Transcription of a Satellite DNA in the Newt

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Abstract. Satellite 2 is an abundant, 330-bp tandemly repeated sequence in the genome of the newt, *Notophthalmus viridescens*. This sequence is distributed throughout the genome on each of the 11 chromosomes. Both strands of satellite 2 are transcribed on the lampbrush chromosomes during oogenesis, probably as a result of readthrough from upstream structural gene promoters. In addition to these heterogeneous nuclear transcripts, satellite 2 is homologous to stable, strand-specific cytoplasmic transcripts in a variety of different tissues. The majority of these transcripts correspond in size to the entire satellite 2 repeat unit, or to whole multiples of the repeat. The transcripts present in the ovary have been sequenced by primer extension and were found to be more homogeneous than eight independently cloned satellite 2 DNA repeats. We propose that the stable cytoplasmic transcripts are encoded by a small subset of genomic satellite 2 sequences.

A large portion of the eukaryotic genome consists of repetitive DNA sequences, which are broadly classified according to their degree of repetition and to the extent of their interspersion with single-copy sequences. While there is ample evidence for transcription of some interspersed repeats (e.g., Davidson et al., 1977; Long and Dawid, 1980; Zuker and Lodish, 1981; Anderson et al., 1982), tandemly repetitive "satellite" sequences are generally inert transcriptionally (reviewed by Brutlag, 1980). Speculations concerning the functions of satellite DNAs have been confined largely to aspects of chromosome structure (Walker, 1971; Brutlag, 1980). However, transcription of satellite sequences on amphibian lampbrush chromosomes has been demonstrated (Macgregor, 1979; Varley et al., 1980; Diaz et al., 1981; Jamrich et al., 1983). Discrete cytoplasmic transcripts have also been found that are homologous to satellite DNA. These include transcripts which map entirely within a satellite DNA in *Xenopus* (Ackerman, 1983) and a potentially translatable satellite transcript that is expressed in a developmental and sex-specific manner in mice and *Drosophila* (Singh et al., 1984). While the functions of these transcripts are not known, it seems probable that at least some satellite DNAs play more than a simple structural role in the chromosome.

We have studied the transcription of satellite DNA in the eastern newt, *Notophthalmus viridescens*. In earlier reports we described the transcription of satellite 1, an abundant 220-bp tandemly repeated sequence (Diaz et al., 1981; Gall et al., 1983). Satellite 1 is found in the pericentric heterochromatin, where it is not generally transcribed, and in the spacer regions between the histone gene clusters, where it is actively transcribed during the lampbrush chromosome stage. Despite their synthesis on lampbrush chromosomes, satellite 1 transcripts do not accumulate in the cytoplasm (Mahon, K., unpublished observations).

We have now isolated and characterized a second satellite sequence from *N. viridescens*, which we call satellite 2. Like satellite 1, this 330-bp sequence is transcribed on lampbrush chromosomes, but in addition it is homologous to a set of cytoplasmic transcripts found in a variety of tissues. Most of the transcripts correspond precisely in size to the monomer repeat unit or to whole multiples of that unit. A novel mechanism of RNA transcription or processing is implicated in the formation of satellite 2 transcripts.

Materials and Methods

Materials

Adult female *Notophthalmus viridescens* were purchased from Lee's Newt Farm, Oak Ridge, TN. Restriction enzymes were purchased from Bethesda Research Laboratories, Gaithersburg, MD, and used according to recommendations. Boehringer-Mannheim Biochemicals, Indianapolis, IN, was the source of DNA ligase, DNA polymerase I, RNA polymerase, T4 polynucleotide kinase, and AMV reverse transcriptase. Labeled nucleotides were obtained from Amersham Corp., Arlington Heights, IL.

Newt DNA

The livers from five animals were diced, homogenized in 10 ml of homogenization buffer (100 mM NaOAc, pH 5.0, 5 mM EDTA, 0.5% SDS) and extracted five times with phenol. Nucleic acids were precipitated with ethanol, and DNA and RNA were separated by centrifugation on a CsCl cushion consisting of 1.67 and 1.74 g/cm^3 layers. The DNA was recovered from the interface and precipitated. When necessary, the DNA was further purified by equilibrium centrifugation in CsCl.

Newt RNA

Total ovary RNA was prepared as follows. The ovaries from five animals were homogenized in 18 ml of lysing medium (5.8 M guanidine-HCl, 0.1 M KOAc, pH 5.0) which was precooled to −20°C and to which 180 μl of diethyl pyrocarbonate was added before use. The homogenate was extracted with an equal volume of phenol/chloroform (3:2), then with an equal volume of phenol/chloroform (1:1), and finally with an equal volume of chloroform. Further purification of the RNA was as described by Strohman et al. (1977).

For the purification of germinal vesicle RNA, germinal vesicles were isolated by hand in 83 mM KCl, 17 mM NaCl, 1 mM EDTA, 10 mM Tris, pH 7.2, and dissolved in 100 mM NaOAc, pH 5.0, 5 mM EDTA, 0.25% SDS. After extraction with phenol/chloroform (1:1) and then chloroform, the RNA was recovered by ethanol precipitation.

