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Estrogen induces tissue specific changes in the chromatin conformation of the vitellogenin genes in Xenopus

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ABSTRACT

Nuclei from male Xenopus liver were digested extensively with DNase I and the residual amount of the four vitellogenin genes measured by hybridization with a moderate excess of vitellogenin cDNA. The saturation value was about twofold lower in chromatin isolated from liver cells of estrogen treated than from untreated males or from erythrocytes. Analyzing the disappearance of several defined restriction fragments specific for the A1 and A2 vitellogenin genes, after limited digestion with DNase I, suggested that the entire A1 and A2 vitellogenin genes are about twofold more sensitive to DNase I in chromatin of hepatocytes isolated from estrogen treated than from untreated males. Using the same assay no change in the DNase I sensitivity of the two vitellogenin genes in erythrocyte chromatin was observed. Analysis of the β_1-globin and an albumin gene demonstrated that the DNase I sensitivity of these genes in both cell types is not altered by estrogen. All these data indicate that estrogen stimulation results in an increased DNase I sensitivity specific for the vitellogenin genes in hepatocytes.

INTRODUCTION

The synthesis of vitellogenin, the precursor of the major yolk proteins, is controlled by estrogen in livers of oviparous vertebrates (reviewed in 2-5). The level of vitellogenin synthesis is controlled by the level of its mRNA and estrogen leads to a dramatic accumulation of the vitellogenin mRNA (6, 7). In Xenopus laevis, vitellogenin is encoded in a small family of four related genes which is composed of two pairs (A1 and A2; B1 and B2) of more closely related genes (8-10). Analysis of the structural organization of the A1 and A2 vitellogenin genes has demonstrated that these genes have a total length of 21 and 16 kb, respectively, and are interrupted by 33 introns at homologous positions (11).

The synthesis of all four vitellogenin mRNAs can be induced by estrogen even in livers of male Xenopus which normally do not produce vitellogenin or any transcripts of the vitellogenin genes (5). It was of great interest, the-

refore, to investigate whether estrogen induces some change in the chromatin conformation of the vitellogenin genes. One approach which has successfully been used to analyze the conformation of specific genes in chromatin is digestion with DNase I which preferentially degrades transcriptionally active gene sequences to non hybridizable fragments (reviewed in 12). A refinement of this technique has recently been described in which digestion of defined restriction fragments of specific genes is measured following DNase I treatment of chromatin (13-19).

In this report we have investigated the chromatin conformation of the Xenopus vitellogenin genes in their inactive and active state in hepatocytes, and in their inactive state in erythrocytes, using both the approaches described above. These experiments showed that estrogen treatment increases the DNase I sensitivity of the vitellogenin genes only in nuclei of hepatocytes and we suggest that this is the result of an altered configuration of these genes in the chromatin.

MATERIALS AND METHODS

Cell preparation

Livers of untreated or estrogen treated (6) male Xenopus laevis were perfused in situ to remove erythrocytes and then disaggregated with collagenase (19). All the solutions used during the perfusion of estrogen treated male livers contained 2 x 10^{-6} M 17-β-estradiol. The purity of liver cell suspensions prepared in such a way was determined in a haemocytometer after staining the nuclei with 0.1% cristal violet in 0.1 M citric acid. The erythrocyte and liver cell nuclei could easily be distinguished by their size, shape and heterochromatin content.

For experiments in which the digestion of the vitellogenin genes was measured by hybridization in solution, nuclei were prepared directly from such liver cell suspensions. In experiments measuring the disappearance of defined restriction fragments, hepatocytes were first purified on Metrizamide gradients as described (20).

DNase I digestion

For experiments using solution hybridization, nuclei were digested extensively so that 10-15% of the DNA became acid soluble (8.6 µg DNase I/mg DNA in RSB) and the TCA solubility was monitored (21). In experiments measuring the disappearance of defined restriction fragments, nuclei were prepared

and digested as described (19).

Determination of DNA length

To determine the length distribution of the DNA after extensive DNase I digestion the DNA was denatured with glyoxal and electrophoresed on a 2.5% acrylamide - 0.5% agarose composite gel (22). This method resolves fragments smaller than 2 kb. The DNA length after less extensive DNase I digestion was accurately determined by measuring the length of the molecules under the electron microscope (19). Their mean length provides a measure of the extent of DNase I digestion, defined as number of breaks per 10 kb of DNA.

Solution hybridization

DNA purified from DNase I digested liver cell or erythrocyte nuclei was hybridized with a moderate excess (23) of ^{3}H-cDNA, synthesized from total vitellogenin mRNA (6). 0.1 ng vitellogenin ^{3}H-cDNA (= 1,000 cpm) and 16.5 µg DNA were hybridized in 10 µl, in triplicate, for various lengths of time and the hybrids formed detected by S_1 nuclease digestion (24). As controls DNA from Xenopus erythrocytes and from E.coli (Sigma) were also hybridized. This DNA was fragmented to the same size as the DNA from DNase I digested nuclei. 1 mg/ml of DNA in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 20 mM NaCl, was fragmented twice in a French Press at 40 kp/cm^{2}, treated with RNase and pronase, extracted with phenol and its length determined as described above. Contamination of the vitellogenin ^{3}H-cDNA with reverse transcripts of rRNA was estimated from its hybridization to a plasmid containing rRNA sequences (0.5% of the input cDNA). Self-annealing of the vitellogenin ^{3}H-cDNA was measured from its hybridization in the presence of E.coli DNA (2% of the input cDNA). The saturation values given in the figures are corrected accordingly. Fragmented erythrocyte DNA hybridized to 15% of the vitellogenin ^{3}H-cDNA at saturation showing that the ^{3}H-cDNA was present in a sevenfold excess in the reaction mixture. This saturation value was defined as 100% vitellogenin sequences hybridized.

