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The organisation and expression of histone genes from *Xenopus borealis*

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ABSTRACT

We have isolated genomic clones from Xenopus borealis representing 3 different types of histone gene cluster. We show that the major type (H1, H2B, H2A, H4, H3), present at about 60-70 copies per haploid genome (1), is tandemly reiterated with a repeat length of 15 kb. In situ hybridization to mitotic chromosomes shows that the majority of histone genes in Xenopus borealis are at one locus. This locus is on the long arm of one of the small sub-metacentric chromosomes. A minor cluster type with the gene order H1, H3, H4, H2A is present at about 10-15 copies. The genome also contains rare or unique cluster types present at less than 5 copies having other types of organisation. An isolate of this type had the gene order H1, H4, H2B, H2A, H1 (no H3 cloned). Microinjection of all of the clones into Xenopus laevis oocyte nuclei shows that most of the genes present are functional or potentially functional and a number of variant histone proteins have been observed. S_1 mapping experiments confirm that the genes of the major cluster are expressed in all tissues and at all developmental stages examined.

INTRODUCTION

The histone genes of animals are arranged in two extreme kinds of way (2). In one, the coding sequences of the 5 histone types (H1, H2A, H2B, H3, H4,) are located close to each other and this quintet is repeated tandemly many times. At the other extreme the genes are arranged in apparently random order (except for H2A and H2B genes tending to be in transcriptionally divergent pairs), some clustered, some dispersed throughout the genome. The closest to the first arrangement is seen in Drosophila melanogaster, where about 100 genes are arranged as tandemly repeated, highly conserved quintets. Two quintet types exist differing only by the presence of a small insertion (3). There are, however, a few dispersed representatives of these genes, called orphons, though there is no evidence that they are expressed (4). At the other extreme are chickens where there are about 6 H1 genes and 8-10 of each core histone gene, mostly scattered on two 50 kb sections of DNA (5), but some variant genes, the $H2A_F$ and H5, are present elsewhere (6,7).

Quite separate from the macro organisation of the histone genes is their individual sequence and pattern of expression. As discussed in several recent reviews (2,8,9,10), histone genes may be restricted in expression to the S-phase, or expressed

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independently of DNA synthesis. In addition they may be restricted to a particular part of the life cycle, like the early genes of sea urchins, or to a particular cell type, like the H5 gene. Only in sea urchins is there a clear correlation between structure and function of their histone genes. These several hundred early genes, expressed between oocyte maturation and gastrulation, are of the conserved, tandemly repeated, quintet type. The genes expressed at later stages are of the disorganised type, like those of chickens and mammals.

We have recently made a preliminary analysis of the histone genes of Xenopus borealis (1). In terms simply of organisation they fit the sea urchin pattern. Genomic Southern blotting shows that about 60% of the genes are present in a single kind of quintet, though it was not clear that they were tandemly repeated. The other genes had some other kind of organisation. Independently it was shown that the same major H4 mRNAs were present throughout the life cycle and in a number of adult cell types (11), though it was not known from which kind of gene they came.

In this paper we establish the detailed organisation of the major gene clusters of X. borealis and establish when and where they are expressed.

MATERIALS AND METHODS

Animals

Xenopus laevis were obtained from the South African Snake Farm, Fish Hoek, South Africa. Xenopus laevis borealis were the first generation raised at Warwick from animals collected in the Kibwezi Forest, Kenya.

Library Construction and Screening

High molecular weight genomic DNA was prepared as previoulsy described from the blood of a single X. borealis female (lane 8, Fig. 3, Ref. 1) and partially digested with Sau 3A. Sucrose gradient fractions containing fragments in the 15-20 kb size range were pooled and ligated to purified Bam HI arms of the vector λ L47.1 (12). Recombinant phage were selected by plating on the P2 lysogenic strain WL 95. Approximately 1 million recombinant phage were screened by the method of Benton and Davis (13) using a mixed H1 and H4 hybridization probe. The H4 probe was the 381 bp Bam HI insert from the H4 cDNA clone pcXlH4W1 (14) and the H1 probe was a 424 bp Msp I fragment from the X. laevis genomic clone XLHW19 containing 266 bp of the coding region and 158 bp of 3' non-coding region (15). An approximately equimolar mixture of these two DNAs was nick-translated with 32P-dCTP and dGTP (16).