Total liver RNA was obtained during the preparation of liver DNA (see above). The RNA, which pelleted during centrifugation through the CsCl cushion, was dissolved in TE (10 mM Tris, pH 8.0, 1 mM EDTA) and precipitated with ethanol.

Clones

mTcS2 consists of a single repeat of satellite 2 from *Triturus cristatus carnifex* inserted into the single-stranded DNA phage vector M13mp9. The insert in mTcS2 was derived from pTcS275-1 (Varley et al., 1980), a pBR322 clone obtained from Herbert Macgregor (University of Leicester).

mNv15-47 consists of a single repeat of satellite 1 from *N. viridescens* cloned into M13mp2 (Diaz et al., 1981).

N. viridescens satellite 2 clones were constructed and sequenced by two slightly different procedures. First, genomic DNA was digested with Bgl II and electrophoresed on a 1.5% low gelling temperature agarose gel. After staining with ethidium bromide, the 330-bp band was excised and purified from the gel. This DNA was cloned into the Bam HI site of pBR322, and satellite 2 clones were identified by hybridization to Southern blots of genomic digests. Sequencing of the clones was accomplished by the chemical cleavage method of Maxam and Gilbert (1980). The inserts were subsequently subcloned, in both orientations, into the Bam HI site of M13mp9 (mNv13-A, mNv13-B, mNv14-A, mNv14-B). These clones were used for the generation of strand-specific satellite 2 probes. For the purpose of clarity, the strands of satellite 2 have arbitrarily been designated the A and B strands, and the clone nomenclature reflects which strand is contained in the single-stranded form of the phage. Thus, single-stranded DNA from mNv13-A and mNv13-B contain the opposite strands of the same cloned satellite 2 repeat.

The remainder of the satellite 2 clones were obtained by cloning gel-purified satellite 2 DNA directly into M13mp10. Plaques were screened by the method of Benton and Davis (1977) using nick-translated double-stranded mNv13-A DNA as a probe. The inserts from selected clones were subcloned into M13mp11, which enabled both strands of each clone to be sequenced by the Sanger dideoxynucleotide method (Sanger et al., 1977).

Blots and Filter Hybridizations

Northern and Southern blots were prepared as described by Forney et al. (1983), except that 1.75% formaldehyde-agarose gels were used in the Northern analysis. Filters were hybridized at 42°C in 10% dextran sulfate-50% formamide and washed at 65°C.

Chromosome Preparations and In Situ Hybridizations

Oocyte lampbrush chromosome preparations were made as described by Gall et al. (1981). Mitotic chromosomes were prepared from the gut epithelium of colchicine-treated newts as described by Macgregor and Andrews (1977). ^{3}H-labeled cRNA probes for in situ hybridizations were prepared using *Escherichia coli* RNA polymerase and single-stranded DNA templates from the M13 clones. The specific activities of the probes were in the range of 10^8 dpm/μg, and 5×10^5 cpm in a 5-μl volume was hybridized per slide (Gall et al., 1981).

Primer Extensions and RNA Sequencing

Oligonucleotide primers were synthesized using a 380A DNA synthesizer (Applied Biosystems, Inc., Foster City, CA). The primers were labeled in 0.4-μg batches in the presence of 50 μCi of [γ-^{32}P]ATP and 10 U of T4 polynucleotide kinase at 37°C for 60 min. Primers were annealed to homologous transcripts in total ovary or liver RNA preparations as follows. Labeled primers (0.015 μg/reaction) and RNA (25 μg/reaction) were denatured together at 65°C for 1–2 h in 250 mM KCl, 1 mM EDTA, 10 mM Tris, pH 8.0 in a total volume of 15 μl. The solution was then slowly cooled to room temperature. The primers were extended with 5 U of AMV reverse transcriptase at 37°C for 1 h in a 45-μl reaction with 80 mM KCl, 0.3 mM EDTA, 18 mM Tris, pH 8.3, 10 mM $MgCl_2$, 5 mM dithiothreitol, and 0.2 mM of each deoxynucleotide triphosphate. The extension products were precipitated with ethanol, pelleted, and analyzed on 9% acrylamide-7 M urea gels.

Satellite 2 transcripts were sequenced in total ovary RNA by adding 0.1 mM dideoxy ATP, GTP, CTP, or TTP to the primer extension reaction. A control reaction with no added dideoxynucleotides was included for each primer.

Results

Characterization of Genomic Satellite 2 Sequences

Fig. 1, lane 2, shows an ethidium bromide-stained gel of liver DNA from *N. viridescens* digested with the restriction enzyme Bgl II. Several discrete bands are evident against a background of heterogeneously sized DNA. Some of these bands represent monomers, dimers, and larger multimers of a 220-bp repeated sequence, satellite 1, which we previously characterized (Diaz et al., 1981; Gall et al., 1983). Fig. 1, lane *3*, shows a Southern blot of the gel probed with a genomic clone of satellite 1. A ladder-like hybridization pattern is typical for tandemly repeated, simple-sequence DNA.

