Chromosomal mapping of *Xenopus* 5S genes: somatic-type versus oocyte-type

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ABSTRACT

Xenopus 5S RNA genes exhibit a pattern of differential expression during development in which some members (oocyte-type) are transcribed only in oocytes, while others (somatic-type) are expressed in both oocytes and somatic cells. Using cloned DNA probes specific for each gene type, we determined the positions of these genes on *Xenopus* metaphase chromosomes by *in situ* hybridization. Somatic-type 5S genes in both *X. laevis* and *X. borealis* are located at the distal end of the long arm of only one chromosome (number 9). The oocyte-type 5S RNA genes are found at the distal ends of the long arms of most *Xenopus* chromosomes, including chromosome 9. Thus, large scale differences in chromosomal location cannot explain the selective expression of these genes, as suggested previously.

INTRODUCTION

Xenopus and other amphibians exhibit a "dual" 5S ribosomal RNA system (1). One class of 5S RNA genes codes for somatic-type 5S RNA and is expressed in both oocytes and somatic cells (2). The other class, which codes for oocyte-type 5S RNA, is expressed in oocytes but not in somatic cells (3). In *X. laevis*, there are approximately 20,000 copies of the oocyte-type 5S RNA genes and 400 copies of the somatic-type genes per haploid genome (4). In *X. borealis*, there are 9,000 and 700 copies of the oocyte- and somatic-type genes, respectively (4). Both oocyte- and somatic-type 5S RNA genes are organized in long contiguous DNA segments consisting of tandemly repeated units of 5S RNA coding regions (genes) separated by non-transcribed spacer DNA (5). To date, no interspersion of the two types of genes has been found.

Chromosomal mapping studies were previously carried out in which ³H-labelled 5S RNA was hybridized *in situ* to *X. laevis* metaphase chromosome preparations. Label was observed on the distal ends of the long arms of most, if not all, chromosomes (6,7). Since the oocyte- and somatic-type genes exhibit 95% nucleotide sequence homology (3,8), the probe used could

detect both types of genes. However, it is likely that these experiments localized primarily the oocyte-type genes due to their 50-fold higher copy number relative to the somatic-type genes.

One major unexplained feature of 5S RNA synthesis in Xenopus concerns the mechanism governing the selective expression of the 5S RNA genes during development. It has been suggested that the distal ends of chromosomes, which contain the oocyte-type genes, might be in a chromatin structure available for transcription in oocytes, but not in somatic cells (9). Such a model predicts that the somatic-type 5S RNA genes have a location distinct from the oocyte-type genes. In order to test this prediction, we mapped the somatic-type 5S RNA genes in relation to the oocyte-type genes by in situ hybridization of somatic-specific and oocyte-specific probes to Xenopus chromosome preparations.

MATERIALS AND METHODS

Somatic-Specific 5S DNA Probes

Somatic-specific probes pXls459 and pXbs250 were constructed from clones pXls11 and pXbs1, each of which contain one repeat unit of somatic 5S DNA from X. laevis and X. borealis, respectively (4). pXls459 was constructed by replacing the 829 base pair (bp) Hind III-Bam HI fragment of pBR322 with the 459 bp Hind III-Rsa I spacer fragment of pXls11 (Fig. 1A). pXbs250 was constructed by inserting the 250 bp Ava I spacer fragment of pXbs1 into the Pvu II site of pBR322 by blunt end ligation (Fig. 1A).

Oocyte-Specific 5S DNA Probes

pXlospl, which contains a 312 bp insert of X. laevis oocyte-type 5S spacer DNA, was obtained from M. Wormington. pXltsp103, containing a 128 bp insert of X. laevis trace oocyte (Xlt) 5S spacer DNA (4), was obtained from D. Brown.

Southern Blot Hybridization

Genomic DNAs from X. laevis and X. borealis were prepared from erythrocytes by lysis in 10 mM EDTA, 0.5% SDS, followed by proteinase K treatment (25 ug/ml, 30 min, 20°C), phenol-chloroform extraction, and ethanol precipitation. Genomic and plasmid DNAs were digested with Hind III, electrophoresed through 1% agarose, transferred to nitrocellulose by Southern blotting (10), and hybridized with ³²P-labelled probes.