Filter hybridization

DNA was purified from nuclei after limited DNase I digestion, restricted with Hind III (Biolab) or Eco RI (Boehringer) and electrophoresed on 1% agarose gels. It was then transferred to nitrocellulose filters and hybridized with nick translated cloned probes (19).

Structures of the A1 and A2 vitellogenin genes used for the analysis of their DNase I sensitivity

Fig. 1 summarizes the structures of the A1 and A2 vitellogenin genes. The cloned cDNA, pXlvc 23 (8) specific for the A1 vitellogenin gene hybridizes, in Hind III digested genomic DNA, to two internal fragments of the gene which are 8.5 and 1.6 kb long. Other Hind III fragments of the A1 gene cove-

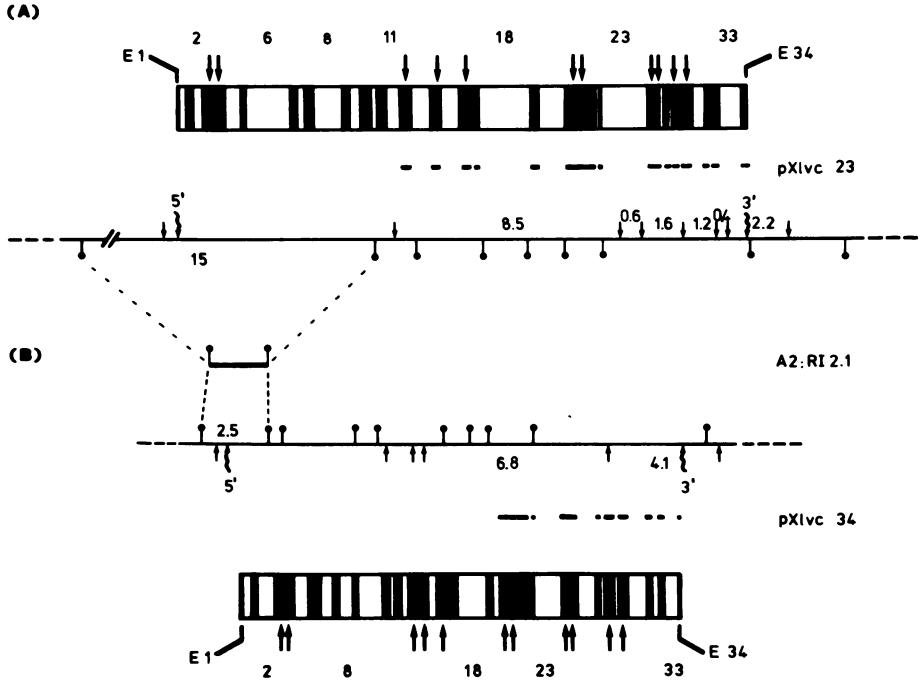

Fig. 1: Structure of the A1 and A2 vitellogenin genes

A) The exon-intron map of the A1 vitellogenin gene is given at the top (11). Large introns are numbered, small introns are indicated by an arrow and the first and last exon indicated by E1 and E34, respectively. Exon sequences contained in the cDNA clone pXlvc 23 (8) are underlined. The restriction sites for Eco RI (⚲) and Hind III (⚲) are aligned to the exon-intron map. Restriction fragments analyzed in this report are designated with their size in kb. The exact size of the 15 kb Eco RI fragment is not known, since the 5' end Eco RI site lies outside the cloned region. The presumed 5' and 3' ends are indicated with an arrow.
B) The exon-intron map of the A2 vitellogenin gene is also aligned to the restriction sites (11). Exon sequences contained in the cDNA clone pXlvc 34 are marked with a line. The position of the genomic subclone A2:RI 2.1 is indicated and the broken line traces the Eco RI restriciton fragments hybridizing with the subclone in the A1 and A2 vitellogenin genes.

red by pXlvc 23 were hardly detected as these fragments contain mostly if not exclusively intron sequences (19). The cloned cDNA, pXlvc 34 (8) specific for the A2 vitellogenin gene hybridizes to the 6.8 kb internal and 4.1 kb 3' end Hind III fragments of the A2 vitellogenin gene. The 4.1 kb fragment showed only weak hybridization since about half of this fragment represents 3' flanking region.

As our existing cDNA clones did not correspond to the 5' ends of the A1 and A2 vitellogenin genes, it was necessary to construct new probes to analyze their 5' ends. Because there are numerous repetitive DNA sequences whithin the introns and the flanking regions of the A1 and A2 vitellogenin genes (5, 25), the genomic clones cannot be used as hybridization probes specific for these two vitellogenin genes. Subclones containing only single copy sequences were constructed (Gerber-Huber, unpublished data), therefore, from the A2 vitellogenin genomic clone λXlv 128 (11) and a subclone, A2:RI 2.1, containing the 2.1 kb Eco RI fragment, corresponding to the 5' end of λXlv 128, was isolated as a probe for the 2.5 kb Eco RI fragment at the 5' end of the A2 vitellogenin gene (see Fig. 1). Under our standard hybridization conditions, exons of the A1 and A2 vitellogenin genes cross hybridize (8); this subclone was therefore also used as a probe for the 15 kb Eco RI fragment at the 5' end of the A1 vitellogenin gene (see Figs. 1 and 4).