Screening of the EMBL 3 partial Sau 3A genomic library was performed using a 0.8 kb Hind III/Sac I fragment from clone λXBH302, marked as probe A in Fig. 1A. DNA was prepared from positive plaques by the rapid plate lysate method (17).

Southern Blot Hybridizations/Gel Analysis/Nuclear Microinjection

The nuclear microinjection procedure, DNA and protein gel analysis and the Southern blot hybridizations were exactly as described in Old et al. (18). The probes used for the Southern blots were as described above for H1 and H4. The H3 probe was a 165 bp Sau 3A/Bam HI fragment from XLHW23 encoding amino acids 74-128 of the H3 protein (19). The H2A probe was a 798 bp Sac I/Xba I fragment from XLHW8 encoding the 3' half of the H2A protein and 549 bp of 3' non-coding region (15,18,20). The H2B probe was a 153 bp Bam HI/Eco RI fragment from XLHW11 encoding amino acids 13-63 of the H2B protein (18,20).

In Situ Hybridization

The hybridization probe was the X. laevis H4 cDNA clone pcXlH4W1 (14) nick-translated (16) using ^{3}H-TTP (40-50Ci/mmole, Amersham). The labelled DNA was phenol-chloroform extracted and ethanol precipitated using E. coli tRNA as carrier. Specific activities varied from 4-8 x 10^6 cpm/μg.

Mitotic chromosomes were made from gut epithelial cells of animals previously injected with colchicine (21).

Prior to hybridization the chromosome preparations were treated with ribonuclease A (100μg/ml in 2 x SSC) for 1 hour at 37°C, washed in 2 x SSC and dehydrated in ethanol. The chromosomal DNA was denatured by submerging the slides in 0.07M NaOH for 3 minutes followed by washes in 70%, 95% and 100% ethanol and air drying. The hybridization reaction contained 40% formamide in 4 x SSC, 0.1M Na_3PO_4, pH7 and a probe concentration of 1-2 x 10^5 cpm/μl. 5-10μl of probe was placed on a mitotic chromosome preparation, a coverslip was added and the edges were sealed with rubber solution. The slides were incubated at 37°C for 19-20 hours. After hybridization and removal of the rubber solution and coverslips the slides were washed at 65°C for 1 hour in 2 x SSC to remove non-specifically bound radioactivity. The dried slides were coated with Kodak NTB_2 diluted 1:1 with H_2O, and left at 5°C for 18-21 days. The autoradiographs were developed in Kodak D19 for 2½ minutes, at room temperature, fixed for 5 minutes, washed in H_2O for 30 minutes and stained with Giemsa.

S_1 Nuclease Assays

RNA was made from oocytes, tadpoles and adult tissues as described previously (11). The hybridization probes were all single-stranded M13 subclones of λXBH302 uniformly labelled with ^{32}P-dCTP and DNA polymerase 1 (Klenow fragment) using the M13 universal primer (22).

Hybridization reactions using the amounts of RNA given in the figure legends were carried out essentially according to Berk and Sharp (23) and gel analysis was as performed in our earlier work (11,24).

Figure 1 Genomic Clones Containing Histone Genes from X. Borealis. (A) Organisation of λ L47.1 clones of the major histone cluster type. In λXBH302, the position of a DNA fragment used as a probe for the EMBL3 library and for the experiment of Fig. 2 is marked as Probe A. (B) Organisation of EMBL3 clones of the major histone cluster type. (C) Organisation of λ L47.1 clones of the minor cluster type. (D) A rare/unique histone cluster in λL47.1. The restriction enzymes used have been abbreviated as follows: B, Bam HI; E, Eco RI; H, Hind III; X, Xba I; S, Sal I; T, Sst I/Sac I; M, Sma I; K, Kpn I; O, Xho I; V, Pvu II; P, Pst I; Sp, Sph I. Not all enzymes listed have been used to map all clones. Clone λXBH152 is thought to contain a double insert.

RESULTS

Organisation of X. borealis Histone Clones

On initial screening of the λ L47.1 genomic library with a mixed H1/H4 probe, 11 independent clones were isolated. Restriction mapping and subsequent probing of Southern blots with specific gene probes as detailed in materials and methods and DNA sequence analysis (not shown) gave rise to the data shown in Fig. 1.

