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Quantitation of DNase I sensitivity in Xenopus chromatin containing active and inactive globin, albumin and vitellogenin genes

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ABSTRACT

The disappearance of defined restriction fragments of the β_1-globin, an albumin and the A1 vitellogenin gene was quantitated after DNase I digestion and expressed by a sensitivity factor defined by a mathematical model. Analysis of naked DNA showed that the gene fragments have similar but not identical sensitivity factors. DNase I digestion of chromatin revealed for the same gene fragments sensitivity factors differing over a much wider range. This is correlated to the activity of the genes analyzed: the β_1-globin gene fragment is more sensitive to DNase I in chromatin of erythrocytes compared to hepatocytes whereas the albumin gene fragment is more sensitive to DNase I in chromatin of hepatocytes. The A1 vitellogenin gene has the same DNase I sensitivity in both cell types. Comparing the DNase I sensitivity of the three genes in their inactive state we suggest that different chromatin conformations may exist for inactive genes.

INTRODUCTION

To understand the regulation of gene expression in eukaryotes a detailed analysis of the higher order structure of DNA in chromatin is essential since in most cases the primary DNA sequence is the same in different cell types. Digestion with nucleases, such as pancreatic DNase I, is a promising approach for analyzing the conformation of specific genes within chromatin as this enzyme seems to preferentially degrade transcriptionally active gene sequences (reviewed in 2). Recently the DNase I sensitivity of specific genes in the chromatin has been investigated using a more refined technique in which the digestion of specific restriction fragments of defined genes was measured following DNase I treatment of chromatin (3, 4). Qualitative evaluation of such experiments supported the general hypothesis that an active gene and its flanking sequences are more rapidly digested by DNase I than an inactive gene (5, 6, 7, 8) and furthermore demonstrated the existence of hypersensitive DNase I sites in the chromatin of some of the genes analyzed (3,

4, 7, 9, 10).

Using DNase I digestion of chromatin of erythrocytes and hepatocytes we have analyzed the chromatin structure of the β_1-globin, an albumin and the A1 vitellogenin genes of Xenopus laevis which are expressed tissue specifically. To quantitate and compare the digestion with DNase I of these three genes in two different cell types we propose a mathematical model and define a sensitivity factor which can be used to describe the DNase I sensitivity of the chromatin fragments containing these genes.

MATERIALS AND METHODS

Preparation of hepatocytes and erythrocytes

Male Xenopus laevis were anaesthetized with MS 222 (Sandoz AG, Switzerland) and 0.15 ml of heparin (Liquémine, Hoffmann-LaRoche AG, Switzerland) was injected into the heart to prevent coagulation of the blood. The liver was perfused essentially as described by Stanchfield and Yager (11). The erythrocytes were collected from the perfusate. The liver was then perfused with 0.025% collagenase (type I, Sigma) and hepatocytes were purified on Metrizamide (Nyegaard, Oslo, Norway) gradients (12).

Preparation of nuclei

The cells were washed three times in reticulocyte standard buffer (RSB; 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 5 mM $MgCl_2$) containing 250 mM sucrose, then gently homogenized in a Potter-Elvehjem homogenizer in RSB containing 0.5% Triton X-100 and vortexed to disrupt the cells. The nuclei were then washed twice in a large volume of RSB.

Digestion of nuclei and naked DNA with DNase I

Intact nuclei (1 mg DNA/ml in RSB) were digested to a limited extent with DNase I (0.2-3.0 µg/mg DNA; DNase I DN-CL, Sigma D 5010) for 10 min at 25° C. Digestion was stopped by addition of nine volumes of 0.15 M NaCl, 50 mM Tris-HCl pH 8.0, 5 mM EDTA and 2% sodium dodecyl sulphate (SDS) and the DNA extracted (13).

Naked DNA was prepared from undigested erythrocyte nuclei and the purified DNA (70 µg/ml in RSB) was digested to a limited extent with DNase I (4-20 ng DNase I/ml DNA) as described above.

Determination of the extent of DNase I digestion

The extent of DNase I digestion was determined accurately by measuring

the length of the digested DNA under the electron microscope. The DNA was dissolved in 2 M urea, 40% formamide, 30 mM Tris-HCl pH 8.5, 1 mM EDTA, 40 µg/ml of cytochrome C and spread onto distilled water as described (14). 60-200 molecules were measured on photographs of randomly selected fields and the average length determined.

Filter hybridization

Purified hepatocyte and erythrocyte DNA was digested with Hind III (Biolab) or Bam HI (Boehringer) and 5 or 10 µg of DNA was loaded onto each slot (7 x 5 mm) of an 1% agarose gel. After electrophoresis, the DNA was transferred to nitrocellulose filters using the Southern technique (15). The filters were incubated at 37° C for 4 h in prehybridization solution and hybridized for 4 days in hybridization solution (50 µl/cm^2) as described previously (16). The hybridization solution contained 10^5 cpm/cm^2 of ^{32}P-labelled cloned cDNA nick translated to a specific activity of 1-4 x 10^8 cpm/µg (17). In all cases, only cloned cDNA which had been isolated from the recombinant plasmid following S_1 nuclease or Pst I digestion (17) was used. After hybridization the filters were washed twice at 37° C for 1 h in 50% formamide, 5 x SSC (1 x SSC: 150 mM NaCl, 15 mM trisodium citrate), 0.2% SDS, 0.1% sodium pyrophosphate and 10 µg/ml denatured calf thymus DNA and then twice at 37° C for 30 min in 2 x SSC, 0.2% SDS, 0.1% sodium pyrophosphate and 10 µg/ml denatured calf thymus DNA and then stringently at 37° C for 30 min in 50% formamide, 0.1 x SSC and 0.2% SDS. Filters hybridized with the albumin cDNA probe were not washed stringently. The filters were then rinsed in 2 x SSC, 0.2% SDS and exposed to preflashed X-ray film at -80° C, using intensifying screens, for a limited time so that the blackening of the autoradiogram was still linearly related to the radioactivity in a band.

