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Histone gene number and organisation in *Xenopus: Xenopus borealis* has a homogeneous major cluster

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ABSTRACT

Using a Xenopus laevis H4 cDNA clone as a probe we have determined that the numbers of H4 histone genes in Xenopus laevis and Xenopus borealis are approximately the same. These numbers are dependent on the hybridization stringency and we measure about 90 H4 genes per haploid genome after a 60°C wash in 3 X SSC. Using histone probes from both Xenopus and sea urchin we have studied the genomic organization of histone genes in these two species. In all of the X.borealis individuals analyzed about 70% of the histone genes were present in a very homogeneous major cluster. These genes are present in the order H1, H2B, H2A, H4 and H3, and the minimum length of the repeated unit is 16kb. In contrast, the histone gene clusters in X.laevis showed considerable sequence variation. However two major cluster types with different gene orders seem to be present in most individuals. The differences in histone gene organization seen in species of Xenopus suggest that even in closely related vertebrates the major histone gene clusters are quite fluid structures in evolutionary terms.

INTRODUCTION

In all eukaryotes so far analyzed the great majority of histone genes are grouped into structures which we shall refer to as clusters. A cluster need not contain all of the five histone gene types nor need equal numbers of each gene type be present. In most of the eukaryotic organisms studied, the histone genes are reiterated, and in some cases a simple tandem repetition of a particular cluster accounts for most of this reiteration. To date, wherever a repeating histone cluster has been found, it contains one gene for each of the five types of histone.

Yeast contains two unlinked copies of H2A-H2B gene pairs, which are distant from the H3 and H4 genes (1). Sea urchins contain 300-600 more or less identical copies of a short histone repeat and the genes in this major repeat are expressed in early development (2,3,4,5). There are also about 10-20 copies of other different clusters which are expressed later in development (6). The histone gene organisation in Drosophila is similar to

that in sea urchins in that the 100 copies of the major cluster are tandemly linked in a short repeating unit (7).

In mammals and birds there are only 10-20 histone genes (8,9,10) and although they are clustered, there is no major type of cluster appparent (11,12,13,14). Furthermore, cloned clusters from chicken, mouse and man often contain more than one copy of a particular histone type (11,12,13,14).

Recent studies of X.laevis suggest that frogs may have an intermediate type of organisation, in that they contain only 20-50 copies of each histone gene and some of these genes are present in a major type of cluster with a constant gene order (15). However clones of differing gene order have been isolated (16). This intermediate type of organisation does not apply to Amphibia as a whole since it has been found that urodeles contain very large numbers of histone genes, for example 600-800 in newts and 2500 in axolotls (17), and in the newt, Notophthalmus viridescens, most of these are present as a homogeneous major repeat. However, these 9kb clusters are separated by at least 50kb of sequence that is predominantly satellite (18).

We have now found that X.borealis, a frog that can interbreed with X.laevis, has its histone genes organized in a homogeneous major cluster like that found in N.viridescens.

MATERIALS AND METHODS

Animals

Adult Xenopus laevis laevis were obtained from the South African Snake Farm, Fish Hoek, South Africa. All except one of the adult Xenopus borealis were the first generation raised in our own laboratory from various frogs collected in the Kibwezi forest, Kenya. The exception was a female (Fig. 3, lane 9) given by Dr. P.J. Ford, which was from a separate isolate from the wild, and derived originally from Dr. A.W. Blackler.

Preparation and Purification of DNA

High molecular weight genomic DNA was prepared from blood by RNAse and proteinase digestion of red cell nuclei, followed by phenol extraction (19). Supercoiled plasmid DNAs were isolated by the method of Colman et al. (20) and inserts prepared by elution from agarose gel slices followed by DE52 chromatography. 32P-labelled hybridization probes were made by nick-translation (21) or, for the H2A probe, by the exonuclease/repair reaction of T4 DNA polymerase (22).

Hybridization Probes

pcXlH4W1 and pcXbH4W1, X.laevis and X.borealis H4 cDNA clones in the

vector pAT153, were used as probes for H4 sequences (23). The purely coding Stronglyocentrotus purpuratus subclone pSp3-1 was used as an H3 probe. It contains 235bp of the coding region from amino acid no. 51-129 in pBR322 and was a generous gift of Prof. L.H. Kedes. An H2A probe was made by labelling 50-100 nucleotides around the unique Hind III site in the S.purpuratus H2A gene in the clone pSp117 (3) using the exonuclease repair reaction of T4 DNA polymerase (22). The H2B probe was a 1kb Eco RI/Bam HI fragment from the S.purpuratus clone pSp102 which contains all the H2B gene and some spacer sequence (3). The H1 probe was a X.laevis genomic subclone containing the 3'- end of an H1 gene and less than 100bp of spacer sequence (our unpublished results).