Xenopus Metaphase Chromosome Preparations

Mitotic cells were prepared from brain tissue of stage 41 tadpoles (11). Tadpoles were incubated in 0.4% colchicine (Sigma) in Holtfreter's solution

(12) at 20°C for 2 hr. Heads were removed, immersed in distilled water for 12 min, and fixed in 3:1 ethanol:acetic acid. Brains were removed by dissection and squashed in 45% acetic acid between subbed slides and siliconized coverslips. After freezing on dry ice, coverslips were removed and preparations were dehydrated in ethanol, dried and stored at room temperature. Chromosome preparations were used within 1 week for in situ hybridization.

In situ Hybridization

Chromosomes were firmly affixed to slides by heating in 2XSSC (0.3 M NaCl, 0.03 M Na citrate) at 70°C for 30 min, followed by dehydration in ethanol. Hybridization was carried out as described by Harper et al. (13,14), with the addition of an acetylation step to decrease background (15). Preparations were ribonuclease-treated at 37°C for 1 hr and then acetylated by suspension in 0.5% acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 10 min. Chromosomal DNA was denatured by a 2 min treatment in 70% formamide, 2XSSC at 70°C. DNA probes were ³H-labelled by nick translation (16) to specific activities ranging from 2.1-5.5 x 10⁷ cpm/ug and purified by Sephadex G-75 chromatography. Each probe, at a concentration of 7-63 ng/ml and contained in 50% formamide, 2XSCP (0.24 M NaCl, 0.03 M Na citrate, 0.04 M Na phosphate, pH 5.6), 10% dextran sulfate (Pharmacia), pH 7.0, and 500-fold excess E. coli carrier DNA, was denatured at 70°C for 5 min, quick-cooled and applied to the chromosome preparations. After incubation at 37°C for 12 hr, slides were rinsed well in 50% formamide, 2XSSC at 39°C and then 2XSSC at 39°C, and dehydrated in ethanol. Preparations were exposed to Kodak NTB2 nuclear track emulsion for 3-22 days, developed in Dektol, stained with Wright Stain (17), and analyzed by light microscopy.

RESULTS

Oocyte- and somatic-type 5S RNA gene families are both organized in long arrays consisting of repeating units of 5S RNA genes separated by non-transcribed spacer DNA (5). Within each species, the nucleotide sequences of the oocyte- and somatic-type genes exhibit 95% homology (3,8), whereas the two types of spacer regions have minimal sequence homology and do not cross-hybridize (4,5,18,19). Somatic-specific DNA probes for in situ hybridization were therefore constructed by subcloning DNA fragments from the somatic 5S spacer regions of two species of Xenopus (Fig. 1A). pXls459 contains a 459 bp fragment from the spacer region of X. laevis somatic-type 5S DNA. Similarly, pXbs250 contains a 250 bp spacer fragment

Figure 1(A). Construction of somatic-specific 5S DNA probes. Each diagram represents a previously cloned 5S DNA repeat unit from *X. laevis* (top) and *X. borealis* (bottom). Spacer-specific fragments (lengths indicated in bp) were subcloned in pBR322, as described in Materials and Methods, to give pXls459 and pXbs250. H, Hind III; R, Rsa I; A, Ava I. **(B).** Specificity of cloned spacer probes for somatic-type 5S DNA. As discussed in Results, pXls459 and pXbs250 each hybridize to a single band of Hind III-digested *Xenopus* genomic DNA corresponding exclusively to the somatic-type 5S repeat unit. In contrast, hybridization of Hind III-digested genomic DNA with pXls11, which contains an entire *X. laevis* somatic-type repeat unit, results in detection of many bands due to cross-hybridization between somatic- and oocyte-type 5S coding regions. Total genomic DNA and 5S plasmid DNA lanes contain 5 μ g and 10 ng DNA, respectively.

from the *X. borealis* somatic-type 5S repeat.

