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Molecular cloning and sequencing of OAX DNA: an abundant gene family transcribed and activated in *Xenopus* oocytes

Eric J.Ackerman

Laboratory of Molecular Biology, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH, UK

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OAX DNA codes for a 181 nucleotide long RNA whose transcription is strongly activated in somatic nuclei after their injection into a *Xenopus* oocyte nucleus. OAX RNA can be transcribed *in vitro* using an extract of *Xenopus* oocyte nuclei and total genomic DNA. Hybridization with OAX RNA as a probe indicates that OAX DNA is abundant in the *Xenopus* genome (at least 10^4 copies per genome). OAX DNA is present in tandemly repeated *Hind*III units of 752 bp. The complete DNA sequence of one of these OAX *Hind*III units is reported here. The OAX RNA transcript has been mapped within the OAX *Hind*III unit using S1 nuclease. Microinjection into *Xenopus* oocyte nuclei of either the OAX *Hind*III unit or a subclone containing only the RNA coding portion of the OAX *Hind*III unit both produce OAX RNA transcripts. This shows that the OAX promoter lies within the coding region of the RNA. The OAX RNA sequence has two elements which fit the RNA polymerase III promoter consensus sequence, and shows homology with dispersed RNA polymerase III transcription units in mammals.

Key words: gene family/small RNA/transcriptional activation/*Xenopus* oocytes

Introduction

OAX RNA, for oocyte activation in *Xenopus*, is strongly transcribed by somatic nuclei following its injection into the nucleus of an oocyte (Wakefield *et al.*, 1983). Previous work has shown that OAX RNA is not derived from rRNA, is transcribed by RNA polymerase III and is not of mitochondrial origin. OAX RNA is slightly larger than 5.8S rRNA and its *in vivo* function is currently unknown. OAX RNA transcripts can be detected in uninjected oocytes, indicating that OAX is not merely an artifact of nuclear injection experiments.

The transcription of OAX genes following injection of somatic nuclei into oocyte nuclei is substantial. In order to investigate OAX genes more fully, and in particular the mechanism of their transcriptional activation by oocytes, we have cloned and sequenced an OAX DNA repeat unit. The transcribed region has been identified, and a characteristic RNA polymerase III promoter recognized.

Results

OAX RNA is transcribed in vitro from total genomic DNA

It was necessary to produce a radioactive OAX RNA probe in order to clone its gene. Although the injection of somatic nuclei into oocytes can produce OAX RNA of high specific activity ($\sim 10^6$ c.p.m./μg), only 5 x 10^3 total c.p.m. result from 40 oocytes with injected nuclei. To avoid the need for hundreds of nuclear injection experiments and lengthy autoradiographic exposures while screening for OAX clones, *Xenopus* total genomic DNA was transcribed *in vitro* as shown in Figure 1. The most abundant stable transcript was 5S RNA; but a band co-migrating with OAX RNA was also transcribed. This band was shown to be OAX RNA (see below).

Approximately 5 x 10^5 c.p.m. of OAX RNA could be obtained by incubating 75 manually isolated oocyte nuclei and recovering the RNA from denaturing gels. This was sufficient probe to screen a library of *Xenopus* total genomic DNA in bacteriophage λ.

OAX DNA is abundant in the Xenopus genome and sometimes present in tandem repeats

Approximately 10^4 λ plaques were screened (Davis *et al.*, 1980) and 54 potential OAX clones were plaque purified and used for a second round of hybridization. 5S RNA made in the *in vitro* transcription reaction was also used to screen the same nitrocellulose filters. Only two plaques reacted with 5S RNA. Since 5S is present in 20 000 copies in the *Xenopus* genome, this indicates that OAX DNA is relatively very abundant. After a third round of plaque purification, seven of the positive-for-OAX λ clones were digested with *Eco*RI or *Eco*RI plus *Hind*III and electrophoresed on 1% agarose gels (Figure 2A). The gels were then hybridized to the OAX RNA probe (Figure 2B) as described by Southern (1975). Figure 2B shows that OAX DNA is present in tandem repeats of a *Hind*III unit ~0.7 kb in length in some of the λ clones, e.g., lanes 12 and 14, because bands appear at 1.4 kb and close to