Structure of the globin, albumin and vitellogenin sequences used for the DNase I experiments

The β_1-globin cDNA clone pXGA2, recently prepared by Widmer et al., contains the structural sequences complementary to the major β-globin gene expressed in erythroblasts of adult Xenopus (18) and is identical to the β_1-globin cDNA clone pXG 8D2 described previously by Kay et al. (19). By analyzing genomic clones containing this gene, Patient et al. (20) showed that the α_1- and the β_1-globin genes are closely linked (Figure 1A). Using the β_1-globin clone pXGA2 we can identify the 5.8 kb restriction fragment carrying the

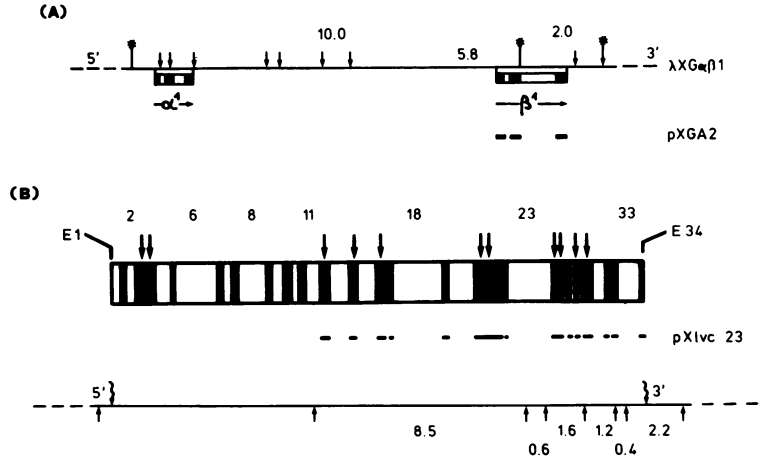

Fig. 1: Structure of the β_1-globin gene and the A1 vitellogenin gene

A) The Hind III (↓) and Bam HI (↑) restriction map of the β_1-globin genomic clone XGαβ1 published by Patient et al. (20) is shown at the top. Those fragments detectable by hybridization in genomic DNA with the β_1-globin cDNA clone pXGA2 (13) are given in kb. Furthermore the exons (black boxes) and introns (white boxes) are indicated.
B) The exon-intron map of the vitellogenin gene A1 is given (21, 22). The large introns are numbered, the small introns are indicated by arrows (↓) and the first and last exon sequences are indicated with E1 and E34. The Hind III sites are marked (↑) and the size of the fragments are given in kb. The exon sequences contained in the cDNA clone pXlvc 23 (23) are underlined.

entire gene in Hind III digested genomic DNA (see Fig. 1A and compare it to the hybridization in Fig. 3, slot 8).

To analyze an albumin gene we used a cDNA clone (pcXa13) constructed and characterized by Westley et al. (manuscript in preparation). R-loop analysis and translation of positively selected RNA in Xenopus oocytes have shown that this clone contains sequences derived from the middle 1,100 nucleotides of the mRNA coding for the 68,000 dalton Xenopus albumin. Using this albumin cDNA clone we can identify a 4.6 kb and a large, >20 kb fragment in Hind III digested genomic DNA. These fragments have recently been mapped to the 5' and 3' halves, respectively, of the gene coding for the 68,000 dalton albumin by analyzing genomic clones (May et al., unpublished data).

The third gene analyzed in this paper is the A1 vitellogenin gene of Xenopus which we have isolated from a gene bank and characterized (16,21, 22). Fig. 1B summarizes the relevant features of the structure of this gene. We

detect the A1 vitellogenin gene 8.5 kb and 1.6 kb fragments in Hind III digested genomic DNA using the vitellogenin cDNA clone pXlvc 23 which contains around 60% of the mRNA sequence starting at the 3' end (23). The 2.2 kb and 1.2 kb fragments could hardly be detected and the 0.6 kb and 0.4 kb fragments could not be detected since they contain mainly intron sequences (see Fig. 1B and compare to the data shown in Fig. 3, slot 1).

RESULTS

DNase I sensitivity of defined restriction fragments of the globin, albumin and vitellogenin genes in naked DNA

To test whether different regions of specific genes show similar sensitivity to DNase I in naked DNA, we analyzed the disappearance of defined restriction fragments in purified DNA subjected to various degrees of DNase I digestion. To visualize the extent of digestion, the DNA was analyzed on an agarose gel and Fig. 2A illustrates the separation of naked DNA without (slot 1) and with increasing DNase I digestion (60-285 ng DNase I/mg DNA, slots 2-7). Comparison with the marker DNA showed that DNA digested with the highest

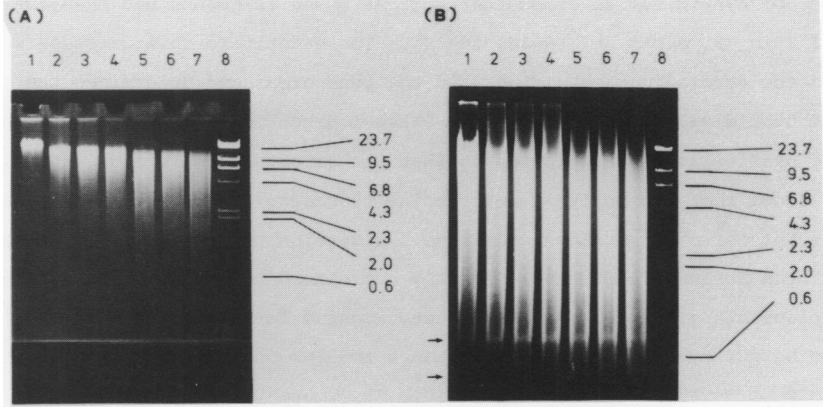

Fig. 2: Length distribution of naked DNA after DNase I digestion alone and after DNase I digestion followed by Hind III digestion