A group of related clones were isolated that had exactly the organisation predicted from our earlier genomic mapping studies (1) and are therefore

representatives of the major cluster type in X. borealis (Fig. 1A). The gene order of this major cluster is H1, H2B, H2A, H4, H3 and from sequencing data (not shown) the H2B and H4 genes have the opposite polarity to the other 3 genes. We have previously shown that the copy number of this major cluster type is 60-70 copies per haploid genome (1).

A second group of clones were obtained (Fig. 1C) that contain a cluster of histone genes that are organised differently to the major cluster type. The gene order of this minor type is H2A, H4, H3, H1. Several of the restriction fragments that make up this minor cluster were observed as minor bands of hybridization on genomic Southern blots in our earlier studies (1). From the relative intensities of the bands we can now estimate that this minor cluster type is present at approximately 10-15 copies per haploid genome. None of the clones isolated contains an H2B gene as judged by mapping and sequencing experiments (not shown), but the position of the four coding regions near one end of each isolate makes it likely that this cluster type contains at least one copy of each of the 5 histone classes.

Two independent isolates of a third cluster type were also obtained. Only one of these was analysed in detail as initial mapping showed them to be very similar (Fig. 1D). This cluster has the following organisation: H1, H4, H2B, H2A, H1 (no H3 gene present) with the H2A and H2B genes having opposite polarity. Again restriction fragments that comprise this clone were observed in our earlier genomic mapping experiments and we can now conclude that this cluster type has a copy number of less than 5 per haploid genome. This gene organisation is unusual in that 2 H1 genes are present but no H3 gene has been cloned and thus this cluster type cannot be a simple quintet. Some genomic clones isolated from X. laevis do not have a quintet structure in that the cluster contains 2 H4 genes (26).

We note that the gene-containing, restriction fragments for the major and minor cluster types generated by Eco RI cleavage of X. borealis genomic DNA are not easily resolved on gels but are clearly separable when Bam HI is used. This highlights the pitfalls of attempting to map and analyse repetitive gene families using genomic blots and it may account for some of the discrepancies in the literature regarding histone gene organisation in X. laevis (25,18,26).

Tandem Repetition of the Major Cluster

To try to establish whether the major cluster is tandemly linked, another X. borealis genomic library, constructed in EMBL 3 (a gift of C. Wilson) was screened using a small fragment from the left-hand end of clone λXBH302 (probe A, Fig. 1A).

The restriction maps of two clones from this library, λXBH9 and 11 (Fig. 1B), suggest that at least one pair of major clusters are contiguous. Clone λXBH9 contains a single Sph 1 site in the intergenic region which allowed us to ask if the majority of the H4 genes in the genome were present in this organisation. If most of the major repeats

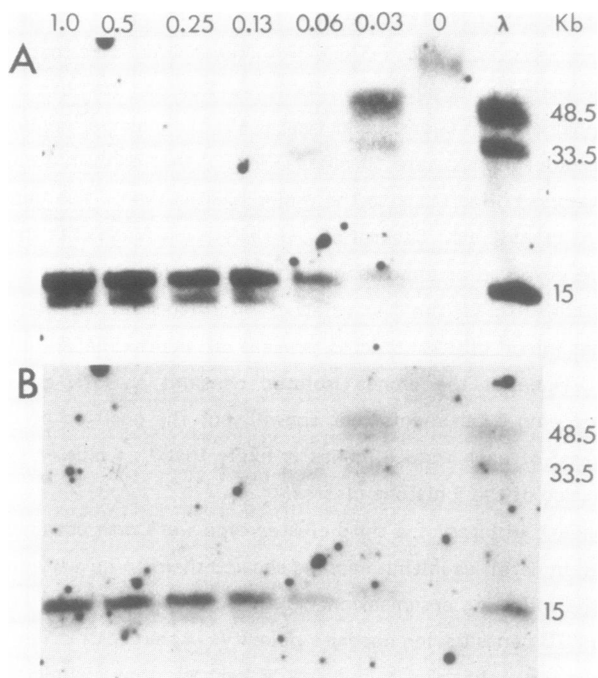

Figure 2 Southern Blot of Totally and Partially Sph 1 - digested Genomic DNA. Panel A was probed with the H4 cDNA clone, pcXlH4W1. Panel B was probed with the HindIII/SstI intergenic probe, specific to the major histone gene cluster, marked as probe A in Fig. 1/A. 2 µg of genomic DNA was digested for 1h at 37°C at from 1.0-0 units of Sph 1/µg DNA, as shown above the lanes. Mixed radioactive markers of digested and undigested λDNA were also run, their sizes being indicated on the right. The DNA was electrophoresed on a 0.4% agarose gel for 24 hours which was acid-treated before blotting.