Southern Blot Hybridizations of Genomic DNA

After digestion with restriction enzymes the genomic DNA (usually 2µg) was electrophoresed on 0.7% agarose gels for 16 hours at 20mA in 40mM Tris pH 8.3, 20mM sodium acetate, 2mM EDTA, 0.1µg/ml ethidium bromide. After Southern transfer (24) filters were prehybridized in 3 x SSC containing 0.2% each of bovine serum albumin, Ficoll, polyvinyl pyrollidone, 0.1 mg/ml poly A and 0.2mg/ml E.coli nucleic acid for 16 hr at 68 or 65°C, depending on the probe.The denatured probe was added in fresh buffer and hybridized for 24 hr at the temperature of the pretreatment. The filters were then given several 10 min washes, firstly in 3 x SSC and then once in 1 x or 0.1 x SSC at either 55, 60, or 65°C, dried and exposed to Fuji RX film at - 70°C with an intensifying screen. Size markers were radiolabelled Eco RI/Hind III double digests of lambda DNA, and/or Hinf I digests of pAT153.

Filter Hybridizations to Determine Gene Numbers

Stock solutions of sonicated DNA (either Xenopus genomic or reference cloned H4 histone DNA with E.coli carrier) in 1 x SSC were divided into 0.4ml aliquots (approx. 25µg),an equal volume of 1M NaOH added and the samples incubated at 37°C for 30 min. The denatured DNA samples were neutralised and then passed through numbered, prewashed (6 x SSC) 1cm^{2}circles of nitrocellulose in a vacuum filter apparatus. After a further wash in 6 x SSC, the filters were dipped in 70% ethanol and baked at 80°C for 2 hr. In control experiments it was shown that, providing the total amount of DNA passed through the filter was constant throughout, a fixed proportion of the DNA was bound. Hybridization conditions were the same as those for the Southern blots, except that the filters were treated batchwise.The filters were placed in scintillation vial inserts in 1ml of either 3 x or 0.1 x SSC and Cherenkov counted. For the thermal melts the filters were washed twice in

0.1 x SSC at the appropriate temperature and counted at 4°C in fresh 0.1 x SSC.

RESULTS

(a) Numbers of H4 genes in the X.laevis and X.borealis genomes.

Using known amounts of a linearized X.laevis H4 cDNA plasmid, pcX1H4W1 (23), as a reference,a calibrated Southern blot experiment was performed to estimate the number of H4 genes in the genomes of X.laevis and X.borealis (Fig.1A). The autoradiograph shows that while the majority of the H4 genes in X.borealis fall into a single size class of 2.2kb on digestion with Bam HI, those in X.laevis are split among 3 major Bam HI fragments of 5.1, 3.5, and 2.7kb. Many minor bands are visible after longer exposures, especially in X.laevis (see Fig.3). Densitometric scans of this autoradiograph indicate that for these individuals, about 70% of the H4 genes in X.borealis fall in the major band while the 3 major bands represent roughly 10, 20 and 30% of the H4 genes in X.laevis.

Fig.1B shows the calibration graph derived and the corrected haploid H4 gene number obtained at this hybridization stringency (1 x SSC wash at 65°C). This is 73 genes for X.laevis and 68 genes for X.borealis in both major and minor bands.

For several reasons these values are likely to be underestimates. Firstly, it is difficult to produce an exposure which allows detection of the minor bands without saturation of the major bands. Secondly, hybridization of the probe to the reference cDNA on the filter will be more efficient, since it can form a perfect hybrid while some of the genomic H4 genes may mismatch and therefore hybridize less well. Thirdly, since the probe was from X.laevis, mismatching might be a more serious problem in the case of X.borealis.

To circumvent these problems the H4 gene numbers were also determined by filter hybridization experiments. The problem of mismatching was addressed by doing the experiments at a range of hybridization stringencies, which was facilitated using this method, since many filters could be processed at one time. This ease of handling permitted each measurment to be done in triplicate and more controls could be included. In particular, a X.borealis H4 cDNA clone, pcXbH4W1 (23), was used as the reference for the X.borealis genomic hybridizations (Figs.1C and 1D).

This experiment indicates that the haploid copy numbers of H4 genes at this stringency (3 x SSC wash at 60°C) are 87±5 for X.laevis and 89±5 for

