Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3364–3367, June 1983
Developmental Biology

Transcription of repetitive sequences on *Xenopus* lampbrush chromosomes

(repetitive DNA/*in situ* hybridization/oocyte RNA)

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Contributed by Joseph Gall, February 22, 1983

ABSTRACT **We reinvestigated the lampbrush chromosomes of *Xenopus laevis* and found them well suited for the study of transcription by *in situ* hybridization to nascent RNA transcripts. Using this technique, we analyzed the transcription of three repetitive sequences that do not show any sequence homology and that differ in their degree of interspersion. We found that they are located on different parts of the chromosomes: two are clustered, one is interspersed. All three of these sequences are transcribed at the lampbrush chromosome stage and transcripts from both strands of each sequence can be detected. The amount of transcription is apparently not proportional to the number of copies of the repetitive sequence at a given chromosomal locus, suggesting that other sequences are involved in the regulation of their transcription.**

Studies of amphibian lampbrush chromosomes during the last 30 years have clarified many features of their structure (refs. 1–6; for review see refs. 7 and 8). It was found that the characteristic lateral loops represent regions of active RNA synthesis and that each loop consists of one or a few transcription units. Because the average loop in the newts *Triturus* and *Notophthalmus* is 50 μm long, the DNA in many transcription units must be hundreds of kilobase pairs in length (1 μm = 3 kilobase pairs). The average size of transcription units on a lampbrush chromosome is therefore believed to be much greater than in somatic cells. This suggests that initiation, termination, or both are regulated differently in oocytes and somatic cells. Most of this information has been obtained from studies on newt lampbrush chromosomes, which are especially large and easy to study. The large size of the newt chromosomes is correlated with a very large genome, which, however, is a distinct disadvantage for experiments involving cloning and characterization of specific DNA sequences. For this reason we felt that it would be useful to search for an experimental system with a considerably smaller genome but with cytologically usable lampbrush chromosomes. The obvious choice was *Xenopus laevis*. The *Xenopus* genome is under investigation in several laboratories and the lampbrush chromosomes have already been described (9, 10), although they have been generally considered poorly suited for detailed cytological studies. We reinvestigated these chromosomes and found them quite adequate for the study of transcription and protein distribution by *in situ* hybridization and indirect immunofluorescence, respectively. In this report, we demonstrate that some of the long transcription units on *Xenopus* lampbrush chromosomes contain repetitive sequences. Transcripts of repetitive sequences have been reported in a variety of tissues, including the lampbrush chromosomes of newt oocytes (11–15).

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MATERIALS AND METHODS

***In Situ* Hybridization to Mitotic Chromosomes.** Mitotic chromosome squashes were prepared from gut cells of adult *Xenopus* and hybridized *in situ* according to Pardue and Gall (16).

***In Situ* Hybridization to Lampbrush Chromosomes.** ^{3}H-Labeled complementary RNA (cRNA) was prepared from single-stranded probes and was hybridized *in situ* to nascent RNA chains on the chromosomes according to Diaz *et al.* (12) and Pukkila (17).

Preparation of Cloned DNA and Radioactive Probes. *Xenopus* DNA was cut with restriction endonuclease *Hin*dIII and fractionated on a malachite green gel (18). The fractions were then electrophoresed on an agarose gel. The 750-base-pair band that is visible in the first fractions of the gradient was eluted and cloned in plasmid pBR322 by standard procedures. The identity of the cloned DNA was confirmed by hybridization to genomic DNA in a Southern blot. The *Hin*dIII fragment was then recloned in a phage M13 vector. In the course of this work we learned that the same repetitive DNA fragment was independently cloned by Lam and Carroll (19). The X-132 clone in pBR322 was kindly provided by G. Spohr, Geneva (20). X-132 DNA was then digested with *Eco*RI and the three individual *Eco*RI fragments (A, B, and C) were recloned in M13. Cloning experiments were carried out under P2 and EK1 conditions specified by the National Institutes of Health guidelines.

RESULTS

Lateral loops displaying polarity of matrix characteristic of amphibian lampbrush chromosomes can be readily recognized on the *Xenopus* lampbrush chromosomes of Fig. 1. The size of the loops varies in individual animals, but generally animals kept at room temperature (21–24°C) have larger loops than those kept in the cold. In some cases maintaining frogs for 16–36 hr at higher temperature (28–32°C) helped to bring about maximal expansion of the loops. Preparations similar to that in Fig. 1 were used for studying transcription of repetitive DNA, whereas mitotic chromosomes were used to localize these sequences on the chromosomes. Fig. 2 shows the distribution of silver grains after hybridization of X-132A cRNA to a preparation of mitotic chromosomes. X-132A is a highly repetitive sequence consisting of a basic repeat of 77–79 base pairs present in about 10^5 copies per genome (20). The terminal regions of all chromosomes show roughly equivalent hybridization. Fig. 3 shows the hybridization of one of the strands of X-132A to *Xenopus* lampbrush chromosomes. One of the chromosomes displays intense label on a cluster of terminal loops, indicative of strong tran-

Abbreviation: cRNA, complementary RNA.