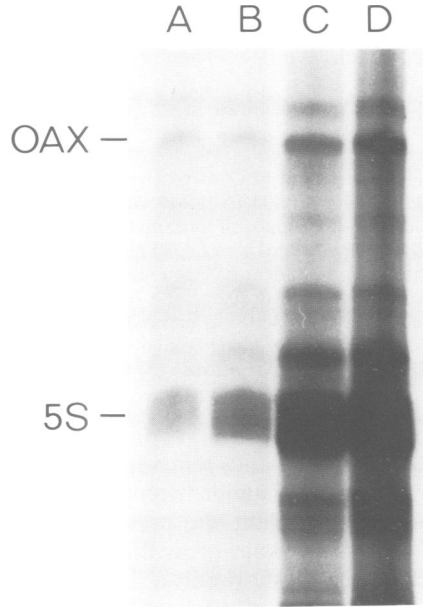

Fig. 1. *In vitro* transcription of total genomic *Xenopus* DNA produces OAX RNA. *Xenopus* total genomic DNA was prepared and transcribed as described in Materials and methods. The reactions were stopped by phenol extraction, ethanol precipitated, electrophoresed on denaturing gels (Lerner and Steitz, 1979) and autoradiographed. **Lanes A – D** represent 1, 2, 4 and 6 h of incubation, respectively; in the transcription extract.

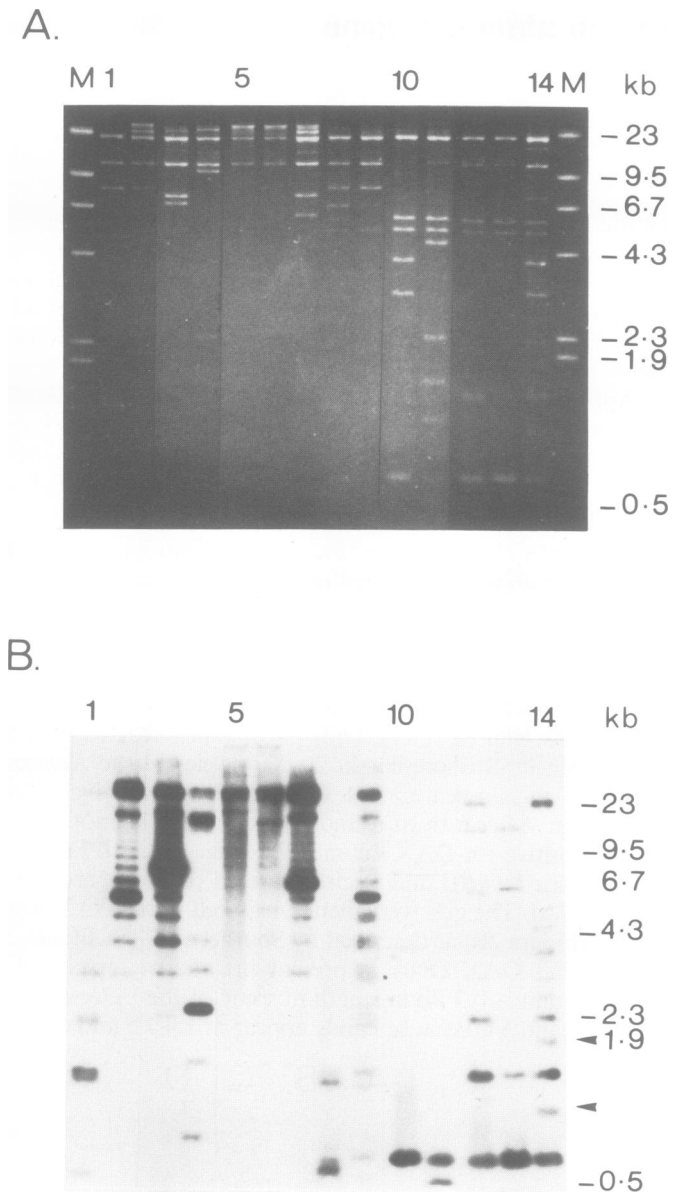

Fig. 2. Hybridization analysis of OAX DNA clones in bacteriophage λ. **(A)** Approximately 0.5 μg of seven λ DNA clones which hybridized with OAX RNA were digested to completion with three units of *Eco*RI **(lanes 1–7)** or the same seven clones were digested with *Eco*RI plus 3 units of *Hind*III **(lanes 8–14)**, electrophoresed on 1% agarose gels and stained with ethidium bromide. Size markers are *Hind*III digests of λ DNA (New England Biolabs). **(B)** The gel was then blotted to nitrocellulose and hybridized with OAX RNA probe as described in Materials and methods. The arrows next to **lane 14** indicate additional bands between the 0.7, 1.4, 2.1 etc., OAX repeats.

2.1 kb. We believe these bands represent a loss of the *Hind*III site in some of the OAX tandem repeats. Although partial digestion could also explain the presence of the 1.4- and 2.1-kb OAX bands, it is noteworthy that some of the λ clones (e.g., Figure 2B, lane 10) show only a single 0.7-kb band. This implies that conditions were sufficient for complete digestion. Further evidence that the 0.7-kb *Hind*III unit is present in multiple copies in some of the λ clones is provided by the molar excess of the OAX 0.7-kb band relative to the bands from the λ vector (seen between the 4.3- and 6.7-kb markers; Figure 2A, lanes 10–14). Notice that small bands such as the 0.5-kb λ-marker stain with ethidium bromide less efficiently than larger marker bands (Figure 2A, lane M). This less efficient ethidium staining of smaller bands further argues that OAX DNA must be in large molar excess to the λ vector. We interpret the presence of additional hybridizing bands between the 0.7-kb repeat units (e.g. Figure 2B, lane 14; denoted by arrows) as additional arrangements of OAX DNA.