RESULTS

Sensitivity to extensive DNase I digestion of the vitellogenin genes in liver chromatin

As a first approach the sensitivity of all four vitellogenin genes to extensive DNase I digestion was studied. Liver cell and erythrocyte nuclei were digested with DNase I to about 10-15% TCA-solubility (2.5-3.5 min incubation with DNase I for liver cell nuclei and 3.5-4.5 min incubation for erythrocyte nuclei). The extent of digestion ranged from 8.5-17.6% TCA-solubility (see Table I). The length of the DNA after DNase I digestion was analyzed on acrylamide-agarose composite gels. As shown in Fig. 2 the DNA from nuclei of erythrocytes and liver cells of untreated and estrogen treated animals as well as the fragmented erythrocyte and E.coli DNA all had an average size of 100 to 700 nucleotides. The DNA could therefore be directly compared in hybridization experiments. The amount of vitellogenin genes in the chromatin preparations was quantitated by hybridization with a moderate excess of vitel-

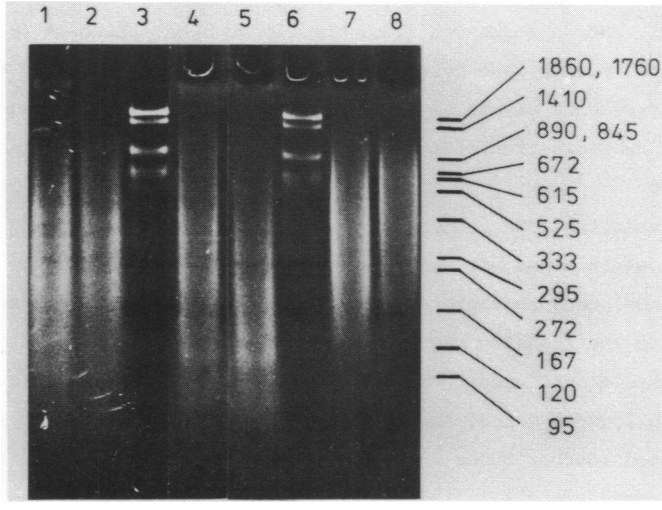

Fig. 2: Length distribution of DNA from extensively DNase I digested nuclei

DNA was isolated from nuclei digested extensively with DNase I or fragmented in a French Press, denatured, separated on an acrylamide-agarose composite gel and stained with acridine orange. Erythrocyte nuclei from untreated (slot 1) and estrogen treated (slot 2) males were digested to 9.1 and 9.8% TCA-solubility respectively. Liver cell nuclei from untreated (slot 4) and estrogen treated (slot 5) males were digested to 12.6 and 10% TCA-solubility respectively. Fragmented DNA of Xenopus erythrocytes (slot 7) and E.coli (slot 8) are shown. PM2 DNA digested with Hae III was used as marker (slots 3 and 6) and the sizes of the fragments are indicated in nucleotides.

logenin cDNA synthesized on all four vitellogenin mRNAs which are known to be expressed to a similar extent (8, 9). Representative saturation curves are compared to the control curves obtained with fragmented erythrocyte DNA in Fig. 3 and, to demonstrate their reproducibility, the saturation values from different experiments are shown in Table I. The saturation curves for DNA from liver cells and erythrocytes of untreated males are the same as for control DNA (Fig. 3B). After three days of estrogen treatment, the saturation values of liver cell DNA from male Xenopus were reduced to 50% while those for erythrocyte DNA remained the same as for control DNA (Fig. 3A).

These results imply that the vitellogenin genes are more sensitive to DNase I digestion in chromatin from liver cells of estrogen treated males than in chromatin from liver cells of untreated males or in erythrocyte chromatin. Thus, estrogen induced increase in DNase I sensitivity of the vitello-

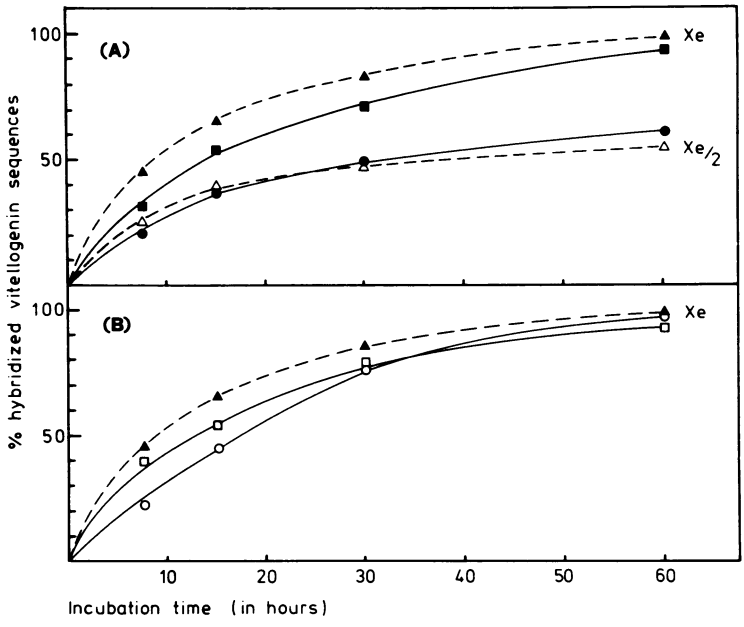

Fig. 3: Quantitation of the remaining vitellogenin genes, after extensive DNase I digestion of the nuclei, by hybridization of the DNA to a moderate excess of vitellogenin ^{3}H-cDNA

DNA was isolated from DNase I digested nuclei and hybridized to a moderate excess of vitellogenin ^{3}H-cDNA. The saturation curves of liver cell (●) and erythrocyte (■) DNA from estrogen treated males (Fig. 3A) and of liver cell (○) and erythrocyte (□) DNA from untreated males (Fig. 3B) are shown. As a standard fragmented Xenopus erythrocyte DNA (▲) was hybridized under identical conditions to vitellogenin ^{3}H-cDNA (Xe). A second standard reaction was carried out using fragmented Xenopus DNA diluted by a factor of two with fragmented E.coli DNA (△, Xe/2).

genin genes is observed in chromatin from liver cells, in which vitellogenin synthesis is induced by estrogen, but not in erythrocytes, which do not synthesize vitellogenin.

