosomal repeat of the repressed gene is shown ('c'; 11% acid-soluble DNA). Nuclei of heat-shocked ('hs') and control cells ('c') were prepared as in Fig. 2 except that the Mg^{2+}-buffer was replaced by spermine-spermidine buffer[34] (0.33 M sucrose, 15 mM Tris-HCl, *p*H 7.5, 60 mM KCl, 15 mM NaCl, 0.5 mM spermidine, 0.15 mM spermine) and the concentration of Triton X-100 was raised to 0.4%. Digestion of heat-shocked nuclei (1.2×10^9 ml^{-1}) with 4 U ml^{-1} of micrococcal nuclease and digestion of control nuclei with 120 or 360 U ml^{-1} rendered <0.2%, 6% and 11% of the DNA acid-soluble, as indicated below the corresponding lanes. The DNA was extracted, and 50-μg samples were subjected to electrophoresis in 1.7% agarose gels, transferred to DBM-paper and hybridized to labelled *Sal*-O DNA as described in Fig. 2. The 'nt' lane shows nick-translated DNA of the digest generating 6% acid-soluble DNA. The second lane from the right contains the same markers as in Fig. 2. The two lanes on the left show the same marker DNA and DNA of the digest of less than 0.2% acid-solubility after staining with ethidium bromide.

We also compared the initial rates of digestion of the active and repressed genes. The average DNA size of the active gene after digestion to less than 0.2% acid-solubility is about the same as that of the repressed gene after digestion to 6% acid-solubility for which 30 times more enzyme is required (Fig. 3). Thus the active gene is degraded 30 times more rapidly.

In addition, we conclude from a comparison of the amounts of hybridizable material in denaturing conditions in control and heat-shock DNA (for example ~1.9 kilobases in lanes of *b* or ~1 kilobase in lanes of *c* in Fig. 4) that both DNA strands of the active gene are more sensitive to micrococcal nuclease than those of the repressed gene.

As genes may be part of large loops of domains[9,10,20,21], it was interesting to examine whether the sensitive region of the active state extends beyond the coding region to include such larger domains. A comparison of the hybridization pattern of the 3'-flanking region (Fig. 2*D*) with that of the coding region (Fig. 2*B*) shows that the enhanced sensitivity does not extend much beyond the 3' end of at least one of the *hsp 70* genes (a similar study with the 5'-flanking region is complicated by the presence of repetitive sequences). The pattern obtained by hybridization to the 3'-flanking-region probe (Fig. 2*D*) is very similar to that of the bulk (Fig. 2*A*, *C*). Thus, for a first approximation, the region downstream from the right *Sal* site (Fig. 1) is organized similarly to bulk chromatin in heat-shock conditions. However, the patterns revealed by these two probes do differ slightly. First, the heat-shock lanes of *b* and *c* in Fig. 2*D* show more hybridization in the regions between monomer and dimer and between dimer and trimer DNA than the corresponding non-heat-shock controls or than the same lanes representing bulk chromatin (Fig. 2*A*, *C*). This suggests that the *Sal–Bgl*II probe overlapped slightly with the chromatin region containing the transcription-specific structure (Fig. 2*B*). Second, there is less hybridization after extensive digestion of the heat-shock-treated nuclei ('hs' lanes of *d*) in the flanking region (Fig. 2*D*) compared with bulk DNA (Fig. 2*C*) when normalized to the control. We infer that the sensitive portion of the gene ends close to the right-hand *Sal* site. As the 3' end of the mRNA coding region also maps close to this site[16], the 3' end of the mRNA coding region and the right boundary of the nuclease-sensitive chromatin region must be very close to each other.

Altered sensitivity to nuclease of actively transcribed genes may result from several structural changes: (1) changes in the