are present as a tandemly repeated 15 kb unit, then an Sph 1 digest of genomic DNA, probed with an H4 probe would be expected to show a single strong band of 15kb. as well as some very minor bands. The actual experiment is presented in Fig. 2A and shows a predominant 15 kb band. Partial digests also generate a 30kb band, which is entirely consistent with tandem repitition. Larger molecules are not resolved on the gel. There is also a more minor band of 14.2kb in the limit digest. This is probably, at least partly, a minor variant of the major cluster, since it hybridizes, as seen in Fig. 2B, with a major cluster-specific probe, probe A of Fig. 1A. It is not the minor cluster, since a probe from between the H3 and H4 genes of λXBH61 reveals a band slightly smaller than 14kb (data not shown); this is sufficiently close to the lower H4 band of Fig. 2A for it to be subsumed into it in the H4 probing experiment.

Thus the intercluster organisation seen in clone λXBH9 of Fig. 1B seems the

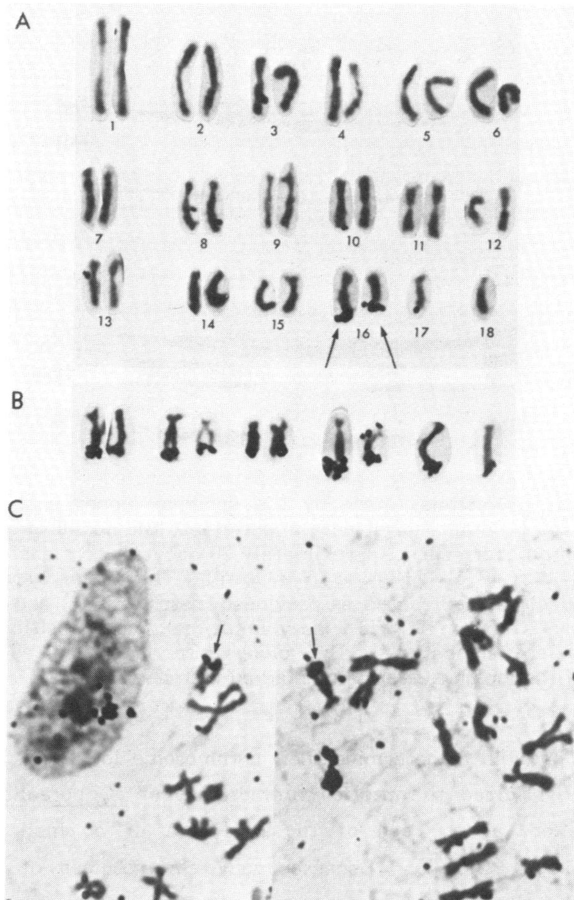

Figure 3 In situ Hybridization Experiments. (A) A mitotic complement of X. borealis after in situ hybridization with ^{3}H-labelled, H4 coding sequences. One pair of chromosomes is labelled at the end of the long arm (arrows). Because of similar length and centromeric position the labelled pair could occupy any one of the positions 14 to 17. (B) Several examples (from the same animal as in A) of terminally labelled chromosomes after in situ hybridization with the same histone probe as in A. (C) A partial mitotic complement of X. borealis after in situ hybridization as above. The arrows indicate the two labelled chromosomes. The interphase nucleus (top left) shows a typical labelling pattern (see text).

predominant arrangement in the genome, and the majority of the major clusters must therefore be tandemly linked with a repeat length of 15 kb. This value fits well with our previous genomic mapping data (1).