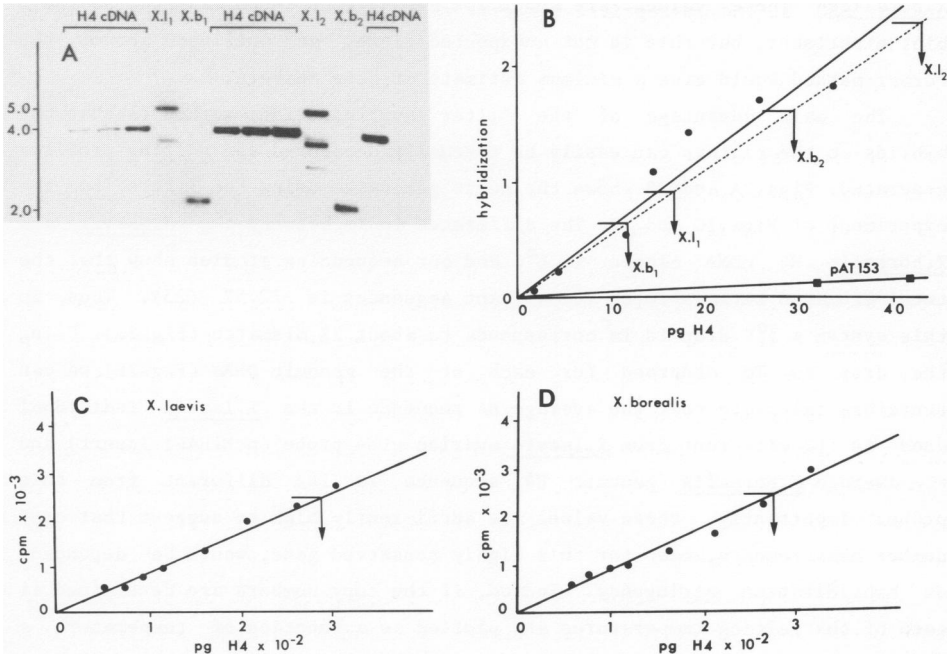

Figure 1, H4 Histone Gene Number Determinations. (A) Two accurately known amounts of X.laevis ($X.l_1$, $X.l_2$) and X.borealis ($X.b_1$, $X.b_2$) genomic DNA were digested with Bam HI and run on a 1% agarose gel together with Eco RI linearized pcX1H4W1 (23) and linear pAT153 (not shown). The gel was blotted, probed with insert from pcX1H4W1 and washed at 65°C in 1 x SSC. (B) From the gravimetrically determined dilution factors and the sequence composition of these plasmids the amount of histone DNA can be determined for each calibration track. The reference tracks of the autoradiograph were scanned to construct standard curves of radioactivity hybridized to H4 genes (in arbitrary units), versus pg H4 gene loaded for pcX1H4W1 (●) and versus pg H4 gene equivalent for pAT153 (■). To account for the hybridization due to contamination, the pAT153 curve has been subtracted from the pcX1H4W1 curve to give the corrected calibration curve (--). The mean haploid H4 gene numbers are 73 for X.laevis and 68 for X.borealis. Molecular weights in all figures are in kb. (C) Eight different gravimetrically determined amounts of pcX1H4W1, pcXbH4W1 (23) and pAT153 were loaded in triplicate onto nitrocellulose filters. Twelve filters were made for each of the genomic DNA samples and control filters of both lambda DNA and E.coli DNA were included to measure non-specific hybridization. The filters were hybridized to nick-translated insert from pcX1H4W1 at 65°C and washed at 60°C in 3 x SSC. The diagrams show cpm hybridized to pcX1H4W1 and (D) pcXbH4W1 sequences, after subtraction of background hybridization to the same amount of pAT153 and E.coli DNA present on each filter. The lines were fitted by linear regression. The amount of hybridization to the genomic DNA samples was converted to H4 sequence present as indicated by the arrows. The mean haploid H4 gene number for X.laevis is 87±5 and for X.borealis is 89±5.

X.borealis. These values are about 25% higher than those from the Southern blot experiment, but this is not unexpected since, as outlined above, the former method would give a minimum estimate of gene numbers.

The main advantage of the filter hybridization method is that the hybrids on the filters can easily be thermally denatured and melting profiles generated. Figs.2A and 2B shows the melts generated using the flters from the experiment of Figs.1C and 1D. The difference in Tm between the X.laevis and X.borealis H4 cDNA clones is 6°C and our sequencing studies show that the total mismatch between these recombinant sequences is 12.5% (23). Thus in this system a 1°C drop in Tm corresponds to about 2% mismatch (Fig.2A). Using the drop in Tm observed for each of the genomic DNAs (Fig.2B) we can therefore calculate that the average H4 sequence in the X.laevis individual used is 6% different from X.laevis ovarian cDNA probe (pcX1H4W1 insert) and the average X.borealis genomic H4 sequence is 14% different from this probe. Importantly, these values are sufficiently high to suggest that copy number measurements, even for this highly conserved gene, would be dependent on hybridization stringency. Indeed, if the copy numbers are determined at each of the melting temperatures and plotted as a function of temperature a sharp reduction in gene numbers is observed as the temperature is raised above 50°C in 0.1 x SSC (Fig.2C).

This marked dependence on hybridization stringency can account for the different gene numbers measured by the two methods described here and for the

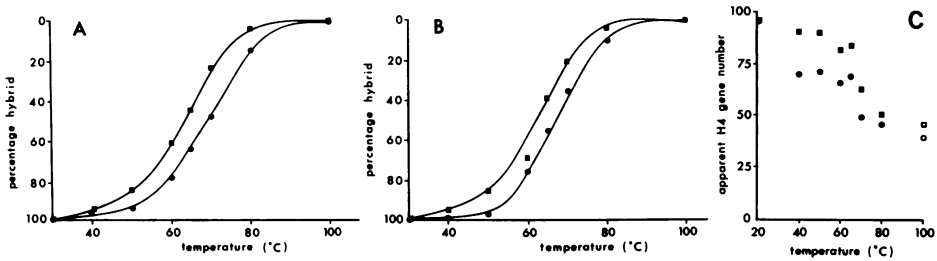

Figure 2. Stringency and H4 Gene Numbers. At each temperature the filters from the experiment of Fig. 1C and 1D were given two 10 min washes in 0.1 x SSC before being Cherenkov counted in fresh 0.1 x SSC at 4°C, correction being made for any pAT153 contamination of the probe. (A) Melting of nick-translated pcX1H4W1 insert from the H4 cDNA plasmids pcX1H4W1 (●) and pcXbH4W1 (■). (B) Melting of the same probe from X.laevis genomic DNA (●) and from X.borealis genomic DNA (■). (C) The apparent H4 gene numbers determined at each wash temperature. Some radioactivity remained bound at 100 °C (open symbols). X.laevis (●), X.borealis (■).