Sequence analysis of OAX DNA

The OAX clone shown in Figure 2A, lane 12 was digested with *Hind*III and the 0.7-kb fragment was subcloned into the unique *Hind*III site of M13mp8 (Messing and Vieira, 1982) and sequenced by the dideoxy nucleotide method (Sanger *et al.*, 1980; Staden, 1982a, 1982b). Figure 3 shows the complete insert DNA sequence of one of these M13mp8 OAX clones, called mp80AX3. This *Hind*III OAX unit is 752 bases long. Two other M13mp8 OAX clones generated from the same λ 0.7-kb *Hind*III fragment have been partially sequenced and reveal at least four nucleotide changes within the *Hind*III DNA unit (data not shown). This shows that OAX DNA sequences are microheterogeneous.

Numerous stem-loop structures can be found within the OAX RNA transcript using the computer programs HAIRPN and HAIRGU (Staden, 1978). The longest of these potential stem structures is 8 bp at the 3′ end of the RNA, containing a loop of 25 nucleotides (positions 497-505 paired with 531–539).

There is a short potential open reading frame within the OAX RNA transcript which initiates with methionine at base pairs 400–402, continues for 25 amino acids, and terminates with a UAA stop codon, at base pairs 478–480. There are two more in-phase stop codons within the remainder of the OAX transcript, UGA at 496–498 and UAG at 517–519. We do not know the significance, if any, of this open reading frame.

The sequence of mp8OAX3 has a RNA polymerase III termination site (Bogenhagen and Brown, 1981) at base pairs 537–542: ATTTTG followed 7 bp later by a second termination site, at base pairs 549–555: CTTTTTC.

S1 mapping of OAX transcripts within the OAX HindIII unit

It was necessary to prove that the OAX-sized RNA transcribed *in vitro* from total genomic DNA is the same as the OAX RNA made in nuclear injection experiments. Therefore, the RNA resulting from a nuclear injection experiment was hybridized with a radioactive DNA probe made from the OAX clone whose sequence is shown in Figure 3. After treatment with S1 nuclease and electrophoresis on a polyacrylamide gel (see Figure 5, lane B), an OAX-sized S1-resistant hybrid was produced (Materials and methods). We then showed that the hybridization was due to OAX RNA resulting from the injection experiment and not to high levels of an endogeneous OAX-sized RNA in the oocyte. This was done by electrophoresing RNA resulting from a nuclear injection experiment as described previously (Wakefield *et al.*, 1983), eluting the OAX RNA band from the gel, and hybridizing this OAX RNA to the same probe. An OAX-sized S1-resistant band resulted as in Figure 5, lane B, and an equivalent analysis of uninjected oocytes did not produce an OAX-sized band (data not shown). Therefore the OAX *Hind*III unit shown in Figure 3 represents a true OAX RNA clone and OAX-sized RNA made by the *in vitro* transcription system is indeed OAX RNA.

The 5′ and 3′ termini of OAX RNA were determined by generating subclones of the OAX *Hind*III unit and mapping

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        10         20         30         40         50         60         70         80         90
AAGCTTAACAAAAACAGGCATTTGAGAAGCTGACTAAGCGAGTGCAGCTTTGGTGCGATTCTCGCCGAAAAGAAAGTAGAGTACGCTCTT
Hind III                     Alu I              Alu I                                  Rsa I

       100        110        120        130        140        150        160        170        180
GATTGCAGATTGCAGATCTGCATAGAATAAAGGGTTCTGTACAGCCATAAAGAAACCTGAAAAGTCACCCACCCGGGATTTGAACTCAAG
                                     Rsa I                                  Xma I

       190        200        210        220        230        240        250        260        270
ACCCCACGGTCAGAAACTTGCGTTTTAACCATTACACCACAGGAAACTAGACCTTATGCTACTGGTTCTGGATGACTGTAATAACCCATT

       280        290        300        310        320        330        340        350        360
GTCCTCTTGTGCCTCTGCATTGACCGGCAGGGCTGGAAGTCAATCTTCTGCCTAATAAAGTCAATACCAACCTGAGGCCCATTCCTAAAA
                                                                                Hae III

       370        380        390        400        410        420        430        440        450
GAGCCTGCTTGAATAGCTCAGTTGGTAGAGCGCGCGGCTATGGTTGATAACCTGGTCCAAAAGGTTGTGGGTTCAATTCCCACCTCTGCC
          Alu I                                                Bst NI,Ava II

