Volume 9 Number 10 1981 Nucleic Acids Research

The organization of the histone genes in the genome of Xenopus laevis

Walter van Dongen, Lia de Laaf, Rob Zaal, Antoon Moorman and Olivier Destrée

Department of Anatomy and Embryology, University of Amsterdam, Mauritskade 61, 1092 AD Amsterdam, The Netherlands

Received 9 March 1981

ABSTRACT

We have studied the organization of the histone genes in the DNA from several individuals of Xenopus laevis. For that purpose, Southern blots of genomic DNA, that was digested with several restriction enzymes, were hybridized with radioactively labeled DNA fragments from clone Xl-hi-1 (14), containing genes for Xenopus histones H2A, H2B, H3 and H4. In the DNA of all animals that were screened we found a major repeating unit of 14 kilobasepairs, which contains genes for histones H2A, H2B, H3 and H4 (H1 not tested) and is represented up to 30 times in the genome. The orde of the genes in this major repeating unit is: H4 - H3 - H2A - H2B. This order is different from that in the histone DNA of clone Xl-hi-1, i.e. H3 - H4 - H2A - H2B. In addition to the genes in the major repeating unit, histone genes are present in unique restriction fragments in numbers that vary from one animal to another. The restriction patterns for the histone genes in these unique fragments were found to be different for all eight Xenopus individuals that were screened. The cloned Xenopus histone gene fragment Xl-hi-1 represents such a unique fragment and is not present in the DNA of each single individual. The total number of genes coding for each of the nucleosomal histones is 45-50 per haploid genome.

INTRODUCTION

Histone genes are repeated several times in the DNA of most organisms that have been studied thusfar. Yeast contains only two copies of the genes for histones H2A and H2B (1), while the reiteration frequency for the histone genes in chicken DNA is 10 fold (2), in mouse 10 - 20 fold (3), in Drosophila melanogaster 110 fold (4), and several hundredfold in different species of sea urchin (5, 7, 8). For Xenopus laevis a reiteration frequency of 20 - 50 fold has been determined (6).

The most extensive studies on the organization of histone genes have been reported on several species of sea urchin and Drosophila (for a review, see 7). In the DNA of these species, genes coding for the different histone proteins are closely linked to one another on the DNA. In sea urchin, several hundreds of these clusters, or repeating units are tandemly linked in the

genome in an identical repetition. Each unit contains the coding regions for each of the five histone proteins, alternating with non-coding spacer sequences. The major repeat unit for the sea urchin species L. pictus (8), S. purpuratus (9) and P. miliaris (10) have been isolated, cloned and analysed in detail by restriction endonuclease mapping and nucleotide sequencing. There is good evidence, that the histone genes in the major repeating unit are expressed in early embryogenesis (11, 12). In addition histone genes have been found in sea urchin DNA, that are repeated only a few times and might code for variant proteins. The histone DNA in clone h19, for example, is repeated only approximately 5 times in the P. miliaris genome (13) and contains coding sequences for a set of histone variants.

In previous experiments (14) a DNA fragment from Xenopus laevis was cloned, that contains genes coding for histones H2A, H2B, H3 and H4, but not for one of the H1 histones. We have used this cloned DNA as a hybridization probe to obtain information on the organization of the histone genes in the Xenopus laevis genome. This knowledge will be helpfull for further cloning of Xenopus histone DNA for the search of developmentally regulated histone genes, if present.

MATERIALS AND METHODS

Materials. Restriction enzymes Pst I, Bgl I, Bgl II, BamH I and EcoR I and DNA polymerase I were purchased from Boehringer (Mannheim); Hinf I was a generous gift of T. Veltman. DNase I was from Worthington. α-^{32}P dATP and dCTP (400 or 2 - 3000 Ci/mmol) were from The Radiochemical Centre (Amersham). DEAE-cellulose was from Whatman, agarose (type II) from Sigma, nitrocellulose filter from Millipore and dextran sulphate from Pharmacia.

Young animals sacrified for DNA purification were raised in the laboratory. Old animals had been purchased from a supplier. They were selected on sex (4 males and 4 females) and age (4 animals of about 2 years old and 4 of about 10 years old).

Electrophoresis and blotting of DNA. High molecular weight DNA (> 50,000 bp) was purified from the blood of individual Xenopus laevis laevis animals as described before (14). All endonuclease digestions were under conditions as described by the suppliers. In some digestions, bacteriophage λ DNA was included, both to monitor the completeness of the digestion and the blotting efficiency and as a molecular weight marker after electrophoresis. 0.2 to 1 μg of the digested DNA was separated at 3 V/cm on 0.7 or 1% agarose gels in 1 x E-buffer (35 mM Tris, 35 mM NaH_2PO_4, 1 mM EDTA, pH 7.6), containing

1 μg ethidium bromide per ml. Thereafter, gels were soaked for 45 min. in 0.5 M NaOH, 1.5 M NaCl, then in 1.0 M Tris-HCl, 3.0 M NaCl, pH 6.0 and the DNA was transferred overnight to a sheet of nitrocellulose filter as described by Southern (15). After blotting, filters were heated for 2 hours at 80°C in a vacuum exsiccator and stored at -20°C until use.