Estrogen induced increase in DNase I sensitivity in chromatin at the 5' ends of the A1 and A2 vitellogenin genes

To extend the findings described in the previous section we characterized the DNase I sensitivity of two of the four vitellogenin genes in detail. We have analyzed the disappearance of defined gene fragments after limited digestion of nuclei with DNase I. This approach was first developed by Wu et

Table I Relationship between cell type, extent of DNase I digestion of chromatin and abundance of hybridizable vitellogenin sequences in chromatin

Source of chromatin		Net content of corresponding celltype (in %)	Extent of DNase I digestion (in %)	Hybr. vitellogenin sequences (in %)
Erythrocytes untreated animals	1	1oo	8.5	91.9
	2	1oo	1o.o	92.3
	3	1oo	9.2	92.2
Erythrocytes estrogen treated animals (3 days)	1	1oo	15.8	95.2
	2	1oo	13.8	92.6
	3	1oo	9.5	83.o
	4	1oo	8.9	88.2
Liver untreated animals	1	7o	17.6	91.7
	2	68	15.6	87.6
	3	78	12.6	1oo.o
	4	7o	8.9	86.6
Liver estrogen treated animals (3 days)	1	72	1o.o	49.4
	2	73	9.3	61.9
	3	72	13.1	62.3

al. (13, 14) and has since been used by several groups (15-19). Since much less DNA is required in this method, we were able to purify the hepatocytes on Metrizamide gradients (19). Such hepatocyte preparations are at least 95% pure (20).

To analyze whether estrogen treatment affects DNase I sensitivity of the 5' ends of the A1 and A2 vitellogenin genes, nuclei isolated from purified hepatocytes of untreated or estrogen treated males were digested with DNase I. DNA was then extracted from nuclei treated with various concentrations of DNase I and restricted to completion with Eco RI. The digested DNA was transferred to nitrocellulose filters. Such filters were hybridized with the nick translated genomic subclone, A2:RI 2.1 which hybridizes to fragments at the 5' ends of the A1 and A2 vitellogenin genes (compare Fig. 1). The autoradiogram shown in Fig. 4 illustrates that when hepatocyte DNA is isolated from untreated animals, both the 15 kb fragment of the A1 and the 2.5 kb fragment of the A2 vitellogenin gene disappear with increasing DNase I digestion (slots 2-5). But these fragments seem to disappear to a greater extent when the hepatocyte DNA is isolated from estrogen treated animals (slots 7-

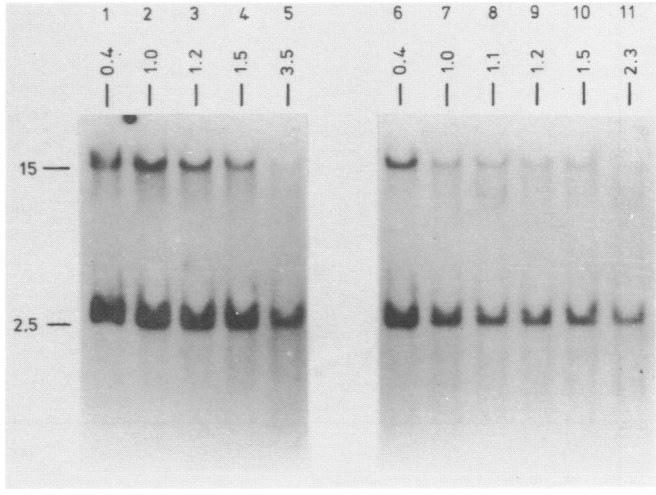

Fig. 4: Sensitivity of the 5' ends of the A1 and A2 vitellogenin genes to DNase I in hepatocyte chromatin of untreated and estrogen treated males

Nuclei from hepatocytes of untreated (slots 2-5) and estrogen treated (slots 7-11) males were digested with increasing amounts of DNase I. The purified DNA was further digested with Eco RI, separated on a 1% agarose gel and transferred to nitrocellulose filters. The filters were then hybridized to the nick translated genomic subclone A2:RI 2.1 . DNA not digested with DNase I is shown in slots 1 and 6. For each DNA sample the measured number of DNase I introduced breaks per 10 kb of DNA is given underneath the slot number.

11). As a comparison we have also analyzed the DNase I sensitivity of these gene fragments in the chromatin of erythrocytes where these genes are not expressed (autoradiogram not shown).

To quantitate these observations we scanned the autoradiograms from several independent experiments and compared these values to the value obtained with DNA extracted from nuclei which had not been digested with DNase I. The fraction which continued to hybridize was calculated and plotted against the extent of DNase I digestion, measured by electron microscopy as the number of breaks per 10 kb of DNA (Fig. 5).

The sensitivity factors s of the two restriction fragments from the 5' ends of these two vitellogenin genes were calculated and are listed in Table II (for a definition of the sensitivity factor s see 19). Comparing the sensitivity factor s obtained with hepatocyte chromatin of untreated males and erythrocyte chromatin we observed that the 15 kb A1 vitellogenin gene fragment is