A 330-bp fragment is also evident in the ethidium bromide-stained gel which does not hybridize to satellite 1 sequences. To determine if this fragment represents another tandemly repeated sequence in the newt genome, DNA from this band was cloned into the phage vector M13mp9 as described in Materials and Methods. One of the resultant clones, mNv13-A, was used to probe a duplicate blot of the gel shown in Fig. 1, lane 2. This probe hybridized to a series of fragments, each a whole multiple of the 330-bp fragment (Fig. 1, lane *4*). When newt DNA was partially digested with Bgl II before blotting and hybridizing to the mNv13-A probe, the intensity of hybridization to the monomer and smaller multimers was reduced, while that to the larger multimers was increased (data not shown). These results demonstrate that the 330-bp band represents a tandemly repeated sequence in the newt genome, which we designate satellite 2.

The distribution of satellite 2 sequences in the genome was determined by in situ hybridization. ^{3}H-labeled cRNA was prepared using single-stranded DNA from mNv13-A as a template, and the cRNA was then hybridized to denatured mitotic chromosomes from newt intestine (Fig. 2). Hybridization was distributed evenly over the entire chromosome set. The distribution of satellite 2 is unusual since tandemly repeated simple sequences are typically localized in discrete heterochromatic regions in the genome.

Eight independent genomic satellite 2 clones were constructed and sequenced as described in Materials and Methods. The consensus sequence of these clones is shown in Fig. 3. The strand shown has been arbitrarily designated the B strand and is orientated so that the first base corresponds to the 5′ end of the major transcript in ovary RNA, as determined by primer extension (see below). This orientation puts the Bgl II site, which marks the actual ends of the M13 clones, at position 201 of the sequence. The individual clones were between 81 and 97% homologous to the con-

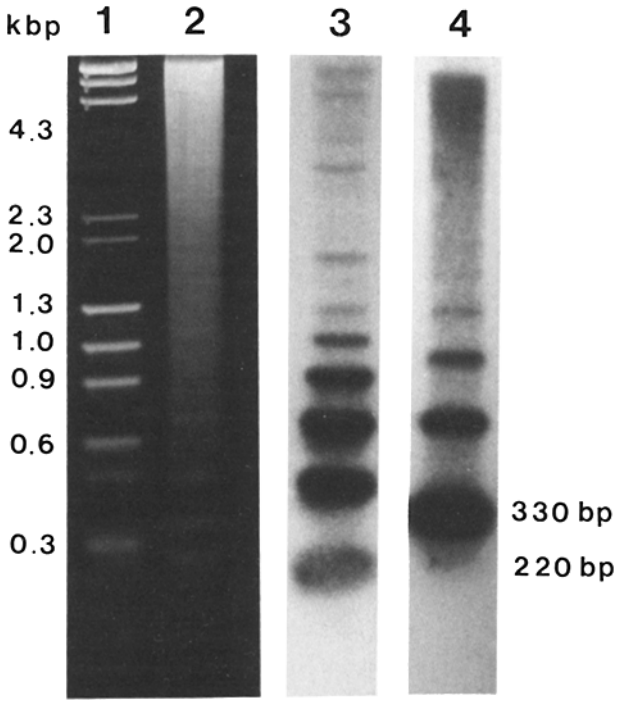

Figure 1. Southern blots with *Notophthalmus* cloned satellite DNA probes. DNA size markers (lane *1*), and 7.5 μg of Bgl II–digested *Notophthalmus* liver DNA (lane *2*) were electrophoresed on a 2% agarose gel and stained with ethidium bromide. Replicate blots of lane *2* were prepared and probed with nick-translated, double-stranded DNA from clones of satellite 1 (lane *3*) or satellite 2 (lane *4*).

sensus sequence. Also included in Fig. 3 is the sequence of mTcS2, a cloned segment of repetitive DNA from the European newt, *Triturus cristatus carnifex* (Varley et al., 1980). The *Triturus* sequence shows 75% homology to the *Notophthalmus* consensus sequence. Other European *Triturus* species and the American *Taricha granulosa* all possess a related satellite DNA (Mahon, K., Z. Wu, H. C. Macgregor, G. Barsacchi-Pilone, unpublished observations).

Transcription of Satellite 2 on Lampbrush Chromosomes

The transcription of satellite 2 sequences was investigated by hybridizing ^{3}H-labeled cRNA probes to the nascent RNA transcripts on lampbrush chromosomes. Using strand-specific probes, we found variable intensity of hybridization to a large number of loops located throughout the chromosome set. Some examples of hybridization are shown in Fig. 4. These results are consistent with the readthrough model of lampbrush transcription (Gall et al., 1983) which predicts that a sequence distributed throughout the genome can be abundantly transcribed due to fortuitous placement downstream from structural gene promoters. Since both strands are transcribed to a similar extent on lampbrush chromosomes, satellite 2 repeats do not have a preferred orientation relative to the active promoters.