Purification and labeling of DNA fragments. Total cloned 5.8 kb Xenopus histone DNA fragment (Xl-hi-1) or restriction fragments of this DNA were used as probes for hybridizations (14). Fig. 1 gives the position of the genes for histones H2A, H2B, H3 and H4 on the 5.8 kb DNA (A), the restriction sites for the enzymes used in this study (B), and the fragments used for hybridization (C). The 5.8 kb DNA was isolated from the recombinant plasmid by EcoR I digestion, followed by agarose gel electrophoresis and electroelution (16), and further purified on CsCl equilibrium gradients. Restriction fragments of this DNA were separated on agarose gel; bands were cut out of the gel, the agarose was finely dispersed through a small gauge

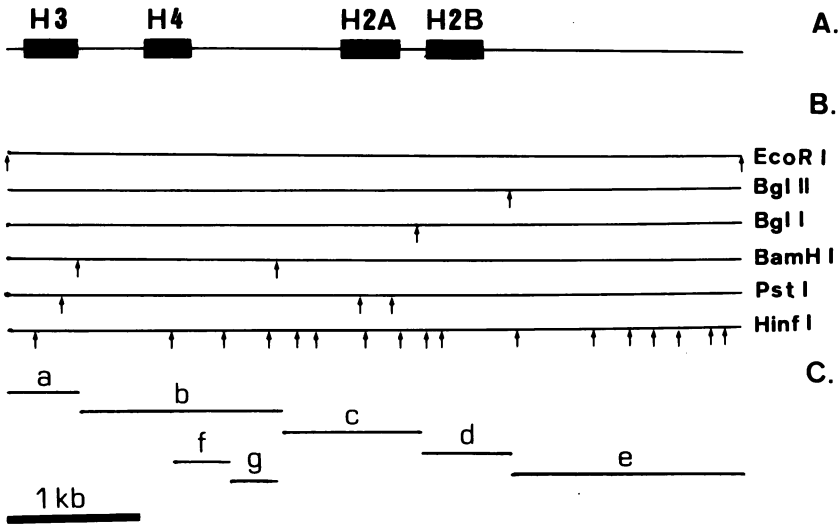

Fig.1 Organization of the genes on the Xenopus histone DNA of clone pXl-hi--1 (5.8 kb). A. shows the position of the genes in this cloned DNA. B. gives the restriction sites for the endonucleases used in this study. C. shows the fragments that were used for hybridization with digested genomic DNA, i.e. an EcoR I x BamH I fragment (a) as probe for H3 genes; a BamH I x BamH I fragment (b) and a Hinf I x Hinf I fragment (f) as probes for H4 genes; a BamH I x Bgl I fragment (c) as probe for H2A genes; a Bgl I x Bgl II fragment (d) as probe for H2B genes and a Bgl II x EcoR I fragment (e) and a Hinf I x Hinf I fragment (g) as probes for non-coding spacer DNA sequences.

needle and the DNA was extracted overnight with 10 ml 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. After centrifugation to remove the bulk of the agarose (15 min. 35,000 rpm Spinco SW-41) the DNA was bound to a 0.2 ml column of lysine--Sepharose, washed with 2 ml 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, eluted with 0.3 M NaCl and precipitated by addition of 2.5 vol. ice-cold ethanol. Precipitates were collected by centrifugation (30 min. 40,000 rpm Spinco SW-41 at -5°C), washed twice with cold 70% ethanol and dissolved in a small volume of sterile water and stored at -20°C until use.

The purity of the restriction fragments of the 5.8 kb histone DNA was tested by electrophoresis on agarose gel, followed by blotting onto nitrocellulose filter and hybridization with other fragments under the conditions described in the next section. All fragments appeared to be pure (i.e. not more than about 1% contamination), except fragment b, that showed about 3% contamination with a 1750 bp BamH I x Bgl II fragment containing the genes for histones H2A and H2B. Furthermore, no crosshybridization was visible between the different fragments, indicating, that there are no regions of homology between them.

Fragments were labeled to high specific activity (2 - 5 x 10^5 cpm/ng DNA) by nick-translation. Nick-translation incubations contained in 50 μl: 20 μCi of each α-^{32}P dCTP and dATP (2 - 3000 Ci/mmol), 20 μM dGTP, 20 μM dTTP, 50 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 5 μg bovine serum albumin (macaloid treated), 50 - 200 ng DNA, 0.05 ng DNase I and 4 units DNA polymerase I. Probes containing the gene for histone H4 were labeled with α-^{32}P dATP and dCTP of 400 Ci/mmol. The reaction was for 2 hours at 11 - 14°C and was stopped by addition of EDTA to 20 mM and 10 μg sheared bacterial DNA. Labeled DNA was separated from unincorporated nucleotides on a small column with Sephadex G-50. The DNA was directly used for hybridization after 5 min. heating at 100°C.