       460        470        480        490        500        510        520        530        540
AAAAACGCAGCTCCAGTCTGCTGTGCCTAAACCGGCTCACGAGAGTGATGGAAGGGCAAGTGCCTGTAGTCCATCATCCGCCTTCCATTT
        Alu I

       550        560        570        580        590        600        610        620        630
TGCGGCCCCTTTTTCCAACTGGGGCTGTCCTTCTAAGCTACGCCGCTATGTACTTCTACTTCAGGCCAGGTCTTTCCCATCTAGCACCTT
  Hae III                          Alu I         Rsa I          Hae III,Bst NI

       640        650        660        670        680        690        700        710        720
GCCCTTTGCATCAACTTGGGTATTTTTTCCCCATACAGAACTCTTTTTGGGGTTTGCCTAGCATCAGCCTGAAGTTGACCTTGGCAAATT

       730        740        750
GATTAATGAGGACAGTGTTCTGCGCCAAGCTT
                   Hind III
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Fig. 3. Complete DNA sequence of an OAX *Hind*III unit coding strand. Restriction enzyme sites for *Ava*II, *Alu*I, *Bst*N1, *Hae*III, *Hind*III, *Rsa*I and *Xma*I are indicated. The underlined portion shows the region which codes for OAX RNA.

with S1. These subclones and fragments resulting after S1 are shown schematically in Figure 4. All of the subclones used for S1 mapping were sequenced as described in Materials and methods. Since the resulting S1-resistant bands are slightly heterogeneous in size (Figure 5), the termini are mapped to within ±2 nucleotides. The exact sizes of all the S1-protected fragments were determined on sequencing gels using *Msp*I-digested pBR322 and sequencing reactions as markers (data not shown). Transcription of OAX RNA ends in the first termination site, base pairs 557–561, rather than the second termination site at base pairs 568–575.

OAX RNA promoter elements

If either the OAX *Hind*III unit or the OAX *Hae*III subclone (348-545) described in Figure 4, part B, is injected into the nuclei of *Xenopus* oocytes, an OAX-sized RNA transcript is made (data not shown). Although we have not yet carefully quantitated the transcription efficiency of these clones relative to other RNA polymerase III genes such as 5S or tRNAs, the oocyte appears to transcribe these OAX M13 clones well.

Since the OAX *Hae*III subclone is only slightly larger than OAX RNA and is transcribed after microinjection, we conclude that the primary promoter for OAX RNA lies within its coding region. Figure 6 shows that OAX RNA has two regions which exactly fit the two consensus RNA polymerase III A box/B box promoter elements derived by Traboni *et al.* (1982). We have not determined if the sequence homology of OAX to the RNA polymerase III consensus promoter elements is biologically relevant as it appears to be for other

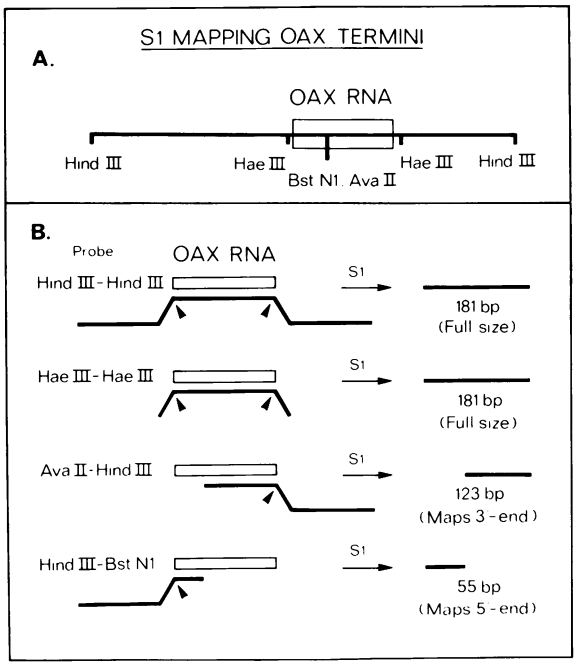

Fig. 4. Schematic diagram for S1 mapping OAX RNA within the OAX DNA *Hind*III unit. **(A)** OAX DNA *Hind*III unit with restriction sites necessary for producing subclones and probes for S1 mapping. **(B)** Probes used for S1 mapping and their resulting S1-resistant bands. *Hind*III-*Hind*III (base pairs 1–752) and *Hae*III-*Hae*III (base pairs 348–545): protect full size OAX RNA transcript of 181 nucleotides; *Ava*II-*Hind*III (base pairs 418–752): maps the 3′ end of OAX RNA and protects 121 nucleotides; *Hind*III-*Bst*N1 (base pairs 1–413): maps the 5′ end of OAX RNA and protects 55 nucleotides of OAX RNA.

