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Histone Genes are Located at the Sphere Loci of Newt Lampbrush Chromosomes

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Abstract. In situ hybridization experiments on mitotic and lampbrush chromosomes show that histone genes and an associated 222 bp repeated sequence, satellite 1, are located at or near the sphere loci of chromosomes 2 and 6 of the newt *Notophthalmus viridescens*. During the lampbrush chromosome stage, transcripts of the histone genes and of satellite 1 occur on loops associated with the spheres. Histone genes are located at sites on the mitotic chromosomes of *Triturus cristatus* and *T. alpestris* which are consistent with the known positions of spheres on their lampbrush chromosomes.

Introduction

Because of their large size and special morphology newt lampbrush chromosomes provide unique opportunities for correlating molecular and cytological aspects of RNA transcription (see reviews by Sommerville, 1977; Macgregor, 1980; Scheer et al., 1979). In particular, in situ hybridization of labeled DNA probes to the nascent RNA transcripts of the lateral loops permits identification of sequences which are being synthesized in the lampbrush stage of oogenesis. Probes used to date include *Xenopus* 5 S genes (Pukkila, 1975), *Xenopus* rDNA (Morgan et al., 1980), *Triturus* simple sequence DNAs (Macgregor and Andrews, 1977; Macgregor, 1979; Varley et al., 1980a, b) and sea urchin histone clones (Old et al., 1977). In order to make full use of both the cloning and in situ hybridization methodologies, however, one needs clones of homologous structural genes which are transcribed during the lampbrush chromosome stage. Genomic clones derived from the organism whose chromosomes are used for in situ hybridization are necessary for examining transcription of gene and spacer regions, coding and non-coding strands, RNA processing and other important molecular features. The histone genes seemed a logical choice, since both histone mRNA and histones are synthesized in amphibian oocytes (Woodland and Adamson, 1977). Moreover, in other organisms these genes are reiterated and clustered (reviewed in Kedes, 1979) making them relatively easy to isolate from a genomic clone library. The enormous size of the newt genome, up to

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50 pg in some species, would make selection for single-copy sequences a much more formidable task.

We have cloned a 9 kb restriction fragment from genomic DNA of the eastern newt, *Notophthalmus viridescens viridescens*, which represents the major histone gene repeat of this organism. It contains sequences encoding the five major histones as well as spacers between the genes (see Fig. 1). Details of the clone are presented elsewhere (Stephenson et al., 1981 a, b). By in situ hybridization we show here that histone sequences are located primarily or exclusively at two sites on mitotic chromosomes, and we identify these as the sphere loci of the lampbrush chromosomes. We show that the sphere loci also contain copies of an abundant simple sequence DNA, which we call satellite 1. Satellite 1 is found in the centromeric regions as well. Both the histone genes and satellite 1 sequences are transcribed on loops associated with the spheres. The locations of the histone genes on mitotic chromosomes of two other newts, *Triturus cristatus carnifex* and *Triturus alpestris apuanus* are consistent with the known positions of spheres on the lampbrush chromosomes of these species.

Materials and Methods

Animals. Notophthalmus viridescens viridescens were collected from a pond near Wells State Park, Worcester County, Massachusetts or were purchased from Lee's Newt Farm, Oak Ridge, Tennessee. *Triturus cristatus carnifex* and *Triturus alpestris apuanus* were collected from ponds in Pisa, Italy.

Chromosomes. Mitotic chromosome squashes were made from gut epithelial cells of animals previously injected with colchicine (Macgregor and Andrews, 1977).

Lampbrush chromosome preparations were made as previously described (Gall, 1966) with several important modifications. Nuclei were isolated manually from oocytes of 0.8–1.3 mm diameter in "5 : 1 plus PO_4" (83 mM KCl, 17 mM NaCl, 6.5 mM Na_2HPO_4, 3.5 mM KH_2PO_4, pH 7.2). In this solution the nuclear contents remain as a gelatinous ball, greatly facilitating the removal of yolk and the nuclear envelope. After the envelope was removed the nuclear contents were transferred by pipette to 1/4 strength "5 : 1 plus PO_4" containing 0.1 % paraformaldehyde. After a brief rinse the nuclear contents were placed in a well slide containing the same solution. The well slide consisted of a standard $3'' \times 1''$ microscope slide attached by paraffin-vaseline (1 : 1) to another slide in which was bored a 1/4″ round hole. In the dilute saline-paraformaldehyde the nuclear gel rapidly dispersed, the loop matrix expanded and the nucleoli partially dissolved. A coverslip was added to the top of the well slide and the chromosomes were allowed to settle for about one hour. The entire well slide was then centrifuged for 30–45 min at 2500 g in a special swinging bucket carrier in the Sorvall HS-4 rotor. At the end of centrifugation the slide was placed horizontally under 70 % ethanol and the coverslip was pushed away. After several hours the lower slide, bearing the centrifuged chromosomes, was separated from the top bored slide. The preparation was then dehydrated in an ethanol series, soaked in xylene to remove traces of paraffin, rinsed in 100 % ethanol and air dried from acetone. The dried preparations were baked in a 65° C oven for 1–3 h before further processing, a modification of a suggestion by Bonner and Pardue (1976) to prevent loss of material from the slide during later steps. Slides were stored for up to several months before use.