Conditions for hybridization. Initial attempts to hybridize blotted genomic DNA from Xenopus with ^{32}P-labeled cloned 5.8 kb Xenopus histone DNA or purified restriction fragments of this DNA were performed in a buffer containing 2 x SSC (0.3 M NaCl, 0.03 M Na_3-citrate), 10 x Denhardt solution (0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin). Even after extensive washing of the filters in 0.05 x SSC at 65°C, autoradiographs of the filters showed homogeneous blackening over the complete lanes. Allthough we did not study this phenomenon in detail, a possible reason for it might be, that the 5.8 kb cloned histone DNA contains regions that have a great homology to stretches of basepairs that are highly repeated in the

Xenopus genome. When we applied more stringent hybridization conditions by the addition of formamide to the hybridization buffer, as suggested by Howley et al. (17), discrete bands appeared on the autoradiographs, however, with a very low intensity. The intensity was increased by addition of 10% dextran sulphate to the hybridization buffer (18). To obtain optimal results, a range of formamide concentrations was tested. Highly reproducible results were obtained by hybridization of the filters in 2 x SSC, 20 mM Na-phosphate, pH 6.8, 10% dextran sulphate, 30% formamide (deionized) and 10 x Denhardt solution at 65°C, and finally washing them in 0.1 x SSC at 60°C. The pattern of hybridizing bands formed under these conditions did not change, when we applied even more stringent conditions, i.e. hybridization with a 36% formamide buffer, or washing with 0.1 x SSC at 65°C; this suggests, that the hybrids formed under these conditions are stable. Hybridization of the 5.8 kb DNA with blotted heterologous DNA, like bacteriophage λ or bacterial DNA was not found under these circumstances.

For the experiments presented in this paper, 1 - 5 filters containing the blotted DNA were prehybridized in 50 ml buffer (20 mM Na-phosphate, pH 6.8, 2 x SSC, 0.1% SDS, 10 x Denhardt solution, 10% dextran suphate, 30% deionized formamide, 20 g/ml sheared, heat-denaturated bacterial DNA) in a thermostated gyrotory shaker (65°C, 50 rev/min) during 4 - 5 hours. After prehybridization the buffer was renewed and the labeled DNA added (50 - 200 ng, 2 - 5 x 10^5 cpm/ng DNA). The mixture was covered with a layer of thin paraffin oil to prevent evaporation. Hybridization was for 18 hours at 65°C at 50 rev/min. Filters were washed twice with 2 x SSC (200 ml per filter) at room temperature and three times with 0.1 x SSC (200 ml per filter) at 60°C. Autoradiography of the filters was on pre-exposed Kodak XR I film at -70°C. With this method 0.1 pg of homologous, filter bound DNA could be detected within four days. Although filters are slightly more fragile after this procedure, they can easily be used for a second hybridization.

RESULTS

Analysis of the histone genes in several Xenopus laevis individuals

DNA from the blood of several Xenopus laevis animals was digested with EcoR I, electrophoresed, blotted onto nitrocellulose filter and hybridized with ^{32}P-labeled 5.8 kb cloned Xenopus histone DNA (fig. 2). The autoradiograph shows a prominent band of hybridization at a position of 8.9 kilobasepairs, and two somewhat less pronounced bands at about 5 kilobasepairs, that are present in the DNA digests of all individuals tested.

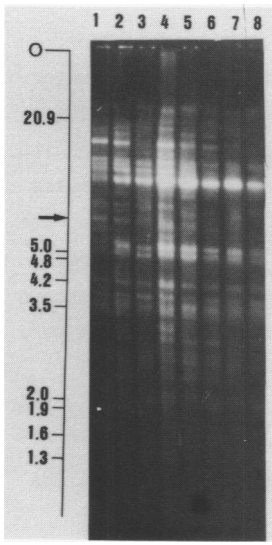

Fig. 2 Hybridization patterns of histone genes in EcoR I digested DNA from several Xenopus individuals. EcoR I digested DNA from four older (about 10 years, lanes 1 - 4) and four younger (about 2 years, lanes 5 - 8) animals was separated on a 0.7% agarose gel, blotted onto nitrocellulose filter and hybridized with 32P-labeled 5.8 kb Xl-hi-1 DNA. Each lane contained 0.5 μg of DNA, except lanes 4 and 5 that contained 1 μg. Sizes of EcoR I x Hind III digestion products of bacteriophageλDNA, separated on the same gel, are indicated on the left. The arrow indicates the position of a DNA band 5.8 kb in length. o represents the origin in this and other figures.

Besides these major bands, a pattern of weak bands of hybridization is visible over the entire length of the lanes. These minor bands differ in number from one animal to another (we count 16 of these bands in the DNA of animal no. 7 to 26 in the DNA of animal no. 4). Some of these minor fragments are represented in the digested DNA of all individuals, many other fragments are not present in the DNA of all individuals, while others can only be found in the DNA of one or two animals (see also fig. 3 for these differences). For example, a fragment corresponding in size to the cloned 5.8 kb histone DNA fragment cannot be detected in the hybridization profiles of genomic DNA from individuals 6, 7 and 8, while individuals 1 - 5 have only a minor hybridizing EcoR I fragment of this size.