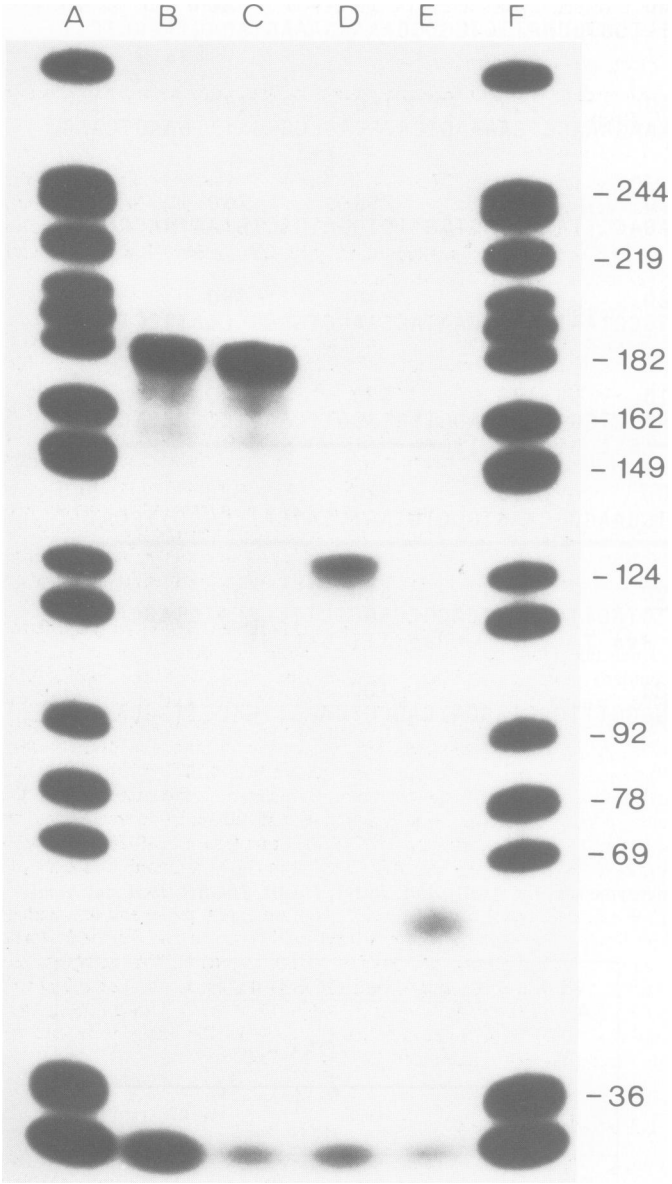

Fig. 5. S1 mapping of the OAX transcripts within the OAX *Hind*III unit. The OAX subclones and probes used for S1 mapping are shown schematically in Figure 4 and were prepared as described in Materials and methods. **Lanes A** and **F**: markers prepared from pBR322 digested with *Msp*I and labeled by end fill-in with [α-^{32}P]CTP and Klenow DNA polymerase; **lanes B** and **C**: full size OAX RNA transcripts protected by the entire *Hind*III-*Hind*III (base pairs 1–752) OAX probe and the *Hae*III-*Hae*III (base pairs 348–545) probe; **lane D**: 123 nucleotides of OAX RNA transcript protected by the *Ava*II-*Hind*III (base pairs 418–752) subclone which maps the 3′ end of OAX RNA; **lane E**: 55 nucleotides of OAX RNA transcript protected by the *Hind*III-*Bst*N1 (base pairs 1–413) subclone which maps the 5′ end of OAX RNA. Because the S1 hybrid for the *Hind*III-*Bst*N1 probe was only 55 nucleotides and has a high adenosine-thymidine content, the S1 hybridizations were done at 42°C instead of 49°C. Since the first 2 bp in the M13 vector were the same as the following 2 bp of OAX DNA (GG at base pairs 414–415), the S1-resistant hybrid was therefore 55 rather than 53 nucleotides.

RNA polymerase III genes such as tRNAs and 5S (Galli *et al.*, 1981; Ciliberto *et al.*, 1983).