Probes. Histone gene probes used in this study were subclones of ES 6 Nv 51, which consists of the 9 kb genomic histone cluster of *Notophthalmus* inserted in a lambda phage vector. Details are given in Stephenson et al. (1981 a, b). Satellite 1 probes used here consisted of λNv 111, an 8 kb genomic fragment from *Notophthalmus* inserted in the lambda vector Charon 4A (Blattner et al., 1977); or in one case of a 1.25 kb subclone from λNv 111 in the single-stranded phage M 13. Details of satellite 1 and its clones are given in Diaz et al. (1981).

Nick translation and cRNA synthesis were carried out as described in Diaz et al. (1981). Nick translated DNA had a specific activity of $2–5 \times 10^7$ dpm/μg (^3H) or 8×10^8 dpm/μg (^{125}I); ^3H-cRNA had a calculated specific activity of $7–9 \times 10^7$ dpm/μg.

In situ Hybridization and Autoradiography. The mixture for in situ hybridization contained 40% formamide, 4 × SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 0.1 M Na_3PO_4, pH 7.0, and *E. coli* DNA at 300 µg/ml. The radioactive probe was evaporated to dryness and taken up in the hybridization mix at a concentration of 5×10^4–5×10^5 cpm/µl (counting efficiency on filters about 35% for both ^{125}I and ^3H). Nick-translated probes were heated to 65° C for 5 min in the hybridization mix to denature the DNA unless they had been previously denatured by boiling in 0.1 × SSC for 5 min.

5 µl of probe were placed on a lampbrush chromosome preparation, an 18 × 18 mm coverslip was added and the edges were sealed with paraffin-vaseline (1 : 1). The slides were held at 37° C for 12–18 h. After hybridization the coverslips were removed under 2 × SSC, excess paraffin was scraped off, and the slides were incubated for 1 h in 1 × SSC at 65° C to remove non-specifically bound radioactivity. They were then dehydrated through an ethanol series, washed in xylene to remove traces of paraffin and air dried from acetone.

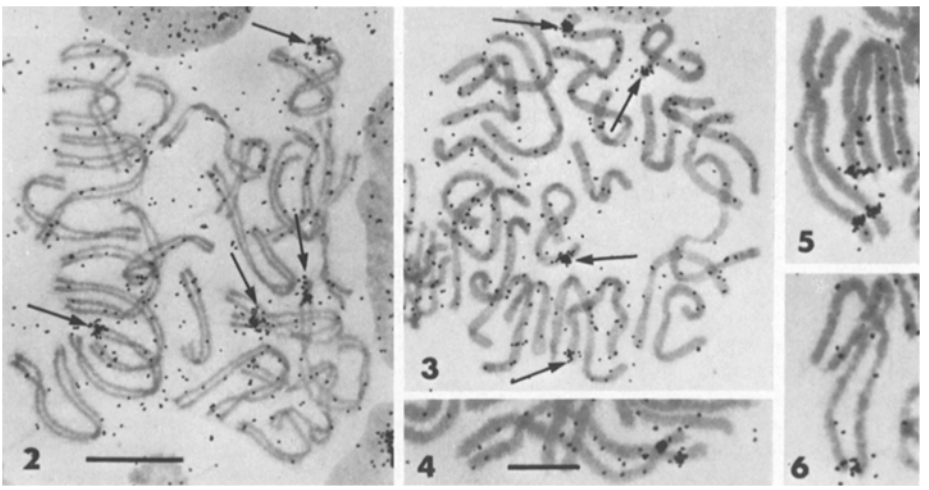
Autoradiography was with Kodak NTB-2 diluted 1 : 1 with H_2O. Slides were developed in Kodak D-19 for 2 min at 15° C. Low temperature development, as recommended by Kodak, markedly reduced background. After fixing (1 min) and washing (10 min) at 15° C, the slides were allowed to dry. Remaining steps were at room temperature. The slides were then passed quickly through 50% methanol into 0.1% Coomassie blue R 250 in 50% methanol, 10% acetic acid for 10 min. The stained slides were dipped in 50% methanol, washed in H_2O for 10 min and air dried. The drying step before staining was important in order to minimize the danger of film reticulation. Coomassie blue stained lampbrush chromosome loops much better than Giemsa, which had been the standard stain for several years.

Results

Mitotic Chromosomes

As probes for hybridization to mitotic chromosomes we have used subclones of the H1, H2A, H2B and H3 genes and associated spacers, as indicated in Figure 1. These subclones are in the vector pBR 322 and are designated pNv51-3 (coding regions of histones H3 and part of H1), pNv51-4 (coding regions of histone H2A and part of H2B) and pNv51-5 (coding region of part of H2B). When a mixture of pNv51-3 and pNv51-4 sequences was hybridized to mitotic chromosomes of *N. viridescens*, two loci were prominently labeled. One locus was near the end of the longer arm of a long submetacentric chromosome (centromere at 0.70); the other