large range of previous values in the literature which were performed at various levels of stringency (8,15,25). Since there is always the possibility that at very low stringencies one may detect fragmented and/or related sequences which may not function as H4 genes, copy numbers for non-identical repeated genes cannot be determined precisely by such hybridization methods. With this proviso, our experiments indicate that X.laevis and X.borealis have similar H4 gene numbers which are around 80-95 copies per haploid genome.

(b) Individual Variation of Histone Genes in Xenopus.

Genomic DNA from several male and female adult X.laevis and X.borealis was digested with Bam HI and run on a 0.7% agarose gel, Southern blotted and then probed with nick-translated insert from pcXlH4W1 to reveal H4 genes. From Fig.3 which presents two exposures of the same blot, it can be seen that all six X.laevis show the same three major Bam HI fragments, although the intensities of these bands relative to each other and to the minor bands shows some variation (lanes 1-6). This is especially pronounced in lane 1. Many of the minor bands vary in size between individuals and these differences do not seem to be linked to the sex of the animal, since the animal of lane 6 was male.

The restriction enzyme Bam HI cannot cut the coding region of H4 genes without a change in the amino acid sequence. Consequently, from these data it is not possible to say whether the three major H4 fragments in X.laevis are restriction enzyme site variants of a similar major cluster or whether they are from totally unrelated genomic clusters. Our efforts to produce a genomic map of the histone genes in X.laevis result in some conflict with the proposals of Van Dongen et al. (15) and we shall return to this problem in the discussion.

Fig.3 also shows the remarkable similarity between four X.borealis individuals in the genomic Bam HI fragments which contain H4 genes. (lanes 7-10). Three of these animals were laboratory bred individuals, derived from a small number of frogs from a single locality in Kenya. It is therefore not possible to say how inbred these animals were. However, the animal of lane 9 was originally derived from Dr. A.W. Blackler's stock and is a completely separate isolate from the wild. The genomic organization of X.borealis histone genes seen in these experiments is therefore likely to be found in many, if not all, members of the species. From Fig.3 it can be seen that the major 2.2kb fragment and many of the next most major bands are conserved. Only the very minor bands (which are probably single copies) show size

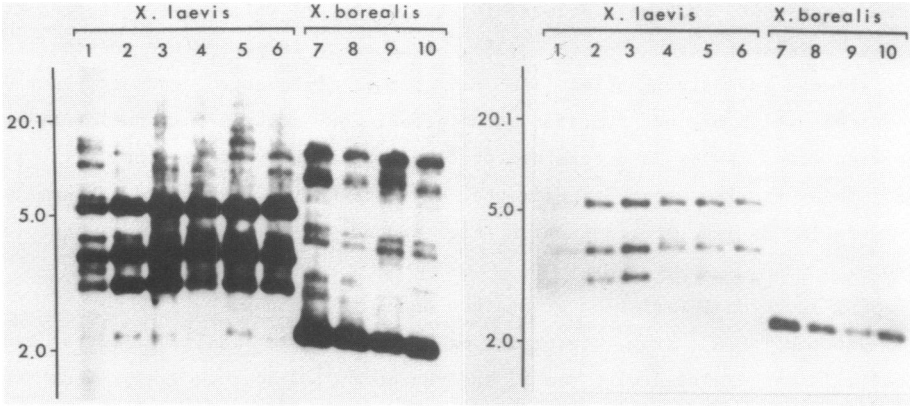

Figure 3. Comparison of Histone Gene Organisation in Several Xenopus Individuals. Approximately 4ug of genomic DNA from the species indicated were loaded in each lane. All animals except No. 6 were female. Exposure times were 1 day and 15 days.

variation. Since digests with several other restriction enzymes show this same high degree of conservation it follows that any map of the major histone gene cluster of one X.borealis individual will be very similar to that of the other individuals. Thus only the DNA from the animal of lane 7 was further analysed in detail. The very strong intensity of the major band facilitated the mapping of this major cluster.

(c) Histone Gene Organisation in X.borealis.

Fig.4 shows the H4-containing fragments after digestion of X.borealis DNA with a variety of other enzymes. Digestion with Hind III shows that the H4 gene in the major cluster is on a 2.05kb fragment (lane 2) and that double digestion with Hind III and Bam HI gives a 1.0kb fragment containing the H4 gene (lane 3). This convenient overlap enables easy mapping in both directions from the H4 gene. As shown in Fig.5A, the H3 gene is on the same Bam HI fragment as the H4 gene but on a different Hind III fragment. The opposite is true for the H2A gene (Fig.5B) which is on the same Hind III fragment as H4 but on a 6.7kb Bam HI fragment. Thus the gene order is H2A, H4, H3. The digests of Figs.5C and 5D map the H2B and H1 genes on the H2A side of the H4 gene with the H2B gene nearest to H2A.