It is unlikely, that these minor bands are products of partial digestion: bacteriophage λ DNA, added to the endonuclease reaction as an internal marker appeared completely digested. In addition, higher concentrations of EcoR I or longer digestion times did not change the digestion patterns (except for DNA of animal 4, where one high molecular weight band represents indeed a partial digestion product, cf. fig. 3).

In general, the digests of DNA from older animals (no. 1 - 4) show a more complex pattern after hybridization with the 5.8 kb DNA than that from younger animals (no. 5 - 8). In the DNA of the younger animals, the highest signal of hybridization is found at a position of 8.9 kb. Hybridization with the 8.9 kb band does not seem to be equally strong in the DNA of all

individuals tested: the more complex the hybridization pattern, the less hybridization seems to coincide with the main band of 8.9 kb.

Genes for the different histones are closely linked in the DNA of sea urchins and Drosophila (see 7 for a review). If this holds also for Xenopus, genes for the different histones should be located on the same restriction fragments of genomic DNA, resulting in similar hybridization patterns of digested genomic DNA with probes for the different histone genes. As shown in fig. 3, the similarity between the hybridization patterns is high, especially in the high molecular weight region of the gel. This result indicates, that many of the histone genes are indeed linked in Xenopus. Some differences in the hybridization patterns can be noticed, mainly in the lower molecular weight range. These differences, however, might reflect cutting of EcoR I within some of the gene clusters.

Is there a major histone gene cluster in Xenopus laevis? Hybridization of EcoR I digested DNA with the 5.8 kb histone DNA fragment and with sub-fragments of this DNA containing the individual histone genes show a major band of hybridization at 8.9 kb. Fragment f, that contains the gene for histone H4, gives a prominent band of hybridization at 5.1 kb as well (fig. 3). We were interested to learn, if these fragments of 8.9 and 5.1 kb are repeated in the Xenopus genome. Therefore, different amounts of the cloned 5.8 kb histone DNA were added to a constant amount of EcoR I digested DNA

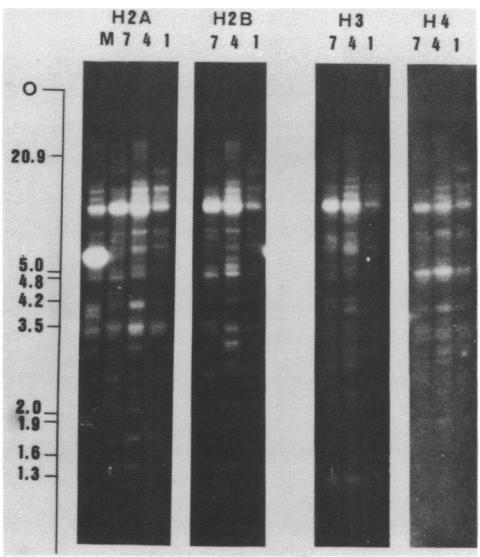

Fig. 3 Hybridization patterns of genes coding for the different nucleosomal histones in EcoR I digested DNA from three individuals of Xenopus. EcoR I digested DNA from animals no. 1, 4 and 7 of fig. 2 was run on 0.7% agarose gels, blotted onto nitrocellulose filter, and hybridized with 32P-labeled DNA probes for the different nucleosomal histone genes. These probes are: fragment c (fig. 1) for H2A genes, fragment d for H2B genes, fragment a for H3 genes and fragment f for H4 genes. Each lane contains 0.5 μg DNA from animals no. 4 and 7 and 0.2 μg DNA from animal 1; in the lane marked M 100 pg 5.8 kb DNA from Xl-hi-1 was added to EcoR I digested genomic DNA.

from animal no. 7. The amounts of added 5.8 kb DNA corresponded to a 0.5,1.5,5 and 15-fold repetition of this fragment per haploid genome (3.1×10^9 bp). After electrophoresis and blotting, the DNA was hybridized with the probes for the different histone genes (fig. 4). The intensity of the bands of hybridization in genomic histone DNA was compared with the intensity of the bands due to the added 5.8 kb histone DNA by scanning the autoradiographs in a gel scanner (scans not shown; a correction was made for the blotting efficiency throughout the gel by addition of small amounts of EcoR I digested λ DNA to some of the samples before electrophoresis, and hybridization of this DNA with nick-translated λ DNA after electrophoresis and blotting).

From this experiment, it appears, that the minor bands in the hybridization patterns of genomic DNA correspond to histone DNA fragments, that are present only once or twice per haploid genome of animal no. 7.

The numbers of genes determined in the major genomic histone bands by use of this method are shown in column 4 of table I. Different numbers of each of the nucleosomal histone genes seem to be present in these bands.