Fig. 1. Restriction enzyme map of a histone gene cluster of *Notophthalmus viridescens* (Stephenson et al., 1981 a). A 9 kb Bgl II genomic DNA fragment was cloned in a lambda vector; newt DNA is represented by a single line, lambda DNA by hatched boxes. Below the restriction map are shown fragments which have been subcloned in pBR 322 (51-2 through 51-7). Above the map are shown the positions and directions of transcription of the five histone coding regions. Double arrows, designated Nv 111, indicate the maximum limits of satellite 1 DNA found at each end of the cloned histone cluster. The probes used for determining the chromosomal location of histone genes came entirely from the region covered by subclones 51-3, 51-4 and 51-5

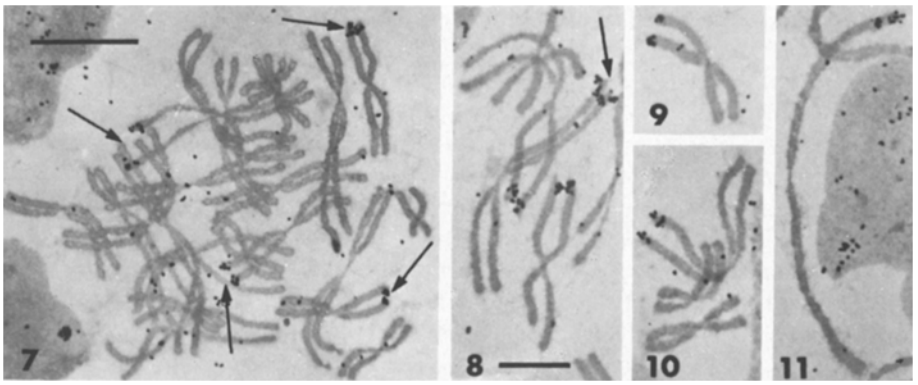


Figs. 2–6. Fig. 2. A complete mitotic complement of 22 chromosomes from *Notophthalmus viridescens*, after in situ hybridization with ^{125}I-labeled nick-translated histone gene clones pNv 51-3 and pNv 51-4. Both homologues of chromosome 2 (lower right) and chromosome 6 (left and upper right) are labeled. Probe sp. act. 8×10^8 dpm/μg. Exp. 7 days. Bar = 20 μm.; also for Fig. 3. **Fig. 3.** A complete mitotic complement of *N. viridescens* after in situ hybridization with ^{3}H-labeled cRNA copied from histone gene clones pNv51-3 and pNv51-4. Chromosome 2 (center and upper left) and 6 (below and upper right) are labeled. Probe sp. act. $= 7 \times 10^7$ dpm/μg. Exp. 104 days. **Figs. 4–6.** Individual labeled chromosomes of *N. viridescens* after in situ hybridization as in Fig. 3. Bar = 10 μm. **Fig. 4.** Chromosome 2. **Fig. 5.** Chromosome 2 (left) and chromosome 6 (*right*). **Fig. 6.** Chromosome 6

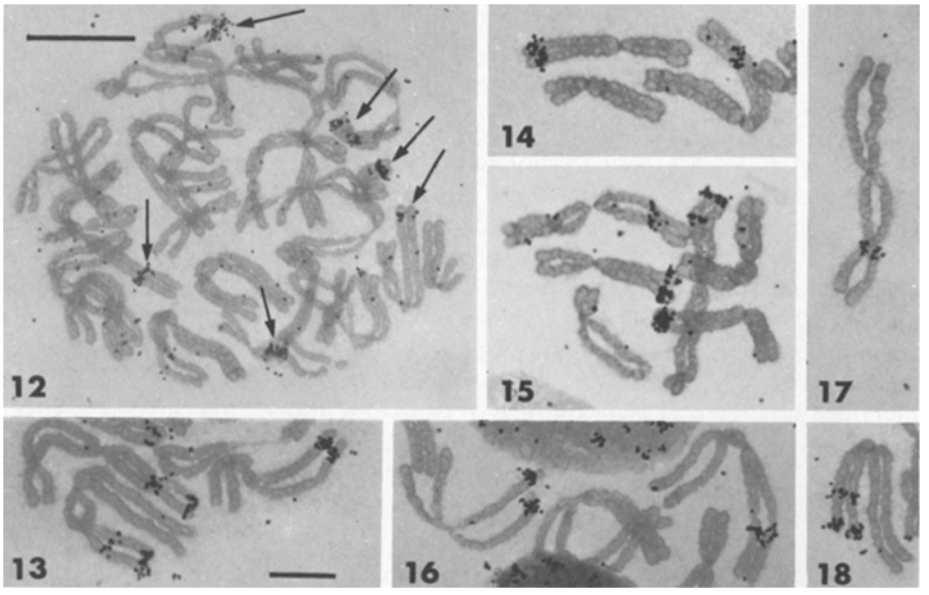
appeared to be strictly terminal on a medium length metacentric chromosome (centromere at 0.50). Three different probes gave the same localization. One consisted of the two clones nick-translated with ^{125}I-dCTP (Fig. 2), the second of the two clones nick-translated with ^{3}H-TTP (not shown), and the third of cRNA synthesized from a mixture of the two clones using ^{3}H-UTP (Figs. 3–6). The ^{3}H-labeled probes gave slightly better spatial resolution but the ^{125}I-labeled probe permitted shorter autoradiographic exposures.