All the data of Figs.4 and 5, together with other information such as partial digests (not shown) are compiled in Fig.6 which shows a complete map of the X.borealis major histone cluster.

(d) Properties of a X.borealis Minor Histone Cluster.

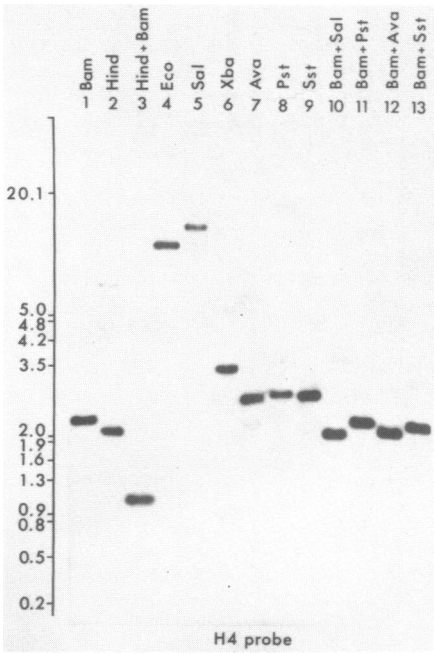

Figure 4. Mapping of the H4 Gene in the Major Cluster of X.borealis. A Southern blot of X.borealis genomic DNA digested with a variety of restriction enzymes was hybridized with the H4 probe, (pcX1H4W1 insert). In Figs. 4 and 5 the following abbreviations are used for restriction enzymes: Ava for Ava I, Bam for Bam HI, Eco for Eco RI, Hind for Hind III, Pst for Pst I, Sal for Sal I, Sst for Sst I and Xba for Xba I.

In a previous publication we described a X.laevis ovarian cDNA clone, pcX1H3W1, which contains 22bp of the 3′ coding region and the entire 3′ non-coding region of an H3 gene (23). If the Southern blot of Fig. 5A is reanalyzed using this probe then the strongest band visible is not that of the major cluster, which hybridizes only faintly, but one of the minor clusters. This observation shows that a small number of X.borealis H3 genes have 3′ non-coding regions which are more similar to that of a X.laevis oocyte H3 mRNA than they are to the H3 genes in the major X.borealis cluster. From the sizes of the minor bands visible in the Southern blots of Fig.5 it is clear that other histone genes are linked to these minor H3 genes and while a complete gene order cannot reliably be deduced one can confidently say that these minor H3 genes are part of a histone cluster.

DISCUSSION

(a) Histone Gene Reiteration Frequency

We find that X.laevis and X.borealis have the same numbers of H4 genes. Since both are tetraploid species, with similar DNA contents (26,27), this is not surprising. The value of 80-95 that we obtain for X.laevis is similar to Sommerville's value of 50-86 (25), but greater than the estimates