Chromosome Location

We reasoned that if the 60-70 copies of the major cluster were tandemly linked

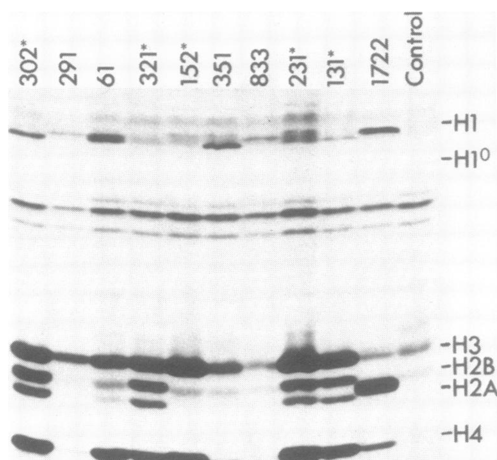

Figure 4 Analysis of histones made by the genomic clones analyzed by SDS gel electrophoresis. The genomic clones were injected into the nuclei of X. laevis oocytes, which were incubated overnight. Approximately 20 oocytes from each group were then incubated overnight in 40 µl ^{3}H-lysine (5 mCi/ml). The nuclei were dissected from these oocytes, selectively extracted as previously described (18) and one third of the sample was run on a 40 cm SDS/18% acrylamide gel. The positions of stained X. borealis histones are indicated. The clones are identified above the tracks, representatives of the major cluster being marked with an asterisk.

then it would be possible to determine their chromosome location. When H4 coding sequences were hybridized to mitotic chromosomes of X. borealis one locus was prominently labelled, at the end of the long arm of a small sub-metacentric chromosome (Fig. 3A and B). Because many chromosomes of the X. borealis complement have similar length and centromere position, precise identification of the labelled chromosome pair is not possible without quinocrine banding. Although Fig. 3 shows the labelled chromosome pair at the position 16, it can be any one from 14 to 17.

The interphase nuclei also showed only 1 or 2 labelled regions (Fig. 3C, top left) which is suggestive of a single locus containing the majority of the histone sequences. However, because the total number of silver grains in the autoradiographs is low, scattered histone sequences would not be detected in our preparations.

Expression of the Genes Following Microinjection into X. laevis Oocytes

To establish whether the genomic clones encoded functional histone genes that corresponded to the histone proteins extracted from X. borealis tissues. the linear DNAs were microinjected into X. laevis oocyte nuclei. This experiment would also give a preliminary indication of the degree of diversity of the histone genes which is valuable since analysing them all in detail would be a major undertaking. After overnight incubation the oocytes were labelled with ^{3}H-lysine and ^{35}S-methionine for