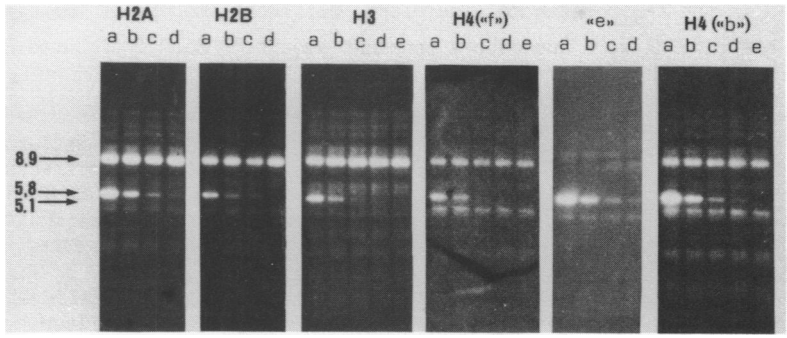

Fig. 4 Quantification of the genes coding for nucleosomal histones in EcoR I digested DNA from an individual Xenopus. 0.5 μg EcoR I digested DNA from animal no. 7 (fig. 2) was mixed with 0 (lanes e), 0.5 (lanes d), 1.5 (lanes c), 5 (lanes b) or 15 (lanes a) pg 5.8 kb Xl-hi-1 DNA. These quantities of added 5.8 kb DNA represent a 0.5, 1.5, 5 or 15 fold repetition of the histone genes per haploid genome of 3.1×10^9 basepairs. The DNA was separated on 1.0% agarose gels, blotted onto nitrocellulose filters and hybridized with 32P-labeled DNA probes for the different nucleosomal histone genes. These probes are: fragment a for H3 genes, fragments b and f for H4 genes, fragment c for H2A genes, fragment d for H2B genes and fragment e for non-coding spacer DNA. In some cases, shorter exposed autoradiographs were used for scanning. Positions of the 8.9 and 5.1 kb bands in genomic DNA and of the added 5.8 kb band are indicated.

TABLE I. ESTIMATION OF THE NUMBERS OF GENES FOR HISTONES H2A, H2B, H3 AND H4 IN THE MAJOR GENOMIC HISTONE FRAGMENTS IN XENOPUS DNA.

1	2	3	4		5
Gene	Probe used for detection [a]	Percentage coding sequences in probe [b]	Number of genes counted [c]		Corrected number of genes in 5.1 + 8.9 kb fragments [d]
			8.9 kb fragment	5.1 kb fragment	
H2A	c	40	12		30
H2B	d	90	25		28
H3	a	75	22		29
H4	f	50	10	5	30
H4	b	30	5	1.5	26

[a] See fig. 1.

[b] Determined by nucleotide sequencing (Moorman et al., in preparation).

[c] Numbers were counted by comparing intensities of hybridization with those of standard 5.8 kb DNA by scanning of the autoradiographs in fig. 4.

[d] Numbers were corrected as follows: Assumed that all the non-coding spacer sequences in the probes do hybridize with the 5.8 kb DNA standard, but not with the DNA in the major genomic DNA fragments of 8.9 and 5.1 kb, numbers shown in column 4 are underestimated. Values in column 5 were obtained after multiplication of the numbers in column 4 with 100/x (x is the percentage of coding sequences in the probes, column 3).

Moreover, the number of genes coding for histone H4 determined in the genomic 8.9 and 5.1 kb bands varies depending on the probes used for hybridization: after hybridization with fragment b a lower number of H4 genes was counted than after hybridization with fragment f. This difference can only be explained, if not all sequences in the probes that were used for hybridization are equally represented in the major genomic histone DNA fragments.

We tested this hypothesis by hybridization of genomic DNA digests with two fragments, that contain only non-coding spacer sequences (fragments e and g, fig. 1). It appears, that these fragments do not hybridize with the major genomic histone bands, whereas they do with control 5.8 kb DNA (result only shown for fragment e). These results point to an extensive divergence between the spacer sequences in the DNA of clone Xl-hi-1 and that of the major histone DNA fragments in genomic DNA. Such heterogeneity in the spacer regions between the histone genes of Xenopus has been reported very recently (20).

Because the probes that were used for gene counting contain non-coding spacer sequences, numbers of genes in the major genomic DNA bands are probably underestimated. If we assume, that all the non-coding spacer sequences in these probes do hybridize with the added 5.8 kb standard DNA in the experiment in fig. 4, but not with the 8.9 or 5.1 kb fragments of genomic

DNA, measured numbers of genes in these fragments have to be corrected. These corrected values are shown in table 1, column 5.

If our assumptions are right, the 8.9 kb genomic DNA band contains 28 - 30 copies for each of the genes coding for histones H2A, H2B and H3. However, as 28 - 30 genes for each of these histones can not be fit on one DNA fragment of 8.9 kb in length, this result indicates that the 8.9 kb fragment is repeated itself several times in genomic DNA, each piece containing one or two sets of genes coding for at least all four nucleosomal histones. The total number of genes for each of the nucleosomal histones, those on the unique restriction fragments included, is approximately 45 per haploid genome.

Two major bands are visible after hybridization of EcoR I digested genomic DNA with probes for the histone H4-gene, one of 8.9 kb, the other of 5.1 kb (fig. 4). After partial EcoR I-digestion of the DNA from animal no. 7, one major band of 14 kb shows up after hybridization with all the different gene- specific probes from Xl-hi-1 DNA (fig. 5, only hybridizations with H4 and H2B probes shown). No other major partials are visible after hybridization with the H4 probe. This result suggests, that the 8.9 kb and 5.1 kb EcoR I fragments are linked in the Xenopus genome, resulting in a 14 kb unit that contains the genes for all the nucleosomal histones and that may be repeated up to 30 times.