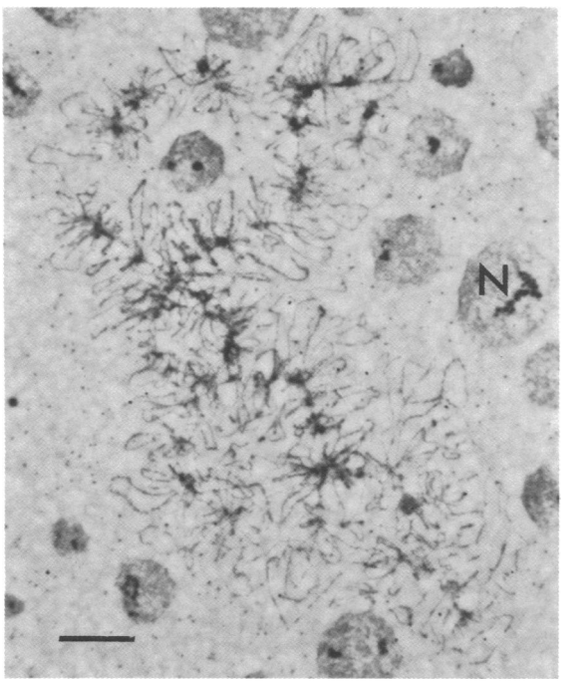

FIG. 1. Lampbrush chromosome from oocyte of *X. laevis* displaying lateral loops with gradients of matrix. N, nucleolus. This and other lampbrush chromosome preparations were stained with Coomassie blue (21). Bar represents 5 μm.

scription. Only weak label can be observed on the rest of the chromosomes. What is probably the same terminal region shows a relatively strong hybridization with the second strand of X-132A, although never so intense as with the first strand (Fig. 4). In a long exposure all of the terminal regions display labeled loops regardless of the strand that was hybridized (Fig. 5). Thus all of the chromosome ends contain this sequence and transcribe both strands of it during the lampbrush stage. However, the intensity of transcription is higher on one of the chromosomes than on all others.

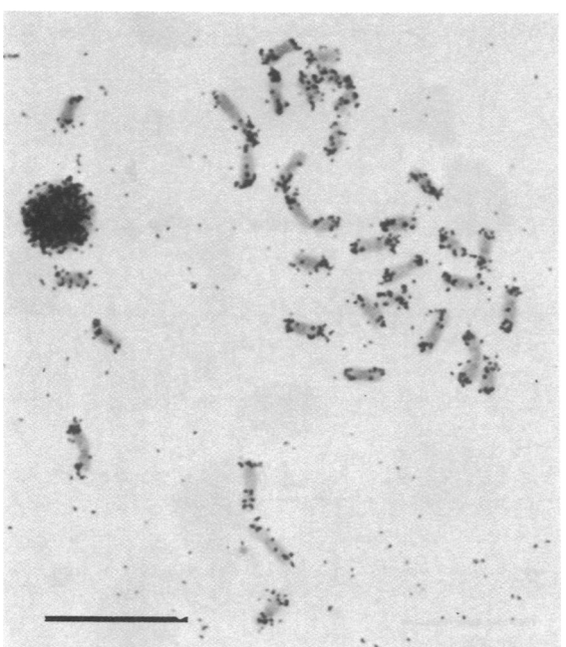

FIG. 2. Mitotic chromosomes of *X. laevis* hybridized with ^{3}H-labeled X-132A cRNA. Note the concentration of grains on terminal regions of the chromosomes. Probe specific activity, 8×10^7 dpm/μg; exposure, 3 weeks. Bar represents 5 μm.