Stable Transcripts from Satellite 2

Although both strands of satellite 2 are transcribed on lampbrush chromosomes, only one strand produces stable transcripts. Fig. 5 shows the hybridization of strand-specific satellite 2 probes to DNA and to various RNA preparations from *Notophthalmus*. As expected, both strands hybridized to the 330-bp ladder in Bgl II–digested DNA. However, only the A strand hybridized to transcripts in the various RNA populations. The most prominent transcript was seen in ovary RNA and had the same mobility as the monomer DNA repeat unit. Less abundant transcripts of about 460, 355, 240, and 180 nucleotides were also present in ovary RNA. Germinal vesicle RNA contained monomer-sized transcripts but was enriched for the 460- and 355-nucleotide transcripts; it lacked detectable amounts of the smaller transcripts.

Figure 2. In situ hybridization of satellite 2 to the DNA of mitotic chromosomes. Mitotic chromosomes were prepared from newt intestine, denatured, and hybridized to ^{3}H-labeled cRNA to mNv13-B. 12-d exposure. Bar, 30 μm.

The transcripts found in liver RNA were especially interesting because they all corresponded in size to the monomer, dimer, or larger multimers of the DNA repeat unit. This same array of transcripts was found in other non-ovarian tissues such as the spleen, oviduct, and intestine (data not shown). Although the pattern of hybridization to liver RNA was essentially the same as to genomic DNA, the hybridization was not due to DNA contamination in the liver RNA preparation. Three arguments support this conclusion. First, the hybridization was completely sensitive to RNase but not to DNase (data not shown). Second, if DNA were contaminating the liver RNA preparation, both strands of satellite 2 would be present in the contaminating DNA. This is not the case since hybridization to liver RNA was strand

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                10         20         30         40         50
CONSENSUS  GATTCAAGCTAGCCTGGCTGATGAaGGGTGATA-CCCTGAAACCGGTCCTAGGATGCttT
   mTcS2   GATTCXAGCAAGCCTGGCTGAAGACGGCTGAAAXGCCAXAAACCGGTCCTAGGAAGCTTX
     RNA   XXXXXXXXCTAGCCTAGCTGATGAAGGGTGATA-CCCTGAAACCGGTCCTAGGATGCTTT
                    ---------23mer----------

                70         80         90        100        110
CONSENSUS  GTTTCcGGTCCAGGGAaGACCTGGCcTGGCAGTTCGGGCTGGACTGttCCCATGAGGcaC
   mTcS2   -TTTCCGGTCCAGGGAAGGCATGGCTTGGCAGTTCXAGCTGGACTGTTCCCATGAGGCAC
     RNA   GTTTCCGGTCCAGGGAAGACCTGGCCTGGCAGTTCGGTCTGGACTGTTCCCATGAGGCAC
                              --------21mer--------

               130        140        150        160        170
CONSENSUS  AGGGTCAAGACTGATTTGCATATGGCTGGGTCCAAACTGGGGTGGCttGGTGAGCAgAAG
   mTcS2   AGGGTCA-GACTGATTTGCATATGGCCATGTCTXAACTGGGGTGGCAXAGTGGGCTAAAX
     RNA   AGGGTCAAGACTGATTTGCATATGGCTGGGTCCAAACTGGGGTGGCTTGGTGAGCAGAAG

               190        200        210        220        230
CONSENSUS  AAtgATGATGGATTAAACCCAGATCTGTGACTGGGGGTGAgTGTCTGTACTttTTGccaG
   mTcS2   AACGGTGATGGATCAAACXTAGATCTXTTGXXTGGTXTXAGTGT-TTXATTGGTT-CTGA
     RNA   AACGATGATGGATTAAACCCAGATCTGTGACTGGGGGTGAGTGTCTGTACT-GTTGCCAG
               ------17mer------

               250        260        270        280        290
CONSENSUS  CATTCCGTCCATCATCACCTTTTgTGtTTcTaAaTGTTCgCCCTAAGTGGCA-tGGGTAT
   mTcS2   TGCTCCGTCCATCACCACCCTCTTTTXTACCGCTTTXTTTGCCATAAXTTGGXAGGGTAX
     RNA   CACTCCGTCCATCATCACCTTTTGTGTTTGTAATTGTTCGCCCTAAGTGGCXXXXXXXXX
                                                                -----

               310        320        330
CONSENSUS  GCCCAGACGTGGGTCCCTTGCTCACTGaGCCACTG
   mTcS2   GCTXAGACGTGAGTCCCTTGCTCACTTTGCCACTG
     RNA   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
           ----22mer--------
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Figure 3. Sequences of satellite 2 DNA and satellite 2 transcripts. The consensus sequence is derived from eight independent *Notophthalmus* satellite 2 clones. The noncoding strand (B strand) is shown and is aligned to the RNA. Capital letters in the consensus sequence indicate a 75% or better consensus, lower case letters indicate a majority, but less than 75% consensus. mTcS2 is a *Triturus* satellite clone and is 75% homologous to the *Notophthalmus* consensus. The RNA sequence is the major satellite 2 transcript in total ovary RNA and was sequenced by a modification of primer extension as described in Materials and Methods. Underlined nucleotides in the mTcS2 and RNA sequences diverge from the *Notophthalmus* consensus sequence. *X*, undetermined nucleotide; −, deletion relative to other sequences. The regions to which complementary primers were synthesized are indicated below the sequences.