From these experiments with partially digested DNA we cannot decide,

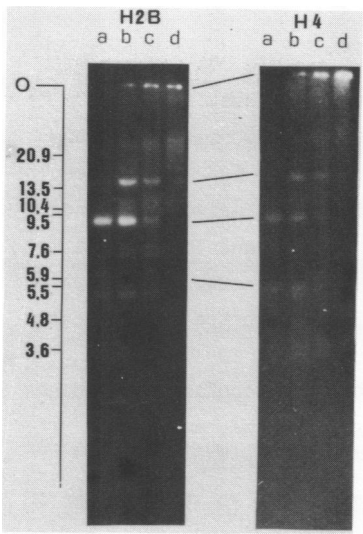

Fig. 5 Hybridization pattern of genes coding for different nucleosomal histones in Xenopus DNA after partial digestion with EcoR I. DNA from animal no. 7 was completely (lanes a) or partially (lanes b - d) digested with EcoR I (the extent of digestion decreases from b to d). 0.5 μg of this DNA was run per slot on 0.7% agarose gels, transferred to nitrocellulose filter, and hybridized with 32P-labeled DNA of fragment d (see fig. 1) for H2B genes or fragment f for H4 genes. Size markers are products of partial digestion of λ DNA with EcoR I.

whether the 14 kb units are tandemly linked in the Xenopus genome. Some weak bands of hybridization show up in the high molecular weight part of the gel after partial digestion, but the separation here is insufficient to determine their corresponding molecular weights.

Gene order on the major 14 kb histone gene unit in Xenopus DNA To determine of the order of the genes coding for the nucleosomal histones in the major 14 kb histone gene unit DNA from animal no. 7 was digested with different restriction endonucleases (fig. 6). After hybridization of the electrophoretically separated and blotted digests with the probes for the different histone genes, it appears, that genes for histones H2A, H2B and H3 are on the same major EcoR I fragment (8.9 kb), while the gene for histone H4 is divided between the 8.9 kb and 5.1 kb major EcoR I fragments (fig. 6A, lanes d). Genes for histones H2A and H2B both lie on the same major Bgl II

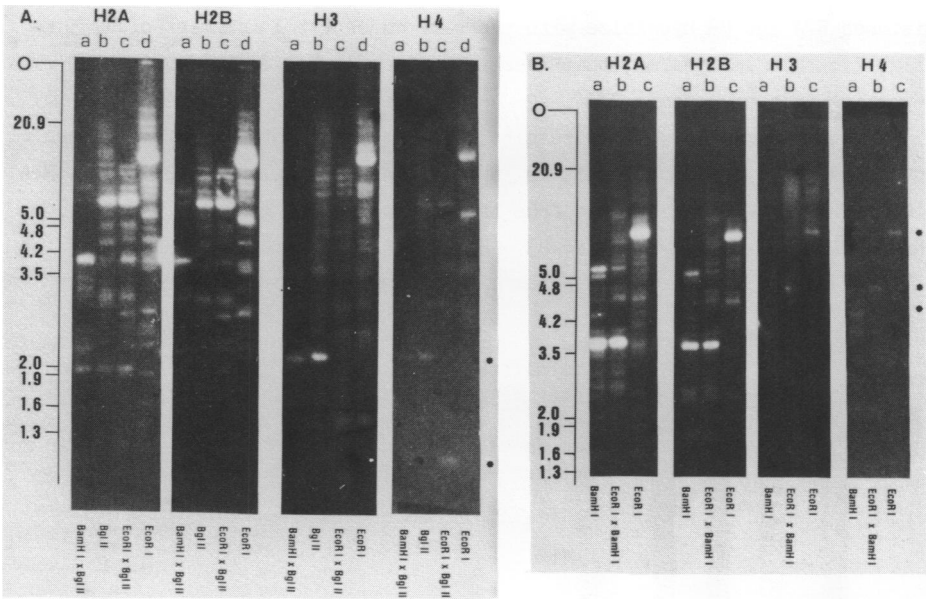

Fig. 6 Hybridization patterns of genes coding for the different nucleosomal histones in Xenopus DNA after digestion with several restriction enzymes. DNA from animal no. 7 was digested with EcoR I (fig. A, lanes d and fig. B, lanes c), Bgl II (fig. A, lanes b), Bgl II and EcoR I (fig. A, lanes c), Bgl II and BamH I (fig. A, lanes a), BamH I (fig. B, lanes b) or BamH I and EcoR I (fig. B, lanes a). 1 μg of the EcoR I digested DNA in fig. A, 0.2 μg of the BamH I x Bgl II digested DNA and 0.5 μg of the other digests were separated on 0.7% agarose gels, transferred to nitrocellulose sheets and hybridized with the same probes as used in fig. 3.