A) Naked DNA was digested with 55, 85, 115, 145, 215 and 285 ng DNase I/mg of DNA (slots 2-7 respectively) and after separation on a 0.8% agarose gel stained with ethidium-bromide. Undigested DNA is shown in slot 1. λDNA digested with Hind III (slot 8) was used as marker and the size of the fragments are indicated in kb.
B) Samples of the DNase I digested DNA shown in Fig. 2A were digested with Hind III, separated on a 1% agarose gel and stained with ethidium-bromide.

enzyme concentration was still longer than 2.3 kb. Because the large DNA fragments (more than 10 kb) are poorly resolved by gel elctrophoresis, we measured the length distribution of the DNA using electron microscopy. From the average length of the molecules we calculated then the mean number of breaks per 10 kb of DNA. The DNA samples shown in Fig. 2A (slots 1-7) had an average number of breaks of 0.4, 1.7, 1.8, 2.3, 2.8, 2.9, and 3.6 per 10 kb of DNA. These DNA preparations were then digested with Hind III and analyzed on an agarose gel. Fig. 2B shows that after Hind III digestion the DNA becomes much shorter and that previous digestion with DNase I (Fig. 2B, compare slot 1 with slots 2-7) results in a decrease in the length of the DNA. Furthermore digestion with Hind III generates two discrete bands (indicated by arrows) which probably represent some repetitive DNA. To detect the amount of defined restriction fragments of the genes of interest the DNA was transferred from these gels to nitrocellulose filters which were then hybridized with the various cloned cDNAs.

Autoradiography of these filters showed the expected 5.8 kb globin, 4.6 kb albumin and the 8.5 and 1.6 kb A1 vitellogenin gene fragments (Fig. 3, slots 1, 8 and 15). In all cases increasing DNase I digestion resulted in a decrease of hybridization demonstrating that these fragments had been cut by DNase I (Fig. 3, slots 2-7, 9-14, 16-19). To quantitate this decrease we scanned the autoradiograms, integrated the peak areas and determined the fraction of hybridization remaining after DNase I digestion. The fraction of remaining hybridization was plotted against the extent of DNase I digestion. Fig. 4 shows that the analyzed restriction fragments do not disappear to the same extent for a given amount of DNase I digestion but rather that the decrease of a defined fragment is roughly correlated to its size. This finding seems plausible since it would be expected that a large fragment is more likely to be cut by DNase I digestion than a smaller one.

Mathematical model to describe DNase I digestion of DNA

To evaluate the data shown in Fig. 4 and to quantitate the disappearance of defined DNA fragments after DNase I digestion we propose the following mathematical model.

If a large number, N, of identical DNA molecules is digested with DNase I, we obtain digestion products whose mean length, observed by electron microscopy, allows calculation of the mean number, b, of breaks per 10 kb of DNA. Let F be a well defined DNA fragment of known length, e.g. a specific re-

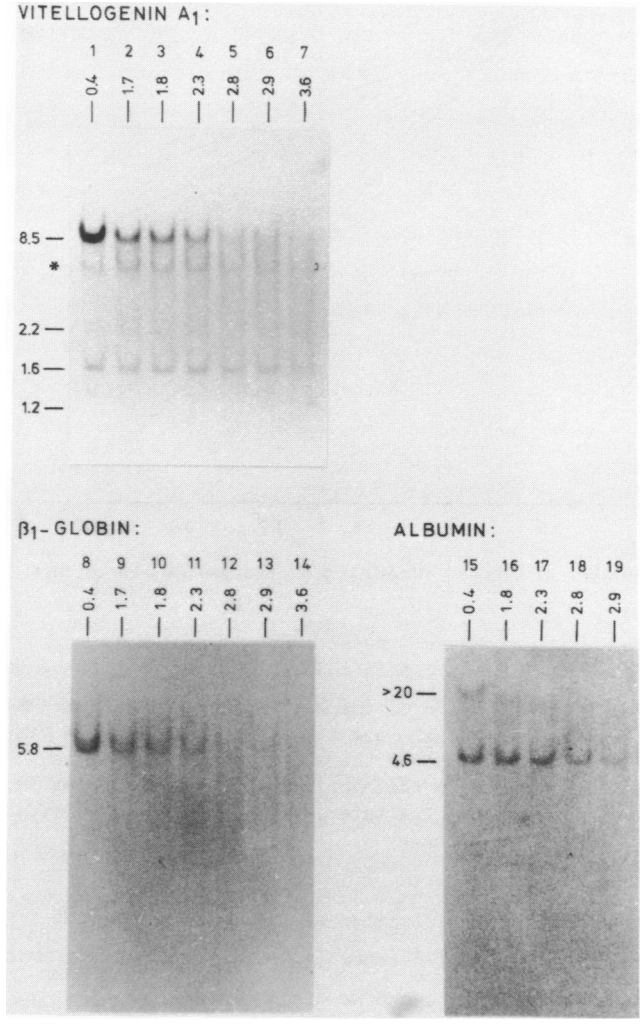

Fig. 3: DNase I sensitivity of the A1 vitellogenin, β_1-globin and albumin genes in naked DNA

Naked DNA was digested with increasing amounts of DNase I as shown in Fig. 2. The DNA was then digested with Hind III, separated on a 1% agarose gel and transferred to nitrocellulose filters. Identical filters were hybridized to the nick translated cloned cDNA sequences specific for the A1 vitellogenin (slots 1-7), the β_1-globin (slots 8-14) and an albumin (slots 15-19) gene. DNA not digested by DNase I is shown in slots 1, 8 and 15 and the size of the expected fragments (see Fig. 1 and text) are indicated in kb. For each DNA sample the number of DNase I introduced breaks per 10 kb of DNA are given underneath the slot number. The asterisk denotes a fragment not present in the genomic clones.