To ensure the specificity of pXls459 and pXbs250 for somatic-type 5S DNA, the recombinant plasmid DNAs were ³²P-labelled and hybridized to Southern blots (10) of Hind III-digested genomic DNAs (Fig. 1B). Hind III cuts genomic DNA once within each somatic-type repeat unit to give fragments of homogeneous length, 880 bp for *X. laevis* and 850 bp for *X. borealis* (4). In contrast, oocyte-type 5S DNA exhibits variability in spacer region length and in the number of Hind III sites (18). Therefore, Hind III digestion results in oocyte repeats of heterogeneous length (~700 bp in *X. laevis*) as well as dimers, trimers, etc. of the basic repeat. When hybridized to Hind III-digested *X. laevis* genomic DNA, pXls11, which contains an entire somatic 5S DNA repeat (spacer DNA plus a 5S RNA gene), hybridized to many bands due to cross-hybridization between the somatic- and oocyte-type 5S coding regions. In contrast, the somatic-type spacer probe pXls459 detected a single 880 bp band, corresponding to somatic-type 5S DNA (Fig. 1B). Similarly, subclone pXbs250 hybridized specifically to the 850 bp somatic-type repeat unit from *X. borealis* genomic DNA (Fig. 1B).

In situ hybridization of ³H-labelled pXls459 to *X. laevis* chromosomes resulted in silver grains located almost exclusively at the distal ends of the long arms of chromosomes 9. Hybridization at a relatively low probe concentration (7-20 ng/ml) resulted primarily in single or double grains on one or both chromosomes 9 and few grains on other chromosomes (Fig. 2A, B). Hybridization at a higher probe concentration (63 ng/ml) resulted in relatively large clusters of grains on both chromosomes 9, as well as single grains (not more than 5 per cell) scattered randomly throughout the chromosome complement (Fig. 2C). Similar results were obtained with the ³H-labelled *X. borealis* somatic-type probe pXbs250. After *in situ* hybridization to *X. borealis* chromosomes, clusters of grains were consistently observed on the distal ends of the long arms of chromosomes 9 (Fig. 3). The chromosome containing the somatic-type 5S RNA genes was identified as chromosome 9 by two criteria: arm ratio (short/long) and length relative to chromosome 1 (the largest chromosome). The *X. laevis* and *X. borealis* karyotypes (n=18) were previously characterized in detail with regard to arm ratio and relative length (20,21). *X. laevis* chromosome 9 is the largest metacentric chromosome with an arm ratio of 0.90 and a length relative to chromosome 1 of 0.72. All other chromosomes in the karyotype have an arm ratio $\leq$ 0.70 except for chromosomes 10 and 11, which

Figure 2. Localization of *X. laevis* somatic-type 5S genes. In situ hybridization of ^3H -labelled somatic-specific 5S DNA probe pXls459 to *X. laevis* chromosomes resulted in typical labelling of the distal ends of the long arms of chromosomes 9 (arrows). (A) Probe concentration of 7 ng/ml; 12 day exposure. (B) Probe concentration of 20 ng/ml; 5 day exposure. (C) Probe concentration of 63 ng/ml; 9 day exposure.

exhibit a length relative to chromosome 1 of ≤ 0.21 , making chromosome 9 easily distinguishable. In an analysis of 12 photographed *X. laevis* mitotic cells, 19 labelled metacentric chromosomes exhibited an average arm ratio of $0.97 \pm .03$ and an average length relative to chromosome 1 of $0.73 \pm .03$.

Our finding that the somatic-type 5S RNA genes are located on one pair

Figure 3. Representative *X. borealis* mitotic spread hybridized with somatic-specific probe pXbs250, illustrating typical labelling of the distal ends of the long arms of chromosomes 9 (arrows). Probe concentration of 33 ng/ml; 8 day exposure.

of chromosomes, and in the same chromosomal position as the 5S DNA detected previously by Pardue *et al.* (6,7), raised the question of whether oocyte-type 5S RNA genes are in fact present at this particular site. Since the probe used in their experiments, 5S cRNA made from 5S DNA, hybridizes to both types of genes, a cluster of somatic-type genes would have been indistinguishable from oocyte-type genes in their analysis. Therefore, we used the oocyte-specific probe pXlosp1, which contains a 312 bp oocyte-type spacer DNA fragment, to locate the oocyte-type 5S genes by *in situ* hybridization to *X. laevis* metaphase chromosomes. As seen in Fig. 4, the distal ends of the long arms of most chromosomes, including chromosome 9, were labelled. Thus, oocyte-type 5S DNA is present in the same chromosomal region as the somatic-type 5S genes.