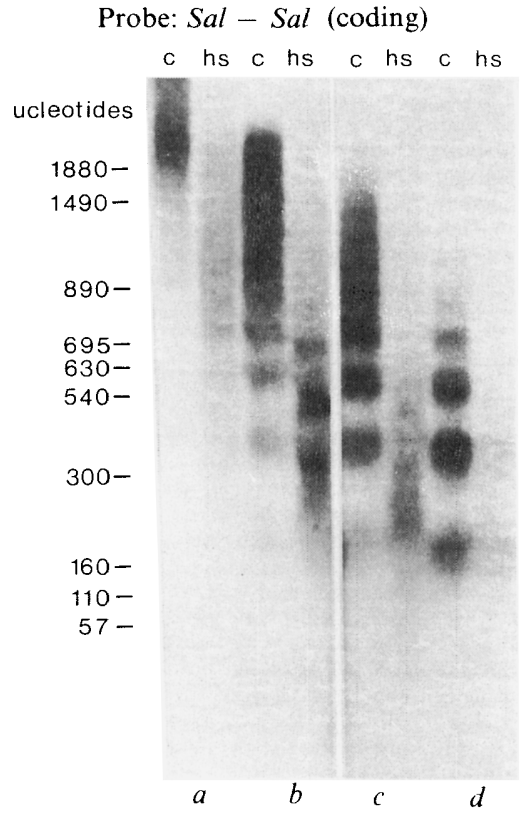


Fig. 4 Analysis of DNA fragments of the *hsp 70* gene in denaturing conditions. DNA samples (50 μg) of the same digests of control ('c') and heat-shocked nuclei ('hs') shown in Fig. 2 were subjected to electrophoresis in an alkaline 1.7% agarose gel[35]. After electrophoresis, the DNA was neutralized, transferred to DBM-paper, hybridized to *Sal*-O DNA and autoradiographed as described in Fig. 2 legend. On the left, the positions of *Hae*III fragments of PM2 DNA, used for calibration, are indicated.

higher structural orders, (2) changes in the structure of the nucleosomes, and (3) the absence of histones from transcribed DNA. Therefore, earlier suggestions made solely on the basis of DNase sensitivity that active genes contain structurally modified nucleosomes are invalid[22,23]. More detailed information with respect to the structure of active genes may be gained by analysing the size distribution of the DNA fragments produced by the action of DNase, and this study has revealed transcription-specific structures not previously observed. Micrococcal nuclease was found to discriminate between a gene, regardless of its state, and the rest of the chromatin. The repressed genes were protected and the active genes were highly sensitive compared with bulk chromatin or the neighbouring non-transcribed region. The levels of sensitivity of the gene are reversible and depend on its transcriptional activity. Hence, models of gene repression need not be based solely on the interaction of regulatory proteins with the 5′ end. Similarly, the association of bulk-type nucleosomes with the gene seems, in itself, insufficient to keep the gene in the inactive form. Some mechanism seems to exist which, on repression, alters the chromatin structure of the gene so as to reduce its accessibility (and that of a region of about 1 nucleosome at its 5′ end) to the nuclease considerably below that of the flanking, non-transcribed DNA sequence.

The mechanism of protection of the repressed genes could be explained on at least two structural levels. Each linker region joining adjacent nucleosomes could be modified in such a way as to reduce its sensitivity to micrococcal nuclease. The known sequence of the *hsp 70* gene[24,25] rules out the trivial explanation that all linker regions are less sensitive to micrococcal nuclease because of a high G + C content. In a more attractive model, all the linker regions of the entire domain become collectively resistant to the nuclease by some change in higher order of chromatin structure. The transition points between the protected domain of the repressed state and the flanking, bulk-like structure might then be sequences of regulatory significance. Specific nonhistone protein(s) interacting with these sequences may associate with each other to bring the two ends of this chromatin segment into tight proximity, producing a loop containing 14 nucleosomes. Such a loop may form a supercoiled, more compact and less accessible form of chromatin. In this model, activation of a gene would require at least two steps. First, the protected domain is unfolded, and only then does initiation of transcription start by the modification or removal of the nucleosomes.

We thank P. Schedl, S. Artavanis-Tsakonas, M.-E. Mirault, W. Gehring and A. Tissières for the hybrid plasmids 132E3 and 56H8, M. Goldschmidt-Clermont for the *Sal*-O subclone of 56H8, R. Jack for the subclone containing the *Bgl*II–*Bgl*II fragment of 132E3, R. Steward for the Kc cell line adapted to low serum, and E. Frei, M. Goldberg, R. White and H. Noll for criticism of the manuscript. This work was supported by Swiss NSF grants 3.719.76 and 3.466.79.

The results of this paper have been presented previously at the Annual Meeting of Swiss Societies of Experimental Biology in Basel, on March 13–14, 1980[36] and at the Gordon Conference on Chromatin and Nuclear Proteins (June 15–20, 1980).