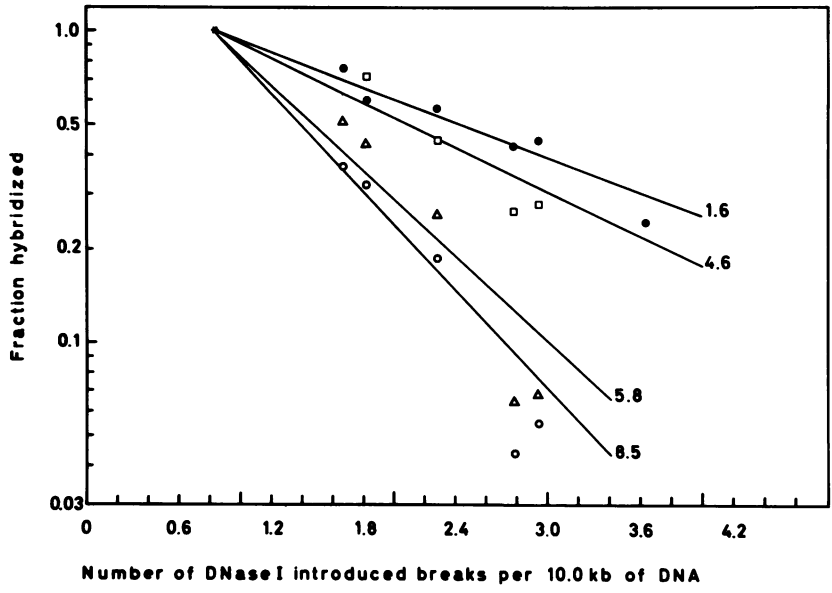

Fig. 4: Quantitation of DNase I sensitivity of restriction fragments of the A1 vitellogenin, β_1-globin and albumin genes in naked DNA

The intensity of hybridization to the 8.5 kb (○) and 1.6 kb (●) A1 vitellogenin, the 5.8 kb (△) β_1-globin and the 4.6 kb (□) albumin fragments were measured by scanning the bands on the autoradiograms (see Fig. 3). The integrated peak area representing the hybridization remaining was related to the extent of DNase I digestion. The asterisk indicates 100% hybridization to undigested DNA used as reference.

striction fragment. By hybridization we measure the fraction $m = \frac{M}{N}$ of the remaining intact fragments of F after digestion by DNase I. Obviously we get decreasing m with increasing b and we are interested in a description of the function $m = m(b)$. Suppose that the DNA is digested in a homogeneous way, i.e. the probability of any one site being cut is always the same. Then the mean number of breaks in a specific fragment is proportional to its length L. Each fragment F contains, therefore, an average number, $b_F = Lb$, of breaks and the total number of breaks in all N fragments F is $B = NLb$. We are interested in the probability that a given fragment, F_o (chosen from the N fragments F), is not cut. If there is only one break within all N fragments F, then F_o has the probability $q = 1 - \frac{1}{N}$ of remaining intact. If there are B breaks in the whole population of F the probability of F_o still remaining uncut is $p = q^B =$

$(1 - \frac{1}{N})^B$. Hence, identifying p with $m(b)$ in the case of this homogeneous model, $m(b) = p = (1 - \frac{1}{N})^B$ and, using $B = NLb$, we get $m(b) = (1 - \frac{1}{N})^{NLb}$ or, asymptotically for large N, $m(b) = e^{-Lb}$ [as $e^x = \lim_{N\to\infty} (1 + \frac{x}{N})^N$, for $x = -1$].

We now introduce a sensitivity factor, s, to correct the assumption that a given fragment F behaves like an average fragment of the same length, thus constructing an inhomogeneous model of DNA cutting. Suppose that a specific fragment of length L is cut like an average fragment of length sL in the homogeneous case. Then the above formula has to be modified to $m = m(b) = e^{-sLb}$. This means, e.g. that F is cut twice as often as an average fragment if $s = 2$, or half as often if $s = 0.5$. We use this sensitivity factor, $s = -\frac{1}{Lb} \ln m$, to describe the behaviour of different fragments subjected to DNase I digestion.

The formula has to be modified for the following two reasons:

1) Due to mechanical effects, DNA suffers a considerable number, b_o, of breaks per 10 kb during extraction.

2) What we actually measure is not $m(b)$, but the quotient $\mu(b) = \frac{m(b)}{m_o}$ where $m_o = e^{-Lb_o}$ is the fraction of F surviving extraction. (Note that by lack of more detailed knowledge we work with the hypothesis that mechanical breaks are distributed homogeneously.)

When naked DNA is digested with DNase I, the DNA molecules are broken by mechanical forces before DNase I treatment. The total number b of breaks must, therefore be divided into DNase I introduced breaks b_{DNase} and b_o as defined above. Obviously $b = b_{DNase} + b_o$ and in the former formula $m(b) = e^{-sLb}$ we have to consider that s applies only to breaks introduced by DNase I, thus giving $m(b) = e^{-L(s(b-b_o)+b_o)}$ where b is now the total of all breaks observed regardless of their origin.

Finally, from the measurements of $\mu = \mu(b) = \frac{m(b)}{m_o} = e^{-Ls(b-b_o)}$ we derive the sensitivity factor $s = \frac{-\ln \mu}{L(b-b_o)}$.

We have used this formula to calculate the sensitivity factors, s, for the defined restriction fragments analyzed in Fig. 4. As Table I demonstrates the s-values are similar within the errors of measurement for a given restriction fragment tested and therefore the hypothesis of the model is justified. When the mean s-values for the different fragments are compared, it can be seen that each fragment has a characteristic sensitivity value. Similar sensitivity values have also been obtained for other vitellogenin gene fragments

Table I Values of the sensitivity factor, s, for different restriction fragments analyzed in naked DNA digested with DNase I

Breaks per 10 kb of DNA	8.5 kb Vitellogenin A_1	5.8 kb β_1 - globin	4.6 kb Albumin	1.6 kb Vitellogenin A_1
1.67	1.41	1.40	n.d.	2.06
1.82	1.37	1.46	0.72	3.25
2.28	1.37	1.62	1.22	2.43
2.78	1.76	2.43	1.49	2.74
2.94	1.23	2.21	1.33	2.45
3.64	n.d.	n.d.	n.d.	3.15
Mean	1.43	1.82	1.19	2.68
SD	± 0.20	± 0.47	± 0.33	± 0.46

n.d. indicates that this measurement was not made.