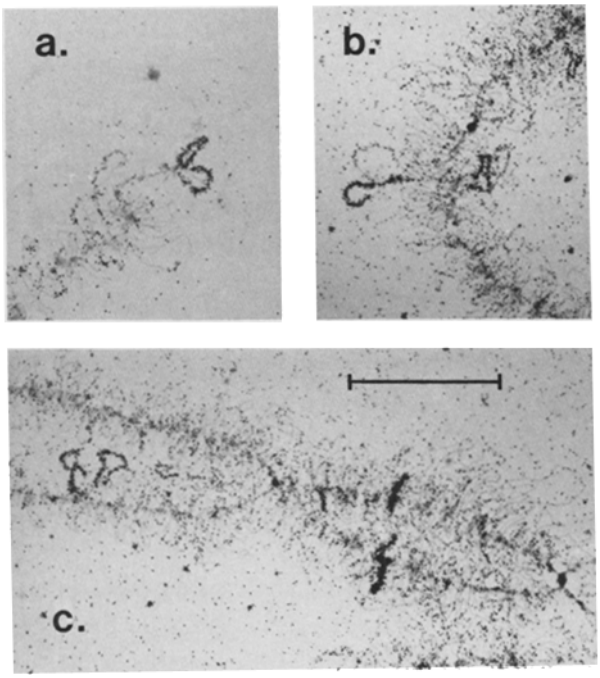

Figure 4. Examples of in situ hybridization of strand-specific satellite 2 probes to the nascent RNA transcripts on lampbrush chromosomes. (*a*) A pair of fully labeled loops at the end of a chromosome after hybridization with ^{3}H-labeled cRNA to mNvl3-B. 30-d exposure. (*b*) A pair of partially labeled loops after hybridization with cRNA to mNvl3-A. 120-d exposure. (*c*) A pair of homologous chromosomes, with two chiasmata in view, labeled at two loci with cRNA to mNvl3-A. Loops on both homologues are transcribing satellite 2 sequences at the locus on the right, while only one homologue is transcribing satellite 2 sequences at the locus on the left. 120-d exposure. Bar, 50 μm.

specific. Finally, in order to obtain multiple bands of hybridization, DNA must be cut with a restriction enzyme. The RNA preparations were not digested with any enzymes and if DNA were contaminating these preparations, it would band near the top of the gel.

Hybridization to these or any other transcripts was not seen when the B strand of satellite 2 was used as a probe (Fig. 5). The apparent hybridization to a low molecular weight RNA in the germinal vesicle lane is due to nonspecific binding of the probe to an excess of yeast tRNA used as a carrier. When germinal vesicle RNA was prepared without carrier tRNA, binding to this lower band was not seen (data not shown).

Characterization of Satellite 2 Transcripts by Primer Extension

To determine the 5′ end of satellite 2 transcripts relative to the DNA repeat unit, four synthetic oligonucleotides were used for a primer extension analysis. These oligonucleotide primers were complementary to different segments of the genomic satellite 2 consensus sequence, and their positions in the repeat unit are indicated in Fig. 3.