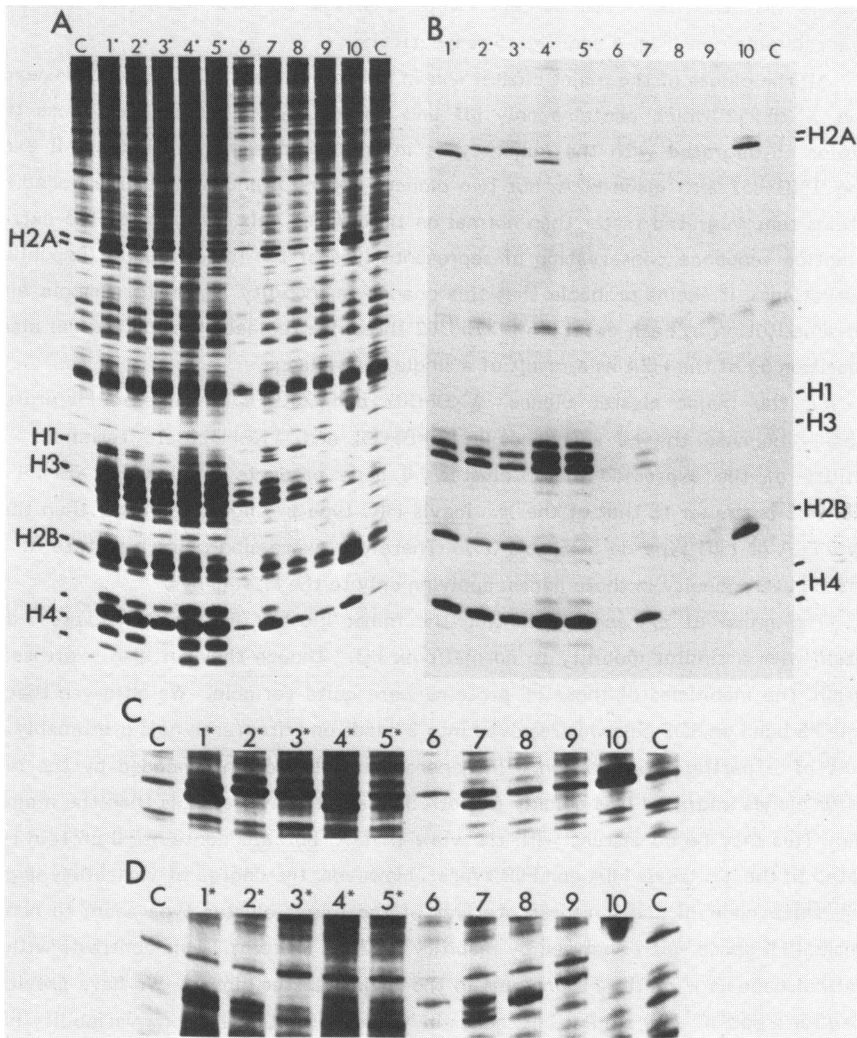

Figure 5 Analysis of histones made by the genomic clones analyzed on Triton/Acetic acid/Urea gels. One third of the same samples as in Fig. 4 was run on a 40 cm triton/acetic acid/urea gel. A shows a 2 month exposure. That in B is 6 hours. C and D are enlargements of the H2A and H1 regions of the longer exposure. The clones injected were as follows: 1, λXBH302; 2, λXBH131; 3, λXBH321; 4, λXBH231; 5, λXBH152; 6, λXBH291; 7, λXBH61; 8, λXBH351; 9, λXBH833; 10, λXBH1722; C, control oocytes. The positions of marker X. borealis erythrocyte histones are marked. Representatives of the major cluster are marked with an asterisk.

24 hours and the extracted proteins were analysed on both SDS and triton/urea polyacrylamide gels (Fig. 4 and Fig. 5 respectively).

All the clones of the major cluster shown in Fig. 1A made H2B, H3 and H4, except clone λXBH152 which contains only H3 and H4 sequences, and in every case these proteins co-migrated with the major types in bulk histone preparations. All except clone λXBH152 also made H2A, but two clones, λXBH231 and λXBH302 encoded H2A proteins that migrated faster than normal on triton/urea gels. In view of the extreme nucleotide sequence conservation of representatives of the major cluster (unpublished observations), it seems probable that this change in mobility is due to a single amino acid substitution in each case. In λXBH302 threonine replaces the more usual alanine at position 53 of the H2A as a result of a single base change.

Of the major cluster clones, λXBH302 and λXBH131 expressed H1 proteins, probably because the H1 sequences in λXBH231 and λXBH321 are terminal. The mobility of the expressed major cluster H1 gene products in clones λXBH131 and λXBH302 is similar to that of the X. laevis H1C type in migrating faster than the X. laevis H1A or H1B type on SDS gels (15). Therefore expression gives a picture of very limited heterogeneity in these genes, applying only to the H2A genes.

The group of clones representing the minor cluster (Fig. 1C) all expressed H3 protein with a similar mobility to normal/bulk H3. Though they all also expressed H1 protein, the mobilities of these H1 proteins were quite variable. We observed that the single H1 band on SDS gels was resolved into 2 bands on triton/urea gels presumably as a result of a partial modification. In general, the H1 proteins encoded by the minor cluster clones migrated less quickly on both SDS and triton/urea gels than the major H1 type. This may be consistent with the view that X. borealis contains H1 protein types related to the X. laevis H1A and H1B types. However, the degree of variability is quite large since none of the 4 representatives of the minor cluster type seem to contain identical H1 genes when analysed by mobility on 2 gel systems. This contrasts with the identical behaviour of the H1 proteins in the major cluster clones. We have previously noted for a pair of very similar H1C genes in X. laevis (15) that H1 gene variability is not restricted to amino acid substitutions but includes signficant insertions and deletions.

Clone λXBH61 seemed to generate two labelled protein bands in the H1 position on SDS gels (and 3 on triton/urea). Further analysis of this clone reveals only one H1 coding region present which must therefore give rise to these two bands. The most likely explanation is a post-translational modification of some of the newly synthesised protein. Nucleotide sequence analysis will hopefully explain this observation.

Clone λXBH1722, containing a rare cluster type, expressed a normal H4 protein but variant H1, H2A and H2B proteins. Since only one H1 protein band is visible by gel analysis, either the two H1 genes present are identical or one is not expressed. If the