(24). Since the various sensitivity values differ at most by a factor of 2.5, we conclude that DNase I cuts the analyzed restriction fragments representing the three different genes with similar, but not identical probability in the naked DNA.

Taking the logarithm from the above formula we have plotted the linear functions $log\ \mu = (-log\ e)Ls(b-b_o)$ in Fig. 4 . It can be seen that the graphs obtained fit well to the measurements and therefore the hypothesis of our model is justified.

DNase I sensitivity of the β_1-globin gene in the chromatin of erythrocytes and hepatocytes

We first analyzed whether the adult β_1-globin gene of Xenopus is in a more DNase I sensitive conformation in erythrocytes where this gene has been activated than in hepatocytes where it is never expressed. Nuclei prepared from these two cell types were digested with various concentrations of DNase I to a similar extent as the naked DNA (see Fig. 2A). Considerably more enzyme was required to obtain a similar degree of digestion. Moreover, hepatocyte nuclei needed about three times more enzyme than erythrocyte nuclei to reach the same degree of digestion. The extent of DNase I digestion was quantitated by length measurement under the electron microscope as described in Materials and Methods. The various samples of DNase I digested DNA were then digested

with Hind III, transferred to nitrocellulose filters after electrophoresis and hybridized with the β_1-globin specific cDNA (see Fig. 1A).

The autoradiogram shown in Fig. 5 demonstrates that increasing DNase I digestion leads to a loss of hybridization to the 5.8 kb fragment in Hind III digested DNA from erythrocytes (slots 1-4) whereas it only results in a slight disappearance from similarly digested hepatocyte DNA (slots 5-8).

From several independent experiments the intensity of hybridization to the 5.8 kb fragment was quantitated by scanning the bands on the autoradiograms, integrating the peak areas and the fraction of hybridization remaining after various DNase I digestion plotted against the extent of DNase I digestion. Fig. 6A shows that the hybridization to the 5.8 kb fragment decreases to a greater extent in DNA from erythrocyte than hepatocyte nuclei showing that the adult β_1-globin gene is more sensitive to DNase I in the chromatin of erythrocytes.

To quantitate the difference in the sensitivity of the 5.8 kb fragment in erythrocyte and hepatocyte nuclei to DNase I we applied the same mathematical model as proposed for the digestion of naked DNA. In this case, DNA extraction is made after DNase I digestion of the nuclei and we have assumed that, since the breakdown products after DNase I digestion are relatively small, they will not be degraded further during DNA extraction. Our formula for the sensitivity factor s is therefore derived from the equation $\mu = \mu(b) = \frac{m(b)}{m_o} = \frac{e^{-sLb}}{e^{-Lb_o}} = e^{-L(sb-b_o)}$ to $s = \frac{1}{b}\left(b_o - \frac{1}{L} \ln \mu\right)$, where all breaks

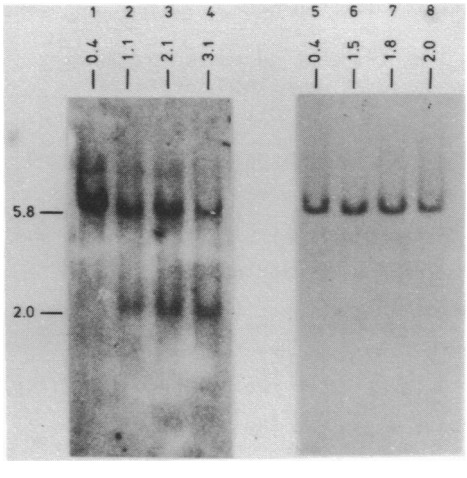

Fig. 5: Sensitivity of the β_1-globin gene to DNase I in erythrocyte and hepatocyte chromatin

Nuclei from erythrocytes (slots 2-4) and hepatocytes (slots 6-8) were treated with increasing amounts of DNase I. The DNA was purified, digested with Hind III, separated on a 1% agarose gel and transferred to nitrocellulose filters. Such filters were hybridized to the nick translated cloned β_1-globin cDNA. DNA not digested by DNase I is shown in slots 1 and 5. To clearly visualize the DNase I introduced fragment of 2 kb the autoradiogram of slots 1-4 has been overexposed.

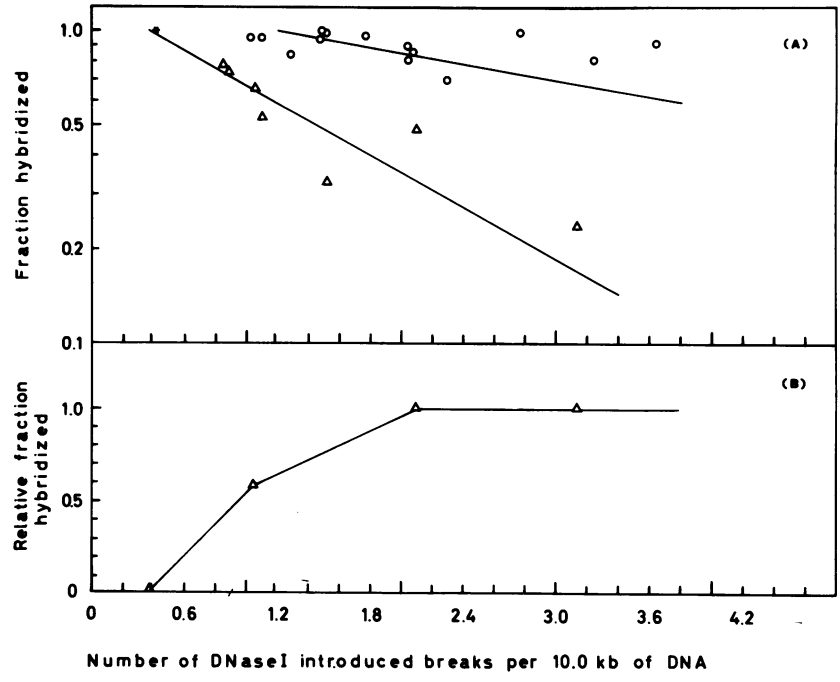

Fig. 6: Quantitation of the DNase I sensitivity of the 5.8 kb Hind III fragment of the β_1-globin gene in chromatin and the generation of a new 2 kb fragment by DNase I