Discussion

These results reveal the DNA sequence for a new class of gene, called OAX, which is abundant in the *Xenopus* genome

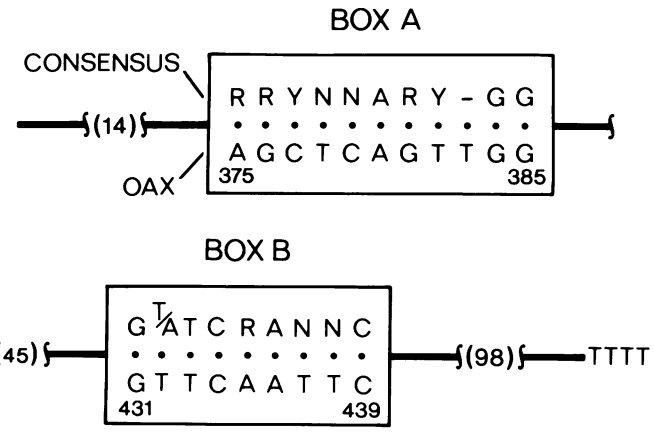

Fig. 6. OAX DNA contains the RNA polymerase III A box/B box promoter consensus sequence. The consensus is from Traboni *et al.* (1982) where R = purine, Y = pyrimidine, N = purine or pyrimidine and - = zero, 1 or 2 bp. The numbers in parentheses are the base pairs of the OAX RNA portion of the *Hind*III unit shown in Figure 3 which lie before, between and after the consensus promoter elements.

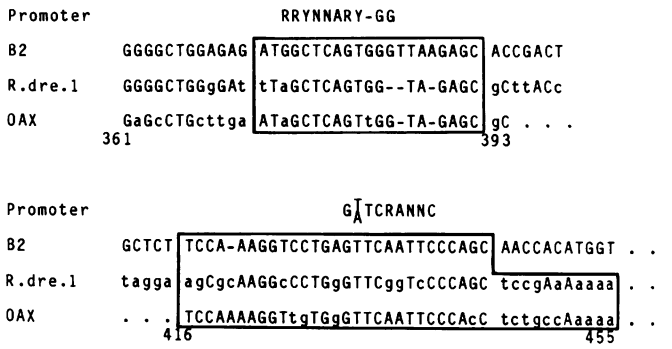

Fig. 7. OAX RNA is related to the rodent B2 and R.dre.1 families of repetitive DNA. The B2 consensus is from Rogers (personal communication) and is similar to that of Jelinek and Schmid (1982) and Krayev *et al.* (1982). R.dre.1 is a dispersed, abundant RNA family found in rats (Lemischka and Sharp, 1982). The promoter consensus is that of Traboni *et al.* (1982). The numbers under OAX bases correspond to those in Figure 3. Boxes indicate the principal regions of homology. Upper and lower case letters indicate homology or non-homology with B2; respectively.

and strongly activated when somatic nuclei are injected into the nucleus of an oocyte. We show here that OAX RNA can also be transcribed *in vitro* using *Xenopus* total genomic DNA and an extract derived from the nucleus of an oocyte.

OAX RNA is not homologous to *Alu* DNA and unlike *Alu* DNA (Singer, 1982; Jelinek and Schmid, 1982), OAX contains its own RNA polymerase III termination signals. OAX DNA is also different from the putative *Xenopus* replication origin (Chambers *et al.*, 1982) or the repetitive DNA sequenced by Spohr *et al.* (1981, 1982), which is present in dispersed copies $\sim 10^5$ times in the *Xenopus* genome. The non-homology of OAX to both DNA strands in the above examples was established using the computer program DIAGON (Staden 1982a; data not shown). OAX RNA is related (Figure 7) to the rodent B2 family of repetitive DNA (Jelinek and Schmid, 1982; Krayev *et al.*, 1982) and to the rat dispersed repetitive family R.dre.1 (Lemischka and Sharp, 1982). A relationship between B2 and R.dre.1 has been reported by Barta *et al.* (1981). R.dre.1 is postulated to be a tissue-specific identifier (ID) sequence and hybridizes to a 160

nucleotide RNA species which is present in rat brain, but not in liver or kidney (Sutcliffe *et al.*, 1982). This 160 nucleotide RNA has not yet been sequenced, so its exact homology to OAX RNA is unknown. The two regions of homology between OAX and B2 or R.dre.1 both occur around the two RNA polymerase III promoter sites but extend beyond them (Figure 7). Although all RNA polymerase III transcripts seem to contain the same promoter signals, OAX does not show as much homology to other RNA polymerase III transcripts such as *Xenopus* tRNAs (Kressmann *et al.*, 1979; Mueller and Clarkson, 1980; Galli *et al.*, 1981), *Alu* DNA or mouse B1 DNA (Jelinek and Schmid, 1982), (DIAGON comparisons, data not shown). The homology between OAX and B2 or R.dre.1 may represent the first example of a stable, discrete RNA related to these repetitive families, parts of which have been conserved from amphibia to rodents. An example of a functional RNA related to a repetitive DNA family has been reported in humans between 7S RNA and *Alu* DNA (Ullu *et al.*, 1982).

The S1 experiments using the entire OAX *Hind*III DNA unit as a probe for OAX RNA show that OAX RNA is not found in long transcription units and instead exists as a discrete transcript of 181 ± 2 nucleotides. This means that OAX RNA transcripts from the *Hind*III repeat units are not members of the class of repetitive transcripts described by Anderson *et al.* (1982) or Davidson and Posakony (1982) which can be found as double-stranded RNA moieties in the oocyte cytoplasm.