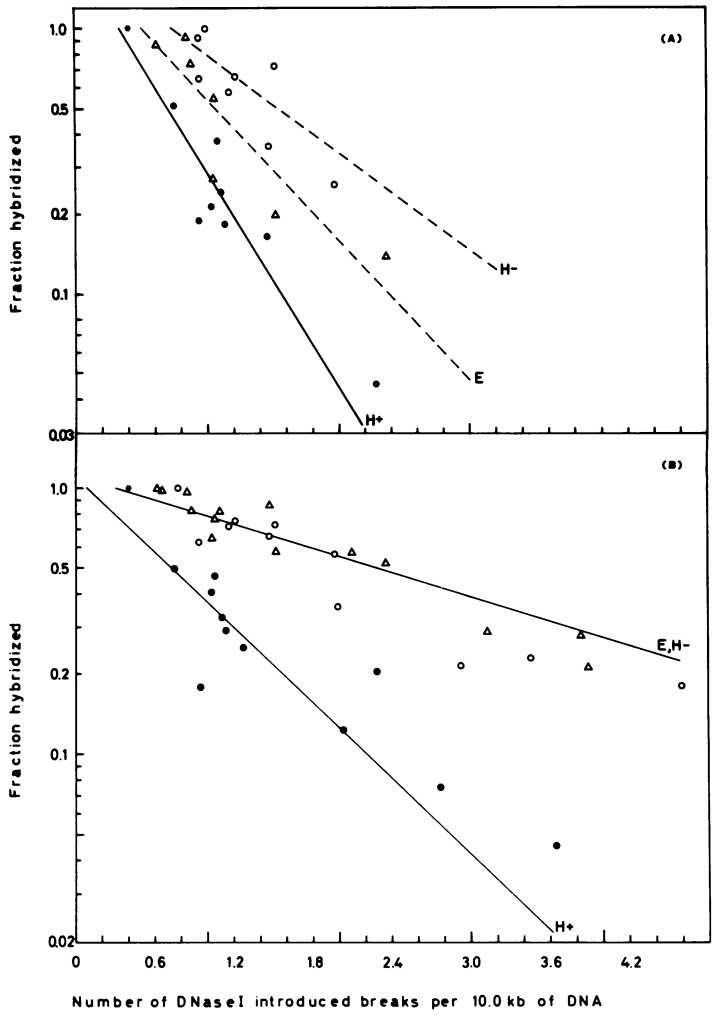

Fig. 5: Quantitation of the DNase I sensitivity of the 5' end fragments of the A1 and A2 vitellogenin genes

The intensity of hybridization to the 15 kb Eco RI fragment of the A1 (Fig. 5A) and to the 2.5 kb Eco RI fragment of the A2 (Fig. 5B) vitellogenin gene were measured and correlated to the extent of DNase I digestion. The asterisk indicates hybridization to DNA without preceding DNase I digestion used as reference. The data shown are taken from several experiments using hepatocytes of untreated (○) and estrogen treated (●) males or male erythrocytes (△). The graphs in Fig. 5A were plotted using the linear function describing DNase I sensitivity of chromatin from untreated (H-) and estrogen treated (H+) hepatocytes and from erythrocytes (E). In Fig. 5B measurements made on erythrocytes and on untreated hepatocytes are combined for plotting the linear function (E, H-) since they do not differ significantly.

significantly more DNase I sensitive in erythrocytes than in hepatocytes of untreated males (Wilcoxon test, P =0.975 level; 26), whereas the DNase I sensitivity of the 2.5 kb A2 vitellogenin gene fragment is not significantly different in these two cell types.

Statistical analysis (26) of the sensitivity factors *s* for hepatocyte chromatin from untreated and estrogen treated males revealed that estrogen treatment increases the DNase I sensitivity of the 5' region of both vitellogenin genes 2.2 to 2.7 fold.

In Fig. 5 we plotted the linear functions describing the DNase I sensitivity of these fragments in the different chromatin preparations. The results for the 15 kb fragment of the A1 vitellogenin gene are shown in Fig. 5A and for the 2.5 kb fragment of the A2 vitellogenin gene in Fig. 5B. Comparison of these graphs clearly illustrates that estrogen increases the DNase I sensitivity of the 5' end fragments of both vitellogenin genes in hepatocyte chromatin.

Estrogen increases the DNase I sensitivity of the entire A1 and A2 vitellogenin genes

To assess whether estrogen causes a similar alteration in the DNase I sensitivity of the entire vitellogenin gene as it does at the 5' end we analyzed the effect of estrogen treatment on the DNase I sensitivity of the rest of the A1 and A2 vitellogenin genes. For analysis of the A1 vitellogenin gene, DNA was extracted from nuclei treated with different DNase I concentrations, restricted with Hind III, electrophoresed on agarose gels, transferred to nitrocellulose filters and hybridized with the nick translated cloned A1 vitellogenin cDNA pXlvc 23 (Fig. 1A). The autoradiogram shown in Fig.6 illustrates that hybridization to the 8.5 and 1.6 kb Hind III fragments from the middle of the A1 vitellogenin gene (Fig. 1A) decreases with increasing DNase I digestion of estrogen treated hepatocytes. The disappearance of the 8.5 kb fragment in several independent experiments, which analyzed chromatin from erythrocytes and hepatocytes of untreated and estrogen treated males, was quantitated as shown in Fig. 7. The disappearance of the 1.6 kb fragment was quantitated in the same way (data not shown) and the sensitivity factors *s* for both fragments were calculated and are listed in Table II. Comparing the sensitivity factors obtained from untreated and estrogen treated hepatocytes we conclude that estrogen treatment increases the DNase I sensitivity of the middle of the A1 vitellogenin gene 1.6 to 2.0 fold. A similar increase in DNase I sen-

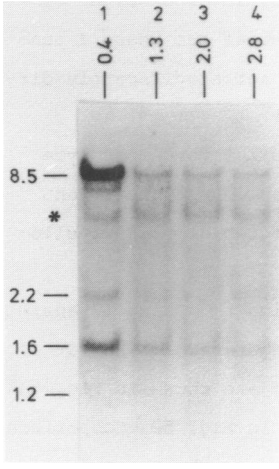

Fig. 6: DNase I sensitivity of the internal part of the A1 vitellogenin gene in chromatin

Nuclei from hepatocytes of estrogen treated males (slots 2-4) were digested with increasing amounts of DNase I. The purified DNA was further digested with Hind III, separated on a 1% agarose gel and transferred to nitrocellulose filters. Such filters were then hybridized to the nick translated A1 vitellogenin specific cDNA clone. DNA from nuclei not digested with DNase I is shown in slot 1 and the size of the expected fragments (see Fig. 1A) are indicated on the autoradiogram in kb. The asterisk denotes a fragment not found in the genomic clones.