A) The intensity of the hybridization to the 5.8 kb β_1-globin fragment was measured as described in the legend to Fig. 4. The digestion of eryhtrocyte (△) and hepatocyte (○) nuclei is shown from several experiments. The results include experiments with chromatin of untreated and estrogen treated males and since they have no altered DNase I sensitivity for the gene analyzed (24) they have the same symbols.
B) The intensity of hybridization to the 2 kb fragment generated by DNase I digestion of erythrocyte chromatin was measured as described in Fig. 4.

b result from DNase I digestion, while b_o is the number of breaks introduced by extraction in undigested DNA.

Using this equation we can calculate the sensitivity factor, s, for the adult β_1-globin gene in the chromatin of erythrocytes and hepatocytes. The s-values given in Table II show that the sensitivity factor is about 3.5 fold increased in DNA prepared from erythrocyte than from hepatocyte nuclei. This difference is significant at the P = 0.975 level using the Wilcoxon test (25). By taking the logarithm of the above formula we obtain the linear function $log\ \mu = (-log\ e)L(sb+b_o)$, which was used to plot the functions for the measu-

rements shown in Fig. 6A. These graphs fit well to the measurements made and justify the hypothesis used to construct our model.

The experiment shown in Figure 5 also revealed that a new fragment of 2 kb appeared after DNase I digestion of erythrocyte nuclei followed by Hind III digestion of the DNA (slots 2-4). This fragment was never detected after DNase I digestion of hepatocyte nuclei (Fig. 5, slots 5-8) or naked DNA (Fig. 3, slots 8-14) even after overexposure of the hybridized filters. We presumed that the 2 kb fragment is generated as a result of digestion by Hind III at a site close to the 3' end of the gene and by DNase I at a hypersensitive region close to the 5' end. The 5.8 kb Hind III fragment was therefore cut into a 2 kb fragment which can be detected and a 3.8 kb fragment lacking hybridizable DNA sequences (Fig. 1A). This interpretation was shown to be correct by analyzing the hybridization of Bam HI restricted DNA with the β_1-globin cDNA clone. DNA prepared from nuclei which had not been digested with DNase I revealed the expected 10 and 2 kb fragments (see Fig. 1A for map), whereas DNase I digestion of nuclei resulted in an additional 0.5 kb fragment generated by the expected DNase I cut within the 10 kb fragment (data not shown). This finding therefore located the hypersensitive region close to the 5' end of the β_1-globin gene. Quantitation of the hybridization to the 2 kb fragment generated by DNase I (Fig. 5, slots 2-4) revealed that the increasing intensity of hybridization to this new fragment with increasing DNase I digestion (Fig. 6B) correlates well with the decrease of hybridization to the 5.8 kb fragment (see Fig. 6A).

In our experiments the DNase I hypersensitive region of the β_1-globin gene could only be detected in erythrocytes but not in hepatocytes. In fact, all available data on DNase I hypersensitive regions suggest that such sites are either present in cells actively expressing the gene, e.g. the conalbumin gene of the chick oviduct (9), or in cells which have previously expressed the gene, e.g. the globin gene of erythrocytes (7), or in cells where the gene can be activated, e.g. the heat shock genes of Drosophila (10). These findings may suggest that these regions represent a specific chromatin conformation reflecting the ability of a tissue to express a particular gene.

DNase I sensitivity of the albumin gene in chromatin of hepatocytes and erythrocytes

To extend our findings on the chromatin conformation of the adult β_1-globin gene, we analyzed the albumin gene which is constitutively expressed in

hepatocytes but is not expressed in erythrocytes. The structure of the albumin gene analyzed is described in the Materials and Methods. Hind III digested DNA prepared from DNase I digested erythrocyte and hepatocyte nuclei was electrophoresed on agarose gels, transferred to nitrocellulose filters and hybridized with nick translated cloned albumin cDNA.

Figure 7 shows an autoradiogram of such a filter of erythrocyte (slots 1 -4) and hepatocyte DNA (slots 5-7). The expected Hind III fragments of >20 kb and 4.6 kb could be detected (slots 1 and 5). Since the fragment >20 kb is too large to be analyzed in our digestion experiments, we only analyzed the 4.6 kb fragment. Fig. 7 shows that progressive DNase I treatment of erythrocyte nuclei results in only a small reduction of hybridization to the 4.6 kb fragment (slots 1-4) whereas DNase I digestion of hepatocyte nuclei results in a much more pronounced loss of hybridization (slots 5-7). These decreases of hybridization observed in several independent experiments were quantitated and the sensitivity factors, s, calculated from these data are shown in Table II. The mean s-values for erythrocytes and hepatocytes differ significantly (25) and indicate that the albumin gene is about twice as sensitive in hepatocytes where it is actively expressed than in erythrocytes where it is not expressed. This difference in DNase I sensitivity is illustrated by Fig. 8 where the graphs, using the linear function, clearly differ.

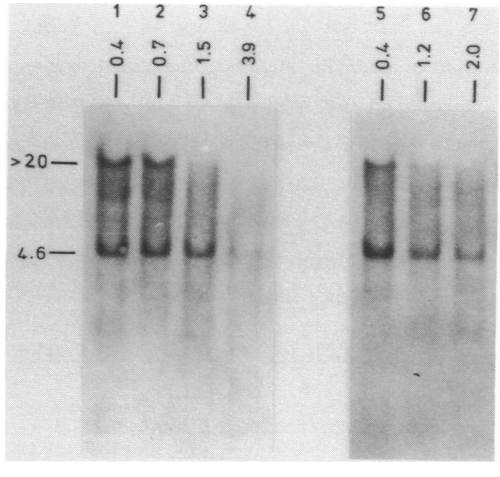

Fig. 7: DNase I sensitivity of an albumin gene in erythrocyte and hepatocyte chromatin

Nuclei from erythrocytes (slots 2 -4) and hepatocytes (slots 6-7) were digested with increasing amounts of DNase I. Nitrocellulose filters prepared as described for Fig. 5 were hybridized to the nick translated cloned albumin cDNA. DNA from nuclei not digested with DNase I is shown in slots 1 and 5.