We do not yet know the functional role of OAX RNA in the cell. Its sequence does not reveal any obvious secondary structure which might provide a clue to its role. We have already shown that OAX transcripts exist in uninjected oocytes and can be found in the cytoplasm of some somatic cells and we are currently investigating the developmental regulation of OAX transcription (Ackerman and Gurdon, unpublished results).

Materials and methods

Oocytes, cells and nuclei

The preparation of OAX RNA from nuclei injected into *Xenopus* oocytes was as described previously (Wakefield *et al.*, 1983). All nuclear injections were done by J.Gurdon as described (Gurdon, 1977). Cultured cells of *X. laevis* were from a stable line maintained in this laboratory and derived from a kidney explant.

Preparation of Xenopus genomic DNA

Approximately 20 μg of genomic DNA was prepared from 10^7 cultured *Xenopus* cells by the following procedure, with all steps carried out at 0°C. Cells were washed with 0.9% NaCl, harvested by centrifugation at 400 *g* for 5 min and then swollen in 6.3 ml of isolation buffer (10 mM Tris pH 7.5, 10 mM NaCl, 5 mM $MgCl_2$) for 30 min. Cells were then broken by the addition of 0.7 ml of 5.0% (v/v) Nonidet P-40 (NP-40) in isolation buffer and 20 strokes with a tight dounce homogenizer. The mixture was underlaid with an equal volume of 0.8 M sucrose in isolation buffer with 0.5% NP-40 and centrifuged at 700 *g* for 5 min. The purified nuclei were resuspended in 2 ml of lysis buffer (40 mM Tris pH 7.5, 300 mM NaCl, 1.7% SDS) containing 2 mg/ml proteinase K for 5 min at 37°C. The DNA was precipitated by addition of 2.5 volumes of 95% ethanol at 4°C for 5 min. The DNA was then removed by spooling with a drawn capillary and transferred to 10 ml of 10 mM Tris pH 7.5, 1 mM EDTA. After redissolving, the DNA was extracted with phenol and precipitated with ethanol.

In vitro transcription

20 μg/ml of total genomic DNA was transcribed according to Birkenmeier *et al.* (1978) using [α-^{32}P]GTP (400 Ci/mmol) from Amersham. Reaction times were generally for 2 h except as noted.

Xenopus total genomic library in λ

A *Hae*III-*Alu*I library of *Xenopus* total genomic DNA in λ was constructed and provided by R.Patient (Kings College, London) and was described previously (Patient *et al.*, 1980).

Cloning and hybridizations

All screenings and hybridizations were carried out as described in Davis *et al.* (1980).

DNA sequencing

The sequence of the OAX *Hind*III fragment was determined using the M13/dideoxy nucleotide method (Sanger *et al.*, 1977, 1980; Heidecker *et al.*, 1980; Messing and Vieira, 1982). The OAX *Hind*III DNA was reduced to smaller sizes using subclones generated by *Rsa*I, *Alu*I, *Hae*III, *Bst*NI or *Ava*II digestions and cloned into M13mp9.

S1 mapping

Single-stranded probes for S1 mapping were prepared by the 'prime, cut' method. This method produces probes of ~10^8 c.p.m./μg with [α-^{32}P]dATP. The sequencing primer and Klenow DNA polymerase under ATP-limiting conditions are used to produce an internally labeled DNA strand on the single-stranded M13 template DNA. The probe is then removed from the template DNA using a restriction enzyme which cuts only on the downstream end of the probe, followed by fractionation on a denaturing gel. In order to avoid a strand-separation step in preparing the single-stranded probe, it is essential not to cut the 5′ end of the probe. This is because the 5′ end of the probe is already determined by the sequencing primer. Since the complementary cold strand is much larger than the probe, the probe is easily separated on the gel.

Approximately 1 μg of single-stranded M13 clone DNA is hybridized to the sequencing primer in a 22 μl reaction volume; i.e., double the volume of a standard sequencing annealing reaction. Then 10 μCi of 30 μCi [α-^{32}P]dATP (400 Ci/mmol), 17 μM each of dGTP, dCTP and dTTP is added and the complementary strand synthesis begun by addition of 0.2 units of Klenow DNA polymerase (Boehringer). After incubation for 30 min at 20°C, the synthesis is stopped by adding 2 μl of 0.5 mM each of dATP, dCTP, dTTP and dGTP and heating at 70°C for 2 min. The reaction is then digested with the appropriate restriction enzyme, precipitated with ethanol, and fractionated on a denaturing 5% polyacrylamide gel (Sanger and Coulson, 1978). After autoradiography the probe is eluted by soaking in 0.5 M ammonium acetate pH 5.3, 5 mM EDTA.