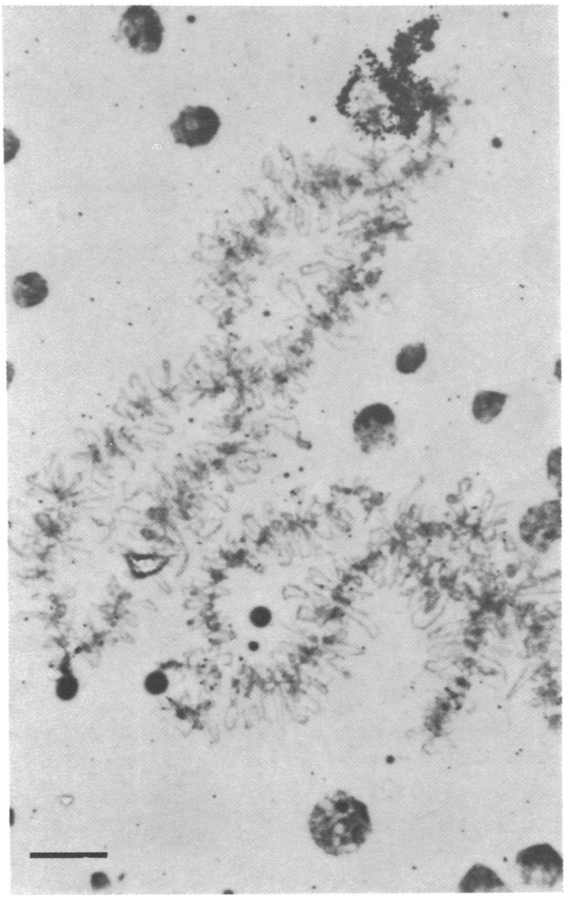

FIG. 3. *In situ* hybridization to lampbrush chromosomes of *X. laevis* of ^{3}H-labeled cRNA complementary to one of the strands of the X-132A sequence. Probe specific activity, 8×10^7 dpm/μg; exposure, 4 days. Bar represents 5 μm.

A similar disproportionality between number of copies and transcription is apparent in the case of the X1-741 clone. X1-741 is the name given to the *Hin*dIII fragment cloned by us and by Lam and Carroll (19). It is 741 base pairs in length, constitutes about 1% of the total genome, and is visible as a discrete band when total *Hin*dIII-digested genomic DNA is electrophoresed on an agarose gel. Like X-132A, it is a tandemly repeated, simple sequence DNA of the sort usually designated "satellite" DNA. The distribution of X1-741 on mitotic chromosomes is shown in Fig. 6. Most of this DNA is located in clusters on slightly more than half of the chromosomes. Unlike many satellite DNAs, X1-741 seems not to be clustered near the centromeres or telomeres. Both strands of this sequence hybridize to lampbrush chromosomes but to only a very few pairs of loops (one to four per preparation). Fig. 7 shows such label on one of the chromosomes. Thus only a limited set of copies of this sequence is transcribed, while the rest remain inactive.

The third clone, X-132C, contains a moderately interspersed sequence present in about 10^3 copies per genome. Hybridization of this sequence to mitotic chromosomes gave generalized labeling (not shown), consistent with its wide interspersion. The hybridization of either strand of this sequence to nascent RNA transcripts on lampbrush chromosomes resulted in a similar picture (Fig. 8). Many loops show weak labeling, as would be

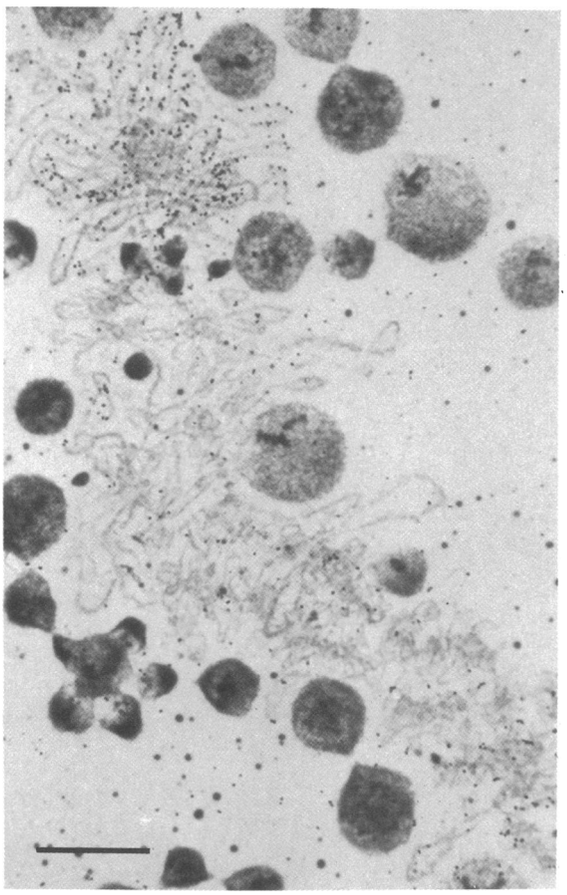

FIG. 4. *In situ* hybridization to lampbrush chromosomes of ^{3}H-labeled cRNA complementary to the opposite strand of the X-132A sequence. Probe specific activity, 7×10^7 dpm/μg; exposure, 4 days. Bar represents 5 μm.