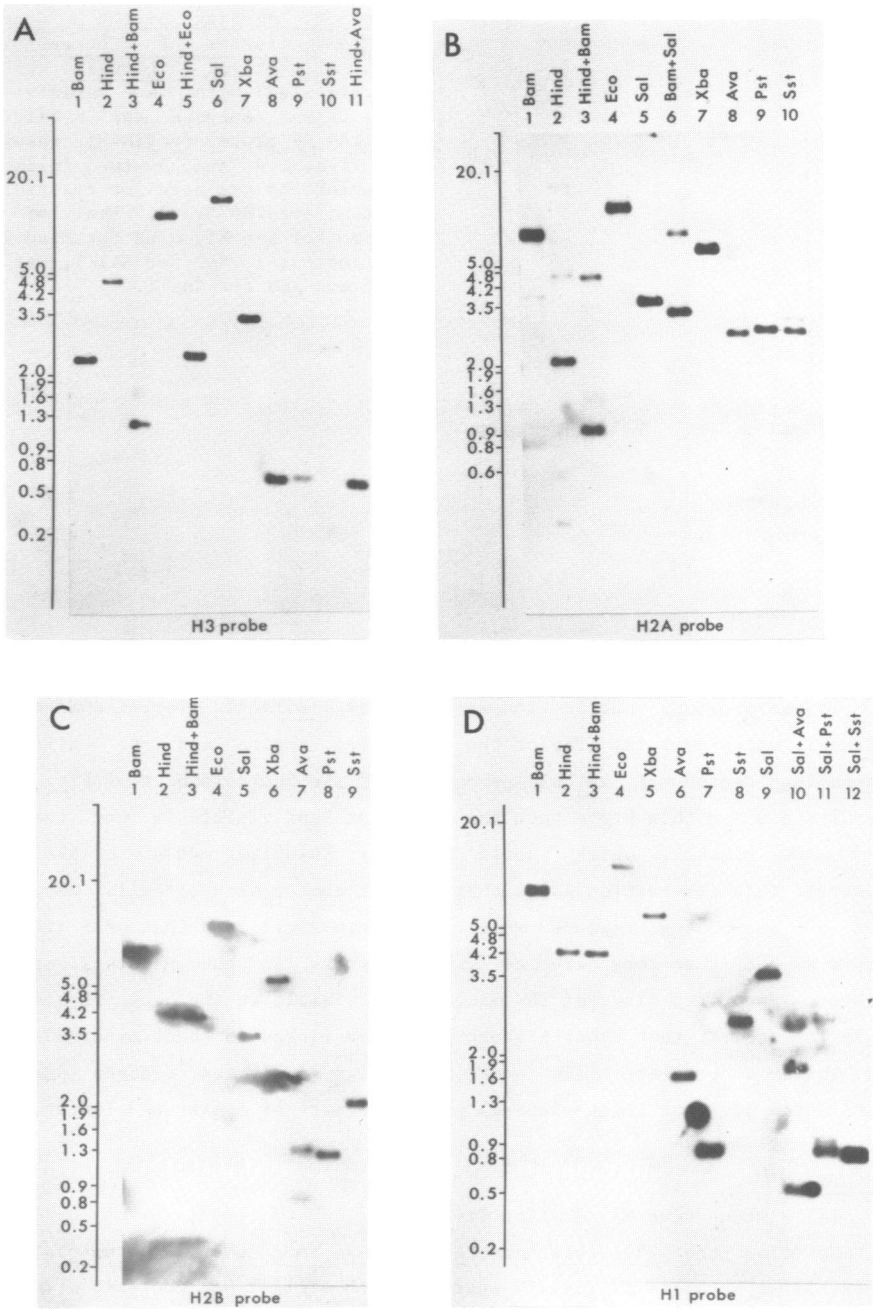

Figure 5. Mapping of the H3, H2A, H2B and H1 Genes in the *X.borealis* Major Cluster.

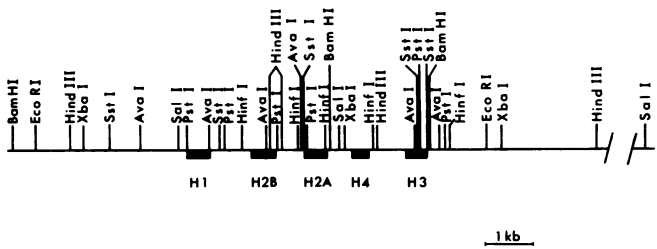

Figure 6. The Major Histone Cluster of X.borealis. This map shows 20kb encompassing the histone genes. In the regions outside the outer histone genes there could be extra sites for the enzymes marked since it is only practical to map the site which is nearest to the gene probe used. Consequently the region to the left of the H1 gene could also be represented at the extreme right of the map.

of 20-50 by Jacob et al. (28) and 45-50 by Van Dongen et al. (15). The standard deviation of the data of Jacob et al. is sufficiently large to regard their results as compatible with ours. In the case of Van Dongen et al. the discrepancy almost certainly arises from the difference in the stringency of the hybridization reaction. These workers used very high stringency to minimize the reaction of a repetitive element in their probe which was a minor genomic cluster. The H2B and H3 genes in this cluster do not appear to be expressed in oocytes (29) and the H4 gene diverges from the major sequences expressed in oocytes (16,23,30) and most other organs (30). It is therefore likely that Van Dongen et al. underestimated the abundance of certain subsets of the histone genes. In contrast, our melting experiments (Fig.2) indicate that the ovarian cDNA clones used here are not highly diverged from the major H4 genes in the genome.

(b) Organization of Histone Genes in Xenopus

In X.borealis at least 70% of the histone genes are present in a very homogeneous major cluster which has the gene order H1, H2B, H2A, H4, H3 (Fig.6). This gene order is the same as that in D.melanogaster (7), but different from the major cluster in sea urchins (4,5) and N.viridescens (31). The last comparison shows that the histone gene orders of the major clusters are not conserved in Amphibia.

Due to their large size, we have been unable to determine if the X.borealis major clusters are tandemly linked. However, the minimum length of the major cluster is 16.1kb (i.e. the sum of the Sal I fragments of 12.8 and 3.3kb, Fig.6) and of this, the five histone genes occupy less than 6kb. Thus within the major cluster there is a continuous length of DNA of at least

10kb which does not code for histone and is probably spacer. If the major clusters are tandemly linked this large spacing between gene regions resembles the organization in N.viridescens, where there are also long stretches of non-histone coding sequence in the clusters (18). However, we have as yet no evidence that highly repetitive sequences are associated with the X.borealis major cluster as in N.viridescens (18). Another similarity with this newt is that we observe a small degree of restriction site heterogeneity in the major cluster (i.e. a 6.5/6.7kb doublet seen in the Bam HI digests of Figs.5B, C and D could be explained by an insertion or deletion of about 200bp between the Bam HI and Eco RI sites in some representatives of the major cluster).

The great homogeneity between the representatives of the X.borealis major cluster is in sharp contrast to that in X.laevis. This marked difference in histone cluster homogeneity between two species which are thought to have diverged only 8-10 million years ago (32) presents a puzzle in respect of the mechanisms of evolution of the histone genes. To add to this puzzle Van Dongen et al. (15) have proposed that about 60% of the histone genes in X.laevis are present in a major cluster which has the gene order H4, H3, H2A, H2B, ie. with the H3 and H4 genes reversed with respect to the X.borealis major cluster. It would seem that we have to explain how the histone clusters have diverged in these two species in less than 10 million years. To do this we should consider the available facts.