In a separate experiment pNv51-5 was nick-translated with ^{125}I-dCTP and hybridized to mitotic chromosomes. It hybridized to the same two loci (not shown). The insert in pNv51-5 is 365 bp in length and consists of about 150 bp of the H2B coding region and 215 bp of the 3′ flanking nontranslated mRNA and spacer.

Mitotic chromosomes of two other newts, *Triturus cristatus carnifex* and *Triturus alpestris apuanus*, were examined. Some preparations were hybridized with the ^{3}H-labeled cRNA described above and some with a mixture of all three clones nick-translated with ^{125}I-dCTP. In *T. cristatus* two chromosomes were labeled (Figs. 7–11). One was a short metacentric in which the label appeared to be strictly terminal on the (slightly) longer arm. The other was a medium length metacentric with label on the end of one arm. In this chromosome the label was somewhat diffuse, and in some cases there were clearly two separate clusters of silver grains. In *T. alpestris* there were four labeled sites on three chromosomes (Figs. 12–18). A long metacentric had label slightly distal to the middle of one arm. A long submetacentric had two labeled sites on the shorter arm; one site was essentially terminal and the second was about 1/3 of the way from the end to the centromere.



Figs. 7–11. Fig. 7. A mitotic complement of *Triturus cristatus carnifex* after in situ hybridization with the same histone gene probe as in Figure 3. The four labeled chromosomes are presumed to be the homologous of chromosome 5 (upper left and upper right) and chromosome 8 (center and lower right). Exp. 138 days. Bar = 20 μm. **Figs. 8–11.** Chromosomes of *T. c. carnifex* labeled as in Figure 7. Bar = 10 μm. **Fig. 8.** Two examples of chromosome 5 (longer) and one of chromosome 8 (shorter). Two labeled sites are resolved on each chromatid of one chromosome 5 (arrow). **Fig. 9.** Chromosome 8. **Fig. 10.** Chromosome 5, two labeled sites resolved on upper chromatid. **Fig. 11.** A stretched chromosome 5 with two clearly resolved sites of label



Figs. 12–18. Fig. 12. A mitotic complement of *Triturus alpestris apuanus* after in situ hybridization with the same histone gene probe as in Figure 3. The arrows indicate six labeled chromosomes, giving a total of eight labeled sites, since the two homologues of chromosome 2 (topmost arrows) each have two labeled sites. Exp. 73 days. Bar = 20 μm. **Figs. 13–18.** Chromosomes of *T. a. apuanus* labeled as in **Fig. 12.** Numbered as discussed in the text. Bar = 10 μm. **Fig. 13.** Two examples of chromosome 2, each with two labeled sites (*left*), and one of chromosome 4 (right). **Fig. 14.** Chromosome 4 (*left*) and chromosome 1 (*right*). **Fig. 15.** Two examples of chromosome 4 (*below*) and one each of chromosome 1 (*upper left*) and chromosome 2 (*upper right*). **Fig. 16.** Chromosome 4 (left) and chromosome 1 (right). **Fig. 17.** Chromosome 1. **Fig. 18.** Chromosome 2

The third labeled chromosome was a medium length metacentric in which the label was subterminal on the (slightly) longer arm. As discussed later, the labeled sites in both *T. cristatus* and *T. alpestris* correspond well to the positions of spheres in the lampbrush chromosomes.

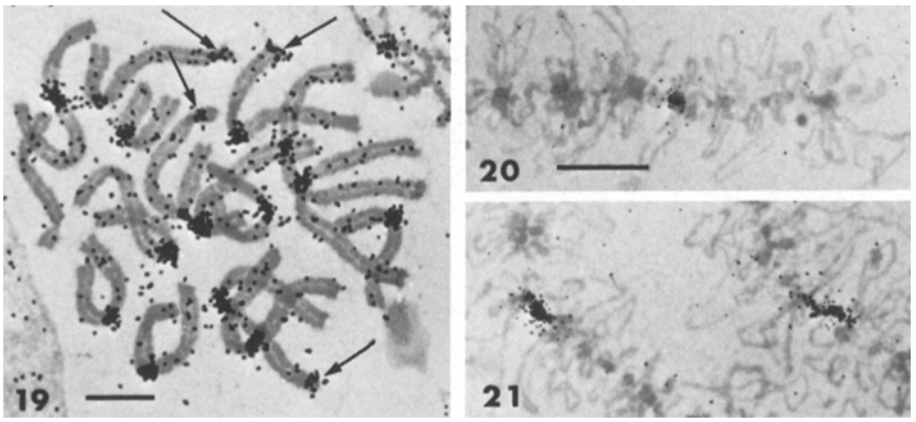
In studies originally unrelated to our examination of the histone genes, members of our laboratory analyzed a clone of genomic DNA from *N. viridescens* which contains copies of an abundant simple sequence DNA (Diaz et al., 1981). This clone, designated λNv111, contains about 8 kb of newt DNA and was selected from a library of random genomic fragments cloned in the lambda vector Charon 4A (Blattner et al., 1977). Its most important feature in the present context is that it consists largely, if not exclusively, of a 222 bp serially repeated sequence, which we call satellite 1. We have shown by filter hybridization experiments (Southern, 1975) that the 222 bp repeat in λNv111 is identical to a prominent ladder of fragments visible by ethidium bromide staining when genomic DNA is digested with certain restriction enzymes (Alu I, Bgl II, Hae III, Sac I). The monomer of this repeated sequence has been cloned in pBR 322 and sequenced (Diaz et al., 1981).