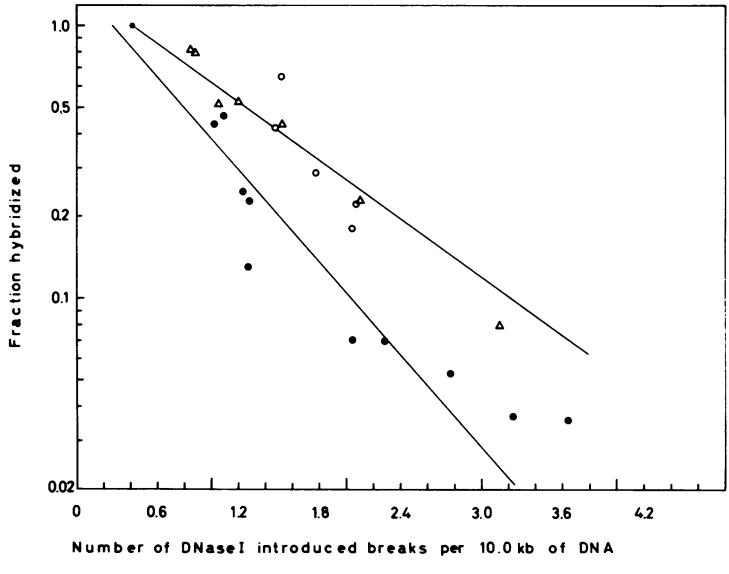

Fig. 7: Quantitation of the DNase I sensitivity of the 8.5 kb Hind III fragment of the A1 vitellogenin gene in chromatin

The hybridization intensity of the 8.5 kb Hind III fragment (see Fig. 6) was determined and correlated to the extent of DNase I digestion as described. The data for hepatocytes from untreated males and for erythrocytes are taken from (19). For further details see legend to Fig. 5.

sitivity was observed for the 5' end of this gene.

The cDNA clone, pXlvc 34 specific for the A2 vitellogenin gene, was used as a probe for the internal and the 3' region of the A2 vitellogenin gene (Fig. 1B). The DNA isolated from DNase I treated chromatin was digested with Hind III, electrophoresed on agarose gels, transferred to nitrocellulose filters and hybridized to nick translated pXlvc 34. This probe hybridized to an internal fragment of 6.8 kb and to a 4.1 kb fragment containing the 3' end of the A2 vitellogenin gene, as shown in Fig. 8. The data was analyzed as described above and the results from several independent experiments are shown in Fig. 9A (6.8 kb fragment) and 9B (4.1 kb fragment). The DNase I sensitivity of both these fragments is greater in hepatocyte chromatin from estrogen treated animals than from untreated animals or than in erythrocyte chromatin. The calculated sensitivity factors (see Table II) for both these fragments indicate that estrogen treatment increases the DNase I sensitivity of the middle of the A2 vitellogenin gene 2.2 fold and of its 3' end 2.7 fold. A similar increase in DNase I sensitivity was observed for the 5' end of this gene. Taken to-

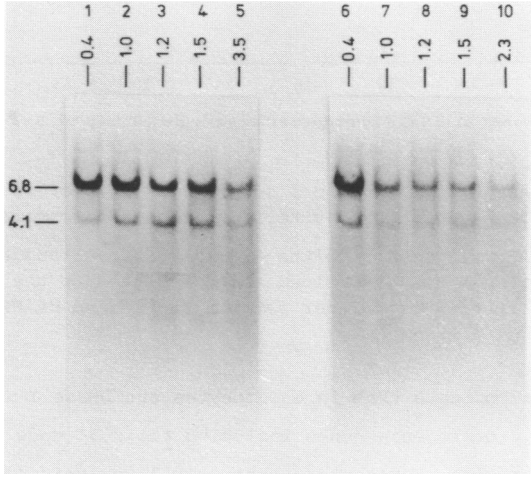

Fig. 8: DNase I sensitivity of the internal part and the 3' end of the A2 vitellogenin gene in chromatin of untreated and estrogen treated hepatocytes

Nuclei from untreated (slots 2-5) and estrogen treated (slots 7-10) male hepatocytes were digested with increasing amounts of DNase I. Nitrocellulose filters prepared as described for Fig. 6 were hybridized to the nick translated A2 vitellogenin specific cDNA clone. DNA from nuclei not digested with DNase I is shown in slots 1 and 6.

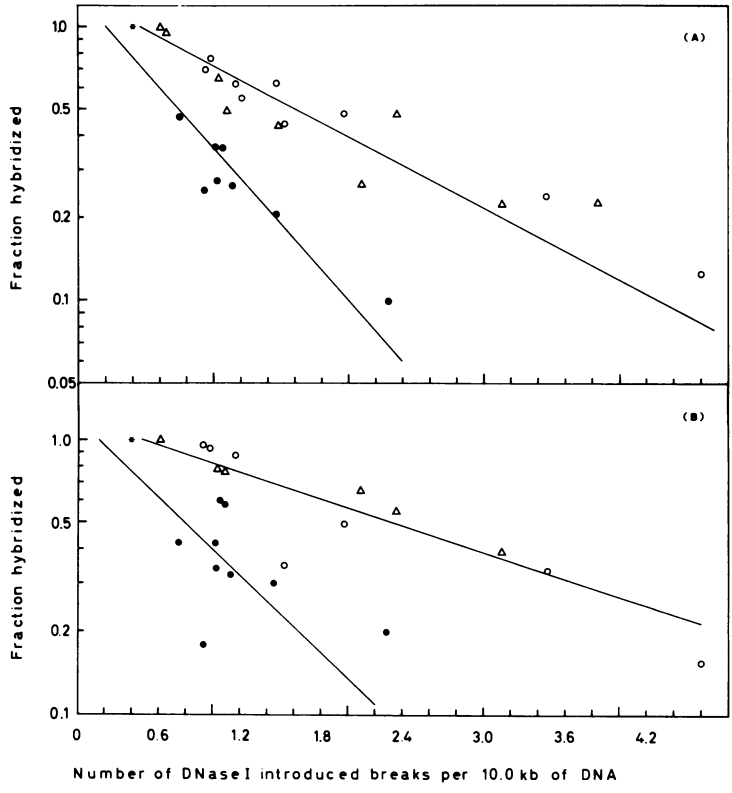

Fig. 9: Quantitation of the DNase I sensitivity of the 6.8 kb and 4.1 kb Hind III fragments of the A2 vitellogenin gene in chromatin