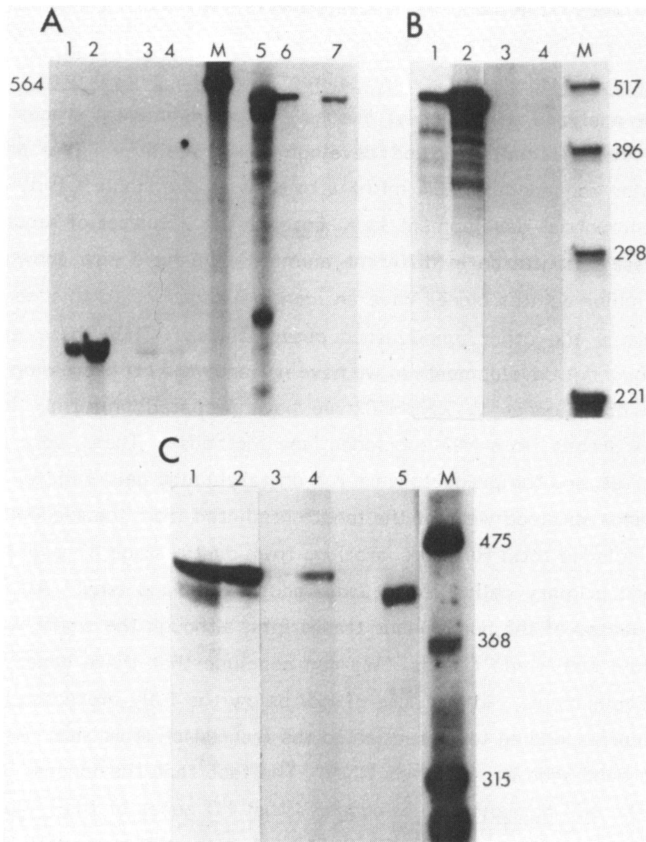

Figure 6 S_1 nuclease analysis of histone gene transcripts from the major cluster clone λXBH302 in X. borealis tissues. (A) Analysis of H2B (lanes 1-4) and H2A expression (lanes 5-7). For H2B we used an M13 subclone from a Hind III site within the gene, through the 3' end of the gene. Protection to the 3' end of the mRNA would give 206n. For H2A we used an M13 subclone spanning the entire transcribed region from an upstream Hind III site to a downstream Bam HI site. Protection of the entire mRNA would give 501 n. Lanes 1 and 5, 63 µg ovary RNA; 2 and 6, 10 µg tadpole RNA; 3, 21 µg liver cell RNA; 4 and 7, 2.5 µg heart cell RNA; M is λHind III marker. (B) Analysis of H1 expression using an M13 subclone from a Sal I site within H1 through the 3' end of the gene. Protection to the 3' end of the mRNA would give 469n. Lane 1, 54 µg ovary RNA; 2, 8 µg tadpole RNA; 3, 12.5 µg lung cell RNA; 4, 10 µg heart cell RNA; M is pAT153 Hinf I marker. (C) Analysis of H3 expression using an M13 subclone from a Bam HI site through the 5' end of the gene. Protection to the 5' end of the mRNA would give 432n. Lane 1, 63 µg ovary RNA; 2, 10 µg tadpole RNA; 3, 21 µg liver cell RNA; 4, 12 µg lung cell RNA; 5, 42 µg X. laevis ovary RNA; M is pBR322 Taq I marker.

latter is true, this would probably be the H1 gene near the end of the clone which may lack some essential sequence. The very anomalously migrating H2A and H2B proteins result from a 2 amino acid deletion (ala-pro) relative to the major H2B at position 10/11

and 2 substitutions of threonine in H2A relative to the major H2A at positions 124 and 126. (Z. Frearson unpublished results).

Developmental and Tissue Specific Expression of the major quintet genes

We have analyzed a number of tissues and developmental stages to see if the major cluster histone genes showed developmental regulation. We previously used primer extension sequencing of H4 mRNAs to show that a single family of transcripts was present throughout development in X. borealis (11). This set of sequences differed by a single base substitution in different animals. We have now shown that the H4 genes of the major quintet clones have an identical sequence to this some having one variant and some the other (unpublished observations). Thus these genes must be expressed throughout development so we have not analysed H4 expression further.

For the other genes in λXBH302 we have prepared uniformly labelled single stranded DNA probes from M13 subclones (see Methods). These were utilized in S_1 nuclease analyses, and the products were run on acrylamide gels (Fig. 6). In every case the longest bands obtained were of the length predicted from the sequence of the gene. The analysis included total RNA preparations from ovary. stage 47 swimming tadpoles, adult liver and primary cell cultures from adult heart and lung. All generated the fragments expected of the homologous transcripts, although the signal was lower from adult than from embryonic tissues. We can conclude that these genes are expressed throughout development. In the case of H3, below the fully protected band is seen a weaker band corresponding to protection to the translation start site. As expected this is the largest band seen in X. laevis RNA. The fact that the longest band is always strongest in the X. borealis tissues suggests that H3 genes of the major cluster type always make up the bulk of the H3 mRNA, at least in the tissues tested. For each gene there are a range of more minor. smaller bands which will certainly represent transcripts with sequence differences (noteably for H1), partially degraded RNA molecules, internal cuts in the RNA/DNA hybrids and probe radiolysis.