Hybridizations (15 μl) contained 2 – 10 x 10^5 c.p.m. probe and one tenth of the RNA from a single oocyte injected with somatic nuclei in 50% formamide, 0.5 M NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA. Samples were then treated with 2000 units S1 nuclease (Miles) after addition of 200 μl cold S1 buffer, 30 mM sodium acetate pH 4.5, 0.25 M NaCl, 1 mM $ZnSO_4$ (Berk and Sharp, 1978). After digestion for 30 min at 37°C, samples were analyzed on denaturing gels (Sanger and Coulson, 1978).

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References

Anderson,D.M., Richter,J.D., Chamberlin,M.E., Price,D.H., Britten,R.J., Smith,L.D. and Davidson,E.H. (1982) *J. Mol. Biol.*, **155**, 281-309.
Barta,A., Richards,R.I., Baxter,J.D. and Shine,J. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 4867-4871.
Berk,A.J. and Sharp,P.A. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1274-1278.
Birkenmeier,E.H., Brown,D.D. and Jordan,E. (1978) *Cell*, **15**, 1077-1086.
Bogenhagen,D.F. and Brown,D.D. (1981) *Cell*, **24**, 261-270.
Chambers,J.C., Watanabe,S. and Taylor,J.H. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 5572-5576.
Ciliberto,G., Raugei,G., Costanzo,F., Dente,L. and Cortese,R. (1983) *Cell*, **32**, 725-733.
Davidson,E.H. and Posakony,J.W. (1982) *Nature*, **297**, 633-635.
Davis,R.W., Botstein,D. and Roth,J.R. (1980) in *Advanced Bacterial Genetics*, published by Cold Spring Harbor Labortory Press, NY.
Galli,G., Hofstetter,H. and Birnstiel,M.L. (1981) *Nature*, **294**, 626-631.
Gurdon,J.B. (1977) *Methods Cell Biol.*, **14**, 125-139.
Heidecker,G., Messing,J. and Gronenbourn,B. (1980) *Gene*, **10**, 69-73.
Jelinek,W.R. and Schmid,C.W. (1982) *Annu. Rev. Biochem.*, **51**, 813-844.

Krayev,A.S., Markusheva,T.V., Kramerov,D.A., Ryskov,A.P., Skryabin, K.G., Bayev,A.A. and Georgiev,G.P. (1982) *Nucleic Acids Res.*, **10**, 7461-7475.
Kressmann,A., Hofstetter,H., di Capua,E., Grosschedl,R. and Birnstiel, M.L. (1979) *Nucleic Acids Res.*, **7**, 1749-1763.
Lemischka,I. and Sharp,P. (1982) *Nature*, **300**, 330-335.
Lerner,M.R. and Steitz,J.A. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5495-5499.
Messing,J. and Vieira,J. (1982) *Gene*, **19**, 269-276.
Mueller,F. and Clarkson,S.G. (1980) *Cell*, **19**, 345-353.
Patient,R.K., Elkington,J.A., Kay,R.M. and Williams,J.G. (1980) *Cell*, **21**, 565-573.
Sanger,F. and Coulson,A.R. (1978) *FEBS Lett.*, **87**, 107-110.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5468.
Sanger,F., Coulson,A.R., Barrell,B.G., Smith,A.J.H. and Roe,B. (1980) *J. Mol. Biol.*, **143**, 161-178.
Singer,M.F. (1982) *Cell*, **28**, 433-434.
Spohr,G., Reith,W. and Sures,I. (1981) *J. Mol. Biol.*, **151**, 573-592.
Spohr,G., Reith,W. and Crippa,M. (1982) *Dev. Biol.*, **94**, 71-78.
Staden,R. (1978) *Nucleic Acids Res.*, **5**, 1013-1015.
Staden,R. (1982a) *Nucleic Acids Res.*, **10**, 2951-2961.
Staden,R. (1982b) *Nucleic Acids Res.*, **10**, 4731-4751.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
Sutcliffe,J.G., Milner,R.J., Bloom,F.E. and Lerner,R.A. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4942-4946.
Traboni,C., Ciliberto,G. and Cortese,R. (1982) *EMBO J.*, **1**, 415-420.
Ullu,E., Murphy,S. and Melli,M. (1982) *Cell*, **29**, 195-202.
Wakefield,L., Ackerman,E.J. and Gurdon,J.B. (1983) *Dev. Biol.*, **95**, 468-475.

Note added in proof

After submission of this manuscript, Lam and Carroll (1983, *J. Mol. Biol.*, **165**, 567-585) described a tandemly repeated DNA sequence from *Xenopus laevis* which is related to OAX DNA. These authors were unable to detect any transcripts homologous to their DNA family.