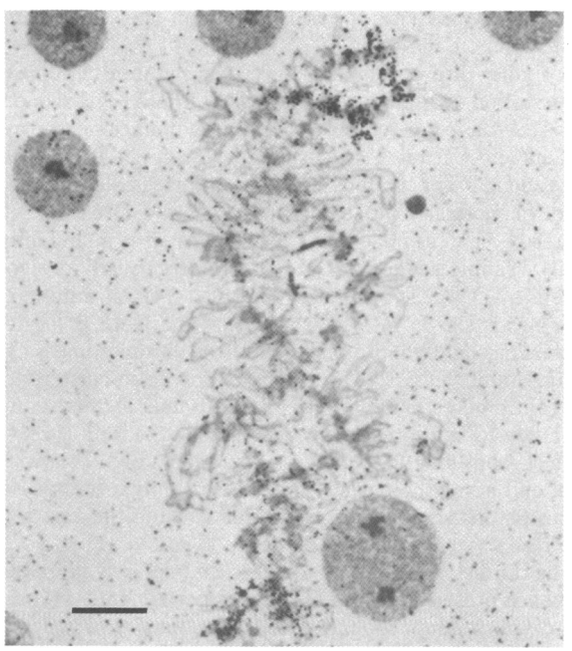

FIG. 5. *In situ* hybridization of ^{3}H-labeled cRNA to the lampbrush chromosomes. cRNA as in Fig. 3; exposure, 30 days. Bar represents 5 μm.

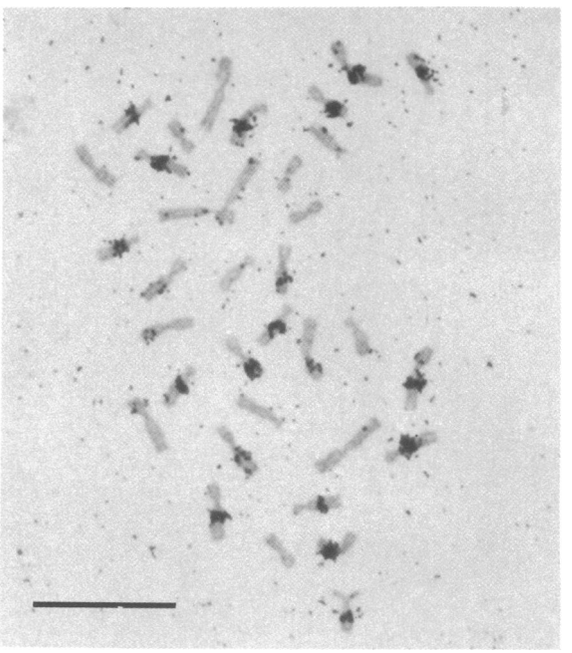

FIG. 6. *In situ* hybridization to the mitotic chromosomes of *Xenopus* of cRNA complementary to one of the strands of X1-741 sequence. Probe specific activity, 9×10^7 dpm/μg; exposure, 2 weeks. Bar represents 5 μm.