Labeled primers were hybridized individually to unfrac-

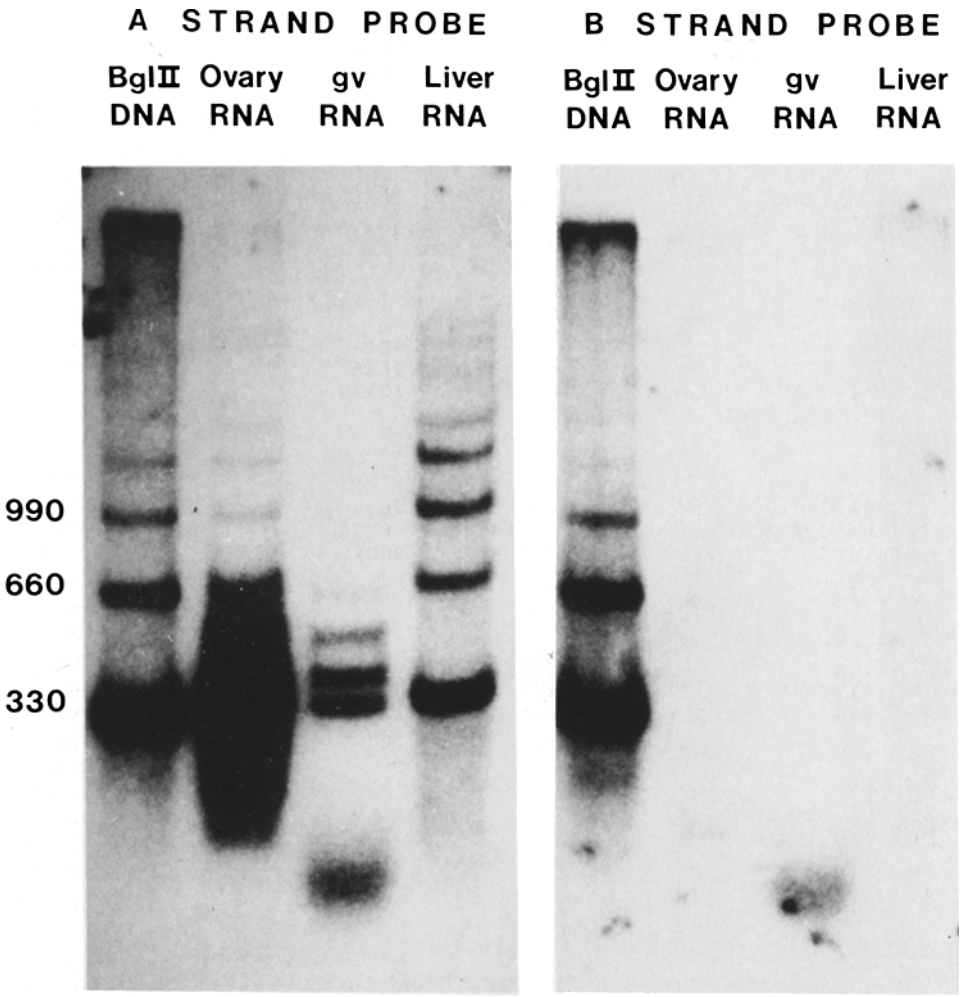

Figure 5. Northern analysis of satellite 2 transcripts. Blots were prepared from identical gels containing 0.3 μg of Bgl II–digested *Notophthalmus* DNA (lanes *1*), 15 μg of total ovary RNA (lanes *2*), 55 ng of germinal vesicle RNA (lanes *3*), and 15 μg of total liver RNA (lanes *4*). The blots were hybridized to unlabeled, single-stranded DNA from mNv13-A (A strand probe) or mNv13-B (B strand probe), and then to nick-translated, double-stranded DNA from M13mpl0 to visualize the unlabeled probes. Sizes in bases.

tionated ovary RNA preparations. Primer molecules that hybridized to satellite 2 transcripts were extended with reverse transcriptase in the presence of deoxynucleotide triphosphates. Fig. 6 *a* shows an acrylamide gel of the extension products from the 17-base primer. Two bands are evident with sizes of ~195 and 200 nucleotides. The formation of two products could be an artifact of the reverse transcriptase reaction. However, evidence presented below suggests that there are true differences in the 5′ ends of ovary transcripts.

The four oligonucleotide primers gave four independent estimates of the 5′ end of the major ovary transcript (data not shown). In each case, the longest extension product predicted the 5′ end shown in Fig. 3. These results argue for the colinearity of the major ovary transcript with the satellite 2 repeat unit, at least throughout the region analyzable with these primers.

The formation of more than one major extension product, as seen with the 17-base primer, was noted with each of the other primers. Evidence that this heterogeneity is not an artifact of the system, but results from the presence of more than one 5′ end on satellite 2 transcripts in ovary RNA, is presented in Fig. 6 *b*. When the 23-base primer was used with ovary RNA, the expected 37-nucleotide extension was evident along with shorter products (lanes *3* and *4*). When the same primer was used with liver RNA several of the shorter products were not seen (lanes *1* and *2*). If the shorter products were due to failure of the reverse transcriptase to reach the 5′ end of the template, similar results should be seen in both ovary and liver RNA. Since the products differ in the two samples, we believe there is genuine heterogeneity of 5′ ends in ovary RNA.

Sequencing by Primer Extension

By adding the appropriate dideoxynucleotides to the primer extension reactions, it was possible to sequence the major satellite 2 transcripts in ovary RNA. An example using the 22-base primer is shown in Fig. 6 *c*. As is evident from this gel, the sequence was clearly readable. Ambiguities could be interpreted by reference to the control lane included on each sequencing gel. This control was an extension reaction without added dideoxynucleotides; it indicated positions where the reverse transcriptase reaction terminated regardless of the nucleotide at that position.