The intensity of hybridization to the 6.8 kb (Fig. 9A) and the 4.1 kb (Fig. 9B) Hind III fragments was determined and correlated to the extent of DNase I digestion as described. For further details see legend of Fig. 5.

gether these data indicate that in hepatocytes the DNase I sensitivity of the entire A1 and A2 vitellogenin genes including their 5' ends changes uniformly in response to estrogen. Furthermore statistical analysis (26) of the data shown in Table II indicates that the DNase I sensitivity of the 1.6 and 8.5 kb A1 and the 4.1 and 6.8 kb A2 vitellogenin gene fragments in hepatocyte chromatin of untreated males and in erythrocyte chromatin does not differ.

Estrogen treatment does not alter the DNase I sensitivity of the vitellogenin genes in erythrocyte chromatin

Hepatocytes are the only cells in which vitellogenin synthesis is indu-

Table II Values of the sensitivity factor s for different restriction fragments in erythrocyte and hepatocyte chromatin and in naked DNA

Fragment analyzed		Source of chromatin: Erythrocytes	Hepatocytes: untreated	Hepatocytes: estrogen treated	Hepatocytes: ratio	Naked DNA
Vitellogenin A_1	15 kb	0.82 ± 0.22 (7)	0.56 ± 0.13 (7)	1.24 ± 0.21 (8)	2.2	1.39 ± 0.39 (2)
	8.5 kb	[b] 0.96 ± 0.15 (8)	[b] 0.98 ± 0.22 (5)	1.53 ± 0.31 (10)	1.6	[a] 1.43 ± 0.20 (5)
	1.6 kb	[b] 1.65 ± 1.14 (3)	[b] 2.51 ± 0.62 (5)	5.00 ± 1.33 (10)	2.0	[a] 2.68 ± 0.41 (6)
Vitellogenin A_2	2.5 kb	1.25 ± 0.48 (14)	1.59 ± 0.59 (11)	4.34 ± 1.28 (11)	2.7	2.73 ± 0.72 (6)
	6.8 kb	0.90 ± 0.25 (9)	0.88 ± 0.15 (9)	1.90 ± 0.46 (9)	2.2	1.59 ± 0.35 (5)
	4.1 kb	0.90 ± 0.25 (6)	0.96 ± 0.49 (7)	2.63 ± 1.04 (9)	2.7	1.69 ± 0.30 (5)
β_1-globin	5.8 kb	[b] 1.10 ± 0.26 (7)	[c] 0.32 ± 0.04 (5)	[c] 0.34 ± 0.15 (9)	1.0	[a] 1.82 ± 0.47 (5)
Albumin	4.6 kb	[b] 0.82 ± 0.35 (6)	[c] 1.38 ± 0.36 (7)	[c] 1.62 ± 0.65 (9)	1.2	[a] 1.19 ± 0.33 (4)

The mean of the sensitivity factors s, the corresponding standard deviations (SD) and the sample numbers (in brackets) are indicated.

[a] data taken from Table I in Felber et al. (19)

[b] data taken from Table II in Felber et al. (19)

[c] data taken from Table II in Felber et al. (19), but the values for hepatocytes from untreated and estrogen treated males have been separated.

cible by estrogen. We therefore investigated whether the DNase I sensitivity of the vitellogenin gene fragments is altered by estrogen treatment of male Xenopus in erythrocytes which do not synthesize vitellogenin in response to this hormone.

Using solution hybridization we observed an estrogen induced increase in the DNase I sensitivity of the vitellogenin genes in hepatocyte chromatin but not in erythrocyte chromatin (see Table I). In our analysis of the DNase I sensitivity of defined restriction fragments we have shown that both vitellogenin genes (A1 and A2) are more sensitive to DNase I digestion in hepatocyte chromatin prepared from estrogen treated animals than in hepatocyte chromatin

from untreated animals. We have also analyzed the DNase I sensitivity of the A1 and A2 vitellogenin gene fragments in erythrocyte chromatin from untreated and estrogen treated males using the methods described above.

The sensitivity factors s obtained for the three fragments of the A2 vitellogenin gene are listed in Table III. The means of the sensitivity factors obtained for these three fragments in erythrocyte chromatin from untreated and estrogen treated males are not significantly different (26). We therefore conclude that the sensitivity of the A2 vitellogenin gene to limited DNase I digestion in erythrocyte chromatin prepared from untreated males is not altered in erythrocyte chromatin prepared from estrogen treated males. The same conclusions were drawn from a similar analysis of the A1 vitellogenin gene (data not shown).