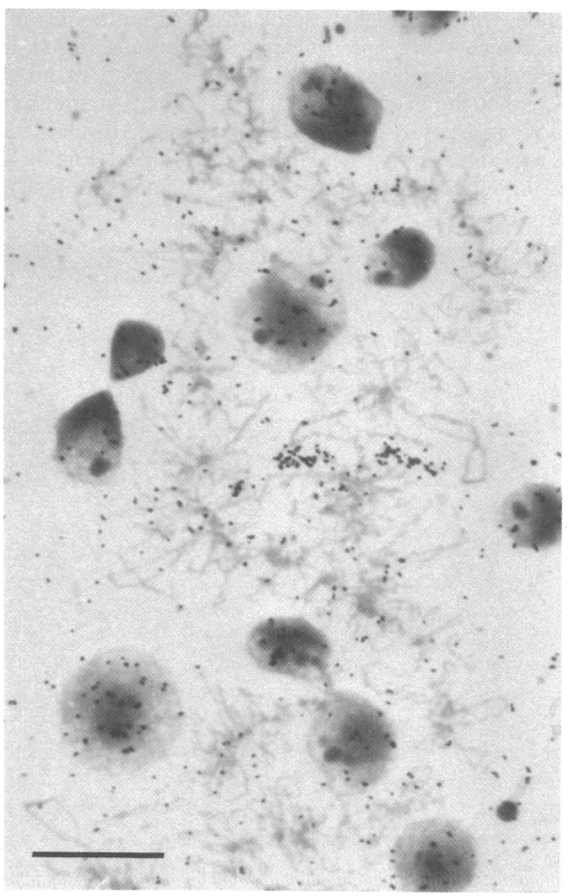

FIG. 7. *In situ* hybridization to the lampbrush chromosomes of *Xenopus* of cRNA complementary to one of the strands of X1-741 sequence. Probe specific activity, 9×10^7 dpm/μg; exposure 3 weeks. Bar represents 5 μm.

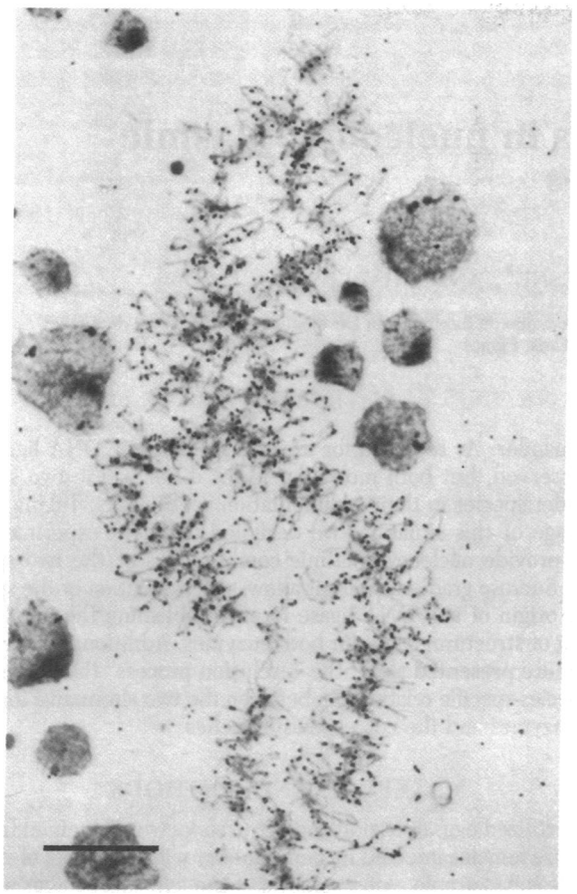

FIG. 8. *In situ* hybridization to lampbrush chromosomes of ^{3}H-labeled cRNA complementary to the X-132C sequence. Probe specific activity, 9×10^7 dpm/μg; exposure 3 weeks. Bar represents 5 μm.

expected for the transcription of interspersed repetitive sequences. Because there are about 10^3 copies of this sequence per genome and several hundred loops become labeled, we can estimate that a significant fraction of this sequence is transcribed in the lampbrush chromosome stage.

DISCUSSION

The very large size of transcription units on amphibian lampbrush chromosomes has prompted several studies addressing the nature of the transcribed sequences. *In situ* hybridization has demonstrated that repetitive sequences are widespread in the transcripts of newt chromosomes. Our finding that three different repetitive sequences are transcribed on *Xenopus* lampbrush chromosomes confirms these results and extends their general validity. It is clear, however, that no direct correlation exists between the number of copies of a repetitive sequence and the abundance of its transcripts at the lampbrush chromosome stage. In the case of clone X1-741, for example, only a few members of this family are transcribed. The implication is that these sequences do not regulate their own transcription or that only a very limited number of copies have this ability in the oocytes. It seems more likely that the transcription of the *Xenopus* repetitive sequences is coincidentally regulated by other sequences. An instructive example was provided by satellite 1 in the newt *Notophthalmus*. Copies of this satellite DNA are located downstream from histone gene clusters, and are transcribed coordinately with the histone genes. Most probably transcription initiates at histone gene promoters, but it does not terminate at the end of the gene. Instead, long transcripts are produced that contain histone sequences and the downstream satellite sequences (12). If such failure of termination is widespread at the lampbrush chromosome stage, then most or all primary transcripts would contain repetitive sequences. Biochemical data suggest that up to 70% of poly(A)$^+$ RNA from *Xenopus* oocytes contains repetitive sequences (11). We hope that the use of *Xenopus* lampbrush chromosomes will make possible a more detailed examination of transcription of repetitive sequences in this species.

R.S. is supported by a National Institutes of Health Postdoctoral Fellowship. M.J. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research. This work was supported by Research Grant GM 12427 from the National Institutes of Health.

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