When λNv111 sequences were hybridized in situ to mitotic chromosomes of *N. viridescens*, label was seen in the centromere region of all chromosomes and at two loci which appeared to be identical to those labeled by the histone probes. The best localization was obtained when the probe consisted of cRNA synthesized from λNv111 with ^{3}H-UTP (Fig. 19), but essentially identical results were given by a probe nick-translated with ^{125}I-dCTP (not shown).

Lampbrush Chromosomes

Histone gene sequences were hybridized to the DNA of *Notophthalmus* lampbrush chromosomes in order to define more precisely the two loci identified on mitotic chromosomes. A probe consisting of pNv51-3 and pNv51-4, nick-translated with ^{125}I-dCTP, hybridized at or near the spheres of chromosomes 2 and 6 (Fig. 22). The spheres are prominent landmarks on these two chromosomes (see the maps in Callan and Lloyd, 1975). They vary considerably in size depending upon the individual and stage of oogenesis, and they may be perfectly spherical, irregular in outline, or studded with smaller hemispherical protuberances (Gall, 1954, where they are described as "knobs"; Callan and Lloyd, 1960). It is difficult to determine whether the label is on the spheres themselves or on adjacent small chromomeres. The positions of the spheres and associated label, 0.08 on chromosome 2 and 0.02 on chromosome 6, correspond well with the labeled sites on the mitotic chromosomes. The DNA of the sphere loci was also labeled by a probe which contained the repeated sequence of λNv111 (Fig. 20). As expected from the studies with mitotic chromosomes, the pericentromeric heterochromatin (centromere bars) of the lampbrush chromosomes labeled with the same probe (Fig. 21).

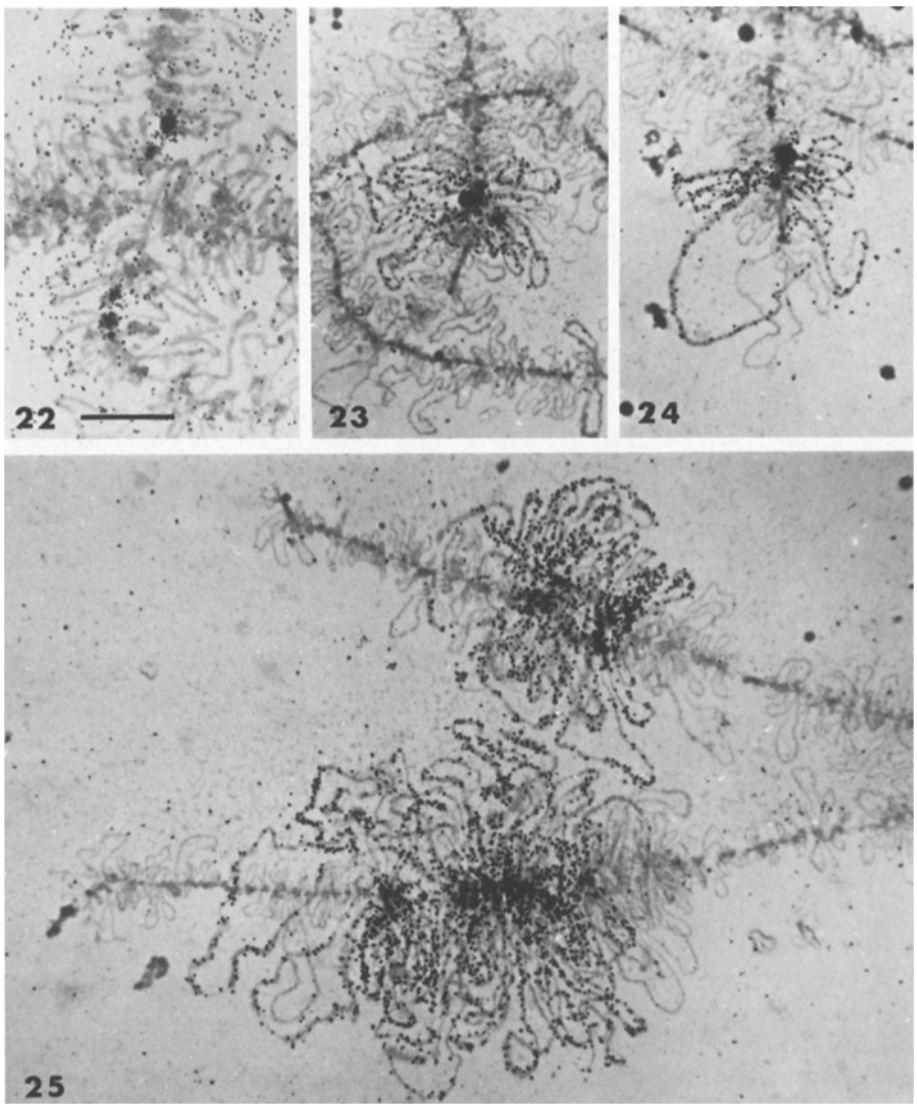
The labeling of DNA at the sphere loci with both histone and satellite 1 probes confirmed the impression gained from the mitotic studies that these sequences are close together on the chromosomes. In fact they are interspersed at the molecular level and are transcribed together during the lampbrush stage on loops associated