fragment of 5.4 kb (fig. 6A, lanes b) and on the same major BamH I fragment of 4.0 kb (fig. 6B, lanes b); genes for histones H3 and H4 can both be detected on a major 5.3 kb BamH I and a major 2.1 kb Bgl II fragment (figs. 6B and 6A, lanes b). This indicates, that genes for histones H2A and H2B are linked in the DNA of the major 14 kb unit, just like genes for histones H3 and H4. Double digestion of the DNA with BamH I and Bgl II results in a major 4.0 kb fragment that hybridizes with H2A and H2B probes, and a 2.0 kb fragment that hybridizes with H3 and H4 genes (fig. 6A, lanes a). The BamH I and Bgl II fragments with genes for histones H3 and H4 are both cleaved after a second digestion with EcoR I (fig. 6A, lanes c and fig. 6B, lanes a). This results in a 4.5 kb BamH I x EcoR I and a 1.2 kb Bgl II x EcoR I fragment containing sequences for histone H4 genes. Fragments with genes for histone H3 are not visible after double digestion with EcoR I and Bgl II or BamH I, and probably ran out of the gel.

Finally, after digestion of the DNA with Pst I, probes with genes for histones H2A and H3 hybridize with the same major Pst I fragment of 1.35 kb (fig. 7); this implicates, that these genes are linked in the major repeating unit of genomic DNA.

It is indicated by these results, that many, if not all, of the genes in the major 14 kb histone unit in Xenopus DNA have the order: H4 - H3 - H2A - H2B. This order is different from that found for the genes in the histone DNA of clone X1-hi-1, which is: H3 - H4 - H2A - H2B. From the results of the

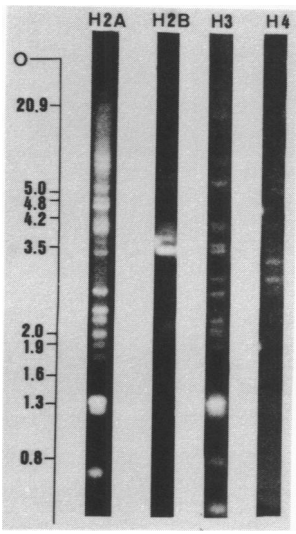

Fig. 7 Hybridization patterns of genes coding for the different nucleosomal histones in Xenopus DNA after digestion with Pst I. Pst I digested DNA from animal no. 7 was separated on 1% agarose gel (1 μg per lane), blotted onto nitrocellulose filter and hybridized with the same probes as used in fig. 3.

experiments in figs. 6 and 7, a concise map of the 14 kb histone DNA unit of Xenopus laevis was constructed (fig. 8).

DISCUSSION

The very sensitive hybridization procedure that we developed enabled us to study the organization of the histone genes in the Xenopus genome. The genes coding for the different nucleosomal histones appear to be linked in the DNA of Xenopus like in sea urchins and Drosophila (reviewed in ref. 7). Very recently it has been shown (20), that genes for histones H1A and H1B are arranged close to the nucleosomal genes in the DNA of some cloned Xenopus histone DNA fragments. A majority of the histone genes in Xenopus appears to be organized in a repeating unit that is 14 kb in length. Up to 30 copies of the genes for each of the nucleosomal histones are located on these 14 kb fragments. The rest of the genes lies on unique restriction fragments; the DNA of clone Xl-hi-1 belongs to this latter family. This kind of organization of histone genes shows some resemblance to that found in sea urchins, where the majority of the histone genes is organized in tandemly linked identical (or nearly identical) repeating units of approximately 6 kb in length (7, 8). At least some other histone genes belong however to smaller clusters (for example the histone DNA in clone h19, ref. 13).
A remarkable finding is, that the order of the genes for the nucleosomal histones is not uniform in Xenopus DNA. Although we are not sure, if all the genes in the major 14 kb unit are arranged in the same order, at least many of them have the order: H4 - H3 - H2A - H2B. In the DNA of clones Xl-hi-1 (14) and λX1h1 (20) this order is: H3 - H4 - H2A - H2B. Finally, a fragment was isolated by Zernik et al. (20), that only contains coding

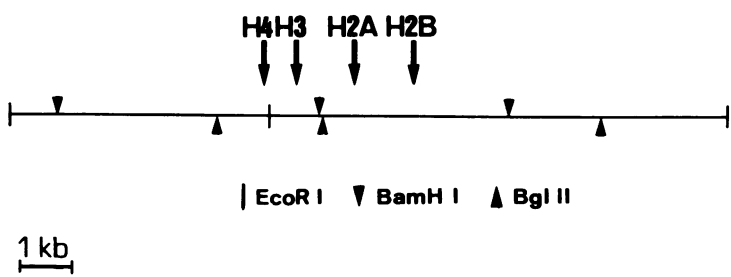

Fig. 8 Organization of the histone genes in the major 14 kb unit in genomic Xenopus DNA. This map is a compilation of the results shown in figs. 6 and 7. Restriction sites for EcoR I, BamH I and Bgl II are shown. The composition of the map is discussed in the text.

sequences for histones H4, H1A and H2B. Besides of this non-uniform arrangement of the genes, we also found an extensive divergence in the non--coding spacer DNA that surrounds the genes. For example, two fragments of clone Xl-hi-1 consisting completely of non-coding spacer DNA do not hybridize with the major genomic histone bands, but they do hybridize with three minor EcoR I fragments of genomic DNA (these fragments hybridize also with probes for histones H2A, H2B, H3 and H4).