Received 17 September; accepted 27 November 1980.

1. Kornberg, R. D. *A. Rev. Biochem.* **46,** 931–954 (1977).
2. Chambon, P. *Cold Spring Harb. Symp. quant. Biol.* **42,** 1209–1234 (1978).
3. McGhee, J. D. & Felsenfeld, G. *A. Rev. Biochem.* **49,** 1115–1156 (1980).
4. Filip, D. A., Gilly, C. & Mouriquand, C. *Humangentik* **30,** 155–165 (1975).
5. Finch, J. T. & Klug, A. *Proc. natn. Acad. Sci. U.S.A.* **73,** 1897–1901 (1976).
6. Ris, H. & Korenberg, J. in *Cell Biology* Vol. 2 (eds Prescott, D. M. & Goldstein, L.) 267–361 (Academic, New York, 1979).
7. Hozier, J., Renz, M. & Nehls, P. *Chromosoma* **62,** 301–317 (1977).
8. Ris, H. & Kubai, D. F. *A. Rev. Genet.* **4,** 263–294 (1970).
9. Benyajati, C. & Worcel, A. *Cell* **9,** 393–407 (1976).
10. Paulson, J. R. & Laemmli, U. K. *Cell* **12,** 817–828 (1977).
11. Panet, A. & Cedar, H. *Cell* **11,** 933–940 (1977).
12. Bellard, M., Gannon, F. & Chambon, P. *Cold Spring Harb. Symp. quant. Biol.* **42,** 779–791 (1978).
13. Wu, C., Wong, Y.-C. & Elgin, S. R. C. *Cell* **16,** 807–814 (1979).
14. Ashburner, M. & Bonner, J. J. *Cell* **17,** 241–254 (1979).
15. Mirault, M.-E., Goldschmidt-Clermont, M., Artavanis-Tsakonas, S. & Schedl, P. *Proc. natn. Acad. Sci. U.S.A.* **76,** 5254–5258 (1979).
16. Alwine, J. C., Kemp, D. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5350–5354 (1977).
17. Lis, J. T., Prestidge, L. & Hogness, D. S. *Cell* **14,** 901–919 (1978).
18. Artavanis-Tsakonas, S., Schedl, P., Mirault, M.-E., Moran, L. & Lis, J. *Cell* **17,** 9–18 (1979).
19. Levy, A., Frei, E. & Noll, M. *Gene* **11,** 283–290 (1980).
20. Wensink, P. C., Finnegan, D. J., Donelson, J. E. & Hogness, D. S. *Cell* **3,** 315–325 (1974).
21. Igó-Kemenes, T. & Zachau, H. G. *Cold Spring Harb. Symp. quant. Biol.* **42,** 109–118 (1978).
22. Weintraub, H. & Groudine, M. *Science* **193,** 848–856 (1976).
23. Garel, A. & Axel, R. *Proc. natn. Acad. Sci. U.S.A.* **73,** 3966–3970 (1976).
24. Török, I. & Karch, F. *Nucleic Acids Res.* **8,** 3105–3123 (1980).
25. Ingolia, T. D., Craig, E. A. & McCarthy, B. J. *Cell* **21,** 669–679 (1980).
26. Schedl, P. *et al. Cell* **14,** 921–929 (1978).
27. Moran, L. *et al. Cell* **17,** 1–8 (1979).
28. Noll, M., Zimmer, S., Engel, A. & Dubochet, J. *Nucleic Acids Res.* **8,** 21–42 (1980).
29. Echalier, G. & Ohanessian A. *In Vitro* **6,** 162–172 (1970).
30. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1184–1188 (1975).
31. Denhardt, D. T. *Biochem. biophys. Res. Commun.* **23,** 641–646 (1966).
32. Laskey, R. A. & Mills, A. D. *FEBS Lett.* **82,** 314–316 (1977).
33. Philippsen, P., Kramer, R. A. & Davis, R. W. *J. molec. Biol.* **123,** 371–386 (1978).
34. Hewish, D. R. & Burgoyne, L. A. *Biochem. biophys. Res. Commun.* **52,** 504–510 (1973).
35. McDonell, M. W., Simon, M. N. & Studier, F. W. *J. molec. Biol.* **110,** 119–146 (1977).
36. Levy, A. & Noll, M. *Experientia* **36,** 750 (1980).