DISCUSSION

In this paper we have proven that our earlier map of the major histone cluster in X borealis based on genomic Southern blots is correct being H1, H2B. H2A. H4, H3. In addition we have determined that the H2B and H4 genes have the opposite polarity to the other 3 genes. The repeat unit length is 15 kb and the chromosomal location of this tandemly repeated major cluster is near the end of one of the shortest sub metacentric chromosomes. The gross organisation of the major cluster is similar to that in Drosophila (3,4), both in terms of gene order and polarity but not in repeat length yet different from sea urchin (27,28), newt (29), X. laevis (18 26 30) and X tropicalis (31). This is not very likely to occur by chance. However. considering the relatively small

number of species so far investigated there seems to be no need to invoke horizontal gene transfer to explain this observation (32).

The more unusual finding is that in the most studied Xenopus species. X. laevis no clone has yet been isolated with the same organisation as this major X borealis cluster. The H1B and H1C X. laevis clusters (15,18,26) are circular permutations of the gene order of this X. borealis major cluster but the gene polarities are not the same. In X. laevis, the H1B/C clusters have the H2A gene as the only one of opposite polarity whereas in X borealis the H4 and H2B genes are of reverse polarity. It is therefore clear that these cluster types are not simply related. In fact nucleotide sequence data (unpublished) suggests that the X borealis major cluster is more closely related to the X. laevis H1A type than the H1B/C type. In addition, the two X. tropicalis clones described in the literature (31) are not easily fitted to the X. borealis major type. Clearly there have been considerable changes in cluster organisation since the divergence of these species. The very high homogeneity in this cluster, together with its marked difference to that in X. laevis is comparable to phenomena identified in other genes. Its origin is an interesting evolutionary problem, but before adding to existing speculation it would be wise to thoroughly examine population variation in the major histone cluster across the full range of the species.

The X. borealis minor cluster H1, H3, H4, H2A, (H2B?) has some very strong similarities to the H1B cluster type in X. laevis. The gene order, polarity and preliminary sequence information support this. In fact this is a rather surprising observation considering the marked differences noted above for the major cluster.

The unique X. borealis cluster λXBH1722 has no direct similarities to any X. laevis clones yet isolated. However, the X. laevis H1A cluster type contains two H4 genes per cluster (26), whereas the unique X. borealis cluster has two H1 genes present. This feature of histone clusters containing more than one copy of certain histone gene types is common in higher eukaryotes such as chicken (5) and man (2).

Taken together, these observations suggest that the processes contributing to the evolution of histone genes are diverse. For some clusters gross organisation such as gene order, polarity and even nucleotide sequence have been conspicuously conserved although gene spacing has altered somewhat. In other cases the gene polarities have been dramatically changed suggesting that evolutionary processes such as gene inversions have acted at the level of individual genes and not on whole clusters. More detailed nucleotide sequence information will be needed to clarify these events, however, transposon like sequences have been observed in X. laevis histone gene clusters (26).

The in situ hybridization data support our earlier conclusions (11) that the major and minor X. borealis clusters evolve independently. Since in X. laevis the H1B cluster

type (to which the X. borealis minor type is related) is tandemly reiterated we are hopeful that by choosing a suitable cluster specific probe we can discover more about the chromosomal organisation of the minor types in X. borealis

The microinjection experiments presented here illustrate an unexpected degree of protein diversity among X borealis histone proteins. In the case of the most anomalously migrating H2A and H2B proteins in λXBH1722 the preliminary sequence data (Z Frearson unpublished results) indicate that there is a two amino substitution in the H2A and a two amino acid deletion in the H2B The protein gels indicate that at least 3 different H1 species are present in our clones This is not entirely surprising in view of the H1 gene diversity we have found in X laevis (15) and a detailed sequence analysis is underway.

Our S_1 nuclease assays did not detect regulation in the developmental or tissue specific expression of genes in the major cluster

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