In constructing the 14kb major histone cluster in X.laevis from Southern blot experiments, Van Dongen et al. (15) have assumed that the H4 gene is split between two adjacent Eco RI fragments of 5.1 and 8.9kb. Since Eco RI cannot cut the coding region of H4 genes which have the calf thymus amino acid sequence, and since the 5.1kb Eco RI fragment is a minor fragment in some X.laevis individuals (29), one must conclude the following: firstly, that the 5.1kb Eco RI fragment is not necessarily adjacent to the 8.9kb fragment in the genome but is either an unrelated cluster or part of a similar cluster. Secondly, that the major cluster in X.laevis is probably not 14kb in length.

It now seems certain that in all X.laevis individuals there is an 8.9kb major Eco RI fragment (15,33,34) and this contains all 5 histone gene types (15,35). In our laboratory, double digestion of X.laevis genomic DNA with Bam HI and Eco RI produces a 4.1kb fragment containing both the H1 and H4 genes. This is a different observation from that of Van Dongen et al. (15) and shows that X.laevis individuals seem to be quite variable in the

detailed structure of their histone gene clusters and we must turn to genomic cloning for further information.

There are now 21 genomic histone clones from X.laevis in the literature (15,16,33,34) and 14 of these are sufficiently well characterised to show the following:

That a 14kb Eco RI fragment comprising linked 8.9 and 5.1kb fragments has not yet been cloned, in agreement with the idea that it is not a common feature of X.laevis histone gene clusters (29).

That a great variety of Eco RI fragments have now been cloned, none fitting exactly the major 8.9kb fragment with all 5 histone gene types. However, the most frequent isolate from the library of Old et al. (33) was an 8.6kb Eco RI fragment containing one copy of each of the 5 histone genes. This type of clone shares some of the characteristics of the major X.laevis cluster observed in Southern blot experiments. Indeed, if some such clusters were tandemly linked in the genome and showed a small degree of sequence change, it would be possible to generate several other clones represented in the libraries of Old et al. (33) and Zernik et al. (16).

The most frequent histone gene order seen in these genomic clones is H1, H2B, H2A, H4, H3 or circular permutations of this, which is the same order as in the X.borealis major cluster. The H4, H3, H2A, H2B order proposed by Van Dongen et al. (15) is represented only once to date.

By analysing the data from the genomic clones and the genomic mapping, we can resolve the problems of X.laevis histone gene organization by proposing a minimum of two major cluster types which have the two different gene orders given immediately above. Representatives of these two cluster types would have both length and sequence heterogeneity, despite being similar in overall structure to other members of the same cluster type. If parts of the overall structure of these two cluster types were similar these combined features would produce the spectrum of differences seen in the genomic clones and in the genomic mapping from different individuals. In this connection Ruberti et al. (34) have isolated from X.tropicalis clones of varying gene order. It may be that X.borealis is an unusual frog, in having a large proportion of one homogeneous histone cluster type.

(c) Evolution of Histone Gene Clusters

Whatever mechanisms may be involved in histone gene evolution they must explain both the remarkable differences between the organisation of these clusters in different organisms and the relative rapidity of the X.laevis versus X.borealis changes.

Initial cloning experiments indicated that histone gene clusters were highly divergent in vertebrates, particularly in their spacer sequences. Zernik et al. (16) suggested that this reflected a difference in selection pressures between vertebrates and invertebrates. This seems unlikely, since we now know that N.viridescens and X.borealis have major, homogeneous clusters. The former organism also has a high histone gene reiteration frequency, which lead Stephenson et al. (18) to argue that the homogeneity and high copy number are linked. However, in Xenopus it is clear that two species can have the same copy number and a marked difference in homogeneity. In fact this copy number is also similar to that in D.melanogaster, which has a highly homogeneous, major histone repeat.

The picture that is emerging of histone gene organization in animals is that all contain a set of variant clusters in low copy number (6,11,12,13,14), and all except mammals and birds also contain a reiterated, homogeneous family of clusters. In the case of sea urchins it is known that the highly reiterated genes are expressed in early development (2,3), when the high copy number is demanded by the requirement for rapid mRNA synthesis (36,37). The rare diverse genes provide the histones of later development (6).

Calculations of histone mRNA content in X.laevis eggs indicate that a large number of histone genes must function in the oocyte (38,39). It is therefore very likely that the reiterated histone gene clusters of amphibians are expressed in oogenesis, and are analogues of the early histone genes of sea urchins. If so, in those organisms which have reiterated histone clusters it is clear that proper functioning of the genes in these clusters does not require a specific gene order, a fixed gene polarity, a fixed spacer length or extensive spacer sequence conservation, since sea urchins, Drosophila, newt and Xenopus differ in these respects.

One way of explaining the differences in the major clusters in different organisms is to suppose that what is needed is a large number of genes, and that in different organisms different representatives of the rare, variable clusters were selected and multiplied at random during evolution. If so, in different species one original cluster might be multiplied, or several might increase to commensurately lesser extents. These may be the two situations in X.borealis and X.laevis. Even in the second case, random drift could eventually lead to fixation of one or other type, particularly if they were allelic. It is to be expected that only a cluster containing all five histone types would be suitable for evolutionary amplification.