The entire ovary transcript was sequenced in this manner except for the extreme 5′ end and 44 nucleotides at the 3′ end. All four primers were used, and where the sequences overlapped, they were in agreement. Fig. 3 shows the sequence of the ovary transcript compared to the consensus sequence of satellite 2 DNA. These sequences differ by only 7 bases out of the 282 positions where both sequences are known. Homology between the RNA and the DNA consensus se-

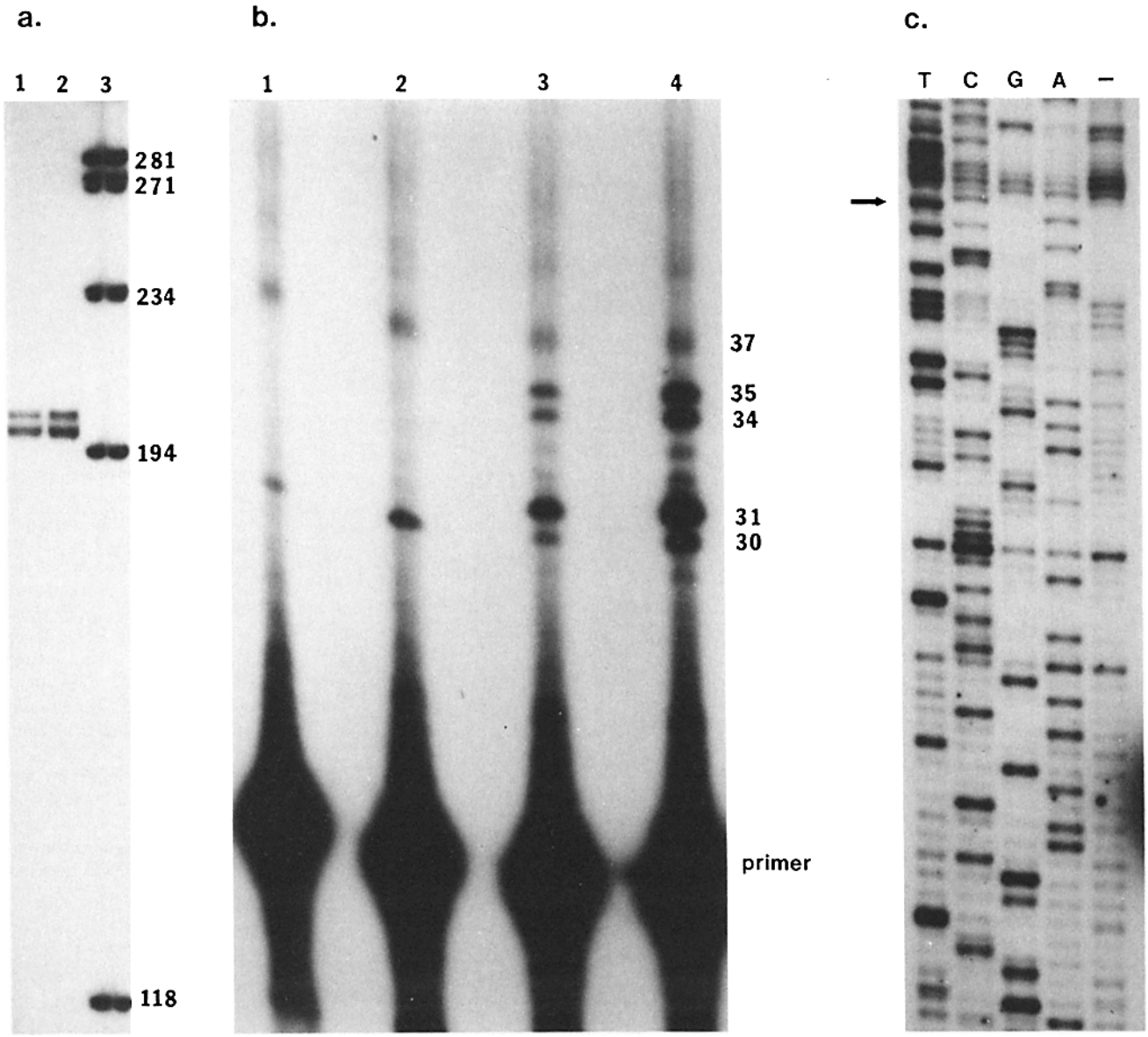

Figure 6. Primer extension analysis of satellite 2 transcripts. Primer extensions and RNA sequencing were performed as described in Materials and Methods. (*a*) Primer extension using 25 μg of total ovary RNA with the 17-base primer (lanes *1* and *2*); Hae III–digested, 5′ end-labeled ΦX DNA fragments (lane *3*). Sizes in bases. (*b*) Primer extension using 10 μg of two independent liver RNA preparations (lanes *1* and *2*) or 3 μg (lane *3*) or 10 μg (lane *4*) of ovary RNA with the 23-base primer. Sizes in bases. (*c*) Sequencing reactions (*T*, *C*, *G*, and *A*) and primer extension standard with no added dideoxynucleotides (−), using 25 μg of ovary RNA per lane with the 22-base primer. Arrow points to a 130-base extension product.

quence has important implications concerning the derivation of satellite 2 transcripts, as will be discussed later.

Discussion

Satellite 2 is a simple sequence DNA that occurs in tandemly repeated clusters throughout the genome of the newt, *N. viridescens*. In this respect it differs from many tandemly repeated satellite DNAs, which are localized in specific heterochromatic regions. It is also unusual in the degree of its sequence conservation among the salamandrid species so far tested (Fig. 3; Mahon, K., Z. Wu, H. C. Macgregor, G. Barsacchi-Pilone, unpublished results). These properties are not shared by satellite 1 of *Notophthalmus*, which is found predominantly in pericentric heterochromatin (Gall et al., 1981) and is not highly conserved among newt species (Mahon, K., and Z. Wu, unpublished results).