Figs. 19–21. Fig. 19. A partial mitotic complement of *N. viridescens* after in situ hybridization with ^{3}H-labeled cRNA synthesized from λNv111, a phage clone containing satellite 1 sequences. Centromeric heterochromatin is labeled on all chromosomes except one homologue of chromosome 11 (extreme left). Four non-centromeric sites of label are indicated by arrows, two on chromosome 2 (two arrows at upper center) and two on chromosome 6 (upper right and lower right). Probe sp. act. 7×10^{7} dpm/μg. Exp. 7 days. Bar = 10 μm. **Fig. 20.** The sphere locus on one homologue of lampbrush bivalent 6 after in situ hybridization with ^{125}I-labeled nick-translated λNv111, a clone containing satellite 1 sequences. Before hybridization the preparation was treated with RNase to remove RNA and with 0.07 N NaOH to denature the DNA, as in the case of the mitotic preparations. The label therefore demonstrates the existence of satellite 1 sequences at the sphere locus. Probe sp. act. 8×10^{8} dpm/μg. Exp. 1 day. Bar = 20 μm in Figs. 20 and 21. **Fig. 21.** Labeled centromere bars (pericentromeric heterochromatin) from the same preparation as Fig. 20. This hybridization confirms the centromeric localization of satellite 1 seen in the mitotic preparation (Fig. 19)

with the spheres. Their coordinate transcription will be described here, but detailed evidence for their interspersion is being pulbished separately (Stephenson et al., 1981b). It may be noted, as shown in Figure 1, that each end of the cloned histone repeat contains one or a few copies of the 222 bp sequence found in λNv111.

Coordinate transcription was demonstrated by hybridizing appropriate cloned sequences to the nascent RNA chains on lampbrush chromosome loops (Pukkila, 1975). Loops associated with the spheres of both chromosomes 2 and 6 were labeled by probes which contained either histone or satellite 1 sequences. Hybridization of histone sequences was observed in lampbrush chromosomes of 5 individual newts. In our initial experiments we used pNv 51-5 and a mixture of pNv 51-3 and pNv 51-4, both nick-translated with ^{125}I-dCTP. Although specific labeling was evident after a few days of autoradiographic exposure, the non-specific background with these probes was high, precluding the long exposures needed for detailed analysis of individual loops. Since we were interested in studying hybridization of both strands of histone genes, we prepared subclones in the single-stranded phage M 13. Two such subclones, mNv51-9-13 and mNv51-9-18 contain opposite strands of the 4.1 kb Bam H 1 fragment covered by 51-3, 51-4 and 51-5 (Fig. 1). Because the single-stranded probes could not be labeled by nick-translation, we prepared ^{3}H-labeled cRNA from them using *E. coli* RNA polymerase. The cRNA was then hybridized to the RNA of the loops to give RNA/RNA hybrids. Although the specific activity of



Figs. 22–25. Fig. 22. Label at the sphere loci on lampbrush chromosome 2 of *N. viridescens* after in situ hybridization with histone gene clones pNv 51-3 and pNv 51-4 which had been nick-translated with ^{125}I-dCTP. Before hybridization the preparation was treated with RNase to remove RNA and with 0.07 N NaOH to denature the DNA. The hybridization therefore demonstrates histone gene sequences at the sphere loci. Each sphere locus is double in this preparation; at the center of the photograph the two homologues are joined by a chiasma. Note the much longer exposure needed to demonstrate histone sequences than satellite 1 sequences at the sphere locus (cf. Fig. 20). Probe sp. act. 8×10^8 dpm/μg. Exp. 90 days. Bar = 20 μm. **Figs. 23–25.** Hybridization of a histone gene probe to the nascent RNA transcripts on lampbrush chromosome loops at the sphere loci of *N. viridescens*. The probe was ^{3}H-labeled cRNA copied from single-stranded mNv51-9-18, an M13 phage containing one strand of segments 51-3, 51-4 and 51-5 of the *N. viridescens* histone cluster (Fig. 1). The hybrid is therefore RNA/RNA and demonstrates sequences in the transcript identical to those in the single-stranded clone. Probe sp. act. 9×10^7 dpm/μg. Exp. 5 days. **Figs. 23 and 24.** A relatively few labeled loops at the sphere loci on the two homologues of chromosome 6. **Fig. 25.** The two homologous sphere loci on chromosome 2 from the same preparation. This animal had an unusually large number of labeled loops. Bars in Figs. 22–25 = 20 μm