Estrogen treatment does not alter the DNase I sensitivity of an albumin or the β_1-globin gene in hepatocyte chromatin

Albumin genes are transcribed in the liver whereas globin genes are not. Both genes are not generally thought to be influenced by estrogen, though it has been reported that chronic estrogen treatment can decrease albumin synthesis (27). Using specific cloned cDNA probes for the β_1-globin and an albumin gene (19) we analyzed whether estrogen treatment influences their DNase I sensitivity in hepatocytes. We measured the DNase I sensitivity of a 5.8 kb Hind III fragment which contains the adult β_1-globin gene (see 19, Fig. 1A) and of a 4.6 kb Hind III fragment from an albumin gene (19). The sensitivity factors s describing the DNase I sensitivity of these fragments, which were derived from data of Felber et al. (19), are shown in Table II. The means of the sen-

Table III Values of the sensitivity factor s of different A2 vitellogenin restriction fragments in erythrocyte chromatin from untreated and estrogen treated males

Fragment analyzed	Source of chromatin: Erythrocytes	
	untreated *	estrogen treated
2.5 kb	1.17 ± 0.48 (6)	1.30 ± 0.50 (8)
6.8 kb	1.02 ± 0.25 (5)	0.74 ± 0.17 (4)
4.1 kb	0.83 ± 0.14 (3)	0.80 ± 0.15 (3)

* To calculate these values for the A2 vitellogenin gene fragments a distinction has been made between erythrocytes from untreated and estrogen treated males whereas in Table II no such distinction was made.

sitivity factors obtained for either the globin or the albumin gene fragments in hepatocyte chromatin from untreated or estrogen treated males are not significantly different (26). We conclude, therefore, that estrogen treatment of male Xenopus alters the DNase I sensitivity, in hepatocyte chromatin, of the vitellogenin genes, which are activated by the hormone, but does not affect the DNase I sensitivity of the globin or albumin genes, whose transcription is not thought to be dependent on estrogen.

DISCUSSION

Extensive DNase I digestion of liver nuclei, from estrogen treated males, degraded 38-51% of the vitellogenin genes to non-hybridizable fragments whereas at most 13% were lost when nuclei were prepared from untreated males, a value also found for erythrocyte chromatin. The chicken β-globin (28) and ovalbumin (21) genes were completely degraded to non-hybridizable fragments by a similar extent of DNase I digestion of nuclei from tissues in which these genes were being transcribed. The 30% contamination of the liver cell suspension with erythrocytes (Table I) and the fact that the liver contains 25-50% non-parenchymal sinusoidal cells (20) could explain the incomplete degradation of the vitellogenin genes in our preparations. If the vitellogenin genes are only degraded by extensive DNase I digestion of hepatocyte nuclei from estrogen treated animals, then our results agree with those reported for the chikken (21, 28). The vitellogenin genes were not degraded by extensive DNase I digestion of nuclei from tissues in which they were not being transcribed suggesting that, as has been reported for other genes (reviewed in 2), the vitellogenin genes are inaccessible to DNase I in their inactive state.

Using the method developed by Wu et al. (13, 14) we analyzed the sensitivity of defined gene fragments in chromatin to mild DNase I digestion. Our results show that the A1 and A2 vitellogenin genes are accessible to some degree to DNase I even in chromatin from which they are not being transcribed. All the fragments of both genes tested, however, are about twofold more sensitive to DNase I digestion in hepatocytes which are transcribing than in hepatocytes which are not transcribing the vitellogenin genes.

For all fragments tested we measured the DNase I sensitivity of naked DNA, i.e. of DNA which has been extracted and purified prior to DNase I digestion. Table II shows that fragments with relatively high sensitivity factors in naked DNA (e.g. 2.68 ± 0.4 for the 1.6 kb A1 vitellogenin gene Hind

III fragment and 2.73 $\pm$ 0.7 for the 2.5 kb A2 vitellogenin gene Eco RI fragment) also have high sensitivity factors in chromatin. This implies that these high sensitivity factors reflect the DNA sequence rather than the chromatin conformation of these fragments.

The presence of DNase I hypersensitive regions around specific genes has been reported (13, 14, 17, 29, 30). We did not detect any DNase I hypersensitive regions around the A1 and A2 vitellogenin genes, although using the same approach we consistently identified a DNase I hypersensitive region at the 5' end of the β_1-globin gene (19).

Analysis of chicken chromatin showed that the DNase I sensitivity of the ovalbumin (18, 31) and ovomucoid (31) genes extends into their flanking regions. Similar conclusions were drawn from experiments on the chicken globin genes (17). The 15 kb fragment which contains the 5' end of the A1 vitellogenin gene and the 4.1 kb fragment which contains the 3' end of the A2 vitellogenin gene, both contain a large proportion of flanking region. The DNase I sensitivity of both these fragments is increased, in hepatocyte chromatin from estrogen treated males compared to untreated males, to a similar extent as the DNase I sensitivity of the intragenic fragments (see Table II) suggesting that the increased DNase I sensitivity includes the flanking regions.

Estrogen treatment induces vitellogenin synthesis in the liver of adult Xenopus (2-5) but premetamorphic larval liver cells are not competent to synthesize vitellogenin in rêsponse to estrogen (32, 33). One could have speculated that an increased DNase I sensitivity of the vitellogenin genes would be a prerequisite for competence to synthesize vitellogenin in response to estrogen. This can now be excluded as the DNase I sensitivity of the vitellogenin genes in the chromatin of the potential target cells, i.e. the hepatocytes from an untreated adult male and of non-target cells, i.e. eryhtrocytes, does not differ.

Recent experiments with micrococcal nuclease (34) could not reveal any significant effect of hormone treatment on the chromatin conformation of the Xenopus vitellogenin genes. We have now shown that estrogen treatment increases the DNase I sensitivity of the vitellogenin genes in hepatocyte chromatin. Since estrogen treatment does not induce liver cell proliferation (35), we conclude that a conformational alteration of the vitellogenin genes occurs within this stable cell population in response to estrogen. This conclusion is

similar to that drawn for the heat shock genes of Drosophila, which alter their chromatin structure, as measured by DNase I digestion, within a few minutes after heat shock (13, 14). This change in DNase I sensitivity was correlated at the cytological level to alterations in the puffing pattern of the chromomeres containing the heat shock genes (reviewed in 36).

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