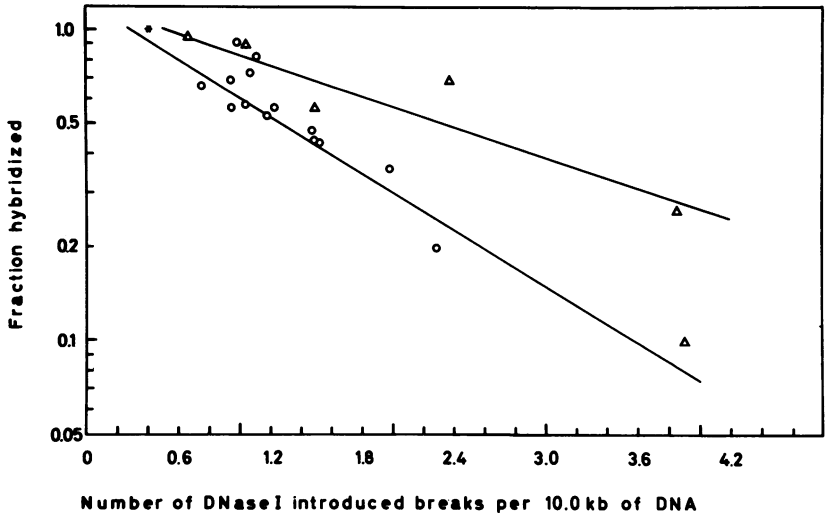

Fig. 8: Quantitation of the DNase I sensitivity of the 4.6 kb Hind III fragment of the albumin gene in chromatin

The intensity of hybridization to the 4.6 kb fragment was measured as described in the legend for Fig. 4. The DNase I sensitivity for erythrocyte (△) and hepatocyte chromatin (○) is shown for several experiments.

DNase I sensitivity of the A1 vitellogenin gene in chromatin from erythrocytes and hepatocytes from male Xenopus

The previous experiments have clearly shown that the albumin gene is in a more DNase I sensitive chromatin conformation in hepatocytes than in erythrocytes, whereas the β_1-globin gene is in a more DNase I sensitive chromatin conformation in eryhtrocytes than in hepatocytes. It was therefore of interest to analyze the DNase I sensitivity of the A1 vitellogenin gene which is normally inactive in erythrocytes and male hepatocytes but which can be activated in hepatocytes by estrogen (reviewed in 16).

Hind III digested DNA prepared from DNase I digested nuclei of erythrocytes and hepatocytes was electrophoresed on agarose gels, transferred to nitrocellulose filters and hybridized with nick translated cloned vitellogenin cDNA (see Fig. 1B). Autoradiograms of such filters show , Fig. 9, that the cloned cDNA hybridized mainly to the 8.5 and 1.6 kb fragments. The effect of DNase I digestion on the reduction of hybridization to these two fragments derived from erythrocyte (Fig. 9, slots 1-4) or hepatocyte (Fig. 9, slots 5-8)

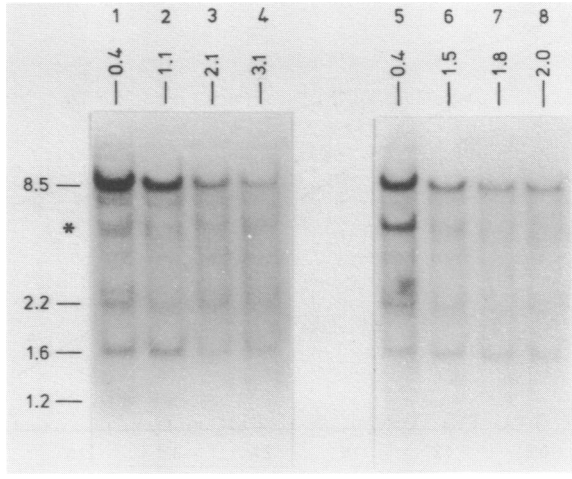

Fig. 9: DNase I sensitivity of the A1 vitellogenin gene in erythrocyte and hepatocyte chromatin

Nitrocellulose filters prepared as described for Fig. 5 were made from DNase I digested nuclei of erythrocytes (slots 2-4) and hepatocytes (slots 6-8) and hybridized to the nick translated cloned A1 vitellogenin cDNA. DNA from nuclei not digested by DNase I is shown in slots 1 and 5. For the asterisk see Fig. 3.

nuclei was then quantitated and is shown in Fig. 10. In contrast to the result with the globin or the albumin gene, the effect of DNase I digestion on the reduction of hybridization was the same in both, erythrocyte and hepatocyte nuclei.

The calculated sensitivity factors s for both fragments, given in Table II, are not significantly different (25) between erythrocytes and hepatocytes. We therefore conclude that the A1 vitellogenin gene which is inactive in both, erythrocytes and hepatocytes of males, has a similar chromatin conformation in both cell types.