Differences exist in the EcoR I digestion patterns for the minor histone DNA fragments in the individual animals. For example, a DNA fragment that corresponds in size to the cloned 5.8 kb histone DNA is not present in EcoR I digests of genomic DNA of at least 3 of 8 animals tested. Such variation in restriction sites has also been found for the globin genes in the DNA of different Xenopus individuals (21). Interestingly, the hybridization patterns obtained with the DNA from older animals are more complex and variable than those with the DNA from younger animals, i.e. more genes are on unique EcoR I restriction fragments in older animals, while less genes seem to be present in the major EcoR I fragments. These differences might be intrinsic to the genetic material of the individuals and caused by allelic polymorphism for the histone genes. They might also be caused by increased methylation of DNA bases. It will be interesting to know if the forementioned differences are related to ageing. More detailed studies will be necessary to clarify these points.

We have previously measured the histone mRNA content of various stage oocytes of Xenopus (Van Dongen et al., submitted). We found that Xenopus oocytes contain a store of about 5×10^8 copies of messenger RNA for histone H3, that is accumulated within a few months. We calculated, that this rapid mRNA accumulation is only possible, if several sets of the histone genes are transcribed at the same time during oogenesis. In two other amphibian species, Notophthalmus and Triturus, histone gene transcription in oocytes was indeed detected on large chromosomal loops (19), that might contain several sets of genes. If the major repeating unit in Xenopus contains identical sets for the histone genes, these sets might by transcribed coordinately, i.e. the transcription of these sets might be switched on and off in the same stages of development. Further study has to reveal, if the genes in the major 14 kb repeating histone gene unit are indeed candidates for coordinate transcription during oogenesis in Xenopus

ACKNOWLEDGEMENT

The authors thank prof. P. Borst and Drs. R. Charles and W.H. Lamers for helpful and stimulating discussions.
This work was supported in part by a grant to O.D. from the Netherlands Foundation for fundamental Medical Research (FUNGO) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO) (grant no. 13-50-09).

REFERENCES

1. Hereford, L., Fahrner, K., Woolford jr., J., Rosbash, M., Kaback, D.B. (1979) Cell 18, 1261-1271
2. Crawford, R.J., Krieg, P., Harvey, R.P., Hewish, D.A., Wells, J.R.E. (1979) Nature 279, 132-136
3. Jacob, E. (1976) Eur. J. Biochem. 65, 275-284
4. Lifton, R.P., Goldberg, M.L., Karp, R.W., Hogness, D.S. (1977) Cold Spring Harbor Symp. Quant. Biol. 42, 1047-1051
5. Kedes, L.H., Birnstiel, M.L. (1971) Nature New Biol. 230, 165-169
6. Jacob, E., Malacinsky, G., Birnstiel, M.L. (1976) Eur. J. Biochem. 69, 45-54
7. Kedes, L.H. (1979) Ann. Rev. Biochem. 48, 837-870
8. Cohn, R.H., Kedes, L.H. (1979) Cell 18, 843-853
9. Kedes, L.H., Chang, A.C.Y., Housman, D., Cohen, S.N. (1975) Nature 255, 533-538.
10. Clarkson, S.G., Smith, H.O., Schaffner, W., Gross, K.W., Birnstiel, M.L. (1976) Nucleic Acids Res. 3, 2617-2632
11. Sures, I., Lowry, J., Kedes, L.H. (1978) Cell 15, 1033-1044
12. Schaffner, W., Kunz, G., Daetwyler, M., Telford, J., Smith, H.O., Birnstiel, M.L. (1978) Cell 14, 655-671
13. Busslinger, M., Portman, R., Irminger, J.C., Birnstiel, M.L. (1980) Nucleic Acids Res. 8, 957-977
14. Moorman, A.F.M., De Laaf, R.T.M., Destrée, O.H.J., Telford, J., Birnstiel, M.L. (1980) Gene 10, 185-193
15. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517
16. Wienand, U., Schwarz, Z., Feix, G. (1979) FEBS Letters 98, 319-323
17. Howley, P.M., Israel, M.A., Law, M.F., Martin, M.A. (1979) J. Biol. Chem. 254, 4876-4882
18. Wahl, G.M., Stern, M., Stark, G.R. (1976) Proc. Natl. Acad. Sci. USA 76, 3683-3687
19. Callan, H.G. and Gall, J. in lectures at the Sec. Int. Congress on Cell Biology, Berlin, sept. 1980
20. Zernik, M., Heintz, N., Boime, I., Roeder, R.G. (1980) Cell 22, 807-815
21. Jeffreys, A.J., Wilson, V., Wood, D., Simons, J.P., Kay, R.M., Williams, J.G. (1980) Cell 21, 555-564