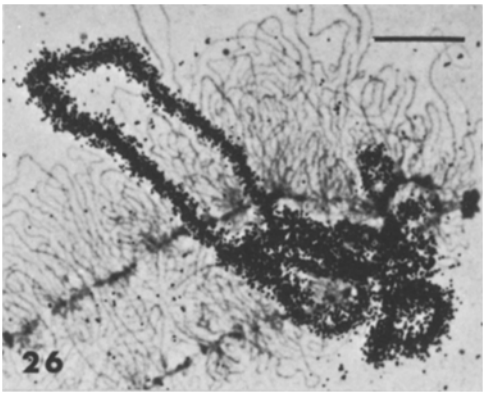


Fig. 26. Hybridization of a satellite 1 probe to the nascent RNA transcripts on loops at the sphere locus of lampbrush chromosome 6 of *N. viridescens*. The probe was ^{3}H-labeled cRNA copied from mNv111-1-1, a single-stranded M 13 phage which contains 1.25 kb of satellite 1. Probe sp. act. 7×10^{7} dpm/μg. Exp. 4 days. Bar = 20 μm

the ^{3}H probes was 10 times lower than that of the ^{125}I probes, the level of labeling in the autoradiographs was not vastly different. Because the cRNA copied from single-stranded DNA cannot reassociate in solution, as can the two strands of nick-translated DNA, the hybridization reaction is probably more complete with the single-stranded probes.

Figures 23–25 show hybridization of cRNA made from mNv51-9-18. Loops at the sphere loci of chromosomes 2 and 6 were moderately well labeled after five days exposure. It is evident that some loops or parts of loops were more heavily labeled than others and some loops probably contained both labeled and unlabeled segments. Details of the labeling patterns will be presented in a separate publication. In this paper we stress only the fact that histone gene sequences are found in transcripts associated with the spheres of chromosomes 2 and 6.

Hybridization of satellite 1 sequences to the RNA of lampbrush loops has been studied in over 25 animals. As probes we have used the original λNv111 clone in Charon 4A, subclones consisting of a 1.25 kb Eco RI fragment of λNv111 in pBR322 or in M 13, and a separately cloned Bgl II fragment from genomic DNA which contains a dimer of the 222 bp repeat. All showed hybridization to one or more loop pairs at the spheres on chromosomes 2 and 6. Since the hybridization of satellite 1 sequences has been described in detail elsewhere (Diaz et al., 1981) we present here only a single example (Fig. 26). In this case several small loops and one giant loop pair on chromosome 6 were labeled. The giant loops showed a conspicuous thin-thick asymmetry with the amount of label at any point on the loop being roughly proportional to the amount of RNA matrix. Labeling of loops with satellite 1 probes was much stronger than with histone probes, other factors such as specific activity and concentration of the DNA or RNA being the same. For instance ^{3}H-labeled cRNA copied from single-stranded satellite 1 produced complete film blackening above labeled loops in 3–5 days.

In lampbrush chromosomes of some animals hybridization of satellite 1 sequences was seen on small loops associated with the pericentromeric heterochromatin of one or a few chromosomes (Diaz et al., 1981). We presume that rare transcription of satellite 1 sequences occurs on loops near the centromeres in addition to the regular transcription at the sphere loci.

Discussion

The major point we have established here is that histone genes and an associated 222 bp repeated sequence are located at or near the sphere loci of chromosomes 2 and 6 of the newt, *Notophthalmus viridescens*. We have demonstrated this by hybridizing homologous cloned histone gene probes to the DNA of both mitotic and lampbrush chromosomes. Furthermore we have shown that the same probes, when hybridized to the RNA of the lampbrush chromosomes, label a set of loops intimately associated with the spheres. From a detailed analysis of the cloned histone repeat and of genomic DNA (Stephenson et al., 1981 a, b) we know that the histone gene clusters are separated by long stretches of a 222 bp repeated sequence. Hybridization experiments established the existence of this repeat at the sphere loci of both mitotic and lampbrush chromosomes and its transcription on loops associated with the spheres. The same repeated sequence was also found in the pericentromeric heterochromatin of all the chromosomes. We presume that transcripts of the histone genes and the 222 bp repeat occur together as long RNA molecules on the same loops, although rigorous evidence for this model has not yet been obtained.

Structures identical in most respects to the spheres of *N. viridescens* are found at a few loci on the lampbrush chromosomes of many other amphibians. Callan and Lloyd (1975) published chromosome maps for eleven urodeles and one anuran, and in all but one (*T. marmoratus*) the spheres provide easily recognizable landmarks. Spheres are also present on the lampbrush chromosomes of the toad *Xenopus* (Müller, 1974) and undoubtedly other species. In order to determine whether lampbrush chromosome spheres are generally characteristic of histone genes, we hybridized *N. viridescens* histone clones to the DNA of mitotic chromosomes of *T. cristatus carnifex* and *T. alpestris apuanus*.

In *T. c. carnifex* two sites were labeled. One was near the end of a medium length metacentric. The silver grains were often somewhat strung out at this locus and in some cases two closely spaced loci were resolved (Figs. 8, 10, 11). The second locus was nearly terminal on the longer arm of a short metacentric. As shown on the maps of Callan and Lloyd two spheres occur close together near one end of chromosome 5 and another occurs near the end of the longer arm of chromosome 8. The mitotic positions of histone genes are therefore compatible with the known positions of lampbrush chromosome spheres. Since chromosomes 5 and 8 cannot be identified unequivocally in mitotic preparations, the correlation must be considered tentative until in situ hybridizations are carried out on lampbrush chromosomes.