REFERENCES

1. Hereford, L., Fahrner, K. Woodford, J. and Rosbash, M. (1979) Cell 18, 1261-1271.
2. Schaffner, W., Kunz, G., Daetwyler, M., Telford, J., Smith, H.O. and Birnstiel, M.L. (1978) Cell 14, 655-671.
3. Sures, I., Lowry, J. and Kedes, L.H. (1978) Cell 15, 1033-1044.
4. Kedes, L.H. (1979) Ann.Rev.Biochem. 48, 837-870.
5. Hentchel, C.C. and Birnstiel, M.L. (1981) Cell 25, 301-313.
6. Busslinger, M., Portman, R., Irminger, J.C. and Birnstiel, M.L. (1980) Nucl.Acids Res. 8, 957-977.
7. Lifton, R.P., Goldberg, M.L., Karp, R.W. and Hogness, D.S. (1977) Cold Spring Harbour Symp.Quant.Biol. 42, 1047-1051.
8. Jacob, E. (1976) Eur.J.Biochem. 65, 275-284.
9. Scott, A.C. and Wells, J.R.E. (1976) Nature 259, 635-638.
10. Seiler-Tuyns, A. and Birnstiel, M.L. (1981) J.Mol.Biol. 151, 607-625.
11. Engel, J.D. and Dodgson, J.B. (1981) Proc.Natl.Acad.Sci.USA 78, 2856-2860.
12. Heintz, N., Zernik, M. and Roeder, R.G. (1981) Cell 24, 661-668.
13. Sittman, D.B., Chui, I., Pan, C., Cohn, R.H., Kedes, L.H. and Marzluff, W.F. (1981) Proc.Nat.Acad.Sci.USA 78, 4078-4082.
14. Sierra, F., Lichtler, A., Marashi, F., Rickles, R., Van Dyke, T., Clark, S., Wells, J., Stein, G. and Stein, J. (1982) Proc.Nat.Acad.Sci.USA 79, 1795-1799.
15. Van Dongen, W., De Laaf, L., Zaal, R., Moorman, A. and Destree, O. (1981) Nucl.Acids Res. 9, 2297-2311.
16. Zernik, M., Heintz, N., Boime, I. and Roeder, R.G. (1980) Cell 22, 807-822.
17. Hilder, V.A., Livesey, R.N., Turner, P.C. and Vlad, M.T. (1981) Nucl.Acids Res. 9, 5737-5746.
18. Stephenson, E.C., Erba, H.P. and Gall, J.G. (1981a) Cell 24, 639-647.
19. Humphries, P., Old, R., Coggins, L.W., McShane, T., Watson, C. and Paul, J. (1978) Nucl.Acids Res. 5, 905-924.
20. Colman, A., Byers, M.J., Primrose, S.B. and Lyons, A. (1978) Eur.J.Biochem. 91, 303-310.
21. Rigby, P.W.J., Deichmann, M., Rhodes, C. and Berg, P. (1977) J.Mol.Biol. 113, 237-251.
22. O'Farrell, P.H., Kutter, E. and Nakanishi, M. (1980) Molec.Gen.Genet. 179, 421-435.
23. Turner, P.C. and Woodland, H.R. (1982) Nucl.Acids Res. 10, 3769-3780.
24. Southern, E.M. (1975) J.Mol.Biol. 98, 503-517.
25. Sommerville, J. (1979) Proc. FEBS Meet. 1978 51, 265-276
26. Dawid, I.B. (1965) J.Mol.Biol. 12, 581-599.
27. Thiebaud, C.H. and Fischberg, M. (1977) Chromasoma 59, 253-257.
28. Jacob, E., Malacinsky, G. and Birnstiel, M.L. (1976) Eur.J.Biochem. 69, 45-54.
29. Van Dongen, W. (1982) Ph.D. Thesis, University of Amsterdam.
30. Woodland, H.R. and Warmington, J.R. unpublished.
31. Stephenson, E.C., Erba, H.P. and Gall, J.G. (1981b) Nucl.Acids Res. 9, 2281-2295.
32. Bisbee, C.A., Baker, M.A., Wilson, A.C., Hadji-Azimi, I. and Fischberg, M. (1977) Science 195, 785-787.
33. Old, R.W., Woodland, H.R., Ballantine, J.E.M., Aldridge, T.C., Newton, C.A., Bains, W.A. and Turner, P.C. (1982) Nucl.Acids Res. 10, No.23.
34. Ruberti, I., Fragapane, P., Pierandrei-Amaldi, P., Beccari, E., Amaldi, F. and Bozzoni, I. (1982) Nucl. Acids Res. in press

35. Turner, P.C. and Woodland, H.R. unpublished.
36. Maxson, R.E. and Wilt, F.H. (1981) Dev.Biol. 83, 380-386.
37. Goustin, A.S. (1981) Dev.Biol. 87, 163-175.
38. Woodland, H.R. and Wilt, F.H. (1980) Dev.Biol. 75, 214-221.
39. Woodland, H.R. (1980) FEBS Lett. 121, 1-7.