The stable transcripts homologous to satellite 2 DNA are also unusual. In most previously studied cases, transcripts from repetitive DNAs are either heterogeneous in size and contain both repetitive and single-copy elements (e.g., Costantini et al., 1980; Anderson et al., 1982), or, if discretely sized, are copied from only a part of a longer repeat unit. This latter organization is true for the rRNA genes transcribed by RNA polymerase I (Long and Dawid, 1980), the small nuclear U1 RNA genes transcribed by RNA polymerase II (Lund and Dahlberg, 1984), and the 5S RNA genes transcribed by RNA polymerase III (Long and Dawid, 1980). To our knowledge, the production of transcripts corresponding precisely in size to the repeat length of a satellite

DNA, or to whole multiples of the repeat, is unique and must result from a novel mechanism of transcription or RNA processing.

The stable, strand-specific transcripts from the ovary were sequenced with almost no ambiguities by primer extension in the presence of dideoxynucleotides (Fig. 6 *c*). This result implies that the RNA is homogeneous in sequence and the templates for this RNA must be equally homogeneous. By contrast, the cloned genomic repeats of satellite 2 diverge by as much as 19% from the consensus DNA sequence. Of particular importance is the fact that the differences between the eight clones include numerous single base insertions and deletions. If the stable transcripts were as heterogeneous as the DNA clones, it would not be possible to sequence the ovary RNA transcripts as a population because the distance from a primer to a specific base would vary among the individual transcripts.

We suggest, therefore, that the stable ovary transcripts are derived from a homogeneous subset of satellite 2 repeats. The location in the genome of these functional satellite 2 sequences is not known. To explain the close similarity between the RNA sequence and the DNA consensus sequence, we postulate that satellite 2 repeats spread throughout the genome at some time in the past, giving rise to many nonfunctional copies by the gradual accumulation of mutations. If the mutations were more or less random, the consensus sequence of the diverged copies would closely approximate the conserved functional sequence and the RNA it encodes.

Although the majority of satellite 2 sequences may now be nonfunctional in the sense that they do not code for stable, strand-specific transcripts, we believe that they are transcribed on the loops of lampbrush chromosomes. In earlier papers we have discussed the evidence from in situ hybridization that repetitive elements in the genome are extensively transcribed on lampbrush chromosome loops, and that such transcripts probably arise by readthrough from upstream structural gene promoters. Because satellite 2 repeats are both abundant and widely dispersed in the genome, they will be located downstream from many genes that are transcribed in the oocyte. Furthermore, since satellite repeats can have either orientation relative to upstream promoters, both strands should be transcribed with roughly equal probability. We showed by in situ hybridization that probes from both strands of satellite 2 hybridize to numerous lampbrush chromosome loops. It is our belief that the transcripts demonstrated by this hybridization are confined to the nucleus and do not contribute to the stable, strand-specific RNA population.

In summary, we postulate two kinds of satellite 2 transcription. The first, detected on the lampbrush chromosome loops, produces heterogeneous transcripts from both satellite 2 strands. This transcription is probably the result of RNA polymerase II, since this polymerase is responsible for the majority of the nascent transcripts on lampbrush chromosomes (Schultz et al., 1981). The second type of satellite 2 transcription must involve a homogeneous subset of the DNA repeats, and gives rise to stable, strand-specific RNA, most of which is in the cytoplasm.

It is not known which polymerase transcribes the stable satellite 2 transcripts, or what is the nature of the functional transcription units. If monomer-sized transcripts are produced by the transcription of individual DNA repeats within tandem arrays, internal transcription signals are required, such as those involved in RNA polymerase III transcription. The satellite 2 DNA clones lack certain characteristics of polymerase III genes; specifically, they do not have the conserved termination signal (Bogenhagen and Brown, 1981) and they do not serve as polymerase III templates in *Xenopus* oocyte nuclear extracts (Birkenmeier et al., 1978) (data not shown). But transcription by polymerase III, or the use of internal transcription signals, cannot be ruled out until a functional satellite 2 transcription unit is identified.

An alternative mechanism for the production of the smaller transcripts (monomers, dimers, etc.) is that they are formed by the processing of longer, multimeric transcripts. Currently we are testing the ability of multimeric transcripts to be cleaved either in vitro or in vivo (by injection into oocyte nuclei). We have evidence that synthetic dimer transcripts are self-cleaving under appropriate in vitro conditions.

We are especially grateful to Jennifer Varley and Herbert Macgregor (University of Leicester), who shared with us their original unpublished observations showing that the related satellite 2 of *Triturus cristatus* is homologous to a set of low molecular weight transcripts. We thank Christine Murphy for her skilled technical assistance.

This work was supported by National Institutes of Health research grant GM 33397. J. G. Gall is American Cancer Society Professor of Developmental Genetics.

Received for publication 13 June 1986.

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