In *T. a. apuanus* the correlation between histone label in mitotic chromosomes and lampbrush chromosome sphere positions (Mancino and Barsacchi, 1965; Callan and Lloyd, 1975) is especially convincing. Lampbrush chromosome 1 is a long metacentric with a sphere slightly distal to the middle of one arm. A long mitotic metacentric was labeled at a similar position (Figs. 14–17). Lampbrush chromosome 2 is a submetacentric with two spheres on the shorter arm. A long mitotic submetacentric was labeled in comparable positions (Figs. 13, 15, 18). Lampbrush chromosome 4, a medium length submetacentric has a nearly terminal sphere on the longer arm, and label occurs at a similar position on a medium length

mitotic chromosome (Figs. 13–16). One sphere, on lampbrush chromosome 9, was not represented by label on a mitotic chromosome in our preparations. Although once again positive identification will require hybridization to lampbrush chromosomes, all detected sites of histone genes on mitotic chromosomes probably correspond to sphere loci.

Approximately 600–800 copies of the histone gene repeat occur in the genome of *N. viridescens* (Stephenson et al., 1981 b). Since we detected roughly equal labeling at the sphere loci of chromosomes 2 and 6, each locus could contain 300–400 histone gene repeats. However, because the total number of silver grains in the autoradiographs is low, scattered histone clusters would not be detected in our preparations. Hence the number of histone clusters at the spheres could be somewhat lower than this estimate. In sea urchins different sets of histone genes are expressed during different stages of development and the major cloned repeats represent genes expressed during early cleavage stages (see Kedes, 1979). It is conceivable that the histone clusters we detect at the sphere loci, and which are expressed in the lampbrush chromosomes, represent oocyte specific genes. In this case transcription in other tissues might take place from genes at other chromosomal sites.

In an earlier study on lampbrush chromosomes of the newt *T. c. carnifex* Old et al. (1977) used various cloned sequences to identify the sites of histone gene transcription. In no case did they find hybridization to loops at the spheres of chromosomes 5 and 8, which we believe represent the major histone gene loci in this species. Their study made use of four sea urchin histone clones, two containing the entire repeats of *Psammechinus miliaris* and *Echinus esculentus* and two containing parts of the repeat of *Strongylocentrotus purpuratus*. Nick-translated DNA from all but one of the *S. purpuratus* clones gave consistent labeling of seven or eight loop pairs: four on the heteromorphic arm of chromosome 1, one or two on chromosome 6, and one each on chromosomes 10 and 11. More recent experiments (Callan and Old, 1980; Callan et al., 1980) show that RNA on these loops hybridizes to a probe which contains only the repeated sequence poly d(C–T): poly d(A–G). The reason for trying this simple sequence probe is that histone gene repeats of both *S. purpuratus* (Sures et al., 1978) and *P. miliaris* (Schaffner et al., 1978) contain stretches of poly d(C–T): poly d(A–G) in spacer regions between histone coding sequences. A careful analysis with new probes, three of which contain only coding sequences of histones H2A, H2B or H4 of *S. purpuratus*, suggests that a small subset of loops, including a prominent site on chromosome 1, contains histone gene transcripts. However, most of the hybridization in the original experiments was due to the simple repeated sequence.

The question remains why Callan and his coworkers did not see hybridization of sea urchin histone probes to loops at the sphere loci. One possibility is that sea urchin sequences differ too much from those of the newt to form stable hybrids. We believe this is unlikely on the basis of our studies on *Notophthalmus* histone genes (Stephenson et al., 1981 a), in which we find good cross reactions between the H2A, H2B and H3 genes of *P. miliaris* and the corresponding genes of *Notophthalmus*. Another possibility is that the major histone gene clusters of *Triturus* were not in an actively transcribing state in the animals examined by Callan and coworkers. As mentioned, we found extreme variation in the number and sizes of hybridizing

loops at the sphere loci of *Notophthalmus*. If this explanation is correct, the site on chromosome 1 could represent a minor cluster of genes which was transcriptionally active in the oocytes examined by Callan.

A possibly analogous situation was described recently by Morgan et al. (1980) for the rDNA of *T. c. carnifex*. The major clusters of ribosomal RNA genes occur on chromosomes 6 and 9 as shown by *in situ* hybridization to mitotic chromosomes. Although these sites sometimes transcribe in the oocytes and form nucleoli attached to the lampbrush chromosomes (Mancino et al., 1972), they usually do not. Thus rDNA probes will not usually hybridize to RNA at the known major sites of the ribosomal genes. Nevertheless, a few loops at other sites do hybridize with rDNA probes, suggesting that isolated gene clusters are being transcribed.

Clearly the sphere loci in *Triturus* lampbrush chromosomes require further study. Our experience with *Notophthalmus* suggests that cRNA probes copied from homologous single-stranded templates may be necessary to achieve adequate autoradiographic sensitivity. Such probes have the advantage over nick-translated DNA that they cannot reassociate during the hybridization reaction. In addition their specific activity is equivalent to that of the precursors used, whereas the specific activity of nick-translated DNA is usually somewhat lower. Finally because the spacers within the gene cluster are also transcribed (unpublished observations), homologous probes prepared from genomic clones will contain longer hybridizing sequences than heterologous probes. The combination of these factors made it possible for us to detect histone gene transcripts after only a few days of autoradiographic exposure (Figs. 23–25).

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