In addition to the major class of oocyte-type 5S RNA genes in *X. laevis*, there is a minor class of approximately 1300 trace oocyte-type (Xlt) genes per haploid genome (4,22). The expression of these genes parallels that of the major class of oocyte-type 5S genes, although the two types of oocyte-type genes differ slightly in nucleotide sequence. We localized the trace oocyte-type genes by hybridization of ³H-labelled pXltsp103, which contains a 128 bp Xlt-specific spacer DNA fragment (4), to *X. laevis* metaphase chromosomes. This resulted in labelling of the distal ends of the long arms of a single pair of chromosomes within group 13-18 (Fig. 5).

Figure 4. In situ hybridization of oocyte-specific 5S DNA probe pXlospl to X. laevis mitotic cell. Distal ends of the long arms of most chromosomes, including those of chromosomes 9 (arrows) were typically labelled. Probe concentration of 30 ng/ml; 11 day exposure.

This chromosome has been identified as chromosome 13, which exhibits an arm ratio of 0.34 and a length relative to chromosome 1 of 0.70. The other most submetacentric chromosomes, 14-18, have arm ratios $\leq .30$ except for chromosome 18, which exhibits a length relative to chromosome 1 of 0.52 (20). An analysis of 8 labelled chromosomes hybridized with the trace oocyte-type probe exhibited an average arm ratio of $0.35 \pm .03$ and a length relative to chromosome 1 of $0.69 \pm .04$. Thus the trace oocyte-type genes were localized to the distal end of the long arm of chromosome 13.

DISCUSSION

We have localized the somatic-type 5S genes in two species of Xenopus to a single major site on the distal end of the long arm of chromosome 9. This chromosomal region also contains some oocyte-type 5S RNA genes, with the remainder distributed on the distal ends of most and possibly all other Xenopus chromosomes. Our experiments cannot eliminate the possibility that some somatic-type 5S RNA genes are located on chromosomes other than chromosome 9, as suggested by limited segregation analysis of backcross progeny between X. laevis and X. borealis (4). However, since hybridization to one particular chromosomal region was highly significant (at least 10-fold greater than any other site), we are confident that no major secondary site was undetected.

Figure 5. Representative in situ hybridization of *X. laevis* trace oocyte-type 5S DNA probe pXltsp103 to *X. laevis* chromosomes. Cell exhibits labelling on distal ends of long arms of chromosomes 13. Probe concentration of 100 ng/ml; 22 day exposure.

The trace oocyte-type (Xlt) genes, a minor component of *X. laevis* oocyte-type 5S genes, were also localized to a single major site on the distal end of the long arm of chromosome 13. This region also contains a portion of the major class of oocyte-type 5S genes, as evidenced by hybridization of the major oocyte-specific probe to chromosome 13 (data not shown). The function of the trace oocyte-type 5S genes is not known, although their expression follows that of the major class.

Models concerning the regulation of 5S genes in *Xenopus* have been proposed to explain the differential expression of the oocyte- and somatic-type genes during development. These include (a) differences in chromosomal location between the oocyte- and somatic-type genes (9), (b) repression of oocyte-type 5S RNA transcription in somatic cells via a system analogous to that found in prokaryotes (discussed in 23) and (c) positive regulation by specific activating factors (24-27). A protein factor, which specifically reactivates quiescent oocyte-type 5S ribosomal RNA genes, has been partially purified (25; P. Farnham, J. Price and L. Korn, unpublished results). Another activating factor may be TFI_{IIA}, a protein that binds to the 5S intragenic control region and is required for transcription (26,27). These factors may act independently by exhibiting different affinities for oocyte- and somatic-type 5S genes (28), or in conjunction with other

activating factors (25,29). Our finding that chromosome 9 contains both oocyte- and somatic-type 5S genes in the same region provides evidence against the first model (a) and makes it unlikely that differential expression of the two types of genes is simply a consequence of large scale differences in their chromosomal location.

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