DISCUSSION

The sensitivity factor s as a useful parameter to characterize the DNase I sensitivity of a DNA fragment

Our analysis of the DNase I sensitivity of defined restriction fragments of globin, albumin and vitellogenin genes in naked DNA and chromatin made it necessary to propose a mathematical model to compare the cutting of defined fragments with increasing DNase I digestion. The definition of a sensitivity

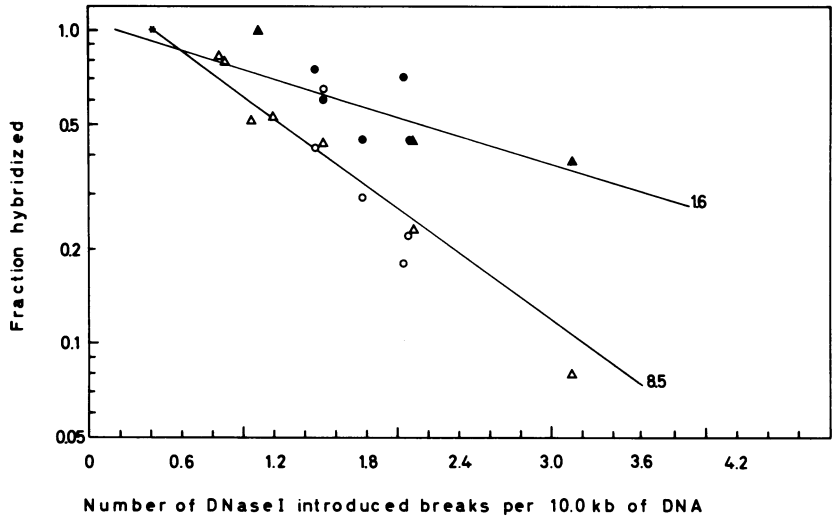

Fig. 10: Quantitation of the DNase I sensitivity of the 8.5 and 1.6 kb Hind III fragments of the A1 vitellogenin gene in chromatin

The intensity of hybridization to the 8.5 and 1.6 kb fragments was determined as described in the legend to Fig. 4 and related to the extent of DNase I digestion. The DNase I sensitivity is shown for the 8.5 kb fragment (erythrocyte △ and hepatocyte ○ chromatin) and the 1.6 kb fragment (erythrocyte ▲ and hepatocyte ● chromatin) from several experiments.

factor, *s*, proved to be extremely useful for comparing data on different fragments as well as on the same fragment in different cell types and in different states of activity. Furthermore, statistical analysis of the data became more feasible. Other authors in previous studies failed to analyze their data in such a quantitative way.

By definition the sensitivity factor *s* is independent of the length of the analyzed fragment. Therefore it is also a tool to compare fragments of different size. Each DNA-fragment has its own characteristic *s*-value, e.g. our analysis of 8 different restriction fragments (24) revealed values between *1.2* and *2.7*. There is no obvious reason why all these values should be larger than the value $s = 1$ of the average fragment. It should be considered, however, that there are more than 100,000 different fragments one could analyze in the genome of Xenopus and that our sample may not be representative.

Nevertheless our analysis of the DNase I sensitivity of these three genes in chromatin showed that the sensitivity factor, *s*, has a characteristic

value for a given fragment in a given cell type and ranges from *0.3* to *2.5* suggesting that specific sequences can be more or less sensitive to DNase I than an average fragment in chromatin. This variation was also evident from analysis of the A1 and A2 vitellogenin genes (24).

Active globin and albumin genes are more sensitive to DNase I digestion

Our experiments clearly demonstrate that the β_1-globin gene of Xenopus is more accessible to DNase I in erythrocytes than in hepatocytes. This finding agrees with previous reports on the chicken and the sheep (reviewed in 2) globin genes. In these experiments the DNase I sensitivity has been investigated by measuring the residual globin sequences still hybridizing with globin cDNA after extensive DNase I digestion. Applying the same approach which we have used, recent experiments made on chicken chromatin have demonstrated that defined globin restriction fragments were preferentially digested by DNase I in nuclei from erythrocytes compared to non-erythropoietic tissues (5, 6, 7). We have also analyzed the DNase I sensitivity of an albumin gene as this gene is expressed in hepatocytes but not in erythrocytes and found that the albumin gene is more sensitive to DNase I in chromatin of the tissue in which it is expressed. These findings strengthen the hypothesis that the chromatin conformation of any gene has an increased DNase I sensitivity in the tissue where this gene is expressed actively.

Different DNase I sensitivity of the inactive globin gene compared to the inactive albumin and vitellogenin genes

Comparison of the sensitivity factors s (see Table II) of the three genes in chromatin of tissues in which they are not expressed suggests that the globin gene is less sensitive to DNase I ($s = 0.3$) than an average sequence ($s = 1$), whereas the albumin and vitellogenin genes behave more like average DNA sequences. [The small 1.6 kb fragment of the A1 vitellogenin gene seems to behave slightly different ($s = 1.65 - 2.51$) but this fragment also has the most aberrant sensitivity factor in naked DNA].

We suggest that in chromatin the inactive β_1-globin gene is about threefold less accessible to DNase I than the inactive albumin and vitellogenin genes. Since in naked DNA the globin fragment is more sensitive to DNase I than the albumin and vitellogenin fragments we conclude the the preferential resistance of the globin fragment is due to some structure of the chromatin and does not reflect a high DNase I resistance of this DNA sequence itself. This

Table II Values of the sensitivity factor, s, for the different restriction fragments analyzed in chromatin of erythrocytes and hepatocytes digested with DNase I

Fragment analyzed	Source of chromatin		Naked DNA
	Erythrocytes	Hepatocytes	
β_1 - globin 5.8 kb	1.10 ± 0.26 (7)	0.34 ± 0.12 (14)	1.82 ± 0.47 (5)[a]
Albumin 4.6 kb	0.82 ± 0.35 (6)	1.52 ± 0.54 (16)	1.19 ± 0.33 (4)[a]
Vitellogenin A_1 8.5 kb	0.96 ± 0.15 (7)	0.98 ± 0.22 (5)	1.43 ± 0.20 (5)[a]
1.6 kb	1.65 ± 1.14 (3)	2.51 ± 0.62 (5)	2.68 ± 0.46 (6)[a]

The mean sensitivity values and the corresponding standard deviations are listed and the number of digestion points analyzed (in brakkets) are given.

[a] taken from data shown in Table I.

different chromatin conformation of the globin gene in chromatin could be due to the fact that the 5.8 kb fragment contains a high proportion of flanking sequence which might be in a different conformation in the chromatin . Such an argument, however, could also be made for the A1 and A2 vitellogenin gene fragments which also contain a high proportion of flanking sequence but which do not show a preferential DNase I resistance (24).

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